THESIS ON NATURAL AND EXACT SCIENCES B222

Development of Point of Care Applications for Capillary Electrophoresis

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Declaration:
Hereby I declare that this doctoral thesis, my original investigation and achievement, submitted for the doctoral degree at Tallinn University of Technology has not been submitted for doctoral or equivalent academic degree before.

/Eeva-Gerda Kobrin/

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EEVA-GERDA KOBRIN

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CONTENTS

LIST OF PUBLICATIONS

This thesis is based on the following publications, which are referred to by Roman numerals within the text:

I. Kuban, P., Kobrin, E-G., Kaljurand, M. Capillary electrophoresis – A new tool for ionic analysis of exhaled breath condensate. *Journal of Chromatography A* **2012**, 1267, 239–245.

II. Kuban, P., Kobrin, E-G., Kaljurand, M. Potential of exaled breath condensate analysis in point of care diagnostics. *9-th International Interdisciplinary Meeting on Bioanalysis*, **2012**, 128–133.

III. Kobrin, E-G., Lees, H., Fomitšenko, M., Kuban, P., Kaljurand, M. Fingerprinting postblast residues by portable capillary electrophoresis with contactless conductivity detection. *Electrophoresis* **2014**, 35, 1165–1172.

IV. Jõul, P., Lees, H., Vaher, M., Kobrin, E-G., Kaljurand, M., Kuhtinskaja, M. Development of a capillary electrophoresis method with direct UV detection for the analysis of thiodiglycol and its oxidation products. *Electrophoresis* **2015**, 36, 1202–1207.

THE AUTHOR'S CONTRIBUTION

The contribution made by the author to the publications included is following:

I. The author was mostly responsible for sample collection and assisting in experimental and methodological optimization of dual-end injection and simultaneous ion analysis. The author participated in the interpretation of the results and analysis of data and also assisted in preparing the manuscript and preparation of figures.

II. The author was partially responsible for sample collection, assisted with experiments, participated in the interpretation of the results and analysis of data.

III. The author optimized the experimental setup and performed major part of sample collection, preparation and electrophoretic analysis. She conducted the pre-processing of electrophoretic data and interpreted the results with the aim to develop a rapid and reliable tool for evaluating the authenticity of explosives based on their unique ionic composition pattern-"fingerprint". The author wrote the manuscript and is the first and corresponding author.

IV. The author conducted preliminary experiments and identified a suitable electrophoretic methodology and participated in the preparation of the manuscript

ABBREVIATIONS

ANAL – ammonium nitrate-aluminium (mixture)
ARDS – acute respiratory distress syndrome
BGE – background electrolyte
C^4D – capacitively-coupled contactless conductivity detector
CE – capillary electrophoresis
CF – cystic fibrosis
COPD – chronic obstructive pulmonary disease
CTAB – cetyltrimethylammonium bromide
CWA – chemical warfare agents
DAD – diode array detector
DI – deionized
DMS – differential mobility spectrometry
ELISA – enzyme-linked immunosorbent assay
EOF – electroosmotic flow
ESI-DMS – electronspray ionization - differential mobility mass spectrometry
FTIR – Fourier transform infrared spectroscopy
GC – gas chromatography
GC-DMS – mass spectrometry-differential mobility spectrometry
GC-MS – gas chromatography-mass spectrometry
GC-nciMS – gas chromatography-ion chemical ionisation mass spectrometry
GC-TEA – gas chromatography thermal energy analyser
GERD – gastroesophageal reflux disease
HMTD – hexamethylene triperoxide diamine
HMX – high melting explosive
HPLC – high-performance liquid chromatography
HPLC-MS – high performance liquid chromatography–tandem mass spectrometry
IC – ion chromatography
IEDs – improvised explosive devices
IMS – ion mobility spectrometry
IS – internal standard
LC – liquid chromatography
LC-MS – liquid chromatography mass spectrometry
LED – light emitting diode
LoD – limit of detection
LoQ – limit of quantification
LOVA – low vulnerable ammunition
ME – matrix effect
MS – mass spectrometry
NMR – nuclear magnetic resonance
OSA – obstructive sleep apnea
PCA – principal component analysis
PETN – pentaerythritol tetranitrate

POC(T) – point-of-care (testing)
PP – polypropylene
PTR-MS – proton transfer reaction with mass spectrometry
PTR-TOF-MS – proton transfer reaction with time-of-flight mass spectrometry
RDX – cyclotrimethylenetrinitramine
SIFT-MS – selected ion flow tube mass spectrometry
SEM-EDEX – scanning electron microscope-energy-dispersive X-ray spectroscopy
TATP – triacetone triperoxide
TDG – thiodiglycol
TDGO – thiodiglycol sulfoxide
TDGOO – thiodiglycol sulfone
TNT – trinitrotoluene
UV – ultraviolet

INTRODUCTION

The rapid development in modern technology has created the opportunity to decrease the size of some indispensable instruments that were invented decades ago. As a result, compact versions of many benchtop instruments are now available, which above all economizes on space and makes the devices in question more easily portable. This remarkable development in analytical equipment comes with previously unavailable opportunities, such as the possibility of bringing the instrument to the sample, rather than *vice versa*, as well as taking the device to remote locations.

Moreover, being often simpler to use and more price-competitive makes them more accessible and affordable, even to non-scientists. Taking the sophisticated (high-level) instrument out to the sampling site or point of care makes it closer to the problem and, in turn, to the response and further counteraction.

Simple point-of-care (POC) instruments or devices for personal use and health monitoring such as pH and glucometers and heartrate monitors are already widely known and in general use. There are in fact many more specific portable and point-of-care instruments in daily use in the medical/clinical, forensic, defense, scientific, and ecological sectors. Nevertheless there remain many gaps waiting to be filled and the demand to fill them with new innovative ideas of technological platforms to be used in equipment development for simple analyzers is rising.

Human health is without doubt the number one priority. Along with physical parameters, human body fluids are one of the most measurable health-describing characteristics of interest. Noninvasive sampling of body fluids is preferred for obvious reasons – no stress to the patient – in point-of-care and clinical diagnostics. Exhaled breath is one possible matrix, attractive since breathing is completely natural for all people and sampling could be performed with no material discomfort to the patient. First reported as a human body fluid by Sidorenko et al [1] in 1980, exhaled breath condensate (EBC) has since been studied mostly in the field of respiratory medicine research. Though markers found in EBC can indicate some more or less serious health issues, clinical testing of only very few chemical analysis based diagnostic methods are approved [2]. Therefore, there is enough of a gap and scientific challenge in developing reliable methods for EBC analysis.

In recent years the number of terrorist attacks has risen, taking many lives, wounding and putting at risk many others. The 2013 Boston Marathon attack was rather the exception at the time. Today explosions happen on an almost monthly basis: the 2016 twin explosions in Hun Hin, Thailand; and the August 2016 bombing at a wedding in Gaziantep, Turkey was, as a matter of fact, not the only explosion to take place in that region during the present year.

Finding explosives before their detonation would, of course, be preferable. Nevertheless, analysis of post-blast explosive residue may be viewed as part of an *early warning* system as it could help ultimately to track down the source and origin of the chemical compounds based on the composition of the detonated device. The chemical fingerprint that is left after detonation can reveal the human factor, a "special recipe" that could assist in identifying suspects, serving as evidence for arrest and preventing future attacks.

Turning to another issue, dumped but not forgotten, large quantities of chemical munitions left after World War II, acting as environmental legacy contaminants, are reported to be leaking [3]. This is a serious issue as a large number of dumping contain the vesicant yperite, also known as sulfur mustard (HD), which, even degrading to the less harmful thiodiglycol, still possesses an immeasurable threat to human health and the environment. Commonly used methods for this type of contamination determination are rather difficult to operate in the field. Therefore, there is a demand for the implementation of simple and innovative techniques.

Most commonly known analyzers (pH meter, glycometer) are able to measure only the properties of one certain analyte at a time. On the other hand, portable instruments that are based on separation techniques often enable the simultaneous determination of multiple species.

Considering the above, methodologies for determination of ionic content of EBC, post-blast explosive residues as well as sulfur mustard degradation products in seawater were developed. Furthermore, all three methodologies were based on one single method – capillary electrophoresis (CE) – though different detection modes were applied depending on the study and the analytes of interest. For determination of ionic content of EBC and post-blast explosives residues a capacitively-coupled contactless conductivity detector (C^4D) was used, and ultra violet (UV) detection was used for analysis of thiodiglycol and its oxidation products.

All four studies in the present work share one crucial point – human health and employment of a single method as a platform for a portable on-site and point-of-care device in three different applications: medical, forensic-safety-defense and environmental.

1 LITERATURE OVERVIEW

Point-of-care analysis

Point-of-care (POC) testing (POCT), also referred to as 'near-patient' is often used in medicine and similarly to '*in situ*' or 'point-of-need' testing, signifies on site or in position testing. Though POC technology presupposes that small devices are brought for testing at or near the site of administering of patient care[4-5], the term POC was extended in context of particular dissertation to onsite analysis of environmental and forensic, safety and defense parameters that may affect human health directly and indirectly.

The goal is to collect the sample and obtain the results as quickly as possible at or near the location of the patient or at an events venue in order to speed up turnaround time, the treatment of the patient or any further action plan development, with the ability to adjust the latter as necessary, sometimes even before leaving the scene. Besides being often less invasive than hospital testing and more user-friendly, POC testing has many other benefits, such as lower patient care costs, with savings in consultation and waiting time in clinics and also laboratory effort and time.

Similarly in medicine, using portable instruments in forensic, environmental, safety and defense investigations to perform analysis on-site in a contamination area removes the requirement to transport collected samples and is therefore considered to be time saving, seeing as there is also no need to wait for laboratory results. Moreover, in some cases, a professional and accredited on-field analysis might be the safest option for the quick determination of pollutants and subsequent decontamination.

Aspects of the clinical, environmental, safety and defense (closely related to forensic) applications of POC tests and devices formed part of the basis of this study.

1.1 Exhaled breath condensate – a valuable body fluid

The main matrixes used in daily clinical analyses are body fluids such as blood serum, and amniotic and cerebrospinal fluid, including body secretions such as saliva, sweat, urine and tear fluid. However, there are also body fluids that are not used or are limited in their applications in clinical analysis, an example being exhaled breath condensate. Compared to other, mostly invasive methods commonly used for the detection of airway inflammation, such as bronchial biopsies [6] bronchoalveolar lavage [7] or sputum induction [8] EBC collection cause minimum stress to patients. Although EBC is especially attractive due to its noninvasive sampling nature, only a few clinical applications of EBC have been approved, for example, analysis of fractional exhaled nitric oxide in asthma diagnosis [9, 10].

EBC is obtained by the cooling and condensing of exhaled air. Consisting mainly of water vapor, exhaled breath content is in fact rich in many other compounds, such as volatile (oxygen, ethane, carbon dioxide, pentane nitric oxide) and non-volatile units, bioaerosols (viruses, bacteria, fungi) and liquid particles (i.e. droplets) that emanate from the respiratory tract.

The composition of EBC corresponds, albeit in significantly lower concentrations, to that of respiratory tract lining fluid – a thin liquid film covering the respiratory epithelium and released as droplets from the surfaces of the airways. Divergent opinions on the exact anatomical origin and the specific formation mechanism of EBC still exist [11, 12].

Over the past three decades EBC analysis has attracted the interest of many researchers precisely due to the large number of mediators present in this matrix and the fact that they can serve as biomarkers for respiratory diseases. Biochemical biomarkers can indicate specific health conditions or pathogenic biological process present in the respiratory system, specifically in the lower respiratory tract. The ultimate goal of the analysis is the interpretation of the values of the specific biomarker or their pattern in EBC samples. Several EBC constituents, such as H_2O_2, pH, nitric oxide and its metabolites, purines, various eicosanoids (prostanoids, leukotrienes) and proteins (interleukins, cytokines, cytokeratins), electrolytes, urates have already been extensively investigated by scientists. Both healthy as well as clinical patients with respiratory diseases have participated in the studies [13].

Total analytical testing of EBC has been divided in three phases of activities: pre-analytical, analytical and post-analytical. The pre-analytical phase involves: EBC formation, sampling, handling, dilution, environmental factors and storage.
The analytical phase involves analytical methods which have to be sufficiently sensitive considering the very low concentrations of compounds in EBC. The post-analytical phase involves the interpretation of the values of the specific biomarker or pattern in EBC samples. Those three phases and specific steps involved, including the analytical methods for EBC analysis, are well described in *Review* by Dodik S. and Cepelac I. [13] and are summarized below in Table 1.

Typical analytical issues faced in EBC analysis involve long sampling time (10-15 min), small volumes (time dependent, ~100 μL/min), unknown degree of dilution, possible contamination with saliva, unstable pH value, extremely low concentrations (ppb, ppt levels) and incomparable results between laboratories. Therefore, EBC is still mainly used as a research tool and in order for it to be used in the clinical practice, further investigations and collaborations between laboratory scientists are required in order to explore other biomarkers and develop new technological platforms that will meet all pre-analytical, analytical and post-analytical standards.

Table 1. Pre-analytical, analytical and post-analytical phases of exhaled breath condenste analysis (Data based on [13-14]).

Phase	
Pre-analytical	
Formation	
Collection	
Sample handling	Environmental temperature and relative humidity Condenser temperature Collection time Collection volume Dilution Sample contamination Pre-analytical procedures Gas standardization Isolation / extraction / lyophylization Storage
Analytical	
Methods selection	
Single-analyte determination	pH-metry spectrophotometry spectrofluorometry enzymatic assay ELISA* fluoroimmunoassay radioimmunoassay
Multi-analyte determination	2-dimensional protein gel electrophoresis immunoassays GC*, MS*, HPLC* GC-MS*, LC-MS*, HPLC-MS* DMS*, GC-DMS*, ESI-DMS* PTR-MS*, PTR-TOF-MS*, SIFT-MS*
Method standardization	optimization, validation LoD, LoQ
Post-analytical	
Reference values	
Clinical use and interpretation	
Biomarker selection – Diagnose and monitoring	asthma COPD* CF* OSA* GERD* ARDS* lung cancer non-respiratory diseases

*abbreviations are defined in the ABBREVIATIONS list

1.2 Explosives and post-blast residue

According to the United States Bomb Data Center, a total of 912 explosive incidents and 642 bombings, including 5 church and 15 school bombings, were recorded by the Bomb Arson Tracking System only during the year 2014 alone[15]. The number of incidences has been rapidly increasing during recent years with terrorist attacks involving explosive devices occurring with increased frequency, raising the need for improvements in national safety and defense and requiring forensic investigations at uttermost level. Next to identification of illegal drugs and accelerants employed in arson cases, explosive and gunshot residue is making a major contribution to crime investigation and prevention. Information (chemical trace) acquired from analysis of explosive residues may serve as a "fingerprint" indicating the origin of the device, helping to trace the source or even the marker of the device. Faster identification of possible suspects may in turn prevent additional attacks.

An explosive is a device or substance that releases a large amount of energy very fast when subjected to impact, heat, detonation or friction. The energy released, causes extreme increases in pressure and temperature and therefore all materials present are turned into hot compressed gases, which in turn expand rapidly and initiate a pressure wave – "shock wave". Different types of explosives could be categorized in several ways. Figure 1 summarizes two possible classifications and includes also some important examples of each class [16], as well as a common classification based on structure, dividing explosives to organic and inorganic [17].

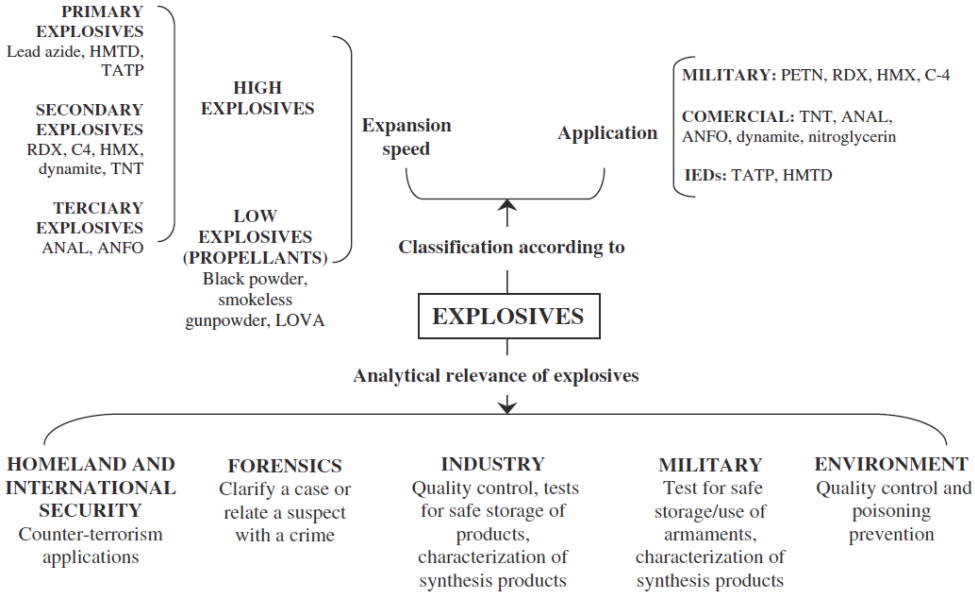

Figure 1. Classification of explosives and fields where the analytical analysis of explosives has material importance [16] (Copyright © 2016 by the Elsevier B.V. Reprinted with permission). Abbreviations are defined in the ABBREVIATIONS list

Although in terrorist attacks improvised inorganic explosives consisting of easily accessible chemicals such as ammonium nitrate fuel oil (ANFO), sodium chlorate-type or black powder devices are mainly used, other types of explosives (i.e. organic high explosives) should not be excluded [16]. The chemical trace that each explosive device leaves after its detonation can assist investigators to more quickly identify possible suspects and prevent additional attacks. Most inorganic explosives produce an ionic detonation residue; however, traces of organic high explosives residues are not so well-known.

With regard to public safety and the subsequent police investigation, fast and reliable identification of the explosive type used in an act of terrorism is of the utmost importance.

1.3 Thiodiglycol and its oxidation products from leaking chemical warfare agents dumped at sea

Following World War II, large quantities of conventional and chemical munitions were left over from German and allied stocks. Decision was made to dispose these at sea, seemingly overcoming the hurdle of complicated and dangerous destruction. Around 50,000 tons of chemical munitions (mostly bombs and shells) were discretely dumped in the Baltic Sea [18]. Now, almost 70 years after, corrosion of the shells have reached a point where the increasing release of highly toxic compounds into the sea's waters is putting human health and the entire Baltic marine ecosystem at serious risk [19].

Much of the dumped munitions contain yperite, also known as 'sulfur mustard (HD)'. HD is a blister agent that causes chemical burns on the skin and is a lung and eye irritant[20]. In aqueous environments, HD easily hydrolyzes (Figure 2) to produce non-toxic thiodiglycol (TDG) and then slowly oxidizes to more stable thiodiglycol sulfoxide (TDGO) and thiodiglycol sulfone (TDGOO).

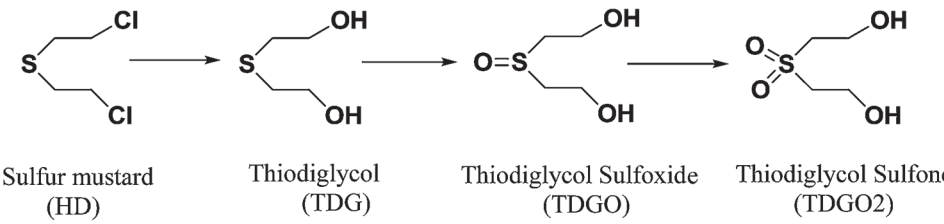

Figure 2. Hydrolytic pathways of sulfur mustard

Moreover, formation of cyclic and open chain compounds as degradation products occur during HD hydrolysis [21, 22].

The reader should note that potential health and ecological risks are associated primarily with HD itself and the identification of degradation products as markers is very suitable for indirect determination of the HD leakage locations, even if HD itself has already degraded.

Several analytical methods are widely used for the determination of HD markers, such as TDG and TDGO and TDGOO. Nevertheless, development of other rapid, sensitive and selective analytical methods and identification procedures to identify HD degradation products at trace levels directly on-site is an ongoing process.

1.4 On-site detection methods for EBC, explosives and their residues, sulfur mustard and its degradation products

Portable analytical instruments are especially valuable and widely used in medicine. Devices used in hospitals, clinics and surgeries are mostly required to be at least mobile (on wheels) for quick and easy transportation to the point of patient care. Time is a crucial factor and decreasing turnaround time increases the likelihood of quicker clinical management, based on rapidly available results. A second considerable advantage is the opportunity of self-monitoring, which lets patients become more aware and involved in the monitoring of their own health. The emphasis of POC is, however, often on prevention and monitoring, rather than on a cure.

The demand to have equipment taken out of the laboratory and brought to the sampling site has initiated the drive to reduce in size the conventional instrumentation also in the fields of forensics and environmental studies as well as in the safety and defense sector.

Point-of-care and on-site testing is usually attained through the use of portable and handheld miniaturized versions of common analytical instruments. Regardless of the field of application, portable instruments must be easily movable and therefore convenient for manual carrying and transportation and adaptable in altered circumstances. Many portable instruments are designed to work on both batteries and from the mains, thus not diminishing their capability of being used back in the laboratory. When used in the laboratory they in turn save space due to their more compact size and also often cost less to run and maintain than their benchtop counterparts. Moreover, a more competitive price and lower sample/reagent consumption, leading to a reduction in waste, and their being designed for use also by nonscientists are additional valuable advantages of those kind of instruments[23].

1.4.1 Methods for EBC sampling and analysis

1.4.1.1 Sampling technologies

EBC sampling is attractive due to its noninvasive nature. Sampling usually consists of the subject breathing for a number of minutes into a specially-designed device. Commercially available devices such as the bench top EcoScreen[24] or portable Rtube[25], Maddison Product Design[26] and the Maastricht Instrument sampler[27] are available. Most scientific practitioners have used home-made devices[28] that typically perform similarly to the commercial ones and are also all based on the principle of freezing exhaled breath, but are lower in cost. Devices consist usually of a mouthpiece with a one-way valve that is connected to a collecting system, which is cooled either by an ice bath or liquid nitrogen. The disadvantage of home-made devices is that they are often not portable and may require rather bulky accessories.

1.4.1.2 Analysis

The analytical phase of the EBC study is a very important methodological issue which occurs due to very low concentrations of biomarkers. Thus conventional assays must be carefully adjusted.

Methods for EBC analysis are usually traditional analytical methods, the same ones used for other biological samples and intended for single-analyte determination, e.g. pH-metry, biosensors[29], spectrophotometry[30,31], spectro-fluorimetry[32], enzymatic assay[33], immunoassays (ELISA, radioimmunoassay, immunoluminometry, immunosensors)[34].
Some of those small devices are not really instruments and are based on chemical reactions and colorimetry with the required reagents already embedded and dried into the device. The fact that this kind of device is often single use and easily disposable may be regarded as an advantage. However, the problem with commercial immunoassay kits is that they are neither validated for extremely low concentrations nor for the matrix of the EBC sample itself [35], and are therefore affected by poor sensitivity and selectivity. Immunoassays have to be validated with reference methods like high performance liquid chromatography (HPLC) or mass spectrometry (MS), that allow precise determination of different markers and their concentrations in EBC [1].

Included amongst the multi-analyte methods for the quantitative and qualitative analysis of EBC are two-dimensional protein gel electrophoresis (2-D PGE)[1] and chromatography for micro-analysis of protein spots. The following chromatography methods are used: GC, HPLC, MS, LC-MS, GC-MS[36], HPLC-MS[37], GC-DMS[38], ESI-DMS[39].

Though multi-analyte methods enable recognition of singular components in nanomolar concentrations even small contaminations (ng) may cause different

results. Contamination of the sample with saliva is quite probable due to the long sampling time (10 min) required to obtain a sufficient amount of sample (1–2 mL). Furthermore, most of the above mentioned techniques are not easily miniaturized and the sample must thus be taken to the laboratory, increasing again the risk of contamination. There are also significant delays in the analysis and data processing because of loss of time due to transportation. Due to the fact that the analysis of EBC displays limitations regarding the optimization and validation of quantitative analytical procedures, it is not possible to compare results arrived at by different laboratories [32].

1.4.2 Analysis methods for explosives residues

Forensic laboratories and law enforcement are more often meeting new challenges, one example being terrorist threats. There is also continuously increasing demand for rapid analysis of forensic evidence. Proactive agencies are providing police with portable forensic analysis tools in order to raise the forensic intelligence of field personnel when conducting investigations.

Presumptive tests of post-blast residues generally involve color reaction (Greiss, Nessler)[40,41]. To ensure valid results, these tests require careful application of blanks and also positive controls, and while rapid, are not conclusive. Samples that yield promising results require further examination and analysis in the laboratory.

The most favored analysis methods of forensic explosive laboratories are LC-MS[42] and GC with either a thermal energy analyzer (GC-TEA)[43, 44] or positive and/or negative ion chemical ionization mass spectrometry (GC-nciMS)[41,42]. Ion chromatography (IC) is the preferred method for the analysis of inorganic ions [45]. Other methods for the analysis of explosives and their residues include spectroscopic techniques, such as atomic absorption spectroscopy (AAS)[46], scanning electron microscopy, energy dispersive X-ray (SEM-EDX)[47], X-Ray diffraction[48,49], MS[50], ion mobility spectrometry (IMS)[51] and laser-induced breakdown spectroscopy (LIBS)[52] and as another chromatographic techniques HPLC[53, 54]. Almost all of the described methods suffer from minor to considerable limitations, some of which are outlined below in Table 2. Nevertheless, the majority of the described techniques are not easily portable.

Commercially available portable instruments for explosives analysis include miniaturized instruments based on Fourier transform infrared spectroscopy (FTIR), Raman spectroscopy, IMS and fluorescence [55]. However, portable instruments have limitations like their benchtop versions. For example, FTIR is challenged with mixtures due to overlapping spectra preventing a clean library match (unless the mixture is in the library already). Raman faces issues with fluorescence interferences and also, since a laser (heat) is involved, dark substances like gunpowder may ignite. IMS requires confirmation technique.

