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Behaviour of inorganic and organic cations in the Debye–Hückel layer of DNA

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Abstract

Inorganic, monovalent cations (Li, Na, K, Rb, Cs), when present in the Debye–Hückel layer of DNA, are found to bind to the negatively charged groups of the helix solely on the basis of their charge/mass ratio. Thus, when an electric field is applied, the free mobility of the DNA is seen to increase from Li- to Cs-equilibrated DNAs, since the latter cation, having a weaker surface charge distribution and a larger physical size (in the non-hydrated state), is more loosely bound to the DNA helix, thus providing less screening of its negative charges. On the contrary, organic amines (Tris and a number of Good's buffers) are found to bind not only via electrostatic interactions, but by additional bonds, notably H-bonds. In particular, Tris can form two H-bonds, with a purine and pyrimidine, respectively, and a third H-bond shared between the –OH groups of two adjacent Tris. Hence, these buffer components may be unwitting participants in reactions carried out in in vitro systems.

Keywords: Electrophoretic mobility; Inorganic cations; Amines; DNA

1. Introduction

According to Hückel, the mobility (μ) of a spherical particle, small enough to be considered as a point charge, but large enough to move in a liquid according to Stoke's law, in an electric field, is given by:

$$\mu = \zeta\varepsilon/6\pi\eta$$

where ζ is the zeta potential, ε the dielectric constant and η the viscosity of the solution. Since ζ is related to the charge (q) divided by the radius of the ion, it follows that the electrophoretic mobility is proportional to the charge/mass ratio (q/M_r) of a particle. For peptides, ranging in length from two to ca. 50 amino acid residues, the power law relating μ to the q/M_r ratio was found by Offord [1] to be:

$$\mu \propto q/M_r^{2/3}$$

(It is of interest to note that, although a variety of other relationships have been proposed [2], the Offord model appears to be the one with the best data fitting, provided that electrophoresis is conducted at quite acidic pH values, where the polypeptide coil is in a fully extended conformation).

For DNAs, the situation is more complex, due to the well-known fact that, above a certain length

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[recently found to be around 400 base pairs (bp)] [3], the charge/mass ratio becomes constant, so that the free solution mobility reaches a plateau value (assessed as $3.75 \pm 0.04 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1} at 25°C, in Tris–acetate–EDTA buffer, pH 8.2, independent of DNA composition, sample size and electric field strength). Over the years, two buffer systems have emerged as the most popular in DNA electrophoresis: Tris–acetate–EDTA (TAE) and Tris–borate–EDTA (TBE), both titrated at pH values in the range 8.0–8.3. Curiously, up to the present, very few studies have explored the effect of background electrolyte on the mobility of DNA, either in terms of pH variations or in terms of cation composition. This is possibly due to the fact that, in gel slab electrophoresis, potential effects of these parameters on the DNA mobility would go undetected, since the separation is essentially driven by contour length (size), rather than charge (or q/M_r) discrimination. Capillary zone electrophoresis (CZE) has now emerged as a powerful tool for evaluating with precision any potential effect of pH and buffer composition on the migration behaviour of macromolecules.

2. Materials and methods

2.1. Materials

Fused-silica, 75 μm I.D. capillaries were from Polymicro Technologies (Phoenix, AZ, USA). The phosphodiester 20-mer oligonucleotide mainly used in this study (5′-TCTGAAAGTGCTCTACTGAG-3′) was synthesized by Primm (Milan, Italy). After synthesis, it was purified by gel filtration on a NAP-25 (Sephadex G-25) column (Pharmacia Biotech, Uppsala, Sweden) equilibrated (and eluted) with 20% ethanol in water. According to the HPLC chromatograms supplied by the producer, the 20-mer was >95% pure. Other 20- to 40-mer oligonucleotides (of widely differing base composition) were a kind gift from Dr. A. Domenici, GlaxoWellcome (Verona, Italy). All other chemicals (Tris, LiCl, NaCl, KCl, RbCl and CsCl) were from Sigma (St. Louis, MO, USA).

