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Non-aqueous capillary electrophoresis using non-dissociating solvents
Application to the separation of highly hydrophobic oligomers

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Abstract

The application of non-aqueous capillary electrophoresis for the separation of very hydrophobic oligomers has been studied. *N*-Phenylaniline oligomers having degrees of polymerisation (*n*) of 2, 4, 6, and 8 were taken as model compounds. Capillary electrophoresis could be performed using a mixture of non-aqueous solvents with a high percentage of solvents with a low dielectric constant. These solvents, such as tetrahydrofuran (THF), chloroform or dichloromethane, are needed to solubilise the hydrophobic solutes in the electrolyte. The composition of the solvent mixture and the nature of the acid added to the electrolyte, which is needed to obtain electrophoretic motion of the *N*-phenylaniline oligomers, are discussed in detail. Next, other parameters such as ionic strength, injection time, electric field, and temperature were investigated too and their influence on the separation is discussed as well. The existence of a reversed (anodic) electroosmotic flow in a fused-silica capillary containing a THF–methanol mixture under acidic conditions is reported.

Keywords: Non-aqueous capillary electrophoresis; Background electrolyte composition; Polyaniline; Poly(phenylaniline); Oligomers

1. Introduction

Non-aqueous capillary electrophoresis (NACE) is a promising separation method [1–3]. The potential application of non-aqueous media is not only of significant value in the separation of solutes that are not soluble in water, but additionally, allows for separation of solutes with very similar electrophoretic mobilities in water-based electrolytes. Although NACE allows for numerous solvents as potential candidates, separation is only possible if the solute is charged or interacting with a charged additive combined with a sufficient selectivity in the non-aqueous electrolyte. The selection of solvents and additives not only requires knowledge of their physical and

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chemical properties, but also of the solutes and of their solute–additive interactions as well.

As pointed out by Bowser et al. [3], the variety of interactions that can take place in non-aqueous solvents is larger compared to aqueous or hydro–organic solvents. In non-aqueous solvents electrostatic and donor–acceptor interactions dominate. This in contrast to aqueous media, where hydrophobic interactions play a major role. Thus, commonly used additives in water, like, e.g., surfactants or cyclodextrins, are only useful in non-aqueous solvents with high cohesion energies, like, e.g., formamide or *N*-methylformamide. In these latter solvents solvophobic interactions can also take place. Consequently, new additives which interact with the solutes in the non-aqueous medium, through, e.g., electrostatic (purely electrostatic, ion–dipole or dipole–dipole) or donor–acceptor interactions, must be found to enhance selectivity. Examples of additives already used in NACE are: ammonium ions as an electron acceptor in methanolic solutions [4,5], poly(ethylene glycol) as an hydrogen bonding donor–acceptor in acetonitrile [6], and tropylium ions with charge-transfer and dipolar interactions in an acetonitrile buffer used for the separation of polyaromatic hydrocarbons [7,8].

N-Phenylaniline oligomers having degrees of polymerisation (*n*) of 2, 4, 6, and 8 were taken as model compounds to investigate the possible application of NACE for the separation of very hydrophobic oligomers. Poly(*N*-phenylaniline) belongs to the family of polyanilines (PANIs) which are known as conductive and electroactive polymers [9,10]. PANI applications concern a variety of fields such as electrochromic devices, corrosion resistant coatings and electrostatic dissipation coatings. Here, the *N*-phenylaniline oligomers have been studied for their magnetic properties. Indeed, high-spin properties have been observed due to intramolecular ferromagnetic interactions between the radical cations in the oxidised *N*-phenylaniline oligomers in dichloromethane [11].

PANIs exceeding a certain degree of polymerisation (*n*) are usually insoluble in commonly used solvents. Therefore, they are often polymerised in the presence of water soluble polymers such as poly(vinylpyrrolidone) or poly(vinyl alcohol), leading to a dispersion of stabilised particles [12]. The *N*-phenylaniline oligomers ($n=2$–8) used in this study are relatively small, so a polymeric stabiliser is not needed. Very few work has been done in the analysis of PANI particles or oligomers by capillary electrophoresis (CE). To our knowledge, only two reports dealing with the CE analysis of PANI particles [13,14] exist, however no experimental data on the use of NACE for the separation of PANIs or poly(*N*-phenylaniline)s are reported. This work shows that it is possible to perform CE on highly hydrophobic oligomers in mixtures of non-aqueous solvents containing a high percentage of non-dissociating solvents such as tetrahydrofuran (THF), chloroform or dichloromethane.

2. Materials and methods

2.1. CE instrumentation

Electrophoresis experiments were performed on a Biofocus 3000 CE system (Bio-Rad, Hercules, CA, USA) with a positive polarity on the inlet side of the capillary. The samples were introduced hydrodynamically by application of a positive pressure (1 psi, 7.1 kPa) on the inlet side of the capillaries. The *N*-phenylaniline oligomers were monitored spectrophotometrically.

2.2. Measurement procedure

The *N*-phenylaniline oligomers were dissolved in pure chloroform. Sample concentration of 0.5 g/l for each oligomer. Electroosmotic mobilities were determined either in positive or negative polarity mode. Acetone was used as electroosmotic flow (EOF) marker. The following set of experimental conditions were used unless otherwise stated. Fused-silica capillary, 24 cm (19.4 cm to the detector)×50 μm I.D. Electrolyte: 10 m*M* perchloric acid in a THF–MeOH (75:25) mixture. Applied voltage: 20 kV (835 V/cm, $I=8.8$ μA). Hydrodynamic injection: 2 psi s. UV detection at 200 nm. Temperature: 25°C.

