

Analytical Methods

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1 Simultaneous separation of cations and anions in capillary electrophoresis - Recent applications
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Abstract

In this review, the simultaneous determination of anionic and cationic species in capillary electrophoresis for different applications such as water quality analysis, medical diagnosis, pharmaceutical analysis, forensic science and food control is discussed. The simplicity and electronic nature of capillary electrophoresis allows the easy modification of custom made set-ups in order to realise various techniques of simultaneous separations of different ionic analytes. As a continuation of our earlier review, in which the details of the working principles were described [1], this report is focused on the reported applications of the simultaneous electrokinetic separation methods during the last five years (2011-2015).

Introduction

Capillary electrophoresis is an analytical technique for the separation and detection of ionic species. While traditionally only one type of charged species (positive or negative ions) is determined in a single run, simultaneous determination of both cations and anions is often desirable, saving time and costs. Simultaneous determination of anions and cations is useful in many types of applications, as it is common that not all target ions are of the same type. For example, in environmental monitoring applications, potentially hazardous species may be cationic as well as the anionic.

As discussed in a recent review [1], the different approaches to accomplish simultaneous separation of cations and anions can be divided into “chemical approaches” -in which ion mobilities are modified by some chemical additive- and “instrumental approaches” -which depend on some special instrumental setup-. One of the chemical ways to modify ion mobilities is to use complexing agents than can form anionic complexes with transition metal cations, allowing their detection alongside native anions in the sample [2]. Another way is to use cationic micelles to sweep anionic compounds towards the detector end of the capillary [3]. In counter electroosmotic flow (EOF) mode CE, an increase in the pH of the background electrolyte is created, which increases the magnitude of the EOF to such an extent, that the migration direction of slow anions is reversed and they can be detected alongside analytes of opposite charge [4].

It is also possible to achieve this instrumentally by applying an external pressure during the CE separation [5]. Two more common instrumental approaches are dual opposite end injection (DOEI) and dual single end injection (DSEI), in both of which two sample plugs are placed in the two opposite ends of the capillary prior to the separation and a single detector is placed somewhere between them. In the former method [6] each plug is injected from its respective side, and in the latter method [7] both of the plugs are injected from a one side, and one of them is hydrodynamically transported to the other side. Alternatively, it is also possible to employ two detectors which are placed at both ends of the capillary and hydrodynamically transport a single sample plug to the centre of the capillary, so that when the HV is turned on anions and cations are simultaneously de-

1 terminated at different detectors [7]. Finally, another approach employing two detectors is dual chan-
2 nel CE, or dual CE (DCE). In this approach, which was first reported in 1992 [8], the CE instrument
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4 employs two separate capillaries and two detectors for cations and anions respectively.
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9 More details on the principles of the different approaches can be found in our previous review [1].
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11 In this article, the different analytical questions that were solved by the simultaneous determination
12 of cations and anions in CE, covering the period from 2011 to 2015, are reviewed. Our aim is to
13 provide a guide reviewing the latest applications where simultaneous separations of differently
14 charged analytes were used. This applications are wide in nature and cover such diverse fields as
15 environmental, medical, pharmaceutical, and forensic analyses.
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24 **CE applications with simultaneous separation of cations and anions**

