



# Effect of background electrolyte anions on markers of electroosmotic flow in capillary electrophoresis

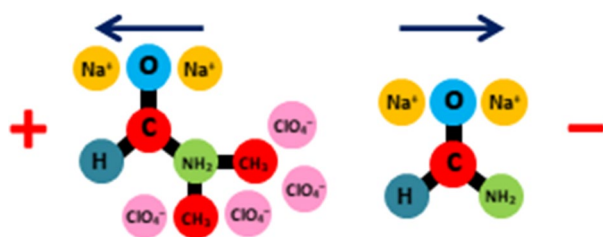
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## Abstract

Accurate knowledge of the electroosmotic flow mobility is crucial for the determination of effective electrophoretic mobilities in capillary electrophoresis. The addition of a neutral marker to the sample is a common method for electroosmotic flow mobility measurement. Nevertheless, the neutral marker can also exhibit electrophoretic mobility due to its interactions with background electrolyte constituents. In our previous study, we have shown the effect of different cations in background electrolytes with a fixed anion. In this work, the relative mobility of neutral compounds, either those commonly used as electroosmotic flow markers or their structurally related molecules, was measured in the presence of various anions in the background electrolyte with sodium as a fixed cation at pH 4.5. The mobility of eight compounds, formamide, *N*-methylformamide, *N,N*-dimethylformamide, acrylamide, *N,N*-dimethylacrylamide, *N*-methylacetamide, mesityl oxide, and dimethyl sulfoxide, was related to the mobility of thiourea. The studied anions were chloride, perchlorate, sulfate, and acetate. The results revealed a dramatic effect of perchlorate. The interaction with perchlorate strengthens significantly with the increasing number of non-polar moieties, such as methyl or acryl, present in the marker molecule. On the other hand, sulfate showed a very weak effect on all the studied compounds.

## Graphic abstract



**Keywords** Capillary electrophoresis · Electroosmotic flow · Electrophoretic mobility · Neutral markers

## Introduction

Accurate electroosmotic flow (EOF) measurement is extremely important in capillary electrophoresis because the value is used in various physicochemical calculations [1–3]. It is also important in analytical chemistry because the identification of analytes can be based on their effective mobility

values. The commonly used method of EOF mobility measurement is the addition of an electrically neutral substance to the injected sample. The EOF mobility is then calculated from the migration time of the marker, which is expected to move with the EOF velocity. Although several methods for EOF mobility determination are known [4–8], the method of addition of an electrically neutral substance to the sample is inexpensive, simple, and fast. Using this method the electroosmotic flow mobility can be easily measured for each analysis and thus the method is still most used. However, it turns out that neutral substances do not necessarily move exactly at the EOF velocity because they can acquire their

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own electrophoretic mobility through their interactions with background electrolyte constituents.