2.3. Chemicals

N-Phenylaniline oligomers ($n=2$, 4, 6, 8) were synthesized as described elsewhere [11,15,16]. The

purity of each oligomer was checked by nuclear magnetic resonance (NMR) and matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF-MS). Methanol, THF (Biosolve), chloroform, dichloromethane, 1,4-dioxane, perchloric acid, hydrochloric acid, acetic acid, trifluoroacetic acid (TFA), sulfuric acid, phosphoric acid (Merck), tetrafluoroboric acid (50% in water, Fluka), hexafluorophosphoric acid (Fluka), methanesulfonic acid (Acros), camphorsulfonic acid (Aldrich) were at least of analytical grade and used as received.

2.4. Capillaries

Separation capillaries, 24 cm (19.4 cm to the detector)×50 μm I.D. prepared from bare silica tubings, were purchased from Composite Metal Services (Worcester, UK). New uncoated capillaries were conditioned by performing the following washes: 1 *M* NaOH for 30 min, 0.1 *M* NaOH for 15 min, and water for 2 min. Between each run, the electrolyte in the inlet and outlet vials was replaced in order to limit any siphoning effects. This is due to eventual differences in the liquid levels of the in- and outlet vials, resulting from solvent evaporation during the run. The capillary was rinsed between each run with plain electrolyte for 1 min under a pressure of 10 psi (71.4 kPa).

3. Results and discussion

3.1. Choice of the solvent

The general chemical structures of the *meta–para*-*N*-phenylaniline oligomers in the neutral form (A), the fully protonated form (B), and the radical cationic form (C) are depicted in Fig. 1. The neutral compounds are basic and highly hydrophobic and therefore good candidates for NACE. Despite their low degrees of polymerisation ($n=2–8$), the solubility of the *N*-phenylaniline oligomers is a serious limiting factor in the choice of the solvent. The better solvents for these oligomers are generally chlorinated solvents like chloroform, dichloromethane, but also toluene, THF, and dioxane are potential candidates. Some important physical and chemical properties of these solvents are listed in Table 1. In terms of electron acceptor/donor numbers as defined by Gutmann [21,22], tetrahydrofuran, due to its rather high donor capability, should be a good solvent for the protonated (cationic) form of the *N*-phenylaniline oligomers. On the other hand, the chlorinated solvents have better acceptor capabilities, and therefore should better solubilise the neutral form of the oligomers with the nitrogen lone pairs.

To ensure oligomeric ionisation in such non dissociating solvents ($\epsilon_r<10$), a possible approach is to mix them with a certain amount of an amphiprotic solvent like methanol (MeOH) [23]. In addition, perchloric acid has strong acidic properties in non-aqueous solvents and was expected to sufficiently protonate the analytes. Therefore, THF–MeOH mixtures containing 10 m*M* of perchloric acid were investigated as candidate electrolytes.

Fig. 2 shows the separation of the *N*-phenylaniline oligomers obtained in different THF–MeOH mixtures. Since the dielectric constant decreases upon lowering the percentage of MeOH from 75% (Fig. 2A) to 5% (Fig. 2E), it causes an increase in analysis time as well as a decrease in current intensity. As

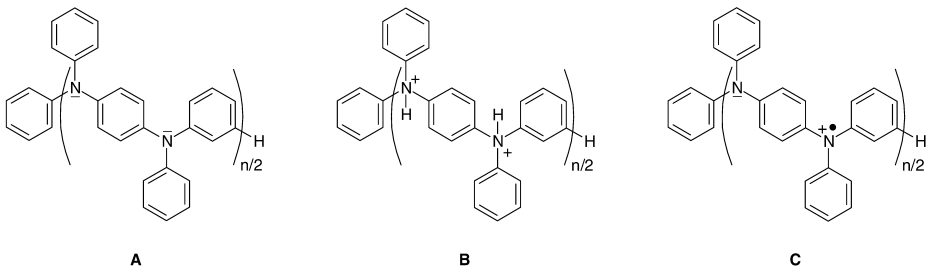

Fig. 1. Chemical structures of the *N*-phenylaniline oligomers studied. (A) Neutral form. (B) Fully protonated form. (C) Cation radical form.

Table 1
Important physical properties of the solvents used in this work

Solvent	ϵ_r	η (cP) at 25°C	UV cut-off (nm)	μ (C m·10^{30})	Polarity	AN[a]	DN[b]
Water	78.4	0.89	<190	6.07	100	54.8	18.0
Methanol	32.7	0.54	205	5.7	76.2	41.3	19.0
Tetrahydrofuran	7.6	0.46	212	5.8	21	8.0	20.0
Chloroform	4.8	0.55	245	3.8	25.9	23.1	n/a
Dichloromethane	8.9	0.44	233	5.2	30.9	20.4	n/a
Dioxane	2.2	1.2	215	1.5	16.4	10.8	14.8

Water is given for comparison. ϵ_r, Dielectric constant; η, viscosity, data from Ref. [17]. UV cut-off data from Ref. [18]. μ, Permanent dipolar moment, data from Ref. [19]. Polarity data from Ref. [20] where water has a reference value of 100.

[a] AN, Acceptor number, as defined by Gutmann, for values and definition see Ref. [20]. By definition the AN of *n*-hexane is 0.

