

**CAPILLARY ELECTROPHORESIS METHOD FOR DETERMINATION OF  
BISPHENOL A IN DRINKING WATER AND IN BRITISH COLUMBIA MUSSELS**

by

Elizabeth Marie Andrucson

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR  
THE DEGREE OF MASTER OF SCIENCE IN ENVIRONMENTAL SCIENCE

In the Department of Physical Sciences (Chemistry)

Thesis Examining Committee:

Kingsley Donkor (PhD), Professor and Thesis Supervisor, Physical Sciences (Chemistry)

Heidi Huttunen-Hennelly (PhD), Associate Professor and Committee Member, Physical  
Sciences (Chemistry)

Dipesh Prema (PhD), Associate Professor (Teaching) and Committee Member, Physical  
Sciences (Chemistry)

James Kariuki (PhD), External Examiner, Professor, University of Alberta, Augustana  
Campus

August 2021

Thompson Rivers University

(Kamloops)

© Elizabeth Marie Andrucson, 2021

## **ABSTRACT**

Bisphenol A has been used around the world for over 50 years and traces of it can be found everywhere: in the air, soil, living things, and water. It is very useful for manufacturing polycarbonate plastics, epoxy resins, and countless other products but there are concerns about negative effects, especially on human health since it has been classified as an endocrine disruptor. As such, knowing exactly where it is found is important information. Municipal water supply from various locations in Kamloops, British Columbia were tested by a capillary electrophoresis method developed in this study to detect bisphenol A as low as 5 parts per million. Mussel samples from coastal British Columbia were also tested. None of the water samples tested showed the presence of bisphenol A at the concentrations that were detectable by this method. Mussel tissue analysis was inconclusive and requires further investigation to confirm BPA concentrations around 5 parts per million. Other future work will include refining the capillary electrophoresis method to detect lower concentrations and to attempt to detect bisphenol analogues which are being used to replace bisphenol A in many products.

**Keywords:** capillary electrophoresis, bisphenol A, water, mussel

**TABLE OF CONTENTS:**

Abstract .....	ii
Table of Contents .....	iii
Acknowledgements .....	v
Dedication .....	vi
List of Abbreviations .....	vii
List of Figures .....	viii
List of Tables .....	ix
Chapter 1. Introduction .....	1
Bisphenol A .....	2
Microplastic Particles and Mussels .....	4
Chapter 2. Capillary Electrophoresis .....	6
Factors Influencing CE Analysis .....	10
Chapter 3. Materials and Methods .....	14
Experimental .....	15
Reagents .....	15
Instrumentation .....	15
Solution and Buffer Preparation .....	16
Capillary Electrophoresis Method .....	17
Sample Collection and Preparation .....	18

Water Samples .....	18
Mussel Samples .....	19
Chapter 4. Results and Discussion .....	20
BPA Standards .....	21
Samples .....	22
Water Analysis .....	22
Mussel Sample Analysis .....	23
Chapter 5. Conclusions and Future Work .....	27
Conclusions .....	28
Future Work .....	29
References .....	30
Appendix .....	33
Mussel Sample Data .....	33
Sample Electropherograms .....	34

## ACKNOWLEDGEMENTS

Unending gratitude to my supervisor, Dr. Kingsley K. Donkor. With his wisdom and compassion, he has provided invaluable guidance and support in my research and in my life. He has been there for me when the lab results were discouraging and when life got in the way of going forward. I have learned by example how to be a better chemist, scholar, teacher, and person.

I wish to acknowledge the amazing support I have received from so many at Thompson Rivers University. The chemistry department, my committee members and the MSc committee and coordinators went above and beyond to help me make this a reality. My instructors have taught me so much both in the classroom and out, including much appreciated concern and advice offered during chance encounters in the community.

My family and friends are everything to me in all I do. Support for my further education has been especially strong from my parents, my children Dennis, Thomas, and Rebecca (even though you are mostly adults) and Jacqueline. You keep me grounded in my sense of self and my place in the world. Never underestimate my appreciation for when you asked how I was doing, or you made me a meal or a coffee. Thank you.

I gratefully acknowledge funding that supported me throughout my studies which include the Natural Sciences and Engineering Research Council (NSERC) of Canada, TRU Graduate Teaching Assistant program, and the Donkor Research Group. I also gratefully acknowledge Canada Foundation of Innovation (CFI) for funding for the purchase of the capillary electrophoresis instrument. Thanks to Dr. John Church and TRU ALNA group for the support in the early part of my program. The work of Mae Frank and Dr. Louis Gosselin was fundamentally important to my research; thank you both.

*To my Dad and to David with love*

*For where I started and what the future holds*

**LIST OF ABBREVIATIONS**

AU	Absorbance Unit
BGE	Background Electrolyte
BPA	Bisphenol A
CE	Capillary Electrophoresis
CMC	Critical Micelle Concentration
EFSA	European Food Safety Authority
EOF	Electroosmotic flow
FDA	Food and Drug Administration
M	Molarity (moles/litre)
MEKC	Micellar Electrokinetic Capillary Chromatography
MP	Microplastic Particles
NSERC	Natural Sciences and Engineering Research Council of Canada
PC	Polycarbonate
R <sup>2</sup>	linear correlation coefficient
SDS	Sodium Dodecyl Sulphate
TDI	Tolerable Daily Intake
UV	ultraviolet

**LIST OF FIGURES**

Figure 1.1. Chemical structure of bisphenol A.....	2
Figure 2.1. Double layer formed within capillary.....	7
Figure 2.2. A diagram depicting EOF.....	8
Figure 2.3. Schematic diagram of capillary electrophoresis.....	9
Figure 2.4. Ohm's law plot.....	12
Figure 3.1. Picture of capillary electrophoresis system used.....	16
Figure 4.1. Calibration curve for BPA concentrations from 5 ppm to 50 ppm.....	21
Figure 4.2. The electropherogram of the 10 ppm BPA standard.....	22
Figure 4.3. Electropherogram for CE analysis of water sample.....	23
Figure 4.4. Electropherogram of mussel sample #109.....	24
Figure 4.5. CE electropherogram of mussel sample #116.....	25
Figure 4.6. CE electropherogram of mussel sample #116, basic pH.....	26
Figure B.1. Water sample electropherograms.....	34
Figure B.2. Expanded view of Figure B.1. electropherograms.....	34
Figure B.3. Mussel sample electropherograms.....	35
Figure B.4. Mussel sample electropherograms.....	35



**LIST OF TABLES**

Table 3.1. Preparation of BPA standards for calibration. ....	17
Table 3.2. Optimized CE instrumentation parameters for BPA analysis .....	18
Table 3.3. Sample sites around Vancouver Island, BC.....	19
Table 4.1. Potential BPA concentration in mussel samples.....	26
Table A.1 Mussel sample locations and microplastic particle counts .....	33

# **CHAPTER 1**

## **INTRODUCTION**

## CHAPTER 1. INTRODUCTION

### Bisphenol A

Bisphenol A (BPA) is ubiquitous in the modern world and has been shown to have negative effects on human health. This small organic monomer (Figure 1.1) was first described and synthesized in 1891 by a Russian chemist (Jalal et al., 2018; Xiao et al, 2020). It was investigated and later abandoned as a possible synthetic estrogen replacement in the 1930s due to its hormone effects; it is classified as an endocrine disruptor (Rubin, 2011). Since the 1940s, it has been used in manufacturing plastics and today it is produced in huge amounts in making of polycarbonate (PC) plastics and epoxy resins; these account for roughly 95% of BPA usage (Rubin, 2011). Other products that use BPA include thermal paper, water-pipes, electronics, toys, and food packaging. Yearly worldwide production of BPA is over 3.8 million tons (Michałowicz, 2014). Use in Canada is reported from 100 000 to 1 million kg annually (Environment and Climate Change Canada, 2018).

BPA is not found naturally; the only source is from manufacturing, and it is now detectable almost everywhere. It is seen in the atmosphere, dust, water, and in human tissues, organs, and fluids (Michałowicz, 2014). BPA concentrations have been measured in the atmosphere across the entire globe over cities, rural areas, oceans, and polar regions (Fu & Kawamura, 2010).

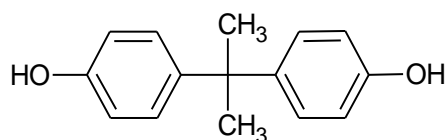


Figure 1.1. The chemical structure of bisphenol A, (2,2-bis(4-hydroxyphenyl)propane, CAS registry number 80-05-7).

Primary concern about the safety of BPA stems from its known disruption of human hormones. There is a large body of research into the effects on human health and the environment. Human health effects include disruption of DNA strands and signaling pathways, dysregulation of calcium ion homeostasis of cells (Jalal et al., 2018), social impairments of 4 year old children

with measured pre-and post-natal BPA exposure (Lim et al., 2017), and promotion of ovarian cancer cell development (Shi et al., 2017). The risks of obesity, diabetes, liver cell damage, immunotoxicity, and coronary heart diseases may also increase with BPA exposure (Michałowicz, 2014). Animal studies show changes in neonatal brain development, development of reproductive organs in males and females, disruptions in sperm production and prostate health (Talsness et al., 2009). Susceptibility to drug addiction has also been linked to BPA exposure (Jones & Miller, 2008).

BPA is found in most people tested (Rubin, 2011). Oral ingestion is the primary source of exposure through food and drink, primarily due to the linings of containers for foods and drinks made using BPA and epoxy resins (Rubin, 2011). BPA levels are higher in women than men and higher in younger age brackets than older. Levels have been found as high as 1-104 ng/g of tissue in placenta and fetus (Jalal et al., 2018). Maximum daily exposure has been estimated at about 1 µg/kg BW/day but negative effects from BPA may be experienced at daily exposure as low as 0.025 µg/kg BW/day (Kang et al., 2006).

