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Electrophoretic mobilities of cationic analytes in non-aqueous methanol, acetonitrile and their mixtures

Influence of ionic strength and ion-pair formation

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Abstract

The mobilities of cationic analytes in organic solvents and water are compared, and the reasons for differences in the mobilities are discussed in detail. Actual mobilities (at background electrolyte concentration 10 mmol/l) of anilinium ions were determined by capillary zone electrophoresis in water, methanol, acetonitrile and mixtures of methanol and acetonitrile (in volume ratios 1:1, 1:3 and 3:1). The actual mobilities correlated with the viscosity of the organic solvent: the products of actual mobility and viscosity were constant within 7%. However, these products were significantly larger in water. Larger products of mobility and viscosity in water were also found for unsubstituted anilinium when the absolute mobility (at zero ionic strength) was taken into consideration. Thus, ion–solvent interactions must be responsible for the seemingly high mobility in water compared with that in organic solvents. This finding can be explained by the effect of the ion on the water structure. Based on equilibrium constant for ion-pair formation given in the literature, about 20% of the main background electrolyte constituent (tetrapropylammonium perchlorate) is associated at 10 mmol/l concentration in acetonitrile. Comparison of the plot of the measured mobilities of the analytes vs. the square root of the corrected ionic strength of the background electrolyte in acetonitrile with the prediction based on the Debye–Hückel–Onsager theory showed the measured mobilities deviate negatively from the theoretical line. This is apparently due to ion pairing, which takes place for the analytes as well. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Methanol (MeOH) and acetonitrile (ACN) are the most commonly used solvents for background electrolytes (BGEs) in non-aqueous capillary electrophoresis (CE). These solvents are inexpensive and easily available at high purity, and they have low UV

absorption at wavelengths commonly used with UV detection. MeOH, with its equal capability as acceptor and donator of proton is a neutral amphiprotic solvent, has moderate autoprotolysis ($pK_{\text{auto}} 17.2$ at 25°C) and dielectric (32.63 at 25°C) constants. The viscosity of MeOH (0.544 cP at 25°C) is lower than that of water (0.890 cP). In general, MeOH is a solvent rather similar to water, and for that reason often is the first choice for non-aqueous CE. Not only used to increase the solubility of more lipophilic analytes, MeOH also changes (as most organic

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solvents) the ionisation properties of both acids and bases compared to water (see Refs. [1–8]).

ACN is a very different type of solvent from both water and MeOH, although its dielectric constant (36.01 at 25°C) is similar to that of MeOH. It has a large dipole moment (3.92 Debye units, water 1.85, MeOH 1.70) and is traditionally classified as a protophobic dipolar aprotic solvent because of its poor hydrogen bond acceptor ability (its hydrogen bond donor ability is even lower). It has much weaker basic properties than water. The ionisation behaviour of acids and bases in ACN is very different from their behaviour in MeOH and water, cf. e.g., Refs. [1,5]. The autoprotolysis constant of ACN is extremely low (pK_{auto} over 33), and the viscosity is 0.345 cP.

From the above, it is clear that the separation selectivity in CE is considerably different in mixtures of MeOH and ACN than in the pure solvents, and this indeed has been demonstrated in several recent papers. Electrophoretic mobilities and separations in MeOH–ACN mixtures have been investigated, for example, with alkali and alkaline earth metal ions [10], alkanesulfonates and alkyl sulfates [11], triazine herbicides [12], fatty acids [13] and cationic drugs [14–17] as model analytes. In many cases, however, the basic physico-chemical properties of both the BGE components and the analytes have not been known. This lack of information makes it difficult to explain the results, as they could stem from the effect of ion–solvent or ion–ion interactions, affecting the mobilities and the pK_a values as well. Clearly to distinguish the different effects of the solvent systems on the electrophoretic behaviour of analytes, it is necessary to pay careful attention to the selection of the experimental parameters.

In the present work the mobilities of cationic analytes were investigated under well-defined conditions. As such, this is a systematic continuation of previous investigations of similar analytes in non-aqueous solvents [18–20], where the main interest was the change in pK_a values. In this work anilinium and anilinium derivatives with closely related molecular structures were selected as analytes; the organic solvent systems consisted of pure MeOH or ACN, or of their mixtures covering the entire composition range. Under the conditions used, the analytes were fully protonated in all solvents due to the addition of perchloric acid.

The ionic mobility is known to be determined by several factors. To begin with, (i) ion–solvent interactions determine the size of the solvated ions, and thus the Stokes radii for frictional resistance and (ii) the moving ion undergoes ion–dipole interactions with the solvent molecules, resulting in an additional, so-called dielectric friction. Two important simplifications are introduced here, by considering the fluid as a continuum: one neglects the solvent structure around the ion and the other the microdynamics of the molecules. (iii) A third factor that may influence the mobility is ion–ion interactions, which reduces the mobility in solutions with finite ionic strength relative to the limiting case at infinite dilution. These ion–ion interactions were initially described by the theory of Debye, Hückel and Onsager, based on the model of the ion cloud with the introduction of electrophoretic and relaxation effects. The theory has been expanded by Pitts [21], Fuoss and co-workers [22–24], with further development of the dielectric friction model by Fuoss [23], Boyd [25], Zwanzig [26,27], Hubbard and Onsager [28,29], and Turq and co-workers [30,31] (for a detailed discussion, see Refs. [32,33]). (iv) Reinforcing electrostatic interaction with the counter-ions, a phenomenon often observed in organic solvents of intermediate or low dielectric constant, is a fourth factor that may influence the mobility, even more so than the first three. Aggregates of ion and counter-ion, formed by ion association (with solvent separated ions) or ion-pair formation (with direct contact of the ions), have a reduced or even zero net charge, which means that the extent of mobility reduction depends on the degree of formation of the aggregate.

