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Determination of electroosmotic flow mobility with a pressure-mediated dual-ion technique for capillary electrophoresis with conductivity detection using organic solvents

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Abstract

A method is described for the indirect determination of the mobility of the electroosmotic flow (EOF), which can be carried out within a few minutes even for very low mobilities. It is independent of the direction of the EOF. It is based on the comparison of the measured mobilities of two oppositely charged reference ions (tetraphenylphosphonium and tetraphenylborate) with given mobilities in different organic solvents (methanol, acetonitrile, *N,N*-dimethylformamide, *N,N*-dimethylacetamide, propylene carbonate) at ionic strengths between 5 and 50 m*M*. The method is based on the sequential movement of the reference ions in a three-step process: first by a laminar flow to a certain position in the separation capillary, followed by electromigration due to application of voltage, and pressurised migration towards the detector. In this way the total mobilities of the reference ions can be determined from their residence times, and the difference to their known actual mobilities gives the mobility of the EOF. The method avoids misinterpretations caused by system- and eigen-peaks, which often bias the results especially when a conductivity detector is used. The method is suitable for all solvents, and is an advantage especially for organic and mixed aqueous–organic background electrolytes with high UV absorbance.

Keywords: Electroosmotic flow; Conductivity detection; Pressurised mobilisation; Organic solvents; Tetraphenylphosphonium; Tetraphenylborate; Methanol; Acetonitrile; *N,N*-Dimethylformamide; *N,N*-Dimethylacetamide; Propylene carbonate

1. Introduction

Electroosmotic flow (EOF) is an important phenomenon in capillary electrophoresis as it affects both separation efficiency and selectivity, and influences the resolution of the compounds to be separated depending on its magnitude and direction. It determines the reproducibility of the migration of the samples and the accuracy of measuring their mobility. Its determination is not a trivial matter as one would assume at first sight, and thus several methods have been worked out to measure it accurately. The simplest method, introduced early [1,2] and still most commonly used in practice, takes the peak or jump of the baseline caused by the disturbance, which is induced by sample introduction into the originally uniform distribution of the back-

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ground electrolyte (BGE) in the separation column. More specifically, it is a disturbance in the Kohlrausch regulating function at the site of the sample introduction causing such peaks, which are often called solvent peaks or water peaks and which are in fact a jump in concentration of the BGE. At first approximation the solvent peak is simply shifted by the EOF in the separation column and serves for the determination of its velocity. However, this method has a number of limitations, which can often hardly be evaluated. So-called system eigen-peaks [3,4], especially those caused by hydrogen or hydroxide ions [5–7], might mimic the solvent zone, and it is often difficult to decide whether the indicator signal stems from the solvent peak or is caused by the eigen-peak. In addition, the concentration boundaries of the solvent peak have their own additional motion and their profiles are deformed during movement [8]. This occurs when the transference numbers of the cation and the anion of the BGE, respectively, depend on its concentration. It causes a bias in the determination of the EOF mobility when the solvent peak is taken as reference [9] or even can cause misinterpretation of changes in the baseline signal.

An alternative to this simple method uses an electrically neutral marker substance instead of the solvent. In fact, a remarkable difference in the migration of the neutral marker zone and the solvent peak can be observed in many cases. The neutral marker must have certain properties fitting to the demands of the measurement: it must be non-chargeable in the BGE and sufficiently soluble. It must have high UV absorbance when the UV detector is used. Otherwise the high concentration needed for detection, changes the composition of the BGE, thus leading to the undesirable concentration boundaries. Finally, low mobilities of the EOF would result in inconveniently long measuring times. Both techniques described so far are named the ''common'' method.

Thus, a number of alternative methods to determine the magnitude of the EOF have been worked out in the past. Many of them are time consuming or need special equipment or modified instrumentation [10–17], which might hinder their routine application in practice. However, some of the methods can be carried out more easily, with normal experimental conditions, and avoid many of the disadvantages of the direct solvent peak method [18–20].

