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Capillary zone electrophoresis of basic analytes in methanol as non-aqueous solvent
Mobility and ionisation constant

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Abstract

The electrophoretically relevant properties of monoacidic 21 bases (including common drugs) containing aliphatic or aromatic amino groups were determined in methanol as solvent. These properties are the actual mobilities (that of the fully ionised weak bases), and their pK_a values. Actual mobilities were measured in acidic methanolic solutions containing perchloric acid. The ionisation constants of the amines were derived from the dependence of the ionic mobilities on the pH of the background electrolyte solution. The pH scale in methanol was established from acids with known conventional pK^*_a values in this solvent used as buffers, avoiding thus further adjustment with a pH sensitive electrode that might bias the scale. Actual mobilities in methanol were found larger than in water, and do not correlate well with the solvent's viscosity. The pK^*_a values of the cation acids, HB^+, the corresponding form of the base, B, are higher in methanol, whereas a less pronounced shift was found than for neutral acids of type HA. The mean increase (compared to pure aqueous solution) for aliphatic ammonium type analytes is 1.8, for substituted anilinium 1.1, and for aromatic ammonium from pyridinium type 0.5 units. The interpretation of this shift was undertaken with the concept of the medium effect on the particles involved in the acid–base equilibrium: the proton, the molecular base, B, and the cation HB^+.

Keywords: Electrophoretic mobility; Ionisation constant; Buffer composition; Dissociation constants; Methanol; Amines; Basic drugs

1. Introduction

Separation in capillary electrophoresis is based on differences in the ionic mobilities of the separands. Beside the non-specific mobility of the electroosmotic flow (if occurring) the migration of the solute is governed by the mobility of the fully charged particle (the actual mobility) and its degree of ionisation. The degree to which a particle is charged depends on chemical equilibria, mostly protolysis in capillary electrophoresis (CE). In this case the charge depends on the pK_a of the solute and on the pH of the buffering background electrolyte (BGE) solution.

Although water is the most common solvent, organic solvents are applied as well in order to adjust separation selectivity (cf. e.g., Refs. [1–4]). Con-

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cerning the solute the solvent can affect both parameters, the actual mobility and the pK_a. It is well known that methanol, the non-aqueous solvent used for the present investigation, shifts the pK_a values of acids and bases to a different extent. Whereas neutral acids of type HA exhibit an increase by up to 5 pK_a units related to water as solvent, the effect on a cation acid of type HB^+ (the conjugated acid of base, B) is not as straightforward; however, it is much less pronounced [5–9]. pK_a values are measured in organic solvents by several methods, e.g., by spectrophotometry, conductimetry, or, most common, by potentiometry. In non-aqueous solvents, the potentiometric measurement might be rather crucial, because the solutions of reference buffer and salt bridges are normally aqueous. Therefore unknown liquid junction potentials occur, which bias pH values derived from the EMF measured. Capillary electrophoresis, on the other hand, offers an elegant method to determine pK_a values in such solvents directly by measuring the dependence of the effective mobility of an analyte as a function of the pH of the background electrolyte solution [10–15]. It is clear that in this case a pH scale should be used based on known pK_a values of the BGE in this solvent. If the pK_a can be taken from the literature, no further measurement of the buffering pH is needed, taking an equimolar mixture of the acid and its salt: it is identical with the pK_a. However, attention must be directed onto the kind of pH scale, and on the type of pK_a expressed by this scale. It is clear that other equilibria than protolysis, especially ion pairing must not be present, otherwise the EMF data lead to biases pK_a values as well. For methanol as amphiprotic solvent with high dielectric constant (see the classification common in the literature [9,16] these preconditions are more or less (whereas not necessarily always) fulfilled.

As the effective mobility reflects the degree of ionisation, the application of the Henderson–Hasselbalch equation allows to derive the pK_a value of an analyte. In principle only two measuring values of the mobility would suffice for the determination of the pK_a of monovalent bases: one at low pH, where the base is fully protonated, and the second at a pH where it is only partially ionised.