2.2. Capillary zone electrophoresis

CZE was carried out using a Beckman P/ACE 2100 apparatus (Fullerton, CA, USA), with UV detection at 254 nm. Capillaries of 75 μm I.D., coated with poly(*N*-acryloylaminopropanol) [4] to minimize the electroendoosmotic flow (EOF), were used. In such coated capillaries, the EOF was found to be negligibly small ($1.6 \cdot 10^{-6}$ cm^2 V^{-1} s^{-1} at 25°C). The DNA samples were injected by pressure for 1 s (10 nL plug). Between runs, the capillaries were flushed with running buffer for a few minutes at high pressure. The injected DNA had a concentration of 30 μg/mL. Runs were carried out at 25°C, with 100 V/cm and a current of 9 μA.

2.3. Molecular modelling

In order to evaluate the potential interactions of Tris with the DNA helix, the Insight program was utilized. The programs O [5] and Molscript [6] were used for the model, which was drawn on a Silicon Graphics workstation.

3. Results

We investigated how the mobility of DNA could be affected by replacing, in the Debye–Hückel layer, the conventional Tris with monovalent cations of the first group of the Periodic Table, namely Li^+, Na^+, K^+, Rb^+ and Cs^+. The initial experiments were performed under iso-conductivity conditions (2.1 mS/cm, and thus equal current densities in all runs), but it was soon found that this was not a proper approach, since iso-conducting buffers did not guarantee iso-ionic strength (I) when the Tris moiety was progressively replaced by the monovalent cations. The apparent mobility data could thus be erroneous, since the thickness of the diffuse double layer is known to depend on the square root of the ionic strength [7,8]. Thus all experiments reported here were run at an iso-ionic strength of $40 \cdot 10^{-3}$ equivalents L^{-1}. Fig. 1 shows four selected panels of the migration behaviour of an oligonucleotide (a 20-mer) in a control buffer (0 m*M* Li^+) and in the presence of increasing amounts of Li^+ (up to 15.5 m*M*). It can be seen that the migration velocity of

