

**High Performance Size-Exclusion Chromatography
(HPSEC) as a Natural Organic Matter (NOM) property
indicator in Ion-Exchange (IX) applications**

by

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MASTER OF SCIENCE

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Declaration of Authorship

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Abstract

Civil Engineering

Master of Science

High Performance Size-Exclusion Chromatography (HPSEC) as a Natural Organic Matter (NOM) property indicator in Ion-Exchange (IX) applications

by Kenneth BREZINSKI

The main objective of this research was to develop an algorithm that would be able to relate ultraviolet absorbing moieties in potable water to disinfection by-products (DBP) and other water quality parameters. The separation was carried out using high performance size exclusion (HPSEC) chromatography to separate water samples based on size, while the developed algorithm used multi-spectral information extracted from 7 Manitoba source waters, and from those treated with ion-exchange (IX). Apparent molecular weight components between 2.2-4k Da were strongly associated with the formation of chlorinated by-products; as tested using Spearman and Pearson coefficients. Associations were found not to improve upon removal of the raw samples from the dataset; indicating that the applied methodology is not specific to the IX treatment used. Strong associations were also found between initial wavelengths of 226-239 nm and final wavelengths of 257-273 nm, which suggests that absorbing moieties in these ranges are of prime importance in the reaction mechanism to form DBPs.

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Contents

Declaration of Authorship	ii
Abstract	iii
Acknowledgements	iv
List of Figures	viii
List of Tables	x
List of Abbreviations	xi
List of Symbols	xiv
1 Research Objectives	1
1.1 Problem Statement	1
2 Literature Review	4
2.1 Natural Organic Matter Characterization	4
2.1.1 Natural Organic Matter Functionalization	5
2.1.2 Disinfection By-products Formation	6
2.1.3 Natural Organic Matter Fractionation	8
2.2 Absorbances Indices	12
2.2.1 Specific Ultraviolet Absorbance and Excitation-Emission Matrices .	15
2.3 Instrumental Analysis of NOM	17

2.3.1	High Performance Size Exclusion Chromatography	17
2.3.2	Apparent Molecular Weight Determination	21
2.4	Ion-Exchange Resins and Applications	26
2.4.1	Resin Properties	27
2.4.2	IX Applications	30
	IX in DBP removal	31
	IX in Membranes	32
3	Materials and Methods	35
3.1	High-performance Chromatography	35
3.1.1	Instrumental	35
3.1.2	Source Water and Resin Preparation	38
3.1.3	Signal Analysis	39
3.2	Laboratory Analysis	46
3.3	Statistical and Correlation Parameters	47
3.3.1	Molecular Weight Distribution	47
3.3.2	Correlation Coefficient	48
3.3.3	Cluster Analysis	50
4	Results and Discussion	51
4.1	2-D HPSEC Chromatograms	51
4.1.1	pH Effects	51
4.1.2	Basic Water Quality Parameters and Associations	54
4.1.3	Molecular Weight Determination	58
4.2	3-D HPSEC Chromatograms	62
4.2.1	Contour Plots	62
4.2.2	Apparent Molecular Weight and Wavelength Intervals	64
	Basic Water Quality Trends	64
	Trihalomethanes	67

4.2.3	Wavelength Interval Array	70
	Basic Water Quality Trends	70
	Trihalomethanes	73
4.2.4	Cluster Analysis	76
5	Conclusions	79
5.1	Main Conclusions	79
5.2	Engineering Significance	83
5.3	Future Work and Recommendations	84
A	Appendix	89
B	Appendix	93
C	Appendix	100
	Bibliography	102

