Contactless conductivity detection for analytical techniques – developments from 2012 to 2014

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Contactless conductivity detection for analytical techniques – developments

from 2012 to 2014

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Abbreviations:

C⁴D – capacitively coupled contactless conductivity detection / detector
DOI – dual opposite end injection
EC – electrochemical cell
EME – electromembrane extraction
FIA – flow injection analysis
26 MCE – microchip electrophoresis
27 μ-EME – micro-electromembrane extraction
28 PDMS – poly(dimethylsiloxane)
29 PMMA – poly(methylmethacrylate)
30 SIA – sequential injection analysis
31 SLM – supported liquid membrane
32 SPE – solid phase extraction
Abstract

The review covers the progress of capacitively coupled contactless conductivity detection over the two years leading up to mid-2014. During this period many new applications for conventional capillary electrophoresis as well as for microchip separation devices have been reported; prominent areas have been clinical, pharmaceutical, forensic, and food analyses. Further progress has been made in the development of field portable instrumentation based on capillary electrophoresis with contactless conductivity detection. Several reports concern the combination with sample pretreatment techniques, in particular electrodriven extractions. Accounts of arrays of contactless conductivity detectors have appeared which have been created for quite different tasks requiring spatially resolved information. The trend to the use of contactless conductivity measurements for applications other than capillary electrophoresis has continued.
1 Introduction

The development of applications of capacitively coupled contactless conductivity detection (C^4D) has kept its pace during the period covered by this review (approximately from July 2012 to June 2014) with over a hundred new publications. Again, as for the 2-year period covered in the previous review [1], most of these concern conventional capillary zone electrophoresis, and the detector now appears to be well established for this application. A number of reports have once more appeared on C^4D in microchip electrophoresis, with several of these concerning the analysis of relatively complex real samples, but a majority still dealing with design issues.

Several accounts of projects in which C^4D was an enabling technique have appeared. These include field portable instruments, a robotic vehicle for air testing, the in-situ study of chromatographic columns, and CE with hydrodynamic pumping. In order to lower the limits of detection, CE-C^4D has also been combined with preconcentration methods, in particular with electrodriven membrane extraction. New applications of C^4D include the monitoring of two-phase flows or the proposal of larger cells for conductivity monitoring in industrial systems. Some more fundamental studies on the impedance characteristics of the detector cell and on its modification have also been carried out.

This review is the last in a series of updates written by the authors [1-4]. The field has also been summarized by other authors, starting with the early reviews by Zemann, one of the protagonists of CE-C^4D, in 2001 and 2003 [5, 6]. This was followed by reviews by Gujit et al. in 2004 [7] and Šolínová and Kašička in 2006 [8]. Pumera in 2007 discussed C^4D on microchip devices [9] and Matysik in 2008 discussed C^4D along with amperometric detection [10]. Trojanowicz [11] discussed C^4D in the context of electrochemical detection methods in
flow analysis in 2009. Coltro et al. in 2012 summarized the developments of the use of capacitive coupled conductivity detection on microchip devices [12] and Elbashir and Aboul-Enein in 2010 and again in 2012 summarized applications of CE-C\textsuperscript{4}D [13, 14]. During the period covered by this review (2012-2014), Opekar et al. published a summary of some fundamental aspects of C\textsuperscript{4}D [15] and Matysik and coworkers published an extensive review on the combination of electrochemical methods in general with capillary electrophoresis, including microchip devices [16]. Newcomers to the field who want to gain an understanding of the basics of C\textsuperscript{4}D may also wish to consult the earlier fundamental publications by do Lago and coworkers [17, 18], Jorgenson and coworkers [19], Opekar et al. [15], or publications from our group [20-23].

The review is broken down to different aspects concerning more fundamental developments, applications of CE-C\textsuperscript{4}D implemented with conventional capillaries and on microchip devices, and new applications other than in capillary electrophoresis. The accompanying tables provide a summary of applications with more detailed information than discussed in the text. Note that some publications may be quoted more than once, if they are relevant in different contexts. We apologize for any oversights.

2 Fundamental aspects

2.1 Improved characterization and cell designs

The basics of the capacitively coupled contactless conductivity cell are fairly well understood, but more details on some aspects are still emerging. It is, however, sometimes difficult to obtain a comprehensive picture, as different authors focus on divergent aspects, use cells with varying characteristics, and use distinct operating conditions. Results reported for different
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studies therefore sometimes even appear to be contradictory. It is a bit like the story of the
blind men and the elephant.

A schematic drawing of an axial capacitively coupled conductivity cell is shown in Fig. 1
together with a simplified equivalent circuit diagram. Shen and coworkers [24, 25] carried out
fundamental studies of this standard cell configuration with an impedance analyzer, and found
that the measured wall capacitances are significantly smaller than the values calculated from
the formula for a coaxial capacitor. This confirms earlier studies with a different cell in which
the capacitances were experimentally determined differently, namely from Bode plots (plots
of signal vs. frequency) [20]. Shen and coworkers also studied in detail the effect of solution
conductivity on wall capacitance and found a clear correlation, i.e. the wall capacitance was
higher for solutions of higher conductivity [24, 25]. The authors proposed a model for a likely
explanation, namely that the field lines between the electrodes are following different paths
for materials of different conductivity inside the tubing.

Liu et al. [26] discussed a detailed model for a planar contactless detector cell for microchip
devices based on the early description by da Silva and do Lago for a tubular cell [27]. This
treats the contactless electrodes not as simple capacitors, but as a series of smaller capacitors
connected with resistors. Liu et al. could show that this more detailed model could better
predict their experimental results than the simple model as shown in Fig. 1.

Further publications have appeared on the use of C+D cells which were operated in series with
a large inductor [28, 29] or a piezoelectric quartz crystal [24, 30]. In the latter case the crystals
were employed also for their high intrinsic inductance values [30, 31]. The effect of a series
inductance is illustrated in Fig. 2, where the modelled frequency response (Bode plot) of a cell
is given according to the simple equivalent circuitry (as shown in Fig. 1) and typical values for wall capacitances and solution resistance for a cell used in CE [20]. In the Bode plot for the cell without inductor the plateau at the high frequency end corresponds to the usual working range where the cell impedance is determined only by the solution resistance and the wall capacitances are negligible. As can be seen, the introduction of the inductor modifies the frequency response so that a maximum is obtained at a lower frequency. However, the maximum signal is still equivalent to that obtained in the plateau region for the cell without inductor as the current is nonetheless limited by the solution resistance. So the series inductor does not give a real gain in sensitivity when it is otherwise possible to work with optimized frequencies. This might not always be the case though. The cell might require operating frequencies which are beyond the bandwidths easily achieved with detector circuitries. The frequency at which the plateau is reached is higher for smaller coupling capacitances and therefore dependent on the cell geometry. It is also dependent on the inner diameter and the conductivity of the solution as lower values of cell resistance will push up the required minimum frequency. Another reason for wanting to move to lower frequencies might be the presence of a significant stray capacitance (direct coupling between the electrodes), which has a more pronounced effect at high frequencies.

2.2 Expanded scope

Several studies have been published which either concern an improvement of CE-C\textsuperscript{4}D or extend the application of C\textsuperscript{4}D beyond electrophoresis. Referenced C\textsuperscript{4}Ds have been reported by two groups. Shen et al. described a differential system consisting of two cells with separate pick-up circuitries which were placed at the two ends of the separation capillary [24]. The referenced system reported by Stojkovic et al. [32] consisted of a single cell through which the detection end of the capillary was looped back. Both approaches automatically subtract
the background signal and can thus compensate for baseline drifts due to temperature and other reasons.

Mai and Hauser reported a detailed further study on the effects of capillary diameter in the range from 10 to 50 µm and buffer concentration on the detection sensitivity [33]. Note that the use of the standard absorbance detectors is not readily possible with diameters of less than 50 µm. Also investigated was the effect of a concurrent hydrodynamic flow. The study confirmed that narrowest capillaries should be used when employing C\textsuperscript{4}D as they give best separation efficiency without loss of sensitivity with buffers of optimized concentrations. Hydrodynamic pumping may be employed with capillaries of less than 50 µm diameter for optimization of separation and analysis time without incurring significant band broadening. Stojkovic et al. [34] constructed an array of 16 cells which enabled the visualization of the development of the separation of ions along the length of the capillary.

Tůma et al. investigated the utility of a multichannel capillary [35]. The fused silica tubing with a standard outer diameter of 360 µm contained 7 channels with round cross-sections of 28 µm diameter. In a comparison with standard capillaries of 25 µm and 75 µm diameter it was found that for the multichannel capillary the sensitivity of a C\textsuperscript{4}D was approximately proportional to the total cross-sectional area of the channels, which corresponds to the expected behaviour. It can also be expected that the narrower channels lead to higher separation efficiencies. While this was found to be true for the also investigated UV-detection, it could, however, not be confirmed for the C\textsuperscript{4}D set-up.

Buglione and coworkers [36, 37] investigated the use of non-aqueous solvents in CE-C\textsuperscript{4}D for the determination of poorly water soluble organic cations (quaternary amines) and anions...
(fatty acids) and found that the sensitivity and baseline stability was strongly dependent on the solvent and the electrolytes used, but that for optimized conditions good detection limits below 1 µM could be achieved.

Huang and coworkers [28, 29] produced scaled up versions of the usual dual axial electrode arrangement to tubings with outer diameters of 3.2 mm, 4.9 mm, 7.5 mm and 10 mm and found that these cells were suitable for conductivity measurements of KCl solutions. Thus it appears that the overall behaviour of larger cells is similar to the better studied capillary cells.

Wang et al. [38] described a conductivity sensor based on five axially arranged electrodes, the outer two for applying AC voltage, the 3 inbetween for differential conductivity measurement via the determination of the voltages at the electrodes rather than current as usual. This was used for flow rate measurements in millimeter scale tubings by determination of the velocity of introduced bubbles between the gaps of the inner three electrodes. In a further publication a similar approach for flow rate measurement was used in which the signal was created by introduction of solutions of different conductivity [39].

Wang et al. [40, 41] designed an array of 12 contactless electrodes arranged radially on a tubing of 55 mm diameter for the study of inhomogeneous flows. All electrodes can be switched between excitation and pick-up mode and with an appropriate data-acquisition system preliminary results on conductivity distribution inside the tubing could be obtained.

Newill et al. [42] probably are the first authors to develop a 2D grid of contactless electrodes. 60 electrodes were arranged on a plane and again a switching circuitry allows the selection of the desired electrodes for measuring of the adjacent impedance.
Coltro and coworkers have designed a microfluidic device with three isolated electrodes, one of which was functionalized with biotin [43]. This electrode acted as an impedance sensor for the binding of avidin. The third electrode served as a reference for the solution conductivity and allowed to obtain the net signal via subtraction.