Table 2. Analytical methods for explosive residue analysis [56]

Analytical method	Limitations
TLC	Low resolution susceptible to contamination
GC-ECD	Requires volatile analytes insensitive to hydrocarbons
GC-TEA	Requires volatile analytes expensive limited to nitro compound
HPLC-UV or DA	Low selectivity
IR	Residues require separation prior to analysis
MS	
LC-MS	Requires interface between HPLC and MS
GC-MS	Limited to volatiles
SEM-EDEX	Expensive, requires expert operators

Addressing the cost effectiveness of more specific, more complicated and often multipurpose devices, the higher the effectiveness of the device the higher is its cost. Nevertheless, it is worth noting that portable technologies allow the users to conduct presumptive analyses and identification of unknown compounds outside of the conventional laboratory. Moreover, instruments miniaturized into portable or semi-portable versions (e.g. Griffin™ 460 by Flir System) may be transported and used directly on site in a specially equipped vehicle or in a deployable forensic laboratory [57, 58]. Along with expediting the development of intelligence for leading the investigations it can also dramatically reduce the burden on forensic laboratories.

1.4.3 Analysis methods for CWA degradation products

The most frequently used methods for the analysis and identification of a sulfur-containing precursor and decomposition products in aqueous samples are based on GC in combination with MS and/or tandem MS [59].

Next to GC analysis, LC coupled with MS [60], nuclear magnetic resonance (NMR) spectrometry [61] or sulfur flame photometric detection [62] are maintaining their position in the analysis of water soluble degradation products. However, most routinely used chromatographic methods are difficult to use for onsite analysis, mainly due to often required derivatization and complicated sample preparation, long analysis and difficulties in miniaturization.

Existing commercial portable instruments also include GC-MS, FTIR, RAMAN, IMS and photo ionization detection (PID) [55]. Instruments based on GC/MS suffer mainly from maintenance difficulties since the MS part needs a high vacuum to operate. The limited range of chemicals comes from the demand for both sides of the GC and MS portion to be optimized for the chemicals of interest.

The challenges for PID devices is their inability to distinguish between hazardous and non-hazardous organic vapors and the dependence on the operator's knowledge (one must choose the correct lamp in order to be able to detect the chemicals of interest).

Taking the above into consideration, it is reasonable to expect development of portable instruments based on a simple detection method, if not possible for all-purpose, then multipurpose capable of detection of at least one of specific marker in each previously described fields of interest.

1.5 Capillary electrophoresis as a promising platform for POC analysis

Capillary electrophoresis (CE) has challenged commonly used technologies in portable instruments in being an emerging technology that is easily miniaturized and simple to use.

CE holds several advantages over the other methods mainly due to its simple construction that requires only a separation capillary, detector and a high voltage (HV) power supply. Another advantage is the small solution volumes (~10 nL) suitable to perform a separation. The price for the components, e.g. fused silica capillaries, is relatively low, as is the running cost. Noteworthy is that CE does not require use of complex mechanical high-pressure pumps such as those used in LC or a vacuum like in MS. Another noteworthy advantage of CE is that it may be combined with different detection methods.

Among other separation techniques CE has found its unique place due to its robustness towards complex matrices, unique separation efficiency and high speed of analysis. This outstanding performance has been demonstrated particularly in the analysis of biomolecules, proteins and DNA sequencing[63-64]. Capability of CE to analyze small sample volumes is of clear benefit when analyzing samples/matrices available in small volumes such as biological samples. It is clear, however, that not all biological samples are limited (e.g. urine), though invasively obtained samples (venipuncture, biopsy, lumbar puncture, etc.) are limited in amounts, for obvious reasons. Biological samples analyzed by CE are for example the analysis of tear fluid[65], cerebrospinal fluid (CSF)[66-67], blood serum[68], saliva[69-70] and even exhaled breath[71], though surprisingly little research has been conducted on the latter. Moreover, since CE can provide efficient separation of small ions it has even been regarded as displacing ion chromatography (IC) as the method most preferred for the analysis of inorganic ions [42].

Though requiring smaller sample volumes, CE has been less successful as a separation method compared to its competitor HPLC and, though it has been claimed that it lacks robustness, unlike HPLC it can, however, be made portable

and thus employed for point-of-care analysis. Despite it falling out of favor with many analysts because of difficulties associated with obtaining reproducible migration times, those issues can be surmounted to achieve acceptable detection limits and resolution of ions with an extraordinary number of theoretical plates.

In conclusion, CE satisfies most of the key parameters and requirements for POC analysis, such as dimensions, weight and power consumption, portability and ease of deployment, and, furthermore, mechanical rigidity, ease of operation, simplicity of sample preparation with minimal consumables, short analysis times and thereby sufficient analytical performance. This, along with an easy sampling technique, makes CE especially attractive as a technological platform to develop equipment for simple portable on-site and clinical analyzers.

1.5.1 General aspects of capillary electrophoresis

Electrophoresis was first described by Tiselius in 1937, who earned a Nobel Prize in Chemistry a decade later (1948) for using U-tube with a buffer solution under an electric field for protein separation [72]. Noteworthy contributors to the development of CE are Hjerten[73], Terabe[74], Jorgenson and Lukacs[75].

The separation of substances by CE is achieved due to application of high voltage across the capillary (25–100 μm) filled with a background electrolyte (BGE) and is based upon the different migration mobility of the charged compounds under the influence of an electrical field *E*.

The electrophoretic mobility (μ_{ep}) depends of the several parameters of analyte and BGE that act simultaneously – the high voltage, the charge, molecular size, shape of the analyte, temperature, ionic strength, pH and viscosity. The charged molecules migrate from one end to the other end of the capillary, usually towards an electrode with the opposite sign. The mobility is given by the charge to the radius ratio of an ion. In a typical CE separation, highly charged ions of a small size (having a higher charge-to-size ratio) migrate faster than larger ions, or ions of a lower charge. Assuming a spherical shape the electrophoretic velocity (v_{ep}) is calculated according to the following equation:

$$v_{ep} = \mu_{ep} E = \left(\frac{q}{6\pi\eta r} \right) \left(\frac{V}{L} \right), \qquad \textbf{(1)}$$

where, q is an effective charge of the solute, η is the viscosity of BGE, r is the Stoke radius of the solute, V is the applied voltage and L is the total capillary length.

Electrophoretic separation is performed under a very high electric field (≥100 – 500 V/cm), usually inside the narrow-bore fused silica but also in a borosilicate glass or polytetrafluoroethylene (PTFE) capillary. The detractive effects of Joule heating are minimized by the high electrical resistance of capillaries and due to

heat dissipation over the large surface area to volume ratio. The high field strength in capillaries, along with suitable BGE for analytes of concern, allows achieving rapid separation with high resolution and efficiency [76].

Effective separation is affected not only due to migration of ions from the solution, but also because the background electrolyte starts moving inside the capillary when the voltage is applied. The bulk movement, i.e electro-osmotic flow (EOF), is caused by the properties of the surface of the fused silica capillary. In contact to an aqueous solution, silica surface groups hydrolyze and are typically negatively charged (as SiO^-), depending on the pH of the surrounding electrolyte solution. At the range of pH>2 to pH<9, silanol groups (SiOH) are mild to fully deprotonated, resulting in a net negative charge of the inner surface of the capillary. A double layer, consisting of a rigid and a diffuse layer, is formed due to the positively charged ions from the buffer attaching tightly to the negatively charged inner surface of the capillary wall. With application of the separation voltage, solvated protons in the diffuse part of the double layer start to migrate towards cathodes pulling along the whole buffer.

Therefore, the extent of EOF (μ_{EOF}) depends upon the charge on the capillary inner surface (zeta potential, ζ), the background electrolyte (BGE) viscosity (η) and dielectric constant of BGE [76].

$$\mu_{EOF} = \left(\frac{\varepsilon \zeta}{\eta} \right), \qquad \textbf{(2)}$$

EOF allows the separation of positive and negative ions within a single CE run, affecting also the overall migration time (t) of analytes. The apparent mobility (μ_{app}) of analytes is calculated by the following equation [76]:

$$\mu_{app} = \mu_{ep} + \mu_{EOF} = \left(\frac{lL}{tV} \right), \qquad \textbf{(3)}$$

where l is an effective length of capillary.

The advantages of CE are high efficiency (N $<10^5$ to 10^6), short analysis time, small required sample, reagents and solvents volumes (1 to 50 nL) and low mass detection limits. There are many CE modes, such as capillary zone electrophoresis, capillary isotachophoresis, micellar electrokinetic capillary chromatography, capillary gel electrophoresis, non-aqueous capillary electrophoresis and capillary isoelectric focusing [77]. The modes are easily changed on the same instrument only by changing the BGE.

The weaknesses of CE are related to poor sensitivity. However, it can be improved by utilizing suitable detection techniques or by applying stacking techniques such as high-field stacking, isotachophoresis and transient isotachophoresis, hydrodynamic injection stacking, ACN-salt mixture stacking, etc.[78,79].

1.5.2 Controlling the electro-osmotic flow

Usually, the co-electroosmotic separation principle is preferred in CE due to migration velocities of small, charged ions that exceed the velocity of the electro-osmotic flow (EOF). The direction of the EOF in uncoated capillaries is from the anode to the cathode, thus without any modification of the capillary wall only cations can be separated in reasonable time, in fact, small and fast anions may never reach the detector and bigger species will arrive at the detector after a long time as a very broad peak. In order to achieve separation and reasonable analysis times for small anions, the direction of the EOF can be decreased or reversed. Cetyltrimethylammonium bromide (CTAB) and other EOF modifiers can be employed for this purpose [80].

1.5.3 Detection

A wide variety of detection methods such as UV-vis absorbance, fluorescence, conductometry, amperometry, potentiometry and mass spectrometry have been used in CE to determine different compounds in biological and environmental matrices.

UV detection is by far the most common and well known detection technique for liquid separation with a high efficiency (reduced band broadening and higher concentration in the sample bands). On the other hand, the C^4D detector provides simplicity, flexibility and enhanced sensitivity compared to UV detection especially in the case of small inorganic molecules. Though several approaches to enhance the sensitivity of detection are known (laser induced fluorescence, electrochemical detection, sample stacking), C^4D is also well suitable for small ions with limited absorption in the UV-Vis region. Small ions such as inorganic cations and anions, have a high electrophoretic mobility and also corresponding conductivities of migrating zones, which makes C^4D sensitive regardless of analytes optical properties.

1.5.3.1 Capacitively-coupled contactless conductivity detection

Capacitively-coupled contactless conductivity detection (C^4D) originates in the 1970s, when capillary isotachophoresis of inorganic species was monitored using a high frequency to inductively monitor changes in the conductance of the separated zones. Today, contactless conductivity detection is used for detection of various charged species: inorganic anions and cations, as well as organic ions, such as carboxylic acids, amines and amino acids. Using C^4D detectors does not require tagging or modifying of the analytes. The limits of detection, especially for small ions are often comparable or even better than UV-visible absorption detection techniques.

Signal intensity is dependent on conductivity – the higher the conductivity differences between the analyte molecules and background electrolyte, the larger the detector response. As conductivity is measured axially along the capillary

(over the length of the gap between electrodes), rather than across the capillary diameter, the capillaries of different diameters therefore give similar signals [81]. C^4D is a universally applicable detection method as it belongs to the non-selective detection modes. Besides the determination of inorganic ions in the sub-ppm range, it is also possible to apply C^4D for the detection of small organic ions, amino acids, HIV therapy drugs and other analytes [82]. CE with C^4D is known for its capability to analyze low sample volumes [81, 83,84], but obtained significant attention recently when C^4D was used in analysis of biological samples [85,86,70].

Although C^4D adapts poorly to high-conductivity samples, there are a number of advantages to using this technique over optical detection modes and galvanic contact conductivity detection that include greater flexibility in capillary handling. One advantage is that analysis can be performed in an on-capillary mode without the need to modify the capillary. The simple construction of this detector allows it to be used in combination with a second, additional detector. Moreover, this detection principle can also be used with capillaries either made of other materials than fused silica (such as PEEK or Teflon) or that have very small inner diameters, as well as with chip based separation technologies.

As the detector works without needing to be in direct contact with the solutions, the electrodes do not easily deteriorate and therefore cleaning or flushing of the detection cell is unnecessary [87].

1.5.4 Data preparation

Due to aging of the capillary, shifting of migration times of the peaks may occur. As a result, the data analysis and interpretation by chemometric techniques can be affected. Alignment of chromatographic or electrophoretic data in order to improve the quality of the final result may be required. To identify the analyte of interest in CE the reproducibility of migration time is essential. Using the internal standard prior to the analysis of CE data is one of the most popular methods [88].

Two internal substance components can be used for evaluating the correction coefficient for each electropherogram/chromatogram against the standard by using the following equation:

$$\gamma = \frac{\frac{1}{\hat{t}_I} - \frac{1}{\hat{t}_P}}{\frac{1}{t_I} - \frac{1}{t_P}} \qquad \textbf{(4)}$$

The new migration time (t_x) is found using the calculated correction coefficient Υ:

$$t_x = \left[\frac{1}{t_I} - \frac{1}{\gamma} \left(\frac{1}{\hat{t}_I} - \frac{1}{\hat{t}_x} \right) \right]^{-1} \quad \textbf{(5)}$$

where Υ is the correction coefficient; t_I, t_P are migration times of the first and second internal standards, respectively; $\hat{t}_I$ and $\hat{t}_P$ are migration times of the first and second internal standards, respectively, in the electropherogram under correction; t_x is the corrected migration time for the corrected electropherogram and $\hat{t}_x$ is the migration time of the electropherogram under correction.

1.6 Data analysis

Interpretation of acquired data is an essential part of analytical chemistry, and therefore pattern recognition techniques or chemometric tools are often applied. They are particularly useful for data pre-processing and chemical fingerprint analysis and searching for variables that join variations, enabling further interpretation of hidden similarities or dissimilarities between groups, within samples. Most common pattern recognition techniques are unsupervised methods, such as principal component analysis (PCA), parallel factor analysis (PARAFAC) and cluster analysis (CA). The goal of such methods is to categorize observations, samples, and chemical fingerprints into groups without former knowledge of the sample class [89].

1.6.1.1 Principal Component Analysis

Amongst the traditional methods used for data analysis PCA is one of the most popular [90]. PCA compresses the large data, keeping only the important information via extraction and simplification. Linear combinations of original variables created by PCA are principal components (PCs). PCs describe the systematic patterns of variation between the samples. PCs are orthogonal, their number is smaller than or equal to the number of original variables. The first PC coordinate has the greatest possible variance. PCA is a breakdown of the original 2D-matrix X and is therefore representative of it as the product of two 2D-matrices, T and P:

$$X = TP^T + E, \quad \textbf{(6)}$$

where T is a matrix of scores, P is a matrix of loadings and E is a matrix of residuals.

2 AIMS OF THE STUDY

As follows from the literature overview, instruments in point-of-care and on-site analysis are expected to be simple, rapid and sensitive with performance parameters similar to conformation techniques. The critical limitations are also set to the sample volumes, especially concerning body fluids, and in this case CE satisfies the requirements. CE possesses several advantages over the other methods, as one needs only a high voltage (HV) power supply, small volumes of solutions, detector and a separation capillary in order to perform a separation in a short period of time. Moreover, CE can be made portable via miniaturization of the components, which is valuable, though frequently overlooked, advantage. Along with an easy sampling technique CE can be used for point-of-care analysis, making it (CE) attractive as a technological platform in equipment development for simple clinical analyzers.

The main goal of the present dissertation is the adaption of a CE instrument for POC and on-site analysis and the development of relevant sample preparation methods by taking advantage of the robustness and amenability inherent to the miniaturization of CE technology. A further goal is to provide proof of the principle and demonstrate the capabilities of various analysis situations.

The aims of the study may then be split into three major tasks:

- Development of a protocol for CE analysis of exhaled breath condensate (EBC) collected via a dedicated breath analyser and analysis of real samples

- Development of a protocol for collection and CE analysis of post-blast explosive residues and analysis of real samples

- Development of a protocol for CE analysis of thiodiglycol and its oxidation products

3 EXPERIMENTAL

3.1 Reagents and samples

All reagents were of analytical grade. Ultrapure water (Milli-Q) was obtained using a Milli-Q Water System (Millipore S. A, Molsheim, France) and used for the preparation of all standard solutions, stock solutions of inorganic anions and cation BGEs as well as for the dilution of samples.

Reagent grade chemicals listed below were mainly purchased from Sigma–Aldrich, Steinheim, Germany (unless specified otherwise).

In **Publications I to III** 100mM stock solutions of inorganic anions and cations were used, and in addition to those, 5 mM stock solution of lithium formate (98% purity), was used in order to spike the sample and standard solutions. BGE, prepared daily for CE measurements, was made by diluting 100 mM stock solutions of 2-(N-morpholino) ethanesulfonic acid (MES), L-histidine (HIS) and 18-crown-6 until the required concentrations were achieved. Cetyltrimethylammonium bromide (CTAB) - prepared as a 10 mM stock solution in 5% acetonitrile (ACN), was added to the BGE.

In **Publication IV**, sodium hydroxide, boric acid, ACN, imidazole and sinapinic acid (internal standard) were used. Phthalic anhydride and pyridine were obtained from Merck KGaA (Darmstadt, Germany). Thiodiglycol, thiodiglycol sulfoxide and thiodiglycol sulfone (97%) were synthesized by Envilytix GmbH (Wiesbaden, Germany). All chemicals were of analytical grade and used as received without further purification.

3.1.1 EBC samples in Publication I and II

For the first study described in the **Publication I**, EBC samples were collected from fifteen, non-smoking volunteers, within an age-range of 22 and 75, both male and female. In total, five studies were performed for each of the individual test subjects and a total of 75 samples were analyzed. Additional EBC samples from two heavy smokers were also analyzed in order to detect the salivary contamination and one of the EBC samples was intentionally spiked with 1 µL of saliva collected from the same person.

EBC samples for the second, an additional study, described in **Publication II**, added two more volunteer sample donations to the investigation. One of the subjects had been diagnosed with a mild form of COPD and the second subject was healthy, but was sampled during a period of acute upper respiratory tract infection and other symptoms of common cold.

For the quantitative analysis in both studies, 1 µL of the internal standard stock solution (5 mM lithium formate) was added to the 99 µL aliquot of all samples.

Three voluntary test subjects also participated in an experimental study of lactate level measurements (in EBC) prior and immediately after an exhaustive cycling exercise. As a result, 11 samples per person were collected, spiked with 50 μM of IS (Li-formate) and analyzed.

3.1.2 Post-blast explosive samples and reagents in Publication III

All used explosives were commercial products, regulated by national authorities and were kindly provided by Forcit OY, (Hanko, Finland). Electrically initiated detonators (No. 8 Al, Sellier & Bellot JSC, Czech Republic) were used to trigger the explosive devices.

3.1.3 Samples and reagents in Publication IV

For the experiments one liter of seawater was collected from the Baltic Sea and spiked with thiodiglycol (TDG), thiodiglycol sulfoxide (TDGO) and thiodiglycol sulfone (TDGOO).

3.2 Methods

3.2.1 Capillary electrophoresis

Three different CE instruments were used in the studies – one a commercially available device and two other, in-house built, instruments. The time required for a complete analysis of a single sample in Publications I and II did not exceed 3 minutes, in case of the Publication III, 4 minutes and in Publication IV recorded analysis time remained below 8 minutes.

3.2.1.1 EBC analysis

The measurements for the **Publications I** and **II** were made in same conditions and set-up on a purposely-built capillary electrophoresis instrument equipped with a capacitively coupled contactless conductivity detector (C^4D). The system consisted of a high voltage power supply unit (Spellman CZE2000R Start Spellman, Pulborough, UK), for the electrodes two Pt wires (0.5 mm I.D., 3 cm length, Advent Research Materials Ltd., Eynsham, England) were used.

The C^4D used for the detection of the separated analytes consisted of an external function generator (GW Instek GFG-8219A, New Taipei City, Taiwan) providing a sinusoidal excitation signal (frequency 290 kHz, amplitude 20 V peak-to-peak) for the in-house built detector cell [91] equipped with a pre-amplifier (OPA655, Burr Brown, TX, USA). The amplified cell current was led to an external detector circuitry for further processing. Data was collected using software coded in-house and a 20 bit sigma-delta data acquisition card (Lawson Labs Inc., Malvern, PA, USA).

Fused silica capillaries (50 µm i.d., 375 µm o.d., Polymicro Technologies, Phoenix, AZ, USA) with a total length of 35 cm were used. As a BGE, a described mixture [92] was modified to 20 mM MES, 20 mM HIS, 30 µM CTAB and 2 mM 18-crown-6 (pH 6). The applied voltage for the separation was −18 kV. Prior to the first use, preconditioning of the capillary was carried out by flushing it with 0.1 M NaOH solution for 30 min, followed by DI water and BGE solution, 10 min for each. Between the injections, capillary was flushed with the BGE solution for 1 min. After finishing work for the day, the capillaries were washed with DI water for 10 min, vacuum dried for 5 min and stored overnight in dry conditions.

The standard solutions and samples were introduced to the capillary hydrodynamically by elevating the sample vial to a height of 10 cm, whilst the injection capillary end was immersed in the sample solution. Sample vial was then held in a position for a specified interval of time. For dual opposite end injection (DOEI), the sample was introduced to the capillary from both ends. Two injection techniques were utilized – with and without time delay. The second - without a time delay approach, is less time consuming and is based on a principle that the sample is first injected into one end of the capillary, followed by the injection of BGE and then injection occurs the same way from the other end of the capillary. Application of the same injection duration for both of the samples and BGE ensures that both injected samples are located at the opposite capillary ends before the separation is initiated. For timely displaced injection, the sample was first injected from one end of the capillary, followed by high voltage (HV) application for a specified interval of time, interruption of the HV, injection of the sample from the other end of the capillary and resuming the HV for the final electrophoretic separation. The recording of electropherogram started following the second injection. The total duration of the injection procedure during the DOEI was 1 min on average. All CE experiments were performed at the room temperature.

3.2.1.2 Post-blast analysis of explosives

Publication III employed an in-house built instrument equipped with a C^4D detector suitable for separate as well as simultaneous analysis of cations and anions in post-blast residue samples.

This miniaturized CE instrument (Figure 3) was fitted into a watertight, dust- and crush-proof case (Peli 1200 Case C©, Peli Products, Barcelona, Spain). The instrument was equipped with an HV safety interlock and a negative (up to −25 kV) high-voltage power supply (EMCO, Sutter Greek, CA, USA), an in-house built C^4D detector operating at 200 kHz with a voltage of 60 Vp-p, and a data acquisition system. As a control, an in-house coded software was used and the signal was obtained through a USB connection to a notebook or desktop computer.

Fused-silica capillaries (50 µm i.d, 375 µm o.d, Polymicro Technologies, AZ, USA) with a total length of 50 cm were used. Capillary rinsing was performed by manually applying pressure to the syringe as the appropriate solution was inserted in the splitter interface S-II with the shut-off valve (P-733, Upchurch Scientific) closed. BGE was the **same as in Publication I**.

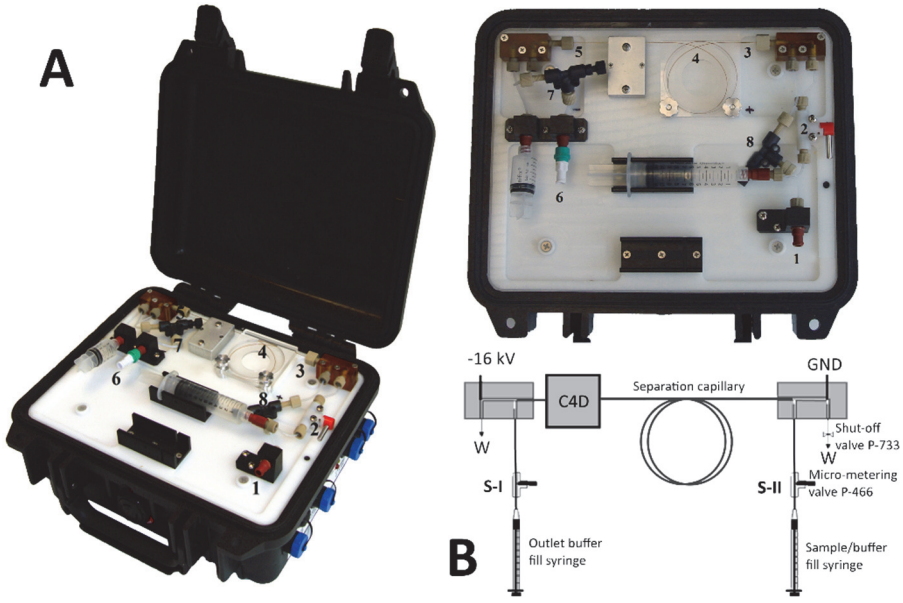

Figure 3. In-house built portable CE device coupled with C^{4}D- (TUT and Ministry of Defense, Estonia) (A) A photo of the portable instrument: (1) syringe socket, (2) shut off valve, (3) inlet end of the capillary, (4) the separation capillary, (5) outlet end of the capillary, (6) syringe socket, (7, 8) metering valve. (B) A scheme of the separation compartment of the portable instrument ["W"-output to waste, GND-grounding, S-I and S-II-interfaces, micro flow-metering valves (P-446)].

For DOEI, 500 µL of sample was injected manually into the first splitter interface (S-I) using a 1 mL disposable plastic syringe (Omnifix 100 Duo, Braun, Melsungen, Germany) followed by an injection of 1500 µL of the BGE solution. Then another sample aliquot of 500 µL was injected into the second splitter interface (S-II), followed by an injection of 500 µL of the BGE solution. This sequence allows for the simultaneous injection of the sample into both capillary ends, as well as removing any remaining sample from the splitter interfaces before the separation takes place. Preconditioning of the capillaries prior first use was the **same as described in the Publication I**. Between injection sequences, the capillaries were washed manually with DI water (amounting to ~150 times of the column volume) and BGE (similarly amounting to ~150 times of the column volumes). Between the two injections, the capillary was flushed with the BGE solution (1 min, ~100 column volumes). At the end of each day, the capillaries were washed with DI water (at least 150 column volumes) as well as kept in it overnight.

3.2.1.3 Analysis of thiodiglycol and its oxidation products

The measurements for the **Publication IV** were made on a commercial CE instrument, Agilent 3D (Agilent Technologies, Waldbronn, Germany) equipped with a diode array UV/Vis detector (DAD). Agilent ChemStation software was used to record and integrate all electropherograms.

Uncoated fused-silica capillaries (50 µm i.d, 375 µm o.d, Polymicro Technologies, Phoenix, AZ, USA) with a total length of 60 cm and (effective length of 52 cm) were used in the experiments. The sample solutions were introduced to the capillary hydrodynamically, at a 50 mbar pressure for 5 s. DAD monitoring took place at 200 nm wavelength.