It follows from the above discussion that the change in the mobility upon change in the solvent often cannot be related to a single parameter, e.g., to the solvent bulk viscosity as done by Walden's rule. Walden's rule is, in fact, highly qualitative approach, with sharp discrepancies between theory and experiment in many cases. Despite its simple assumptions it is nevertheless often obeyed, especially when ions are large and univalent. Since many univalent organic analytes are of relatively large size, we considered that solvent viscosity might be a useful parameter to describe the effect of solvents on the mobility in CE at least to the accuracy obtainable in CE. In addition, since the analyte and BGE concentrations are normally low in CE, the simple theoretical

approaches to treat the influence of the counter-ions in CE might be appropriate to elucidate the role of the BGE composition. It is thus the goal of this paper to discuss several aspects that are decisive for the mobility of ions in solvents, and to evaluate their significance. This discussion will be carried out in the context of anilinium ions as analytes, and with MeOH and ACN and their binary mixtures as solvents. Special emphasis will be placed on a comparison with aqueous solutions.

2. Materials and methods

2.1. Chemicals

Aniline, 2-methylaniline (*o*-toluidine), 3-methylaniline (*m*-toluidine), 4-methylaniline (*p*-toluidine), 2,6-dimethylaniline, *N*-isopropylaniline, 70% perchloric acid, tetraethylammonium perchlorate (TEAP) and electrochemical grade tetrapropylammonium perchlorate (TPAP) were purchased from Fluka (Buchs, Switzerland). Nitromethane was from Aldrich (Steinheim, Germany). HPLC-grade ACN (Lab-Scan, Dublin, Ireland) and HPLC-grade MeOH (J.T. Baker, Deventer, The Netherlands) were stored under nitrogen to avoid the uptake of moisture from air during the storage. All chemicals were used as received. Distilled water was further purified with a Water-I system from Gelman Sciences (Ann Arbor, MI, USA).

2.2. Sample and background electrolyte solutions

Stock solutions (50 mmol/l each) of anilines were prepared in either MeOH or ACN and were stored at 7°C. Photosensitive analytes were stored in brown bottles. The stock solutions were diluted 500-fold with BGE immediately before the electrophoretic measurements. Nitromethane, used as electroosmotic flow (EOF) marker, was dissolved in BGE at concentration of 5 mmol/l. Non-aqueous BGE consisted of 1 mmol/l perchloric acid and 4–29 mmol/l TPAP dissolved in MeOH, ACN or their mixtures. Aqueous BGE consisted of 5 mmol/l perchloric acid and 5–25 mmol/l TEAP in water. The water content of freshly prepared non-aqueous BGEs was between 0.01 and 0.02% (w/w) as measured by Karl Fischer titration. The water content of the non-aqueous BGE

in the running vials was determined after electrophoretic measurements, and the maximum water content was 0.05% (w/w). Fresh BGE and sample solution were introduced in every run. All the BGEs were prepared daily at room temperature ($25.0 \pm 0.5^\circ\text{C}$).

2.3. CE instrument and related parameters

The CE instrument was a HP ^{3D}CE system (Hewlett-Packard, Waldbronn, Germany) with a slightly modified pressure control system in order to apply the constant pressure needed for pressure mediated capillary electrophoretic experiments (see below). Untreated fused-silica capillaries [58.5 cm (50.0 cm effective length) \times 375 μm O.D. \times 50 μm I.D.] were from Composite Metal Services (Hallow, UK). Polyimide coating at each end of the capillary was removed by burning. Sample injection was done at 50 mbar pressure for 0.9–1.5 s (non-aqueous BGEs) or 2.4 s (aqueous BGE). The capillary cassette temperature was set to 25.0°C, and the tray temperature was maintained at $25.0 \pm 0.5^\circ\text{C}$ with external water-cooling. UV detection was carried out with a diode-array detector at 200 nm. Running voltage was either +2.5 or +5 kV, and the resulting electric current was below 5 μA in every case. Before use the capillary was rinsed with 0.1 mol/l sodium hydroxide (dissolved in MeOH or water) for 10 min, then with pure solvent (MeOH or water) for 10 min, and finally with BGE for 30 min. Before every run the capillary was rinsed with BGE for 2 min. After use the capillary was flushed with pure solvent and dried with air.

All electrophoretic mobilities were measured by the pressure mediated capillary electrophoretic method [34] as follows. In the first step, analyte and EOF marker (nitromethane) were moved into the thermostated region of the capillary by application of injection pressure for 60–150 s. After that, a normal capillary zone electrophoretic step was carried out (with programmed linear ramp-up and ramp-down time of 0.17 min), and the analyte and nitromethane were separated during 5–6 min. A second nitromethane zone was then injected into the capillary, and finally the data acquisition was started and all three zones were mobilised through the detector window. When the mobilisation time of the second nitromethane peak is known, it is possible to calcu-

late the distance between the analyte peak and the nitromethane peak from the first injection, and thus the electrophoretic mobility of the analyte. All electrophoretic mobilities presented in this work are averages of three replicate measurements with relative span less than 0.5%.

