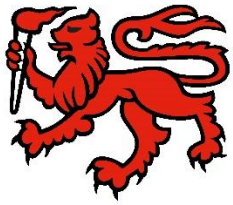

AUTONOMOUS FILTRATION SYSTEM FOR PORTABLE CAPILLARY ELECTROPHORESIS INSTRUMENTS

By

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Submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

University of Tasmania

June 2022

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María Gabriela Paniagua Cabarrús

24 September 2021

ACKNOWLEDGMENTS

I am extremely grateful to my supervisor, Professor Michael Breadmore for making me believe in myself, taught me there is no box. No matter how wrong, disappointed, or frustrated results I brought Michael always had kind and inspiring words for me. My deepest admiration to Professor Rosanne Guijt for academic and personal support and encouragement. My sincere gratitude to my co-supervisor Dr. Fernando Maya, for taking me through the complications to practical solutions on this journey. Thanks to Dr Marni Amuno for taking me through the art of writing a paper, your patience and guidance help me across this difficult task. To Dr. Min Zhang for the practical knowledge shared at the start of my PhD. Special thanks to Dr. Petr Smejkal for the enormous contribution to my project, not only in the academic area but also as a friend.

Thanks to 434 officemates for making an enjoyable environment where we could share every stage of the journey, we have laughed, cried, and survived. Feng, Laurel, Leo, Elizabeth, Zaidon, James, and my misery buddies Sepideh and Monju, this journey could not be finished without you.

There is a special place in my heart for my colleagues and friends Ibraam, Umme, Cecil, Estrella, Laura, Eli, Joan Marc, Mostafa, Prashant, Brenda, Ethan, Natalia, Thisara, Bahador. Thanks for all the good memories.

I cannot thank enough for the people who has become my family in Tasmania, Paula, Jessica, Petra, Fran, Mario, Jary, Camila, Marion, Angus and Jose. Melisa thank you for always been there for me. To my favorite loca, Silvia, it seems crazy we found each other in madness, in the furthest place in the world.

Thanks to Dan, for showing me a brighter side of the world, and make every moment happier and simpler. I am deeply grateful for my chosen family, Ana Luisa, Manuel y Silvio, always there for me. Thanks to my family, to my dad, to Valentina, Emma and Giulia, to Edda, to my sister and mom, all what I am is because of you.

BROKEN FLOW

Oh my dear syringe pump, I just did everything wrong;

I replaced you because you sink the particles I gave to you;

I shouldn't leave you alone,

I replace you for a younger peristaltic pump;

I believed it would give me a better flow;

Just leaks and extra pulsing it's what I got;

Oh my dear syringe pump,

Please forgive me for doing everything wrong;

Going through 3D printed chips was not good for me;

You showed me PDMS chip was the right way for my PhD;

Oh my dear syringe pump;

Forgive me for doing everything wrong;

I wish I could go back to you;

But is too late for my project too;

Your flow is not anymore pumping for me;

But peristaltic pump can't help me;

I am pump broken;

My fluids do not match anymore;

Oh my dear syringe pump;

Forgive me for doing everything wrong.

STATEMENT OF CO-AUTHORSHIP

The following people and institutions contributed to the publication of work undertaken as part of this thesis:

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Paper 1, Portable capillary electrophoresis with online continuous particulate removal [Research Article: in progress].

Located in Chapter 2 of the thesis. Candidate was the first author and conducted all the experimental work. P. Smejkal contributed with electronics, engineering and designs. R. Alexander assisted with engineering and design. M.C. Breadmore, R. Guijt, and M. Zhang contributed to the idea and the experiments design. M.C. Breadmore, R. Guijt, F. Maya and M. Amuno assisted with refinement and presentation.

We the undersigned agree with the above stated "proportion of the work undertaken" for each of the above published (or submitted) peer-reviewed manuscripts contributing to this thesis:

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LIST OF PUBLICATIONS AND PRESENTATIONS

Patents

AU 2021901319: An analytical instrument, a method for controlling an analytical instrument and a filter system (filed 04 May 2021)

Conference presentations

Continuous particle removal

Gabriela Paniagua Cabarrús, Michael C. Breadmore

December 2017, 25th annual Royal Australian Chemical Institute Research and Development Topics Conference - (poster presentation)

Continuous particulate removal for capillary electrophoresis applications

Gabriela Paniagua Cabarrús, Michael C Breadmore

December 2019, 27th annual Royal Australian Chemical Institute Research and Development Topics Conference - (oral presentation)

LIST OF ABBREVIATIONS

3D	Three-dimensional
AAS	Atomic absorption spectroscopy
BGE	Background electrolyte
C^4D	Capacitively coupled contactless conductivity detection
CE	Capillary electrophoresis
CHES	2-(cyclohexylamino)-ethanesulfonic acid
CNC	Computer numerical control
DAQ	Data acquisition module
EOF	Electroosmotic flow
Fb	Buoyant force
Fc	Centrifugal force
Fd	Drag force
GC	Gas chromatography
HPLC	High performance liquid chromatography
HV	High voltage
IC	Ion chromatography
ICP-MS	Inductively coupled plasma mass spectroscopy
ICP-OES	Inductively coupled plasma-optical emission spectroscopy
ID	Internal diameter
IS	Internal standard
LED	Light-emitting diode
LOD	Limit of detection

LOQ	Limit of quantification
MS	Mass spectrometer
OD	Outside diameter
OES	Optical emission spectroscopy
OI	Optical interface
OSR	Outlet solution reservoir
PAR	4-(2-pyridylazo)resorcinol
pCE	Portable capillary electrophoresis
PEEK	Polyetheretherketone
RSD	Relative standard deviation
TAPS	N-tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid
TPM	Three-port F style manifold
TRIS	Tris-(hydroxylmethyl) amino-methane
µ-TAS	Micro total analysis system
UV	Ultraviolet
XRF	X-ray fluorescence spectroscopy

ABSTRACT

Environmental pollution has become of global concern as the population grows, and industrial and agricultural activities increase. These directly influence human wellbeing, because pollutants are carried by soil, water and air into waters and farms into animals and crops that are consumed by people. These contaminants can also alter atmospheric conditions, the hydrological cycle, and the greenhouse effect. The environment as we know it is prone to changes on a long-term basis, but it is difficult to measure and correlate unless implementing long term monitoring strategies to establish trends and patterns of change. So far this is one of the most complicated topics in science. Amongst these pollutants, there is a special interest in nutrient enrichment which can be correlated with the perception of over fertilization. These can influence the growth of toxic algal blooms along with hypoxia in freshwater and marine environments and human health.

Nutrients monitoring currently relies in samples that are taken on the field and transport to the laboratory, making environmental monitoring a laborious, complex, expensive, and slow task. The development of field deployable analytical instrumentation is crucial to overcome this issue. Monitoring strategies should include the analysis of several samples per day, for several months or even years, and be done often in remote locations. In recent decades scientists have dedicated significant time and resources to the study of miniaturized field deployable instruments. The frequent challenges faced while developing these instruments are sample filtration, changes of weather conditions in the field, like temperature and humidity, size and portability of the devices and excessive cost to produce and run the instruments.

This thesis is focused on addressing the challenge of continuous sample filtration of liquid samples in order to make a fully autonomous field deployable system capable of monitoring water chemistry. This includes considerations to control the temperature of analysis while on site, power consumption, portability, cost, and versatility of the target analytes.

Chapter 1 gives an overview of capillary electrophoresis systems and the path towards automatization and portability. The main challenges encountered while developing field deployable instruments and the invention of microdevices are to overcome the problems of sample preparation, excessive use of reagents, sample incompatibility within detectors and fast response of the systems.

In Chapter 2 the aim was to develop a continuous particle removal device as a solution for automated filtration. In this work an H-filter, a passive microdevice, has been considered because it is a simple structure, low cost, easy to replicate and does not consume extra energy compared to active methods. The H-filter was designed, manufactured, and integrated to a portable capillary electrophoresis (pCE) system, developed previously at University of Tasmania. The fabricated microdevice allowed continuous removal of particles over 1 µm size in water samples without the necessity for a membrane filter that requires cleaning or replacement. The integrated system was tested continuously for 6 weeks, using a soil slurry water sample with particulate matter up to 100 µm. The portable capillary electrophoresis (pCE) coupled with a capacitively-coupled contactless conductivity detector (C^4D), separated, and detected chloride (Cl^-), nitrate (NO_3^-) and sulphate (SO_4^{2-}) with detection limits (LOD) from 500 to 1000 ppb, and relative standard deviations (RSD) of 12% for Cl^-, 13% for NO_3^- and 14% for SO_4^{2-}, for a total of 1575 samples analysed.

Due to the dimensions of the H-filter particulate matter over 100 µm could not be analysed. In Chapter 3 the aim was to remove particles above this size to allow the analysis of real water samples. To achieve this, a 3D printed mini

cyclone was incorporated into the system. The microdevice was printed in a PolyJet printer and connected to a peristaltic pump with a flow rate of 4 mL/min. The mini cyclone has two outlets, the underflow where the bigger particles will follow through and the overflow, where the smaller particles will out. The sample collected from the overflow was analysed by a particle sizer and particles up to 90 µm size were found indicating that particles above this size were completely removed. By connecting the mini cyclone to the H-filter on-line to a portable capillary electrophoresis was demonstrated that all the particles above 1 µm were removed from a water sample. To test the flexibility of the instrument the C^4D was switched for an LED detector. The method allowed to detect zinc (Zn^{2+}) from 10 different environmental water samples, with variable particulate matter contents, with no clogging or blockage of the system. The capillary electrophoresis instrument was tested in an industrial water treatment plant, detecting Zn^{2+} in real time.

The instrument developed was able to produce a clean sample to analyse contaminants in water continuously for several weeks but concerns about portability arose from the field deployment as detailed in Chapter 3. In Chapter 4 the objective was to incorporate all the necessary pieces to perform the separations in a truly portable and autonomous analytical apparatus. First considerations were how to maintain stable and controlled temperature. To achieve this all the electronic parts, microdevices and solutions were placed into an insulated box. A 3D printer was used to produce a platform where the pumps, microdevices, electronics, solutions and even waste containers were mounted to avoid mobile parts. An exterior submersible pump was connected to provide sample from a water stream with a distance over 10 m. The sensitivity of the instrument was tested performing a calibration curve. The limit of quantification (LOQ) was 91 ppb for Cl^-, 403 ppb for NO_3^- and 227 ppb for SO_4^{2-}, and LOD was 30 ppb for Cl^-, 121 ppb for NO_3^- and 75 ppb for SO_4^{2-}. The instrument variability was 10% for Cl^-, 10% for NO_3^- and 8% for SO_4^{2-}, for n=3. Two different field deployments were completed. First, a pond in Margate, Tasmania, Australia where the pCE worked for 10 days, analysing a total of 241

samples, separating, and detecting chloride, nitrate, and sulphate. For a second deployment the pCE was taken to a secure location next to Plenty River in Tasmania, Australia.

A total of 974 samples were analysed continuously for 1 month. The insulated box contains all the elements needed to perform analysis from a water stream, including sample intake, sample pre-treatment, separation and detection of various analytes, and data collection. The insulated box is easily transportable by 1 operator and does not needed any maintenance during the time deployed.

Current portable instrumentation has been developed and tested for numerous environmental samples, but many of them still perform the sample clean up by filters, membranes, or cartridges that must be replaced constantly. While other on-site instrument like sensors are capable to detect a single analyte at the time. The pCE developed in this work, can detect a mix of different analytes, the detection method can be easily changed, it is truly portable and automated, and able to work for several weeks without the need of maintenance or parts replacement.

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CHAPTER 1 : INTRODUCTION AND LITERATURE REVIEW

1.1. INTRODUCTION

The environment can be extremely affected by human activities, which also modify the distribution of chemicals that are essential for many aspects in life. Monitoring the environment is crucial to comprehend, for example, chemical cycles of key elements, the impact of intensive farming and value of wastewater treatments [1]. When monitoring the environment, the location and time where the samples are collected is important. The concentration of analytes can fluctuate according to the day, season, from natural events such heavy rains or storms, among others. To overcome and ponder all these variations it is essential to have extended sampling practices of environmental monitoring [2].

Established methodologies for environmental testing require the sample to be collected manually and transferred to the laboratory for examination. This typical labour-intensive process increases the time and cost and generates inherent risks of cross-contamination and potential degradation [3]. Moreover, laboratory analysis can be costly when using specialized procedures and handling processes. Consequently, the results are delayed particularly when coming from remote areas.

To reduce these challenges, portable analytical instruments for on-site analysis with quick response times have been researched [4]. The benefits of portable instrumentation include of the reduction of tasks such as sampling, transportation, and storage which contribute to green methodologies, and provide fast decision-making. Portable devices are, in general, cheaper than bench equipment. Additionally, this low-cost makes analytical technology accessible for larger sectors of population [5].

For field measurements, significant progress has been made with the construction of chemical sensors [6]. Detection levels of sensors can reach nM concentrations, are operated on-site, involve small volume of reagents, are low-

cost, portable, and automated. Still, some of the downsides of sensors are the consistency for long term use in complex environmental conditions, because their response can be perturbed by the matrix effect, have slow time response, use membranes or filters that should be replaced regularly, and/or they typically can detect only one analyte per analysis [6–8]. As an alternative to generate sensor-like-data separation techniques such as chromatography and electrophoresis have been used. Portable ion chromatography (IC) systems have been developed and used to analyse inorganic ions in environmental samples with relative standard deviation (RSD) under 5%, in some cases for several days [9–12].

Capillary electrophoresis (CE) presents several advantages over IC with regards to miniaturization. CE instruments are generally simpler, faster, use smaller amounts of reagents, and operate at low pressure. They can also be easily modified by changing the chemistry of the background electrolyte, to achieve different separation mechanisms for different types of compounds and there is a range of different detectors that can be used. Various research groups are dedicated to develop and improve portable CE systems for environmental monitoring [13]. Some scientists reported the use of CE instruments with UV-VIS, electrochemical detectors, and capacitively coupled contactless conductivity detection (C^4D), to measure anions and cations in environmental samples, for up to 48 hours of continuous use, but all of them employ filter membranes to prepare the samples prior analysis [14–16].

These studies emphasize the major limitation for systematic analysis is the sample preparation. As a solution microdevices have been used to minimize these challenges. Researchers have been exploring different materials and manufacturing processes to improve these microdevices. Microdevices have gained popularity across the analytical workflow because they are small and easy to operate. Additionally, some fabrication techniques allow for rapid prototyping in the laboratory at low costs. These microdevices have been used for many steps of the sample preparation, including sample clean-up, and mixing of reagents. The main challenge for sample clean-up in environmental analysis is the particle separation. Generally, this is achieved by filtration using

membranes which are typically prone to clogging. Therefore, two membrane-free particle separation methods, the H-filter and then cyclone have been selected for this work. By modifying the dimension of these microdevices it is possible to manipulate the size of the particulate matter that can be removed from a liquid sample. These microdevices can be connected easily with a portable CE.

1.2. HISTORY OF CAPILLARY ELECTROPHORESIS

Electrophoresis term refers to the movement of charged particles under the influence of an applied electric field, the development of this technique dates back to 1807, when Reuss experiment led to the discovery of electroosmotic flow (EOF). By using a glass U tube packed with water and insoluble powered material like sand, he created a porous barrier to separate the ends of the tube, then he applied voltage to the water, and could observed how started to permeate the barrier. Michaelis, in 1909, established the term electrophoresis to describe this phenomenon which derived from the Greek words elektron = amber (i.e. electric), and phore = carrier [17]. Following this trials, Tiselius in 1930 demonstrate the use of electrophoresis to separate proteins but, his work didn't get enough attention until, one of his students, Hjerten included the use of a capillary in 1965 and created the first completely autonomous capillary electrophoresis [18].

The first instruments created dealt with some challenges in terms of separation efficiency, required specialised operator and were expensive to produce and maintain. Moreover, all moving boundary methodologies have an inherent disadvantage where only partial separations can be achieved. As a result, Hjerten devised a zone electrophoresis method that could be applied for both analytical and micro preparative applications, leading to the development of the so-called free zone electrophoresis equipment [19]. Using a quartz capillary of 1-3 mm of internal diameter (ID) Hjerten created a smaller instrument which was as well easier to operate [19]. The smaller ID of the capillary decreased the convection resulting in smaller band width, increased the sensitivity, reduced

the time of analysis and allowed the use of higher voltages [20]. Continuing with his investigations, Hjerten used a layer of polymer to coat the internal wall of the capillary, reducing the electroosmotic flow. This was rapidly used for numerous researchers with the aim of creating smaller ID capillaries. But was not until 1981 when Jorgenson and Lukacs used a 75 µm flexible open tube made form fused silica, borosilicate, and Teflon, coated with a polymer layer. By this experiment they accomplish high resolution separations, something that appeared unachievable at that time [21].

The use of capillaries in electrophoresis resolved some of the difficulties that were frequent in classical electrophoresis. The narrow size of the capillaries, for example, enhanced the surface to volume ratio, reducing the overheating caused by high voltages. Because higher voltages could be used, capillary electrophoresis had improved efficiency and outstanding separation capabilities, which created a rising interest in the scientific community to carry out additional advances in the technology [22]. CE has shown advantages compared to classic separation techniques such as gel electrophoresis and liquid chromatography: it is a simpler setup and can be miniaturized easily, quick separation, high resolution, high efficiency, minimal sample and low solvent consumption. Therefore, CE has been broadly used to separate varied analytes ranging from small ions, macromolecules and microorganisms in different research areas such as biomedical, forensic, environmental and food quality, among others [23].

1.3. OVERVIEW OF CE OPERATION

Capillary electrophoresis instrumentation can be relatively simple, consisting of two electrodes placed in separated vials, the “inlet” and the “outlet” vial, an interchangeable sample vial, a high voltage power supply, and a detector connected to a data collection system. A basic set up of an instrument is shown in Figure 1-1. Some instruments incorporate sensors to control the temperature, which provide more reproducible measurement. The capillary is made of fused silica and often coated with a polymer. The ends of the capillary are placed in

the vials, and those are filled with an electrolyte buffer solution. The sample is introduced by placing the capillary into the sample vial and applying pressure/vacuum (hydrodynamic injection) or voltage (electrokinetic injection) [24]. Each electrode is connected to one side of the high voltage power supply. These electrodes generate an electric field, which initiates the migration of the sample from the anode to the cathode via the capillary tube. The ions, either positive or negative, move across the capillary and because of their electrophoretic mobility, the analytes are separated and detected at the end of the capillary. The detector output is sent to a data acquisition system, like an integrator or computer [25]. The data is presented as an electropherogram, showing detector response versus time. In the electropherogram each analyte is represented by a peak, with a characteristic migration time [26].

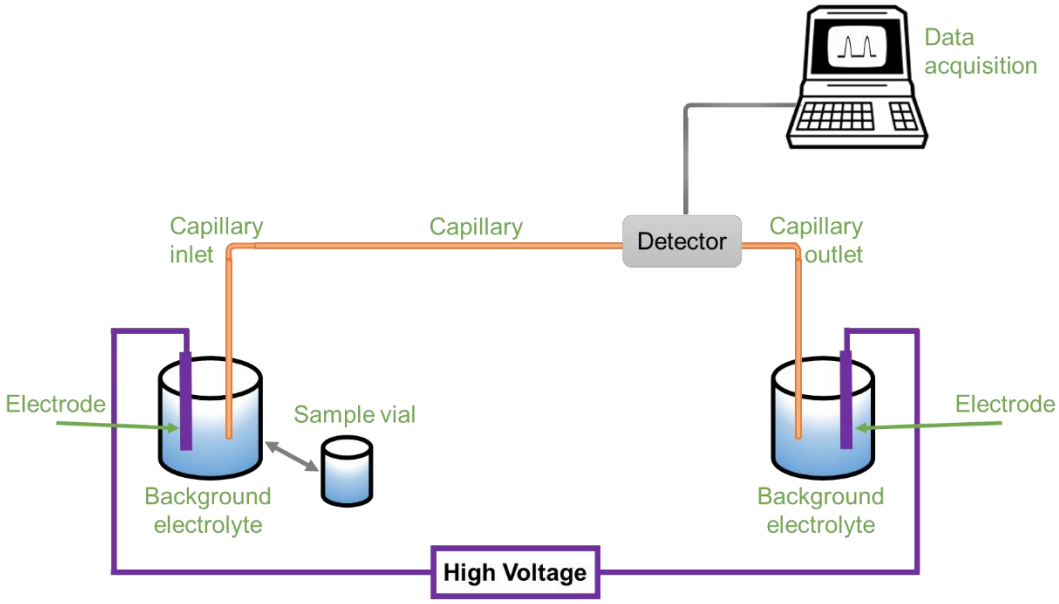


Figure 1-1 Basic set up for a capillary electrophoresis instrument.

1.3.1. Injection method

There are two methodologies to inject a sample in a capillary electrophoresis instrument, those are hydrodynamic and electrokinetic. The hydrodynamic injection is achieved by a difference of pressure between the inlet and the outlet vial. This method can be highly reproducible, and practical. Electrokinetic

injection, on the other hand, employs electrophoresis to introduce the sample into the capillary. Analytes enter the capillary by a combination of electrophoretic mobility and electroosmotic flow, whereas neutral components enter merely by electroosmosis process. This is the simplest approach because it uses the same principle as the separation just by changing the inlet vial containing the electrolyte to the vial containing the sample [27]. However, the quantity of injected sample with these techniques is relatively small. Additionally, some detection method used, like optical detectors, may limit the amount of sample that go through the pathway. As a result, the sensitivity can be poor, making necessary the use of preconcentration methods to increase the detection limit of the technique [28].

1.3.2. Capillary coatings

The EOF defines the movement of a buffer solution when an electric field is applied. This movement can be affected by interactions between silanol groups (negatively charged) on the surface of a fused silica capillary [29]. Matrix components and analytes can also interact with the capillary walls, causing poor resolution, wider peaks, changes in the baseline, variations in the EOF, and low migration time reproducibility. Since Towns and Regnier studied the influence of cationic polymer analyte adsorption to the inner wall of fused silica capillaries on electroosmotic flow and CE separation performance, researchers have dedicated time to investigate different coating materials to minimize these problems [30]. Monteferrante *et. al.* described a method to predict EOF changes as function of pH, for different polymer coating conditions. The proposed method was used to analyse quantitatively the EOF and mobility by determination of the charge in the near-wall of the coating [31]. Fu *et. al.* were able to control EOF by a hybrid functional coating method, using polydopamine and polyethyleneimine at different mass ratios and stabilised with $FeCl_3$. They obtained RSDs lower than 5% when analysing aromatic acids, in different days and different coated columns [32]. Poulsen *et. al.* presented a polyethylene glycol covalent coating methodology were EOF was supressed almost completely and coating was stable in analysis of proteins at a pH range of 3.4

to 8.4 for 100 days of use [33]. Stock *et. al.* separated and detected proteins and peptides on the same capillary by coating a capillary with a successive multiple ionic polymer layers. The method employed poly-(acrylamide-co-2-acrylamido-2-methyl-1-propansulfonate) to decrease migration time of EOF by lowering its charge density [34].

1.3.3. Background electrolyte

In CE, analytes are separated based on their charge-to-size ratio, and effective separation is highly dependent on the choice of the correct separation buffer solution, which should provide enough differential in the electrophoretic mobility of the analytes. Several resources have been focussed on finding the appropriate separation media and the correct additives to analyse different groups of chemicals [22]. For example, neutral surfactants such as Tween20 can reduce adverse interactions within the capillary, improving the sensitivity of in electrophoresis separations [28]. Even though aqueous solutions are the common solvent utilized in CE, there are not always compatible with other detection methods, like mass spectrometry. In this case organic solvents can be used to dissolve hydrophobic analytes. As described by Scriba, nonaqueous CE can be used as an alternative to couple with mass spectrometry (MS) due to the low surface tension, solvent volatility and reduced electrophoretic currents [35]. Because of their particular ionic and solvation properties, ionic liquids can also be used as buffer additives. They can be used as modifiers or surfactants in micellar electrokinetic chromatography to increase separation efficiency and selectivity. Xue *et. al.* demonstrated the use of ionic liquids to separate a group or enantiomers, the method successfully determined a group of enantiomeric impurities in a real sample of amino acids [36].

1.3.4. Detection system

Different detection methodologies can be applied with CE techniques. The selection of a detection method includes some considerations such as the properties of the target compounds, the sensitivity required, and the matrix of

the samples [37]. The main detection modes include optical, electrochemical, including C^4D and MS. Optical methods, like UV absorption, are widely used because they are simple, flexible and can detect a wide variety of chemical compounds. One of the drawbacks of optical detection is the pathlength is usually small, due to the dimensions of the capillary, causing poor sensitivity. Additionally, some of the analytes must be derivatised to be able to detect them, increasing the sample preparation process. Electrochemical detectors can detect μM to pM concentrations, but the sensitivity may change with time because the surface of the electrodes adsorb components form the samples. In addition, these methods need all the analytes to be electrochemically active at the same potential to be detected simultaneously [38]. Mass spectrometry detection can reach high levels of sensitivity, with low variability. The challenge is to find the appropriated interface to make the separation technique compatible with the detector. Capacitively coupled contactless conductivity detection can be used in detection for CE for organic and inorganic compounds. The technique is not dependant on electrochemical reactions occurring at the electrode interface, hence there is no deterioration or contamination of electrodes, meaning it can be used reliably for extended periods of time, as a technique it is power efficient and can be low cost when compared with other techniques [39].