[b] DN, Donor number, as defined by Gutmann, for values and definition see Ref. [21]. By definition the DN of 1,2-dichloroethane is 0.

can be seen from Fig. 2, the best baseline separation was obtained for the 25% MeOH mixture (Fig. 2C). For the *N*-phenylaniline dimer often two separated peaks were found: peak 2 and peak 2* (Fig. 2B–E). At present, there is no clear explanation for this phenomenon. However, the following observations

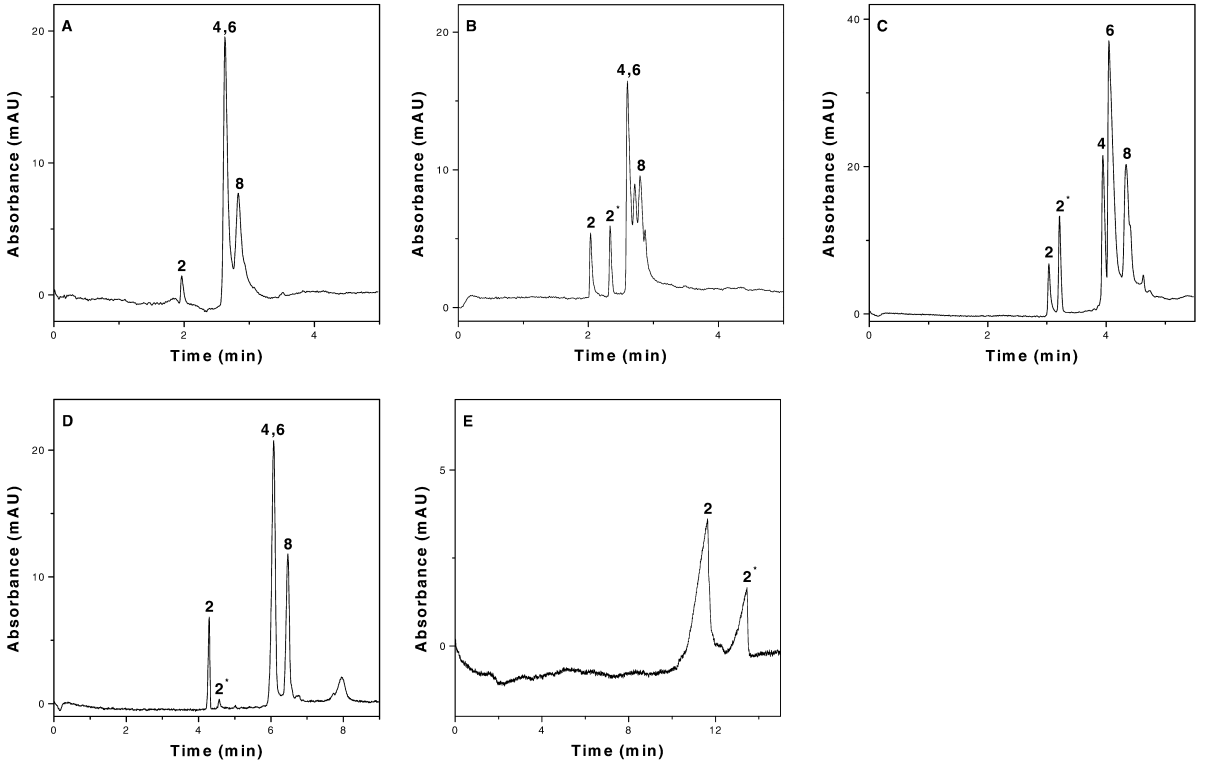

Fig. 2. Electropherograms of the *N*-phenylaniline oligomers obtained with different percentages of MeOH in the THF–MeOH electrolyte. Electrolytes: 10 m*M* perchloric acid in a solvent consisting of: (A) THF–MeOH (25:75) (I=22.6 μA), (B) THF–MeOH (50:50) (I=15.0 μA), (C) THF–MeOH (75:25) (I=8.8 μA), (D) THF–MeOH (85:15) (I=3.3 μA), (E) THF–MeOH (95:5) (I=0.7 μA). Fused-silica capillary, 24 cm (19.4 cm to the detector)×50 μm I.D. Applied voltage: 20 kV (835 V/cm). Sample: 0.5 g/l for each oligomer in chloroform. Hydrodynamic injection: 2 psi s. UV detection at 200 nm. Temperature: 25°C. Identification: *N*-phenylaniline oligomers with *n* is the number of monomeric units. See the text for the peak annotated 2*.

were made for the ratio between the areas of the two peaks. The areas were found to be dependent on the age of the sample (kinetic aspect), since peak 2* tended to disappear in older samples. In addition the nature of the solvent mixture also influenced this ratio.

Next, THF was replaced by chloroform, dichloromethane or 1,4-dioxane. Fig. 3 shows the separations of the *N*-phenylaniline oligomers in chloroform–MeOH (75:25) (Fig. 3A) and in a dichloromethane–MeOH (75:25) (Fig. 3B). Surprisingly, in both cases, peak 2* has completely disappeared. Also no improvement on the resolution in these latter solvents compared to THF was observed. For the 1,4-dioxane–MeOH mixture, no separation could be obtained in a reasonable timeframe (results not shown). This is probably due to a very low ionisation of the oligomers in this electrolyte, since the measured current intensity was only approximately 0.4 μA. As a comparison, the current intensity values in the dichloromethane–MeOH (75:25), THF–MeOH (75:25) and chloroform–MeOH (75:25) mixtures were 12.2, 8.8 and 5.6 μA, respectively. This is in agreement with the dielectric constant of the solvents (Table 1), where the highest current intensity is obtained for the solvent with the highest dielectric constant. It is important to note that electrophoretic migration of solutes is even possible in mixtures containing as high as 95% of solvents usually not considered as potential candidates for CE due to their low dielectric constants.

