A Method to Characterize Natural Organic Matter using Size Exclusion Chromatography with Charged Aerosol Detector

by

Licai Hu

Submitted in partial fulfilment of the requirements
for the degree of Master of Applied Science

at

Dalhousie University
Halifax, Nova Scotia
May 2017

TABLE OF CONTENTS

LIST OF TABLES v
LIST OF FIGURES vi
ABSTRACT viii
LIST OF ABBREVIATIONS AND SYMBOLS USED ix
ACKNOWLEDGEMENTS xi
CHAPTER 1 INTRODUCTION 1
1.1 Research Rationale 1
1.2 Research Objectives 3
1.3 Research Process 4
CHAPTER 2 BACKGROUND 5
2.1 NOM in Source Water 5
2.2 NOM Characterization methods 8
2.3 Detection Methods for Hydrophilic Compounds 13
2.3.1 Refractive index detector (RI or RID) 13
2.3.2 Evaporative light-scattering detector (ELSD) 14
2.3.3 Charged aerosol detector (CAD) 15
2.3.4 Comparison between IR, ELSD, and CAD detectors 16
CHAPTER 3 PRELIMINARY METHOD DEVELOPMENT 18
3.1 Introduction 18
3.2 Materials and Methods 20
3.2.1 Water samples and chemical reagents 20
3.2.2 Analytical techniques 21
3.2.3 Molecular weight calibration curve 22
3.3 Result and Discussion 22
3.3.1 Preliminary method operation parameters 22
3.3.2 Calibration curve 27
3.3.3 Model compound experiment 29
3.4 Conclusion 32
CHAPTER 4 METHOD APPLICATION – UFC TEST 33
4.1 Introduction 33
4.2 Materials and Methods 34
4.2.1 Materials 34

4.2.2 Experimental Methods 34
4.3 Result and Discussion 35
4.3.1 UFC test of phenylalanine 35
4.3.2 Peak identification 39
4.4 Conclusion 41
CHAPTER 5 METHOD APPLICATION – RESIN FRACTIONATION 42
5.1 Introduction 42
5.2 Materials and Methods 43
5.2.1 Source water 43
5.2.2 Resin fractionation 43
5.2.3 Water quality parameters and analytical methods 45
5.3 Result and Discussion 46
5.3.1 DOC and SUVA of each fraction 46
5.3.2 UV chromatogram 47
5.4 Problem Analysis 50
5.4.1 Baseline noise 50
5.4.2 Ion interference 52
5.5 Conclusion 53
CHAPTER 6 METHOD IMPROVEMENT 54
6.1 Introduction 54
6.2 Source Water Enrichment 55
6.2.1 Introduction 55
6.2.2 Materials and methods 55
6.2.3 Results and discussions 56
6.3 HPLC-UV-CAD 58
6.3.1 Introduction 58
6.3.2 Materials and methods 58
6.3.3 Results and discussions 59
6.4 Anion Precipitation 64
6.4.1 Introduction 64
6.4.2 Materials and methods 64
6.4.3 Results and discussions 64
6.5 Anion Exchange Resin 66
6.5.1 Introduction 66

6.5.2 Materials and methods 67
6.5.3 Results and discussions 68
6.6 Conclusion 71
CHAPTER 7 CONCLUSION 72
7.1 Conclusions 72
7.2 Recommendations 73
REFERENCES 74
APPENDIX A – Resin Fractionation Process 79

LIST OF TABLES

Table 2.1. Chemical and physical features of each NOM fraction. Table obtained and modified from thesis of Montreuil, 2011 6

Table 2.2 (a) Percentage of NOM fraction for different studies (Montreuil, 2011)..... 7

Table 2.3. Methods used to characterize features of NOM, with short comment on their advantages and disadvantages (modified from Matilainen *et al*. 2011)..... 8

Table 2.4. Comparison between RI, ELSD and CAD detection methods 17

Table 3.1. Properties, chemical structure and UV absorbance of selected primary model compounds. 19

Table 3.2. Types of mobile phase solutions tested for CAD detector 21

Table 3.3. Performance comparison between four mobile phases. 26

Table 4.1. Inorganic solution used during experiments. 39

Table 5.1. DOC measured for each NOM fractions from water samples collected at Lake Major, Nova Scotia. 46

Table 5.2. Peak's retention time and their average molecular size 49

Table 6.1 Dose for precipitation experiment. 64

Table 6.2. Chloride and sulfate removal rate at different dosages of silver acetate and barium acetate. 65

Table 6.3. Buffer solution test groups 67

LIST OF FIGURES

Figure 1.1. Flow chart of research procedures 4

Figure 2.1. Principle of size exclusion chromatography (ChemPep, 2005) 11

Figure 2.2. Effects of molecular weight on NOM properties and behavior. (Cabaniss, 2000) 12

Figure 2.3. General mechanism of refractive index detector (Kazakevich, 2007) 14

Figure 2.4. Mechanism of ELSD detector (Shimadzu, n.d.) 15

Figure 2.5. Principle of charged aerosol detection (Thermo Fisher Scientific, 2013) 16

Figure 3.1. UV absorbance spectra of phenylalanine, tryptophan, and tyrosine (Wetlaufer, 1962) 19

Figure 3.2. UV Chromatograms of Pockwock raw water sample using ammonium formate as mobile phases from 5mM to 20mM 25

Figure 3.3. UV chromatograms of Pockwock raw water sample with ammonium formate (red), ammonium bicarbonate (blue), ammonium acetate (light blue), and acetonitrile (green) as mobile phase. 25

Figure 3.4. Calibration curve for TSK G3000 SW Column. Using MW standard of 210, 1500, 5000, 7500, and 17000 Da. 28

Figure 3.5. Calibration curve for TSK G3000 SW Column. Using MW standard of 1500, 5000, 7500, and 17000 Da. (Do not contain 210 Da standard) 28

Figure 3.6. SEC-UV-CAD chromatograms of UV detector (a) and CAD detector (b). 20 mgL^{-1} tryptophan (peak 1), phenylalanine (peak 2), tyrosine (peak 3), and alanine (peak 4, CAD only) were analyzed. 31

Figure 4.1. Free chlorine residual over 24-hour of the duplicated UFC test with 5 mgL^{-1} phenylalanine. 36

Figure 4.2. UV chromatograms (top image) and CAD chromatograms (bottom image) for first six hours of phenylalanine UFC test. 38

Figure 4.3. CAD chromatograms for inorganic solution used during UFC test 40

Figure 5.1. Resin fractionation procedure from Marhaba et al. (2003). 44

Figure 5.2. UV chromatogram of Lake Major raw water (red) and HON fraction (blue) 48

Figure 5.3. UV chromatogram of Lake Major raw water (red) and HOA fraction (blue) 48

Figure 5.4. UV chromatogram of Lake Major raw water (red) and HIN fraction (blue) .49

Figure 6.1. CAD chromatogram of PW raw water before (red) and after (blue) evaporation/enrichment experiment.....57

Figure 6.2 UV chromatogram (Red) and CAD chromatogram (Blue) after evaporation/enrichment experiment.....57

Figure 6.3. HPLC-UV-CAD with isocratic method's chromatogram. 5 mgL^{-1} of alanine, tryptophan, tyrosine, and phenylalanine were analyzed 60

Figure 6.4. HPLC-UV-CAD gradient method's chromatograms. 5 mgL^{-1} and 10 mgL^{-1} of alanine, tryptophan, tyrosine, and phenylalanine were analyzed. 61

Figure 6.5. The area under peak vs. Concentration plot of model compounds (ranged from 0.5 mg/L to 10 mg/L). R-squared values calculated for UV detector (blue) and CAD detector (red). 62

Figure 6.6. Log area under peak for model compounds measured at 10 mg/L. 63

Figure 6.7. CAD chromatograms of 20 mgL^{-1} Cl- in ultrapure water with 75, 150, and 200 mL of 0.1 mol/L silver acetate added. 65

Figure 6.8. NOM fractions elute by 1M ammonium bicarbonate. Top chromatogram: UV detector Bottom chromatogram CAD detector 68

Figure 6.9. NOM fractions elute by 0.01M ammonium acetate. Top chromatogram: UV detector Bottom chromatogram CAD detector 70

ABSTRACT

The main objective of this research was to develop a new detection method to characterize natural organic matter (NOM) using size exclusion chromatography (SEC) with charged aerosol detector (CAD). Conventional UV detectors installed with SEC system has difficulty in detecting hydrophilic NOM due to the lack of UV-light absorbing structure. Attaching the CAD to the SEC system to form an SEC-UV-CAD detection series is a possible solution to detect hydrophilic NOM in water samples. The preliminary SEC-UV-CAD method was first developed from original SEC method and tested by using 0.5 to 10 mgL^{-1} of phenylalanine, tyrosine, and tryptophan as selected model compounds. The CAD showed similar performance as the UV detector, and the CAD showed beneficial capabilities for detecting low UV-absorbing compounds such as phenylalanine. The amino acids' chlorination experiments and the raw water resin fractionation experiments were performed to evaluate the preliminary SEC-UV-CAD method further. Both experiments indicated the CAD chromatograms have severe baseline noise which could cover weak peak signals of targeted hydrophilic NOM, and the presence of ionic signals (sulfate and chloride) greatly interfered the hydrophilic NOM peaks. Several short experiments were attempted to improve these issues: CAD showed potential to detect desired hydrophilic NOM with concentrated raw water; replacement of SEC analytical column to C18 analytical column significantly reduced the baseline noise when detecting selected amino acids; precipitated anions with silver acetate and barium acetate removed sulfate and chloride peak signals from CAD chromatograms; strong anion exchange resin (i.e. DSC-SAX) can remove unwanted anions and separate NOMs by their affinity to the resin. The results from the C18 analytical column and SAX resin experiments have shown a great potential to characterize hydrophilic NOM samples. Further studies using C18 analytical column and SAX analytical column on detecting hydrophilic NOM were recommended.

LIST OF ABBREVIATIONS AND SYMBOLS USED

µg	Microgram
°C	Celsius Degree
AAs	Amino acids
CAD	Charged aerosol detection
Cl_2	Chlorine
Da	Daltons
DBP	Disinfection by-product
DOC	Dissolved Organic Carbon
ELSD	Evaporative light-scattering detector
GC-MS	Gas Chromatography – Mass Spectrometry
HIA	Hydrophilic acid
HIB	Hydrophilic base
HIN	Hydrophilic neutral
HOA	Hydrophobic acid
HOB	Hydrophobic base
HOCl	Hypochlorous acid
HON	Hydrophobic neutral
MDL	Minimum detection limit
MW	Molecular weight
NaCl	Sodium Chloride
NOM	Natural organic matter
pH	Potential hydrogen

RI	Refractive index
SEC	(High pressure) Size exclusion chromatography
SO_4^{2-}	Sulfate
SUVA	Specific ultraviolet absorbance
TOC	Total organic carbon
UFC	Uniform formation conditions
UV	Ultraviolet

ACKNOWLEDGEMENTS

I would like to thank my supervisor Dr. Graham Gagnon for his insightful guidance through my entire master program. This work would not complete without his extensive knowledge and great support. I would also like to thank him for encouraging me to attend conferences and present my work to the public. I would also like to thank my supervisory committee members Dr. Margaret Walsh and Dr. Rob Jamieson for their advice and encouragement.

I would like to thank the NSERC/Halifax Water Industrial Research Chair program for funding this research.

I would also like to express my appreciation for the contributions made by Dr. Yuri Park for her technical guidance and enthusiasm throughout my program. Her help and patience in the laboratory are indispensable to my program.

At last, I would like to thank my family, friends, peers, and supervisor for their support and encouragement throughout my entire master program.

CHAPTER 1 INTRODUCTION

1.1 Research Rationale

Natural organic matter (NOM) is a complex mixture of naturally occurring organic compounds in water. The major source of NOM is from the decaying of animal and plant tissues around the water system (Korth *et al.*, 2004). The presence of NOM in source water impacts the water treatment industry, especially, the drinking water industry, because:

- NOM's characteristics affect coagulation performance (Edwards *et al*., 1997);
- NOM is the precursor of disinfection by-products (DBPs) (Singer, 1999);
- NOM is found to be the main foulant in membrane system (Cabaniss *et al.*, 2000).

The best way to mitigate the impact of NOM is to remove as much NOM as possible before the disinfection/membrane filtration process. Characterization of NOM is required to achieve optimal removal of it. However, to determine the exact composition of NOM could be very challenging and costly since the nature of NOM is very complex, and the composition of NOM may vary on season and location (Delpla, 2009). Alternatively, to use a combination of convenient analytical techniques to obtain certain characteristics of NOM of a specific site is a more cost-effective and provide more information about NOM. These commonly use analytical techniques include total/dissolved organic carbon test (TOC/DOC), UV_{254} absorbance, specific UV absorbance (SUVA), resin fractionation, fluorescence excitation-emission matrix (FEEM), high-performance liquid chromatography (HPLC), and size-exclusion chromatography (SEC) (Abbt-Braun *et al.*, 2004).

The conventional HPLC or SEC systems usually use a UV absorbance detector. Many studies indicate this detector has a limitation in that: it only detects compounds that absorb UV light. If a compound does not UV light, it will be "invisible" by this type of detector (Her *et al*., 2002). Part of NOM, mainly the hydrophilic compounds, lack aromatic structure which absorbs UV light (Matilainen *et al.*, 2011), which can hardly be detected by HPLC system with the UV detector installed. Hydrophilic NOM contributes a relatively

high portion (30-50%) of total NOM, and hydrophilic compounds are the main foulant of low-pressure membrane since they have lower molecular weight (MW) than hydrophobic compounds (Cabaniss *et al.*, 2000). Moreover, the hydrophilic NOM is harder to be removed by coagulation process, since it is less polar than hydrophobic NOMs that hydrophilic NOM has less attraction to coagulants. Thus, the hydrophilic NOM is an important part of NOM. An alternative analytical technique is necessary to characterize hydrophilic NOM in water.

The charged aerosol detector (CAD), a universal detector, is one of the possible solutions to detect the "invisible" hydrophilic NOM in water. The CAD detector "charge" analyte particles with positive charge, and measures the charge carried by the particles. The greater the mass of analyte, the stronger signal is measured by CAD. The CAD detector is capable of measuring any analyte that is less volatile than mobile phase, and CAD is sensitive enough to detect pg-level at the ideal situation (Thermo Fisher Scientific Inc., 2013). However, the CAD detector is a relatively new detector, and its main applications are in pharmaceuticals, food science, and polymer studies. Very limited information about source/treated water analysis using CAD detector can be found. A new method for CAD detector to characterize hydrophilic NOM in source water was the focus of this research.

1.2 Research Objectives

The optimum objective of this study was to develop a method for SEC system with CAD detector that can be used to characterize NOM, especially hydrophilic NOM, in source water. Since the CAD detector is new to drinking water studies, it is expected to encounter unknown problems and difficulties during the method development. Three steps of method development were conducted within this study as follow:

1) To develop a preliminary method of SEC-UV-CAD based on existing SEC system and CAD operation menu. Test, adjust and decide the operating parameters including system flow rate, sample injection volume, analytical time, and mobile phase. Test the preliminary method with selected model compounds that represents NOM in water. Evaluate the performance of the preliminary method of detecting both hydrophobic and hydrophilic model compounds.
2) To apply the preliminary method on an amino acid chlorination experiment. Evaluate preliminary method's performance on detecting the kinetic of chlorination and formation of DBPs.
3) To apply the preliminary method to an actual source water and its resin fractionated portions. Evaluate the result of resin fractionation of source water, and the performance of the preliminary SEC-UV-CAD method analysis of these fractions.
4) Base on the results from step 2) and step 3), adjust and improve the preliminary method. If any critical problems were found, attempt to solve these problems as the main objective. If possible, advice for future study to further improve method developed.

1.3 Research Process

The research procedure can be summarized in the following flow chart (**Figure 1.1**): The introduction and background chapters (blue) introduced the importance of NOM, the limitation of conventional SEC method, and the CAD detector as a possible solution. **Chapter 4** (green) introduced the development of the preliminary SEC-UV-CAD method. **Chapter 4** and **Chapter 5** (orange) introduced the application of the preliminary method and the two issues that encountered during the experiments and their possible causes. **Chapter 6** (purple) introduced the possible solutions and improvement applied to these issues. **Chapter 7** (gray) summarized this study and provided recommendations for future studies.

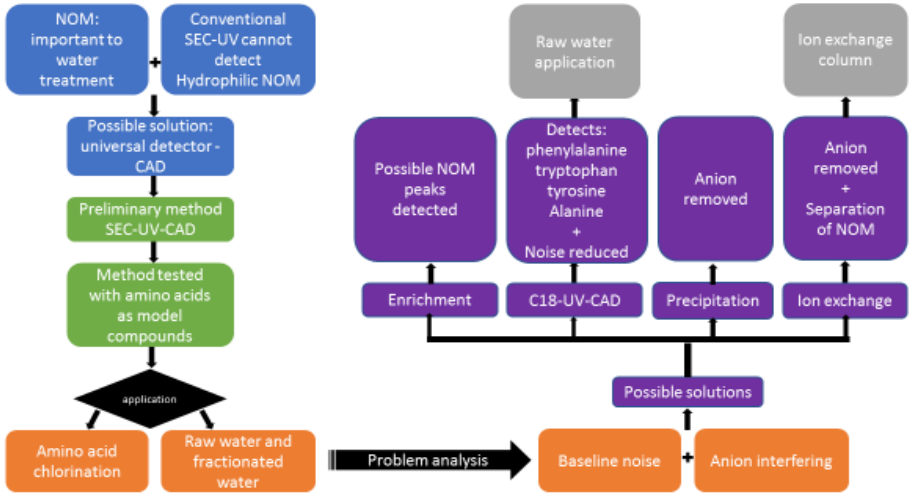


Figure 1.1. Flow chart of research procedures

CHAPTER 2 BACKGROUND

2.1 NOM in Source Water

Natural organic matter (NOM) is a complex mixture of naturally occurring organic compounds. It contains humic acid-like, fulvic acid-like and protein like organic matters (Delpla *et al.*, 2009). The major source is the decaying of plant and animal tissues around the water system. The change of weather affects composition of NOM since natural force like rainfall and wind blow carry substance into the water system. Combine with these factors, the composition of NOM has seasonal trend and it is highly depended on the location of the water system (Korth *et al.*, 2004; Delpla *et al.*, 2009).

The NOM presence in the source of drinking water brings impacts to water treatment: NOM contributes to membrane fouling during the membrane filtration process. Hydrophilic part of NOM is found to be the primary foulant of nano-filtration (Lamsal *et al.*, 2012); NOM is the precursor of disinfection by-products (DBPs) during the disinfection process. Chlorine is widely used disinfectant in drinking water industry. When disinfectant contact with NOM in water, it will oxidize the NOM and from DBPs (Singer, 1999). DBPs could bring impact to human health, and it is regulated by Health Canada (2009).

Since NOM is a complex mixture, NOM has been classified into different categories based on chemical or physical features, for example, hydrophobic and hydrophilic. Resin fractionation is a technique that separates NOM into six fractions base on NOM's affinity to different resins at different pH. This technique was first described by Leenheer (1981) and modified by Marhaba *et al.,* (2003). After a complete resin fractionation, NOM is separated into six fractions: hydrophobic neutral (HON), hydrophobic acid (HOA), hydrophobic base (HOB), hydrophilic acid (HIA), hydrophilic base (HIB) and hydrophilic neutral (HIN). **Table 2.1** summarizes the features of NOM fractions.

Table 2.1. Chemical and physical features of each NOM fraction. Table obtained and modified from thesis of Montreuil, 2011

Organic Fraction	Chemical Compounds	DBP forming potential	Relative Molecular Weight	Biological Activity	Transport of Metals	Color	Taste and Odor
Hydrophobic Neutral (HON)	Hydrocarbons, pesticides, Carbonyl Compounds, aldehydes, ketones, alkyl alcohols	Moderate	High to moderate	High	Low	None	None
Hydrophobic Acid (HOA)	Humic acids, Fluvic acids, aromatic acids, high MW carboxyl acids, phenols.	High	Moderate	Low	High	High	Moderate
Hydrophobic Base (HOB)	Aromatic amines, Proteins, Amino acids, Amino-Sugars	Moderate	High to Moderate	High	Moderate	High	None
Hydrophilic Acid (HIA)	Sugar Acids, Fatty Acids, Hydroxyl Acids, Low MW Carboxylic acids	N/A	Low to moderate	N/A	N/A	Moderate	N/A
Hydrophilic Base (HIB)	Polysaccharides, Aromatic Amines, Proteins, Amino acids, Amino-Sugars	Moderate	Low to moderate	High	Moderate	High	None
Hydrophilic Neutral (HIN)	Oligosaccharides, Polysaccharides, aldehydes, ketones, low MW aldehydes, ketones, low MW alkyl alcohols	N/A	Low to moderate	N/A	N/A	N/A	N/A
References	(Imai, 2001)	(Croue, 2000)	(Montreuil, 2011)	(Croue, 2000)	(Croue, 2000)	(Croue, 2000)	(Croue, 2000)

NOM fraction composition results of different studies at different sites are shown in **Table 2.2 (a)&(b).** By comparing the result from 13 different sites, NOM fraction composition of different water sources can be very different. In general, hydrophobic acid and hydrophilic neutral fractions are the most abundant fractions. A study from Lamsal *et al.* (2012) showing that hydrophobic fraction can be removed effectively by ultrafiltration process while the ultrafiltration primate still contains relatively high hydrophilic NOM since hydrophilic fraction is smaller than hydrophobic fractions. Fan *et al.* (2001) found that hydrophilic neutral compound has the highest fouling potential to membrane filtration treatment. Therefore, a complete understanding of NOM fractions, especially the hydrophilic fractions, is critical in optimizing NOM removal for drinking water treatment.

Table 2.2 (a) Percentage of NOM fraction for different studies (Montreuil, 2011)

Reference	Swietlik and Sikorska, 2005	Marhaba and Van, 1999	Marhaba and Van, 2000	Kanokkanta pong et al., 2005	Kanokkanta pong et al., 2006	Korshin et al., 1997
HON	12%	21.5%	10%	0 - 12%	5.7 - 12%	0 - 25%
HOB	> 1%	5.6%	7%	0.8 - 6.8%	0.8 - 5.7%	0 - 22%
HOA	73%	11%	12%	31 - 38%	31 - 34%	19 - 68%
HIB	5%	3.4%	5%	1.4 - 5.5%	3.3 - 5.5%	1.5 - 10%
HIA	7%	44%	53%	5.9 - 18%	8 - 18%	8 - 50%
HIN	3%	19%	13%	20 - 56%	25 - 44%	1 - 35%

Table 2.2 (b) Percentage of NOM fraction for seven different sites across North America (Kent *et al.*, 2014)

Site	A	B	C	D	E	F	G
HON	3%	3%	4%	4%	1%	3%	2%
HOB	3%	1%	2%	4%	5%	1%	7%
HOA	30%	22%	35%	40%	28%	58%	63%
HIB	2%	2%	1%	1%	1%	1%	1%
HIA	9%	51%	6%	13%	12%	3%	12%
HIN	54%	21%	50%	38%	53%	34%	15%

2.2 NOM Characterization methods

Many analytical techniques are capable of characterizing different features of NOM with advantages and disadvantages. Part of commonly used analytical techniques is summarized and compared in the following **Table 2.3**.