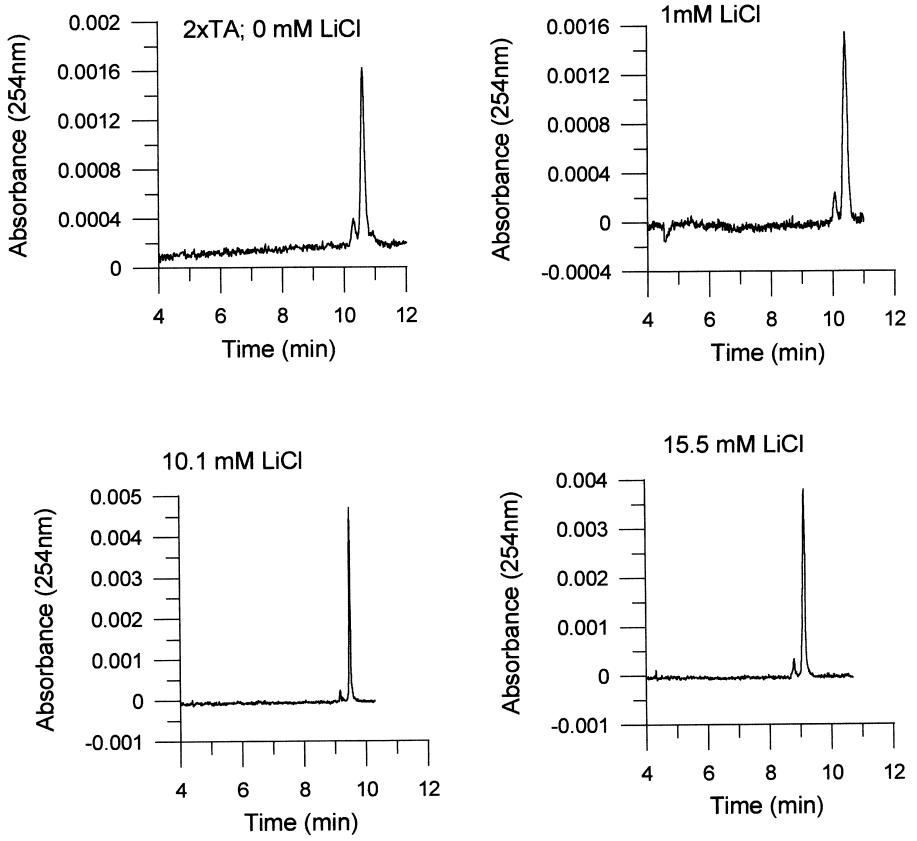

Fig. 1. CZE profiles of a 20-mer double-stranded (ds) DNA in TAE (40 m*M* Tris, 20 m*M* acetate, 1 m*M* EDTA, pH 8.2) buffer. Upper left panel: control, 2× TAE, pH 8.3; upper right: with 1 m*M* LiCl added; lower left: with 10.1 m*M* LiCl; lower right: with 15.5 m*M* LiCl. All CZE runs in iso-ionic strength background electrolytes ($I = 40 \cdot 10^{-3}$ equiv. L^{-1}).

DNA is markedly accelerated at progressively higher Li^+ concentrations. Fig. 2 summarizes all the CZE data collected for four cations (excluding Rb^+) in the same TAE buffer in which the Tris moiety had been progressively replaced by Li^+, Na^+, K^+ and Cs^+, in all cases by maintaining $I = 40 \cdot 10^{-3}$ equivalents L^{-1} of all buffer solutions and guaranteeing adequate buffering power (this is the reason why no further addition of cations could be performed, since the buffering ion could not be diluted much below 20 m*M* concentration). One can see progressive increments of mobility ($\Delta\mu$) in going from the smallest to the largest ion, over the standard, reference mobility of the 20-mer in plain TAE buffer (assessed as $3.18 \pm 0.04 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1}, averaged over daily and weekly measurements; on a daily basis, the error was barely ± 0.02) (note that the value of 3.75 given in the Introduction refers to the plateau value reached for 400 bp and larger fragments!). It can also be appreciated that the changes in mobility become progressively smaller as the ion radius becomes larger, indicating that this phenomenon could reach a saturation value. By plotting the values of $\Delta\mu$ vs. the q/M_r ratio (which in our case equals the reciprocal of the atomic mass of the ion, since the charge is unity), and by fitting the data to an exponential, the curve shown in Fig. 3 is obtained, described by the following equation:

$$y = y_0 + a e^{-bx}$$

where $y_0 = 0.256$, $a = 0.291$ and $b = 23.7$.

A possible interpretation: since Li^+ has a higher surface charge density, it clings more tightly to the

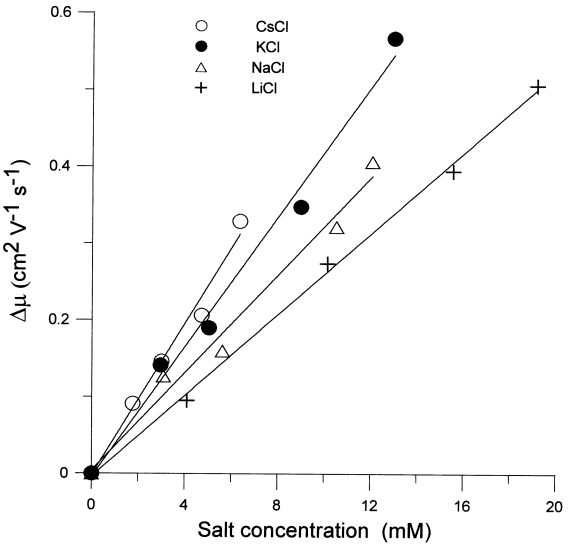

Fig. 2. Mobility increments ($\Delta\mu$) of a 20-mer dsDNA in the presence of increasing molarities of LiCl, NaCl, KCl and CsCl.