The final optimized BGE was a 30 mM borate solution (pH 8.5) and the separation was performed at the voltage of 15 kV and temperature of 25 °C (that being the capillary temperature). During the optimization, different voltage (15 kV-30 kV), temperature (15°C -25 °C) and pH ranges (pH 7.5-10) were investigated. The pH value of the BGE solution was measured using a Metrohm 744 pH meter (Metrohm, Herisau, Switzerland), calibrated with commercial buffer solutions at pH 7.00 (±0.01), pH 10.00 (±0.01) and pH 12.00 (±0.01) (Sigma-Aldrich). Empty SPE tube (polypropylene (PP), 3 mL, Phenomenex) was used for preparing the SPE cartridge.

Derivatizing mixture was prepared according to the literature [93]. Phthalic anhydride (1.61g) was dissolved in pyridine (10 mL) and imidazole (0.24 g) was added to catalyze the reaction. The mixture was stored with a sealed septum inside a dark desiccator. For derivatization, 100 µL of the described mixture was added to the each 2.5 mg of analyte, sealed and heated at 45 °C for 20 minutes. After cooling the mixture, equal amount of water (100 µL) was added in order to stop the derivatizing reaction.

3.3 Sample collection

3.3.1 EBC sampling

EBC collection device (Figure 4) described in **Publication I and II**), as described in **Publications I and II**, was constructed using commonly available laboratory consumables, utilizing maximum simplicity and minimal cost. The outer container was made of 50 mL PP tube with a skirted base (TYPE 62.559, Sarstedt, Germany) and a 5 mm diameter hole was drilled to the bottom of the tube. A 5 mL PP pipette tip (Brandt GmbH, Wertheim, Germany) was inserted through the tube and tightly pressed into the hole. The pipette tip was protected from contamination by sealing it with a GC septum (HTLB 0.281, Hamilton, Reno, NV, USA), while the top was covered in a thin sheet of Parafilm® (Bemis, Neenah, WI, USA). The cooling of a pipette tip was achieved by placing the PP tube, after filling it with water, in a vertical position into the deep freezer (at −20 °C) for

several hours. Device was ready to use when the water in the PP tube was frozen. The device could be reused multiple times by changing the pipette tip inside the PP tube. The top protective film had to be removed from the pipette tip to enable sampling. A regular PP straw (Rimi, Tallinn, Estonia) was inserted into the pipette tip and as the slow tidal breath moved through the cooled pipette tip (Figure 4), the sample was collected. The exhaled breath, exiting the straw, was cooled by being in contact with the cooled pipette tip walls and eventually accumulated as condensation at the bottom of the pipette tip. During 1–2 min of tidal breathing, 100–200 μL of the EBC was collected. The liquid remaining on the pipette tip walls was then collected by gently scraping the straw towards the bottom of the tip. It was possible to transfer EBC into a 1.5 mL Eppendorf vial for further manipulations after removing the septum.

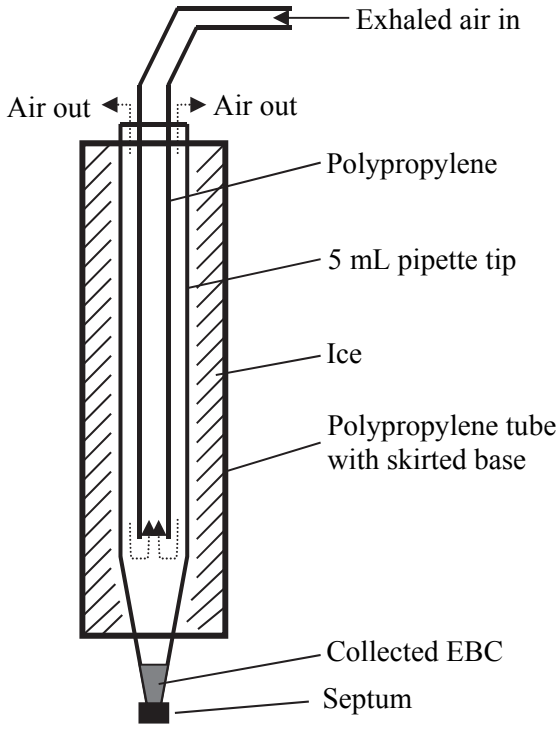

Figure 4. Scheme of an in-house constructed EBC collection device.

3.3.2 Arrangement of explosion and post-blast sample collection from different matrices

Three different surfaces were used to simulate the explosions on: sand, metal plate, and concrete. Each explosion was carried out on a fresh sand bed, a concrete

plate (25 × 25 cm, 5 cm thick) or a metal witness plate (10 × 10 cm, 8 mm steel). Each surface had three explosions carried out on and two parallel samples were collected after each explosion. Only one blank sample was taken from each of the matrices.

Following each explosion, 4 g of the post-blast sand was collected. Approximately half of the total weight of the sample was gathered from the center and the other half was collected from at a 10–20 cm distance to the center or from an obvious trace, such as light or dark ash etc.

Sampling of the metal witness plate and concrete plate were done by wiping one half of the plate with one cotton pad and second half with another, resulting in two samples for each plate. In case of the concrete plate shattering, (caused by the force of the explosion) the pieces were first collected and then wiped.

4 RESULTS AND DISCUSSION

This dissertation is based on the results of four studies.

The first three studies, as discussed in **Publications I – III**, cover the application of CE-C^4D with DOEI supported by pattern recognition technique (PCA):

- for the analysis of ionic content of the EBC;
- for the separation of post-blast explosive residues on different matrices.

In addition, **Publication I** included the screening of the inorganic nitrogen reactive species during an acute and chronic airway inflammation as well as monitoring lactate contents.

Publication III focused on previously unreported systematic study of the matrix-effects arising from the use of different surfaces. More importantly, this study incorporated improvised inorganic devices as well as the organic explosives.

Fourth study, described in **Publication IV** focused on:

- the development and validation of CE-UV based method for the analysis of TDG and its oxidation products;
- the determination of the low amounts of TDG and its oxidation products in seawater.

All four studies have one common feature, namely human health, which obviously does not only depend on the state of one's individual health, but also concerns public safety and a secure environment as a whole. Furthermore, analyzing all those aforementioned topics using a single method is put forward in this thesis.

4.1 Non-invasive sampling of exhaled breath condensate and rapid electrophoretic analysis (Publication I)

Analysis of EBC's ionic content by capillary electrophoresis using capacitively coupled contactless conductometric detection is discussed in **Publication I**. The aim of **Publication I** was to develop a simple device for non-invasive EBC collection and use a CE method for simultaneous determination of inorganic cations and anions, as well as organic anions from the samples whilst applying the DOEI principle.

EBC condensate was deemed the most interesting sample matrix and was also chosen as a human body fluid matrix, thus, making the desired process of a noninvasive sampling possible. Since it has been demonstrated that home-made devices typically perform similarly to the commercially available ones, whilst

costing significantly less, it was essential for the study to come up with a suitable sample collection method prior to carrying out the analysis.

4.1.1 Sampling device for the collection of EBC

The innovative and rather simple device for collecting EBC, as described previously in Chapter 3.3.1 and depicted in action in Figure 5**,** allowed to collect in a relatively short amount of time (1-2 min), 100–200 μL of EBC, which is more than enough for the subsequent CE analysis.

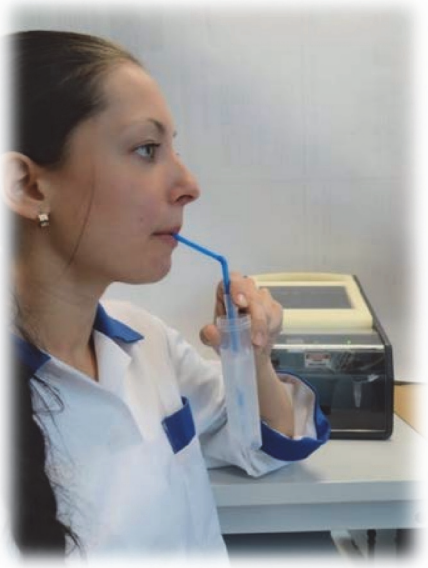

Figure 5. Picture of EBC sample collection.

4.1.2 DOEI for simultaneous analysis of ions in EBC

The one-end injection approach was not applicable for simultaneous separation of all analytes of interest – anions or cations from the separated mixture either never reached the detector or arrived at the detector after a long time-lapse in a very broad peak. As the chosen electrolyte was suitable for the analysis of both, anionic and cationic species, the choice to employ simultaneous separation using conductivity detection and the dual-opposite end injection approach was a rather straightforward one.

Optimization procedure for the separation conditions of simultaneous separation of anions and cations was done by the means of individual separations of both charged groups (injected from the opposite capillary ends) and then placing the detection cell at different positions along the separation capillary. With no need to burn in an optical window, which is required for UV detection, C^4D cell can be easily repositioned along the capillary and was best suited for this application.

Though the C^4D detection cell can be placed at nearly anywhere along the length of the capillary, the mobility of the capillary end during the hydrodynamic injection has to be ensured, therefore limiting the possible placements to a minimal practical effective length.
Constructing a plot of migration times vs. effective capillary length for each group (Figure 6) enabled an easy way to optimize the separation conditions.

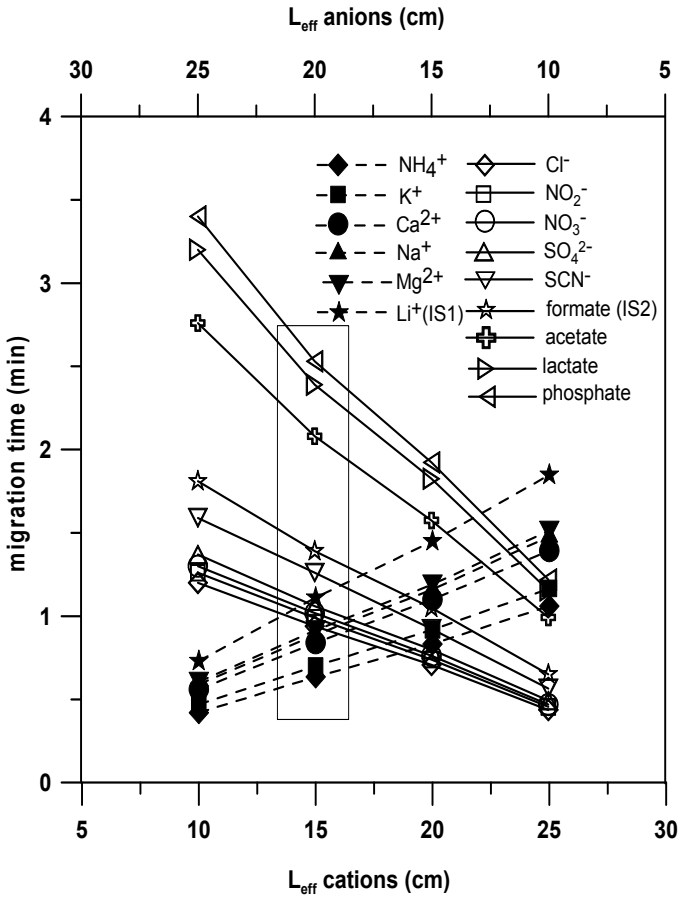

Figure 6. Dependence of migration times of anions and cations vs. separation capillary length. Anions – solid lines, cations – dotted lines. (Figure 2 in Publication I).

If there is no suitable position for the detector cell to allow full separation, such as in case of co-migration of chloride and sulfate with sodium, the separation can still be and was achieved by timely delaying of injections from both ends. Despite the analysis time being longer by some 0.7 min, the delayed injection of anions by 25 s (Figure 7A) was used in all experiments contributing to the simpler operation methodology.

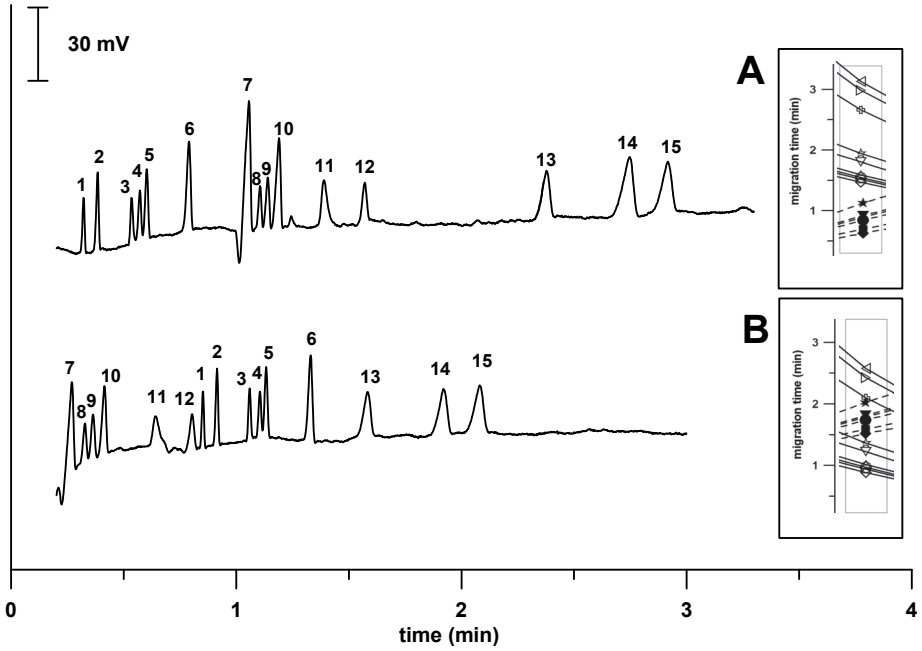

Figure 7. Simultaneous separation of anions and cations using different injection sequences. (A)-timely displaced injections of cations (20 s, HD injection from 10 cm), HV application 25 s, followed by anions (20 s, HD injection from 10 cm). (B)-timely displaced injections of anions (20 s, HD injection from 10 cm), HV application 55 s, followed by cations (20s, HD injection from 10 cm). Inlays: Example of simulated migration times vs. detector position (15 cm from anodic side). (A)-delayed injection of anions by 25 s. (B)-delayed injection of cations by 55 s. CE conditions: -18kV, contactless conductivity detection. Peaks: (1)-NH_4^+, (2)-K^+, (3)-Ca^{2+}, (4)-Na^+, (5)-Mg^{2+}, (6)-Li^+, (7)-Cl^-, (8)-NO_2^-, (9)-NO_3^-, (10)-SO_4^{2-}, (11)-SCN^-, (12)-formate, (13)-acetate, (14)-lactate, (15)-phosphate. (Figure 3 in Publication I)

4.1.3 Method validation and quantitative analysis of EBC

During initial CE screening of EBC for its ionic content, it was determined that measurable concentrations of seven anions (inorganic: Cl^-, NO_2^-, NO_3^-, SO_4^{2-},$H_2PO_4^-$, organic: acetate, lactate) and five inorganic cations (NH_4^+, K^+, Ca^{2+}, Na^+, Mg^{2+}) were present.

Analytical parameters, such as repeatability of peak areas (n=7), linearity and LoDs of developed CE method, are listed in Table 3. The LoDs were acceptable for the determination of most ions typically present in EBC at concentration levels. The last column in Table 3 presents concentration ranges of all analyzed compounds found in the EBC of 75 volunteers – the average values can be found in Table 2 of **Publication I**.

Table 3. Analytical parameters of the developed CE-C^{4}D method for simultaneous determination of inorganic anions and cations in EBC, n=7 and determined concentrations n=75

Ion	RSD (%) P.A.	Calibration range (μM)	LoD (μM)	r^2	Concentration range (μM) [a]
NH_4^+	3.6	1–1000	0.54	0.9995	0–1000
K^+	6.2	1–250	0.33	0.9975	1–150
Na^+	3.5	1–250	0.55	0.9936	9–200
Ca^{2+}	5,4	1–250	0.75	0.9992	20–300
Mg^{2+}	1.9	1–250	0.47	0.9974	0–10
Cl^-	8.7	1–250	0.34	0.9979	0–200
NO_2^-	4.0	1–25	0.63	0.9958	0–10
NO_3^-	5.5	1–25	0.56	0.9951	0–10
SO_4^{2-}	3.5	1–25	0.38	0.9966	0–10
SCN^-	5.3	1–250	1.25	0.9958	–
acetate	3.0	1–250	1.25	0.9977	5–200
lactate	4.6	1–250	1.07	0.9971	5–250
phosphate	4.0	1–250	1.15	0.9980	1–150

[a] Concentration range found in the analyzed samples in this study (n=75)

No correlation between the detected ionic concentrations and age, sex, or physical fitness were found. High concentration of ammonium, detected in most samples that is clamed to represent mouth contamination[94-95]. All samples except purposely spiked with saliva were proved to be not contaminated with saliva as no thiocyanate (SCN^-) was observed (usually 1–2 mM in saliva). Although in the contaminated sample a clearly distinguished peak of SCN^- could be observed, no additional measurements are required when using our developed method.

Moreover, analyzing EBC samples of a volunteer in full health and when they had a common cold (an acute infection of the upper respiratory tract), confirmed the results of previous studies that demonstrated that increased levels of nitrogen-reactive species can be found in the EBC of persons with serious lung condition. The corresponding electropherograms are represented in Figure 4 in **Publication I**. Though they appear very similar, significantly elevated level of nitrite was found in EBC trace collected when the subject had a cold. No measurable concentration of nitrogen was detected before, though 7.3 μM of the latter was observed during the illness of the subject, which is about twice as high compared to the determined average concentration in the initial study. Significantly elevated levels of nitrite were obtained from the EBC of a person with a diagnosed mild form of chronic obstructive pulmonary disease (COPD). Furthermore, elevated chloride, nitrate, sulphate, lactate and potassium were observed and also the ratio of calcium/sodium was 7.7–11.5 times higher in EBC of a subject during an illness than that of person sampled during full health.

4.1.4 Determination of lactate in EBC

In the last phase of the study described in **Publication I**, major attention was payed to the determination and sensitive detection of lactate in EBC among other anions and cations. Lactate concentration may be an indicator of physical fitness as well as cardio-respiratory or metabolic diseases. In fact, an elevated level of lactate was found in the EBC of a person with COPD (see **Publication I**, Figure 4 trace C) and also Figure 5 in **Publication I** shows the results of lactate determination, before and after exhaustive cycling. Likewise, about a 3–4 fold increase in lactate concentration in the EBC was observed.

Further, observable correlation of the changes of other ions was noted, such as a constant increase in ammonium throughout the resting period, while acetate and phosphate remained rather constant. Potassium, sodium and chloride, on the other hand, followed closely the concentration curve of lactate.

Therefore, besides the obtained promising results that underscore the diagnostic potential of a combination of non-invasive EBC sample collection and subsequent CE-C^4D analysis, the current methodology also helped us to outpace two major claimed issues: first, the concerning relatively voluminous sample amounts (several milliliters), and second, long sampling times (10–60 min).

4.2 Comparison of sampling devices (Publication II)

Since the sampling device developed in the first study, described in **Publication I**, ended up rather bulky with regards to carrying it to the site or point-of-care without additional equipment such as a small icebox, the supplementary survey was conducted.

In this trial two EBC collection devices were compared: the previously described tube in tube cooled sampler and a simple low density polyethylene zip-lock bag. Though employing same sampling time (2 min) of tidal breathing, either through the straw to the tube in tube sampler or directly into the zip-lock bag, the tube in tube device showed about a 4-fold higher collection efficiency. Nevertheless, the zip-lock bag is a hygienic, single use, cheap and easily disposable alternative that can be used in remote areas, at mass events or as an emergency sampler. Because it can be tightly sealed it can eventually be sent by regular mail to the laboratory.

4.3 Fingerprinting of post-blast explosives (Publication III)

The same ions mentioned in first two publications may reveal entirely different information. The aim of the next study, described in **Publication III**, was to use a portable CE-C^4D system for fingerprint analysis of post-blast explosive residues based on their ionic content. Commercial organic and improvised inorganic explosives were detonated for this purpose on several surfaces, such as sand,

concrete and metal witness plates. The extraction methods were developed jointly with simple sample collection techniques for each of the surfaces and analyzed by simultaneous CE analysis of cations and anions.

4.3.1 DOEI for simultaneous analysis of ions in post-blast residues

Since the instrumentation that was used in **Publication III** differed from that which was described in **Publications I and II**, the optimization of the simultaneous separation of anions and cations for the current system was performed. Considering the specific construction of the instrument the minimum possible effective length of capillary was 6 and 13 cm from anodic and cathodic end respectively. The injection was performed manually from both ends, bearing in mind that when injecting second sample from other side the part of the first is pushed out and have to be a priory compensated by two extra portions of BGE. The graph showed in **Publication III**, Figure 2 showing the migration times vs. the effective separation capillary length (from the respective injection point to the detector) was conducted similarly to the one described in previous study (**Publication I**). The optimum position of the detector was found to be 14 cm from the anodic side and 36 cm from the cathodic side, particular configuration was used throughout further study.

4.3.2 Method validation and quantitative analysis of post-blast residues

The developed CE method was validated using a set of standard solutions prepared in DI water. The LODs between 12.2 to 35.7 µM for anions and 3.8 and 7.3 µM for cations were determined. Those and other important analytical parameters are more specifically listed in Table 4 below.

Table 4. Figures of merit of the developed CE-CCD method for simultaneous determination of inorganic anions and cations in explosives residues, n=3. (Publication III)

Ion	RSD (%) P.A.	Calibration range (µM)	r^2	LOD (µM)
NH_4^+	5.5	1–500	0.9960	7.3
K^+	4.4	1–250	0.9969	3.7
Na^+	5.0	1–250	0.9928	7.1
Ca^{2+}	5.7	1–250	0.9982	3.8
Mg^{2+}	3.6	1–250	0.9968	5.8
Cl^-	7.1	1–250	0.8707	35.7
NO_2^-	9.9	1–75	0.9900	11.0
NO_3^-	7.8	1–75	0.9889	15.6
SO_4^{2-}	7.1	1–75	0.9953	12.2
N_3^-	9.1	1–250	0.9900	12.8

An optimized and validated CE method was used for further detailed screening of the ionic content of 8 different explosives: Dynamite, PETN, TNT, RDX, PENO, ANFO, V40, C4 on three different surfaces, with the exception of V40, which was not tested on concrete (for safety reasons).

4.3.3 Fingerprinting the post-blast explosive residues

The observations of this study confirmed that not all organic explosives leave inorganic residues; however, some of them leave a significant inorganic ion trace that may be used to identify them.

The bar graphs of cation and anion concentrations in each of the tested explosives and the matrix shown in APPENDIX 1 demonstrate the complexity of the ionic traces. A little, but nonetheless sufficient, information can be gathered from the separate observation of each bar-plot – for instance the anionic graphs clearly show that high concentrations of nitrate are present in dynamite and ANFO traces. The same can be seen for ammonia cation. Though other ions did not demonstrate such a clear difference they were still successfully used to partially characterize post blast residues. The use of a flow chart depicted in Figure 8 was suggested for explosive identification in a metal matrix.

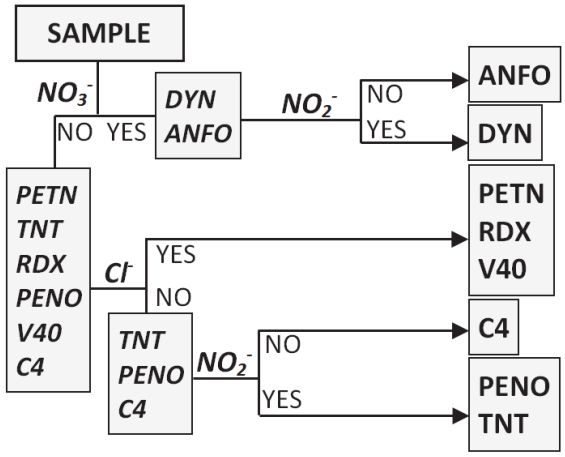

Figure 8. Flow chart for explosive identification in a metal matrix, based on the simultaneous separation of anions and cations. DYN- dynamite; PETN; TNT; RDX, PENO, DE - detonator; ANFO; V40; C4. (Supporting information, Publication III)

It should be noted that the flow chart is valid only for the selected group of explosives, however, and only applies to the particular matrix, while two other matrices (sand and concrete) did not provide similar results.

4.3.4 PCA analysis and clustering of the explosives

Furthermore, PCA was applied to the measured data in order to eliminate matrix effects, at least to some extent. It was suggested that this approach could eventually simplify the identification. The set of electropherograms of post blast samples formed the data matrix. As a feature, peak areas of the identified ions were used (10 ions). Altogether 70 samples were measured. Thus the data matrix has a dimensionality of 70x10.

Since standard PCA was not enough to establish if the pattern of ions on electropherogram was characteristic of the explosive used, the data were subjected to the mean centered PCA procedure. As a result of the "leave-one-out" cross-validation procedure, 4 significant principal components were revealed. Results of two first, as most vivid components – PC1 and PC2, were shown in Figure 4 in **Publication III** and also a color copy can be found in Appendix 2. After data interpretation it was clear that definite patterns could be identified. From score plots of all samples (see Figures A and B in Appendix 2), at least two big clusters were recognized. Moreover, the loading vectors plot (Figure A) shows that the separation of the clusters is due to the domination of Cl^- ion. By applying data normalization in PCA, the sample matrix influence on the clustering was reduced. However, the nature of the explosive correlates loosely with the location of clusters. Further, three distinctive clusters were observed in the distribution of the ions on different matrices (Figures 4C, 4D and 4E, Appendix 2). The first cluster, which is separated from the rest by the dominance of the Cl^- ion, included: V40, PETN and RDX. The second cluster was formed by the Dynamite and ANFO, mainly due to the dominance of the NH_4^+ ion, while other loadings were matrix dependent. The third cluster was formed by C4 and PENO; the positon of the cluster is however also matrix dependent. The location of Detonator and TNT seemed to be matrix dependent as well.

In conclusion, it was found that though matrix influence on the post blast sample electropherogram pattern is strong and clusters are not well "focused" due to bad reproducibility, the matrix can be considered in a particular situation; therefore, if clusters are well defined on the corresponding scores plot then it means that the electropherograms are characteristic of the detonated explosive on the given matrix and can thus be used for their identification.

4.4 Development of a CE-UV method for the analysis of thiodiglycol and its oxidation products (Publication IV)

The aim of the study described in **Publication IV** was to develop and validate the CE method with direct UV detection for analysis of thiodiglycol and its oxidation products. Although the proof of the principle was arrived at by being demonstrated on a commercial instrument (Agilent), the future goal was to create a portable format of it.