3.2. Nature of the acid

A major drawback of using perchloric acid as a protonation agent, is the degradation of the *N*-phenylaniline oligomers. Therefore other strong acids in a THF–MeOH (75:25) mixture were also investigated. The values for the current intensity for the various different acids (10 m*M*) are given in Table 2. These results indicate that the current intensity can be used, in first approximation, as an estimation for the strength of the acid dissociation constant in the involved organic solvents. From the investigated acids, only perchloric acid sufficiently protonated under these conditions the *N*-phenylaniline oligomers, resulting in an acceptable separation of these compounds. For the other acids a

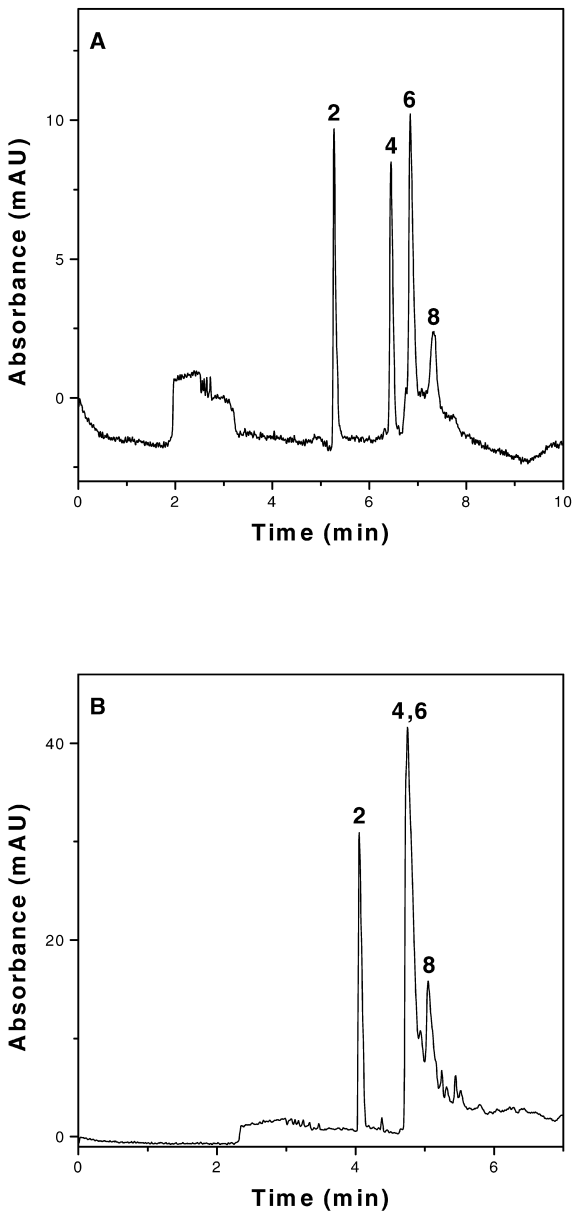

Fig. 3. Influence of non-dissociating solvents in a mixture with methanol on the selectivity of the *N*-phenylaniline oligomers. Electrolytes: 10 m*M* perchloric acid in a solvent consisting of: (A) chloroform–MeOH (75:25) (I=5.6 μA), (B) dichloromethane–MeOH (75:25) (I=12.2 μA). Fused-silica capillary, 24 cm (19.4 cm to the detector)×50 μm I.D. Applied voltage: 20 kV (835 V/cm). Sample: 0.5 g/l for each oligomer in chloroform. Hydrodynamic injection: 4 psi s. UV detection at 210 nm. Temperature: 25°C. Identification: *N*-phenylaniline oligomers with *n* is number of monomeric units. See the text for the peak annotated 2*.

Table 2
Current intensity measured in an electrolyte containing 10 m*M* of the acid in a THF–MeOH (75:25) mixture

	Acid									
	H_3PO_4	TFA	Acetic acid	Camphorsulfonic acid	$CH_3–SO_3H$	HPF_6	HBF_4	HCl	H_2SO_4	$HClO_4$
Current intensity (μA)	0.06[a]	0.08[a]	0.12	0.22	0.36	0.46[b]	0.68	0.76	1.48	8.8
t_{eo} (min)	2	4	3	6	8		5.5	12	20	–[c]

Fused-silica capillary 24 cm (19.4 cm to the detector)×50 μm I.D. Applied voltage: 20 kV (835 V/cm). Temperature: 25°C.
[a] The current intensity is of the same order as the sensitivity limit of the apparatus.
[b] Not completely soluble in the solvent mixture.
[c] The electroosmotic flow is reversed (anodic). For more details, see the text.

large peak was detected close to the EOF peak (t_{eo}) (see Table 2) corresponding to the migration of the oligomers in their neutral form (results not shown). Except for acetic acid and HBF_4, t_{eo} is an increasing function of the current intensity measured under the same conditions. Lister et al. [24] did not report any correlation between measured current intensity and electroosmotic mobility in various pure solvents. However, in the present work, higher current intensities correspond to stronger acids implying lower densities of silanolate on the silica capillary wall. As a consequence, higher current intensities correspond to lower electroosmotic mobilities. From all investigated acids, perchloric acid showed the best results and was used further on this study.

3.3. Reversed electroosmotic flow in THF–methanol mixtures

The electroosmotic mobility in a THF–MeOH (75:25) mixture containing 10 m*M* perchloric acid shows an average value of $-6.5\cdot10^{-9}$ m^2 V^{-1} s^{-1} for a capillary equilibrated for 1 h in this electrolyte. The apparent (m_{ap}) and electrophoretic (m_{ep}) mobilities of the *N*-phenylaniline oligomers were calculated under the same conditions and are listed in Table 3. Obviously, the reversed EOF due to the protonation of the silanol groups cannot be caused by the adsorption of cationic *N*-phenylaniline oligomers on the capillary wall. Since this phenomenon occurs on new capillaries without injection of the oligomer sample. Though theoretically expected in an acidic medium, such a reversed EOF is not commonly observed in absence of a cationic surfactant. A possible explanation might be that, in THF–MeOH, the average pK_a of the silanol groups is shifted to higher values facilitating the protonation of silanols under strong acidic conditions. As a comparison, in electrolytes containing 10 m*M* perchloric acid, Carabias-Marinez et al. [25] reported cathodic EOFs either in methanol or in acetonitrile. In pure THF, Valko et al. [26] also observed a cathodic EOF. Reversed EOFs in NACE are generally observed in methanolic electrolytes and poly(ethylene glycol)-