Table 2.3. Methods used to characterize features of NOM, with short comment on their advantages and disadvantages (modified from Matilainen *et al*. 2011)

Method	Detected features	Advantage	Disadvantage	References
Spectroscopic methods				
UV absorbance	Quantitative measurement of all compounds that absorb UV-light, conjugated C-C multiple bonds, aromatic carbon, -COOH and -OH	Simple and fast	The compound must absorb UV-light to be detected. Not all of NOM can be detected. Wavelength specific absorption. Sensitive to chemical environment, e.g. pH and ionic strength	(Korshin *et al.*, 1999) (Hur *et al.*, 2006) (Roccaro, 2009)
Fluorescence	Molecules of the sample are excited by irradiation at a certain wavelength, and the emitted radiation is measured at a different wavelength. Conjugated double bonds and aromatic rings. Three major groups: tryptophan-, humic- and fulvic-like fluorophores	Sensitivity, specificity, speed	Not all of NOM can be detected. Sensitive to chemical environment, e.g. pH	(Liu *et al.*, 2007) (Wu *et al.*, 2007) (Peiris *et al.*, 2010)
Chromatographic method				
HPLC	With C18 analytical column installed,	Fast, sensitive, and no pre-	Not all NOM can be detected by	

Method	Detected features	Advantage	Disadvantage	References
	NOM is fractionated base on polarity. Conventional UV-absorbance detector	extraction needed Separate NOM before measurement.	conventional UV detector.	
SEC	NOM is fractionated base on MW. MW distribution can be obtained	Fast, sensitive, and no pre-extraction needed Separate NOM.	Charge effects during measurement. Not all NOM can be detected by conventional UV detector.	(Wu *et al.*, 2007) (Her *et al.*, 2002) (Zhou *et al.*, 2000) (Chow *et al.*, 2008)
Mass spectrometric methods				
LC-MS	Chemical formulas of compounds in NOM	Qualification of NOM, knowing the composition of NOM	Dependent on chemical properties of the analyte. Not so sensitive to all species (e.g. High MW). Matrix effects. Expensive equipment	(Reemtsma & These, 2005) (Mawhinney *et al.*, 2009)
Bulk parameters				
TOC/DOC	Total/dissolved organic carbon in water	Easy to use	Give only information on quantity of NOM, not quality	(Liu *et al.*, 2007) (Seredynska-Sobecka *et al.*, 2007) (Spencer *et al.*, 2007)
SUVA	High SUVA-value >4 refers to hydrophobic, aromatic compounds; low SUVA <3 indicates mainly hydrophilic material	Easy to determine	High nitrate content in low DOC waters may interfere the measurement	(Edzwald & Tobiason, 1999) (Archer & Singer, 2006)

Determine a detailed composition of NOM is achievable with analytical techniques such as chromatography/mass spectrophotometry (GC-MS) and carbon-13 nuclear magnetic resonance (^{13}C-NMR) (Leenheer, 2003). However, this composition of NOM could be costly and restrictive to extraction techniques which may not capture many of the unique characteristics of NOM. Alternatively, to characterize NOM using several effective analytical techniques to obtain chemical and physical features of NOM is a cost-effective approach (Leenheer J. a., 2003). The SEC method is the focused analytical technique in this thesis.

The SEC method is one kind of HPLC methods that provides molecular weight distribution profile of analyte. SEC method uses an analytical column that separates analyte base by their size (molecular weight). The principle of SEC column is demonstrated by **Figure 2.1**: SEC column is filled with packing material containing many pores. Larger molecules cannot get into the smaller pores, so they travel through the column faster; smaller molecules will get into the smaller pore, so they will take a longer time to pass the column. A UV detector will quantify the separated analyte. Therefore, on the chromatogram, early peaks represent larger molecule while later peaks represent smaller molecule. With the help of SEC method, a molecular weight distribution profile of NOM can be obtained (SHIMADZU, 2010).

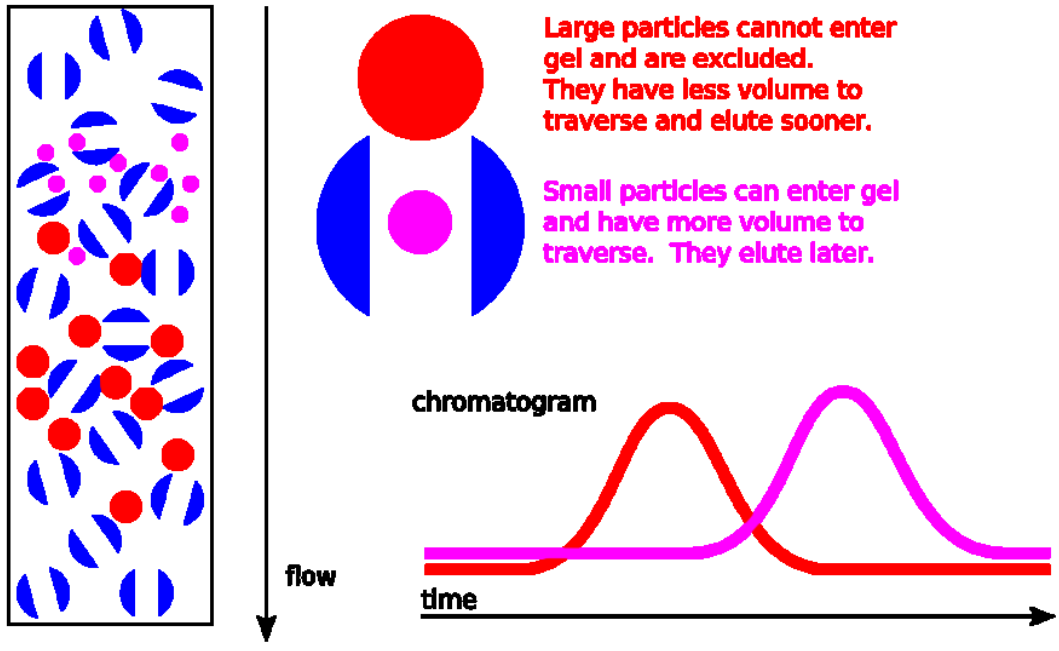


Figure 2.1. Principle of size exclusion chromatography (ChemPep, 2005)

Certain chemical and physical features can be predicted with the molecular weight distribution profile obtained from SEC. Effect of molecular weight on NOM is shown by **Figure 2.2**: Low molecular NOMs are more hydrophilic, faster adsorption kinetics, more water-soluble and higher bio-available; high molecular NOMs are more hydrophobic, greater metal binding with more aromatic structure (Cabaniss *et al.*, 2000).

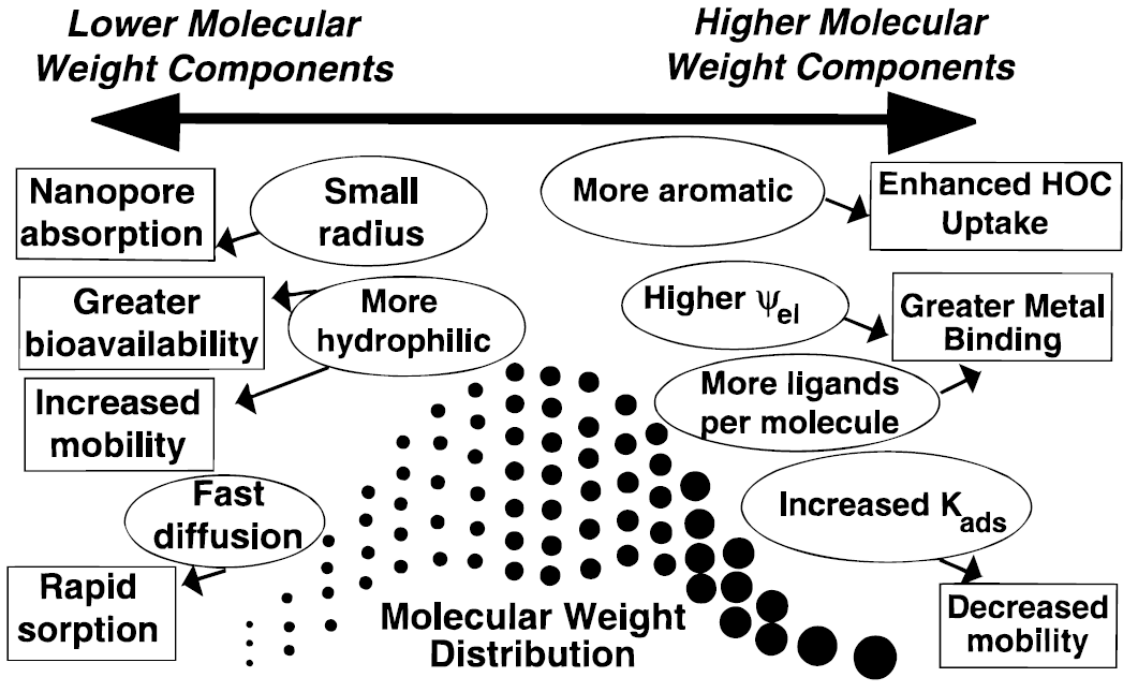


Figure 2.2. Effects of molecular weight on NOM properties and behavior. (Cabaniss, 2000)

The SEC method also has several drawbacks. Studies found that there are non-ideal interactions between the stationary phase, mobile phase, and analyte, which will cause the separation process not based on molecular size only (Allpike *et al.*, 2005; Her *et al.*, 2002). In the paper of Pelekani *et al*. 1999, they pointed out the existence of solute-gel interactions. Analytes may absorb to the gel surface due to van der Waals and electrostatic forces, which will cause an increase in apparent molecular weight (apparent molecular weight is the measured molecular weight by SEC); in the contract, analytes repelled by the gel, which will cause a decrease in apparent molecular weight. Therefore, the SEC interpreted molecular weight of a sample might differ to its actual molecular weight. A term "apparent molecular weight" is used in some studies to describe the SEC results (Allpike *et al.*, 2005).

The other drawback is the detection method of SEC. The conventional SEC system usually has a UV detector as default. UV detector is capable of measuring analyte with C-C conjugated double bonds that absorbing UV light. If analyte absorbs little UV light, it can be hardly detected by UV detector (Her *et al.*, 2002). Also, different compounds absorb different amounts of UV light at different wavelengths, so the UV detector's response is

not directly relevant to the concentration of the analyte. The combination of these factors makes the conventional UV detector not adequate for analyte quantitative analysis; it can be used for limited qualitative analysis (Her *et al.*, 2002).

In theory, hydrophilic NOM absorbs less UV light due to lack of aromatic structure. Due to the feature of hydrophilic NOM fractions, NOM cannot be fully detected and quantified by UV detector. Results from the studies of Kent *et al.*, (2014) and Lamsal *et al.*, (2012) suggested UV detection method has difficult to detect hydrophilic NOM: Resin fractionation technique was used to analyze seven surface water sources across North America. TOC measurement results indicate HOA and HIN fractions are the two major fractions; however, HIN fraction absorbs little UV light so that its SUVA is less than 0.1. Moreover, the SEC chromatograms showing very weak signals at low MW range and hydrophilic fractions. Therefore, it is suggested to develop a new detection method to analyze NOM fully, especially hydrophilic NOM.

2.3 Detection Methods for Hydrophilic Compounds

Universal detectors are the possible solution to detect both hydrophobic and hydrophilic NOM. The common types universal detector available on the market are refractive index detector (RI), an evaporative light-scattering detector (ELSD), and charged aerosol detector (CAD). The principle of universal detection, advantages, and disadvantages of each detector are discussed and compared in the following section.

2.3.1 Refractive index detector (RI or RID)

The refractive index (RI) detector is one of the universal detectors for HPLC. The principle of RI detector is to measure the difference between samples' refractive index and the reference cell's. The greater the difference between sample and reference, the higher signal will generate on the by the detector. However, when measuring complex mixture, the analytes may have a broad range of RIs so that some of the analytes may have a very close RI as the mobile phase. In this case, those analytes are not detectable by IR detector. (Kazakevich & Lobrutto, 2007)

As shown in **Figure 2.3**, a beam of the laser is shooting through both sample cell and the reference cell. If both sample cell and reference cell contain the same mobile phase, the laser will pass straight through since the refractive index is the same in both cells. However, when sample elutes from the column and passes through the sample cell, it will cause the change in refractive index that will move the laser. The changing angle of refraction will be measured by the sensor and generate an electric signal (Kazakevich Lobrutto, 2007).

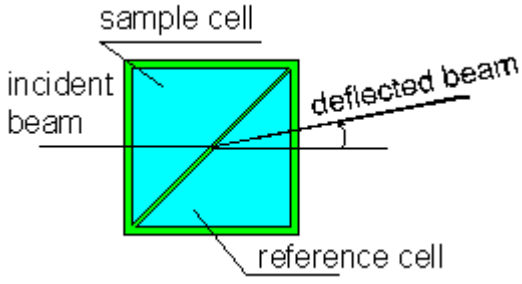


Figure 2.3. General mechanism of refractive index detector (Kazakevich, 2007)

Song *et al.,* (2010) had a study using SEC-UV-RI system to analyze humic acid. They pointed out the weakness of conventional UV detector – cannot detect analyte absorb no UV light. In the study, three HA peaks were detected with RI detector while UV detector only detected two out of three peak signals. The peak cannot be detected by the UV detector is the latest peak which has relatively low MW. The study suggested that HA represented by that latest peak is lacking conjugated double bond that absorbing UV light. Also, the results showing the RID/UVAD response ratio is consistently greater than 10, which indicates the RI detector is more sensitive than UV detector for measuring HA (Song *et al.,* 2010).

2.3.2 Evaporative light-scattering detector (ELSD)

The mechanism of ELSD is shown by **Figure 2.4**: ELSD detector can attend to the end of HPLC system. The eluent of the analytical column will enter a nebulizer to from minute droplets, and the droplets will be carried to a drift tube. In the drift tube, the mobile phase will be fully evaporated by heat, and the remaining analyte particle will be carried to the detection unit. In the detection unit, when the analyte particle passes through a light beam

will scatter the light. A photomultiplier will measure the scattered light. However, the analyte must have lower evaporation temperature than the mobile phase to be detected by ELSD detector; otherwise, the analyte will be fully vaporized before it enters the detection unit (Shimadzu, n.d.). Condensation nucleation light scattering detection (CNLSD) is an improved method of ELSD. CNLSD has an additional water condensation step to increase analyte particle size before the detection, which enhances the detection process. Thus, CNLSD is considered as the same type of detection method as ELSD in later discussion.

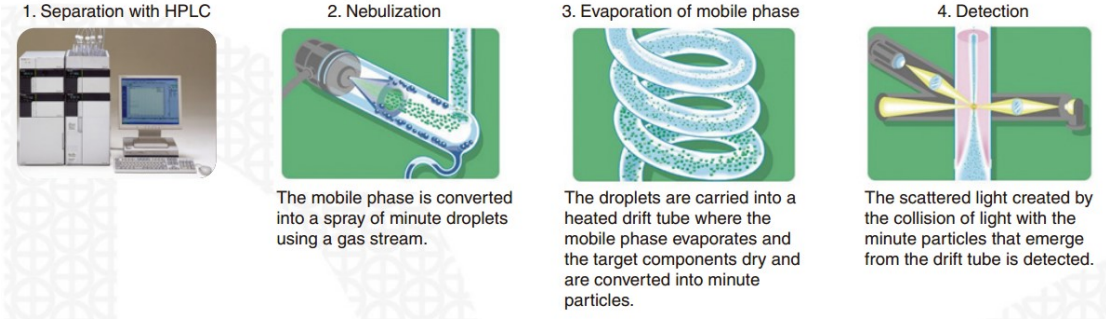


Figure 2.4. Mechanism of ELSD detector (Shimadzu, n.d.)

2.3.3 Charged aerosol detector (CAD)

The mechanism of CAD is similar as ELSD since they all belong to evaporation universal detectors. The charged aerosol detector also use the same evaporation method as ELSD does to remove mobile phase and obtain analyte particles, so it has similar advantages and disadvantages: capable to gradient method, but analyte must be more volatile than mobile phase. The difference between CAD and ELSD are the detection method. The ELSD measures the light scattered by analyte particles while the CAD measures the charge carried by the analyte particles. As shown in **Figure 2.5,** after the nebulization, the mobile phase is evaporated, and the analyte is dried to ultra-fine particles. The analyte particles will enter a collision chamber where filled with positively charged nitrogen gas. The positive charge on nitrogen gas will transfer to analyte particles after the collision between each other. The exceed positively charged nitrogen gas will be removed by the magnetic filter and the positively charged analyte particles will be measured by the sensitive electrometer. The greater the mass of analyte particle the greater the charge it can carry which results in a greater signal by CAD.

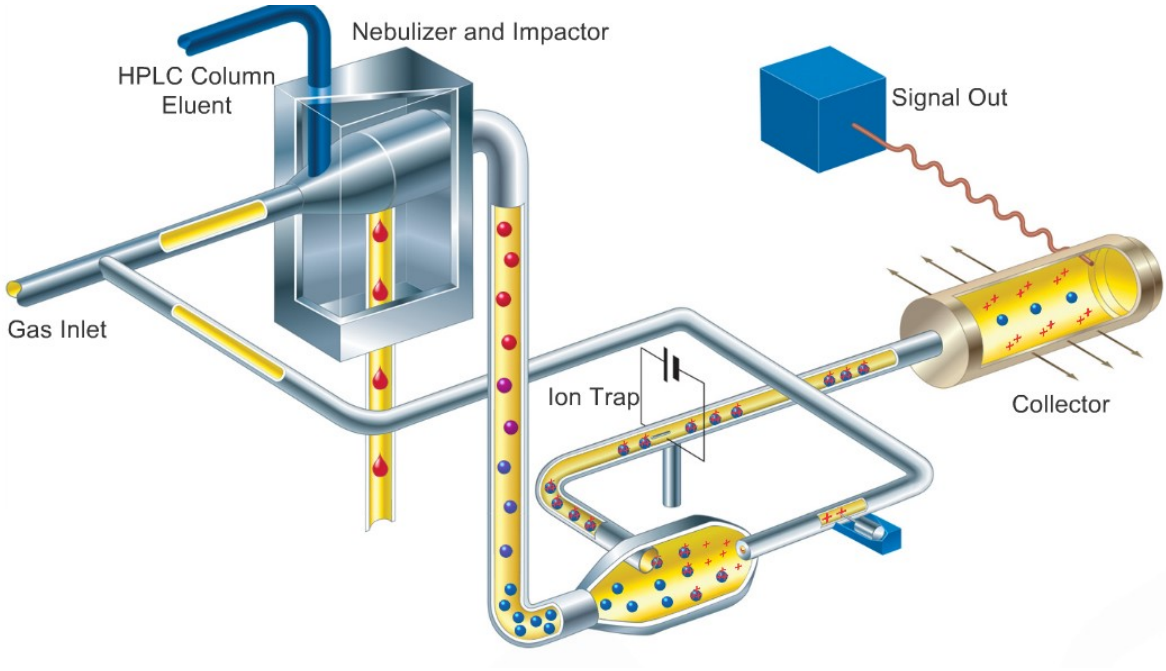


Figure 2.5. Principle of charged aerosol detection (Thermo Fisher Scientific, 2013)

2.3.4 Comparison between IR, ELSD, and CAD detectors

The performance of IR, ELSD, and CAD detectors are listed and compared in the following **Table 2.4.** The CAD detector is showing advantages over other two detectors: it is more sensitive and shows better response base on the mass of analyte. However, the feature of universal detection is a double-edged blade: it can detect targeted hydrophilic compound, but it will detect all other unwanted compounds that could cause interference to the result. (Almeling *et al.*, 2012) Moreover, the CAD detection method is a relatively new technique that CAD detector was first introduced in 2004 (Thermo Fisher Scientific Inc., 2013). CAD detector has mainly been used in pharmaceutical and food science related studies, but a little study using CAD has been found on surface water or water treatment field.

Table 2.4. Comparison between RI, ELSD and CAD detection methods

Detection method	**Detection limit**	**Advantages**	**Disadvantages**	**Reference**
Refractive index	100 ng – mg level	Universal response; Easy to use; Not affected by dirt and air bubbles in the cell	Not capable of gradient method; Temperature sensitive; Relatively low sensitivity; Need cleaning when film or clog formed inside of cell	(Kazakevich & Lobrutto, 2007)
ELSD	ng level (for non-volatile compound)	Support gradient method; 5-10 times more sensitive than RI; Minor affected by change in ambient temperature	Analyte must be less volatile than mobile phase; The evaporating temperature affects the result; The detection sensitivity is based on analyte's volatility (less volatile is preferred)	(Shimadzu, n.d.) (Mourey & Oppenheimer, 1984)
CAD	pg-ng level	Support gradient method; more sensitive than ELSD; Detection base on mass Simple to use.	Analyte must be less volatile than mobile phase; The evaporating temperature affects the result	(Thermo Fisher Scientific Inc., 2013) (Almeling *et al.*, 2012)

After considered all the benefits and challenges, the Dalhousie Water Studies research group decided to choose the CAD detector as a possible solution to detect hydrophilic NOM. Therefore, the goal of this thesis is to develop a method for CAD coupled with HPLC system to detect hydrophilic NOM in a water sample.

CHAPTER 3 PRELIMINARY METHOD DEVELOPMENT

3.1 Introduction

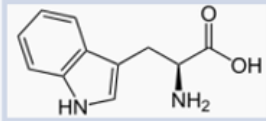
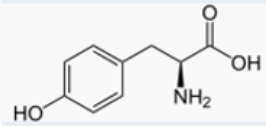
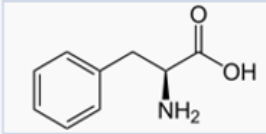
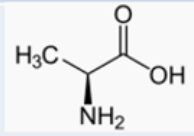
The CAD detector was installed and attached to the outlet of HPLC unit to form an SEC-UV-CAD detector series. The first step was to test and determine the following operating parameters for the preliminary method: system flow rate, the injection volume of sample, evaporation temperature within CAD detector, run time, and mobile phase to carry samples. Since CAD detector is new to drinking water study, experiment set up for the CAD detector cannot be found from literature. Each of the parameters listed above was tested, and the values yielded optimized result were chosen to form the preliminary method. After the preliminary SEC-UV-CAD method had developed, globular proteins molecular weight standards were used to generate a calibration curve for sample's molecular weight estimation.