List of Figures

2.1	Summary of AMW ranges used by researchers investigating potable/wastewater treatment using HPSEC	22
2.2	Ammonium groups typically found in strong and weak anionic exchange resins	28
3.1	Calibration curve for the AMW profiling of NOM	37
3.2	Detrending of an Absorbance Peak	40
3.3	3D chromatogram subdivision by (a) wavelength, and (b) apparent molecular weight.	43
3.4	Identified AMW peaks as significant contributors to chromophoric organic matter	44
4.1	Removal of SRNOM standard using a series of SBA resins	52
4.2	Removal of SRNOM standard at a variety of initial pH levels using DOW TAN-1 SBA resin	53
4.3	Water quality trends for 7 source waters treated with various doses of DOW TAN resin	55
4.4	Paired plot for the removal of basic water quality parameters	57
4.5	Number-averaged molecular weight of a sample of DB Raw as a function of wavelength	61
4.6	Effective removal for water sources treated with DOW TAN doses of 3 mgL ⁻¹ and 5 mgL ⁻¹	63

4.7	Pearson correlation parameters for a series of integrated peak trends with common water quality and THM species measurements	65
4.8	Spearman correlation parameters for a series of integrated peak trends with common water quality and THM species measurements	68
4.9	Pearson correlation coefficient evaluated for the integral array described in Eq. 3.5 for basic water quality parameters	71
4.10	Spearman correlation coefficient evaluated for the integral array described in Eq. 3.5 for basic water quality parameters.	72
4.11	Pearson and Spearman correlation coefficients evaluated for the integral array described in Eq. 3.5 for THM species	74
4.12	Cluster analysis based on squared Euclidean distance for a series of raw waters tested.	77
5.1	Illustration of the peak identification techniques used to deconvolute the HPSEC peaks	87
5.2	Clustering using K-means for the 7 water samples, and treated with 3 mgL ⁻¹ and 5 mgL ⁻¹ doses of SBA resin	88
A.1	HPSEC peak profile as a function of ionic strength of the sample	89
A.2	HPSEC PSS standard profiles as a function of measured wavelength.	90
A.3	HPSEC peak profile for SAN Raw as a function of measured wavelength	91
A.4	Apparent molecular weight determinations, and polydispersity indices for all 19 samples treated with DOW TAN resin, displayed as a function of wavelength	92
C.1	Sample collection locations in Manitoba, Canada.	101

List of Tables

2.1	Common structural characteristics of humic and fulvic acids obtained using NMR/FTIR analysis	6
2.2	Component analysis of various water constituents based on hydrophilicity and acidity	10
2.3	Summary of UVA indices used to predict NOM properties	14
3.1	Properties of strong base ion-exchange resins	38
4.1	Weight-averaged, number-averaged and polydispersity index for 7 water sources contacted with DOW TAN SBA resin	59
B.1	Adjusted correlation coefficients and Pearson coefficients of skewness	94
B.2	Measured basic water quality parameters for the raw and IX treated samples	95
B.3	Measured Trihalomethane species for the raw and IX treated samples	96
B.4	<i>p</i> values determined to test no correlation versus the alternative null hypothesis	97
B.5	Pearson and Spearman correlation parameters for a series of integrated peak trends with common water quality measurements	98
B.6	Pearson and Spearman correlation parameters for a series of integrated peak trends with 3 THM species	99

List of Abbreviations

A	A bsorbance
AC	A ctivated C arbon
AF4	A symmetric F low field fractionation
AHS	A quatic H umic S ubstances
AMW	A pparent M olecular W eight
AOP	A luminum O xide P articles
DB	D uck B ay
DBP	D isinfection B y P roduct
DCAA	D i C hloro A cetic A cid
DCAN	D i C hloro A ceto N itriles
DI	D e- I onized
DL	D etection L imit
DOC	D issolved O rganic C arbon
DOW	D O W ex
EC	E lectro- C oagulation
EEM	E xcitation E mission M atrix
EO	E lectro- O xidation
FA	F ulvic A cids
FFF	F low- F ield F ractionation
FTIR	F ourier T ransform I nfra R ed S pectroscopy
GC	G as C hromatography
GLEN	G LE N ella

