

Determination of electroosmotic flow in nonaqueous capillary electrophoresis

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Abstract

Mobility of the electroosmotic flow (μ_{EOF}) in fused-silica capillaries strongly depends on the nature of the background electrolyte. In this study, 27 solvent systems were investigated, namely water, methanol, ethanol, 2-propanol, 1-butanol, acetonitrile (MeCN), formamide, *N*-methylformamide (NMF), *N,N*-dimethylformamide and dimethyl sulfoxide, as well as 8 hydroorganic and 9 organic mixtures. For each system, six μ_{EOF} were determined at a different ionic strength in basic conditions, and an absolute electroosmotic flow mobility ($\mu_{\text{EOF},0}$) was extrapolated according to the Debye–Hückel Onsager model. The obtained $\mu_{\text{EOF},0}$ values were correlated with the solvent's relative permittivity (ϵ) and viscosity (η). A good correlation ($r^2 = 0.867$) between $\mu_{\text{EOF},0}$ and the solvent's ϵ/η ratio was demonstrated, except for two solvents (MeCN and NMF). Furthermore, the donor number (DN) of a solvent took into account the possible zeta potential modification in the electric double layer near the capillary wall. Consequently, the relationship between $\mu_{\text{EOF},0}$ and $\epsilon/(\eta \times \text{DN})$ was superior, with a r^2 of 0.943 for 10 pure solvents.

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1. Introduction

Capillary zone electrophoresis (CZE) using either nonaqueous or hydroorganic background electrolytes (BGEs) is an interesting alternative to usual CZE performed in aqueous media. The main reasons for this success are different selectivity as well as better solubility and stability of compounds in organic solvent than in water. Several reviews provide comprehensive overviews of the status of nonaqueous capillary electrophoresis (NACE) and cover main application in this field [1–4]. Many NACE applications have been reported in pure acetonitrile (MeCN), lower alcohols [e.g. methanol (MeOH), ethanol (EtOH)] as well as in mixture. Furthermore, many solvent systems can be used as BGE for CZE, as long as they fulfill different criteria [1]: (i) suitable liquid range, (ii) solubilization of components, (iii) chemical stability, (iv) not too high volatility, (v) compatibility with instrumental demands, (vi) availability at reasonable cost and

(vii) sufficient relative permittivity (ϵ) and (viii) relatively low viscosity (η). The two latter are closely related to the intensity of the electroosmotic flow (EOF) and thus to the analysis time. Indeed, the effect of ϵ and η on EOF mobility (μ_{EOF}) can be described as [5]:

$$\mu_{\text{EOF}} = - \frac{\epsilon_0 \epsilon_r \zeta_{\text{wall}}}{\eta} \quad (1)$$

where ζ_{wall} is the zeta potential of the capillary wall, ϵ_0 is the permittivity of vacuum, ϵ_r and η are the solvent's dielectric constant and viscosity, respectively. Considering this equation, μ_{EOF} of two solvent systems can be compared by taking into account their ϵ/η ratio, ϵ being solvent's relative permittivity ($\epsilon = \epsilon_0 \epsilon_r$). However, other parameters have to be considered for μ_{EOF} estimation [1]. In fact, ζ_{wall} may also differ from one solvent system to another, because of different dissociation constants of the silanol groups. Moreover, Eq. (1) considers that ϵ and η values are identical near the wall as they are in a bulk solution. However, these values may diverge due to the orientation of solvent molecules in the vicinity of

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the capillary wall. For example, ε was reported to be lower in the electrical double layer than in the bulk solution [5,6].

In addition to previous theoretical limitations, practical difficulties occur when μ_{EOF} is compared with ε/η ratio of pure solvents. While EOF has been reported to be performed in pure solvent without addition of ionic species [7,8], results depend on factors barely under control. As pointed out by the authors [7,8], the pH may fluctuate from one solvent to another and ionic impurities in “pure” solvents might generate or affect the EOF. Some studies avoid these problems by using BGEs containing similar salt concentrations. However, these salts reduce mobility compared to pure solvent.