Due to its ubiquitous presence in the environment, there have also been studies into its effects there. Aquatic species studied by Alexander et al in 1988 showed toxic effects from BPA at levels between 1 and 10 ppm of water and a study by Mihaich et al (2009) found no-effect concentrations (NOECs) as low as 0.025 ppm. Xiao et al (2020) have recently written a review in the Journal of Hazardous Materials of scores of articles studying how BPA effects growth and development of plants and some research into using plants for bioremediation of BPA pollution. BPA does break down but there is always more entering the environment, so it appears as a persistent contaminant.

Evidence that there is widespread concern about the effects of BPA on human health and on the environment can be seen in the existence of government regulations and guidelines. In Canada, there are guidelines and one regulation regarding BPA. The Government of Canada through Environment and Climate Change Canada has set a Federal Environmental Quality Guideline (FEQG) as a measure of acceptable levels of BPA in environmental waters at 3.5 µg/L (Environment and Climate Change Canada, 2018). FEQGs are not regulatory or binding; they are merely set to provide a guide for what is deemed to be safe. No maximum acceptable concentration (MAC) has been set in the Guidelines for Canadian Drinking Water

Quality as BPA has not been found in drinking water in levels that are considered “of possible human health significance” (Health Canada, 2020). The first regulation concerning BPA was set in Canada in the Canada Consumer Product Safety Act of 2010 which includes a prohibition against the sale of PC baby bottles made with bisphenol A (Government of Canada, 2010). Infants up to six months old fed formula from polycarbonate bottles were seen to have the highest BPA dietary exposure per kilogram of body weight (Srivastava et al., 2015) so there was sufficient concern to mandate reduced sources of BPA. The United States Environmental Protection Agency (US EPA) established a chronic oral Reference Dose (RfD) and the European Food Safety Authority (EFSA) set a Tolerable Daily Intake (TDI) of BPA. They are both at 50  $\mu\text{g}/\text{kg}\cdot\text{bw}/\text{day}$  (Shelnutt et al., 2013). These measurements are both estimates of safe daily exposure over a lifetime. In 2015 the EFSA reduced that to 4  $\mu\text{g}/\text{kg}\cdot\text{bw}/\text{day}$  because of concerns that the data did not conclusively show that the higher limit was a reasonable level of exposure; in September 2018, the EFSA put out a “Call for data relevant to the hazard assessment of Bisphenol A (BPA)” (European Food Safety Authority, 2018) to better assess the effects of BPA and set a TDI based on current research. There have been no changes to the guidelines reflecting new information from this research.

### **Microplastic Particles and Mussels**

Microplastics are small, less than 5 mm, bits of plastic (Anderson et al., 2016) that are found in water everywhere including the ocean and other water systems (Frank, 2018). There are an estimated 15 to 51 trillion particles, up to 236 thousand metric tons, of microplastics in the ocean (Van Sebille et al., 2015). Some microplastic particles are intentionally manufactured but much of the pollution arises from larger plastics breaking down into tiny pieces through use, such as plastic fibers breaking off fabrics being laundered (Frank et al., 2016) or degradations in the environment (Anderson et al., 2016; Desforges et al., 2014). Microplastics found in the ocean are composed of many types of plastic including ones made with BPA (Tang, Rong, et al., 2020) and microplastics may even be absorbing BPA from the water (Tang, Zhou, et al., 2020).

One of the concerns is that these particles are about the size of food particles for phytoplankton and other marine creatures, especially filter-feeders (Desforbes et al., 2014). Filter-feeding shellfish are marine animal that includes mussels, clams, and oysters. They ingest food, mainly phytoplankton, by siphoning in water which contains phytoplankton but can also have varying levels of pollutants, including microplastics and chemicals that adsorb to microplastics such as BPA, (Davidson & Dudas, 2016; Desforbes et al., 2014; Frank et al., 2016). Thus, microplastics are ingested as particles and as contaminants in the phytoplankton (Van Cauwenberghe & Janssen, 2014). Many of these filter-feeders are shellfish species which are economically significant. Farmed and wild shellfish production in Canada was over 3 billion dollars in 2018 (Canada, 2019). Understanding and protecting shellfish is critical to the economy and the environment.

The risks to marine life that ingest microplastics are both physical and chemical. Physical hazards include obstructions, deformations, malnutrition, and others (Tang, Rong, et al., 2020). Chemically, there is some research that suggests increased toxic effects from other pollutants in the presence of microplastics (Tang, Rong, et al., 2020) and there is speculation that the presence and possible release of BPA from these plastics could have deleterious effects beyond the physical trauma (Frank et al., 2016; Tang, Zhou, et al., 2020).

Davidson and Dundas (2016) found plastic microparticles in all samples of wild and cultured Manila Clams (*Vererupis philippinarum*) collected from Baynes Sound on the east coast of Vancouver Island, British Columbia. Research has also confirmed the presence of microplastic particles in the majority, 28 of 36, of wild *Mytilus trossulus* samples collected various locations between the mainland and the west coast of Vancouver Island, British Columbia (Frank et al., 2016). In Frank's work, there were zero to six particles counted in each specimen with an average of 2 microplastic particles in each of the mussels examined.

## **CHAPTER 2**

# **CAPILLARY ELECTROPHORESIS**

## CHAPTER 2. CAPILLARY ELECTROPHORESIS

Capillary electrophoresis (CE) is a relatively new analytical separation technique that is based on a solute's ability to move through a solution, usually an aqueous buffer, under the influence of an electric field (Harris, 2007). In CE, the separations take place within a tubular capillary with internal diameter ranging from 25 to 100  $\mu\text{m}$ . Before the capillary can be used for analysis, the inner walls of the capillary need to be electrically charged. This is accomplished by flushing the capillary with sodium hydroxide (NaOH) solution followed by a buffer solution. On passing the NaOH solution, the silanol (Si-OH) groups lining the inner walls of the capillary are ionized to negatively charged silanoate (Si-O<sup>-</sup>) groups. The cations within the buffer get attracted towards the negatively charged silanoate groups. This results in the formation of a double layer composed of a fixed layer and a mobile layer (Figure 2.1).

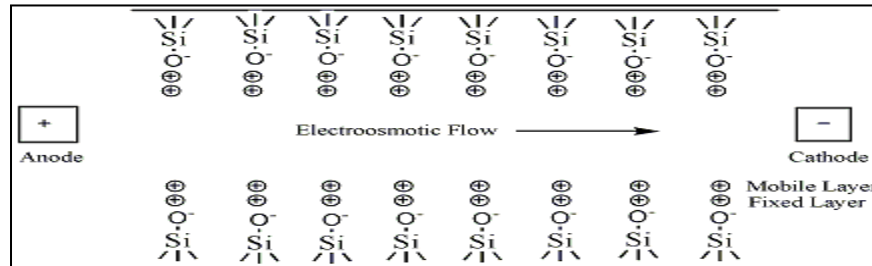


Figure 2.1. Double layer formed within capillary (Baker, 1995)

The fixed layer is composed of cations held tightly to the silanoate groups. The mobile layer is composed of cations further away from the silanoate groups, and thus can slowly migrate towards the cathode if a voltage is applied. Solvation of anions to cations forming the mobile layer causes the bulk buffer to flow towards the cathode. The flow of the bulk buffer towards the cathode is called the electroosmotic flow (EOF) (Baker, 1995).

Once the capillary is electrically charged, the sample containing the analyte can be injected into the buffer filling the capillary. As the electric current is applied, the buffer moves towards



the cathode and the sample containing the analyte will move within the buffer and be separated based on differing electrophoretic mobilities, which are related to size-to-charge ratios. Once the analyte reaches the UV detector, it is detected and processed by a computer in the form of an electropherogram (Figure 3). The electropherogram shows peaks, representing different analytes, at different migration times. The area under the peak is proportional to the amount of analyte and the migration time is unique to each compound.

Capillary electrophoresis (CE) is an analytical method that differentiates and separates charged species on the basis of mobility under the influence of an electric field gradient (Chu et al., 1995) or electrophoretic mobility. The analytes are separated and analysed based on size and charge. Smaller ions travel more easily through the buffer resulting in faster migration within the buffer toward the respective electrode. Increased charge on the ion increases attraction and thus the flow toward the respective electrode. Therefore, small positive ions are detected before small negatively charged ions. Neutral species migrate at the rate of the EOF. This process can be seen below in Figure 2.2.

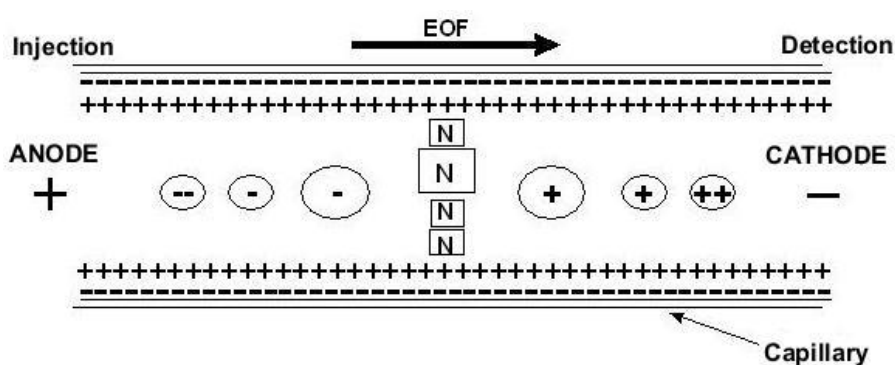


Figure 2.2. A diagram depicting EOF including the separation of ions based on size and charge between the anode and cathode (Laboratoire Suisse d'Analyse du Dopage, 2008) .