1.4. FIELD-DEPLOYABLE INSTRUMENTATION

Portable instrumentation has its origin in 1934 when Beckman was trying to improve the sensitivity and robustness of glass electrodes to measure the acidity in citric samples. He used his knowledge on vacuum tubes to build an electrode with high input resistance. Beckman invented an amplifier that was novel due to its sensitivity, and put it in a wooden-box creating the first portable instrument, a pH meter that revolutionised the chemical instrumentation industry [40,41].

The rationale behind a portable instrument is simple: take the laboratory to the sample instead of taking the sample to the laboratory. This is especially

important in chemical monitoring, a series of data points can provide information about changes in sample related with time that single sampling cannot reflect. Chemical instrumentation has been traditionally large-sized, expensive, and complicated to use, transforming it into a smaller, affordable, sturdy, and simple to use instrument, will significantly improve the efficiency in the analytical workflow [42]. Additionally, in situ analysis can provide near real-time information, allowing to solve specific problems which involve instant response of information for an active solution.

According to Overton *et. al.* field-deployable analytical chemistry is the comprehensive practice of collection and analysis of samples on-site with high sensitivity, selectivity, robustness, and whit a high analysis throughput. They state a field deployable instrument must provide (1) analytical capability which includes selectivity, resolution, sensitivity, detection limits, precision and accuracy; (2) operational parameters like speed, size/transportability, power and utilities consumption, ease of use, level of operator skill required for effective use, reliability/ruggedness and cost; (3) the ability to produce chemical information, use of peer accepted procedures, reliability of manufacturer, accessibility (lots of devices in use), wide range of applications (2 or 3 possible applications per device) and adaptability to other applications [43].

1.5. PORTABLE CAPILLARY ELECTROPHORESIS

CE is a separation technique with high performance and have numerous advantages that make it suitable to miniaturize and developed into a field portable instrument. CE can detect multiple analytes simultaneously, separation is fast, has high sensitivity, operates at low pressure, consume low sample and reagents, the separation mode can be flexible, and the instrumentation is relatively simple [13]. Table 1.1 summarized the development of portable CE, including novelty of the development, application, detection limits and sample preparation procedures.

The first field portable CE (pCE) reported was developed by Kappes *et. al.* in 1998. The authors describe a CE with a potentiometric detector able to detect

anions and cations from 8 µM from a river sample. The instrument used coated-wire electrodes as detectors, the data acquisition was integrated into the unit and weighted only 7.5 kg, making it easy to transport for 1 person [44]. The same year Gerhardt *et. al.* reported a portable CE to analyse dopamine by square-wave voltammetry detection method using end-capillary detection. By adjusting the high-frequency sample rate required by CE, researchers achieved detection limits of 0.5 µM for dopamine samples [45]. From there, Kappes research group focused on modifications to the detection systems to improve their pCE. These modifications include a double amperometric/potentiometric detection system [46], the use of a single electrode [47] and the use of three electrochemical detection methods to increase versatility on the analysis [48]. In early 2000's the use of C^4D as a detector method for pCE became popular, opening the pCEs to new applications. By changing the chemistry of the separation methods, pCE-C^4D its been used to analyse on site environmental samples [14,49–53], illicit drugs [54,55], plasma and biological fluids [56,57], explosive residues [58], and various food matrices [59–63]

Efforts to improve the injection system started when Wang *et. al.* incorporated a flow injection (FI) system into a pCE-C^4D for the analysis of anions and cations in water samples, achieving RSDs under 2% and LODs in the order of low µM [64]. A cross sample injection system automated by pressure was incorporated by Seiman *et. al.* to analyse phosphonic acids from soil samples [65]. Kuban *et. al.* presented a method to improve the reproducibility by machined splitter interface onto a block of polyimide, together with a "smart" software for data processing, and the use of two internal standards (IS) [66]. To facilitate the operation in the field Mai *et. al.* adapted a valve-injection system, operated on compressed air to deliver solutions, and the use of a micromembrane pump for sample aspiration. Their system showed the potential to work autonomously for hours of monitoring operations [67]. Further, this system was modified adding a 4-way manifold to introduce automatically up to four different solutions into the capillary to allow an automated pre-run capillary conditioning [68]. The next improvement from the researcher's group was to replace the split injectors for an engraved flow cell interface, to achieve autonomous performance of the system [69]. Koenka *et. al.* developed a dual pCE-C^4D for the concurrent

separation of anions and cations in parallel channels. The system includes an automated mini-syringe pump, which can apply positive and negative pressures, the system incorporates a thermostat compartment to enhance the reproducibility on the field conditions [70]. Fuiko *et. al.* demonstrated the simultaneous measurement of anions and cations by the superimposition of hydrodynamic pumping with the electrokinetic motion. Additionally, this system was coupled to a membrane filter designed to sample directly, by aspiration, from a wastewater treatment plant [16].

By the incorporation of a laser induced fluorescence detector, Lee *et. al.* used the difference on DNA mobilities to separate and detect genes from cattle breeds with high sensitivity. This method was proposed for fast gene analysis in the lab and on the field [71]. Further modification to the system was to combine the pCE with a polymerase chain reaction (PCR) to analyse influenza A virus, providing an improvement on the methodology for fast and on-site molecular genetic diagnostic [72]. Saar-Reismaa *et. al.* reported a pCE coupled to a UV fluorescence detector (FD) for the study in situ of illicit drugs in oral fluids. The pCE-FD was validated using samples collected previously from suspects of drug abuse during a music festival, and achieved a similarity with the official screening method of 80% [73]. One of the latest, and more sophisticated pCEs was presented by Drevinskas, *et. al.*, which comprises a fully automated chemical analysis apparatus with sampling capacity, small enough to be installed in a drone. The instrument can analyse volatile and non-volatile analytes in air samples, weight less than 800 g and can be controlled remotely opening the possibility to reach chemical analysis in hazardous and inaccessible locations [74].

Table 1.1 development of portable CE and its applications

Detector type	Target analyte	Sample	LOD	Novelty	Dimensions (weight)	Sample preparation	Year	Ref.
Pot.	Anions and cations $^{-}$	Natural waters	8 µM NO_3^- 9 µM Ca^{2+}	first pCE custom built	34 x 17.5 x 17.5 cm (7.5 kg)	dilution, filtration 0.2 µm Nylon filter	1998	[44]
Amp. and volt.	Dopamine	No reported	0.5 µM	square-wave voltammetry, end-capillary det.	not reported	dilution	1998	[45]
Pot. and amp.	Cations and amino acids	Natural waters	0.1 to 10 µM	double detection system to extend applicability	34 x 17.5 x 17.5 cm (7.5 kg)	dilution, filtration 0.2 µm Nylon filter	1999	[46]
Amp.	Catecholamines, ascorbic acid, carbohydrates, and heavy metals	Road dust	from 0.5 µM	detection with a single electrode	34 x 17.5 x 17.5 cm (7.5 kg)	sample heated in HNO_3, filtered, washed, and diluted	1999	[47]
Amp., pot. and cond.	Anions, cations, heavy metals, carbohydrates, amino acids	Food matrices and natural waters	from 0.1 µM	uses three electrochemical detection methods	34 x 17.5 x 17.5 cm (7.5 kg)	dilution, filtration 0.2 µm Nylon filter	2001	[48]
C^4D	Cations	Natural waters	2.5 to 3.5 µM	incorporates flow injection system	not reported	No treatment	2004	[64]
C^4D	Anions and cations	Environmental samples	0.2 to 1 µM	enhanced sensitivity	31 x 22 x 26 cm	filtration 0.2 µm syringe filters	2007	[14]

C^4D	Phosphonic acids	Soil samples	2.5 to 9.7 µM	uses a cross-sampler injection system	33 x 18 x 13 cm (< 4 kg)	extraction with phosphonic acids for 1 h, sonication for 10 min, filtration 0.45 µm filter	2009	[65]
LIF.	DNA	Animal tissue	4.4×10^{-3} to 1.3×10^{-4} ppm	incorporates a LIF detector	44 x 27 x 13 cm (8 kg)	DNA extraction and amplification by PCR	2010	[71]
C^4D	Nerve agents	Teflon, ceramic tile, and concrete matrices	15.2 to 26 µM	incorporates milled polyimide blocks to facilitate sample injection	30 × 30 × 15 cm (5 kg)	extraction and filtration 0.45 µm filter	2011	[66]
C^4D	Anions and cations	Wastewater	1.5 to 17 µM	valve-based injection system, including a micromembrane pump for sample aspiration	not reported	filtration 0.45 µm membrane filter	2013	[67]
C^4D	Nitrogen mustard	Natural waters	5 µM	4-way manifold for automated introduction of sample and solutions	similar to [11]	no treatment	2013	[68]
C^4D	Cations	Porewater from sediment cores	sub µM range	incorporates MicroRhizon filter tubes for fast sampling	similar to [7]	filtration tubes, hydrophilic membrane of 0.15–0.20 µm	2013	[49]

C^4D	Scopolamine	Food matrices	600 ppm	development of simple sample pre-treatment methods	similar to [12]	vortex-mixed and dilution	2013	[75]
C^4D	Explosive residues	Sand, concrete, metal witness plates matrices	12 to 36 µM for anions 3.8 to 7.3 µM for cations	uses a dual-opposite end injection principle for fast separation	not reported	SPE, filtration with 0.45 µm filter	2014	[76]
C^4D	Beta-agonists	Pig-feed sample matrices	0.7 ppm	semi-automated CE system using pneumatic operation	40 × 28 × 21 cm (6 kg)	sonication, centrifugation and filtration 0.45 µm membrane	2014	[59]
C^4D	Anions and cations	Fireworks	lower µM range	incorporates an engraved flow-cell interface for autonomous injection	45 × 35 × 15 cm (8 kg)	dilution, stirred, filtration 0.45 µm syringe filter	2014	[69]
LIF.	DNA of influenza virus	Animal tissue	6.3×10^{-3} to 7.2×10^{-3} ppm	method combined PCR and pCE	similar to [9]	DNA extraction and amplification by PCR	2014	[72]
C^4D	Amphetamines	Tablets and urine	0.5 ppm	miniaturized high-voltage C^4D	similar to [16]	tablets: liquid extraction (LE), ultrasonication, filtration 0.2 µm Urine: pH 10–11, LE centrifugation, evaporation, re-dissolution	2015	[54]

C^4D	Rare earth elements	Ore samples	0.24 ppm	new arrangement of the pCE-C^4D for adaptation to the local infrastructure	not reported	digestion and filtration 0.45 µm ash-free filter paper	2016	[50]
C^4D	Anions and cations, artificial sweeteners	Food matrices	µM range	triple-channel pCE with different BGE to determine three different categories of charged analytes	45 x 35 x 15 cm (15 kg)	filtration 0.02 µm PTFE membrane filter, dilution and ultrasonication.	2016	[60]
C^4D	Anions and cations	Sediment porewater samples	2.8 to 18 µM	miniature automated syringe pump, can apply positive and negative pressures for full automation	52 × 34 × 18 cm (< 15 kg)	filtration tube of 1 mm diameter and 0.20 µm pore size	2016	[70]
C^4D	Formate	Biological fluids	0.319 µM	incorporates a PMMA interface for semi-automated hydrodynamic sample injection	20 × 33 × 17 cm (< 5 kg)	thawing and dilution	2016	[57]
C^4D	Cations	Tablets	0.10 to 1.25 µM for cations 0.13 to 1.03 µM for anions	include two C^4D detectors	not reported	ground into a coarse powder, dissolution in water	2016	[55]
C^4D	Pharmaceuticals	Natural waters and wastewater	0.2 to 0.8 ppm	dual-channel setup for high throughput operation	45 x 35 x 15 cm	filtration 0.45 mm membrane filters	2016	[51]

C^4D	Anions and cations	Rock surface	sub µM range	new technique for the analysis of available ions and ATP	similar to [13]	drop-on-rock extraction method	2016	[52]
C^4D	Food additives	Food matrices	0.7 to 5.0 ppm	manual or semi-automated operation, solution	similar to [16]	ultrasonication, centrifugation, filtration 0.45 µm PTFE membrane	2017	[77]
C^4D	Paraquat	Plasma	0.5 ppm	siphoning for sample injection	similar to [16]	solid phase extraction	2017	[56]
LIF	Illegal drugs	Oral fluids	0.5 µM	application of a deep UV excited fluorescence detector	20 × 10 × 30 cm (3 kg)	preconcentration, and extraction with Salivette tube	2018	[73]
C^4D	Inorganic nitrogen compounds	Wastewater	0.03 to 0.11 ppm	modified sequential injection analysis system	not reported	membrane filter system developed for direct sample aspiration	2019	[16]
C^4D	Taurine and choline	Food matrices	0.27 to 0.45 ppm	concurrent determination of analytes using two independent BGEs	similar to [25]	ultrasonication and filtration 0.2 µm PTFE membrane filter	2019	[61]
C^4D	Volatile and non-volatile chemicals	Air samples	1 µM	autonomous analytical system with sampling capability on a drone	16 x 12 x 12 cm (< 0.8 kg)	automated sample carrousel	2020	[74]

C^4D	10-hydroxy-2-decenoic acid (10-HDA) and free amino acids	Food matrices	39 to 90 ppm	dual-channelled CE-C4D instrument	similar to [25]	centrifugation and filtration 0.2 µm Nylon filter	2020	[62]
C^4D	Anions and cations	Environmental waters	2.1 to 6.8 µM	pressure-driven flow through injection setup	(20 kg)	on-line filtration 0.45 µm pore Nylon membrane	2021	[53]
C^4D	Ophiocordyceps sinensis-based products	Food matrices	11.2 to 22.0 ppm	dual-channelled CE-C^4D instrument	similar to [25]	ultrasonication, centrifugation and filtration 0.2 µm PTFE membrane	2021	[63]

Pot. = potentiometric, Amp. = ampherometric, Volt. = voltametric, Cond. = conductivity, LIF = laser induced fluorescence

1.5.1. Limitations

One of the main bottlenecks developing portable and on-site instrumentation is the sample preparation. In most cases, matrix components need to be removed from the analytes to make sample compatible with the injection and separation method, a process sometimes accompanied by concentration enhancement. Clean-up sample procedures can be a risk of contamination, loss of analyte, a source of systematic errors, and is often the most time-consuming step of the analytical process[78]. Some steps for sample preparation are difficult/impossible to automate and require manual operators [79,80]. These steps include extraction techniques which hold disadvantages like the use of fibres, membranes, and stationary phases, this can be expensive, fragile, and have limited commercial availability [81–83]. Other extraction techniques report the use of instrumentation for physical/mechanical processing such as centrifuge, vortex, microwave, stirrers, sonication, high pressure, temperature and/or an electric fields. Additionally, to render the analytes compatible with the detection method it may be necessary to derivatize the samples prior analysis. All these techniques make the sample preparation process long and reliant on operator intervention [54,69,76,84–86].

To deal with these disadvantages scientist have developed microdevices able to help with separation and preconcentration tasks. Laboratory instrumentation has been changing rapidly and new analytical devices are developed every day. The trend is to generate instruments and devices smaller, simpler, and easier to handle.

1.6. HISTORY OF MICRODEVICES

The story of miniaturized devices has been started last century when Terry, in 1975, created the first miniaturized instrument, a gas chromatography (GC). This GC instrument was fabricated in a 5 cm silicon wafer. The system included an open-tubular capillary column of 1.5 m long, sample injection valve, and a thermal conductivity detector. This GC was able to separate a simple mixture of compounds in less than a minute [87]. Regardless of the fast separation abilities and miniature size of this GC instrument, the adoption by the scientific community was minimal. Alternately, the research work associated to miniaturization on silicon was dedicated

to the production of components as micropumps, microvalves, and chemical sensors [88].

In 1990, Manz *et. al.* proposed the micro total analysis system (μ-TAS) concept and open a new era in analytical chemistry. In this experiment a CE was integrated on a glass chip, creating what is considered the first example of modern microfluidic technology [89]. The μ-TAS includes in one single platform, all the required steps for analysis such as loading and transferring the sample, chemical reactions, separation, and detection of analytes. Harrison *et.al.* demonstrated CE in micro channels made by etching a glass chip, in which they demonstrated the separation of six fluorescent-labelled amino acids with excellent separation efficiency, using lower voltage, faster analysis time, and a reduced platform size [90]. Following the μ-TAS conceptualisation several researchers and industries started to use and improve manufacturing process for a cheaper, flexible and more complex designs by modifying the fabrication with different materials like fused quartz, ceramics, plastics, polymers, paper, and 3D printed items [91–96]. Subsequently, the lab on a chip devices has been incorporated in a wide range of disciplines, mainly in biomedical, food and environmental fields [97].

1.7. MICRODEVICES TECHNIQUES FOR PARTICLE SORTING AND SEPARATION

Microfluidic systems have emerged as a technology solution in many laboratory applications, but current devices still need significant off-chip sample preparation procedures, which limit their applicability for in-field applications including chemical monitoring activities [98]. Perhaps, the most critical step for analysis in μ-TAS and miniaturized instruments, is the removal of particulate matter, which typically takes place before introducing the sample into the analytical system. Due to the small dimensions of the system, particles can cause blockages and other serious operational problems. The simplest way to avoid particulate matter is by filtering all samples and reagents, however, as filters need to be replaced when blocked this process may require regular human intervention [99]. Numerous efforts have been made to develop particulate removal steps that are less prone to blockages. As an alternative, microfluidic sorting devices have been studied widely for filter-free sample

preparation for µ-TAS and other field deployable instrumentation. Microfluidic sample preparation devices have been developed for matrix elimination, particle sorting, analyte pre-concentration and derivatisation, and for instrument interfacing [100–103].

Microfluidic separation and sorting techniques can be classified in two main particle separation methods, active and passive. Active methods use external forces such as electric and magnetic field, acoustic wave, and optical interaction, using the particle's specific dielectric, magnetic or optical properties, to separate different size particles. Passive methods depend on internal forces created by different geometries in the fabricated device [104], in addition to specific physical characteristics of the particles like size and density [105]. While active methods can provide higher selectivity, and sensitivity compared with passive methods, active methods require external energy input and hard/software control interfaces for operation [106]. These can increase the complexity of an automated portable instrument; hence the aim of this study will focus on the use of passive microdevices. A comparison of microdevices form microdevices efficiency, particle size and application, from the past 5 years is presented in Table 1.2.

1.7.1. Microfiltration methods

Microfiltration is the simplest method and is used to separate particles and cells according to size and deformability. It offers several advantages, including high separation efficiency, a simple geometry, and accurate control. Microfiltration can be classified into four categories based on the filter structures in the microchannel: weir, pillar, cross-flow, dead-end and membranes. Figure 1-2 present schematic of four type of microfiltration systems and its sorting mechanisms [106].

Weir filters consist of a barrier obstructing the flow path, meaning particles that cannot pass through a narrow window on the top of the barrier are blocked. Dead-end or pillar microfiltration includes a line of pillar structures, placed with specific spacing in between preventing the particles to pass, according to their size. In cross-flow pillar microfiltration, a series of pillars is placed perpendicular to the flow stream, allowing smaller particles to pass through the pillar spaces, while the bigger particles will be flushed away with the main flow stream. Membrane-based microfilters consist of a flat

layer substrate with specific pore size, where particles below the cut-off will be able to pass the membrane while bigger particles will be retained. Membranes can be operated in dead end or cross flow mode.

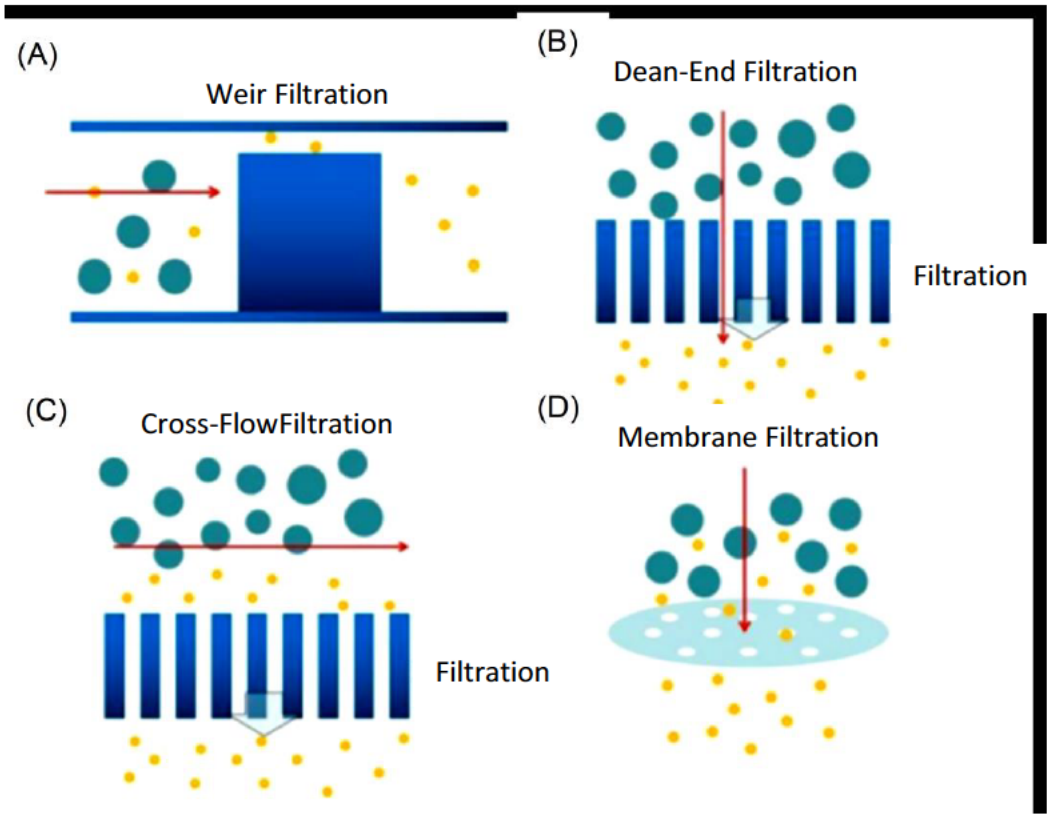
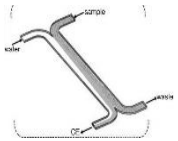
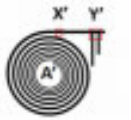
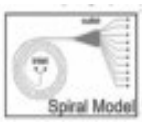
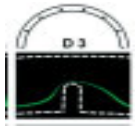
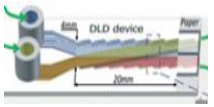
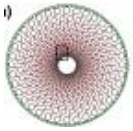
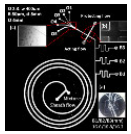
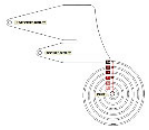
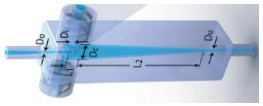
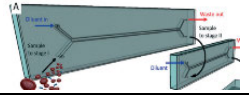
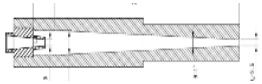


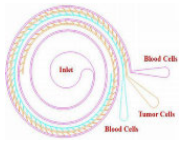
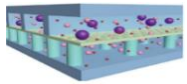
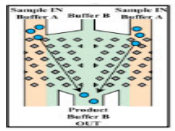
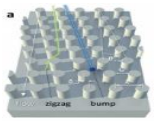
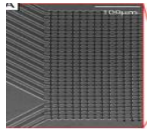
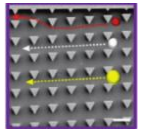
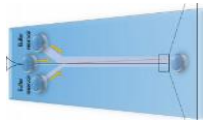
Figure 1-2 Schematic of microfiltration techniques (A) weir, (B) pillar, (C) cross-flow, (D) membrane, taken from [107].

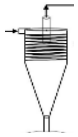
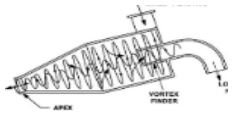
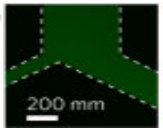
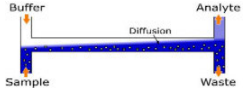
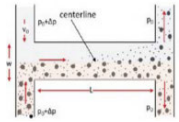
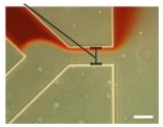
For microfabricated filtration devices, the design also needs to consider physical properties of the particles to separate, such as density, shape and deformability, in the case of cells. Physical filtration microstructures are simple to fabricate, allow continuous separation, are non-destructive separation method, and permit the integration with other separation techniques, making them crucial in the passive process. However, particles can accumulate on the membrane faces or between the pillars, causing low separation efficiency, clogging, and poor durability.