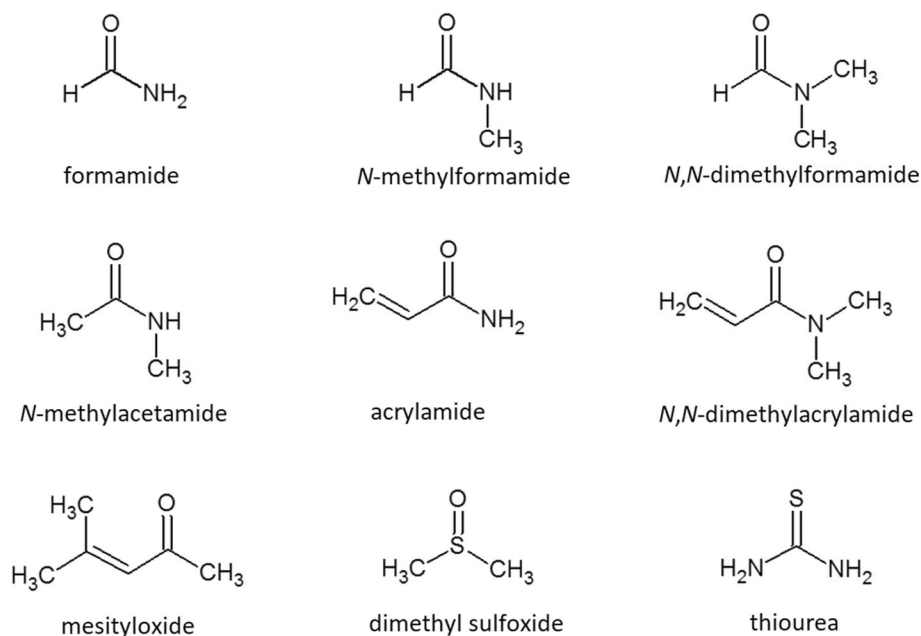
Several studies concerning such mobilization of large neutral molecules have already been published [9–11]. On the other hand, Müllerová et al. observed effective mobility of small EOF markers caused by their interactions with charged complexing agents [12]. It turns out that, apart from these rather specific cases, even small neutral molecules interact with inorganic ions commonly used as background electrolyte constituents. In one of our previous studies, we showed that the commonly used EOF markers, thiourea and dimethyl sulfoxide, exhibit non-zero electrophoretic mobility values and we measured their relative electrophoretic mobility in a set of Hofmeister series anions [13]. In our last work, we have studied the effect of background electrolyte cations on the mobility of 11 EOF markers [14]. We measured their relative electrophoretic mobilities in combination with five background electrolyte cations and revealed the effect of cation's charge-to-size ratio, the effect of methylation of the marker molecule, and the effect of the marker's central atom and geometry.

In this study, we turned our attention towards the effect of anions. We measured relative mobilities of eight neutral compounds in four background electrolytes consisting of sodium acetate buffer, pH 4.5 and its mixtures with solutions of sodium chloride, perchlorate, and sulfate.

## Results and discussion

Relative mobilities of eight neutral compounds were studied in this work, for their structures, see Fig. 1. Electrophoretic measurements were performed in background electrolytes of pH 4.5, which suppressed the EOF and thus allowed the studied compounds to reside inside the capillary during the whole 1-h experiment. Due to the lack of a simple buffering cation for pH 4.5, acetate anion was used to maintain the acidity. Background electrolytes were thus composed of four volume parts of the studied salt solution and one volume part of sodium acetate buffer, pH 4.5. As the mobilization effects strengthen with increasing concentration of the background electrolyte ions [13], a relatively high ionic strength of  $250 \text{ mmol dm}^{-3}$  was used to emphasize the observed effects. It is important to note that, as discussed in our previous work [14], all the mobility values reported were related to thiourea as a reference marker. Regarding the fact that sodium cation was used in all background electrolytes, the mobilization effects of the studied anions can be compared with each other as well as the relative strength of the mobilization effect on individual markers can be evaluated even though the absolute mobilities of the studied neutral compounds remained unknown. The measured values of the relative mobilities are given in Table 1. In most cases, the relative mobilities were positive, i.e., the studied marker passed through the detector before thiourea. All the studied markers contain the highly electronegative oxygen atom in their structures and thus are mobilized by sodium cations more than thiourea, in which the oxygen atom is replaced by a less electronegative sulfur atom. In several cases, the

**Fig. 1** Structures of the studied electroosmotic flow markers and related molecules



**Table 1** Relative mobilities of studied neutral compounds in pure sodium acetate buffer and sodium acetate buffer mixed with sodium chloride, perchlorate, and sulfate in a ratio of 1:4 (v/v), pH=4.5,  $I=250 \text{ mmol dm}^{-3}$ 