A basic CE system is shown in Figure 2.3. It consists of several main components namely a controllable high voltage power supply, inlet and outlet buffer vials, a capillary with optical viewing window, a detector, and a data display device like a computer. Most CE units also are equipped with a cooling ability to control or dissipate heat inside the capillary. The power supply can be set at a constant voltage up to 30 kV or a constant current up to 300  $\mu\text{A}$  as well

as a constant power up to 6 W. The polarity can be also reversed. Because the migration time varies if the voltage changes, it is very common to operate at constant voltage. The most important variable in CE is the composition of the buffer, which influences the EOF. Any small change in pH or concentration of buffer can affect the migration time of solutes. Capillaries in CE system usually have very small inner diameters, around 25-100  $\mu\text{m}$ . It is very common to use a fused silica capillary instead of the glass ones because they are still transparent at shorter wavelengths than 280 nm. An external coating of polyimide covers the capillary, making them stronger, more flexible, durable, and not easily broken.

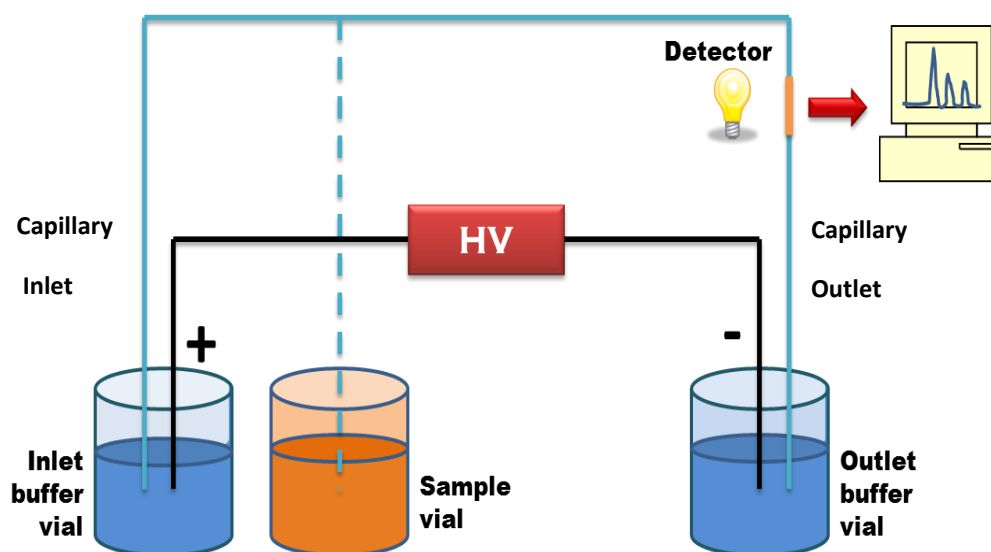


Figure 2.3. Schematic diagram of capillary electrophoresis (Donkor, 2021).

UV detection or PDA detection can be used for direct or indirect detection of an analyte. Direct detection is used for compounds that can absorb UV light strongly, resulting in high positive peaks in the electropherogram without any software manipulation. For solutes that do not possess strong UV absorbance, indirect UV detection can be used. In this approach, the buffers would contain UV absorbing compounds, i.e., chromophoric ions, that serve as visualization agents to make the background electrolyte absorbent with indirect UV detection. In the electropherogram, the absorbance of buffer with those UV absorbing compounds will appear

as the baseline with a high background signal. When the non-UV absorbing solutes pass through the detector, their peaks would be a negative dip compared with the baseline. These negative peaks will be flipped to become positive peaks in the electropherogram by a software that reverses detector's output polarity (Baker, 1995).

Micellar Electrokinetic Capillary Chromatography (MEKC) is a mode of capillary electrophoresis that allows for interactions with micelles and the separation of electrically neutral compounds becomes possible (Harris, 2007). A surfactant is added to the buffer above the critical micelle concentration (CMC), the threshold where micelles can form. These micelles form a pseudostationary phase. Very hydrophobic molecules spend all their time in the micelles and migrate at the same rate as the micelles. Molecules that are partially soluble in the micelles will partition between the buffer and the micelles at a rate proportional to their hydrophobicity. Hydrophilic, water-soluble molecules stay in the aqueous buffer and do not interact with the micelles. The interactions with the pseudostationary phase are rapid compared to interactions with a stationary phase and do not increase analysis time because the EOF dominates the migration rate. Sodium dodecyl sulphate forms anionic micelles above the CMC of 8.2 mM; cationic, non-ionic, and zwitterionic surfactants form corresponding micelles. Anionic micelles migrate within the buffer toward the anode but the EOF is stronger and, ultimately, the micelles flow toward the cathode.

### **Factors Influencing CE Analysis**

#### **(i) Capillary Inner Diameter**

The capillary diameter influences the convective diffusion and it is important to minimize it in analysis. The smaller the capillary radius is, the less convective diffusion is generated due to the smaller temperature difference. This leads to narrower zones, and better separation of the zones. The temperature difference,  $\Delta T$ , between the center and the wall of the capillary, is given by the equation below (Baker, 1995):

$$\Delta T = \left(\frac{0.239Q}{4k}\right)r^2$$

Q: power density (Watt/m<sup>3</sup>)  
k: thermal conductivity  
r: capillary radius

A reduced capillary diameter offers higher electrical resistance and less current for the applied voltage, resulting in less Joule heat. In addition, a small capillary dissipates heat faster because of a larger inner surface area-to-volume ratio. In a smaller capillary, the solutes tend to move to the detector as single zones. In a larger capillary, the solutes around the warm center will move through the tube with different speeds compared to the ones at the cooler outer wall, leading to two distinct zones for the same solute. Therefore, for CE analysis, it is desirable to use a capillary with the smallest diameter possible, as it can provide good separation as well as reduce Joule heat and increase heat dissipation (Baker, 1995).

#### (ii) Chemical Modification of the Capillary Wall (Coating)

The capillary wall can be altered by coating or chemical bonding to reduce or eliminate the EOF. These modifications reduce the zeta potential by shielding the surface charges on the inner wall, thereby resulting in a reduction or elimination of EOF. The modifications also increase the viscous drag on the buffer at the wall, thus reducing the rate of EOF. A coating can also minimize the adsorption of solute to the capillary wall and leading to better results in some cases. By chemically treating the capillary, a reasonable detection time is obtained., but adequate EOF is still needed and the level of EOF required depends on the analytes (Baker, 1995).

#### (iii) Voltage

High voltages of up to 30 kV can be used because the capillary has a small diameter and can dissipate heat quickly. The EOF is proportional to the electrical field which depends on the applied voltage. The higher the voltage applied, the shorter the migration time of samples because of the increase in EOF. High voltage also provides faster separation and better efficiencies. Theoretically, it is better to use the highest voltage because of faster analysis time and narrow peaks. Nevertheless, Joule heat will be increased by the higher voltage, a decrease of the buffer's viscosity or an increase of flow rate. The Joule heat may cause broader peaks, unstable migration times, solution decomposition or denaturation, or buffer boiling which is a cause of electrical discontinuity. Therefore, the applied voltage should be reasonable so that the heat can dissipate well in capillary. The maximum optimized voltage can be investigated and determined based on a plot of Ohm's law, which is a plot of the observed currents versus

the applied voltages as shown in Figure 2.4. The point at which nonlinearity starts represents the maximum voltage that can be applied to prevent Joule heating (Baker, 1995).

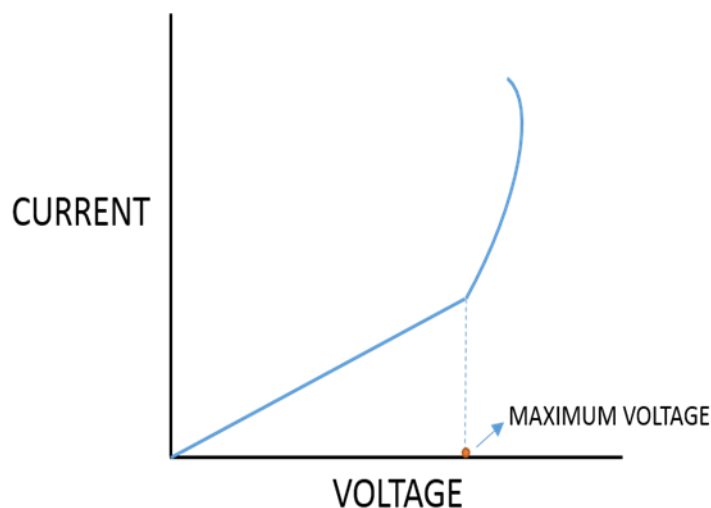


Figure 2.4. Ohm's law plot (Baker, 1995).

#### (iv) Source and Destination Vials Buffers

Source and destination vials are filled with the same buffers with even levels to prevent changes in migration time or laminar flow due to the siphoning of the buffer. Even with that, repeated analysis might also change the concentration and pH of the buffer because of the electrolysis of water, where hydrogen ions are formed at the cathode and the hydroxyl ions at the anode. Moreover, after repeated analysis, the buffers in outlet vial may have a different composition because solute ions in the capillary might elute and accumulate into the outlet buffer vial, which can change the electrical field and lead to non-reproducible migration times. Therefore, buffer replenishment is very important by rinsing and refilling the vials and capillary to achieve good reproducibility. Especially in indirect absorbance detection, any change in the chromophore's concentration or composition might lead to a drifting baseline and more noise (Baker, 1995).

There are some criteria for selecting the buffer or chromophoric ion in CE analysis. Firstly, the mobility of a chromophoric ion or any ions in the buffers should be similar to the solute's mobility to prevent asymmetrical peaks. Secondly, in indirect absorbance detection, the

chromophore should have a high molar absorptivity at the specific wavelength so that when non-absorbing solutes pass through the detector, there will be a large decrease, indicated by a negative dip peak. In other words, the chromophoric ions should absorb light strongly at the wavelength that the solutes cannot absorb. Lastly, the buffer or chromophoric ion must be stable and nonreactive with the capillary or any components in the samples (Baker, 1995).

