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Journal of Chromatography A, 741 (1996) 99–113

JOURNAL OF CHROMATOGRAPHY A

Self-consistent framework for standardising mobilities in free solution capillary electrophoresis: applications to oligoglycines and oligoalanines[1]

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Received 22 January 1996; revised 6 February 1996; accepted 15 February 1996

Abstract

A theoretical analysis of deviations from ideality in ionic transport is presented to correct mobilities, μ, measured in free solution capillary electrophoresis (CE) to mobility at infinite dilution, μ° (limiting mobility). Non-ideality is treated at the same level of approximation as in equilibrium, using a correction factor for the sum of the analyte and counter-ion radius originally suggested by Robinson and Stokes (Electrolyte Solutions, 1961). Unlike previous corrections using Debye–Hückel–Onsager theory, which are strictly applicable only at very low ionic strengths, this treatment is expected to be valid for univalent ions migrating in a uni-univalent background electrolyte for ionic strengths up to 0.075 mol kg^{-1}, a range typical of CE experiments. The analysis is applied to the determination of μ° in acidic and basic buffers for oligoalanines and oligoglycines with degree of polymerisation 2 to 6. Limiting mobilities for the fully protonated and deprotonated peptides are found to be numerically equal but opposite in sign, consistent with a change in charge from +1 to −1. In all uni-univalent buffers studied (borate, citrate, low pH lithium phosphate and sodium phosphate) μ° values established using data over a range of pH and ionic strength are found to be identical and in excellent agreement with previous values from isotachophoresis. Values of μ° in high pH sodium phosphate buffer are systematically $0.2 \cdot 10^{-8}$ m^2 V^{-1} s^{-1} higher than those in other buffers; this may be attributed to limitations of the model for a buffer with 1+:2− and 1+:3− ions. This self-consistent framework for standardising mobilities in free solution CE is expected to be widely applicable to univalent analytes migrating in a 1:1 background electrolyte.

Keywords: Electrophoretic mobility; Buffer compositon; Oligoglycines; Oligoalanines; Peptides

1. Introduction

In seeking to systematise data for analyte migration in capillary electrophoresis (CE) [1–3], a primary objective must be to bring electrophoretic mobilities determined in a variety of background electrolyte (BGE) solutions over a range of pH values and ionic strengths into a rational theoretical framework. For an analyte involved in a dynamic equilibrium process (e.g. protonation [4,5], complexation [6,7], micellar partition [8,9]) the mobility is a weighted average over the equilibrating species. Beckers et al. [4], have developed a treatment of acid–base equilibria which takes into account non-

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[1]Part of this work was presented at the 3rd International Symposium on Capillary Electrophoresis, York, 24–26 August, 1994.

PII S0021-9673(96)00151-3

ideality in both ionic equilibria and transport. They determined pK_a values for twelve weak acids using both free solution capillary electrophoresis (FSCE) and isotachophoresis (ITP), and results from both techniques were shown to be in good agreement. Comprehensive tables of mobilities and dissociation constants measured by capillary ITP have also been given [10].

The aim of the present paper is to provide a rational approach to peptide mobilities measured in solutions of different BGE ions and ionic strengths. Previous work on peptide mobility and mobility modelling has been performed in citric acid [11,12], glycine [13] and phosphoric acid [14] buffers at low pH, and tricine/morpholine at pH 8 [13]. The correlation devised by Grossman et al. [11] is frequently quoted [2] and often assumed to be generic. However, it has been shown to offer only an approximate fit for two sets of oligopeptides [12], and is specific to a particular buffer system and pH. Differences in parameters used to fit mobilities are found in citrate and phosphate buffers. Part of the problem is that effects of incomplete dissociation at the pH of measurement were accounted for using known or calculated pK_a values, but no corrections were made for deviations from ideality. Here we outline a theoretical analysis of deviations from ideality which is expected to be valid over the BGE ionic strength range up to 0.075 mol kg^{-1}, typical for CE. Rather than using the Onsager equation as in [4], non-ideality in ionic transport is treated at the same level of approximation as that in equilibrium, as proposed by Robinson and Stokes [15]. The analysis is applied to the determination of limiting mobilities of oligoglycines and oligoalanines, and data in four different buffer solutions of both low and high pH shown to give concordant results for the fully ionised peptides: The treatment can cope with data obtained in buffer solutions at any pH and ionic strength, and provides a self consistent framework for standardising mobilities of peptides in free solution.

2. Theoretical

2.1. Non ideality in ionic equilibria

For the equilibrium between an acid HA and its conjugate base A^-

$$\underset{1-\alpha}{HA} \rightleftharpoons \underset{\alpha}{H^+} + \underset{\alpha}{A^-} \quad (1)$$

with α the degree of dissociation and the acid dissociation constant K_a defined in terms of the activities of the species involved.

$$pH = pK_a + \log \frac{\alpha}{1-\alpha} + \log \frac{\gamma_{A^-}}{\gamma_{HA}} \quad (2)$$

where γ_{A^-} and γ_{HA} are activity coefficients. Eq. (2) uses $pH = -\log a_{H^+}$ and differs from Eq. (7) in [4], where the incorrect substitution $pH = -\log [H^+]$ was made. A full discussion of the definition of pH and measurement of pK_a is given elsewhere [16,17]. Rearrangement of Eq. (2) allows calculation of the degree of dissociation at any pH

$$\alpha = \frac{\gamma_{HA}}{\gamma_{HA} + \gamma_{A^-} 10^{pK_a - pH}} \quad (3)$$

Activity coefficients for neutral species may be taken to be unity, and for charged species calculated from Debye–Hückel theory. For aqueous solutions at 298 K

$$-\log \gamma = \frac{0.509 z^2 I^{1/2}}{1 + 3.28 a I^{1/2}} \quad (4)$$

where z is the charge, a the sum of the radius of the ion and its counterion (nm), and I the ionic strength of the solution (mol kg^{-1}). For other temperatures and solvent compositions the constants in the numerator and denominator of Eq. (4) scale with $(\rho/\epsilon^3 T^3)^{1/2}$ and $(\rho/\epsilon T)^{1/2}$ respectively, where ρ is the density and ϵ the relative permittivity and T the absolute temperature [18,19].

The effective electrophoretic mobility, μ_{eff}, of the mixture of HA and A^- [Eq. (1)] is [4]

$$\mu_{eff} = (1-\alpha)\mu_{HA} + \alpha\mu_{A^-} \quad (5)$$

where μ_{HA} and μ_{A^-} are the electrophoretic mobilities of the acid and its conjugate base.

Two ionization equilibria may be probed for the oligopeptides with neutral side chains considered in this study. Symbolising the zwitterionic form present in neutral solution as HP,

$$H_2P^+ \overset{K_1}{\rightleftharpoons} HP + H^+ \overset{K_2}{\rightleftharpoons} 2H^+ + P^- \quad (6)$$

The first equilibrium is ionisation of the C-terminal

COOH group and the second the ionisation of the N-terminal NH_3^+ group, with pK_a values pK_1 and pK_2 respectively. At low pH since $z_{H2P^+}=1$, $z_{HP}=0$, $\mu_{HP}=0$,

$$\mu_{eff}=(1-\alpha)\mu_{H_2P^+} \quad (7)$$

whilst at high pH

$$\mu_{eff}=\alpha\mu_{P^-} \quad (8)$$

since $z_{HP}=0$, $z_{P^-}=-1$, $\mu_{HP}=0$.

