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Influence of solvent properties on separation and detection performance in non-aqueous capillary electrophoresis–mass spectrometry of basic analytes

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Abstract

The versatility of non-aqueous capillary electrophoresis (NACE) results mainly from the variety of physico-chemical properties of the different solvents. They provide solubility for a wide range of analytes, enable to control electrophoretic selectivity, but affect in some cases UV absorbance detection. The coupling of NACE to electrospray mass spectrometry (ESI-MS) allows to cope with the high UV cut-off of some CE relevant solvents (e.g., formamides). In this paper the pure organic solvents methanol, acetonitrile, dimethylsulfoxide, formamide, *N*-methylformamide and *N*,*N*-dimethylformamide are evaluated against water for the preparation of ammonium acetate electrolytes to separate the basic model substances 2-aminobenzimidazole, procaine, propranolol and quinine with NACE–MS. MS coupling is assisted with the sheath liquid water–isopropanol (1:4, v/v) with 0.1% formic acid. The goal of the paper is to assess the influence of the solvent on selectivity, separation speed, and peak efficiency for a given set of model compounds on a simple empirical basis. It should give the user an idea how the separation quality is changed when nothing but the running solvent is altered. The obtained efficiency results were discussed with respect to physico-chemical models described in literature (assuming longitudinal diffusion as the only source of band broadening), but no satisfying correlations with solvent properties could be traced. The feasibility of all six organic solvents for MS coupling was demonstrated and the influence of the separation solvent on the MS detection performance was compared. In the seven different solvents, the shortest run time was obtained with acetonitrile, the best peak resolution with the amphiprotic solvents (especially methanol) best peak efficiency with methanol and formamide, and the most sensitive ESI-MS detection with acetonitrile and methanol, but with only slight advantage to water.

Keywords: Solvents properties; Nonaqueous capillary electrophoresis; Selectivity control; Separation speed; Peak efficiency

1. Introduction

1.1. General

Non-aqueous capillary electrophoresis (NACE), i.e. the use of organic solvents to prepare the CE electrolyte has been published for the first time in 1984 [1], shortly after the introduction of the basic technique high-performance capillary electrophoresis (HPCE) [2,3]. The feasibility of NACE has been demonstrated in numerous publications, most of them are summarized in review papers [4–7]. Several publications on the theoretical advantages over the aqueous mode have pointed out the physico-chemical basis for alterations of efficiency, speed, and selectivity when the electrolyte solvent is altered [8–10].

In separation sciences, method selectivity is the crucial parameter, but this property is hardly predictable from physico-chemical characteristics of the solutes and the separation system, neither in electrophoresis, nor in chromatography. In HPLC, such approaches are mostly based on linear free energy relationships (LFERs) and are becoming increasingly successful in recent years, especially for stationary phase characterization [11]. This concept is not readily transferable to CE, where the separation normally takes place in one single phase. Changes of the electrolyte solvent, however, can also have tremendous impact on electrophoretic selectivity. The ion mobility can be correlated with solvent

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properties like the ratio of solvent permittivity and viscosity (ε_r/η) [8], as well as for solvent mixtures when the von Smolukowski equation [12] is applied. Based on that concept, Salimi-Mossavi and Cassidy [13] reported on the change of mobility of alkyl sulfates and alkane sulfonates in different methanol/acetonitrile mixtures. Whilst the correlation of the mobility of the sulfates with the solvent composition was as expected, the sulfonates behaved inversely. At higher acetonitrile concentration, a pronounced selectivity between both surfactant groups was encountered. Such selectivity effects can be attributed to a specific influence of the solvent on dissociation constants, as well as to solute specific association phenomena like homoconjugation [14], but mostly heteroconjugation [15,16] in the separation electrolyte, thus changing the effective charge to size ratio. Roy and Lucy [17] discussed the solvent influence on selectivity on the basis of dielectric friction. However, this concept is not very common and primarily applies for the separation of differently charged ions. In spite of all successful physico-chemical interpretations of experimental results, a general and accurate prediction of selectivity in different solvents is not yet possible and empirical method optimization is thus unequivocal.

Unlike separation selectivity, the theoretical description of peak efficiency or band broadening in separation science can be accomplished in a more concise way. Jorgenson adopted the Van Deemter concept from chromatography to peak height equivalents in CE [18], based on the assumption that band broadening is only due to longitudinal diffusion. Even under these simplified conditions, different approaches to describe the solvent effect on band broadening can be found in literature. Jansson and Roeraade pointed out the theoretical advantage of *N*-methylformamide (NMF) due to its outstanding value of ε_r^2/η, on which the efficiency per unit time N/t should directly depend and demonstrated very efficient separations of carboxylic acids using this solvent [8]. Following this theory, NMF should improve peak efficiency per unit time three-fold relative to water. A systematic experimental proof for this relation using a variety of solvents has never been published to our best knowledge. Geiser et al. reported a systematic study on the influence of the solvent on peak efficiency of β-blockers in NACE [19]. Applying six different solvents, they demonstrated for one compound (celiprolol) that rather the plate number N than the plate number per unit time N/t depended on ε_r^2/η, a finding that is not in accordance with theory. In fact, they obtained the best efficiency with NMF, but included no data on the plate number with water (second solvent in the ε_r^2/η ranking after NMF) in their paper.

In a publication on a firm theoretical background by Muzikar et al., "the principle cause for lower plate numbers in CZE with most organic solvents" (CZE = capillary zone electrophoresis) is addressed [20]. From the conductance theory of Debye (DHO) [21] and the diffusion theory of Onsager et al. [22], they theoretically predicted and practically demonstrated a decrease of plate numbers with increasing ionic strength of the electrolyte. Increasing the ionic strength to $0.08\,\mathrm{mol\,L^{-1}}$, this effect was three times higher with methanol (MeOH) than it was with water (iodide as sample ion).

Besides the solvents typically applied in aqueous, hydro-organic, and non-aqueous CE [like water, acetonitrile (ACN) or methanol] different amides [formamide (FA), acetamide, *N*-methylformamide (NMF) and *N*,*N*-dimethylformamide (DMF)] and dimethyl sulfoxide (DMSO) are applicable in NACE, as they provide a high capacity to dissolve ions. However, they are not easily applicable with UV detection, because of UV cut off wavelengths of 270 nm or higher. Electrospray ionization mass spectrometry (ESI-MS) detection is a way to overcome this inconvenience. It is mostly carried out with the so-called shield–flow interface [23], but sheathless nanospray [24], and liquid junction [25,26] is also described for interfacing. Especially the use of volatile solvents like lower alcohols or ACN is considered to be favorable to improve sensitivity and spray stability [27].