#### (v) pH of the Buffer

Buffer pH can change the zeta potential, thus affecting strongly the EOF. At higher pH, the inner wall of the capillary will be highly negatively-charged because of the high dissociation of Si-OH to Si-O<sup>-</sup>. The surface's high charge is proportional to the zeta potential, resulting in increased EOF or electroosmotic velocity. In contrast, at low pH (< 2), the EOF is eliminated because most of the silanol groups cannot be deprotonated. In addition, buffer pH will also affect the electrophoretic mobility of the solutes. Depending on the analysis, the required buffer pH can be selected by providing the best separation or optimum EOF flow (Baker, 1995).

#### (vi) Buffer Concentration

For a stable capillary temperature, a high buffer concentration will decrease the zeta potential and result in lower EOF. Analysis time will be shorter with lower concentration of buffers, but unreasonably low concentration may lead to broader and asymmetric peaks as well as cause adsorption of solutes. Another noticeable distortion of electrical field can occur when the buffer concentration is not higher than the solute concentration. This results in broad, skewed peaks. As a general rule, the run buffer concentrations are in the range of 10-100 mM (Baker, 1995).

#### (vi) Temperature

Temperature is one of the important factors in CE analysis. Unstable temperature can lead to various migration times, zone spreading, and sample decomposition. The EOF rate also increases when the temperature elevates. It is always advisable to control the temperature. There are many different cooling systems to help the heat dissipate quickly and keep the temperature stable, either by air or liquid cooling. The most common method is liquid cooling in which a coolant is circulated through a jacket placed around the capillary (Baker, 1995).

## **CHAPTER 3**

### **MATERIALS AND METHODS**

## CHAPTER 3. MATERIALS AND METHODS

### Experimental

#### Reagents

Bisphenol A, 97+%, was purchased from Alfa Aesar, Ward Hill, MA, USA. Sodium tetraborate decahydrate,  $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ , and sodium dodecyl sulfate (SDS) were purchased from Sigma-Aldrich Chemical Co, St Louis, MO, USA. Sodium dodecyl sulfate (SDS) was obtained from BDH Biochemicals, Toronto, Canada. Sodium hydroxide was purchased from EMD Chemicals, Gibbstown, New Jersey, USA.

#### Instrumentation

The Beckman Coulter P/ACE MDQ capillary electrophoresis system (Beckman Coulter, Brea, CA, USA) equipped with ultraviolet detector and interfaced with the 32-Karat software for data acquisition is shown in Figure 3.1. An uncoated fused-silica capillary with 50  $\mu\text{m}$  internal diameter and total length of 60 cm was used. The separation is done in fused silica capillary from Polymicro Technologies, AZ, USA. The 25 mm Nylon® 0.45  $\mu\text{m}$  syringe filter was purchased from Canadian Life Science, Ontario, Canada. The pH meter used was Mettler Toledo FE20- FiveEasy™ from Greifensee Switzerland, purchased from USA.





Figure 3.1. Picture of capillary electrophoresis system used in the Donkor research laboratory.

### **Solution and Buffer Preparation**

All water used for standards and buffer preparation and capillary rinsing was 18 M $\Omega$  prepared by the TRU Chemistry department. Glassware was all cleaned with manual detergent in hot water, rinsed with tap water three times, deionized water three times, followed by three rinses with 18 M $\Omega$  water. They were dried either in an oven at 100°C or at ambient temperature.

The buffer used for analysis was 60 mM borate, 20 mM SDS prepared in 18 M $\Omega$  water and pH adjusted to 9.5 with 1.0 M NaOH. Buffer solutions were prepared in a volumetric flask using 18 M $\Omega$  water and sonicated when necessary to fully dissolve the solids. The pH of the buffer solutions was adjusted with 0.1 M NaOH. All stock solutions were filtered when prepared and before injection into the CE instrument using 0.45  $\mu$ m Nylon syringe filters. Sodium hydroxide solutions (0.1 M and 1.0 M) were prepared in a volumetric flask using 18 M $\Omega$  water.

Standards were prepared in 10 % methanol to allow the BPA to fully dissolve and keep the organic solvent to a minimum to avoid solvent effects in the analysis. A 50 mM BPA stock solution was prepared in a 50 mL volumetric flask by adding 5.00 mL methanol by volumetric pipette. When the BPA was fully dissolved, the flask was topped up with 18 M $\Omega$  water, thoroughly mixed and then filtered with a 0.45  $\mu$ m Nylon syringe filter. Dilutions were made directly in 2 mL CE vials by using the 50 mM standard and 18 M $\Omega$  water using micropipettes of the appropriate volume range. No precipitation of BPA was observed upon dilution with the water. Table 3.1 shows the volumes of BPA and 18 M $\Omega$  water used to prepare the standards.

Table 3.1. Preparation of BPA standards for calibration.

Standard Concentration	Volume of 50 ppm BPA	Volume of 18 M $\Omega$ water
50 ppm	2 mL	0 mL
40 ppm	1.6 mL	0.4 mL
30 ppm	1.2 mL	0.8 mL
20 ppm	0.8 mL	1.3 mL
10 ppm	0.4 mL	1.6 mL
5 ppm	0.2 mL	1.8 mL

### Capillary Electrophoresis Method

All analysis was performed using a Beckman P/ACETM MDQ capillary electrophoresis instrument (Beckman Coulter, Inc., Fullerton, CA) using an ultraviolet (UV) detector. The capillary used was uncoated fused-silica with 50  $\mu$ m inner diameter, 365  $\mu$ m outer diameter, 60 cm total length with 50 cm to the detector (Polymicro Technologies, Phoenix, AZ). Galden® HT-110 perfluoropolyether coolant (Ideal Vacuum Products LLC, Albuquerque, NM) was used in the capillary cartridge to keep the buffer and samples at 25 °C during migration through the capillary. Nylon filters, 0.45  $\mu$ m, from Canadian Life Science, Peterborough ON, were used to filter all solutions prior to injection. The temperature was held constant at 25 °C. Direct UV detection wavelength was set at 214 nm.

New capillaries were conditioned at 20 psi for 10 min with water, 60 min with 1M NaOH, 30 min with 0.1 M NaOH followed by 20 min with water again. Each day the capillary was initially rinsed with 0.1 M NaOH for 15 min and then with the buffer that would be used in the analysis for another 15 min. This daily rinse was also at 20 psi. Capillaries were always stored with both ends immersed in 18 M $\Omega$  water when not in use.

Bisphenol A analysis was ultimately conducted with the method shown in Table 3.2.

Table 3.2. Optimized CE instrumentation parameters for BPA analysis

Rinse Pressure	20 psi	5 min	0.1 M NaOH	
Rinse Pressure	20 psi	3 min	18 M $\Omega$ water	
Rinse Pressure	20 psi	8 min	buffer	
Injection	0.5 psi	5s		
Separate voltage	20 kV	20 min	0.17 min ramp	normal polarity

## Sample Collection and Preparation

### Water Samples

Water for analysis was collected from the Thompson River at Riverside Park and from the Kamloops city water supply. Tap water was from the north end of the city, Rayleigh, the south end, Aberdeen, and central to Kamloops at Thompson Rivers University. At TRU, samples were collected from water taps in the Ken Lepin Science building and from the oldest campus building, Old Main, in a staff kitchen and two washrooms.

Water was collected from taps after the water was allowed to run for a full one minute. All water collection followed the same procedure; amber glass vials were rinsed three times with the sample, filled to the shoulder of the sample bottle and sealed with a screw top. The only exceptions were the domestic tap water provided from Rayleigh in a clear glass, nylon stoppered sample vial. Samples were left unopened at room temperature until they were filtered immediately before analysis.

## Mussel Samples

Mussel samples, *Mytilus trossulus*, were collected from 12 sites around the southern half of Vancouver Island (Table 3), all of which were accessible by vehicle. All mussels were between 3 and 4 cm and their tissues were digested with nitric acid to remove organic matter while leaving non-tissue materials, such as plastics. Microplastic particles were documented and the samples were stored at 4°C until they were pH adjusted with 1M NaOH for analysis by CE. (Frank, 2018).

Table 3.3. Sample sites around Vancouver Island, BC. (Frank, 2018).

Site	Community	Latitude/ Longitude	Coast	Environment	Human population
1	Tofino	49°09.161'N, 125°54.196'W	West	Dock	1,932
2	Bamfield	48°50.081'N, 125°08.144'W	West	Dock	155
3	Port Renfrew	48°33.338'N, 124°24.818'W	West	Dock	144
4	Jordan River	48°25.254'N, 124°03.299'W	West	Intertidal	100
5	Sooke	48°22.978'N, 123°42.344'W	South	Intertidal	367,770
6	Colwood	48°20.030'N, 123°27.230'W	South	Intertidal	367,770
7	Oak Bay	48°27.065'N, 123°17.856'W	South	Dock	367,770
8	Duncan	48°47.795'N, 123°36.145'W	East	Dock	44,451
9	Departure Bay	48°11.396'N, 123°56.983'W	East	Dock	104,936
10	Parksville	49°20.899'N, 124°21.516'W	East	Dock	28,922
11	Comox	49°40.319'N, 124°55.699'W	East	Dock	54,157
12	Campbell River	50°01.952'N, 125°14.650'W	East	Dock	37,861

## **CHAPTER 4**

### **RESULTS AND DISCUSSION**

## CHAPTER 4. RESULTS AND DISCUSSION

### BPA Standards

The capillary electrophoresis analysis of water and mussel samples for the presence of bisphenol A (BPA) was undertaken. A CE method was developed and optimized until it was able to detect BPA concentrations in standard solutions between 5 ppm and 50 ppm.

Following the optimization, a calibration curve was developed to quantify any BPA that was detected in the samples. The calibration curve obtained is shown in Figure 4.1. The calibration curve showed reasonable linearity with a  $R^2$  of 0.9867.