It was the main goal of this paper to investigate the change of the pK_a values of a number of nitrogen bases in non-aqueous methanol as solvent, using capillary zone electrophoresis (CZE). Acids with defined conventional pK^*_a values taken from the literature were used as buffers. Note that the asterisk (*) indicates that the value is referred to infinite dilution as selected standard state. The buffers covered the pH* range between 4.9 and 9.7. No further adjustment of the pH* in methanol was necessary, and a pH* scale was established in this way, which is not biased by the use of aqueous reference solutions. These buffers enable to measure the effective mobilities. For the determination of the actual mobility (of the fully protonated analyte) in acidic solution, on the other hand, perchloric acid was used, because it is a strong acid also in methanol, where it fully dissociates. Under these circumstances the application of the Henderson–Hasselbalch equation allows the calculation of the pK^*_a values. In order to facilitate an interpretation of the results, only monoacidic bases were selected as analytes.

2. Experimental

2.1. Chemicals

Aniline, *N*-methylaniline, *N*,*N*-dimethylaniline, *N*-isopropylaniline, 2-methylaniline (*o*-toluidine), 3-methylaniline (*m*-toluidine), 4-methylaniline (*p*-toluidine), 2,6-dimethylaniline, 2-methylpyridine (2-picoline), 3-methylpyridine (3-picoline), 4-methylpyridine (4-picoline) and isoquinoline were from Fluka (Buchs, Switzerland). Ephedrine hydrochloride and quinoline were from Sigma (St. Louis, MO, USA). Pyridine was from J.T. Baker (Deventer, The Netherlands), 4-Methoxypyridine from Aldrich (Milwaukee, WI, USA). Amphetamine sulphate, dipipanone hydrochloride and methadone hydrochloride were kindly donated by the National Bureau of Investigation Crime Laboratory (Vantaa, Finland). Noscapine and papaverine hydrochloride were gifts from the Department of Pharmacy, University of Helsinki, Finland.

Acetic acid (glacial), chloroacetic acid, potassium dichloroacetate, sodium chloroacetate and sodium trichloroacetate were from Aldrich. Dichloroacetic acid, 70% perchloric acid, sodium acetate and tri-

chloroacetic acid were from Fluka. Solid buffer reagents were dried over phosphorus pentoxide in vacuum before use. All buffer reagents were of highest grade of purity commercially available.

HPLC grade methanol (MeOH) was from J.T. Baker; it was stored in nitrogen atmosphere in order to avoid the uptake of moisture from air during storage. Analytical-grade dimethyl sulphoxide (DMSO) was from Lab-Scan (Dublin, Ireland). Hydranal-Coulomat AD Karl Fischer reagent was from Riedel-de Haën (Seelze, Germany), sodium hydroxide from Merck (Darmstadt, Germany). Water was distilled with an Aquatron A4S apparatus (Bibby Sterilin, Staffordshire, UK) and further purified with a Water-I system from Gelman Sciences (Ann Arbor, MI, USA).

2.2. Instrumentation and operational conditions

Capillary electrophoretic experiments were carried out with HP ^{3D}CE system (Hewlett-Packard, Waldbronn, Germany) equipped with an on-column diode-array detector. Instrument control and data analysis were performed with HP Chemstation software. Uncoated fused-silica capillaries (Composite Metal Services, Hallow, UK) of 50 μm I.D.×375 μm O.D. were used with an effective length of 50.0 cm and a total length of 58.5 cm. Polyimide coating at each end of the capillary was removed by burning. The capillary cassette temperature was maintained at 25.0°C with air cooling system, and the autosampler temperature was adjusted to 25.0±0.5°C with an external water bath (Heto, Birkeröd, Denmark). Running voltage was +10 kV and the resulting electric currents were 2–2.3 μA for acetate buffers, ca 4.9 μA for methanolic perchloric acid solution, and ca 11.6 μA for aqueous perchloric acid solution. Sample injection was made by 50 mbar pressure for 1.5 s (MeOH) or 2.4 s (water).