This paper reports the characteristics of the electrophoretic separation of four basic model compounds (2-benzimidazole, procaine, propranolol, and quinine) in seven different solvents (water, acetonitrile, methanol, formamide, *N*-methylformamide, *N*,*N*-dimethylformamide, and dimethylsulfoxide) applying sheath-flow MS detection. To each solvent 10 mM NH_4OAc was added as electrolyte and the pH (or pH^*) was not adjusted. The experimental conditions for both CE and MS were kept constant at standard values in all solvents (only solvent and injection protocol was varied) to enable a meaningful comparison of the solvent effects. The aim of the study was to directly compare the analysis speed, selectivity, peak efficiency, as well as the MS detection performance for the given set of model compounds. The observed efficiencies are evaluated for a possible correlation with solvent properties based on theoretical models reported in literature.

1.2. Theory on efficiency in CE

A discussion of the complexity of solvent influences on efficiency in NACE that includes all possible band broadening origins is given in a series of papers by Palonen et al. [28–31]. Their studies are restricted to short-chain alcohols as solvents and mainly focus on the application of high field strength. Engelhardt and Cuñat-Walter [32] demonstrated the influence of capillary dimension, field strength and injection conditions on the obtained plate number with proteins in aqueous CZE. Under optimized conditions they achieved 400 000 plates on a 20 cm capillary for cytochrome *c*, but almost 10-fold decrease in efficiency with modifications of the injection protocol, field strength, and capillary diameter. The following discussion will be restricted to longitudinal diffusion, since it is an inevitable phenomenon in CE and cannot be suppressed by experimental precaution.

As mentioned in the introduction, there are different approaches in literature to theoretically describe the influence

of solvent properties on peak efficiency in CE. In an early theoretical discourse on NACE, Jansson and Roeraade [8] derived the following equation for the plate number per unit time in CE:

$$\frac{N}{t} = \frac{6\pi r}{kT\eta}(2\zeta_{\text{ion}} - 3\zeta_{\text{wall}})^2(\varepsilon_0\varepsilon_r E)^2 \quad (1)$$

where r is the Stokes radius of the solvated solute ion, k the Boltzmann constant, T the thermodynamic temperature, η the solvent viscosity, ζ_{ion} the ζ-potential of the dissolved analyte ion, ζ_{wall} the ζ-potential of the inner capillary wall, ε_0 the permittivity of the vacuum, ε_{r} the relative permittivity of the solvent and E the electric field strength. In the derivation of this equation, several assumptions are made. The band broadening in CE is considered to be only due to longitudinal diffusion, the relative permittivity and the viscosity close to the ion surface and in the electrical double layer at the capillary wall are assumed to be equal to each other and to that of the bulk solvent, since only the bulk values are available. The derivation is based on the description of the diffusion coefficient D by the Stokes–Einstein relation (Eq. (2)).

$$D = \frac{kT}{6\pi\eta r} \quad (2)$$

This relation, however, accounts for the diffusion of uncharged solutes, whilst for ionic solutes, the electrostatic interactions with the other ions present in the electrolyte would have to be considered. Another inconvenience of Eq. (1) is that the Stokes radius r is a solute related parameter that is not readily available for a distinct ion in a given electrolyte solution. The correlation of N/t with the $\varepsilon_{\text{r}}^2/\eta$ neglects the influence of the solvent and the electrolyte on the ζ-potentials of sample ions and the capillary wall.

Based on Eq. (1), the dependence of the plate number N on solvent parameters can be derived as follows:

$$N = \frac{\varepsilon_0\varepsilon_r\pi r L_{\text{eff}} E}{kT}(2\zeta_{\text{ion}} - 3\zeta_{\text{wall}}) \quad (3)$$

with L_{eff} being the length from capillary inlet to the detection window. This relation predicts a linear dependence of N on the solvent permittivity ε_{r}.

Under the approximation that $L_{\text{eff}} \approx L_{\text{tot}}$, with L_{tot} being the total capillary length, Eq. (3) can be transformed into Eq. (4):

$$N = \frac{\varepsilon_0\varepsilon_{\text{r}}\pi r U}{kT}(2\zeta_{\text{ion}} - 3\zeta_{\text{wall}}) \quad (4)$$

where U is the applied voltage. A clear advantage of this concept is that the influence of the electroosmotic flow (EOF) on the efficiency is considered in the derivation of Eq. (1).

A different approach published by Muzikar et al. [20] considered the strict interrelation between $D_{0,\text{ion}}$, the diffusion coefficient of an ion at zero ionic strength and $\mu_{0,ion}$, the mobility of this ion at zero ionic strength (Nernst–Einstein relation). From this relation, Giddings [33] derived a general calculation for the ultimate plate number in CE to be

$$N = \frac{zeU}{2kT} = 19.47zU \approx 20zU \quad (5)$$

where z is the effective charge of the ion in solution, e the electron charge and the constant factor ~20 is calculated for a temperature of 25 °C. This equation expresses no solvent dependence of efficiency, because the influence of the solvent on the ion diffusion coefficients compensates for its influence on ion mobility, which is valid for an ion in solution at zero ionic strength. Hence, N solely depends on separation voltage and analyte charge. When the ionic strength is increased, ion mobility is decreased due to the electrophoretic and the relaxation effect described by the DHO theory. At the same time the diffusion coefficient is decreased, but only due to the relaxation effect, since the diffusive ion needs to renew its counterion sphere, whilst no electrophoretic countermigration (electrophoretic effect) occurs with diffusion. Consequently mobility decreases faster with increasing ionic strength than diffusion and thus the plate number N decreases as well when the ionic strength is increased. The influence of the ionic strength I on the N is solvent dependent and can be described for $z = 1$ as follows:

$$N = 19.47U\left(1 - \frac{(0.2476/\mu_{0,\text{ion}}\eta\varepsilon_{\text{r}}^{1/2})\sqrt{I}}{1 - (1.5936/\varepsilon_{\text{r}}^{2/3})\sqrt{I}}\right) \quad (6)$$

According to Eq. (6), the decrease of N with increasing I is in a first approximation proportional to $1/(\mu_0\eta\varepsilon_{\text{r}}^{1/2})$. The solvent parameters $\eta\varepsilon_r^{2/3}$ for a series of common solvents vary between 13.7 mPa s for DMSO and 2.1 mPa s for ACN (ratio is 6.3). The mobility μ_0 (at zero ionic strength) of, e.g. iodide (sample ion used by Muzikar et al.) varied inversely between 24.5×10^{-9} m^2 V^{-1} s^{-1} for DMSO and 106.3×10^{-9} m^2 V^{-1} s^{-1} for ACN (ratio is 4.3). These relations point out that solvent effects on efficiency do not clearly rule over solute specific phenomena. Following Eq. (6), the decrease of N for iodide with the square root of the ionic strength is approximately proportional to the factor 1.6 in water, 2.9 in DMSO, 4.5 in ACN and 4.9 in MeOH. A principle disadvantage of organic solvents in terms of efficiency at finite ionic strength is thus obvious.