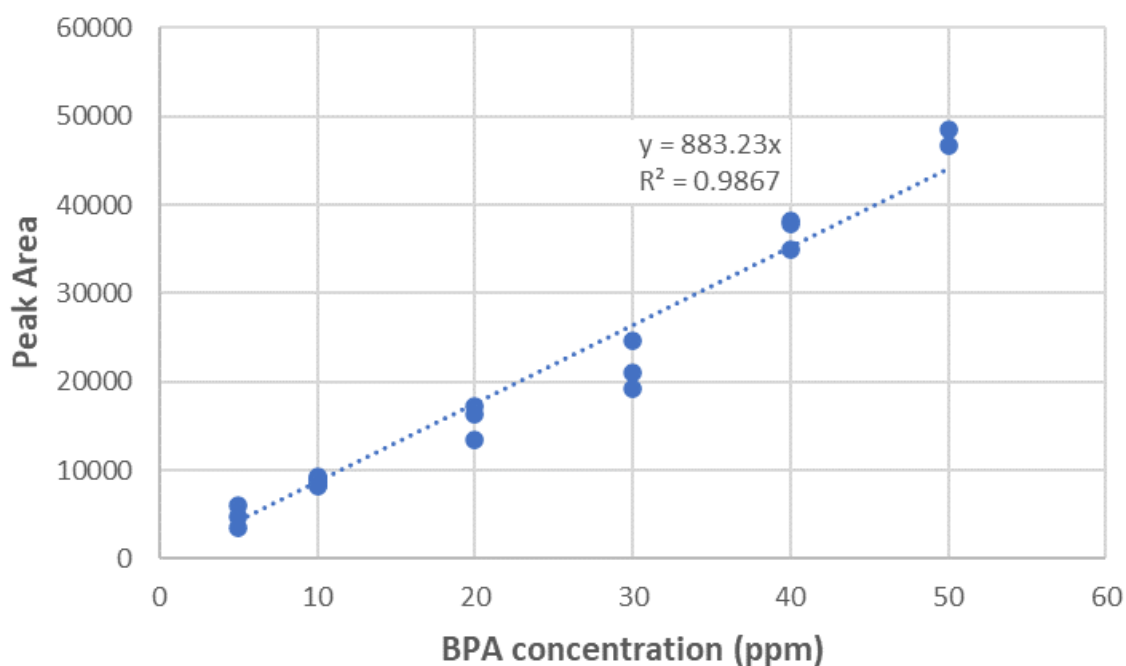


Figure 4.1. The calibration curve for BPA concentrations from 5 ppm to 50 ppm developed with the method used in this study.

The BPA identifying peak in standard solutions from 5 ppm to 50 ppm using ultraviolet direct detection appeared in the electropherograms between 13.5 and 14 min. Below, in Figure 4.2, is an electropherogram of the 10 ppm BPA standard.

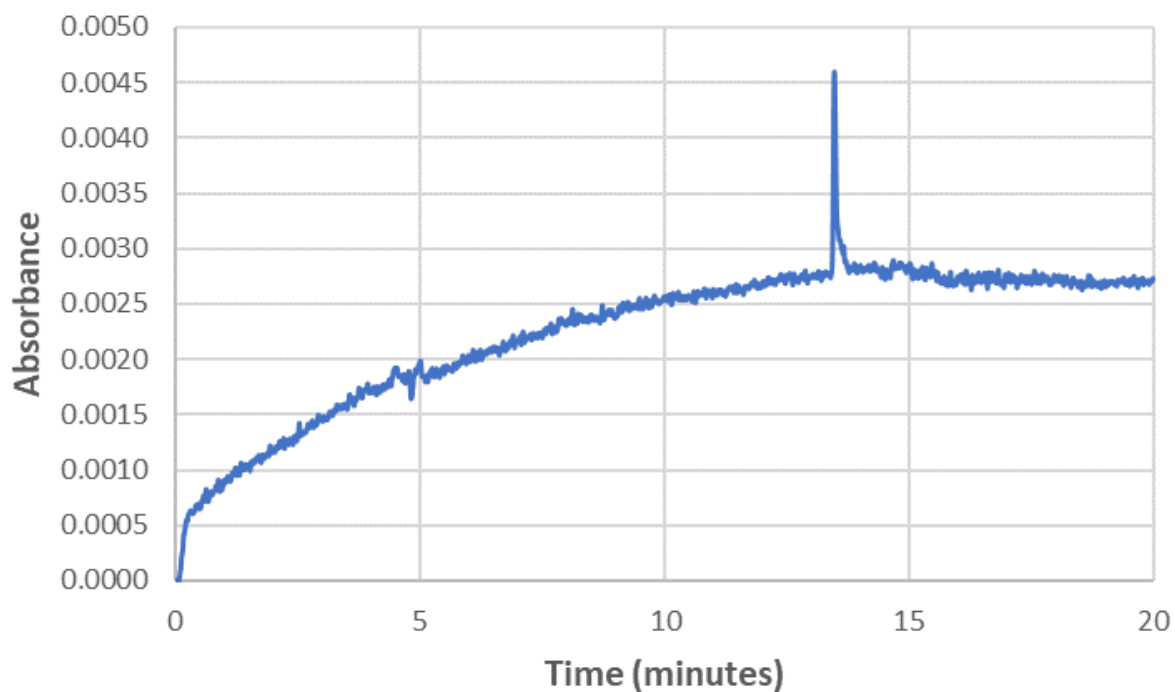


Figure 4.2. The electropherogram of the 10 ppm BPA standard as a sample of the data used to generate the calibration curve shown in Figure 7.

## Samples

### Water Analysis

Water samples from municipal water supply from several sources around Kamloops were analysed. No BPA was detected in these samples. A representative electropherogram shows no peak in the BPA region. An example of the electropherogram of water sample taken from TRU Old Main Staff lunchroom kitchen cold water tap is shown in Figure 4.3. As it can be seen no significant peak was obtained in the region for the detection of BPA. Electropherograms for other water samples displayed similar results. This observation indicates that there was no measurable amount of BPA in any of the Kamloops municipal water supply that was analysed or the amounts were below the detection limit of the developed method.

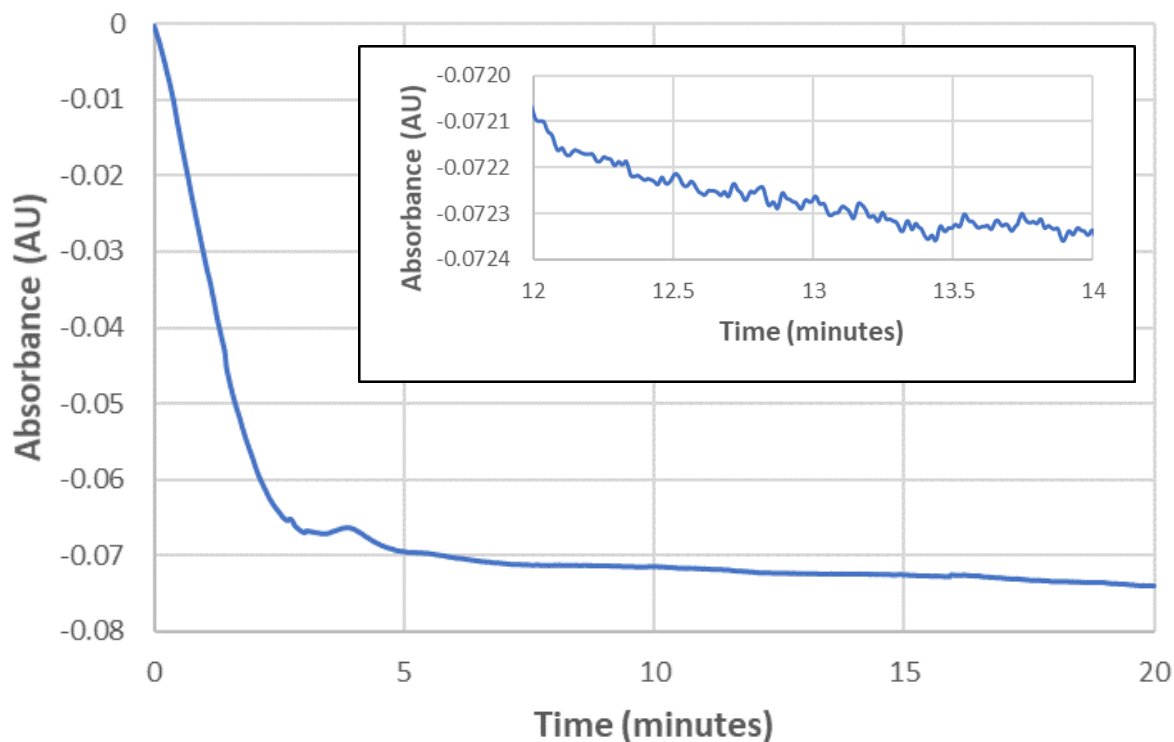


Figure 4.3. Sample electropherogram for CE analysis of Kamloops municipal tap water taken from the staff lunchroom in the Old Main building on the Thompson Rivers University campus showing no BPA detected. The peak for BPA is expected at about 13 min. The insert shows an expanded view from 12 min to 14 min.

### Mussel Sample Analysis

The samples of mussel tissue tested were identified by area of collection and the number of microplastic particles (MP) counted per mussel. There were up to three MPs in each of the samples tested and up to 6 MPs across all of the samples available. On the chromatograms of the mussel samples, the absorbance units (AU) scale is very much smaller than the other graphs and no peaks appeared at the expected migration time for BPA. Small peaks in the electropherograms for the mussel samples had inconsistent and shorter migration times. This may be due to a change in the EOF of the analysis for the mussel samples. The samples were provided in nitric acid with pH less than one. Different pHs of the mussel samples were also investigated. One mussel sample was subjected to extreme acidic ( $\text{pH} < 1$ ) and basic conditions, and then analysed. Electropherograms are shown in Figures 4.4 to Figures 4.6. The



BPA was detected at approximately 7.5 min, 11 min for the acidic samples and 10 min for the basic mussel sample. Adjusting the pH of the sample did not greatly affect the results. The sample that was adjusted to a basic pH had a peak detected at 10 min, representing 1 min shorter migration time than when the same sample was acidic. This is within the variation seen for the detection of the standards and does not necessarily represent any significance.

