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Determination of pK_a values of organic bases in aqueous acetonitrile solutions using capillary electrophoresis

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Abstract

Capillary electrophoresis (CE) was used for the determination of ionisation constants (pK_a) of a variety of organic bases in aqueous acetonitrile solutions over the range 0–60% (v/v) acetonitrile. These bases are used as test compounds in HPLC column evaluation, thus knowledge of their pK_a in hydro–organic solutions is useful. The base pK_a decreased with acetonitrile concentration and significant shifts from the aqueous pK_a (up to −0.8) were found using 60% acetonitrile. The CE application was confirmed to be very suitable for fast and accurate pK_a measurement in aqueous organic solutions.

Keywords: Ionisation constants; Organic bases

1. Introduction

The pK_a of an ionogenic compound is a valuable parameter, since its knowledge enables estimation of the state of protonation of the compound dissolved in aqueous solution at given pH [1]. Knowledge of the state of protonation of organic bases is essential in RP-HPLC separations in order to explain changes in retention, peak shape or overloading behaviour of these substances. For example, the state of protonation of a base determines its potential interaction with ionised silanols; also, we have shown that HPLC columns exhibit much higher loadability for unprotonated bases than for corresponding partially protonated or completely protonated species [2,3].

RP-HPLC often utilises mobile phases consisting of organic solvents, such as methanol (MeOH) or acetonitrile (ACN), mixed with aqueous buffers. It is common practice to measure the pH of the aqueous buffer prior to combining with the organic modifier. Although this method is a reproducible means for mobile phase preparation, the organic modifier can alter the pK_a of the buffer and thus the pH of the mobile phase, in addition to altering the pK_a of the basic analyte (e.g. [4,5]). For example, an aqueous phosphate buffer of pH 3.1 when mixed with MeOH in the ratio 45:55 (v/v) is necessary to half-protonate pyridine, whereas its aqueous pK_a is about 5.1 [6].

Many buffers used in HPLC are neutral or anionic acids (e.g. phosphate or borate), which in general show increasing pK_a with organic modifier concentration [7–11]. A pH shift occurs for the buffer, relative to the aqueous pH, due to a change of dissociation of the buffer (i.e. pK_a change) with organic modifier concentration. This pH shift depends on the buffer type. For example, a 0.05 *M* dihydrogen phosphate buffer has an aqueous pH of 6.75, which in a mobile phase containing 50% ACN increases by about 0.78 pH units to pH 7.53; 0.05 *M*

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boric acid increases by 1.72 pH units from pH 9.15 in aqueous solution to pH 10.87 in a solution with 50% ACN content [10]. Multiprotic compounds have several pK_a values, each of which may experience an individual shift with organic solvent. This was shown for phthalic acid to result in individual pH increases with % ACN dependent on the original aqueous pH [9]. Buffers prepared from cationic acids on the other hand (e.g. butylamine or ammonia) show decreases in pH with % ACN, relative to the aqueous pH [9,11], in line with the result for pyridine above. Note that our considerations of pH variations of the buffer with % ACN in the mobile phase (this paragraph) refer to the rigorous $^{s}_{w}$pH scale (see below).

Capillary electrophoresis is a method which can be used for fast, accurate pK_a determination and has been applied mainly in aqueous solution or for completely non-aqueous solvents [12–29]. These studies have shown CE to be superior to conventional methodologies used for pK_a determination, such as UV spectrometry, potentiometric titrations or LC [30]. The advantages of CE include good sensitivity of the technique, wide applicability, and separation power, which obviates the need for the use of highly pure compounds. The general aims of the present study were:

(a) To investigate a CE procedure for pK_a determination of bases in both aqueous and aqueous–organic buffers.

(b) To study pK_a shifts in aqueous–ACN solvents for those bases used in our current test procedures for RP-HPLC columns.

(c) To establish any possible regularity in these pK_a shifts.

(d) To investigate the possibility of accurate pK_a prediction for bases by CE using a minimum number of experiments.