3 Instrumentation

3.1 Portable CE-C4D-instruments

Portable instrumentation represent an attractive alternative to bench-top analytical systems and the combination of CE with C4D lends itself very easily for portable applications since the instrumentation is simple and has low power requirements. A new partly automated portable CE-C4D instrument employing compressed air for automated BGE distribution and hydrodynamic sample injection was reported by Mai et al. [44]. A photograph of this instrument can be seen in Fig. 3. The publication included a demonstration that capillary zone electrophoresis may be optimized either for high separation efficiency, low limits of detection, or fast separations, but that not all can be achieved at the same time, and compromises have to be made. In Fig. 4 a baseline separation of 4 ions is shown which was achieved on this instrument in less than 20 seconds. This affirms that fast separations can be carried out in conventional capillaries and that this is not a feature unique to microchip devices. Da Costa et al. [45] described an unmanned mobile platform employing CE-C4D (called lab-on-a-robot) for air sampling and analysis and demonstrated the determination of formic-, acetic- and propionic acid vapours. Applications of portable CE-C4D systems were further reported for the analysis of warfare degradation products [46], scopolamine in forensic studies [47], determination of inorganic and heavy metal ions in environmental samples [48,
and the analysis of post blast residues [50]. Portable CE-C\textsuperscript{4}D was also used as a scanning device for fraction collection prior to CE-MALDI-MS analyses of peptides [51].

### 3.2 On-line coupling of CE-C\textsuperscript{4}D to flow-injection systems

Flow-through techniques, such as flow injection (FIA) and sequential injection analysis (SIA), enable easy and automated operation and their coupling to CE-C\textsuperscript{4}D has been shown to be beneficial for liquid handling and sample injections in several contributions during the last two years. Mai and Hauser have published a series of manuscripts on coupling a SIA manifold to CE-C\textsuperscript{4}D and have examined the hyphenated systems for flexible manipulations of sample plugs and BGE solutions before and during CE analyses [33, 44, 52, 53]. Stojkovic et al. [53] demonstrated how the application of hydrodynamic pumping during an electrophoretic separation in narrow capillaries could be used to compensate the electroosmotic flow and to optimize the analysis time in the analysis of artificial sweeteners.

Mai and Hauser demonstrated different schemes of concurrent anion and cation separations in a single capillary aided by hydrodynamic pumping [52]. For example, by placing the sample into the centre of the separation capillary, simultaneous separations of anions and cations were possible using two C\textsuperscript{4}Ds at the ends of the capillary. The simultaneous separation of anions and cations was also demonstrated in a flow-through system with two CE capillaries (one for the anion and the other for cation separations) and two C\textsuperscript{4}Ds by Gaudry et al. [54].

Alhusban et al. [55] used a similar instrumental set-up, with a single capillary, for on-line lactate monitoring in cell culture media. Automated handling of solutions and samples is also very attractive for separations in short capillaries since manual operations (BGE flushing, sample injection) with short capillaries is rather delicate. Vochyánová et al. designed an instrument employing flow-through electrokinetic injection into 10 cm long capillaries (total...
length) and demonstrated the rapid analysis of saccharides [56] and human activity stimulants [57] in energy drinks.

### 3.3 Combination of CE-C⁴D with sample pretreatment techniques

Many samples are not suitable for direct injection into a CE-C⁴D instrument, either due to low concentrations of target analytes below the detection limit, or a matrix which leads to overload and inadequate separation. As a consequence, sample pretreatment is then required prior to their analysis. In some applications, filtration and considerable dilution of samples might be sufficient to overcome the matrix effects for analyses of major components. On the other hand, in analyses of minor components (especially in clinical applications), such dilutions are not acceptable and other pretreatment techniques, which usually combine removal of matrix components with analyte preconcentration are applied. Standard procedures, such as denaturation, deproteinization, centrifugation and micro-dialysis have been part of the procedures for some of the reports of the last two years reported for the pretreatment of complex and biological samples [58-61].

The recently developed microscale extraction technique of electromembrane extraction (EME) has received particular focus for the pretreatment of complex samples prior to CE-C⁴D [62-66]. In EME, ionic analytes are electrophoretically transferred from an aqueous complex sample across a thin layer of a water immiscible organic solvent (in form of a supported or free liquid membrane) into an aqueous acceptor solution. A key characteristic of the extraction technique is its selectivity (i.e. elimination of matrix components and transfer of analytes). C⁴D is a universal detection technique and in combination with CE enables determination of a broad range of analytes in one run. CE-C⁴D has, for the first time, evidenced that EME strictly eliminates proteins, salts and most biochemical compounds and
efficiently transfers small pharmaceutical analytes by simultaneous determination of human serum albumin, inorganic cations, amino acids, creatinine and basic drugs in one single CE run [62]. Further developments of EME, such as application of polymer inclusion membranes in selective transfers of inorganic and organic anions [66, 67] and of organophosphorous herbicides [65] were demonstrated by CE-C\textsuperscript{4}D. A further down-scaling of EME to a micro format (sub-\(\mu\)L to \(\mu\)L volumes of respective solutions) was demonstrated by CE-C\textsuperscript{4}D for the recently developed \(\mu\)-EME across free liquid membranes [63, 64].

Sample pretreatment is normally performed in an off-line fashion and the resulting extract is then manually transferred to the analytical system for injection and analysis. Direct coupling of sample pretreatment to CE-C\textsuperscript{4}D represents an attractive alternative to the off-line approach, since the manual handling of the sample by the operator is minimized and some tasks or even complete analytical procedures are fully automatized. Santos et al. [68] have demonstrated an on-line system coupling an electrochemical cell (EC) to CE-C\textsuperscript{4}D, which was capable of electrooxidation of otherwise neutral (and therefore for CE not accessible) alcohols, unattended injection, and electrophoretic separation in beverage samples with analytical frequency of 12 analyses per hour. The hyphenated EC-CE-C\textsuperscript{4}D system was also shown to be suitable for the simultaneous electrooxidation and CE analyses of cationic, anionic and neutral analytes [69]. Kubáň and co-workers have shown that direct coupling of CE-C\textsuperscript{4}D to thin planar membranes is suitable for direct injection of undiluted biological fluids into separation capillaries. Micro-dialysis membranes [70] and supported liquid membranes [71] were sandwiched between a sample of biological fluid and acceptor solution and CE separation capillary was touching the membrane surface (at the acceptor side) for direct electrokinetic injection of analytes through the membrane. The analytes were transferred by electrokinetic means directly into the capillary whereas matrix components, such as particulate matter,
proteins, lipids and other large molecular compounds were retained by the membranes and did
not interfere with subsequent CE measurements. The process is illustrated in Fig. 5, and the
determination of formate in blood samples following the direct extraction in Fig. 6.

3.4 C\textsuperscript{4}D on microchip devices

A number of reports concern the construction of embedded sensing electrodes for microchip
devices. While it has previously been demonstrated that it is possible to work with external
electrodes [72], embedded electrodes have larger coupling capacitances due to the thinner
insulating layers, which can be a benefit because it leads to lower required operating
frequencies (see the discussion in section 2.1). Liu and co-workers [73] prepared a PDMS
microchip electrophoresis device with embedded electrodes covered with a 0.6 µm thick layer
of PDMS acting as the insulating layer. They demonstrated substantially lower limits of
detection compared to microchips with the same design but higher insulating layer
thicknesses (15 and 50 µm). Coltro \textit{et al.} [43] presented a separation device with electrodes
which were isolated with a SiO\textsubscript{2} layer of only 50 nm thickness. Sensing electrodes can be also
fabricated by direct injection of molten alloys into microchannels, which are prepared during
microchip fabrication, and the technique represents a very economical way for precise
electrode fabrication [74, 75]. An alternative is the use of non-metal materials; a conductive
polymer (polyaniline, PANI) was shown as a suitable substitute for low-cost fabrication of
C\textsuperscript{4}D as well as of the high voltage electrodes in MCE devices [76].

Different authors reported embedded electrode designs in microchip devices which were not
intended for electrophoretic separations. Blaszcyk \textit{et al.} [77] designed a device which
employed electrolyte filled channels as contactless electrodes and demonstrated the
measurement of conductivity for standard solutions. A new highly stable insulating material
was used for the fabrication of a C\textsuperscript{4}D sensor, consisting of a 120 nm thick layer of perovskite oxide deposited over Pt electrodes [78]. The sensor was then used for measurements of salt solutions with various conductivities. In microchip devices, planar electrodes are predominantly used, which means that the capacitive coupling in general cannot be as good as for conventional capillaries where tubular electrodes are the norm. Lima \textit{et al.} [79] have thus also implemented concentric electrodes in a microfluidic flow-through device, which encompass the whole channel and have demonstrated a strong improvement in sensitivity compared to conventional planar electrodes [79]. A limit of detection of 344 pM was reported for flowing stream of LiClO\textsubscript{4} using the concentric electrodes, which was almost 4 orders of magnitude lower compared to planar electrodes.

Breadmore and coworkers [80] presented an instrument based on a dual channel microchip device with two separation channels and two C\textsuperscript{4}D cells for the concurrent separation of cations and anions. The microfluidic device was connected to an external manifold consisting of pumps and valves for automated sampling and flushing. Hydrodynamic injection was employed in order to avoid the injection bias which occurs for the otherwise often used electrokinetic injection mode. A photograph of the chip device with attachments is shown in Fig. 7 and electropherograms for the automated repetitive determination of cations and anions are shown in Fig. 8.

A portable, battery powered system was reported by Ansari \textit{et al.} [81], which is very small (14 × 25 × 8 cm) and light (1.2 kg). It operates with detection electrodes which are external to the separation chips. These are therefore much easier and cheaper to manufacture than chip devices with embedded electrodes. In order to ensure high sensitivity, a dual top-bottom electrode configuration was used, which encompasses the separation channel from both sides.
Electrodes are positioned in exchangeable cartridges with various designs (i.e. cells with
detection gaps from 0.5 to 2 mm), which can be replaced instantly and thereby enable
selection of an appropriate detection system optimized either for resolution or for sensitivity.
The replaceable C4D cells were shown suitable for analyses of standard solutions as well as of
food and clinical samples and detection limits below 1 µM were achieved.

In-line coupling of solid phase extraction (SPE) to MCE was demonstrated by Zhai et al. [82].
In their set-up a short segment (27 mm) of a monolithic SPE column was coupled to a
glass/PDMS microchip and all analytical procedures were carried out on the same device. The
sample was first injected and pretreated on the SPE column, then washed with methanol into
the injection channel and finally separated and detected using C4D.

A method for fabrication of cheap microchips based on printing the microchannel structure on
a thin polyester film using a laser printer and laminating it with a second polyester film, which
acts as the microchip cover, was described by Coltro and co-workers [83–85]. C4D electrodes
were fabricated from thin printed circuit boards and were placed on a chip holder underneath
the chips. Different processes were used for chip fabrication employing black and white [83, 84]
and colour [85] printing of the microchip structures, which showed a surprisingly
significant effect of the toner characteristics on separation efficiencies and detection
sensitivities.