To examine the performance of the preliminary method, simple compounds with confirmed chemical characteristics that can represent part of NOM were selected. Four amino acids were selected as model compounds, since:

1) Selected amino acids represent the protein like NOM in the water;
2) Selected amino acids' chemical and physical characteristics are clear;
3) Selected amino acids should be detected by both UV and CAD detectors (except for alanine).

Amino acids selected were: alanine, tryptophan, tyrosine, and phenylalanine, and their physical and chemical characteristics are shown in **Table 3.1.** These amino acids have different molecular weight and size that can be separated by SEC column. Tryptophan, Tyrosine, and Phenylalanine are hydrophobic compounds with an aromatic structure that absorbs UV light, while alanine with no aromatic structure should not be detected by the UV detector. The **Figure 3.1** shows the study results by Wetlaufer (1962) also concluded: from wavelength 200-300 nm, tryptophan has greater UV absorbance than tyrosine than phenylalanine. These amino acids have a maximum UV absorbance at 210 nm.

Table 3.1. Properties, chemical structure and UV absorbance of selected primary model compounds.

Amino Acids	Properties	Chemical structure	UV absorbance
Tryptophan	204.23 g/mol $C_{11}H_{12}N_2O_2$ white powder		High UV abs.
Tyrosine	181.19 g/mol $C_9H_{11}NO_3$ white powder		Medium UV abs.
phenylalanine	165.19 g/mol $C_9H_{11}NO_2$ white powder		Low UV abs.
Alanine	89.09 g/mol $C_3H_7NO_2$ white powder		No UV abs. Lack of aromatic structure

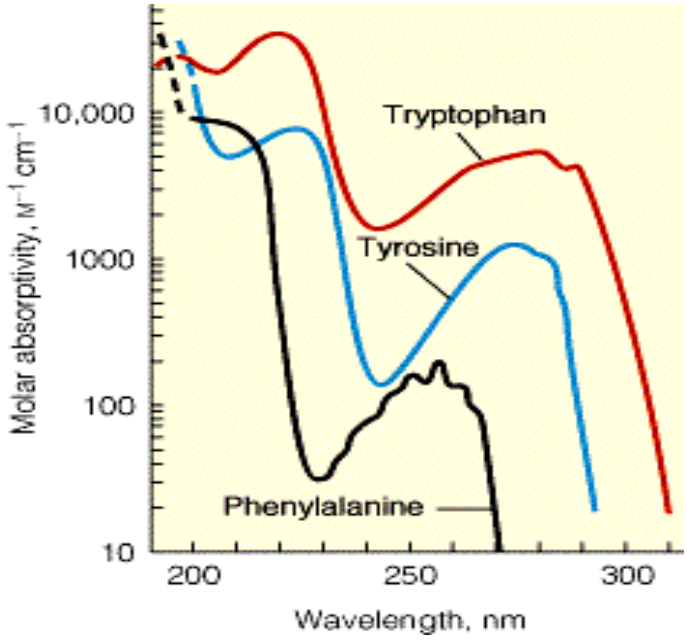


Figure 3.1. UV absorbance spectra of phenylalanine, tryptophan, and tyrosine (Wetlaufer, 1962)

All amino acids were prepared at 0.5 mgL^{-1}, 1 mgL^{-1}, 3 mgL^{-1}, 5 mgL^{-1}, 10 mgL^{-1}, and 20 mgL^{-1} individually in ultrapure water; the mixture solution contains all selected amino acids were made at 5 $mgL^{-1}$and 10 mgL^{-1}. The preliminary SEC-UV-CAD method was tested using selected model compounds, and the performance of UV and CAD detectors were evaluated and compared in this chapter.

3.2 Materials and Methods

3.2.1 Water samples and chemical reagents

Water samples were taken from Pockwock Lake, Halifax, NS. The Pockwock Lake is the source water of J.D Kline Drinking Water Treatment Plant. The reason to choose this sampling location was: water samples from Pockwock Lake were frequently measured by the laboratory. Experiment data such as TOC/DOC, UV_{254}, and SEC can be used as a reference to validate the new data obtained from the preliminary method. The water collected from Pockwock Lake has very good quality on average: very low alkalinity (<1.0 mgL^{-1}), low color (15 True Color Unit), low TOC (2.5 mgL^{-1}) and low turbidity (0.25 NTU) (Halifax Regional Municipality, 2014).

Amino acids: phenylalanine, tryptophan, and tyrosine (purchased from Sigma-Aldrich) were selected as model compounds. Mobile phases tested were acetonitrile (purchased from Fisher Scientific), ammonium acetate, ammonium formate (purchased from Sigma-Aldrich), and ammonium bicarbonate (purchased from Fluka). All chemical solution and water samples were filtered by polyether sulfonate (PSS) 47mm 0.45μm membrane before entering HPLC system.

3.2.2 Analytical techniques

3.2.2.1 Size exclusion chromatography (SEC)

Samples were collected in headspace free in pre-cleaned and baked (100 °C for 24 hours) 2 mL vials. Samples were injected through high-pressure size exclusion chromatography (Perkin-Elmer Series 200) using a TSK G3000SW column (7.5 mm × 300 mm, 250Å). The original method of SEC system to characterize NOM was using a flow rate of 0.7 mL min^{-1}, 20 µL injection volume, and 30 min process time. These operation parameters were tested and adjusted from the original SEC method to form the preliminary method.

Mobil phases showed the critical impact to chromatograms. The original SEC method was using sodium acetate as mobile phase; however, CAD detector requires new mobile phase that is more volatile (Thermo Fisher Scientific, 2013). The following mobile phase solutions **(Table 3.2)** were tested. The mobile phase provided a smooth baseline, more peak signal and better separation of peaks for both UV and CAD detector would be chosen.

Table 3.2. Types of mobile phase solutions tested for CAD detector

Mobile Phase	Concentrations
Acetonitrile	100% ACN
Ammonium Acetate	10mM
Ammonium Bicarbonate	5mM, 10mM
Ammonium Formate	5mM, 10mM, 20mM

After series of testing, the following operating parameters were confirmed for the preliminary SEC-UV-CAD method: 0.01M ammonium acetate as mobile phase, flow rate of 0.45 ml min^{-1}, 45-minute analysis time, 100 µL injection volume, and UV detection wavelength at 210 nm.

3.2.2.2 Charge aerosol detection (CAD)

The Thermo Corona Charged Aerosol Detector was attached to the outlet of the HPLC system (Perkin-Elmer Series 200) to from a detection series. During CAD Operation, the

ion trap voltage was 20.4V, and gas pressure was 59.8-60.0 psi. The evaporation temperature was set to default (35℃). The charger voltage must be lower than 2.99 kV, and the charger current must be no greater than 1.00 μA during sampling. Otherwise, the nebulizer needs to be pulled out and cleaned. The run mode current indicated the instant reading of signal, and the lowest range (0-100 Pa) were selected. Automatic noise filter was set to be 5 seconds as default.

3.2.3 Molecular weight calibration curve

After the preliminary SEC-UV-CAD method had developed, globular proteins standards (from Aldrich-Sigma) of 210, 1500, 500, 7500 and 17000 Da were used to generate a molecular weight calibration curve. 10 mgL^{-1} of each molecular weight standard were injected twice with the preliminary method. Average retention time was calculated from each standard.

3.3 Result and Discussion

3.3.1 Preliminary method operation parameters

All the operation parameters listed below were tested and adjusted from the original SEC method. A series of duplicate experiments were performed to ensure the consistency of experimental results for each parameter tested.

3.3.1.1 System flow rate

Since the CAD connection tubing has different inner diameter than origin HPLC system, the attachment of the CAD detector caused an increase of pressure within the system. The current installed analytical column is designed to work under 370 psi pressure. Otherwise, the column might be damaged by the high pressure. To protect the column, an automatically system pause was set at 360 psi. Considering a long sequence of samples will build up residual in the column which will increase system pressure as well. A flow rate of 0.45 mL min^{-1} was determined. With the 0.45 mL min^{-1} flow rate, the system started

at 300-320 psi and ended at 330-350 psi and the end of 30-hour sequence. The flow rate of 0.45 mL min^{-1} can maintain the system running for more than 24 hours.

3.3.1.2 Sample injection volume

Increase injection volume will provide bigger peak signal in the result; however, increase injection volume will also increase the speed of residual build up inside of analytical column, which will increase system pressure during processing. To remove residuals and maintain the column pressure, more cleaning steps must add to the analytical sequence. Higher injection volume will eventually shorten the column's lifetime. However, the objective of this experiment was to develop a new method that detects hydrophilic material in water. Higher injection volume gives bigger peaks of signal, which could cause targeting compound easier to be found. Injection volumes from 20 to 180 μL were tested. Balancing the consequences of bigger signal and faster pressure build-up, the100 μL of injection volume was chosen for the preliminary method.

3.3.1.3 Evaporation temperature (CAD)

Evaporation temperature controls the temperature inside of nebulizer. Volatile samples might be lost during the evaporation process. Different evaporation temperatures at 30℃, 35℃, 40℃, 45℃ and 50℃ were tested with Pockwock water sample. Two unknown peaks signal were detected at 29 minute and 43 minute. The area of both peak decrease as evaporation temperature rise; however, the area of the first peak appears to drop faster than the second one. Therefore, increasing in evaporation temperature will cause the loss of analyte, and the severity of loss could be related to analyte's volatility. The preliminary method should keep as much analyte as possible so that the default evaporation temperature 35℃ was used for the preliminary method.

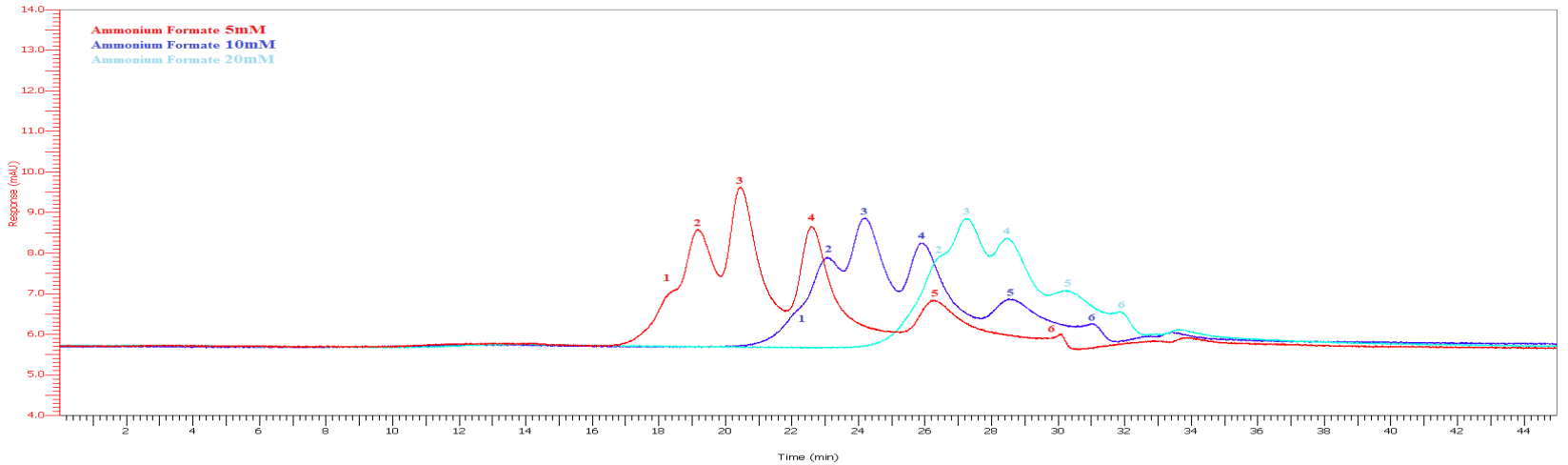
3.3.1.4 Mobile phases comparison and selection

Mobile phase carries analyte through the entire HPLC system. The selection of mobile phase shows a critical impact to the resulted chromatograms (Mourey, 1984). The original

SEC method was using sodium acetate as mobile phase; however, CAD detector requires new mobile phase that is more volatile and avoids salt accumulation inside of the device (suggested by Thermo's technician). Therefore, a more volatile mobile phase must be found for the CAD detector.

By comparing the chromatograms of all four types of the mobile phases, a phenomenon can be found in common – higher mobile phase concentration will compress peak signals which lead to merging of peaks or missing peak signal. **Figure 3.2** is showing a typical example. Water samples from Pockwock Lake were analyzed by the HPLC system using ammonium formate as mobile phases. Six peaks were measured between 16 minute to 31 minute using 5mM ammonium formate (red line) as mobile phase while six peaks were measured between 20 minute to 32 minute using 10 mM ammonium formate (blue line) as the mobile phase. The distance between peaks was shortened, while peak 1 almost merged into peak 2. When the concentration of ammonium formate further increased to 20 mM (light blue), five peak signals were measured between 24 minute to 32 minute. Peak 1 had completely merged into peak 2, and the distance between peaks was compressed further compared to 10mM. This phenomenon can be observed from all four types of mobile phases that were tested. Thus, lower mobile phase concentration provides a better separation of peaks as a result.

With the conclusion above, the UV chromatograms of each type of mobile phase were selected at optimum concentrations and compared with each other (**Figure 3.3**). The CAD chromatograms, on the other hand, all showing two or three unknown peaks with high baseline noise. Peak identification of these unknown peaks will be discussed in future chapters. The possible causes of the baseline noise were: the rotation of pump's piston cause pressure change in a certain period, which caused the system flow rate change in the same frequency as well. The change in flow rate might have been amplified at the connecting point between CAD and original system since the tubing diameter has sudden changed (large to small). The baseline noise has a certain period which matches to the pump's rotation period. However, this hypothesis needs further investigation.



25

Figure 3.2. UV Chromatograms of Pockwock raw water sample using ammonium formate as mobile phases from 5mM to 20mM

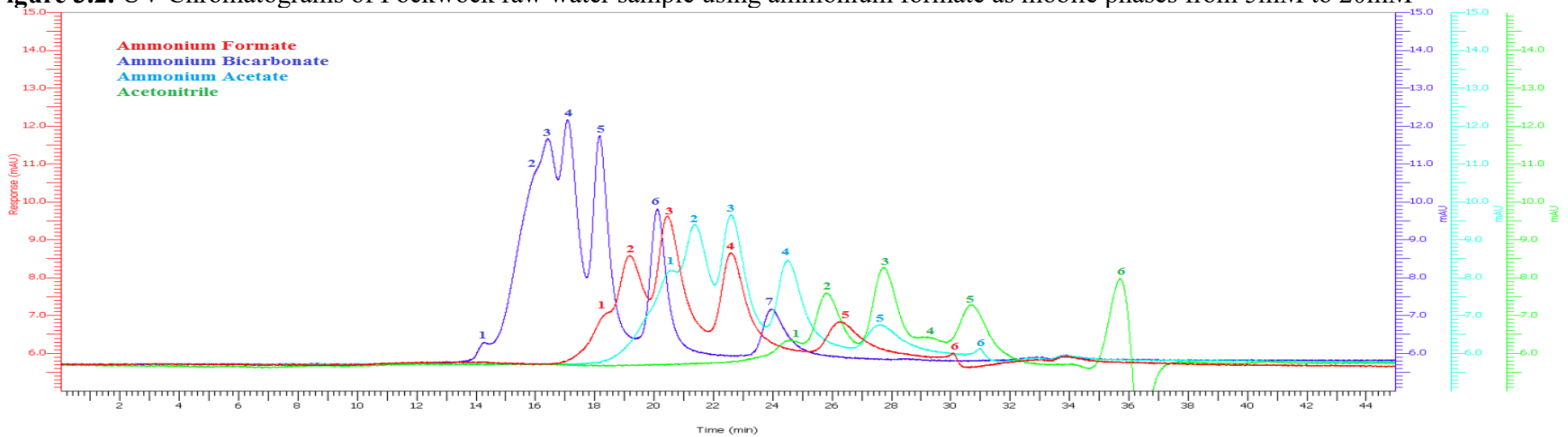


Figure 3.3. UV chromatograms of Pockwock raw water sample with ammonium formate (red), ammonium bicarbonate (blue), ammonium acetate (light blue), and acetonitrile (green) as mobile phase.

The overall performance of four mobile phases is compared in **Table 3.5.** After considering all factors, 10mM ammonium acetate had shown advantages over other mobile phases, and it was selected for the preliminary method. Thus, the preliminary method was set to:

- 10mM ammonium acetate as mobile phase
- 0.45 ml min^{-1} flow rate
- 45-minute analysis time
- 100 µL injection volume
- UV detection wavelength at 210 nm for maximum UV absorption

Table 3.3. Performance comparison between four mobile phases.

Mobile Phase	Peaks detected UV/CAD	Short Comment
Ammonium Formate (red)	6/2	Both UV and CAD chromatogram's performance were similar as ammonium acetate's.
Ammonium Bicarbonate (blue)	7/0	Most peaks detected compared to other mobile phases, and the peak shape is also sharper. The UV chromatogram's performance was the best out of four. However, when using ammonium bicarbonate, the CAD detector was reading "0" most of the time, so it was obsoleted from the list.
Ammonium Acetate (light blue)	6/2	Since the original SEC method was using sodium acetate as mobile phase, ammonium acetate with similar chemical characteristic provides the closest result to the original method. From the UV chromatograms, the separation of peak 1 and peak 2 was slightly better than ammonium formate's performance.
Acetonitrile (green)	6/3	The acetonitrile as pure organic content mobile phase is different from other three. However, this pure organic mobile phase has brought significantly high load to the analytical column. The system flow rate must be dropped below 0.3 mL min^{-1} to maintain a safe pressure for the column. Since the chromatogram performance was not improved, while the system pressure was a lot higher, acetonitrile was obsoleted from the list.

3.3.2 Calibration curve

Globular proteins standard of 210, 1500, 5000, 7500, and 17000 Da were analyzed by the preliminary SEC-UV-CAD method. Each standard had been injected twice and the average retention time was calculated. The average retention time vs. Log molecular weight was plotted to generate the calibration curves. The two calibration curves presented in **Figure 3.4** and **Figure 3.5**.

Figure 3.4 has an R-squared value of 0.8975 which is less than 0.95. The data point of 210 Da locates at 35-minute is the out layer causes the calibration curve loses its linearity. **Figure 3.5** is showing the calibration curve if data point of 210 Da was excluded. The R-squared value of the second calibration curve rises to 0.9879 which has higher linearity.

For a broader range of molecular size, the calibration curve for SEC column will have an "S" shape. Calibration curve will lose its linearity at either extremely low or extremely high molecular size range (Sigma-Aldrich, 1996). The 210 Da standard is extremely low in molecular weight, so it was expected the calibration curve generated from 210-17000 Da has a low R-squared value (loss of linearity). Using this calibration curve to estimate the molecular weight of unknown substance will have greater error. However, the molecular weight of NOM is mostly within the range from 1.0×10^2 to 1.0×10^4 Da suggested by Montreuil (2011). Therefore, it is necessary to include the 210 Da standard in the calibration curve which covers the molecular weight range of NOM.

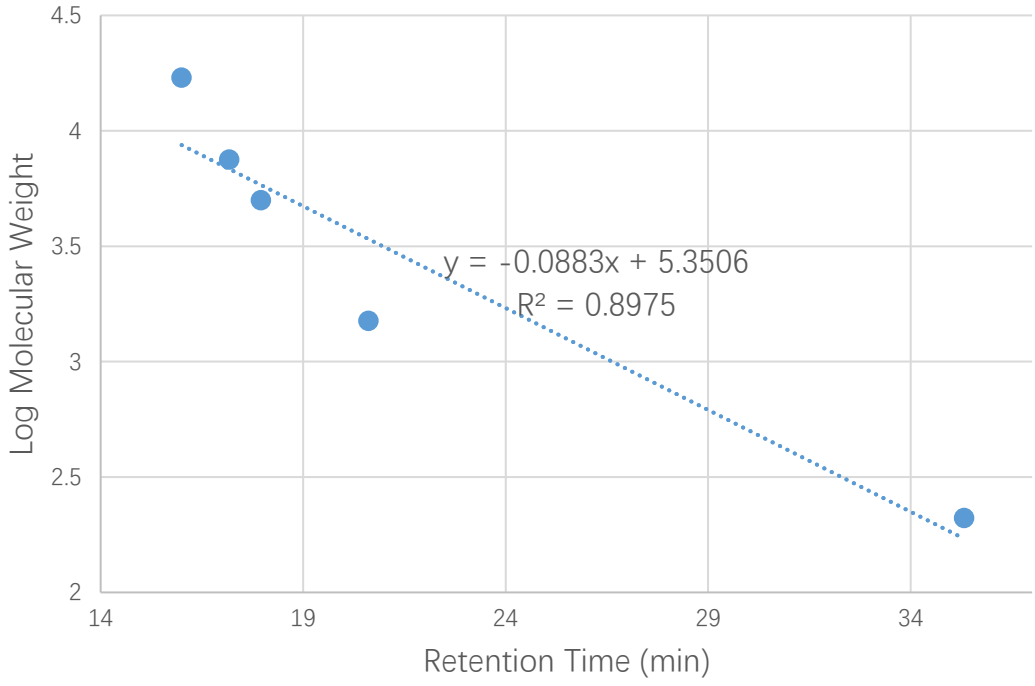


Figure 3.4. Calibration curve for TSK G3000 SW Column. Using MW standard of 210, 1500, 5000, 7500, and 17000 Da.