In fact, the actual mobility of an ion (μ_i) is maximal at zero ionic strength (absolute mobility, $\mu_{i,0}$). For a 1:1 electrolyte, this mobility can be expressed by the Falkenhagen and Pitts (FP) model [9,10], as:

$$\mu_i = \mu_{i,0} - \left(\frac{8.20 \times 10^5 \mu_{i,0}}{(\varepsilon T)^{3/2}} + \frac{42.75}{\eta \sqrt{\varepsilon T}} \right) \times \left(\frac{\sqrt{I}}{1 + 50.29a\sqrt{I/\varepsilon T}} \right) \quad (2)$$

where $8.20 \times 10^5/(\varepsilon T)^{3/2}$ and $42.75/\eta(\varepsilon T)^{1/2}$ describe relaxation and electrophoretic effects, respectively. For unit consistency, μ_i and $\mu_{i,0}$ are in $10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$, T in $^\circ\text{K}$, η in Pas and ε is dimensionless. The term a (in \AA) is the distance of the closest approach between ion i and its counter-ion. The ionic strength (mol L^{-1}) depends on the concentration (c_i , in mol L^{-1}) and charge (z_i) of ions in BGE. It is calculated as:

$$I = 0.5 \sum_i z_i^2 c_i \quad (3)$$

In theory, measurements of μ_i at different electrolyte concentrations should allow to determine $\mu_{i,0}$, whatever the concentration. However, in order to achieve good $\mu_{i,0}$ estimation, it is suitable to determine μ_i at low electrolyte concentrations, where the response function is almost linear. Actually, for low ionic strength, the last term denominator between brackets is

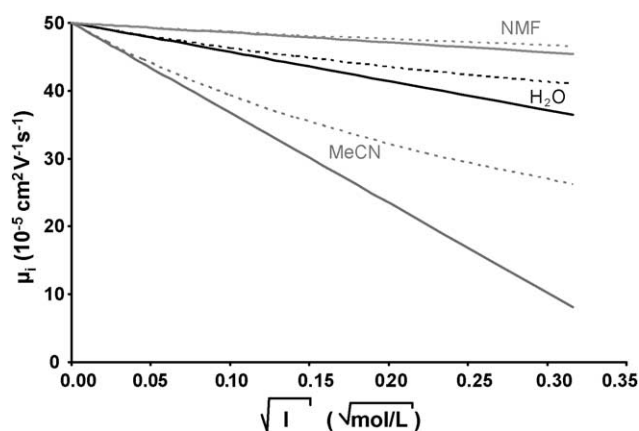


Fig. 1. Theoretical correlation of actual mobility with ionic strength square root in water, MeCN and NMF at 25 $^\circ\text{C}$. Absolute mobility ($\mu_{i,0}$) is considered similar in all solvents, with a $50 \times 10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ value. Broken lines are drawn according to FP model (Eq. (2)) with $a = 5 \text{ \AA}$, and solid lines according to DHO model (Eq. (4)).

close to unit and Eq. (2) is simplified into Eq. (4). Likewise, mobility decreases linearly with \sqrt{I} if ions are considered as point charges ($a = 0$). Hence, a simplified model can be used, the so-called Debye–Huckel Onsager (DHO) [11,12] limiting slope:

$$\mu_i = \mu_{i,0} - \left[\frac{8.20 \times 10^5 \mu_{i,0}}{(\varepsilon T)^{3/2}} + \frac{42.75}{\eta \sqrt{\varepsilon T}} \right] \sqrt{I} \quad (4)$$

Fig. 1 illustrates the theoretical relationship between μ_i and \sqrt{I} for a single charged ion in water, acetonitrile (MeCN) and *N*-methylformamide (NMF). Relative permittivity and viscosity values were taken from Table 1 [13], and the absolute mobility of an ion was estimated similar in all three solvents, $\mu_{i,0} = 50 \times 10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$. The term a was estimated at 5 \AA in Eq. (2) and neglected in Eq. (4). As exhibited, the nature of the solvent had a great influence on the response function. In fact, the slope was less significant for a solvent possessing a high ε value (NMF) than for a solvent with a low ε value (MeCN).

Table 1
Physico-chemical properties of pure selected solvents [13]

Solvent	Abbreviation	Boiling point ($^\circ\text{C}$)	Dipole moment (Debye)	Relative permittivity	Viscosity (mPas)	$\text{p}K_{\text{auto}}$	Acceptor number	Donor number (kJ mol^{-1})
Water	H ₂ O	100.0	1.85	78.36	0.8903	14.0	54.8	138
Methanol	MeOH	64.5	2.87	32.66	0.551	16.91	41.3	126
Ethanol	EtOH	78.2	1.66	24.55	1.083	19.1	37.1	134
2-Propanol	2-PrOH	82.2	1.66	19.92	2.044	21.08	33.5	151
1-Butanol	1-BuOH	117.6	1.75	17.51	2.571	20.89	36.8	126
Acetonitrile	MeCN	81.6	3.92	35.94	0.341	32.2	18.9	59
Formamide	FA	210.5	3.37	109.5	3.302	16.8	39.8	151
<i>N</i> -Methylformamide	NMF	199.5	3.86	182.4	1.65	10.74	32.1	205
<i>N,N</i> -Dimethylformamide	DMF	153.0	3.82	36.71	0.802	23.1	16.0	111
Dimethyl sulfoxide	DMSO	189.0	4.06	46.45	1.991	31.8	19.3	121

Acceptor and donor numbers were from refs. [14,15].