Due to weak electroosmotic flow (either anodic or cathodic) in some cases, all the electrophoretic mobilities were measured according to the method by Williams and Vigh [17] as follows: in the first step, the injected analyte and electroosmotic flow marker (DMSO) were transferred into the thermostatted region of the capillary by applying the injection pressure for 2–3 min. Then, a running voltage of +10 kV was applied, and the analyte and DMSO were allowed to separate for 5 min. The separation was carried out inside the thermostatted region of the capillary. Next, the second DMSO band was injected into the capillary, and finally all three bands were mobilised through the detector window by applying the injection pressure. The electrophoretic mobility of the analyte was calculated using the distance between the analyte peak and the DMSO peak from the first injection. Pressure delay time (ca 0.05 min), pressure ramp-up time (ca. 0.06 min), linear voltage ramp-up time (0.17 min) and voltage ramp-down time (0.17 min) were considered in the calculations of the electrophoretic mobilities. Because the method used for electrophoretic measurements relies on constant pressure transport, it was necessary to slightly modify the pressure control of the HP ^{3D}CE system.

UV detection of analytes and DMSO was carried out at 200 nm except when analyte mobilities were so low that analyte peak and DMSO peak could not be separated from each other well enough. In those cases the analytes were detected at wavelengths (240 or 254 nm) where DMSO has no significant UV absorbance.

Water content of the BGE solutions was measured by Karl Fischer titration using 756 KF Coulometer without diaphragm (Metrohm, Herisau, Switzerland). Measured pH values of buffer solutions were obtained with a PHM220 Lab pH Meter and a pHC2401-8 combined glass electrode (both from Radiometer, Lyon, France) at 25.0±0.1°C. The pH equipment was calibrated with aqueous standard buffer solutions.

2.3. Procedures

The stock solutions of analytes were prepared at concentrations of 50 m*M* or 5 m*M* in methanol or in water, respectively. The stock solutions were diluted to final concentration (0.5 m*M* each) with the BGE right before analysis. When limited solubility in water prevented the use of higher concentrations, the analyte was prepared directly at concentration of 0.5 m*M*. DMSO was used at concentration of 10 m*M*; this solution was prepared either together with the analyte under investigation or directly diluted with the BGE. The stock solutions of light-sensitive analytes were stored in brown bottles.

Buffer solutions were prepared by adding equimolar amounts of the acids and their respective sodium (or potassium) salts at concentrations of 7.9 m*M* except for acetate buffer (pH* 10.7) which was prepared with 1:10 as concentration ratio of acetic acid and sodium acetate. The pH* values were taken without further adjustment. Perchloric acid was used at concentration of 7.9 m*M*. The BGE solutions were prepared daily and they were filtered through 0.45-μm Acrodisc filters (Pall, Ann Arbor, MI, USA) prior to use. All these solutions were prepared at room temperature (25.0±0.5°C).

Before use the capillary was rinsed with 0.1 mol/l sodium hydroxide (dissolved in respective solvent), with pure solvent, and with the BGE, respectively. Between runs the capillary was rinsed for 2 min with the BGE. The BGE solution in the vials was replaced after every third run except for unbuffered perchloric acid solution, which was replaced after every run. After use the capillary was rinsed with pure solvent and dried with air by flushing from an empty vial.

3. Results and discussion

3.1. BGE and pH scale

The pH scale, which is common in water, has limited applicability in organic solvents and thus restricts the specification of the acidity of a solute. It is obvious that the pH scales are related to the activity of the solvated proton in the respective solvent. An analogue acidity scale like in water is seemingly applicable to those solvents for which the Brønsted acid–base theory is compatible. The applicability decreases with decreasing dielectric constant (the ''polarity'') and with increasing aprotic character of the solvent. Whereas for the definition of the acidity scale a problem does not occur in theory, the approximation of the measured values of the proton activity to the specifications defined might be difficult in practice.