4.4.1 Sample derivatization

Since target analytes have no UV chromophore, derivatization with phthalic anhydride was deployed. Further optimization of derivatization conditions (Figure 2, **Publication IV**) set a standard that 100 µL of reactant per each analyte is required and derivatization at 45 °C for 20 min; therefore, those conditions were implemented for further experiments in order to obtain reproducible results. It was demonstrated that the TDG, TDGO and TDGOO remain relatively stable when kept refrigerated, as well as at room temperature (RSD P.A 3.2%), with the exception of TDGOO at room temperature (RSD P.A 5.7%).

Since improvement of the method for the analysis of sulfur compounds of interest involved derivatization with phthalic anhydride that affected the pK_a value of the formed derivatives, charging the molecules and allowing use of a borate buffer for separation optimization of the BGE conditions was required. Investigation of the effect of the buffer concentration, pH, applied voltage and capillary temperature on separation efficiency showed that the optimal conditions for the purpose of separation of the three derivatives are 30mM borate, pH 8.5, 15 kV at 25 °C.

4.4.2 CE method validation for quantitative analysis of TDG and its oxidation products

The study of precision of the developed method showed that run-to-run precision resulted in maximum RSD values of 0.6% (n=6) for the migration time and 3.1% (n=6) for the peak area. Moreover, day-to-day results showed RSD values of 1.2% (n=6) and 3.6% (n=6) for the migration times and the peak areas, respectively. No systematic changes were observed in peak shape during the precision tests.

The developed CE method was validated based on optimized BGE and a standard mixture of derivatives. LoD as low as 98 ng/mL(S/N=3) and LoQ 305 ng/mL (S/N= 10) for TDG was obtained. This and other important analytical parameters are listed in Table 5 below.

Table 5. Analytical parameters of the developed CE-UV method for determination of TDG and its degradation products (Publication IV).

Derivative	Linear range (µg/mL)	r^2	LoD (µg/mL)	LoQ (µg/mL)
TDG	0.10–2.44	0.9996	0.10	0.31
TDGO	0.14–2.76	0.9997	0.14	0.42
TDGOO	0.15–3.08	0.9994	0.15	0.46

Though the detection limits obtained are outperformed by GC-MS, they can still be considered acceptable based on real concentrations present in environment and point-of-care.

4.4.3 Spiked seawater analysis

For simulation of real-world conditions in order to demonstrate the method's applicability for real sample analysis, analysis of seawater spiked with a standard mixture of three underivatized analytes (TDG, TDGO and TDGOO) was performed. The derivatization procedure was performed after extraction. Figure 9 below represents the electropherograms of the separation performance of seawater and as a comparison distilled water samples.

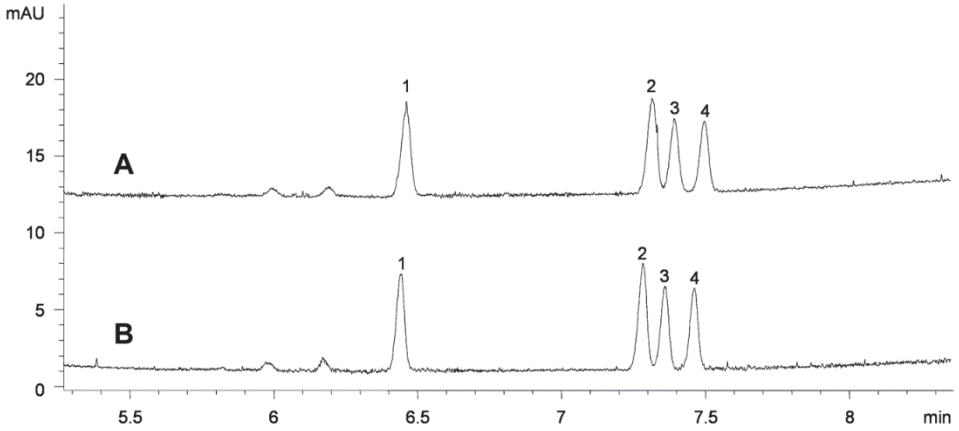

Figure 9. Electropherograms of (A) distilled water and (B) seawater samples. Peaks: IS (1), TDGO (2), TDG (3), TDGOO (4). Separation conditions: BGE – 30mM borate, pH 8.5, 15 kV, 25 °C (Publication IV)

In both cases, TDG, TDGO and TDGOO were baseline separated. The RSD of the migration times was 0.4% between all analytes in the spiked sea and distilled water samples and the RSD of the peak areas was below 5%. Therefore, no evident influence of seawater matrix on the derivatization and extraction processes was observed.

5 CONCLUSIONS

The principal aim of the present thesis was adapting a CE instrument for POC and on-site analysis by taking advantage of the inherent robustness and amenability of the miniaturization of CE technology and secondly providing proof of the principle and demonstrating the capabilities of various analysis situations.

The results of this work have shown that CE is a very promising technique, which should be considered for the rapid on-site detection in medical, environmental and safety and defense applications. It was shown to the reader that CE can be employed in order to analyze exhaled breath condensate (EBC) as a complicated body fluid as well as explosive residues in different matrices and thiodiglycol and its degradation products in seawater.

It was demonstrated that CE results can be further improved by applying chemometric tools.

The results of the study can be summarized as follows:

- ✓ A portable CE method with C^4D using DOEI for the rapid analysis of the ionic content of EBC in less than 2 min was successfully validated.
  - o A simple and cost-effective sampling device was developed for fast (2 min) and effective EBC sample collection.
  - o EBC sample analysis of 75 volunteers was successfully performed.
  - o Markers of acute airway inflammation and mild COPD were assessed.
  - o Elevating levels of lactate were detected during resting period after exhaustive exercising.
- ✓ The same method, modified to suit other portable instrument, was successfully optimized and validated for rapid (4 min) analysis of ionic content of explosives residue in various matrices.
- ✓ Explosives were detonated and post-blast residues collected and tested in "real-world" conditions.
  - o Classification of post-blast residues, regardless of the matrices in question, was achieved by means of PCA application.
  - o Identification of explosives based on unique "fingerprint" in soil matrix was proposed.
  - o The matrix effects were accessed and eliminated by PCA.
- ✓ A CE method with direct UV detection after pre-capillary derivatization with strong UV chromophore (phthalic anhydride) for separation of TDG, TDGO and TDGOO in 8 min was validated.
  - o The developed method was used to determine the low amounts of thiodiglycol and its oxidation products in seawater.
  - o The proof of the principle was demonstrated to the reader on a laboratory-scale commercial instrument.

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ABSTRACT

Nowadays, developing portable point-of-care and on-site instruments is much easier due to the rapid technical evolution of compounds that form the basis of POC devices and common analytical instrumentation. Modern technologies display cleaver design through the incorporation of smaller components, such as optical fibers, liquid crystal displays (LCD) and light emitting diodes (LED). The drive towards the miniaturization of instruments created decades ago is based on the demand to have the instruments taken out of the lab and into the field. Whether it is a medical device such as pH- or glucometers or a specific instrument or a kit for pollution detection in the environment or an X-ray at the airport, it is clear that many compact versions of benchtop instruments are already available and present in our daily life. The small size of instruments economizes on space in the laboratory and means that they are easily portable and can therefore be brought closer to the problem and thus closer to solution as well, saving time and money with regards to the transportation of samples. Moreover, often simpler to use and more price-competitive, they are affordable and easy to handle even by those lacking scientific or technical training. Nevertheless, with increasing demand and issues in medical, environmental and safety and defense fields, there remain many unfilled niches and unresolved issues.

This study explored the possibility of using CE technology for the development of portable instruments for on-site and point-of-care applications. Methodologies for the determination of the ionic content of exhaled breath condensate (EBC), post-blast explosive residues and sulfur mustard degradation products in bottom seawater were developed. Different detection modes were applied for the determination of the ionic content of EBC and for post-blast explosives residues a capacitively-coupled contactless conductivity detector (C^4D) was used, and ultra violet (UV) detection for thiodiglycol (TDG) and its oxidation products analysis. Their connection to the same platform, however, was left outside of this work.

A portable CE method with C^4D using DOEI for the rapid analysis of the ionic content of EBC was developed and applied together with innovative, simple and cost-effective sampling technique for EBC sample collection. Promising results were achieved in real sample analysis; however, further clinical study is required in order to evaluate the possible justification of their use in point-of-care applications.

Applying the same/similar to previous, slightly modified methodology for the rapid analysis of the ionic content of explosives residue in various matrices showed that at least partial classification of post-blast residues, regardless of the matrices in question, is achievable by using portable CE-C^4D and application of simple chemometrics (PCA).

In the last section, the validated CE method with direct UV detection for separation of TDG, thiodiglycol sulfoxide (TDGO) and thiodiglycol sulfone (TDGOO), along with pre-capillary derivatization with phthalic anhydride, showed reliable results in the detection of those compounds in seawater.

CE has been frequently underestimated, but is a powerful and flexible tool in the separation and identification of the individual components of a mixture. In fact, at the time when this study started the issues tackled by the study had not yet piqued the interest of CE practitioners – only one study of EBC and just a handful on explosive residues were published at the time. The number of studies have since risen, proving that the developed methods for the analysis of the ionic content of EBC and post-blast residues, and thiodiglycol and its degradation product are reliable and of current relevance in the field.

KOKKUVÕTE

Tänapäeval on tänu vastavate instrumendidetailide kiirele tehnilisele täiustumisele portatiivsete instrumentide ja tavapärase analüütilise aparatuuri väljatöötamine muutunud palju lihtsamaks. Kaasaegsed tehnoloogiad võimaldavad nutikat disaini, kasutades kompaktsemaid detaile, sealhulgas optilisi kiude, vedelkristallekraane (LED) ja valgusdioode (LCD). Juba aastakümneid tagasi tekkinud suund aparatuuri miniaturiseerimisele tugineb vajadusele instrumentide järele, mida saaks tuua laborist välitingimustesse. Olgu tegemist meditsiiniseadmega nagu pH- või glükomeeter, instrument või seadmete komplekt keskkonnareostuse määramiseks või ka lennujaama röntgenseade – ilmne on, et paljud kompaktversioonid tavapärastest lauainstrumentidest on juba saadaval ja kasutusel meie igapäevaelus.

Väikesemõõdulised instrumendid säästavad laboriruumi ja on kergesti ümberpaigutatavad, seega saab neid tuua lähemale probleemile ja selle lahendusele, säästes proovide teisaldamisega seotud kulutusi ning aega. Lisaks on selliseid instrumente lihtsam kasutada, nad on kuluefektiivsemad ning kergesti käsitletavad ka ilma teadusliku ja tehnilise väljaõppeta inimese poolt. Seoses kasvava nõudluse ja üha areneva probleemistikuga meditsiini, keskkonna- ja riigikaitse ning turvalisuse valdkondades on siiski jäänud veel palju täitmata nišše ja lahendamata küsimusi.

Käesolevas töös uuriti võimalusi rakendada kapillaarelektroforeesi (CE) tehnoloogiat uuringupaigas kasutatavate portatiivsete instrumentide väljatöötamiseks. Uurimustöö käigus töötati välja metoodikad väljahingatava õhu kondensaadi ja plahvatusjärgsete jääkide ioonkoostise määramiseks ning ipriidi laguproduktide määramiseks. Töös kasutati erinevaid detekteerimise meetodeid. Väljahingatava õhu ning plahvatusjärgsete jääkide ioonse koostise määramiseks kasutati mahtuvuslikku kontaktivaba juhtivusdetektorit (C^4D) ja tiodiglükooli (TDG) ning selle oksüdatsiooniproduktide analüüsiks UV-detektorit. Kahe meetodi ühendamine jäi siiski antud töö raamidest välja.

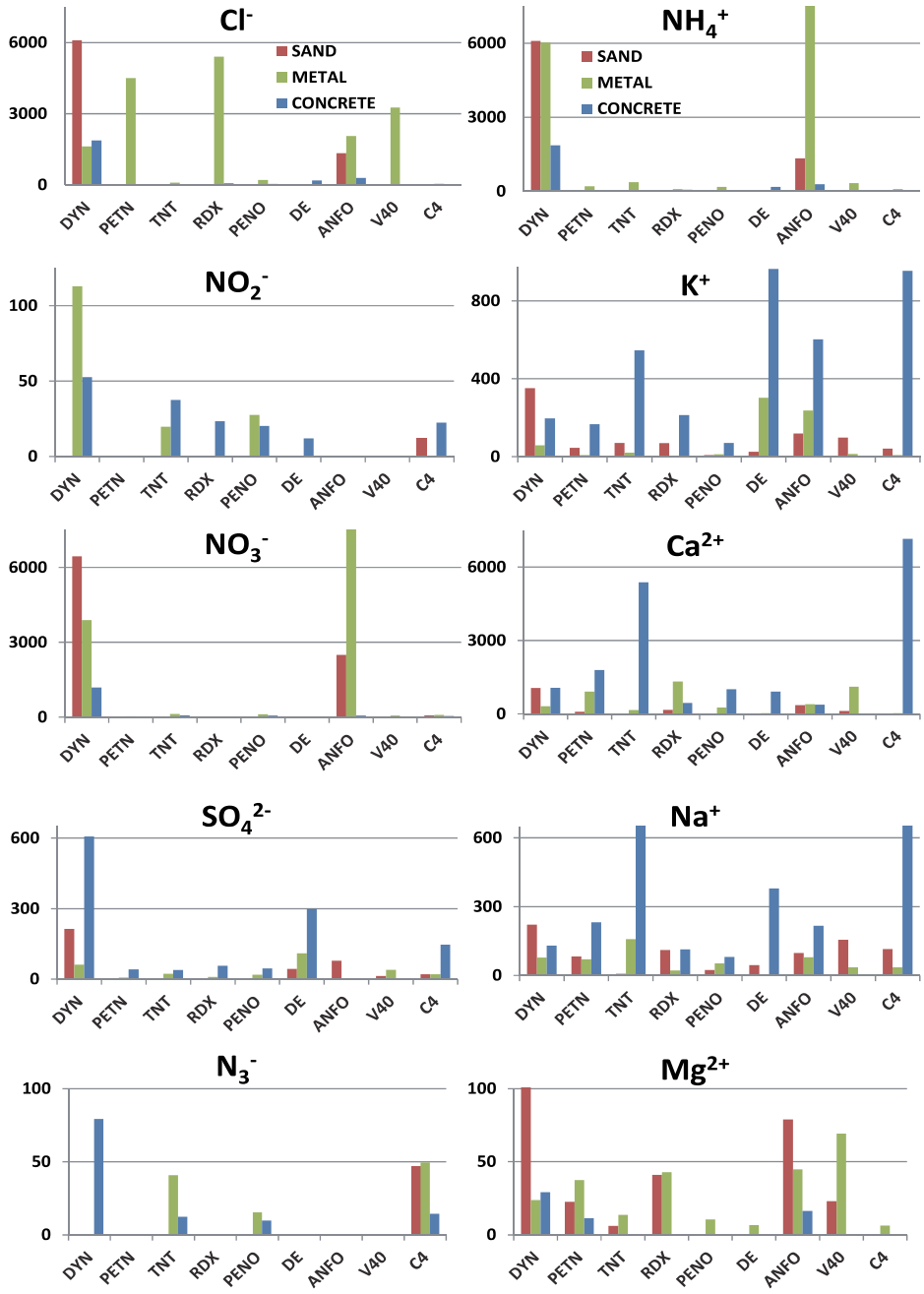
Töö käigus töötati välja portatiivne CE-C^4D meetod, kasutades analüüsiks kahepoolset proovi sisestamise metoodikat (DOEI) ning proovi kogumiseks innovatiivset, lihtsat ja kuluefektiivset proovivõtu seadet ja tehnikat. Vaatamata reaalsete proovide analüüsi paljulubavatele tulemustele on siiski vajalikud põhjalikumad kliinilised uuringud õigustamaks meetodi kohapealset kasutatavust.

Eelnevaga sarnast, kuid veidi modifitseeritud metoodikat kasutati plahvatusjärgsete jääkide ioonse koostise määramiseks erinevates maatriksites. Tulemused näitasid, et maatriksile vaatamata on võimalik plahvatusjääkide osaline klassifitseerimine, kasutades portatiivset CE-C^4D instrumenti ja tulemuste tõlgendamiseks kemomeetriat (PCA).

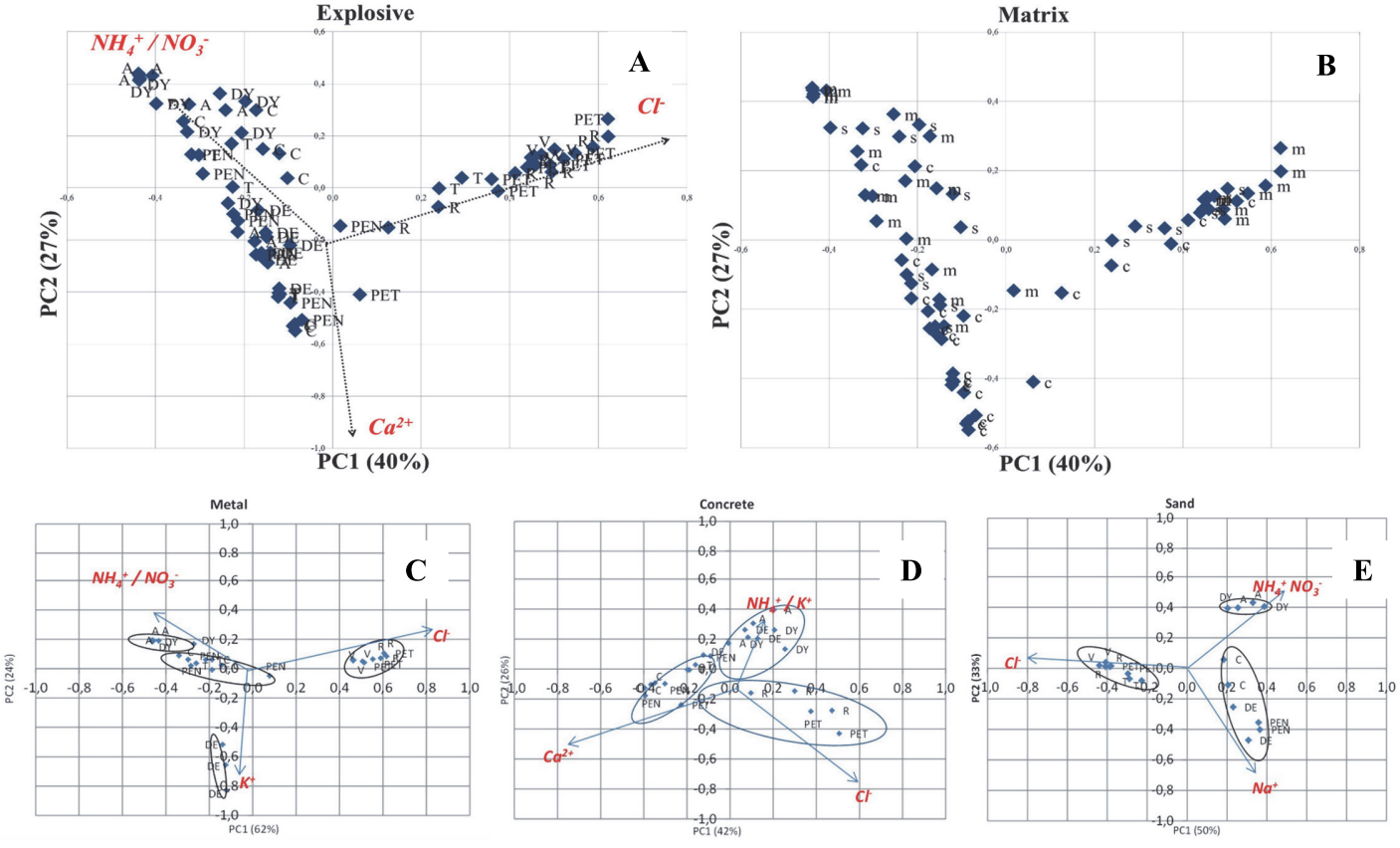
Uurimustöö viimases etapis valideeriti CE-UV meetod TDG, tiodiglükool sulfoksiidi (TDGO) ja tiodiglükool sulfooni (TDGOO) lahutamiseks pärast eelnevat ftaalanhüdriidiga derivatiseerimist ning saavutati usaldusväärsed tulemused nende ühendite detekteerimisel merevees.

Kapillaarelektroforeesi on sageli alahinnatud, kuid see on efektiivne ning paindlik vahend segu üksikute komponentide lahutamisel ja identifitseerimisel. Vajab erilist märkimist, et uurimustöö alguses ei olnud kõne all olevad objektid äratanud erilist huvi CE praktiseerijate seas (vaid üks avaldatud artikkel väljahingatava õhu ja vaid mõni lõhkeainete kohta). Nüüdseks on publikatsioonide arv mitu korda kasvanud, mis tõestab, et väljatöötatud metoodikad väljahingatava õhu ning plahvatusjärgsete jääkide ioonse koostise määramiseks ja TDG ning selle laguproduktide määramiseks CE abil on usaldusväärsed ning teema on jätkuvalt aktuaalne.

APPENDIX 1. Bar plot of the ionic content of the studied explosives. X-axis: DYN- dynamite; PETN; TNT; RDX, PENO, DE- detonator; ANFO; V40; C4. Y-axis: concentration (µM) (Supporting information, Publication III)



Appendix 2. A- score plots of all samples labelled by matrix type (s- sand, c-concrete, m-metal). B- score plots of all samples labelled by explosive: Dynamite (DY); PETN (PET), TNT (T), RDX (R), PENO (PEN), ANFO (A), V40 (V), C4 (C), DETONATOR (DE). C, D, E- distribution of the ions on metal, concrete, sand respectively. (Publication III, Figure 4)



LIST OF ORIGINAL PUBLICATIONS

1.1 – 4 publications

Kuban, P., Kobrin, E-G., Kaljurand, M. Capillary electrophoresis – A new tool for ionic analysis of exhaled breath condensate. *Journal of Chromatography A* **2012**, 1267, 239–245.

Kobrin, E-G., Lees, H., Fomitšenko, M., Kuban, P., Kaljurand, M. Fingerprinting postblast residues by portable capillary electrophoresis with contactless conductivity detection. *Electrophoresis* **2014**, 35, 1165–1172.

Jõul, P., Lees, H., Vaher, M., Kobrin, E-G., Kaljurand, M., Kuhtinskaja, M. Development of a capillary electrophoresis method with direct UV detection for the analysis of thiodiglycol and its oxidation products. *Electrophoresis* **2015**, 36, 1202–1207.

3.1. – 1 publication

Kuban, P., Kobrin, E-G., Kaljurand, M. Potential of exaled breath condensate analysis in point of care diagnostics. *9-th International Interdisciplinary Meeting on Bioanalysis*, **2012**, 128–133.

5.2. – 6 publications

Kobrin, E-G., Kubáň., P., Kaljurand, M. Determination of post-last explosive residuesin various matrices using portable capillary electropherograph with contactless conductivity detection. TU and TUT graduate school "Functional materials and technologies" 5th science conference, Tartu, **2014**

Kobrin, E-G., Kubáň., P., Kaljurand, M. Ionic analysis of exhaled breath condensate using capillary electrophoresis. TU and TUT graduate school "Functional materials and technologies" 4th science conference, Tallinn, **2013**

Kobrin, E-G., Kubáň., P., Kaljurand, M. Determination of post-last explosive residuesin various matrices using portable capillary electropherograph with contactless conductivity detection. TU and TUT graduate school "Functional materials and technologies" 3rd science conference, Tartu, **2012**

Kobrin, E-G., Knjazeva, T., Kuban, P., Seiman, A., Vaher, M., Kaljurand, M. Keskkonnaproovide laboriväline ekstraktsioon ja kapillaarelektroforeetiline analüüs In Abstract book: 32th Estonian Chemistry Days, Tartu, **2011**

Knjazeva, T., Makarõtševa, N., Kobrin, E-G., Helmja, K., Seiman, A., Vaher, M., Kaljurand, M. Portatiivne kapillaarelektroforeesi aparaat keskkonnareostuse analüüsiks In Abstractbook: 31st Estonian Chemistry Days, Tallinn, **2010**.

Knjazeva, T., Kobrin, E.-G., Kaljurand, M. Capillary electrophoresis frontal analysis for the study of flavonoid noncovalent interactions with human serum albumin In Abstract book: 5th Conference on Separation and Related Techniques by Nordic Separation Science Society, Tallinn, **2009.**

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1. Personal data

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2. Education

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Tallinn University of Technology	2011	Applied chemistry and biotechnology, MSc
Tallinn University of Technology	2009	Applied chemistry and biotechnology, BSc
Tallinn Sikupilli Gymnasium	2006	Secondary education

3. Language competence/skills (fluent, average, basic skills)

Language	Level
Estonian	Native
English	Fluent
Russian	Fluent

4. Special courses

Period	Educational or other organisation
03.03.2014 - 13.04.2014	Estimation of Measurement Uncertainty in Chemical Analysis

5. Professional employment

Period	Organisation	Position
01.11.2015 – ….	AS Laser Diagnostic Instruments	Researcher
01.06.2006 – 31.10.2014	AS Chemi-Pharm	Sales agent
01.02.2014–31.12.2014	TUT, Department of Chemistry,	Engeneer
01.01.2008 – 31.12.2103	TUT, Faculty of Science, project SF01140023s08	Investigator
01.01.2012 – 31.05.2012	TTU, Faculty of Science, project ETF8986	Investigator
01.01.2009 – 31.12.2010	TUT, Department of Chemistry project ETF7818	Investigator

6. Research activity, including honors and thesis supervised

Theses supervised

Heidi Lees MSc, 2015, (sup) Eeva-Gerda Kobrin, Analysis of post-blast explosive residues by portable capillary electrophoresis with contactless conductivity detection, Department of Chemistry Tallinn University of Technology

Honors and activities

Defense related masters and doctoral thesis competition- Ist prize (master's thesis) Ministry of Defense, 2012

ELULOOKIRJELDUS

1. Isikuandmed

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2. Hariduskäik

Õppeasutus (nimetus lõpetamise ajal)	**Lõpetamise aeg**	**Haridus (eriala/kraad)**
Tallinna Tehnikaülikool	2011	rakenduskeemia ja biotehnoloogia/ magistrikraad
Tallinna Tehnikaülikool	2009	rakenduskeemia ja biotehnoloogia/ bakalaureusekraad
Tallinna Sikupilli Keskkool	2006	Keskharidus

3. Keelteoskus (alg-, kesk- või kõrgtase)

Keel	**Tase**
eesti keel	emakeel
inglise keel	kõrgtase
vene keel	kõrgtase

4. Täiendusõpe

Õppimise aeg	**Täiendusõppe korraldaja nimetus**
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01.01.2008 – 31.12.2103	TTU, Matemaatika- loodusteaduskond, projekt SF01140023s08	täitja
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Capillary electrophoresis – A new tool for ionic analysis of exhaled breath condensate

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ABSTRACT

Exhaled breath condensate has been analyzed for its ionic content by capillary electrophoresis with capacitively coupled contactless conductometric detection. A simple device for collection of small volumes (100–200 μL) of exhaled breath condensate in less than 2 min was developed. A method for simultaneous determination of inorganic cations, inorganic anions and organic anions from the samples using dual-opposite end injection principle with a short fused silica capillary (35 cm, 50 μm I.D.) was developed. A background electrolyte composed of 20 mM 2-(N-morpholino)ethanesulfonic acid, 20 mM L-histidine, 30 μM cetyltrimethylammonium bromide and 2 mM 18-crown-6 was used. The analysis time was less than 3 min with limits of detection reaching low μM levels for most of the anions and cations. It has been shown that changes of nitrite could be observed in acute inflammation of upper airways and in a person with diagnosed mild chronic obstructive pulmonary disease, while changes of other ions could also be observed. Lactate concentrations could also be monitored and about 4-fold increase of lactate concentration in exhaled breath condensate was determined following an exhaustive cycling exercise. The developed non-invasive sampling of exhaled breath condensate, followed by rapid capillary electrophoretic analysis, could be very useful in lung inflammatory disease screening as well as in monitoring fast metabolic processes such as lactate build-up and removal.

