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Determination of ionisation constants of organic bases in aqueous methanol solutions using capillary electrophoresis

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Abstract

The pK_a of eight organic bases was determined in aqueous and aqueous methanol solutions of 0–70% (v/v) methanol using capillary electrophoresis. The bases investigated include compounds commonly used to test the activity of RP columns in HPLC. The variation of pK_a with temperature in aqueous methanol solutions was also investigated and found to closely resemble temperature coefficients reported for bases in purely aqueous solutions. pK_a values determined by CE were compared to those reported using NMR spectroscopy. The good agreement of the results is evidence that either technique is suitable to perform pK_a measurements.

Keywords: Dissociation constants; Triptylines; Diphenhydramine; Quinine; Codeine; Procainamide; Benzylamine

1. Introduction

In HPLC, the protonation state of ionogenic solutes can have considerable influence on retention, peak shape and mass overload properties [1–4]. Solute protonation can be estimated from pK_a values [5]. However, determination of pK_a in mobile phases commonly used in RP-HPLC is not trivial because of the presence of organic solvents, which can affect both the pK_a of buffer compounds (leading to pH changes in the mobile phase) and that of the solute itself [6,7]. In general, the pK_a of acids increases as organic solvent concentration increases, whereas that of bases decreases compared with the value in aqueous solution [8–14]. The pK_a of acid silanol groups on the surface is likely to be affected in a similar fashion.

In a previous publication, we showed that capillary electrophoresis (CE) was suitable for measurement of the pK_a of a number of bases commonly used to test RP-HPLC columns in hydro–organic solutions containing 0–60% acetonitrile [14]. We observed substantial compound-dependent differences in the pK_a shift for bases from their aqueous values at a given solvent composition. For example, benzylamine and quinine gave shifts of about −0.8 and −0.2 pK_a units, respectively (using the $^{s}_{w}$pH, scale defined further below) in 60% acetonitrile compared with aqueous values. Thus, while related compounds may give similar shifts allowing reliable prediction of the effect of organic solvent [11,15,16], empirical measurements may be required for structurally different solutes.

Methanol may be preferred over acetonitrile as the organic modifier in routine HPLC analysis for cost, safety and environmental reasons. The peak shape of bases is affected by substitution of methanol for acetonitrile; for example, some compounds give less tailing and higher efficiency at pH 7 when using buffered methanolic solutions compared with acetonitrile [1,2,17]. Knowledge of pK_a in both methanolic and acetonitrile solutions might be useful in the interpretation of such data. Methanol is a neutral amphiprotic solvent, whereas acetonitrile is a protophobic dipolar aprotic solvent [18]. Thus, the ionisation behaviour of individual bases could change dependent on which modifier is used [8,16]. Previously, we have shown that the peak shape of bases in RP-HPLC can improve substantially at elevated temperature [19]. The pK_a of bases is known to decrease with temperature and could be a factor in explaining this improvement [5]. Thus, an additional aim was to measure pK_a values of some bases at elevated temperature (40 °C) and to determine whether the percentage of methanol in the background

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electrolyte (BGE) could affect the reduction rate of pK_a which occurs for these compounds with temperature.

Some of us have used NMR spectroscopy to study the pK_a of bases in hydro–organic solutions [20]. In the present paper, we have compared values determined by CE and NMR thus investigating any possible bias of either technique.

pK_a determination by CE is not at all new, and has been described previously more than 10 years ago by authors such as Beckers et al. [21] and Smith and Khaledi [22]. Many further references to the use of CE for pK_a determination are given in our previous publication [14]. However, many of these studies have used aqueous, or completely non-aqueous solvents whereas our major interest is measurement in aqueous–organic solvents as used in RP-HPLC. pK_a determination by CE is based on the principle that at sufficiently high buffer pH, the unprotonated, uncharged base has no electrophoretic mobility (μ_{base}) and thus migrates with the electroosmotic flow (EOF), whereas at low pH, a fully protonated (positively charged) base exhibits maximum mobility and elutes faster than the EOF due to electrostatic attraction to the cathode. Intermediate mobility is a function of the dissociation equilibrium of the base. μ_{base} is obtained from Eq. (1) by measurement of solute migration time (t_{base}) and EOF (t_{EOF}, e.g. using acetone); L_{cap} and l_{eff} are capillary length (inlet to outlet) and effective capillary length (inlet to detection window), respectively, and V the voltage applied across the capillary:

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

Plotting μ_{base} versus 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 [23]:

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

where z is the charge number of the ion (in our case $z = 1$). A and B are Debye–Hückel parameters, which are functions of temperature and dielectric constant of the solvent medium. a_0 expresses the distance of closest approach of ions in solution (i.e. the sum of effective radii). These values were obtained from the literature [24,25]: quoted A values range from 0.53 to 1.01 and Ba_0 from 1.50 to 1.88 when going from purely aqueous to 70% methanol solutions, respectively. These values lead to calculated I-correction terms of 0.09–0.16, respectively, for the range of methanol concentrations that we used in our study. We used the I-correction for better comparability of our results to literature pK_a values. However, we remain cautious about the accuracy of the correction since calculations assume a constant value for a_0 independent of the variety of ions present and the different hydro–organic solvent compositions used.

