

Nitromethane as solvent in capillary electrophoresis

Xavier Subirats^{a,b}, Simo P. Porras^{c,*}, Martí Rosés^b, Ernst Kenndler^{a,*}

^a Institute for Analytical Chemistry, University of Vienna, Währingerstrasse 38, A-1090 Vienna, Austria

^b Departament de Química Analítica, Universitat de Barcelona, Diagonal 647, E-08028 Barcelona, Spain

^c Laboratory of Analytical Chemistry, Department of Chemistry, P.O. Box 55, FIN-00014, University of Helsinki, Finland

Available online 16 March 2005

Abstract

Nitromethane has several properties that make it an interesting solvent for capillary electrophoresis especially for lipophilic analytes that are not sufficiently soluble in water: freezing and boiling points are suitable for laboratory conditions, low viscosity leads to favourable electrophoretic mobilities, or an intermediate dielectric constant enables dissolution of electrolytes. In the present work we investigate the change of electrophoretically relevant analyte properties – mobilities and pK_a values – in nitromethane in dependence on the most important experimental conditions determined by the background electrolyte: the ionic strength, I , and the pH. It was found that the mobility decreases with increasing ionic strength (by, e.g. up to 30% from $I=0$ to 50 mmol/L) according to theory. An appropriate pH scale is established by the aid of applying different concentration ratios of a buffer acid with known pK_a and its conjugate base. The mobility of the anionic analytes (from weak neutral acids) depends on the pH with the typical sigmoidal curve in accordance with theory. The pK_a of neutral acids derived from these curves is shifted by as much as 14 pK units in nitromethane compared to water. Both findings confirm the agreement of the electrophoretic behaviour of the analytes with theories of electrolyte solutions. Separation of several neutral analytes was demonstrated upon formation of charged complexes due to heteroconjugation with chloride as ionic constituent of the background electrolyte.

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Keywords: Capillary electrophoresis; Nitromethane; Organic solvent; Contactless conductivity detection; Non-aqueous; UV detection; Heteroconjugation; pK_a shift; Mobility

1. Introduction

Although water is by far the most common solvent in capillary electrophoresis (CE), it has the disadvantage that lipophilic compounds may exhibit a low solubility in it, and the amount of analytes dissolved often does not reach the limit of detection. In such cases it is favourable to substitute water by aqueous–organic mixtures or organic solvents. It is obvious that in some cases these solvent systems might also improve the separation selectivity (though the case can be vice versa as well). Most probably the effect of organic solvents on separation efficiency is overestimated, as has been discussed in detail in a previous paper [1]. Methanol and acetonitrile are certainly the most common members of

the class of organic solvents for solutions of analytes and of the constituents of the background electrolyte (BGE). However, not only are these two solvents used in CE, but also a number of other protic or dipolar aprotic solvents (see, e.g. ref. [2]).

Nitromethane (NM) has not been applied as solvent to CE so far; only one recent application dealt with the separation of chlorophenols in binary mixtures of water with NM [3]. However, NM is widely used, e.g. as extraction solvent or as a reaction medium. It has a broad application range in organic synthesis (e.g. pharmaceuticals, pesticides, fibres, etc.) and as stabilisation agent, e.g. for halogenated hydrocarbons. It is also used as a fuel for high performance engines (e.g. in drag racing) because of the low amount of air it needs to burn. In addition, NM is also used for cleaning electronic circuit boards and in explosive industry. It should be noted, however, that NM itself is not classified as an explosive, but an explosive is formed only when it is mixed together with inorganic nitrite [4].

* Corresponding authors. Tel.: +43 1 4277 52305; fax: +43 1 4277 9523 (E. Kenndler)/+358 9 19150 253 (S.P. Porras).

E-mail addresses: porras@cc.helsinki.fi (S.P. Porras), ernst.kenndler@univie.ac.at (E. Kenndler).

For potential use in CE it has a number of interesting properties, e.g. its freezing (-28.6°C [5]) and boiling (101.2°C [5]) points, its moderate relative permittivity (36.3 at 25°C [5]), low dynamic viscosity (0.614 cP at 25°C [5]), and wide pH range of at least 24 pH units (autoprotolysis constant, $\text{p}K_{\text{auto}} \geq 24$ [6]). It should also be noted that NM is a solvent where hetero- and homoconjugation [6–9] as well as ion pair formation [10,11] might be present.

It is therefore the goal of the present work to investigate the suitability of NM for CE. This investigation will not be directed to a special application, from which often no general conclusions can be drawn. This work rather deals with fundamental physicochemical parameters, which describe the main electrolyte properties CE is based on. Therefore, we consider the influence of the solvent on the electrophoretic mobility and the acid–base properties of electrolytes, being either analytes or constituents of the BGE. We treat the influence of the solvent on the mobility as function of the ionic strength by the extended Debye–Hückel–Onsager (DHO) conductivity theory. We further prove the applicability of the Henderson–Hasselbalch relation to nitromethane as solvent, describing the dependence of the effective mobility on the pH and the $\text{p}K_{\text{a}}$ via the degree of dissociation. Such dependence could give the tool to establish a pH scale in nitromethane without the use of an electrode for the pH measurement, avoiding in this way the experimental bias introduced by liquid junction potentials.