1. Introduction

Capillary electrophoresis has found an important niche among separation techniques, especially in the analysis of biomolecules, proteins and DNA sequencing [1,2] due to its unprecedented separation efficiency, high speed of analysis and robustness toward difficult matrices. However one of the main and often overlooked advantages of CE is the ability to analyze minute sample volumes. A volume of sample introduced into a CE separation capillary is typically less than 10 nL, thus even a sample having volume as low as 1 μL can be repeatedly analyzed by CE. This contrasts significantly with chromatographic techniques such as HPLC, where the introduced sample volume may be up to thousand fold higher. On the downside is comparably lower sensitivity and reproducibility of CE. Nevertheless, the low sample volume requirement of CE becomes a clear benefit when analyzing (mainly) samples of biological origin, because they are often not available in large quantities as most of them are obtained by invasive sampling (lumbar puncture, venipuncture, biopsy etc.). Examples of CE analysis of biological samples include the analysis of cerebrospinal fluid (CSF) [3–6], tear fluid [7–9], saliva [10–12] or blood serum [13].

The biological samples are predominantly analyzed for their protein and biomolecular content, however, analysis of small molecules and ions is also important [13]. CE can provide efficient separation of small ions and it was the introduction of capacitively coupled contactless conductivity detection (C4D) more than a decade ago [14,15] that has significantly advanced the popularity of CE in this field. C4D detector provides simplicity and enhanced sensitivity compared to the commonly used UV–vis detection. CE with C4D has been mainly used for the analysis of samples which are abundant in volume [16–18], however recently the analysis of biological samples has obtained significant attention as demonstrated by CE–C4D analysis of biological fluids including saliva [12,10], blood plasma [19–24] and urine [25–27]. Nevertheless, as a separation method, CE has been less successful compared to its competitor HPLC. The main argument from practitioners' side has been that CE is not as robust as HPLC. Whether it is true or not the one feature of CE has been frequently overlooked: contrary to HPLC it can be made portable and as such can be used for point of care analysis. This makes CE attractive as a technological platform to develop equipment for simple clinical analyzers.

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In this contribution we would like to point to a new matrix that (surprisingly) has not attracted any attention of the practioners' of CE – exhaled breath condensate (EBC). EBC was first reported as human body fluid in 1980 by Sidorenko et al. [28] and since then has been intensely studied especially in the area of respiratory medicine research [29–31]. EBC is the liquid obtained upon cooling and condensation of exhaled air. It mainly consists of water and CO_2, but contains also aerosolized particles of airway lining fluid. The aerosolized particles contribute to the non-volatile EBC constituents, such as inorganic ions, small organic molecules and proteins. Additionally EBC may contain water soluble volatile gases as well. The analysis of EBC may offer a simple way of monitoring lung inflammation and provide insights into the pathophysiology of inflammatory lung diseases. Several inflammatory mediators, such as hydrogen peroxide, NO-reactive species, nitrosothiols, prostaglandins, and leukotrienes have been identified and determined in EBC [29–31].

EBC sampling is attractive due to its noninvasive nature, compared to other, invasive methods commonly used for quantifying airway inflammation, such as bronchoalveolar lavage [32], bronchial biopsies [33] or sputum induction [34]. It consists of simple breathing into a specially designed device: commercially available devices can be used, such as bench top EcoScreen [35] or portable Rtube [36], but home made devices typically perform similarly to the commercial ones at a fraction of the cost of the former ones. They typically consist of a mouthpiece with one-way valve connected to a collecting system which is placed in either liquid nitrogen or ice bath. For instance Mutlu et al. have used jacketed cooling pipes or tubes in buckets of ice [37]. One disadvantage of home made devices is that they are not portable and may require rather bulky accessories.

Another significant problem with conventional EBC sampling is the required sample volume which is typically several milliliters with sampling times between 10 and 60 min. The large sample volume requirement depends mainly on the subsequent analytical method used. For the analysis of nitrogen reactive species, colorimetric assays including Griess reaction [38–40], enzymatic reaction [41] or fluorimetric measurements [42,43] are widely used. These techniques, apart from being time consuming and requiring relatively large sample volumes can only analyze one analyte at the time. Alternatively ion chromatography [44–47] can be employed, however in any case minimum EBC sampling time to obtain sufficient sample volume no less than 10 min. Extended sampling may increase risk of salivary contamination of the EBC, which then requires one to perform additional tests to avoid false positive results. It would be thus important to develop a simple, portable device for collecting EBC in a shortest possible time and have a suitable analytical method ready for multianalyte screening and analysis. In this regard CE fulfills most of the criteria, i.e. it is fast, requires only very small sample volume and can be used to analyze multiple species in one electrophoretic run. It is thus one of the most promising techniques of choice for fast point of care analysis.

In this contribution we show for the first time that CE with C4D can be used for analysis of ionic content of EBC. For this purpose, we have developed a simple, portable, EBC collection device that can be used to collect sufficient volume of EBC (typ. 100–200 μL) in 1–2 min. We employ an optimized separation system with dual-opposite end injection and C4D detection for analysis of anions and cations in EBC simultaneously in less than 3 min. Practical examples of EBC analysis by CE–C4D include screening of inorganic nitrogen reactive species during acute and chronic airway inflammation and lactate monitoring. The promising results obtained underscore the diagnostic potential of CE–C4D combined with non-invasive EBC sampling.

2. Experimental

2.1. Materials and methods

2.1.1. Electrophoretic system

A purpose-built CE instrument was employed for all electrophoretic separations. The separation voltage of −18 kV was provided by a high voltage power supply unit (Spellman CZE2000R Start Spellman, Pulborough, UK). Two Pt wires (0.5 mm I.D., 3 cm length, Advent Research Materials Ltd., Eynsham, England) were used as electrodes. The separation capillaries used were fused-silica (FS) capillaries (50 μm I.D., 375 μm O.D., 35 cm total length, Polymicro Technologies, Phoenix, AZ, USA). Prior to the first use, the separation capillary was preconditioned by flushing with 0.1 M NaOH for 30 min, deionized (DI) water for 10 min and background electrolyte (BGE) solution for 10 min. Between two successive injections, the capillary was flushed with BGE solution for 1 min. At the end of a working day, the capillaries were washed with DI water for 10 min, followed by applying a vacuum for 5 min to remove any liquid from inside and stored dry overnight. All CE experiments were performed at ambient temperature.

2.1.2. Injection

Injection of standard solutions and EBC samples was carried out hydrodynamically. The injection capillary end was immersed in a sample vial and elevated to a height of 10 cm for a specified time interval. Dual opposite end injection (DOEI) was accomplished by injecting the sample from both capillary ends. When injection without a time delay was applied, the sample was first injected into one capillary end, followed by the injection of BGE. The sample was then injected from the other capillary end. The same injection duration was applied for both samples and BGE, resulting in both samples located at the opposite capillary ends before the separation was started. When timely displaced injection was applied, the sample was injected first from one capillary end, followed by an application of the high voltage for a specified time interval, interruption of the HV, injection of the sample from the other capillary end and resuming the HV for electrophoretic separation. The electropherogram recording was started after the second injection. The total duration of the injection sequence during the DOEI was typically 1 min.

2.1.3. Detection system

A capacitively coupled contactless conductivity detector (C4D) was used for the detection of the separated analytes. It consisted of an external function generator (GW Instek GFG-8219A, New Taipei City, Taiwan) providing a sinusoidal excitation signal (frequency: 290 kHz, amplitude: 20 V peak-to-peak) to an in-house built detector cell [48] with a pre-amplifier (OPA655, Burr Brown, TX, USA). The amplified cell current was led to an external detector circuitry for further processing. Data were collected using in-house written software and a 20 bit sigma-delta data acquisition card (Lawson Labs Inc., Malvern, PA, USA).

2.1.4. Construction of the EBC collection device

The EBC collection device is depicted in Fig. 1. It was constructed with maximum simplicity and minimal cost using commonly available laboratory consumables. A 50 mL polypropylene (PP) tube with skirted base (TYPE 62.559, Sarstedt, Germany) was used as the outer container. A 5 mm diameter hole was drilled to the bottom of the tube and a 5 mL PP pipette tip (Brandt GmbH, Wertheim, Germany) was inserted from the top and tightly pressed into the hole. To protect the inside of the pipette tip from contamination the bottom of the pipette tip was closed by using a GC septum (HTLB 0.281, Hamilton, Reno, NV, USA), while the top was closed with a thin sheet of Parafilm® (Bemis, Neenah, WI, USA). The PP tube was filled with DI

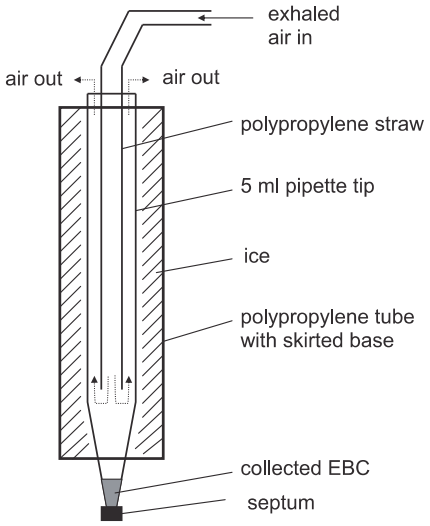

Fig. 1. Schematic of an in-house constructed EBC collection device.

water to provide the cooling of the pipette tip walls upon freezing. The prepared sampler was placed in a vertical position in a deep freezer at −20° C for several hours. When the water in the PP tube froze to ice, the device was ready to use. After each use, the pipette tip was discarded, but the device itself could be reused multiple times simply by inserting a new pipette tip inside the PP tube, as described above.

2.1.5. Sampling of the EBC

Before sampling the top protective film was removed from the pipette tip and a PP straw purchased in local store (Rimi, Tallinn, Estonia) was inserted into the pipette tip with its end being positioned in its lower half. The sampling was performed by slow tidal breathing through the straw (see Fig. 1) into the cooled pipette tip. The exhaled breath exiting the straw, as indicated by the arrows in Fig. 1, was cooled down when contacting the pipette tip walls and eventually accumulated in the bottom of the pipette tip. Approximately 1–2 min of tidal breathing was sufficient to collect 100–200 μL of the EBC. At the end of the sampling, the remaining liquid on the pipette tip walls was collected by gentle moves of the straw on the inside walls. The septum was carefully removed and the EBC was transferred into a 1.5 mL Eppendorf vial for further CE analysis. For quantitative analysis, 99 μL aliquot of the sample was pipetted into a separate vial and 1 μL of the internal standard stock solution of lithium formate (5 mM) was added.

2.2. Chemicals

2.2.1. Reagents, standards, electrolytes

All chemicals were of reagent grade and DI water (MilliQ Water System, Millipore, Molsheim, France) was used for stock solution preparation and dilutions. Stock solutions of inorganic anions and cations (100 mM) were prepared from reagent grade chemicals (Sigma–Aldrich, Steinheim, Germany). Lithium formate (98% purity) was purchased from Aldrich and its 5 mM stock solution was used to spike the sample and standard solutions. BGE for CE measurements was prepared daily by diluting 100 mM stock solutions of 2-(N-morpholino)ethanesulfonic acid (MES, Sigma–Aldrich), L-histidine (HIS, Sigma–Aldrich) and 18-crown-6 (Sigma–Aldrich) to the required concentration. Cetyltrimethylammonium bromide (CTAB, Sigma–Aldrich) was prepared as 10 mM stock solution in 5% acetonitrile and was added to the BGE. The optimized BGE composition used in this work was 20 mM MES, 20 mM HIS, 30 μM CTAB and 2 mM 18-crown-6 at pH 6.

3. Results and discussion

3.1. Optimization of the BGE for simultaneous separation of anions and cations in EBC

Initial CE screening of the ionic content of the EBC using a previously described BGE [49] (20 mM MES, 20 mM HIS, 20 μM CTAB and 1 mM 18-crown-6) has shown that it contains measurable concentrations of seven anions (inorganic anions: Cl^-, NO_2^-, NO_3^-, SO_4^{2-}, $H_2PO_4^-$, organic anions: acetate, lactate) and five inorganic cations (NH_4^+, K^+, Ca^{2+}, Na^+, Mg^{2+}). A slightly modified BGE consisting of 20 mM MES, 20 mM HIS, 30 μM CTAB and 2 mM 18-crown-6 at pH 6 was found suitable for simultaneous separation of selected anions and cations in EBC using the DOEI principle [50,51]. The modification consisted of increasing the concentration of 18-crown-6 to 2 mM to allow separation of large concentrations of NH_4^+ from K^+ in the analyzed samples. Further, by employing a slightly higher concentration of CTAB (30 μM) as BGE additive, the analysis time for simultaneous analysis of selected ions was decreased below 3 min.

Except the ions found in the EBC, thiocyanate, formate and lithium (as lithium formate) were added to the model mixture for optimization of the CE conditions. Thiocyanate was added as an indicator of salivary contamination (see the discussion later), while lithium and formate were the internal standards for the respective groups of cations and anions. When sample is injected from both capillary ends, the anions migrate toward anode, while the cations migrate toward the cathode. The optimization of the separation conditions for simultaneous separation of anions and cations typically includes individual separations of both groups injected from opposite capillary ends, with the detection cell positioned at different positions along the separation capillary. A plot of migration times vs. effective capillary length from the injection point to the detection point (L_{eff}) for each group, shown in Fig. 2, was constructed and used for the optimization of the separation conditions. The C4D detection cell can be placed at virtually any place along the separation capillary; the only restriction is the need for capillary handling during the hydrodynamic injection limiting the minimum practical L_{eff} to 10 cm. The 10 cm distance from the anodic capillary end was also the only position of the detection cell that allowed separation of the two groups of ions, anions and cations, from one another using DOEI at the same time. Unfortunately full separation of all cations was not possible as the resolution of critical analyte pair sodium/magnesium was less than 0.6. Thus a minimum length to the detection cell was set to 15 cm. This is the case marked with a rectangle in Fig. 2. The resolution of sodium/magnesium increases above 1.0, however, a full simultaneous separation of all anions and cations is still not possible, because there is a co-migration of chloride and sulfate with sodium, magnesium and lithium cations migrating from opposite direction. By carefully studying Fig. 2, it can be concluded that it is not possible to place the detection cell at any suitable distance to allow full separation of all 15 analytes.

The separation can still be achieved by injecting the sample from both ends with a time delay, as demonstrated by Kubáň and Karlberg [50]. Two injection sequences are possible to achieve full separation of all ions. In the first one, the cations are injected first from cathodic capillary end and high voltage is applied for 25 s, followed by the injection of anions. By delaying the injection of anions by 25 s, the cations are separated first, followed by the anions without any overlap. Conversely, by reversing the injection order

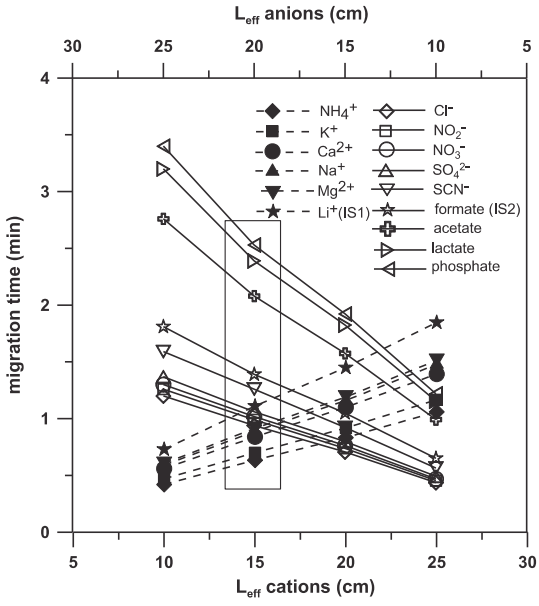

Fig. 2. Dependence of migration times of anions and cations vs. separation capillary length. Anions – solid lines, cations – dotted lines.

and injecting anions first and applying the high voltage for 55 s before the cations are injected, the cations can be "fitted" to migrate between the formate and acetate peak. Apparently there would be numerous other possibilities on how to combine the detection cell position and the injection delays, however the two above mentioned cases, were optimized with respect to the minimum analysis time. The two possible separations with timely displaced injections are shown in Fig. 3. The inlays in Fig. 3 also show simulated

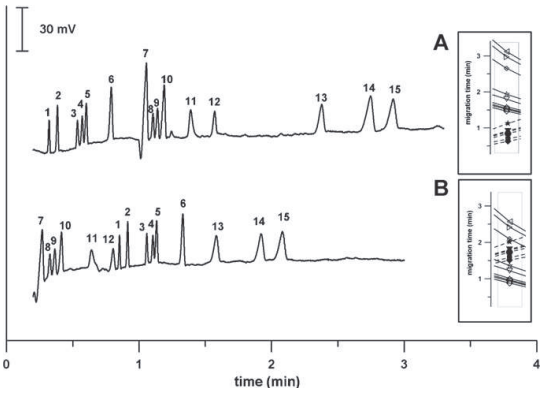

Fig. 3. Simultaneous separation of anions and cations using different injection sequences. (A) Timely displaced injections of cations (20 s, HD injection from 10 cm), HV application 25 s, followed by anions (20 s, HD injection from 10 cm). (B) Timely displaced injections of anions (20 s, HD injection from 10 cm), HV application 55 s, followed by cations (20 s, HD injection from 10 cm). Inlays: example of simulated migration times vs. detector position (15 cm from anodic side). (A) Delayed injection of anions by 25 s and (B) delayed injection of cations by 55 s. CE conditions: −18 kV, contactless conductivity detection. Peaks: (1) NH_4^+, (2) K^+, (3) Ca^{2+}, (4) Na^+, (5) Mg^{2+}, (6) Li^+, (7) Cl^-, (8) NO_2^-, (9) NO_3^-, (10) SO_4^{2-}, (11) SCN^-, (12) formate, (13) acetate, (14) lactate, and (15) phosphate.

cut-outs of the graph from Fig. 2 in which the injection of anions was delayed by 25 s or the injection of cations was delayed by 55 s. Note that for the delayed injection of anions, a precise timing of the second injection is very important as the cations need to be "fitted" tightly between the formate and acetate peaks (peaks no. 12 and 13 in Fig. 3). Therefore, even when the total analysis time is about 0.7 min longer, the delayed injection of anions by 25 s (Fig. 3A) was used in all experiments.

3.2. Method validation and analytical parameters

The developed CE method for simultaneous determination of anions and cations was validated using a set of standard solutions prepared in DI water (a matrix that is very similar to the EBC matrix). Table 1 lists the most important figures, such as repeatability of peak areas ($n = 7$), linearity and LODs. The calibration curves were constructed by using lithium formate as an internal standard (IS). 50 μM lithium formate was added to the cationic and anionic standard solution and the ratio of peak areas of each analyte to peak area of IS was plotted against the analyte concentration. The same amount of IS was also added to the EBC samples for qualitative and quantitative analysis. The linearity was measured in the range of the concentrations of ions found in most of the EBC samples. The LODs were between 0.34 and 1.25 μM for anions and 0.33–0.75 μM for cations, which is adequate for the determination of concentration levels of most ions typically found in EBC.

The optimized and validated CE method was then used for detailed screening of EBC ionic content of 15 healthy volunteers, non-smokers, age between 22 and 75 years, both males and females. In total 5 studies have been performed from the tested individuals resulting in 75 samples the content of which was analyzed. No special care was taken to monitor the eating habits, daytime of the sampling, ingestion of food or drink before the sampling, as the goal of this initial screening was to asses the possible concentration ranges of all analyzed compounds found in EBC. Principal component analysis was used to identify any possible cluster formations and trends within the analyzed sample population. Preliminary investigation did not reveal any correlation between the found ionic concentrations and any parameters that were initially thought to have some importance (such as age, gender, and physical fitness), so the results are not shown here.

Table 2 summarizes the average concentrations and the concentration ranges of all anions and cations found during this study. Note that nitrite, nitrate, sulphate and magnesium were only detected at very low concentration levels. On contrary, high concentration of ammonium was detected in most samples, much of which represents mouth contamination [52–54]. For instance, NH_4^+ was not detectable in EBC collected from tracheostomies in three subjects with obstructive sleep apnea [52]. Other ions were found in moderate concentrations. It is worthwhile noting that no thiocyanate was observed in any of the analyzed samples. Thiocyanate is present in high concentrations (1–2 mM) in saliva. The fact that no detectable concentrations of thiocyanate were found in EBC samples makes this anion suitable as a possible salivary contamination indicator. As salivary concentrations of SCN^- are typically elevated in smokers [55,56], additional EBC samples from two heavy smokers were also analyzed with no SCN^- anion detected in any of these samples. To test the measurable level of salivary contamination, one sample was intentionally contaminated with 1% saliva by spiking 100 μL of the EBC sample with 1 μL of saliva collected from the same individual. Clearly distinguished peak of SCN^- could be observed in the contaminated sample. SCN^- anion may thus serve as a simple indicator of whether the salivary contamination should be suspected. Contrary to a commonly adopted procedure, in which salivary α-amylase assay kits are used to check for salivary

Table 1
Analytical parameters of the developed CE–C4D method for simultaneous determination of inorganic anions and cations in EBC, $n = 7$.

Ion	RSD (%) P.A.	Calibration range (μM)	r^2	LOD (μM)
NH_4^+	3.6	1–1000	0.9995	0.54
K^+	6.2	1–250	0.9975	0.33
Na^+	3.5	1–250	0.9936	0.55
Ca^{2+}	5.4	1–250	0.9992	0.75
Mg^{2+}	1.9	1–250	0.9974	0.47
Cl^-	8.7	1–250	0.9979	0.34
NO_2^-	4.0	1–25	0.9958	0.63
NO_3^-	5.5	1–25	0.9951	0.56
SO_4^{2-}	3.5	1–25	0.9966	0.38
SCN^-	5.3	1–250	0.9958	1.25
Acetate	3.0	1–250	0.9977	1.25
Lactate	4.6	1–250	0.9971	1.07
Phosphate	4.0	1–250	0.9980	1.15

P.A. – peak area.

contamination [40,42], by using our developed method, no additional measurement is required.

3.3. Analysis of EBC using DOEI CE with C4D

The initial study, in which we have analyzed 75 samples of healthy, non-smoker, population for the ionic content of EBC (Table 2) may provide a starting base for an investigation of using EBC-CE–C4D analysis as a possible screening method to recognize and monitor respiratory tract inflammation or other (chronic) respiratory disease. It has been shown in several studies that increased levels of nitrogen-reactive species can be found in EBC of persons with serious lung condition. For instance, elevated levels of nitrite were found in individuals with severe asthma [40,57,58], cystic fibrosis [59], and in acute lung injury [60]. The developed CE–C4D method could evolve into an alternative point of care analytical method and device, giving an extended information through simultaneous determination of anionic and cationic EBC content. A detailed study performed in a hospital facility including a statistically significant group of patients with diagnosed respiratory tract disease and the same large control group would be required to validate the usefulness our developed method.

In here, we present some first promising results. Fig. 4, trace A, shows an example of a simultaneous separation of anions and cations in EBC sample from a healthy volunteer with no record of any respiratory disease. Consequently, the levels of all ions fall within the ranges measured in the screening study. The trace B shows an electropherogram of the same person during an acute infection of upper respiratory tract with serious cough, elevated temperature and other symptoms of common cold. The traces A and B look very similar, except that significantly elevated level of nitrite can be found in trace B. This is also shown in the insert of figure, with zoomed portion of the electropherogram, showing the separation of chloride, nitrite, sulphate and formate (IS). The concentration of nitrite in the trace B has been determined using spiking with IS and the concentration was 7.3 ± 0.5 μM, which is significantly higher than the level of nitrite before the acute respiratory tract inflammation, where nitrite was not detected and is about twice as high as the average nitrite concentration determined during the initial screening study.

The trace C in the same figure, shows an electropherogram of a person with a diagnosed mild form of chronic obstructive pulmonary disease (COPD). Again, significantly elevated levels of nitrite were observed (9.6 ± 0.8 μM). Additionally,

Table 2
Average concentrations and concentration range of anions and cations found in EBC samples ($n = 75$).

Ion	Average concentration (μM)	Concentration range (μM)[a]
NH_4^+	303.5 ± 221.7	0–1000
K^+	38.3 ± 34.2	1–150
Na^+	76.1 ± 72.0	9–200
Ca^{2+}	120.9 ± 87.3	20–300
Mg^{2+}	4.2 ± 2.6	0–10
Cl^-	72.1 ± 60.6	0–200
NO_2^-	3.7 ± 2.6	0–10
NO_3^-	3.2 ± 2.2	0–10
SO_4^{2-}	5.0 ± 2.5	0–10
Acetate	55.9 ± 47.3	5–200
Lactate	55.1 ± 47.1	5–250
Phosphate	34.8 ± 37.0	1–150

[a] Concentration range found in the analyzed samples in this study ($n = 75$).