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28 · Water quality analysis
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32 Many human activities, such as agriculture and industry, affect the quality of surface and ground
33 water. For this reason, the analysis of water is highly important and a variety of techniques are cur-
34 rently used to study the composition and characteristics of the water.
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40 In recent years, research groups have also focused their efforts on the analysis of water by the
41 simultaneous separation of certain anions and cations with CE. Wang et al. [9] developed a meth-
42 od for the analysis of river water with simultaneous UV determination of calcium and some anions,
43 such as phosphate, which is abundant in fertilisers and directly impacts water quality when dis-
44 charged to rivers via surface run off. The authors of this study used 2,6-pyridinedicarboxylic acid
45 (PDCA) as a complexing agent for creating an anionic metal chelate with calcium $[\text{Ca}(\text{PDCA})_2]^{2-}$,
46 which can be separated together with the anions in the sample in a single capillary without modifi-
47 cation of a standard commercial instrument. Moreover, since PDCA can be used for on-column
48 complexation of calcium, there is no need for a specific sample pre-treatment. Neaga et al. [10]
49 made use of the DOEI approach for the analysis of ground water from 51 domestic wells. The ca-
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pillary was coated with polyvinyl alcohol to precisely control the EOF and avoid possible interaction of analytes with the capillary wall. They were able to separate 10 inorganic anions and cations, including nitrate and nitrite, which are of great concern among the inorganic pollutants because they can induce methemoglobinemia in infants and may be causing different types of cancers on chronic exposure. Contactless conductivity detection (C^4D) was used due to the lack of absorbance of some analytes. Mai and Hauser [7] also studied the simultaneous separations of major inorganic cations and anions in tap water. In this case, the authors used dual single-end injection CE in one capillary with two C^4D -cells to obtain separate electropherograms for negatively and positively charged species (Figure 1). Gaudry et al. [11] designed a new system for the simultaneous determination of cations and anions using DCE. The construction of customised instruments allows different features that cannot be obtained on conventional equipment. For example, in this case, the authors were able to automatically monitor tap water over a period of 48 h, since the system was able to collect and analyse the sample from a water flow unattendedly. The authors of this work also analysed other environmental samples, including river and lake water samples, determining up to 23 cations and anions. A final application reported in the same work was the analysis of water samples from a zinc processing plant, where samples were taken from various stages of production. Pham et al. [12] also used a custom designed system with C^4D for the automated monitoring of water during the biological removal of ammonium from contaminated groundwater in a sequencing batch reactor, where NO_3 and NO_2 are formed as intermediate products. The authors could experimentally follow the decrease of the ammonium concentration and the formation of the oxidation products nitrite and then nitrate.

The simultaneous separation of cations and anions in natural water samples by CE has frequently been reported in the last years. One reason is that these samples do not need special pre-treatment before injection in CE and, when needed, this usually consists of a simple dilution or filtration. Moreover, CE is especially useful for the analysis of aqueous samples.

· Medical diagnosis

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CE may also be used in clinical laboratories for diagnostic purposes. The simultaneous separation and determination of cations and anions by CE has been proven to be suitable in tests for medical diagnosis of certain diseases. Kubán et al. [13] conceived a small device allowing to collect 100-200 μL of exhaled breath condensate in less than 2 min. Samples obtained in this non-invasive way were analysed for their ionic content by DOEI-CE with C^4D . The authors determined up to 15 inorganic cations and anions and organic acids in less than 3 min and observed changes in the concentration of nitrite and other ions in a person with diagnosed mild chronic obstructive pulmonary disease and a person suffering from acute cold and serious cough. Moreover, as the authors mentioned, the method could also be useful for monitoring fast metabolic processes such as lactate build-up following an exhaustive cycling exercise and subsequent elimination (Figure 2). Kubán et al. [14] recently developed an approach for non-invasive collection of sweat samples with a moisturised cotton swab and its analysis by DOEI-CE with C^4D . The samples were analysed for the quantification of chloride, sodium and potassium, whose concentrations are affected in individuals suffering from cystic fibrosis. The method was able to indicate differences between healthy individuals and cystic fibrosis patients. The results were evaluated with principal component analysis (PCA) in order to render the diagnosis more accurate, i.e. to reduce the number of false positive or negative findings.

Because CE can easily be miniaturised and packed into a compact, low power format, and produced as inexpensive instrument [15] it may be used as a powerful point-of-care screening tool to be easily used by the user at home. The references reviewed in this section demonstrate this possibility. Healthy people and patients with different diseases are already demanding such tools for their everyday lives. The development of smaller and less expensive instruments and of new methods which offer information about the health status of the user in a short time may be of high importance in the near future.