In this paper, 27 solvent systems with known ϵ and η values were used in untreated fused-silica capillaries. For each system, μ_{EOF} was determined in six BGEs with different ionic strengths, and the absolute electroosmotic flow mobility ($\mu_{\text{EOF},0}$) was extrapolated according to the DHO model (Eq. (4)). The latter should be appropriate for the determination of μ_{EOF} , since the term a is not significant for neutral molecules. This procedure was performed with two electrolytes (formate/ammonia and acetate/ammonia), and results were compared with those obtained by other authors [7,8] in pure solvents. Next, these $\mu_{\text{EOF},0}$ values were correlated to ϵ/η ratios of the different solvent systems. Finally, an estimation of ζ_{wall} variations was examined, considering the donor number of investigated solvents.

2. Materials and methods

2.1. Chemicals

Analytical reagent grade sodium hydroxide, ammonium formate, ammonium acetate, EtOH, 1-BuOH, FA, NMF and DMF were obtained from Fluka (Buchs, Switzerland), DMSO was obtained from Merck (Darmstadt, Germany). Ammonia solution 30% for analysis was from Carlo Erba (Rodano, Italy). HPLC-grade MeOH, MeCN and 2-PrOH were supplied by Romil (Kölliken, Switzerland). Ultra-pure water was supplied by a Milli-Q RG purification unit from Millipore (Bedford, MA, USA).

2.2. Electrolyte preparation

All the investigated solvents and solvent mixtures contained ammonia with either formate or acetate ammonium. The same ammonium formate–ammonia and ammonium acetate–ammonia ratios of 100:38 were selected to obtain a pH of about 9.3 in water. Electrolytes with different salt concentrations were prepared: 0.5, 1, 2, 3, 4 and 5 mM of either ammonium formate or ammonium acetate. For sake of clarity, ammonia concentration is not specified and always adjusted to attain the stated ratio (concentration between 0.19 and 1.9 mM).

Acetone was used to determine the electroosmotic flow. Different 5:95 acetone:solvent (v/v) solutions were prepared by dissolution in the pure investigated solvent (or solvent mixture).

2.3. Instrumentation

Experiments were performed using a HP^{3D}CE system (Agilent Technologies, Waldbronn, Germany) equipped with an on-column diode-array detector, an autosampler and a power supply able to deliver up to 30 kV. A CE Chemstation (Agilent Technologies) was used for CE control, data acquisition and data handling. Separation was performed in a fused silica capillary (Polymicro, Phoenix, AZ, USA)

with an inner diameter of 50 μm and 64.5 cm total length (56 cm to the UV detector). All experiments were carried out using the cationic mode (anode at the inlet and cathode at the outlet). A constant voltage of 30 kV, with an initial ramping of 2.5 kV s⁻¹, was applied during analysis. The capillary was thermostated at 25 °C. Samples were kept at ambient temperature in the autosampler and 1% of the total capillary length was injected by applying a pressure of 50 mbar for a few seconds, depending on solvent viscosity. UV detection was carried out at 270 nm with a bandwidth (bw) of 10 nm (reference at 350 nm, bw 40 nm).

Before its first use, the fused silica capillary was sequentially washed with MeOH, 0.1 M NaOH, water and BGE for 5 min each. Because of the possibility of memory effect, a new capillary was used for each investigated solvent. Between analyses, the capillary was flushed with the electrolyte for 5 min. At least two runs were performed for each condition to determine EOF intensity.