2. Theory

2.1. Principle of pK_a determination by CE

At high buffer pH, the unprotonated, uncharged base has no electrophoretic mobility (μ_{base}) and thus migrates with the electroosmotic flow (EOF). Alternatively, a fully protonated i.e. positively charged base exhibits maximum mobility and elutes faster than the EOF due to electrostatic solute attraction by the cathode. Intermediate mobility is a function of the dissociation equilibrium of the base [30]. The μ_{base} is obtained from Eq. (1) by a straightforward measurement of migration time of the base (t_{base}) and EOF (t_{EOF}); the latter can be obtained from any convenient neutral marker e.g. acetone. L_{cap} and l_{eff} are capillary length (inlet to outlet) and effective capillary length (inlet to detection window), respectively and V is the voltage applied across the capillary:

$$\mu_{base} = \frac{L_{cap} l_{eff}}{V} \cdot \left(\frac{1}{t_{base}} - \frac{1}{t_{EOF}} \right) \quad (1)$$

Plotting μ_{base} vs. pH gives a sigmoidal curve, whose inflection point reflects the apparent base pK'_a, which may be corrected for ionic strength, I, using Eq. (2) in order to obtain the thermodynamic pK_a value in the respective solvent composition (e.g. [22]).

$$pK_a = pK'_a - \frac{Az^2\sqrt{I}}{1 + Ba_0\sqrt{I}} \quad (2)$$

Parameters A and B are Debye–Hückel parameters, which are functions of temperature (T) and dielectric constant (ε) of the solvent medium. For the buffers we used, $z = 1$ for all ions. a_0 expresses the distance of closest approach of the ions, i.e. the sum of their effective radii in solution (solvated radii) [31]. Rosés and co-workers [9,10] have calculated A and Ba_0 ($T = 25\,^{\circ}C$) for pure water and ACN and ACN–water compositions using a constant value of $a_0 = 4.56$ Å. Ions were assumed to be spheres. I correction terms give rather small values (around 0.1) for our buffers using these A and Ba_0 values.

However, the assumption of a constant value of a_0 in our experiments is questionable, considering the number of different ions involved in our measurements ($R_3NH^+_{sample}$, $R_3NH^+_{buffer}$, K^+, Cl^-, H^+, OH^-). Also, a_0 could vary significantly in ACN–water systems of different % ACN, where preferential solvation effects on the microsphere of the ion could play a role [32]. Due to these factors, and the small size of the term, the pK_a values were not corrected for I.

2.2. pH scale

pH is the negative logarithm of the hydrogen ion activity ($-\log a_H$) [33]. In pure water, w, the standard state for a_H is infinite dilution of hydrogen ions in water. In a mixed solvent, such as a mobile phase in HPLC, a second standard state for a_H can exist; the one referring to infinite dilution of the hydrogen ion in the solvent mix, s. Different pH scales are obtained, where measured pH is either relative to aqueous calibration buffers or referred to buffers, which were prepared in the same solvent in which the pH is measured. To distinguish these pH scales IUPAC [33] recommends: lowercase superscript is the solvent in which the pH is measured; lowercase subscript is the solvent of standard state [31]. For example, ${}^{s}_{s}$pH means that the pH meter calibration and pH measurement were performed in identical solvent compositions. Replacing s by w (${}^{w}_{w}$pH) gives the absolute pH scale in water. ${}^{s}_{w}$pH is obtained by measuring the pH in the aqueous–organic solvent mixture but with the electrode calibrated in aqueous buffers.

pK_a determination of bases requires the use of a wide pH range and thus the use of several buffers. However, the varying pH shifts of different buffer types with organic modifier was shown to give severe deviation of sigmoidal plots of retention time vs. pH, using HPLC for pK_a determination, when the ${}^{w}_{w}$pH scale was the reference scale [10,11]. Deviation was greatest in the pH region where the base is only partially protonated; recall at conditions pH = pK_a the base is 50% protonated [1]. It is very likely that such deviations occur for μ_{base} vs. ${}^{w}_{w}$pH, since μ_{base} is a function of pH. In addition, pK_a values obtained in aqueous organic buffered solutions and referred to the ${}^{w}_{w}$pH scale are only meaningful for the particular buffer types utilised.