· Pharmaceutical applications

1 Approximately, 50% of the active pharmaceutical ingredients of drugs are administered as salts.
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3 Although chloride and potassium are the preferred counter-ions for the salt formation, different
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5 counter-ions are sometimes chosen in dependence on the administration path of the drug [16]. The
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7 availability of numerous counter-ions makes the salt selection process complex and, for a basic
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9 drug, a wide variety of inorganic and organic anions can be chosen. The selection of the correct
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11 salt determines the physicochemical stability and bioavailability, which is why it is important to con-
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13 firm the composition of pharmaceutical salts by analytical methods [17]. As both the cation and the
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15 anion in the salt have to be analysed, the analysis of a drug by conventional CE methods would
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17 require two different analyses, one for each ionic species. Therefore, in recent years, new method-
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19 ologies for the simultaneous determination of drugs and their counter-ions have been developed.
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21 Lopez et al. [17] developed a method using DOEI-CE with C⁴D for the analysis of five drugs (chlor-
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23 pheniramine, metoprolol, clomiphene, catharanthine and vinorelbine) and their respective counter-
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25 ions (maleate, tartrate, citrate, sulphate and ditartrate). With this method, the authors were able to
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27 analyse each pharmaceutical composition in less than 10 min. Cunha et al. [16] focussed their re-
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29 search on the simultaneous determination of diclofenac and its common counter-ions (potassium,
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31 sodium, and diethylammonium). In this case, the authors chose an EOF of high intensity to per-
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33 form counter-EOF mode CE with C⁴D using short capillaries for short analyses (less than 1 min).
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35 With this method, it was possible to provide information about the nature of the salt, to study the
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37 presence of impurities and to monitor the degradation of the active pharmaceutical ingredient after
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39 exposure to solar radiation.
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44 These works show the applicability of the different approaches for the simultaneous separation of
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46 drugs and their counter-ions in the quality control of pharmaceutical formulations. The methods
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48 have the potential for use in quality control of pharmaceutical formulation batches before release.
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52 · Forensic applications
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56 The simultaneous determination of cations and anions by CE has also been applied to the analysis
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58 of forensic samples. In particular, the study of explosives has recently attracted the interest of sci-
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entists. In these cases, the aim is to identify the nature of the explosive device from the results of the analysis of residues. Improvised explosive devices (IEDs), are usually made of inorganic salts containing perchlorate, chlorate, or nitrate, which can be identified by CE. Certain organic explosives can also leave significant traces of inorganic ions which may aid in their identification [18]. Kobrin et al. [18] analysed different kinds of post-blast residues on different matrices, such as sand, a metal plate and concrete. Organic explosives and inorganic explosives, including IEDs, were detonated and the residues sampled and analysed by DOI-CE with C^4D in less than 4 min. After PCA analysis the authors were able to identify the type of each detonated explosive.

Fireworks are also suitable for this type of analysis since they are mostly composed of inorganic salts which are soluble in water, and the analysis allows to prove if the device meets the manufacturing regulations. Sáiz et al. [19] studied the cationic and anionic compositions of several consumer fireworks by DCE with two C^4D -cells, providing new information about the composition of the charges and fuses of these explosives devices, and exposing some inaccuracies in the declarations of the compositions given by the manufacturers.

In both cases, the systems employed were inexpensive, purpose made, portable instruments able to simultaneously separate cations and anions. This highlights the applicability of portable instrumentation for field analysis, where rapid results are needed.

Other applications

Apart from the analysis of water, other environmental applications have attracted the interest of researchers. Huang et al. [20] analysed airborne particulate matter (PM), which is directly related to mortality and morbidity. The authors of this work studied fine particles with diameters smaller than $2.5 \mu\text{m}$, which reach the alveolar region acting as carriers of toxic species. Using the DOI-CE approach in a system with two C^4D -cells, the authors were able to determine ten water-soluble ions in PM, which can be carried into the respiratory system. In the same article, the authors re-