Throughout this work we have used recommended IUPAC designations of pH. The subscript is the solvent of standard state; the superscript is the solvent in which the pH is measured [14,26,27]. We have used ${}^{s}_{w}$pH measurements, which are obtained by measuring the pH in the respective aqueous–organic solvent mixture but with the electrode calibrated in aqueous buffers. These measurements can be related to the rigorous ${}^{s}_{s}$pH-scale through the δ term, which depends on the solvent composition; δ can be found in the literature for any methanol–water mixture [24,25].

Our major aim in the present study was to generate pK_a data for a number of compounds that we and other workers have used as test probes in HPLC, in order to aid in the interpretation of retention and peak shape data. This interpretation is difficult when pK_a values only in water are known. The rationalisation of the changes in pK_a of compounds with organic solvent composition is another complex matter. We have made reference to the work of other research groups, who have made more detailed studies in this area, for those whose main interest lies in the theoretical interpretation of these pK_a changes.

2. Experimental

The buffers tris(hydroxymethyl)aminomethane (Tris, ${}^{w}_{w}pK_a = 8.06$), ethanolamine (${}^{w}_{w}pK_a = 9.5$), and potassium acetate (${}^{w}_{w}pK_a = 4.76$) were used to cover the pH range used in this study. In our previous work [14], we showed that there was little influence of the nature of the buffer salt on μ_{base}, as long as ${}^{s}_{w}$pH is measured and ionic strength in the BGE is held constant. This assumption does not hold at all for ${}^{w}_{w}$pH measurements, since the addition of organic solvent to different buffers having the same ${}^{w}_{w}$pH can give rise to different ${}^{s}_{w}$pH values. However, an alternative approach would be to use mixed buffers to allow constant buffer composition throughout. Stock solutions of each aqueous buffer of concentration 125 mM l^{-1} were prepared and adjusted to an ionic strength $I = 250$ mM using KCl. ${}^{w}_{w}$pH was adjusted using 100 mM HCl. Each buffer was used in a pH interval ±1 pH unit of the ${}^{w}_{w}pK_a$ of the respective buffer compound. The stock solutions were diluted five times with appropriate quantities of methanol and water in order to give BGE containing 0, 20, 30, 40, 60, and 70% methanol. Thus, buffers were nominally of concentration 25 mM with $I = 50$ mM. All running buffers were prepared fresh daily and were ultra-sonicated for 10 min prior to use. The ${}^{s}_{w}$pH value was measured at the same temperature (T) at which analysis was performed, i.e. either at 25 or 40 °C. Analyte solutions (~3 g l^{-1}) were prepared in methanol–water (50:50, v/v) and diluted by about 25 times in the respective running buffer. Buffer solutions and samples were filtered through 0.45 μm filters from Chromacol (Hertshire, UK).

A ^{3D}CE system (Agilent, Waldbronn, Germany) was used with three different set-ups: (1) $T_{instrument} = 25$ °C, negative CE mode (short-end injection); (2) $T_{cap} = 25$ °C, positive CE mode; (3) $T_{cap} = 40$ °C, positive CE mode; in (3) in addition to the capillary the autosampler carousel was

thermostatted using a water circulator C-85A from Techne (Cambridge, UK). Note that in the normal "positive" CE mode, sample is introduced at the anodic end of the capillary and migrates through the long length of capillary l_{eff} to the detector, under the influence of the electroosmotic flow. In the "short-end" procedure, the polarity of the system is reversed and the sample is injected at the other end of the capillary which now becomes the anode. The separation is accomplished using the short length of capillary to the detector (this length is equivalent to $L_{cap} - l_{eff}$, where l_{eff} is the effective length of the capillary as used in the positive CE mode). All experiments used untreated fused-silica capillaries, from Esslab (Hadleigh, UK); capillary diameters: i.d. = 50 μm, o.d. = 365 μm; capillary lengths: set-up (1) L_{cap} = 64.5 cm, however, l_{eff} = 8.5 cm in the negative CE mode, allowing us to use the same capillaries as used in our previous work. Note it is not possible to accommodate a capillary shorter than about 30 cm in the cartridge of the Agilent system (with the object of reducing analysis time), giving a minimum possible l_{eff} in the normal positive CE mode of about 22 cm; this is why we studied the short-end procedure. For set-ups (2) and (3), the capillary was cut to L_{cap} = 34.1 cm in order to reduce analysis time, l_{eff} = 25.6 cm. Before use each day the capillary was flushed for 10 min with 0.1 M NaOH, 20 min with water and 10 min with the running buffer. Run conditions: preconditioning flush—1 min water, 2 min buffer; electrokinetic injection V_{inj} = 5 kV for t = 10 s; running voltage V_{run} = 10 kV (stated when different); pressure (8 bar) was applied across the capillary (inlet and outlet) in order to overcome bubble formation (outgassing) within the capillary when using aqueous–methanol buffers. Detection was with a diode array system at wavelengths: 214 nm (bases), 254 nm (acetone). Acetone (∼6%) or benzyl alcohol (∼3%) were used as EOF markers. At high methanol concentrations, acetone gave rather small peaks and benzyl alcohol was used instead. t_{EOF} measured using either compound was very similar. Each value of μ_{base} was calculated as the average of at least three measurements; the average values of the relative standard deviations of electrophoretic mobility measurements, calculated over the range of different methanol concentrations and for the different solutes was 0.3% or less. After use the capillary was flushed with water. Sigmoidal plots of electrophoretic mobility against ${}^{s}_{w}$pH (five to six 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 (Poole, UK) with a single nylon junction containing saturated KCl, calibrated as described previously [14].