2.2. Non-ideality in transport behaviour

The Debye–Hückel–Onsager equation for variation of molar conductivity, λ, with concentration in a 1:1 electrolyte solution for aqueous solutions at 298 K is [4,15,18,19]

$$\lambda=\lambda^\circ-(0.229\lambda^\circ+3.01\times10^{-3})c^{1/2} \quad (9)$$

where λ° is the limiting molar conductivity ($m^2\ \Omega^{-1}\ mol^{-1}$). The non-ideality correction term for the relaxation effect, $-0.229\lambda^\circ c^{1/2}$, scales with $(\epsilon T)^{-3/2}$. The term for the electrophoretic effect, $-3.01\times10^{-3}c^{1/2}$, scales with $\eta^{-1}(\epsilon T)^{-1/2}$, where η is the viscosity.

Eq. (9) has been used in [4] to account for non-ideality in FSCE mobility of a dilute solution of an ion in excess of the background electrolyte. It should be noted that the change from treatment of a 1:1 electrolyte to that of an ion migrating in a 1:1 electrolyte, typically with a co-ion of significantly different mobility, is justified theoretically only in the case when the co-ion mobility is identical to that of the analyte [20].

For solutions at concentrations typical of CE experiments, rather than using Eq. (9) it is more appropriate to introduce an additional ion-size correction term to provide a level of approximation analogous to that in Eq. (4). Whereas the Onsager limiting law is valid only up to concentration ~1 m*M*, division of the non-ideality term in Eq. (9) by the denominator of Eq. (4) has been shown to give a very fair account of the conductivities of aqueous 1:1 electrolytes up to 50 or 75 m*M* [15]. Making this correction to Eq. (9) as proposed by Robinson and Stokes [15], and converting from conductivity to mobility using

$$\lambda=F\mu \quad (10)$$

where F is Faraday's constant, gives for a 1:1 electrolyte in aqueous solution where c (M) is numerically equal to I (mol kg^{-1})

$$\mu=\mu^\circ-\frac{(0.229\mu^\circ+3.12\times10^{-8})I^{1/2}}{1+3.28aI^{1/2}} \quad (11)$$

where μ° ($m^2\ V^{-1}\ s^{-1}$) is the limiting mobility (also termed the absolute mobility [4]).

In using Eqs. (4,11) to correct for non-ideality in equilibrium and transport behaviour it is necessary to estimate the ionic radius sum, a (nm). The radius r of the migrating ion may be related to its mobility by the Stokes' law expression

$$r=\frac{e}{6\pi\eta\mu^\circ} \quad (12)$$

where e is the charge per electron. For aqueous solutions at 298 K

$$r=\frac{9.54\times10^{-9}}{\mu^\circ} \quad (13)$$

The sum of the ionic radii a is obtained by addition with the appropriate radius of the hydrated counter-ion. In this paper, these are Na^+(0.18 nm) and Li^+(0.24 nm) as reported by Burgess [21].

2.3. Experimental and computational strategy for determination of μ°

The effective electrophoretic mobility is determined from measurement of migration times of analyte and neutral marker [4]. In all experiments mesityl oxide was present as electroosmotic flow marker, and μ_{eff} calculated as the difference between the observed mobility and the electroosmotic flow mobility. Other quantities which must be known are pH, concentrations of BGE ions, temperature, analyte and buffer pK_a values. Four steps are used in the calculation of the limiting mobility.

1. The *ionic strength*, I, of the BGE is calculated knowing the total concentrations of the buffer ions, the pH and the buffer pK_a. Two cycles of iteration using Eqs. (3,4), together with equations for mass and charge balance, are used to determine the concentration and activity coefficients of all ionic species present. In evaluating γ values

for buffer and hydrogen ions the denominator in Eq. (4) is assumed equal to $(1+I^{1/2})$ (cf. [18]).
2. The *degree of dissociation*, α, of the peptide is calculated knowing pH, the peptide pK_a and I. For self-consistency with mobility calculations an initial estimate of μ° is made, and Eq. (13) used to calculate a for substitution in Eq. (4) to calculate γ for the charged form of the peptide.
3. The *electrophoretic mobility*, μ, follows using Eq. (7) or Eq. (8).
4. The *limiting mobility*, μ°, is determined from Eq. (11), and used to refine the estimate in (2). Two iterations of steps (2)–(4) are generally sufficient to define μ° to the third decimal place.

In basic media, where the peptide is over 90% in the charged form, mean values of μ° are obtained by averaging results at all pH and ionic strengths for a given buffer. In acidic media, where the degree of dissociation varies between 35 and 80% for studies in citrate buffers and between 50 and 92% for studies in phosphate buffers, a limiting effective mobility μ°_{eff} is obtained at each value of α, using Eq. (7) to process the results from step (4). μ° is then determined as the intercept at $\alpha=0$ from a plot of μ°_{eff} versus $1-\alpha$. This procedure is used to avoid overweighting data taken where the peptides exist largely in the uncharged form.

3. Experimental

3.1. Reagents and chemicals

Compounds used were oligoglycine and oligoalanine di-, tri-, tetra-, penta-, hexa-peptides, sodium hydroxide, citric acid (Sigma, Dorset, UK), phosphoric acid, lithium hydroxide (Fisons, Loughborough, UK) and mesityl oxide (Aldrich, Dorset, UK). Solutions of all compounds were prepared in deionised water (Elgastat UHQ, High Wycombe, UK) at concentration 1 mg ml^{-1} with mesityl oxide as neutral marker. When injected as mixtures concentrations were normally 0.1 mg ml^{-1} of each component and neutral marker was always included. Citrate buffer was prepared from 20 m*M* citric acid and adjusted to a range of pH values (from 2.57 to 3.24) using 1 *M* lithium hydroxide. Sodium phosphate and lithium phosphate buffers were prepared from 20 m*M* phosphoric acid and adjusted to a range of pH values (from 2.03 to 3.04 for sodium phosphate and 2.01 to 3.10 for lithium phosphate) using 1 *M* sodium hydroxide and lithium hydroxide respectively. Borate buffers were prepared from 20 m*M* disodium tetraborate and adjusted to a range of pH values (pH 9.33 to 10.70) using 1 *M* sodium hydroxide. High pH sodium phosphate buffers at pH>11 and concentrations 1, 2, 5, 10, 20, 50, 70 and 100 m*M* were prepared from the appropriate concentration of phosphoric acid and adjusting the pH with 1 *M* NaOH. pH was measured using a Ross combination electrode (BDH, Poole, UK) and pH meter (Corning ion analyzer 150, Corning, Halstead, UK) standardised with phthalate, phosphate and borate buffers at pH 4.00, 7.00 and 9.20 respectively. All samples and buffers were filtered through a 0.2 μm filter (Millipore, Watford, UK) prior to analysis.

3.2. Electrophoretic conditions

All experiments were performed using an automated CE apparatus (Beckman P/ACE with System Gold software, Beckman, High Wycombe, UK) thermostatted at 25.0°C, with UV absorbance detection at 200 nm. The capillary, 27 cm×50 μm (Composite Metal Services, Hallow, UK), was conditioned with either 0.1 *M* sodium hydroxide or lithium hydroxide and background electrolyte prior to analysis. Samples were injected for 1 s under pressure (2.2 nl) and separated using an applied potential of 7.5 kV for all high pH buffers. For low pH phosphate buffers a potential of 17.5 kV was used, and for studies in citrate buffers the potential was 15 kV.