2. Experimental

2.1. Instrumentation

Capillary electrophoresis was carried out with an HP^{3D}CE instrument (Hewlett-Packard, Waldbronn, Germany) using photometric diode-array detection (DAD) and a dual cell contactless conductivity detector (CCD) mounted in the capillary cassette as described elsewhere [12]. DAD signals were recorded at 240, 254 and 340 nm.

CCD signals were processed by a Hewlett-Packard 35900E dual channel A/D converter. Data collection from detectors was performed with ChemStation software. Uncoated fused-silica capillaries (Composite Metal Services, Ilkley, UK) of $25\ \mu\text{m}$ I.D. \times $375\ \mu\text{m}$ O.D. were used. Total capillary length was 58.5 cm, and effective capillary lengths for normal/short end-injection were 50.0/8.5 cm (DAD), 43.4/15.1 cm (CCD, cell 1) and 44.9/13.6 cm (CCD, cell 2). The capillary cassette was thermostatted at 25°C with forced air-cooling. Samples were hydrodynamically injected at 50 mbar for 4 s. The applied positive voltage was 29,880 kV (set at 30 kV), as averaged from the recorded voltage signal.

The water content of pure nitromethane and BGEs was measured with a coulometric Karl Fischer titrator (756 KF Coulometer, Metrohm, Herisau, Switzerland).

2.2. Reagents

Nitromethane (HPLC grade 96+%; the purity of the lots used was 99.43%) was from Aldrich (Steinheim, Germany). Analytes injected were *p*-nitrophenol (98%), pyrene (99%, both Aldrich), *p*-toluic acid (4-methylbenzoic acid, 98%), *p*-chlorobenzoic acid (both EGA-Chemie, Steinheim, Germany), phenylacetic acid (99%, Fluka, Steinheim, Germany), tetramethylammonium (TMA) chloride (97%, Aldrich), tetraethylammonium (TEA) chloride (98%, Sigma, Steinheim, Germany) and tetrabutylammonium (TBA) chloride ($>97\%$, Fluka). BGEs were prepared with benzoic acid ($>99\%$, Fluka), tetramethylammonium benzoate ($>98\%$, Fluka) and tetrapropylammonium (TPA) perchlorate ($>98\%$, Fluka). Hydranal-Coulomat AD Karl Fischer reagent from Riedel-de Haën (Seelze, Germany) was used for coulometric titrations of the water content. All chemicals were used as received. Water was double distilled from a quartz apparatus.

2.3. Procedures

Water uptake experiments were performed in two open vessels containing 20 mL of pure nitromethane each, both exposed to atmospheric moisture. One of these vessels was subjected to gentle stirring with a magnetic bar. Relative humidity during the measurements was $44 \pm 2\%$, temperature was $21.7 \pm 0.3^{\circ}\text{C}$. These parameters were measured with a Rotronic Hygroskop DV-2 meter (Bassersdorf, Switzerland).

The BGEs used to measure the mobilities of several analytes at different pH were prepared by mixing the required amount of benzoic acid ($\text{p}K_{\text{a}}$ of 19.5 at $25.0 \pm 0.3^{\circ}\text{C}$ [6]) with tetramethylammonium benzoate in NM. The BGEs used to determine the mobility of the analytes at varying ionic strength were prepared from tetrapropylammonium perchlorate. All BGEs were degassed after preparation in an ultrasonic bath.

Several neutral solutes were tested as electroosmotic flow markers (pyrene, phenanthrene, naphthalene, benzene, aniline and pyridine) and they all exhibited the same behaviour. Pyrene was finally selected due to its high molar extinction coefficient in a range of wavelengths used for UV detection of the analytes.

3. Results and discussion

Despite nearly not used so far, NM can be considered as a suitable solvent for CE due to its favourable thermal properties (its melting and boiling point allow its use under laboratory conditions), its relative permittivity (which is high enough to dissociate electrolytes in solutions), and its low viscosity favourable for fast analysis and comfortable manipulation of the solutions. One possibly restrictive aspect that has to be considered as well is related to its optical properties, because it absorbs light in the UV range. According to the literature the UV cut-off is 380 nm [5] (the cut-off is defined as