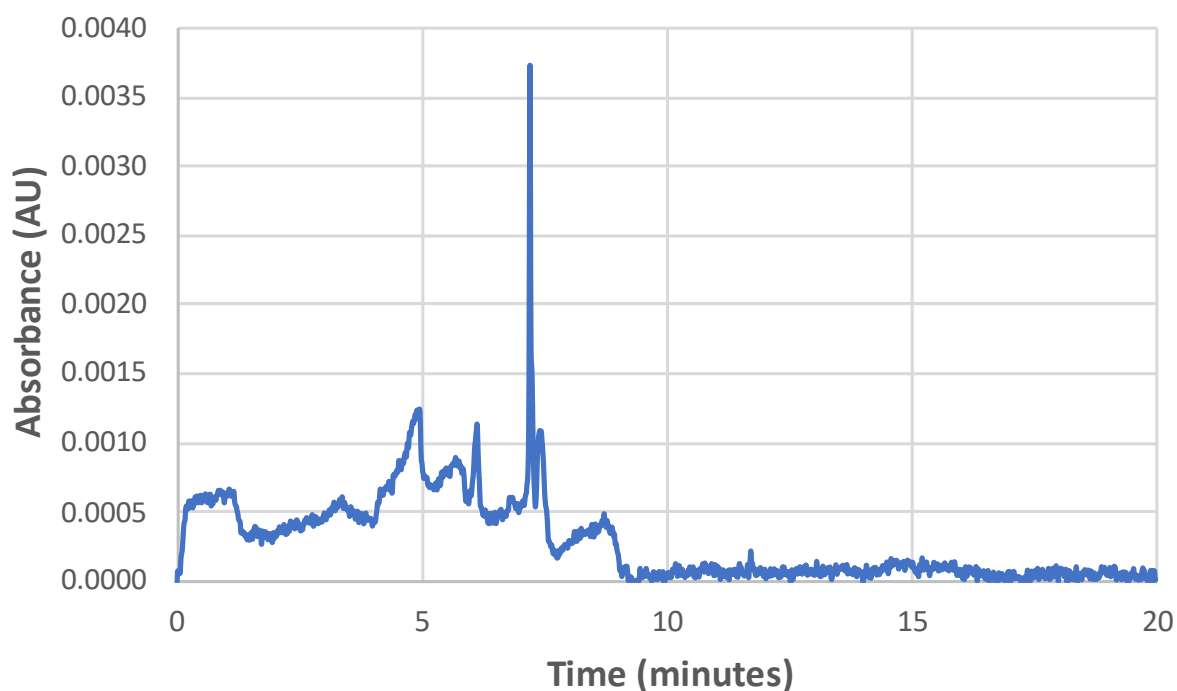


Figure 4.4. Electropherogram of mussel sample #109 collected from Oak Bay, BC. The sample was digested in nitric acid for microplastic particle analysis (2 MP count) and analysed by CE without pH adjustment. The large peak at 7.3 min was labeled by the CE analysis software with an area of 7472.

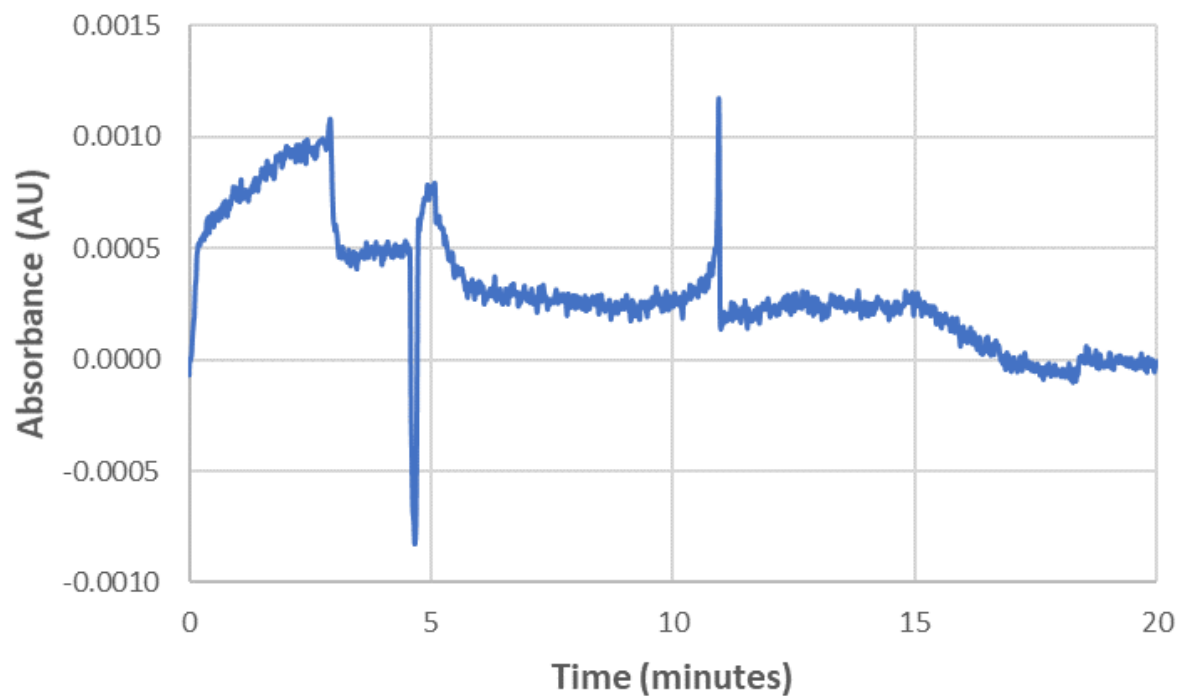


Figure 4.5. CE electropherogram of mussel sample #116 (3 MP count) collected from Duncan, BC, acidified for microplastic particle analysis and provided for BPA analysis. The pH of the sample was less than one and left unadjusted for CE analysis. The area of the peak at approximately 11 min has an area of 2617.

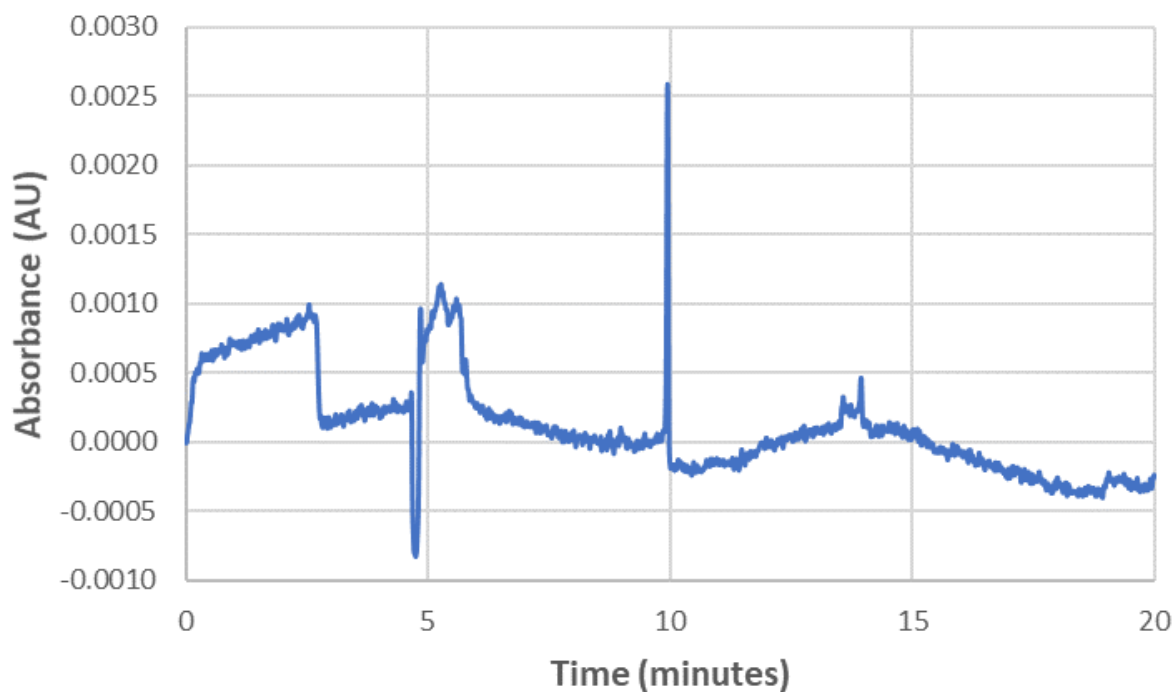


Figure 4.6. CE electropherogram of mussel sample #116 (3 MP count) collected from Duncan, BC, acidified for microplastic particle analysis and provided for BPA analysis. The pH of the sample was adjusted with NaOH and tested to be basic. The area of the peak at approximately 10 min was found to be 6957.

In the mussel analysis, the peaks of interest which may indicate the presence of BPA can be interpreted to indicate low levels of BPA. Table 4.1 shows calculated concentration from the peak areas.

Table 4.1 Potential BPA concentration in mussel samples.

<b>Figure</b>	<b>Area of CE peak</b>	<b>BPA concentration (ppm)</b>
<b>4.4</b>	7472	8.5
<b>4.5</b>	2617	3.0
<b>4.6</b>	6957	7.9

## **CHAPTER 5**

### **CONCLUSIONS AND FUTURE WORK**

## CHAPTER 5. CONCLUSIONS AND FUTURE WORK

### Conclusions

Bisphenol A (BPA) was not detected in any of the water samples tested by the capillary electrophoresis (CE) method developed and used in this study. The lowest concentration standard used was 5 ppm. With safe levels of BPA exposure set between 4 µg/kg·bw/day by the EFSA and 50 µg/kg·bw/day by the US EPA (Shelnutt et al., 2013), a BPA detection at 5 ppm is not sensitive enough to determine if the BPA levels are of concern unless the levels were extremely high. Walpole et al (2012) estimated the average adult body mass at 62 kg across the world and 80.7 kg in North America; therefore, a total exposure for an adult could be considered safe between (62x4=248 µg/day and 4035 µg/day ) 0.248 and 4.035 mg/day. If BPA were detected at 5 ppm in drinking water, as little as 0.05 to 0.8 L per day could result in an unsafe level of exposure. Considering all the other sources of BPA exposure, a level that could be detected and quantified with the current method would be very alarming.