2. Experimental

2.1. Chemicals and solvents

Water was purified by a Milli-Q system from Millipore (Eschborn, Germany). *N*,*N*-Dimethylformamide, *N*-methylformamide, formamide, 2-aminobenzimidazol, procaine, propranolol and quinine were from Fluka (Neu Ulm, Germany), methanol was from Merck (Darmstadt, Germany), acetonitrile was from Bischoff Chromatography (Leonberg, Germany) and dimethylsulfoxide from Riedel-de Haen

(Seelze, Germany). All solvents and reagents had the highest purity available.

2.2. Instrumentation and settings

CE experiments were carried out on an Agilent $3D^{CE}$system (Waldbronn, Germany). Instrument control and data processing was by an Agilent Chemstation software (Rev. A. 06.03). The fused silica capillaries (50 μm i.d. × 360 μm o.d.) were from Polymicro Technologies (Phoenix, AZ, USA). For MS detection, a Bruker Esquire LC (Bremen, Germany) ion trap spectrometer was used and controlled by the Bruker Esquire control software (ver. 4.0, build 37). MS data were processed using the Bruker Data Analysis software (ver. 3.0, build 49). To interface the CE capillary to the MS system, the standard Agilent MS-cassette and the standard coaxial triple tube sprayer were used.

The CE temperature control was switched off (about half of the capillary was outside the cassette), but laboratory temperature was controlled at 23 °C. The capillary length from the inlet to the sprayer outlet was 75 cm. No simultaneous UV-detection was carried out. The separation voltage was 30 kV (ESI needle is at ground voltage).

The ESI parameters were 4 kV needle voltage (positive mode), 10 p.s.i. nebulizer gas pressure (1 p.s.i. = 6894.76 Pa), and 4 L min^{-1} dry gas flow at 200 °C. The sheath liquid was isopropanol–water (4:1, v/v) with 0.1% formic acid, the sheath flow was deliverd by a Cole Parmer 74900 series syringe pump (Vernon Hills, IL, USA) at 4 μL min^{-1}. The tuning of the ion trap and ion optic parameters was performed automatically ("smart" mode of Bruker software) for the *m*/*z* range of 200, which was the average of the 4 solute masses. Scan was at 13 000 *m*/*z* s^{-1} from 80 *m*/*z* to 340 *m*/*z*, seven scans were averaged.

2.3. Procedure

The running electrolytes were prepared by dissolving 10 mM NH_4OAc in each solvent. Although non buffering, this electrolyte was soluble at the given concentration in all seven solvents and enabled a stable electric current. A model compound mixture from 2-aminopyridine, procaine, propranolol, and quinine each at 25 μmol L^{-1} was dissolved in the seven running electrolyte solutions. Injection was performed hydrodynamically at 50 mbar. Based on a 5 s injection time for the aqueous system, the injection time was adopted for the other solvents according to their viscosity (18 s for FA, 9 s for NMF, 5 s for DMF, 11 s for DMSO, 2 s for ACN and 3 s for MeOH) in order to inject the same amount of sample into all systems. The nebulizer gas pressure was switched off during the injection to avoid errors by a suction effect.

For each solvent/electrolyte system, a new piece of CE capillary was used and conditioned for 15 min with 0.1 M NaOH, 15 min with water, and 15 min with the running electrolyte before the first run. Between each repetition it was rinsed 5 min with running buffer. Data processing was carried out with the selected-ion monitoring (SIM) extracts for each compound [2-aminobenzimidazole, $(M + 1)/z = 134$; procaine, $(M + 1)/z = 237$; propranolol, $(M + 1)/z = 260$; quinine, $(M + 1)/z = 325$]. Three repetitive runs were performed and the migration time and plate number results were averaged. The span of the three values relative to the mean did not exceed 5% for the migration times or peak heights and 15% for the plate numbers. For the sake of clarity, the plotted results do not show error bars. To determine the EOF, a plug of water was injected (see injection method above) into the different solvent/electrolyte systems. The arrival of the water plug at the capillary end was detected by a steep increase of the current. The peak migration times were recorded automatically, the plate numbers at half peak height and the signal to noise ratios were determined manually. To determine the detection limits, the samples were diluted to an expected S/N of ∼10 and re-injected. From the related concentrations, the limit of detection (LODs) were calculated for S/N = 3.

3. Results and discussion

3.1. Selectivity, resolution and speed of analysis

The electropherograms obtained with the four model compounds 2-aminobenzimidazole (1), procaine (2), propranolol (3), and quinine (4) in seven different solvents under standardized conditions are depicted in Fig. 1. The overlaid four lines represent the four SIM tracks from MS detection and enabled to distinguish non-resolved compounds. Electrophoretic conditions like capillary dimensions and field strength were kept constant. As can be deduced from the electropherograms in Fig. 1, peak resolution and run time varied tremendously between the different solvents, and migration orders changed. The results will be discussed in detail in the following.

An appropriate way to describe electrophoretic selectivity, which does not directly correspond to peak resolution, is to depict the effective ion mobilities of each compound of the sample mixture, because differences in this parameter account for it. This is done in Fig. 2 where the solutes are ordered by increasing molecular mass. The data on molecular mass and aqueous pK_a of the four solutes are given in Table 1. Solely in the aqueous system the migration order followed the molecular mass, whilst in the organic solvents it was rather inverted, except for quinine which was always among the slower migrating compounds. 2-Aminobenzimidazole is the weakest base in the set (pK_a in water is 7.2). With the aprotic solvents DMSO, DMF and ACN, this weak base showed the smallest mobility in spite of its low molecular mass. Propranolol is the strongest base of the four model compounds (pK_a in water is 9.5) and exhibited the highest mobility in the amphiprotic solvents MeOH and NMF, in contrast to the results with the also amphiprotic media FA and water. Procaine (pK_a in water is 8.9) is a slightly weaker base with slightly lower molecular weight than propranolol. In water, the selectivity between both was hence not very pronounced. In contrast,