3. Results and discussion

3.1. Physico-chemical properties of investigated solvents

Water and nine organic solvents were investigated at first. Table 1 exhibits some of their physico-chemical properties, namely boiling point, dipole moment, relative permittivity, viscosity, autoprotolysis constant ($\text{p}K_{\text{auto}}$), acceptor number (AN) and donor number (DN). A solvent's autoprotolysis constant gives information about both accepting and donating a proton. Amphiprotic solvents possess low $\text{p}K_{\text{auto}}$ values and are good proton acceptor and donor, while aprotic solvents are either bad proton acceptor or donor. Thus, water, MeOH, FA, NMF, EtOH, 2-PrOH and 1-BuOH can be classified as amphiprotic solvents, and MeCN, DMSO and DMF as aprotic solvents. Furthermore, acceptor and donor numbers (AN and DN) were introduced by Gutmann [14] to describe the acidic and basic strength of a solvent. A solvent with a high DN possesses a strong basic strength. DN is a quantitative measure for a solvent's ability to donate electrons, i.e. to bind a proton. DNs were first determined from the formation enthalpy of SbCl_5 complexes in dilute dichloroethane solutions. Later, DN values of some solvents (e.g. water, NMF) were indirectly measured in bulk solutions to take into account their intermolecular interactions. More details about this topic are given in different articles [4,14,15]. AN is the solvent's quantitative measure to accept electrons.

Beside the 10 pure solvent systems, 17 solvent combinations with known ϵ and η were investigated: eight hydroorganic and nine organic mixtures (Table 2). It can be noted that donor numbers of solvent mixtures were not found in the literature. In order to estimate these values, a linear relationship was used with the proportion of each solvent, which is probably an oversimplification.

Table 2
Physico-chemical properties of studied solvent systems from references [8,18,19]

	Relative permittivity	Viscosity (mPas)	Estimated donor number (kJ mol ⁻¹)
Water–MeOH 20:80	42.8	1.01	128
Water–MeOH 40:60	51.8	1.40	131
Water–MeOH 60:40	61.1	1.42	133
Water–MeOH 80:20	70.0	1.26	136
Water–MeCN 80:20	67.5	0.91	122
Water–MeCN 60:40	56.0	0.98	106
Water–MeCN 40:60	46.5	0.76	90.6
Water–MeCN 20:80	41.0	0.58	74.8
MeCN–MeOH 20:80	33.9	0.440	113
MeCN–MeOH 61:39	35.3	0.337	85.8
MeCN–MeOH 76:24	35.6	0.328	75.8
DMF–MeOH 88:12	37.8	0.766	113
DMF–MeOH 59:41	38.3	0.693	117
DMF–MeOH 21:79	36.4	0.596	123
MeCN–DMSO 95:5	36.6	0.376	62.1
MeCN–DMSO 61:39	40.3	0.663	83.2
MeCN–DMSO 17:83	44.9	1.493	110

Donor numbers were estimated according to solvent's proportion.

Table 3
Slopes, intercepts and determination coefficients (r^2) calculated from DHO model (Eq. (1)) for ten solvents with different ionic strength

	Formate			Acetate		
	Slope (10 ⁻⁴)	Intercept $\mu_{\text{EOF},0}$ (10 ⁻⁵)	r^2	Slope (10 ⁻⁴)	Intercept $\mu_{\text{EOF},0}$ (10 ⁻⁵)	r^2
Water	-40.0 ± 8.0	109.5 ± 3.7	0.9251	-29.7 ± 2.3	103.8 ± 1.1	0.9880
MeOH	-40.5 ± 2.1	44.8 ± 1.0	0.9945	-36.8 ± 4.0	41.0 ± 1.9	0.9769
EtOH	-15.5 ± 2.2	14.6 ± 1.0	0.9608	-13.6 ± 2.8	14.3 ± 1.3	0.9217
2-PrOH	-4.9 ± 0.7	5.2 ± 0.3	0.9586	-5.8 ± 1.3	6.8 ± 0.6	0.9149
1-BuOH	-3.8 ± 0.9	3.7 ± 0.4	0.8994	-3.1 ± 0.5	3.9 ± 0.2	0.9486
MeCN	-231.4 ± 12.9	198.4 ± 4.7	0.9883	-96.8 ± 19.7	189.6 ± 9.2	0.9230
FA	-15.1 ± 1.2	22.5 ± 0.6	0.9878	-21.4 ± 1.4	30.8 ± 0.7	0.9910
NMF	-10.1 ± 0.7	50.4 ± 0.3	0.9899	-10.5 ± 0.3	50.7 ± 0.1	0.9987
DMF	-28.8 ± 2.9	53.4 ± 1.3	0.9806	-23.6 ± 1.4	64.7 ± 0.6	0.9933
DMSO	-11.3 ± 1.0	24.5 ± 0.5	0.9877	-9.5 ± 2.4	29.7 ± 1.2	0.9146

Intercepts correspond to the estimated μ_{EOF} at zero ionic strength (infinite dilution).

3.2. Determination of absolute electroosmotic flow mobility

For each solvent listed in Table 1, six different salt concentrations were prepared with ammonium formate–ammonia mixtures, as with ammonium acetate–ammonia. As discussed previously, low ionic strength concentrations were selected to prevent important deviation from the DHO model (Eq.