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2 peated the same application using a system conceived for DCE, with two C⁴D-cells, in order to re-
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4 duce the superposition of peaks.
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8 The simultaneous determination of anions and cations was also covered in the work of Stojkovic et
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10 al. [21] in which the authors placed a sample plug of NaCl and KNO₃ in the middle of a capillary,
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12 fitted with an array of 16 C⁴D-cells. The authors demonstrated the simultaneous separation of cation-
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14 ons and anions while they studied the evolution of peaks originating from the sample plug, offering
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16 such a set-up as a novel way for studying the dynamics of electrophoretic separations.
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20 Food quality control and the detection of adulteration in food are of high concern in certain coun-
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22 tries. Food and infant formula have been adulterated with melamine with the aim of increasing the
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24 apparent protein content. Long-term exposure to melamine and its by-products (ammeline, am-
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26 melide and cyanuric acid) can reduce fertility, produce kidney stones and renal failure, and result in
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28 fetal toxicity. For this reason Kohler et al. [22] developed a method for the simultaneous determina-
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30 tion of melamine, ammeline, ammelide and cyanuric acid, being able to determine the cationic and
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32 anionic species using a high EOF in CE with mass spectrometric detection. The method was suc-
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34 cessfully applied to the analysis of melamine-contaminated powdered milk.
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38 Ito and Okada [23] developed a novel and simple analyte pre-concentrating method in which only
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40 the freezing of the sample is needed. When an aqueous sample is frozen, solutes are expelled
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42 from the ice core while the remaining liquid is becoming more concentrated. This makes this pre-
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44 concentrating technique especially suitable for the analysis by CE, as it is possible to analyse vol-
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46 ume-limited samples. The authors were able to simultaneously separate anions and cations using
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48 a high EOF, while a maximum enrichment factor of 1000 was achieved by simple freezing of sam-
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54 Finally, Santos et al. [24] developed a new method for the simultaneous determination of cationic,
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56 anionic and neutral alcohols by CE. The method consists of coupling electrochemical derivatization
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58 with CE-C⁴D, employing a strong EOF. The alcohols were electrochemically oxidised to carboxylic
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1 acids which then can be separated together with other anions. The use of a strong EOF permitted
2 to reverse the mobilities of these anions and simultaneously separate them with cations. The
3 method was successfully applied to the analysis of an antiseptic mouthwash sample in which cationic
4 ions (Na^+), anions (benzoate, saccharinate) and neutral alcohols (in acidic form after derivatization)
5 such as ethanol and *n*-pentanol, were all determined by a single detector.
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12 **Conclusions**

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18 Different applications of simultaneous separations of anionic and cationic species have been re-
19 ported for capillary electrophoresis in the last years. In many cases customised instrumental set-
20 ups had to be developed and employed for these purposes as the conventional commercial in-
21 struments are not always suitable. However, due to the relative simplicity of CE this is not a signifi-
22 cant complication and fewer compromises have to be made when specially developed systems
23 can be employed. This is particularly true for dual capillary instruments which are still not overly
24 complex, and indeed an increasing number of applications has been observed for DCE. C^4D was
25 the detection technique of choice in most of the cases. This is due to its suitability for inorganic
26 species, and its relatively low cost and small size also allows the use of multiple detectors. The
27 breadth of reported applications, including among others, pharmaceutical applications, food analy-
28 sis and forensic tests, indicates a great but largely untapped potential of simultaneous analysis by
29 CE.
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Figure captions:

Figure 1. Simultaneous separations of anions and cations with dual single-end injection, single capacitively coupled contactless conductivity detection (C^4D) and with pressure-assisted capillary electrophoresis (CE). {Reprinted from [7] with permission from Elsevier}.