The vial of BGE was generally used for only two runs at 25 °C, although experiments showed that failure to replace the vial had little effect on the reproducibility of mobility measurements at this temperature. However, at 40 °C considerable changes in μ_{base} could occur if one BGE vial was used for more than one or two runs. For example, the 1st run using BGE containing 30% methanol at ${}^{s}_{w}$pH 10.16 gave μ_{base} (in 10^{-4} cm^2 V^{-1} s^{-1}) 0.16 and 0.38 for benzylamine and nortriptyline, respectively; 2nd run: 0.18 and 0.41; 3rd run: 0.20 and 0.45; 4th run: 0.22 and 0.48; 13th run: 0.36 and 0.70; 14th run using fresh buffer: 0.17 and 0.40. It is conceivable that these variations are caused by solvent evaporation from the thermostatted vial after it has been punctured during the first run or that chemical changes could occur in the inlet buffer vial at 40 °C compared with 25 °C. Thus, the vial of BGE was replaced after every run at 40 °C.

3. Results and discussion

The ${}^{w}_{w}pK_a$ and ${}^{s}_{w}pK_a$ values for eight organic bases (structures given in Fig. 1) were determined in aqueous and aqueous–methanol buffers over the range 0–70% (v/v) methanol. Fig. 2A and B show representative sigmoidal plots of μ_{base} against ${}^{s}_{w}$pH obtained using 60% methanol. The inflection point of these plots gives the apparent pK'_a of the base. These pK'_a values were converted by means of Eq. (2) to give the thermodynamic pK_a values, shown in Table 1 for each solvent composition. In addition, literature values in aqueous solution (${}^{w}_{w}pK_a$(literature)) are given, which were determined using a range of methods, e.g. potentiometric titration, CE, HPLC or NMR [20,23,28–33]. Also shown in Table 1 are the correlation coefficients (R values), of the fits of the three-parameter sigmoidal curves, which were obtained by non-linear regression from Sigma Plot 5.0. The ${}^{s}_{w}pK_a - {}^{w}_{w}pK_a$ values illustrate pK_a-shifts for the bases in the respective aqueous–methanol composition, relative to the aqueous pK_a.

The R values, which are close or equal to unity, show very good fits of the data points to the sigmoidal non-linear regression plots for each composition of water and methanol in the BGE. The ${}^{w}_{w}pK_a$ values we determined by CE agree well with ${}^{w}_{w}pK_a$(literature), which indicates the reliability of our CE procedure. In addition, we estimated by interpolation from the second-order polynomial regression curve shown in Fig. 3, a value of ${}^{s}_{w}pK_a$ 9.08 for benzylamine in 50% methanol, which compares well with values reported by Rived et al. (${}^{s}_{w}pK_a$ 8.94–9.16) [16]. Note, ${}^{s}_{s}pK_a$ values given in [16] were converted to ${}^{s}_{w}pK_a$ using $\delta_{50\%\,methanol}$ = 0.13 [24,25].

Significant changes in μ_{base} were obtained with varying methanol composition in the BGE, e.g. with methanol 70 and 0% (v/v), nortriptyline gave maximum μ_{base} of 1.28 and 1.92×10^{-4} cm^2 V^{-1} s^{-1}, respectively. It is well known that such changes in mobility are mainly attributable to a change in viscosity resulting from different organic modifier content in the BGE [34–36]. However, our method of pK_a determination does not require comparison of mobility in solutions of different methanol concentration. Indeed, viscosity effects would influence all measurements at a given BGE composition of constant ionic strength to the same extent, and thus only deflect the sigmoidal plots up or down