Jummpanen and Riekkola [19] used several reference ions of the same charge with mobilities differing as much as possible, which were directly injected into the capillary. Pressurised movement of a reference zone consisting of neutral marker substances, combined with electroosmotic motion upon application of an electric field was applied by Williams and Vigh [18]. The authors demonstrated that even very small EOFs could be determined within a short measuring time. Although this ingenious technique is routinely applied in our laboratory when using UV detection, above-mentioned disturbances due to heterogeneous composition of sample and BGE, and caused by system peaks recorded by conductivity detection cannot be avoided, and migration of concentration boundaries may occur as well.

The use of a neutral marker is connected with limitations especially when organic solvents with UV absorbance, or when a conductivity detector are used. In this latter case a marker zone must have a different conductivity than the BGE, otherwise no detection signal would be obtained. Consequently, inhomogeneities in conductance are introduced which lead to concentration boundaries and in most cases to system peaks. These disturbances are thus nearly inevitable for conductivity detection, with all the uncertainties accompanying the measurement of the marker zone. Thus, an alternate method was sought; this is the topic of the present paper.

This method, named the dual-ion method, is fast, applicable under normal instrumental conditions, and independent of the solvent of the BGE. The determination of the mobility of the EOF is based on the comparison of the measured, total mobility (that is composed from its own electrophoretic mobility and that of the EOF) of two oppositely charged reference ions with their known electrophoretic mobility. The difference between these mobilities gives the mobility of the EOF.

Tetraphenylphosphonium (Ph_4P^+) and tetraphenylborate (Ph_4B^-) were selected as reference ions because due to their similar size and their low degree of solvation they have nearly equal electrophoretic mobility in many organic solvents [21,22]. Their electrophoretic mobilities are given in all solvents over a concentration range of the BGEs of practical interest. These tabulated values can be used to derive the mobility of the EOF from the measured total mobility of the reference ions.

2. Experimental

2.1. Instrumentation

For all experiments a ^{3D}CE capillary electrophoresis instrument (Hewlett-Packard, Waldbronn, Germany) equipped with both diode array and contactless conductivity detection systems [23] was used. For each particular solvent, individual fused-silica capillaries (Composite Metal Services, Hallow, UK) were used, with an I.D. of 50 μm, total length of 30.5 cm, and a length to contactless conductivity detector of 16.5 cm, and to the UV detector of 22.0 cm.

2.2. Chemicals

N,N-Dimethylformamide (99.8%) and *N,N*-dimethylacetamide (99.9%) were obtained from Aldrich, (Steinheim, Germany), propylene carbonate (99%) from Fluka (Buchs, Switzerland), methanol (LiChrosolv, 99.8%) from Merck (Darmstadt, Germany) and acetonitrile (HPLC Ultra Gradient Grade, 99.8%) from Mallinckrodt Baker (Deventer, The Netherlands).

Tetraethylammonium hydroxide (20%), acetic acid (glacial, 99.7%), salicylic acid (99.7%) and 2,6-dihydroxybenzoic acid (98%) were from Aldrich, dimethylsulfoxide (purum) from Loba (Fischamend, Austria) and anthracene (p.a) from Merck. Tetraethylammonium acetate, tetrahydrate (99%) was obtained from Fluka.

Tetraethylammonium salicylate and 2,6-dihydroxybenzoate were prepared as follows: the acid was titrated in methanol with tetraethylammonium hydroxide to neutralisation, the solvent was evaporated under vacuum and the residue dried by lyophilisation.

2.3. Procedures

The mobility of the EOF was determined as described in detail in Results and discussion in the BGE systems described in Table 1. For comparison the traditional method was carried out using a UV detector and a neutral marker (either anthracene or DMSO), which led to uncomfortably long measuring times of up to 30 min. The determination of the mobility of the reference ions was carried out independently in the usual way by the use of a neutral marker to correct for the EOF [27].

3. Results and discussion

3.1. Description of the measuring procedure

The detailed procedure for the determination of the EOF mobility is illustrated by Fig. 1. It has some similarity with the method of Williams and Vigh [18]. First the zone of the reference ions (Ph_4B^- and Ph_4P^+) is shifted by pressure to a certain position into the capillary. Then voltage is applied, leading to the migration of the ions towards their respective electrodes. After a certain time the voltage is switched off, a marker zone injected, and the zones are pressurised towards the detector and registered.