4 Applications of electrophoresis methods with conventional capillaries

A comprehensive list of CE-C4D publications in various application fields and of CE-C4D
hyphenations with sample pretreatment and analytical techniques reported in the last two
years is given in Table 1. Additionally, information on recent CE-C\textsuperscript{4}D applications can also be found in a review article published on general aspects of electrochemical detection methods in CE by Matysik and coworkers in 2012 [16] and in a review article specifically devoted to applications of CE-C\textsuperscript{4}D by Elbashir and Aboul-Enein also published in 2012 [13].

4.1 Pharmaceutical, clinical and forensic analysis

There is a strong interest in CE-C\textsuperscript{4}D for the determination of pharmaceutically relevant compounds. The development of new drugs, which possess no chromophores and are therefore not easily detectable by conventional UV-Vis detection, and a general acceptance of CE-C\textsuperscript{4}D as an economic, rapid and efficient method for the analyses of complex samples resulted into an increased number of CE-C\textsuperscript{4}D applications in the field.

A range of adulterants, usually synthetic compounds added to natural pharmaceutical formulations, was evidenced in several herbal formulations by recent CE-C\textsuperscript{4}D studies [86-88]. Various painkillers [89-92], antibiotics [93, 94], antihypertensives [95], muscle relaxants [96] and enzyme inhibitors [97, 98], were determined in pharmaceutical formulations in order to prove their composition and content of active ingredients. CE-C\textsuperscript{4}D also enables determination of the active ingredient and its counter-ion, which may often reveal counterfeit medicines, which is a serious problem for medicine markets in poor countries. Vidal \textit{et al.} [98] developed a rapid method for determination of sildenafil, vardenafil and their anionic counter ions (chloride, citrate) using a dual-C\textsuperscript{4}D electrophoretic system. Determination of diclofenac and its counter-cations was also demonstrated by Cunha \textit{et al.} [92].

CE-C\textsuperscript{4}D has also often been reported for the determination of ionic analytes in clinical samples, such as in urine, serum, plasma and whole blood. A range of analytes, \textit{e.g.} inorganic
cations, amino acids, human serum albumin and basic drugs were determined in a single run in various complex matrices [62]. Perchlorate was determined after direct electrokinetic injection of several body fluids across supported liquid membranes [71]. A method for collection of a novel biological fluid, exhaled breath condensate, and subsequent analysis of small inorganic ions therein, was demonstrated [99]. Other applications, such as the determination of lactate in cell cultures [55], glycosidic antibiotics in bronchial epithelial lining fluid [59], free/total valproic acid in human plasma [60], and neurotransmitters in periaqueductal gray matter [61], were also reported. The latter application is illustrated in Fig. 9.

Analyses of ionic analytes may also be necessary in forensic/toxicological science and several applications of CE-C4D were reported in the reviewed period. Scopolamine, a tropane alkaloid, is often used for recreational and predatory purposes. A simple CE-C4D method for determination of scopolamine and atropine, a related alkaloid, in seeds, drinks and body-lotions was presented by Sáiz et al. recently [47]. Methanol poisonings are often reported as a consequence of ethanol adulteration by methanol and subsequent application of the toxic mixture in alcoholic beverages production. In human body, methanol is enzymatically dehydrogenated to formic acid, which is responsible for the serious methanol toxicity. Several reports on CE-C4D determination of formic acid in body fluids of methanol-intoxicated patients were reported after the recent “Methanol affair” in the Czech Republic [70, 100, 101]. Moreover, CE-C4D methods can easily be applied to the simultaneous determination of formic acid and other ionic substances, for example, oxalic and glycolic acids, which are the toxicological markers of ethylene glycol poisoning [101].
4.2 Food analysis

Application of CE-C\textsuperscript{4}D in analyses of food samples is of high relevance, since many analytes are small ions and samples need to be analysed rapidly with minimum sample pretreatment. Determination of short chain aliphatic alcohols (ethanol – 1-pentanol) was reported after electrochemical oxidation of alcoholic and non-alcoholic beers [68]. Fatty acids were determined in margarine and vegetable oil samples using conventional CZE [102] and non-aqueous CE [36]. The separation of the enantiomers of tartaric acid in wine and grapes were carried out by ligand-exchange CE [103]. Eight biogenic amines (e.g. spermine, spermidine, putrescine, cadaverine, etc.), which are often reported for their benign biological characteristics, were determined in several liquor samples [104]. A rapid CE-C\textsuperscript{4}D method for determination of saccharides in energy drinks utilizing short capillaries was described recently by Vochyánová et al. [56]. The content of other major components of energy drinks, namely caffeine and taurine, was also examined [57]. Stojkovic et al. reported the determination of artificial sweeteners [53].

4.3 Environmental analysis and other applications

The determination of inorganic anions, cations and heavy metals has been reported for environmental samples, namely in lake sediment porewater [48, 49], soil extracts [105] and extracts of aerosol samples (PM2.5) [106]. Nie et al. [107] reported the use of CE-C\textsuperscript{4}D in the analysis of solutions used in corrosion studies [107]. An important reason for choosing CE-C\textsuperscript{4}D for these applications is its applicability for small sample volumes in the low µL-range. Another consideration was the need to analyse both, cations and anions. This usually requires two runs to be performed, in which the positive and negative ions are determined separately. The simultaneous determination of anions and cations is possible through dual-opposite end injections (DOI) in one run, where cations are injected into one end and anions into the
opposite end of the separation capillary and detection is performed approximately in its
centre. Due to its universality this method is greatly facilitated by the use of C^4D.
Applications of DOI in CE-C^4D of small inorganic anions and cations in environmental water
samples were reported by Kobrin et al. [50] and by Naega et al. [108].

CE-C^4D analyses of environmental waters were further demonstrated on determination of
warfare agent (nitrogen mustard) degradation products [46] and of organophosphorous
pesticide glyphosate and its major metabolite aminomethyl phosphonic acid [65]. Volatile
organic acids were determined in air after conversion into liquid samples using a tubular
porous polypropylene sampling device [45].

Other applications include the determination of three polyphenols in tobacco leafs [109], a set
of 14 lanthanides in simulated spent nuclear reactor fuel [110], peroxycarboxylates in
commercial peracetic acid [111] and PCR products in genetically modified soybeans [58].
Enzymatic assays of myrosinase were evaluated on the basis of sulphate production and its
subsequent CE-C^4D determination [112]. Effective mobilities of non-charged EOF markers
were determined in BGE solutions containing sulphated-β-cyclodextrins, which may complex
the EOF markers and induce their non-zero effective mobilities [113].

5 Applications of microchip electrophoresis

MCE with C^4D has proved to be useful in the analyses of complex samples. Inorganic cations
were determined in rabbit blood serum and urine [81]. Other applications involve
determination of ofloxacin and its enantiomers in eye drops [114, 115] and determination of
lactate in synovial fluids [116]. A MCE method for determination of partition coefficients of
selected pharmaceuticals, which was based on phase distribution between 1-octanol and water, was also described [117]. Detail on applications of C\textsuperscript{4}D in MCE reported in the last two years is given in Table 2.

6 Other analytical applications of C\textsuperscript{4}D

Conductivity measurements in the contactless mode are non-invasive and for this reason have been employed in the examination of narrow-bore chromatographic columns. C\textsuperscript{4}D was used for the monitoring of the development of a monolithic stationary phase during its \textit{in situ} fabrication [118], the characterization of monolithic capillary columns with integrated gold nano-particles [119], and the characterization of iminodiacetic acid functionalised monolithic columns [120, 121].

In industry applications, important information may be gathered by measurements of flowing streams. In order to obtain measurements at the industrial scale, significantly larger IDs of the tubing are required than can be accommodated with the C\textsuperscript{4}D cells for CE or LC. Li and co-workers have designed various C\textsuperscript{4}D measuring cells, which are able to perform conductivity measurements in pipes with IDs up to 7.8 mm. These cells were then shown to be suitable for flow-through measurements via bubble velocity in a two phase (gas-liquid) system [38, 39] and for flow-through measurements of solution conductivities [28, 29].

A microfluidic device with electrochemical cell and C\textsuperscript{4}D was developed, which combines label-free isothermal amplification of nucleic acids with subsequent real-time monitoring [122]. By using this approach, pure DNA can be determined down to 0.1 pg/mL. Emaminejad \textit{et al.} [123] have demonstrated the use of C\textsuperscript{4}D on chip for cell counting, and interesting and
potentially very useful application derived from the well-established Coulter counter. When a cell passes between the two electrodes, a drop in conductivity occurs, showing up as a peak when recording the signal vs. time, which allows the counting of single cells.

A formerly developed reagent-free SIA system with C^4D was used by Mantim and coworkers for determination of dissolved carbon dioxide in beverages [124]. Newill et al. [42] employed their planar 2D grid of contactless electrodes mentioned above for the determination of moisture distribution in the soil of the root area of plants in a special laboratory growth container.

7 Concluding remarks

A solid number of applications of CE-C^4D with conventional capillaries has been reported, which further demonstrates the growing maturity of the method. Frequently commercial detectors are used and C^4D is becoming an accepted part of the toolbox. In comparison to other methods CE-C^4D has the advantage of being universal for all ionic species. It also tends to be more tolerant to the sample matrix and often only a dilution is necessary as pretreatment to avoid an overload. However, for complex samples appropriate clean-up/preconcentration are necessary just as for other analytical methods. In particular, the determination of concentrations below about 1 µM is not possible without preconcentration. For C^4D in microchip devices relatively few real applications have been reported, the majority of publications are still dealing with design issues. This is a somewhat curious situation considering that it is often claimed that microchip methodology is revolutionizing the analytical sciences. One shortcoming of MCE-C^4D might be the limited separation efficiency. The trend to new uses of C^4D outside CE has continued. C^4D dates back about 70 years and
was not new when introduced to CE. However, it is good to see that CE-C4D has inspired new investigations of the wider merits of C4D.