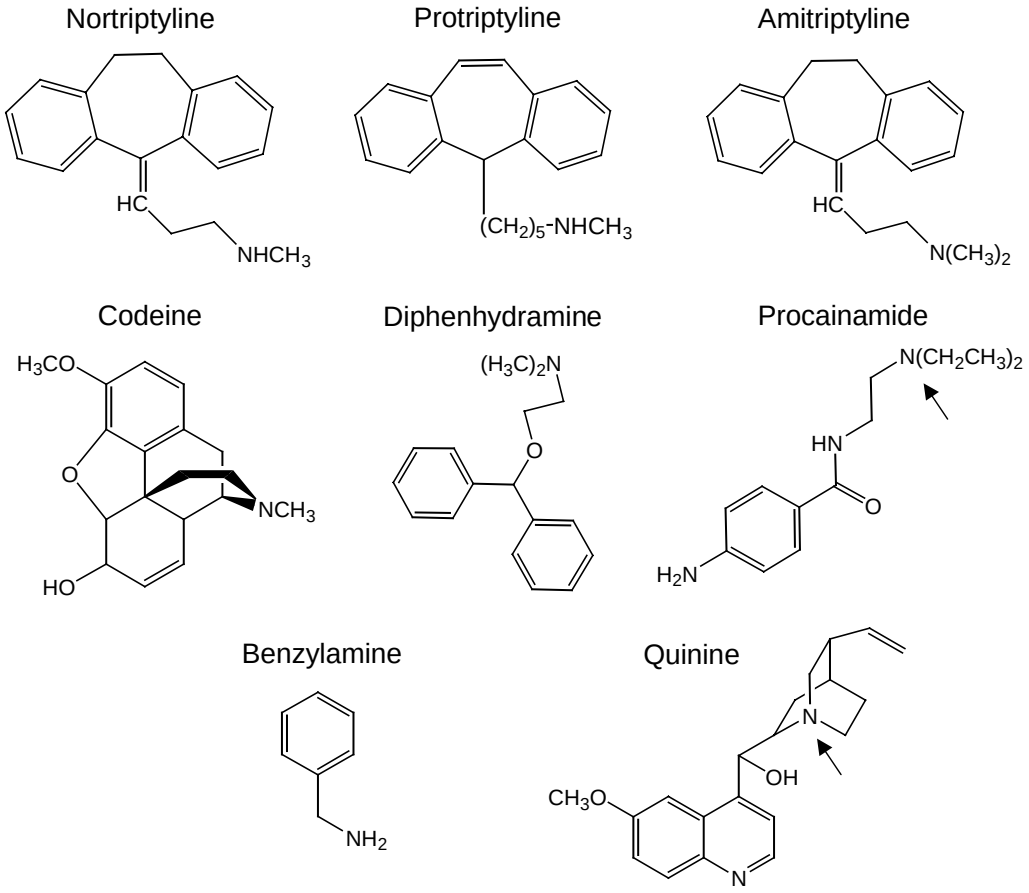

Fig. 1. Structures of the organic test bases. Arrows indicate groups whose pK_a was determined in molecules containing several ionisable centres.

along the μ_{base} axis, without affecting the position of the inflection point in respect to the pH axis. The same argument applies for changes in dielectric constant with percentage of methanol in the BGE [37].

Joule heat is generated by electrical current within the capillary. Differences in temperature could lead to erroneous pK_a estimations, since the pK_a is temperature dependent [5]. As shown later in this paper, the pK_a of bases can decrease by about 0.03 pK_a units K^{-1}. Temperature can also affect the pK_a measurement since it affects viscosity of the BGE by about 2–3% K^{-1} [38,39]. In earlier studies [14], we calculated the temperature difference ($T_{capillary\ centre} - T_{environment}$) to be negligibly small (<1 °C) under conditions (purely aqueous buffers, 20 kV) where the highest currents (about 45 μA), were produced. In the present work we used 10 kV for most experiments, which gave currents slightly above 20 μA in aqueous BGE, as expected from theory [38,39], when using a capillary of the same dimension as in [14]. As the percentage of methanol was increased, currents decreased to about 10 μA in 60–70% methanol. Thus, we expect the generation of Joule heat to be even less than in [14]. Nevertheless, for some measurements (results in Table 1) in this study we used the "short-end" injection procedure (experimental set-up (1)) in order to increase sample throughput. In the Agilent instrument, part of the capillary is not inside the thermostatted cassette in this set-up; heat which dissipates through the capillary wall may not be removed so efficiently. In order to eliminate concerns, we repeated the pK_a determinations for seven bases in 60% and 30% methanol using the positive CE mode (set-up (2) in Section 2) where l_{eff} was inside the thermostatically controlled capillary cassette. Table 2 gives the results of these repeat measurements at points over almost the entire range of solvent composition studied in Table 1, and ${}^{w}_{w}pK_a$ values from [14], which employed a positive CE mode similar to set-up (2). Only very small differences $\Delta^{(2)-(1)}$ were obtained between pK_a values determined using CE set-ups (1) and (2), showing that in our case, the short-end procedure had not affected the results.