DNA helix, thus providing greater shielding of the negative charge during electrophoretic migration. With progressively larger ion radii, two concomitant phenomena occur: due to the lower surface charge density (and to their higher inertia at the instant at which the field is applied), the ions are more loosely bound to the DNA helix and, upon application of a voltage gradient, the Debye–Hückel layer is more readily dispersed in front of the migrating macroion and rebuilt on its back. This greater distortion of the Debye–Hückel layer diminishes the shielding of the negatively charged phosphate groups in DNA. The situation can, however, be more complex than this, as shown in Table 1. Although the ratio of atomic radii Cs/Li is ca. 3:1, when such ions are in solution, and thus hydrated, Li^+ appears to be considerably larger than Cs^+, as shown by the μ and D values. Thus, paradoxically, Cs^+ would appear to have twice the mobility of Li^+. Even in this latter case, though, our data would fit this new scenario: under dynamic conditions, i.e. when applying an electric field, as the DNA coil and Cs^+ would tend to move in opposite directions, the Cs cation would tend to retro-migrate faster than Li^+, with the net result of leaving the phosphate negative charges in DNA less shielded, resulting in a higher mobility of this macro-ion.

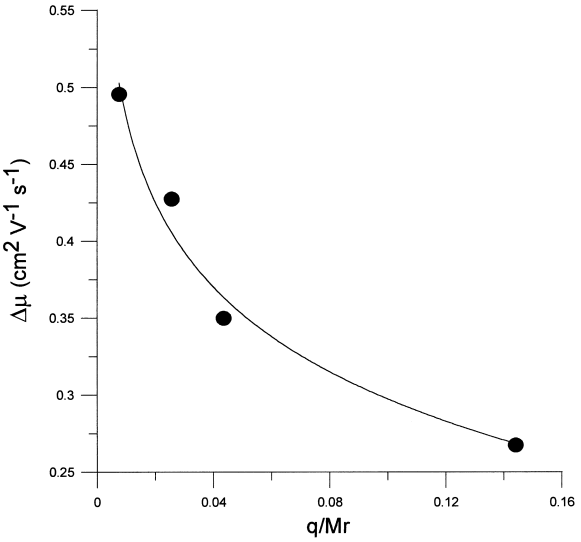

Fig. 3. Relationship between mobility increments ($\Delta\mu$) of a 20-mer dsDNA and the charge/mass ratio of the four cations in Fig. 2. The $\Delta\mu$ values plotted here represent the values at 10 m*M* concentrations of each cation.

This phenomenon, however, tends to plateau, since it can be seen that, with progressively larger ion radii, the $\Delta\mu$ values become progressively smaller (cf. Figs. 2 and 3). This is also consistent with the known notion that, no matter how high the voltage gradient is, the macro-ion cannot strip itself free from the cloud of counterions surrounding it. Such electrophoretic stripping of the Debye–Hückel layer (called the Wien effect), were it to occur, would require voltage gradients in excess of 100 000 V/cm [9].

Table 1
Some physico-chemical parameters of inorganic and organic cations

Species	Radius (Å)[a]	Mass	λ°	μ	D
Li	0.60	6.94	38.7	4.01	1.03
Na	1.00	22.99	50.1	5.19	1.33
K	1.37	39.09	73.5	7.62	1.96
Rb	1.48	85.47	77.8	8.00	2.07
Cs	1.70	132.91	77.3	8.01	2.06
Tris	2.50[b]	121.14	–	–	–

Symbols: λ°, equivalent conductivity, in $(S/cm)/(equiv./cm^3)$; μ, free electrophoretic mobility, units 10^{-4} cm^2 V^{-1} s^{-1}; D, diffusion coefficient, units 10^{-5} cm^2/s.

[a] Atomic for all metals, molecular for Tris.

[b] Estimated from mean atomic distances.