Acknowledgements

The authors would like to thank the Swiss National Science Foundation (Grant No. 200020-149068/1), the Academy of Sciences of the Czech Republic (Institutional Support RVO:68081715) and the Grant Agency of the Czech Republic (Grant No. 13-05762S) for partial financial support, as well as Boris Schlesky for useful discussions on the fundamentals of C4D.
8 References


### Table 1. Applications of C\(^4\)D in conventional CE.

<table>
<thead>
<tr>
<th>Analytes</th>
<th>BGE composition</th>
<th>C(^4)D parameters</th>
<th>Mode</th>
<th>Sample type</th>
<th>LODs</th>
<th>Ref.</th>
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<tbody>
<tr>
<td><strong>Food analysis</strong></td>
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<td></td>
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<tr>
<td>Aliphatic alcohols</td>
<td>50 mM Tris, 10 mM HCl, pH 8.6</td>
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</tr>
<tr>
<td>Amines, biogenic</td>
<td>150 mM 18-crown-6, 500 mM acetic acid</td>
<td></td>
<td></td>
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<tr>
<td>Artificial sweeteners</td>
<td>150 mM CHES, 400 mM Tris, pH 9.1</td>
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<tr>
<td>Caffeine, taurine</td>
<td>40 mM CHES, 15 mM NaOH, 50 mM sodium dodecyl sulfate, pH 9.36</td>
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<tr>
<td>Fatty acids</td>
<td>100% MeOH + 10 mM deoxycholic acid sodium salt</td>
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<tr>
<td>Inorganic cations and anions</td>
<td>12 mM His, 2 mM 18-crown-6, adjusted to pH 4 with acetic acid</td>
<td></td>
<td></td>
<td></td>
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<td>Saccharides</td>
<td>75 mM NaOH</td>
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<tr>
<td>Scopolamine</td>
<td>10 mM HEPES, Tris, pH 7.6</td>
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<tr>
<td>Tartaric acid enantiomers</td>
<td>7 mM CuCl(_2), 14 mM trans-4-hydroxy-L-proline, 100 mM ε-aminoacaproic acid, adjusted to pH 5 with HCl</td>
<td></td>
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<tr>
<td><strong>Pharmaceutical, clinical and other complex sample analysis</strong></td>
<td></td>
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<tr>
<td>Amikacin, kanamycin</td>
<td>20 mM MES, adjusted to pH 6.6 with His, 0.3 mM CTAB</td>
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<tr>
<td>Amikacin, urea</td>
<td>30 mM malic acid, adjusted to pH 4.1 with Arg, 10 mM 18-crown-6</td>
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</tr>
<tr>
<td>Anorectics, antidepressants (adulterants)</td>
<td>50 mM phosphate buffer, 50% (v/v) ACN, pH adjusted by 0.1 M H(_2)PO(_4)</td>
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<tr>
<td>Caffeine, dipyrone, aceytylsalicic acid</td>
<td>10 mM 3,4-dimethoxycinnamate, 20 mM Tris, pH 8.4</td>
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<td></td>
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<tr>
<td>Ciprofloxacin</td>
<td>1.8 mM oxalic acid, 12 mM triethanolamine, pH 8.5</td>
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<tr>
<td>Creatinine, choline</td>
<td>5.2 M acetic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diclofenac + counter-cations</td>
<td>10 mM Tris, 10 mM TAPS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diclofenac, codeine</td>
<td>1.8 mM oxalic acid, 10 mM triethanolamine, pH 8.4</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Substance</td>
<td>Conditions</td>
<td>Equipment</td>
<td>Notes</td>
<td></td>
<td></td>
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<tr>
<td>-----------</td>
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<td></td>
</tr>
<tr>
<td>Diuretics, laxatives (adulterants)</td>
<td>20 mM H₃PO₄, 40 mM NaOH, 30% (v/v) MeOH, pH 9.2</td>
<td>4 V&lt;sub&gt;pp&lt;/sub&gt;, 1.1 MHz</td>
<td>CZE</td>
<td>Food supplements 1.5 – 3.3 mg/kg [86]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA ladder fragments</td>
<td>20 mM Tris, 20 mM CHES, 5% PVP, pH 8.5</td>
<td>380 V&lt;sub&gt;pp&lt;/sub&gt; 200 kHz</td>
<td>CZE</td>
<td>Bacterial plasmid DNA, soybeans Serum, plasma, whole blood Serum</td>
<td>1.5 µM [70]</td>
<td></td>
</tr>
<tr>
<td>Formate</td>
<td>20 mM His, 25 mM glutamic acid, pH 4.8</td>
<td>Agilent, 50 V&lt;sub&gt;pp&lt;/sub&gt;, 1.84 MHz</td>
<td>µD-EKI-CZE</td>
<td>CZE</td>
<td>Periaqueductal grey matter</td>
<td>9 – 15 nM [61]</td>
</tr>
<tr>
<td>Glycine, glutamate, GABA</td>
<td>20 mM acetic acid, pH 1.9</td>
<td>Agilent</td>
<td>µD-LVSS-CZE</td>
<td>CZE</td>
<td>Food supplements 1.5 – 3.3 mg/kg [86]</td>
<td></td>
</tr>
<tr>
<td>Hypoglycemics (adulterants)</td>
<td>20 mM sodium acetate, pH 10.0</td>
<td>2 V&lt;sub&gt;pp&lt;/sub&gt;, 600 kHz</td>
<td>CZE</td>
<td>Artificial biological fluids</td>
<td>2.0 – 5.8 µg/mL [87]</td>
<td></td>
</tr>
<tr>
<td>Inorganic anions</td>
<td>5.2 M acetic acid</td>
<td>Agilent, 50 V&lt;sub&gt;pp&lt;/sub&gt;, 1.84 MHz</td>
<td>µ-EME-CZE</td>
<td>CZE</td>
<td>Artificial biological fluids</td>
<td>n.r. [63]</td>
</tr>
<tr>
<td>Inorganic cations</td>
<td>1 M, 3 M or 6 M acetic acid</td>
<td>Agilent, 50 V&lt;sub&gt;pp&lt;/sub&gt;, 1.25 MHz</td>
<td>EME-CZE</td>
<td>CZE</td>
<td>Artificial biological fluids</td>
<td>n.r. [63]</td>
</tr>
<tr>
<td>Inorganic cations, inorganic and organic anions</td>
<td>20 mM MES, 20 mM His, 30 µM CTAB, 2 mM 18-crown-6</td>
<td>20 V&lt;sub&gt;pp&lt;/sub&gt;, 290 kHz</td>
<td>CZE</td>
<td>DOI</td>
<td>Exhaled breath condensate</td>
<td>0.33 – 0.75 µM [99]</td>
</tr>
<tr>
<td>Lactate</td>
<td>25 mM Tris, 35 mM CHES, 0.02% poly(ethyleneimine), pH 8.65</td>
<td>TraceDec</td>
<td>SIA-CZE</td>
<td>ITP</td>
<td>Simulated spent MOX fuel</td>
<td>3 µM [55]</td>
</tr>
<tr>
<td>Lanthanides</td>
<td>LE: 14 mM HIBA or 14 mM HMBA, 10 mM acetic acid, adjusted to pH 4.5 with ammonia</td>
<td>TraceDec</td>
<td>SIA-CZE</td>
<td>ITP</td>
<td>Simulated spent MOX fuel</td>
<td>n.r. [110]</td>
</tr>
<tr>
<td>Muscle relaxants</td>
<td>30 mM ammonium acetate, 20 mg/mL HP-β-CD, pH 5.75</td>
<td>TraceDec</td>
<td>CZE</td>
<td>Pharmaceutical samples</td>
<td>26 – 28 µM [96]</td>
<td></td>
</tr>
<tr>
<td>Myrosinase kinetics (via SO₄²⁻ analysis)</td>
<td>His/acetic acid, I = 40 mM, pH 4.6</td>
<td>TraceDec</td>
<td>CZE</td>
<td>Enzymatic assays</td>
<td>15 µM (LOQ) [112]</td>
<td></td>
</tr>
<tr>
<td>Na&lt;sup&gt;+&lt;/sup&gt;, HSA</td>
<td>5.2 M acetic acid</td>
<td>Agilent, 50 V&lt;sub&gt;pp&lt;/sub&gt;, 1.84 MHz</td>
<td>µ-EME-CZE</td>
<td>CZE</td>
<td>Urine, serum</td>
<td>n.r. [64]</td>
</tr>
<tr>
<td>Na&lt;sup&gt;+&lt;/sup&gt;, saccharine, benzoate, ethanol</td>
<td>30 mM Tris, 10 mM HCl, pH 8.6</td>
<td>4 V&lt;sub&gt;pp&lt;/sub&gt;, 1.1 MHz</td>
<td>Agilent, 50 V&lt;sub&gt;pp&lt;/sub&gt;, 1.84 MHz</td>
<td>µ-EME-CZE</td>
<td>CZE</td>
<td>Mouthwash antiseptic sample</td>
</tr>
<tr>
<td>Oxalate, formate, glycolate</td>
<td>50 mM MES, 50 mM His, pH 6.1</td>
<td>4 V&lt;sub&gt;pp&lt;/sub&gt;, 1.1 MHz</td>
<td>Agilent, 50 V&lt;sub&gt;pp&lt;/sub&gt;, 1.84 MHz</td>
<td>µ-EME-CZE</td>
<td>CZE</td>
<td>Urine, serum</td>
</tr>
<tr>
<td>Perchlorate</td>
<td>15 mM nicotinic acid, 1 mM TDAPS, pH 3.3</td>
<td>Agilent, 50 V&lt;sub&gt;pp&lt;/sub&gt;, 1.84 MHz</td>
<td>SLM-EKI-CZE</td>
<td>CZE</td>
<td>Commercial peracetic acid</td>
<td>8 – 24 µM [111]</td>
</tr>
<tr>
<td>Performate, peracetate, perpropionate</td>
<td>20 mM Li/CHES, pH 9.8; 20 mM Li/β-alanine, pH 10.2; 20 mM Li/CHES, pH 9.0; 20 mM Li/TAPS, pH 8.5; 20 mM Li/TAPS, pH 8.0; 20 mM Li/TAPS, pH 8.0</td>
<td>4 V&lt;sub&gt;pp&lt;/sub&gt;, 1.1 MHz</td>
<td>CZE</td>
<td>Commercial peracetic acid</td>
<td>8 – 24 µM [111]</td>
<td></td>
</tr>
<tr>
<td>Substances</td>
<td>Buffer/Method</td>
<td>Detection Method</td>
<td>Concentration/Unit</td>
<td>Reference</td>
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</tr>
<tr>
<td>Polyphenols</td>
<td>150 mM 2-amino-2-methyl-1-propanol, pH 11.2</td>
<td>20 V&lt;sub&gt;pp&lt;/sub&gt;, 3 – 180 kHz</td>
<td>550 kHz</td>
<td>SPE CZE</td>
<td>Tobacco leaves</td>
<td>0.08 – 0.15 µg/g (LOQ)</td>
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<tr>
<td>Sildenafil, vardenafil + their counteranions</td>
<td>0.5 M acetic acid</td>
<td>4 V&lt;sub&gt;pp&lt;/sub&gt;, 1.1 MHz</td>
<td></td>
<td>CZE</td>
<td>Pharmacuticals</td>
<td>0.75, 0.9 µM</td>
</tr>
<tr>
<td>Terbinfine</td>
<td>10 mM acetic acid, sodium acetate, pH 4.7</td>
<td>4 V&lt;sub&gt;pp&lt;/sub&gt;, 1.1 MHz</td>
<td></td>
<td>CZE</td>
<td>Pharmacuticals</td>
<td>0.11 mg/L</td>
</tr>
<tr>
<td>Trimethoprim, sulfamethoxazole</td>
<td>Lithium phosphate, pH 7.1</td>
<td>4 V&lt;sub&gt;pp&lt;/sub&gt;, 1.1 MHz</td>
<td></td>
<td>CZE</td>
<td>Pharmacuticals</td>
<td>1.1, 3.3 µM</td>
</tr>
<tr>
<td>Valproic acid (free and total)</td>
<td>10 mM His, 10 mM MES, 10 µM CTAB, pH 6.5</td>
<td>380 V&lt;sub&gt;pp&lt;/sub&gt;, 200 kHz</td>
<td></td>
<td>CZE</td>
<td>Plasma</td>
<td>80 µg/L</td>
</tr>
</tbody>
</table>