We therefore distinguish different acidity scales: the standard, conventional, operational and absolute (thermodynamic) scale, respectively (cf. e.g., Ref. [18]). Briefly, the *standard* scale uses a measuring cell without liquid junction potential, refers, e.g., to infinite dilution as standard state, and assumes that the activity of the solvated proton and the counter-ion is the same. The *conventional* scale tries to circumvent the problem associated with the single ion activity coefficient by deriving the activity coefficient of chloride as counter-ion from its Debye-Hückel parameters. More practical is the *operational* scale using buffer solutions with known conventional pH for the calibration of cells with liquid junction, e.g., the convenient glass electrode. Unknown samples are measured in the cell calibrated in this way. For the standard buffers the conventional and the operational pH is the same, which is not necessarily the case for the unknown samples. The *absolute* or *thermodynamic* scale would allow to establish a universal scale valid for all solvents, based on the transfer activity coefficient on the proton. Unfortunately, this transfer activity coefficient is not derivable by thermodynamic methods. Results obtained by the different approximation methods vary widely (see below), and therefore no unequivocal absolute scale has been established yet.

As most convenient for electrophoretic practise, whereas probably least accurate, might be an *apparent* pH, measured in the usual way e.g., with a glass electrode using aqueous solutions and aqueous calibration buffers as well. All scales might lead to different pH values of the buffer solution, and thus different pK_a values of the solutes. It is therefore of importance to differentiate what kind of pH is taken as basis of the measurement, and define the conditions under which the pK_a of the analyte is derived. For a comparison of the pK_a values in different solvents they must be specified. Unfortunately, a clear distinction is not always made in the literature.

For the determination of the pK^*_a of the analytes from the mobility dependence on the pH*, the pK^*_a values of the acids used as buffer constituent in methanol must be given. Therefore the following acids were selected: acetic, chloroacetic, dichloroacetic and trichloroacetic acid with known conventional pK^*_a values of 9.7, 7.8, 6.3 and 4.9 [19–21], respectively. These acids are only weakly UV absorbing at the wavelengths normally used in CZE, and are also favourable from this point of view. The free acid and the corresponding salt in equimolar proportion were taken as reference buffering systems in methanol without any further calibration, because it follows from the fundamental relations of thermo-

dynamics that this solution has pH* values equal to the pK^*_a. In this way the problems related to unknown liquid junction potentials, deviations due to calibration with purely aqueous buffers, etc., is circumvented. It is obvious that these acids must be weak at least in methanol, otherwise their solutions have no buffering ability.

An interesting aspect is how the *apparent* pH values of solutions containing equimolar mixtures of free acids and their salts deviates from the *conventional* pK_a of the corresponding acids. A comparison of these values both in water and in methanol is given in Table 1. It can be seen that the pH values in water for acetic acid and chloroacetic acid agree with the conventional pK_a values. It is clear that dichloroacetic acid and trichloroacetic acid are too strong in water as their acid–salt mixtures would obey the conditions for the Henderson–Hasselbalch equation.

Interestingly, in methanol the corresponding *apparent* pH is about 2.7 units lower than the conventional pK^*_a value. If the glass electrode is used as common in practice to measure pH values in methanol, the resulting pK_a values obtained from potentiometric titrations are thus about 2.7 units too low, at least in the considered pH range. It should be noted that this problem is seemingly more pronounced in non-aqueous solvents. In mixed aqueous–organic solvents with not too low water content the deviation of the apparent from the conventional value is much lower, and even nearly negligible in some cases. For aqueous ethanolic solvents, e.g., with an ethanol content up to 80%, indeed a good agreement between the apparent and conventional pK_a values was found [22].

3.2. Mobilities and pK^*_a values

3.2.1. Mobility and viscosity

We can expect that all analytes are fully protonated in a sufficiently acidic BGE. To obtain actual mobilities a solution of 7.9 m*M* perchloric acid (without buffering capacity) in the solvent was used (the pH* value is around 2). Data in both, the aqueous and the methanolic solution are given in Table 2. All electrophoretic mobilities are averages of three replicate measurements with typical relative span less than 1% (except for some very low mobilities below 10^{-10} m^2 V^{-1} s^{-1}, for which the span was about one order of magnitude larger). It can be seen that the actual mobilities are higher in methanol than in water in all cases. The difference varies strongly, between about 10 and 50%. However, a trend is observable from the data: roughly, the difference between the actual mobilities is higher, the smaller the mobility is.