The rigorous ${}^{s}_{s}$pH scale gives physically meaningful pK_a values obtained in the solvent mixture, allowing thermodynamic pK_a values to be calculated using Eq. (2) [34]. A major shortcoming of the ${}^{s}_{s}$pH scale is the rather time-consuming calibration procedure, which requires buffers of known ${}^{s}_{s}$pH value for each particular solvent composition used (e.g. [34]). However, the rigorous ${}^{s}_{w}$pH scale circumvents this calibration problem and ${}^{s}_{s}$pH and ${}^{s}_{w}$pH only differ in a delta term, δ [10]. The δ term includes primary medium effect for the transfer of H^+ from solvent w to s and the difference between the liquid junction-potentials of the electrode system in s and w. However, the latter can be neglected by using pH electrodes containing a salt bridge of an equitransferent binary salt, at much higher concentration than sample and standard solutions (e.g. 3 *M* KCl) [10]. Thus the ${}^{s}_{w}$pH scale was recommended to be the most suitable [34], and was used as reference scale in the present study. It is sometimes argued that glass electrodes give poor reproducibility, or are damaged when used in aqueous organic solvents. However, RP-HPLC mobile phases usually have high water contents and glass electrodes have been used successfully, even in neat MeOH [35].

3. Experimental

3.1. Chemicals

A variety of bases of different aqueous pK_a values (literature) and stereochemistry, of highest available grade, were used. These and acetone were obtained from Sigma–Aldrich (Poole, UK). The buffers Tris [Tris(hydroxymethyl)-aminomethane] (${}^{w}_{w}$pK_a = 8.06), ethanolamine (${}^{w}_{w}$pK_a = 9.5), diethylamine (${}^{w}_{w}$pK_a = 10.98) and far UV HPLC grade ACN were from Fisher Scientific (Loughborough, UK). HCl and KCl were obtained from BDH (Poole, UK). Doubly deionised water from a Purite Still plus system (Oxon, UK) was used for all experiments.

3.2. Sample and background electrolyte solutions

All buffers were prepared fresh daily. Stock solutions (125 m*M*) of each buffer compound (Tris, ethanolamine or diethylamine) were prepared in water and ${}^{w}_{w}$pH was adjusted using 0.1 *M* HCl. Each buffer was used in a pH interval ±1 pH unit of the ${}^{w}_{w}$pK_a of the respective buffer compound. The ionic strength *I* was adjusted to a constant value of 250 m*M* using KCl. Stock solutions were diluted five times with respective aliquots of water and ACN in order to give buffers containing 0, 20, 40, 60% ACN. The solely aqueous solutions had a concentration of buffer compound of 25 m*M* and *I* = 50 m*M*. The ${}^{s}_{w}$pH value was measured at 20–23 °C and corrected to

25 °C using the aqueous T coefficient for the individual buffer compound (Tris, ethanolamine, diethylamine in pH units/K: −0.028, −0.029, −0.034, respectively) [1,36]. Samples (~3 g/l) were prepared in ACN–water (50:50, v/v) and diluted 30 times in the respective running buffer. Buffer solutions and samples were filtered through 0.45-μm filters from Chromacol (Herts, UK).

3.3. Instrumentation and related parameters

An HP ^{3D}CE-system (Agilent, Waldbronn, Germany) was used. Untreated fused-silica capillaries [64.5 cm (effective length 56 cm)×50 μm I.D.×365 μm O.D.] were from Esslab (Hadleigh, UK). Before use each day the capillary was flushed for 20 min with 0.1 *M* NaOH, 20 min with water and 20 min with buffer. Buffers were ultrasonicated for 10 min prior to use. Run conditions: $T_{cap}=25$ °C; preconditioning flush: 2 min water, 4 min buffer; injection hydrodynamic $P=50$ mbar for $t=1$ s; running voltage $V=20$ kV; pressure applied across capillary (in- and outlet): $P=8$ bar in order to overcome bubble formation (''outgassing'') within the capillary when using aqueous–ACN buffers; detection using a diode array detection system at wavelengths: 214 nm (bases), 254 nm (acetone). Acetone (~6%) was chosen as EOF marker. Each value of μ_{base} was calculated as the average of at least $n=3$ μ_{base} measurements; typically standard deviations ($n=3$) were 0.01 (10^{-4} cm^2 V^{-1} s^{-1}). After use the capillary was flushed with water. Sigmoidal plots of electrophoretic mobility against $^{s}_{w}$pH (15–18 data points), corresponding trendlines and inflection points were obtained from SIGMA PLOT 5.0 by non-linear regression.