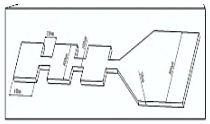
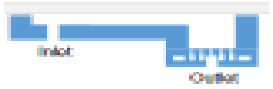
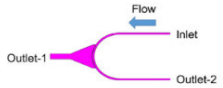
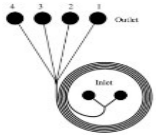
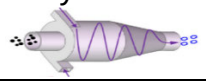
Table 1.2. Passive microdevices application form the last 5 years

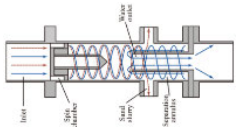
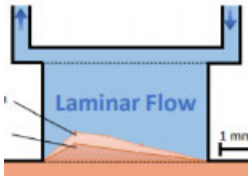
Type of microdevice	Sample matrix	Particle size	Flow rate	Efficiency	Application	Reference
H-filter 	Cells	10-16µm	8 µL/min	-	Clinical	[108]
Inertial 	Fluorescent beads	7, 10 and 15 µm	1800 µL/min	90%	Clinical	[109]
Inertial 	Polystyrene micro-beads	10-25 µm	0.5, 2 µL/min	90%	Clinical	[110]
Inertial 	Polystyrene particles	7.3, 9.9, 15.5 µm	3000 µL/min	98%	Clinical	[111]
DLD 	Blood cells	5.1-12.2 µm	4 µL/min	95%	Clinical	[112]
DLD 	Polystyrene particles	2, 10 µm	3000 µL/min	90%	Clinical	[113]

Inertial 	Polystyrene particles	1-5.5 µm	800 µL/min	95%	Food safety	[114]
Inertial 	Polystyrene beads	4-7 µm	1500 µL/min	90%	Clinical	[115]
Hydrocyclone 	Poly(methyl methacrylate) microspheres	5-20 µm	3800 µL/min	-	Microalgae harvesting	[116]
H-filter 	Bacteria from blood sample	1-2 µm	10 µL/min	79%	Clinical	[117]
Hydrocyclone 	Rare earth particles	2.45-34.67 µm	1406 L/h	92%	Slurry water sample	[118]
Hydrocyclone 	Metal organic frame (MOF-199)	10-500 µm	650 L/h	99%	Removal of sulphur from fuel	[119]
Inertial 	Polystyrene microparticles	10, 15, 20 µm	400, 2700 µL/min	-	Clinical	[120]

Inertial 	MCF-7 cells (human breast adenocarcinoma)	20-25 µm	50, 167 µL/min	90%	Clinical	[121]
Microfilter 	Polystyrene bead	4.5 µm	55, 167 µL/min	93%	Clinical	[122]
DLD 	Cells	4 µm	1167 µL/min	80%	Clinical	[123]
DLD 	Vesicles	0.095 µm	15 µL/min	50%	Clinical	[124]
DLD 	Bacteria	1.24 µm	0.017 µL/min	100%	Clinical	[125]
DLD 	Cells	6 µm	200 µL/min	80%	Clinical	[126]
DLD 	Polymer beads	0.05, 1 µm	0.05 µL/min	-	Clinical	[127]

Hydrocyclone 	Fly ash	0.1-1000 µm	3000 L/h	66%	Removal of azo dyes	[128]
Hydrocyclone 	Plastics	2800-8000 µm		40-58%	Environmental waste	[129]
H-filter 	Cow's milk, whole human blood	1-10 µm	0.5, 5 µL/min	-	Cell enrichment	[130]
H-filter 	Polymer beads	20 µm	80-250 µL/min	-	Clinical	[131]
H-filter 	Gold nanoparticles	24 µm	6 µL/min	93%	Clinical	[132]
PFF 	Spermatozoa from erythrocytes	45 * 4*1 um 7.5-8.7 1.7-2.2 um	3.3 µL/min	95%	Clinical	[133]
Inertial 	Sperm cells and micro-beads	3-5 µm	0.3 µL/min	-	Clinical	[134]

PFF 	Plasma	5-20 µm	10, 500 µL/min	96%	Clinical	[135]
Membrane microfilter 	Polystyrene particles	0.4, 0.8, 1, 2.2 µm	2, 10 µL/min	87%	Clinical	[136]
Cross-flow filtration 	Polystyrene microparticles	3, 8, 12, 25 µm	9, 45 µL/min	90%	Clinical	[137]
Inertial 	Polystyrene particles	0.81, 2.29, 4.70 µm	33 µL/min	98%	Clinical	[138]
H-filter 	Protein mixtures	0.001-0.006 µm	0.5, 5 µL/min	-	Clinical	[139]
DLD 	Cells	7.5 µm	15, 30 µL/min	-	Clinical	[140]
Hydrocyclone 	Homemade EMAH adsorber	0.5-6 µm	20 L/h	95%	Wastewater pollution	[141]

Inertial 	Polystyrene microspheres	8, 15 µm	3, 30 µL/min	95%	Clinical	[142]
Hydrocyclone 	Sand from slurry sample	27, 80, 200 µm	7000 L/h	32-95%	Mining	[143]
H-filter (midified) 	Mineral slurry Dissolved paracetamol tablets	40-300 µm 1-1000 µm	7-25 µL/min 8-25 µL/min	- -	Mineral monitoring Pharmaceutical	[144]

1.7.2. Hydrodynamic microfluidic separation and sorting techniques

At the microfluidic scale, the flow pattern is characterized by low Reynolds number, forcing particles in a liquid solution to follow the streamline. The particles subjected to shear flow, experience a lift force perpendicular to the streamline as well as forces from the channel wall. The equilibrium of these two forces is responsible for particle migration and will be affected by a number of variables, including channel shape, flow rate, rheological characteristics of the carrier fluid, and mechanical properties of the components. Size and shape based separation of the particles will be possible by modifications in the flow path, such as increase or decrease the flow rate, adding inlets and/or outlets, and by changing the geometry of the channels [145]. Different hydrodynamic microdevices have been created in the last decades, such as, deterministic lateral displacement, mini hydrocyclone, H-filter, pinch flow fractionation, viscoelastic and inertial. Schematics of some of these microfluidic devices are shown in Figure 1-3.

1.7.2.1. Deterministic lateral displacement

Deterministic lateral displacement (DLD) relies on microfabricated structures to manipulate the fluidic path of particles [146]. The array structures can be arranged to allow particles smaller than a critical radius ($a < Rc$) to migrate with the flow, but particles larger than the critical radius ($a > Rc$) will deviate under an angel as defined by the arrays. DLD can separate particles and cells not only based on their size but also shape and deformability [147].

1.7.2.2. Mini hydrocyclone

At the macroscale, hydrocyclones are simple and robust devices used to separate particles (for example sand) from a fluid without the need for moving parts other than a pump to drive the flow [116]. Previous studies have shown the separation efficiency will increase when the hydrocyclone size decreases,

opening opportunities for mini hydrocyclones [148]. The mini hydrocyclone uses the centrifugal force to separate solid particles form liquid samples. They consist of a cylinder-shaped chamber with a conical bottom and two inlets placed tangentially to the cylindrical compartment [106]. The device is fed through two inlets creating a rotational flow and generating two vortices. Three forces are imposed on the particles based on their shape, size and density: the centrifugal force (Fc), the buoyant force (Fb) and the drag force (Fd). Fc is related to the tangential velocity, Fb arises from the difference in density between the liquid and the particle, and Fd is caused by the fluid viscosity been opposite to the particle movement. Fd depends on the particle shape and size, along with the turbulence force of the flow [149]. Hence, if a particle is denser than the fluid will follow the primary vortex to the underflow. While for particles with smaller density, the centrifugal force will be eliminated by the drag forces, and the particles will remain disperse inside the cyclone and follow the flow to both outlets [116].

1.7.2.3. H-filter

The H-filter works based on the difference in diffusion between small molecules and particles. The size and shape of the particles control the diffusion process. As a result, small particles will have bigger diffusion coefficient and will travel a longer distance per time than large particles which present smaller diffusion coefficient. These difference in diffusion coefficient it is used to separate analytes form particulate matter over time. The size of the channel inside the H-filter control the time a particle will flow, so appropriate design of the micro channel allows to control the extraction of molecules with different diffusion coefficients [150]. The main factors affecting the diffusion are the size and shape of the particles; the viscosity of the solution; and the temperature [151].

1.7.2.4. Pinch Flow Fractionation

Pinch flow fractionation (PFF) microdevices feature two inlets, one to introduce the sample with the particulate matter and the second one to introduce particle

free buffer. The channels merge into a pinched section where the particles align to the side wall, following a centre line with a distance equivalent to their radius, moving across streamlines. After the narrow/shallow sector, larger particles locate at the centre, while smaller particles will exit at the side of the channel. The channel is then split into multiple outlets for collection of the fractionated particles [152].

1.7.2.5. Viscoelastics

Most of microfluidic systems are developed for biological and clinical applications, hence the majority operates on Newtonian fluid behaviour. But, for untreated biological samples with a viscoelastic nature the separation of cellular components become a challenge. Therefore, in recent decades the manipulation of particles suspended in viscoelastic fluids has been widely studied. The studies include simple channel geometries with single stream focusing. The particles in a viscoelastic flow migrate because the difference in stress produced by the arrangement of the molecules along the flow path [153].

1.7.2.6. Inertial microdevices

Another type of passive microfluidic device for particle separation is based on inertial forces. A microchip with curved channels can be used to force particles to migrate between streamlines by using the hydrodynamic forces of the fluid. Normally, a lift is induced by inertial forces created from the border effect of fluid stream next to the walls of a microfluidic channel. This process follows spiral patterns to order separate particles or cells [146]. While at the microscale the inertial forces should be consider negligible in fact, inertial separations work in an intermediate range amongst Stokes and turbulent systems, where both inertial and viscous forces in the fluid are finite. The finite inertial forces the fluid carries offers several interesting inertial effects that constitute the foundation of inertial microfluidics including inertial migration and secondary flow [154]. The inertial lift force focuses particles into an equilibrium position dictated by their shape and size and is the base principle to control and sort particles.

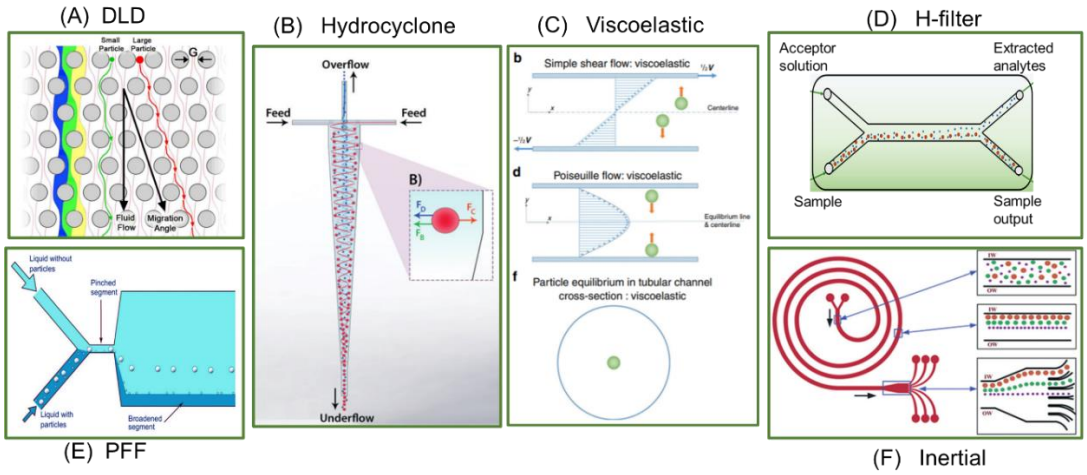


Figure 1-3 (A) DLD [155], (B) Hydrocyclone [116], (C) Viscoelastic [153] (D) H-filter, (E) PFF [152], (F) Inertial [156]

1.7.3. Limitations

Microdevices can be portable, easy to operate and cheap; but their operation may need complex, and precise pumps and systems to control the flow/pressure precisely. In addition, techniques to fabricate functionally integrated microfluidic devices can be complicated and expensive, and not many are cost viable for mass production. This makes some of the most sophisticated ‘‘first generation’’ microfluidic devices have not found a commercial use so far [157]. Here, 3D printing technology can be an effective tool to overcome the manufacturing challenges currently facing the microdevices market, allowing mass production in short time and at affordable cost.

Most of the applications of microfluidic cell/particle separation can be found for biological samples, including the separation of blood components and cells, and limited applications can be found for sample preparation of environmental waters. In environmental monitoring researchers continue to rely on paper filters and membranes. The focus of this research is to develop an alternative solution, building on the progress made in cell separation to identify the best methodology for sampling environmental water samples.

1.8. PROJECT AIMS

The literature overview presents the progress towards portable and field deployable instrumentation. Several capillary electrophoresis instruments have been developed to assist with monitoring tasks. While sophisticated microdevices have been developed for most steps of sample treatment, on-line particulate removal is under-developed and often relies on a dead-end membrane filtration. Driven by demands in medical and biological applications, the field of microfluidic particle separation techniques has rapidly developed. By separating the particles away from the target analytes, passive microfluidic devices provide an alternative to traditional filtration step. While single mode devices like the H-filter have been successfully coupled to on-line monitoring systems, a combination of complementary microdevices conducting orthogonal particle removal steps has yet to be reported.

The general aim of this work was to create a field deployable capillary electrophoresis instrument, which -built form low-cost components- can operate autonomously without operator intervention for extended periods of time. To achieve this, Chapter 2 presents a H-filter to extract target analytes from a sample containing small particulate matter. Following optimization of the flow conditions, the particle removal efficiency was evaluated over time.

In Chapter 3, the incorporation of a mini hydrocyclone is added to aid the removal of larger particulate matter, allowing for direct introduction of the flow into the H-filter. The versatility of the portable capillary electrophoresis (pCE) was then evaluated, by developing two different methodologies, one for the analysis of nutrients as chloride, nitrate and sulphate and other for the analysis of zinc, both in water samples. The instrument portability and autonomy are evaluated in Chapter 4 where a modified instrument is deployed at a remote location. This deployment will provide insight in the practicality of the pCE and in the technical challenges still to overcome.

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CHAPTER 2 : THE H-FILTER

2.1. ABSTRACT

A portable capillary electrophoresis (pCE) instrument was developed for environmental monitoring by online coupling of a H-filter to remove particulate matter larger than 1 µm. The acceptor flow from the H-filter was coupled to an automated sequential injection CE system for monitoring of water samples. The system was operated continuously for six weeks without the need for cleaning or replacement. Using a contactless conductivity detector (C^4D), the prototype was used for the detection of inorganic anions including Cl^-. NO_3^-, and SO_4^{2-} in a water sample containing particulate matter up to 100 µm. Limit of detection (LOD) values of 0.5 to 1 ppm of the anions were obtained. A total of 1575 separations was performed over a period of 6 weeks. Normalised with an internal standard, the relative standard deviation (RSDs) calculated for the peak height were 12%, 13% and 14% for Cl^-, NO_3^- and SO_4^{2-}, respectively; and 5% Cl^-, 1% NO_3^-, and 5% for SO_4^{2-}, respectively, for migration time. The developed device is simple to fabricate, low cost, easy to operate and can work autonomously for an extended period of time without the need for maintenance.

2.2. INTRODUCTION

Water quality has become a global issue of concern as human populations grow, industrial and agricultural activities expand, and climate change threatens to cause major changes to the hydrological cycle [1]. Monitoring nutrients/pollutants in the environment provides data that can be used to ensure our health and safety and to underpin measures to preserve the natural ecosystem. Environmental monitoring is vital to understand chemical cycles of important elements, the effect of exhaustive farming (e.g. nutrients such as nitrogen and phosphorous), and effectiveness of wastewater treatments (composition of effluents). Furthermore, early detection of a crisis, natural or anthropogenic, is important to facilitate an effective response [2]. Among these contaminants, there is a particular interest in nutrients as these can influence

the growth of harmful algal blooms, and cause hypoxia in freshwater and oceanic environments [3] along with human health effects [4,5].

Researchers around the world currently rely on spectroscopic and chromatographic techniques to detect and quantify nutrients in water samples [6,7]. These traditional methodologies require sample to be collected manually and transferred to the laboratory for analysis. Preservation of the sample is of vital importance to avoid degradation of target analytes between sampling and analysis. Additionally, treatment steps are required to protect the analytical instrumentation and/or avoid changes in method performance [8–10]. Sample treatment techniques include membranes and filters for particle removal [11–13], the use of fibres or resins for extraction [14,15], and manual processing for pH adjustment, and derivatization [16,17]. This complex workflow means that the analytical data is only reported days or weeks after samples are taken, lag time that compromises the ability for fast and informed decision making [18].

To address these issues, portable and on-site instrumentation has been developed to provide near-real-time information. An important advance has been the creation of chemical sensors for in-field measurements [19]. Sensors can achieve nM detection levels, can be used on-site, require small volume of reagents, are cheap, portable, and can be automated. However, some of the drawbacks of sensors are the reliability for long term use in complex environments, as their response can be perturbed by matrix effect, the use membranes or filters that need to be replaced, and they mostly detect one analyte at the time [19–21].

An alternative approach to produce sensor-like-data is to use a separation method such as chromatography or electrophoresis. There have been a number of reports of portable capillary electrophoresis (pCE) systems of varying degrees of complexity that have been used for environmental monitoring [8]. Kuban *et. Al.* developed a portable capillary electrophoresis instrument with capacitively coupled contactless conductivity detection (C^4D) to monitor cations and anions. Limit of detection (LODs) ranged from 0.2 to 1 µM in environmental samples, treated off-line by passing it through a 0.2 µm syringe filter [22]. Gaudry *et. Al.* developed a dual-capillary sequential injection-capillary

electrophoresis separation method coupled whit a C^4D to simultaneously separate and detect cations and anions from water samples. They reported the analysis of 900 samples of tap water continuously for a period of 48 hours, without a filter [23]. Fuiko *et. Al.* reported a CE-C^4D system to analyse inorganic nitrogen compounds in waste water samples, which was used continuously for a 24 hours periods using a 0.2 µm size membrane to filter the sample [24].

There have also been recent reports of portable ion chromatography (IC) systems. Boring *et. Al.* created a portable and computer-controlled IC, able to operate for more than 5 hours with a single battery. Using a pre-concentrator column, LODs of chloride 30 nM, sulphate 120 nM, and phthalate 250 nM, were obtained with RSDs up to 3.2% [25]. Kiplagat *et. Al.* reported the use of a portable IC using open tubular capillary columns and gravity-based eluent delivery. The instrument was operated continuously for more than 8 hours with repeatability under 2% and LODs in the micromolar range for common inorganic cations including Na^+, NH_4^+, K^+, Cs^+, Ca^{2+}, Mg^{2+} for river water, mineral water and snow samples [26]. Elkin also report the development of a portable, fully autonomous IC system. With a reproducibility up to 1% for a working period of 14 days the instrument detected anions including Cl^-, NO_3^{2-}, PO_4^{3-}, SO_4^{3-}, with sensitivity in the micromolar range [27]. Murray *et. al.* created a miniaturized capillary ion chromatograph with UV light-emitting diode to detect anions in potable and environmental waters. The system was able to analyse F^-, Cl^-, NO_2^- and NO_3^- with RSDs no higher than 1.25%, its robust, sensitive and uses off-the-shelf low-cost, miniaturized components [28].

Despite the potential of these instruments, all were either deployed for the analysis of relatively clean samples or required filtering, which if implemented on-line, is prone to clogging and blockages, decreasing reliability and increasing maintenance costs. Here, the focus is to improve the first and most critical part of sample collection for an autonomous analytical system – that of removing particulate matter. In this work, a H-filter is used as a sample preparation device for the removal of particles between 1-100 µm size present in environmental water samples prior to CE-C^4D analysis. Brody *et.al*, first demonstrated the use of silicon based H-filter for the separation of fluorescein dye from polystyrene

spheres from a sample stream [29]. Owing to its small channel size, the flow in a H-filter is typically low Reynold's number flow (Re<<1), implying transport is driven by diffusion rather than convection. As the rate of diffusion is inversely proportional to the size of the analyte/particle, a small particle can travel a longer distance than a large particle in a set time interval. This differential can be used to separate particles and molecules of different sizes [30]. The H-filter has mainly found applications for cell separation and clean-up of biological and medical samples [31–36]. Here, the simple structure, which can be manufactured at low cost, is used for the extraction of analytes from a particle rich sample and applied for the quantitative detection of chloride (Cl^-), nitrate (NO_3^-) and sulphate (SO_4^{2-}). Using perchlorate (ClO_4^-) as internal standard, the system was operated continuously for 6 weeks. The only operator intervention was to refill the electrolyte solutions.

2.3. EXPERIMENTAL

2.3.1. Materials and Chemicals

Background electrolyte (BGE) solution was prepared using analytical reagent grade Tris-(hydroxylmethyl) amino-methane (TRIS), 2-(cyclohexylamino)-ethanesulfonic acid (CHES) and sodium hydroxide (NaOH), obtained from Sigma-Aldrich (New South Wales, Australia). Solutions of chloride, nitrate, sulfate and perchlorate were prepared from analytical reagent grade potassium or sodium salts purchased from Sigma-Aldrich (New South Wales, Australia). Solid polymer microspheres (composed of polystyrene or polystyrene divinylbenzene or polymethylmethacrylate) of 1, 7, and 15 µm were purchased from Bang laboratories (Indiana, USA). Solutions were prepared in water from a Milli-Q Water Plus system from Millipore (Bedford, MA, USA), with a resistivity of 18.2 MΩ cm.

All fused silica capillary was obtained from Polymicro Technologies (USA). The capillary was coated in-house using a procedure provided by Eco Detection, Australia (https://www.ecodetection.com/). The coated capillary is also commercially available from Eco Detection (Melbourne, Australia).

2.3.2. Instrumentation

2.3.2.1. Capillary electrophoresis design

A miniaturized CE was assembled using mini peristaltic pumps purchased from Takasago (RP-Q1.2N-P20Z-DC3V Series, Takasago, Japan) to deliver liquid through a cross connector. The cross and other fittings are made of polyetheretherketone (PEEK) material and purchased form IDEX, USA. PEEK nuts and ferrules were compatible with 1.59 mm outside diameter (OD) tubing. Teflon tubing of 0.75 mm internal diameter (ID) was used for fluidic connections (Cole-Parmer, USA). A check valve (EW-30505-92, Cole-Parmer, USA) was placed between Pump 3 and the cross PEEK. A photograph and schematic design are shown in Figure 2-1.

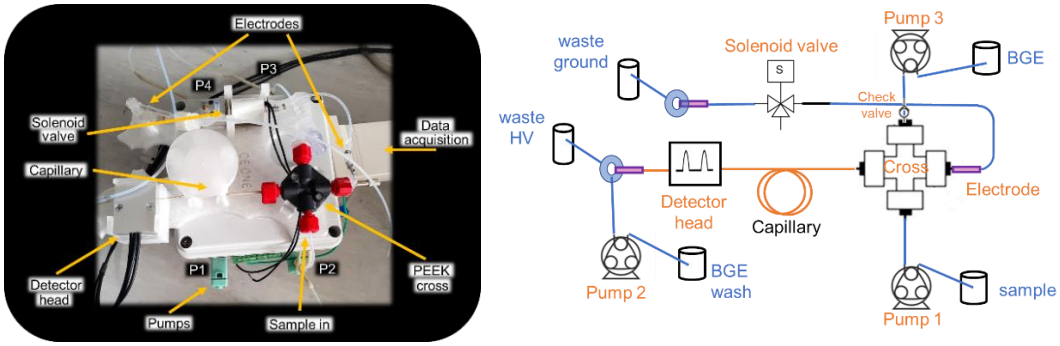

Figure 2-1 Schematic of the homemade portable capillary electrophoresis. In the left a picture of the instrument. In the right a fluidic schematic, explanation in the text.

Fused silica capillary of 10 µm ID was connected to the cross by a PEEK sleeve for sealing. The other end of the cross was connected to a 23G needle, with blunt tip. The metal needle was grounded externally to create a circuit loop which enable the electrophoresis process.

The grounded needle was connected to a mini solenoid valve (SV, MA332-VA11-L200, Gems Sensors & Controls, USA). The other end of the solenoid valve was connected to a Three-port F Style Manifold (part number 3PF230-6 Cole-Parmer, USA) where liquid enter from the lower port through the upper port connected to a waste reservoir (waste ground). The reservoir was levelled

to reduce hydrodynamic flow in the system. The capillary was inserted into a detector head Tracedec C^4D detector (Strassahof, Austria) and the end of the capillary was connected to a Three-port F style manifold (TPM) where BGE wash was pumped through, and solution exited to a waste high voltage reservoir, placed at the same height as the ground reservoir. A stainless-steel needle, 23G, was used as the high voltage (HV) electrode, sealed with epoxy glue and by clamping the tip. The HV power supply (Q101-5, EMCO, USA) provide 8 kV. The BGE and BGE wash were stored in separate reservoirs to avoid bypass circuit from the HV. Similarly, the waste reservoirs for both BGE solutions were separated. The system was connected and controlled through a 24-bit data acquisition module (DAQ, EMANT 300, Singapore) which converted the data from analog-to-digital. The electronic system, DAQ, and the data processing were controlled by an in-house program written in LabVIEW (National Instruments, USA)

Visualization of fluorescent particles was performed using Nikon high-definition colour CCD camera head (Digital Sight DS-Fi1c, Nikon, Japan) operated with NIS-Elements BR 3.10 software (Melville, NY, USA) mounted on an inverted fluorescence microscope (Ti-U, Nikon, Tokyo, Japan).

Particulate matter of the sample was analysed by particle sizer instrument Malvern, Saturn DigiSizer 5200.

2.3.2.2. H-filter Fabrication

The H-filter was designed by using drawing software SolidWorks 2017 (Dassault Systèmes, S.A., Vélizy, France). The H-filter was drawn in 2 separated parts, the base contained the channels geometry, and the top part contained the inlet holes. The design was transferred to a computer numerical control (CNC) machine, and H-filter was produced using a speedy 100 laser engraver (Trotec, Marchtrenk, Austria) over 2 mm thick cast acrylic sheet (Resiplex, Geelong, VIC, Australia). The parts were produced using a Datron M7 HP CNC milling machine (Datron, Mühltal, Germany). The 1 mm deep channels in the H-filters were machined using a polished single fluted 1 mm

endmill with a 0.3 mm depth of cut with feeds and speeds of 400 mm/min and 48,000 RPM, respectively. The last 0.1 mm was cut using a 0.2 mm stepover to improve the surface finish. The top layer of the chip containing the inlet holes was cut directly from the 2 mm cast acrylic sheet which had been pre laminated with Tesa 4965 transparent double-sided tape (RS Components, NSW, Smithfield, Australia) using the laser engraver. Finally, the parts were deburred, washed, dried, and assembled.