When using high methanol concentrations (60–70%, v/v) in the BGE t_{EOF} became very long at lower pH values, due to suppression of silanol ionisation. For instance, using experimental set-up (1), t_{EOF} was satisfactory at about 10 min with ${}^{s}_{w}$pH 9.94 but increased to about 50 min at ${}^{s}_{w}$pH 7.06. To accelerate analysis time, the voltage was increased from 10 to 20 kV for BGE with 70% methanol content and ${}^{s}_{w}\text{pH} < 7.1$. The voltage used for the determination of μ_{base} for quinine and codeine at 60% methanol at ${}^{s}_{w}$pH 5.18 was 20 kV (latter results are shown in Fig. 2A). μ_{base} should theoretically not be affected by changes in running voltage due to

Table 1
Thermodynamic ${}^{s}_{w}pK_a$ and ${}^{w}_{w}pK_a$ of eight organic bases in aqueous–methanol solutions (0–70% (v/v) methanol)

	70% MeOH		60% MeOH		40% MeOH		30% MeOH		20% MeOH		Water	
	${}^{s}_{w}pK_a$	${}^{s}_{w}pK_a - {}^{w}_{w}pK_a$	${}^{s}_{w}pK_a$	${}^{s}_{w}pK_a - {}^{w}_{w}pK_a$	${}^{s}_{w}pK_a$	${}^{s}_{w}pK_a - {}^{w}_{w}pK_a$	${}^{s}_{w}pK_a$	${}^{s}_{w}pK_a - {}^{w}_{w}pK_a$	${}^{s}_{w}pK_a$	${}^{s}_{w}pK_a - {}^{w}_{w}pK_a$	${}^{w}_{w}pK_a$	${}^{w}_{w}pK_a$ (literature)
nor	9.25 (0.9999)	−0.94	9.43 (1)	−0.76	9.72 (0.9997)	−0.47	9.84 (0.9998)	−0.35	9.96 (0.9997)	−0.23	10.19 (0.9994)	10.0–10.11
diph	8.24 (0.9998)	−0.92	8.48 (0.9999)	−0.68	8.78 (0.9998)	−0.38	8.89 (0.9999)	−0.27	9.00 (0.9999)	−0.16	9.16 (0.9994)	9.00–9.40
quin	7.94 (0.9997)	−0.54	8.11 (0.9999)	−0.37	8.33 (0.9998)	−0.15	8.38 (0.9993)	−0.10	8.44 (0.9987)	−0.04	8.48 (0.9983)	8.39–8.52
cod	–	–	7.56 (0.9998)	−0.64	7.80 (0.9999)	−0.40	7.90 (0.9998)	−0.30	8.06 (0.9995)	−0.14	8.20 (0.9998)	7.83–8.21
proc	8.45 (0.9997)	−0.87	8.68 (1)	−0.64	8.93 (0.9996)	−0.39	9.05 (0.9999)	−0.27	9.15 (0.9998)	−0.17	9.32 (0.9992)	9.20–9.40
benz	8.86 (0.9989)	−0.59	9.01 (0.9994)	−0.44	9.15 (0.9980)	−0.30	9.23 (0.9978)	−0.22	9.33 (0.9989)	−0.12	9.45 (0.9963)	9.33–9.73
protr	9.73 (0.9996)	−0.98	9.88 (0.9998)	−0.83	10.21 (0.9993)	−0.50	10.31 (0.9989)	−0.40	10.43 (0.9990)	−0.28	10.71 (0.9948)	10.70
amitr	8.27 (0.9999)	−1.05	8.53 (0.9999)	−0.79	8.92 (0.9999)	−0.40	9.09 (0.9998)	−0.23	9.27 (0.9994)	−0.05	9.32 (0.9983)	9.40–9.45

Numbers in parentheses are correlation coefficients obtained for sigmoidal plots from Sigma Plot 5.0 (see Fig. 2). Conditions as in set-up (1) in Section 2. nor = nortriptyline; diph = diphenhydramine; quin = quinine; cod = codeine; proc = procainamide; benz = benzylamine; protr = protriptyline; amitr = amitriptyline.

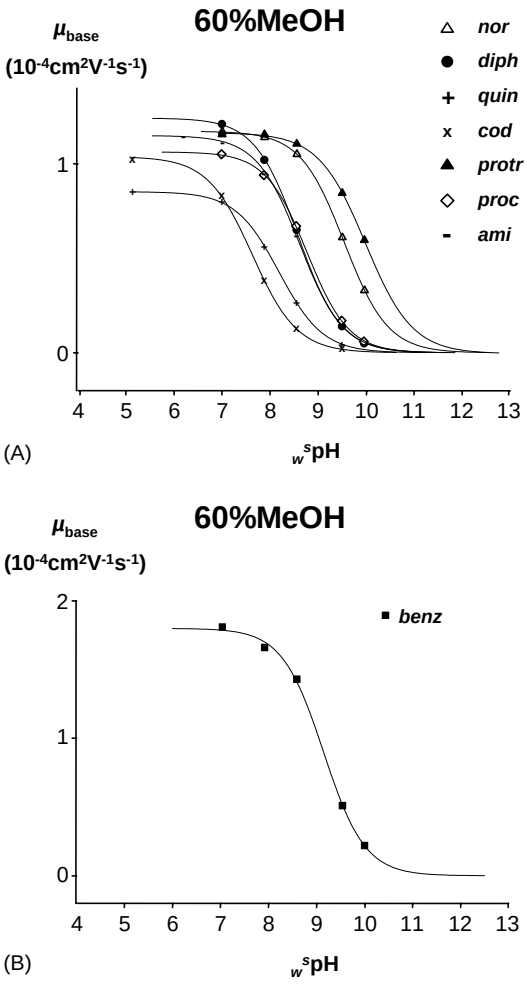

Fig. 2. Plots of electrophoretic mobility (μ_{base}) vs. $^{s}_{w}$pH in methanol–water–buffer (60:20:20, v/v/v). Sigmoidal curves through data points calculated by non-linear regression (Sigma Plot 5.0). Detection wavelength (bases): 214 nm; T = ambient 25 °C; V_{run} = 10 kV except for cod and quin at $^{s}_{w}$pH 5.18, where V_{run} = 20 kV; typical electrical currents were about 10 and 20 μA for 10 and 20 kV, respectively. For other conditions, see Section 2.