### Environmental analysis

- **Amines, aliphatic (nitrogen mustard degradation products)**
  - 20 mM MES, adjusted to pH 6.0 with His
  - eDAQ, 100% V<sub>pp</sub>, 1200 kHz
  - Portablon CZE
  - River, well water
  - 5 µM
  - [46]

- **Amines, biogenic**
  - 150 mM 18-crown-6, 500 mM acetic acid
  - eDAQ, 60 V<sub>pp</sub>, 550 kHz
  - CZE
  - River, tap, lake water
  - 44 – 149 mg/mL
  - [104]

- **Glyphosate, AMPA**
  - 12 mM His, 8 mM MOPS, 50 µM CTAB, pH 6.3
  - eDAQ, 100% V<sub>pp</sub>, 300 kHz
  - EME-CZE
  - River water
  - 43, 64 pg/mL
  - [65]

- **Heavy metals**
  - 10 mM His, 50 mM acetic acid, 2.5 mM 18-crown-6, pH 4.2, linear polyacrylamide coated capillary
  - TraceDec
  - SIA-CZE
  - Water samples
  - 5 – 61 µg/L
  - [54]

- **Inorganic anions**
  - 10 mM His, 50 mM acetic acid, 2.5 mM 18-crown-6, pH 4.2, linear polyacrylamide coated capillary
  - 20 mM MES, 20 mM His, 20 µM CTAB, pH 6.1
  - 4 V<sub>pp</sub>, 300 kHz
  - Portablon SIA-CZE
  - Tap water
  - 0.7 µM
  - [44]

- **Inorganic anions and cations**
  - 11 mM His, 50 mM acetic acid, 1.5 mM 18-crown-6, 0.1 mM citric acid
  - TraceDec
  - Portablon CZE
  - Sediment porewater
  - 0.28 – 0.98 µM
  - [48]

- **Inorganic cations**
  - 10 mM His, 50 mM acetic acid, 0.5 mM 18-crown-6, pH 4.1
  - Agilent
  - EME-CZE
  - Water samples
  - n.r.
  - [126]

- **Inorganic cations**
  - 10 mM His, 50 mM acetic acid, 2.5 mM 18-crown-6, pH 4.2, linear polyacrylamide coated capillary
  - TraceDec
  - SIA-CZE
  - Water samples
  - 16 – 40 µg/L
  - [54]

- **Inorganic cations**
  - 30 mM MES, 30 mM His, 2 mM 18-crown-6, pH 6.1
  - 4 V<sub>pp</sub>, 1.1 MHz
  - CZE
  - Soil samples
  - 7 – 91 µM
  - [105]

- **Inorganic cations**
  - 11 mM His, 50 mM acetic acid, 1.5
  - TraceDec
  - Portablon
  - Sediment
  - 0.46 – 0.65 µM
  - [48]
and heavy metals

| and heavy metals | mM 18-crown-6, 0.1 mM citric acid, 11 mM His, 50 mM acetic acid, 1.5 mM 18-crown-6, 0.1 mM citric acid | TraceDec | e CZE | Portabl e CZE | Sediment porewater | n.r. | 1.55 µM | [49] |

Organic anions

| Organic anions | 20 mM MES, 20 mM His, 0.2 mM CTAB, pH 6.1, 0.1 mM citric acid | 4 V_pp, 1.1 MHz | Portabl e CZE | Sediment porewater | Air samples | n.r. | 5 µM | [44] |

Phosphate

| Phosphate | 1 mM His, 25 mM acetic acid, pH 3.47 | 20 V_pp, 300 kHz | Portabl e SIA-CZE | Sediment porewater | Air samples | 10 µM | [45] |

Phosphonic acids, inorganic, organic anions

| Phosphonic acids, inorganic, organic anions | 30 mM MES, 30 mM His, 0.2 mM CTAB, pH 10.2 | 4 V_pp, 1.1 MHz | CZE | Air samples | n.r. | [45] |

Industrial applications

| Industrial applications | Chloride | 10 mM 2,6-pyridinedicarboxylic acid, 0.5 mM CTAH, pH 4 | TraceDec | CZE | Industrial waters | 10 µg/L | [107] |
| Industrial applications | Heavy metals | 10 mM 2,6-pyridinedicarboxylic acid, 0.5 mM CTAH, pH 4 | TraceDec | CZE | Industrial waters | 100 µg/L | [107] |

Standard solutions

<p>| Standard solutions | Acid orange 7 + degradation products | 20 mM acetic acid | n.r. | CZE | Standard solutions | 0.013 – 0.047 µM | [127] |
| Standard solutions | Alkylsulfonates | 0.5 M acetic acid | eDAQ | EME-CZE | Standard solutions | n.r. | [67] |
| Standard solutions | Amino acids | 2 M acetic acid, 0.1% hydroxyethylcellulose, pH 2.1 | DRC^4D, 20 V_pp, 200 kHz | CZE | Standard solutions | n.r. | [24] |
| Standard solutions | Angiotensins I-IV | LE: 10 mM ammonium acetate, pH 4.5; TE: 10 mM acetic acid | Csense One | ITP | Standard solutions | n.r. | [128] |
| Standard solutions | CT, NO_3^- | 0.5 M acetic acid | C^4D array, 20 V_pp | CZE | Standard solutions | n.r. | [34] |
| Standard solutions | Dextran ladder | 100 mM formic acid or 100 mM acetic acid, pH 2.9 | TraceDec | t-ITP-CZE | Standard solutions | 10 nM | [129] |
| Standard solutions | Dopamine, adrenaline, noradrenaline | 100 mM acetic acid | 18 V_pp, 320 kHz | CZE | Standard solutions | n.r. | [35] |
| Standard solutions | Glucose, ribose | 37.5 mM NaOH, pH 12.5 | 18 V_pp, 320 kHz | CZE | Standard solutions | n.r. | [35] |
| Standard solutions | Inorganic and organic anions | 70 mM Tris, 70 mM CHES, 0.2 mM CTAB, pH 8.5 | 20 V_pp, 300 kHz | Portabl e SIA-CZE | Standard solutions | n.r. | [44] |
| Standard solutions | Inorganic and organic anions, inorganic cations, amines and aminoalcohols | MES/His at pH 6.1, 90/90 mM, 60/60 mM, 30/30 mM | 20 V_pp, 300 kHz | SIA-CZE | Standard solutions | 0.4 – 10 µM | [33] |
| Standard solutions | Inorganic cations | 50 mM acetic acid, 20 mM Tris, pH 4.5 | 18 V_pp, 320 kHz | CZE | Standard solutions | n.r. | [35] |
| Standard solutions | Inorganic cations and heavy metals, inorganic anions | 0.5 M acetic acid | C^4D array, 20 V_pp | CZE | Standard solutions | n.r. | [34] |
| Standard solutions | K^+, Na^+ | 12 mM His, 2 mM 18-crown-6, adjusted to pH 4.0 with acetic acid | 380 V_pp, 200 kHz | SIA-CZE | Standard solutions | 1 – 3 µM | [32] |</p>
<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration Details</th>
<th>Instrument</th>
<th>Method</th>
<th>Standard Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobility measurements</td>
<td>20 mM succinic acid, 30 mM LiOH, 60 g/L sulfated-β-CD, pH 5.5</td>
<td>Agilent</td>
<td>CZE</td>
<td>n.r. [113]</td>
</tr>
<tr>
<td>Monoalkyl carbonates</td>
<td>10 mM NaHCO₃, pH 8.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nicotine, cotinine</td>
<td>45 mM acetic acid, pH 3.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO₂⁻, NO₃⁻, SO₄²⁻</td>
<td>30 mM MES, 30 mM His</td>
<td>RC⁺D, 200 V&lt;sub&gt;pp&lt;/sub&gt;, 250 kHz</td>
<td>CZE</td>
<td>n.r. [32]</td>
</tr>
<tr>
<td>Peptides</td>
<td>0.75 M acetic acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perchlorate, inorganic anions</td>
<td>7.5 mM His, 40 mM acetic acid, pH 4.05</td>
<td>Portabl e CZE</td>
<td>EMEx</td>
<td>2 nM [66]</td>
</tr>
<tr>
<td>Quaternary ammonium ions</td>
<td>MeOH/ACN (90%/10%), 10 mM deoxycholic acid sodium salt 0.2 M acetic acid</td>
<td>eDAQ</td>
<td>NACE</td>
<td>0.1 – 0.7 µM [37]</td>
</tr>
</tbody>
</table>

**Abbreviations:**
- AMPA – aminomethyl phosphonic acid
- CTAB – cetyl trimethylammonium bromide
- CTAH – cetyl trimethylammonium hydroxide
- DRC⁺D – differential resonant C⁺D
- EKI – electrokinetic injection
- HIBA – α-hydroxyisobutyric acid
- HMBA – 2-hydroxy-2-methylbutyric acid
- LVSS – large volume sample stacking
- µD – micro-dialysis
- MEKC – micellar electrokinetic chromatography
- NACE – non-aqueous capillary electrophoresis
- RC⁺D – referenced C⁺D
- TDAPS – 3-(N,N-dimethylmyristylammonio) propanesulfonate
- Tween 20 – Polyethylene glycol sorbitan monolaurate
- n.r. – not reported
### Table 2. Applications of C\(^{13}\)D in microchip electrophoresis.