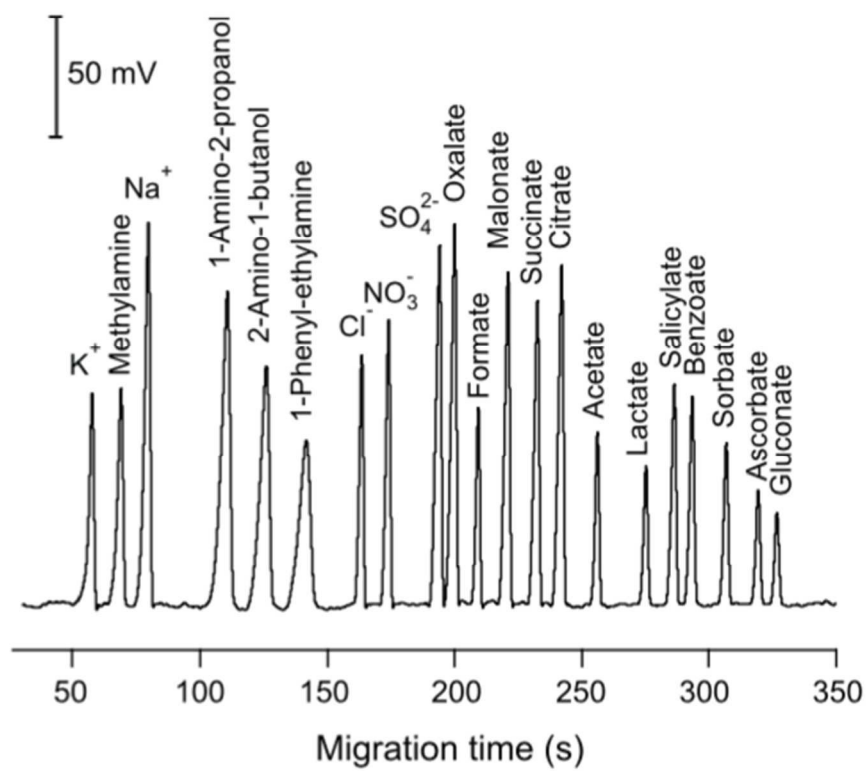
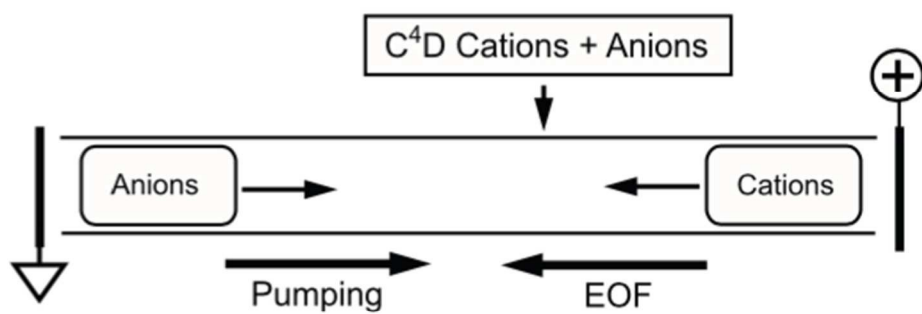
Figure 2. Electropherogram of lactate screening together with other cations and anions in exhaled breath condensate before (A) and after 4 (B), 10 (C), 25 (D) and 60 (E) minutes of exhaustive cycling exercise (B). {Reprinted from [14] with permission from Elsevier}.

Figure 3. Dual electropherograms for the analysis of a firecracker. Cations (A-a) and anions (A-b) were determined in the pyrotechnic charge of the firecracker and, similarly, cations (B-a) and anions (B-b) were also determined in the fuse of the same firecracker. {Reprinted from [19] with permission from Elsevier}.