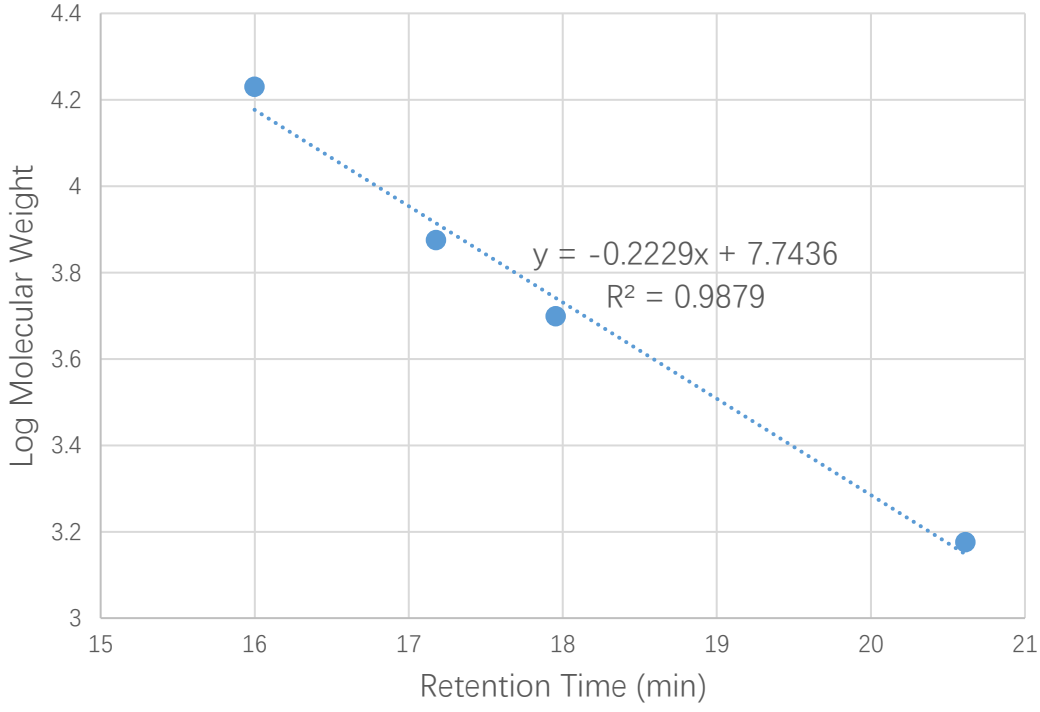


Figure 3.5. Calibration curve for TSK G3000 SW Column. Using MW standard of 1500, 5000, 7500, and 17000 Da. (Do not contain 210 Da standard)

3.3.3 Model compound experiment

3.3.3.1 UV chromatogram

The chromatogram (a) of **Figure 3.6** indicated UV detector detects tryptophan (peak 1) at 36 minute, phenylalanine (peak 2) at 33 minute, tyrosine (peak 3) at 31.5 minute. The retention time of these amino acids measured by the preliminary method kept consistent (less than 15-second difference) for all different concentration in the repetitive experiment. The chromatogram of mixture solution showing three separated peaks representing each amino acid, which proves the SEC column can separate selected model compounds efficiently.

Peaks area were different even all three model compounds had the same concentration. The amino acid with higher UV absorbance will have larger peak area because of the principle of UV detector: tryptophan with higher UV absorbance than tyrosine and phenylalanine has much larger peak area; alanine with no UV absorbance cannot be detected by UV detector from 0.5-20 mgL^{-1}. Since the UV response depended on analyte's UV absorbance, the UV detector has limited performance on detecting analyte with low or no UV absorbance, phenylalanine and alanine in this case. The phenylalanine peaks of 0.5 mgL^{-1} concentration of model compounds have a very low height that is close to the baseline, which can cause difficulty to observe the peak or to calculate peak area precisely. Thus, the UV detector was suggested to detect no less than 1 $mgL^{-1}$of phenylalanine. The actual minimum detection limit (MDL) of this method was not tested and calculated at this point since the current preliminary method might be modified as it has been improved as the research going.

Based on SEC column's mechanism, larger particles cannot enter gel in the SEC column will have less volume to traverse and elute sooner; smaller particles can enter gel will have less volume to traverse and elute later (ChemPep, 2005). The expected the order of peaks being observed on chromatogram should be tryptophan, tyrosine and then phenylalanine; however, tyrosine was the latest amino acid being eluted based on the result obtained. This

might due to a non-ideal interaction between analyte and column packing material (Allpike *et al*., 2005). The model compounds have relatively high polarity (tryptophan and tyrosine are polar amino acids) so that these model compounds might be attracted or repelled by the packing material due to van der Waals and electrostatic forces (Pelekani *et al*., 1999).

3.3.3.2 CAD chromatogram

The chromatogram (b) of **Figure 3.6** shows the CAD detector detected tryptophan (peak 1) at 36 minute, phenylalanine (peak 2) at 33 minute, tyrosine (peak 3) at 31 minute, and alanine (peak 4) at 30.5 minute. The CAD detector demonstrated its ability to detect alanine, a non-UV-absorbing compound. With the same concentration, the peaks area measured for different types of amino acids were similar to each other (with less than 15% of difference), which indicated the CAD detector gives a universal response to selected amino acids at the same concentration. However, all chromatograms of CAD detector showed severe baseline noise, and the baseline problem could not be solved by changing mobile phase nor flow rate. Due to the baseline noise problem, samples with 5 mgL^{-1} concentration reached the limit of observation; sample signals with 0.5, 1, and 3 mgL^{-1} concentration were hidden in the baseline noise. Therefore, MDL of CAD detector was suggested to be 5 mgL^{-1} as this point. Also, the baseline of tryptophan reached 0 from 4 to 29 minute, showed a complete flat baseline. This was due to the system software cannot record a negative single for CAD chromatograms. The CAD detector's monitor was showing negative reading while the chromatogram was recorded as zero. In other words, the CAD detector itself is capable of negative reading, but the system software cannot recognize the negative signal, which caused a zero baseline of the CAD detector.

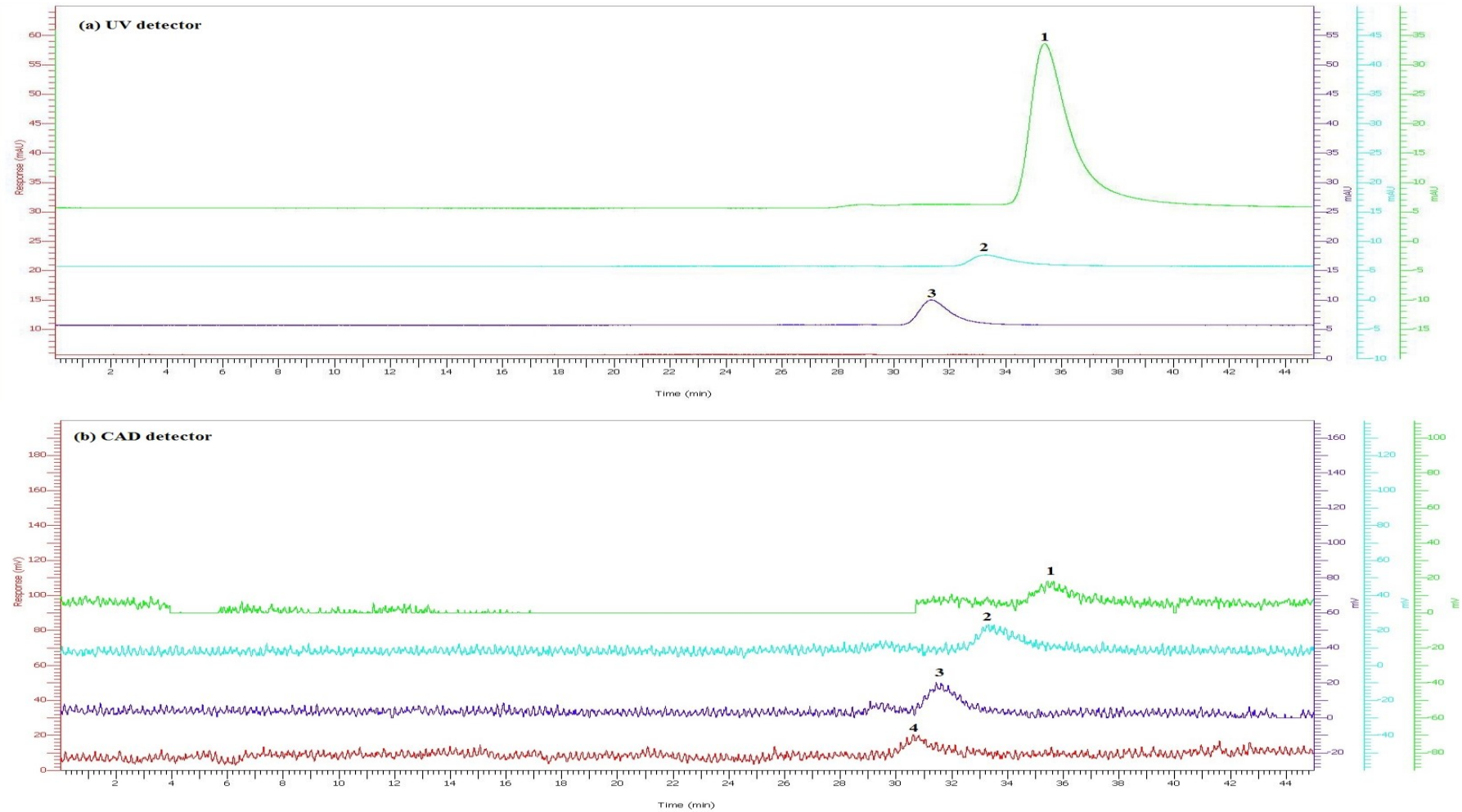


Figure 3.6. SEC-UV-CAD chromatograms of UV detector (a) and CAD detector (b). 20 mgL^{-1} tryptophan (peak 1), phenylalanine (peak 2), tyrosine (peak 3), and alanine (peak 4, CAD only) were analyzed.

3.3.3.3 Comparison between UV and CAD detector

Both UV and CAD detector had successfully detected tryptophan, phenylalanine, and tyrosine. The UV detector responded based on analyte's UV absorbance, while the CAD detector had a universal response for analytes with the same concentration. The UV detector demonstrated limitations in detecting low UV-absorbing compound such as phenylalanine, and UV detector could not detect compound with no UV absorbance such as alanine; while the CAD detector had detected all non-volatile and semi-volatile analyte and provided a universal response base on analyte's mass. The CAD detector experienced a heavy baseline noise issue which might cover weak peak signals.

3.4 Conclusion

After several test and comparison, operation factors for the preliminary SEC-UV-CAD method were successfully set up. The calibration curve of this preliminary method using MW standard was generated for future use.

The SEC-UV-CAD method was first examined by four selected amino acids as model compounds. The CAD detector showed advantages over traditional UV detectors: the CAD detector successfully detected alanine as a non-UV-absorbing compound which the UV detector failed to detect; the CAD detector provided a universal response based on the analyte's mass while the UV detector's response is depended on analyte's UV absorbance which is variable across different analytes. During the preliminary method test, the CAD chromatogram experienced a heavy baseline noise issue that might cover weak peak signals.

In conclusion of this chapter, the preliminary SEC-UV-CAD demonstrated the CAD detector's performance and benefits over conventional UV detector. The CAD detector showed its ability to detect hydrophilic compounds that absorb little UV light.

CHAPTER 4 METHOD APPLICATION – UFC TEST

4.1 Introduction

The CAD detector's performance was tested by four different amino acids as model compounds in **Chapter 3**. The result from the previous chapter has shown that the CAD detector has comparable performance as UV detector. Two series of experiments were carried through to further investigate the preliminary method under more complex conditions: uniform formation condition (UFC) test of model amino acids (in this **Chapter 4**); raw and fractionated water sample test (**Chapter 5**).

The UFC test was first developed by Summers *et al.*, (1996) to assess formation of chlorination disinfection by-products in drinking water. The UFC test simulates chlorination conditions within the drinking water distribution system, and it allows to compare the DBP formation potential along different waters under uniform formation conditions. However, the DBP formation potential was not the focus of this study. The concept of UFC test was used, and the procedure of UFC test was modified to fit the focus of this experiment – test the CAD detector's ability to monitor entire kinetic of chlorination reaction, which included:

1) Test the CAD detector's ability to monitor the decay of selected amino acids;
2) Test the CAD detector's ability to monitor the formation of disinfection by-products (DBPs) during chlorination reaction

The challenges of this experiment were: more complex experiment conditions compared to the last chapter; low concentrations of targeting compounds; and unknown retention time for new forming DPBs which will require peak signal identification.

4.2 Materials and Methods

4.2.1 Materials

The amino acids selected was 5 mgL^{-1} of phenylalanine. The experiment used 130 ml amber bottles (chlorine demand-free glassware). The pH of samples was adjusted to 7 with 1M sulfuric acid (H_2SO_4) or 1M sodium hydroxide (NaOH), and the pH was buffered by anhydrous boric acid. Standard hypochlorite (5-6%) solution was used to preserve samples after 24-hour chlorination. All the amino acids were obtained from Sigma-Aldrich, and other chemical solutions were obtained from Fisher Scientific.

4.2.2 Experimental Methods

The uniform formation conditions for the entire chlorination experiment were set to pH 7.0 ± 0.2 (with buffer), room temperature (20.0 °C), 24-hour incubation time, and 1 mgL^{-1} free chlorine residual at the end of 24 hours. Before chlorination, all chemical used and 130-ml chlorine demand-free amber bottles were prepared followed Summer's method (Summers *et al.*, 1996).

4.2.3.1 Preliminary chlorine dosage test

Preliminary chlorine dosage test was used to determine the initial dosage of chlorine to achieve targeted 1 mgL^{-1} chlorine residual after a 24-h period. Samples were buffered to pH 7 and incubated in the dark at 20.0 °C. A series of three chlorine dosages based on Cl_2:TOC ratios of 1.2:1, 1.8:1 and 2.5:1 were used to test the chlorine residual. From the results of these tests, the initial chlorine dose that yielded a 24-h residual closest to 1.0 mgL^{-1} free chlorine was selected.

4.2.3.2 UFC test of amino acids

Before chlorination, adjusted and buffered 1.5 L of sample to 7.0 ± 0.2 in 2L Erlenmeyer flask with stir. When timing had started, added chlorine solution and mixed for 30 seconds, then distributed water sample into ten 130 mL amber bottles and capped with head space

free. At the time of 15 minute, 30 minute, 1 hour, 2 hour, 4 hour, 8 hour, 12 hour, and 24 hour after chlorination, took one amber bottle of sample and measured its free chlorine with HACH DR 5000 spectrophotometer (Method 8021, followed instruction provided by HACH, 2014). After free chlorine measurement, added three drops of standard hypochlorite solution (5-6%) to remove the rest of free chlorine and preserved the sample for future measurement. After 24-hour chlorination, all collected samples were measured by the preliminary SEC-UV-CAD method.

4.3 Result and Discussion

4.3.1 UFC test of phenylalanine

After a series of preliminary experiment, starting chloride concentrations were determined to be 6.2 mg Cl^-/L for 5 mgL^{-1} of phenylalanine, which led to a 1 ± 0.4 mgL^{-1}free chlorine residual at the end of 24-hour incubation. The kinetic change of free chlorine residual is shown in **Figure 4.1:** duplicated experiments showed the starting free chloride of 6.2 mgL^{-1} was being consumed quickly in the first 6-hour period, and then the free chloride level dropped slowly to 1 ± 0.4 mg Cl^-/L within the rest of 18 hours. Since the reaction speed was rapid within the first six hours, 5 out of 8 sampling points were set within the first 6-hour period to have a better observation of the reaction kinetic. A duplicated UFC test of phenylalanine had successfully reached designed 24-hour chloride residual of 1 ± 0.4 mg Cl^-/L.

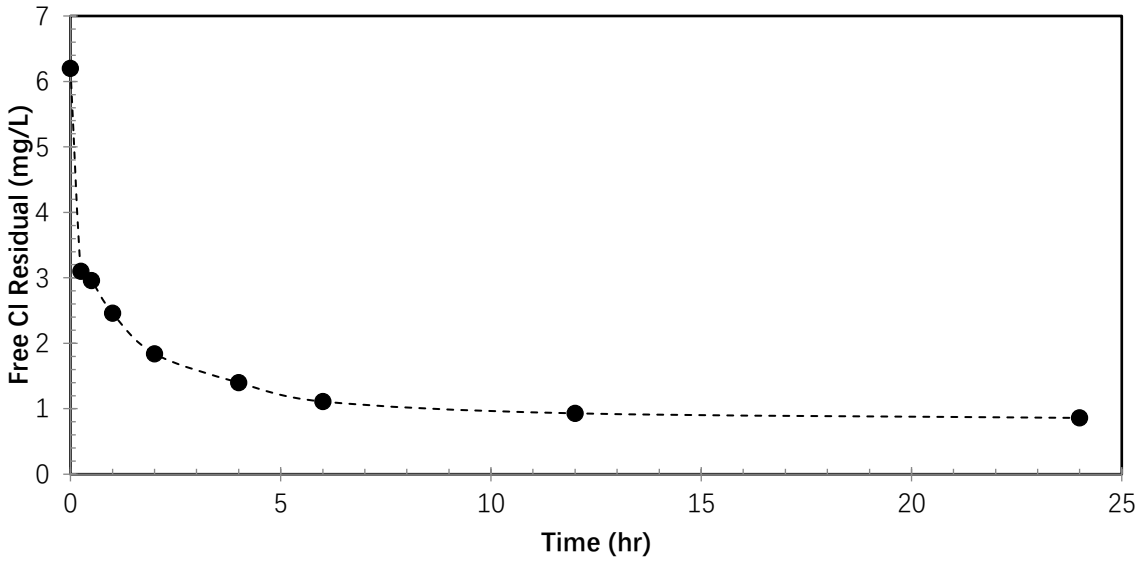


Figure 4.1. Free chlorine residual over 24-hour of the duplicated UFC test with 5 mgL^{-1} phenylalanine.

Samples collected at each sampling points were analyzed with the SEC-UV-CAD method. By running a sample of 5 mgL^{-1} phenylalanine in ultrapure water alone, a single peak was found at 34 minute on both UV and CAD detector. Thus, the peak at 34 minute was assumed to be phenylalanine.

Figure 4.2 (top) shows the overlapped UV chromatograms of samples taken from first 6 hours since the chlorination reaction started. The reduction of the peak at 34 minute indicates phenylalanine was being consumed during the first 6 hours of the reaction. By the end of the 6^{th} hours, the peak can be barely observed, so most of the phenylalanine was consumed at the first 6 hours during the experiment. Also, a series of new peaks at 25 minute were observed by UV detector. If these newly formed peaks could represent the new forming DPBs during the chlorination reaction, an increasing trend of peaks should be observed. In fact, these new peaks' area was gradually increasing from 15 minute to 2 hour since reaction started; however, the peak of 6 hour after reaction dropped to a significantly lower level. Therefore, no clear trend of increase in peak area was observed. Chemical represented by this peak at 25 minute remains unknown, a further experiment would be required to identify the new peak at 25 minute.

Figure 4.2 (bottom) shows the overlapped CAD chromatograms of samples taken from first 6 hours since the chlorination reaction started. Similar to the UV detector, the CAD detector also detected peaks of phenylalanine at 34 minute and a group of new peaks at 25 minute. For the peaks at 25 minute, they were showing the exact same trace as on the UV chromatograms. Again, these results proved that CAD detector provided a similar result as the UV detector. Also, two extra new groups of peaks were found by the CAD detector: peaks at 28 minute and peaks at 39 minute. Since these peaks were not detected by UV detector, they could be the compounds absorbing no UV light. Neither peaks at 28 minute nor peaks at 39 minute showing an increasing trend, so the peak area was irrelevant to the time of chlorination. Further experiments would be needed to identify these peaks.

Repetitive experiments under the same conditions confirmed the retention time of peak detected at 25 minute, 28 minute, and 34 minute kept consistency. However, the peak at 39 minute moved to 44 minute after an analytical column maintain process for unknown reason.

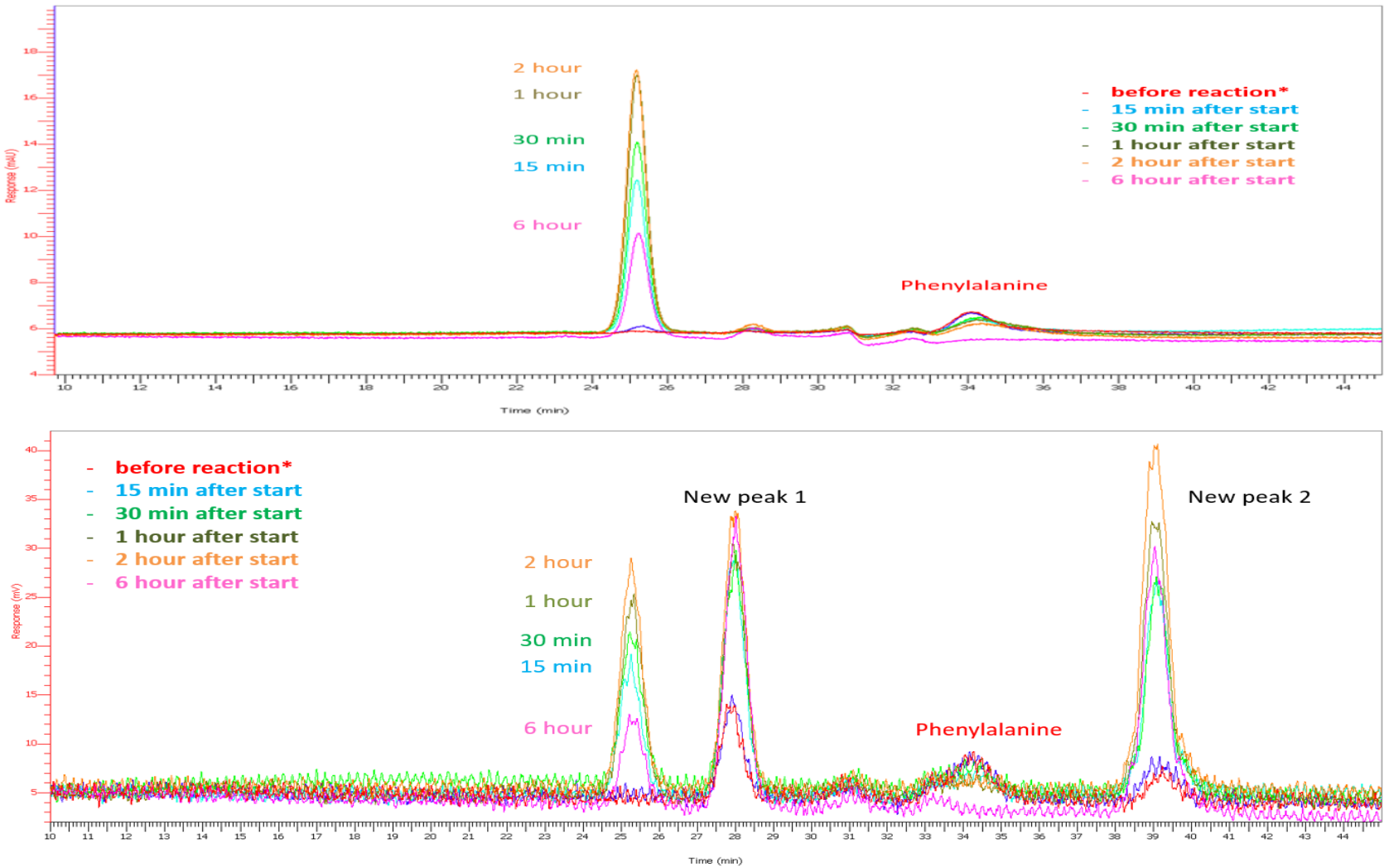


Figure 4.2. UV chromatograms (top image) and CAD chromatograms (bottom image) for first six hours of phenylalanine UFC test.

