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# Electrophoretic separations with polyether ether ketone capillaries and capacitively coupled contactless conductivity detection

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## Abstract

Polyether ether ketone (PEEK) capillaries were found to be compatible with capacitively-coupled contactless-conductivity detection (C<sup>4</sup>D). Detection limits in the order of  $10^{-7}$  M were obtained with C<sup>4</sup>D employing a high excitation voltage (HV-C<sup>4</sup>D) for inorganic cations and anions. The organic polymer capillary shows a relatively low electroosmotic flow of  $2.6 \cdot 10^{-4}$  cm<sup>2</sup> V<sup>-1</sup> s<sup>-1</sup>. Thus inorganic anions and slower organic anions can be separated with a PEEK capillary in a single run without flow modifier. This feature also enables the analysis of both, cations and (fast and slow) anions, in a sample in two subsequent runs just by reversing the polarity or in a single run if dual opposite end injection is employed.

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## 1. Introduction

Capillary electrophoresis is commonly carried out in capillaries made from fused-silica, and it might be even argued that the commercial introduction of these capillaries enabled electrophoresis in its modern form. Fused silica capillaries however, possess charged silanol groups on the surface which causes a pronounced electroosmotic flow (EOF) in the cathodic direction. Therefore only anions with sufficiently high mobility to overcome the electroosmotic flow can be determined by migration to the anode. For anionic species with low mobility the separation in untreated fused-silica capillaries is only possible

by allowing them to be swept towards the cathode by the electroosmotic flow. The charged surface sites can also lead to unwanted interactions with the analytes (e.g. with proteins). The degree of deprotonation of the silanol groups is dependent on the pH-value and this poses a particular problem when analytes are to be determined which require basic conditions.

In order to reduce, remove or reverse the surface charge on fused-silica, different methods to modify the capillary permanently via in situ polymerization procedures have been described [1]. These modifications are fairly elaborate and therefore costly and avoided if possible. For the determination of anions, dynamic coating procedures are routinely employed in order to obtain conditions useful for a broad range of species with different mobilities. These methods rely on the inclusion of a surface active compound

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(such as a quaternary amine) in the running buffer to reverse the polarity of the surface charge and hence the direction of the electroosmotic flow.

This approach has the consequence that if in a single sample both cations and anions are to be determined an intervening change of buffer solutions in the capillary is necessary. In practice this not only requires flushing of the capillary and all containers but also a prolonged conditioning period. Several approaches have been described to address this limitation of fused-silica. These include the use of complexing agents to form negatively charged chelates with metal ions which are separated with other anions (see for example Refs. [2,3]). Bächman and coworkers achieved the concurrent detection of anions and cations by loading the sample simultaneously into two capillaries, each of which is fitted with its own detector [4]. A related, but instrumentally simpler, approach is the use of dual injection into a single capillary from both ends with a single detector located at the centre of the capillary [5–9]. Detection was carried out by indirect UV-absorption and in order to make this method suitable for more than just a few anions with the highest mobilities, it was necessary to suppress the electroosmotic flow.

Unlike fused-silica, polymer capillaries do not possess intrinsically charged surface groups. In an early publication Lukacs and Jorgenson reported that the electroosmotic flow is indeed much reduced, albeit not eliminated, in capillaries made from polytetrafluoroethylene [10]. The remaining electroosmotic flow in the polymer was attributed to the establishment of a zeta-potential by adsorption of anionic species on the surface. More recently, capillaries made from poly(methyl methacrylate) (PMMA) [11–13] and polyether ether ketone (PEEK) [13,14] and other materials [11,12] have been investigated. These capillaries also show a relatively low electroosmotic flow, but detection by the usual method of UV-absorption is impaired because of the lack of optical transparency at short wavelengths. In any case, polymeric capillaries have not been widely available and have rarely been used.

In a different development, capacitively-coupled contactless-conductivity detection ( $C^4D$ ) has recently been introduced to capillary electrophoresis [15,16]. In comparison to conventional conductivity detection, the arrangement has the advantage of easier

alignment with the capillary and the inherent prevention of electrode fouling [17]. It has been shown that by the use of a high a.c. voltage (several hundred volt) for excitation (HV- $C^4D$ ), detection limits in the order of  $10^{-7} M$  can be achieved for small ions [18]. Unterholzner et al. [19], as well as Kuban et al. [20] have very recently also demonstrated that contactless conductivity detection may be used for concurrent cation and anion determination when using the dual opposite end injection approach.