2.3.2.3. Dimensions

Figure 2-2A depicts the dimensions of the H-filter channel and Outlet Solution Reservoir (OSR) used in this work.

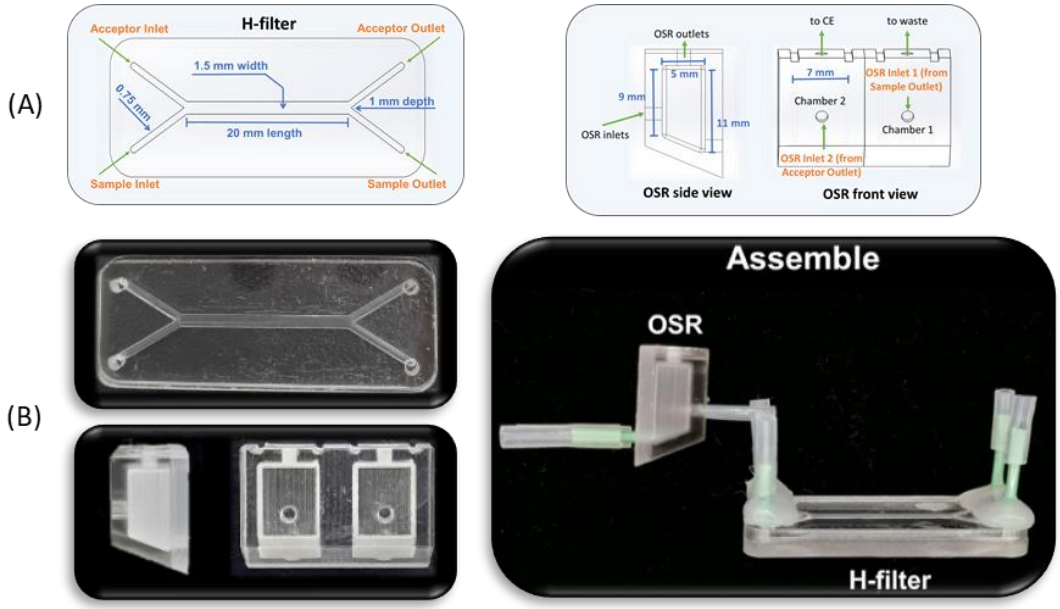

Figure 2-2 (A) Schematic of the H-filter and Outlet Solution Reservoir (OSR); (B) Picture of H-filter, OSR and H-filter-OSR assemble.

The H filter is designed with two inlet channels of 0.75 mm width by 1 mm depth. The two inlet channels meet in the middle to create a main channel of 20 mm x 1.5 mm x 1 mm length, width, and depth, respectively. The H-filter inlet and outlet channels are labelled as Sample inlet, where the sample is pumped into

the H-filter, Acceptor inlet, where an acceptor solution is introduced, Sample outlet, connected to the waste reservoir, and Acceptor outlet, connected to the CE for analysis. An Outlet Sample Reservoir (OSR) is connected to Acceptor outlet and Sample outlet of the H-filter to ensure that the height at the outlets is the same and the back pressure in the channels will not disturb the laminar flow as a small fraction of the acceptor outlet is taken by the CE instrument. The OSR connects the Sample outlet and Acceptor outlet into OSR chamber 1 and OSR chamber 2, which are connected to the waste reservoir and to the CE instrument, respectively. Figure 2-2A also depicts the OSR dimensions and the two chambers of 350 µL volume each. Figure 2-2B show pictures of the H-filter, OSR and the assembled system.

2.3.2.4. Integration of H-filter to the CE system

The H-filter was integrated into a portable homemade CE instrument developed at the University of Tasmania by our group (see section 2.3.2.1), with few modifications.

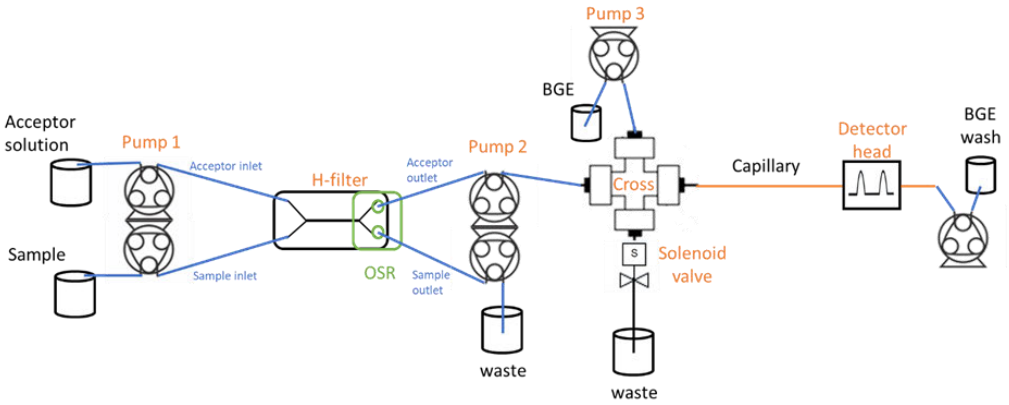

Figure 2-3. Schematic diagram of the homemade CE instrument. Sample and acceptor solutions are introduced to the H-filter by Pump 1 and exit to the OSR. There Pump 2 takes the Sample Outlet solution to a waste reservoir and the Acceptor Outlet solution into the PEEK cross towards the CE. The cross is connected to a BGE solution pumped by Pump 3 and a solenoid valve which switch between BGE and sample. These connects to the capillary and leads to the detector head, connected to the C^4D.

Figure 2-3 shows the schematic diagram of the portable homemade CE instrument and the position of the H-filter. In short, the CE instrument is equipped with four peristaltic pumps. Pump 1 and Pump 2 have been modified with a longer pump head to fit two separate tubes providing synchronization of the flow in the H-filter for both inlets (Pump 1) and outlets (Pump 2). Pump 1 is used to introduce the sample and acceptor solutions into the H-filter, Pump 2 is used to pump out the fluid from the H-filter to the instrument inlet and waste, Pump 3 and Pump 4 are used for the BGE solutions. OSR is connected to the H-filter Acceptor outlet and Sample outlet, prior to connecting them to the CE instrument and the waste reservoir, respectively. The BGE and the sample are introduced into the CE instrument through a PEEK cross which is connected to the capillary. Separation is quantified by a C^4D.

2.3.3. Testing procedures

2.3.3.1. Particle size diffusion testing

A fluorescein solution of 20 µM in Milli-Q water containing particle mix of 1, 7 and 15 µm size, was pumped through the H-filter from the Sample Inlet and Milli-Q water from the Acceptor inlet, at different flow rates of 50, 100, 200, 300, 400, 500, 750 and 1000 µL/min, for each inlet. The test was performed using a double syringe pump in continuous mode to control the flow rate. Visualization of the diffusion inside the H-filter was conducted using a Nikon high-definition colour CCD camera head (Digital Sight DS-Fi1c, Nikon, Japan) operated with NIS-Elements BR 3.10 software (Melville, NY, USA) mounted on an inverted fluorescence microscope (Ti-U, Nikon, Tokyo, Japan). Fluorescein was quantified measuring the colour intensity of the stream, particles were counted, both using ImageJ software. To verify the efficiency of particle diffusion inside the H-filter, the solution coming out from the 2 outlets was collected in separate vials and 1 µL of each solution was placed in a glass slide and was observed under a fluorescent microscope. This procedure was repeated 3 times. The number of particles in each fraction was counted with the aid of ImageJ software.

2.3.3.2. Electrophoretic analysis

Separation was performed using BGE of 200 mM TRIS−CHES solution (pH 8.0), 8 kV, and 600 s separation time. A coated capillary of 10 µm inner diameter and 25 cm length was used for the CE separation with detection via a C^4D detector. The parameters in the C^4D were: Frequency High; Voltage 0 dB; Gain 50% and Offset 000.

The electropherograms were analysed by an in-house LabView program to obtain the height of the peak and OriginLab software was used to obtain the area of the peak.

2.3.3.3. Soil slurry sample for continuous monitoring

A particulate laden sample for continuous monitoring was prepared by mixing 3 L of tap water from Chemistry building at University of Tasmania with 50 g of soil collected outside the Chemistry building (Sandy Bay, Tasmania, Australia). This sample was spiked with a solution of perchlorate to make a final concentration of 10 ppm. The sample was filtered offline using a 100 µm sieve to remove bulky materials like leaves, sticks, pebbles, stones, etc. During continuous analysis, the sample was stirred and inspected daily.

2.4. Results and Discussion

2.4.1. H-filter particle separation

The principle of particle removal using the H-filter is dependent on the absence of turbulent mixing in the micro channel. The flow of liquid from the 2 inlets moves in parallel with each other at the main channel of the H-filter by laminar flow [33].

Within the H-filter, molecules diffuse from zones of high concentration (sample solution) to zones of low concentration (acceptor solution). The diffusion coefficient, which governs the extent of diffusion, is inversely proportional to the size of the molecule/particle. The diffusion coefficient of a 0.5 µm size particle

is 0.5 $\mu m^2/s$, while the diffusion coefficient of a small inorganic ion such a Na^+ is 1000 $\mu m^2/s$, therefore we can assume the anions will diffuse to the acceptor stream at the proposed H-filter dimensions while diffusion of a 0.5 µm size particle will be significantly less [14,16].

In order to design the adequate geometry for the H-filter channels a river water sample was observed under the microscope and compared with a mix of 1, 7 and 15 µm particles. Depicted in Figure *2-4* On the left a river water sample observed under the microscope, on the right a solution containing a mix of 1, 7 and 15 µm particle size. The particles found in the river water sample can be compared with the size of the particles in the commercial mix.Figure 2-4 is the comparison of the river water sample and the commercial particle mix. It can be observed the main component are particles of 1 µm size, but particles of sizes 7 and 15 µm can be comparable between the two solutions. Based on these observations, the H-filter geometry was designed to be able to receive several particles of 15 µm without clogging.

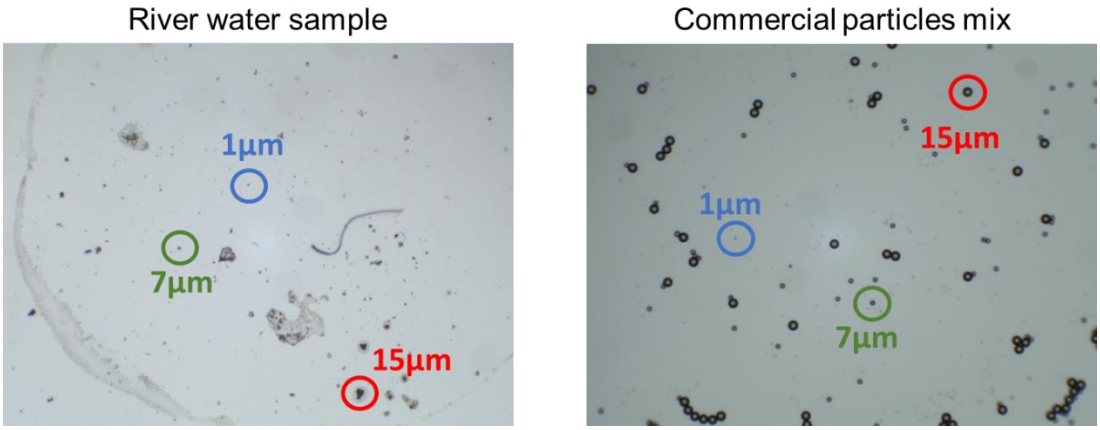

Figure 2-4 On the left a river water sample observed under the microscope, on the right a solution containing a mix of 1, 7 and 15 µm particle size. The particles found in the river water sample can be compared with the size of the particles in the commercial mix.

2.4.2. Optimisation of flow rate in the H-filter for particle separation

Figure 2-5A visualises the flow of fluorescein solution through the H-filter at different flow rates. The flow rate is the same for sample inlet and acceptor inlet for each experiment. The microscope was focused on the middle channel

immediately before the sample and acceptor outlet branch. As shown in Figure 2-5A, when the flow rate is 100 µL/min fluorescein travelled 86 µm for a residence time of 9 s. The value of residence time was calculated using the H-filter dimensions: 1.5 x 1 x 20 mm, hence a volume of 30 µL divided by the flowrate represents the residence time for a set flowrate. When the flow rate increases and the resident time decreases, the distance of fluorescein diffused decreased respectively.

The diffusion equation $\sqrt{2Dt}$ was used to calculate the theoretical travel distance for particles of 1 µm size at different flowrates. Where fluoresceine diffusion is D = $4.20E^{+02}$ $\mu m^2/s$, and t is the residence time in seconds for each flow rate. The experiment, test and recorded the distance travelled for fluorescein and 1 µm size particles for flow rates of 50, 100, 200, 300, 400, 500, 750, and 1000 µL/min. The results are shown in Figure 2-5B. Particles of size 7 and 15 µm were tested, but quantification was not possible, the reason might be sedimentation on the syringe while conducting the experiment. The theoretical migration distance travel for fluorescein is plot against the experimental results in Figure 2-5B. the results differ from 1 to 19%.

The fluorescein was quantified measuring the middle channel, near to the outlets. To determine the number of particles, sample was collected from the outlets and counted under fluorescent microscope, using ImageJ software.

As shown in Figure 2-5B, at the lowest flowrate of 50 µL/min fluorescein diffuses, from the middle of the H-filter into the acceptor channel, up to 123 µm and around 10% of the particles were observed in the acceptor stream. As the flow rate is increased, the residence time decreases and hence the number of particles in the acceptor decreases, as does the amount of fluorescein. The optimum flowrate was chosen at 300 µL/min as fluorescein still diffuses into the acceptor channel, travelling 54 µm form the middle of the channel, but less than 4 % of the particles have diffused into the acceptor.

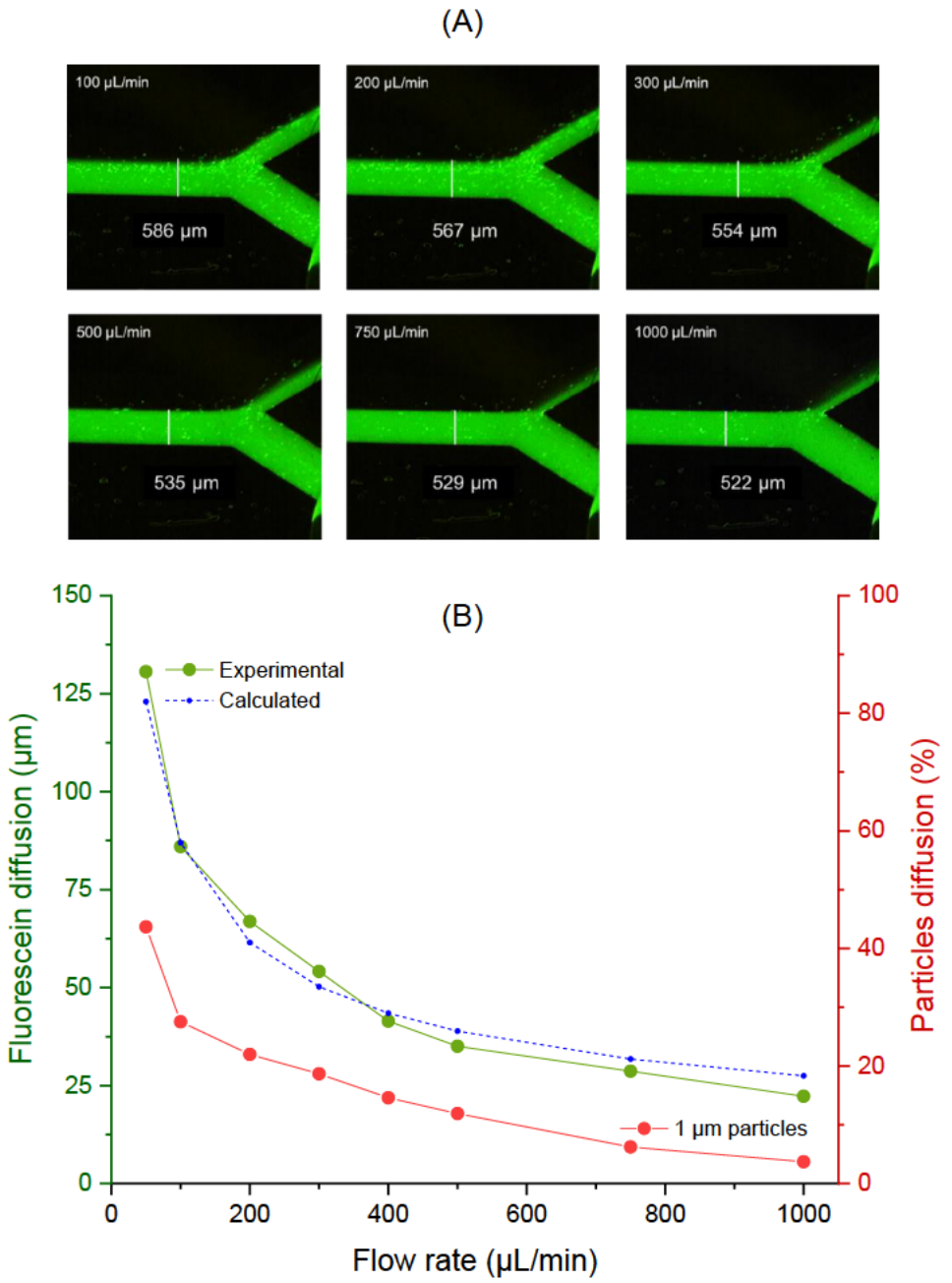

Figure 2-5 (A) Fluorescein solution inside the H-filter at different flow rates from 100 to 1000 µL/min, the flow rate is the same for both inlets. Each photo shows the measurement of the distance occupied by fluorescein at different flow rate and resident time. (B) Graph shows a mix solution of 1 µm particles and fluorescein, flow rates for sample and acceptor inlet are 50 to 1000 µL/min. The x-axis is the flow rate and, in the left y-axis (green plot) the distance travelled by fluorescein in µm. In the right y-axis (red plot) the diffusion of particles in %.

After optimization of the flow rate, the particle removal performance of the filter was tested with a mixture of 1, 7 and 15 µm fluorescent particles passed through the H-filter, entering form the sample inlet at a flow rate of 300 µL/min. From the

acceptor inlet water was introduced at a flow rate of 300 µL/min. The number of particles in sample outlet and acceptor outlet were counted. Figure 2-6Figure 2-5 shows fluorescent microscope images of the sample before the H-filter (Sample in (A)), and the sample outlet (B) and acceptor outlet (C). The Sample outlet contains the 1, 7 and 15 µm sized particles, while the acceptor outlet only 1 µm size were found. This confirms the target analytes will diffuse into the acceptor, while the particulate matter, bigger than 1 µm, will remain in the sample stream. For the purpose of this work, we are satisfied that the 1 µm particles do not pose a threat to the analytical system, hence we decided not to further optimise the fluidic system to also remove these particles.

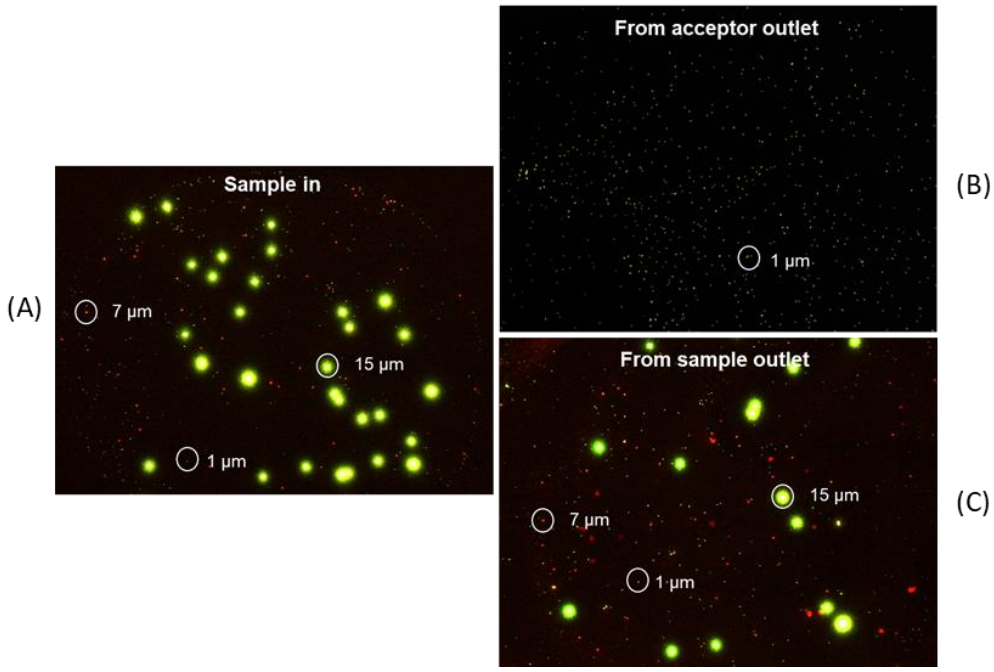

Figure 2-6. A solution mix of 1, 7 and 15 µm particles was pumped into the H-filter at a flowrate of 300 µL/min for the sample inlet and 300 µL/min for the acceptor inlet (A) shows the mix of particles before passing through the H-filter from Sample inlet. (B) picture of the solution collected from Sample outlet; (C) picture of the sample collected from Acceptor outlet. 4X objective was used to visualise the particles.

2.4.3. CE Comparison of standard solution with and without the H-filter

A quantitative evaluation of the H-filter was conducted by CE measurements using standard solutions of Cl^-, NO_3^-, and SO_4^{2-} at 10 ppm concentration. Perchlorate was used as an internal standard. Analysis with and without the H-filter was performed in triplicate and the relative response calculated by dividing the peak areas of the analytes with the H-filter by the peak areas of the analytes without the H-filter, multiplied by 100%. For the tested anions, the response was 35% when compared with the direct analysis without the H-filter, which is appropriated for the diffusion-based extraction. Recognising an efficient H-filter would result in a response of 50%, this gives a relatively recovery of 70%. The relative standard deviation (RSDs) of peak areas of all analytes performed without the H-filter were 7%, 7% and 5% for Cl^-, NO_3^- and SO_4^{2-}, respectively and with the H-filter 5% for Cl^-, 9% for NO_3^- and 6% for SO_4^{2-}. With the H-filter, the limit of detection (LOD) was calculated at 0.6 ppm for Cl^-, 0.5 ppm for NO_3^- and 1 ppm for SO_4^{2-} and the limit of quantification (LOQ) was 2.1 ppm for Cl^-, 1.7 ppm for NO_3^- and 3.4 ppm for SO_4^{2-}.

2.4.4. Application to real sample – Continuous monitoring

To evaluate the efficacy of the H-filter for long-term removal of small particles (Section 2.3.3.3), a soil slurry (17 g/L) was spiked with 10 ppm of perchlorate as internal standard. The IS was added directly to the sample to control and correct the fluctuation due to fluidic process inside the H-filter. The target analytes Cl^-, NO_3^- and SO_4^{2-}, were chosen based on the impact nutrients can have on the crops, aquatic animals, oceanic environment, and collateral effects in human health.

The photographs of the sample in, sample out and acceptor out solutions shown in Figure 2-7 illustrate the efficacy of the H-filter in the removal of particulate matter. The sample outlet solution is similar colour to the original sample. No particulate matter was visually detected in the vial collecting the Acceptor

effluent, an observation in agreement with the study conducted with the fluorescent beads.

To characterise the efficacy of the H-filter removing particulate matter, the solutions were characterised by a particle sizer.

Figure 2-8 shows solids load in mg/mL (y-axis) of particle size in µm (x-axis) found in the sample.

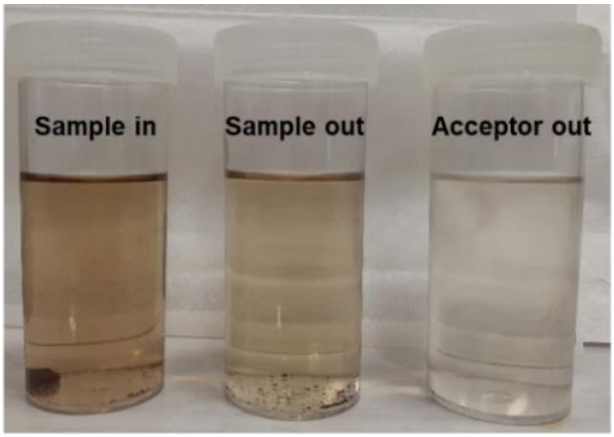

Figure 2-7. Photograph shows, from left to right, Sample introduced in the H-filter; sample collected from Sample Outlet, sample collected from Acceptor Outlet.

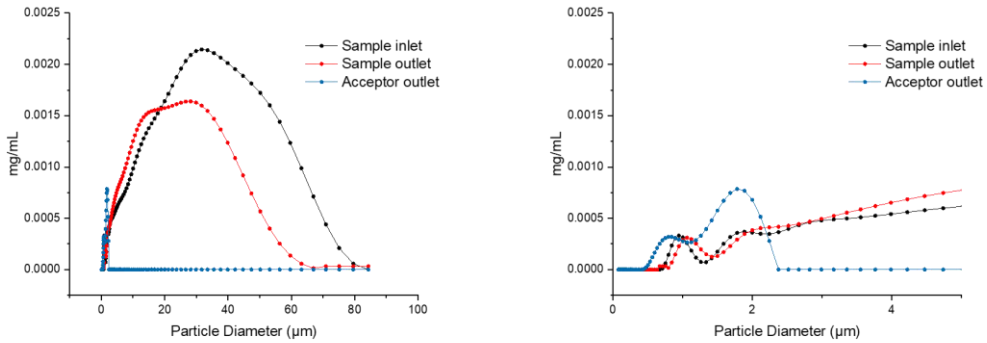

Figure 2-8 Distribution of the particle size of suspensions at the sample inlet, sample outlet and acceptor outlet. A zoomed section of the graphic (right) shows particles leaving the H-filter through the Acceptor Outlet are 1 to 2 µm in size while the sample collected from Sample Outlet reflects the particle range present in the original sample.