The pH meter was an MP 220 from Mettler (Toledo, Spain) equipped with a Gelplas combination pH electrode from BDH with a single nylon junction containing saturated KCl. The maximum solubility of KCl at 25 °C is ~4 mol/l [31]. Thus, the residual liquid junction potential between solvents s and w can be neglected [10]. The meter was calibrated in aqueous buffer solutions of hydrogen phthalate and phosphate, (pH 4.00, 7.00), and the validity of higher pH measurements was checked with borax and phosphate buffers (pH 9.18, 11.00) [1,36].

4. Results and discussion

The electrophoretic mobility of bases (μ_{base}) can decrease with increasing ionic strength (I) in the running buffer. This effect has been shown to be considerable in neat ACN compared with that in water [37]. For example, as the concentration in the background electrolyte (BGE) was increased (BGE: perchloric acid–tetraalkyl ammonium perchlorate), over the range 10–30 m*M* a decrease in μ_{base} for *N*-isopropylanilinium was reported about $1.3\cdot10^{-4}$ cm^2 V^{-1} s^{-1} in ACN and about $0.2\cdot10^{-4}$ cm^2 V^{-1} s^{-1} in water. This difference in μ_{base} decrease was proposed to be caused by preferred ion–ion interaction of bases in solutions with high I in solvents of small dielectric constant (ε) such as ACN ($\varepsilon_{ACN}=36.01$), compared to water ($\varepsilon_{water}=80.18$) [37].

In our present study we used relatively water rich hydro–organic buffers (20–60% ACN or 0.079–0.339 ACN in mole fraction), in which ion-pairing was reported to be negligible [7]. Nevertheless, if there is any strong effect of I upon μ_{base}, this should be most pronounced in the buffers containing the highest organic solvent concentration (60% ACN in our case). Thus, we investigated the μ_{base} in 25 m*M* Tris buffers containing 60% ACN vs. I. I was 116, 250, 300, and 400 m*M* in aqueous stock buffers prior combination with aliquots of ACN and water to give a 5-fold dilution (i.e. I is nominally=23, 50, 60, and 80 m*M* in the running buffer). The pH was measured in the final solvent composition and found to be constant at about $^{s}_{w}$pH 6.9 for all buffers. This indicates that there is probably no marked effect of I difference on ion-activity in the range 23–80 m*M*, recall pH$=-\log a_H$. Fig. 1 shows the plot obtained for μ_{base} vs. I for (mostly protonated) diphenhydramine and procainamide, and (partially protonated) codeine and nicotine. The plots exhibit small negative slopes, showing a decrease in mobility with increasing I. Shifts in μ_{base} were largest for procainamide with a reduction of about 11% ($-0.26\cdot10^{-4}$ cm^2 V^{-1} s^{-1})) at $I=80$ m*M* compared with its highest mobility ($2.43\cdot10^{-4}$ cm^2 V^{-1} s^{-1}) at $I=23$ m*M*.

We conclude that the effect of I in the range 23–80 m*M* on μ_{base} is small but nevertheless significant. As a precaution we used a constant $I=50$ m*M* throughout our pK_a investigations. Also, by addition

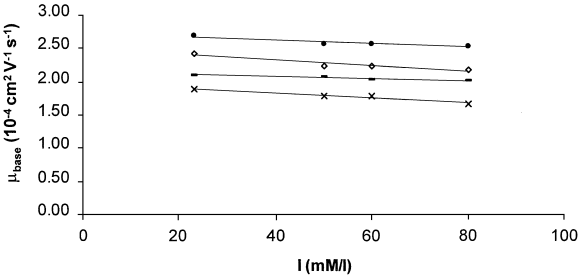

Fig. 1. Electrophoretic mobility of bases (μ_{base}) vs. ionic strength (I) in the running buffer 25 mM Tris ($^{s}_{w}$pH 6.88) in ACN–aqueous buffer (60:40, v/v). Diphenhydramine (•), procainamide (◇), codeine (×), nicotine (–). Detection wavelength (bases): 214 nm; T_{cap} = 25 °C; V = 20 kV; typical electrical currents were about: 10 μA for I = 23 mM, 26 μA for I = 50 mM, 31 μA for I = 60 mM, 42 μA for I = 80 mM. For other conditions, see Experimental Section.