Our data leave something unexplained, though: if the q/M_r ratio is the main parameter regulating the behaviour of the ions in the Debye–Hückel layer of a macromolecule, how is it that Tris, which has essentially the same q/M_r ratio as Cs^+, does not follow the Cs^+ curve shown in Fig. 2 (in this figure the mobility in the Tris-cation buffer is the control value of μ and thus it is coincident with the *x*-axis)? Some other interactions must occur, which have been simulated in Fig. 4, obtained by molecular modelling and energy minimization: assuming that only the fully charged Tris molecules bind to DNA, one such molecule is seen docking onto the negatively charged oxygen group in phosphate via its positively charged amino group. The cloud of –OH groups in the Tris moiety begins to seek hydrogen bonds in the surroundings, to be found in the carbonyl group of a neighbouring pyrimidine and in the ring nitrogen of an adjacent purine (neither of these groups is engaged in the normal AT and CG H-bonds in a DNA double helix), the third H-bond being shared between two neighbouring Tris groups (T4–T7) (Table 2). This extensive H-bond formation, coupled to the salt bridge, forces the Tris ions to cling more tightly to the DNA helix; this could be the reason for the (relatively) lower mobility of Tris-saturated DNA as compared to any of the monovalent cations entering the Debye–Hückel layer of the DNA helix (see Fig. 2). Notice that, in the model of Fig. 4, it is hypothesized that the Tris ion penetrates into the