3. Results and discussion

Accurate determination of the mobility in capillary zone electrophoresis from the apex of the analyte peak requires that non-linear effects are not present. In other words, the peaks need to be symmetrical, Gaussian shaped. The experimental conditions were accordingly selected so that the triangulation of the peaks due to electromigration dispersion was minimised. Clearly the analyte should be fully charged as well. This second requirement was fulfilled in the present experimental set-up by adding perchloric acid to the BGE. This acid is fully dissociated even in non-aqueous ACN [35]. We accepted the fact that the BGE was unbuffered because the analyte concentration was chosen to be much lower than the acid concentration, and no other components that might interfere with the protonation of the analytes were present in the samples. Note that BGE in the running vials was replaced by fresh BGE after each run. The analytes (anilines) have pK_a values (as cation acids, BH^+) in the range 3.9 to 5.8 in water [4,5,18] between 5.5 and 6.6 in MeOH [1,4,5,18] and about 6 pK units higher in ACN than in water [1,36].

Thus we can assume that the analytes are fully protonated in all solutions (for 2,6-dimethylaniline, see footnote in Table 1).

Non-linear effects can be avoided by appropriate selection of the co-ion of the cationic analytes. However, the mobility of H^+ as co-ion does not match the mobility of the analytes, and we therefore applied a neutral electrolyte (TPAP) at higher concentration (9 mmol/l) than the perchloric acid (1 mmol/l), and used it as main constituent of the BGE in the organic solvents. TEAP was used in water. The use of tetraalkylammonium ion resulted in sufficiently symmetrical peaks in all cases, enabling accurate determination of the mobilities of the analytes.

3.1. Mobilities of analytes in the different solvents

Table 1 presents the actual mobilities of the anilinium ions in the different solvents (BGE concentration 10 mmol/l, analyte concentrations 0.1 mmol/l). The mobilities in the organic solvents are lowest in pure MeOH, and increase with ACN content until they reach a nearly constant, maximum value at 75% and higher ACN content. In a particular organic solvent composition the mobilities of the analytes are closely similar, differing by less than 10% within a single solvent (with anilinium having the highest mobility). This behaviour is in strong contrast to that in water (Table 1) where the mobilities span a considerable broader (relative) range than in the organic solvents. Evidently the organic

Table 1

Actual mobilities ($\mu_{act,i}$) of anilinium ions, i , in solvents consisting of different proportions of MeOH and ACN. Mobilities measured in water are included for comparison

Analyte	Abbreviation	$\mu_{act,i}$ ($\cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$)					
		Water	MeOH–ACN (100:0)	MeOH–ACN (75:25)	MeOH–ACN (50:50)	MeOH–ACN (25:75)	MeOH–ACN (0:100)
Anilinium	An	34.24	39.93	48.63	55.59	60.08	59.93
2-Methylanilinium	2MeAn	31.19	37.48	46.35	53.36	58.1	57.99
3-Methylanilinium	3MeAn	31.51	38.54	47.09	53.82	58.31	57.82
4-Methylanilinium	4MeAn	31.19	37.85	46.34	53.1	57.54	56.97
2,6-Dimethylanilinium	2,6diMeAn	28.87 ^a	36.69	45.51	52.61	57.66	57.44
<i>N</i> -Isopropylanilinium	<i>N</i> -iPrAn	26.33	36.48	45.85	53.33	58.18	55.45

The solvent compositions are given in % (v/v). BGE in MeOH–ACN: 9 mmol/l TPAP, 1 mmol/l perchloric acid. BGE in water: 5 mmol/l TEAP, 5 mmol/l perchloric acid. Temperature 25°C.

^a Effective mobility of the ion with 0.973 as degree of protonation (pK_a value in water is 3.89, pH 2.34).

solvents introduce a certain levelling effect on the mobilities of the analytes.

The ACN content cause some changes in the sequence of the actual mobilities. These changes can be better visualised by relating the mobility to that of unsubstituted anilinium, as shown in Fig. 1. For 3-methylanilinium and 4-methylanilinium with methyl groups in *meta* and *para* positions, respectively, relative mobilities remain constant within 1% over the entire composition range of the organic solvent. Here the sterical shielding of the amino group by the methyl substituent is not possible, and thus ion–dipole interactions between the charged nitrogen and the solvent molecules are probably the same as with the unsubstituted anilinium. In contrast, for the two analytes with the methyl substituent in the *ortho* position, a slight increase in the relative mobility with increasing ACN content is seen up to 75% ACN. At higher ACN content, the relative mobility remains constant. A more pronounced effect is seen for the *N*-substituted analyte (with the sterically most protected positive charge). Its relative mobility reverses at 75% ACN, and decreases significantly upon further addition of ACN. The mobility in pure ACN is nearly the same as in pure MeOH. Nevertheless, the entire change of the relative mobilities in all organic solvents is very small.