HA	Humic Acids
HAA	HaloAcetic Acid
HAAfp	HaloAcetic Acid formation potential
HAN	HaloAcetoNitrile
HK	HaloKetone
HPI	HydroPhilic
HPIA	HydroPhilic Acid
HPLC	High Performance Liquid Chromatography
HPO	HydroPObic
HPOA	HydroPhobic Acid
HPON	HydroPhobic Neutral
HPSEC	High Performance Size Exclusion Chromatography
HS	Humic Substances
IC	Inorganic Carbon
I-DBP	Iodated Disinfection ByProduct
IX	Ion-Exchange
LET	LETellier
LOD	Limit Of Detection
LMW	Low Molecular Weight
m	mass
MIEX	Magnetic Ion-EXchange
MOR	MORris
MW	Molecular Weight
N-DBP	Nitrogenous Disinfection ByProduct
NF	Nano-Filtration
NOM	Natural Organic Matter
PAC	Poly Aluminum Chloride

PC	Pine Creek
PEG	PolyEthylene Glycol
PSS	PolyStyrene Sulfonated salts
PVDF	PolyVinylidene Fluoride
Py-GC-MS	Pyrolysis Gas Chromatography Mass Spectrometry
RR	Red River
RSD	Relative Standard Deviation
SAN	SANford
SBA	Strong Base Anionic Exchange
SEC	Size-Exclusion Chromatography
SMB	Soluble Microbial Products
SRFA	Suwannee River Fulvic Acid
SRNOM	Suwannee River Natural Organic Matter
SUVA	Specific UltraViolet Absorbance
THM	TriHalogenatedMethanes
THMfp	TriHalogenatedMethane formation potential
THMfp	TriHalogenatedMethane formation potential
TIC	Total Inorganic Carbon
TN	Total Nitrogen
TOC	Total Organic Carbon
TOX	Total Organic Halide
TPI	TransPhIllic
TTHM	Total TriHalogenatedMethane
USEPA	United States Environmental Protection Agency
UVA	UltraViolet Absorbance
UV	UltraViolet
VPO	Vapor Phase Osmometry

List of Symbols

centimeter	<i>cm</i>	1×10^{-2} m
concentration	<i>C</i>	mgL^{-1}
dalton	<i>Da</i>	1.660539×10^{-27} kg
degree celcius	$^{\circ}\text{C}$	273.16 K
equivalent	<i>eq</i>	1 eq
meter	<i>m</i>	1 m
litre	<i>L</i>	1 L
micrometer	μm	1×10^{-6} m
miliequivalent	<i>meq</i>	1×10^{-3} eq
milligram	<i>mg</i>	1×10^{-6} kg
millilitre	<i>mL</i>	1×10^{-3} L
millimeter	<i>mm</i>	1×10^{-3} m
milimolar	<i>mM</i>	1×10^{-3} molL
molarity	<i>M</i>	1 molL^{-1}
nanometer	<i>nm</i>	1×10^{-9} m
ounce	<i>oz.</i>	31.1034×10^{-3} kg
path length	<i>I</i>	1×10^{-2} m
polydispersivity	ρ	unitless
revolutions per minute	<i>rpm</i>	unitless

1 Research Objectives

1.1 Problem Statement

Natural Organic Matter (NOM) are some of the most diverse components found within natural ecosystems. From the reference point of aquatic ecosystems, NOM can be autochthonous (aquatic) or allochthonous (terrestrial) sourced. The components, and therefore the properties of NOM are source specific, which lends to the difficulty in predicting their behavior and fate within an ecosystem. NOM removal is a prime consideration for water treatment operators, being responsible for a wide array of water quality issues including taste and odour; and is responsible for reactions involving metal-chelation, redox, and the formation of disinfection by-products [31, 183, 193, 335]. Researchers and engineers are looking for new tools and methods to better describe NOM, as a better understanding will ultimately lead to a better prediction of their effects and removal.