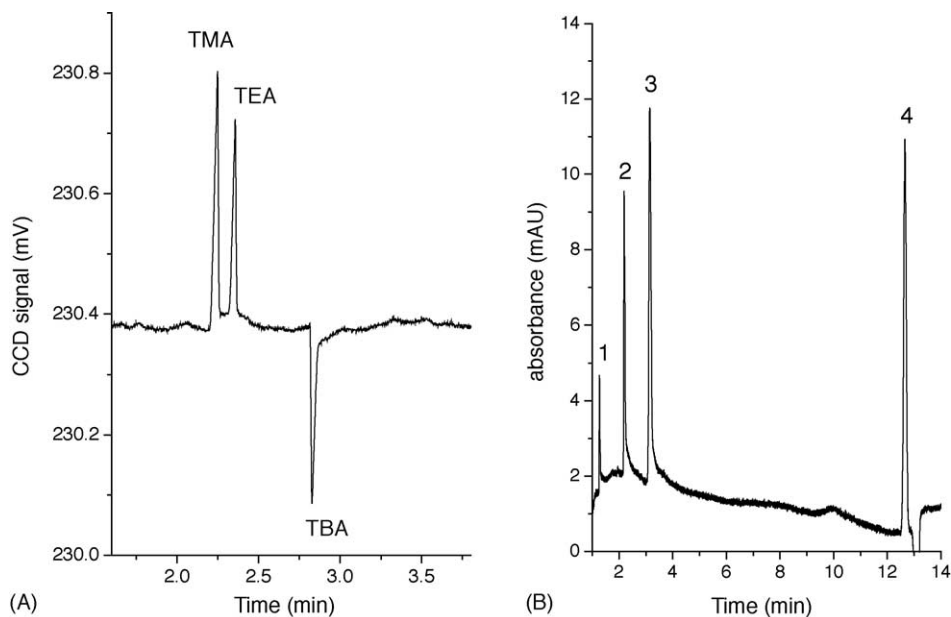


Fig. 1. Electropherogram of (A) tetraalkylammonium cations: TMA, TEA and TBA obtained with contactless conductivity detector (CCD). BGE: unbuffered, 30 mmol/L tetrapropylammonium perchlorate. (B) Neutral analytes: (1) *p*-nitrophenol; (2) *p*-chlorobenzoic acid; (3) *p*-toluic acid; (4) pyrene; obtained with UV absorbance detection at 240 nm. BGE: unbuffered, 20 mmol/L TMA chloride. Sample components 1, 2 and 3 are injected from the short end, sample 4 from the normal end of the capillary. Experimental conditions: uncoated fused silica capillary, I.D./O.D. 25/375 μm , total length 58.5 cm, effective lengths for normal/short-end injection 50.0/8.5 cm (DAD), 43.4/15.1 cm (CCD). Temperature 25 $^{\circ}\text{C}$. Voltage +30 kV.

the point where the absorbance in a 1.0 cm light-path-length cell against dry air is 1.0). However, this absorptivity does not exclude its use as solvent in CE (as will be shown below) and because conductivity detection is not interfered by the optical behaviour of the solvent. Note that the use of the conductivity detector extends not only the applicability range of the solvents, but also that of the potential components for the background electrolyte: UV absorbing BGE components can be applied without any restriction. Note also that the conductivity detector has a further advantage being able to detect non-UV absorbing analytes. Its disadvantage in many cases is related to the tendency for triangulating peaks, which has the cause in the relatively high analyte concentrations often needed for detection (the same holds, by the way, for indirect UV detection).

An example for an electropherogram obtained in NM with such separands is given in Fig. 1A. It shows the separation of tetraalkylammonium ions in a non-buffered BGE consisting of tetrapropylammonium (TPA) perchlorate. Tetramethylammonium (TMA) and tetraethylammonium (TEA), both exhibiting a higher mobility than TPA, give positive peaks, whereas tetrabutylammonium (TBA) gives a negative one due to its lower mobility. A second example (Fig. 1B) demonstrates the possibility of UV detection at 240 nm even with this light-absorbing solvent. Separation was carried out in a non-buffered salt solution (20 mmol/L TMA^+Cl^-), in which the analytes should not be ionised by protolysis (the analytes are very weak acids). However, although being not dissociated, they are migrating as anions (note that the sample is injected from the short end of the capillary). Their anionic form

indicate that complex formation with the chloride ion of the BGE due to heteroconjugation is the cause for their charge (for detailed discussion, see Ref. [13]). This phenomenon will be discussed below in more detail.

3.1. Mobility, ionic strength, viscosity

The actual mobility of the ions (i.e. the mobility of the fully charged ion at a certain ionic strength) depends on the ionic strength. According to the extended DHO theory this dependence is formulated for a monovalent 1:1 electrolyte as

$$\mu_{\text{act},i} = \mu_{0,i} - \left[\frac{8.204 \times 10^5}{(\varepsilon T)^{3/2}} \mu_{0,i} + \frac{4.275}{\eta(\varepsilon T)^{1/2}} \right] \times \frac{\sqrt{I}}{1 + 50.29a(\varepsilon T)^{-1/2}\sqrt{I}} \quad (1)$$

where $\mu_{\text{act},i}$ is the actual mobility, $\mu_{0,i}$ the absolute mobility (i.e. the mobility of fully charged ion at zero ionic strength), ε the relative permittivity, T the absolute temperature, η the dynamic viscosity (in Pa s or cP) and a is the ion size parameter or distance of closest approach (in \AA). All mobilities are in $10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$.