The solvent can affect the actual mobilities in two ways: due to the viscosity, and due to the size of the ion determined by its solvation shell. Indeed the viscosity of methanol (0.545 cP at 25°C) is smaller than that of water (0.8903 cP). The effect of the solvent viscosity on the (absolute) mobility is reflected by Walden's rule, which states constancy of the product $\mu_0\eta$. Strictly, the rule is defined for limiting concentration. As all analytes under consideration have the same charge $(+1)$ and a similar size, the finite ionic strength should have the same effect on the actual mobilities for all analytes. Therefore we can assume that an analogue to Walden's rule is applicable to the present conditions.

Table 1
pK_a values of acids used as buffers in the methanolic BGE, and pH values of solutions consisting of equimolar mixtures of the free acids and their salts (ionic strength 7.9 m*M*)[a]

Acid	Water		Methanol	
	pK_a	pH	pK^*_a	pH
Acetic	4.756 [41]	4.73	9.7 [21]	6.99
Chloroacetic	2.87 [41]	2.92	7.8 [21]	5.10
Dichloroacetic	1.35 [41]	2.23[b]	6.3 [21]	3.72
Trichloroacetic	0.63 [42], 0.70 [19,20]	2.16[b]	4.90 [19,20]	2.26

[a] The pH was measured with a combined all aqueous glass electrode, calibrated with aqueous buffers. Temperature, 25°C.
[b] Note that these solutions are composed from a strong acid (in aqueous media) and its salt; it does not follow the Henderson–Hasselbalch equation, and its pH is not equal to the pK_a of the acid.

Table 2
Actual mobilities, $\mu_{act,i}$, of the analytes in water and methanol as solvent of the BGE (7.9 m*M* perchloric acid, pH~2.1): temperature 25°C

Analyte	$\mu_{act,i}$ (10^{-9} m^2 V^{-1} s^{-1})	
	Water	MeOH
Aliphatic N		
Amphetamine	29.58	35.71
Ephedrine	26.34	32.13
Methadone	20.19	29.39
Dipipanone	18.59	27.63
Noscapine	19.39	23.47
Aniline N		
Aniline	36.51	41.95
N-Methylaniline	35.16	44.33
N,*N*-Dimethylaniline	34.65	44.29
N-Isopropylaniline	28.52	38.53
2-Methylaniline	33.75	39.62
3-Methylaniline	33.66	40.50
4-Methylaniline	33.95	39.68
2,6-Dimethylaniline	31.64	38.68
Aromatic N		
Pyridine	47.87	53.10
2-Methylpyridine	40.73	50.41
3-Methylpyridine	42.53	51.51
4-Methylpyridine	42.67	51.01
4-Methoxypyridine	40.20	48.59
Quinoline	36.27	45.69
Isoquinoline	37.20	45.59
Papaverine	19.00	25.68

However, it is known that this rule overestimates the role of the viscosity, which means that a more appropriate expression describes its influence by the value of the exponent, p, being <1:

$$\mu_{act}^{W}(\eta^{W})^{p} = \mu_{act}^{MeOH}(\eta^{MeOH})^{p} \quad (1)$$

For 26 anionic aromatic compounds in mixed aqueous–organic solvents consisting of lower alcohols or acetonitrile, respectively, as co-solvent (with an organic content up to 60%, v/v) an exponent of 0.9 leads to accordance with the extended Walden product within only few percent [23]. In the present work the values of p are far from unit, and most of them are even far from 0.9. They are in a wide range between 0.8 and 0.2. Note that an exponent of zero means that the mobility is independent of the solvent. A rough tendency for the exponents is observable: the smaller the mobility, the larger is the exponent. However, the results indicate again that an interpretation of the role of the solvent on mobilities must take into account the effects of specific ion solvation.