4.3.2 Peak identification

Different from UV detector, the CAD detects any mass that passes through it, so many chemicals other than targeting compound will be detected as well. For example, inorganic compounds can be detected by CAD. In this experiment, several inorganic solutions were used for different purposes (as shown in **Table 4.1**). The presence of inorganic material might interfere the result measured by CAD, and misleading the peak identification process. Thus, it is important to identify the signal of these inorganic solutions as a reference.

Table 4.1. Inorganic solution used during experiments.

Inorganic Solution	**Chemical formula**	**Purpose**
Hypochlorous acid	HClO	Initiate chlorination reaction
Sodium Hydroxide	NaOH	Adjust pH
Boric acid	B_3BO_3	Buffer pH
Sodium Thiosulfate	$Na_2S_2O_3$	Stop chlorination/preserve sample

A series of reference samples including of these inorganic solutions were prepared in ultrapure water and analyzed with the SEC-UV-CAD method. The UV detector detected a peak of sodium thiosulfate at 25 minute. Sodium thiosulfate solution absorbs UV light since it has the S-O bond that absorbs UV light. The CAD chromatogram is shown in **Figure 4.3**. All inorganic solutions had a peak signal on CAD's chromatogram: sulfate had a peak signal at 25 minute, chloride had a peak signal at 29 minute, and sodium ion had a peak signal at 44 minute.

However, these detected peaks of inorganic anions were found overlapped with the three new peaks found by the SEC-UV-CAD method. The sulfate peak overlapped with the peak found at 25 minute, and the chloride peak also overlapped with the peak found at 29 minute. The sodium peak found at 44 minute overlapped with the peak found at 39 minute even there is a 5-minute difference since this peak was found moving from 39 minute to 44 minute after several times of device maintenance.

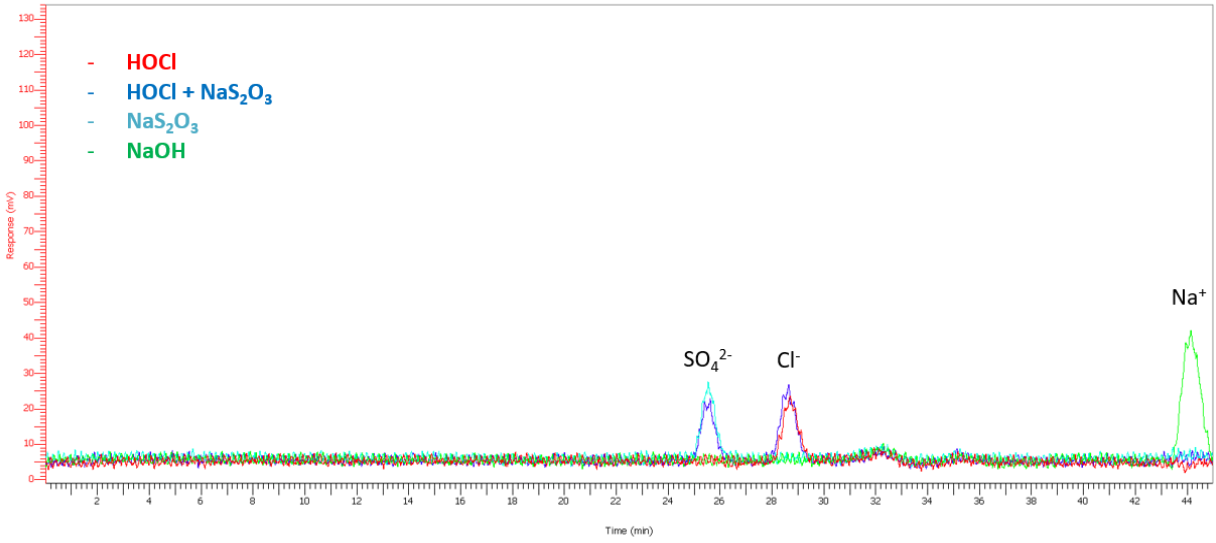


Figure 4.3. CAD chromatograms for inorganic solution used during UFC test

Since all three new peaks signal found by the SEC-UV-CAD method overlapped with the inorganic signals, and no other clear signal was found, the following possible assumptions could be made:

- the new forming material did not absorb UV light so it cannot be detected by UV detector;
- the signal of new forming material might have been detected by the CAD, but its signal overlapped with the signals of inorganic; or
- the signal of new forming material did not overlap with the signals of inorganic, but the concertation was too low that the detected signal was covered by the baseline noise.

Due to the interfering of inorganic, it is impossible to determine which assumption is correct, unless there is a method to eliminate the interference with inorganic anions.

4.4 Conclusion

The experiment of using the preliminary SEC-UV-CAD method to analyze phenylalanine's UFC test was interfered by inorganic ions. This method detected and tracked the consumption of phenylalanine; however, the peak identification experiment showed the new peaks signals were greatly interfered by signals from inorganic solutions used during the UFC test. A method must be found to eliminate the interfering effect of inorganic to future investigate signal of new forming material (DBPs in this case). The baseline noise issue needs to be solved to discover peak signals of low concentration compounds.

CHAPTER 5 METHOD APPLICATION – RESIN FRACTIONATION

5.1 Introduction

In this chapter, the preliminary SEC-UV-CAD method was used for raw water analysis and NOM fractions analysis. The resin fractionation technique divides NOM in surface water into six different fractions. Based on NOM's affinity to different types of resin, NOM will be divided into hydrophobic neutral (HON), hydrophobic base (HOB), hydrophobic acid (HOA), hydrophilic neutral (HIN), hydrophilic base (HIB), and hydrophilic acid (HIA) after the entire resin fractionation process. Information of each NOM fraction, such as MW distribution profile, TOC/DOC, SUVA, and UV absorbance can be studied with the preliminary SEC-UV-CAD method.

As stated in the research background (**Chapter 2**), the hydrophobic material absorbs more UV light, while hydrophilic material can be hardly detected by UV detector due to the absence of conjugated double bond (Her *et al*., 2002; Cabaniss *et al*., 2000). Therefore, the main focus of this chapter is to use the preliminary SEC-UV-CAD method on analyzing raw water sample and fractionated samples to investigate:

1) The preliminary method's performance on analyzing actual water sample
2) The preliminary method's detectability on hydrophilic NOM fraction

Serval challenges this experiment might encounter: raw water sample contains inorganic ions that the same interfering issue (in **Chapter 4**) might happen again; concentration of NOM is low in raw water (about 4.2 mg/L as TOC) that NOM's signal might not overcome the CAD detector's baseline noise.

5.2 Materials and Methods

5.2.1 Source water

The sample water used for resin fractionation was taken from Lake Major, Dartmouth, NS, Canada. Lake Major is the water supply of Lake Major Water Supply Plant. Water samples took from Lake Major has good quality in average: very low alkalinity (<1.0 mgL^{-1}), moderate color (42 True Color Unit), low TOC (4.7 mgL^{-1}) and low turbidity (0.25 NTU) (Halifax Regional Municipality, 2014). The reason to choose Lake Major as water sample (other than Pockwock Lake) was: seasonal resin fractionation data of Pockwock Lake had been done by other colleagues from the laboratory. Also, the resin fractionation process is labor intensive and time consuming that a complete fractionation takes approximately a month to finish. The researcher believed to obtain new data from a new water source (Lake Major) could provide more valuable data. Therefore, the water sample for resin fractionation was chosen to be Lake Major.

5.2.2 Resin fractionation

The fractionation procedure was first developed by Leenheer, (1981) and later modified by Marhahba *et al.* (2003). Superlite DAX-8 (Sigma-Aldrich), Diaion WA-10 (Sigma-Aldrich) and AG MP-50 (Bio-Rad) were the resin used for fractionation. The new DAX-8 resin passed a 500μm sieve to remove large resin. All resins were sequentially cleaned with hexane and acetone using a soxhlet extractor for 24-hour. The cleaned resins were then packed into five 2.5 x 120 cm Kontes Chromaflex chromatography columns and further cleaned by passing 0.1M NaOH, 0.1M HCl and ultrapure water. The ion exchange resin WA-10 and AG MP-50 reached breakthrough by passing 500 ml of 3N NH_4OH until breakthrough of NH_4. Detailed resin cleaning and preparing method can be found in the attached appendix or in Leenheer, (1981).

Before passing water sample through the columns, ensured the ultrapure water effluent had conductivity <10 μs/cm and UV_{254} absorbance < 0.001 cm^{-1}. Filtered 10L of water sample

by Polycap 0.45 μm filter capsules before passing water through the resin columns. The general resin fraction procedure is demonstrated by **Figure 5.1**: through this procedure, sample's pH was adjusted to 7, then pumped through the first column of DAX-8 resin. HON compounds would be absorbed to the first column. The eluent was then adjusted to pH 10 and pumped through the second column of DAX-8. At pH 10, HOB compounds would be absorbed to the second column of DAX-8. The eluent from the second column would be adjusted to pH 2 and pumped through the third column. At pH 2, HOA compounds would be absorbed to the third column of DAX-8. Then, the eluent was pumped through the fourth column with AG-MP 50 resin and the fifth column with WA-10 resin. HIB compounds would be taken by AG-MP 50 resin, and HIA compounds would be taken by WA-10 resin during the weak anion exchange process. The final eluent out of the fifth column would contained only HIN compounds.

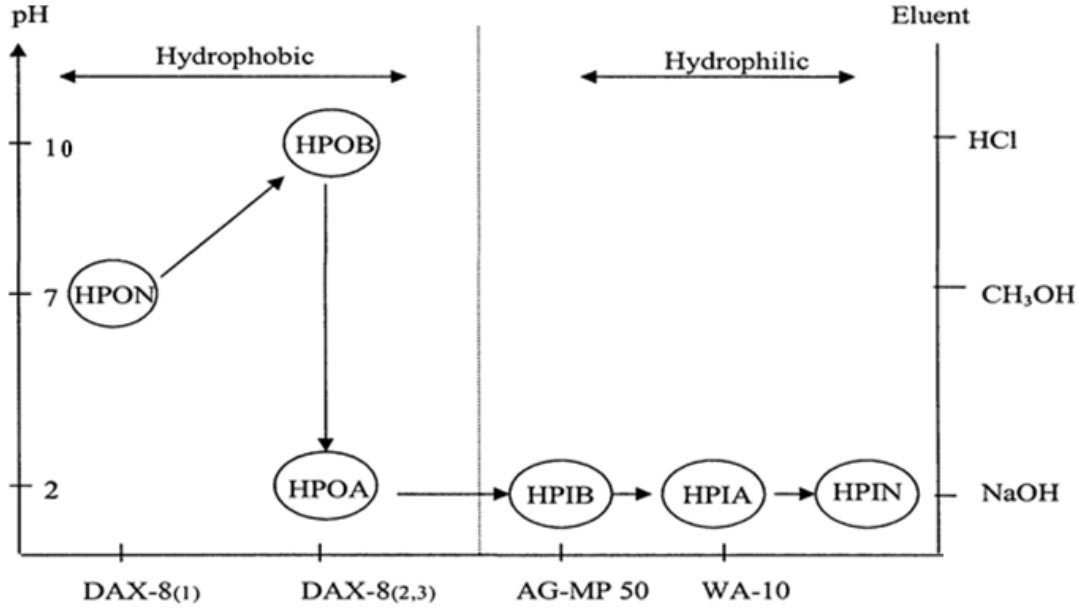


Figure 5.1. Resin fractionation procedure from Marhaba *et al.* (2003).

5.2.3 Water quality parameters and analytical methods

5.2.3.1 Total and dissolved organic carbon (TOC/DOC)

TOC and DOC samples were measured by Shimadzu TOC-Vcph Total Organic Carbon Analyzer. TOC samples were prepared in 50mL head space free vials and acidified to a pH < 2 with phosphoric acid. DOC samples were first filtered by 0.45 mm filter paper before TOC analysis.

5.2.3.2 UV_{254} absorbance and special UV absorbance (SUVA)

The HACH DR 5000 UV-Vis Spectrophotometer was used to measure UV254 absorbance for water samples. Method 10243 was used, and detailed instruction was followed by (HACH, 2014). The SUVA value is equal to sample's UV_{254} absorbance normalized with its DOC concentration.

5.3 Result and Discussion

5.3.1 DOC and SUVA of each fraction

The DOC result **(Table 5.2)** indicates that the resin fractionation experiment was successful, 74.8% of NOM was recovered after the entire fractionation. The lost 25.2% of NOM might be caused by: evaporation during the experiment (the fractionation process took one week at room temperature); loss during desorption and extraction of NOM out of resins. After a week of evaporation and 5 times of absorption/desorption processes, 25% of the carbon was unaccounted for.

The DOC result also indicates 65.6% NOM from the sample water was hydrophobic, while 34.4% of NOM from sample water was hydrophilic. Hydrophobic neutral (39.3%), hydrophobic acid (23.9%) and hydrophilic neutral (29.4%) were the dominant fractions; the rest fractions contributed less than 10% of total DOC. The SUVA values of hydrophilic fractions were very low due to hydrophilic NOM absorb little UV light due to lack of aromatic structure.

The DOC and SUVA measured for each fraction were in agreement with Kent et al. (2014): HOA and HIN fractions were the dominating fractions, and hydrophilic fractions had very low SUVA value. Composition wise, the Lake Major water sample had three dominating fractions: HON, HOA, and HIN fraction. So the composition of Lake Major NOM fractions was similar to the results found by Marhaba *et al.* (2003).

Table 5.1. DOC measured for each NOM fractions from water samples collected at Lake Major, Nova Scotia.

NOM Fractions	DOC (mgL^{-1})	SUVA	Percentile	
HON	1.339	2.158	39.3%	
HOB	0.081	3.751	2.4%	65.6%
HOA	0.815	4.866	23.9%	Hydrophobic
HIB	0.059	-	1.7%	
HIA	0.112	1.472	3.3%	34.4%
HIN	1.002	<0.1	29.4%	Hydrophilic
Sum of all fraction	3.408		Recovery rate:	
Raw water	4.559	4.436	74.8%	

5.3.2 UV chromatogram

Each NOM fraction was measured and compared with raw water sample using the SEC-UV-CAD preliminary method. The dominating HON, HOA, and HIN fractions were chosen to be discussed in this section. The rest of NOM fractions were not selected since they were less important (contributed less than 8% as total DOC). **Figure 5.2** shows the UV chromatogram of Lake Major (red) overlap with HON fraction (blue) as a comparison while **Figure 5.3** shows the UV chromatogram of Lake Major (red) overlap with HOA fraction (blue). The raw water sample of Lake Major showing seven peak signals at 14 minute, 20minute, 21 minute, 22 minute, 24 minute, 27 minute and 30 minute were marked from 1 to 7 on the chromatogram. As shown in **Table 5.3** below, detected peaks represent compounds of 500-13000 Da molecular size. Both of HON and HOA fraction showed peaks from 20 to 31 minute, which contains compounds with 500-3800 Da molecular size. Neither of HON fraction nor HOA fraction showing any signal from 14 to 20 minute, which means 3800-13000 Da compounds were not detected. However, the chromatogram of water shows a strong signal within the range of 16-20 minute (4000-10,000 Da). This missing part of NOM compound might contribute to the 25.2% loss of NOM after fractionation so that larger NOM compound might not be recovered from resin absorption during fractionation process.

The chromatograms of raw water, HOA, and HON fraction are similar in shape – chromatograms of HOA and HON fraction look like a compressed version of raw water's chromatogram, which means they contain the similar composition of NOMs. The difference between HOA and HON fraction is: the HOA had higher peaks of 3, 4 and 5 while the HON fraction had a higher peak of 6 and 7, so that the HOA fraction contained larger NOM than the HON fraction.

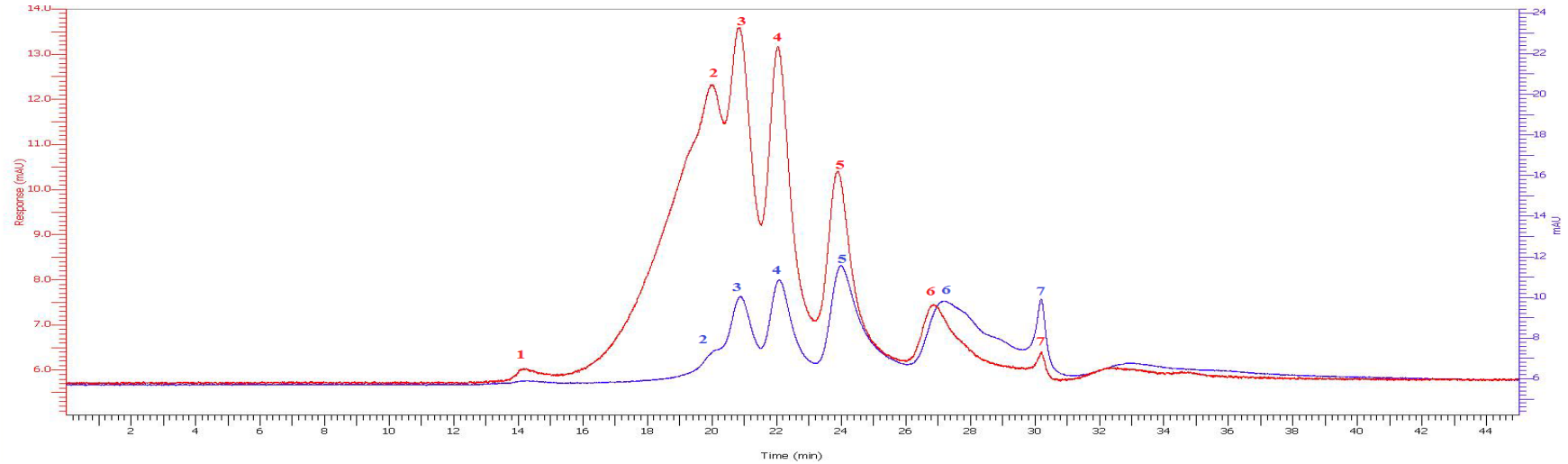


Figure 5.2. UV chromatogram of Lake Major raw water (red) and HON fraction (blue)

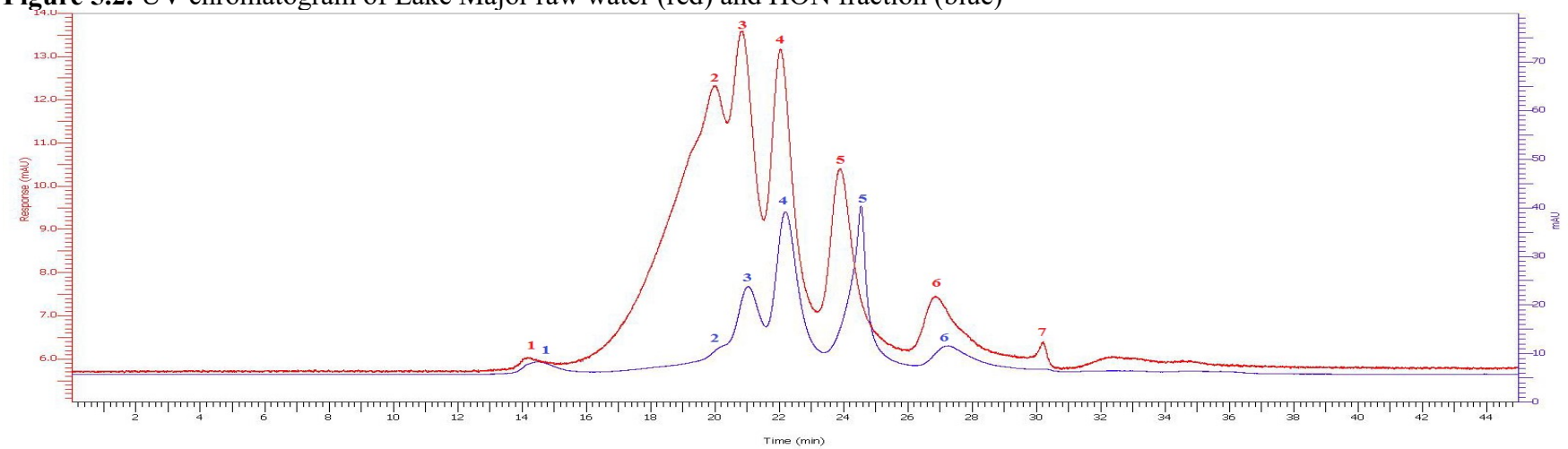


Figure 5.3. UV chromatogram of Lake Major raw water (red) and HOA fraction (blue)

Table 5.2. Peak's retention time and their average molecular size

Peak No.	Retention Time (min)	Log(Molecular Size)	Apparent Molecular Size (Da)
1	14	4.1144	13014
2	20	3.5846	3842
3	21	3.4963	3135
4	22	3.408	2559
5	24	3.2314	1704
6	27	2.9665	926
7	30	2.7016	503

Unlike hydrophobic fractions, the HIN fraction shows in **Figure 5.4** indicates the HIN fraction is missing more earlier peaks, most of peak signals were detected between 22-29 minute. So, it contains NOM with 700-2600 Da. The shape of HIN chromatogram is different from raw water sample's. Very limited peak signals were detected by UV detector for HIN fraction, and the peaks signals are weaker compared to chromatograms of hydrophobic compounds. Therefore, hydrophilic peaks signal might not have been fully detected by UV detector. This results agreed with Lamsal *et al.*, (2012).

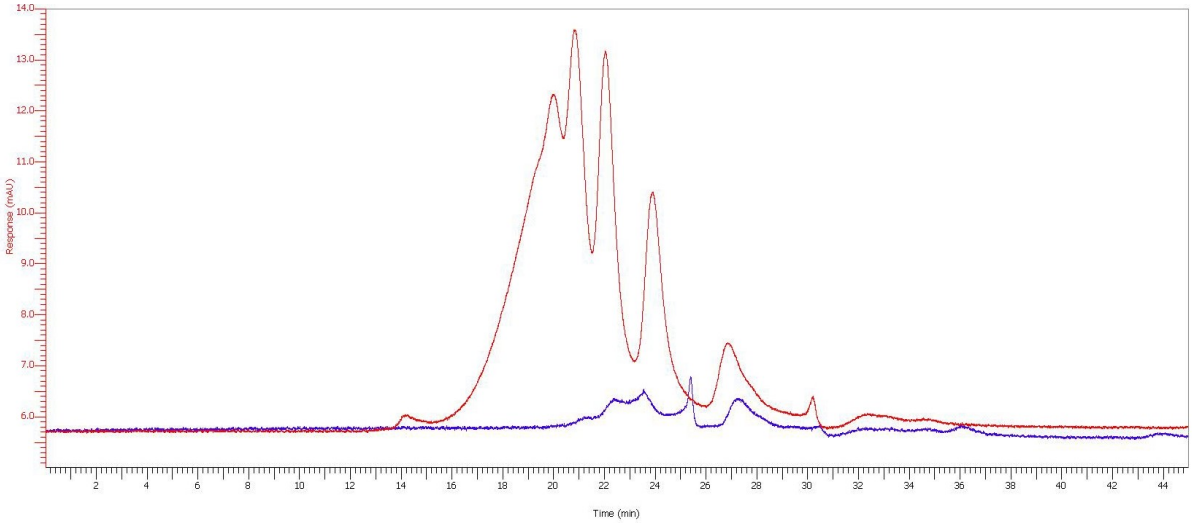


Figure 5.4. UV chromatogram of Lake Major raw water (red) and HIN fraction (blue)

Since the desorption process of resin fractionation requires using extremely high concentration of inorganic solutions such as 3N sodium hydroxide. The CAD chromatograms were interfered by huge peaks of inorganic ions. All the CAD chromatograms did not provide meaningful result due to the presence of an extremely high concentration of inorganic compounds. Methods that remove or reduce the interfering of inorganic must be found to continue this experiment.