There is a high level of similarity for the particulate size between the prepared soil slurry sample before was it was passed through the H-filter and that collected from the Sample outlet. Particles larger than 40 µm are less represented, but as they are also not in the acceptor phase, this finding suggests the larger particles do not readily enter the H-filter. In the Acceptor outlet, only particles from 1 to 2 µm size are observed. These results are in good agreement with the findings with the fluorescent particles.

The longevity of the CE instrument when combined with the H-filter for particle removal was examined by monitoring the soil slurry sample continuously for 6 weeks following to the procedure in Section 2.3.3.2. A total of 1575 separations were performed with a sample-to-sample time of around 40 min. Representative electropherograms are presented in

Figure 2-9A which shows the first electropherogram at the start of the monitoring test (blue), the middle of the experiment (red) and at the end of the experiment (black). These electropherograms show the inorganic anions remain well resolved throughout the experiment, with only some shifts in migration times observed. Importantly, at no point was the CE instrument blocked by the particles present in the soil slurry.

Automated data analysis was conducted for every run, with the resulting data for the 1575 separations recorded over the 6 weeks shown in Figure 2-9B. The RSDs calculated for the peak height are 20% for Cl^-, 21% for NO_3^-, 16% ClO_4^- and 21% for SO_4^{2-}. Normalising to the IS, these reduced to 12%, 13% and 14% for Cl^-, NO_3^- and SO_4^{2-}, respectively. The migration time RSDs are 11% Cl^-, 9% NO_3^-, 9% ClO_4^- and 5% SO_4^{2-} for raw data and 5% Cl^-, 1% NO_3^-, and 5% for SO_4^{2-} when normalized against ClO_4^-. While these RSDs are higher than the data obtained without the H-filter, they are still acceptable due the limitations of the portable system, including the absence of temperature regulation, and the use of a data analysis algorithm developed in-house that is not optimised for this application. We anticipate improved performance as some of these issues are addressed in the future.

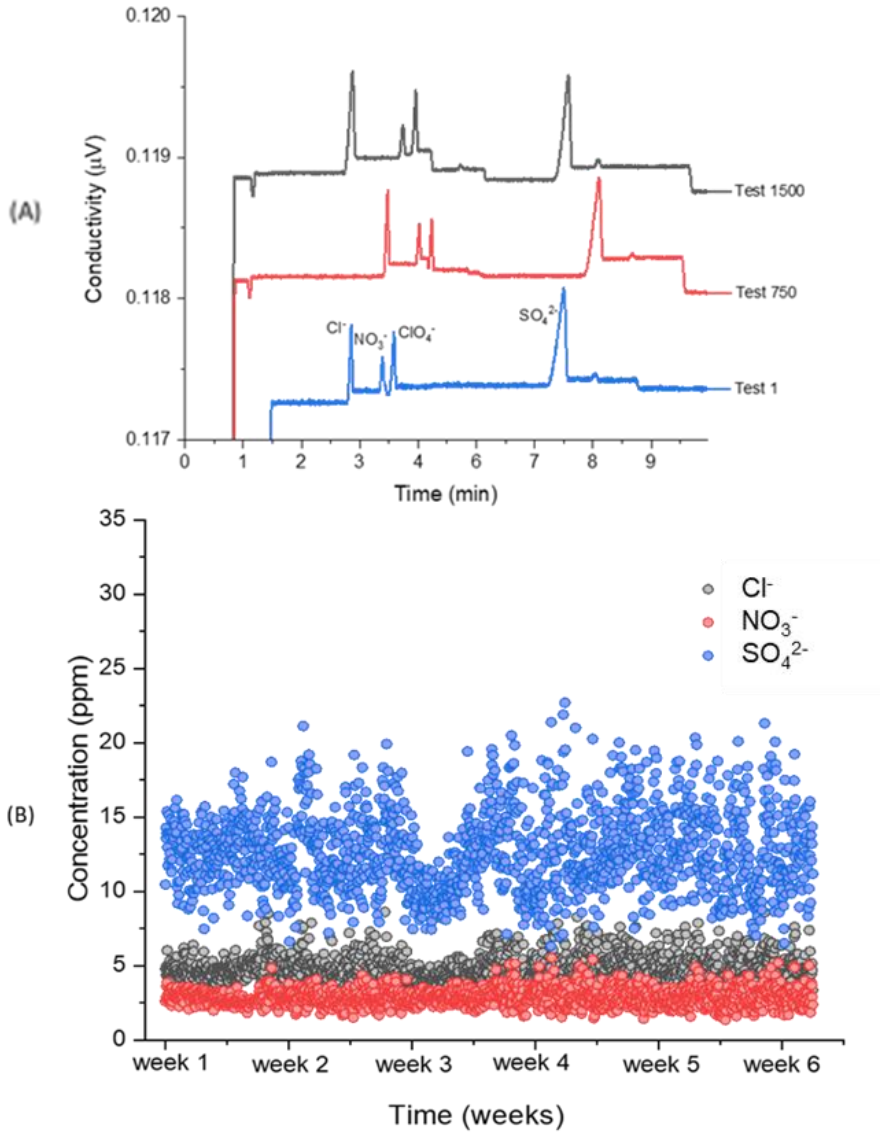

Figure 2-9 (A) Representative electropherograms of the slurry sample during continuous monitoring from Test 1, Test 750, and Test 1500. (B) Analysis of Cl^-, NO_3^-, and SO_4^{2-} in soil slurry sample. The CE was in operation continuously for 6 weeks. Data was normalized using ClO_4^- as IS. Capillary was 25 cm long, 10 µm ID, BGE 200 mM CHES/TRIS.

2.5. CONCLUSIONS

A machined microfluidic H-filter was successfully interfaced with an in-house built CE system as a particle removal microdevice prior to CE analysis. The optimized flowrate significantly contributed to the efficiency of the removal of particles >1 µm continuously from a “dirty” sample. The setup was utilised for a continuous monitoring of inorganic anions for 6 weeks, using a sequential

injection CE system with conductivity detector to separate and quantify Cl^-, NO_3^-, and SO_4^{2-} analytes in the water sample. The obtained RSDs of the results for the inorganic analytes demonstrated the robustness of the system and the possibility of autonomous operation in the field. Future directions arising from this work will focus on the deployment of the system in the field and will require technical improvements including operation from a battery. Taking advantage of the versatility of CE as separation method, the separation could be adapted for the analysis of different analytes such as heavy metals.

2.6. REFERENCES

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CHAPTER 3 : THE HYDROCYCLONE

3.1 ABSTRACT

Removal of particulate matter is crucial in the development of autonomous portable instrumentation. The pCE reported in Chapter 2 was modified by coupling a mini hydrocyclone for the removal of particulate matter over 100 µm in size. The hydrocyclone was produced in a PolyJet printer and used to remove particles larger than 90 µm. To improve the versatility of the pCE instrument reported in previous chapters, the C^4D was replaced with an LED detector. Various environmental water samples with different particulate solid load were analysed and Zn^{2+} was separated and detected with concentrations of 0.46 and 0.98 ppm. The pCE was tested on site in an industrial water treatment plant with an 82% of recovery, results were comparable from the test on the lab.

3.2 INTRODUCTION

Aquatic environments are affected by different sources of pollution, including many hazardous chemicals. Among environmental contaminants special concern is given to metals. Metals can enter freshwater ecosystems from atmospheric precipitation, geologic weathering or industrial and domestic discharges [1]. Presence of metals at high concentrations in water bodies is alarming as they are toxic, non-degradable and bio accumulative – metals can be absorbed by plants and animals, thus reaching humans. [2].

Metals are commonly detected using spectroscopic, electrochemical, and optical methods. Spectroscopic detection for metals include atomic absorption spectroscopy (AAS) [3–6], inductively coupled plasma mass spectroscopy (ICP-MS) [7–9], X-ray fluorescence spectrometry (XRF) [10,11], and inductively coupled plasma-optical emission spectrometry (ICP-OES) [12,13]. Those techniques can detect a range of different metals simultaneously with low limit of detection. The drawbacks are the necessity of specialised personnel and high instrumentation cost, increasing the cost per sample [14]. Electrochemical methods, compared with spectroscopic techniques, are faster, lower cost, and

user-friendly [15,16]. Though, these methodologies often present lower sensitivity and habitually require improvements and adjustments in the design to increase their performance in detection of metal ions [17]. Optical techniques which include fluorescence, colorimetry, and spectral change, are highly selective, sensitive, low cost, and had valuable applications in environmental monitoring and biological sciences. Disadvantages for optical techniques are poor selectivity, performance is affected by pH of the matrix, and often need additional reagents to perform [18,19]. Separation-based techniques like capillary electrophoresis (CE) and high-performance liquid chromatography (HPLC) including ion chromatography (IC) also play an important role in metal analysis. These techniques are sensitive, robust, and can be coupled to different detector systems. The drawbacks of separation-based analytical techniques include lengthy and complex samples needing long and complicated preparation procedures, which may include filtration, extraction, enrichment and derivatization. Instruments also tend to be expensive, and require skilled operator [20].

Sample preparation is a crucial step for environmental analysis and analytical laboratories require multiple sample preparation steps when analysing environmental samples [21]. Hence, for in-field detection miniaturization and automation of sample preparation techniques is important [22]. Peng *et. al.* reported a battery-operated portable optical emission spectroscopy (OES) able to determine Cd, Hg and Pb simultaneously, with limit of detection (LOD) between 33 to 253 ppm [23]. Unfortunately, the workflow included a complex sample preparation procedure where the sample was dissolved in acid, heated for several hours, and filtered. Other researchers implemented laser-induced breakdown spectroscopy (LIBS) as an in-situ analytical method for multi-elemental analysis, reporting a LOD for Zn^{2+} of 5 ppm [24]. Wang *et. al.* used similar instrumentation to analyse Cd, Cr, Cu, Ni, Pb and Zn, at ppb levels, continuously for 5 days but, with high variability when compared with traditional techniques (up to 20%) [25]. Li *et. al.* developed a portable six-electrode electrochemical sensor to detect heavy metals in liquid samples. The instrument

was equipped with a disposable plastic pipette, the authors reported the analysis of Pb, Hg, Cu, and Zn with no interferences, and LODs from 0.0022 to 0.0155 ppm [26]. Among many other miniaturised instrumentation for analysis and detection of metals in environment, these techniques still present low reproducibility and stability, limited capability of simultaneous analysis, the use of toxic interface materials as well as the challenge of mass production, reusability and most important sample pre-treatment are usually not provided in the miniaturised analysis system, which hinders its practicability [27–29].

In the previous chapter, a portable capillary electrophoresis (pCE) instrument was coupled with a H-filter and shown to operate continuously for several weeks without operator intervention. The dimensions of the H-filter allowed for the system to operate with samples containing particulate matter <100 µm size. The target of this work is to monitor environmental water samples which will include an extended size range of particulate matter. In this work the aim was to increase the particle size tolerance through the addition of a mini hydrocyclone.

Hydrocyclones have been widely used at a macro-scale device for fluid/particle separations in different industries. Cyclones use the centrifugal force to separate solid particles from liquid samples. They consist in a cylinder-shaped chamber with a conical bottom and two inlets placed tangentially to the cylindrical compartment. Inside the device, the pressure of the fluid coming through two feed inlets creates rotation of the liquid generating two vortices. The primary vortex is the outer one which pushes particles toward the wall and makes them follow the flow to the bottom outlet (underflow). The secondary vortex is a low-pressure zone across the vertical axis which drives the particles to the centre of the hydrocyclone and they flow to the top outlet (overflow) [30]. Complementing the applications at the macroscale, their use in microfluidic applications started only in the last decade [31]. One of the obstacles when producing mini hydrocyclones is that they are difficult to make by the traditional fabrication techniques. Recently the fabrication of a mini hydrocyclone by 3D printing technology was reported, opening the possibility for low-cost, large, precise, and reliable production technique [32].

In this research, 2 sample clean-up microdevices, a hydrocyclone and a H-filter, are combined in series for the removal of all particulate matter in environmental water samples. The hydrocyclone and a H-filter remove all particles above 90 µm and 1 µm, respectively, producing a clean sample for the analysis of Zn^{2+} by pCE with no clogging or blockage of the instrument.

3.3 EXPERIMENTAL

3.3.1 Materials and Chemicals

Background electrolyte (BGE) solution was prepared using analytical reagent grade N-Tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid (TAPS), 4-(2-Pyridylazo resorcinol monosodium salt hydrate (PAR), and sodium hydroxide (NaOH), obtained from Sigma-Aldrich (New South Wales, Australia). Co^{2+} and Zn^{2+}, were prepared from analytical reagent grade chloride or nitrate salts purchased from Sigma-Aldrich (New South Wales, Australia). Solutions were prepared in water from a Milli-Q Water Plus system from Millipore (Bedford, MA, USA), with a resistivity of 18.2 MΩ cm. Veroclear-RGD810 print material and SUP707 water-soluble support were purchased from Stratasys, Ltd. (Eden Prairie, MN, USA).

Commercial Solid polymer microspheres particles (composed of polystyrene, polystyrene divinylbenzene or polymethylmethacrylate) of 1, 7, and 15 µm were purchased from Bang laboratories (Indiana, USA). Commercial polyethylene microspheres of 90 and 250 µm were obtained from Cospheric (Santa Barbara, CA, USA).

All fused silica capillary was obtained from Polymicro Technologies (USA).

Detection was achieved with a homemade visible LED absorbance detector. The polyimide coating of the capillary was removed by burning it in a small section to obtain a detection window ~4.5 cm from the end. The capillary was inserted into an optical interface (OI, alignment interface, coded Green, Agilent, USA).

Light-emitting diode (LED) 500 nm, (Nichia, Japan) was used as the light source and a light-to-voltage sensor (PD, TSL257, AMS AG, Austria) as the photodetector.

The H-filter was fabricated as indicates in Chapter 2 of this thesis. For the mini hydrocyclone, the STL file from Syed *et. al.* [32] was 3D printed by Object Eden 260VS, in transparent material.

Samples were collected from different locations in Tasmania, Australia. The samples were not treated before injection into the instrument. To find the solid loading of each sample, 100 mL was placed in a glass vial, evaporated and the solid was weighed.

3.3.2 Instrumentation

The homemade pCE instrument was developed at University of Tasmania. The system schematics, parts and functioning are explained in detail in Chapter 2 of this thesis.

Visualization of fluorescent particles was performed using Nikon high-definition colour CCD camera head (Digital Sight DS-Fi1c, Nikon, Japan) operated with NIS-Elements BR 3.10 software (Melville, NY, USA) mounted on an inverted fluorescence microscope (Ti-U, Nikon, Tokyo, Japan).

Particulate matter of the sample was analysed by particle sizer instrument Malvern, Saturn DigiSizer 5200.

For devices fabrication an Objet Eden 260VS professional 3D printer (Stratasys, Ltd. Eden Prairie, MN, USA) was used.

3.3.3 Integration of the mini hydrocyclone to the pCE system

Modifications to the H-filter-pCE instrument reported in Chapter 2 of this thesis were made to attach the hydrocyclone on-line with the pCE. The schematics of the instrument are shown in Figure 3-1. The hydrocyclone was feed by a 12-volt pump and the overflow was collected in a vial, while the underflow was sent

to waste. The collected overflow was used as the sample inlet in the H-filter, with the other inlet being the acceptor solution. The Acceptor outlet is connected to a PEEK Tee which joins PAR outlet from Pump 6, incorporating PAR solution to complex the metals post-filtering. The outlet of the PEEK Tee connects to a mixing coil to next enter the PEEK cross which leads to the capillary. The C^4D detector used for experiment in Chapter 2 was replaced for a LED absorbance.

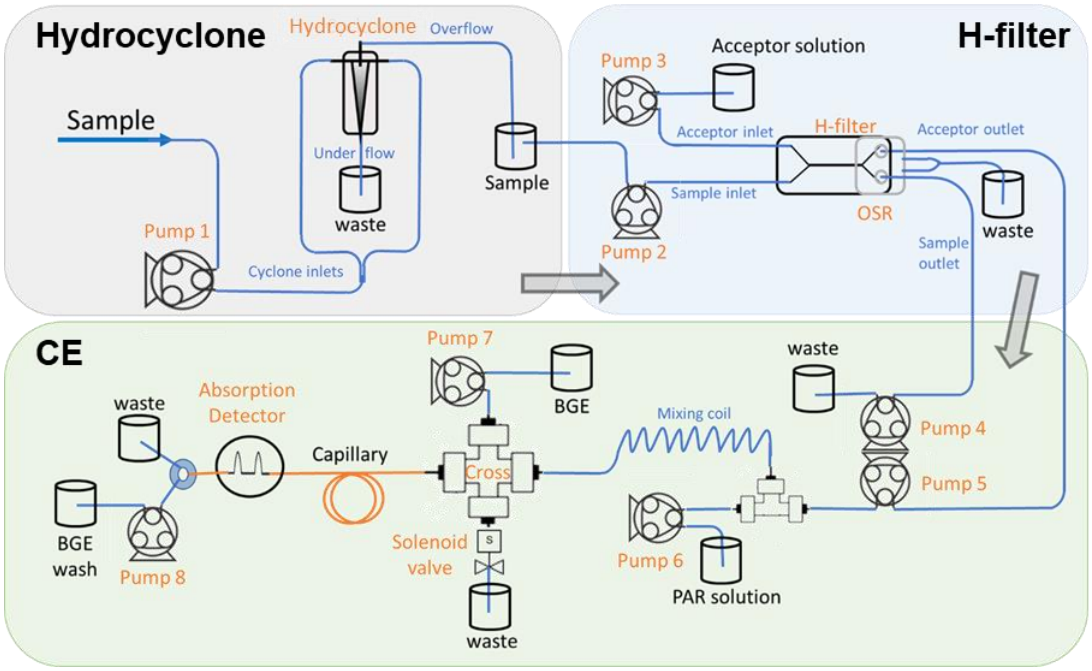

Figure 3-1 Schematics of the hydrocyclone-H-filter-pCE online system. Explanation in the text.

3.3.4 Particle size sorting analysis

To verify the efficiency of particle sorting in the hydrocyclone, a solution of commercially sourced particles of 90 and 250 µm was used. The particles were redispersed in a 1% Tween20 solution. The solution was introduced into the hydrocyclone from the hydrocyclone inlets. The experiment was performed using a 12-volt peristaltic pump at a flowrate of 4 mL/min. The solution coming out from the underflow and overflow were collected in separate vials and 1 mL of each solution was placed on a glass slide and was observed under a microscope. This experiment was repeated 3 times. The number of particles that goes to both outlets was counted with the aid of ImageJ software.

A second test was performed using 1, 7 and 15 µm particles size. Similar to the previous experiment, underflow and overflow solutions were collected and particles were counted manually, using ImageJ software.

The soil slurry sample prepared (section 2.3.3.3) was tested by passing through the hydrocyclone and collecting the underflow and overflow. The experiment was repeated until 2000 mL of each sample was gathered. The solutions were evaporated to get the weight of the solid contents on each fraction, including a solution of the soil slurry sample before passed through the hydrocyclone. The solid contents were redissolved in 600 mL of milliQ water and analysed by a particulate sizer instrument Malvern, Saturn DigiSizer 5200.

3.3.5 Electrophoretic analysis

Separation was performed using BGE of TAPS 50 mM + 0.1 mM PAR pH 8.2 (adjusted with NaOH), 8 kV at negative polarity and 500 s separation time. Capillary of 25 µm inner diameter (ID) and 40 cm length was used for the CE separation. Solution of 4 mM PAR in 10 mM TAPS at pH 8.2, was used as complexation reagent. Detection was perform using a 500 nm LED absorbance detector.

The electropherograms were analysed by OriginLab software to obtain the migration time, height, and area of the peaks.

3.4 Results and Discussion

3.4.1 Sample particle sorting

In Chapter 2 the H-filter was used to separate particles below 100 µm in size. Water samples from a natural stream contain particulate matter over this value, hence the need for a microdevice with a larger particle size removal is imperative. The minicyclones have been used in the particle separation field widely. The addition of a new particulate matter microdevice represent a challenge on the previous design of the pCE instrument. The system has been

modified as explained in the experimental section. The use of 2 different microdevices improve the particulate removal process and make it possible to work in a continuous manner avoiding blockages and clogging of the microdevices.

3.4.2 Hydrocyclone particle sorting

Hydrocyclone devices are a cylinder-shaped inner chamber with a conical bottom and two inlets placed tangentially to the cylindrical compartment, and two outlets located at the bottom and at the upper part of the chamber. The device is feed by two inlets, creating rotational flow and two vortices. The hydrocyclone mechanism is similar to a centrifuge, but the device is feeding constantly with the sample to separate. The separation capability of a minicyclone is dependent on its design parameters. The main parameter to consider is the diameter of the cylinder. Bradley and Rietema's ratios are the most used designs. The diameter of the outlets also play an important role in the separation efficiency. Inside the hydrocyclone, the separation is influenced by the shape, size, and density of the particles. The forces involved in this process are the centrifugal force, the buoyant force, and the drag force. The centrifugal force is caused by the flow tangential velocity. The buoyant force is the result of the different densities between liquid and particles in the sample. The drag force is produced for the opposed forces between the fluid viscosity against the particle movement [31]. Hence, if a particle is denser than the fluid will follow the primary vortex to the underflow. While, for smaller particles the centrifugal force will be insufficient to overcome the drag forces, then the particles will remain disperse inside the hydrocyclone, following the stream out to the underflow and overflow evenly.

To test the separation efficiency a solution containing a mix of different particle size was introduced to the cyclone a at a flow rate of 4 mL/min. Figure 3-2A depicts the solution collected from underflow and overflow of a mixture containing 90 and 200 µm size particles. The particles were counted manually using ImageJ software. For 200 µm particle size 100% follow the stream to the underflow outlet, while 57% of the 90 µm particles are in the underflow (43% in

the overflow). This is similar to the 55% of 1, 7 and 15 µm size particles that were also observed in the underflow (Figure 3-2 B) and confirms no dilution or segregation in hydrocyclone other than the targeted larger particles. The H-filter will prevent the particles >1 µm from entering the pCE.

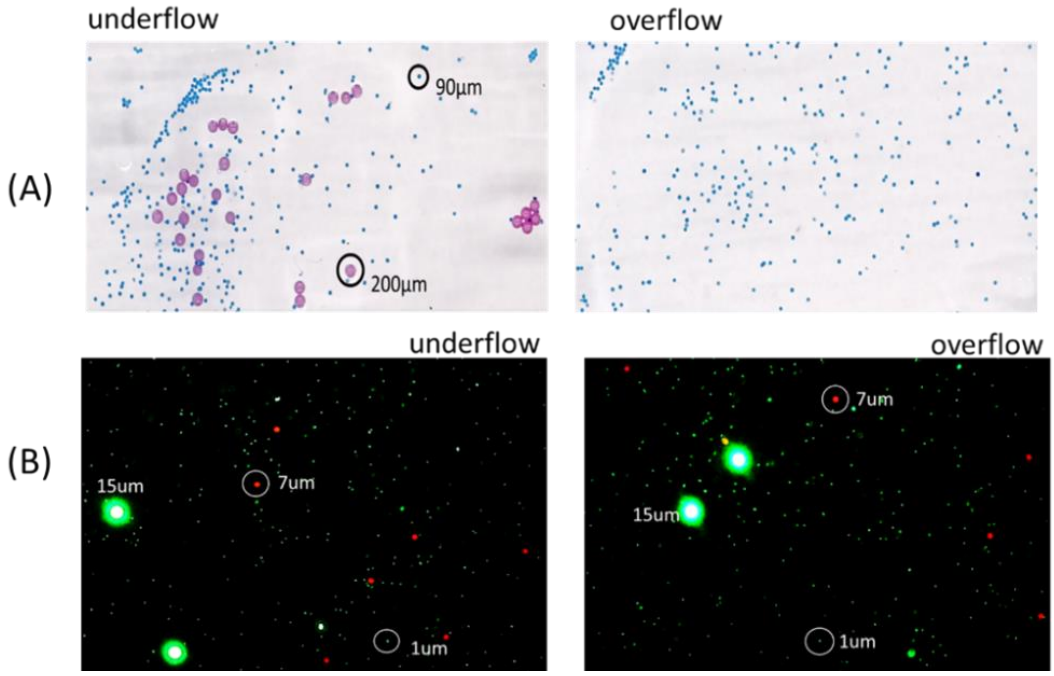

Figure 3-2 (A) Particles collected after the underflow and the overflow hydrocyclone outlets. Both, 90 and 200 µm size particles follow the stream to the underflow (left) while only particles of 90 µm size passed to the overflow (right) (B) Particles collected after the underflow and the overflow outlets shown and even distribution of 1, 7 and 15 µm size particles passed through the hydrocyclone microdevice.

3.4.3 System integration test

A soil slurry sample was passed through the hydrocyclone with the solution from both outlets collected and analysed in a particle sizer.

Figure 3-3 shows discrepancies on the content form the original soil slurry sample introduced into the hydrocyclone and the results from the underflow and overflow. These differences can be attributed to sedimentation of the particles in the separation process, due to large volumes used in the experiment. The results show the particles above 150 µm in size were efficiently removed and that there is also a slight reduction in the concentration of the particles down to

25 µm. Given the effective removal of particles from 1-100 µm with the H-filter, these findings confirm sequential operation of the hydrocyclone and H-filter would produce a particle free sample to connect online with the pCE.