Table 3
Apparent (m_{ap}) and electrophoretic (m_{ep}) mobilities of the *N*-phenylaniline oligomers

N-Phenylaniline oligomer (*n*)	m_{ap} ($\cdot10^{-9}$ m^2 V^{-1} s^{-1})	m_{ep} ($\cdot10^{-9}$ m^2 V^{-1} s^{-1})
2	13.8	20.3
2*	12.8	19.3
4	9.8	16.3
6	9.3	15.8
8	8.5	15.0

Fused-silica capillary, 24 cm (19.4 cm to the detector)×50 μm I.D. Electrolytes: 10 m*M* perchloric acid in a THF–MeOH (75:25) mixture. Applied voltage: 20 kV (835 V/cm, I=8.8 μA). Sample: 0.5 g/l for each oligomer in chloroform. Hydrodynamic injection: 1 psi s. UV detection at 200 nm. Temperature: 25°C. See text for the peak annotated 2*. The electroosmotic mobility was measured under the same conditions by injection of an acetone–electrolyte (50:50) mixture as an average value of $-6.5\cdot10^{-9}$ m^2 V^{-1} s^{-1}.

coated capillaries. This phenomenon is explained by the preferential adsorption of ammonium cations present in the electrolyte onto the capillary wall [27].

3.4. Ionic strength

After optimisation of the solvent mixture and finding the proper acid, the next step was to optimise the ionic strength for the THF–MeOH (75:25) mixture. Fig. 4 shows the oligomer separations obtained in this mixture containing different concentrations of perchloric acid. Fig. 5A shows the relationship between the apparent mobility (m_{ap}) of the oligomers as a function of the ionic strength. Obviously from these results the best resolution for the oligomer mixture is obtained at a concentration of 10 m*M* perchloric acid. This is also in agreement with Fig. 5B where the relationship of the $\Delta m_{ap}/m_{ap}$ selectivity term as a function of the ionic strength is plotted [28]. A detailed examination of the electropherograms of Fig. 4D–F shows a slight perturbation of the baseline, indicated with an arrow. This increase of the baseline at higher acid concentrations is due to a stepwise increase of the current intensity. The higher the ionic strength, the longer it takes for the current intensity to reach a steady state. Therefore, it is assumed that the EOF decreases with increasing perchloric acid concentration. A possible explanation might be that the equilibrium time required to reach a stable current intensity corresponds to the time needed to push the non-conductive chloroform sample zone out of the capillary due