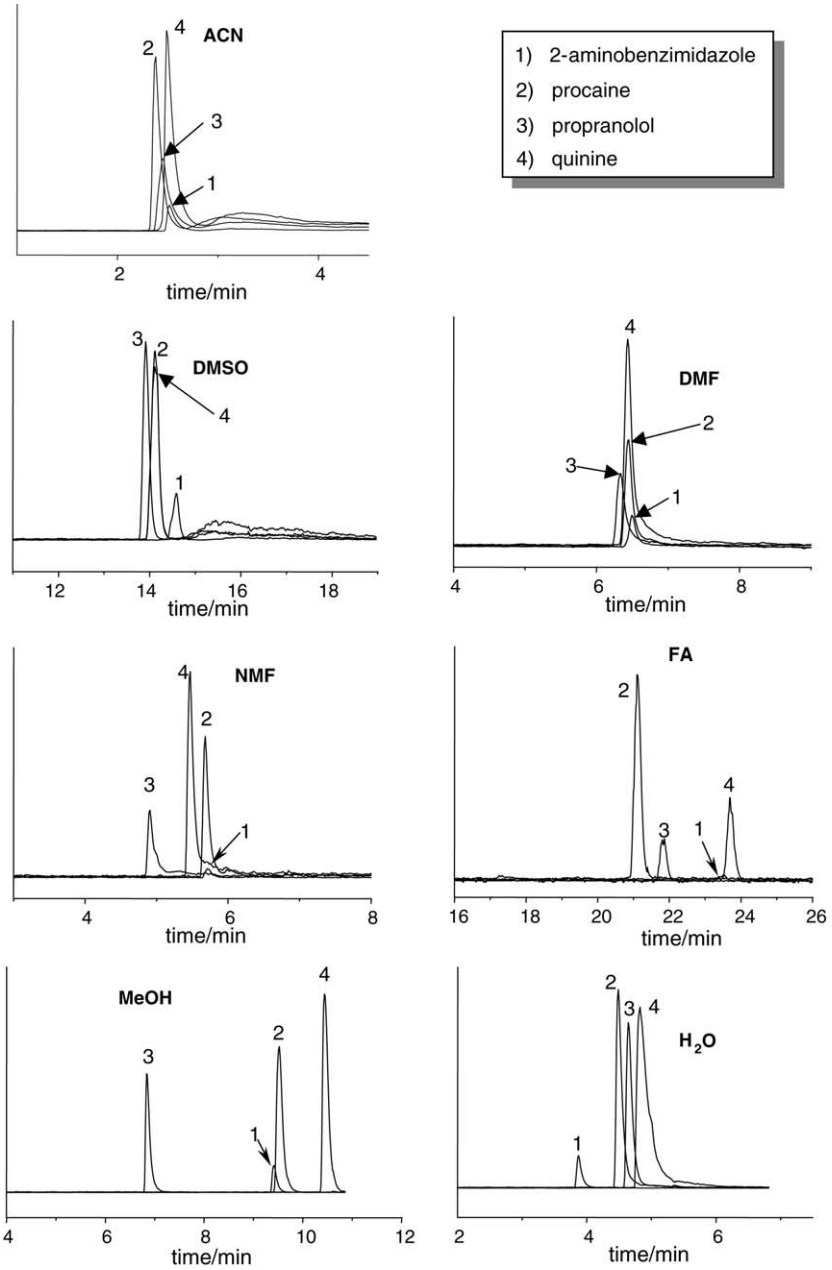

Fig. 1. Electropherograms of four basic solutes obtained with CE–MS in seven different solvents. Individual lines represent specific SIM tracks for each solute. For conditions see materials and methods section.

Table 1
Molecular masses, aqueous pK_a values and basic functionalities of probe solutes

	Solute			
	2-Aminobenzimidazole	Procaine	Propranolol	Quinine
M (g mol^{-1})	133	236	259	324
pK_a (water)	7.2	8.9	9.5	7.7
Strongest basic functionality	Aromat. $-NH_2$	*tert*.-Amine	*sec*.-Amine	*tert*.-Amine (bridge head-N)

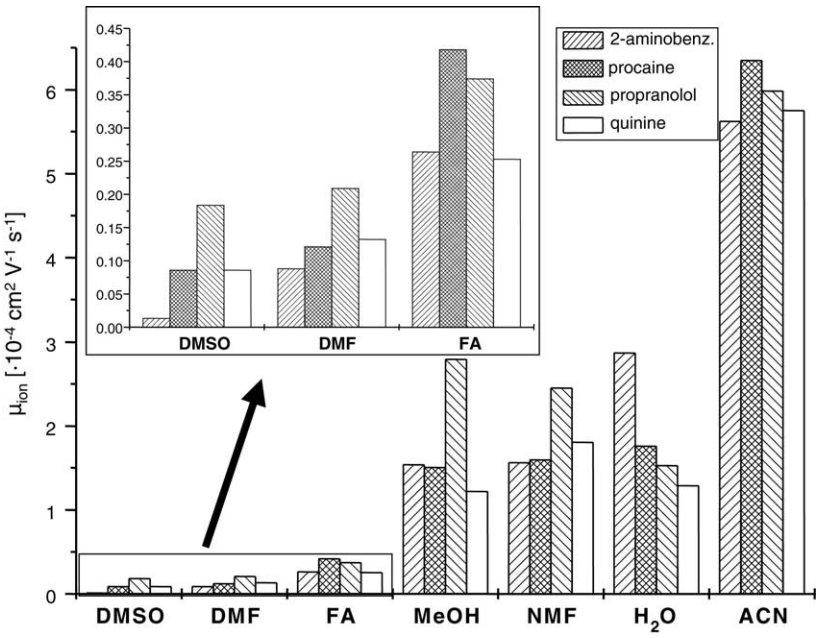

Fig. 2. Effective ion mobilities of the four basic solutes in each solvent calculated from the electropherograms in Fig. 1.

both the two amphiprotic solvents MeOH and NMF and the two aprotic solvents DMSO and DMF generated a high selectivity between these two solutes. These results can only be attributed to specific interactions like solvent depending conjugation phenomena or an amplification of the small differences in pK_a values (referring to the "leveling" aqueous system).

Selectivity in CE is defined similar to chromatography and calculated by Eq. (7) with t_{eo} being the migration time of the electroosmotic flow and t_{ion1} and t_{ion2} the migration times of two successively migrating ions. In order to obtain α-values higher than 1, ion 1 must be selected to migrate faster than ion 2 in case of a co-electroosmotic mode and for the counter-electroosmotic mode vice versa.

$$\alpha = \frac{t_{ion1} - t_{eo}}{t_{ion2} - t_{eo}} \quad (7)$$

For each solvent, the selectivity for a pair of successive peaks was calculated by Eq. (7). The average of the three α-values in each separation was made for each solvent and the resulting data are plotted in Fig. 3. From this it can be deduced that marked differences in electrophoretic selectivity occur in the different solvents. As pointed out in the introduction, the influence of the solvent on the effective ion mobility is due to alterations of solvation, conjugation effects and its influence on the effective charge of the solute. According to the pronounced influence of the solvent properties on pH* with a given electrolyte and the pK_a of a given solute, the alteration of the solute charge is supposed to play the dominant role when acids and bases are separated with CE.