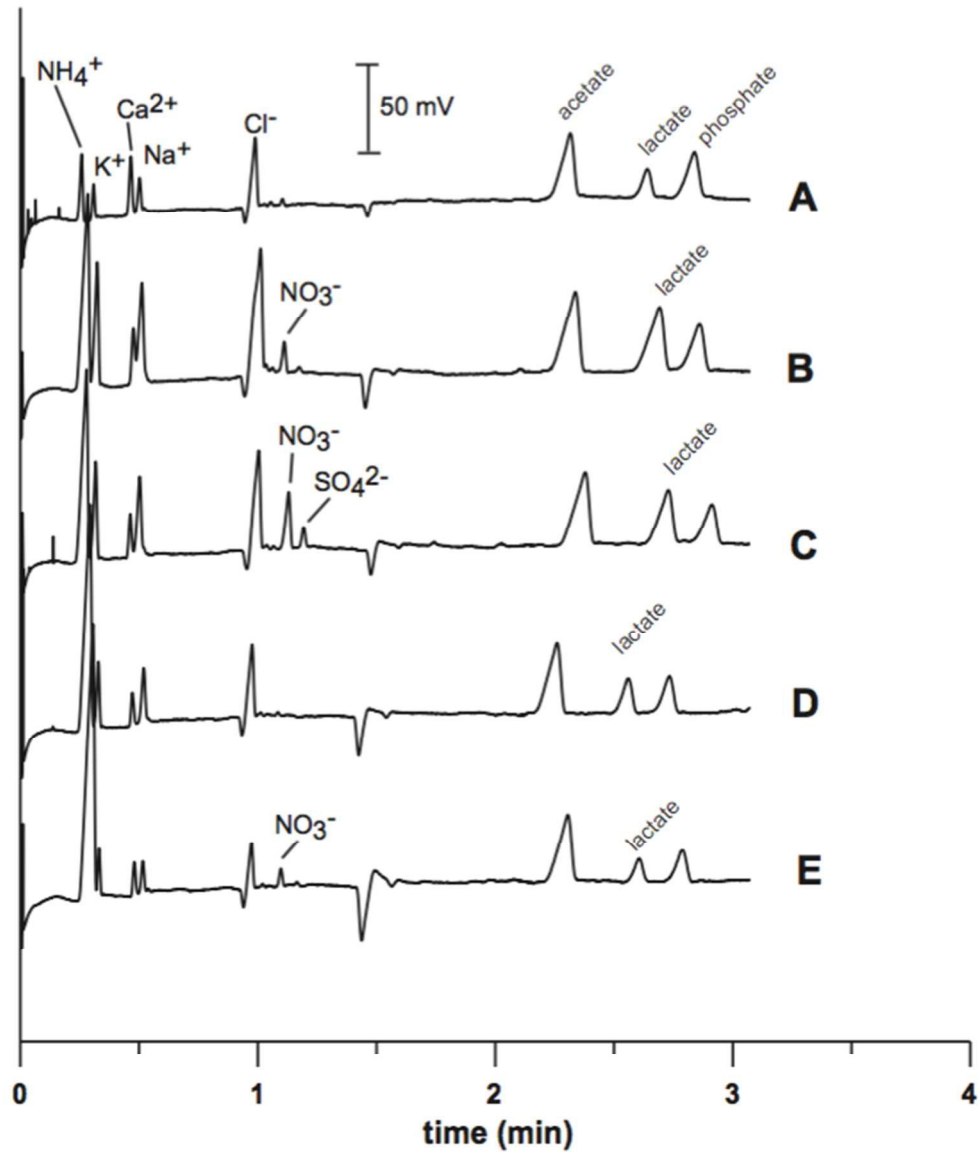
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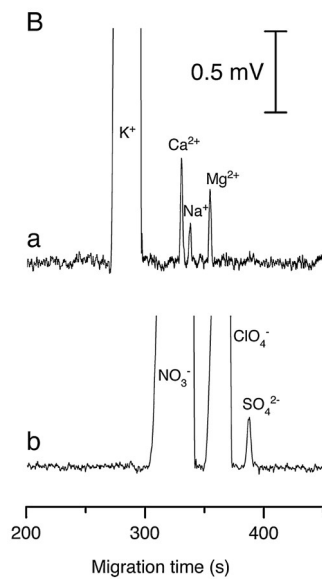
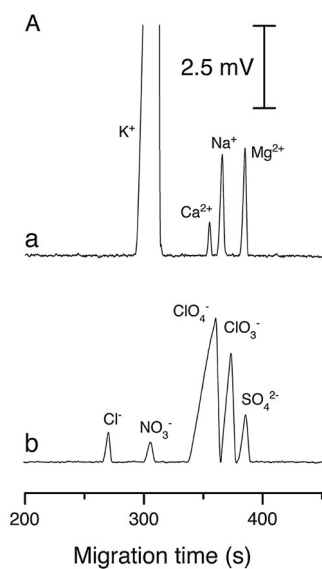


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