The performance and ease of use establishes  $C^4D$  as a serious contender to the now prevalent optical detection methods, in particular for the determination of small inorganic ions. Also, PEEK capillaries of dimensions suitable for capillary electrophoresis have recently become readily available. Lack of optical transparency is not a hindrance in the use of this material with conductivity detection. The aim of the work presented herein was therefore to investigate the performance of the combination of the HV- $C^4D$  system with PEEK capillaries. It was hoped that it would be possible to carry out anion separations without an EOF modifier. This then would allow to use the same set of conditions for the determination of cations and anions by simply changing the polarity of the separation voltage.

## 2. Experimental

### 2.1. Instrumentation

Separations were performed on a purpose made capillary electrophoresis set-up comprising of an injection compartment made of Perspex and a detection section housed in a Faraday cage in order to minimise electrical interferences. A high-voltage power supply with reversible polarity (CZE1000R, Start Spellman, Pulborough, UK) was used for the electrophoretic separations. The detector cell is based on two steel tubes of 4 mm length which form the excitation and pick-up electrodes on the separation capillary. These are housed in a small metal case and are separated from each other by a gap of 2 mm. An internal Faraday shield is mounted between the two electrodes in order to prevent direct capacitive coupling. An operational amplifier (OPA 627, Texas Instruments, Dallas, TX, USA) in the current-to-

voltage configuration (feedback resistor: 820 k $\Omega$ ) is mounted in close proximity to the pick-up electrode. The external electronics consist of a conventional stand-alone function generator and a high voltage operational amplifier (PA94, Apex Microtechnology, Tucson, AZ, USA), to obtain a sine-wave with a peak-to-peak voltage of 450 V at 100 kHz for excitation, and a synchronous detector as well as gain and offset stage at the pick-up side. The detector arrangement is a slight modification of an earlier design employed in our laboratory and more details may be found in our previous report [18].

## 2.2. Materials and methods

All chemicals were of analytical reagent grade and were supplied by Fluka (Buchs, Switzerland) with the exception of potassium bromide, potassium fluoride and tri-sodium phosphate, which were purchased from Merck (Darmstadt, Germany). The electrolyte solution was composed of 2-morpholinoethanesulfonic acid (MES), DL-histidine (His) in equimolar portions and 18-crown-6, which was required to resolve the ammonium and potassium peaks. This buffer was used for all separations. A fresh solution was prepared immediately before use and was used without adjustment at its natural pH value of 6. Stock solutions of the cations were prepared from their chloride salts. Those of bromide, chloride, nitrate and oxalate were prepared from potassium salts. Sulphate, acetate and phosphate stock solutions were prepared from magnesium, lithium and sodium salts respectively. Samples were diluted 1:20 in the buffer solution. Volumetric flasks made of poly(methylpentene) (PMP), supplied by Semadeni (Ostermundigen, Switzerland), were used for the preparation and dilution of working standards. The dilutions were carried out immediately before use. All solutions were degassed in an ultrasonic bath and filtered through 0.2  $\mu\text{m}$  nylon filters before analysis. All capillaries were of 75  $\mu\text{m}$  I.D.  $\times$  360  $\mu\text{m}$  O.D. except where stated otherwise. The lengths quoted refer to the total and the detector cell was placed approximately 5 cm from the capillary end except where stated otherwise. The fused-silica capillaries were purchased from Polymicro Technologies (Phoenix, AZ, USA). PEEK polymer capillaries were supplied by Ercatech (Berne, Switzerland), a dis-

tributor for Upchurch Scientific (Oak Harbor, WA, USA). Capillaries were preconditioned with a 0.1 M sodium hydroxide solution before flushing with deionised water followed by running buffer. All standards were contained in the background buffer in order to carry out electrokinetic injections under non-stacking conditions.