<table>
<thead>
<tr>
<th>Analytes</th>
<th>BGE composition</th>
<th>C(^{13})D parameters</th>
<th>Material</th>
<th>Mode</th>
<th>Sample type</th>
<th>LODs</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auramine O</td>
<td>5 mM lactic acid, 15%</td>
<td>40 (V_{pp}), 60 kHz</td>
<td>Glass/</td>
<td>SPE-CZE</td>
<td>Shrimp</td>
<td>2.5 (\mu g/mL)</td>
<td>[82]</td>
</tr>
<tr>
<td></td>
<td>(v/v) MeOH</td>
<td></td>
<td>PDMS</td>
<td></td>
<td></td>
<td>9 – 24 (\mu M)</td>
<td>[80]</td>
</tr>
<tr>
<td>Cl(^{-}), F(^{-}), HPO(_{4})(^{2-})</td>
<td>50 mM acetic acid, 10 mM</td>
<td>TraceDec</td>
<td>PMMA</td>
<td>SIA-CZE</td>
<td>Standard solutions</td>
<td>45 – 70 (\mu M)</td>
<td>[84]</td>
</tr>
<tr>
<td></td>
<td>His, pH 4.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glyphosate, AMPA</td>
<td>80 mM CHES/Tris, pH 8.8</td>
<td>4.5 (V_{pp}), 320 kHz</td>
<td>Polyester</td>
<td>CZE</td>
<td>Environmental samples</td>
<td>12.5 – 45 (\mu M)</td>
<td>[81]</td>
</tr>
<tr>
<td>Inorganic and organic anions</td>
<td>10 mM His, 7 mM glutamic acid, pH 5.53</td>
<td>20 (V_{pp}), 300 kHz</td>
<td>PC</td>
<td>CZE</td>
<td>Standard solutions</td>
<td>1.6 – 12.4 (\mu M)</td>
<td>[81]</td>
</tr>
<tr>
<td>Inorganic cations</td>
<td>30 mM MES, 30 mM His, 2 mM 18-crown-6, pH 6.0</td>
<td>20 (V_{pp}), 300 kHz</td>
<td>PC</td>
<td>CZE</td>
<td>Urine, serum</td>
<td>n.r.</td>
<td>[81]</td>
</tr>
<tr>
<td></td>
<td>6.5 mM maleic acid, 7.5 mM Arg, 1.5 mM 18-crown-6, pH 4.6</td>
<td>20 (V_{pp}), 200 kHz</td>
<td>PDMS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K(^{+}), Na(^{+})</td>
<td>20 mM MES, 20 mM His</td>
<td>30 (V_{pp}), 120 kHz</td>
<td>PDMS</td>
<td>CZE</td>
<td>Standard solutions</td>
<td>0.07 (\mu M)</td>
<td>[73]</td>
</tr>
<tr>
<td>K(^{+}), Na(^{+}), Li(^{+})</td>
<td>20 mM MES, 20 mM His, 3% (v/v) EtOH, pH 6.1</td>
<td>10 (V_{pp}), 400 kHz</td>
<td>PDMS</td>
<td></td>
<td>Energy drinks, pharmaceuticals</td>
<td>4 – 23 (\mu M)</td>
<td>[83]</td>
</tr>
<tr>
<td>K(^{+}), Na(^{+}), Li(^{+})</td>
<td>20 mM MES, 20 mM His, pH 6.1</td>
<td>5 or 10 (V_{pp}), 400 kHz</td>
<td>Polyester</td>
<td>CZE</td>
<td></td>
<td>n.r.</td>
<td>[85]</td>
</tr>
<tr>
<td>K(^{+}), Na(^{+}), Li(^{+})</td>
<td>50 mM acetic acid, 10 mM His, pH 6.2</td>
<td>TraceDec</td>
<td>PMMA</td>
<td>SIA-CZE</td>
<td>Standard solutions</td>
<td>5 – 16 (\mu M)</td>
<td>[80]</td>
</tr>
<tr>
<td>K(^{+}), Na(^{+}), Li(^{+})</td>
<td>45 mM MES, 55 mM His, pH 5.9</td>
<td>TraceDec</td>
<td>PMMA</td>
<td>CZE</td>
<td>Standard solutions</td>
<td>26 – 73 (\mu M)</td>
<td>[76]</td>
</tr>
<tr>
<td>K(^{+}), Na(^{+}), Li(^{+})</td>
<td>15 mM MES, 15 mM His</td>
<td>5.5 (V_{pp}), 220 kHz</td>
<td>PDMS</td>
<td></td>
<td>Standard solutions</td>
<td>6.1 – 8.5 (\mu M)</td>
<td>[74]</td>
</tr>
<tr>
<td>K(^{+}), Na(^{+}), Li(^{+})</td>
<td>20 mM MES, 20 mM His, pH 6.1</td>
<td>6 (V_{pp}), 90 kHz</td>
<td>Glass</td>
<td>CZE</td>
<td>Standard solutions</td>
<td>n.r.</td>
<td>[132]</td>
</tr>
<tr>
<td>Lactate</td>
<td>10 mM Tris, 1 mM HCl, 0.1 mM CTAB, pH 9.1</td>
<td>90 (V_{pp}), 60 kHz</td>
<td>PMMA</td>
<td>CZE</td>
<td>Synovial fluid</td>
<td>6.5 (\mu M)</td>
<td>[116]</td>
</tr>
<tr>
<td>NH(_{4})(^{+}), Na(^{+}), Li(^{+})</td>
<td>50 mM acetic acid, 10 mM His, pH 4.2</td>
<td>TraceDec</td>
<td>PMMA</td>
<td>CZE</td>
<td>Standard solutions</td>
<td>86 – 326 (\mu M)</td>
<td>[75]</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>1 mM MES, 1 mM His, pH 6.5</td>
<td>22 (V_{pp}), 60 kHz</td>
<td>PMMA</td>
<td>CZE</td>
<td>Eye drops</td>
<td>21 (\mu g/mL)</td>
<td>[115]</td>
</tr>
<tr>
<td>Ofloxacin enantiomers</td>
<td>1 mM MES, 1 mM Tris, pH 8.0</td>
<td>22 (V_{pp}), 60 kHz</td>
<td>PMMA</td>
<td>CZE</td>
<td>Eye drops</td>
<td>18 – 21 (\mu g/mL)</td>
<td>[114]</td>
</tr>
<tr>
<td>Partition coefficients – berberine</td>
<td>1 mM acetic acid, 3 mM sodium acetate</td>
<td>60 (V_{pp}), 60 kHz</td>
<td>PMMA</td>
<td>CZE</td>
<td>Standard solutions</td>
<td>5.6 (\mu g/mL)</td>
<td>[117]</td>
</tr>
<tr>
<td>Partition coefficients – lidocaine</td>
<td>1 mM acetic acid, 2 mM sodium acetate, 1% (v/v) EtOH</td>
<td>60 (V_{pp}), 60 kHz</td>
<td>PMMA</td>
<td>CZE</td>
<td>Standard solutions</td>
<td>4.0 (\mu g/mL)</td>
<td>[117]</td>
</tr>
<tr>
<td>Partition coefficients – lysine</td>
<td>15 mM boric acid, 5 mM ethanediamine</td>
<td>60 (V_{pp}), 60 kHz</td>
<td>PMMA</td>
<td>CZE</td>
<td>Standard solutions</td>
<td>3.1 (\mu g/mL)</td>
<td>[117]</td>
</tr>
<tr>
<td>Partition coefficients – procaine</td>
<td>1 mM acetic acid, 4 mM sodium acetate</td>
<td>60 (V_{pp}), 60 kHz</td>
<td>PMMA</td>
<td>CZE</td>
<td>Standard solutions</td>
<td>2.5 (\mu g/mL)</td>
<td>[117]</td>
</tr>
</tbody>
</table>

AMPA – aminomethyl phosphonic acid

CTAB – cetyl trimethylammonium bromide
PC – polycarbonate

TDAPS – 3-(N,N-dimethylmyristylammonio) propanesulfonate

n.r. – not reported
Figure Captions

Fig. 1 Schematic drawing of the standard capillary cell (A) and its simplified equivalent circuit diagram (B). $C_{\text{coupling}}$ are the wall capacitances for coupling the excitation voltage into the cell, and the resulting current out to the amplifier. $R$ is the solution resistance. $C_{\text{stray}}$ is due to unwanted, parasitic, direct coupling between the electrodes, and can be minimized by including a shield between the two half-cells.

Fig. 2 Predicted cell currents in dependence of the applied frequency for typical values of $C_{\text{coupling}}$ (0.1 pF) and $R$ (10 MΩ) for a cell as used for capillary electrophoresis [20] without a series inductor, and series inductors of 1 H and 10 H. The freeware circuit simulator Qucs was employed for the modelling.

Fig. 3 On-site measurement in a sewage treatment plant with the portable CE instrument with automated hydrodynamic injection described in [44].

Fig. 4 Fast separation of 4 anions carried out in a conventional capillary and on a portable CE-C$^4$D instrument. Reprinted with permission from [44]. Copyright (2013) American Chemical Society.

Fig. 5 Electrokinetic injection across micro-dialysis membrane for direct injection of blood samples reported by Kubáň and Boček [70]. Reproduced with permission from Elsevier.
Fig. 6 Direct analysis of formate in blood samples of a healthy person (a) and a patient intoxicated with methanol (traces b-f) reported in [70]. Reproduced with permission from Elsevier.

Fig. 7 Photograph of the dual channel microchip electrophoresis devices reported by Breadmore and coworkers [80]. Reprinted with permission from [80]. Copyright (2014) American Chemical Society.

Fig. 8 Concurrent electropherograms acquired in the two channels of the device shown in Fig. 7 for automated repetitive injections. Reprinted with permission from [80]. Copyright (2014) American Chemical Society.

Fig. 9 Determination of 1) γ-aminobutyric acid (GABA), 2) glycine, and 3) glutamate in a micro-dialysate of grey brain matter reported by Tůma et al. [61]. Reproduced with permission from Elsevier.
Fig. 1

(A) Actuator Electrode and Pick-up Electrode

(B) Circuit diagram with R, C_{coupling}, and C_{stray}
Cell Current (µA)

Fig. 2

- Inductor of 10 H
- No inductor
- Inductor of 1 H

Frequency (Hz)
318x212mm (300 x 300 DPI)
Inorganic Cations and Anions in the presence of 2 mM of 18-crown-6. Capillary: 25 μm I.D., 36 cm effective length and 60 cm total length. Separation voltage: +15 kV.  

<table>
<thead>
<tr>
<th>ion</th>
<th>linear range (μM)</th>
<th>r²</th>
<th>LODa (μM)</th>
<th>intraday reproducibility of peak area (%RSD)b</th>
<th>intraday reproducibility of migration time (%RSD)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₄⁺</td>
<td>6–100</td>
<td>0.9994</td>
<td>2</td>
<td>1.1</td>
<td>0.5</td>
</tr>
<tr>
<td>K⁺</td>
<td>6–100</td>
<td>0.9996</td>
<td>2</td>
<td>1.7</td>
<td>0.4</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>6–100</td>
<td>0.9983</td>
<td>2</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>Na⁺</td>
<td>10–100</td>
<td>0.9994</td>
<td>3</td>
<td>1.4</td>
<td>0.5</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>10–200</td>
<td>0.9992</td>
<td>3</td>
<td>1.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Li⁺</td>
<td>10–1000</td>
<td>0.9999</td>
<td>5</td>
<td>1.8</td>
<td>0.3</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>10–1000</td>
<td>0.9999</td>
<td>3</td>
<td>0.9</td>
<td>0.6</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>10–450</td>
<td>0.9999</td>
<td>3</td>
<td>1.0</td>
<td>0.4</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>5–450</td>
<td>0.9999</td>
<td>1.5</td>
<td>0.6</td>
<td>0.3</td>
</tr>
<tr>
<td>NO₂⁻</td>
<td>10–1000</td>
<td>0.9999</td>
<td>3</td>
<td>0.7</td>
<td>0.6</td>
</tr>
<tr>
<td>F⁻</td>
<td>27–2000</td>
<td>0.9998</td>
<td>8</td>
<td>2.1</td>
<td>0.5</td>
</tr>
<tr>
<td>H₂PO₄⁻</td>
<td>57–500</td>
<td>0.9991</td>
<td>17</td>
<td>2.4</td>
<td>0.3</td>
</tr>
</tbody>
</table>

aPeak heights corresponding to 3 × baseline noise. bDetermined for 50 μM, n = 9, over a period of 8 h.