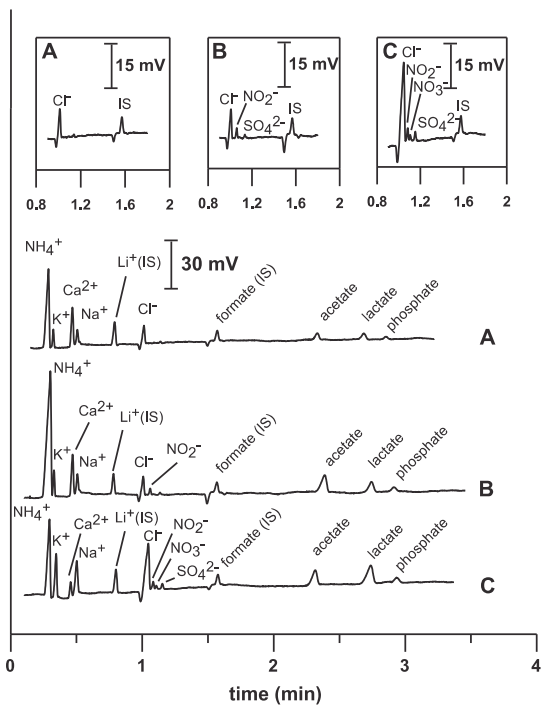

Fig. 4. Analysis of EBC. Electropherogram of simultaneous determination of anions and cations in: (A) a healthy male, 38 years old, (B) the same person as in (A) suffering from acute cold and serious cough, (C) female, 67 years old, diagnosed with mild form of COPD. CE conditions are the same as in Fig. 3A. The concentration of internal standard was 50 μM.

elevated chloride, nitrate, sulphate, lactate and potassium were observed. The determined concentrations of these anions were: Cl^- (212.9 ± 4.6 μM), NO_3^- (4.5 ± 0.3 μM), SO_4^{2-} (3.1 ± 0.3 μM), lactate (308.7 ± 27.2 μM), K^+ (52.3 ± 2.4 μM). The concentration of chloride and lactate were significantly higher than the average level determined in the initial screening study (72.1 ± 60.6 and 55.1 ± 47.1 μM, respectively) and even falls outside the "normal" concentration range (0–200 and 5–250 μM, respectively). Note also that the ratio of calcium/sodium in the trace C ((P.A.[Na^+]/P.A.[Ca^{2+}]) = 2.3) is significantly different from the traces A ((P.A.[Na^+]/P.A.[Ca^{2+}]) =0.2) and B ((P.A.[Na^+]/P.A.[Ca^{2+}]) = 0.3). To verify whether these observations are indeed related to COPD and the acute inflammation would however require a much more through and detailed study which is currently under way.

3.4. Determination of lactate in EBC

The developed CE method allows a sensitive detection of lactate among other anions and cations in EBC. Typical average lactate concentration in resting condition found in our screening study was around 55 μM. Lactate concentration is often taken as an indicator of physical fitness but can also be found at elevated levels in patients with cardio-respiratory or metabolic diseases [61]. Indeed a relatively high level of lactate was found in the person with COPD (see Fig. 4 trace C). In athlete training the levels of arterial lactate increase significantly during an exhausting exercise. Monitoring of lactate during and after such exercise can be used to determine the maximum load and recovery period and find effective ways to decrease the resting period. Lactate sampling is typically done by invasive ear-lobe or fingertip blood sampling and subsequent analysis [62,63], thus EBC sampling provides a suitable substitute non-invasive method. There is evidence that increased levels of lactate in blood will induce increase in lactate in the airway lining fluid that can be measured in EBC. In a study by Marek et al. [63] lactate in EBC has been determined after maximal exercise using an enzymatic conversion of lactate into H_2O_2 and amperometric measurement of the released H_2O_2. About 4 times higher levels of lactate in EBC after the exercise were found compared to the resting condition. Corresponding increase of lactate concentrations in arterial blood was about 10-fold. Recently Pormsila et al. [22] have determined lactate in blood plasma samples during workout on an exercise bicycle using CE–C4D method. The levels of lactate in blood plasma increased up to 7-fold after the exercise.

In our experiments the EBC levels of lactate of three volunteers were measured prior and immediately after an exhaustive, 15 min cycling exercise using an exercise bicycle. After the end of the exercise, the volunteer was allowed to rest for 2 min. The EBC was then collected at 2 min intervals, i.e. at 2, 4, 6, 8 and 10 min, followed by 5 min sampling intervals at 15, 20, 25 and 30 min, with two additional samplings at 45 and 60 min after the exercise. The sampling time for each measurement was 2 min and approximately 100–150 μL of the EBC was collected. For quantitation, 50 μM of IS (Li-formate) was added to the samples. Only anionic trace was quantitated in this study. Fig. 5 shows the results of lactate determination. The lactate concentration at 0 min corresponds to the lactate level before the exercise. It can be observed that the concentrations of lactate before the exercise vary from 10 to 40 μM and peak between 8 and 10 min after the end of the exercise, with concentration levels between 90 and 120 μM. Thus, about 3–4 fold increase in lactate concentration in EBC has been observed that is consistent with the measurements of Marek et al. [63].

The advantage of the present sampling and determination method is that samples can be taken non-invasively with very short time intervals (possibly as short as 1 min) and a detailed curve showing rapid increase and decrease of lactate can be measured.

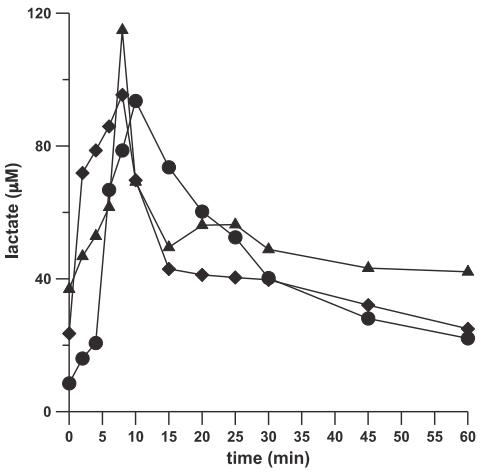

Fig. 5. Concentration profile of lactate in EBC following exhaustive cycling exercise in three volunteers. (♦) Trained female, 22 years old, (●) trained male, 38 years old, and (▲) trained female, 25 years old.

Further, as the CE method can give the results of all anions and cations simultaneously, it could be possible to observe the correlation of the changes of other ions. To give an overview of the changes in ionic content of the EBC, selected electropherograms at times of 0 min (A), 4 min (B), 10 min (C), 25 min (D) and 60 min (E) are shown in Fig. 6. Except a significant change in the concentration of lactate, there are observable changes of other ions in the

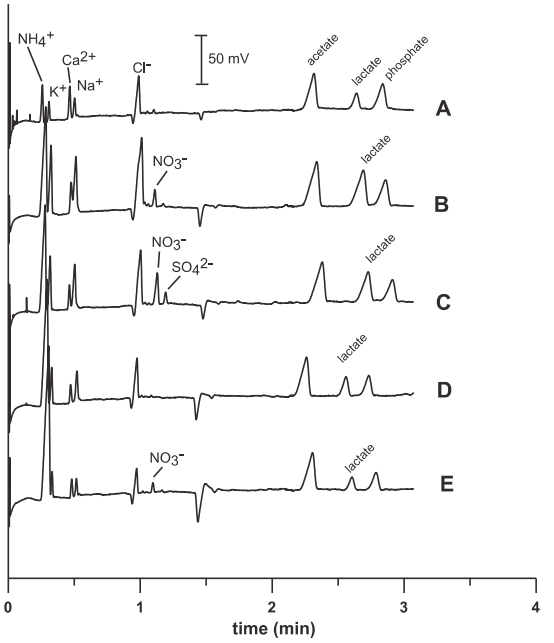

Fig. 6. Electropherogram of lactate screening in EBC before and after exhaustive cycling exercise. (A) Before the exercise, (B) 4 min after exercise, (C) 10 min after exercise, (D) 25 min after exercise, and (E) 60 min after exercise. CE separation conditions the same as in Fig. 3.

electropherograms as well. Some anions and cations followed closely the concentration curve of lactate (potassium, sodium and chloride), while other ions (acetate, phosphate) remain fairly constant. Ammonia increased throughout the measured series of samples, but it is difficult to draw any conclusions about this cation, as its major contribution may come from the mouth contamination (see Section 3.2.). An acidic mouth wash is recommended to remove possible mouth contamination [54], but as the determination of NH_4^+ was not a major focus of this study, this procedure was not adopted during the experiments. A detailed study and quantitative evaluation of concentration changes of analyzed ions after the exercise could be an asset of the currently developed method, as correlations between various ionic constituents could be revealed.

4. Conclusions

In this contribution it has been demonstrated that capillary electrophoresis with C4D using DOEI can be used to rapidly analyze both inorganic cations and inorganic and organic anions present in EBC. The EBC sampling relies on a simple, inexpensive in-house made device that can be constructed in any analytical laboratory. The collection time is significantly reduced; typically 1–2 min is sufficient to collect 100–200 μL of EBC sample for CE analysis. It has been shown that changes of nitrite could be observed during acute inflammation of upper airways and in EBC of a person with diagnosed mild COPD, while the changes of other ions were also observed. Whether this method could possibly be used as a simple, non-invasive, point of care screening tool would however require deeper clinical study. In non-critical applications, for instance in monitoring of lactate in athletes during and after exhaustive exercise, the developed method can be used as a substitute for invasive blood sampling and testing.

Acknowledgements

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PUBLICATION II

Kuban, P., **Kobrin, E-G.**, Kaljurand, M. Potential of exhaled breath condensate analysis in point of care diagnostics. *9-th International Interdisciplinary Meeting on Bioanalysis*, **2012**, 128–133. [2]

P05 POTENTIAL OF EXHALED BREATH CONDENSATE ANALYSIS IN POINT OF CARE DIGNOSTICS

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ABSTRACT

Exhaled breath condensate has been analyzed for its ionic content by capillary electrophoresis with capacitively coupled contactless conductometric detection. Two devices for exhaled breath condensate collection were compared. These include a tube-in-tube cooled sampler and a simple zip-lock bag. The devices allow collection of small volumes (100-200 µL) of exhaled breath condensate in less than 2 min. A method for quick (less than 3 min) simultaneous determination of inorganic cations, inorganic anions and organic anions from the samples using dual-opposite end injection principle with a short fused silica capillary (35 cm, 50 µm i.d.) was optimized with final background electrolyte composition of 20 mM 2-(N-morpholino)ethanesulfonic acid, 20 mM L-histidine, 30 µM cetyltrimethylammonium bromide and 2 mM 18-crown-6 was used. It has been shown that changes of nitrite could be observed in acute inflammation of upper airways and in person with diagnosed mild chronic obstructive pulmonary disease, while the changes of other ions could also be observed.

Keywords: Exhaled breath condensate, capillary electrophoresis, point of care diagnostics.

1 INTRODUCTION

It has been long known that changes of chemical composition in tissues and biological fluids may be indicative of processes occurring in the organism, including an ongoing disease, previous intake of drugs of abuse or signaling other physiological conditions. Analysis of these markers in biological fluids is most commonly performed after so called “invasive” sampling (such as venipuncture during the blood sampling, lumbar puncture to sample cerebrospinal fluid from the spinal column or biopsy for identification and analysis of suspect tissues). On contrary, “non-invasive“ sampling represents an appealing alternative, because it causes minimum stress to the organism and can be easily obtained even far from medical facilities and by non-trained personnel. In particular, non-invasive sampling is attractive in the field of medical prevention, screening, diagnostics and therapy of diseases at an early stage. Unfortunately, in current medical and clinical practice, non-invasive sampling is not very common as there is a lack of approved non-invasive sampling techniques and corresponding analytical methods. This applies for instance for the analysis of exhaled breath condensate (EBC). EBC is the liquid obtained upon cooling and condensation of exhaled air. Exhaled breath is saturated with water vapors that will condensate by breathing through a cooling or freezing system. Although the condensate consists mostly of water vapor, it also contains aerosol particles or respiratory fluid droplets. The aerosolized particles contribute to the non-volatile EBC constituents, such as inorganic ions, small organic molecules and proteins. The analysis of EBC may offer a simple way of monitoring of various diseases related (but not limited) to the function of lung and respiratory tract [1]. EBC sampling consists of simple breathing into a specially designed device: commercially available devices can be used, such as bench top EcoScreen [2] or portable Rtube [3], but home made devices typically perform similarly to the commercial ones. One disadvantage of home made devices is that they are not portable and may require rather bulky accessories. Another significant problem with conventional EBC sampling is the required sample volume which is typically

several milliliters with sampling times between 10 and 60 minutes. The large sample volume requirement depends mainly on the subsequent analytical method used. In this contribution we show that CE with contactless conductivity detection (C4D) can be used for analysis of ionic content of EBC that is collected with very simple collection devices. We employ an optimized separation system with dual-opposite end injection and C4D detection for analysis of anions and cations in EBC simultaneously in less than 3 min. Practical example of EBC analysis by CE-C4D include screening of inorganic nitrogen reactive species during acute and chronic airway inflammation. The promising results obtained underscore the diagnostic potential of CE-C4D combined with non-invasive EBC sampling.

2 RESULTS AND DISCUSSION

2.1 Samplers for EBC collection, comparison of tube-in-tube and bag-type of samplers

Two types of EBC collection devices were used and tested. They are depicted in Figure 1. The EBC collection device is depicted in Figure 1A. It was constructed with maximum simplicity and minimal cost using commonly available laboratory consumables from a 50 mL polypropylene (PP) tube with skirted base and a and a 5 mL PP pipette tip. The PP tube was filled with DI water to provide the cooling of the pipette tip walls upon freezing. The simplest possible devices consisted of a LD-PE bag purchased in local store (Fig 1B). The bags were rinsed with DI water and dried prior use.

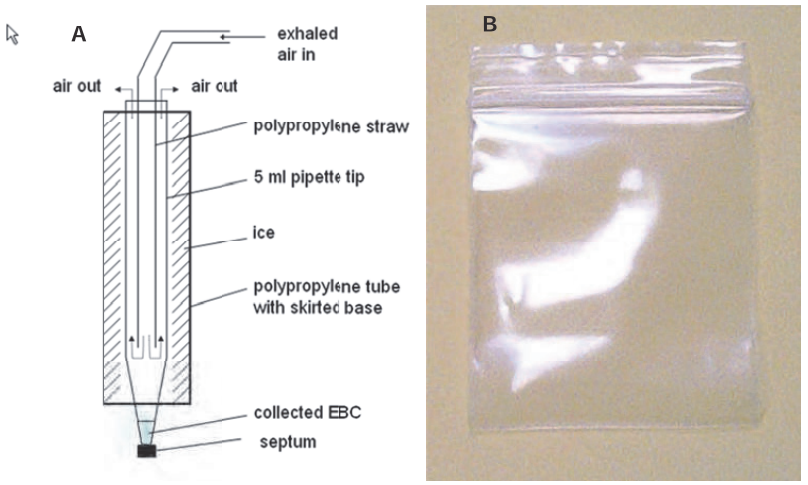

Fig. 1. Schematic of an in-house constructed tube-in-tube EBC collection device (A) and LD-PE zip-lock bag (B) used in the studies.

2.2 Electrophoretic system

A purpose-built CE instrument with C4D detector [4] was employed for all electrophoretic separations. The separation voltage of - 18 kV was provided by a high voltage power supply unit (Spellman CZE2000R Start Spellman, Pulborough, UK). The separation capillaries used were fused-silica capillaries (50 µm I.D., 375 µm O.D., 35 cm total length, Polymicro Technologies, Phoenix, AZ, USA). All CE experiments were performed at ambient temperature. Injection of standard solutions and EBC samples was carried out hydrodynamically by immersing the injection capillary end into a sample vial and elevating it

to a height of 10 cm for a specified time interval. Dual opposite end injection (DOEI) was accomplished by injecting the sample from both capillary ends.

2.3 Comparison of tube-in-tube and bag sampler for anion screening

The samplers were used in an initial suitability study to compare their performance. The test consisted of 2 minute breathing through the straw into the sampling device (A) or directly into the LD-PE bag (B). The collected liquid was subsequently analyzed by CE for anionic content. The results revealed that the data traces are very similar. Although the tube-in-tube sampling device is preferred because the collection efficiency was about 4-fold more efficient, the LD-PE bag can be used in remote or as an emergency sampler. It can eventually be tight sealed and send to the laboratory for analysis by regular mail.

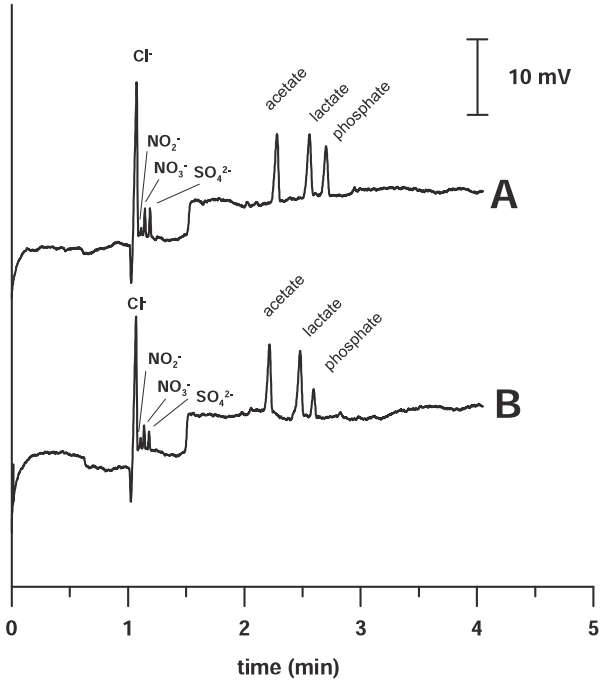

Fig. 2. Comparison of sampling devices: (A) tube-in-tube sampler, (B) zip-lock bag. CE conditions: -18kV, contactless conductivity detection. 20s HD injection from 10 cm.

2.4 Simultaneous separation of ions in EBC

The optimization of the separation conditions for simultaneous separation of anions and cations typically includes individual separations of both groups injected from opposite capillary ends, with the detection cell positioned at different positions along the separation capillary. The separation can be achieved by injecting the sample from both ends with a time delay, as demonstrated by Kubáň and Karlberg [5]. Two injection sequences are possible to achieve full separation of all ions. In the first one, the cations are injected first from cathodic capillary end and high voltage is applied for 25 s, followed by the injection of anions. By delaying the injection of anions by 25 s, the cations are separated first, followed by the anions

without any overlap. Conversely, by reversing the injection order and injecting anions first and applying the high voltage for 55 s before the cations are injected, the cations can be “fitted” to migrate between the formate and acetate peak. The possible separation scenarios are depicted in Figure 3.

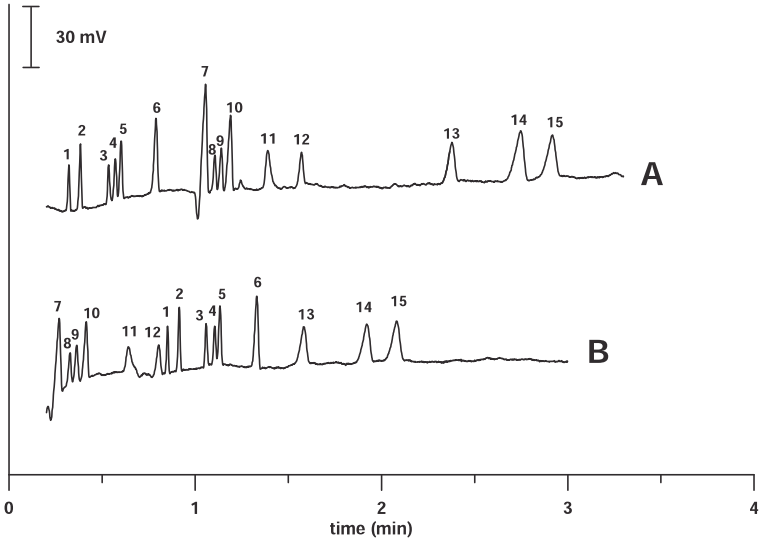

Fig. 3. Simultaneous separation of anions and cations using different injection sequences. (A)-timely displaced injections of cations (20s, HD injection from 10 cm), HV application 25s, followed by anions (20s, HD injection from 10 cm). (B)-timely displaced injections of anions (20s, HD injection from 10 cm), HV application 55s, followed by cations (20s, HD injection from 10 cm). Inlays: Example of simulated migration times vs. detector position (15 cm from anodic side). (A)-delayed injection of anions by 25 s, (B)-delayed injection of cations by 55s. CE conditions: -18kV, contactless conductivity detection. Peaks: (1)-NH_4^+, (2)-K^+, (3)-Ca^{2+}, (4)-$Na^{+,}$ (5)-Mg^{2+}, (6)-Li^+, (7)-Cl^-, (8)-NO_2^-, (9)-NO_3^-, (10)-SO_4^{2-}, (11)-SCN^-, (12)-formate, (13)-acetate, (14)-lactate, (15)-phosphate.

2.5 Analysis of real samples

In an initial study (not shown here) we have analyzed 75 samples of healthy, non-smoker, population for the ionic content of EBC as a possible screening method to recognize and monitor respiratory tract inflammation or other (chronic) respiratory disease. It has been shown in several studies that increased levels of nitrogen-reactive species can be found in EBC of persons with serious lung condition. In here, we present some first promising results. Figure 4, trace A, shows an example of a simultaneous separation of anions and cations in EBC sample from a healthy volunteer with no record of any respiratory disease. The trace B shows an electropherogram of the same person during an acute infection of upper respiratory tract with serious cough, elevated temperature and other symptoms of common cold. The trace C in the same Figure, shows an electropherogram of a person with a diagnosed mild form of chronic obstructive pulmonary disease (COPD). The traces A, B and C look very similar, except that significantly elevated level of nitrite can be found in both traces B (nitrite: 7.3±0.5 μM) and C (nitrite: 9.6±0.8 μM). When comparing the traces A and B, the nitrite concentration is significantly higher in B than the level of nitrite before the acute respiratory tract inflammation and is about twice as high as the average nitrite concentration determined during the initial screening study of healthy volunteers. In trace C, except nitrite, elevated nitrate, sulphate and lactate were also observed. Whether these observations are related to

COPD and the acute inflammation would require a much more through and detailed study which is currently under way.

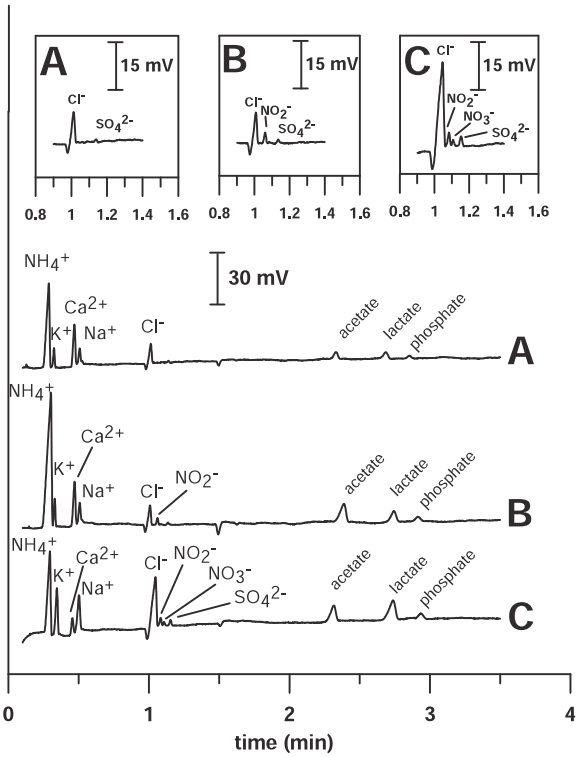

Fig. 4. Analysis of EBC. Electropherogram of simultaneous determination of anions and cations in: (A) - a healthy male, 38 years old, (B)- the same person as in (A) suffering from acute cold and serious cough, (C) – female, 67 years old, diagnosed with mild form of COPD. CE conditions are the same as in Figure 3A, except injection times: cations: 10s, anions 20s.

3 CONCLUSIONS

It has been demonstrated that capillary electrophoresis with C4D using DOEI can be used to rapidly analyze both inorganic cations and inorganic and organic anions present in EBC. The EBC sampling relies on a simple, inexpensive in-house made device that can be constructed in any analytical laboratory. The collection time is significantly reduced; typically 1-2 min is sufficient to collect 100-200 µL of EBC sample for CE analysis. It has been shown that changes of nitrite could be observed during acute inflammation of upper airways and in EBC of a person with diagnosed mild COPD, while the changes of other ions were also observed. Whether this method could possibly be used as a simple, non-invasive, point of care screening tool would however require deeper clinical study.

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P06 SEASONAL VARIATIONS OF METALS AND IONS IN PM1 AEROSOL IN BRNO AND ŠLAPANICE

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ABSTRACT

Submicron aerosol particles in the size fraction PM1 were collected in Brno and Šlapanice in winter and summer of 2009 and 2010. The aerosols were analysed for content of selected metals and ions.

Local traffic in summer and coal and wood combustion during household heating in winter were identified as the main emission sources of aerosols in both towns. Secondary aerosol components formed a significant part of aerosol during the whole year.

Keywords: PM1 aerosols, metals, ions

1 INTRODUCTION

Epidemiological studies [1-3] have suggested a statistical association between health effects and ambient fine particle concentrations, especially the submicron fraction (PM1) that can penetrate deep into the alveolar region of the lungs. Chemical composition of PM2.5 and PM10 is subject of many studies, however, relatively little attention has so far been paid to PM1 aerosols. Determination of their composition is essential to understand their properties and reactivity and hence their environmental and health effects [4].

2 EXPERIMENTS

Aerosol particles in the size fraction PM1 were collected in Brno at balcony on the first floor of the Institute of Analytical Chemistry faced to Veveř Street while the collection of aerosols in Šlapanice was performed in the garden of a family house (Fig. 1). Aerosols in the size fraction PM1 were sampled every day for 24-hours over one week in winter and summer of 2009 and 2010. A total number of 56 filters (28 samples from Brno and 28 samples from Šlapanice) were collected.