The computation of the velocity of electroosmotic flow can be performed simply, as only few parameters must be known: the duration time, $t_{p,1}$, of the pressurised shift of the initial sample zone, the residence times, $t_{Ph_4P^+}$ and $t_{Ph_4B^-}$, of the reference

Table 1
List of background electrolytes used in the different solvents; the BGEs consist of an equimolar mixture of acid and the respective salt

BGE abbreviation	Composition	pH[a]				
		DMF	DMA	PC	MeOH	ACN
2,6-B–TEA	2,6-Dihydroxybenzoic acid–TEA 2,6-dihydroxybenzoate	3.56	–	–	5.3	12.6
2-B–TEA	Salicylic acid–TEA salicylate	8.24	6.9	15.2	7.85	16.7
Ac–TEA	Acetic acid–TEA acetate	13.5	12.6	19.04	9.7	22.3
2,6-B–Na	2,6-Dihydroxybenzoic acid–sodium 2,6-dihydroxybenzoate	3.56	–	–	5.3	12.6
2-B–Na	Salicylic acid–sodium salicylate	8.24	6.9	15.2	7.85	16.7
Ac–Na	Acetic acid–sodium acetate	13.5	12.6	19.04	9.7	22.3

[a] $pH = pK_a$ values taken from Ref. [25], for acetic acid in PC from Ref. [26]; –, no value available.

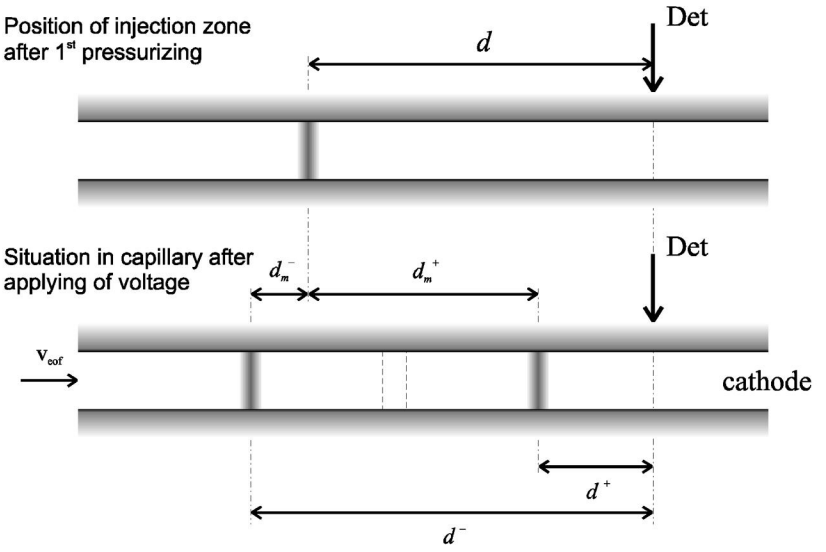

Fig. 1. Schematic drawing of situation in the capillary after positioning of the reference ion zone into the capillary by pressurising (above), and after application of voltage (below). For symbols see text. Det, position of detector.

ions (this is the time of their appearance in the detector measured upon application of the second mobilisation pressure); the residence time, $t_{p,2}$, of the marker zone upon pressurised mobilisation, and the mobilities, μ^+, μ^-, of Ph_4P^+ and Ph_4B^- in the BGEs.

The velocity of the laminar flow, v_{lam}, occurring upon application of mobilisation pressure is determined first. It is calculated from the residence time of marker zone, $t_{p,2}$, according to $v_{lam} = l_d/t_{p,2}$. l_d is the length of the capillary to the detector. Then, the distance, d, of the sample injection zone from the detector after the first application of pressure can be calculated from the pressurising time, $t_{p,1}$, as

$$d = l_d - v_{lam} t_{p,1} \quad (1)$$

where the value $t_{p,1}$ is based on an integration of the pressure record. Next, the distances d^+ and d^- are calculated from the residence times of Ph_4P^+ and Ph_4B^-, respectively. For instance, the distance d^+ is calculated according to $d^+ = v_{lam} t_{Ph_4P^+}$. The beforementioned distances can be derived (compare with Fig. 1) as

$$d^- = d + d_m^- \quad (2a)$$

$$d^+ = d - d_m^+ \quad (2b)$$

$$d_m^- = (v^- - v_{eof}) t_V \quad (3a)$$

$$d_m^+ = (v^+ + v_{eof}) t_V \quad (3b)$$

d^+, d^- are the distances of Ph_4P^+ and Ph_4B^- from the detector before the second mobilisation, d_m^+, d_m^- the distances of Ph_4P^+ and Ph_4B^- from the first injection zone, and t_V is the duration of applying voltage. v^+, v^- are the electrophoretic velocities of Ph_4P^+ and Ph_4B^-.