The first resin fractionation was done in August 2015, and a repetitive resin fractionation was done in December 2015. The results of the second fractionation indicated a seasonal change of NOM fractions: the HON fraction's DOC contribution increased from 39% (in August) to 52% (in December). This phenomenon agreed with the result from Montreuil's thesis, (2011). Since the second resin fractionation still experienced inorganic interfering issue, its chromatograms are not showing in this chapter (results and chromatograms can be found in Appendix).

5.4 Problem Analysis

5.4.1 Baseline noise

Refer to the operating instructions come along with the CAD detector manufacturers, possible causes of heavy baseline and high background current were discussed as follow:

1) The mobile phase is contaminated
   The possibility is very low that all mobile phases during this research are made with ultrapure deionized water and filtered by 0.45 μm filters before use.

2) Highly retained compounds are being eluted from the column
   This may occur when a new mobile phase or column is used. However, during the experimental period, the same type of column has been replaced because of the lift time issue. All the column functioned properly with other studies but experience the same baseline issue when connected to the CAD detector

3) Fittings are leaking
   This is no likely to happen since all the fittings were checked before operation

4) Contamination occurs somewhere in the system
   The baseline noise does not show any improvement after cleaning process.

5) The detector is flooded with solvent (pump flow is turned on while no gas flow is present)
   This situation never happened during operation.

Therefore, no obvious evidence could be found to explain the high baseline noise referring to the operating instructions. The operators noticed: when zooming into the CAD baseline for a short time interval (about 30 seconds), a pattern of baseline noise was repeating within a certain period (about 5 seconds). This repeating period matched the gap between of "ticking" sound made from the pump of SEC system. Therefore, this machinery noise might be originated from the pump operation. The same vibrating frequency was found on the UV baseline when zoomed in; however, the amplitude of noise is much severe at CAD detector.

After reviewed several literatures, the possible cause of baseline noise was found. A study from Hung et al. (2009) also experienced high baseline noise with CAD detector, and they suggest the high baseline noise is caused by column bleeding. Huang used silica-based columns (ZIC-HILIC and Atlantis HILIC) with ammonium acetate with acetonitrile (25:75) as the mobile phase. Hung's silica-based columns showed high background noise while the polymer-based column (SeQuant ZIC-pHILIC) did not have the baseline issue. Huang believes the column bleeding is the cause of high background noise that the dissolved silica was eluted and detected by CAD detector. The studies from Unger *et al*. (1984), Churms (1996) and McCalley (2007) have shown evidence that several types of silica-base analytical columns have column bleeding issue. In the same time, the operators noticed the calibration process of the CAD detector, which used another column, had a significantly

reduced baseline noise. The same phenomenon happened when the system was connected without analytical column installed. The operators of lab convinced that changing the analytical column might result in lower baseline noise. Therefore, the high background noise could be caused by dissolved silica bleeding from the SEC column.

5.4.2 Ion interference

Base on the theory of SEC: the ions, with much lower MW than amino acids' or NOMs', should be eluted later than amino acids or NOMs. Thus, the interfering of ions was not expected at the beginning of the experiment. However, the actual results from the CAD detector indicated the presence of ions, especially anions, had greatly interfered the CAD chromatograms. As more literature review had been done about this issue, the clue was found. The ion interfering issue might be caused by a combination of the "universal detecting" feature of CAD and the non-ideal interactions between the stationary phase and the analyte (Allpike *et. al*., 2005; Pelekani *et. al*., 1999).

The analytical column contains silica-based material which is polar at the micro level (Sigma-Aldrich). The van der Waals and electrostatic force repelled the anions away from stationary phase, which caused the anions eluted earlier and overlapped with NOMs. The CAD detector detected both anions and NOM at the same time, which caused the anion interfering issue. A study of Emmenegger *et al.*, 2007, was done with using SEC combine with ESLD detector to analyze humic compounds. Since ESLD is another type of universal detector, they encountered the same anion interfering issue with a SEC column. Their solution to this problem was: they tried to add barium hydroxide to remove sulfate by precipitation. The sulfate could be successfully removed, but a part of the humic compound had also been precipitated due to the excess of barium.

5.5 Conclusion

Both amino acid chlorination experiment (**Chapter 4**) and raw water fractionation experiment (**Chapter 5**) showed the issues of anions interfering and high baseline noise for the preliminary SEC-UV-CAD method. The high baseline noise was found to be caused by analytical column bleeding, and the anion interference was found to be caused by the non-ideal interaction between anions and analytical column stationary phase. Both the bleeding compounds and the anions were detected by the CAD detector and caused the issues with the current method. Therefore, the next chapter focus on finding possible solutions to high baseline noise and inorganic interference issues.

CHAPTER 6 METHOD IMPROVEMENT

6.1 Introduction

Chapter 4 and **Chapter 5** showed the applications of the SEC-UV-CAD method encountered the ion interfering and the severe baseline noise issues. At the end of **Chapter 5**, the possible causes of these problems were found. Therefore, the goal of this chapter was to provide possible solutions to the existing problems and to evaluate the effectiveness of these solutions.

Four short experiments were discussed in this chapter: the source water enrichment experiment, the HPLC-UV-CAD experiment, the anion precipitation experiment, and the anion exchange resin experiment. The source water enrichment experiment and the HOLC-UV-CAD experiment tried to provide solutions to the baseline noise issue, while the anion precipitation experiment and the anion exchange resin experiment tried to provide solutions to the anion interfering issue.

Since these four experiments used different methods that were irrelevant to each other, they were introduced and discussed separately in the following sections. Duplicate experiments were not applied since the purpose of these experiments was to test and find out the most effective solution within a limited time. Once the most effective solution was confirmed, a new series of duplicate experiments based on the new method were recommended for the future studies.

6.2 Source Water Enrichment

6.2.1 Introduction

The previous chapters showed the heavy baseline noise of the SEC-UV-CAD method might cover existing NOM peak signals. This experiment concentrated the raw water sample and attempted to generate strong NOM signals to overcome the baseline noise by the SEC-UV-CAD method. Therefore, this solution would not solve the baseline noise problem, but it tried to enhance the signal of targetted peaks. The volatile NOM might be lost during the evaporation process. Also, the CAD detector will also vaporize the sample, and the volatile NOM will be lost as well. Therefore, the loss of volatile NOM should be negligible since the volatile NOM will be lost for both processes.

6.2.2 Materials and methods

Water sample used for concentration experiment was collected from Pockwock Lake, Halifax, NS, Canada. Pockwock Lake is the water supply of J.D. Kline Water Supply Plant. The water collected from Pockwock Lake has similar quality as Lake Major, except it has lower color (15 True Color Unit) and slightly lower TOC (2.5 mgL^{-1}) (Halifax Regional Municipality, 2014).

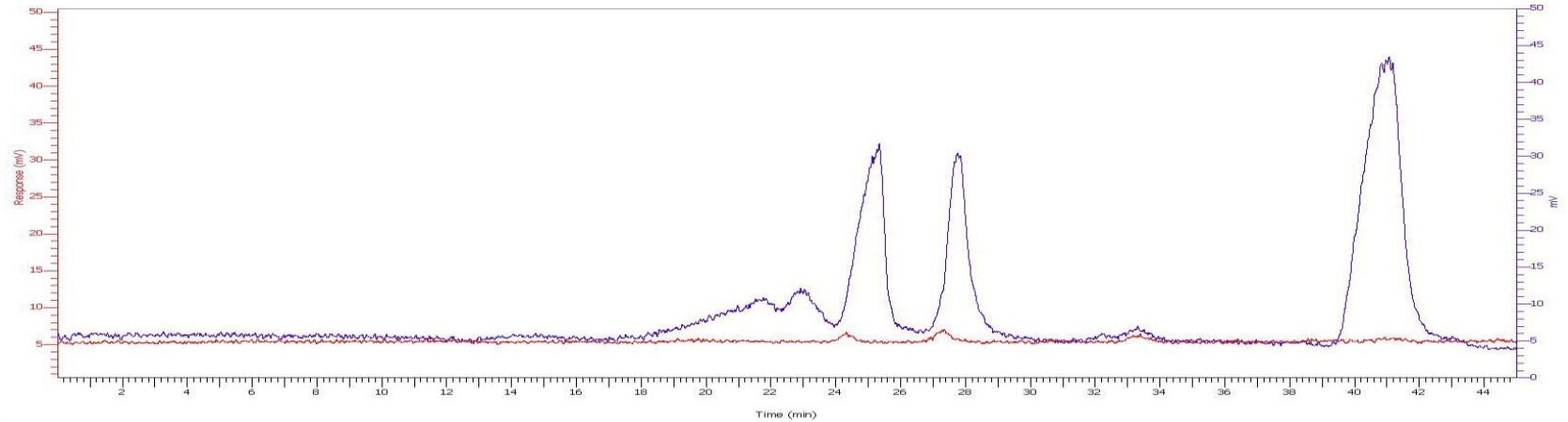
A liter of surface water taken from Pockwock Lake was heated and stirred (to slightly boiled) on a heat plate during the day time, and placed in an oven (100℃) during night time for 48 hours. Record the starting volume and the final volume to calculate the concentration factor. The concentrated water was then filtered by 0.45 mm filter and persevered in a clear bottle for analysis.

6.2.3 Results and discussions

The raw water sample after evaporation concentrated to 1/55 of the original volume. The CAD chromatogram **(Figure 6.1)** shows the peak signal was much stronger after enrichment experiment. **Figure 6.2** shows the chromatograms of both UV (red) and CAD (blue). The UV chromatogram had a similar shape as the un-concentrated water sample that all major peaks had been detected. Thus, the loss of volatile NOM brings minor effect to UV detector's result.

Also, peaks of NOM started to show after enrichment experiment. Refer the inorganic test from previous chapters, the peak number 4, 5, and 8 were interfering by inorganic. After the enrichment, the CAD detector showed broad peak signal under the UV's peak number 1, 2, and 3. These peaks signal detected by CAD other than inorganic interfering zone, and both UV and CAD detector detected these signals at the same time. Thus, peak number 1-3 from CAD detector were possibly the NOM signals. In addition, a small peak number 7 was detected at 33 minute by CAD detector only. This peak might represent the non-UV-absorbing hydrophilic compound.

The source water enrichment experiment had successfully generated peak signals that overcame the baseline noise, and part of these enhanced signals were possibly NOM signals. Further experiment will be required to prove and identify these signals. This experiment provided a possible solution to the baseline noise issue; however, the evaporation of sample water is time-consuming (24-hour for each sample), and the experiment has difficulty on reproducing samples with the same concentration ratio.



57 **Figure 6.1.** CAD chromatogram of PW raw water before (red) and after (blue) evaporation/enrichment experiment

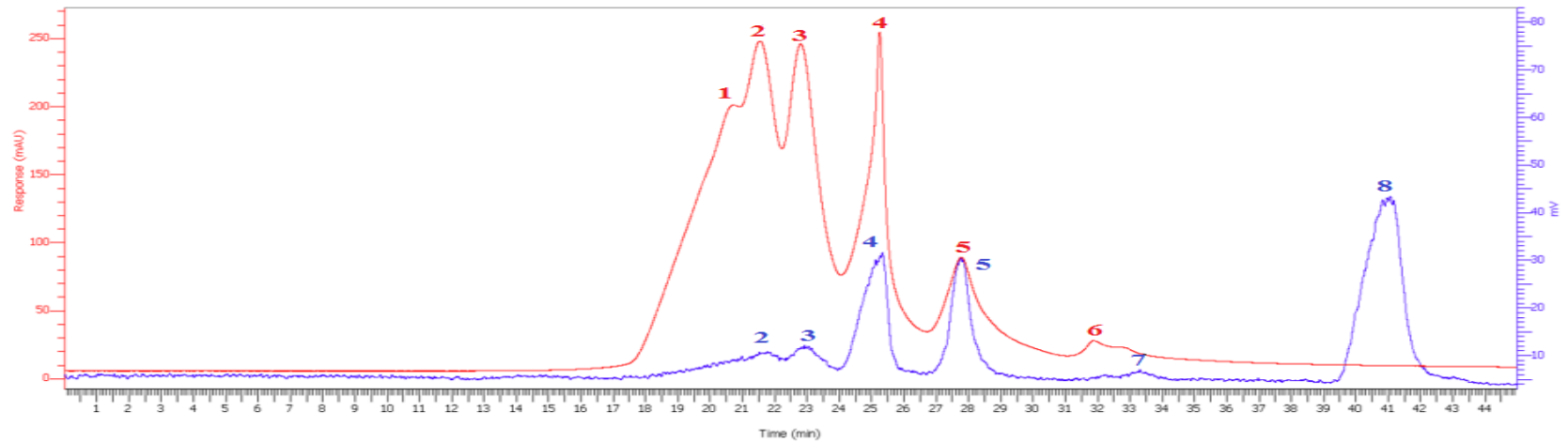


Figure 6.2 UV chromatogram (Red) and CAD chromatogram (Blue) after evaporation/enrichment experiment

6.3 HPLC-UV-CAD

6.3.1 Introduction

The heavy baseline noise might be caused by the bleeding effect from the current SEC analytical column. This experiment replaced the SEC column by a C18 HPLC column and quickly established the preliminary HPLC-UV-CAD method. The same amino acids used in **Chapter 3** were used to evaluate the performance of the HPLC-UV-CAD method. Since the type of analytical column was changed, the method also changed from a SEC method to an HPLC method. Similar to the SEC method, the HPLC method separates sample based on their polarity instead of molecular size. By changing the type of analytical column, the column bleeding issue might be solved. The C18 column was the column suit for NOM analysis in the laboratory, so it was chosen for the test.

6.3.2 Materials and methods

The operation factors of the preliminary HPLC-UV-CAD method were adjusted and tested from original HPLC method (refer to section 3.2). The tyrosine, tryptophan, phenylalanine, and alanine were selected as model compounds to test the performance of the HPLC-UV-CAD method. All amino acids were prepared at 0.5 mgL^{-1}, 1 mgL^{-1}, 3 mgL^{-1}, 5 mgL^{-1}, and 10 mgL^{-1} individually in ultrapure water; the mixture solution contained all selected amino acids were made at 5 mgL^{-1} and 10 mgL^{-1}.

Prepared samples were analyzed by a high-performance liquid chromatography (HPLC, Perkin-Elmer Series 200) using a Nova-Pak C18 column (3.9mm × 150mm). The following conditions for HPLC-UV-CAD (isocratic method) were used: 90:10 Water/Acetonitrile eluent mixtures pH adjusted to 4 with 1M acetic acid as mobile phase, flow rate of 0.7 ml min^{-1}, 10-minute analysis time, 20 μL injection volume, and UV detection wavelength at 210 nm.

The following conditions for HPLC-UV-CAD (gradient method) were used: 0.005M ammonium acetate (pH adjusted to 4 with 1M acetic acid) and 100% acetonitrile eluent mixtures as mobile phase, flow rate of 0.5 ml min^{-1}, 10-minute analysis time, 20 μL injection volume, and UV detection wavelength at 210 nm.

6.3.3 Results and discussions

6.3.3.1 HPLC- UV-CAD isocratic method

With the HPLC- UV-CAD isocratic method, **Figure 6.3** shows both UV detector and CAD detector detected tryptophan elutes at 4 minute, tyrosine elutes at 1.7 minute, and phenylalanine elutes at 2.4 minute. The baseline noise observed at SEC-UV-CAD method had been significantly improved by switching to HPLC method. The unstable bumps no longer exist, and the baseline became flat and smooth. The CAD detector showed a signal of alanine at 1.4 minute while UV detector has no signal detected around 1.4 minute. For the chromatograms of phenylalanine and tryptophan, the "peak tailing" and "broad peak" phenomena were being observed. The peak tailing issue could be caused by column secondary interactions or loss column efficiency. Also, some active compounds like amines and carboxylic acids always tail (Agilent).

Similar to the results from the SEC-UV-CAD method, the CAD detector also showed benefit on low UV absorbance compound with the HPLC-UV-CAD method. For example, CAD detector showed a better response for phenylalanine as shown in **Figure 6.3.** All peak signals of 0.5 mgL^{-1} samples can be clearly identified from CAD chromatogram due to the great improvement of baseline noise. However, the 0.5 mgL^{-1} phenylalanine can be hardly identified from UV chromatogram due to the peak tailing issue and low UV absorbance of phenylalanine. The CAD detector showed an advantage over UV detector after the baseline noise issue was solved. The peak tailing issue caused difficulty in identifying peaks.

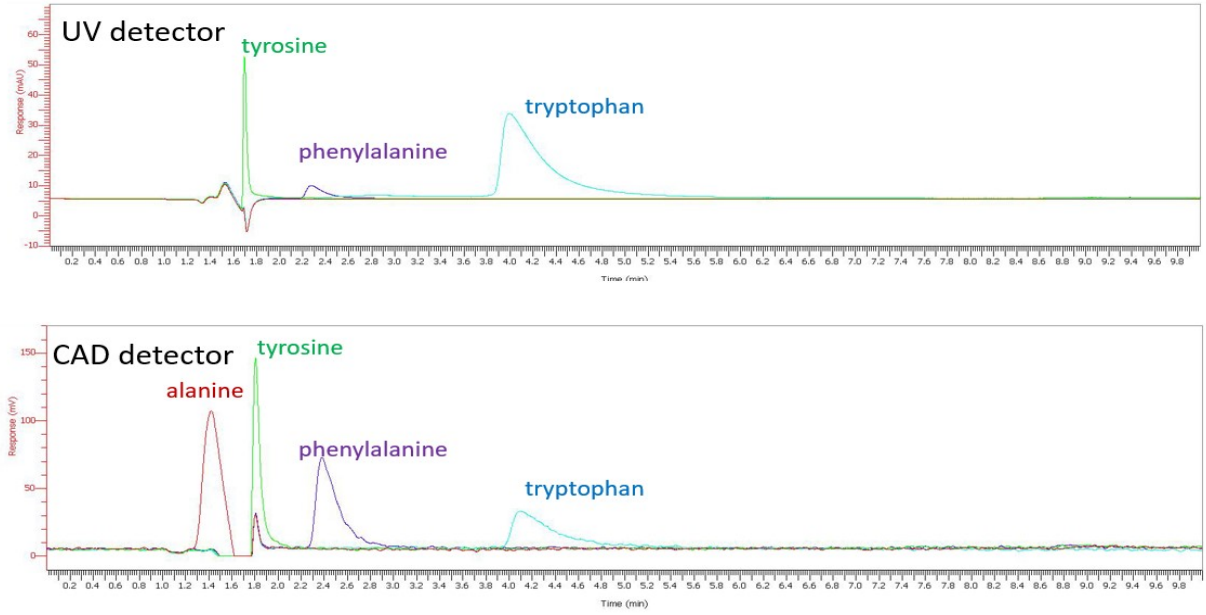


Figure 6.3. HPLC-UV-CAD with isocratic method's chromatogram. 5 mgL^{-1} of alanine, tryptophan, tyrosine, and phenylalanine were analyzed

6.3.3.2 HPLC- UV-CAD gradient method

The gradient method can improve the peak tailing issue by "pushing" the tailing peak to a sharper peak. It increases the pushing force of mobile phase over time, so the tailing peak will be compressed and becomes sharper in shape. As **Figure 6.4** shows, with the gradient method, the elute time for each model compound has been shifted from the isocratic method: tryptophan was detected at 21.5 minute, tyrosine was detected at 6 minute, phenylalanine was detected at 14 minute, and alanine was detected at 2.5 minute (on CAD detector). The tailing peak issue from isocratic method had been improved by switching to gradient method. The side-effect of gradient method is the loss of baseline linearity due to the changing concertation of mobile phase over time. The loss of baseline's linearity would cause a minor problem on peak area calculation (which can be solved by software justification), but the sharper peak shape improved the peak identification process. With the HPLC-UV-CAD gradient method, weak peak signal can be identified easier, and the baseline noise issue was solved.

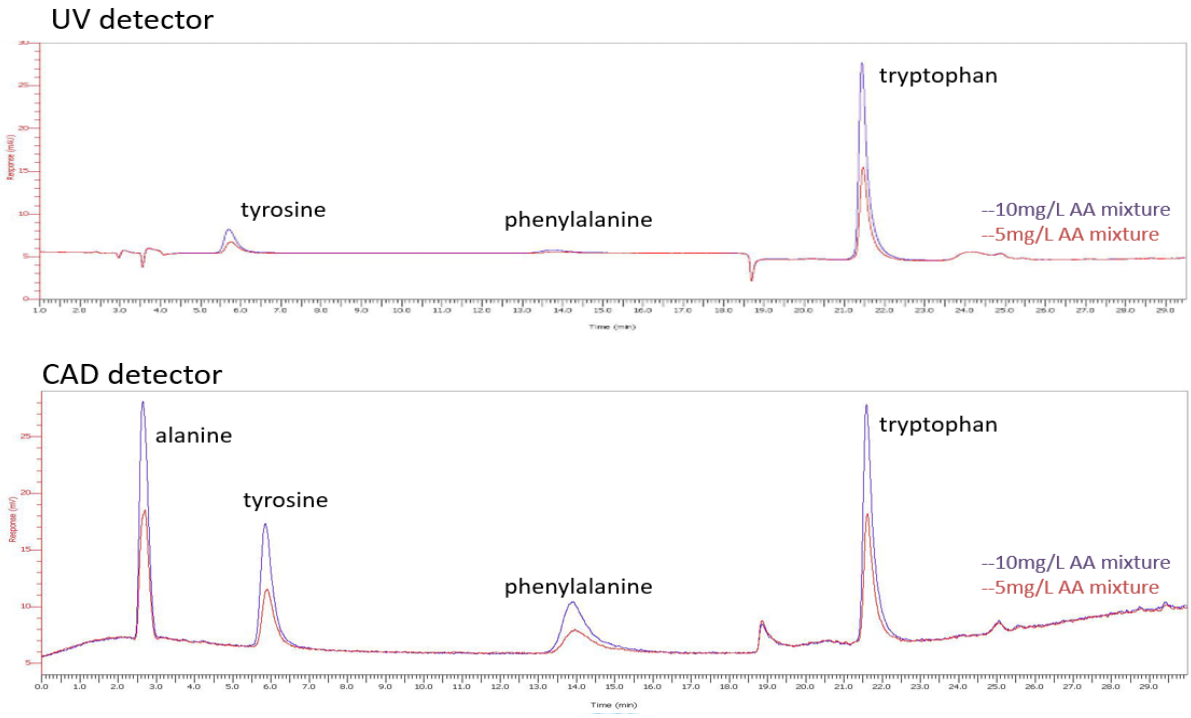


Figure 6.4. HPLC-UV-CAD gradient method's chromatograms. 5 mgL^{-1} and 10 mgL^{-1} of alanine, tryptophan, tyrosine, and phenylalanine were analyzed.