The analysis of mussel tissue had more interesting results that were quantifiable by interpolation using the equation of the calibration line. Assuming the prominent peaks represent BPA, the concentrations ranged from 3.0 ppm to 8.5 ppm in the samples. There is no apparent correlation between BPA concentration and the number of microplastic particles; the sample with the larger concentration had two microplastic particles counted while the smaller area peak, was in a sample with 3 MPs.

The peaks for the mussel samples had shorter and varied migration times which means they cannot be definitively identified as being a response to the presence of BPA. In fact, they were initially assumed to not represent detection of BPA and it is only upon further reflection that they are possibly indications of BPA in the samples. Without confirmation that the peaks actually represent detection of BPA, no definitive conclusions can be made about the presence of BPA in the mussels. The presence of microplastic particles shows there is plastic contamination of the marine environment around Vancouver Island but the present research has not verified BPA as a component of the problem. Given the ubiquitous nature of BPA in the environment, it would be surprising if these filter feeders did not have some BPA in their tissues.

## **Future Work**

Using the current method to further analyse the mussel samples could provide interesting information. Spiking the samples with known concentrations of BPA could reveal if the peaks are due to the presence of BPA. Using the method of standard addition with the mussel samples would be valuable future work given the complex matrix of these samples.

A first approach to detecting lower concentrations of BPA would be using the existing parameters to test lower concentration BPA standards. This might allow development of a lower concentration calibration curve and lower the level of detection. This could allow the smaller area peaks in the mussel samples to be quantified.

One of the limitations for UV detection in CE is the narrow capillary diameter provides a short pathlength for the UV detection and thus limits its sensitivity. There are various other CE techniques that could also be investigated such as large volume sample stacking to enhance the sensitivity.

Using CE to detect analogues of BPA is a natural next step in this line of research. As the health and environmental hazards have been documented, BPA has been replaced with similar compounds such as bisphenol S. It will be important to know where these compounds are and what concentrations are there.

## REFERENCES

- Anderson, J. C., Park, B. J., & Palace, V. P. (2016). Microplastics in aquatic environments: Implications for Canadian ecosystems. *Environmental Pollution*, 218, 269–280. <https://doi.org/10.1016/j.envpol.2016.06.074>
- Baker, D. R. (1995). *Capillary Electrophoresis* (First Edit). John Wiley & Sons, Inc. New York.
- Canada, Fisheries. and Oceans. (2019). *Canada's Fisheries Fast Facts 2019*.
- Chu, Y. H., Avila, L. Z., Gao, J., & Whitesides, G. M. (1995). Affinity Capillary Electrophoresis. *Accounts of Chemical Research*, 28(11), 461–468. <https://doi.org/10.1021/ar00059a004>
- Davidson, K., & Dudas, S. E. (2016). Microplastic Ingestion by Wild and Cultured Manila Clams (*Venerupis philippinarum*) from Baynes Sound, British Columbia. *Archives of Environmental Contamination and Toxicology*, 71(2), 147–156. <https://doi.org/10.1007/s00244-016-0286-4>
- Desforges, J. P. W., Galbraith, M., Dangerfield, N., & Ross, P. S. (2014). Widespread distribution of microplastics in subsurface seawater in the NE Pacific Ocean. *Marine Pollution Bulletin*, 79(1–2), 94–99. <https://doi.org/10.1016/j.marpolbul.2013.12.035>
- Donkor, K. (2021). *Capillary Electrophoresis Schematic Diagram*. Thompson Rivers University.
- Environment and Climate Change Canada. (2018). *Canadian Environmental Protection Act, 1999 Federal Environmental Quality Guidelines Bisphenol A. June*. [http://www.ec.gc.ca/ese-ees/CB1CEFFB-983E-4113-805E-86A74C6824D0/FEQG\\_BPA\\_15-02-2017\\_clean.pdf](http://www.ec.gc.ca/ese-ees/CB1CEFFB-983E-4113-805E-86A74C6824D0/FEQG_BPA_15-02-2017_clean.pdf)
- European Food Safety Authority, Food Ingredients and Packaging Unit. (2018). *Call for data relevant to the hazard assessment of Bisphenol A ( BPA )*. 1–4. <https://doi.org/10.2903/sp.efsa.2017.EN-1354/epdf>
- Frank, M. (2018). *Microplastics in marine invertebrate filter-feeders: development of an isolation method and quantification*. Thompson Rivers University.
- Frank, M., Gosselin, L., & Donkor, K. (2016). *Microplastics in your mussels ? Microplastic particles in *Mytilus trossulus* collected around the coasts of Vancouver Island*. Thompson Rivers University.
- Fu, P., & Kawamura, K. (2010). Ubiquity of bisphenol A in the atmosphere. *Environmental Pollution*, 158(10), 3138–3143. <https://doi.org/10.1016/j.envpol.2010.06.040>
- Government of Canada. (2010). *Canada Consumer Product Safety Act Loi canadienne sur la sécurité des produits de consommation*. <https://laws-lois.justice.gc.ca/PDF/C-1.68.pdf>

- Harris, D. C. (2007). *Quantitative Chemical Analysis* (Seventh Ed). W. H. Freeman and Company.
- Health Canada. (2020). *Guidelines for Canadian Drinking Water Quality Summary Table Health Canada Federal-Provincial-Territorial Committee on Drinking Water of the Federal-Provincial-Territorial Committee on Health and the Environment September 2020*.
- Jalal, N., Surendranath, A. R., Pathak, J. L., Yu, S., & Chung, C. Y. (2018). Bisphenol A (BPA) the mighty and the mutagenic. *Toxicology Reports*, 5(June 2017), 76–84. <https://doi.org/10.1016/j.toxrep.2017.12.013>
- Jones, D. C., & Miller, G. W. (2008). The effects of environmental neurotoxicants on the dopaminergic system: A possible role in drug addiction. *Biochemical Pharmacology*, 76(5), 569–581. <https://doi.org/10.1016/j.bcp.2008.05.010>
- Kang, J. H., Kondo, F., & Katayama, Y. (2006). Human exposure to bisphenol A. *Toxicology*, 226(2–3), 79–89. <https://doi.org/10.1016/j.tox.2006.06.009>
- Laboratoire Suisse d'Analyse du Dopage. (2008). *Capillary Electrophoresis*. [http://www.doping.chuv.ch/en/lad\\_home/lad-prestations-laboratoire/lad%02prestations-laboratoire-appareils/lad-prestations-laboratoire-appareils-ec.htm](http://www.doping.chuv.ch/en/lad_home/lad-prestations-laboratoire/lad%02prestations-laboratoire-appareils/lad-prestations-laboratoire-appareils-ec.htm)
- Lim, Y. H., Bae, S., Kim, B. N., Shin, C. H., Lee, Y. A., Kim, J. I., & Hong, Y. C. (2017). Prenatal and postnatal bisphenol A exposure and social impairment in 4-year-old children. *Environmental Health: A Global Access Science Source*, 16(1), 1–10. <https://doi.org/10.1186/s12940-017-0289-2>
- Michałowicz, J. (2014). Bisphenol A - Sources, toxicity and biotransformation. *Environmental Toxicology and Pharmacology*, 37(2), 738–758. <https://doi.org/10.1016/j.etap.2014.02.003>
- Mihaich, E. M., Friederich, U., Caspers, N., Hall, A. T., Klecka, G. M., Dimond, S. S., Staples, C. A., Ortego, L. S., & Hentges, S. G. (2009). Acute and chronic toxicity testing of bisphenol A with aquatic invertebrates and plants. *Ecotoxicology and Environmental Safety*, 72(5), 1392–1399. <https://doi.org/10.1016/j.ecoenv.2009.02.005>
- Rubin, B. S. (2011). Bisphenol A: An endocrine disruptor with widespread exposure and multiple effects. *Journal of Steroid Biochemistry and Molecular Biology*, 127(1–2), 27–34. <https://doi.org/10.1016/j.jsbmb.2011.05.002>
- Shelnutt, S., Kind, J., & Allaben, W. (2013). Bisphenol A: Update on newly developed data and how they address NTP's 2008 finding of "Some Concern." *Food and Chemical Toxicology*, 57, 284–295. <https://doi.org/10.1016/j.fct.2013.03.027>
- Shi, X. Y., Wang, Z., Liu, L., Feng, L. M., Li, N., Liu, S., & Gao, H. (2017). Low concentrations of bisphenol A promote human ovarian cancer cell proliferation and glycolysis-based metabolism through the estrogen receptor-A pathway. *Chemosphere*, 185, 361–367. <https://doi.org/10.1016/j.chemosphere.2017.07.027>