3.2. Relation of mobility to solvent viscosity, a rough approach

It is clear that the solvent viscosity influences the

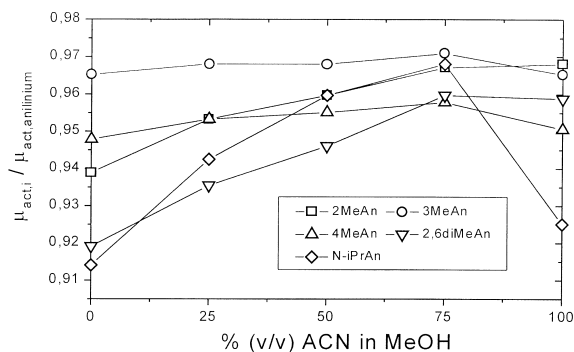


Fig. 1. Actual mobilities, $\mu_{\text{act},i}$, of analytes, i , in the organic solvents relative to the mobility, $\mu_{\text{act},\text{anilinium}}$, of unsubstituted anilinium ion.

migration behaviour of ions. In the simplest approach relating mobility to viscosity, ion movement is described by applying Stokes law of friction. This approach regards the ions as spherical particles moving in a continuum, and leads to the well-known expression:

$$\mu_{0,i}\eta = \text{const.} \quad (1)$$

This relation, known as Walden's rule, states that the product of mobility of ion, i , at infinite dilution (the absolute mobility, $\mu_{0,i}$) and the viscosity, η , of the pure solvent is constant and independent of the solvent. Although the rule is based on some oversimplifications, it is roughly obeyed in many organic solvents. It offers further an appropriate explanation of the temperature dependence of the mobility.

For practical purposes in CE, an analogue of the Walden product can be formulated for mobilities at finite ionic strength, I , according to:

$$\mu_{\text{act},i}f\eta = \mu_{0,i}\eta = \text{const.} \quad (2)$$

where $\mu_{\text{act},i}$ is the mobility of fully charged ion at given ionic strength. In this modified expression f is a correction factor that depends on the ionic strength of the BGE. As mentioned above, Walden's rule is formulated for zero ionic strength where ion–ion interactions do not occur. It is clear that the modified rule expressed by Eq. (2) is valid only when the correction factor f is either close to unity (as it is at very low ionic strength), or changes equally in the different solvents (which indeed is not true, as discussed below).

In Table 2 the products based on Eq. (2) are presented, taking the actual mobilities at BGE concentration of 10 mmol/l. The products differ significantly less than the actual mobilities (Table 1). In the organic solvents taken together, they differ only by about 7% maximum, whereas the actual mobilities differ by nearly one order of magnitude more, namely up to 60%. At first view, then, the good constancy of the modified products in the organic solvents would seem to justify the application of Stokes model of frictional resistance, at least qualitatively.

An interesting finding is that the modified Walden products in water are about 40% higher on average than those in the organic solvents. In other words, on

Table 2

Product of the actual mobility and the solvent viscosity ($\mu_{\text{act},i}\eta$) of the analytes, i , in MeOH–ACN mixtures and water in a BGE with 10 mmol/l concentration

Analyte	$\mu_{\text{act},i}\eta$					
	Water, viscosity 0.890 cP	MeOH–ACN (100:0), viscosity 0.544 cP	MeOH–ACN (75:25), viscosity 0.433 cP	MeOH–ACN (50:50), viscosity 0.370 cP	MeOH–ACN (25:75), viscosity 0.335 cP	MeOH–ACN (0:100), viscosity 0.345 cP
Anilinium	30.47	21.72	21.06	20.57	20.13	20.68
2-Methylanilinium	27.76	20.39	20.07	19.74	19.46	20.01
3-Methylanilinium	28.04	20.97	20.39	19.91	19.53	19.95
4-Methylanilinium	27.76	20.59	20.07	19.65	19.28	19.65
<i>N</i> -Isopropylanilinium	23.43	19.85	19.85	19.73	19.49	19.13

Conditions as in Table 1. Viscosities are from Ref. [49]; dimension of products is $\cdot 10^{-12} \text{ NV}^{-1}$.

the basis of Walden's rule, the mobilities are either too high in water or too low in the organic solvents. In an attempt to explain this discrepancy, we need to examine two possible main causes, namely specific ion–solvent and ion–ion interactions.

(i) Ion–solvent interactions could result in different sizes of solvated ions, and thus in different Stokes radii in the particular solvents. Note that dielectric friction and microdynamics of the solvent molecules also probably differ in the various solvents. All these effects influence $\mu_{0,i}$ in Eq. (2) at infinite dilution (the conditions where Walden's rule is defined), and at finite ionic strength as well.

(ii) Ion–ion interactions lead to a change in the mobility due to the presence of counter-ions, whereby the magnitude of this effect depends on the solvent. The effect on mobility is mediated through the factor f in Eq. (2). Clearly such interactions cannot take place at infinite dilution. This then provides a tool to distinguish between the possible causes of the higher values in water, as they can be elucidated on the basis of the mobility at zero I . Accordingly, we determined the absolute mobilities in pure water, MeOH and ACN. Anilinium and *N*-isopropylanilinium were selected as analytes because the differences in their modified Walden products between water and the organic solvents are the largest and smallest, respectively (see Table 2).

It should be mentioned that there is an additional potential cause for the non-constancy of the modified products, namely the change of the viscosity with electrolyte concentration. However, in the low concentration ranges under consideration this effect is negligible [37].

Fig. 2 depicts the mobilities of the two analytes as a function of square root of the BGE concentration for the pure organic solvents. The mobilities decrease nearly linearly with increasing \sqrt{c} (the linear correlation coefficients are between 0.996 and 0.999). Two types of information can be extracted from these plots. One is the absolute mobilities obtained by extrapolation of the line to $c=0$ and the other is the deviation between the experimentally derived slopes and those obtained from the Debye–Hückel–Onsager theory. The first results allow an evaluation of the ion–solvent effect on the mobilities in the absence of ion–ion interactions, while the second enable assessment of the role of ion–ion interactions and ion-pair formation in the different solvents. These two sets of results are discussed in the following.