By combining Eqs. (3a) and (3b) the velocity of EOF is obtained as

$$v_{eof} = \frac{d_m^+ v^- - d_m^- v^+}{d_m^+ + d_m^-} \quad (4)$$

After division of both sides by the electric field strength the EOF mobility is

$$\mu_{eof} = \frac{d_m^+ \mu^- - d_m^- \mu^+}{d_m^+ + d_m^-} \quad (5)$$

For practical purposes, the distances in Eq. (5) are converted into the measured times giving

$$\mu_{eof} = \frac{\mu^- (t_{p,2} - t_{p,1} - t_{Ph_4P^+}) + \mu^+ (t_{p,2} - t_{p,1} - t_{Ph_4B^-})}{t_{Ph_4B^-} - t_{Ph_4P^+}} \quad (6)$$

It is seen that the electroosmotic flow mobility can be derived from the applied time, $t_{p,1}$, of pressurised shift of the initial sample zone into its position in the capillary, the time of hydrodynamic migration of the flow marker, $t_{p,2}$, and the time, $t_{Ph4P+}$and t_{Ph4B-}, after that the reference cation and anion are detected. Their mobilities, μ^+, μ^-, can be taken from tabulated values at all ionic strength of practical interest as detailed in the next paragraph.

3.2. Mobilities of tetraphenylphosphonium and tetraphenylborate

It was our first intention to use — for simplicity — reference ions with identical mobilities. Tetraphenylphosphonium and tetraphenylborate were candidate ions for this purpose, because they have nearly the same ionic size; in addition they are large and have only a low electrical charge density, so that they are supposed to be unsolvated in many solvents. This would make them suited as reference ions in the present method, as in the case of identical mobilities these values were not needed for the determination of the EOF mobility, which then reads

$$\mu_{eof} = \frac{l_t l_d (2t_{p,2} - 2t_{p,1} - t_{Ph_4P^+} - t_{Ph_4B^-})}{2Ut_{p,2}t_V} \quad (7)$$

where U is the applied voltage. However, although both ions have almost the same mobilities in some BGEs, in others they are different. Although such differences lead to a less simple algorithm for the calculation of the EOF mobility [see Eq. (6)], they do not hinder it as long as the mobilities are known under the experimental conditions.

We have thus determined the mobilities of Ph_4P^+ and Ph_4B^- in all solvents within the ionic strengths commonly used in CE, using an acetate buffer at seven concentrations between 5 and 50 m*M*. It is not the topic of the present work to relate the mobility to the ionic strength based on the theory of conductance. In contrary, we use only a fitting function here in order to enable the calculation of the mobility of both ions under all ionic strengths selected in practice. Thus we express the dependence of the mobility on the ionic strength, $\mu = \mu(I)$, by a polynomial according to

$$\mu = \sum_{k=0}^{n} b_k I^k \quad (8)$$

with $n \leq 3$, where b_k are the coefficients of the polynomial. Note that in Eq. (8) the coefficients b_k have no direct physicochemical meaning and are only fitting parameters.

Table 2 shows that the experimental data fit well to the polynomial: the coefficients of determination, r^2, are between 0.9797 and 0.9986. For an evaluation of the bias by calculating the reference ion mobilities by the aid of the parameters b_k (Table 2) at a certain ionic strength, e.g. at 10 m*M*, a comparison is made with those experimentally obtained. The resulting relative standard deviations for Ph_4P^+ and Ph_4B^-, respectively, are in ACN: −0.50, 0.00; in MeOH:

Table 2
Fitting b_k coefficients for the calculation of the mobilities of Ph_4P^+ and Ph_4B^- at any ionic strength between 5 and 50 m*M*; r^2 is the coefficient of determination; the data the fitting is based on were measured at seven ionic strengths in acetate buffer [27]