With the reported experiments, the averaged selectivity increased in amphiprotic solvents with increasing proton

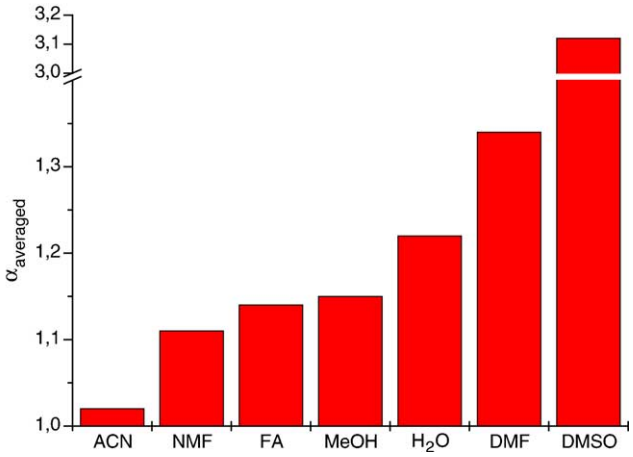

Fig. 3. Electrophoretic selectivities α calculated from the electropherograms in Fig. 1 by Eq. (7). Column bars represent the average of the three α-values obtained for each solvent.

donor ability (deducible from their molecular structures and a decreasing electron acceptor number [10]) in the sequence NMF, FA, MeOH, water. The greatest selectivities for the model compounds were generated in the aprotic solvents DMSO and DMF. However, very low ion mobilities occurred in these solvents (see Fig. 2) and the transport of the solutes to the detector was mainly due to the non-selective EOF. This is the reason for the poor peak resolution with these systems. In spite of the slow ion migration, the quinine/procaine (co-migrating) peak was baseline resolved from that of 2-aminobenzimidazole, as the selectivity for this peak pair was higher than 6. It must be considered however, that the calculated selectivities in this system can be tremendously biased from errors in the EOF determination. A completely

different behavior was obtained with acetonitrile, although it is an aprotic solvent as well. With the given electrolyte, the highest ion mobilities have been encountered in ACN, which can in part be attributed to its high ε_r/η ratio. The peak resolution found with ACN was the poorest among all seven solvents. In contrast to DMSO and DMF, this was due to the weak selectivity with this solvent.

At a given field strength and effective capillary length, the speed of analysis in CE is affected by the mobilities of the analyte ions and that of the EOF, i.e. analysis is accelerated with the increase of both these mobilities, at least in a co-electroosmotic mode. Both the ion and the EOF mobilities depend on the ε_r/η ratio of the solvent [8]. This simple view does not consider the specific influence of the solvent on the solvated ions (for ion mobility) and on the capillary wall (for EOF mobility). Fig. 4a shows a plot of the averaged mobilities of the four model solutes versus ε_r/η of the applied solvent, Fig. 4b likewise for the encountered EOF, Fig. 4c likewise for the linear velocity of the last peak to arrive at the detector (to indicate for speed of separation). All three plots show a clear trend to make solvents of high ε_r/η favorable for fast analysis. Moreover, the experimental data suggest a considerable variation in the individual ζ-potentials. Deviations in the ion mobilities from the trend predicted by the ε_r/η ratio of the electrolyte solvent are the crucial source for selectivity differences due to specific solvent influences. Concerning the EOF, marked differences in the ζ_{wall}-potentials are reported in literature for both electrolyte free and electrolyte containing NACE- and hydroorganic systems [9,34,35]. Valko et al. encountered an outstanding ζ-potential in ACN and gave a theoretical explanation for this. The deviations from the correlation of μ_{eof} with ε_r/η traceable in Fig. 4b (to be attributed to relatively low ζ-potentials in NMF and MeOH, as well as relatively high ζ-potentials in DMSO and DMF) are in good agreement with the data reported in literature [9]. The high expectations on NMF in terms of speed of analysis could not be fulfilled. Fig. 4c demonstrates the combined influence of EOF and ion mobility depending on the solvent. The linear velocity of the slowest migrating compound in each system is plotted versus the solvent ε_r/η ratio. The similarity in the pattern of Fig. 4b and c points out that analysis time is mainly controlled by the EOF. As can be seen from Fig. 4c, the speed of analysis was increased almost 10-fold when switching from FA to ACN. However, the potential of ACN could not be exploited for the given solute mixture, due to the poor selectivity in this solvent. The best compromise between peak resolution and speed of analysis was obtained with the aqueous system. When FA or MeOH based electrolytes are applied, the excellent average peak resolution is compromised by a very long run time. It can be concluded from these results that the EOF is in most cases the dominating transport mechanism, which must be considered in the discussion on band broadening.

3.2. Peak efficiency

As pointed out in the theoretical section, different expectations on the influence of solvent properties on peak efficiency

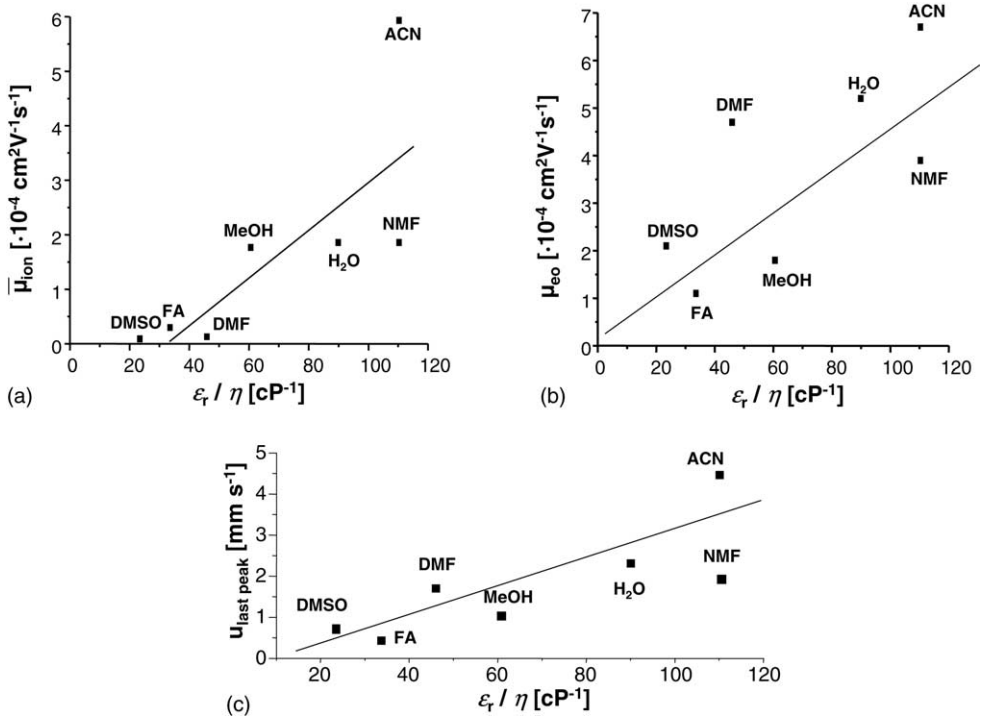

Fig. 4. Correlation of electrophoretic dynamic parameters like averaged ion mobility (a), electroosmotic mobility (b) and linear velocity of the slowest solute band (c) with the physico-chemical solvent parameters (ε_r/η).