3.3. Correction of mobilities for power dissipated

The Beckman P/ACE permits control of external temperature from 5 degrees below ambient up to 60°C. For all experiments in the present study, the programmed temperature was 25°C. The capillary, of total length between 27 cm and 107 cm is housed in a liquid cooled cartridge. Due to the requirement for the capillary ends to be immersed in either rinse (NaOH) or BGE solutions the entire length of the capillary cannot be liquid cooled. For the present

cartridge design, 4 cm lengths are left exposed for immersion, for which at least 3 cm is left to be cooled by ambient air. It was necessary to correct mobilities from a knowledge of the power dissipated as the operating current obtained at high concentrations gave significant heating effects. In order to compensate for the contribution to mobility from the increased power due to Joule heating, a calibration plot was obtained for the mobility of benzoate ion versus power in a pH 9.5 phosphate–borate buffer as shown in Fig. 1. Mobilities were corrected to zero power, μ_{0P} using the relationship

$$\mu_{0P} = \frac{\mu_{\exp(P)} \times \mu_{0B}}{\mu_{P(B)}} \quad (14)$$

where $\mu_{\exp(P)}$ is the experimentally determined mobility of the peptide at power P, μ_{0B} is the benzoate ion mobility at zero power, and $\mu_{P(B)}$ is the mobility of the peptide at power P from the benzoate calibration plot.

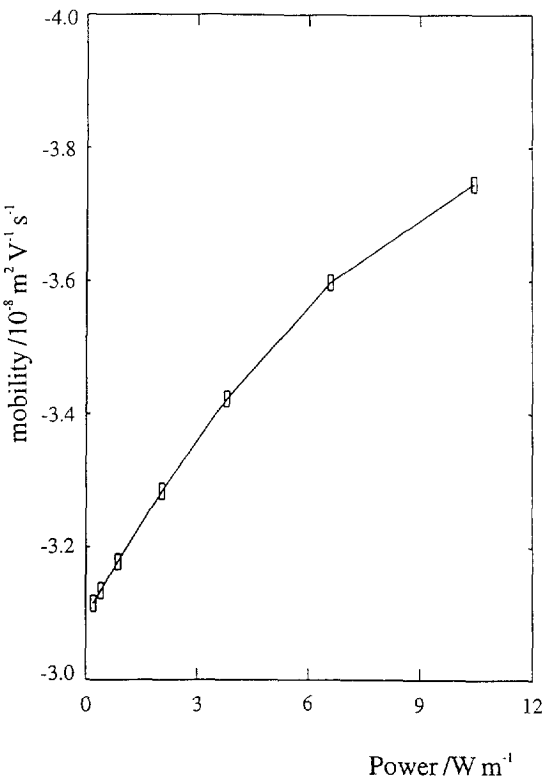

Fig. 1. Calibration plot of benzoate ion mobility versus power dissipated in pH 9.5 phosphate–borate buffer.

4. Results and discussion

4.1. Mobility in basic solutions

pK_a values [22] for the oligoglycines and oligoalanines used, with degree of polymerisation (DP) = 2 to 6, are given in Table 1. Literature values for oligoalanines of DP 5 and 6 were not available and therefore approximated to be 3.42, the same as for tetraalanine. The assumption made was that a similar trend would be followed as for the glycine series where pK_a values were constant after the tetrapeptide. In all studies the ionic concentrations of BGEs and analytes were randomised in order to eliminate any source of systematic error, for example, drift in migration times which may occur throughout the course of each experiment. Migration times of individual peptides in the oligoglycine series (concentration range 8 m*M* for diglycine to 2 m*M* for hexaglycine) were measured in borate buffer solutions over the pH range 9.3 – 10.7 and in phosphate buffer solutions for oligoglycine and oligoalanine mixtures (concentration range 0.8 m*M* to 0.3 m*M* for the glycine series and 0.7 m*M* to 0.07 m*M* for the alanine series) over the range 11.6 to 12.2. At this pH and above, the electroosmotic flow is high, being appproximately $5.7 \cdot 10^{-8}$ m^2 V^{-1} s^{-1}. In all cases the peptides exist over 90% in the −1 charge form [Eq. (5)], and P^- mobilities were obtained by dividing the effective mobility by the degree of dissociation [Eq. (8)].

Since there is frequently some confusion about the nature of the conjugate acid and base species in borate buffer solutions, it is helpful to be reminded

Table 1
pK_a data for oligoglycines and oligoalanines [22]

	pK_1	pK_2
Diglycine	3.12	8.17
Triglycine	3.26	7.91
Tetraglycine	3.05	7.75
Pentaglycine	3.05	7.70
Hexaaglycine	3.05	7.60
Dialanine	3.30	8.14
Trialanine	3.39	8.03
Tetraalanine	3.42	7.94
Pentaalanine	3.42[a]	7.94[a]
Hexaalanine	3.42[a]	7.94[a]

[a]Estimated using tetraalanine values.

[23] that the borax formula $Na_2B_4O_7 \cdot 10H_2O$ can be recast as $2H_3BO_3 \cdot 2NaH_2BO_3 \cdot 5H_2O$, with equivalent amounts of acid and its conjugate base. Assuming no polyborate species are formed, the ionic strength of 20 m*M* disodium tetraborate solution is $4 \cdot 10^{-2}$ mol kg^{-1}. The pK_a of H_3BO_3 at 298 K is 9.234 [15].

Table 2 lists current data and hence power dissipated (*V.I*) for the range of borate and phosphate buffers used. For each BGE solution, values of degree of dissociation, effective mobility, mobility corrected to zero power and limiting mobility were calculated for the five peptides as described below. All values are listed in Table 3, together with the mean and 95% confidence limits [24] for μ°. Comparison of results from our study with those obtained by ITP at high pH [25,26] summarised in Table 4 shows excellent agreement between both data sets.

Measurements in borate buffer solutions covered the ionic strength range 0.048 to 0.075 mol kg^{-1}. A series of measurements were carried out above pH 11 for both series of peptides, where the peptides are fully ionised, using phosphate buffers over the concentration range 1 –100 mM (ionic strength range 0.006 to 0.3 mol kg^{-1}). Fig. 2 shows electropherograms obtained for oligoalanines (DP 2–6) in solutions of increasing phosphate concentration. It can be seen that migration time increases with ionic concentration. This is due to two effects: decrease in electroosmotic mobility, due to screening of charge and reduction of zeta potential at the capillary wall [2], and decrease in electrophoretic mobility due to the relaxation and electrophoretic processes. Peak shape and resolution improved with increasing concentration, however peak widths increased with ionic

Table 2
Resultant powers at varying ionic strengths for high pH borate and phosphate buffers

Buffer type	Ionic strength (mol kg^{-1})	Current (μA)	Power (W m^{-1})
Borate	0.048	19.8	0.55
	0.058	23.5	0.65
	0.070	28.3	0.79
	0.075	38.2	1.06
Phosphate	0.006	2.6	0.07
	0.009	3.8	0.11
	0.021	7.5	0.21
	0.051	16.0	0.45
	0.066	26.0	0.73
	0.179	48.1	1.35
	0.266	64.8	1.81
	0.298	80.9	2.26

Table 3
Degree of dissociation, effective mobility, temperature corrected mobility, μ_{OP} and limiting mobility for oligoglycines (DP 2–6) in borate buffers

Peptide	α	μ_{eff}	μ_{OP}	μ°	$\mu^\circ \pm t_{95} s_{\mu^\circ}/\sqrt{n}$
		(10^{-8} m^2 V^{-1} s^{-1})			
Diglycine	0.947	2.445	2.398	3.099	
	0.971	2.515	2.459	3.136	
	0.990	2.605	2.535	3.225	
	0.998	2.610	2.517	3.170	3.18±0.07
Triglycine	0.970	2.106	2.066	2.669	
	0.984	2.133	2.085	2.693	
	0.995	2.150	2.092	2.730	
	0.999	2.149	2.093	2.686	2.69±0.04
Tetraglycine	0.979	1.853	1.818	2.375	
	0.989	1.869	1.827	2.399	
	0.996	1.888	1.837	2.446	
	0.999	1.863	1.797	2.383	2.40±0.04
Pentaglycine	0.981	1.645	1.614	2.146	
	0.990	1.659	1.622	2.171	
	0.997	1.668	1.623	2.209	
	0.999	1.646	1.587	2.161	2.17±0.04
Hexaglycine	0.983	1.434	1.407	1.910	
	0.990	1.487	1.454	1.982	
	0.997	1.494	1.454	2.019	
	0.999	1.474	1.422	1.969	1.97±0.06

For each peptide, data are tabulated at four pH values 9.3, 9.6, 10.1, 10.7 with BGE composition 48, 58, 70 and 75 m*M* $Na^+H_2BO_3^-$ respectively. Mean values for μ° are given with 95% confidence limits.