Electrolyte of Tris/CHES at a concentration of 70 mM for each compound and in the presence of 200 μM CTAB for reversal of the electroosmotic flow. Three different soft drinks were analyzed as illustrative samples for this demonstration. The electropherograms for a standard mixture and for soft drink samples are shown in Figure 6. Electropherogram b is for a soft drink made from a byproduct of cheese production and for this reason contains a large concentration of lactate besides other anionic species. The cola beverage (electropherogram c) was found to contain phosphate, while the orange juice (electropherogram d), as expected, contained a high concentration of citrate.

**Application Example. Field Measurements of Phosphate at a Wastewater Treatment Plant.** To demonstrate its suitability for field work, the instrument was taken to a local sewage treatment plant and set up for the determination of phosphate. A solution of 1 mM His/25 mM acetic acid (pH 3.5) was found to be an optimal background electrolyte for the determination of this species. Under this condition, the phosphate peak is very well separated from the very broad peak of the major anions (Cl⁻, NO₃⁻, and SO₄²⁻) which are present in the sewage water at very high concentrations (ranging from 1 to 4.5 mM). An electropherogram for separation of phosphate in a sewage water sample is shown in Figure 7. In Table 2 the phosphate concentrations (mg P/L) measured with the new instrument in several samples are given together with the results from the standard photometric molybdenum blue method for validation. The first six samples were determined in the field (single measurements), and the remainder back in the laboratory (in triplicate). As shown in Table 2, the results from the CE method are in good agreement with those obtained from the molybdenum blue reference method (errors between the two methods were less than 10% for measurement done in the lab). However, the on-site...
of 20 various blood samples, and was then disposed of. No deterioration was observed in the membrane performance during this period, however, if preferred, membranes can also be used as disposable units (see Section 3.4). All injections were performed at 23 ± 2 °C.

2.1.2. Capillary electrophoretic system

A purpose-built CE instrument was employed for all electrophoretic runs. The separation voltage was provided by the CZE1000R (Spellman) HV power supply, which was operated at a potential of –16 kV applied at the injection side of the separation capillary for all runs. The same HV power supply was used for sample injections across dialysis membranes and for CE separations. Separation capillary used in these experiments was a fused-silica (FS) capillary (Polymicro Technologies, Phoenix, AZ, USA) with 25 μm ID, 375 μm OD, 36 cm total length and 18 cm effective length. New separation capillary was preconditioned with 1 M HCl for 5 min, DI water for 5 min and with background electrolyte (BGE) solution for 5 min. Between two successive CE runs, separation capillary was flushed with BGE solution for 45 s. Injections of standard solutions during system optimization (Section 3.1) were carried out hydrodynamically by elevating the sample vial to a height of 15 cm above the detection end of the separation capillary for 30 s. All CE-CD experiments were performed at 23 ± 2 °C.

2.1.3. Detection system

A capacitively coupled contactless conductivity detector used was developed and assembled at the Department of Physical Chemistry, Charles University, Prague and it operates at an excitation frequency and voltage of 1,843 MHz and 50 Vpp, respectively [48]. Data were collected using Orca-2800 data acquisition system (Ecom, Prague, Czech Republic) and processed with Clarity software (Data Apex, Prague, Czech Republic).

2.2. Reagents, electrolytes, standards, and real samples

All chemicals (reagent grade) were purchased from Pliva-Lachema, Brno, Czech Republic, Sigma, Steinheim, Germany and Fluka, Buchs, Switzerland, and DI water with resistivity higher than 18 MΩ cm was used throughout. Stock solutions of inorganic and organic anions (100 mM) were prepared from their Na+ and K+ salts or from organic acids. All stock solutions were kept refrigerated at 4 °C. Standard sample solutions of the anions were freshly prepared from these stock solutions and were diluted with DI water. Human serum albumin (HSA) was purchased from Sigma. BGE solutions for CE measurements were prepared weekly from L-histidine (Sigma) and L-glutamic acid (Sigma) and were kept refrigerated at 4 °C. Separation of formate and other inorganic/organic anions was achieved in BGE solution consisting of 20 mM L-histidine and 25 mM L-glutamic acid (pH 4.8). Human plasma and serum samples were purchased by lyophilized powders from Sigma, prepared according to supplier’s instructions and deep-frozen at –20 °C for storage. Additional samples of human serum and whole blood were collected from healthy individuals at the Institute of Analytical Chemistry; a written informed consent was signed by the volunteers before the experiments. Serum samples were prepared from clotted intravenous blood by centrifugation at 6000 rpm for 10 min and were stored at –20 °C. Whole blood was collected either from a finger stick (for instant analysis) or as heparinized intravenous whole blood that was deep-frozen at –20 °C for storage. Serum samples of a patient diagnosed and treated with acute methanol poisoning were donated by Prof. Robert Bocek from Department of Anaesthesiology, Resuscitation and Intensive Care, Hospital and Polyclinics Havířov, Czech Republic and were stored at –20 °C. All blood samples were diluted 1:9 with DI water before analysis.
A rapid method for determination of formate in blood samples was developed, which is based on in-line pretreatment of blood samples using a dialysis membrane, electrokinetic injection of small anions across the membrane directly into a separation capillary and rapid CE analysis of the injected anions. Interchange of individual membranes of the same type had no effect on the analytical system performance; one dialysis membrane was used for up to 100 injections of ten-fold diluted blood samples and its pretreatment efficiency remained unchanged for at least one working day. Formate, the major product of methanol metabolic degradation, was successfully determined in blood samples of a methanol poisoned patient by CE with C4D. As the method is cost effective, uses only microliter volumes of blood samples, injects directly whole blood, serum or plasma, and enables rapid and quantitative analyses, it might be used as a fast diagnostic tool for formate determination in suspected methanol poisonings.

Acknowledgments

Financial support from the Academy of Sciences of the Czech Republic (Institute Research Fund RVO:68081715) and the Grant Agency of the Czech Republic (Grant No. P206/10/1219) is gratefully acknowledged. Dr. Petr Kubáň (CEITEC, Masaryk University, Brno, Czech Republic) and Prof. Robert Bocek (Department of Anaesthesiology, Resuscitation and Intensive Care, Hospital and Polyclinics Havířov, Czech Republic) are acknowledged for donation of serum samples of a patient treated with acute methanol poisoning.

References


Fig. 6. CE-C4D determination of formate in serum of methanol-intoxicated patient after in-line injection across dialysis membrane. CE conditions as for Fig. 3. Full scale view (A) and detailed view (B). (a) Serum of a healthy person, (b) serum of the patient treated for 3 h, (c) same as (b) spiked with 1000 µM of formate, (d) serum of the patient treated for 4 h, (e) serum of the patient treated for 18 h and (f) serum of the patient 7 days after hospitalization. Dilution 1:9 with DI water.

well with formate half-life (~2–3 h) in serum under hemodialysis treatment [51], and remains fairly constant (0.12 ± 0.01 mM) also in the serum sample collected 7 days after hospitalization. Formate concentrations in traces (e) and (f) are within the range of endogenous formate concentrations in healthy individuals. Comparison measurements (CE-C4D) with hydrodynamic injection from 15 cm for 30 s were performed for the two samples (b and d) after deproteinization with acetonitrile, centrifugation, and 1:9 dilution with DI water. 12.2 ± 0.3 mM and 10.2 ± 0.3 mM formate were found in the two samples, respectively, which correlate well with the concentrations determined using the hyphenated method.

4. Concluding Remarks

A rapid method for determination of formate in blood samples was developed, which is based on in-line pretreatment of blood samples using a dialysis membrane, electrokinetic injection of small anions across the membrane directly into a separation capillary and rapid CE analysis of the injected anions. Interchange of individual membranes of the same type had no effect on the analytical system performance; one dialysis membrane was used for up to 100 injections of ten-fold diluted blood samples and its pretreatment efficiency remained unchanged for at least one working day. Formate, the major product of methanol metabolic degradation, was successfully determined in blood samples of a methanol poisoned patient by CE with C4D. As the method is cost effective, uses only microliter volumes of blood samples, injects directly whole blood, serum or plasma, and enables rapid and quantitative analyses, it might be used as a fast diagnostic tool for formate determination in suspected methanol poisonings.

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References


Fig. 6. CE-C4D determination of formate in serum of methanol-intoxicated patient after in-line injection across dialysis membrane. CE conditions as for Fig. 3. Full scale view (A) and detailed view (B). (a) Serum of a healthy person, (b) serum of the patient treated for 3 h, (c) same as (b) spiked with 1000 µM of formate, (d) serum of the patient treated for 4 h, (e) serum of the patient treated for 18 h and (f) serum of the patient 7 days after hospitalization. Dilution 1:9 with DI water.
For a channel with rectangular cross section $R_H$ is well-known, and for channels of width $w$ [m] and height $h$ [m], the following expression is valid when $h < w$ (low aspect ratio channels):

$$R_H = \frac{8\eta L}{\pi r^2}$$  \hspace{1cm} (3)

The hydraulic–electric circuit analogy where Hagen–Poiseuille’s law corresponds to Ohm’s law is well-known, meaning that the pressure drop is analogous to the voltage drop ($V$), the volumetric flow rate to the current ($I$), and the hydraulic resistance to the electric resistance ($R$). In a network of channels, equivalent resistances can be calculated as for electric circuits: $n$ channels in series have an equivalent resistance of $R_H = R_{H1} + R_{H2} + ... + R_{Hn}$, and $n$ channels in parallel have an equivalent resistance of $1/R_H = 1/R_{H1} + 1/R_{H2} + ... + 1/R_{Hn}$.

A schematic overview of the DCSI ME system is given in Figure 1, with an equivalent circuit diagram in the inset of Figure 1a and a photograph of the system at Figure 2. Using eqs 3 and 4 and $\eta = 1.002 \times 10^{-3}$ (Pa·s), $R_H$ was calculated for the major components of the system (the T-piece, feeder tubing, and tubing interconnects were excluded because in this context their resistance can be considered negligible). The results of these calculations are summarized in Table 2.