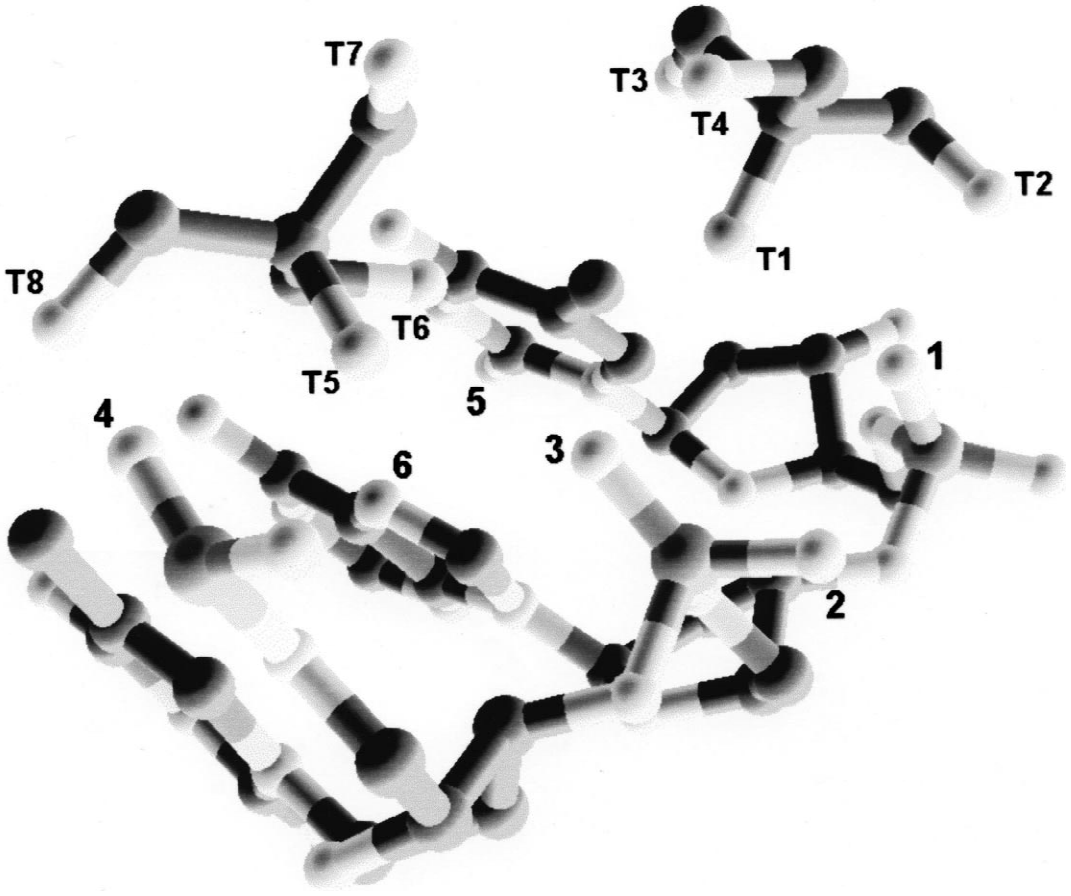

Fig. 4. Molecular model of the binding of Tris to dsDNA. For clarity, only a portion of a single filament is shown, with three bases protruding from the phosphodiester bridge. Above, two Tris molecules can be seen, with the potential binding sites, labelled T1–T8, to the corresponding atoms in the DNA chain, labelled 1–6 (except for T4–T7 shared between two adjacent Tris moieties).

Table 2
Types of potential bonds formed between Tris and DNA (see Fig. 4)

Atom in Tris	Atom in DNA	Type of bond	Distance (Å)
T1	1	Salt bridge	3.32
T2	2	Hydrogen bond	4.94
T3	5	Hydrogen bond	4.26
T4, T7		Hydrogen bond	4.87
T5	3	Salt bridge	3.14
T6	6	Hydrogen bond	3.27
T8	4	Hydrogen bond	3.73

large groove of the DNA double helix. Additionally, this binding is independent of the base composition of the DNA, since the H-bonds would occur with any purine or pyrimidine ring available in the neighbourhood of the phosphodiester bridge. In fact, a series of runs with 20-mers of widely different base composition (as well as with fragments of different lengths, in the 20- to 40-mer range) gave the same $\Delta\mu$ values (unpublished observations). In order to prove this hypothesis, we ran the DNA under denaturing conditions, in 7 *M* urea at 50°C: the control run (2× TAE) gave $\mu = 3.43 \cdot 10^{-4}$ V^{-1} s^{-1}, whereas the run in 10 m*M* CsCl gave $\mu = 3.63 \cdot 10^{-4}$ V^{-1} s^{-1}. The $\Delta\mu$ (=0.20) under these conditions is less than one-half the $\Delta\mu$ (=0.48) under native conditions (cf. Fig. 2), suggesting that H-bonds could play a non-negligible role in the observed mobility of DNA in plain Tris buffer.

4. Discussion

4.1. On the different behaviour of organic and inorganic cations

Our data suggest that there are two, quite different types of behaviour of cations when present in the Debye–Hückel layer of a DNA helix: inorganic (monovalent) cations simply provide a shielding of the charge via electrostatic interaction. Such interactions become weaker and weaker at progressively larger (non-hydrated) ion radii, with the result of an increment of mobility of the DNA in the electric field. Organic cations, on the contrary, bind in a more elaborate manner, via additional hydrogen bonds, resulting in complexes with DNA which can greatly alter not only the electrophoretic behaviour, but possibly even its biological function. Although we have demonstrated this here for the first time with Tris, other evidence is now gathering in the literature pointing towards a general, common behaviour of such cations. For example, by performing electrophoresis of DNA in isoelectric histidine buffer (at pH = p*I* = 7.6, i.e. in the absence of any other foreign ion than plain His; p*I* is the isoelectric point) we found that there is a massive coating of the His moiety on the DNA filaments. This results in a stiffening of the DNA helix and in an increase in its radius of gyration to such an extent that even small DNAs, which would not normally be sieved in dilute liquid polymers, are fully separated by sieving in such solutions [10]. In addition, we have recently found that a number of other amine-based buffers of the Good's family [such as 3-(*N*-morpholino)-propanesulfonic acid (MOPS), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), *N*,*N*-Bis(2-hydroxyethyl)-2-aminoethane sulphonic acid (BES), *N*-Tris(hydroxymethyl)methyl-2-aminoethane sulphonic acid (TES) and Tricine] also interact with DNA, giving a bimodal peak distribution in free CZE [11,12]. Such DNA buffer interactions were recently held responsible for altering the rate of cleavage of plasmid pBR322 by the restriction enzyme *Eco*RV [13]. Another interesting example comes from recent work by Gelfi et al. [14]. It is known that the sensitivity of single-strand conformation polymorphism (SSCP) analysis of polymerase chain reaction (PCR) products is lower (ca. 65%) compared to that of DGGE (denaturing gradient gel electrophoresis) (ca. 95%). These authors examined the effects of buffer pH and type of cations in an attempt at improving the mutation detectability in the SSCP-CZE mode. They noted that there was a large increment in the resolution of conformers by running samples in a low-pH buffer system (pH 6.8 vs. the standard pH 8.3 buffer) and by partially replacing the Tris cation with 2-(*N*-morpholino)ethanesulfonic acid (MES). By using this new buffer system (consisting of 35 m*M* MES, 30 m*M* Tris, 1 m*M* EDTA, pH 6.8), there was a remarkable improvement in the resolution and the sensitivity became comparable to that of DGGE in standard gel slab systems. It was hypothesized that the different binding properties of