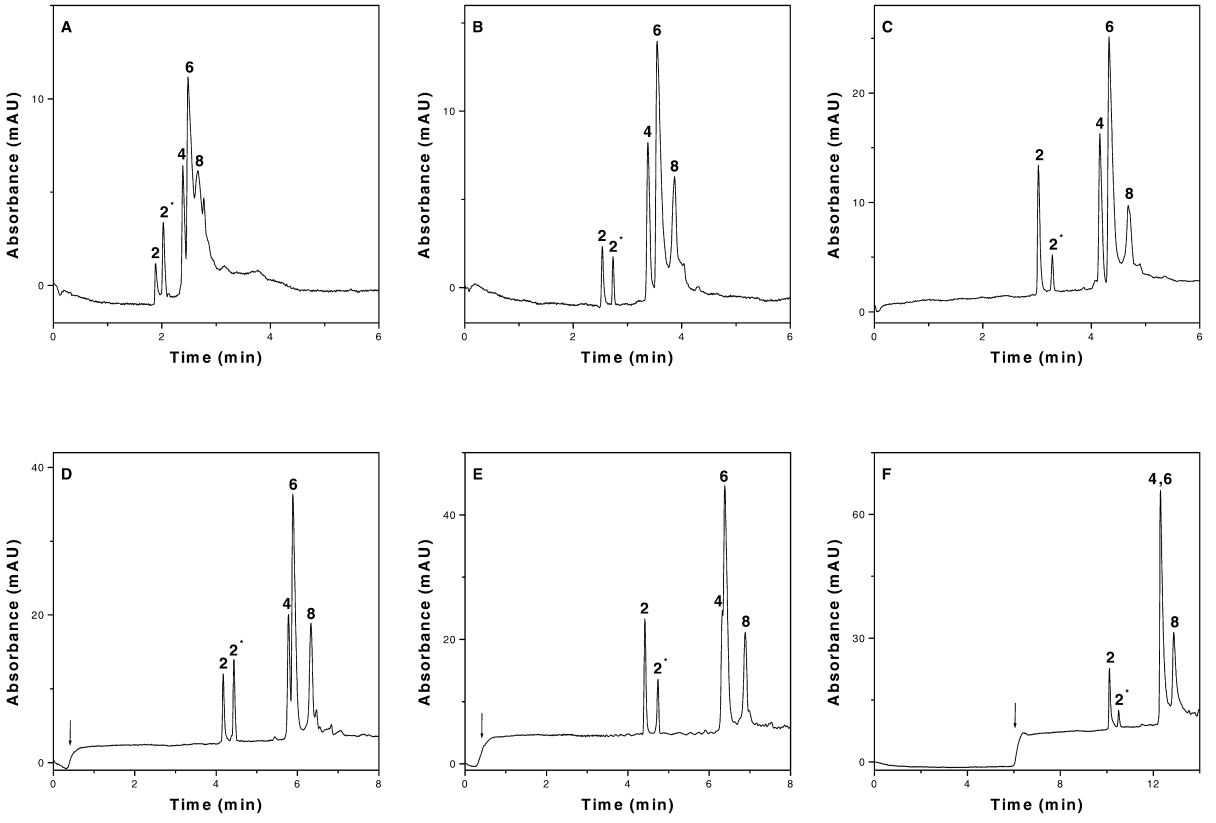

Fig. 4. Influence of the ionic strength on the electropherograms of the *N*-phenylaniline oligomers obtained in a THF–MeOH electrolyte. Electrolytes: THF–MeOH (75:25) mixture containing: (A) 1.3 m*M* perchloric acid (I=1.9 μA), (B) 5.2 m*M* perchloric acid (I=4.7 μA), (C) 10 m*M* perchloric acid (I=8.8 μA), (D) 31 m*M* perchloric acid (I=20 μA), (E) 52 m*M* perchloric acid (I=32 μA), (F) 75 m*M* perchloric acid (I=47 μA). Other conditions as in Fig. 2.

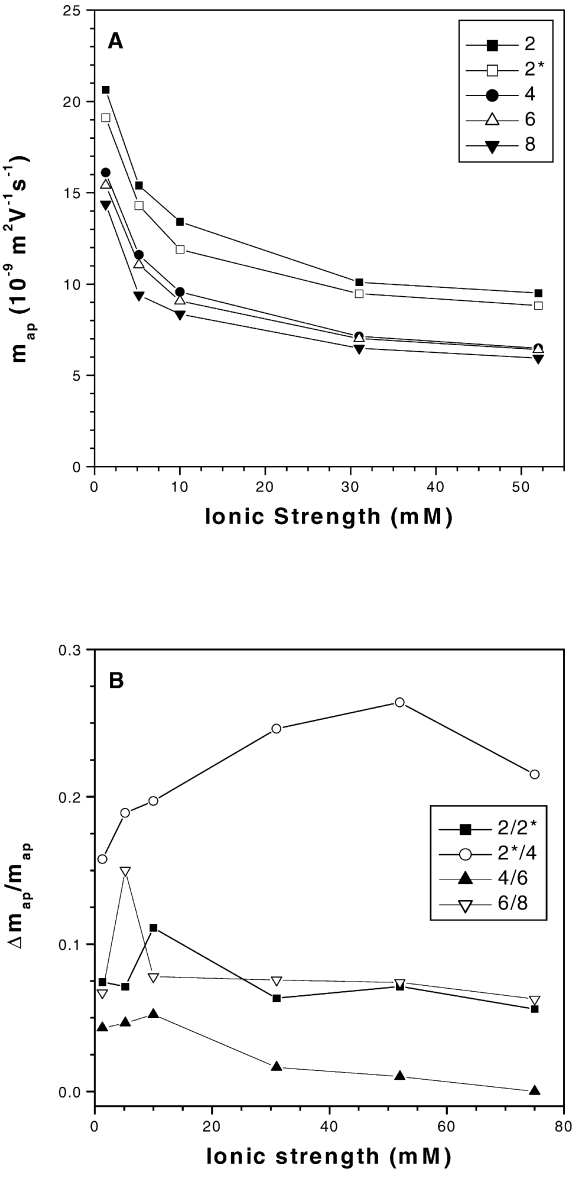

Fig. 5. Relationship between the apparent mobility (A) and the $\Delta m_{ap}/m_{ap}$ selectivity term (B) as a function of the ionic strength of perchloric acid in a THF–MeOH (75:25) electrolyte. For conditions see Fig. 4.

to the anodic EOF. As a consequence this baseline shift was taken as the origin of the time for the calculation of the apparent mobilities in Fig. 4.

3.5. Injection time

Fig. 6 displays the influence of the injected volume on the electropherograms for the *N*-phenylaniline oligomers separation at injection times varying between 1 and 5 s at a constant pressure of 1 psi (7.14 kPa). For an electrolyte viscosity of around 0.55 cP (see Table 1), a 1-s injection into the 24 cm long capillary introduces a volume of about 9 nl (total volume of the capillary=470 nl). Due to the low viscosity of the electrolyte and the short capillary, this injected volume results in a rather long sample zone of 0.5 cm (2% of the capillary length). Unfortunately, it was not possible to use a lower pressure on this instrument. Considering an average reversed electroosmotic mobility of $-6.5\cdot10^{-9}$ m^2 V^{-1} s^{-1}, the linear electroosmotic speed is about 0.055 cm s^{-1} under the applied field of 20 kV. Thus, it takes about 9 s for the EOF to eject the 1s injected zone of the non-conductive chloroform sample zone out of the capillary. For larger volumes, e.g., in the case of a 5-s injection zone, the time needed to push that sample zone out of the capillary amounts more than 45 s. In this latter case the strong perturbation of the EOF is caused by the 2.5 cm long chloroform sample zone. This is also in agreement with the findings of Valko et al., who measured electroosmotic mobilities of nearly zero in inert solvents like chloroform or hexane [26]. It is obvious from Fig. 6 that injections larger than 2 psi s cause a decrease in resolution.

3.6. Electric field

Fig. 7 shows the influence of electric field strengths between 208 V/cm and 1250 V/cm on the separation of the *N*-phenylaniline oligomers. Clearly, at the highest electric field strength of 1250 V/cm, the resolution of the oligomers (n=4–8) is somewhat lower than at 835 V/cm (Fig. 4B). On the other hand, at both low electric field strengths (Fig. 7A and B), the separation ends up in deteriorated peaks. This is probably due to degradation of the analytes under the oxidative conditions of the electrolyte in combination with the longer analysis times. Thus, the separation has to be fast enough to avoid such degradation. The electrophoretic mobilities of the *N*-phenylaniline oligomers as a function of the electric field strength were similar (results not shown), indicating a normal electrophoretic behaviour of the compounds without any orientation of the oligomers in the direction of the electric field.