of KCl to give relatively high I, we hoped to inhibit possible electrostatic adsorption of protonated bases on ionised silanols on the capillary wall, while limiting Joule heating [38]. The protonated form of the amino buffers should also help to shield ionised silanols. Ion exchange interactions were shown to give distorted sigmoidal curves of retention in RP-HPLC against pH, especially for hydrophilic compounds [4]. In addition to ion atmosphere effects, shifts in mobility could partly be caused by ionic strength effects upon the degree of protonation of the base [Eq. (2)]. This would be more crucial for the partially protonated compounds. Currents were about 42 μA at I = 80 mM and 10 μA at I = 23 mM. Temperature gradients across the capillary due to the increase of current with ionic strength (i.e. Joule heating), are likely to be small and are discussed below.

4.1. $^{s}_{w}pK_a$ values for bases in aqueous and aqueous–ACN buffers

Fig. 2 shows sigmoidal plots of μ_{base} against $^{s}_{w}$pH for diphenhydramine; these plots were typical for all the bases investigated. The concentration of ACN in the running buffer ranged from 20 to 60% (v/v). One of the experiments used purely aqueous buffers resulting in plots of μ_{base} vs. $^{w}_{w}$pH. Clearly, the electrophoretic mobility depends on the viscosity of the background electrolyte, which changes depending on the concentration of acetonitrile [39]. However, the pK_a values were determined from the inflection point of the curve using data for a given mobile phase. Our calculation of the pK_a does not involve comparison of mobilities at different organic solvent compositions (i.e. different viscosities). The effect of viscosity will just displace the curves up or down in a vertical direction, but not the position of the inflection point on the x-axis (pH scale). The same argument would apply for changes in the dielectric constant with % ACN in the BGE [40].

Table 1 summarises the pK_a values of six bases obtained in aqueous ($^{w}_{w}pK_a$) and aqueous–ACN buffers ($^{s}_{w}pK_a$). Also given are the correlation coefficients (R values) of the fits of the three-parameter sigmoidal curves, obtained by nonlinear regression from SIGMA PLOT 5.0. The $^{s}_{w}pK_a - {}^{w}_{w}pK_a$ values reflect the pK_a shift for the bases in the respective solvent composition, relative to the aqueous pK_a. The aqueous pK_a values ($^{w}_{w}pK_a$ lit) in the far right hand column were obtained from the literature (e.g. [15,22,26,41–44]) in which both classical and CE methods have been utilised.

The aqueous pK_a values determined by us agree well with those found in the literature, which indicates that our CE procedure gives reliable results. The R values, which are very close to unity, indicate very good fits of the data points to the sigmoidal nonlinear regression plots in each % ACN in the running buffer. The pK_a decrease with increasing concentration of organic solvent in the buffer was in line with findings for other bases such as, amines and pyridines in aqueous–organic solvents (MeOH and ACN) using HPLC for pK_a determination [6,10,11,32].

Joule heat is generated by the passage of electrical current. The electrical currents typically were about 45 μA in aqueous running buffers and decreased by about 5 μA per increase of 20% ACN (v/v). The temperature difference between the centre of the capillary and outer wall at highest electrical current (i.e. 45 μA in aqueous buffers) was calculated at less than 1 °C [45,46] when the capillary was thermostatted at 25 °C. These temperature differences should not lead to serious errors considering typical pK_a shifts with T for organic bases of about 0.03 pK_a units/K (see Experimental). Nevertheless, it is known that small changes in T can change viscosity