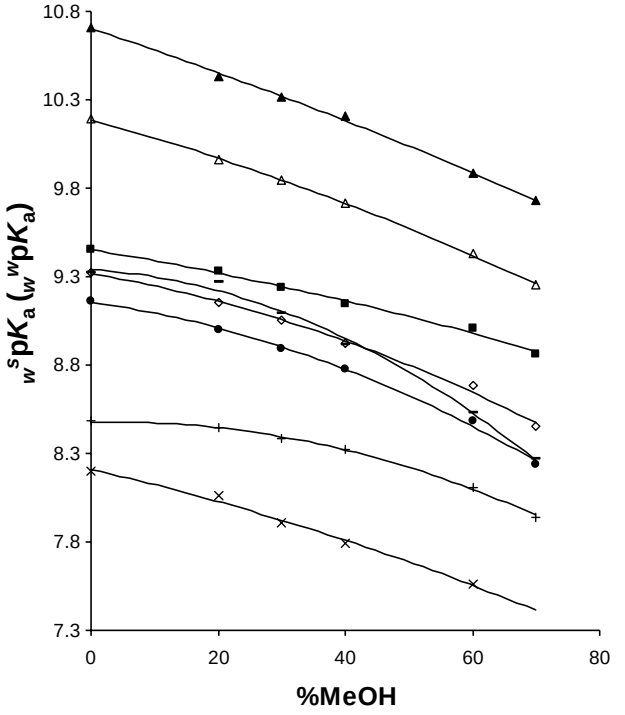

Fig. 3. Thermodynamic pK_a against percentage of methanol in the BGE. Solute identities as in Fig. 2. For conditions, see Section 2.

the equivalent effect on both t_{base} and t_{EOF}. Nevertheless, higher voltage should lead to higher current and thus could produce more Joule heat. We found that application of 20 kV produced currents (about 20 μA) about twice those observed at 10 kV, indicating the validity of Ohm's law when using 60–70% methanol solutions at $^{s}_{w}$pH about 7 [38]. Application of 20 kV instead of 10 kV led to only small differences in μ_{base}, for instance, μ_{base} of nortriptyline at 20 kV was about 2% larger than μ_{base} measured at 10 kV. Thus, the effect of Joule heating appears to be negligible also in these 20 kV experiments when using relatively high methanol content with the short-end procedure. Nevertheless, we recommend that heating effects should be considered very carefully indeed if the short-end procedure is utilised with instruments of the type employed here, since an attempt to use 20 kV in purely aqueous BGE (current around 40 μA) gave an increase of μ_{base} of nortriptyline about 6% from that obtained when using 10 kV.

In the present study we used only five to six data points for each pK_a determination. Excellent agreement was obtained

Table 2
Thermodynamic $^{s}_{w}$pK_a and $^{w}_{w}$pK_a of seven organic bases in aqueous–methanol solutions (0, 30 and 60% (v/v) methanol)

	60% MeOH		30% MeOH		Water	
	$^{s}_{w}$pK_a	$\Delta^{(2)-(1)}$	$^{s}_{w}$pK_a	$\Delta^{(2)-(1)}$	$^{w}_{w}$pK_a [14]	$\Delta^{(2)-(1)}$
nor	9.49 (0.9996)	0.06	9.83 (0.9999)	0.01	10.24 (0.9989)	0.05
diph	8.52 (0.9998)	0.04	8.91 1	0.02	9.16 (0.9997)	0.00
quin	8.14 (0.9998)	0.03	–	–	8.47 (0.9989)	0.01
proc	8.72 (0.9999)	0.04	9.06 1	0.01	9.33 (0.9997)	0.01
benz	9.06 (0.9987)	0.05	9.28 1	0.05	9.46 (0.9992)	0.01
protr	9.88 (0.9991)	0.00	10.29 (0.9993)	0.02	–	–
amitr	8.54 (0.9999)	0.01	9.09 1	0.00	–	–

$^{w}_{w}$pK_a values were obtained from [14]. $\Delta^{(2)-(1)}$ is the difference in pK_a obtained using positive CE mode and negative CE mode (set-ups (2) and (1) in Section 2), respectively. Other details as in Table 1.

between the ${}^{w}_{w}pK_a$ values obtained from this reduced data set and the 15–18 data points as used previously [14] as shown by the very small $\Delta^{(2)-(1)}$ values given in Table 2. Clearly, due to the reliability of the CE procedure, the number of μ_{base} measurements in BGE of different pH can be reduced satisfactorily to these levels giving a useful time saving.