Table 4
Literature values for limiting mobilities for oligoglycines and oligoalanines determined by isotachophoresis

Peptide	μ° (10^{-8} m^2 V^{-1} s^{-1})
Diglycine	3.15[a]
Triglycine	2.61[b]
Tetraglycine	2.33[b]
Pentaglycine	2.12[b]
Hexaglycine	1.93[b]
Dialanine	2.70[a]
Trialanine	2.22[b]

[a] [25].
[b] [26].

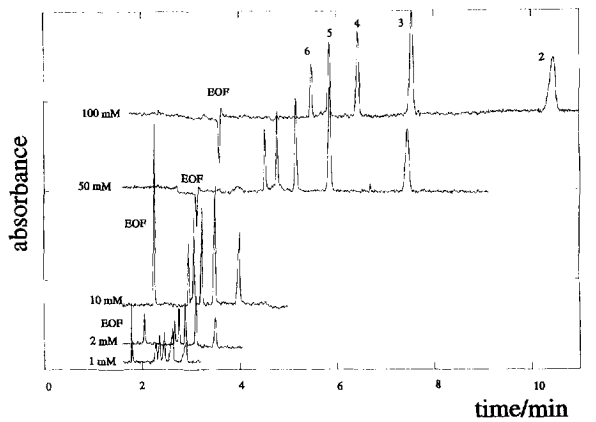

Fig. 2. Electropherograms of oligoalanines in high pH phosphate buffers and of concentrations 1 to 100 m*M*. EOF is mesityl oxide neutral marker. Order of elution at high pH is hexa-(6) to di-glycine (2).

concentration due to diffusion as a consequence of increased residence times of each analyte in the capillary. Data at 1 m*M* phosphate was not included in the subsequent analysis because the effects of electromigration dispersion in the peak shape could lead to a systematic error of ~3% in estimating the mobilities. For all other concentrations use of peak maxima to define migration times reduced this uncertainty to $<1\%$.

Ionic concentrations in the high pH phosphate buffers were calculated from mass balance equations and analysis of the dissociation equilibria of water ($pK_w = 14.00$) and HPO_4^{2-} [Eqs. (3,4), $HA = HPO_4^{2-}$, $A^- = PO_4^{3-}$, $pK_a = 12.38$]. Fig. 3 gives plots of mobility as a function of $I^{1/2}$ for the oligopeptides. As expected at these high ionic strengths (up to 0.07 mol kg^{-1}), the Debye–Hückel–Onsager limiting equation [Eq. (9)] is inapplicable and the variation of μ with $I^{1/2}$ is at all points less than the limiting law predictions. One interesting feature from Fig. 3 is that the mobility differences between peptides are almost invariant with ionic strength. For values of $I < 0.07$ mol kg^{-1}, Eq. (11) was used to account for non-ideality in transport behaviour. Table 5 presents data for the high pH phosphate BGE solutions in a similar format to Table 3 for borate BGE solutions. At the high pH values used (pH > 11.5) all peptides are fully ionised ($\alpha = 1.00$) and the mobility μ_{P^-} is measured directly.

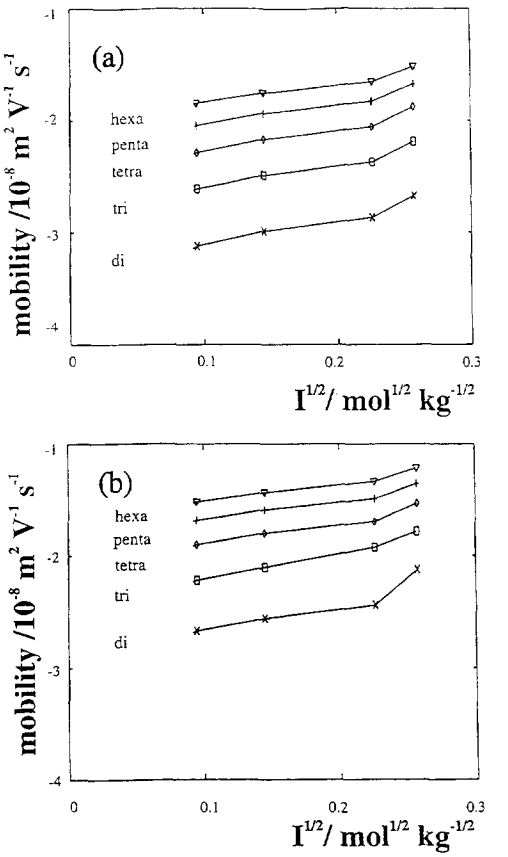

Fig. 3. Plots of mobility as a function of ionic strength in high pH phosphate buffers for (a) oligoglycines and (b) oligoalanines.

Mean values of limiting mobilities for oligoglycines in phosphate and borate buffers lie outside each other's 95% confidence limits. Looking at the series as a whole, values in phosphate buffers are seen to be systematically 8 to 10% higher than those in borate buffers and from ITP. A possible source of systematic error lies in the use of Eq. (11), a mobility treatment devised for 1:1 electrolytes, in a BGE solution containing a mixture of 1:2 (Na_2HPO_4) and 1:3 (Na_3PO_4) electrolytes.

4.2. Mobility in acidic solutions

This study involved the preparation of buffers of sodium citrate, sodium phosphate, (H_3PO_4/NaH_2PO_4) and lithium phosphate, (H_3PO_4/LiH_2PO_4) where changes in buffer type and co-ion

Table 5
Mobility and limiting mobility for oligoglycines and oligoalanines (DP 2–6) in phosphate buffers

Peptide	μ	μ_{0P}	μ°	$\mu^{\circ} \pm t_{95} s_{\mu_0} / \sqrt{n}$
	(10^{-8} m^2 V^{-1} s^{-1})			
Oligoglycine				
Diglycine	3.121	3.106	3.416	
	2.993	2.969	3.403	
	2.868	2.823	3.418	
	2.537	2.474	3.104	3.34±0.17
Triglycine	2.614	2.602	2.898	
	2.495	2.475	2.888	
	2.375	2.338	2.900	
	2.165	2.111	2.712	2.85±0.10
Tetraglycine	2.288	2.277	2.564	
	2.173	2.155	2.553	
	2.059	2.027	2.567	
	1.879	1.832	2.408	2.52±0.08
Pentaglycine	2.046	2.036	2.315	
	1.938	1.922	2.308	
	1.831	1.802	2.324	
	1.673	1.631	2.188	2.28±0.07
Hexaglycine	1.846	1.837	2.111	
	1.751	1.737	2.114	
	1.654	1.628	2.135	
	1.515	1.477	2.017	2.09±0.06
Oligoalanine				
Dialanine	2.674	2.661	2.959	
	2.561	2.540	2.956	
	2.441	2.403	2.970	
	2.127	2.074	2.672	2.89±0.16
Trialanine	2.222	2.212	2.497	
	2.107	2.090	2.485	
	1.929	1.899	2.423	
	1.786	1.741	2.308	2.43±0.09
Tetraalanine	1.904	1.895	2.170	
	1.802	1.787	2.167	
	1.696	1.669	2.180	
	1.533	1.495	2.038	2.14±0.07
Pentaalanine	1.686	1.678	1.946	
	1.593	1.580	1.948	
	1.492	1.468	1.960	
	1.355	1.321	1.844	1.92±0.06
Hexaalanine	1.517	1.510	1.771	
	1.434	1.422	1.780	
	1.337	1.316	1.793	
	1.218	1.188	1.695	1.76±0.05