(4)). Moreover, diluted BGEs are almost not affected by ion-pairing effects. The latter can be significant for solvent systems with ϵ inferior to water, since formate/ammonia and acetate/ammonia ion-pairing can take place in some BGEs [16].

The ionic strength was calculated according to Eq. (3). In experimental conditions, formate and acetate were considered as fully anionic, and ammonia ($\text{p}K_{\text{a}}$ in water at

Table 4
Slopes, intercepts and r^2 determined with a similar procedure (see Table 2) for eight hydro-organic mixtures

	Formate			Acetate		
	Slope (10 ⁻⁴)	Intercept $\mu_{\text{EOF},0}$ (10 ⁻⁵)	r^2	Slope (10 ⁻⁴)	Intercept $\mu_{\text{EOF},0}$ (10 ⁻⁵)	r^2
Water–MeOH 20:80	-24.0 ± 1.5	36.7 ± 0.7	0.9923	-23.7 ± 1.0	36.5 ± 0.5	0.9964
Water–MeOH 40:60	-23.3 ± 1.52	38.7 ± 0.7	0.9915	-21.0 ± 0.8	38.3 ± 0.4	0.9969
Water–MeOH 60:40	-21.1 ± 0.9	45.7 ± 0.4	0.9967	-20.3 ± 1.4	45.4 ± 0.7	0.9902
Water–MeOH 80:20	-23.8 ± 4.8	64.5 ± 2.2	0.9254	-24.0 ± 1.5	64.3 ± 0.7	0.9921
Water–MeCN 80:20	-25.4 ± 0.9	83.9 ± 0.4	0.9975	-23.0 ± 4.8	82.5 ± 2.3	0.9183
Water–MeCN 60:40	-34.5 ± 1.53	79.7 ± 0.7	0.9960	-34.2 ± 1.2	80.1 ± 0.6	0.9976
Water–MeCN 40:60	-43.4 ± 1.75	78.0 ± 0.8	0.9967	-43.3 ± 2.6	78.0 ± 1.2	0.9930
Water–MeCN 20:80	-54.7 ± 2.5	77.5 ± 1.3	0.9968	-58.5 ± 2.2	81.4 ± 1.0	0.9971

Table 5
Slopes, intercepts and r^2 determined with a similar procedure (see Table 2) for nine nonaqueous mixtures

	Formate			Acetate		
	Slope (10^{-4})	Intercept $\mu_{\text{EOF},0}$ (10^{-5})	r^2	Slope (10^{-4})	Intercept $\mu_{\text{EOF},0}$ (10^{-5})	r^2
DMF–MeOH 88:12	-29.8 ± 4.0	47.5 ± 1.9	0.9650	-25.5 ± 1.3	54.3 ± 0.6	0.9959
DMF–MeOH 59:41	-36.5 ± 2.2	51.7 ± 1.0	0.9924	-31.6 ± 4.6	53.4 ± 2.1	0.9597
DMF–MeOH 21:79	-35.3 ± 1.2	50.2 ± 0.6	0.9977	-34.6 ± 2.3	52.0 ± 1.1	0.9911
MeCN–MeOH 20:80	-48.9 ± 3.1	66.1 ± 1.4	0.9921	-48.5 ± 2.5	67.9 ± 1.2	0.9947
MeCN–MeOH 61:39	-70.0 ± 10.2	98.6 ± 4.8	0.9589	-71.9 ± 7.8	109.1 ± 3.6	0.9771
MeCN–MeOH 76:24	-75.4 ± 11.3	110.5 ± 5.3	0.9565	-80.4 ± 9.0	127.5 ± 4.2	0.9755
MeCN–DMSO 95:5	-82.7 ± 2.7	126.4 ± 1.3	0.9979	-58.1 ± 1.5	144.2 ± 0.8	0.9993
MeCN–DMSO 61:39	-27.0 ± 3.2	71.0 ± 1.5	0.9718	-55.1 ± 22.2	102.0 ± 11.2	0.9221
MeCN–DMSO 17:83	-12.5 ± 1.1	34.4 ± 0.5	0.9850	-9.1 ± 2.4	42.0 ± 1.3	0.9351

25 °C = 9.24) as partially protonated (50%) whatever its concentration and the tested solvent. In water, this estimation is in close agreement with the measured pH, between 9.0 and 9.3 for the lowest and highest investigated salt concentrations. Since pK_a values of ammonia were seldom available in the investigated solvents, the same approximation was employed for other solvents. Hence, ionic strengths were similar in all solvents, comprised between 4.2×10^{-4} and 4.2×10^{-3} M for the investigated concentrations (from 0.5 mM up to 5 mM ammonium formate or acetate).