It can be seen from Eq. (1) that the decisive solvent-related physical properties are the relative permittivity and dynamic viscosity. In order to obtain an insight into the extent of the influence of the ionic strength on the mobility in nitromethane, and to compare it with other solvents, we have calculated

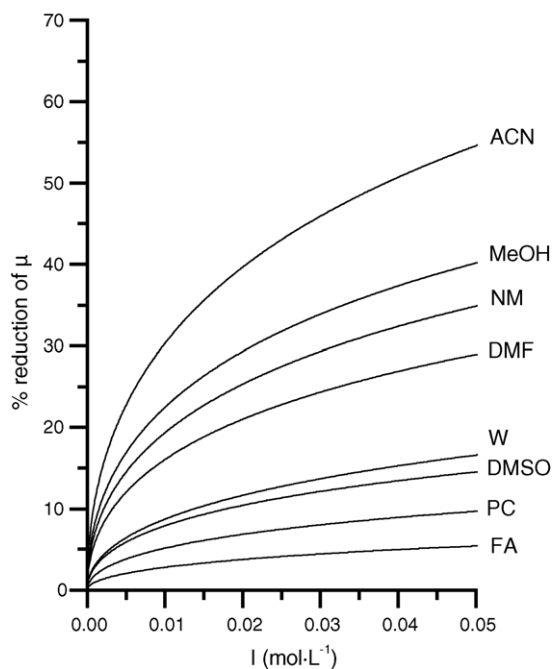


Fig. 2. Theoretical reduction of the mobility, μ , as a function of ionic strength, I , in several solvents according to the extended DHO theory (Eq. (1)). For all solvents a hypothetical absolute ion mobility of $40 \times 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ and a distance of closest approach of 5 \AA was taken. Temperature 25°C . Abbreviations: ACN, acetonitrile; MeOH, methanol, NM; nitromethane, DMF; *N,N*-dimethylformamide; W, water; DMSO, dimethylsulfoxide; PC, propylene carbonate; FA, formamide.

the decrease in mobility according to Eq. (1) with solvent data taken from ref. [5]. For simplicity the calculation was carried out for hypothetical ions with an absolute mobility of $40 \times 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ and 5 \AA as distance of closest approach (we neglect at this stage that a particular ion will exhibit different absolute mobilities in the different solvents).

The relative reduction of the absolute mobility increases with ionic strength (Fig. 2) as predictable by Eq. (1). Only considering monocharged 1:1 electrolytes (for higher charged ions Eq. (1) has to be extended; moreover, the effect is much larger) the following conclusions can be drawn from the plots. The decrease in water is not very pronounced as it reaches, e.g. at $I = 50 \text{ mmol/L}$, not more than 15% compared to infinite dilution. This is in fair agreement with practical daily experience. The influence is less than for water in case of formamide (FA), dimethyl sulfoxide (DMSO) and propylene carbonate (PC), but a much higher effect is predicted for acetonitrile (ACN), methanol (MeOH) and *N,N*-dimethylformamide (DMF). NM behaves rather similar to MeOH, and thus the reduction of the mobility with ionic strength is much more pronounced here than in water.

The theoretical dependence of μ on I was examined experimentally for three permanent monovalent ions, TMA, TEA and TBA. TPA could not be used as analyte because it was a constituent of the BGE (TPA perchlorate). The resulting mobilities decrease nonlinearly for ionic strength between 2.5 and 75 mmol/L (see Fig. 3). The data match to Eq. (1),

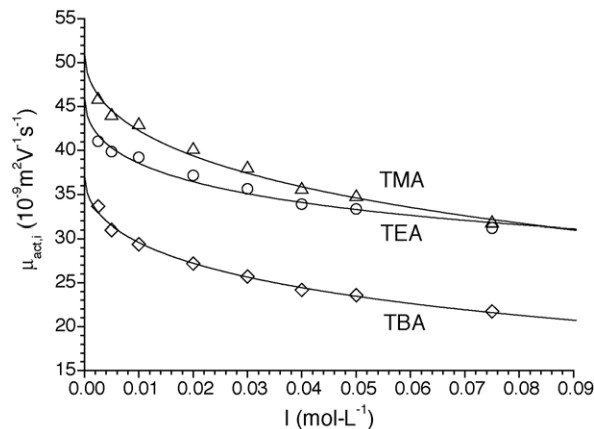


Fig. 3. Experimental actual mobilities, $\mu_{\text{act},i}$, of tetramethylammonium (TMA), tetraethylammonium (TEA) and tetrabutylammonium (TBA) as a function of ionic strength. Temperature 25°C . Relative standard deviation for the measurement of the mobilities ($n = 3$) was typically 0.4%. Solid lines are fitted curves according to Eq. (1).

as seen from the fitted curves in the figure. We can therefore conclude that the mobility dependence on I follows quite well the extended DHO theory.