## 3. Results and discussion

Electropherograms for a standard cation mixture obtained in fused-silica and PEEK capillaries are shown in Fig. 1. As evidenced by the two traces, no

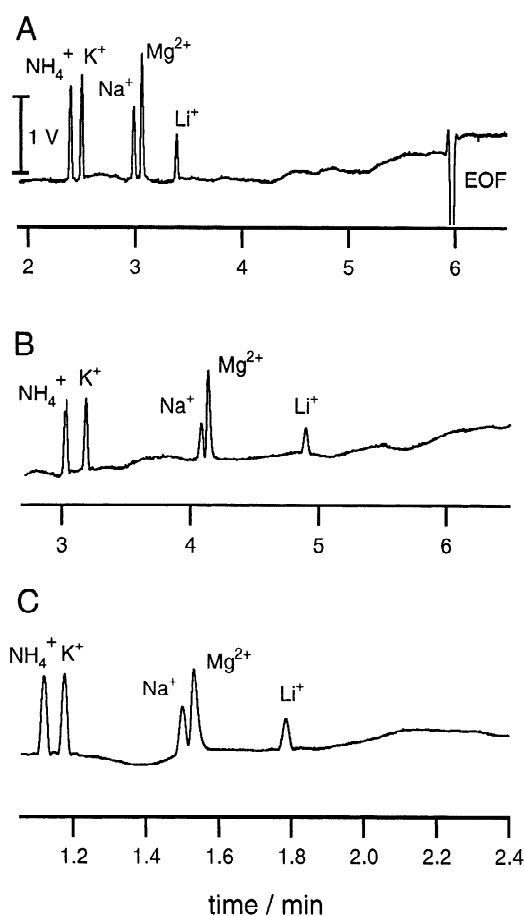


Fig. 1. Electropherograms of cations at 10  $\mu\text{M}$ . (A) Fused silica 75 cm; (B) PEEK 75 cm; (C) PEEK 50 cm. Buffer: 10 mM MES/His, 1 mM 18-crown-6 (pH 6.0). Injection: electrokinetic (non-stacking conditions), 7 s at +5 kV. Separation: +25 kV.

significant differences are apparent except for the migration times. Clearly both types of capillaries are suitable for the separation of the cations and for use with contactless conductivity detection. The indirect peaks obtained for methanol as an EOF marker shows that this is due to the fact that the electroosmotic flow is indeed lower in PEEK. The values were determined to be  $2.6 \cdot 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$  and  $5.8 \cdot 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ , for PEEK and fused-silica respectively (for the 10 mM MES/His-buffer at pH 6). In order to compensate for the effect of the reduced EOF in the PEEK capillary, the same separation was then carried out in a capillary of 50 cm length. As illustrated in Fig. 1C the ions can also be successfully resolved in the shorter capillary in less than 2 min.

It was noted that the EOF in PEEK increased by about 7% over the first 2 days of use. Constant values were obtained afterwards and the value quoted above was determined after stabilization. The peak heights and retention times were found to be highly reproducible. However, the baseline recorded with the PEEK capillary was found to be somewhat less stable than that obtained with the silica capillary. More frequent conditioning of the PEEK capillary was therefore necessary.

In Fig. 2, the electropherograms for anions separated in PEEK capillaries of different lengths are presented. A common buffer, i.e. 10 mM MES/His, 1 mM 18-crown-6, was used for the analysis of both cations and anions. Separation of anions without electroosmotic flow modifier was successful. However, as seen in Fig. 2A, the retention times were found to be fairly long for the 75 cm capillary. The shorter capillary proved to be more suitable. Separation of the same anions was accomplished within 5.5 min in the 50 cm capillary and better resolution of the nitrate and sulphate peaks was even achieved with this capillary (Fig. 2B). As evidenced by Fig. 2C the slow anions fluoride, citrate, succinate and acetate could also successfully separated with the 50 cm PEEK capillary. The EOF in PEEK was sufficiently low to enable, in particular, the determination of the acetate ion. This species cannot be detected in an unmodified silica capillary with a positive applied separation voltage since the EOF is larger and in the wrong direction. The ionic mobility of acetate has been reported as  $4.2 \cdot 10^{-4} \text{ cm}^2 \text{ V}^{-1}$