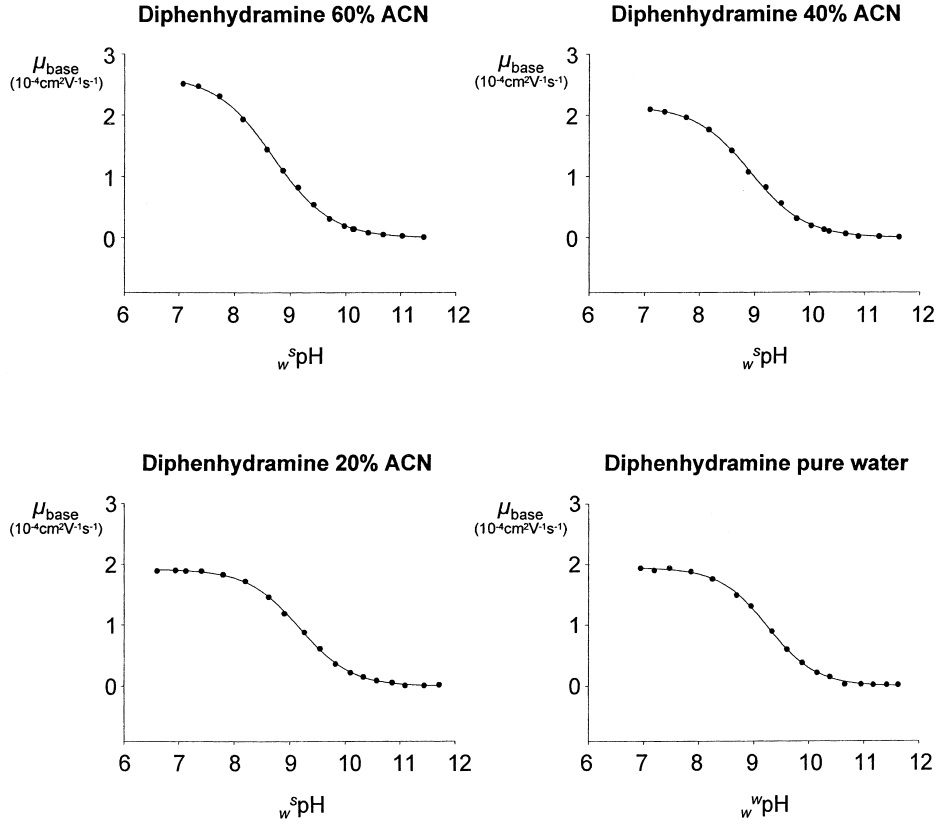

Fig. 2. Electrophoretic mobility of diphenhydramine (μ_{base}) vs. ${}^{s}_{w}$pH in ACN–aqueous buffer (60:40, v/v, 40:60, v/v, 20:80, v/v, 0:100, v/v). Sigmoidal curves through data points calculated by nonlinear regression (SIGMAPLOT 5.0). Detection wavelength (bases): 214 nm; T_{cap} = 25 °C; V = 20 kV; typical electrical currents were about 45 μA in water and reduced by about 5 μA per 20% (v/v) ACN increase. For other conditions, see Experimental Section.

by about 2–3%/K, which in turn can affect μ_{base} [45,47]. However, again these viscosity effects (see above) are likely to merely displace the sigmoidal μ_{base} *vs.* pH curve vertically on the plots.

We used three different buffers to cover the pH range of our investigation. Thus, we examined any consequences for determination of $\mu_{base.}$ We made up and adjusted the pH of the aqueous component of the organic solvent mixture, measuring the resultant ${}^{s}_{w}$pH in the appropriate organic solvent admixture, since this simplified maintenance of constant ionic strength. The diethylamine buffer (DEA) adjusted with HCl to ${}^{w}_{w}pH_{DEA}$ 10.65, and the ethanolamine buffer (EtA) adjusted to ${}^{w}_{w}pH_{EtA}$ 10.38, had almost identical ${}^{s}_{w}$pH (10.13 and 10.15, respectively) when mixed to give a 60% ACN solution. This was due to differences in the ${}^{w}_{w}$pH − ${}^{s}_{w}$pH shift experienced by the buffers DEA and EtA in 60% ACN, (about −0.5 and −0.2, respectively). Clearly, a significant error could be introduced in measurement of μ_{base} if ${}^{w}_{w}$pH values were used in conjunction with different buffer compounds. On the contrary, Fig. 2 shows the data points for μ_{base} vs. ${}^{s}_{w}$pH 10.13 and ${}^{s}_{w}$pH 10.15 for diphenhydramine at 60% ACN content in the two different buffers to be almost congruent. This result also shows no influence of the buffer compound itself on μ_{base} at the same ${}^{s}_{w}$pH. Nortriptyline is more basic than diphenhydramine, and around pH 10 μ_{base} is changing much more rapidly with pH than it is for diphenhydramine (data not shown). Nevertheless, use of different buffers at the same ${}^{s}_{w}$pH values in 60% ACN again produced consistent results: μ_{base} for