Fig. 3 shows that the pK_a of bases decreases as the percentage of methanol in the background electrolyte increases. This decrease is in line with results obtained by other workers, who have in addition shown that the pK_a of acids tends to increase with the addition of organic solvent such as methanol or acetonitrile [8,9,12,14,35,36,40,41]. Those interested in the theoretical interpretation of these findings are referred in particular to the extensive studies in this area by Rosés and Bosch, and by Kenndler and co-workers. The changes in pK_a on addition of organic solvent can be interpreted in terms of the medium effect, which relates the total change to the stabilisation of the individual particles involved in the acid–base equilibrium [35]. For instance, Kenndler and co-workers attribute the increase in the pK_a of acidic compounds with increasing concentration of alcohols like methanol, ethanol or propanol to the lower ability of these alcohols to solvate the acid anion, leading to a loss of stabilisation of the ionised acid. In contrast, they propose that because methanol has a similar basicity to water, the medium effect on the proton is not decisive in determining the pK_a shift; furthermore, the medium effect on the neutral particle is considered of minor significance [42–44]. Rosés and Bosch propose that methanol–water is a better proton acceptor than water, which is in turn a better proton acceptor than methanol. This increased basicity of the methanol–water complex compared with that of water is proposed responsible for the decrease in pK_a of protonated base with increasing methanol content [8,16]. In contrast, they suggest that the pK_a of neutral acids increases with increasing methanol content because the electrostatic contribution to pK_a values (which depends on the charge and radius of the ions and on the dielectric constant of the medium) overwhelms the decrease in pK_a caused by the higher basicity of the methanol–water complex.

The bases we have used are structurally rather diverse, and it would not be expected that their acid–base behaviour in solutions of different methanol composition would necessarily be the same [14]. However, nortriptyline and protriptyline are secondary amines of similar structure (Fig. 1) and both give a similar, almost linear decrease in pK_a with increasing percentage of methanol. Diphenhydramine, procainamide and amitriptyline are structurally distinct compounds but all show more curvature in the plots. All are tertiary amines and it is possible that this factor may contribute to their somewhat similar behaviour. The drop in pK_a for quinine with increasing percentage of methanol is less than for the other bases. It is possible that stereochemistry in vicinity of the nitrogen atom in the particular amine structure could play an important role in the ionisation behaviour of bases in aqueous–organic solutions. However, more investigation of the possibility of such effects, using structurally more related compounds is desirable.

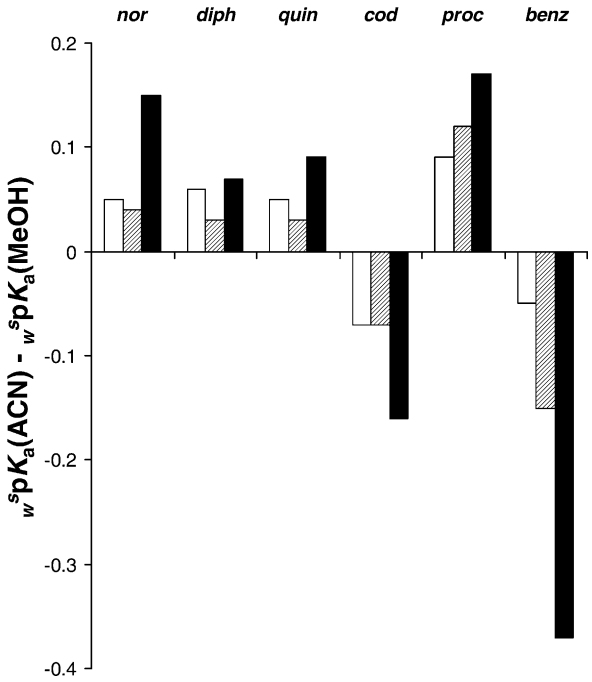

Fig. 4. Values for ${}^{s}_{w}pK_a$ in acetonitrile minus ${}^{s}_{w}pK_a$ for methanolic solutions. Data for acetonitrile mixtures from [14]. Column key: white, 20% (v/v) organic; grey, 40% (v/v) organic; black, 60% (v/v) organic.