are discussed. Plate numbers at half peak height were calculated manually from the SIM-MS tracks of the electropherograms measured in the seven different solvents. Since no UV detection was performed, the contribution of electrospray detection to peak dispersion could not be assessed for the given experimental set-up. The main contribution of the pressure assisted sheath flow interface should come from the so-called suction effect [36,37]. This is the impact of a hyberbolic flow profile generated from an additional pressure gradient along the capillary, which is due to the high linear velocity of the nebulizing gas that sheaths the capillary outlet. To minimize the influence of this effect, a small inner capillary diameter (50 μm) was selected. Geiser et al. studied NACE systems with UV and ESI-MS detection to analyze basic solutes and reported no additional band broadening due to MS [19]. In Fig. 5a, the obtained plate numbers of the four solutes are depicted for the seven different solvents, Fig. 5b shows the plate number per unit time likewise. The various pattern obtained with different solvents point to specific influences of the solvent on the efficiency of distinct solutes. Whilst the plate numbers of the different solutes in NMF, ACN and FA were relatively close, marked differences were encountered when applying DMF, MeOH or water. The average plate number of all four solutes for a distinct solvent increased 10-fold when changing from ACN to MeOH. If the suction effect played a dominant role, then the obtained plate numbers would strictly follow the solvent viscosities. Although the high plate numbers with FA and DMSO would support a prominent suction effect, the highest efficiency with MeOH and the very close efficiencies with NMF and DMF are clearly contradicting. As can be seen in Fig. 5b, the lowest plate numbers per unit time were obtained with FA and the highest again with MeOH, but the difference was only twofold.

To assess the relations between efficiency and solvent parameters predicted by Jansson and Roeraade [8], the average plate numbers N_{av} were plotted versus ε_r, in Fig. 6a, the ratio N_{av}/t versus ε_r^2/η in Fig. 6b. In both cases, no meaningful correlation could be encountered. The high expectations on NMF could not nearly be fulfilled, whilst for DMSO and MeOH, the high plate numbers observed are not consistent with the given theoretical approach. Geiser et al. [19] found a fair correlation of N with ε_r^2/η for the solvents DMF, NMF, ACN and MeOH. However it can be seen from Fig. 6c, that their findings could not be reproduced with our experiments. Besides the altered solutes (β-blockers in [19]), the main experimental difference was the solvent pH (or pH*). Geiser et al. used an electrolyte of 25 mM NH_4HCOO and 1 M formic acid for the non-aqueous solvents and 100 mM formic acid (pH 2.4) for the aqueous system. In the experiments reported here, 10 mM NH_4OAc (aqueous pH was 7) was applied with all 7 solvents. Acidic pH values, as used in [19], enable to suppress silanophilic capillary wall interactions which can have a tremendous impact on peak efficiency. However this effect cannot generally be exploited, since pH (pH*) often is a crucial variable for selectivity adjustment. A possible reason for the deviations from theory may be the influence of the solvent on the ζ-potentials of both the solute ion and the capillary wall. Exemplary for procaine, ζ_{ion} was calculated from the mobility of this drug compound and ζ_{wall} from the obtained EOF mobility. Fig. 6d shows the correlation of $N_{procaine}/t$ versus $\varepsilon_r/\eta(2\zeta_{ion} - 3\zeta_{wall})^2$. Again, no precise correlation was traceable, but unlike the plots without consideration of ζ-potentials, an obvious trend could be observed. The influence of the solvent on the Stokes radius r of the ion in solution is still not considered in this correlation (Fig. 6d). Moreover, it must be assumed that other band broadening effects besides longitudinal diffusion contributed.

According to Muzikar et al. [20], the ionic strength of the electrolyte must be taken into account when efficiency is discussed. As theoretically derived and experimentally proven with iodide as solute in water, ACN and MeOH, the plate numbers should decrease with increasing ionic strength. Assuming the 10 mM NH_4OAc electrolyte as fully dissociated in all solvents (albeit nonrealistic for a weak electrolyte) a maximum molar ionic strength of 0.01 mol L^{-1} is calculated.

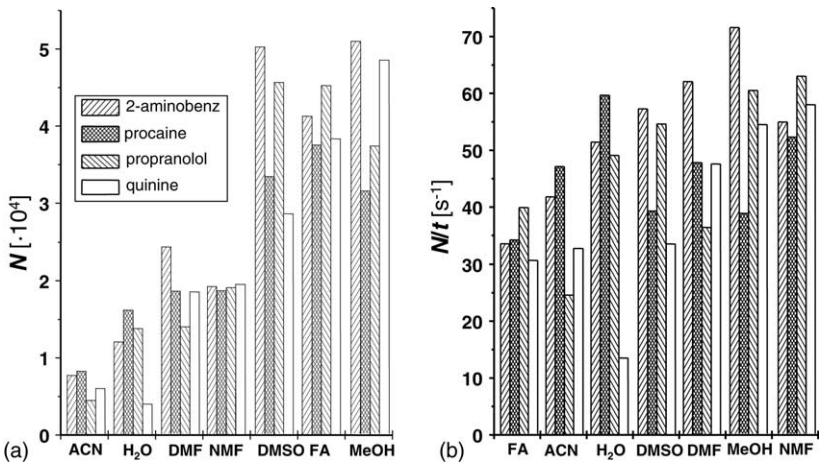

Fig. 5. Plate numbers (a) and plate numbers per unit time (b) calculated at half peak height from the electropherograms in Fig. 1.