Table 1
${}^{s}_{w}pK_a$ values and ${}^{w}_{w}pK_a$ values of six basic compounds in aqueous acetonitrile solutions [0–60% (v/v) ACN]. ${}^{s}_{w}pK_a - {}^{w}_{w}pK_a$ shift for the bases in the respective solvent composition; conditions as for Fig. 2

	60% ACN		40% ACN		20% ACN		Water	
	${}^{s}_{w}pK_a$	${}^{s}_{w}pK_a - {}^{w}_{w}pK_a$	${}^{s}_{w}pK_a$	${}^{s}_{w}pK_a - {}^{w}_{w}pK_a$	${}^{s}_{w}pK_a$	${}^{s}_{w}pK_a - {}^{w}_{w}pK_a$	${}^{w}_{w}pK_a$ H_2O	${}^{w}_{w}pK_a$ lit
Nortriptyline	9.72 $R=0.9991$	−0.61	9.87 $R=0.9998$	−0.46	10.11 $R=0.9997$	−0.22	10.33 $R=0.9989$	10.0 to 10.11
Diphenhydramine	8.69 $R=0.9998$	−0.56	8.92 $R=0.9997$	−0.33	9.16 $R=0.9998$	−0.09	9.25 $R=0.9997$	9.00 to 9.40
Quinine	8.34 $R=0.9996$	−0.22	8.47 $R=0.9994$	−0.09	8.59 $R=0.9995$	+0.03	8.56 $R=0.9989$	8.39 to 8.52
Codeine	7.54 $R=0.9997$	−0.71	7.84 $R=0.9999$	−0.41	8.09 $R=0.9998$	−0.16	8.25 $R=0.9997$	7.83 to 8.21
Procainamide	8.99 $R=0.9994$	−0.43	9.16 $R=0.9995$	−0.26	9.34 $R=0.9997$	−0.08	9.42 $R=0.9997$	9.20 to 9.40
Benzylamine	8.78 $R=0.9982$	−0.77	9.11 $R=0.9990$	−0.44	9.38 $R=0.9995$	−0.17	9.55 $R=0.9992$	9.33 to 9.73

nortriptyline was 0.65 (10^{-4} cm^2 V^{-1} s^{-1}) at ${}^{s}_{w}pH_{DEA}$ 10.13 and 0.66 (10^{-4} cm^2 V^{-1} s^{-1}) at ${}^{s}_{w}pH_{EtA}$ 10.15.

Fig. 3 shows plots of ${}^{s}_{w}pK_a - {}^{w}_{w}pK_a$ vs. % ACN (from values given in Table 1) for the six moderately strong bases we studied. Although, the trends of the pK_a decrease with % ACN for some bases such as nortriptyline, benzylamine, and codeine are similar, the pK_a changes are not identical for each base. Other workers [11,48], using HPLC, showed that for a series of weaker bases (${}^{w}_{w}pK_a$ values <5), ${}^{s}_{w}pK_a - {}^{w}_{w}pK_a$ values varied from −0.17 to −0.45 at 20% ACN, −0.50 to −0.89 at 40% ACN, and −1.00 to −1.25 at 60% ACN. The ${}^{s}_{w}pK_a - {}^{w}_{w}pK_a$ shifts observed by us seem to be at the smaller end of these ranges. However, stronger bases (as in our study) can experience less pK_a shift with increasing % ACN compared with weaker bases [48]. The stronger bases EtA, ammonia, and triethylamine (${}^{w}_{w}pK_a$ 9.48, 9.29, and 10.66, respectively) studied gave ${}^{s}_{w}pK_a - {}^{w}_{w}pK_a$ of −0.24, −0.24, and −0.50, respectively in 40% ACN which are quite similar to the values we found. (Note: we have used $\delta_{40\%\ ACN}=0.14$ [10] to convert ${}^{s}_{s}pK_a$ values quoted in [48] to ${}^{s}_{w}pK_a$). However, EtA, ammonia and triethylamine are simpler, smaller molecules than those we studied, and some caution is necessary in these comparisons. Indeed, *N,N*-dimethylbenzylamine, a relatively strong base (${}^{w}_{w}pK_a$ 8.91) shows quite a large ${}^{s}_{w}pK_a - {}^{w}_{w}pK_a$ shift of −0.76 in 40% ACN [11,48].