3.3. Ion–solvent interactions

In Table 3 the absolute mobilities of the two analytes are given together with their Walden products and Stokes radii. Stokes radii (r_{Stokes}) are calculated according to following equation:

$$r_{\text{Stokes},i} = \frac{z_i e_0}{6\pi\mu_{0,i}\eta} \quad (3)$$

where z_i is the charge number of the ion and e_0 the elementary charge. The Stokes radius is obtained in Å, when the mobility is taken in $\cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ and the viscosity in Poise, with the value of $z_i e_0 / 6\pi$ for a monocharged ion being $0.851 \cdot 10^{-9}$.

The Walden products in the organic solvents differ by only 4%, which is slightly less than for the

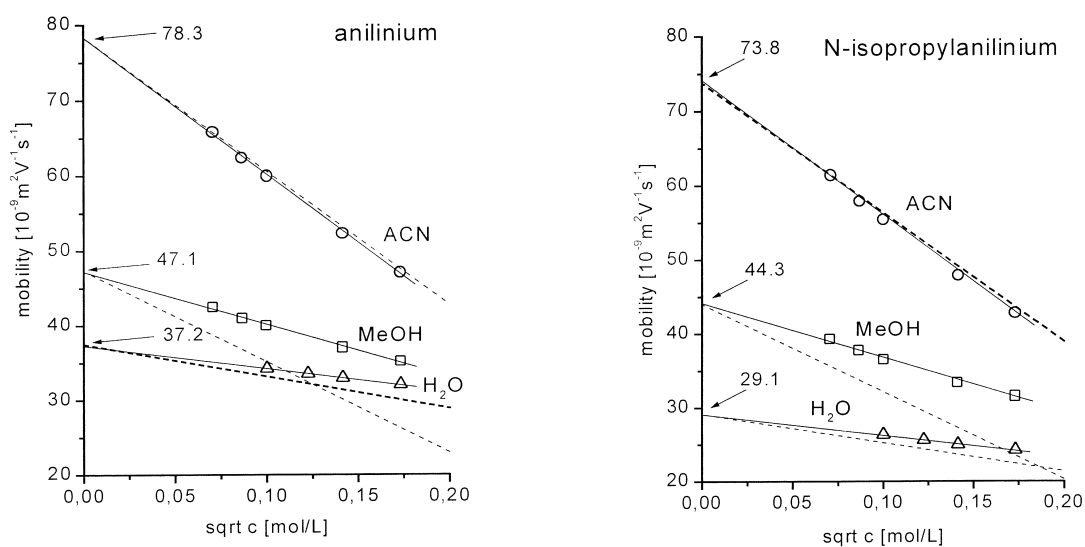


Fig. 2. Dependence of the mobilities of anilinium and *N*-isopropylanilinium in the different solvents on the square root of the BGE concentration. The solid lines result from linear regression of the data, the dotted lines are Onsager limiting slopes according to Eq. (5). Symbols: Δ , water; \square MeOH; \circ , ACN.

modified products (based on the actual mobilities, see Table 2). This is in good agreement with Walden's rule. Interestingly, for the *N*-substituted anilinium derivative, the difference of the products is also small between the organic solvents and water, only a few percent. Accordingly, as can be seen from Table 3, the Stokes radii of *N*-isopropylanilinium are very similar in all three solvents. On this bases we can assume that the analyte is unsolvated, and that its migration is mainly governed by the viscosity of the solvent.

For anilinium ion, on the other hand, the difference of the Walden products (and Stokes radii) between the organic solvents and water is significant.

Table 3

Absolute mobilities, $\mu_{0,i}$, of two ions obtained by linear extrapolation of the actual mobilities to zero concentration (Fig. 2), Walden products ($\mu_{0,i}\eta$) and Stokes radii ($r_{\text{Stokes},i}$) in pure solvents

Solvent	Anilinium			<i>N</i> -Isopropylanilinium		
	$\mu_{0,i}$	$\mu_{0,i}\eta$	$r_{\text{Stokes},i}$	$\mu_{0,i}$	$\mu_{0,i}\eta$	$r_{\text{Stokes},i}$
Water	37.2	33.1	2.57	29.1	25.9	3.28
MeOH	47.1	25.6	3.32	44.3	24.1	3.53
ACN	78.3	27.0	3.15	73.8	25.5	3.34

Absolute mobilities are in $\cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$, Walden products in $\cdot 10^{-12} \text{ NV}^{-1}$ and Stokes radii in \AA .

Several reasons can be suggested for this. First, we may recall that the basic simplification behind Walden's rule is that spherical solutes with a constant radius are moving in a continuum of a certain viscosity. The cationic analytes in this work are not spherical; nor, and more importantly, are their radii necessarily constant in the different solvents. Although we can presume that they are very weakly solvated in the organic solvents (note that ACN has very poor solvation ability for cations), no such an assumption can be made for water. Seemingly, ion-solvent interactions play a more important role in water, as could also be concluded from the large range of mobilities the analytes exhibit in aqueous solutions. However, the high value of the Walden product for anilinium in water indicates a significantly smaller effective fluid-dynamic radius (at infinite dilution) for water than the organic solvents, which is not plausible if solvation is responsible for the difference in the products, because it would imply that water forms a smaller solvation shell for the analytes than the organic solvents do.