For a fixed $\Delta p$, as a result of constant Q (controlled by a constant volumetric flow rate supplied by the pumps) and all three waste outlets at atmospheric pressure $p_{atm}$, Q will be split proportionally between three paths: the two separation channels ($Q1 = Q2$), each of which has equal $R_H$ of $9.3 \times 10^{15}$ Pa·s·m$^{-3}$ (sum of serials 1, 6, and 7 from Table 2 when no external separation channel resistors are fitted) and the main waste outlet leading to the isolation valve (Q3) with an $R_H$ of $1.09 \times 10^{13}$ Pa·s·m$^{-3}$ (sum of serials 5, 6, 7, and 8 from Table 2). Using the hydraulic–electric circuit analogy we can derive the ratio $Q1/Q2/Q3 = 1:8:85$ indicating that 99.677% of the fluid will flow toward the main waste outlet. This ratio is considerably lower than that of the dual-capillary system where two 50 cm lengths of 50 μm i.d. capillary relative to a 10 cm long, 500 μm i.d. flow path to the main waste outlet yield a volumetric flow rate of capillary/capillary/waste of 1:1.5 × 10$^4$ (99.996%). Both of these values lie between the hydraulic resistance ratio of 10$^5$ where hydrodynamic isolation was observed and 94 where it failed as reported by Attiya et al.

Thus, experimental evaluation of the design was undertaken to establish its suitability.

**Sample Introduction and Hydrodynamic Isolation.** To examine the effectiveness of the injection interface design with this hydrodynamic ratio, a study of the sample introduction procedure was done by running sample past the interface without closing the isolation valve and effecting injection. Sample injection was made sequentially, and typical separation sequence steps are detailed in Table 1. A schematic of the sample introduction and injection procedure (corresponding to steps 3–9 of Table 1) is in Figure 3. IV2 is only included to simulate a situation without split injection and is kept closed during all phases for experiments described in the sections on Sample Introduction and Hydrodynamic Isolation through Effect of External Hydrodynamic Separation Channel Resistance. With both isolation valves closed, the separation channels were flushed (step 1) during which the inline pressure relief valve (87.1 psi) would overfill. IV1 was then opened and the BGE flow rate of 4 μL·s$^{-1}$ maintained to equilibrate pressure through the chip (step 2). Sample is then introduced in a way to minimize hydrodynamic leakage and introduce a short well-defined and controlled volume of sample. This was achieved by sheathing the sample flow with BGE during sample introduction (steps 3–5). During this process, there is a risk of the sample bleeding into the separation channels, during the ~1 s where only sample is pumped into the main channel (steps 6 and 7). To examine the extent of hydrodynamic bleed and to ensure no carryover of sample due to incomplete flushing of the interface, control studies were conducted where the pump sequence was conducted as indicated in Table 1, without the closure of IV1 for injection (step 7). Theoretically, any hydrodynamic bleed of sample during this ~1 s period would collect as a sample plug at the entrances of the separation channels which could be detected during the separation step (step 9). At flow rates $\geq 7$ μL·s$^{-1}$, small deviations from the baseline were observed, indicating that the $R_H$ ratio of the DCSI ME system was insufficient to restrict entry of the sample into the separation microchannels. However, at flow rates $\leq 6$ μL·s$^{-1}$, no changes in the baseline signal could be observed, indicating the sample is introduced only by actuation of IV1 and that there was a sufficiently high difference in hydraulic resistance to effectively isolate the separation microchannel during sample introduction.

**Hydrodynamic Control of Injection Volume.** In CE, typically 1–2% of the capillary length is injected with sample. In order to calculate the amount of sample injected when IV1 is closed, we return to the hydraulic–electric circuit analogy. For all steps in the sequence where the valves are open (filling the interface with sample and during separation), the volumetric flow rate is independent of the pressure drop across the inlet and outlet ports of the microchip and the combined flow from
Because K⁺ was resolved under all conditions, the analytical performance is measured by migration times, peak heights, theoretical plate number, and the variability in peak area and plotted in Figure 5a. Representative electropherograms for external hydrodynamic injection channel resistor values of infinite resistance (IV2 shut = 0 μm i.d.), 65, 90, and 125 μm i.d. are given in Figure 5b. With an external hydrodynamic injection channel resistor i.d. of 175 μm, the injection volume was highly variable (>50% RSD) and no injections were observed over 10 consecutive runs when using tubing with an i.d. of 250 μm; hence, the values of 175 and 250 μm resistors were omitted from Figure 5a. As illustrated in the figure, smaller volumes were injected with increasing diameter, and hence decreasing hydrodynamic resistance R3 of the external hydrodynamic injection channel resistor, as a result of the increased flow Q3. This effect is evidenced by the decreasing peak areas with increasing external hydrodynamic injection channel resistor internal diameters. Examination of Figure 5b reveals that the rate of decrease is different for each of the ions. The peak of Li⁺ is higher than K⁺ with no resistor and the 65 μm i.d. tubing connected, but the K⁺ is slightly higher with the 175 μm i.d. resistor. The more significant increase in peak height of Li⁺ as opposed to K⁺ when the diameter of the hydrodynamic resistor is changed is related to the change in hydrodynamic flow through the separation channel which changes the time in which the zone spends in the detector (akin to photon counting for fluorescence). Thus, Li⁺ has a greater change in velocity through the detector than K⁺ (Li⁺ is much slower than K⁺, so the hydrodynamic portion of its velocity is much greater, which changes the detector response). The % RSD of peak areas decreased with decreasing internal diameter, with all diameters ≤90 μm giving %RSD for peak area <5%, which is similar to the repeatability that is typical for conventional CE. Migration times (and SD) did not change significantly over the resistance ranges examined. The separation efficiencies increased with the smaller injection volumes, and while the repeatability decreased, it remained <5% for the 90 μm i.d. tubing. A maximum efficiency of 1129 plates (13 000 plates m⁻¹) was observed with the 90 μm i.d. tubing. All three peaks could be baseline-resolved when the R1:R3:R2 ratio during injection was ≈1:1.870 corresponding to external hydrodynamic separation channel hydrodynamic resistors of 30 cm × 90 μm i.d. PEEK tubing and an external hydrodynamic injection channel hydrodynamic resistor of 10 cm × 90 μm i.d.

**Simultaneous Separation of Cations and Anions.** The optimized conditions from the section on Split Injection Using External Hydrodynamic Injection Channel Resistor were used to run 100 consecutive separations of a mixture comprising K⁺, Na⁺, Li⁺, Cl⁻, F⁻, and PO₄³⁻ (primarily seen as H₂PO₄⁻ at pH 4.2). Figure 6 shows 10 consecutive separations, with the cations separated in a co-EOF mode in the cation channel, while the anions were separated in counter-EOF mode due to the cathodic EOF generated by the PMMA surface, although the magnitude of the EOF is likely to be low at pH 4.2. A BGE consisting of 50 mM acetic acid/10 mM l-histidine (pH 4.2) was chosen as it has been previously demonstrated as an excellent BGE for the separation of these anionic and cationic targets. The choice of BGE has a large impact on the sensitivity of C4D detection for specific analytes as sensitivity is largely determined by the difference in mobilities between the analyte and the BGE counterion. This is demonstrated here whereby the limit of detection (LOD) of H₂PO₄⁻ is approximately 20 times higher than that of Li⁺. Additionally, H₂PO₄⁻ is seen as a negative peak due to the fact that it has a lower mobility than the BGE counterion (acetate). Figures of merit were obtained for 20 runs, analyzing every fifth run, and are given in Table 3. The %RSD for peak areas ranges from 2.3% to 4.5%, which compares very well with our previous work using a dual-capillary system and electrokinetic injection and is comparable to performance data obtained with a conventional commercial CE instrument. While the LODs are about 1 order of magnitude higher when compared with our previous work in capillaries using the same electronics with the capillary head, the LODs are 2 orders of magnitude lower than we reported for the in-plane embedded C4D electrodes as a result of modifications in the grounding and shielding of the microchip system. Furthermore, the LODs of the DCSI ME system are about an order of magnitude lower than those reported for the same analytes using the same electronics in a different electrode arrangement using 4S/5S mm MES/His as BGE and slightly better than LODs recently published for in-plane metal C4D electrodes and a 15 mM MES/His buffer.

The efficiencies obtained with the DCSI ME system at a field strength of 450 V cm⁻¹ (13 300 plates m⁻¹) are slightly lower than those obtained for the same analytes using electrokinetically pinched injection from a standard injection cross (17
tested amino acids and 1.9, 1.4 and 1.0% for the peak areas of GABA, Gly and Glu, respectively. These values are fully comparable with the RSD values for standard CE determinations for which, however, long injection zones and the application of pressure during analyses are not employed.

3.3. Analysis of the CNS microdialysate

CNS microdialysates are complicated mixtures of inorganic and organic ions and low-molecular-mass electroneutral substances and, in CE analysis at about pH 2, the CED recording contains the peaks of almost all the biogenic amino acids. Fig. 5. Consequently, the CE/CED method was modified slightly: to attain higher separation efficiency and separation of GABA from excess Na⁺ ions, the I.D. of the capillary was reduced from 60 to 50 μm. Injection of microdialysate containing 75% (v/v) ACN was performed in an impulse of 50 mbar × 100 s, so that 25% of the total length of the capillary was filled and the time of forcing the zone out by a pressure of 50 mbar was equal to the injection time. The electropherogram obtained, Fig. 5, indicates that, under these conditions, all the monitored neurotransmitters are separated from the other substances. The basic analytical parameters are given in Table 3. The sensitivity and LODs were calculated on the basis of spiking of untreated microdialysate.

### Table 3

Parameters of the calibration dependences determined from the areas of the peaks of model mixtures of neurotransmitters in the concentration range 5 × 10⁻⁷–10⁻⁵ M, for injection of the sample in ACN to 98% of the total length of the capillary.

<table>
<thead>
<tr>
<th></th>
<th>Slope (mV s μM⁻¹)</th>
<th>Intercept (mV/s)</th>
<th>R</th>
<th>LOD (nmol L⁻¹)</th>
<th>LOD (μg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GABA</td>
<td>1.68 (0.03)</td>
<td>−0.31 (0.16)</td>
<td>0.9995</td>
<td>9</td>
<td>0.92</td>
</tr>
<tr>
<td>Gly</td>
<td>1.41 (0.02)</td>
<td>−0.19 (0.09)</td>
<td>0.9997</td>
<td>10</td>
<td>0.75</td>
</tr>
<tr>
<td>Glu</td>
<td>1.78 (0.02)</td>
<td>−0.39 (0.09)</td>
<td>0.9998</td>
<td>15</td>
<td>2.21</td>
</tr>
</tbody>
</table>

![Fig. 5. Electropherogram of PAG microdialysate for injection of the sample into 25% of the length of the capillary. Identification of peaks: GABA (1), Gly (2), Glu (3), 2-aminoisobutyric acid (IS).](image-url)

![Fig. 6. Levels of glycine (A), glutamate (B) and GABA (C) during microdialysis with designation of addition of carrageenan (60 min) and paracetamol (120 min). Identification, physiological solution + water (black), physiological solution + paracetamol (green), carrageenan + water (red), carrageenan + paracetamol (blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)](image-url)