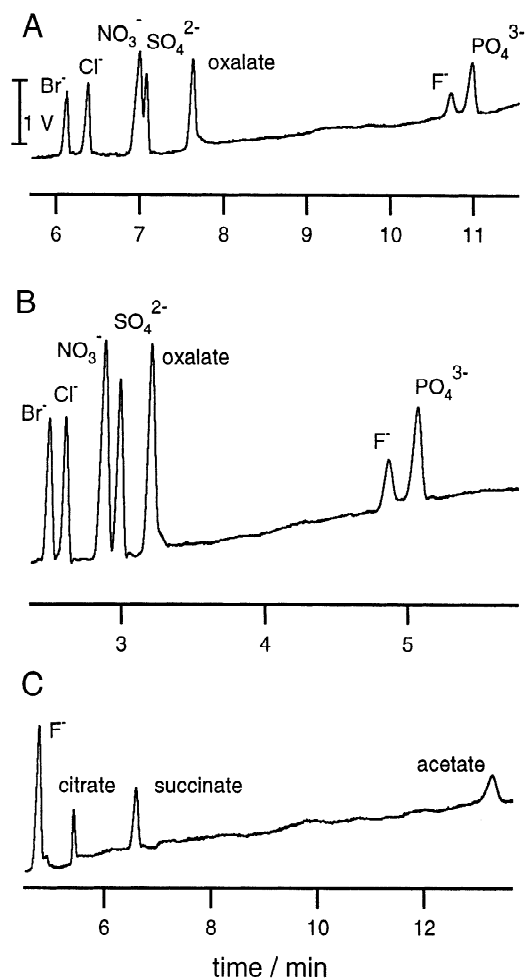


Fig. 2. Electropherograms of anions at 20  $\mu\text{M}$  (A and B) or 30  $\mu\text{M}$  (C) in PEEK capillaries: (A) 75 cm, (B) and (C) 50 cm. Buffer: 10 mM MES/His, 1 mM 18-crown-6 (pH 6.0). Injection: electrokinetic (non-stacking conditions), 7 s at  $-5 \text{ kV}$ . Separation:  $-25 \text{ kV}$ .

$\text{s}^{-1}$  [21], but is expected to be a fraction lower under the conditions used here because of partial protonation. Note, that in fused-silica the slow anions may be determined together with the cations because the stronger EOF pushes them in the cathodic direction, but concurrent determination with the fast anions in unmodified standard capillaries is not possible.

Electropherograms of inorganic cations and anions at 1  $\mu\text{M}$  recorded with the PEEK capillary are presented in Fig. 3A and B, respectively. As shown,

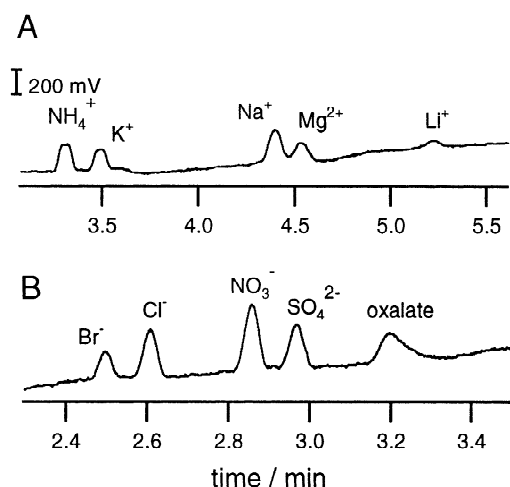


Fig. 3. Electropherograms of  $1 \mu\text{M}$  cations and anions in a PEEK capillary of 50 cm. Buffer: 10 mM MES/His, 1 mM 18-crown-6 (pH 6.0). Injection: electrokinetic (non-stacking conditions), 7 s at +5 kV for cations and -5 kV for anions. Separation: +10 kV for cations and -25 kV for anions.

the detector clearly is sensitive to such low concentrations. A slight baseline drift is evident in both electropherograms and for unknown reasons was found to be more pronounced in the cation determination. As this is also dependent on the level of the applied voltage, presumably because of Joule-heating, the separation of the cations at  $1 \mu\text{M}$  was carried out at the relatively low voltage of 10 kV. The regression equations for the ammonium, potassium, sodium, magnesium and lithium calibration curves were:  $y = 0.2004x + 0.0295$ ,  $y = 0.1597x + 0.1734$ ,  $y = 0.098x + 0.2376$ ,  $y = 0.2455x + 0.0428$  and  $y = 0.075x + 0.0515$  ( $y = \text{peak area}$ ,  $x = \mu\text{M}$ ). The corresponding correlation coefficients were determined as 0.9963, 0.9984, 0.981, 0.9987 and 0.985, respectively. The  $3 \times S/N$  detection limits for ammonium, potassium, sodium, magnesium and lithium in the 50 cm PEEK capillary were determined to be 1, 1.2, 0.73, 0.84 and  $1.6 \cdot 10^{-7} \text{ M}$ , respectively. For the anions, detection limits of 3.3, 1.8, 1.8, 2.1, and  $5.4 \cdot 10^{-8} \text{ M}$  were obtained for bromide, chloride, nitrate, sulphate and oxalate, respectively. There also appears to be a systematic difference between the detection limits achieved for the cations and anions, which may be correlated to the baseline effect

mentioned above. The limits of detection are comparable to the values obtained with HV-C<sup>4</sup>D for the same ions in fused-silica [18].