A second reason for the larger values for anilinium ion in water could be the solvent dynamics in the vicinity of an ion. In particular, solvent molecules with large dipole moments become oriented under

the influence of the electric field associated with an ion, and re-orient themselves, after a certain relaxation time after passage of the ion [38]. The effect on ion motion (leading to so-called dielectric friction) is highly dependent on the solvent, and might lead to a solvent specific change of the mobility that is not related to the viscosity. However, a microdynamic model is hardly suitable for quantitative description of the mobility of such complex ions as the analytes of the present work. It is often not successful even for less complicated conditions. Thus, it is not further considered here.

A third reason for smaller values of the hydrodynamic radii in water could be related to the specific structure of water. Water has a three-dimensional structure that may be severely influenced by ions present, by either amplifying or reducing the initial hydrogen bonding between the water molecules in the bulk liquid. Consequently ions can induce either a structure-making or a structure-breaking effect [9]. Small univalent inorganic ions, but also organic cations like Me_4N^+ , are known to have a structure-breaking effect, whereas larger organic ions like Pr_4N^+ and the higher homologues have a structure-making one. The destruction of the water structure in the vicinity of the ion could lead to a higher mobility due to the reduction of the effective, local viscosity. In some cases this model can result in an even smaller effective fluid-dynamic radius of the ion than its crystal radius.

At first sight one would expect the relative large and lipophilic univalent ions under consideration to lead to a structure-making effect, which would reduce the mobility in water. The higher value of the Walden product for anilinium in water is contrary to this expectation. Hence, the higher products in water cannot be due to ion–ion interactions (because these effects are excluded at infinite dilution), but instead they may reflect an unexpected structure-breaking induced by the ion. Even more unexpected, however, is the comparatively weaker effect of the shielded *N*-isopropylanilinium, as can be concluded from the similar Walden products in all three solvents (Table 3). It should be mentioned that we observed higher modified Walden products in water than in MeOH also for substituted aromatic acids (benzoates) [39,40].

3.4. Ion–ion interactions

From Fig. 2 it was seen that the mobilities decrease with increasing concentration. Such dependence is explained by the theory of electrical conductance as introduced by Debye, Hückel and Onsager, and extended by Fuoss, Pitts and others (see Introduction). In an advanced form the theory expresses the dependence of the conductivity, Λ , of an electrolyte on the concentration, c , including the effect of possible ion-pair formation [38,41,42]. The dependency can be expressed by the following equation [38]:

$$\Lambda = \Lambda_0 - S\sqrt{\alpha c} + E(\alpha c) \ln(\alpha c) + J_1(\alpha c) - J_2(\alpha c)^{3/2} - K_A \Lambda \gamma_{\pm}^2(\alpha c) \quad (4)$$

where the suffix 0 indicates zero ionic strength. The terms S and E describe the effect of the ion atmosphere on the conductance. For low ionic strengths, S approaches the Onsager limiting slope. J_1 and J_2 depend on the same parameters than S and E , and on the distance of closest approach between ion and counter-ion. K_A is the ion-pair formation constant, α is the degree of dissociation of the ion pair and γ_{\pm} is the mean activity coefficient according to the Debye–Hückel limiting law. The extent of the ion-pair formation reaction $\text{A}^- + \text{B}^+ = \text{A}^-\text{B}^+$ is expressed as usual by the equilibrium constant $K_A = [\text{A}^-\text{B}^+]/[\text{A}^-][\text{B}^+]$. The brackets indicate the equilibrium concentrations of the ion pair, the anion and the cation, respectively. The last part of Eq. (4) represents an important feature for the behaviour of the ions, especially in solvents with low dielectric constant that can favour ion-pair formation with the counter-ions. This is in contrast to aqueous solutions with high dielectric constant where ion pairing is negligible in most cases.

3.4.1. Effect of ion atmosphere

In the first theoretical approach (according to the model introduced by Debye and Hückel [43], and applied by Onsager [44] to ion transport), the ion of interest is considered as a point charge (the ionic radius is zero) surrounded by an atmosphere or cloud of counter-ions. The presence of an ionic cloud around an ion reduces its mobility, μ_i , where the

reduction is proportional to the square root of the ionic strength, I , expressed as [45]:

$$\mu_i = \mu_{0,i} - \left[\frac{2.801 \cdot 10^6 |z_+ z_-| q}{(\epsilon T)^{3/2} (1 + \sqrt{q})} \cdot \mu_{0,i} + \frac{42.75 |z_i|}{\eta (\epsilon T)^{1/2}} \right] \cdot \sqrt{I} \quad (5)$$

Here z_+ and z_- are the charge numbers of the anion and the cation, ϵ is the dielectric constant, T the absolute temperature. Note that the expression in brackets corresponds to the S term in Eq. (4).

For the solvents water, MeOH and ACN, the mobilities as function of \sqrt{I} for 1:1 electrolytes (with $z=1$, $q=1/2$) according to Eq. (5) are given in Table 4. As can be seen, the functions have different slope in the particular solvent. For example, the actual mobilities for ions with an absolute mobility of 50 units at ion strength $I=10$ mmol/l are 45.5 in water, 37.7 in MeOH and only 34.2 in ACN. This means that the absolute mobility would be reduced by 9% in water, a value that is commonly found for this solvent. However, the mobility decreases by 25% in MeOH and by as much as 32% in ACN. These changes are surprisingly large for organic solvents, even for BGEs with the relatively low ionic strength of 10 mmol/l.