6.3.3.3 UV and CAD detector performance under HPLC- UV-CAD method

To compare the precision of both detectors, the linearity test was performed for both detectors through the measuring range (0.5-10 mg/L). Data points of the area under peak (Y-axis) versus analyte's concentration (X-axis) were plotted. A trend line was generated through these data points, and the R-squared value was calculated to investigate the linearity of these data points. As shown in **Figure 6.5**, both UV and CAD detector showed greater than 0.99 R-squared value for tryptophan, tyrosine, and phenylalanine. These high R-squared values indicated the data showed high linearity, which means both UV and CAD were highly precise through the measuring range. For the alanine, CAD detector has an R-squared value of 0.974 which drops from 0.99, which was caused by an outlier at 3 mg/L. However, an R-squared value of 0.974 was still considered high in linearity. Therefore, with HPLC-UV-CAD gradient method, the CAD detector showed the same level of precision as the UV detector.

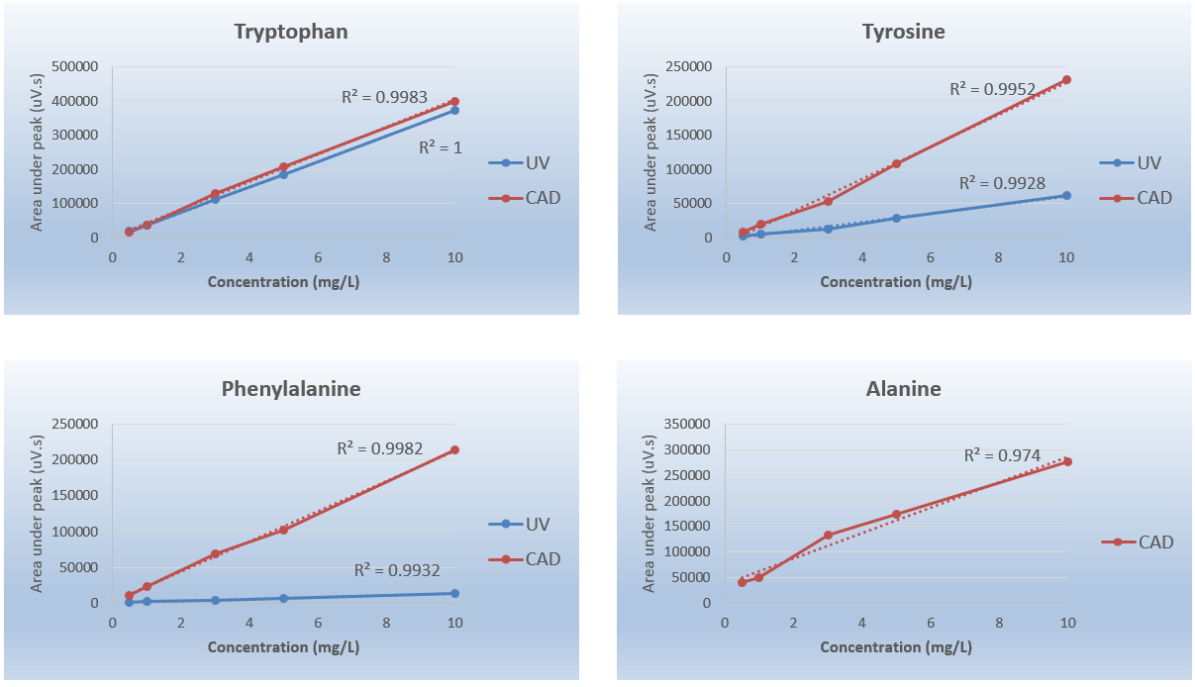


Figure 6.5. The area under peak vs. Concentration plot of model compounds (ranged from 0.5 mg/L to 10 mg/L). R-squared values calculated for UV detector (blue) and CAD detector (red).

When both detectors were measuring model compound with the same concentration, the UV detector responded signals based on model compounds' UV absorbance. So, in **Figure 6.6**, a decreasing trend of log area under peak was observed for tryptophan, tyrosine, and phenylalanine with the same concentration. On the contrary, the CAD detector responded based on analyte's mass (concentration), so the same log area under peak was calculated for all model compounds with the same concentration. This "universal response" feature of CAD detector showed another advantage over the UV detector – sample's concentration can be estimated by measuring its peak area.

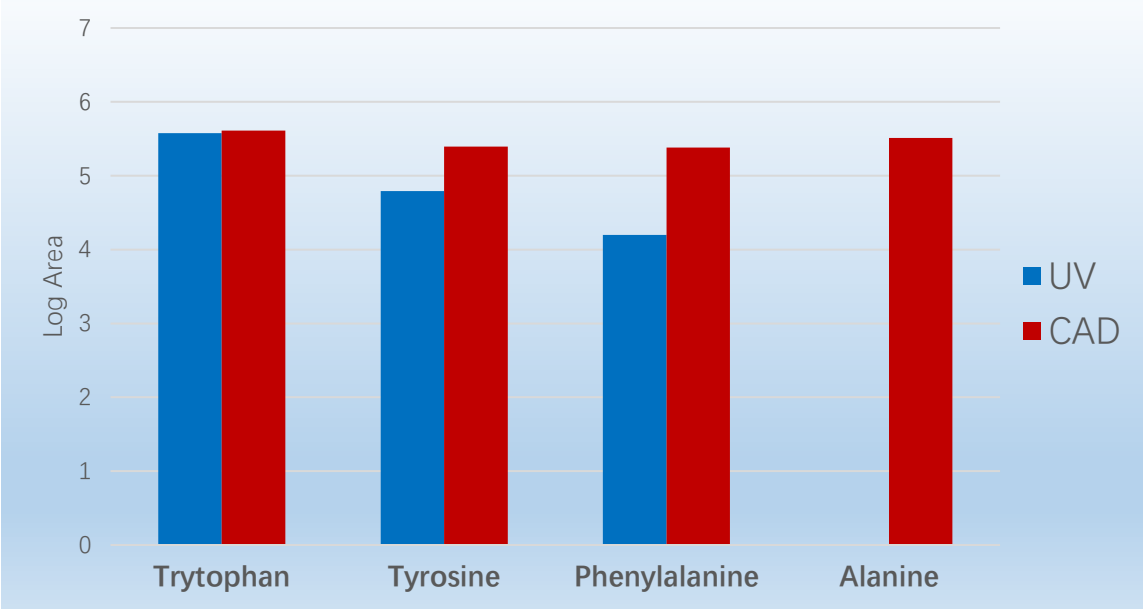


Figure 6.6. Log area under peak for model compounds measured at 10 mg/L.

Therefore, the HPLC-UV-CAD gradient method was convinced to be the best preliminary method so far for the CAD detector on detecting model compounds. Switching the SEC column to the C18 column solved the baseline noise issue. With the HPLC-UV-CAD gradient method, the CAD detector provided the same performance as the UV detector, and the CAD detector showed its advantages on detecting low or non UV-absorbing compounds. Also, the universal response feature was the other advantage of the CAD detector that helped to estimate sample's concentration.

6.4 Anion Precipitation

6.4.1 Introduction

The previous chapters showed the anions interfering issue of the SEC-UV-CAD method. This experiment used chemical solutions to precipitate anions (sulfate and chloride) and removed the precipitates by filtering the water sample. The anion-free samples were then analyzed by the SEC-UV-CAD method.

6.4.2 Materials and methods

The surface water of Pockwock contains approximately 22 mgL^{-1} chloride, and <1 mgL^{-1} sulfate (Halifax Regional Municipality, 2014). The first part of precipitation experiment prepared 10 ml of 20 mgL^{-1} ammonium chloride and 20 mgL^{-1} of ammonium sulfate in ultrapure deionized water to simulate the ion concentration of Pockwock water. The second part of precipitation experiment used 10 ml of Pockwock raw water. Silver acetate and barium acetate were added to precipitate sulfate and chloride since the remain acetate group would not cause further interfering to ammonium acetate as the mobile phase. The dosing procedure is shown in **Table 6.1**. The precipitated samples were filtered by 0.45 μm filter and analyzed by the SEC-UV-CAD method.

Table 6.1 Dose for precipitation experiment.

Water sample	0.1 mol/L silver acetate added	0.1 mol/L barium acetate added
10mL of 20 mgL^{-1} NH_4Cl	75 μL, 150 μL, 200 μL	-
10mL of 20 mgL^{-1} $(NH_4)SO_4$	-	25 μL, 50 μL, 100 μL
10mLof Pockwock water	75 μL, 150 μL, 200 μL	25 μL, 50 μL, 100 μL

6.4.3 Results and discussions

Figure 6.7 shows the CAD chromatograms of water samples of 20 mgL^{-1} Cl^- in ultrapure water. The CAD chromatograms showed the peak area of Cl^- reduce as silver acetate added. Since the CAD detector response base on mass, the chlorine peak area reduced in

percentage is equivalent to the chlorine being removed in percentage. With this feature, the anion removal rate of all dosages was calculated.

The result of precipitation experiment is shown in **Table 6.2**. The removal rate of the anion was similar between simulated water and actual Pockwock raw water. The sulfate was totally removed by adding 25 µL of 0.1M barium acetate added; while chloride reached 50-60% removal with 200 µL 0.1M silver acetate added. Chloride can be further removed by increasing the dose of silver acetate. However, due to the relatively high concentration of chloride in water, the precipitation of silver chloride caused clogging on the filter paper.

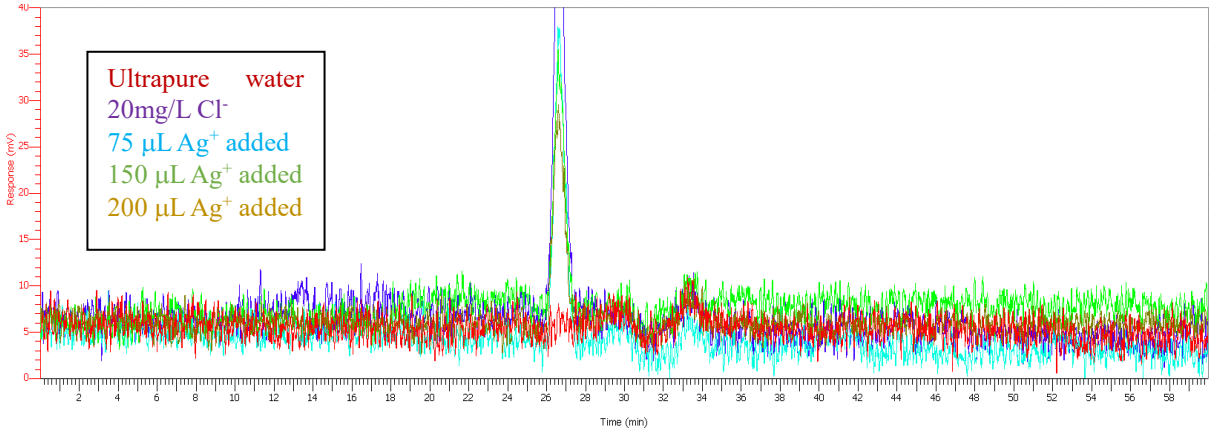


Figure 6.7. CAD chromatograms of 20 mgL^{-1} Cl- in ultrapure water with 75, 150, and 200 mL of 0.1 mol/L silver acetate added.

Table 6.2. Chloride and sulfate removal rate at different dosages of silver acetate and barium acetate.

Dosage added	Ultrapure water sample	PW raw water sample
Silver acetate 75 µL	33.6% Cl removal	29.4% Cl removal
Silver acetate 150 µL	45.3% Cl removal	42.5% Cl removal
Silver acetate 200 µL	51.8% Cl removal	65.3% Cl removal
Barium acetate 25 µL	100% SO_4 removal	100% SO_4 removal
Barium acetate 50 µL	100% SO_4 removal	100% SO_4 removal
Barium acetate 100 µL	100% SO_4 removal	100% SO_4 removal

The experiment result showed the precipitation method removed anion, and the similar precipitation method did by Emmernegeer *et al*., (2007) removed interfering anion as well. However, as Emmernegeer pointed out: the concern of precipitation method is that it might also precipitate and remove NOM at the same time. Therefore, the precipitation method is less recommended as a solution to anion interfering (Emmenegger, 2007).

6.5 Anion Exchange Resin

6.5.1 Introduction

This experiment used an alternative solution – anion exchange resin to remove anions in water samples. Since ion exchange resin method is used by some water treatment plant to remove ions from drinking water, the researchers believed this could be the solution to the anion interfering issue.

The following is the basic principle to use strong anion exchange (SAX) resin to remove anions: both NOMs and anions will be attracted to SAX resin once the sample water passes through the resin. Since anions have a much stronger affinity to the resin than NOMs, NOMs can be washed off with an eluent solution. Thus, after the SAX resin treatment, the anions will remain on the SAX resin, and the NOM will be washed off with the eluent solution.

The eluent solution is critical to the SAX treatment since the type and concentration of eluent solution control the speed of NOM being washed off (Crittenden *et al*., 2012). If this "washing" speed was adjusted to a proper level, the NOM washed off can be separated by its affinity to the SAX resin. NOMs with weak affinity to the resin will be washed off faster, while NOMs with strong affinity to the resin will be washed off slower (Crittenden *et al*., 2012). Therefore, the SAX method has the potential to remove anions from NOMs and to separate NOMs based on their affinity to the resin. The focus of this chapter was to find the optimal eluent solution for the SAX method which helped NOM separation. Since the SAX method leads to another field of NOM characterization, part of the anion exchange experiment was selected to be discussed in this chapter.

6.5.2 Materials and methods

The strong anion exchange resin: DSC-SAX 57214-U SAX resin was used for anion removal experiment. 500mg of DSC-SAX resin was packed into nine 6cc cartridges to perform nine groups of test as shown in **Table 6.3**. Eash cartridge of resin was conditioned by 2mL of methanol followed by 4 column volumes of 1mol/L ammonium acetate. When all cartridges of resin were ready, passed 150 mL of Pockwock Lake raw water through each cartridge. For each test group's cartridge, pass 10 mL of corresponding eluent solution (referred to **Table 6.3)** to collect a fraction of washed off NOM from the resin. Repeatedly passed 10mL buffer solution till 10 fractions of NOM samples were collected. All of the NOM fractions were collected and measured by the SEC-UV-CAD method.

Table 6.3. Buffer solution test groups

Test Group Number	Solvent	Concentration
1	Ammonium Acetate	0.01 M
2	Ammonium Acetate	0.1 M
3	Ammonium Acetate	1M
4	Ammonium Formate	0.01 M
5	Ammonium Formate	0.1 M
6	Ammonium Formate	1M
7	Ammonium Bicarbonate	0.01 M
8	Ammonium Bicarbonate	0.1 M
9	Ammonium Bicarbonate	1M

6.5.3 Results and discussions

The SAX experiment results showed both 0.1M and 1M concentrations were too strong for eluting NOM from the resin. **Figure 6.8** shows an example of 1M ammonium bicarbonate. The first 10 mL of ammonium bicarbonate washed out the majority of NOM (the red chromatogram with highest peak signals). And the rest of washed out fractions contained limited NOM (very low peak signals compared to the first wash).

Also, all types of NOMs were washed out at once that no separation of NOM occurred. All chromatograms shown in **Figure 6.8** had the same peak signals (with different height), which means these fractions contained the same type of NOMs. The goal of separating NOMs was failed with strong eluent solution. Similar situations were observed for other 0.1M and 1M eluent test group. Therefore, the 0.1M and 1M eluent solutions were too strong for the SAX experiment.

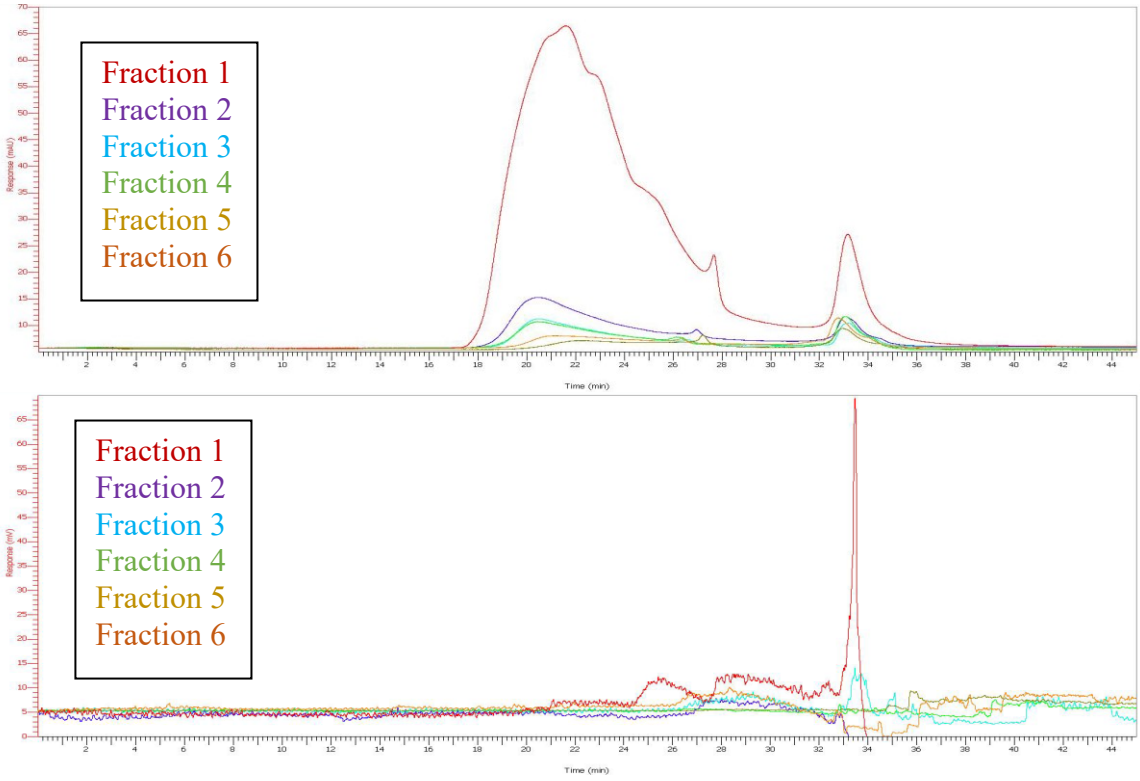


Figure 6.8. NOM fractions elute by 1M ammonium bicarbonate. Top chromatogram: UV detector Bottom chromatogram CAD detector

On the contrary, the 0.01M eluent experiment groups showed their ability to separate NOMs. The eluent solution with the best performance to separate NOMs was the 0.01M ammonium acetate. As shown in **Figure 6.9**, the first NOM fraction (red) washed off contained a peak at 29 minute and 25.5 minute; the second NOM fraction (purple) washed off contained a peak at 24 minute and a smaller peak at 22 minute; the third NOM fraction (blue) washed off contained a peak at 23 minute and 21 minute; the fourth and fifth washes showed peaks eluted at earlier time. A clear trend can be observed: the NOM fractions had been washed out earlier, tend to have lower molecular weight; the NOM fractions had been washed out latter, tend to have higher molecular weight. Therefore, the following conclusion can be made:

1) NOM with low molecular weight is less polar, so it has less affinity to SAX resin;
2) NOM with high molecular weight is more polar, so it has higher affinity to SAX resin

The SAX resin experiment demonstrated the great potential of SAX resin on NOM separation and anion removal. If the SAX resin was packed into an analytical column with 0.01M ammonium acetate as the mobile phase, a separation of NOM could be expected. Also, a SAX analytical column should not have the anion interfering issue.

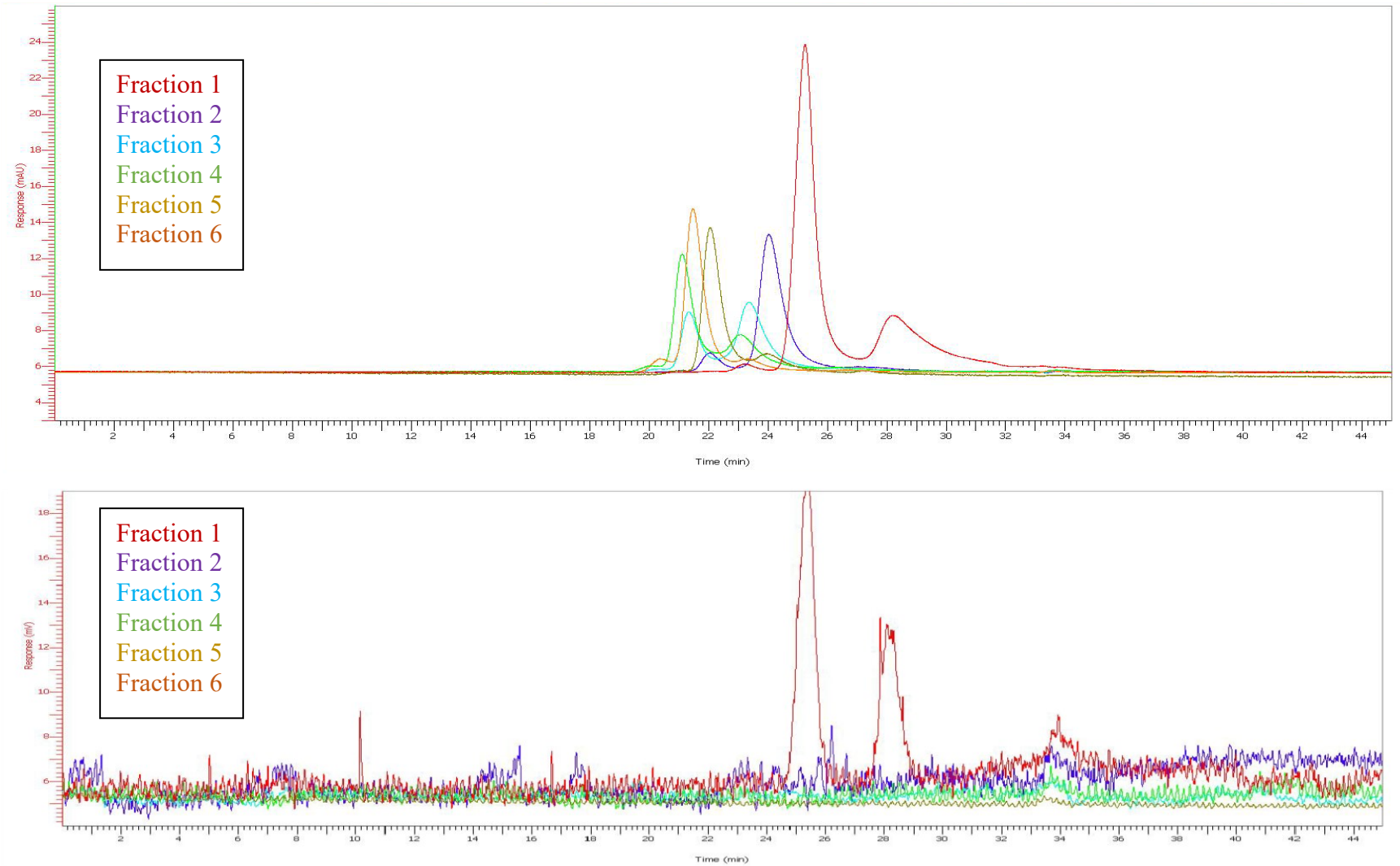


Figure 6.9. NOM fractions elute by 0.01M ammonium acetate. Top chromatogram: UV detector Bottom chromatogram CAD detector

6.6 Conclusion

The four short experiments showed their potential to solve the baseline noise and ion interfering issues:

The source water enrichment experiment showed the CAD detector had detected possible NOM signals by concentrate the raw water. The CAD detector's potential for detecting NOM was proved; however, the source water enrichment experiment is time-consuming and not easily reproducible.