Solvent	Ion	b_0	b_1	b_2	b_3	r^2
DMF	Ph_4P^+	22.37	−196.8	1726	–	0.9932
	Ph_4B^-	25.36	−213.9	1832	–	0.9884
DMA	Ph_4P^+	18.69	−142.2	1195	–	0.9860
	Ph_4B^-	22.39	−224.1	2403	–	0.9673
PC	Ph_4P^+	8.289	−26.85	222.0	–	0.9957
	Ph_4B^-	8.723	−55.92	1125	−9018	0.9994
MeOH	Ph_4P^+	35.03	−191.4	1939	–	0.9669
	Ph_4B^-	34.59	−107.7	–	–	0.9489
ACN	Ph_4P^+	51.88	−409.4	3599	–	0.9939
	Ph_4B^-	55.10	−769.4	17 140	−152 500	0.9986

−1.5, −2.4; in DMF: −0.07, −1.3; in DMA: 0.33, −2.2; and in PC: 0.09, 0.03%.

3.3. EOF mobility obtained with the dual-ion and the common method

It was pointed out in the Introduction that problems might arise when the solvent peak method is used for the determination of the EOF mobility from the recorded solvent jump. Examples for such problems are given in Fig. 2A for conductivity detection. A series of maxima and minima in the record are seen, and it is thus hard to decide which peak or dip in the baseline is indicative for the EOF. In order to get a better insight into the correct position of the EOF, a neutral marker component was taken, and the signals were recorded by UV detection in addition to conductivity detection, see Fig. 2B. The signal of the latter detector does not allow the unambiguous identification of the neutral marker zone. It can be seen from Fig. 2B that the migration time of the neutral marker (shifted solely by the EOF) and the solvent dip differ significantly, by nearly 1.2 min at a migration time of 12 min. The slower migration of the solvent zone most probably stems from the additional movement of the concentration boundaries formed between sample zone and BGE, and it is directed against the EOF. This difference means that more than 10% systematic deviation from the true EOF mobility occurs when the solvent peak is taken as indicator.

Note that the use of the neutral marker zone might be erroneous also, namely when the marker zone and the solvent zone are not so clearly separated as in the example given. In that case the superimposed signals from the two zones might not enable the determi-