Extrapolation of the curves to $I = 0$ leads to the absolute mobilities, which are given in Table 1. They range between 37.2 and $51.1 \times 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$. Although the indeterminate error for measurement of the mobility is only in the range of about 1%, the absolute mobility is possibly more biased due to the asymptotic nature of the extrapolation to zero I . Parameter a , the distance of closest approach, can also be derived from the curve fitting. The resulted values are between 4 and 7 \AA in the case of the selected analytes, which are realistic concerning the sizes of the ions.

Moreover, the obtained absolute mobilities match well to the ones expected from Walden's rule (Table 2). This rule states that the product of dynamic viscosity and absolute mobility is constant ($\eta\mu_{0,i} = \text{const}$) at a certain temperature, independent of the solvent. Although not well obeyed by the smallest ion (TMA), the rule is followed very well especially by the largest ion (with the lowest charge density): for TBA Walden's products agree in such different solvents as NM, ACN, MeOH and FA within 5% relative, despite the viscosities vary nearly by a factor of 10.

In the measurement of the mobilities, the capillary cassette was thermostatted at 25°C . Temperature increase due to Joule heating does not play a role under the working conditions (at 30 kV, the typical current for 10 mmol/L TMA

Table 1
Absolute mobilities, $\mu_{0,i}$, of tetraalkylammonium ions obtained by extrapolation of the fitted curve according to Eq. (1) to zero ionic strength

Solute	$\mu_{0,i}$ ($10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$)
Tetramethylammonium (TMA)	51.1 ± 0.4
Tetraethylammonium (TEA)	46.1 ± 0.5
Tetrabutylammonium (TBA)	37.2 ± 0.3

$T = 25^\circ\text{C}$.

Table 2

Product of absolute ionic mobility, $\mu_{0,i}$, and dynamic viscosity, η , of the solvent (Walden product) of the tetraalkylammonium ions in different solvents

Solute	$\mu_{0,i}\eta$ (10^{-12} N V $^{-1}$)					
	NM ^a	NM ^b	ACN ^b	MeOH ^b	FA ^b	Water ^b
Tetramethylammonium	31.4	34.69	33.44	39.31	43.94	40.94
Tetraethylammonium	28.3	30.32	30.14	34.56	35.73	29.66
Tetrabutylammonium	22.8	21.67	21.81	22.28	22.38	17.84

$T = 25^\circ\text{C}$. For error see Table 1.

^a This work; viscosity 0.614 cP [5].

^b Calculated from conductivities taken from [19] and viscosities from [5]. Viscosities: NM, 0.614; ACN, 0.341; MeOH, 0.551; FA, 3.302; water, 0.8903 cP.

benzoate BGE was 2.5 μA ; for 75 mmol/L TPACIO₄ it was 17.5 μA), because no significant changes of the mobilities were observed when repeating the measurements with lower applied voltages (20 and 10 kV).

It should be mentioned that a strong functional effect of I on μ has the consequence that the separation efficiency, expressed by the ultimate plate number, N^{ult} , is also strongly affected by the ionic strength, and is always decreased. N^{ult} considers the limiting case that only longitudinal diffusion is the source of peak broadening. It depends on the ratio of mobility and diffusion coefficient of the analyte. Both parameters are correlated at zero ionic strength according to the Nernst–Einstein relation, resulting in the well-known equation $N^{\text{ult}} = 19.46zU$, where z is the charge number of the ion, and U is the voltage. Note that at infinite dilution all solvents should have the same ultimate plate number (for a given voltage). However, the ionic strength dependence of the diffusion coefficient and mobility is not the same. As at finite electrolyte concentrations the mobility is decreased by both, the relaxation effect and the electrophoretic effect, but the diffusion coefficient only by the relaxation effect, the former parameter is more reduced with increasing ionic strength than the latter. As a consequence plate number is always lost at finite ionic strength compared to the case with $I = 0$, and it can be predicted that the reduction will be as more pronounced as stronger a functional dependence of μ on I is. This aspect is discussed in detail for various solvents in previous papers [1,2,14]. In practice (at ionic strengths of several tenths of mmol/L) NM should be a less favourable solvent than water when the separation efficiency is considered.

3.2. pH scale and effective mobilities

By the aid of the commonly used glass electrode the pH is derived from the difference in the electrochemical response of pH-sensible electrodes, e.g. according to $\text{pH} = \text{pH}_{\text{st}} + (E - E_{\text{st}})F/RT \ln 10$, where E is the cell potential of the sample of a certain pH, E_{st} the standard cell potential of a buffer of known pH_{st} , F the Faraday constant and R is the gas constant.