As we pointed out above, the plots in Fig. 2 allow a comparison of the magnitude of the effect of the concentration observed experimentally with that predicted by theory. For ACN as solvent, the experimental slope is identical with that predicted by theory. For the other two solvents (water and MeOH) a positive deviation of the measured data from the theoretical line is found. For water the difference is small (4%), for MeOH more pronounced (13%). The positive deviations follow from the assumptions made in the Debye–Hückel–Onsager limiting ap-

proach (Eq. (5)) that the ion is a dimensionless point charge. More advanced theories introduced by Falkenhagen et al. [46] and Pitts [21] take the finite radii of the ions into account and give the expression:

$$\mu_i = \mu_{0,i} - \left[\frac{8.204 \cdot 10^5}{(\epsilon T)^{3/2}} \cdot \mu_{0,i} + \frac{42.75}{\eta (\epsilon T)^{1/2}} \right] \cdot \frac{\sqrt{c}}{1 + 50.29a(\epsilon T)^{-1/2} \sqrt{c}} \quad (6)$$

Here a is the mean diameter of the ions in Ångstrom (more exactly it is the distance of the closest approach between the ion and the counterion). With the appropriate values for a 1:1 electrolyte at 25°C inserted, the denominator of the ratio outside the brackets is as given in Table 4 for water, MeOH and ACN. If we take a value of 4 Å for the mean ionic diameter, we obtain for 10 mmol/l concentration a 12% higher mobility in water than predicted by the Debye–Hückel–Onsager limiting equation (Eq. (5)). For MeOH and ACN the mobility is 17% higher. The value is the same for the two solvents because of their similar dielectric constant. It can be concluded that this more refined theory provides a good explanation for the positive deviation from the limiting slopes in water, and even for the stronger deviation in MeOH. However, it does not explain why such positive deviations were not observed in ACN.

3.4.2. Ion-pair formation

It was pointed out above that ion pairing is favoured in organic solvents with low dielectric constants. In the case of MeOH and ACN, with their intermediate dielectric constants, it is not clear whether ion-pair formation of a particular species takes place or not. K_A values in some organic

Table 4

Equations obtained from Eqs. (5) and (6) after insertion of the physical parameters for the pure solvents

Solvent	ϵ	η (Poise)	Mobility according to Eq. (5)	Denominator of \sqrt{c} term in Eq. (6)
Water	78.39	0.00890	$\mu_i^{\text{water}} = \mu_{0,i}^{\text{water}} - [0.230 \mu_{0,i}^{\text{water}} + 31.4] \sqrt{I}$	$(1 + 0.329a \sqrt{c})$
MeOH	32.63	0.00544	$\mu_i^{\text{MeOH}} = \mu_{0,i}^{\text{MeOH}} - [0.855 \mu_{0,i}^{\text{MeOH}} + 79.7] \sqrt{I}$	$(1 + 0.510a \sqrt{c})$
ACN	36.01	0.00345	$\mu_i^{\text{ACN}} = \mu_{0,i}^{\text{ACN}} - [0.737 \mu_{0,i}^{\text{ACN}} + 119.6] \sqrt{I}$	$(1 + 0.485a \sqrt{c})$

solvents have been determined for inorganic and organic salts, including tetraalkylammonium cations, and perchlorate as anions [38,41,42,47,48]. They are derived from the concentration dependence of the conductance as expressed by the series expanded Eq. (4). Unfortunately the published K_A values are strongly dependent on the assumptions made in the calculations and are considerable different. The deviations are due to different estimations of the closest distance of approach of the ion and counterion, and of the contribution of non-Coulombic interactions. The different approximations introduced for the parameters can lead to values for K_A varying by a factor of four or more, even when they are derived from the same conductance data [42].

The following discussion focuses on ACN, because more reliable data for ion-pair formation was found for this solvent. Before detailed discussion of the expected consequences for the mobilities of the analytes, we look at the effect of ion-pair formation on the BGE. As noted above, perchloric acid is a strong electrolyte even in ACN, and can thus be considered as fully dissociated. This is not necessarily the case for the neutral salt TPA^+P^- , the main electrolyte of our BGE. For this electrolyte a seemingly reliable K_A value of 27 l mol^{-1} in ACN was found in the literature [42]. If we adopt this value, at an initial TPA^+P^- concentration of 10 mmol/l the degree of ion-pair formation is 0.18 (this estimate does not take into consideration the mean ion activity coefficient). This means that 82% of TPA^+ is present as free ions. Thus ion pairing reduces the mobility of both TPA^+ and perchlorate ions by nearly one fifth. One consequence of this is that the ionic strength of the BGE must be accordingly corrected; the BGE solution with 10 mmol/l perchlorate concentration now has an ionic strength of only 8.2 mmol/l . This means that the ionic strength should also be corrected when the Debye–Hückel–Onsager equation (Eq. (5)) is applied to evaluate the deviation of the measured actual mobilities from the theoretical behaviour (see Fig. 2). It is clear that we must take into account the degree of dissociation, α , and the values of αc instead of c must be used in the appropriate equation. α depends on c as well, but it can be calculated by basic thermodynamics.