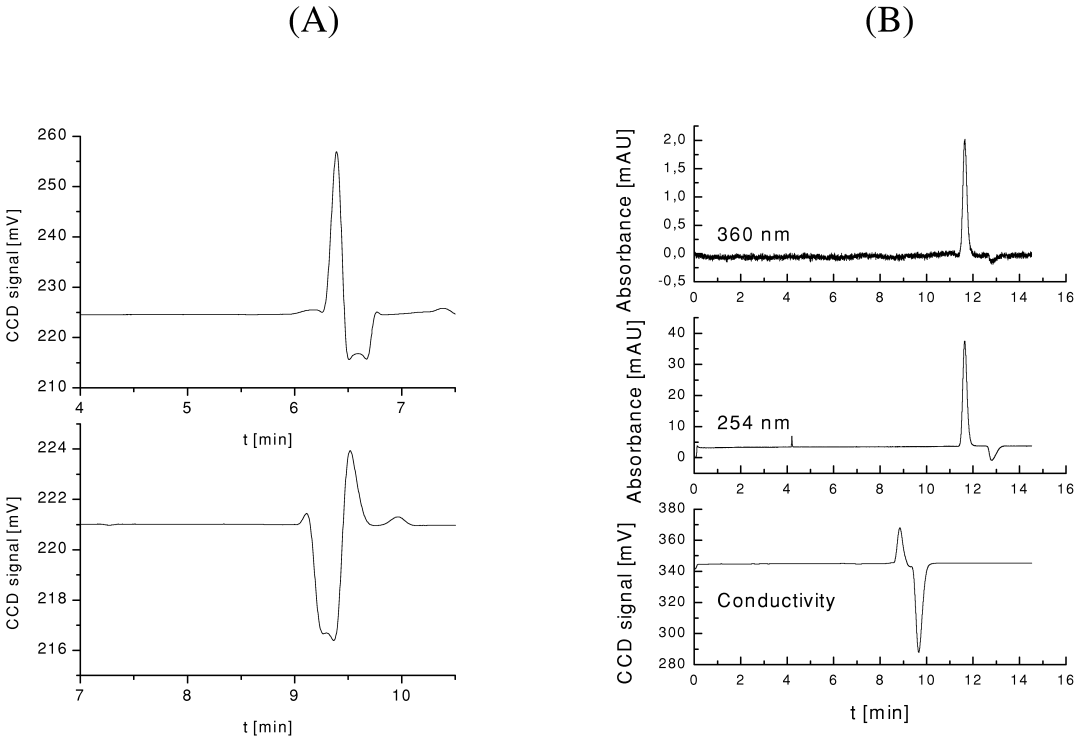

Fig. 2. Examples for the records of the sample zone with conductivity and UV detection in the different BGEs. (A) Top: 0.02 *M* Ac–TEA in DMA, solvent injection with 20 mbar s; driving voltage +10 kV, current ~3.9 μA. Bottom: 0.04 *M* 2-B–TEA in PC, solvent injection with 100 mbar s; driving voltage +8 kV, current ~2.6 μA. (B) 0.08 *M* Ac–TEA in DMF, $2 \cdot 10^{-4}$ *M* anthracene injected with 20 mbar s; driving voltage +10 kV, current ~14.7 μA.

nation of the position of the marker zone unambiguously. Clearly, the present method avoids such biasing records.

The EOF was determined in the different organic solvents with three different BGEs at a certain ionic strength. A typical electropherogram recorded by the conductivity detector with DMF as solvent is shown in Fig. 3. The residence times of Ph_4P^+ and Ph_4B^- together with that of the marker zone upon second pressurisation can be unequivocally identified. Note that the residence time of the sample injection zone is not taken from the electropherogram, as its initial position inside the capillary might have changed during application of voltage. The method is also applicable for BGEs in which the UV detector can be used. This is due to the suitable optical properties of the reference ions. A typical example is shown in Fig. 4 with MeOH as solvent. Note that also in this case the peak of a neutral marker added to the sample, which might be impaired by the superimposed solvent dip, is not used to localise the initial position of the sample after pressurised shift into the capillary.

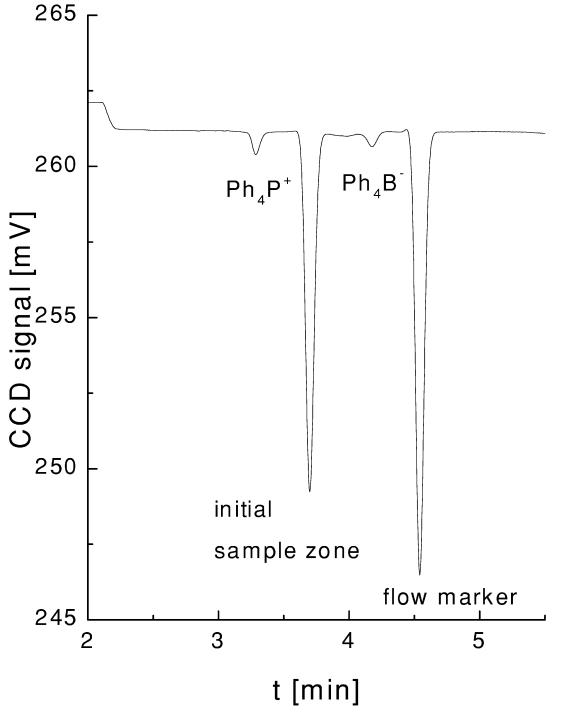

Fig. 3. Example of determination of EOF mobility in DMF with the dual-ion method by conductivity detection. BGE: 2,6-B–TEA (0.02 *M*).