The aerosols were collected using a high-volume sampler (DHA-80, Digitel, 30 m^3 h^{-1}) on cellulose-nitrate filters (150 mm diameter, 3 μm, Sartorius). In parallel, the aerosols were sampled on 47 mm Teflon filters (Zefluor, 1 μm, PALL Corporation) using a low-volume sampler (1 m^3 h^{-1}), consisting of a NILU filter unit (type 9633) and a Teflon coated aluminium cyclone inlet (URG-2000-30EH). To avoid interferences of gaseous pollutants (NH_3, NO_2, HNO_3, HONO, SO_2, O_3, HCl, VOCs, ...), an annular diffusion denuder [5] was placed between

PUBLICATION III

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Research Article

Fingerprinting postblast explosive residues by portable capillary electrophoresis with contactless conductivity detection

A portable capillary electrophoretic system with contactless conductivity detection was used for fingerprint analysis of postblast explosive residues from commercial organic and improvised inorganic explosives on various surfaces (sand, concrete, metal witness plates). Simple extraction methods were developed for each of the surfaces for subsequent simultaneous capillary electrophoretic analysis of anions and cations. Dual-opposite end injection principle was used for fast (<4 min) separation of 10 common anions and cations from postblast residues using an optimized separation electrolyte composed of 20 mM MES, 20 mM L-histidine, 30 μM CTAB and 2 mM 18-*crown*-6. The concentrations of all ions obtained from the electropherograms were subjected to principal component analysis to classify the tested explosives on all tested surfaces, resulting in distinct cluster formations that could be used to verify (each) type of the explosive.

Keywords:
Capillary electrophoresis / Contactless conductivity detection / Explosives / Simultaneous separation of anions and cations / Principal component analysis
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Additional supporting information may be found in the online version of this article at the publisher's web-site

1 Introduction

The analysis of postblast explosive residues is important to trace the origin and source of the chemical compounds in explosive devices that are used in the acts of terrorism. Especially in the latest years, the incidence of terrorist attacks using such devices has increased and targeted major cities, such as Madrid, Spain (2004). London, England (2005), Prune, India (2010), Oslo, Norway (2011), and most recently Boston, USA (2013). Although mainly improvised inorganic explosives based on easily accessible chemicals, such as ammonium nitrate/fuel oil (ANFO), black powder, or sodium chlorate-type devices were employed, the use of other types of explosives (i.e. organic high explosives) cannot be completely ruled out. The chemical trace that each explosive device leaves after detonation can lead to quicker identification of possible suspects and prevent additional attacks. The obtained data may function not only as a "fingerprint" of the explosive itself but also of a particular constructor. It has been shown that most inorganic explosives produce an ionic postblast residue that is composed of a few distinct inorganic anions and cations. On the contrary, however, very little knowledge is available on the chemical traces that organic high explosives leave.

Currently used techniques for the analysis of postblast explosive residues include a wide range of spectroscopic techniques, such as atomic absorption spectroscopy [1], SEM, energy dispersive X-ray detection [2, 3], X-Ray diffraction [4], MS [5], ion mobility spectrometry [6], as well as chromatographic techniques, such as HPLC [7] and ion chromatography [8, 9]. Many of the above mentioned techniques are not easily portable and the sample must be taken to the laboratory for analysis, resulting in increased risk of contamination and significant delays in the analysis and data processing.

CE, on the contrary is easily amenable for field analyses. The analysis of postblast explosive residues has been carried out by conventional CE with indirect absorbance [10], direct [11] and indirect laser induced fluorescence [12]. Electrochemical detection [13], and particularly capacitively coupled contactless conductivity detection (C^4D), [14, 15] have been recently used [16]. For the detection of small inorganic ions, C^4D is more sensitive than indirect UV detection, has

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Abbreviations: ANFO, ammonium nitrate/fuel oil; **DOEI**, dual-opposite end injection; **IS**, internal standard; **PCA**, principal component analysis

simple electronic circuitry, relatively low cost, and low power consumption. It is thus ideally suited for implementation in portable CE instruments. Moreover, CE is even applicable for postblast explosive analysis on a chip [17]. The edge that CE can offer in terms of portability for postblast explosive residue analysis has been first realized by Hutchinson et al. in 2008 [18]. They used a commercially available portable CE instrument (http://www.ce-resources.com) for sensitive determination of inorganic anions and cations in residues from inorganic homemade explosives. Two separate methods for analysis of anions and cations were developed employing two different separation electrolytes at different pH. Hopper and co-workers [19] have proceeded one step further toward a comprehensive ionic analysis and optimized a CE method with indirect UV detection for simultaneous separation of anions and cations in low explosive residues employing the dual-opposite end injection (DOEI) approach [20, 21]. In this mode, the sample is injected sequentially into both ends of the separation capillary and during the electrophoretic run, cations and anions migrate toward the detector placed in an optimized position (typically in the centre of the separation capillary). With indirect UV detection, as used by Hopper et al. [19], the choice of separation electrolyte for DOEI is rather difficult, requiring complex BGE containing anionic and cationic indirect detection probes, cation complexation agents, organic modifier, and an EOF modifier. Despite the BGE complexity, six cations and eight anions could be separated in less than 6 min.

In this work, we show simultaneous separation of postblast explosive residues using portable CE with C^4D. The choice of electrolyte for simultaneous CE separation with C^4D detection is much easier than with indirect UV detection and a portable CE system recently developed in our laboratory [22–24] was shown to be able to differentiate between postblast residues resulting from various commonly encountered explosives on various matrices. While postblast residue analysis by CE has been reported several times there is virtually no systematic study on the effect of background ions from the matrix where the explosion took place. This is an important problem to address since many analytes that can be used in identification of a particular explosive are frequently found also in the matrix itself. More importantly, this study deals not only with inorganic improvised devices, but also with organic high explosives. By applying simple chemometrics (principal component analysis (PCA)) to the obtained data, explosives can be, at least partly, classified regardless of the matrix complexity.

2 Materials and methods

2.1 Electrophoretic system

A purpose-built portable CE instrument was fitted into a watertight, crush-proof, dust-proof case made of durable plastic (Peli 1200 Case©, Peli Products, Barcelona, Spain). The instrument was equipped with an HV safety interlock and included a negative high-voltage power supply (EMCO, Sutter Creek, CA, USA) capable of delivering voltages up to −25 kV, an in-house built C^4D detector operating at 200 kHz and a voltage of 60 Vp-p, and a data acquisition system. The instrument was controlled by an in-house written software and the signal was obtained through a USB connection of a notebook computer.

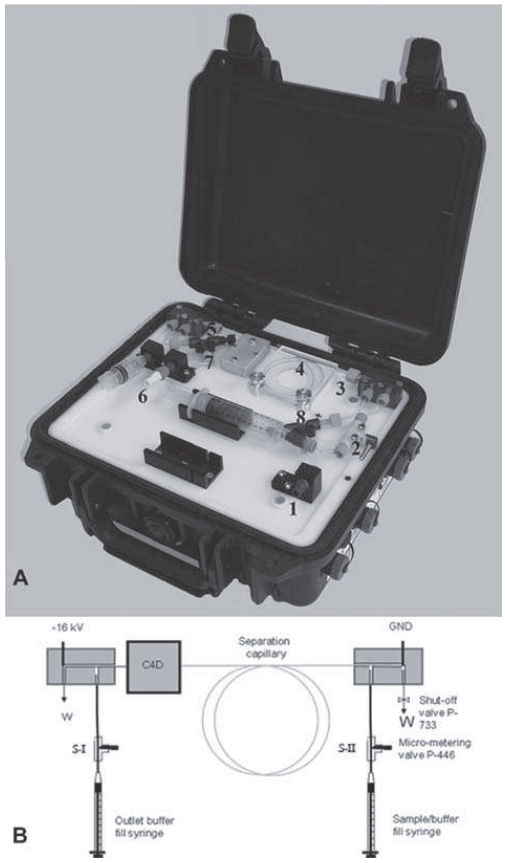

Figure 1. (A) A photo of the portable instrument (1) syringe socket, (2) shut off valve, (3) inlet end of the capillary, (4) the separation capillary, (5) outlet end of the capillary, (6) syringe socket, (7, 8) metering valve. (B) A schematic of the separation compartment of the portable instrument ("W"—output to waste, GND—grounding, S-I and S-II—interfaces, micro flow-metering valves (P-446)).

The schematic of the experimental set up and a photograph of the instrument is shown in Fig. 1. The sample injection units placed at both capillary ends include each a splitter interface machined to 35×15×15 mm from a block of polyimide. Each splitter interface has a 2 cm long

horizontal flow-through channel of 1 mm id. to which two vertical channels of the same diameter are connected. A separation capillary is inserted from the side of the interface with its tip positioned exactly at the cross-section of the vertical and horizontal channel. A grounding and a high voltage electrode (made of Pt wire) are inserted into the second vertical channel of the respective interface. Both the capillary and the Pt electrodes are secured with 1/16″ flangeless fittings (Upchurch Scientific, Oak Harbor, WA, USA). Two in-line micro flow-metering valves (P-446, Upchurch Scientific) are used to precisely regulate the flow rates with manual syringe injection [25].

2.2 Dual opposite end injection

During DOEI process, a 500 μL volume of sample is injected manually into the first splitter interface (S-I) using a 1 mL disposable plastic syringe (Omnifix 100 Duo, Braun, Melsungen, Germany) followed by an injection of 1500 μL of the BGE solution. Then another sample aliquot of 500 μL is injected into the second splitter interface (S-II), followed by an injection of 500 μL of the BGE solution. This sequence allows (i) simultaneous injection of sample into both capillary end and (ii) removal of any remaining sample from the splitter interfaces before the separation takes place.

2.3 Separation capillary conditioning

Fused-silica capillaries (50 μm id, 375 μm od, 50 cm total length, Polymicro Technologies, AZ, USA) were used. Capillary rinsing was performed by manually applying the pressure to the syringe with appropriate solution inserted in the splitter interface S-II with the shut-off valve (P-733, Upchurch Scientific) closed. Prior to the first use, the separation capillaries were preconditioned with 0.1 M NaOH for 30 min, DI water for 10 min, and BGE solution for 10 min. Before each analysis sequence, the capillaries were manually washed with approximately 150 column volumes of DI water and 150 column volumes of the BGE. Between two successive injections, the capillary was flushed with 100 column volumes of the BGE solution (1 min). At the end of each day, the capillaries were washed with at least 150 column volumes of DI water and kept in DI water overnight.

2.4 Chemicals

Stock solutions of standards, 100 mM for each inorganic ion, were prepared from reagent grade chemicals (Sigma-Aldrich, Steinheim, Germany) by dissolving them in DI water (MilliQ Water System, Millipore, Molsheim, France). Lithium formate (Sigma-Aldrich, 98% purity), 5 mM stock solution, was used as internal standard to spike the sample and standard solutions. BGE for CE measurements was prepared daily by diluting 100 mM stock solutions of MES (Sigma-Aldrich), 100 mM L-histidine (HIS, Sigma-Aldrich) and 18-*crown*-6 (Sigma-Aldrich) to the required concentration. CTAB (Sigma–Aldrich) was prepared as 10 mM stock solution in 5% acetonitrile and was added to the BGE. The optimized BGE composition used in this work was 20 mM MES, 20 mM HIS, 30 μM CTAB, and 2 mM 18-*crown*-6 at pH 6.

2.5 Explosives

All used explosives with their approximate chemical composition are listed in Table 1 in the Supporting information. They were commercial products, regulated by national authorities and were kindly provided by Forcit OY, (Hanko, Finland). Electrically initiated detonators (No. 8 Al, Sellier & Bellot JSC, Czech Republic) were used to trigger the explosive devices.

2.6 Field analysis

The explosions were carried out at the test site of Forcit OY company, Hanko, Finland. Typically 50 to 100 g of the selected explosive was placed on the investigated matrix and the explosion was initiated with an electrically connected detonator. The detonators contained lead azide (45 mg) and lead styphnate (105 mg) as primary explosive and small amounts of PETN (0.72 g) and TNT (0.08 g) as secondary explosive. In a detonator the primary explosive is the more sensitive part which is initiated electrically and gives enough energy to ignite the secondary explosive. The secondary explosive is then enough to ignite the charge that is handled.

2.7 Sampling and sample preparation

The explosions were performed on three different surfaces—sand, metal plate, and concrete that represent the three common matrices similar to those that can be encountered in a real terrorist attack. The matrices were selected as they are readily available and were used to test the applicability of the proposed approach to identify the explosive type. However, the matrices used herein should not be considered universal and a more detailed study addressing the effect of other possible matrices will be required. Here, we provide just the initial results and a proof of concept. Thus a fresh sand bead, a concrete plate (25 × 25 cm, 5 cm thick) or a metal witness plate (10 × 10 cm, 8 mm steel) was used for each explosive. Each explosion was done in triplicate. Only one blank sample from each matrix was taken, while two parallel samples were taken after explosion. The total amount of blank samples taken for one explosive and one surface was three and the amount of postblast explosive residue samples was six.

2.7.1 Blank samples

Blank samples were collected from the explosion sites before the detonation of each explosive. For sand matrix, 4 g of the sand directly from the sand bead was transferred into

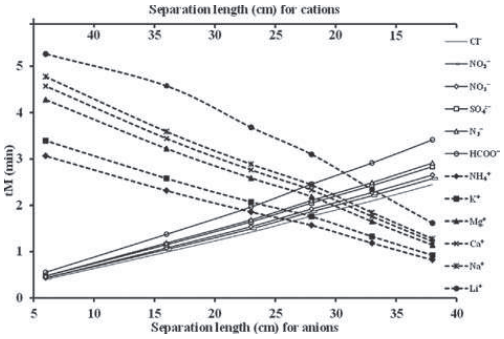

Figure 2. Dependence of migration times of anions and cations on the separation distance from the detector. Total capillary length was 50 cm and the detector was consecutively positioned 6, 16, 23, 28, and 38 cm from one injection end. The separation of anions was carried from the negative end, while the separation of cations was carried from the positive end.

a 50 mL sampling container and 10 mL of DI water was added. For concrete and metal plates, cotton pads (purchased from local pharmacy) were first washed with 30 mL of DI water and dried to remove any background ions. Right before sampling, the pre-processed cotton pads were moisturized by 1 mL DI water and the surface was wiped utilising NIOSH surface wipe sampling technique (http://www.bnl.gov/esh/shsd/sop/pdf/ih_sops/ih75190.pdf). The cotton pad was then transferred into a 50 mL sampling container and 10 mL of DI water was added. All samples were shaken for 1 min and the aqueous extract was filtered through the 0.45 μm filter (Filtropur S (Sarstedt, Germany)) and the filtrate was used for the CE analysis.

2.7.2 Sand sampling after detonation

After each set of explosions, 4 g of postblast sand was collected from the remaining sand and processed in the same way as the blank samples. Typically about one half of the total sample weight was taken from the centre and another half was collected from the areas about 10–20 cm from the centre. When the explosion has left an obvious trace (light ash, dark ash etc.) this type of residue was preferentially collected.

2.7.3 Metal and concrete plate sampling after detonation

Two samples were taken from each metal witness plate or each concrete plate, by wiping one half of the plate surface with one cotton pad and second half with another one. Depending on the explosive strength, the concrete plate either remained whole or was shattered into several pieces. In case of shattering, the pieces were collected together and their surface was wiped. Again, two parallel samples were taken from each concrete block. The cotton pads with collected residues were processed in the same way as blank samples.

3 Results and discussion

3.1 Optimization of the separation electrolyte for DOEI

The optimization of the simultaneous separation of anions and cations consisted of injecting anions and cations separately from opposite capillary ends with a C^4D detector positioned at different positions along the separation capillary [26]. Only those ions that were detected in the samples of explosive residues were separated in the model mixture, thus 12 ions in total, including lithium formate as internal standard were evaluated. Due to the specific construction of the instrument the minimum possible effective length of capillary from anodic and cathodic end is 13 and 6 cm, respectively. The graph in Fig. 2 shows the migration times versus the effective separation capillary length from the respective injection point to the detector. A previously optimized [27] separation electrolyte consisting of 20 mM MES, 20 mM HIS, 30 μM CTAB, and 2 mM 18-crown-6 was used. A suitable position of the detector can be selected from a graph in Fig. 2. The minimum detector distance from the anodic side that allows full separation of selected cations with the shortest analysis time is 14 cm, while for full separation of all selected anions the minimum distance from the cathodic side is around 36 cm. This gives an optimum position of the detector to be 14 cm from the anodic side (right) and 36 cm from the cathodic side (left) and this configuration was used throughout this study. An electropherogram of a standard solution containing all selected ions and two internal standards is shown in Fig. 3A. Note that all ions are fully baseline-separated in less than 4 min.

3.2 Performance data

The developed CE method for simultaneous determination of anions and cations was validated using a set of standard solutions prepared in DI water (a matrix that is very similar to the sample matrix). Table 1 lists the most important figures of merit, such as repeatability of peak areas ($n = 3$), linearity and LODs. The calibration curves were constructed by using lithium formate as an internal standard (IS). Lithium formate was added to the cationic and anionic standard solution to yield final concentration of 50 μM and the ratio of peak areas of each analyte to peak area of IS was plotted against the analyte concentration. The same amount of IS was also added to the postblast explosive residue samples and blank samples for qualitative and quantitative analysis. The linearity was measured in the range of the concentrations of ions found in most of the samples. A variable dilution of the samples (1:2–1:500) was used to fit the measured peak areas within the calibration curve. The LODs were between 12.2 and 35.7 μM

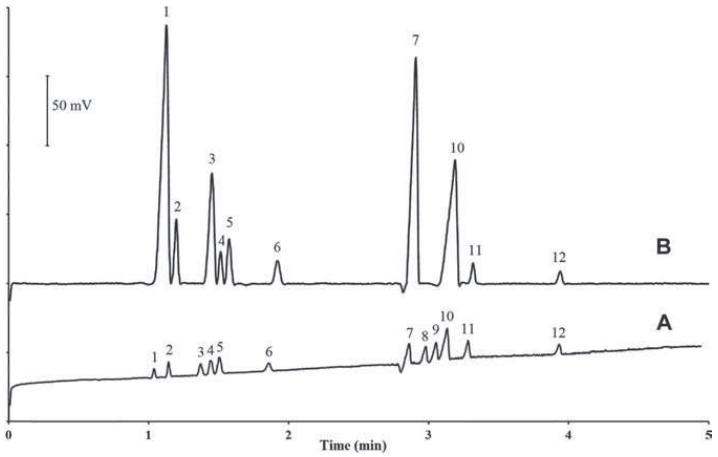

Figure 3. (A) Separation of standard solution with IS using DOE injection. Simultaneous separation of anions and cations. Cations injected first (3 s, hand pressure injection of 500 μL standard solution and 1500 μL BGE), followed by anions (3 s, hand pressure injection of 500 μL standard solution, BGE 500 μL). CE conditions: −16 kV, contactless conductivity detection. Peaks: (1) NH_4^+, (2) K^+, (3) Ca^{2+}, (4) Na^+, (5) Mg^{2+}, (6) Li^+, (7) Cl^-, (8) NO_2^-, (9) NO_3^-, (10) SO_4^{2-}, (11) N_3^-, (12) $HCOO^-$. Detector position: 36 cm from the positive side and 14 cm from the negative side. (B) Simultaneous separation of anions and cations in postblast residue from ANFO explosion. ANFO sand sample diluted 1:2. Simultaneous injection in the following sequence and side: 500 μL sample (positive side)—1500 μL BGE (positive side) 500 μL sample (negative side)—500 μL BGE (negative side). Peaks: (1) NH_4^+, (2) K^+, (3) Ca^{2+}, (4) Na^+, (5) Mg^{2+}, (6) Li^+, (7) Cl^-, (8) NO_3^-, (9) SO_4^{2-}, (10) $HCOO^-$. CE conditions: the same as in 3(A).

for anions and 3.8 and 7.3 μM for cations. The optimized and validated CE method was then used for detailed screening of ionic content of 8 different explosives.

3.3 Postblast explosive analysis

3.3.1 Analysis of blank samples

As most of ions used in this work, are also ubiquitous in nature and on various surfaces it is important to determine the extent of matrix effects and subtract it from the measured data. For this reason blank sample from each matrix was taken and analyzed as described previously. Blank concentration levels of most anions were lower than their LOD, only in concrete blank samples traces of NO_3^- and SO_4^{2-} between 19 and 51 μM were detected. On the other hand all cations (measured in our work) were present in all blank samples and ranged between 6 and 272 μM.

Table 1. Figures of merit of the developed CE-C^4D method for simultaneous determination of inorganic anions and cations in explosives residues, $n = 3$

Ion	RSD (%) P.A.	Calibration range (μM)	r^2	LOD (μM)
NH_4^+	5.5	1–500	0.9960	7.3
K^+	4.4	1–250	0.9969	3.7
Na^+	5.0	1–250	0.9928	7.1
Ca^{2+}	5.7	1–250	0.9982	3.8
Mg^{2+}	3.6	1–250	0.9968	5.8
Cl^-	7.1	1–250	0.8707	35.7
NO_2^-	9.9	1–75	0.9900	11.0
NO_3^-	7.8	1–75	0.9889	15.6
SO_4^{2-}	7.1	1–75	0.9953	12.2
N_3^-	9.1	1–250	0.9900	12.8

P.A., peak area.

3.3.2 Analysis of inorganic and organic explosive postblast residues

Analysis of post blast residues of eight explosives: Dynamite, PETN, TNT, RDX, PENO, ANFO, V40, C4 from three different surfaces (except for V40, which was not tested on concrete due to the safety reasons) showed the majority of inorganic ions described previously in the literature. As an example, simultaneous analysis of postblast residue in an ANFO sample from sand matrix is shown in Fig. 3B.

3.4 Fingerprinting the postblast explosive residues

In a simple case, when improvised inorganic explosive is premixed from raw chemicals, such as perchlorate, chlorate, and nitrate the identification can indeed be based on either anionic or cationic trace. Significant research effort has been devoted to identify these types of explosives. On the contrary, very little knowledge is available on the traces that organic

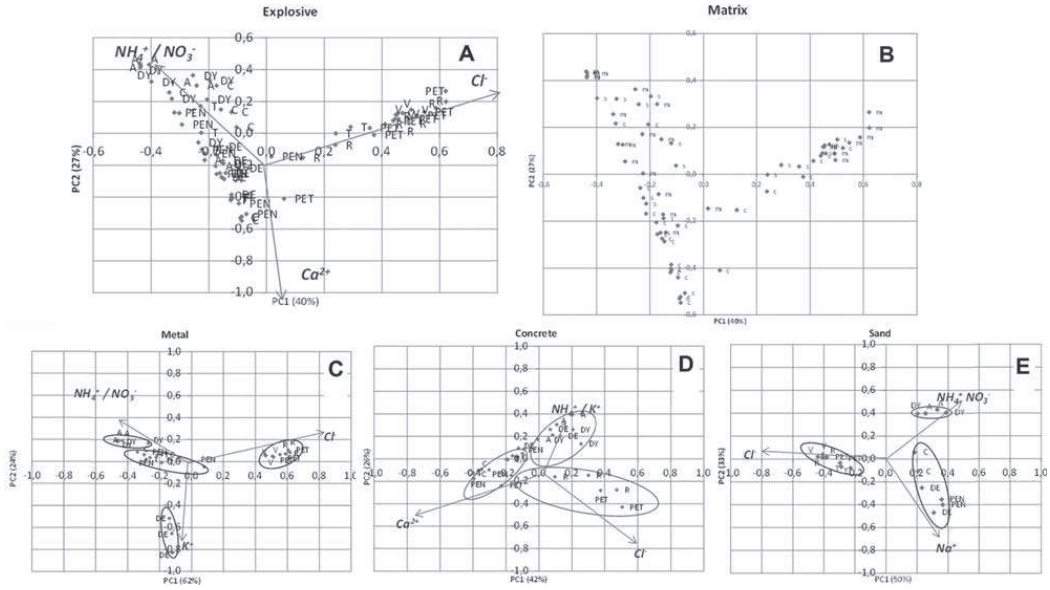

Figure 4. Ion intensity data from all explosives in different matrices analyzed by standard principal component analysis (PCA). (A) Score plots of all samples labeled by matrix type (s, sand; c, concrete; m, metal). (B) Score plots of all samples labeled by explosive: Dynamite (DY); PETN (PET), TNT (T), RDX (R), PENO (PEN), ANFO (A), V40 (V), C4 (C), detonator (DE). (C–E) Distribution of the ions on metal, concrete, and sand, respectively.

high explosives leave. In a paper by Hutchinson et al. [10] two organic devices (a mixture of PETN/RDX and TNT) were tested with no apparent inorganic residues left. This observation is consistent with our initial observations; however, other organic high explosives do leave a significant inorganic ion trace that may help to identify them. Separate analysis of anions or cations may not be sufficient for correct identification of the explosive. The complexity of the ionic traces is demonstrated in Fig. 1 (see the Supporting information) that shows bar graphs of cation and anion concentrations in each of the tested explosives and each matrix. When looking at the anionic graphs it is clearly visible, for instance, that high concentrations of nitrate are present in dynamite and ANFO traces. The same can be seen for ammonia cation. The other ions do not exert such a clear difference, but can be still used to at least partially characterize the postblast residues. This is shown in an example in Fig. 2 (Supporting information) that suggests a flow chart that could be used for explosive identification. The flow chart is however valid only for the selected group of explosives and only applies for metal matrix, while the other two matrices do not provide similar results. It is apparent that the matrix has an influence on the data. Chemometric approaches, such as PCA, are known to eliminate matrix effects, at least to some extent, and eventually may simplify the identification. That is why in the next step, we have applied PCA to the measured data.