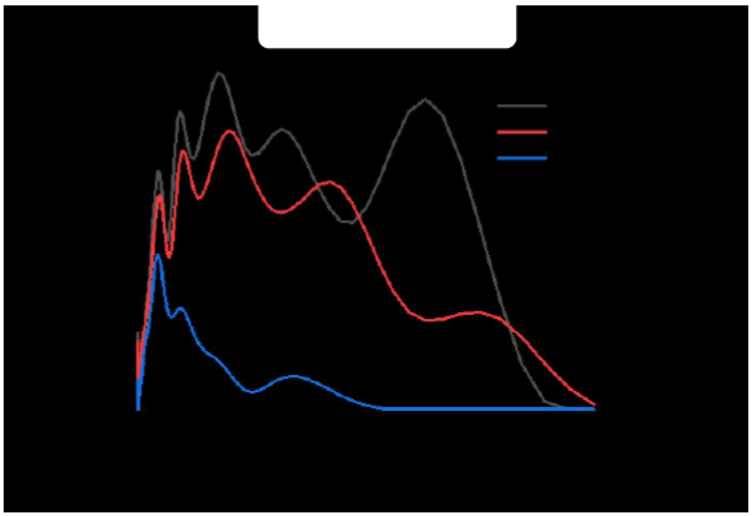

Figure 3-3 shows the size of the particles for the original sample range from 1 to 300 µm. The blue line represents the overflow and reveals particles from 1 to 150 µm size. While in the underflow (red line) particles from 1 to 300 can be observed, a range similar to the sample introduced to the hydrocyclone (black line). Difference in the density and shape of the particles explain the differences between the commercially sourced particles and the soil slurry.

3.4.4 PAR-Zn complex optimization

In this section, a method to analyse metals was optimized by using Pyridylazo resorcinol monosodium salt hydrate (PAR) as a complexation agent. The analysis of metals by complexation with PAR has become popular through the years due to its simplicity and low cost [33]. This method has been chosen for this study because present the highest molar absorption coefficient for zinc complexation, and do not require a complex detection system [34].

Zinc ion was chosen to perform these analysis and optimizations because it is well known there are high levels of the metal in Tasmanian waters. Companies spend large amounts of money in wastewater treatments for this compound, which makes it of big interest.

Three parameters were optimized in the PAR complex formation, first the time of the complex formation, second the response of the LED wavelength and third the place where the mixing occurs.

The first parameter was tested by introducing a Zinc sample immediately after mixing with PAR, the mix after 1 hour of mixing and after 18 hours of the initial mixing. Is shown in Figure 3-4 response of Co varies only 5% from time 0 to time 18. While for Zn the variation is only 8%. For this reason, the PAR-complex formation at time cero is acceptable and is chosen for the present experiment.

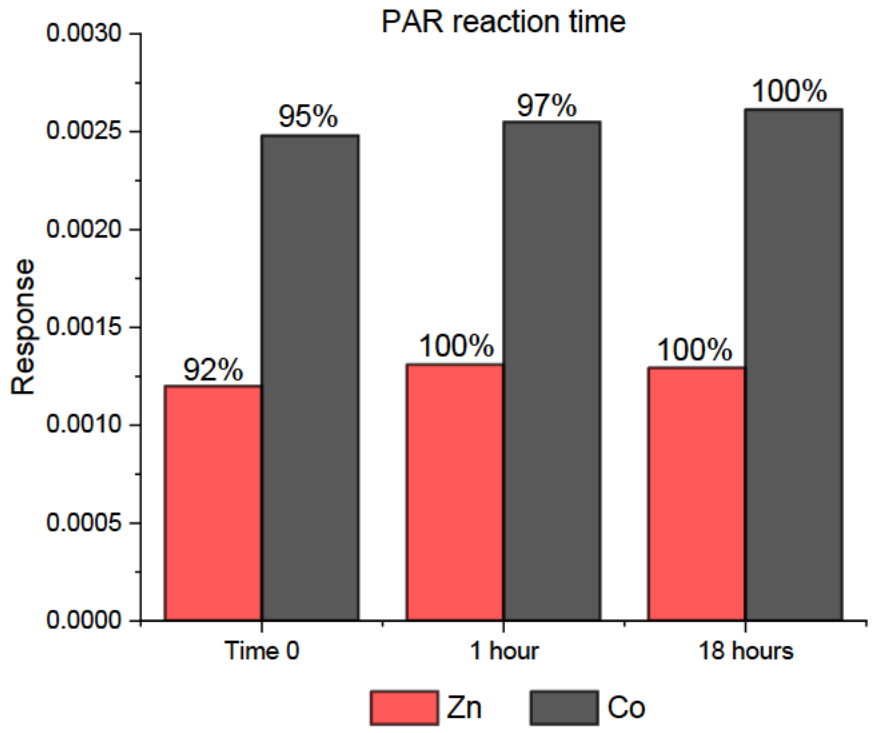

Figure 3-4 Shows the response of the Zinc-PAR complex formation at time 0, 1 and 18 hours.

Two LED were tested to determine the difference response versus wavelength. A 480 and a 505 LED. The results in Figure 3-5 shown a difference of 25% for Co and 45% for Zn when a 428 nm LED is used. For the present work the 505 nm LED has been chosen, increasing the detection limit of the present method.

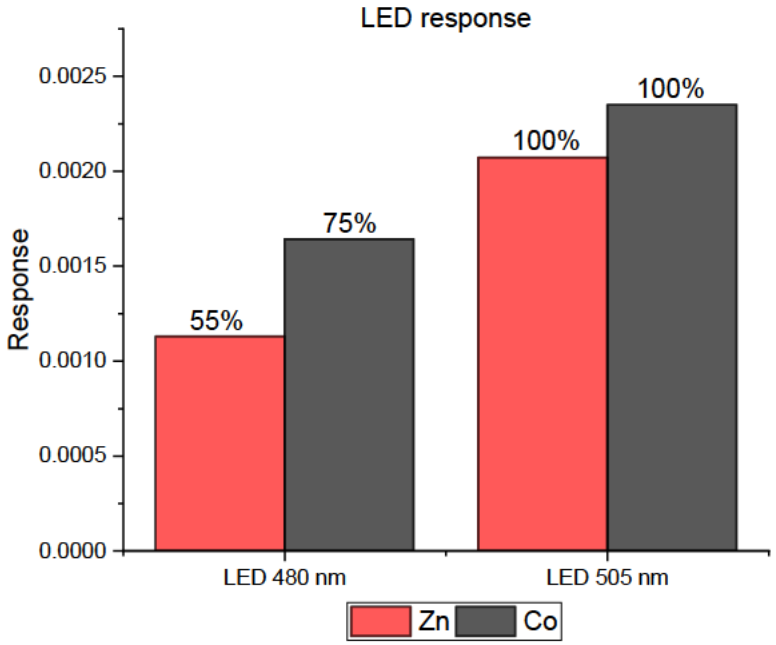

Figure 3-5 Shown the response of PAR complex versus LED wavelength.

The last parameter studied was the influence of the mix location for PAR and Zn sample. Different positions were identified for the PAR reagent introduction: i) in the sample before the H-filter, ii) in the acceptor solution before the H-filter and iii) in the acceptor solution after the H-filter, these are illustrated in Figure 3-6A. These three locations were compared experimentally by determining the difference in detector response for a solution containing 50 µM of Zn^{2+} and Co^{2+} following separation in the pCE instrument, with the results shown in Figure 3-6B. From this, it can be concluded that the largest detector response was obtained when injecting PAR into the acceptor flow after the H-filter, hence this configuration was selected.

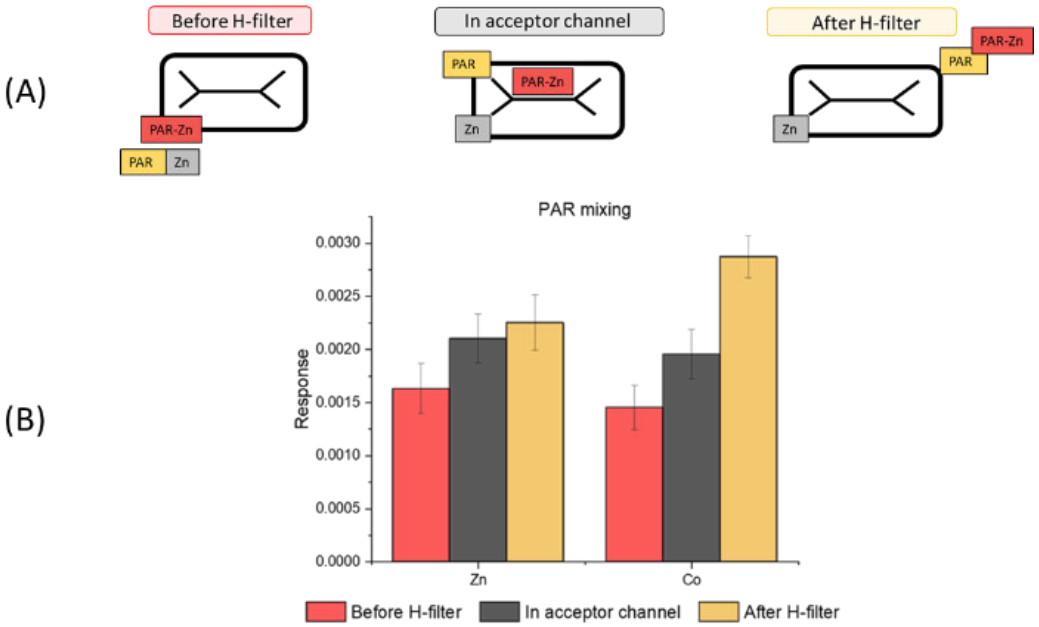

Figure 3-6 PAR-Zn complex formation study. (A) Show where the solutions were mixed. (B) The response of Zn^{2+} and Co^{2+} when mixed before the H-filter, in the acceptor channel, and after the H-filter.

3.4.5 Analysis of repeatability

An increase in variability was expected with the incorporation of the sample clean up microdevices. To corroborate the error due to the incorporation of the microdevices, a standard solution of 50 µM Zn^{2+} was injected i) directly to the pCE, without the H-filter and without the hydrocyclone, ii) with the H-filter but without the hydrocyclone, and iii) with the hydrocyclone and H-filter. RSD (n=10) increased from 3% for the direct analysis, to 7% when H-filter is introduced and 8% when using the cyclone and H-filter together.

3.4.6 Sample testing

A total of 10 water samples were collected from various places around Tasmania. A map of the sampling points is presented in Supplementary Information (3.7.1 Map of sampling points around Tasmania).

Before the analysis, a 5-point calibration curve ranging from 18 to 200 µM of Zn^{2+} was constructed with a linearity of 0.9969. The LOD and LOQ for Zn^{2+} were 5 µM (0.3 ppm) and 35 µM (2.2 ppm) respectively.

Figure 3-7 shows photographs of the 10 different water samples collected, with varying levels of turbidity and sedimentation. The solid loading was determined to range from 0.02 to 9.36 g/L, as indicated in Table 3.1, along with the sampling location and RSDs for migration time and concentration. The samples were pumped directly to the pCE without sample pre-treatment. individual samples were analysed 10 times. Zn^{2+} was detected in samples 2 (7 µM) and 10 (15 µM) with RSDs 11% and 17% for heigh response and 6% and 7% for migration time, respectively. The internal standard was 50 µM of Co^{2+} with RSDs in peak height ranging from 3% to 14% and RSDs in migration time ranging from 1% to 8%, respectively. For sample 1 and 3 to 9, Zn^{2+} was not detected, but the RSDs values obtained for the IS (6 to 14%) show an appropriate system performance.

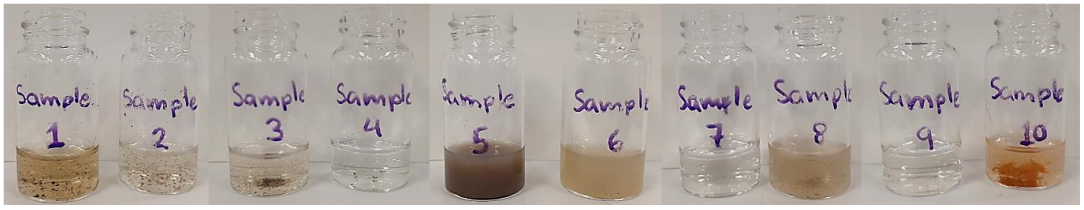

Figure 3-7 Water samples collected from different places around Tasmania. A map of the sampling points is presented in Supplementary Information (section 3.7.1 Map of sampling points around Tasmania).

Table 3.1 Set of samples analysed and their solid load. RSDs for migration time and concentration for n=10, Zn^{2+} present in sample shown in ppm, ND= not detected.

Sample number	Sample site	Solids loading (g/L)	RSD migration time		RSD concentration		Detected Zn^{2+} (ppm)
			Co^{2+}	Zn^{2+}	Co^{2+}	Zn^{2+}	
1	University of Tasmania	0.72	2%	ND	7%	ND	ND
2	TasWater treatment plant	0.58	8%	7%	13%	17%	0.46
3	Sandy Bay, Tasmania	0.46	4%	ND	6%	ND	ND
4	Derwent, Tasmania site 1	1.78	4%	ND	14%	ND	ND
5	Derwent, Tasmania site 2	9.36	1%	ND	6%	ND	ND
6	Boyer, Tasmania site 1	1.34	3%	ND	3%	ND	ND
7	New Norfolk, Tasmania	0.04	3%	ND	13%	ND	ND
8	Boyer, Tasmania, site 2	1.88	2%	ND	6%	ND	ND
9	Derwent, Tasmania, site 3	0.02	5%	ND	9%	ND	ND
10	Queenstown, Tasmania	3.56	5%	6%	4%	11%	0.98

The conditions when analysing on the field can interfere and disturb chemical instrumentation. It seems necessary to test the capabilities of the pCE instrument on-site, before being left for long-term field testing. To test the performance on site, the pCE system with the sequential hydrocyclone and H-filter was taken to a local wastewater treatment plant and a sample of sewage inflow was analysed for Zn^{2+}. Figure 3-8 shows the field setup.

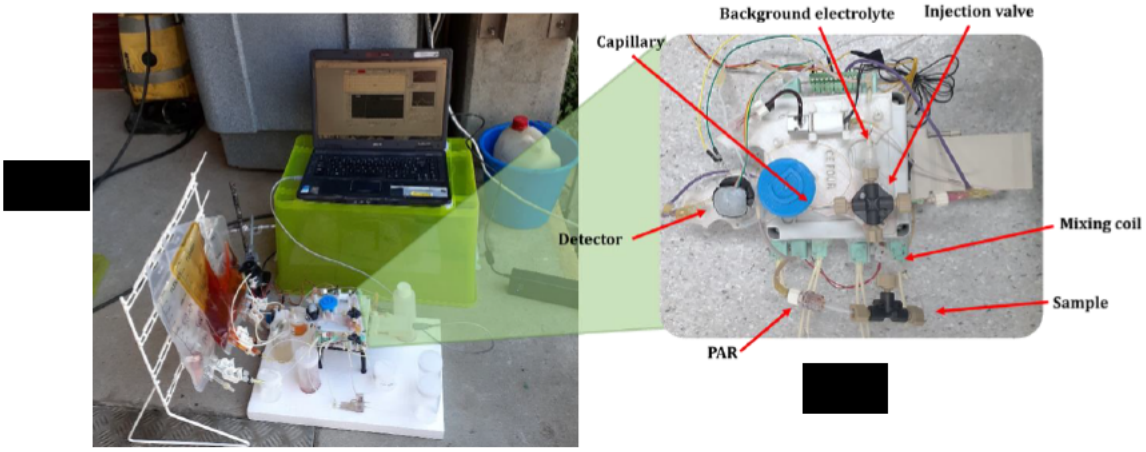

Figure 3-8 (A) pCE system in water treatment plant, (B) close-up of the pCE system

On-site, a standard solution of 150 µm Zn^{2+} was analysed, followed by a sample taken directly from the sewage inflow access point, followed by the same sample spiked with 150 µm Zn^{2+}. The electropherograms obtained on-site are shown in Figure 3-9A. The Zn^{2+} in the sewage inflow was no detectable, hence samples were spiked for proof of concept purposes. The level of Zn^{2+} in the sewage sample are dependable of the discharge from industries in the vicinity, while sometimes Zn^{2+} can go over 700 µM (50 ppm), the levels can be undetectable if the industries have not discharge contaminated wastewaters.

From the spiked sewage sample, the recovery for Zn^{2+} was calculated to be 82%.

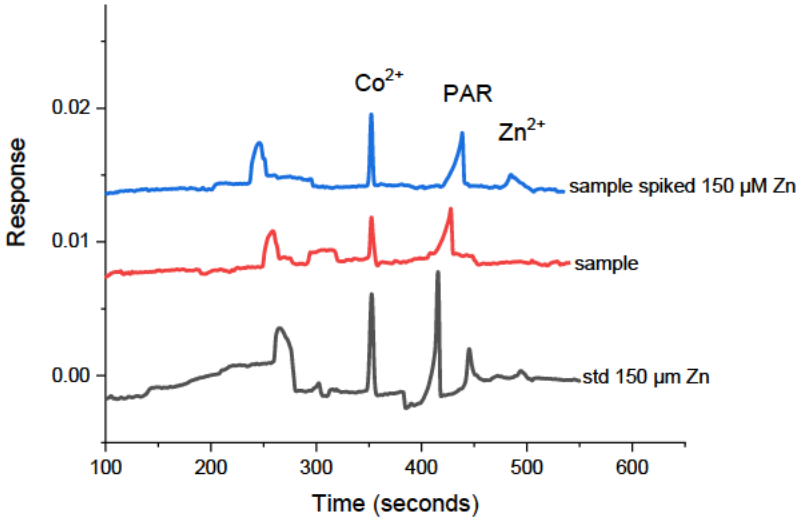

Figure 3-9 Electropherograms correspond to sample analysed on water treatment plant. Black line is the standard, red line is the sample untreated, and blue line is the sampled spiked with 150 µM of Zn^{2+}.

3.5 CONCLUSIONS

In this chapter, a hydrocyclone was introduced to eliminated suspended particulate matter > 150 µM, decreasing the risk of the H-filter blocking during extended periods of operation. Combined upstream to a H-filter, the hydrocyclone was effectively used for the removal of particulate matter from 10 different environmental samples with a solid loading ranging from 0.02 to 9.36 g/L. coupled with a pCE and without further sample treatment, the system was applied for the detection of Zn^{2+} with RSDs for quantitation ranging from 11 to 17%.

An instrument consisting of the pCE coupled with the hydrocyclone and H-filter was taken to on a wastewater treatment plant and shown to be capable to analysed samples from a sewage inflow, worth mentioning the analysis was carried on with the aim of an operator.

Future work is to explore the reliability of the pCE on site by deploying the hydrocyclone-H-filter-pCE homemade instrument on the field for several days or even weeks. Modifications to improve sensitivity has to be performed along with considerations on maintain stable temperature and humidity, as well as power consumption have to be taken prior to deploy the instrument on the field.

3.6 REFERENCES

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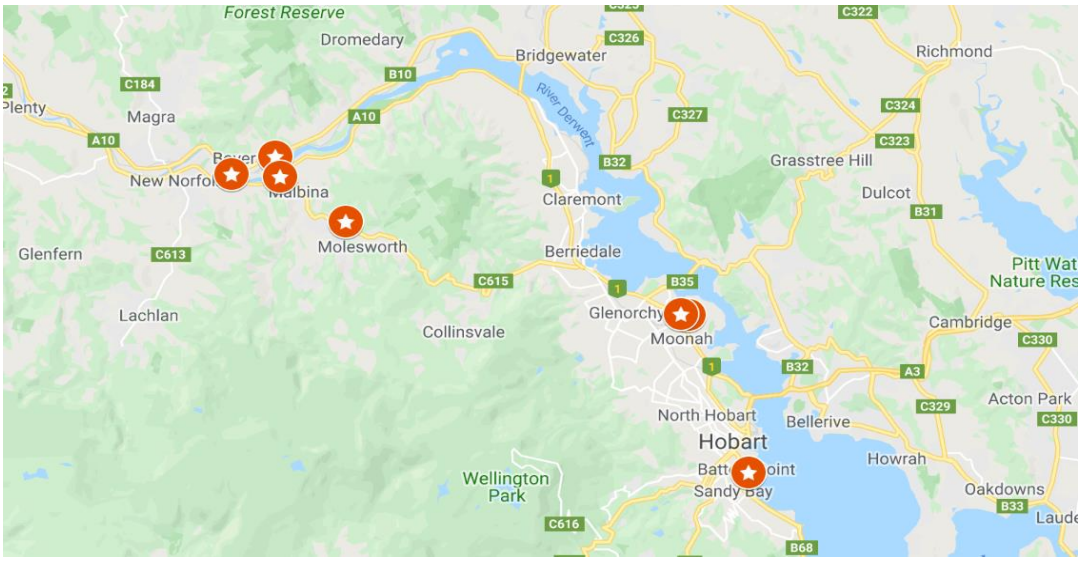
3.7 SUPPLEMENTARY INFORMATION

3.7.1 Map of sampling points around Tasmania



For more detail mas can be accessed at:

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CHAPTER 4 : FIELD TESTING

4.1. ABSTRACT

Portable instrumentation has been widely developed increasing the use of green analytical techniques. A new automated pCE-C^4D has been developed to monitor nutrients in water samples. The instrument has been enclosed in an insulated box with temperature control to enhance repeatability. The pCE system proposed include a cyclone and H-filter coupled online, to allow autonomous sample clean-up from environmental waters. The pCE was tested for 10 days in Margate, Tasmania and in Plenty River, Tasmania for more than 30 days. LODs were 30 ppb for Cl^-, 121 ppb for NO_3^- and 75 ppb for SO_4^{2-}, with RSDs of 10% for Cl^-, 10% for NO_3^- and 8% for SO_4^{2-}. The developed pCE is low cost compared with commercial instrumentation and is a potential fully automated tool for environmental monitoring.

4.2. INTRODUCTION

Environmental pollution directly affects human health; air, water and soil can carry contaminants into crops, drinking water and animals that are later consumed by humans [1]. Changes in climate are affected by human beings and natural causes, changing the environment as we know it in long term basis, but it is difficult to measure and correlate unless we establish trends and patterns of change [2]. In order to see these trends, it is necessary to measure over long periods of time and often, in remote locations. This creates a necessity for autonomous, robust, portable, and easy to manage detection instruments [3]. An enormous amount of time and resources have been devoted to the development of new miniaturized and portable instruments [4]. The main challenges to overcome are the change of conditions in the field like temperature, humidity, and vibration; the limitation of reagents; size and weight of instrument components, cost, sample preparation procedures and energy consumption [5–7].

Among many separation techniques, Capillary Electrophoresis (CE) has gained attention because of its relative short time of analysis, minimal consumption of chemicals, high sensitivity, flexibility to change target analytes, and its high separation efficiency making it a perfect technique to miniaturize [8]. In recent years portable CE instruments have been reported by different research groups. Gregus *et. al.* developed a lightweight and small pCE to analyse biological fluids, using hydrodynamic injection; the instrument was able to work continuously for 10 hours, but the sample required previous preparation prior to injection [9]. Nguyen *et. al.* reported an in-house-made pCE-C^4D to detect several rare earth elements simultaneously, in ore samples with detection limits of 0.24 ppm. The method required a complicated sample preparation were samples were digested, redissolved in water and filtered through 0.45 µm filter, before injecting into the system [10]. Fuiko *et. al.* developed a pCE-C^4D system to detect inorganic nitrogen ions in wastewater samples. The instrument operated continuously over 24 hours using a membrane filter system to introduce the sample directly into the instrument [11]. Saar-Reismaa *et. al.* reported a pCE coupled with an ultraviolet flourescence detector for drug testing in oral fluids. Samples were collected by a cotton pad, analytes were extracted by centrifugation in acetonitrile prior analysis [12].

Despite the demonstrated field of pCE, most of them still use filters, membranes, and extraction procedures prior to analyse the samples. Environmental monitoring requires not only portability but automation of the instruments, these recent developments still lack automation of the sample preparation step [13–15]. Microfluidic devices can offer a solution to deal with the challenges that portable instruments face [16,17]. As presented in prior chapters of this thesis a system of microfluidic devices has been developed to remove particulate matter over 1 µm size from water samples. The devices, an H-filter and a cyclone have been reported in sample preparation procedures, mainly for biological samples, and individually [18–22]. In this work the H-filter and cyclone are coupled together as a solution for sample filtration, allowing the proposed instrument to work continuously for several weeks without operator intervention. This portable and automated instrument for field analysis will reduce labour and

expense consumptions for system maintenance, while producing reliable results.

In this chapter, an inexpensive pCE system including an automated sampling interface was adapted, able to work continuously over 30 days. The instrument uses a 6-channel peristaltic pump to sync the fluids in the H-filter. An external submersible pump was installed to pump sample from a distance over 10 m from a natural water body to the instrument which is placed in an insulated box to control the temperature. The C^4D, pumps, electronics, and chemical solutions were enclosed inside the insulated box. The pCE was taken for 2 field trips. First, it was deployed in a pond in Margate, Tasmania, Australia for 10 days, detecting chloride (Cl^-), nitrate (NO_3^-), and sulphate (SO_4^{2-}) with a total of 241 samples. A second deployment was taken at a secure location next to Plenty river in Tasmania, Australia, to detect chloride, nitrate, and sulphate with a total of 974 samples analysed, working continuously for over 1 month.