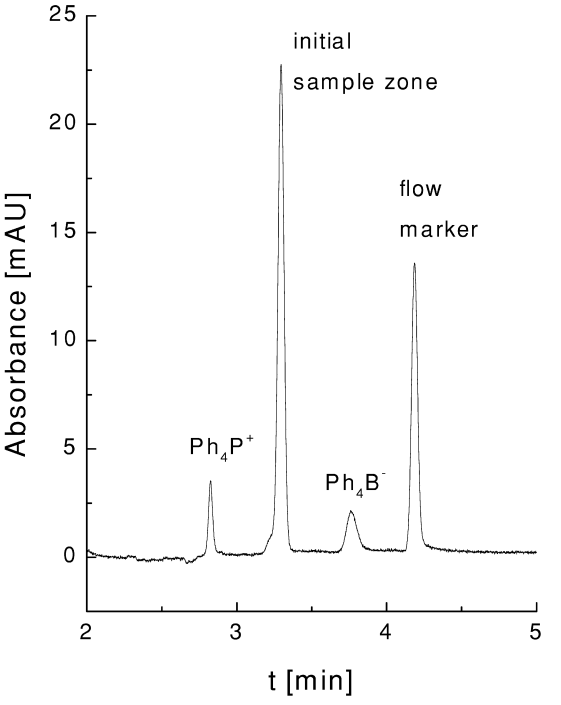

Fig. 4. Example of determination of EOF mobility in MeOH with the dual-ion method by UV detection. BGE: Ac–Na (0.02 *M*).

The electroosmotic mobilities in the different systems and their standard deviations obtained for six consecutive runs are given in Table 3. The mobilities are in the range between $-3\cdot10^{-9}$ and $+53\cdot10^{-9}$ $m^2\ V^{-1}\ s^{-1}$. From the set of fifteen BGEs the precision of the common method is higher in five cases. In the other ten BGEs, no significant difference in precision is found on the 95% confidence level. The slightly lower precision of the present method in the five cases seems to be caused by error propagation on the one hand [note that the EOF mobility with the dual-ion method is the result of the subtraction of two measuring values, see Eq. (4)]. On the other hand, the hydrodynamic flow may vary slightly upon application of pressure at the injection side of the capillary, as it is kept within a wider range (which could be narrowed by external manipulations [24]) than the voltage. Using the true

Table 3
Mobilities of the electroosmotic flow in the organic solvents in different BGEs, obtained with the common and dual-ion method. The mobilities are given in 10^{-9} m^2 V^{-1} s^{-1}. Confidence intervals were calculated from results of six consecutive injections

Solvent	BGE	Conc. (*M*)	μ_{EOF}		SD[a]	Mean[a]	%[b]
			Dual-ion	Common			
DMF	2,6-B–TEA	0.02	3.22±0.33	2.06±0.07	Yes	Yes	36
	2-B–TEA	0.02	14.83±0.29	12.51±0.09	Yes	Yes	16
	Ac–TEA	0.02	19.91±0.08	19.78±0.14	No	No	
DMA	2,6-B–TEA	0.02	−3.28±0.11	−3.04±0.11	No	Yes	7
	2-B–TEA	0.02	9.90±0.46	8.37±0.21	No	Yes	15
	Ac–TEA	0.02	11.27±0.35	11.42±0.09	Yes	No	
PC	2,6-B–TEA	0.04	8.16±0.12	7.58±0.08	No	Yes	7
	2-B–TEA	0.04	12.13±0.19	11.36±0.12	No	Yes	6
	Ac–TEA	0.04	13.89±0.40	13.60±0.09	Yes	No	
MeOH	2,6-B–Na	0.02	6.75±0.19	5.67±0.08	No	Yes	16
	2-B–Na	0.02	11.02±0.27	10.60±0.49	No	No	
	Ac–Na	0.02	16.14±1.02	15.77±0.45	No	No	
ACN	2,6-B–TEA	0.02	44.01±0.42	42.44±0.18	No	Yes	4
	2-B–TEA	0.02	53.13±0.53	51.16±0.30	No	Yes	4
	Ac–TEA	0.02	53.07±0.71	53.30±0.12	Yes	No	

[a] Statistically significant difference between the standard deviations or means of the dual-ion and the common method at the 95% confidence level.
[b] $100\times[\mu_{EOF}$ (dual-ion)$-\mu_{EOF}$ (common)$]/\mu_{EOF}$ (dual-ion).

integrated value of the pressure would result in a more reproducible value.

Comparison of the mean values of the EOF mobilities determined by the two different methods leads to the result that in nine out of fifteen systems the mean values differ significantly on the 95% confidence level. In all nine cases the dual-ion method gives higher mobilities of the EOF.