Considering the simplified DHO model, a regression line was drawn for investigated solvents with formate and acetate BGEs. Slopes, intercepts (with their 95% confidence interval) and determination coefficient are reported in Table 3. The same process was applied for the eight hydroorganic and nine organic mixtures listed in Table 2, and regression line data are reported in Tables 4 and 5, respectively. Results obtained in MeCN, water:MeCN 40:60, DMF:MeOH 59:41 and 1-BuOH are reported in Fig. 2, showing that linear regression was an appropriate model for the investigated conditions. This is corroborated by determination coefficients superior to 0.90 for all responses.

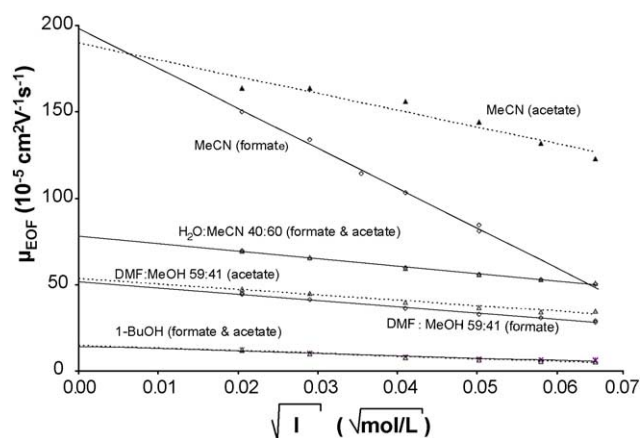


Fig. 2. Practical relationship of μ_{EOF} with the square root of six different ionic strengths in MeCN, water:MeCN 40:60, DMF:MeOH 59:41 and 1-BuOH. Solid lines are drawn for formate, broken lines for acetate.

With the exception of MeCN, profiles were similar with formate and acetate BGEs (Fig. 2 and Tables 3–5). The discrepancy with MeCN may be related to ion-pairing effects, more inclined to occur in such an aprotic solvent. Appar-

Table 6
Comparison of ζ values obtained with $\mu_{\text{EOF},0}$ determined by a DHO model (extrapolation), and ζ values in pure solvents [7,8]

	ζ Values from DHO model (formate and acetate) (mV)	ζ Values from pure solvents [7] (mV)	ζ Values from pure solvents [8] (mV)
Water	-117/-125	-98	-97/-108
MeOH	-69/-76	-84	-108/-127
EtOH	-62/-65	-73	-
MeCN	-189/-199	-195	-207/-226
FA	-67/-93	-46	-96
NMF	-45/-46	-52	-
DMF	-116/-142	-144	-86
DMSO	-104/-128	-155	-85
Water–MeOH 80:20	-115/-117	-118/-120	-122
Water–MeOH 60:40	-105/-107	-123/-125	-117
Water–MeOH 40:60	-103/-105	-115/-117	-103
Water–MeOH 20:80	-86/-87	-111/-113	-92
Water–MeCN 80:20	-111/-114	-	-101
Water–MeCN 60:40	-139/-141	-	-134
Water–MeCN 40:60	-127/-128	-	-142
Water–MeCN 20:80	-109/-116	-	-115

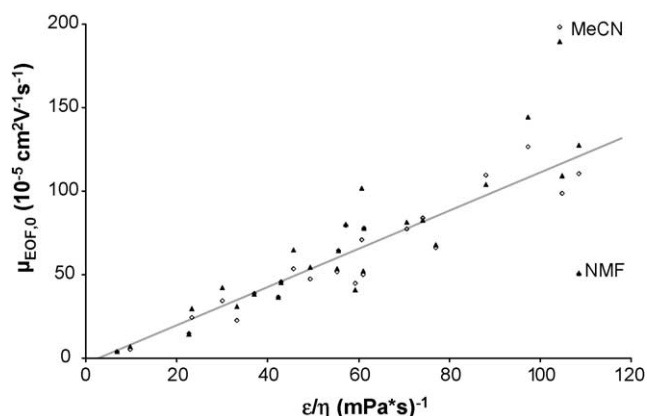


Fig. 3. Correlation of $\mu_{\text{EOF},0}$ with ε/η of solvent systems. (\diamond) $\mu_{\text{EOF},0}$ values from formate BGEs, (\blacktriangle) $\mu_{\text{EOF},0}$ values from acetate BGEs. Equation of the least-squares regression lines on 54 data: $1.17x - 4.26$ ($r^2 = 0.675$).

ently, the ion-pairing between ammonium and acetate was stronger than between ammonium and formate. The same behaviour was reported for higher BGE concentrations in a MeCN:MeOH 80:20 mixture [16]. Despite this discrepancy, similar intercepts (i.e. $\mu_{\text{EOF},0}$) were extrapolated from formate and acetate BGEs in MeCN.