3.4.1 PCA analysis and clustering of the explosives

Before applying the PCA procedure the peak areas of all the ions from blank samples were subtracted from the peak areas of the ions in the corresponding postblast explosive residue samples. The obtained peak areas of all ions from all explosives in different matrices were then combined into a matrix of data which was initially analyzed by standard PCA. However, as the ionic concentrations of the postblast explosive residues are very similar and the matrix (concrete, sand, and metal surface) contains frequently the same ions, the identification was difficult. Moreover, the spread of the ion concentrations is extremely heterogeneous, so the samples taken from different locations differ significantly not only in concentrations levels but also in composition. Thus, to establish whether the pattern of ions in an electropherogram is characteristic of the used explosive or not, the peak areas were normalized to the sum of the total peak areas in each electropherogram. The resulting table consisted of 70 rows (samples of different explosives detonated on different matrices and obtained in different locations around the blast) and 10 columns of corresponding ion peak areas. The table was subjected to mean centered PCA procedure. The "leave-one-out" cross-validation procedure was used to determine the number of significant principal components. The "predicted residual error sum of squares" or PRESS was calculated according

to [28] . If the ratio PRESS(n)/PRESS($n-1$) exceeds one, then the use of $n-1$ principal components in the model is recommended. This procedure revealed four significant principal components that accounted for 94% of the total variance. Results of first two—most vivid components—PC1 and PC2 are shown in Fig. 4. PCA is a tool that allows better visualization of data in exploratory analysis. This is demonstrated in Fig. 4, where definite patterns can be identified, which highlight similarities and differences between the electropherograms of different explosive residues. In Fig. 4A and B are score plots of all samples. The figures are essentially the same except the labeling of points for clarity. The figures reveal the distinctive structure of postblast electropherograms. Two big clusters can be recognized which are located almost orthogonally to each other. Loading vectors plot (Fig. 4A) indicates that the separation of the clusters is due to the domination of Cl^- ion peak on the electropherogram. Figure 4B demonstrates that when using normalized data in PCA, the sample matrix has little influence on the clustering; on the contrary, the nature of the explosive (Fig. 4A) correlates loosely with the location of clusters. The structure of the PCA plots suggests that there is an opportunity to use electropherograms of postblast explosive extracts in classification algorithms for further identification of the explosives. Clustering becomes even more distinctive when one looks at the distribution of the ions on different matrices (Fig. 4C–E). Here again the results are represented in the first most significant PC coordinates. In all score plots the explosives can be divided into three distinctive clusters. V40, PETN, RDX form one cluster which is separated from the rest by the dominance of the Cl^- ion (note that on concrete matrix V40 was not determined). Although the dispersion of this cluster is wide on concrete matrix it still can be easily differentiated from the others. The second cluster is formed by the Dynamite and ANFO and it is separated from the rest by the dominance of the NH_4^+ ion (the other loadings depend on the matrix). The last cluster is formed by C4 and PENO, the position of which is however matrix dependent. Detonator and TNT location seems to depend on the matrix.

To summarize, the matrix influence on the postblast sample electropherogram pattern is strong. Concrete is an especially difficult matrix. Reproducibility is low and due to that, the clusters are not well "focused." Ca^{2+} influence on the distribution of clusters seems to contribute much to the location of clusters. On the other hand, however, the matrix can be considered as such in the particular situation. Well defined clusters on the corresponding scores plot mean that the electropherograms are characteristic for the detonated explosive on the given matrix and can be used for their identification.

4 Concluding remarks

Field detection of postblast residues to identify the origin of explosive devices is of uttermost importance with regard to the civil safety and subsequent police investigation. Portable CE with C^4D and DOEI sample introduction presents a suitable, fast, and sensitive method for the analysis of total ionic trace of the postblast explosive residue. A rapid separation of ten most commonly present ions (both cations and anions) was achieved in <4 min and can be used for preliminary fingerprinting of the explosives. Additionally, PCA analysis shows three main matrix-dependent and ion specific clusters formed from different explosives. By combination of the information obtained from the electropherogram and the PCA analysis, the identification of a specific explosive can be made. The identification has been tested on postblast residues of eight selected inorganic and organic high explosives, which suggests that the methodology may in general be applicable for the sensitive, field-based identification of a wide range of other explosive types.

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The authors have declared no conflict of interest.

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PUBLICATION IV

Jõul, P., Lees, H., Vaher, M., **Kobrin, E-G.**, Kaljurand, M., Kuhtinskaja, M. Development of a capillary electrophoresis method with direct UV detection for the analysis of thiodiglycol and its oxidation products. *Electrophoresis* **2015**, 36, 1202–1207.[4]

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Research Paper

Development of a capillary electrophoresis method with direct UV detection for the analysis of thiodiglycol and its oxidation products

A novel method based on CE with precolumn derivatization and direct UV detection for the determination of thiodiglycol (TDG), TDG sulfoxide, and TDG sulfone in water samples was developed. The lack of a UV chromophore of target analytes was overcome by derivatization with phthalic anhydride. The reactant concentrations, as well as the derivatization dependence on heating temperature and time, were carefully investigated. The baseline separation of three derivatives was achieved in less than 8 min by applying a simple BGE composed of a 30 mM borate buffer at pH 8.5. Several parameters affecting the separation efficiency (buffer pH and concentration, capillary temperature, and applied voltage) were evaluated. Calibration curves of all compounds showed good linear correlations ($R^2 > 0.9994$). The LODs of the TDG and its oxidation products were in the range of 98–154 ng/mL. The precision tests resulted in RSDs for migration times and peak areas of less than 1.2 and 3.6%, respectively. The developed method was successfully applied for the analysis of TDG and oxidation products in seawater, utilizing the carbon aerogel-based adsorbents for sample purification and concentration. Additionally, the method has the potential to be transformed into a portable CE format.

Keywords:
Phthalation / Precolumn derivatization / Sulfur mustard degradation products / UV detection
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1 Introduction

At the end of World War II it was necessary to dispose of large quantities of conventional and chemical munitions left over from German and allied stocks. Dumping at sea was considered the most appropriate solution at this time. Thus, around 50 000 tons of chemical munitions were dumped in the Baltic Sea (mostly bombs and shells) [1]. After almost 70 years of such dumping, human health and the entire Baltic marine ecosystem might be at serious risk due to the corrosion of the shells, which has led to a constantly increasing release of highly toxic compounds into the seawater.

A large number of the dumped munitions contain yperite, commonly known as sulfur mustard (HD). The compound itself is a vesicant that causes chemical burns on skin and is an eye and lung irritant [2]. In an aqueous environment, HD rapidly hydrolyses to nontoxic thiodiglycol (TDG) and then slowly oxidizes to TDG sulfoxide (TDGO) and TDG sulfone (TDGOO). In addition, HD hydrolysis leads to the formation of a variety of degradation products, such as cyclic and open chain compounds [3]. It should be noted that potential ecological and health risks are associated primarily with sulfur mustard itself, and the importance of analysis of HD degradation products is that they act as a markers of the HD leakage locations.

Based on the recommended operating procedure for analysis in the verification of chemical disarmament [4], the most frequently used methods for the identification of a sulfur-containing precursor and breakdown products in aqueous samples are based on GC, in combination with MS and/or MS/MS. Due to the low or nonvolatility of the TDG and its oxidation products, derivatization is an essential step in sample preparation prior to GC analysis. Silylation is a widely used derivatization process for GC and, in the case of HD degradation products, *N*,*O*-bis(trimethylsilyl)trifluoroacetamide [5] and *N*-methyl-*N*-(tertbutyldimethylsilyl)trifluoroacetamide [6] are the most common derivatizing reactants. There are some disadvantages in the application of silylation. The most critical point here is that silyl derivatives tend to be highly moisture sensitive, which leads to derivative decomposition and, thus,

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Abbreviations: CWA, chemical warfare agent; **HD**, sulfur mustard; **TDG**, thiodiglycol; **TDGO**, TDG sulfoxide; **TDGOO**, TDG sulfone

Colour Online: See the article online to view Figs. 2 in colour.

requires strict control under derivatization conditions [7]. Another problem is the appearance of a large number of artificial peaks on the total ion chromatogram caused by the indiscriminate property of these derivatization reactants when they react with hydroxyl as well as carboxylic groups. Moreover, the reactant itself is prone to form clusters and contaminate the total ion chromatogram [8]. 1-(trifluoroacetyl)imidazole also demonstrated its high potential for TDG and TDGO derivatization. Trifluoroacetylation is less demanding, but the derivative is still sensitive to water traces and storage time [9].

Besides GC, LC coupled with MS [10], NMR spectrometry [11] or sulfur flame photometric detection [12] are also used for the analysis of water-soluble degradation products. In this context, the application of new analytical techniques, such as CE, could be the next promising step in the field of chemical warfare agents (CWAs) analysis.

However, the advantages of CE, such as simplicity of instrumentation and operation procedure as well as high separation efficiency, have not fully been realized yet and there is an urgent need to be more closely evaluated regarding, e.g. the screening of seawater quality as pointed out above. To the authors' knowledge, there are a very limited number of scientific articles on the utilization of CE for the analysis of HD and its degradation products. The neutral degradation products of HD can be analyzed by direct UV detection using micellar electrokinetic capillary chromatography (MEKC) [13, 14]. Separation was achieved through a running buffer of 10 mM borate and 100 mM SDS. The moderate sensitivity was due to the lack of UV chromophore sites on analyte molecules.

Moreover, unlike other separation methods (GC and HPLC), the simplicity and robustness of CE (besides its other features) allow for miniaturization of instrumentation and, as a consequence, the design of portable field analyzers [15]. Such instruments can be used in situ, at the point of care. This in turn provides a fast response when information is urgently needed. Although portable GC and HPLC instruments have been developed, the need to use compressed gases or pumps and solvents makes the construction of portable GC and HPLC instruments a difficult task. In contrast, a couple of successful portable CE instruments have been reported [16–18]. In terms of CWA screening, some examples are already available. The excellent separation performance of a portable CE has been confirmed by the separation of alkylphosphonic acids using a contactless conductivity detection system [19, 20]. The separation of other critical compounds of military and forensic interest has been demonstrated by Hauser's group (nitrogen mustard) [21] and Breadmore's group (explosives) [22].

In spite of that the GC-MS/MS protocol still outperforms the CE protocol proposed here on LOD as was pointed above, the portable GC is more difficult to operate in the field and its eventual usefulness of the portable CE instrument will depend on real concentrations that are present at the point of care. The information about the real concentration of TDG and its oxidation products in near-bottom water or sediments is very limited. Thus, the measured concentrations of WW II CWA munitions near the Gotland and Gdansk dumping sites in the Baltic have been reported to be about 20–250 μg/kg of sediment (for sulfur compounds) [9]. In this respect, the proposed CE technology seems to be a very promising and reliable alternative to conventional GC-MS analysis.

In the present study, a CE method with direct UV detection for the analysis of TDG and its oxidation was developed and validated. The proof of the principle was demonstrated on a commercial lab scale instrument (Agilent), with the goal of transforming it further to a portable format. A CZE separation method with direct UV detection after precapillary derivatization with strong UV chromophore (phthalic anhydride) is described. A method validation in terms of specificity, precision, linearity and limits of quantification and detection was performed. The BGE contained only boric acid adjusted with sodium hydroxide, making it simple and easy to use. The developed method was used to determine the low amounts of TDG and its oxidation products in seawater.

2 Materials and methods

2.1 Chemicals

Boric acid, sodium hydroxide, acetonitrile, sinapinic acid (internal standard, IS) and imidazole were purchased from Sigma-Aldrich (Germany). Phthalic anhydride and pyridine were obtained from Merck KGaA (Darmstadt, Germany). TDG, TDGO, and TDGOO were synthesized by Envilytix (Wiesbaden, Germany). All chemicals were of analytical grade and used as received. BGE was prepared in DI water from a Milli-Q water purification system (Millipore S. A. Molsheim, France).

2.2 Instrumentation

An Agilent 3D instrument (Agilent Technologies, Waldbronn, Germany) equipped with a diode array UV/Vis detector (DAD) was used for the separation of TDG and its oxidation products. All electropherograms were recorded and integrated with Agilent ChemStation software. Uncoated fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) with an internal diameter of 50 μm and a length of 52/60 cm (effective length/total length) were employed in the experiments. Samples were injected hydrodynamically by applying a pressure of 50 mbar for 5 s. Separation process was monitored at 200 nm. The pH value of the electrolyte solution was measured with a Metrohm 744 pH meter equipped with a combination electrode (Metrohm, Herisau, Switzerland), which had been calibrated with commercial buffers at pH 7.00 (±0.01), pH 10.00 (±0.01), and pH 12.00 (±0.01) (Sigma-Aldrich). Empty SPE tube (polypropylene, tube volume 3 mL, Phenomenex) was used for SPE cartridge preparation.

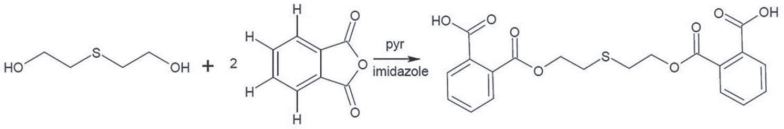

Figure 1. Derivatization reaction of TDG with phthalic anhydride.

2.3 Sample preparation

One liter of seawater (from the Baltic Sea) was spiked with TDG, TDGO, and TDGOO standard solutions to obtain the final concentrations of 0.12, 0.14, and 0.15 μg/mL, respectively. For the target analytes extraction, an SPE cartridge based on powdered carbon aerogels [23] was used (100 mg of powder per cartridge). Fifty milliliters of spiked seawater was run through the SPE cartridge using a vacuum system, then the sorbent was washed with 10 mL of DI water and, finally, the compounds of interest were eluted with 1.5 mL of acetonitrile. Then the solvent was evaporated under a gentle steam of nitrogen till dryness. Finally, 20 μL of derivatizing mixture was added to the solid residue and the sample was treated as described below.

2.4 Derivatization procedure

The derivatizing mixture was prepared in accordance with the literature [24]. Briefly, 1.61 g of phthalic anhydride was dissolved in 10 mL of pyridine, and then 0.24 g of imidazole was added to catalyze the reaction. The mixture was sealed with septum and stored in a desiccator in the dark. For derivatization, 100 μL of phthalic mixture was added to each 2.5 mg of analyte of interest, sealed and heated at 45°C for 20 min. Then the mixture was cooled and the same amount of water was added to stop the derivatizing reaction. Finally, the mixture was diluted by DI water in accordance with need, an IS of sinapinic acid was added, and the sample was introduced into the CE system (Fig. 1).

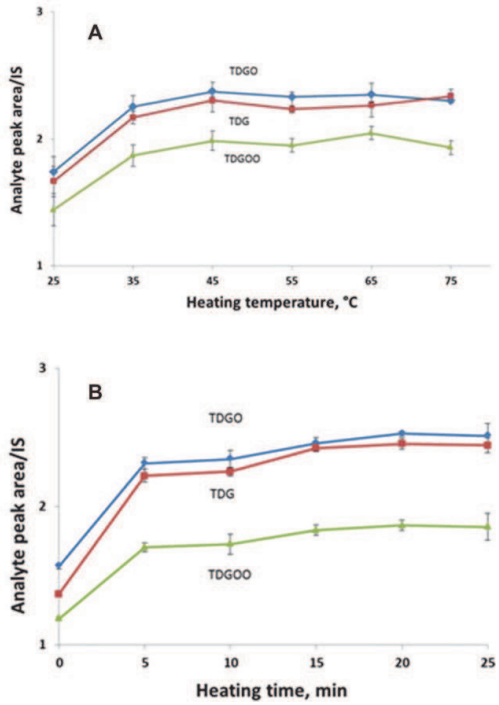

Figure 2. Effect of heating temperature (A) and time (B) on derivatization efficiency.

3 Results and discussion

3.1 Sample derivatization

To obtain reproducible results from the sample derivatization procedure, a careful optimization of several reaction parameters was needed. The effects of the amount of the derivatizating reactant, and the heating time and heating temperature were examined. Each time sample was injected into an electrophoretic system, the average peak area ($n = 3$) was measured and the condition that gave maximum response (peak area) was selected. Based on the stoichiometry of the derivatizing reaction, the required minimum amount of reactant was calculated (~40 μL per 2.5 mg of TDG). Then two-, three-, and fourfold volume excess of reactant was applied for the derivatization of each analyte. Based on the average peak area measurements, the maximum response was achieved applying the twofold volume excess of phthalic mixture. The appearance of the phthalic acid peak on an electropherogram (unreacted phthalic anhydride) acted as an indicator of a sufficient excess of derivatizing reactant. For future experiments, 100 μL of reactant per each 2.5 mg of analyte was selected to avoid the lack of derivatizing reactant in samples.

In the presence of imidazole, the derivatization reaction was quite fast. To find the optimal derivatization temperature and heating time, a set of additional experiments was carried out, varying the temperature in a range of 25–75°C and the heating time in a range of 0–25 min. Keeping the temperature constant (85°C, in accordance with the literature source [24]), the maximum response was achieved within 5 min (Fig. 2A). The effect of the heating temperature was evaluated, keeping the reaction time (20 min) constant. The best response was obtained at 45°C and further temperature increases did not

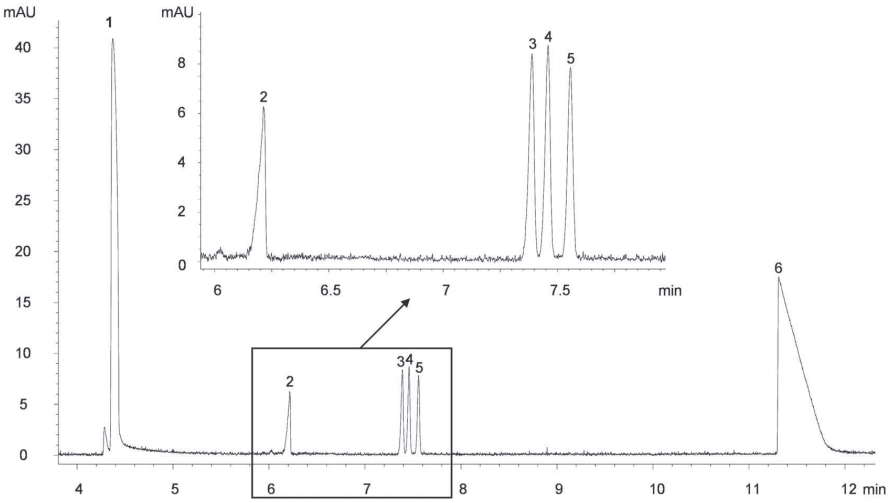

Figure 3. Representative electropherograms obtained after derivatization of 3.05 μg/mL of TDG (4), 3.45 μg/mL of TDGO (3), and 3.85 μg/mL of TDGOO (5) under optimized separation conditions: borate buffer concentration 30 mM, buffer pH 8.5, applied voltage 15 kV, and capillary temperature 25°C. Additional peaks: EOF (1), IS (2), and phthalic acid (6).

affect the result (Fig. 2B). Finally, the derivatization conditions were as follows: the amount of reactant 100 μL per each analyte, derivatization temperature 45°C, and derivatization time 20 min.

The stability of derivatives was investigated. For this, target analytes (3.1, 3.5, and 3.9 μg/mL for TDG, TDGO, and TDGOO, respectively) were derivatizated as described above and after IS addition the mixture was divided into two equal aliquots. The first aliquot was stored at room temperature (23 ± 1°C) and under light (mostly halogen lamps), and the second aliquot was kept in a refrigerator (4°C, in darkness). Systematic sampling over 5 days was performed to measure the peak areas of the derivatives. In the case of TDG and TDGO, there were no systematic changes in the peak areas and shapes during the evaluation period for the first aliquot (kept at room temperature) or for the second one (kept at 4°C). The RSD of the peak areas did not exceed 3.2% ($n = 5$, one analysis per day), which indicated the high stability of the derivatives. TDGOO samples kept at room temperature showed a slight peak area decrease (RSD = 5.7%), but the refrigerated samples demonstrated stability.

3.2 Choice of BGE

Underivatized TDG and its oxidation products are neutral at pH below 9 and, thus, can be analyzed using the MEKC separation technique. Applying 10 mM borate buffer with 100 mM SDS and direct UV detection at 200 nm, TDG and TDGO could be separated within 6 min. The calculated LOD for TDG and TDGO was 10 μg/mL [14]. The high values of LOD are logically justified due to the absence of strong UV-absorbing sites. In this work, an improved method for the analysis of target sulfur compounds involved derivatization with phthalic anhydride incorporating chromophore sites into the analyte structure and, at the same time, affecting the pK_a value of the formed derivatives and giving a charge to the molecules that, in own turn, allows to use borate buffer for analyte separation. The optimization of the separation conditions was carried out by the investigation of the effect of buffer pH, concentration, capillary temperature, and applied voltage on separation efficiency.

It is well known that buffer pH plays the key role in optimization of a separation process affecting the EOF velocity and degree of analyte ionization. So, in the present work, a 30 mM borate buffer with a pH range of 7.5–10.0 was investigated to evaluate the impact on separation results. At buffer pH values below 8.0, the derivatized analytes were unresolved and the peak shapes were asymmetric. An increase in the buffer pH value to 8.5 led to improvement in analysis time and all analytes were baseline separated. Further, pH change to 9.5 resulted in an increase in the electrophoretic mobility of the analytes. Baseline separation was still achieved and the analysis time was the shortest. Unfortunately, starting at pH 9.0, the sulfone peak area was prone to rapid decrease and at pH 10 almost disappeared, which is, obviously, associated with instability of TDGOO at pH 9 and above.

The influence of borate buffer concentration on separation was studied in the range of 20–50 mM at pH 8.5. By increasing the buffer concentration from 20 to 50 mM, the migration times of all analytes were also significantly increased. Nevertheless, the best separation efficiency was achieved at 30 mM and further concentration increase led to peak broadening without a remarkable change in peak separation.

Table 1. Regression data for the calibration curves

Derivative	Linearity range (μg/mL)	Regression equation	Regression coefficient (R^2)	LOD (μg/mL)	Limit of quantification (μg/mL)
TDG	0.10–2.44	$y = 0.1517x + 0.0017$	0.9996	0.10	0.31
TDGO	0.14–2.76	$y = 0.1359x - 0.0161$	0.9997	0.14	0.42
TDGOO	0.15–3.08	$y = 0.1233x - 0.0304$	0.9994	0.15	0.46

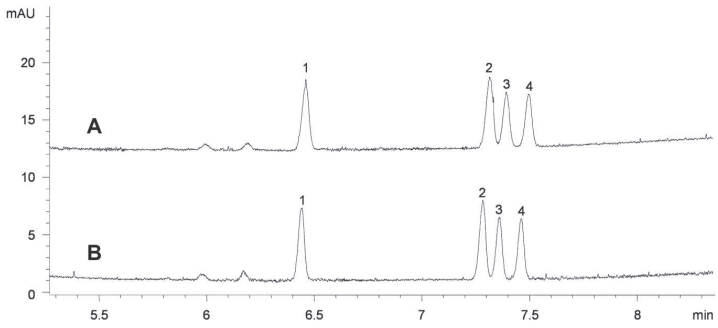

Figure 4. Electropherograms of (A) distilled water and (B) seawater samples. Separation conditions: borate buffer concentration 30 mM, buffer pH 8.5, applied voltage 15 kV, and capillary temperature 25°C. Peaks: IS (1), TDGO (2), TDG (3), TDGOO (4).

Additionally, the effect of the applied voltage over the range 15–25 kV and capillary temperature (15–30°C) was also investigated in terms of separation efficiency and migration times. Voltage values above 20 kV resulted in faster migration times of analytes, but the separation efficiency was not sufficient. All derivatives were baseline resolved at 15 kV, but the analysis time was extended by several minutes. The increase in capillary temperature also noticeably improved the migration time of the derivatives. Thus, the temperature increase from 15 to 30°C decreased the analysis time by a quarter, keeping the separation efficiency at a reasonable level.

Finally, the optimized separation conditions for the separation of three derivatives were as follows: borate buffer concentration 30 mM, buffer pH 8.5, applied voltage 15 kV, and capillary temperature 25°C. The representative electropherogram is shown in Fig. 3.

3.3 CE method validation

The precision of the developed method was investigated. All precision tests were based on optimized BGE and a standard mixture of derivatives. The tests were performed for the run-to-run and day-to-day variations of the migration times and peak areas. Run-to-run precision resulted in maximum RSD values of 0.6% ($n = 6$) and 3.1% ($n = 6$) for the migration time and peak area, respectively. Additionally, day-to-day results showed RSD values of 1.2% ($n = 6$) for the migration times and 3.6% ($n = 6$) for the peak areas. There were no systematic changes in peak shape during the precision tests.

The linearity was evaluated in the range of 0.10–2.44 μg/mL for TDG, 0.14–2.76 μg/mL for TDGO, and 0.15–3.08 μg/mL for TDGOO. Calibration curves were constructed using five concentration levels and were based on the ratio of the corresponding derivative to IS peak area versus concentration. The linearity range, regression equations, and regression coefficients are shown in Table 1.

The LOD and limit of quantitation (LOQ) were obtained experimentally by measuring the S/N. The lowest LOD and LOQ were obtained for TDG and calculated as 98 ng/mL (S/N = 3) and 305 ng/mL (S/N = 10), respectively.

To evaluate the specificity of the developed method, a blank sample was treated as described in Sections 2.3 and 2.4 and then injected into the CE system. There were no interfering peaks observed in the resulting electropherogram.

3.4 Spiked seawater analysis

To demonstrate the method applicability for real sample analysis, seawater was spiked with a standard mixture of free underivatized analytes (TDG, TDGO, and TDGOO) to get the final concentrations of 0.12, 0.14, and 0.15 μg/mL, respectively. The extraction of analytes and the derivatization process are described Sections 2.3 and 2.4. The same sample preparation procedure was carried out for spiked distilled water to evaluate the seawater as a matrix influence on the extraction and derivatization processes. Figure 4 shows the electropherograms of the separation performance of seawater as well as distilled water samples. In all cases, TDG, TDGO, and TDGOO were baseline separated. The RSD of the migration times between all analytes in spiked sea and distilled water samples resulted in 0.4% and the RSD of the peak areas was below 5%. These results show no evident influence of seawater matrix on the extraction and derivatization processes.

4 Concluding remarks

The results of the study show that precolumn derivatization by phthalic anhydride can be used for the significant reduction of LOD of TDG, TDGO, and TDGOO by applying capillary zone electrophoresis with direct UV detection. It also was demonstrated that sample purification and concentration on carbonaceous adsorbent allows for the quantitative analysis of mustard gas hydrolysis and oxidation products in seawater. Nevertheless, it should be noted that the obtained detection limits are less reliable than GC-MS results, but can still be considered acceptable. Moreover, the miniaturization benefits of CE allow translation onto a truly portable instrument, thus making the method more attractive for in-field use.

Transforming the protocol to a portable instrument involves another problem. Contemporary LED sources can generate radiation with wavelengths above 240 nm while the protocol reported in this study requires radiation of 200 nm. Thus, the UV detector implemented in the portable CE instrument must employ a miniature deuterium lamp which somewhat reduces the robustness of the instrument. We believe, however, that this is not a serious obstacle to its design.

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The authors have declared no conflict of interest

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