In a normal glass electrode, the electric contact between the glass sensitive membrane electrode and the reference electrode is produced through the external solution by means of a porous material, normally a frit. A low current is established when the ions of the internal reference filling solution and the external solution migrate across the frit. If these

ions have different mobilities a charge separation is produced and a liquid junction potential is generated. This potential is strongly affected by the nature of the solvent at both sides of the membrane. If the solvent is the same in the filling reference solution and in the external solution this contribution is ideally cancelled, because then it has the same magnitude in calibration and in pH measurement. Consequently, the same solvent as in the sample should be used in the reference solution and in the calibration solutions in order to minimize the liquid junction potential effect. This means that, in the present case, the glass electrode had to be adapted with a reliable NM reference filling solution, and the preparation of trustworthy pH standard solutions with an accurately known and stable pH. However, such special glass electrodes are hard to maintain, and the solutions might not be stable under the habitual working conditions in a chemistry laboratory, especially under atmospheric moisture.

An alternative for the problematic use and maintenance of the glass electrode is the application of a buffer composed from an acid with known pK_a in the given solvent, and its conjugated base, mixed at defined ratios. The Henderson–Hasselbalch equation (with activity correction) allows then calculating the pH of a solution from the composition of the buffer according to

$$\text{pH} = \text{pK}_a - \log \left(\frac{a_{\text{HA}}}{a_{\text{A}}} \right) \quad (2)$$

where pH is the pH value in the organic solvent scale, pK_a the dissociation constant of the buffering acid–base pair, a_{HA} the activity of the acid and a_{A} is the activity of the conjugate base of the acid. Strictly speaking, Eq. (2) considers only the acid–base equilibria, not parallel or side equilibria like ion pairing or homo- and heteroconjugation. It is obvious that the requirement for applying a pH scale with this concept is the knowledge of the accurate pK_a of the acid in the given solvent.

Using this concept, we established a pH scale with benzoic acid (with the known pK_a of 19.5 [6]) and its tetramethylammonium salt in different proportions. Keeping constant the salt concentration at 10 mmol/L led to the same constant ionic strength in all electrolytes. The activity of the anion, a_{A} , was calculated from the product of the anion concentration, c_{A} , and the activity coefficient, f_i . The latter was calculated from $-\log f_i = (Az_i^2 I^{1/2}) / (1 + aBI^{1/2})$ according to the extended theory of Debye and Hückel, taking the mean

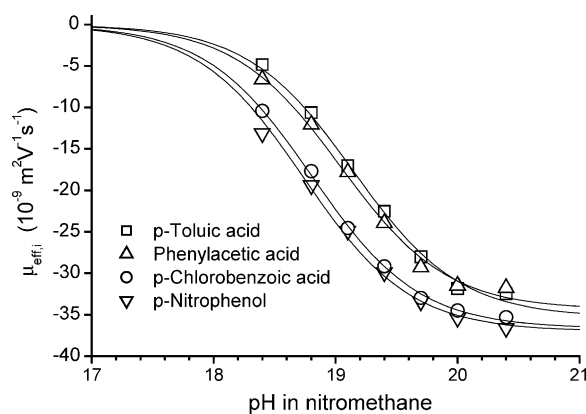


Fig. 4. Measured effective mobilities, $\mu_{\text{eff},i}$, vs. the pH of the BGE. The BGE consisted of tetramethylammonium benzoate (10 mmol/L) and benzoic acid at different proportions. The pH was calculated by means of the Henderson–Hasselbalch equation (Eq. (2)), corrected for the activity of the buffer anion. Relative standard deviation for the measurement of the mobilities ($n \geq 3$) was typically 0.7%. Temperature 25 °C. Solid lines are the fitted curves according to Eq. (3).

distance of closest approach as 5 Å. A and B are the appropriate parameters for NM. The activity coefficient for the ionic strength of 0.010 mol/L was then 0.737. The activity of the molecular acid, HA, was taken equal to its concentration.

Considering this activity correction, the pH scale was shifted by -0.13 units compared to the concentration-based scale. Agreement with the Henderson–Hasselbalch conditions in this solvent system was proved for four different compounds, for which the effective mobilities were determined as a function of the pH.

These effective mobilities, $\mu_{\text{eff},i}$, are depicted in Fig. 4. In the same figure the curves are obtained by fitting the data to

$$\mu_{\text{eff},i} = \frac{\mu_{\text{act},i}}{1 + 10^{\text{p}K_{\text{a}} - \text{pH}}} \quad (3)$$

which gives the effective mobility as a function of the $\text{p}K_{\text{a}}$ of the analyte and the pH of the solution. Fittings were carried out taking a value of zero for the analyte mobility at pH lower than 14 (which is more than three pH units smaller than the $\text{p}K_{\text{a}}$ of the samples, see below). It can be concluded that the fitted mobility versus pH curve is well followed by the measured data points for all analytes.

This agreement is rather surprising when taking into account that strong homoconjugation occurs in NM (see Ref. [6]; for a detailed discussion, see e.g. [15]). In fact, a rather high homoconjugation constant, K^{f} , of 5620 L/mol ($\log K^{\text{f}} = 3.75$) [6] has been reported for the present reference buffer acid, benzoic acid (water content of NM 5 mmol/L). It is known that an increase in water content decreases homoconjugation due the competition between the water and the acid molecules in hydrogen bonding. We can thus expect that under the present conditions with a water content being higher than 5 mmol/L the effect of homoconjugation is most probably less pronounced, whereas we cannot quantify it due to the lack in supporting data in the literature. Anyway, for

a given initial concentration ratio of HA and A^- , homoconjugation would change the pH. Consequently, the mobilities determined in a buffer with given initial $c_{\text{HA}}/c_{\text{A}^-}$ should not match to Eq. (3). It is seen in Fig. 4 that in fact they do, which leads us to conclude that for the BGE under discussion homoconjugation does not play the role it should according to literature data.