The $\mu_{\text{EOF},0}$ values estimated from the DHO model in different solvent systems were comparable in formate and acetate BGEs. Slight differences occurred due to the following approximations: (i) use of a simple DHO model, (ii) formate/ammonia and acetate/ammonia ion-pairing effects were neglected and (iii) ionic strength was considered as equivalent in all systems.

As shown in Tables 3–5, the slopes in all systems were as expected from Eq. (4), completely different (e.g. about two orders of magnitude between 1-BuOH and MeCN), due to their different ε , η and $\mu_{\text{EOF},0}$ values. Therefore, μ_{EOF} values determined at a low concentration can be significantly lower than $\mu_{\text{EOF},0}$ for systems with high slopes. This clearly demonstrates the importance of comparing $\mu_{\text{EOF},0}$, and not an arbitrary μ_{EOF} at a similar electrolyte concentration in diverse solvent systems.

3.3. Relationship between $\mu_{\text{EOF},0}$ and solvent ε/η ratio

Eq. (1) shows that $\mu_{\text{EOF},0}$ is proportional to ε/η ratio, and zeta potential close to the capillary wall (ζ_{wall}). Fig. 3 illustrates the $\mu_{\text{EOF},0}$ variation in function of ε/η ratio of solvent systems. With all the reported values ($n=54$), a good linearity was obtained ($r^2=0.675$) showing that ζ_{wall} (slope of the linear regression) was almost constant. Only two outliers were observed, corresponding to $\mu_{\text{EOF},0}$ determined in MeCN and NMF. Without the latter, the determination coefficient was fully acceptable ($r^2=0.867$) with an average ζ_{wall} of 118 mV.

The demonstrated relationship between $\mu_{\text{EOF},0}$ and ε/η ratio is remarkable, given that numerous approximations were

performed. Firstly, ε and η values were assumed constant in the system. Secondly, organic solvents were considered as pure, without any impurities. It can be noted that bad day to day repeatability was observed with FA and NMF (important and/or illogical μ_{EOF} variations), probably related to an oxidation of these solvents. To prevent this problem, freshly available solvents were always employed. However, some commercially available solvents may contain impurities, such as formate and acetate anions (e.g. hydrolysis product of FA). Moreover, some amount of water was present in the BGE, principally due to the organic solvent hygroscopy and to BGEs' preparation (i.e. small amount of water added with ammonia). This slight water percentage, probably not identical for all investigated solvents, had an influence on BGE physicochemical properties. Finally, the procedure applied for $\mu_{\text{EOF},0}$ determination contained simplifications explained previously.

3.4. Zeta potential comparison

In Table 6, results from the DHO model were compared to the ζ_{wall} values directly and rapidly determined in pure solvents [7,8]. These values are very similar even if they were obtained in different conditions and laboratories. However, the estimation of $\mu_{\text{EOF},0}$ such as reported in this paper is recommended, since it allows a better control of experimental conditions, similar to those generally used in conventional CZE (i.e. with salt additives).

3.5. Influence of the donor number

In a first approximation, the variation of $\mu_{\text{EOF},0}$ with ε/η is fully acceptable. However, this estimation is not justified for NMF and MeCN (Fig. 3). This problem could result from ζ_{wall} variation between solvent systems, which is often related to a solvent's autoprotolysis constant [7]. Indeed, as reported in Table 1, these constants are the highest and lowest for MeCN and NMF, respectively. It can be noted that DMSO did not follow the same behaviour as MeCN (close $\text{p}K_{\text{auto}}$ values) and that $\mu_{\text{EOF},0}$ was perfectly related to ε/η ratio.

It can be noted that considering $\text{p}K_{\text{auto}}$ values of pure organic solvents may be misleading, since they contain a slight water percentage. In fact, up to 0.5% of water may be present, due to solvent purity, solvent hygroscopy and ammonia addition. This small percentage may have a significant impact on amphiprotic solvent such as MeCN. Nevertheless, small water contents in organic solvents have been shown to have a minor effect on the EOF intensity for MeOH, MeCN, DMSO and NMF [17]. Therefore, it is probably not the only reason for the discrepancy between $\mu_{\text{EOF},0}$ and the ε/η ratio.