Marker	Pure buffer	Chloride	Perchlorate	Sulfate
Formamide	$7.7 \pm 0.2$	$8.7 \pm 0.8$	$5.0 \pm 0.5$	$12.0 \pm 0.8$
Acrylamide	$1.3 \pm 0.1$	$3.8 \pm 0.1$	$4.7 \pm 0.2$	$9.8 \pm 0.4$
<i>N</i> -Methylformamide	$3.2 \pm 0.1$	$4.8 \pm 0.8$	<LOO <sup>a</sup>	$10.0 \pm 1.3$
<i>N</i> -Methylacetamide	<LOO <sup>a</sup>	$1.7 \pm 0.2$	$-2.1 \pm 0.2$	$9.5 \pm 0.3$
<i>N,N</i> -Dimethylformamide	$2.3 \pm 0.1$	$4.2 \pm 0.6$	$-6.2 \pm 0.5$	$12.3 \pm 0.3$
<i>N,N</i> -Dimethylacrylamide	<LOO <sup>a</sup>	$2.7 \pm 0.0$	$-7.5 \pm 0.2$	$11.1 \pm 0.7$
Mesityl oxide	$-1.3 \pm 0.0$	$2.7 \pm 0.1$	$-6.9 \pm 0.2$	$10.8 \pm 0.3$
Dimethyl sulfoxide	$3.0 \pm 0.2$	$3.5 \pm 0.4$	$-4.1 \pm 0.1$	$12.1 \pm 0.4$

The values of relative mobilities are expressed as median  $\pm$  95% confidence interval,  $\alpha=0.05$ ,  $n=3$

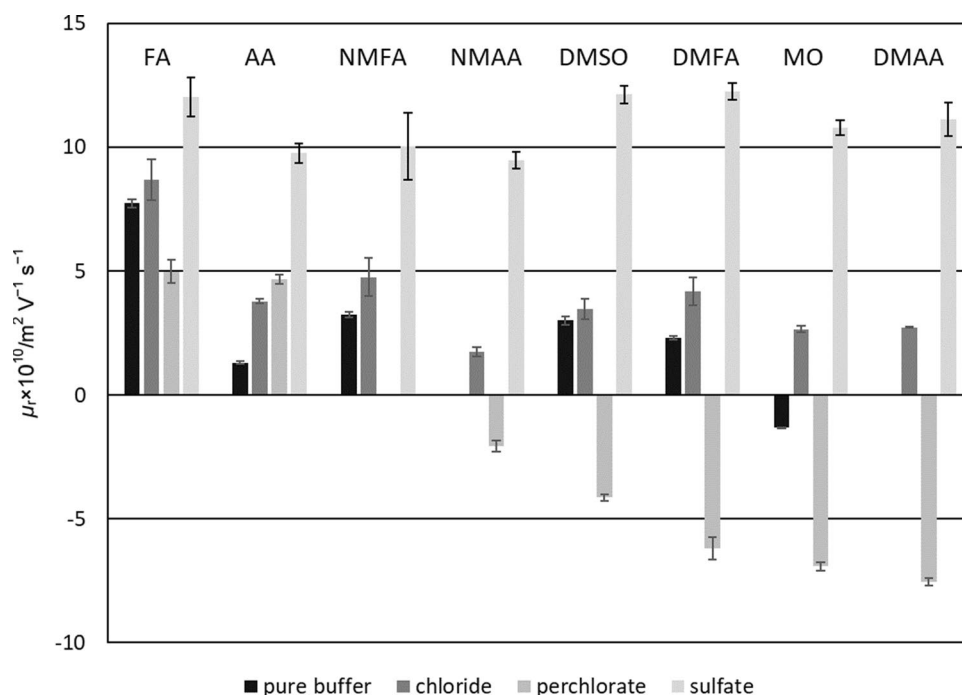
<sup>a</sup><LOO=below the limit of observability, peaks were insufficiently separated

marker passed through the detector after thiourea, which is represented by negative relative mobility values. With one exception, this happened only in the case of perchlorate anions present in the background electrolyte. As can be seen from Fig. 2, the chosen combination of marker and background electrolyte anion has a great influence on the result. The difference of relative mobilities reaches up to  $2 \times 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ .

The most striking results were obtained for the perchlorate-containing background electrolyte. The relative mobility spanned from a relatively high positive value of  $5.0 \pm 0.5 \times 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$  obtained for formamide to  $-7.5 \pm 0.2 \times 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$  for *N,N*-dimethylacrylamide. A clear trend can be observed for the series of amides. The relative mobility value decreased gradually with the increasing number of non-polar moieties, i.e., methyl, acryl, and 2-methylprop-1-enyl. Formamide with none of these moieties showed the highest mobility and thus the weakest interaction with perchlorate anion. Acrylamide containing only one acryl group remained in positive relative mobility values. *N*-Methylformamide with one methyl group on amide nitrogen comigrated with thiourea. The rest of the amides showed negative values in the order of *N*-methylacetamide (two methyls), *N,N*-dimethylformamide (two methyls), *N,N*-dimethylacrylamide (two methyls, one acryl). The two non-amide markers fitted well in this trend. Mobility of dimethyl sulfoxide (two methyls) lies between the mobility of *N*-methylacetamide and *N,N*-dimethylformamide. Mobility of mesityl oxide (one methyl, one 2-methylprop-1-enyl) lies between *N,N*-dimethylformamide and *N,N*-dimethylacrylamide. With the increasing number of methyl or other non-polar moieties and thus with the growing non-polar part of the molecule, stronger interactions with perchlorate ions occur. This is in agreement with the previous findings of the strong affinity of perchlorate anion towards non-polar parts of the molecules [13].

Relative mobilities in the presence of chloride anions are close to those obtained in pure acetate buffer. The results

**Fig. 2** Relative mobilities of studied neutral compounds in pure sodium acetate buffer and sodium acetate buffer mixed with sodium chloride, perchlorate, and sulfate in a ratio of 1:4 (v/v), pH 4.5,  $I=250 \text{ mmol dm}^{-3}$ . Medians of three replicate measurements are plotted. Error bars show 95% confidence intervals. Abbreviations: FA (formamide), NMFA (*N*-methylformamide), DMFA (*N,N*-dimethylformamide), NMA (*N*-methylacetamide), AA (acrylamide), DMAA (*N,N*-dimethylacrylamide), DMSO (dimethyl sulfoxide), TU (thiourea)



suggest that the markers, in general, interact weaker with chloride ions than with acetate ions and thus have slightly higher values of relative mobility. From the tested anions, sulfate exhibits the weakest interactions with all the studied markers as their relative mobilities in the sulfate-containing background electrolyte are similar to each other, close to the value of  $1 \times 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ . Compared to thiourea, all of them are more positively mobilized by sodium cations and thus their relative mobility acquires high positive values in all cases.

It is noteworthy that the order of marker-anion interaction strength shows agreement with the order of the tested anions in the Hofmeister series [15]. The order of the anions in this historical series is  $\text{SO}_4^{2-} > \text{CH}_3\text{COO}^- > \text{Cl}^- > \text{ClO}_4^-$ . Kosmotropic sulfate exhibits the weakest interactions with the studied neutral compounds while chaotropic perchlorate, standing at the opposite side of the series, exhibits the strongest interactions. Acetate and chloride anions, standing in between in the Hofmeister series show moderate interactions with the studied compounds.

## Conclusion

We have studied the mobilization effects of four inorganic anions on eight neutral species, several commonly used electroosmotic flow markers, and their structurally related compounds. We have revealed that the electrophoretic mobilities related to thiourea as a reference marker can span in the range of  $2 \times 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ , which is a value that cannot be neglected. A proper choice of the background electrolyte anion—electroosmotic flow marker combination is thus important for accurate mobility measurements in capillary electrophoresis. The strength of the negative mobilization effect of the studied anions is roughly in agreement with their position in the Hofmeister series. Kosmotropic sulfate exhibits a very weak effect on all the studied compounds while in chaotropic perchlorate the relative mobility shifts from positive values for compounds with a low number of non-polar moieties to negative values for compounds with a high number of methyl or other non-polar groups.