3.2.2. Effective mobility and pK^*_a

The effective mobilities of the analytes were determined at various conventional pH* of the methanolic BGE. The values are given in Table 3. Note that the pH* range was enlarged by preparing acetate buffer with pH* of 10.7. The pK^*_a values were determined from the effective mobility as function of the conventional pH* following the well-known relation:

$$\mu_{eff} = \frac{\mu_{act}}{1 + 10^{pH^* - pK^*_a}} \quad (2)$$

Although in principle a one-point measurement should suffice for the determination of the pK^*_a (the actual mobility given), calculation was carried out using four to six data points by non-linear curve fitting to Eq. (2). Typical curves for four analytes are shown in Fig. 1.

The result of the curve fit is given in Table 4. It should be mentioned that the pH* range is not optimal for the four bases with aliphatic amino group (namely amphetamine, ephedrine, methadone and dipipanone), because due to their large pK^*_a values the pH* region of the inflection point and higher is not reached. It can be seen that for all analytes under consideration the pK^*_a value is higher in methanol than in water. The changes are significantly smaller than those for neutral acids. This result is in accordance with the tendency known from the literature (cf. e.g., Refs. [24–26]). Whereas for neutral acids the increase is about 5 pK^*_a units (see also the data of Table 1), the maximum increase for the cation acids is only 2.7 units. This maximum increase is observed for amines with aliphatic *N*. It can be seen that the average pK^*_a shift differs according to the type of analyte: the amines with aliphatic *N* exhibit the largest change with a mean ΔpK^*_a of 1.8 for the five compounds. The anilinium-related compounds change the pK^*_a in average by 1.1 units, whereas those compounds with the nitrogen atom being part of the aromatic ring show an only slight increase by 0.5 units.

It is of high interest to what extend small amounts or even traces of water affect the pK^*_a values of the

Table 3
Effective mobilities of the analytes at different pH* of the methanolic BGE: temperature 25°C; ionic strength 7.9 m*M*

Analyte	Effective mobility (10^{-9} m^2 V^{-1} s^{-1})				
	pH* 4.9	pH* 6.3	pH* 7.8	pH* 9.7	pH* 10.7
Aliphatic N					
Amphetamine	33.20	33.18	32.54	31.84	27.56
Ephedrine	31.70	30.26	29.68	28.70	24.86
Methadone	29.09	29.44	29.01	27.47	19.53
Dipipanone	27.59	28.26	28.00	26.77	19.16
Noscapine	21.93	18.74	5.08	–[a]	–
Aniline N					
Aniline	36.07	18.52	0.78	–	–
N-Methylaniline	34.82	8.54	0.10	–	–
N,*N*-Dimethylaniline	32.15	5.23	0.02	–	–
N-Isopropylaniline	33.75	23.03	2.18	–	–
2-Methylaniline	33.83	16.17	0.47	–	–
3-Methylaniline	35.31	22.56	1.79	–	–
4-Methylaniline	35.37	28.50	4.22	–	–
2,6-Dimethylaniline	30.92	6.61	0.05	–	–
Aromatic N					
Pyridine	43.17	9.44	0.32	–	–
2-Methylpyridine	46.27	25.73	1.89	–	–
3-Methylpyridine	45.65	18.25	0.92	–	–
4-Methylpyridine	47.01	27.59	2.22	–	–
4-Methoxypyridine	46.10	40.77	10.24	0.03	–
Quinoline	32.53	4.66	0.07	–	–
Isoquinoline	39.52	13.15	0.38	–	–
Papaverine	25.20	23.68	9.88	0.10	–

[a] –, too small for measurement under given conditions.

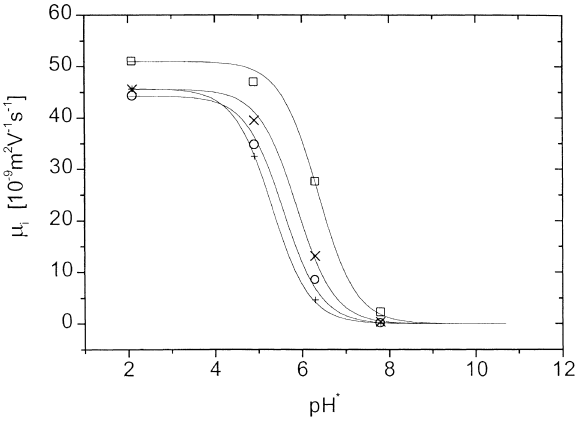

Fig. 1. Mobilities, μ_i, of four analytes in dependence on the conventional pH* of the BGE, with curves fitted according to Eq. (2). Analytes: (□) 4-methylpyridine; (×) isoquinoline; (○) *N*-methylaniline; (+) quinoline. Temperature, 25°C; ionic strength, 7.9 m*M*.