Hence, an attempt was carried out to determine a correction factor considering the ζ_{wall} variation, without taking into account the presence of water. The selected term was the DN of a solvent. As shown in Fig. 4A, by dividing term ε/η ratio

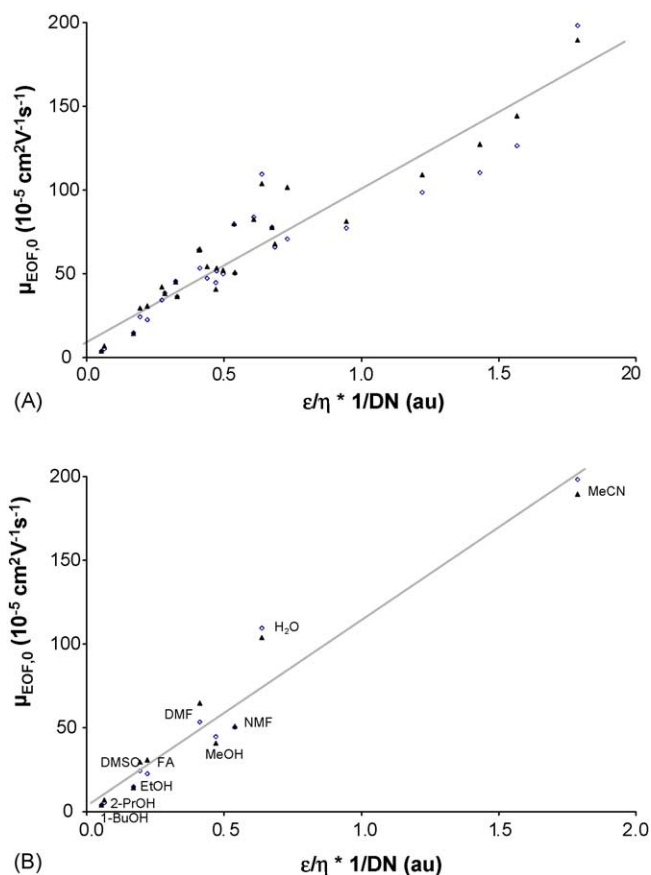


Fig. 4. Correlation of $\mu_{\text{EOF},0} \varepsilon/(\eta \times \text{DN})$ of solvent systems. (\diamond) $\mu_{\text{EOF},0}$ values from formate BGEs, (\blacktriangle) $\mu_{\text{EOF},0}$ values from acetate BGEs. Equation of the least-squares regression lines: (A) $88.6x + 12.1$ ($r^2 = 0.876$) on 54 data and (B) $110.9x + 2.7$ ($r^2 = 0.943$) on 20 data.

by DN, a fully acceptable r^2 of 0.876 was obtained with all 54 data. It is noteworthy that there was a better correlation ($r^2 = 0.943$) for the ten pure solvents (Fig. 4B), using available DN from Table 1.

With these results, the following hypothesis can be formulated: DN probably takes into account zeta potential differences from one solvent to another. If one assumes that the electric diffuse layer (EDL) is of identical dimension in all solvents (which is probably not fully correct), the ζ_{wall} value depends on the quantity of protons within the EDL. If protons are strongly solvated (which is the case for solvents possessing a high DN), their number will decrease due to the presence of solvent, leading to a decrease of charge density inside the layer. Thus, ζ_{wall} is inversely proportional to a solvent DN.

4. Conclusions

This study allowed to answer some questions about the relation between EOF intensity and BGE composition. Despite the large number of applications performed in NACE, few information are available about the μ_{EOF} variations in different BGEs. μ_{EOF} were determined in 27 solvent systems at six different ionic strengths, and $\mu_{\text{EOF},0}$ were estimated according to the DHO model. This procedure was successfully applied with ammonium/formate and ammonium/acetate BGEs, showing excellent linear correlations ($r^2 \geq 0.90$) between μ_{EOF} and \sqrt{I} at low ionic strengths. Consequently, μ_{EOF} can be predicted for the 27 tested solvent systems. Next, comparing the ε/η ratio of a solvent system was shown to be a valuable estimation of $\mu_{\text{EOF},0}$ variation. Furthermore, a better prediction could be carried out by considering the donor number of a solvent, especially between BGEs with different autoprotolysis constants. It would probably be interesting to consider the solvent's donor number more systematically in NACE, as the solvation modification of positive analytes from one solvent to another may also be partially explained by this property.

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