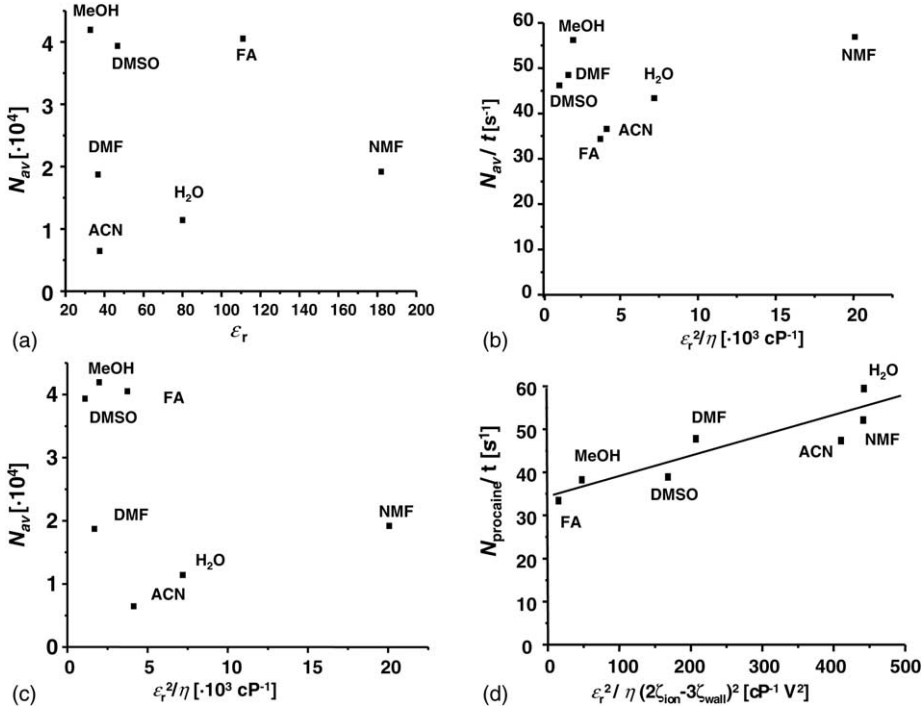

Fig. 6. Averaged plate numbers and averaged plate numbers per unit time (from Fig. 5) plotted vs. solvent parameters. For further details see text.

Following the DHO theory, the dependence of the ion mobility on the ionic strength and eventually the plate numbers can be calculated with the help of Eq. (6), which neglects, however, the influence of EOF.

From such theoretical calculations, the lowest plate numbers were obtained for DMSO (∼200 000) and the highest for water (∼400 000). Besides the fact that such a sequence of efficiencies was not found with our experiments, those figures are 10–50-fold higher than the plate numbers obtained in our system. This result clearly demonstrates that the efficiency is mainly controlled by secondary effects like capillary wall interactions rather than by pure longitudinal diffusion. Regarding the electropherograms in Fig. 1, especially for the solvents that generate low efficiency like ACN, DMF and water, this can already be deduced from the peak asymmetry. In the ideal case of band broadening only by longitudinal diffusion, water should provide the best efficiency followed by ACN and MeOH. The low plate number calculated theoretically for DMS results from the very low ion mobility obtained with this solvent. The order of the experimental plate numbers is completely altered, since ruled by a different band broadening mechanism. The best efficiencies were obtained in DMSO, FA, and MeOH, where wall interactions are obviously mostly suppressed.

It must be expected with cationic solutes that the interactions with the fused silica capillary wall are mainly ionic. Hence, an increase of the electrolyte concentration can partially suppress this effect. In the aprotic solvents, a significant increase of the NH_4OAc concentration was impossible for solubility reasons. In NMF, MeOH, water, and formamide the ammonium acetate concentration was successively increased up to 100 mM and the plate numbers of the four solutes were re-measured. Fig. 7 depicts the tendency of efficiency when the ionic strength was increased. In all solvents, a trend to increase efficiency, obviously by suppressing wall interactions could be observed. The strongest influence was encountered with water, where the poorest efficiencies were obtained (at 10 mM electrolyte concentration) among the amphiprotic solvents. If the theory discussed above applied [20], the plate numbers would be expected to drop with increasing ionic strength. In practice, however, when wall interactions occurred, the inverse behavior was observed.

From the results discussed above, the difficulty to achieve the theoretical peak efficiencies in real life systems is obvious. All the theoretical attempts to correlate solvent characteristics and band broadening were not successful with the obtained experimental data. It is indispensible to consider the individual effects between the solvent and both the capillary wall and the solute, as well as the secondary band broadening effects. Nevertheless, it could be demonstrated practically that the solvent has a marked influence on peak efficiency. Due to the complex origins of band broadening in CE, accurate predictions are mostly not possible. General rules for solvent effects on separation efficiency in NACE can rarely be given.

3.3. Mass spectrometric detection

When a sheath flow interface is applied to couple CE to MS, the composition of the spray conditions can be

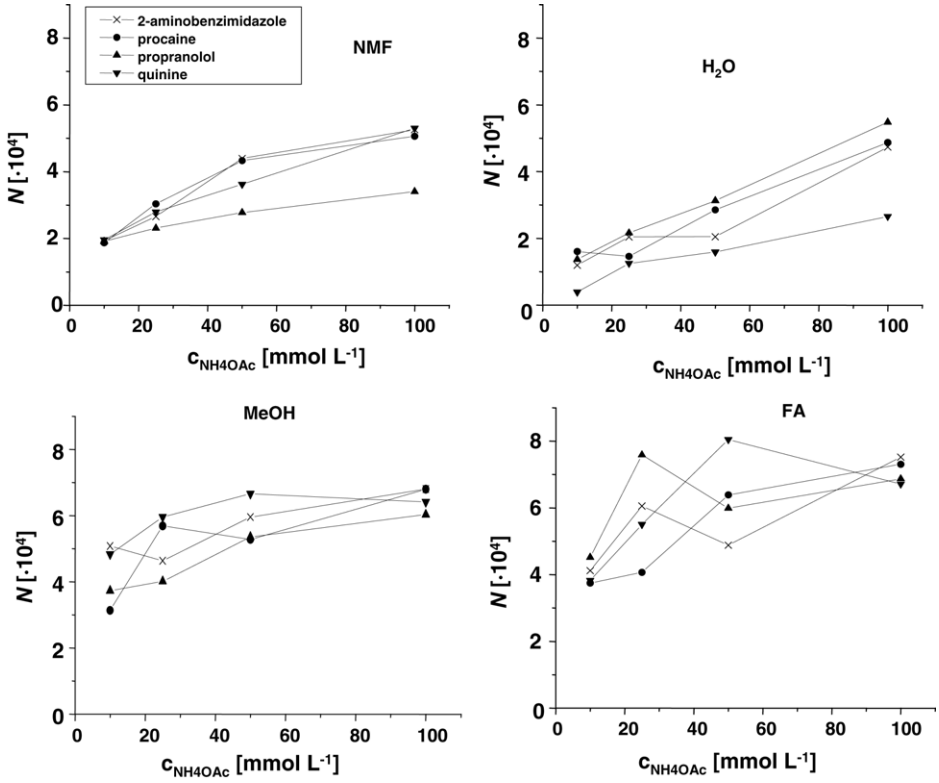

Fig. 7. Influence of electrolyte concentration on peak efficiency in four amphiprotic solvents. For other experimental details see text.

modulated by an appropriate sheath liquid in order to optimize flow rate, volatility, surface tension, and ionic additives for optimum spray stability and detection sensitivity, since the CE running buffer does not necessarily provide this. As the sheath flow rate in our experiments was 4 μL min^{-1} and the EOF in the 50 μm capillary varied between 35 nL min^{-1} (FA) and 290 nL min^{-1} (ACN), the sprayed solution should mainly have consisted of the sheath liquid and the conditions (and results) were thus supposed to be dominated by its composition. Nevertheless, it is not evident that the CE capillary effluent (which contains the analyte solutes) perfectly mixes with the sheath liquid in the spray by convection or diffusion. Aim of the study was to compare the MS detection sensitivity and the detection limits for the four model compounds in seven electrolyte solvents under identical sheath flow and electrospray conditions.