3.4. Electroosmotic flow in different solvents

The present method has been used to determine the EOF in the different organic solvents with acetate buffer at ionic strength between 5 and 50 m*M*. Note that the pH* of the BGEs in the different solvents differ, but in all buffers an excess of acetic acid is present (they are in an ''acidic'' range, whatever this term means in non-aqueous solvents). The results are depicted in Fig. 5. The mobilities of the EOF decrease with increasing ionic strength, as expected. The highest mobility is found for ACN, seemingly a consequence of the low viscosity, η. It is clear that the solvent viscosity is not the only property that is responsible for the magnitude of the EOF at a given ionic strength. The inadequate relation of the EOF mobility on the solvent viscosity is reflected by the very similar mobilities in PC, MeOH and DMF, although the viscosities are 2.51, 0.545 and 0.802 cP. According to the Smoluchowski relation it is rather the ratio ϵ/η, where ϵ is the dielectric constant, which is decisive for the EOF mobility. However,

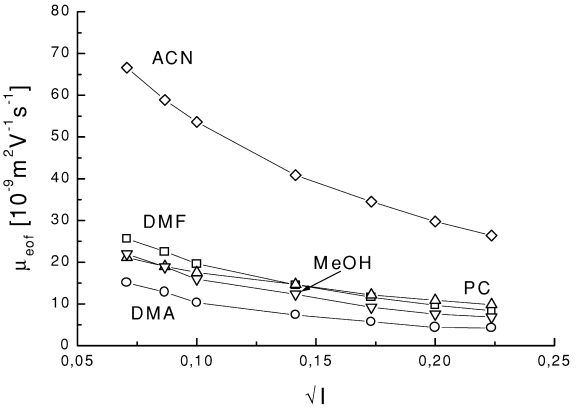

Fig. 5. Dependence of the electroosmotic flow mobility, μ_{eof}, on the square root of the ionic strength, *I*, of the BGE in organic solvents. BGEs, acetic acid–TEA acetate.

these ratios do not reflect the resemblance of the mobilities in these three solvents: with values of 26.3, 60.0 and 45.8, respectively, they are still far from being similar.

At higher ionic strength the mobilities reach values at a few units only. Even in these cases the measurement with the dual-ion method needed only a few minutes (this value depends on v_{lam} that is proportional to the applied pressure), which is in striking contrast to the common method. With the latter, the determination of the migration time of the neutral marker zone under normal conditions (e.g. voltage 10 kV, length of capillary to around 30 cm, mobility of the EOF of $3 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}) would need more than ten times longer.

4. Concluding remarks

The present dual-ion technique for the determination of the EOF mobility is a fast method when the common methods using the solvent dip or a neutral marker peak are only erroneously applicable or take an unacceptable long time (e.g. when the EOF is very slow, e.g. at low pH of the BGE, or when coated capillaries are applied). It is better suited than the common methods e.g. when a conductivity detector is used, although there is no principle limitation concerning the type of the solvents or BGE, or the detector. It is more correct in many practical cases than the common method because it circumvents the uncertainties connected with concentration boundaries. It is based on the accurate determination of the residence times of Ph_4P^+ and Ph_4B^- instead of the non-accurate determination of migration time of the solvent peak or solvent zone, which is often very broad and accompanied by system peaks, that hinder the clear denotation of the migration time of EOF. Even where only a single marker peak appears it is unclear which part of the often irregular record should be taken (cf. Fig. 2).

There are, however, some limitations to the dual-ion method that must be taken into account. One is the stability of the laminar flow, which is influenced by a number of factors, e.g. (i) pressure stability, (ii) height of liquid in vials, that could result in an additional hydrodynamic flow, (iii) capillary properties changing over the period of the experiments (e.g. partial clogging by sample or BGE constituents). A careful selection and control of the experimental conditions could overcome these limitations. A second limitation might be the poor solubility of $Ph_4P^+Ph_4B^-$ in some solvents.

Apparently the most relevant limitation in the context of organic solvents might be ion pair formation, especially in solvents with low dielectric constant. As ion pair formation depends on the type of the counterion, it could occur to different extend in the different BGEs. Thus it is hardly possible to quantify this effect in advance for the appropriate selection of the mobility data needed to calculate the EOF mobility according to Eq. (6).

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