To date, tools such as ultraviolet absorbance at 254 nm (UVA_{254}) and total organic carbon (TOC) have acted as the most commonly used surrogate parameters for NOM [219]. UVA_{254} provides an estimation of the aromaticity of the sample - which is linked to reactivity - while TOC provides an estimation of the total concentration of carbon present in NOM [146]. Both methods are readily available in most analytical laboratories, and UVA_{254} has been developed in portable form factors [22]. The problem with these parameters is that they are one-dimensional, in that they do not encompass the heterogeneity of NOM as they only measure one variable. The use of parameters such as $SUVA_{254}$ - defined as UVA_{254} divided by the TOC - has to an extent partially solved this problem, by using both variables to test

for the aromaticity per unit gram of carbon [135, 287]. Researchers however, have a wider variety of resources available at their disposal, and do not have to be limited to portability or availability. Researchers have utilized chromatographic methods to partition NOM into apparent molecular weight (AMW) fractions, and have tested various pre-treatment methods to provide a better understanding of the underlying effects of their treatment on NOM composition [24, 157, 317, 319, 330]. The use of these techniques has expanded to uses in preventing membrane fouling [339], tracing metal-colloid associations [235], and monitoring the effects of alternative disinfectants [315].

In a potable water setting, a pressing concern is the formation of disinfection by-products (DBP) - a by-product of the disinfection process that is usually formed at the end of the conventional treatment train. These DBP are correlated to their precursor products, which in most scenarios are a function of their local NOM [21]. In order to trace the presence of certain NOM components with their corresponding DBP product, a better appreciation of the nature and scope of those components is required. The use of multi-wavelength detectors, such as photodiode arrays, provides a wealth of information regarding the functionality, reactivity, and presence of these components. Drikas, Dixon, and Morran [79] applied high performance size exclusion chromatography (HPSEC) in a long term investigation of water treatment operation; including tracing a relationship between AMW and hydrophobic acids (HPO) concentration. This information has been used to make predictive outcomes in NOM reactivity, that would have otherwise been unavailable. Awad et al. [14] utilized HPSEC peak identification techniques to model the formation of THM species - a particular type of DBP. The results showed promise, but included the use of sets of arbitrary coefficients, and limited its use of information to UVA_{260} . Greater use of the multi-spectral properties provided by HPSEC will allow for a more encompassing appreciation of NOM reactivity, and a better tool to model its outcomes on potable water systems.

The objective of this research was to develop a methodology that could reliably and effectively extract the peak prominence of UV moieties from a unique 3D spectra using HPSEC.

This would in turn be used to test the correlation between peak prominence and water quality measurements for NOM and select DBPs. With this information researchers and investigators will be able to formulate a water *autopsy* that can be used to make recommendations on the best treatments and pre-treatments necessary to treat the particular water source for potable water consumption. Information related to molecular weight distribution and polydispersity can further highlight the weighted distributions of the source water components; thereby providing an indication of the steric effects for a particular pre-treatment method. This study will further focus on the universality of the methodology, so that the application may be widely used for multiple water sources and treatment methods which are currently outside of the scope of this work. This work limited its scope to 7 water sources in Manitoba, and pre-treatment using strong base anionic exchange resins. The sub-objectives of the developed methodology can be summarized as the following:

- Develop a chromatographic method that could reliably and effectively partition 7 natural water samples from Manitoba, Canada, into a multi-spectral array as a function of AMW;
- Develop an automated script that could extract useful information from the 3D array, including: weight averaged, and number averaged molecular weight (M_n, M_m); polydispersity (ρ); absorbances of wavelength and AMW ranges proposed to be significant towards the reactivity of NOM; and absorbances of a multi-dimensional array consisting of every iteration of wavelength ranges possible between 220-365 nm;
- Carry out a parametric, and non-parametric statistical analysis to test the association between absorbances extracted from the 3-D chromatogram with common water parameters and THM species;
- Perform a cluster analysis to trace the proximity of the variables in Euclidean space in order to classify the 7 water samples with their source water neighbor.