The preliminary HPLC-UV-CAD method had greatly improved the baseline. The CAD detector showed advantages on detecting low or non-UV-absorbing compound. Further experiment using the HPLC-UV-CAD method on detecting actual water samples is recommended to investigate its performance further.

The precipitation method is simple but effective. Sulfate and chloride were removed by the precipitation method. However, accidentally removed targeting compounds could be the drawback of this method. Also, filtering precipitate will cause extra work especially when a large number of water samples were taken.

The SAX resin experiment showed great potential to remove anions and to separate NOM at the same time. Future studies using ion exchange column to analyze NOM with CAD detector can be expected.

CHAPTER 7 CONCLUSION

7.1 Conclusions

CAD detector has potential for water studies

The CAD detector showed its potential for water studies. In this thesis, the CAD showed:

- It provided similar performance as the UV detector;
- It detected compound that the UV detector cannot detect;
- It showed advantage over the UV detector when detecting less UV absorbing compounds;
- Its universal response base on mass helps to quantify analyte.

Therefore, the CAD detector showed its potential to detect hydrophilic NOM in a water sample.

Analytical column selection is important to CAD detector

The SEC-UV-CAD method was found to experience severe baseline noise, and the cause was found to be the column bleeding issue. After the C18 analytical column had replaced the SEC column, the baseline noise had been significantly improved. The original TSK G3000SW SEC column with the column bleeding issue might not suit for the CAD detector. The material washed out from a bleeding column can be detected by the CAD detector and cause high baseline noise. The baseline noise might cover weak peak signal. Therefore, a stable baseline noise can greatly improve the performance of the analytical method when using the CAD detector.

Interference of unwanted material is critical to CAD detector

A universal detector will detect every compound that passes through the sensor. This universal detecting feature is a double-edged sword that it helps to detect targeting

compound that other detectors cannot detect, but it also detects other unwanted compounds in the same time. When the CAD detector is used to measure a mixture sample, such as raw water sample, a reliable filtering method to screen out unwanted compound is critical to the CAD detector.

The SEC-UV-CAD method experienced an unexpected anion interfering issue which was caused by non-ideal interaction between stationary phase and anion. The van der Waals and electrostatic forces repelled and pushed the anions, so their signal overlapped with NOM compounds. The anions interfering issue can be resolved by precipitation method or ion exchange resin method.

7.2 Recommendations

The HPLC-UV-CAD gradient method showed its great performance on detecting selected amino acids as model compounds. Also, the anion interfering issue should not affect the HPLC method in theory. A series of experiments using the HPLC-UV-CAD method to analyze raw water samples and resin fractionated samples will be recommended.

The SAX resin experiment showed its great potential to remove anions while separating NOMs at the same time. Further experiments to enhance the NOM separation will be recommended. Eventually, convert the SAX resin experiment to the SAX analytical column experiment can be expected. A research program using the SAX column combined with CAD detector to analyze NOM in raw water is recommended.

REFERENCES

Abbt-Braun, G., Lankes, U., & Frimmel, F. H. (2004). Structural characterization of aquatic humic substances–The need for a multiple method approach. *Aquatic Sciences, 66(2)*, 151-170.

Agilent. (n.d.). *www.agilent.com.* Retrieved from https://www.agilent.com/cs/library/eseminars/Public/secrets%20of%20good%20peak%20shape%20in%20hplc.pdf

Allpike, B. P., Heitz, A., Joll, C. A., Kagi, R. I., Abbt-Braun, G., Frimmel, F. H., ... & Amy, G. (2005). Size exclusion chromatography to characterize DOC removal in drinking water treatment. *Environmental Science & Technology, 39(7)*, 2334-2342.

Almeling, S., Ilko, D., & Holzgrabe, U. (2012). Charged aerosol detection in pharmaceutical analysis. *Journal of pharmaceutical and biomedical analysis, 69*, 50-63.

Archer, A.D., Singer, P.C. (2006). An evaluation of the relationship between SUVA and NOM coagulation using ICR database. *J. Am. Water Works Assoc, 98 (7)*, 110–123.

Cabaniss, S. E., Zhou, Q., Maurice, P. A., Chin, Y. P., & Aiken, G. R. (2000). A log-normal distribution model for the molecular weight of aquatic fulvic acids. *Environmental science & technology, 34(6)*, 1103-1109.

ChemPep. (2005). *ChemPep Inc.* Retrieved from http://www.chempep.com/ChemPep-Generic-Term_HPLC_MS.htm

Chow, C.W.K., Fabris, R., Van Leeuwen, J., Wang, D., Drikas, M. (2008). Assessing natural organic matter treatability using high performance size exclusion chromatography. *Environ. Sci. Technol, 42*, 6683–6689.

Churms, S. C. (1996). Recent progress in carbohydrate separation by high-performance liquid chromatography based on hydrophilic interaction. *Journal of Chromatography A*(720(1-2)), 75-91.

Crittenden, J. C., Howe, K. J., Hand, D. W., Tchobanoglous, G., & Trussell, R. R. (2012). *Principles of Water Treatment.* John Wiley & Sons.

Croue, J.P., Korshin, G.V., and Benjamin, M. (2000). Characterization of Natural Organic Matter in Drinking Water. *AWWA Research Foundation and AWWA Association*.

Delpla, I., Jung, A. V., Baures, E., Clement, M., & Thomas, O. (2009). Impacts of climate change on surface water quality in relation to drinking water production. *Environment International, 35(8)*, 1225-1233.

Edwards, M. (1997). Predicting DOC removal during enhanced coagulation. *89*, 78-89.

Edzwald, J.K., Tobiason, J.E. (1999). Enhanced coagulation: USA requirements and a broader view. *Water Sci. Technol, 40 (9)*, 63-70.

Emmenegger, C., Reinhardt, A., Hueglin, C., Zenobi, R., & Kalberer, M. (2007). Evaporative light scattering: A novel detection method for the quantitative analysis of humic-like substances in aerosols. *Environmental science & technology, 41(7)*, 2473-2478.

Fan, L., Harris, J. L., Roddick, F. A., & Booker, N. A. (2001). Influence of the characteristics of natural organic matter on the fouling of microfiltration membranes. *Water Research, 35(18)*, 4455-4463.

HACH. (2014). *HACH.* Retrieved from Chlorine, Free: file:///D:/Google%E4%B8%8B%E8%BD%BD/DOC316.53.01023.pdf

Halifax Regional Municipality. (2014). *Halifax Regional Municipality.* Retrieved from http://www.halifax.ca/HalifaxWater/documents/Pockwock-LakeMajor2013-2014-FINAL-KM.pdf

Health Canada. (2009). *Protecting the Water in your Pipes.* Retrieved from Health Canada: http://www.hc-sc.gc.ca/fniah-spnia/pubs/promotion/_environ/pipe-conduite/index-eng.php

Her, N., Amy, G., Foss, D., Cho, J., Yoon, Y., & Kosenka, P. (2002). Optimization of method for detecting and characterizing NOM by HPLC-size exclusion chromatography with UV and on-line DOC detection. *Environmental science & technology, 36(5)*, 1069-1076.

Her, N., Amy, G., Foss, D., & Cho, J. (2002). Variations of molecular weight estimation by HP-size exclusion chromatography with UVA versus online DOC detection. *Environmental science & technology, 36(15)*, 3393-3399.

Huang, Z., Richards, M. A., Zha, Y., Francis, R., Lozano, R., & Ruan, J. (2009). Determination of inorganic pharmaceutical counterions using hydrophilic interaction chromatography coupled with a Corona® CAD detector. *Journal of pharmaceutical and biomedical analysis, 50(5)*, 809-814.

Hur, J., Williams, M.A., Schlautman, M.A. (2006). Evaluating spectroscopic and chromatographic techniques to resolve dissolved organic matter via end member mixing analysis. *Chemosphere, 63*, 387–402.

Imai, A., Fukushima, T., Matsushige, K., and Kim,Y.H. (2001). Fractionation and characterization of dissolved organic matter in a shallow eutrophic lake, its inflowing rivers, and other organic matter sources. *Water Resources, 35:*, 4019-4028.

Kazakevich, Y. V., & Lobrutto, R. (2007). *HPLC for pharmaceutical scientists.* John Wiley & Sons.

Kent, F. C., Montreuil, K. R., Stoddart, A. K., Reed, V. A., & Gagnon, G. A. (2014). Combined use of resin fractionation and high performance size exclusion chromatography for characterization of natural organic matter. *Journal of Environmental Science and Health, Part A, 49(14)*, 1615-1622.

Korshin, G.V., Benjamin, M.M., Li, C.-W. (1999). Use of differential spectroscopy to evaluate the structure and reactivity of humics. *Water Sci. Technol, 9*, 9–16.

Korth, A., Fiebiger, C., Bornmann, K., & Schmidt, W. (2004). NOM increase in drinking water reservoirs-relevance for drinking water production. *Water Science and Technology: Water Supply, 4(4)*, 55-60.

Lamsal, R., Montreuil, K. R., Kent, F. C., Walsh, M. E., & Gagnon, G. A. (2012). Integrating bench-and full-scale nanofiltration testing for two surface waters. *Journal of Water Supply: Research and Technology-Aqua, 61(5)*, 291-305.

Lamsal, R., Chaulk, M., Zevenhuizen, E., Walsh, M. E., & Gagnon, G. A. (2012). Characterization and removal of natural organic matter by an integrated membrane system. *Desalination, 303*, 12-16.

Leenheer, J.A. (1981). Comprehensive approach to preparative isolation and fractionation of dissolved organic carbon from natural waters and wastewaters. *Environmental Science and Technology, 15*, 578-587.

Leenheer, J.A., and Croue, J.P. (2003). Characterizing dissolved aquatic organic matter. *Environmental Science and Technology, 37:*, 18A-26A.

Liu, R., Lead, J.R., Baker, A. (2007). Fluorescence characterization of cross flow ultrafiltration derived freshwater colloidal and dissolved organic matter. *Chemosphere, 72*, 263–271.

Marhaba, T.F., Pu, Y., and Bengraine, K. (2003). Modified dissolved organic matter fractionation technique for natural water. *Journal of Hazardous Materials, B101*, 43-53.

Matilainen, A., Gjessing, E. T., Lahtinen, T., Hed, L., Bhatnagar, A., & Sillanpää, M. (2011). An overview of the methods used in the characterisation of natural organic matter (NOM) in relation to drinking water treatment. *Chemosphere, 83(11)*, 1431-1442.

Matilainen, A., Gjessing, E. T., Lahtinen, T., Hed, L., Bhatnagar, A., & Sillanpää, M. (2011). An overview of the methods used in the characterisation of natural organic matter (NOM) in relation to drinking water treatment. *Chemosphere, 83(11)*, 1431-1442.

Mawhinney, D.B., Rosario-Ortiz, F.L., Baik, S., Vanderford, B.J., Snyder, S.A. (2009). Characterization of fulvic acids by liquid chromatography-quadrupole time-of-flight mass spectrometry. *J. Chromatogr, A 1216*, 1319–1324.

McCalley, D. V. (2007). Is hydrophilic interaction chromatography with silica columns a viable alternative to reversed-phase liquid chromatography for the analysis of ionisable compounds? *Journal of chromatography A*(1171(1)), 46-55.

Montreuil, K. R. (2011). Natural Organic Matter Characterization in Drinking Water. *Dalhousie University*.

Mourey, T. H., & Oppenheimer, L. E. (1984). Principles of operation of an evaporative light-scattering detector for liquid chromatography. *Analytical Chemistry, 56(13)*, 2427-2434.

Peiris, R.H., Hallé, C., Budman, H., Moresoli, C., Peldszus, S., Huck, P.M., Legge, R.L. (2010). Identifying fouling events in a membrane-based drinking water treatment process using principal component analysis of fluorescence excitation–emission matrices. *Water Res, 44*, 185–194.

Pelekani, C., Newcombe, G., Snoeyink, V. L., Hepplewhite, C., Assemi, S., & Beckett, R. (1999). Characterization of natural organic matter using high performance size exclusion chromatography. *Environmental Science & Technology, 33(16)*, 2807-2813.

Reemtsma, T., These, A. (2005). Comparative investigation of low-molecular-weight fulvic acids of different origin by SEC-Q-TOF-MS: New insights into structureand formation. *Environ. Sci. Technol, 39*, 3507–3512.

Roccaro, P., Vagliasindi, F.G.A. (2009). Differential vs. absolute UV absorbance approaches in studying NOM reactivity in DBPs formation: comparison and applicability. *Water Res, 43*, 744–750.

Seredynska-Sobecka, B., Baker, A., Lead, A. (2007). Characterisation of colloidal and particulate organic carbon in freshwaters by thermal fluorescence quenching. *Water Res, 41*, 3069–3076.

Shimadzu. (n.d.). Retrieved from www.shimadzu.com: http://www.ssi.shimadzu.com/products/literature/lc/c190e108.pdf

SHIMADZU. (2010). *Shimadu.com.* Retrieved from http://www.shimadzu.com/an/hplc/support/lib/lctalk/55/55intro.html

Sigma-Aaldrich. (n.d.). *http://www.sigmaaldrich.com.* Retrieved from http://www.sigmaaldrich.com/analytical-chromatography/analytical-products.html?TablePage=9657807

Singer, P. C. (1999). Humic substances as precursors for potentially harmful disinfection by-products. *Water Science and Technology, 40(9)*, 25-30.

Song, J., Huang, W., Peng, P. A., Xiao, B., & Ma, Y. (2010). Humic acid molecular weight estimation by high-performance size-exclusion chromatography with ultraviolet absorbance detection and refractive index detection. *Soil Science Society of America Journal, 74(6)*, 2013-2020.

Spencer, R.G.M., Bolton, L., Baker, A. (2007). Freeze/thaw and pH effects on freshwater dissolved organic matter fluorescence and absorbance properties from a number of UK locations. *Water Res, 41*, 2941–2950.

Thermo Fisher Scientific Inc. . (2013). Retrieved from Thermoscientific.com: https://tools.thermofisher.com/content/sfs/brochures/BR70662-Dionex-Corona-Veo-EN.pdf

Unger, K. K., Kinkel, J. N., Anspach, B., & Giesche, H. (1984). Evaluation of advanced silica packings for the separation of biopolymers by high-performance liquid chromatography: I. Design and properties of parent silicas. *Journal of Chromatography A*(296), 3-14.

Wu, F.C., Kothawala, D.N., Evans, R.D., Dillon, P.J., Cai, Y.R. (2007). Relationships between DOC concentration, molecular size and fluorescence properties of DOM in a stream. *Appl. Geochem, 22*, 1659–1667.

Zhou, Q., Cabaniss, S. E., & Maurice, P. A. (2000). Considerations in the use of high-pressure size exclusion chromatography (HPSEC) for determining molecular weights of aquatic humic substances. *Water Research, 34(14)*, 3505-3514.

APPENDIX A – Resin Fractionation Process

Table A.1. Materials used for resin fractionation

Category	Materials in detail
Soxhlet extractor	• Extraction thimbles 60x180mm pk/25 (Fisher) • Soxhlet extraction apparatus (size large – 200ml extractor vol.)
Resins	• Superlite DAX-8 1000g (Sigma Aldrich) • Diaion WA-10 1000g (Sigma Aldrich) • AG MP-50 500G (Bio-Rad)
Fractionation columns	• Kontes Chromaflex Columns (60x2.5 cm) (Fisher) • Ferrules TEFZEL for 1/8" OD PTFE tubing (Fisher) • Tubing nut CTFE 1/4 28 1/8 (Fisher)
Cole Parmer tubings and pump fittings	• Green PTFE tubing (1/16" ID x 1/8" OD, 12ft/pack) • High-density polyethylene (HDPE) straight barbed connectors (1/16")/10 per pack • Masterflex Precision FDA Viton tubing, L/S 14, 25 ft
Solvents and chemicals	• Polycap 0.45 µm Filter capsules for Raw water filtration • Methanol certified ACS 4L • Acetone certified ACS 4L • Hexane certified ACS 4L • Ammonium hydroxide ACS 500mL • Sodium hydroxide • Conc. Sulfuric acid • Conc. Hydrochloric acid • 0.01N NaOH 2500 mL

	• 0.1N NaOH 2500 mL • 1N NaOH 1500 mL • 0.01N HCl 500 mL • 0.1N HCl 3500 mL • 1N HCl 2100 mL • 2N HCl 1500 mL • 3N NH_4OH 1000 mL

Pre-treatment procedures:

Before the actual fractionation, series of pre-treatment should be done to resin columns and source water. The purpose of pre-treatment is to clean and active resins, and wash out un-wanted materials of the system. The following is the pre-treatment procedure in detail.

XAD-8 column (1^{st}, 2^{nd} and 3^{rd} columns):

1) Soak XAD-8 resin in 0.1N NaOH for 24 hours
2) Rinse well with ultrapure water
3) Soxhlet clean with Acetone for 24 hours
4) Rinse well with ultrapure water
5) Soxhlet clean with Hexane for 24 hours
6) Rinse well with ultrapure water
7) Place glass wool
8) Pump Methanol until no more hexane (500mL)
9) Pump ultrapure water until DOC <1mg, Abs < 0.0001
10) Pump 2.5 Bed Volumes of 0.1N NaOH
11) Pump 2.5 Bed Volumes of 0.1N HCl

12) Pump ultrapure water until conductivity <10us/cm and DOC < 0.2 mgL^{-1}, Abs <0.0001

13) Take 1 TOC sample

AG-MP 50 column (the 4th column):

1) Rinse the resin with ultrapure water
2) Soxhlet clean with Methanol for 24 hours
3) Rinse well with ultrapure water
4) Slurry with ultrapure water and pour resin into column
5) Pump 3N NH_4OH until breakthrough of NH_4 (500mL)
6) Pump 1-2 Liters of ultrapure water
7) Pump 4 Bed Volumes of 2N HCl (1 L)
8) Rinse with ultrapure water until conductivity is < 10us/cm
9) Take 1 TOC sample

WA-10 column (5th column):

1) Rinse with ultrapure water
2) Soxhlet clean with Acetone for 24 hours
3) Rinse well with ultrapure water
4) Slurry with ultrapure water and pour resin into column
5) Pump 1N HCl until DOC< 1 mgL^{-1}
6) Pump 3N NH4OH until breakthrough (500mL)
7) Pump ultrapure water until conductivity is < 10us/cm
8) Take 1 TOC sample

Fractionation procedure:

Step 1: Source water filtration

1) Take and preserve 3 Li water
2) Filter remaining 20L of water through 0.45μm filter cartridge and into a clean carboy
3) Collect 250mL of the filtered water

Step 2: Column #1 – HON Fraction

1) Adjust sample water pH to 7±0.2 with 1N NaOH
2) Pass sample water through column at flow rate less than 12 bed volumes/hour
3) Collet eluent with a clean carboy for next column
4) Pass 1 bed volume of ultrapure water water through column and discard effluent
5) Retrieve resin and store in a desiccator for 24 hours
6) Extract organics with methanol in soxhlet extractor
7) Collect methanol and rotary evaporate at no greater than 40 ℃
8) Continue evaporation until no liquid is left in flask and only a film remains
9) Dissolve the film with ultrapure water and measure the volume of solution
10) Filter the solution (HON) through a 0.45 μm filter store in a glass bottle for further analysis

Step 3: Column #2 – HOB Fraction

1) Adjust sample water pH to 10 ±0.2 with 10N NaOH
2) Pass sample water through column at flow rate less than 12 bed volumes/hour
3) Collet eluent with a clean carboy for next column

4) Pass 1 bed volume of ultrapure water through column and discard effluent

5) Desorb organics by passing 0.25 bed volumes of 0.1N HCl followed by 1.5 bed volume of 0.01N HCl

6) Collect the eluent (HOB) for future analysis

Step 4: Column #3 – HOA Fraction

1) Adjust sample water pH to 2 ±0.2 with conc. H_2SO_4

2) Pass sample water through column at flow rate less than 12 bed volumes/hour

3) Collet eluent with a clean carboy for next column

4) Pass 1 bed volume of ultrapure water through column and discard effluent

5) Desorb organics by passing 0.25 bed volumes of 0.1N NaOH followed by 1.25 bed volume of 0.01N NaOH

6) Collect the eluent (HOA) for future analysis

Step 5: Column #4 – HOB Ensure the pH of water sample is 2

1) Pass sample water through column at flow rate less than 12 bed volumes/hour

2) Collet eluent with a clean carboy for next column

3) Pass 1 bed volume of ultrapure water through column and discard effluent

4) Desorb organics by passing 1 bed volumes of 1N NaOH

5) Collect the eluent (HOB) for future analysis

Step 6: Column #5 – Hydrophilic Acid Fraction

1) Ensure the pH of water sample is 2

2) Pass sample water through column at flow rate less than 12 bed volumes/hour

3) **The final eluent now contains only Hydrophilic Neutral Fraction (HIN)** collect it

for future analysis

4) Pass 1 bed volume of ultrapure water through column and discard effluent

5) Desorb organics by passing 1.5 bed volumes of 0.1N NaOH followed by 1bed volume of 0.01N NaOH

6) Collect the eluent (HIA) for future analysis