4.3. EXPERIMENTAL

4.3.1. Materials and Chemicals

Background electrolyte (BGE) solution was prepared using analytical reagent grade Tris-(hydroxylmethyl) amino-methane (TRIS), 2-(cyclohexylamino)-ethanesulfonic acid (CHES) and sodium hydroxide (NaOH), obtained from Sigma-Aldrich (New South Wales, Australia). Solutions of chloride, nitrate, sulfate and perchlorate were prepared from analytical reagent grade potassium or sodium salts purchased from Sigma-Aldrich (New South Wales, Australia). All solutions were prepared in water from a Milli-Q Water Plus system from Millipore (Bedford, MA, USA), with a resistivity of 18.2 MΩ cm.

Fused silica capillary was obtained from Polymicro Technologies (USA). The capillary was coated in-house using a procedure provided by Eco Detection, Australia (https://www.ecodetection.com/). The coated capillary is also commercially available from Eco Detection (Melbourne, Australia).

4.3.2. Instrumentation

The homemade pCE instrument is based on that described in Chapter 2. The instrument was modified and placed in an insulated box including all the electronic parts, C^4D and bags containing BGE, IS and acceptor solution (Figure 4-1A). The waste reservoirs are attached and secure to the insulated box's outside wall (Figure 4-1B). Hose connectors are placed to connect to a submersible water pump (Ozito PSDW-350; Bunnings, Australia) to introduce the sample from the sampling point. The hose length can be changed according to how far the system is placed from the natural water body. Inside the insulated box a Peltier system was installed to control the temperature. Platform and component stands were designed by Solid Works software and 3D printed by a Prusa I3 MK3S printer (Prusa Research a.s, Czech Republic) (Figure 4-1C). The instrument uses mini peristaltic pumps from Takasago, Japan, Model number: RP-Q1.2N-P20Z-DC3V, and a 6-channel pump RP-6R01S-3P6A-DC10VS (Takasago, Japan). Tracedec C^4D detector (Strassahof, Austria) is employed for analyte detection. The instrument was operated with LabVIEW (National Instruments, USA) and used to control the system, data acquisition (DAQ) and data processing. Schematic of the circuitry is shown in Supplementary Information in section 4.7.2. Circuitry of the pCE instrument.

Analysis to compare the accuracy of the instrument was by ion chromatography using ICS-5000 System from Thermo Scientific Dionex (USA). The method used a column IonPac AS26 (0.4 x 250 mm), 9 µm, guard IonPac AG26 (0.4 x 50 mm), 9 µm, temperature was set at 15˚C, injection volume was 40 µL, flow rate 17 µL/min, eluent was a gradient of KOH in water, conductivity detector, cell temperature was 37˚C, suppressor ACES 300 and applied current of 16 mA.

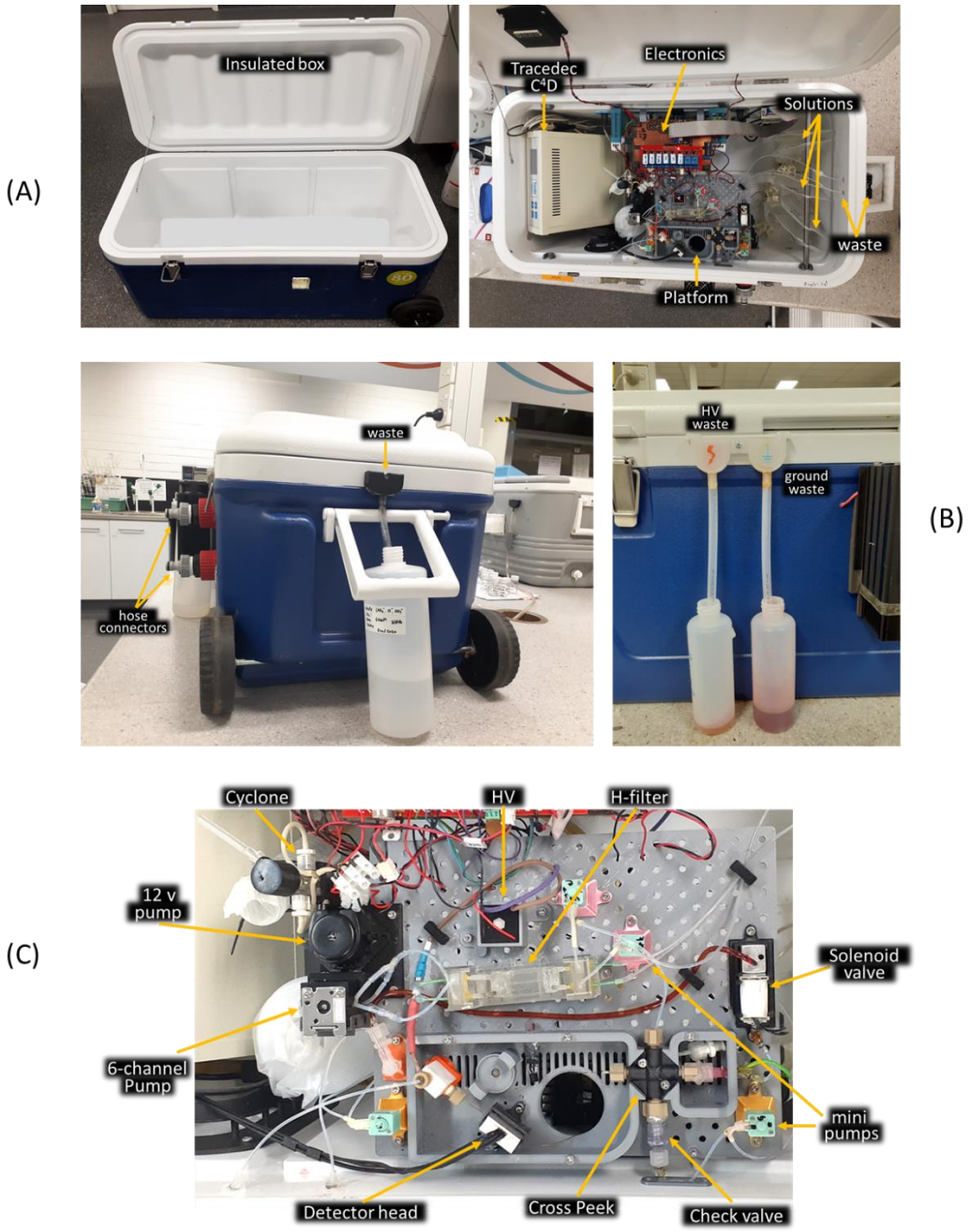

Figure 4-1 (A) Insulated box with the modifies pCE, including Tracedec, solution bags, and electronics, (B) Attached to the insulated box the waste reservoirs and hose connectors, (C) 3D printed platform and stands to secure the pCE parts.

4.3.3. Field Application

The pCE system was deployed for field analysis at a pond at Margate, Tasmania, Australia (Figure 4-2) for 10 days as a control experiment. The pCE was then deployed along the Plenty river, Tasmania, Australia, for 1 month with

periodic visits to check the performance and refill the reagent bags. The system was monitored remotely using TeamViewer software.

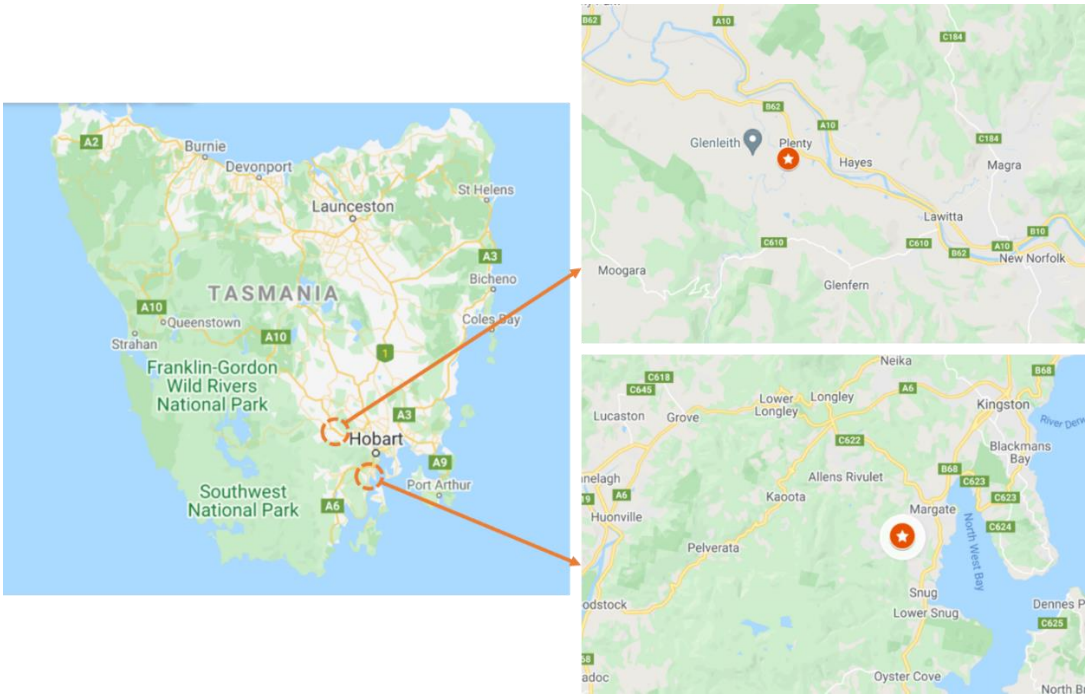

Figure 4-2 Deployment points in Tasmania, Australia. Right up location at Plenty river. Right down pond at Margate (map taken from https://www.google.com/maps).

4.3.4. Sample clean-up microdevices

4.3.4.1. H-filter

The H-filters were first drawn using computer aided design software, SolidWorks 2017 (Dassault Systèmes, S.A., Vélizy, France). The parts were produced using a Datron M7 HP CNC milling machine (Datron, Mühltal, Germany). Complete design and fabrication procedure are explained detailed in Chapter 2 of this thesis.

4.3.4.2. Mini cyclone

An STL file was obtained from Syed *et. al.* [23] and 3D printed by Objet Eden 260VS, in transparent material.

4.3.5. Electrophoretic analysis

Separation was performed using BGE of 200 mM TRIS−CHES solution (pH 8.0), 8 kV, and 480 s separation time. A coated capillary of 10 µm inner diameter and 25 cm length was used for the CE separation with detection via a C^4D detector. The parameters in the C^4D were: Frequency High; Voltage 0 dB; Gain 50% and Offset 000.

The electropherograms were analysed by OriginLab software to obtain the height and area of the peaks.

4.4. Results and Discussion

4.4.1. Modification of the system

The previous version of the homemade pCE, coupled with a cyclone and H-filter, was modified and placed inside an insulated box, to regulate the temperature of the system. The insulated box includes wheels for easy transportation. Schematics of the instrument are shown in Figure 4-3 and photos are shown in Figure 4-1(C). The sample was collected from the cyclone overflow and pumped through a 6-channel pump (Pump 2), together with internal standard, and acceptor solution. The acceptor solution was pumped through 2 tubes and joined to match the flow rate with the sample and IS inside the H-filter. The 6-channel pump was installed to mix the IS and sample before entering the H-filter and deliver equal flow in the H-filter channels from this mix and the acceptor solution. The sample and the acceptor solution then enter to the H-filter and analytes are diffused into the acceptor stream, leading to the acceptor outlet and enter the capillary through a PEEK cross by Pump 4. Pump 3 direct the liquid form Sample outlet to a waste reservoir, to match the dynamic fluids inside the H-filter and conserve diffusion process. BGE is introduce by Pump 5, after the sample, to the capillary where the separation takes place and analytes are detected by a C^4D.

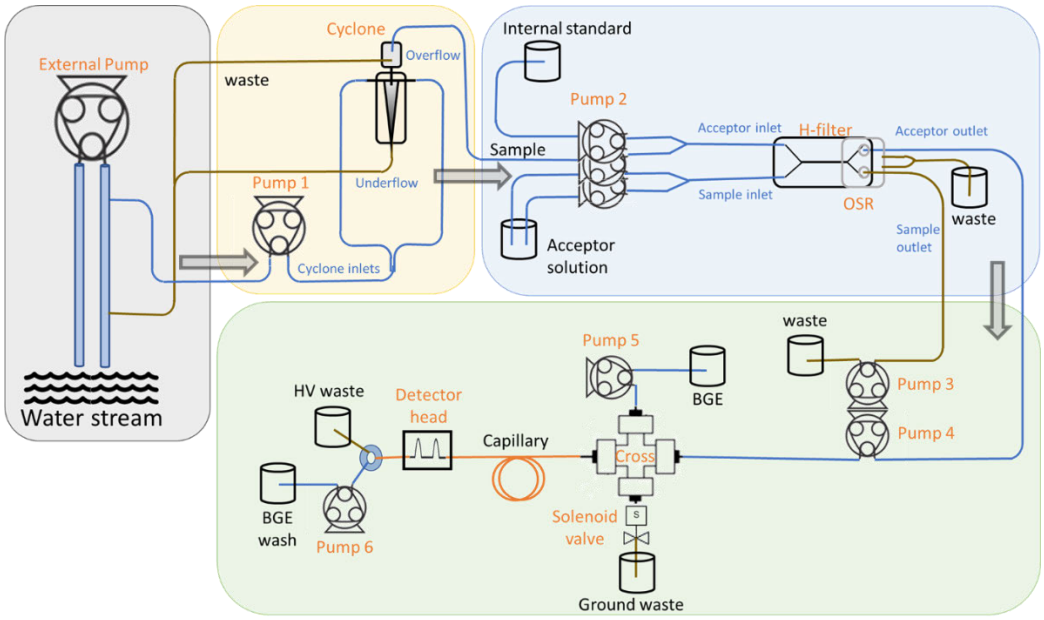

Figure 4-3. Schematic diagram of the portable CE. Sample is taken via submersible pump from a water stream into the cyclone. From the overflow sample is mixed with IS and director to the H-filter. Sample is taken form acceptor outlet into the capillary and analytes are detected by C^4D. Instrument is controlled by a in home developed LabView program.

A submersible pump was attached externally to provide sample from a natural water body to the instrument. Hose connectors were incorporated for easy interchange of the hose length as required, shown in Figure 4-4A. The submergible pump provides water through the hose connector which redirect the sample back to the water body outside the insulated box, allowing a constant flow of fresh sample. The cyclone is feed by a 12 V pump connected to the sampling hose, Figure 4-4B. Excess of sample in the cyclone overflow and cyclone underflow are discarded to a waste reservoir and is redirected back to the water body, Figure 4-4C. Sample and BGE waste coming from solenoid valve are collected in an outside ground waste reservoir and stored for disposal. Waste from the capillary outlet is redirected outside the insulated box to a high voltage waste reservoir. A fan is connected to control the temperature inside the insulated box by a Peltier and managed by LabView software. Two waterproof power points were connected at the back of the insulated box to provide electricity to the Laptop and the external pump via extension cord, Figure 4-4D. At this point the insulated box is still powered by direct connection to the grid.

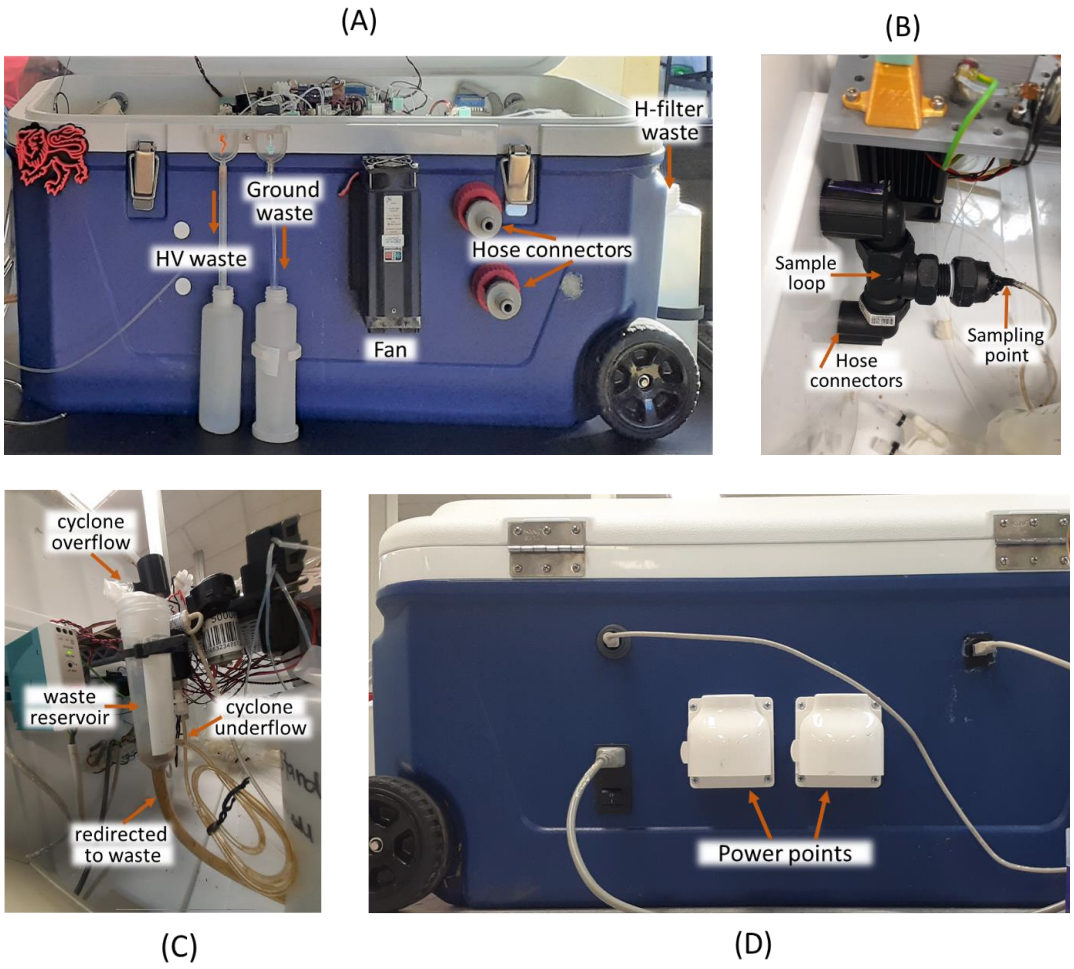

Figure 4-4. Pictures of modified insulated box. (A) hose and waste connections attached to the outer walls of the insulated box. (B) picture of the loop arrangement for taking sample to the cyclone inlets. (C) cyclone waste reservoir. (D) picture of the waterproof power point connectors at the back of the insulated box.

4.4.2. Performance testing

Calibration curves were constructed for Cl^-, NO_3^-, SO_4^{2-} and ClO_4^- from 25 to 400 ppb with a R^2=0.99572. The limit of Detection (LOD) was 30 ppb for Cl^-, 121 ppb for NO_3^- and 75 ppb for SO_4^{2-}. The limit of Quantification (LOQ) was 91 ppb for Cl^-, 403 ppb for NO_3^- and 227 ppb for SO_4^{2-}.

Samples form a pond in Margate, Tasmania (Sample 1) and a sample from a dairy farm in Tasmania (Sample 2) were analysed in the lab to check the effectiveness of the instrument. Photographs of the samples and electropherograms are shown in Figure 4-5. The samples were injected to the instrument with no sample preparation. Sample 2 was diluted 10-fold and

analysed again to corroborate presence of phosphate (Sample 2b). The presence of Cl^-, NO_3^-, and SO_4^{2-} was determined in Sample 1. In Sample 2 Cl^- and SO_4^{2-} were identify. Samples were injected 10 times with no clogging of the instrument or disruption in the baseline. The RSDs were sample 1: Cl^- 9%, NO_3^- 8% and SO_4^{2-} 11%, for sample 2: Cl^- 8% and SO_4^{2-} 12%.

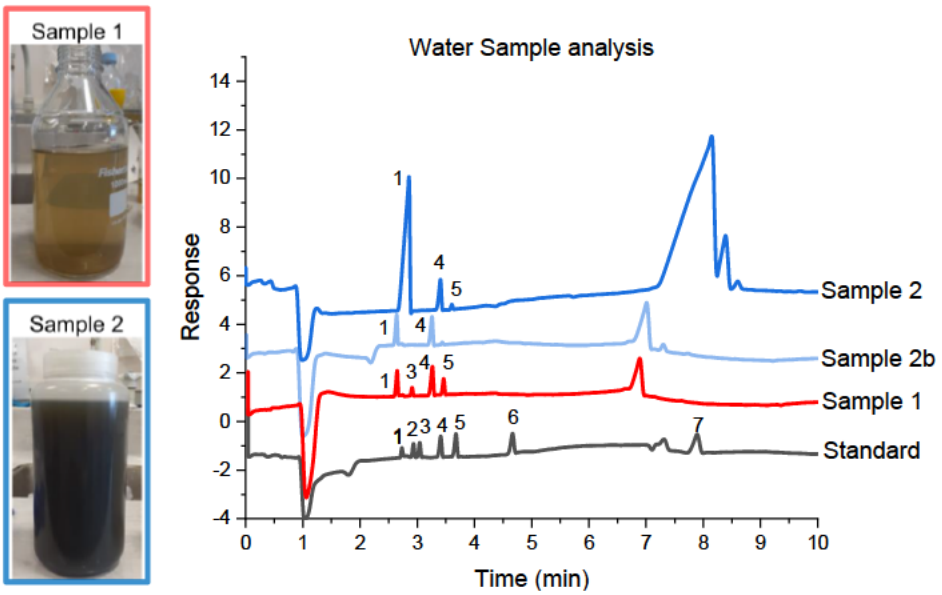

Figure 4-5. Sample 1 was taken from Margate pond; Sample 2 was taken from a dairy farm, Sample 2b is sample 2 diluted 10-fold in MilliQ water. Method: BGE 200 mM TRIS/CHES, coated capillary 10 µm ID, 30 cm length, Standards peaks are (1) Chloride, (2) Nitrite, (3) Nitrate, (4) Perchlorate (IS), (5) Sulphate, (6) Fluoride, (7) Phosphate.

4.4.3. Field deployment

The pCE was deployed next to a pond in Margate Tasmania, Australia. Pictures of the site are shown in Figure 4-6. The laptop was placed in a plastic box to protect from rain. The insulated box was powered via an extension cord from a nearby house. The external pump and Laptop were connected to the insulated box power points. The external pump was placed inside the pond and sample was pumped directly into the instrument for analysis.

Figure 4-6. Sample site at Margate, Tasmania, Australia. The pump is submerged into the water pond, taking the sample directly into the pCE and excess of the sample is redirected back to the pond. Laptop and pump are powered by the insulated box. Laptop is placed in a plastic box to protect form rain.

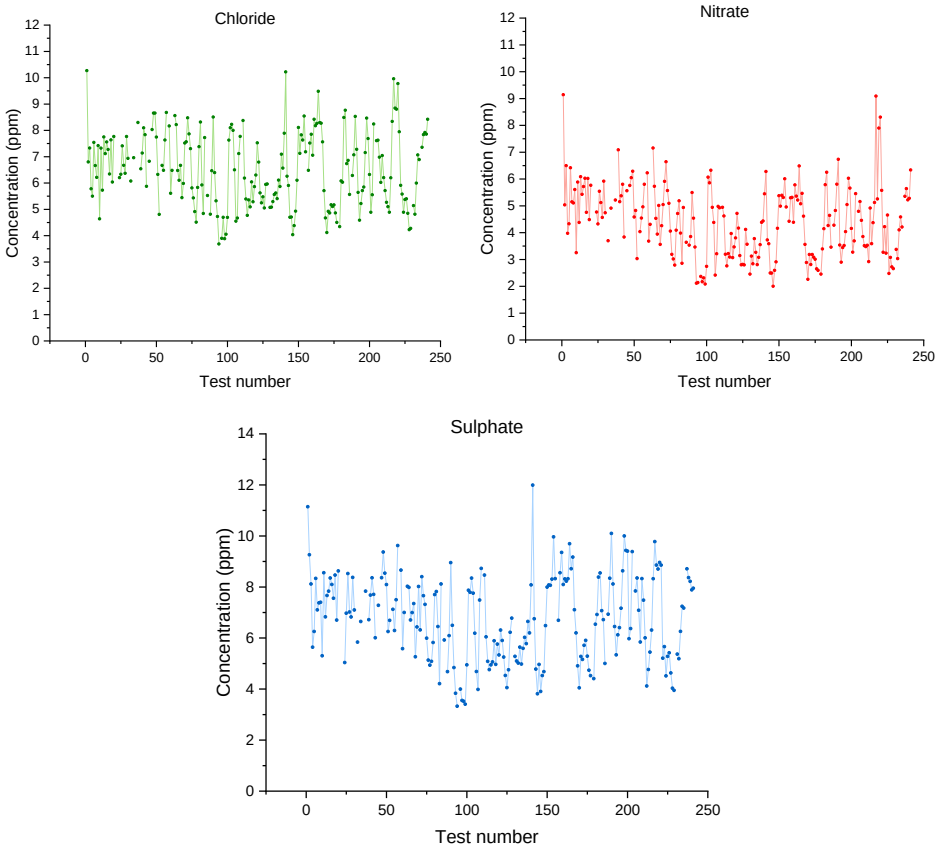

Figure 4-7 Continuous analysis at Margate pond. Chloride (green graph), Nitrate (red), and sulphate (blue) were detected in the sample. Method: BGE 200 mM TRISH/CHES, coated capillary 10 µm ID, 30 cm length, data was normalised by perchlorate response.

The pCE was operated in-field for 10 days, for a total of 241 analysis. Chloride, nitrate, and sulphate were detected. Perchlorate was used as an internal standard with an RSD for migration time of 15% (n=241) and peak height of 18% (n=241). Results of the analysis are shown in Figure 4-7.