The composition of the sheath liquid was optimized by continuous infusion of an aqueous sample (at 200 nL min^{-1}) by a pressure gradient generated with the CE instrument to mimic the EOF. A standard composition of isopropanol–water (4:1, v/v) with 0.1% (v/v) of formic acid was found to be superior to water–MeOH and water–ACN mixtures, as well as to other mixing ratios. To assure a constant amount of sample introduced into the capillary by hydrodynamic injection independent of the solvent properties, the injection times were adopted to the individual solvent viscosities (at 25 °C).

From the different relative peak heights in the electropherograms in Fig. 1, differences in detection sensitivity can be deduced for the various systems. The peak heights were determined by the software and the baseline noise was measured manually (both from SIM tracks). From the obtained signal to noise ratio at 25 μmol L^{-1} electrolyte concentration, the expected concentration for a S/N of 10 was calculated. These solutions were prepared for each solute in each respective solvent individually and injected. The obtained S/N ratio varied from 7 to 12. From these values, the limits of detection (assuming S/N = 3) were calculated. From the signal slope between this low concentration and 25 μmol L^{-1}, the sensitivity of each system (solvent) for each solute was calculated. The results varied considerably for both differing solutes and differing electrolyte solvents. The calculated

Table 2
Detection limits determined in the seven different solvents (S/N = 3)

Solvent	Limit of detection (nmol L^{-1})			
	2-Aminobenzimidazole	Procaine	Propranolol	Quinine
NMF	21000	1000	2350	1500
FA	23500	600	3050	1450
DMF	2550	700	1100	370
DMSO	1310	305	290	330
ACN	70	10	24	7
MeOH	65	16	17	9
Water	75	11	15	14

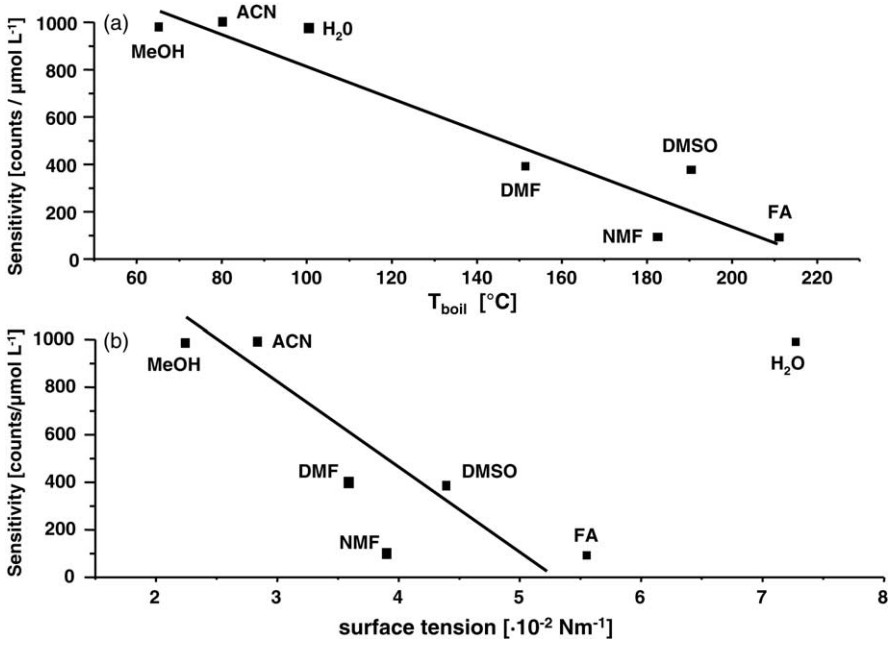

Fig. 8. MS detection sensitivity (average of four solutes) plotted vs. CE electrolyte solvent properties boiling point (a) and surface tension (b). For MS parameters see materials and methods section.

detection limits are listed in Table 2. It can be seen that the LODs for some solutes varied up to 300-fold in the different solvents. Within one solvent, they were similar for procaine, propranolol and quinine (with a slight advantage of quinine), but markedly higher for 2-aminobenzimidazole. FA and NMF running electrolytes generated the poorest performance for trace detection, DMF and DMSO a medium performance, whilst ACN, MeOH and water showed excellent trace detection capability. Detection limits down to 10 nmol L^{-1}, corresponding to mass concentrations between 1 and 3 ppb, were achieved.

Since baseline noise also depended strongly on the running solvent, the determined sensitivities were not exactly inversed to the calculated LODs. The baselines were much noisier when applying the low volatile formamides or DMSO than they were with ACN, MeOH or water. The obtained detection sensitivities for the four solutes were averaged for each solvent and the averaged values were plotted versus the electrolyte solvent boiling temperature T_{boil} (Fig. 8a) and surface tension γ (Fig. 8b). As expected, a trend of decreasing sensitivity with increasing T_{boil} and γ could be encountered. The sensitivity found with water was relatively high considering its T_{boil} and was extraordinarily high with respect to its γ. The sensitivity with DMSO was higher than expected from its T_{boil}, which can be attributed to its low surface tension. NMF generated a low sensitivity considering its surface tension, obviously due to its high boiling point.

Summing up, it is obvious that formamides exhibit serious shortcomings for their use with ESI-MS detection opposite to volatile solvents like MeOH and ACN or water. The clear advantage of ACN and MeOH over water, deducible from their physico-chemical parameters, could not be encountered.

4. Conclusions

NACE–MS is a very versatile method and can be carried out successfully with a wide range of solvents. The selection of the solvent has an impact on efficiency, speed of analysis, selectivity, and detection sensitivity. Since controlled by a large variety of influences, efficiency in real life systems can rarely be predicted from solvent parameters, but plate numbers are influenced by the solvent. Solvents with a high ε_r/η ratio are favorable to speed up analysis, mainly due to a faster EOF. The selectivity for cationic solutes increases with increasing proton donor ability of the solvent, aprotic solvents generated either poor selectivity or very low ion mobilities. MS detection is possible with very different solvents, but the advantage of ACN, MeOH and water over formamide and derivatives for sensitive detection is obvious. The paramount potential in solvent variation for CE is definitely the possible selectivity tuning in order to adopt a method to an analytical task. This is important when using NACE–MS for the analysis of complex samples, were ambiguous masses and ion suppression can occur.

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