2 Literature Review

2.1 Natural Organic Matter Characterization

Natural Organic Matter (NOM) plays an important role in the general ecological makeup of ecological and engineered systems. NOM is a ubiquitous class of complex aquatic organic molecules that are ultimately sourced from the breakdown of plant residues, lignin, cellulose and other complex macromolecules from allochthonous (terrestrial) and/or autochthonous (marine) sources [1, 7, 84]. As a result of its ambiguous composition NOM does not have a set defined size distribution, functional group composition, acid-base functionality, or chelating potential. This makes it extremely difficult to model the behavior of NOM in regards to its impact in potable water treatment processes and distribution systems. This chapter aims to provide an overview on the ways in which researchers have categorized NOM through physical and/or analytical means.

NOM removal is a prime consideration for water treatment operators, being responsible for a wide array of water quality issues including taste and odour; and is responsible for reactions involving metal-chelation, redox, and the formation of disinfection by-products [31, 183, 193, 335]. As a result, the removal of NOM has been extensively explored using different chemical and physical methods, including well described methods such as carbon-based adsorbents [10, 311], oxidation [333], and coagulation [338, 346]; as well as some advanced forms of treatment such as membrane filtration [345], ion-exchange [21], electro-oxidation (EO) [100], electro-coagulation (EC) [47, 82, 227, 228], modified nanotechnologies [59, 95], and biofiltration [24, 128, 281, 306]. NOM has a significant impact on engineered

potable water treatment systems by contributing to membrane fouling [52, 206, 319, 333], trihalomethane formation [21, 173, 183, 252], metal mobility [3, 193, 279, 332], aesthetic concerns [218], and corrosion [88]. A thorough explanation on the remedies for issues concerning NOM by treatment with ion-exchange (IX) can be found in Section 2.4.

2.1.1 Natural Organic Matter Functionalization

Researchers have used various analytical means to better describe NOM composition. Table 2.1 outlines the elemental, and functional composition of two major components of NOM, Fulvic (FA) and Humic Acids (HA), collectively known as Humic Substances (HS). These acids are complex polyprotic acids, with the main operational definition between them being: HA are insoluble at low pH levels, while fulvic acids are soluble at all pH levels [298]. Research carried out by Vance and David [309] also outlines the characteristic pKa values, obtained via potentiometric titration, that distinguishes HA and FA; obtained from a northern hardwood forest floor in Central Maine, USA. Table 2.1 also lays out a series of other notable differences, namely humic acids have a larger proportion of aromatic groups and phenolic groups as a percentage of total level of functionality.

The proportions of these compounds in water bodies varies from source to source. Bolto et al. [27] found that fulvic and humic acids make up approximately 40% and 10% of the water body, respectively; while in other studies these two acids together constitute anywhere between 40-67% [49, 102, 312]. These acidic components are important for water treatment operators and engineers alike because they are considered precursors products towards the formation of disinfection by-products (DBP) [29, 148, 196, 265]. The following section will further illustrate the importance of monitoring and accounting for DBP formation.

TABLE 2.1: Common structural characteristics of humic and fulvic acids obtained using NMR/FTIR analysis, adapted from [50, 73, 116, 121, 159, 166, 167, 190, 193, 196, 214, 218, 222, 287, 334, 336]

Elemental Composition (%)	Humic Acids	Fulvic Acids
Carbon	50-60	40-50
Hydrogen	4-6	4-6
Oxygen	30-35	44-50
Nitrogen	2-4	1-3
Sulfur	1-2	0-2
Functionality (%)		
Aliphatic	74	86
Aromatic	24	16
Carboxyl	14-45	58-65
Phenol	10-38	9-19
Alcohol	13-15	11-16
Carbonyl	4-23	4-11
Methoxyl	1-5	1-2

2.1.2 Disinfection By-products Formation

DBP are a known set of potentially carcinogenic, mutagenic, cytotoxic, genotoxic by-products that form as a result of water contact with a disinfectant such as chlorine [42, 80, 126, 254, 265]. As a result, water treatment plants must abide by regulatory agencies that impose maximum acceptable concentrations (MAC) to their effluent discharges [39, 238]. This has led to the regulation of upwards of 20 different DBP worldwide [263], and a further classification of 600 compounds as new DBPs [264]. As demonstrated in the work of Plewa, Wagner, and Richardson [254], DBP such as trihalomethanes (THM) and haloacetic acids (HAA) are a small component of the overall cytotoxic effect on mammalian cells. This may be for numerous reasons, one of which is the lack of thorough toxicology and quantitative analysis haven been carried out [230, 254, 266]. Another reason is due to the lack of classification of existing total organic halogen (TOX) by-products. This problem was initially addressed by Krasner et al. [182] who noted 50% of TOX formation was not accounted for, or regulated.