analytes. It is known that the curve pK^*_a versus composition of solvent has a steep increase at the side rich of organic solvent. For *o*-chloroanilinium, e.g., a limiting slope of the pK^*_a versus percent solvent curve of about 0.4 pK_a units/percent (w/w) water content is obtained by extrapolation [27]. This seems to be a typical value for cation acids. A similar trend is found also for neutral acids: for example, the addition of 1% (w/w) water to methanol decreases the pK^*_a values of acetic acid and trichloroacetic acid by about 0.4 and 0.2 units, respectively, as approximated from literature data [20].

The following water content of methanolic BGE solutions was measured by titration: in newly prepared BGE it was around 0.010–0.015% (w/w), except for BGE with perchloric acid, which contained about 0.050% (w/w) of water. All the methanolic BGEs in the running vials contained less than 0.070% (w/w) of water after use for electrophoresis. As in the present case the maximum water content

Table 4
pK_a values of the analytes (as cationic acids, BH^+) in water and methanol at 25°C[a]

Analyte	pK_a^W	p$K^*_a{}^{MeOH}$	ΔpK
Aliphatic N			
Amphetamine	9.9	11.2	1.3
Ephedrine	9.6	11.2	1.6
Methadone	8.3	11.0	2.7
Dipipanone	8.5	11.1	2.6
Noscapine	6.2[b]	7.1	0.9
Aniline N			
Aniline	4.87	6.2	1.3
N-Methylaniline	4.85	5.6	0.8
N,N-Dimethylaniline	5.07	5.3	0.2
N-Isopropylaniline	5.77	6.5	0.7
2-Methylaniline	4.45	6.1	1.7
3-Methylaniline	4.71	6.4	1.7
4-Methylaniline	5.08	5.5	0.4
2,6-Dimethylaniline	3.89	5.5	1.6
Aromatic N			
Pyridine	5.23	5.6	0.4
2-Methylpyridine	6.00	6.3	0.3
3-Methylpyridine	5.70	6.0	0.3
4-Methylpyridine	5.99	6.4	0.4
4-Methoxypyridine	6.58	7.1	0.5
Quinoline	4.90[c]	5.3	0.4
Isoquinoline	5.40[c]	5.9	0.5
Papaverine	6.4	7.6	1.2

[a] The error of the calculation of the pK_a by curve fit is less than 0.1 units in nearly all cases. Only for the most basic amines with aliphatic *N* was the error between 0.1 and 0.2 units. Values for water from Ref. [41].
[b] From Ref. [25].
[c] At 20°C.

was less than 0.07% (w/w) a deviation of the pK_a^* due to traces of water present was less than 0.03 units, and is therefore negligible.

3.2.3. Transfer activity coefficients

For the interpretation of the change in pK_a we must consider the stabilisation of all species involved in the thermodynamic equilibrium. The fundament of such an interpretation can be based on the standard free energy of transfer of the single species involved in the acid–base equilibrium, and by the concept of the transfer activity coefficient and the medium effect. We do not go into detail to these approaches, because this has been topic of a number of previous papers [23,28–35]. We refer to this work and the literature cited therein.