For each peptide, data are tabulated at five phosphate concentrations 2,5,10 and 20 m*M* with pH values 11.6, 11.7, 12.2 and 11.5 and ionic strengths 0.009, 0.021, 0.050 and 0.066 mol kg^{-1} respectively. Electrophoretic mobilities are corrected to 25.0°C as described in the text. Mean values for μ° are given with 95% confidence limits.

as well as effects of change of pH can be investigated. In the case of the citrate buffers ($pK_1 = 3.128$, $pK_2 = 4.761$), a typical range for $(1-\alpha)$ is 0.41 to 0.77 for diglycine. For diglycine in lithium phosphate buffers, ($pK_a = 2.148$) the range was 0.51 to 0.92. Studies in acidic solutions covered a relatively narrow range of ionic strength compared to studies in basic solutions, for example in sodium citrate buffer, the range studied was 0.005 to 0.013 mol kg^{-1}. Mobilities for all analytes were corrected for power dissipated. These are summarised in Table 6. The hydrodynamic radius, r, at low pH was taken from the mean values of μ° obtained in high pH phosphate and borate buffers using Eq. (13). Hydrodynamic radii values for the oligopeptides are summarised in Table 7. Small changes in r were found to have negligible effects on values of μ° calculated.

An electropherogram in lithium phosphate at pH 2.03 is given in Fig. 4. Effective limiting mobilities, μ°_{eff}, were determined and plotted as a function of $(1-\alpha)$ to calculate μ°. Fig. 5 plots μ°_{eff} versus $(1-\alpha)$ for diglycine in lithium citrate, lithium phosphate and sodium phosphate. μ° is the value of μ°_{eff} at $(1-\alpha)=1$, and is always reported with 95% confidence limits.

Table 8 collects together results in citrate, whilst Table 9 gives values for lithium phosphate and Table 10 sodium phosphate buffers respectively. Ionic

Table 6
Calculated powers dissipated from varying concentrations of low pH lithium citrate, lithium phosphate and sodium citrate buffers

Buffer	pH	Ionic strength (10^{-3} mol kg^{-1})	Current (μA)	Power (W m^{-1})
Lithium citrate		5	14.0	0.78
		7	15.0	0.83
		8	15.0	0.83
		10	15.4	0.86
		13	17.5	0.97
Lithium phosphate		10	35.5	2.30
		13	27.7	1.80
		16	24.9	1.61
		17	23.9	1.55
		18	23.0	1.49
Sodium phosphate		10	37.3	2.42
		13	30.2	1.96
		16	29.3	1.90
		17	27.8	1.80
		18	24.0	1.56

Table 7
Mean values for hydrodynamic radii, r, obtained for oligopeptides in high pH phosphate and borate buffers

Peptide	r (nm)
Diglycine	0.29
Triglycine	0.35
Tetraglycine	0.38
Pentaglycine	0.42
Hexaglycine	0.46
Dialanine	0.33
Trialanine	0.39
Tetraalanine	0.45
Pentaalanine	0.50
Hexaalanine	0.55

strengths in these acidic buffers are low and corrections for Joule heating effects smaller than in the basic solutions. In addition, corrections from mobilities to limiting mobilities, approximately 20% for hexaglycine at the highest ionic strength studied (0.018 mol kg^{-1}), are substantially less than those in the basic buffers discussed previously.

Comparison of Table 9 and Table 10 shows that there are no significant differences in μ° values found in lithium and sodium phosphate buffers after correction of mobilities to zero power. Values of μ° are within each other's 95% confidence limits for all low pH phosphate and citrate buffer solutions. This supports the treatment of non-ideality in transport developed in this paper, which considers ionic strength to be the major factor influencing the mobility of an analyte ion.

In Fig. 5, the intercept of the plot of μ°_{eff} versus $(1-\alpha)$ for diglycine is zero within experimental error as expected, since at zero charge all species will have no net mobility. This profile was similarly observed for triglycine and the oligoalanines. However, positive intercepts were seen for tetra- to hexaglycine in all acidic solutions. An example is Fig. 6a which plots μ°_{eff} versus $(1-\alpha)$ for pentaglycine in lithium phosphate. This implies that a systematic error may be present which has not been accounted for. One possible source of error is the pK_a values used. Data from various sources [27–29] show good agreement between reported values for the di- and tripeptides and therefore the values for these species in Table 1 can be taken to be accurate.

The trend in pK_a values for the oligoalanines of DP 2 to 4 is as would be expected, that is, the pK_a increases with chain length. The terminal NH_3^+ group will make the loss of a proton in acidic pH easier as a result of electrical charge repulsion [30]. However, the pK_a for the glycine series increases from di- to triglycine from 3.12 to 3.26 as expected but decreases to 3.05 for tetraglycine. Cohn and Edsall [27] stated that the pK_a values for tetra- to hexaglycine were an uncertain assessment. To simulate the possible effects of systematic errors in the pK_a values for these peptides, these were changed from 3.05 to 3.4 to investigate the effects on μ°. An assumed pK_a value of 3.4 causes the intercept in Fig. 6a to go through the origin (Fig. 6b), and the same is observed for tetra- and hexaglycine in citrate and phosphate buffers. Table 11 compares results using $pK_a = 3.4$ with the original analysis ($pK_a = 3.05$). There is little change in μ° for tetra- to hexaglycine in lithium and sodium phosphate buffers with the 2–3% decrease being within the 95% confidence limits. However, the mean values for μ° for studies in sodium citrate buffer which assumes a value of 3.4 for pK_a are systematically $0.2 \cdot 10^{-8}$ m^2 V^{-1} s^{-1} lower than those calculated at pK_a 3.05. The greater sensitivity of data in citrate buffer can be attributed to the spanning of a range of $(1-\alpha)$ less close to the fully protonated peptide than in phosphate buffers.

4.3. Comparison of our model with that proposed by Beckers et al.

Since our treatment for non-ideality in equilibria and ion transport in Eq. (11) differs from the Beckers et al. [4] treatment of Eq. (9), we need to evaluate differences, if any, which may arise between the two theories. As an example we will calculate the value for μ from μ° for the average value of diglycine from all buffers (see Table 12). Eq. (11) differs from Eq. (9) as it consists of a denominator $1+3.28aI^{1/2}$ which has been theoretically justified by Debye and Hückel to account for the radius sum of a species in non-ideal conditions. μ calculated from Eq. (11) for diglycine in a solution of ionic strength 0.058 mol kg^{-1} gave a value of $3.14 \cdot 10^{-8}$ m^2 V^{-1} s^{-1}, whereas μ° calculated from Eq. (9) yields the result $\mu^{\circ} = 3.38 \cdot 10^{-8}$ m^2 V^{-1} s^{-1}. Such calculations show that μ° will be