Heteroconjugation data are not available from the literature for the analytes and buffer constituents depicted in the plots in Fig. 4. We think that we can exclude heteroconjugation between these specific analytes and benzoate, because the mobilities match very well to the curves when values of zero are taken for μ at low pH. If heteroconjugation would play a role, finite (negative) values for the mobilities at low pH would otherwise lead to a better match.

It is, however, evident that in our system heteroconjugation takes place under other conditions, which follows from the electropherogram shown in Fig. 1B. In this case, chloride as constituent of the BGE forms anionic heteroconjugation complexes with the neutral analytes, which are thus electrophoretically separated.

Note that neither homo- nor heteroconjugation influences the ionic strength of the BGE.

3.3. $\text{p}K_{\text{a}}$ values

Although the analyte mobilities are well following the fitted curves (Fig. 4), only for *p*-chlorobenzoic acid the $\text{p}K_{\text{a}}$ derived from curve fitting is identical with the literature data (Table 3). For the other analytes the deviation between our values and those given in the literature (determined by potentiometric method using a glass electrode [6]) is between 0.8 and 1.3 units. This is noticeable because the literature $\text{p}K_{\text{a}}$ values for both, the reference acid and the analytes, are reported by the same authors (see citations given in ref. [6]), and it is assumed that the data is measured under identical experimental conditions. An explanation for the $\text{p}K_{\text{a}}$ difference might be the occurrence of secondary equilibrium, which shifts the measured mobilities of some analytes and thus the $\text{p}K_{\text{a}}$ values. Again, this is hard to confirm due to lack of the literature data for such equilibria. However, the difference between our $\text{p}K_{\text{a}}$ values and published data is even much smaller than the $\text{p}K_{\text{a}}$ discrepancy found in some cases for an individual acid reported in the literature (see the compilation of Izutsu [6]).

Table 3
 $\text{p}K_{\text{a}}$ values of neutral acids in NM derived from the measured mobilities as function of the pH

Solute	$\text{p}K_{\text{a}}$	Literature $\text{p}K_{\text{a}}$ [6] ^a	$\text{p}K_{\text{a}}$ in water [20]
<i>p</i> -Nitrophenol	18.74 ± 0.03	20.1	7.14
Phenylacetic acid	19.04 ± 0.03	20.1	4.31
<i>p</i> -Toluic acid	19.14 ± 0.02	19.95	4.37
<i>p</i> -Chlorobenzoic acid	18.81 ± 0.01	18.8	4.00

Literature values for NM and water are given for comparison. $T = 25$ °C.

^a Water content 5 mmol/L.

The difference in pK_a values, $\Delta pK_a^{NM-W} = pK_a^{NM} - pK_a^W$, of the carboxylic acids in Table 3 between NM and water is 14 units (for phenol, it is 11.6 units only). This total shift is much larger than for methanol, and slightly smaller than for acetonitrile. ΔpK_a^{NM-W} is connected to the stabilisation of the acid–base equilibrium, in particular to the stabilisation of the individual particles involved in the equilibrium in the two solvents (we have discussed about these concepts in a number of previous works (see, e.g. [2,16,17]) and readers are referred to these papers and to the literature cited therein). For the present analytes and buffer acid, which are of type HA, the particles are the proton, the anion and the molecular acid. The standard free energy of transfer, ΔG_t^0 , is related to ΔpK_a^{NM-W} by basic thermodynamics. ΔG_t^0 for the proton is 95 kJ mol^{-1} from water to NM [18]. Positive value means that NM is less basic than water (it is even less basic than ACN, which has a ΔG_t^0 for the proton of 46.4 kJ mol^{-1} [18]). The contribution of the proton destabilization in NM to the pK_a shift is accordingly 4.0 pK_a units. Unfortunately, data for ΔG_t^0 of molecular acids is scant; only for acetic acid data could be found, not for the analytes. However, for acetic acid the pK_a shift is similar (from 4.76 in water to 20.5 in NM [6]). For acetate ΔG_t^0 is positive (56 kJ mol^{-1} [18]), which allows the conclusion that anionic carboxylates are destabilised in NM. The contribution of acetate on the pK_a shift is accordingly 3.2 pK_a units. Both effects contribute to the reduction of the acidity in NM compared to water, but they cannot fully explain the total shift. The remaining contribution must come from the better stabilisation of the molecular acid in NM compared to water. It is expressed by the higher solubility of the relatively lipophilic organic acids in NM.