In the protolysis equilibrium of the cation acid and its conjugated base according to:

$$HB^+ = H^+ + B \tag{3}$$

three species are involved: two cations, the proton and BH^+, and one uncharged particle, the molecular base, B. The model of the transfer activity coefficient expresses the change of the pK_a values of the cation acid as:

$$\begin{aligned}\Delta pK_{a,HB^+} &= pK^S_{a,HB^+} - pK^W_{a,HB^+} \\ &= \log\left(\frac{{}_m\gamma_{H^+}\,{}_m\gamma_B}{{}_m\gamma_{HB^+}}\right) \\ &= \log {}_m\gamma_{H^+} + \log {}_m\gamma_B - \log {}_m\gamma_{HB^+}\end{aligned} \tag{4}$$

where ${}_m\gamma_i$ is the transfer activity coefficient (the logarithm, $\log {}_m\gamma_i$, is named medium effect). The medium effect is a measure for the free energy of transferring one mol of species i from water to solvent, S. It is negative when the particle is more stable in S, and positive, when it is better stabilised in water.

It can be seen that the change in pK_a depends:

(i) on the basicity of the solvent compared to water, reflected by $\log {}_m\gamma_{H^+}$;

(ii) the stabilisation of the protonated base, the cation HB^+, expressed by $\log {}_m\gamma_{B^+}$;

(iii) the stabilisation of the molecular base, expressed by $\log {}_m\gamma_B$.

The contribution of the non-charged particle to the total standard free energy of transfer is often underestimated in the literature. However, for aromatic neutral acid of type *HA* (in mixed aqueous *N,N*-dimethylformamide and *N,N*-dimethylacetamide solvents, probably somewhat unusual in CE) it was found that this effect can contribute significantly to the pK_a shift [30].

The medium effect on B can be related to the solubility, S_B, of this species in the different solvents, according to:

$$\log {}_m\gamma_B = \log \frac{S_B^W}{S_B^{MeOH}} \tag{5}$$

To estimate the contribution of B on ΔpK_a, values for the solubilities of the free base in methanol and water, respectively, are needed. At least for methanol quantitative data are hardly available from the litera-

ture. The few semi-quantitative data allow to assume a larger solubility in methanol. For benzoic acid, e.g., a value or −2.1 was found for the medium effect on the neutral species [36]. If we assume an about one order of magnitude better solubility in methanol as in water for the compounds under consideration, the medium effect, $\log {}_{m}\gamma_{B}$, on the free base is larger than −1. However, even without taking exact numbers for the solubilities it follows, that the medium effect is negative, and the pK_a value should decrease upon transfer from water to methanol. Indeed the contrary is found experimentally. For this change therefore the medium effect either on the proton, or on BH^+, or on both species must overcompensate that on the molecular base.

The medium effect on the proton expresses the different basicity of water and methanol. The determination of the effect on such charged particles is not so straightforward than that on non-charged species, because ions do not occur in solution without their counter-ions. Therefore the value obtained for a particular electrolyte always includes the effect on both, the cation and the anion. Extrapolation methods are thus applied, that try to separate the single ion contributions. Unfortunately the results obtained by the different approximation methods are partially contradictory. For methanol the large number of over 30 different values for $\log {}_{m}\gamma_{H^+}$ have been published. They vary between about +4 and −4 [37–40] (note the logarithmic scale of the medium effect). The average of all these data is approximately zero, indicating that methanol would have about the same basicity than water. However, the values scatter so strongly, that they hardly allow a clear decision, a weak point for the interpretation of the pK_a shift. The restriction is more pronounced for the cation acids, because the medium effect on the other species present (see the discussion on the free base) seems to be in the same order of magnitude. This is in contrast to the neutral acids, where the medium effect on the anion dominates the overall effect in many cases.

For a further discussion of the source of the pK_a shift we follow the selection of Marcus and coworkers [37–39] in order to narrow the number of values for of $\log {}_{m}\gamma_{H^+}$. This selection has been carried out according to reliable extrathermodynamic assumptions. Based on the reliability criteria given by the authors, only 11 values remain, all of them positive. The average from these values is around 1.7, indicating a lower basicity of methanol compared to water. Note that the (absolute) value of this effect is more or less the same than that assumed for the neutral species. It is, on the other hand, also in the range of the ΔpK_a values (that are between 0.3 and 2.7 units, see Table 4). Consequently, it is hardly possible to decide unequivocally whether the pK_a shift of the cation acids stems from the lower basicity of methanol, from the better solubility of the neutral species in methanol, or from the preferred stabilisation of the cation in water or methanol.

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