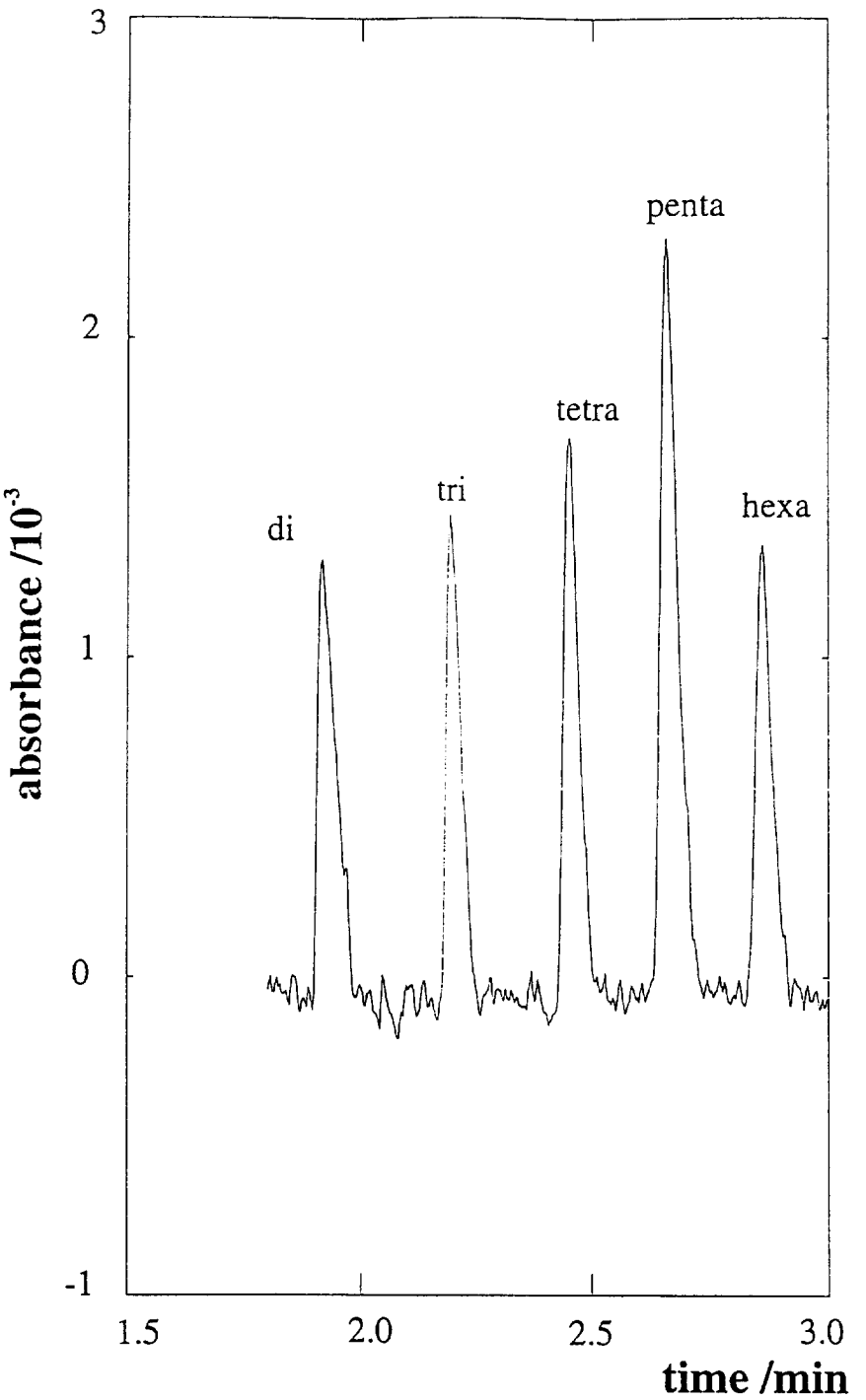

Fig. 4. Electropherogram of oligoglycines in 20 m*M* lithium phosphate buffer, pH 2.03.

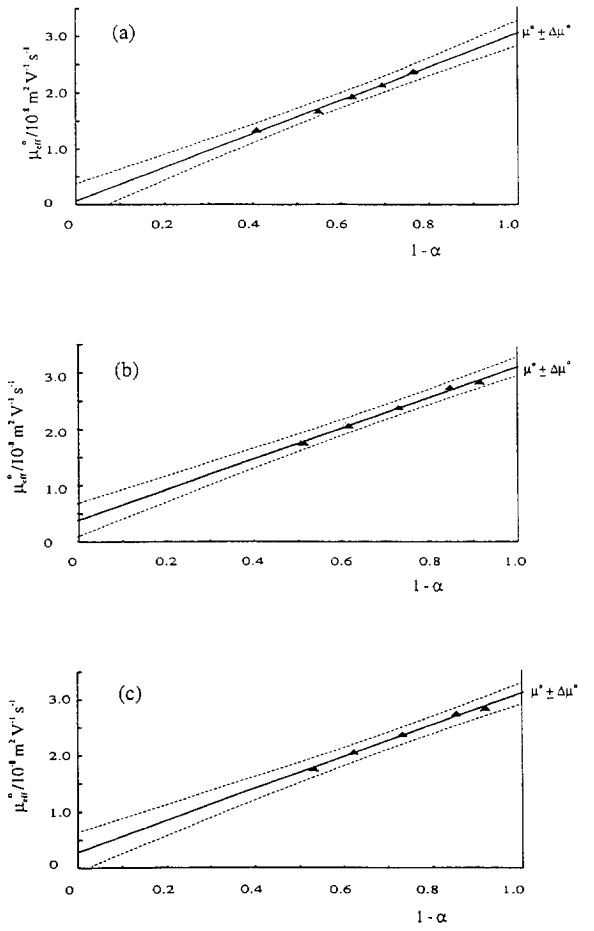

Fig. 5. Plots of μ°_{eff} versus $(1-\alpha)$ for diglycine in low pH (a) sodium citrate, (b) lithium phosphate and (c) sodium phosphate buffers with 95% confidence limits.

systematically $\sim 0.2 \cdot 10^{-8}$ m^2 V^{-1} s^{-1} lower than using the corrections of Beckers et al. [4]. Another difference between the two treatments is that Beckers et al. [4] do not account for changes in mobility with increase in power.

4.4. Correlation of all limiting mobility data

Table 12 summarises data for all studies in acidic and basic solutions. Discounting the results in high pH phosphate buffer, which are systematically $\sim 0.2 \cdot 10^{-8}$ m^2 V^{-1} s^{-1} higher than in the other four buffers used in this study, excellent agreement can be seen for limiting mobilities for each analyte. The

Table 8
Degree of dissociation, μ_{0P}, mobility at infinite dilution for partially charged species, μ°_{eff}, and μ° for oligoglycines (DP 2–6) and oligoalanines in citrate buffers

Peptide	$(1-\alpha)$	μ_{0P}	μ°_{eff}	μ°
		(10^{-8} m^2 V^{-1} s^{-1})		
Oligoglycine				
Diglycine	0.765	2.191	2.390	
	0.694	1.942	2.139	
	0.626	1.743	1.936	
	0.549	1.483	1.664	
	0.407	1.171	1.323	3.06±0.21
Triglycine	0.818	1.939	2.143	
	0.758	1.758	1.964	
	0.698	1.613	1.819	
	0.628	1.404	1.602	
	0.487	1.143	1.316	2.57±0.15
Tetraglycine	0.735	1.689	1.842	
	0.660	1.535	1.712	
	0.588	1.408	1.593	
	0.510	1.228	1.418	
	0.369	1.013	1.177	2.33±0.08
Pentaglycine	0.736	1.522	1.671	
	0.660	1.382	1.555	
	0.589	1.270	1.450	
	0.510	1.110	1.295	
	0.369	0.913	1.072	2.12±0.06
Hexaglycine	0.736	1.387	1.533	
	0.660	1.260	1.430	
	0.589	1.158	1.334	
	0.510	1.011	1.191	
	0.369	0.838	0.993	1.94±0.06
Oligoalanine				
Dialanine	0.831	1.969	2.177	
	0.774	1.807	2.019	
	0.716	1.644	1.856	
	0.647	1.431	1.636	
	0.508	1.170	1.351	2.60±0.17
Trialanine	0.858	1.704	1.911	
	0.809	1.583	1.796	
	0.757	1.474	1.690	
	0.694	1.311	1.523	
	0.560	1.104	1.297	2.19±0.12
Tetraalanine	0.867	1.490	1.693	
	0.819	1.393	1.602	
	0.770	1.302	1.515	
	0.708	1.166	1.376	
	0.577	0.993	1.184	1.92±0.08
Pentaalanine	0.867	1.342	1.541	
	0.819	1.253	1.458	
	0.770	1.180	1.388	
	0.708	1.058	1.263	
	0.577	0.905	1.092	1.74±0.08
Hexaalanine	0.867	1.220	1.415	
	0.819	1.142	1.342	
	0.770	1.079	1.282	
	0.708	0.970	1.170	
	0.577	0.937	1.020	1.59±0.08

For each peptide data are tabulated at five pH values 2.57, 2.73, 2.86, 2.99, 3.24 with BGE ionic strengths 0.005, 0.007, 0.008, 0.010 and 0.013 mol kg^{-1} respectively. Mean values for μ° are given with 95% confidence limits.