With correction for the ion-pair formation of TPA^+P^- , the dependence of the actual mobilities on

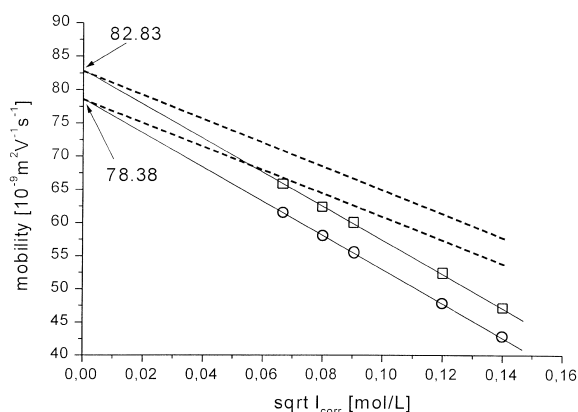


Fig. 3. Mobilities of anilinium and *N*-isopropylanilinium in ACN as a function of the square root of the corrected ionic strengths of the BGE. The ionic strength as calculated from the BGE concentration was corrected by the degree of ion-pair dissociation, α . An ion-pair formation constant K_A of 27 l mol^{-1} was taken for TPAP. Symbols: \square anilinium; \circ , *N*-isopropylanilinium. The solid lines were obtained by linear regression of the data, the dotted lines are the Onsager limiting slopes according to Eq. (5).

the square root of the (corrected) ionic strength is as shown in Fig. 3. The dependency is highly linear (linear correlation coefficients are 0.9998). Note that the degree of ion-pair dissociation, but not the mean ion activity coefficient was taken into account in the correction. Owing to the steeper slope of the regression line compared with that in Fig. 2, the intercepts on the y-axis (corresponding to the absolute mobilities) are about 5% higher. Even more interesting is, in contrast to Fig. 2, the slight negative deviation of the measured mobilities from the Onsager limiting slopes. It may be recalled that mobilities usually deviate positively from the limiting slope. The negative deviation indicates a reduction of the mobility in addition to the effects of the ion atmosphere. Thus, it is most probable that ion-pair formation of the analyte ions with perchlorate takes place in the solvent system under consideration. Although we have no literature data suitable for evaluating the migration behaviour in the methanolic and the mixed systems in more detail, the conclusion is allowed that the effect is similar to that in ACN owing to the isoelectric properties of these solvent systems.

Unfortunately, no K_A values for the analytes and perchlorate ions were found in the literature. How-

ever, comparison with values for compounds with some similarity in chemical properties and molecular size (but not explicitly their shape) should enable the estimation at least of a reasonable order of magnitude. For tetraalkylammonium perchlorates (including TPAP) in ACN, the published values lie between 25 and 30 l mol⁻¹ [42]. For MeOH the respective constants seem to be similar or even higher [41]. Note that these are the maximum values of the literature data for the given perchlorate salts, and that other authors have found much lower values. From the assumed value of K_A and the concentration of perchlorate in BGE, we can derive the maximum degree of ion-pair formation that the analytes undergo. In the present electrophoretic set-up the perchlorate concentration is nearly constant since it is in large excess relative to the analyte concentration. Rearrangement of the equation for K_A given above leads to the ratio of the concentration of the free analyte ion, $[B^+]$, to ion pair, $[A^-B^+]$ as $[B^+]/[A^-B^+] = 1/(K_A[A^-])$. In the present systems the concentration of ions A^- (perchlorate) is about 10 mmol/l. Thus, when the equilibrium constant K_A is 25, about 20% of B^+ is forming ion pairs. If the value of K_A is overestimated, say by a factor of 10, less than 3% of the analyte ions would be indicated as forming ion pairs with perchlorates. Owing to the lack of data for the analytes these estimates are both somewhat speculative.

4. Conclusions

(i) From the actual mobilities of aromatic cations measured in MeOH, ACN, MeOH–ACN mixtures and water it can be concluded that the organic solvents level the mobility range. The mobility window is broader by a factor of four in water. The much smaller range in the organic solvents can be attributed to the low degree of solvation, resulting in a certain constancy of the Stokes radii, independent of the organic solvent. The more pronounced and more specific solvation in water causes a wider variation of the hydrodynamic radii.

(ii) Under the present conditions, actual mobilities correlate well with viscosity in the pure and mixed organic solvents; the modified Walden products of

actual mobility and solvent viscosity differ by less than 7%.

(iii) The modified Walden products are about 40% higher in water. This deviation was evaluated by excluding ion–ion interactions and basing the evaluation on the absolute mobilities of two representative analytes at zero ionic strength in water, MeOH and ACN. The resulting Walden products of *N*-alkylsubstituted anilinium were found to be constant in all three solvents within just 7%, indicating nearly constant Stokes radii. For unsubstituted anilinium, on the other hand, the Walden product is more than 20% higher in water than in the organic solvents. The difference must be due to the different ion–solvent interactions in water and organic solvents. The influence of the ion on the structure of water is considered important.

(iv) The actual mobilities of the two analytes selected for closer investigation depend linearly on the square root of the concentration of BGE. However, the limiting Onsager slope is steeper than the regression line of the measured actual mobilities for water and MeOH. For ACN, full consistency is found between the experimental data and the line predicted from theory. The positive deviations of the measured data from the limiting Onsager slope are due to the assumption the theory is based on, namely considering the ions as point charges. The positive deviation is plausibly explained when radii are considered to be finite.

(v) The literature data on formation constants provides evidence for ion pairing of the main constituent of the BGE in ACN. Taking this ion-pair formation into account, excellent linear correlation is found for the dependence of the actual mobilities in ACN on the square root of the ionic strength. With this correction for the degree of ion-pair dissociation for the BGE the measured mobilities in ACN show a negative deviation from the limiting slope. This means that ion-pair formation of the analyte ions most probably takes place as well.

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