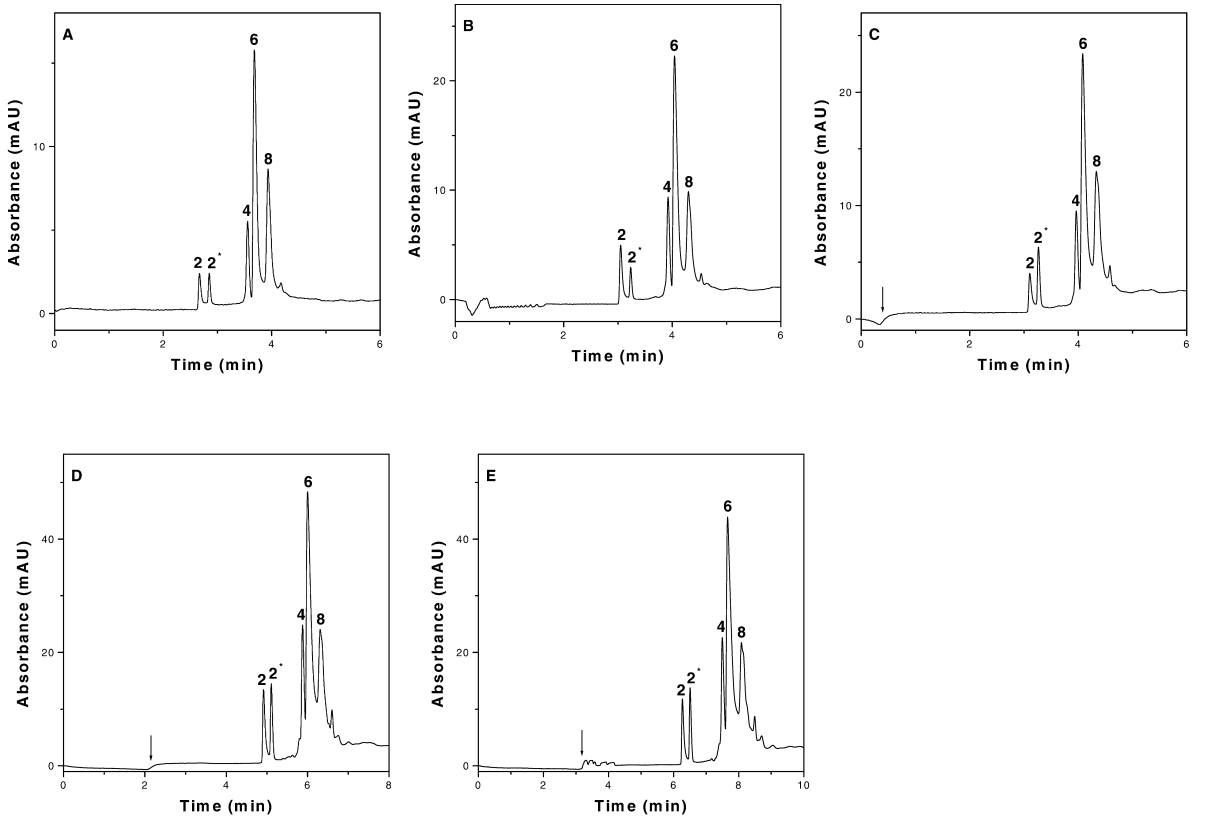

Fig. 6. Influence of the injected volume on the separation of *N*-phenylaniline oligomers. Hydrodynamic injection: (A) 1 psi s, (B) 2 psi s, (C) 3 psi s, (D) 4 psi s, (E) 5 psi s. Electrolytes: 10 m*M* perchloric acid in a THF–MeOH (75:25) solvent. Other conditions as in Fig. 2.

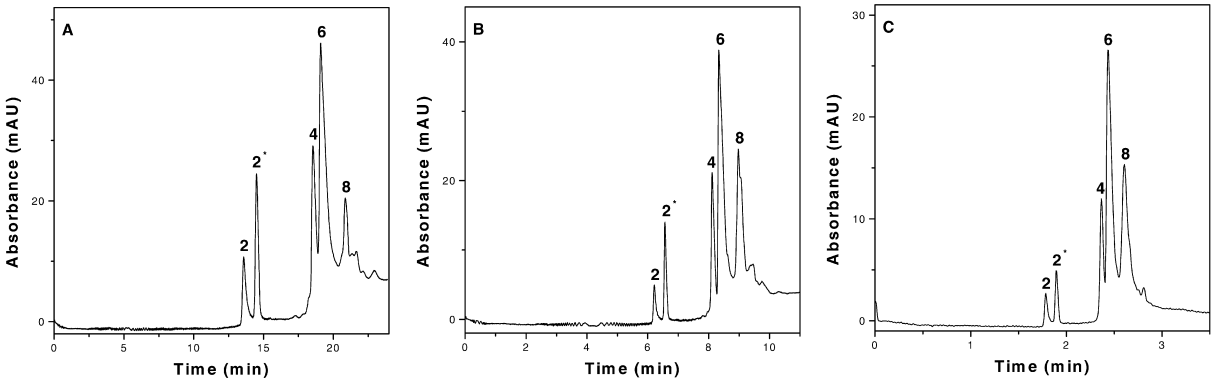

Fig. 7. Influence of the electric field strength on the separation of *N*-phenylaniline oligomers. Applied voltage (electric field): (A) 5 kV (208 V/cm), (B) 10 kV (417 V/cm), (C) 30 kV (1250 V/cm). Electrolytes: 10 m*M* perchloric acid in a THF–MeOH (75:25) solvent. Other conditions as in Fig. 2.