Table 9
Degree of dissociation, μ_{0P}, mobility at infinite dilution for partially charged species, μ°_{eff}, and mobility at infinite dilution, μ° for oligoglycines and oligoalanines in lithium phosphate

Peptide	$(1-\alpha)$	μ_{0P}	μ°_{eff}	μ°
		$(10^{-8}\ m^2\ V^{-1}\ s^{-1})$		
Oligoglycine				
Diglycine	0.919	2.592	2.827	
	0.851	2.491	2.731	
	0.736	2.147	2.372	
	0.615	1.854	2.057	
	0.512	1.557	1.747	3.09±0.16
Triglycine	0.940	2.211	2.442	
	0.887	2.152	2.392	
	0.794	1.915	2.147	
	0.688	1.696	1.913	
	0.592	1.477	1.686	2.61±0.12
Tetraglycine	0.906	1.907	2.091	
	0.830	1.871	2.090	
	0.705	1.656	1.873	
	0.577	1.472	1.684	
	0.473	1.290	1.495	2.28±0.20
Pentaglycine	0.906	1.713	1.893	
	0.830	1.689	1.903	
	0.705	1.487	1.699	
	0.577	1.333	1.540	
	0.473	1.165	1.364	2.07±0.21
Hexaglycine	0.906	1.552	1.729	
	0.830	1.523	1.732	
	0.705	1.352	1.559	
	0.578	1.209	1.410	
	0.474	1.061	1.255	1.88±0.17
Oligoalanine				
Dialanine	0.945	2.274	2.508	
	0.896	2.109	2.351	
	0.809	1.923	2.159	
	0.707	1.726	1.949	
	0.614	1.502	1.718	2.61±0.08
Trialanine	0.955	1.905	2.133	
	0.914	1.776	2.013	
	0.839	1.657	1.893	
	0.749	1.527	1.754	
	0.663	1.370	1.595	2.19±0.08
Tetraalanine	0.957	1.670	1.892	
	0.920	1.541	1.772	
	0.849	1.457	1.688	
	0.762	1.350	1.573	
	0.678	1.218	1.440	1.93±0.10
Pentaalanine	0.957	1.512	1.729	
	0.920	1.385	1.611	
	0.849	1.311	1.537	
	0.762	1.221	1.439	
	0.678	1.109	1.325	1.75±0.12
Hexaalanine	0.957	1.376	1.589	
	0.920	1.255	1.476	
	0.849	1.191	1.412	
	0.762	1.119	1.332	
	0.678	1.017	1.228	1.60±0.12

Data are tabulated at 5 pH values 2.01 2.32, 2.62, 2.86 and 3.04 with BGE composition 0.010, 0.013, 0.016, 0.017 and 0.018 mol kg^{-1} respectively. Mean values for μ° are given with 95% confidence limits.

Table 10
Degree of dissociation, μ_{0P}, mobility at infinite dilution for partially charged species, μ°_{eff}, and mobility at infinite dilution, μ° for oligoglycines and oligoalanines in sodium phosphate

Peptide	$(1-\alpha)$	μ_{0P}	μ°_{eff}	μ°
		$(10^{-8}\ m^2\ V^{-1}\ s^{-1})$		
Oligoglycine				
Diglycine	0.917	2.592	2.830	
	0.852	2.491	2.735	
	0.732	2.147	2.734	
	0.621	1.854	2.062	
	0.531	1.557	1.756	3.11±0.19
Triglycine	0.939	2.221	2.455	
	0.889	2.152	2.395	
	0.791	1.915	2.150	
	0.694	1.696	1.918	
	0.610	1.477	1.695	2.63±0.15
Tetraglycine	0.905	1.907	2.093	
	0.831	1.871	2.094	
	0.700	1.656	1.876	
	0.584	1.472	1.691	
	0.492	1.290	1.507	2.29±0.21
Pentaglycine	0.905	1.713	1.895	
	0.831	1.689	1.906	
	0.700	1.487	1.701	
	0.584	1.333	1.546	
	0.492	1.165	1.376	2.08±0.21
Hexaglycine	0.905	1.552	1.730	
	0.831	1.523	1.736	
	0.700	1.352	1.561	
	0.584	1.209	1.416	
	0.492	1.061	1.266	1.89±0.19
Oligoalanine				
Dialanine	0.944	2.274	2.511	
	0.897	2.109	2.354	
	0.805	1.923	2.162	
	0.713	1.726	1.954	
	0.632	1.501	1.727	2.63±0.08
Trialanine	0.954	1.905	2.136	
	0.915	1.776	2.017	
	0.836	1.657	1.896	
	0.754	1.527	1.759	
	0.679	1.370	1.604	2.20±0.10
Tetraalanine	0.957	1.670	1.895	
	0.920	1.541	1.776	
	0.846	1.457	1.691	
	0.767	1.350	1.578	
	0.695	1.218	1.449	1.94±0.12
Pentaalanine	0.957	1.512	1.732	
	0.920	1.385	1.614	
	0.846	1.311	1.539	
	0.767	1.221	1.444	
	0.695	1.109	1.334	1.76±0.12
Hexaalanine	0.957	1.376	1.591	
	0.920	1.255	1.480	
	0.846	1.191	1.415	
	0.767	1.119	1.337	
	0.695	1.017	1.237	1.61±0.12

Data are tabulated at 5 pH values 2.03 2.31, 2.63, 2.85 and 3.01 with BGE composition 0.010, 0.013, 0.016, 0.017 and 0.018 mol kg^{-1} respectively. Mean values for μ° are given with 95% confidence limits.