this buffer system to the denatured DNA filament allowed a more proper refolding of the single-stranded DNA to find a correct secondary structure. These combined results show that amine-based buffers must be assumed to interact, to different extents, with DNA, unless proven otherwise. Hence, these buffer components may be unwitting participants in reactions carried out in in vitro systems.

4.2. On the importance of ionic strength corrections in free μ measurements

This is an important aspect, often ignored in the current literature. Under dilute conditions (e.g., 1 m*M* solution of a 1:1 electrolyte) the thickness of the ionic atmosphere surrounding a small ion could be as high as 100 times the radius of the ion. However, as the ionic strength of a solution increases, the thickness of the ionic atmosphere decreases, as described in the equation giving the Debye–Hückel reciprocal length [7,8]. Thus, both theoretical and empirical relationships predict that mobility will decrease with the square root of the ionic strength. A few relationships have been derived. In one approach, Reijenga and Kenndler [15] derived the following empirical expression for mobility relative to the infinite dilution mobility (μ_0):

$$\mu = \mu_0 \exp(0.5z^{1.78}\sqrt{I})$$

where z is the charge number of the solute and I is the ionic strength of the buffer. This expression was stated to be valid for solute charge numbers from 1 to 3 and ionic strengths up to ca. 10 m*M*. In another approach, Friedl et al. [16] observed the following empirical relationship:

$$\mu = \mu_0 \exp(0.77\sqrt{zI})$$

valid, apparently, for solutes with charge numbers from 2 to 6 and ionic strengths in the 1 to 100 m*M* range.

Both equations try to eliminate some flaws inherent to the generalized Debye–Hückel–Onsager (DHO) equation, which is valid only for highly dilute buffers (1 m*M*) and for spherical ions of high charge density. In real life, in CZE, one has to deal with much higher molarities (up to 100 m*M* and higher) and, in general, with asymmetric organic molecules of low charge density. Even when using such equations, though, it has been observed that, quite often, plots of mobility vs. square root of buffer ionic strength present significant deviations from linearity, in contrast with the DHO limiting law, which predicts instead a linear decay. In order to correct for this, Li et al. [17] revised a treatment proposed long ago by Pitts et al. [18] and derived the following expression:

$$\mu_e \approx \mu_0 - \frac{|z|K\sqrt{I}}{1 + Ba\sqrt{I}}$$

(μ_e is the effective mobility, μ_0 the absolute mobility, z the ion charge, K is a constant, B is a solvent-dependent parameter, and a is the ion size parameter), which leads to greatly improved linearity for both mono-charged and multi-charged organic ions. It should thus be borne in mind that comparisons of experiments in which mobilities are assessed under different ionic strength conditions will be meaningless, unless proper corrections are made to the different data sets.

Acknowledgements

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