Fig. 4 compares the ionisation behaviour of six bases in 20, 40 and 60% organic solvent content in the BGE using methanol results from the present study, and acetonitrile results from our previous investigation [14]. The apparent pK'_a values given in [14] have been corrected for ionic strength using Eq. (2) so they could be compared with results from the present study. For five of the bases, it appears that the difference in pK_a at constant organic solvent composition ${}^{s}_{w}pK_a$(acetonitrile)–${}^{s}_{w}pK_a$(methanol), is rather small (considerably less than 0.2 pK_a units) even in BGE containing 60% organic solvent. However, a larger difference (about 0.4 pK_a units) exists for benzylamine when 60% organic solvent is used. Nortriptyline, diphenhydramine, quinine and procainamide have higher ${}^{s}_{w}pK_a$ in aqueous acetonitrile than aqueous methanol solutions of the same % (v/v) composition. In contrast, codeine and benzylamine have higher pK_a values in methanolic BGE than in those containing the same % (v/v) acetonitrile. Sarmini and Kenndler noted that the shift of pK_a values of a series of substituted benzoic acids were, rather surprisingly, quite similar for aqueous acetonitrile compared with aqueous methanol or aqueous ethanol mixtures [35]. However, these authors suggest that differences between organic solvents are far less pronounced due to the dominating effects of water in the mixture. Indeed the differences in pK_a of these benzoic acids was found to be very much greater when compared in pure acetonitrile and pure methanol.

Table 3
${}^{s}_{w}pK_a$ of organic bases in aqueous–methanol solutions at 40 °C at 30 and 70% (v/v) methanol

	70% MeOH		30% MeOH	
	${}^{s}_{w}pK_a$	$\Delta({}^{s}_{w}pK_a)/\Delta T$ (K)	${}^{s}_{w}pK_a$	$\Delta({}^{s}_{w}pK_a)/\Delta T$ (K)
nor	8.88 (0.9999)	−0.025	9.39 (0.9998)	−0.030
benz	8.49 (0.9999)	−0.025	8.82 (0.9998)	−0.027

The temperature coefficient, $\Delta({}^{s}_{w}pK_a)/\Delta T$, is the rate of p$K_a$ reduction with T (K). Conditions for CE experiments as in set-up (3) in Section 2. Other details as in Table 1.

Table 4
Comparison of ${}^{s}_{w}pK_a$ values of organic bases in aqueous methanol solutions (30 and 70% (v/v) methanol) determined previously by NMR spectroscopy at 25 and 40 °C and by CE

	70% MeOH		30% MeOH	
	${}^{s}_{w}pK_a$	Δ^{CE-NMR}	${}^{s}_{w}pK_a$	Δ^{CE-NMR}
benz (40 °C)	8.47 (0.9999)	0.02	8.89 (0.9986)	0.07
amitr (25 °C)	8.28 (0.9997)	0.01	–	–

Δ^{CE-NMR} is the difference in pK_a when determined by CE and NMR. Other details as in Table 1.

Table 3 shows ${}^{s}_{w}pK_a$ values for nortriptyline and benzylamine determined at 40 °C in BGE containing 30 and 70% methanol using CE set-up 3 (see Section 2). Note, in the 40 °C experiments both the capillary cassette and also the autosampler carousel were thermostatically controlled. Buffer and sample vials were placed in the carousel at least 30 min prior to use to achieve temperature equilibrium. From these pK_a values and those given in Table 1, the T coefficients ($\Delta({}^{s}_{w}pK_a)/\Delta T$) were calculated. Values for T coefficient were about −0.03 pK_a units K^{-1} for both bases investigated, which is line with findings reported for benzylamine in aqueous solution (−0.030 K^{-1}) using NMR [20] and for other bases such as Tris, ethanolamine or diethylamine −0.028, −0.029, and −0.034 ${}^{w}_{w}pK_a$ K^{-1}, respectively [5,45]. However, the T coefficient can vary for different bases [46]. Thus, it appears that the concentration of modifier has little, if any effect upon pK_a reduction with T.

Some of us have investigated the determination of pK_a using NMR. Table 4 summarises pK_a values for benzylamine (40 °C) and amitriptyline (25 °C) determined in aqueous methanol mixtures containing 30 and 70% methanol using this NMR procedure [20]. The values from [20] were corrected for ionic strength using Eq. (2). The agreement between results obtained by CE (Tables 1 and 3) and NMR is excellent as demonstrated by the Δ^{CE-NMR} values in Table 4. This provides very strong evidence for the accuracy of measurements by either technique.

4. Conclusion

The speed of pK_a measurement of bases can be improved by using only 5–6 μ_{base} measurements, which gave excellent agreement of ${}^{w}_{w}pK_a$ values with those determined previously from 15–18 data points. Instead of the positive CE mode, the short-end injection procedure can be used to increase analyte throughput if Joule heat generated is kept sufficiently low. However, careful monitoring of the effect of Joule heating is necessary if this method is adopted.

The pK_a of bases in hydro–methanolic compositions up to 70% methanol decreases, relative to the corresponding aqueous pK_a. However, the pattern of this decrease with increasing percentage of methanol varies somewhat between individual compounds. Comparison of ionisation behaviour of bases in aqueous methanol solutions with that in acetonitrile solutions of identical composition (v/v) showed that some bases had slightly higher pK_a in methanolic mixtures compared to those in acetonitrile mixtures and others vice versa.

The rate of pK_a decrease with temperature seems little affected by the amount of methanol in the BGE. The T coefficient in solutions containing 30 or 70% methanol were closely similar to those for bases in pure aqueous solution.

The similarity of pK_a values of bases determined in aqueous methanol compositions by CE with values obtained using NMR provides strong evidence for the accuracy of either technique.

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