A second field deployment was carried out at Plenty River in Plenty, Tasmania, Australia. The insulated box was placed in a sheltered place and powered from the dwelling of the local landowner. A 20 m hose was installed, and the pump was placed directly into the river.

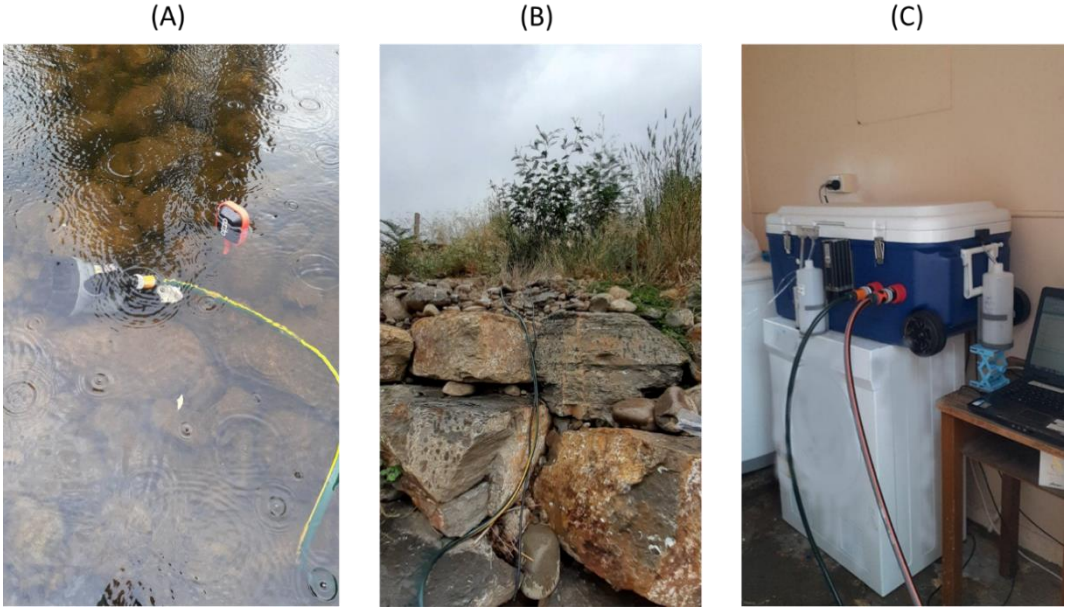

Figure 4-8 Deployment of pCE at river Plenty, Tasmania, Australia. (A) pump was submerged in the river. (B) a 20 m length hose was connected to provide sample to the pCE. (C) The pCE was placed in a secure space to prevent exposure to public.

The pCE worked continuously for 1 month, detecting chloride, nitrate, and sulphate. A total of 974 samples were analysed. The instrument was programmed to take a new measurement every 45 min. Results are shown in Figure 4-9. The concentration of chloride varied from 6000 ppm to 10000 ppm with some days higher or lower. Sulphate detection (blue graph) from 100 to 2000 ppb of concentration was detected on the month period. Nitrate (red graph) was detected at the start of the deployment, followed by no detection, and detected again after day 25.

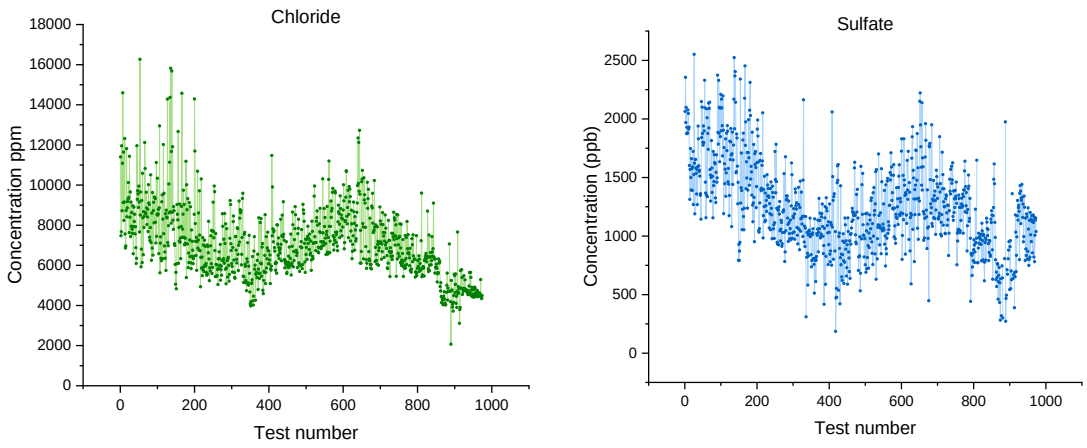

Figure 4-9. Results from the deployment at Plenty river, Tasmania Australia. Green graph shows chloride detection, blue graph sulphate concentration, for a period of 1 month with a total of 974 samples analysed. Method: BGE 200 mM TRIS/CHES, coated capillary 10 µm ID, 30 cm length, data normalised by perchlorate.

In Figure 4-10 Nitrate concentration in ppb (left y-axis) is plotted vs rainfall mm for the corresponding day day (right y-axis), the detection of NO_3^- can be associated to rain event the previous day in the area. During this time, the instrument worked continuously, but experienced some periods of non-operation due to local power outages. This required physical attendance on-site to restart the system. The H-filter and cyclone were not blocked or clogged at any point.

Data was validated against a grab sample analysed by IC in the laboratory. The sample analysed corresponds to 23 of March 2021. The electropherogram from the pCE on site and IC chromatogram for the same sample are shown in Figure 4-11. The sample analysed on site has a concentration of 6.3 ppm of Cl^-, 0.98 ppm of SO_4^{2-}, and NO_3^- was not detected. The concentration obtained by IC in the laboratory was 7.6 ppm for Cl^-, 0.91 ppm for SO_4^{2-}, and 10.4 ppb for NO_3^-, this value is under the LOD in the pCE, therefore nitrate was not detected. The results on site and on lab shown a 17% of difference for Cl^- and 8% for SO_4^{2-}. This difference can be attributed to error in the sample preparation, due sensitivity on the IC instrument, dilution step must be performed, while on site human error was not introduced, sample degradation due to transport and storage of the samples must be also considered.

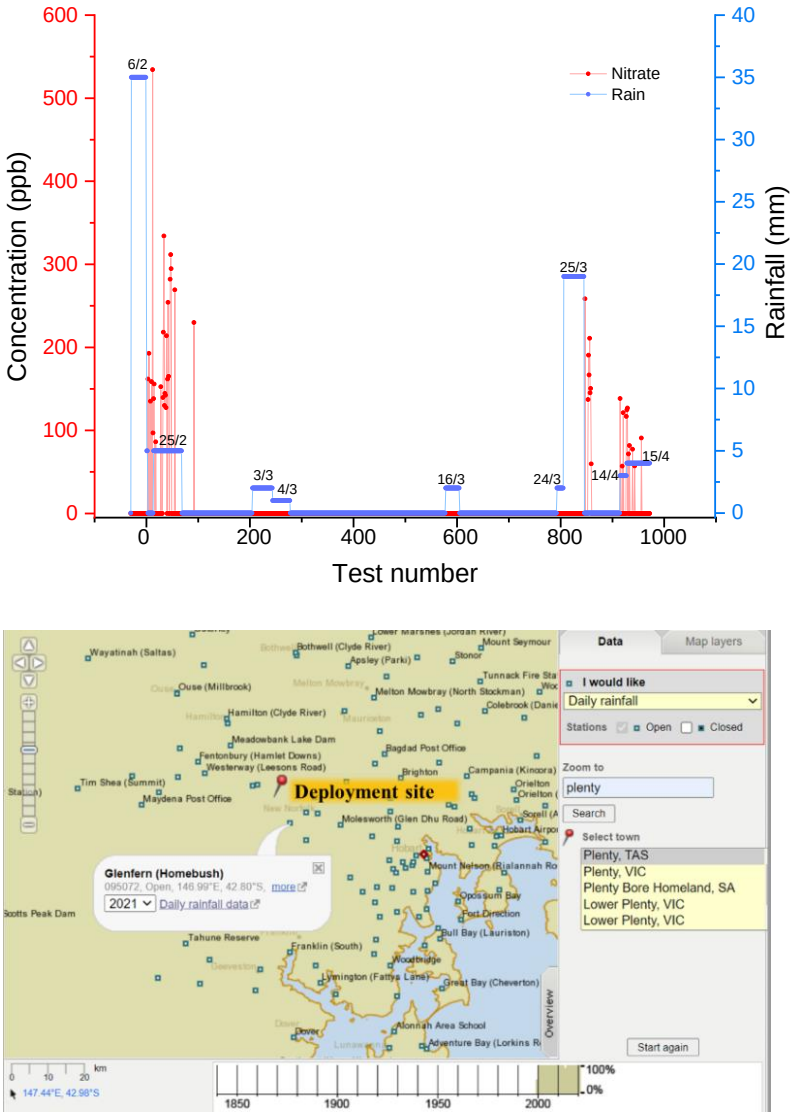

Figure 4-10 (A) Graph shows concentration of nitrate vs rainfall. (B) The rainfall data was taken from Australian government, bureau of metrology, using the nearest weather station: Glenfern.

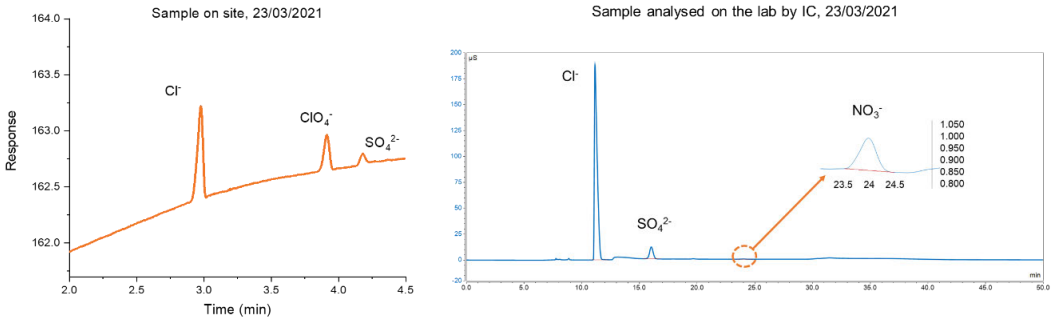

Figure 4-11 Comparison of sample from 23/03/2021, on the left sample analysed on site, chloride and sulphate were detected, on the right sample analysed on the lab by IC, chloride, sulphate and nitrate were detected, nitrate was under LOD for the sample analysed on site.

4.5. CONCLUSION

A fully automated, portable capillary electrophoresis instrument was developed. The pCE was placed in an insulation box which can be transported for 1 single person and is able to maintain controlled temperature conditions. The solutions required for the analysis such as BGE, acceptor solution, and internal standard are placed in bags inside the insulation box, as well as the Tracedec C^4D detector. The insulation box has secure attachments to contain the waste reservoirs. The instrument was able to pump sample directly from a natural water stream, analyse and detect water samples for 1 month without operator manipulation to take or process the samples. The detected concentration for Cl^- and SO_4^{2-} on the field and in the laboratory differ in 17% for Cl^- and 8% for SO_4^{2-} which is acceptable due to error introduced by operator manipulation and the difference in sensitivity of the instruments. The apparatus uses two microdevices to clean the sample from particulate matter under 1 µm of size. The first one a cyclone which preclean the sample, separating particulate matter with size over 100 µm. the second micro device, an H-filter, remove particulate matter above 1 µm size. As review in the literature various portable CE instruments have been deployed previously but manly uses filters or membranes which must be replaced regularly. The pCE was able to detect chloride, nitrate and sulphate in the set up described in this report. But the target analytes can be easily change by small modifications to the instrument like the addition of an LED detector, which will allow to detect metals, as has been shown in a previous chapter of this thesis (Chapter 3). The future direction of this work is to adapt a battery to power the pCE to be able to deploy the instrument in remote locations.

4.6. REFERENCES

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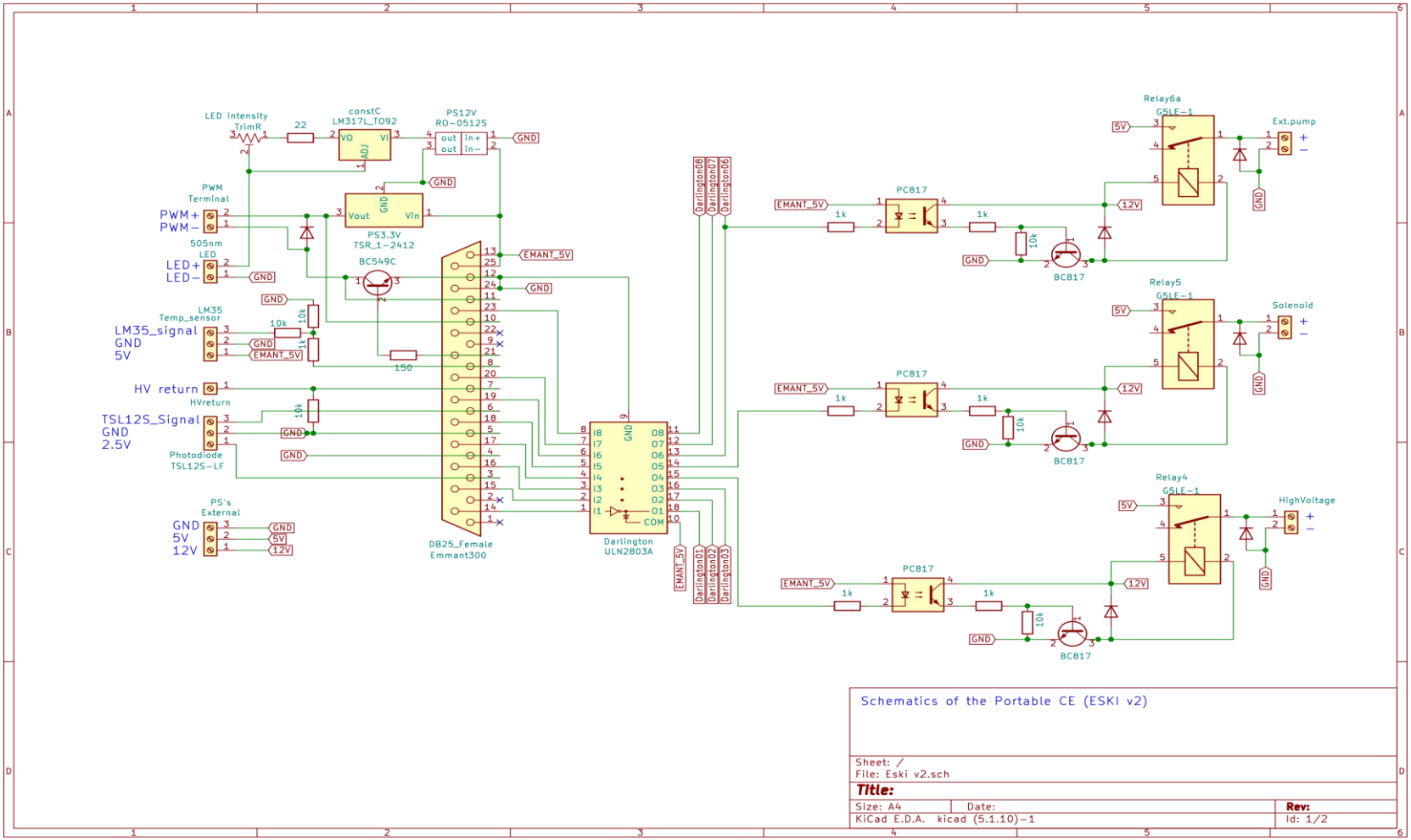
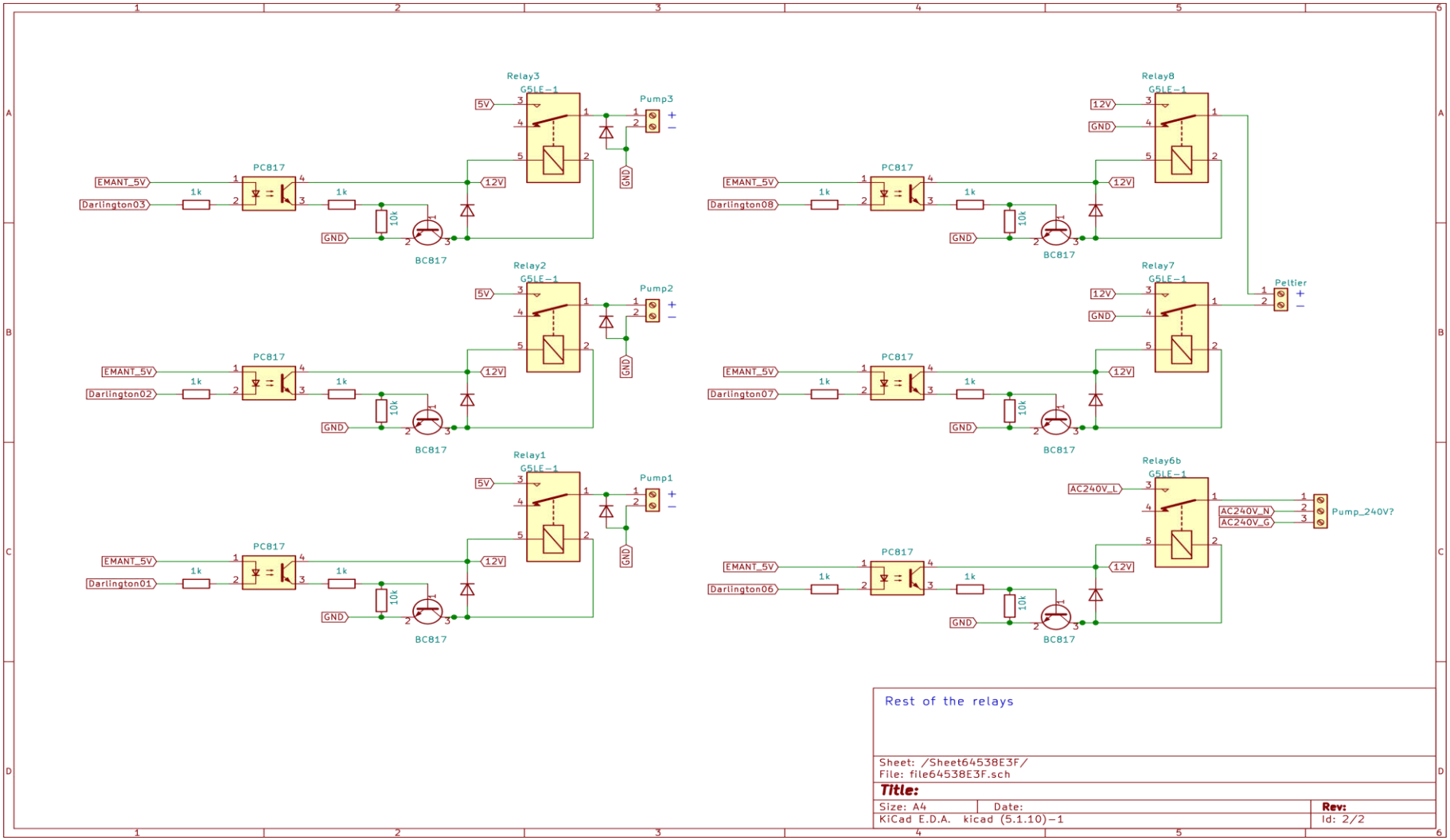
4.7. SUPPLEMENTARY INFORMATION

4.7.1. The capillary modification procedure used is as follows:

- Fused silica capillary was washed with acetone, followed by water.
- A solution of 0.2 M NaOH was flushed through the capillary for 1 h followed by water until effluent was neutral.
- A solution of 0.2 M HCl was flushed through the capillary for 15 min and followed by water until neutral.
- The capillary was then flushed with toluene and then a 25 % solution of 3-glycidoxypropyl-trimethoxysilane prepared in toluene and flushed through the capillary for one hour.
- The capillary was sealed and placed in a water bath at 60 °C for 20 h. Following the reaction, the capillary was washed with toluene and dried under nitrogen.
- To create a hydrophilic surface, the epoxide ring is reacted with acid to open the ring and form two hydroxyl groups on the surface (-OH). A 0.5 M solution of H_2SO_4 was flushed through the capillary overnight.
- The surface modified fused silica capillary was then washed with water and dried and ready for using.

4.7.2. Circuitry of the pCE instrument




Relay3
G5LE-1
Pump3
5V
PC817
EMANT_5V
Darlington03
1k
12V
GND
10k
BC817
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Pump2
Darlington02
Relay1
Pump1
Darlington01
Relay8
Darlington08
Relay7
Peltier
Darlington07
Relay6b
AC240V_L
AC240V_N
AC240V_G
Pump_240V?
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CHAPTER 5: CONCLUSIONS AND FUTURE PERSPECTIVES

Analytical methodologies for chemical quantification have evolved over time, and the scientific community is increasingly focused on these patterns to understand how humans are interacting with the environment. To establish trends of change, portable and in-situ instrumentation has been developed. There is a special interest to detect nutrients and metals in the environment, particularly in water samples. The monitoring of chemicals as metals and nutrients, currently relies on taking samples from the field and transporting them to the laboratory, which makes environmental monitoring a laborious, complicated, costly, and slow task. The focus of this work was to create a portable capillary electrophoresis that can function independently for at least a month, separating and detecting chemicals such as nutrients and metals. The main challenges when developing portable instrumentation are sample preparation, control the temperature, easy transportation, i.e. size and weight, versatility of target analytes, and the production cost. With the aim of solve these challenges, a truly portable and autonomous analytical instrument was developed to analyse nutrients and metals in water samples.

The pCE was assembled in an insulated box to allow portability and temperature control. The cost of the pCE was kept relatively low by using mini-peristaltic pumps, mini-solenoid valve, Peltier to control temperature, stainless steel needle as electrode, 8 kV high voltage power supply and 24-bit data acquisition module. Platform and stands for microdevices, electronics, pumps, and solution containers, were 3D printed. The pCE instrument was controlled by an in-house program written in LabVIEW. A submersible pump and long hoses were incorporated to provide the sample directly to the instrument and automate the sampling process.

To address the challenge of continuous sample filtration the sample preparation step was automatized by coupling 2 particle sorting microdevices. A machined H-filter connected on-line with the pCE demonstrated to separate analytes such as Cl^-, NO_3^- and SO_4^{2-} from a slurry soil sample, continuously for 6 weeks. The

H-filter was capable to separate the particulate matter above 1 µm, providing a clean sample to preserve the capillary used in the analysis. In Chapter 2 the sample was pretreated to remove particulate matter over 100 µm, because the dimensions of the micro device it may possibly blocked with larger particulate matter. This problem was solved as showed in Chapter 3, incorporating a second micro device, a cyclone. The cyclone was 3D printed maintaining low cost and easy replication of the device. The devices can separate particles above 90 µm size, providing, in conjunction with the H-filter, a particulate free sample over 1 µm size.

The developed pCE is a versatile instrument able to switch target analytes by an easy change of the detector system. In Chapter 2 and 4 the pCE was coupled with a C^4D to separate and detected anions, while in Chapter 3 the detector was replaced for a LED, detecting Zn^{2+} in 10 water samples with variable solid loads.

In Chapter 4 the capabilities of portability and automatization of the pCE were demonstrated by deployment in 2 different locations in Tasmania, Australia. The pCE was able to separate and detect Cl^-, NO_3^- and SO_4^{2-}, with LOQ of 91 ppb for Cl^-, 403 ppb for NO_3^- and 227 ppb for SO_4^{2-}, and LOD of 30 ppb for Cl^-, 121 ppb for NO_3^- and 75 ppb for SO_4^{2-}, with RSDs of 10% for Cl^-, 10% for NO_3^- and 8% for SO_4^{2-}, for n=3. In the first deployment location in Margate Tasmania, the pCE worked for ten days, analysing a total of 274 samples, in the second deployment location at Plenty, Tasmania, the pCE analysed a total of 974 samples over one month, both deployment analysis were carried out without operator manipulation to take or pre-treat the samples.

The outcomes of the created pCE, provide a solution for constant and operator free filtration step, for over a month. These reduce labour and cost of system maintenance, while produce reliable results. The automatization of the sampling and analysis process reduce time, decreased operator introduced error, and eliminate sample interferences.

Current deployable instrumentation can monitor environmental samples, but with operator intervention to replace filters or membranes used for sample clean

up, while other in-situ instrumentation like sensors, lack of multi analysis capabilities.

The pCE instrument created in this work, can perform autonomously all the steps to analyse samples from a water body, including sample intake, sample clean-up, separation and detection of different analytes, and data collection. The pCE is relatively light and small, allowing to be transported by 1 person, can be deployed, and can work autonomously for over one month without parts replacement or maintenance.

Some limitations arise from the pCE deployment and have to be addressed in further research. The automated sampling system requires an external mesh to prevent debris, stones, vegetal matter, and any other bulky material to enter into the system. This will avoid the tubing blockages before the particles sorting devices. The filtration system provided can only deal with relatively small solid material. In the case off colloids and biological matrix interferences additional studies are required.

Another significant limitation for the pCE presented in this thesis, is the power consumption. A power supply was connected to the pCE for deployment but was not able to provide all the energy needed for the system. Until now, the pCE still relies on grid connection to function, which represents a major drawback for the automatization of the pCE.

The sensitivity of the methods is another area of research and improvement. For environmental monitoring, the level of analytes can vary and be lower than the LODs presented in this thesis. For a full applicability of the instrument presented these values have to be improved.