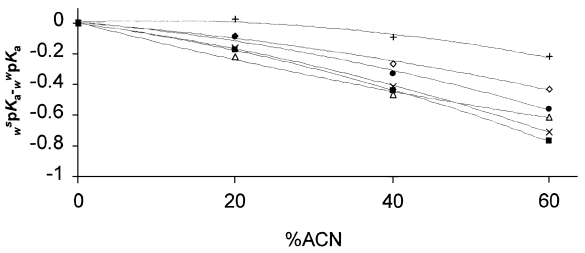

Fig. 3. pK_a shift in ACN–aqueous solvents relative to the pK_a in water illustrated as ${}^{s}_{w}pK_a - {}^{w}_{w}pK_a$ vs. % (v/v) ACN in the running buffer. Nortriptyline (△), diphenhydramine (•), quinine (+), codeine (×), procainamide (◇), benzylamine (■).

The reason for these differences in pK_a shifts for different compounds lies most likely in the fact that solute properties such as pK_a depend on the preferential solvation of the analyte in its microsphere by the co-existing solvents in a mixture. In other words, the acidity of a compound depends on the basicity of its directly surrounding solvent [32,49]. For example, amines and pyridines have been shown to be preferentially solvated by methanol–water complexes rather than water itself; pure methanol is the least preferred solvent [32,49]. It was proposed that the

methanol–water complex is a better proton acceptor than pure methanol and probably a better acceptor than water. This higher basicity of the methanol–water complex in reference to water determines a decrease of pK_a values of protonated bases with increase of methanol content. Note, that for higher methanol concentrations the base is mostly solvated by less basic methanol and the pK_a value can increase up to pK_a values slightly higher than that in water when referred to the ${}^{s}_{s}$pH scale; this was also observed in ACN mobile phases [10,32,49]. The bases used in the current study are different in structure and lipophilicity (e.g. benzylamine is a primary amine and is much less lipophilic with log P~1 compared to the secondary amine nortriptyline with log P~5.5). Therefore, it is very likely that the preferential solvation effect varies for the individual base with solvents or solvent aggregates, which might co-exist in ACN–water compositions, since the solvent composition on the microsphere of solvation of each individual base can be different from the composition of the bulk solution [32].

We conclude that if the accurate pK_a of a base is required in aqueous–organic solvents, it must be determined for each individual compound, although groups of bases with similar structure may behave similarly [48]. Run times are dependent on t_{EOF}, which in our studies was 14.5–24.5 min in 60% ACN solutions and 5–10.5 min in aqueous solutions at pH~10 and ~7, respectively. pK_a values of several bases may be measured at once due to the separation power of CE. Considering the good R values for the sigmoidal curves of μ_{base} vs. ${}^{s}_{w}$pH or ${}^{w}_{w}$pH (see Table 1) it is possible to get reliable results by performing fewer runs than performed in the present study. pK_a values have been predicted using this method for bases in aqueous solutions (e.g. [22]), but also for acids in aqueous–MeOH solutions [23], using the Henderson–Hasselbalch equation modified for CE.

5. Conclusion

${}^{w}_{w}$pK_a and ${}^{s}_{w}$pK_a values were determined by CE for six bases currently used in our test procedures for HPLC columns, in the range of organic modifier ACN 0–60%. Reliability of the CE methodology is shown by agreement between ${}^{w}_{w}$pK_a values determined presently and those found in the literature. The decrease of pK_a of bases with increasing % ACN in the mobile phase observed is in line with findings for other bases using different procedures for pK_a determination. Although trends in pK_a decrease were similar, they were not identical, which may result from distinct stereochemistry of the basic solutes and subsequently a different solvent composition on the microsphere of solvation of each individual base. Hence, we recommend the individual pK_a determination of each base in aqueous–organic solutions, in order to explain phenomena in RP-HPLC, which are dependent upon the state of protonation of the base. However, the good fits reflected by correlation coefficients (R values) of the sigmoidal curves of electrophoretic mobility reported here show that the analysis time could be shortened, since a few sample runs should be sufficient for pK_a determination. A minor inconvenience of the CE method is that a small pressure (typically available in CEC instruments) needs to be applied to both ends of the capillary to prevent outgassing of aqueous–organic solvent mixtures.

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