- Srivastava, S., Gupta, P., Chandolia, A., & Alam, I. (2015). Bisphenol A: a threat to human health? *Journal of Environmental Health*, 77(6), 20–26.
- Talsness, C. E., Andrade, A. J. M., Kuriyama, S. N., Taylor, J. A., & Saal, F. S. V. (2009). Components of plastic: Experimental studies in animals and relevance for human health. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364(1526), 2079–2096. <https://doi.org/10.1098/rstb.2008.0281>
- Tang, Y., Rong, J., Guan, X., Zha, S., Shi, W., Han, Y., Du, X., Wu, F., Huang, W., & Liu, G. (2020). Immunotoxicity of microplastics and two persistent organic pollutants alone or in combination to a bivalve species. *Environmental Pollution*, 258, 113845. <https://doi.org/10.1016/j.envpol.2019.113845>
- Tang, Y., Zhou, W., Sun, S., Du, X., Han, Y., Shi, W., & Liu, G. (2020). Immunotoxicity and neurotoxicity of bisphenol A and microplastics alone or in combination to a bivalve species, *Tegillarca granosa*. *Environmental Pollution*, 265, 115115. <https://doi.org/10.1016/j.envpol.2020.115115>
- Van Cauwenberghe, L., & Janssen, C. R. (2014). Microplastics in bivalves cultured for human consumption. *Environmental Pollution*, 193, 65–70. <https://doi.org/10.1016/j.envpol.2014.06.010>
- Van Sebille, E., Wilcox, C., Lebreton, L., Maximenko, N., Hardesty, B. D., Van Franeker, J. A., Eriksen, M., Siegel, D., Galgani, F., & Law, K. L. (2015). A global inventory of small floating plastic debris. *Environmental Research Letters*, 10(12). <https://doi.org/10.1088/1748-9326/10/12/124006>
- Walpole, S. C., Prieto-Merino, D., Edwards, P., Cleland, J., Stevens, G., & Roberts, I. (2012). The weight of nations: An estimation of adult human biomass. *BMC Public Health*, 12(1), 1. <https://doi.org/10.1186/1471-2458-12-439>
- Xiao, C., Wang, L., Zhou, Q., & Huang, X. (2020). Hazards of bisphenol A (BPA) exposure: A systematic review of plant toxicology studies. *Journal of Hazardous Materials*, 384(October 2019), 121488. <https://doi.org/10.1016/j.jhazmat.2019.121488>

**APPENDIX****Appendix A: Mussel samples**

Table A.1. Mussel samples by location and microplastic particle (MP) count

Sample number	Collection site	MP count	*Analysed by CE
3	Bamfield	0	
7	Bamfield	5	
8	Bamfield	3	
12	Departure Bay	3	
15	Departure Bay	6	
18	Departure Bay	1	
23	Parksville	0	
24	Parksville	3	
30	Parksville	2	
31	Jordan River	1	
34	Jordan River	0	
36	Jordan River	1	
44	Campbell River	1	
45	Campbell River	1	
46	Campbell River	0	*
52	Port Renfrew	2	*
57	Port Renfrew	4	
58	Port Renfrew	3	*
64	Tofino	0	*
65	Tofino	0	
66	Tofino	1	
75	Comox	3	
76	Comox	2	
77	Comox	2	*
84	Sooke	3	
86	Sooke	3	
89	Sooke	2	
91	Colwood	0	
94	Colwood	1	
99	Colwood	5	
107	Oak Bay	1	
108	Oak Bay	0	
109	Oak Bay	2	*
115	Duncan	2	
116	Duncan	3	*
117	Duncan	4	

## Appendix B: Sample Electropherograms

Figure B.1. Water samples from Rayleigh (black), Old Main, hot kitchen tap (grey), Old Main cold kitchen tap (blue), Old Main 2<sup>nd</sup> floor ladies washroom (pink), and Old Main gender neutral washroom automatic tap (green).

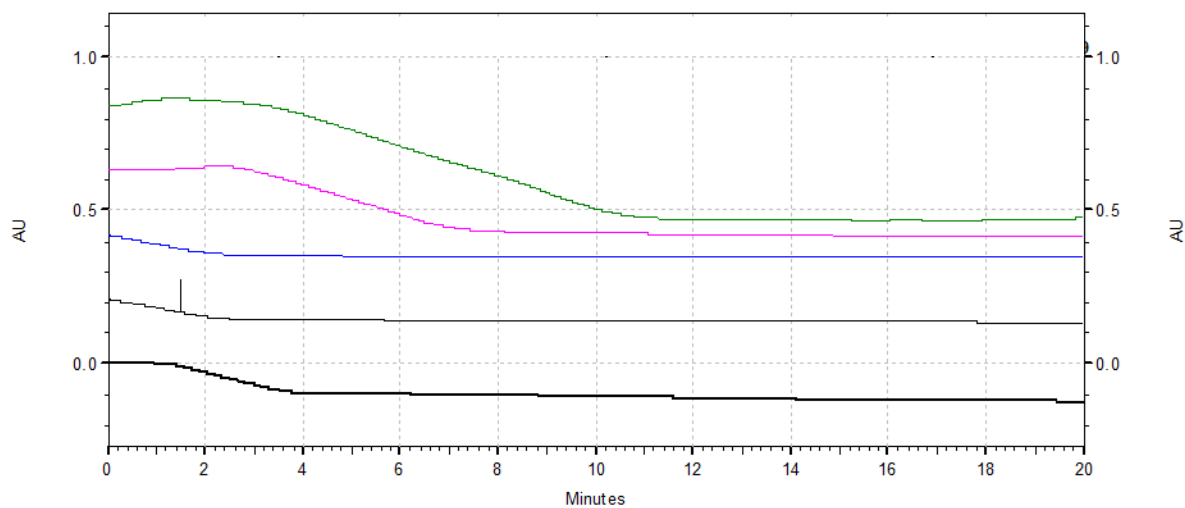


Figure B.2 Water sample electropherograms from Figure B.1 (see above) expanded to show 11.5 min to 15.5 min more closely.

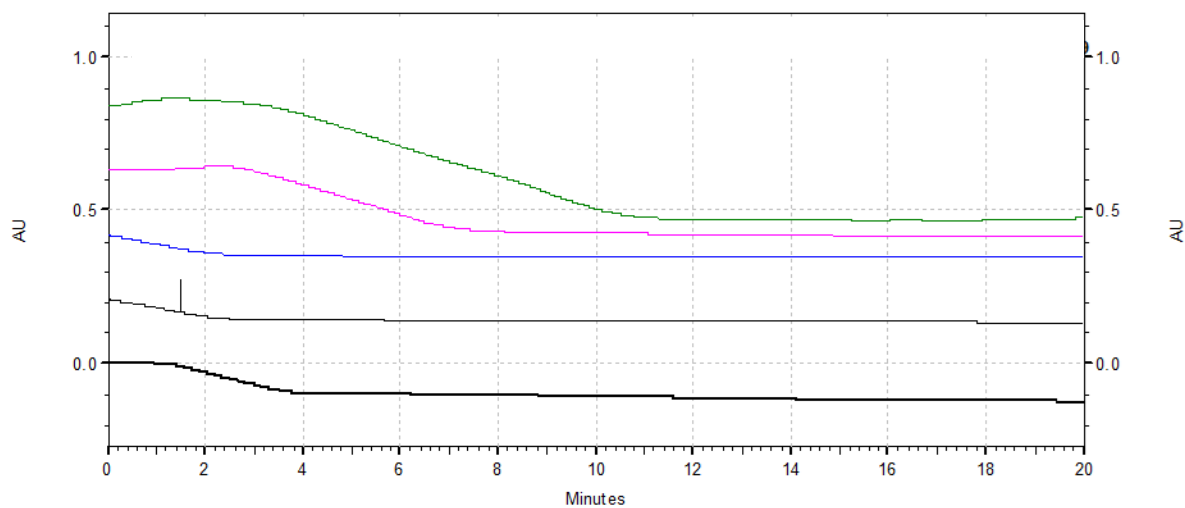


Figure B.3. Mussel sample electropherograms for samples: #58 pH unadjusted <1 (grey); #58 pH adjusted >14 (blue); #64 pH unadjusted (pink); #64 pH adjusted >14 (dark green) and #77 pH unadjusted (bright green). The pH of samples unadjusted were analysed as provided, pH <1.

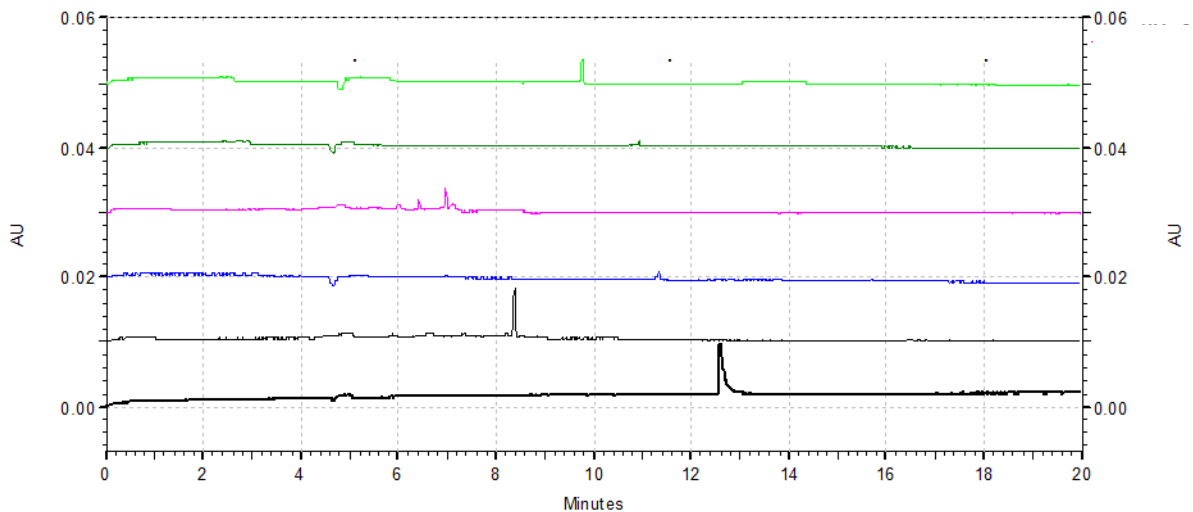


Figure B.4. Mussel sample electropherograms for samples: #109 pH unadjusted (black); #116 pH unadjusted (grey); #116 pH adjusted >6 (blue); #52 pH 6 (pink); #46 pH 3 (dark green); #46 pH 6 (bright green) and #46 pH unadjusted (purple). The pH of samples unadjusted were analysed as provided, pH <1.