3.4. Water uptake

Organic solvents have an intrinsic trend to be hygroscopic. This has to be taken into account because under normal analytical laboratory conditions there is no protective anhydrous atmosphere to prevent from the uptake of water. As the water content can change the properties of organic solvents, it is important to control at least roughly how much water the solvent takes up from the laboratory atmosphere. For this purpose the water concentration of NM stored in an open vessel was measured as a function of time of exposure under conditions of a typical laboratory environment.

It was found that the water content of freshly opened NM was lower than 0.03%, in agreement with the specifications of the manufacturer. As seen in Fig. 5, the water content increases when the solvent is exposed to laboratory air; it is clear that the increase is stronger in the stirred than in the non-stirred vessel. However, the content was less than 0.5% (all water concentrations are given in % w/w) within about 2.5 h, independently whether the solvent was stirred or not. After 5 h of exposure the content reached 1%, after 10 h it was

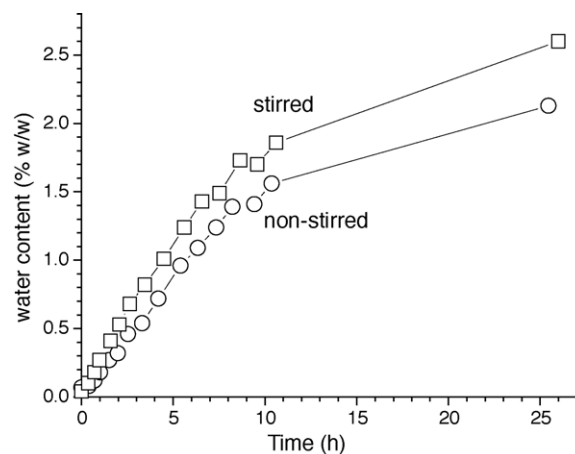


Fig. 5. Water content of pure nitromethane exposed to atmospheric moisture as a function of the time of exposure. Relative humidity $44 \pm 2\%$, temperature $21.7 \pm 0.3^\circ\text{C}$.

less than 2%, and after 24 h the curves flattened at contents lower than 3%.

It is clear that the content itself does mean anything if not related to the possible extent of changes in the properties of the analytes and the BGE constituents. One of the most important influences is on the pK_a values. The prediction of the detailed pK_a shift with water content is not easy to make because the pK_a values do not linearly depend on the composition of the mixed aqueous–organic media. However, it can be assumed that the influence of small amounts of water will be smaller when ΔpK_a^{NM-W} is small. For the carboxylic acids in Table 3, ΔpK_a^{NM-W} is about 14 units. Under the (unrealistic) assumption that the pK_a shift is a linear function of the water content, the decrease of the pK_a values in NM would be about 0.14 pK_a units per % water. It is expected that in reality the decrease is much more pronounced at the side of low water concentrations, because there the slope of the pK_a versus % water curve is normally steeper. However, when the analytes and the reference acid taken for the adjustment of the pH are both neutral acids (of type HA), their behaviour is similar. This means that the change will result in an only parallel shift of the data. For accuracy reasons the change of the pK_a values should be taken into account even for an uptake of say few tenth % water in NM, which might not be exceeded when some care is taken on the protection of the solvent. Although in this case the shift of few tenths pK_a units is perhaps lower than the difference in pK_a data taken from different sources (see, e.g. [6]), its fluctuation with varying water concentrations can lead to a low reproducibility of the electrophoretic behaviour.

4. Conclusions

Nitromethane as solvent in capillary electrophoresis has certainly the advantage over water that it dissolves lipophilic compounds much better. The behaviour of electrolytes in this

solvent can be well described by the models developed for electrochemistry and solution chemistry. Consequently, the decrease of the actual mobility with increasing ionic strength follows the dependence formulated by the extended theory of DHO. The establishment of a pH scale using BGEs with different ratios of an acid and its salt leads to a good agreement between effective mobility and pH according to the Henderson–Hasselbalch equation for the selected experimental conditions. This is surprising because the expected homoconjugation should alter the pH scale.

Conjugation clearly takes place in NM. This is documented by the high values of the homoconjugation constants published in the literature. Also, it is demonstrated in the present work by the separation of neutral analytes upon complex formation with chloride from the BGE.

The pK_a values of the analytes are shifted by about 11–15 units compared to water. Interestingly they are even levelled in NM: whereas the maximum difference in pK_a between the phenolic analyte and the carboxylic acids is more than +3 units in water, it is –0.4 units in NM.

Although the optical properties of NM may restrict its use in CE, it is still possible to measure analytes with UV detection. With the conductivity detector no such restrictions exist.

Acknowledgements

X.S. thanks the MCYT of the Spanish Government (project BQU2001-3226) for financial support. S.P. thanks the Academy of Finland for financial support (grant No. 204829). The authors acknowledge the loan of a KF coulometer from Professor M.-L. Riekkola (University of Helsinki, Finland), and the gift of the CCD from Professor B. Gaš (Charles University, Prague, Czech Republic).

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