## Experimental

Measurements were performed on a 1600 CE instrument (Agilent Technologies, Germany) with a diode-array detector operating at 200, 214, and 254 nm wavelengths. ChemStation software (Agilent Technologies, Germany) was used for instrument control and data evaluation. Measurements were conducted in a fused-silica capillary of 50  $\mu\text{m}$  inner diameter, a total length of 80.0 cm, and an effective length of 71.5 cm (Polymicro Technologies, USA). Capillary was

thermostated at 25 °C with air. Substances were weighted on Precisa 262 SMA-FR (Precisa Gravimetrics AG, Switzerland). For pH measurement, a 3540 pH and conductivity meter (Jenway, Great Britain) was used.

Acetic acid 99% (Lach-Ner, Czech Republic) and sodium hydroxide (Penta, Czech Republic) were used for buffer preparation. All chemicals used for salt solutions, i.e., sodium chloride, sodium sulfate, and sodium perchlorate and all chemicals used as markers, i.e., formamide, *N,N*-dimethylformamide, *N*-methylformamide, acrylamide, *N,N*-dimethylacrylamide, *N*-methylacetamide, mesityl oxide, dimethyl sulfoxide, and thiourea were purchased from Lach-Ner, Czech Republic. All chemicals were of analytical grade purity. Deionized water was prepared by a Milli-Q water purification system (Millipore, USA).

## Background electrolyte and marker solutions

Background electrolytes were prepared by mixing acetate buffer (590 mM acetic acid, 250 mM sodium hydroxide, pH = 4.5,  $I = 250 \text{ mmol dm}^{-3}$ ) with the appropriate salt solution in a 1:4 ratio (*v/v*). The salt solutions used ( $I = 250 \text{ mmol dm}^{-3}$ ) were 250 mM sodium chloride, 250 mM sodium perchlorate, and 83 mM sodium sulfate.

Liquid markers were diluted with water in the 1:500 ratio (*v/v*). Solid markers were dissolved in water and diluted to a concentration of  $1.0 \text{ mg cm}^{-3}$ . Before measurement, the marker solution was further diluted to the final 10 mM concentration.

## Method

The method was adopted from the previous study [14]. Before each measurement, the capillary was washed with 1.0 M sodium hydroxide for 5 min, with deionized water for 5 min, and with BGE for 2 min by a pressure of 93 kPa. A sample containing the studied marker and thiourea was injected hydrodynamically for 5 s by a pressure of 5 kPa. A voltage of 15 kV was applied for 60 min. During each measurement, vials with background electrolyte were changed every 15 min for fresh ones. When the voltage was switched off a reference sample containing thiourea was injected hydrodynamically for 5 s by a pressure of 5 kPa. Then the whole system was mobilized by a pressure of 5 kPa until the zone of tested marker and both zones of thiourea passed through the detector. For each combination of marker and BGE anion measurement was performed three times.

The mobilities of markers are in this work related to thiourea as a reference marker. From each electropherogram recorded at 200 nm wavelength, migration time of the studied marker, thiourea, and the additionally injected thiourea zone were subtracted. Electropherograms obtained at 214 nm and 254 nm were used for confirmation of peak

identity as migration order or the studied marker and thiourea was reversed in some cases. Relative mobilities of markers were calculated according to the following formula:

$$\mu_{\text{rel}} = \frac{(t_{\text{TU1}} - t_{\text{M}})l_{\text{d}}l_{\text{c}}}{t_{\text{TU2}}t_{\text{U}}U} \quad [\text{m}^2\text{V}^{-1}\text{s}^{-1}],$$

where  $t_{\text{TU1}}$  [s] is the migration time of the thiourea injected together with the studied marker,  $t_{\text{M}}$  [s] is migration time of the studied marker,  $t_{\text{TU2}}$  [s] is migration time of the second zone of thiourea,  $l_{\text{d}}$  [m] is the effective capillary length,  $l_{\text{c}}$  [m] is total capillary length,  $t_{\text{U}}$  [s] is time for which the voltage was applied and  $U$  [V] is the value of the applied voltage. The migration time was measured from the moment when the external voltage was first applied.

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