As illustrated in Fig. 4, the simultaneous determination of cations and anions with dual opposite end injection is possible with a PEEK capillary by using the 10 mM MES/His buffer without alteration for EOF suppression, as is required when using fused-silica capillaries [19,20]. Note, that such a buffer modification is likely to compromise the sensitivity in conductimetric detection. It was found that PEEK capillaries are well suited for this approach and slower organic anions could be included as well. However, when using opposite end injection it is necessary to establish a position along the capillary length which yields good separation for both, anions and cations, and at the same time avoids an overlap of the interleaving cation and anion bands moving in opposite direction. Although this adjustment is readily carried out when using contactless conductivity detection, as no optical window has to be created on the capillary, it was found to be a fairly tricky and time-consuming procedure. If the method, once developed, is then used for a longer period of time for similar samples, the set-up procedure may be justified. If however, the analytical

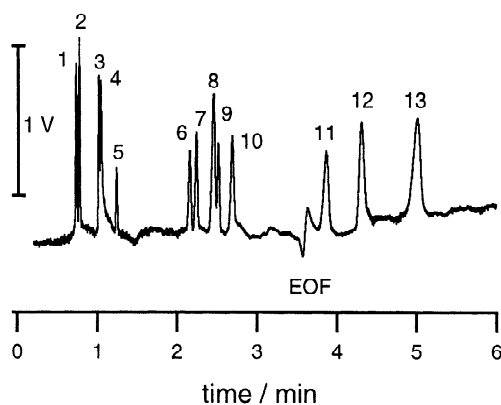


Fig. 4. Simultaneous determination of cations and anions via dual opposite end injection in a PEEK capillary. (1)  $\text{NH}_4^+$ , (2)  $\text{K}^+$ , (3)  $\text{Na}^+$ , (4)  $\text{Mg}^{2+}$ , (5)  $\text{Li}^+$ , (6)  $\text{Br}^-$ , (7)  $\text{Cl}^-$ , (8)  $\text{NO}_3^-$ , (9)  $\text{SO}_4^{2-}$ , (10) oxalate, (11) tartrate, (12) citrate and (13) succinate. Concentrations:  $20 \mu\text{M}$  (except citrate:  $50 \mu\text{M}$ ). Buffer: 10 mM MES/His, 1 mM 18-crown-6 (pH 6.0). Injection: electrokinetic (non-stacking conditions), 5 s at  $\pm 5 \text{ kV}$ . Separation:  $\pm 25 \text{ kV}$ . Capillary: 60 cm (detection 35 cm from cathode)  $\times 50 \mu\text{m}$  I.D.

task changes frequently it is considered that it is more convenient to carry out the analysis in two subsequent runs. As the buffer is identical for both cations and anions, all that is required is a switch of polarity, and the additional instrumental constraint and method development effort is avoided entirely.

As an example for a potential application, the analysis of inorganic ions in a tap water sample was carried out with the single PEEK capillary and the one common buffer. Cations were analysed first. The polarity was reversed after detection of the EOF peak, followed immediately by a second injection for the determination of the anions. In Fig. 5A and B the two electropherograms for the cations and anions in the single sample are illustrated. Potassium, calcium, sodium and magnesium ions were detected within 3 min and the separation of anions took 4 min, so that the complete analysis could be carried out in approximately 8 min. The tap water sample contained approximately 120, 0.4, 4, 9, 8, 10 and 12 mg l<sup>-1</sup> of calcium, potassium, sodium, magnesium,