3.7. Temperature

Finally, the influence of the temperature on the separation was also investigated. Fig. 8 shows two electropherograms showing the separation of three oligomers (n=2, 6, 8) at 15°C (Fig. 8A) and at 45°C (Fig. 8B). At 15°C, the increase of the viscosity results in a longer separation time. Moreover, at the end of the run the shape of the peaks, especially of the octamer, were also deteriorated. As already discussed in Section 3.6 this must be attributed to degradation effects due to the longer analysis times. In contrast, at 45°C, the separation was fast and resulted in well resolved peaks. Clearly higher temperatures are in favour of better resolution.

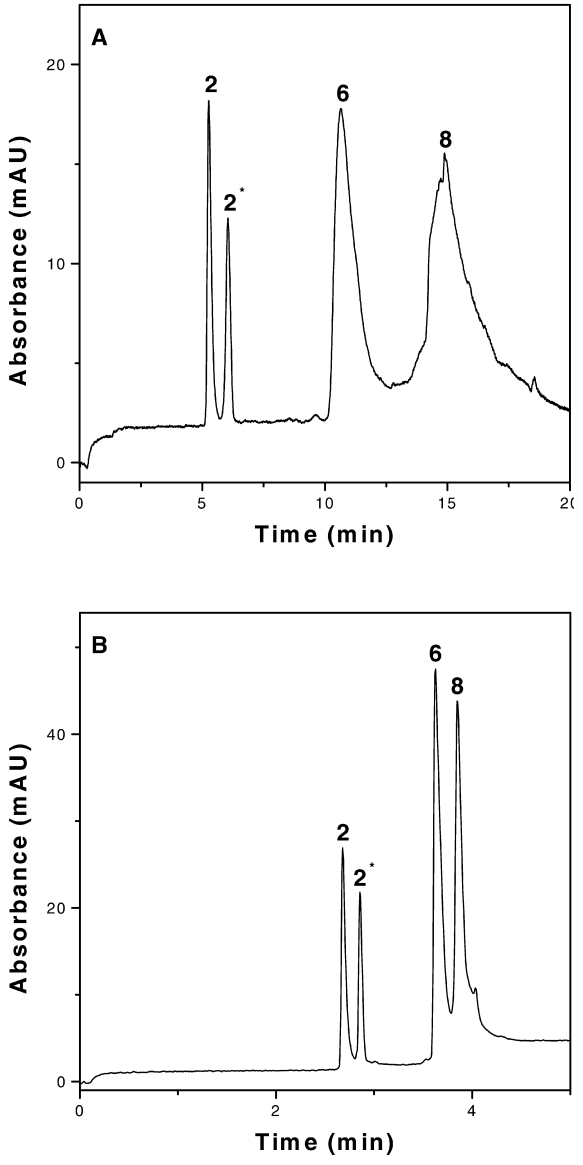

Fig. 8. Influence of the temperature on the separation of *N*-phenylaniline oligomers. Temperature: (A) 15°C, (B) 45°C. Electrolytes: 10 m*M* perchloric acid in a THF–MeOH (75:25) solvent. Other conditions as in Fig. 2.

3.8. Discussion

A possible explanation for the separation obtained in an electrolyte containing perchloric acid might be that free (or solvated) protons of the electrolyte migrate in the capillary in the direction of the outlet end (cathode) passing through the injected chloroform zone containing the *N*-phenylaniline oligomers in their neutral form, and protonate the amines. At the same time, the reversed EOF (anionic) expulses the non-conductive chloroform zone out of the capillary resulting/helping in stabilisation of the current intensity. A similar scenario can be imagined in the case of a large sample volume in which the positively chargeable analytes are stacked. In this case the reversed EOF is due to the addition of a cationic surfactant [29]. The here observed reversed EOF, resulting from the protonation of the silanol groups, can also limit the adsorption of *N*-phenylaniline oligomers onto the capillary wall.

Reconsideration of the issue on protonation of the oligomers in the electrolyte, whether the poly(*N*-phenylaniline) are fully charged as shown in Fig. 1B, or only partially charged, results in the following two extreme cases. If the *N*-phenylaniline oligomers bear only one charge, for example at one end of the molecule, then their electrophoretic mobility is supposed to decrease with the degree of polymerisation (n) as experimentally was observed. But if the oligomers are fully charged, then the electrophoretic mobility is expected to increase with n, as has already been reported for the migration of small ($n<10$) poly(styrenesulfonates) oligomers in water-based electrolytes [30]. Therefore, we assume that the *N*-phenylaniline oligomers are not fully charged under the applied conditions.

4. Conclusion

The results from this study show that NACE can be used to separate hydrophobic aniline oligomers in mixtures consisting mainly of non-dissociating solvents with low dielectric constants. However, to obtain electrophoretic migration an amphiprotic solvent, e.g., methanol must be added to increase the dielectric constant of the electrolyte solution. Solvents in which the oligomers dissolve well, like, e.g., dichloromethane and chloroform, proved to be far from optimal for the preparation of background electrolytes. From the investigated solvents, THF proved to be the most suitable for the separation of this type of oligomers. To create protonation of the compounds the addition of an acid is necessary. From the investigated acids, perchloric acid was the best dissociating agent in THF–MeOH mixtures. Optimal separation of the aniline oligomers was obtained at a concentration of 10 m*M* perchloric acid. However, short analysis times must be used in order to avoid peak deterioration under the oxidative perchloric acid conditions. Therefore, high electric field strengths are preferred to obtain undisturbed electropherograms. It was found out that injection volumes up to 18 nl (4% of the capillary length) of nearly non-conductive sample solvent can be used without sacrificing resolution. Obviously high temperatures are more favourable to obtain fast and especially well resolved non-deteriorated electropherograms. Finally, a qualitative explanation of a migration mechanism is proposed.

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