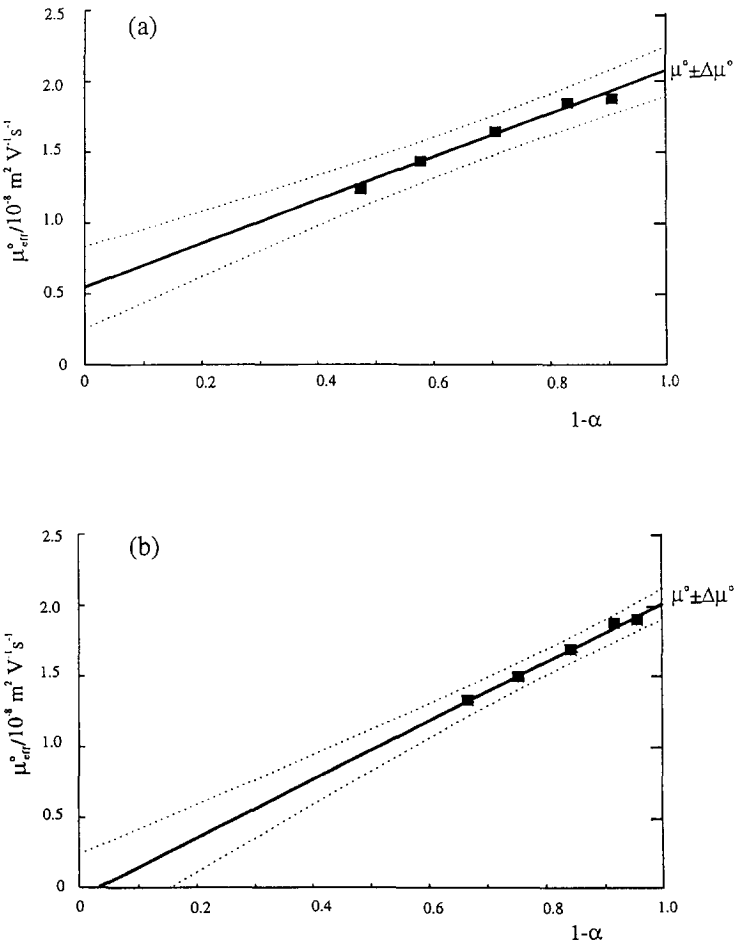

Fig. 6. Plots of μ°_{eff} versus $(1-\alpha)$ for pentaglycine in lithium phosphate buffers assuming pK_a of (a) 3.05 and (b) 3.4.

Table 11
Summary of results for μ° calculated with pK_a of 3.4 for tetra- to hexaglycine

Peptide	Citrate	LiH_2PO_4	NaH_2PO_4
Tetraglycine	2.13±0.08 (2.33±0.08)	2.23±0.14 (2.29±0.21)	2.24±0.15 (2.28±0.20)
Pentaglycine	1.94±0.06 (2.12±0.06)	2.02±0.15 (2.08±0.21)	2.03±0.15 (2.07±0.21)
Hexaglycine	1.63±0.06 (1.94±0.06)	1.84±0.15 (1.89±0.19)	1.85±0.14 (1.88±0.17)

Values in brackets are original data with pK_a 3.05.

fully ionised univalent peptides migrating in 1:1 electrolytes have limiting mobilities which are numerically equal but opposite in sign at low pH and at high pH, consistent with the change of charge from +1 to −1.

Table 13 compares weighted averages (with their weighted errors [31]), for all buffers excluding high pH phosphate with those obtained by isotachophoresis. Excellent agreement can be seen between both data sets, showing the validity of our method for a univalent ion migrating in a 1:1 electrolyte. Phosphate buffers at high pH consist of a mixture of 1:2 and 1:3 electrolytes. Deviations from ideality for such systems cannot be properly treated with the present methods discussed and requires further investigation.

Corrections for non-ideality are found to be substantial at the ionic strengths used, ranging from 21% for diglycine to 34% for the least mobile hexaglycine

Table 12
Summary of results of limiting mobilities

	A	B	C	D	E
	($\mu^\circ/10^{-8}$ m^2 V^{-1} s^{-1})				
Diglycine	−3.18±0.07	−3.34±0.17	3.06±0.21	3.09±0.16	3.11±0.19
Triglycine	−2.69±0.04	−2.85±0.10	2.57±0.15	2.61±0.12	2.63±0.15
Tetraglycine	−2.40±0.04	−2.52±0.08	2.33±0.08	2.28±0.20	2.29±0.21
Pentaglycine	−2.17±0.04	−2.28±0.07	2.12±0.06	2.07±0.21	2.08±0.21
Hexaglycine	−1.97±0.06	−2.09±0.06	1.94±0.06	1.88±0.17	1.89±0.19
Dialanine	–	−2.89±0.16	2.60±0.17	2.61±0.08	2.63±0.08
Trialanine	–	−2.43±0.09	2.19±0.12	2.19±0.08	2.20±0.10
Tetraalanine	–	−2.14±0.07	1.92±0.08	1.93±0.10	1.94±0.12
Pentaalanine	–	−1.92±0.06	1.74±0.08	1.75±0.12	1.76±0.12
Hexaalanine	–	−1.76±0.05	1.59±0.08	1.60±0.12	1.61±0.12

All values are taken are given as mean and 95% confidence limits.
A = H_3BO_3/NaH_2BO_3 — high pH borate buffer.
B = NaH_2PO_4/Na_3PO_4 — high pH phosphate buffer.
C = HCit/NaCit — low pH sodium citrate buffer.
D = H_3PO_4/NaH_2PO_4 — low pH sodium phosphate buffer.
E = H_3PO_4/LiH_2PO_4 — low pH lithium phosphate buffer.

at the highest ionic strength used (0.075 mol kg^{-1}). Insofar as the correction from μ to μ° is dependent upon both mobility and ionic strength [cf. Eq. (11)], any generic correlation between μ and size in a peptide series could be revealed only if all data were first corrected to give μ° values. None of the fitting parameters from previous work on peptide mobility variation with chain length [11–14] can therefore be considered to be unique, since no corrections for non-ideality have been made.

Table 13
Weighted average mobilities and errors, $|\overline{\mu^\circ} \pm \Delta\overline{\mu^\circ}|$ ([31]) for all peptides calculated from studies in high pH borate and low pH citrate and phosphate buffers

Peptide	Weighted averages for μ° from CZE	From ITP
	(10^{-8} m^2 V^{-1} s^{-1})	
Diglycine	3.15±0.06	3.15[a]
Triglycine	2.67±0.04	2.61[b]
Tetraglycine	2.38±0.03	2.33[b]
Pentaglycine	2.15±0.03	2.12[b]
Hexaglycine	1.95±0.04	1.93[b]
Dialanine	2.62±0.05	2.70[a]
Trialanine	2.19±0.06	2.22[b]
Tetraalanine	1.84±0.06	
Pentaalanine	1.75±0.06	
Hexaalanine	1.60±0.06	

[a] [25].
[b] [26].

5. Conclusions

For experiments in CE, we have extended the method of Beckers et al. [4] to account for non-ideality in equilibrium and ion transport. This enables a measure of mobility at infinite dilution, μ°, to be obtained. The Beckers et al. [4] treatment is based on the Debye–Hückel–Onsager approximation and is expected to be theoretically applicable to univalent ions migrating in a uni-univalent background electrolyte up to a concentration of ~1 m*M*. We have shown that by including an ion size correction term as originally introduced by Robinson and Stokes [15], non-ideal effects in ionic mobilities of univalent analytes can be treated in a self-consistent way in 1:1 background electrolyte solutions of concentrations up to 75 m*M*. Before the treatment can be applied, contributions due to Joule heating must be eliminated, for example via an extrapolation of mobility to zero power.

Our self-consistent framework for standardising mobilities in free solution CE has been applied to determine μ^{o} in acidic and basic media for oligoglycines and oligoalanines with DP 2 to 6. In all 1:1 BGE buffer solutions studied, μ^{o} values determined using data over a range of pH and ionic strengths are found to be identical and in excellent agreement with previous values from isotachophoresis.

This treatment of non-ideality in ionic transport at the same level of approximation of ionic equilibrium provides a self-consistent framework for standardising mobilities of peptides in free solution. It permits mobilities determined in any 1:1 BGE at any ionic strength up to 0.075 mol kg^{-1} to be corrected to the standard reference state of infinite dilution. It should be widely applicable to other analytes studied using free solution capillary electrophoresis. Future studies should be directed to testing the generality of the treatment to other univalent analytes migrating in 1:1 BGE solutions, and to establishing phenomenological models applicable to analytes and BGE solutions of higher charge type.

Acknowledgments

We acknowledge support of the SERC and Zeneca Pharmaceuticals towards a studentship (CASE award) to MAS.

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