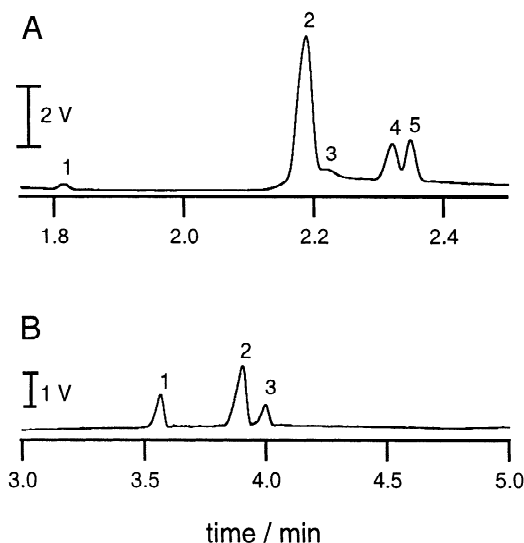


Fig. 5. Subsequent determination of cations and anions in tap water with a PEEK capillary of 60 cm. The sample was diluted 1:20 with buffer. (A) (1) K<sup>+</sup>, (2) Ca<sup>2+</sup>, (3) unknown, (4) Na<sup>+</sup> and (5) Mg<sup>2+</sup>. (B) (1) Cl<sup>-</sup>, (2) NO<sub>3</sub><sup>-</sup> and (3) SO<sub>4</sub><sup>2-</sup>. Buffer: 10 mM MES/His, 1 mM 18-crown-6 (pH 6.0). Injection: electrokinetic (non-stacking conditions), 7 s at +5 kV for cations and at -5 kV for anions. Separation: +25 kV for cations and -25 kV for anions.

chloride, nitrate and sulphate ions, respectively. The analysis of wine is perhaps somewhat more challenging and the rapid subsequent determination of cations and anions is shown in Fig. 6. The detection of the organic anions in the sample illustrates very nicely that contactless conductivity detection is useful not only for the determination of inorganic ions. A number of additional, smaller but quantifiable, peaks were detected in both the cation and anion run, but no effort was made to identify the species.

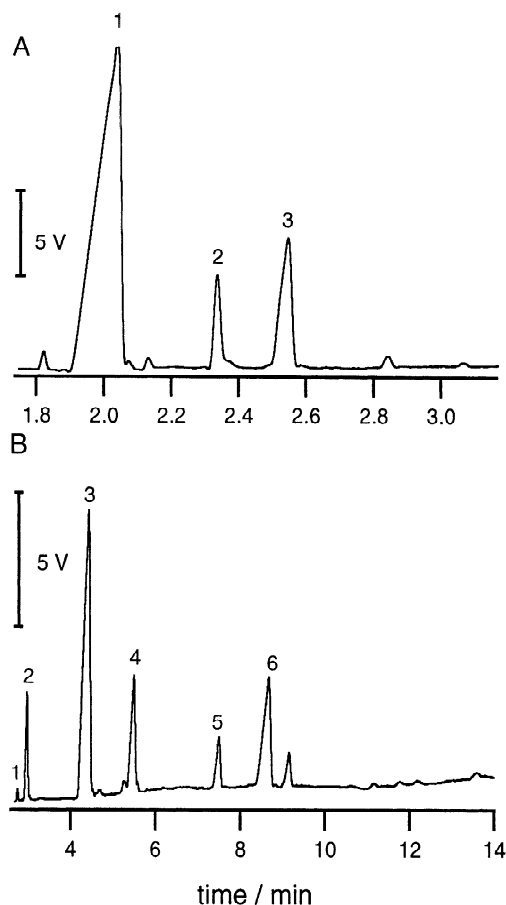


Fig. 6. Subsequent determination of cations and anions in a red wine sample with a PEEK capillary of 60 cm. The sample was diluted 1:20 with buffer. (A) (1) K<sup>+</sup>, (2) Ca<sup>2+</sup> and (3) Na<sup>+</sup>. (B) (1) Cl<sup>-</sup>, (2) SO<sub>4</sub><sup>2-</sup>, (3) tartrate, (4) succinate, (5) acetate and (6) lactate. Buffer: 10 mM MES/His, 1 mM 18-crown-6 (pH 6.0). Injection: electrokinetic (non-stacking conditions), 5 s at +5 V for cations and at -5 kV for anions. Separation: +25 kV for cations and -25 kV for anions.

#### 4. Conclusions

PEEK capillaries were found to be suitable for use with the enhanced capacitively-coupled contactless-conductivity detector. The EOF was found to be sufficiently low to enable the analysis of anions with intermediate mobility without having to add an EOF-modifier to the electrolyte solution. The absence of an EOF modifier and the low buffer concentration allow a low background conductivity of the buffer and hence detection limits for anions are not compromised. Detection of oppositely charged species in a single sample can be accomplished in a straightforward manner with two subsequent runs by simply switching the polarity of the separation voltage. This is considered an attractive alternative to the method of simultaneous detection by dual injection, since it does not require instrumental modifications, nor optimization with regard to detector positioning and buffer composition.

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