This issue is very much a current one, as demonstrated in two papers carried out by Kristiana et al. [186] and Kristiana et al. [185]; among others similar investigations [94, 199, 327]. It is estimated in the work of Kristiana et al. [185] that <20% of TOX by-products are accounted for, while the rest are still yet to be classified [208]. The mechanisms for the formation of DBP from their precursor products is very much a “black box” as described in the literature due to the sheer complexity of the reaction mechanism that is expected to take place [32, 48, 103]. Mechanisms have been proposed to take place, such as in the work of Reckhow et al. [259] and as early as 1974 in the work produced by Rook [274] [273]. Current investigation into modeling DBP formation with an assortment of reaction pathways had been carried out by Zhu and Zhang [354], who were able to trace the formation of chlorinated, brominated, and iodated by-products due to chloramination disinfection with good agreement.

It has been suggested in the earlier work of Reckhow, Singer, and Malcolm [260] that electron-rich aromatics and electron withdrawing groups help to facilitate the reaction mechanism by activation of the aromatic ring; thereby FA and HA are heavily associated with this phenomenon in the literature. Reports by Mohora et al. [227] list the contribution from FA to be responsible for 41% of all THM formation, and 21% of all HAA formation. Investigations have been carried out which aim to find the connection between these properties and other common water characteristics and products. As an example, the work carried out by Wang et al. [315] drew parallels between functional composition of NOM, and the formation of DBP [288]. It was noted in the work of [315] that phenolic and hydroxyl groups, very characteristic of fulvic acids in their work and in literature, was the main contributor to the DBP dichloroacetic acid (DCAA) formation. The authors also highlighted the formation of another DBP, chloroform CHCl_3 , was enhanced in the presence of aromatic, methyl, and guaiacyl lignin groups [315]. This was also corroborated in the work of Kim and Yu [166] that found a direct correlation with phenolic content of HS and THM and HAA formation. Researchers have investigated the presence of lignin phenols on DBP formation, as lignin

derived NOM is responsible for the bulk of the terrestrial NOM pool [89]. In the works of Pellerin et al. [243] and Hua, Reckhow, and Abusallout [135], lignin derived NOM surrogates were heavily correlated with higher DBP yields if they were phenolic derived. In Hua, Reckhow, and Abusallout [135] lignin derived NOM was shown to be responsible for higher DBP yields, but less TOX formation than humic substances. C-DBP and N-DBP formation has been associated with other precursor NOM products, including; β -dicarbonyl acids, amino acids, α -amino acids, purines, and amines [207].

2.1.3 Natural Organic Matter Fractionation

NOM has further been characterized through the classification of water components based on acidity and hydrophilicity [193, 219]. These have become collectively known as *NOM fractionation*, which is the separation of NOM using a combination of strong/weak resins and variations in pH. Earlier adaptations of fractionation were used as early as 1981 by Leenheer and Croué [193], and further popularized in the work by Aiken et al. [1]. It is still very common practice to use a combination of XAD-8/-4 resins [192, 221, 251] in series, as well as XAD-8/-3 [104, 198, 213]. Adaptions have been made to fractionate with disposable solid-phase extraction (SPE) cartridges, with reverse-phase sorbents [71, 105, 258, 331] as well as C-18 sorbents [40, 86, 167, 224]. Table 2.2 lists the commonly fractionated components of NOM, as well the associated molecular weight (MW) ranges and functionality of the fractions. These fractions are important as they provide further classification into the fate and reactivity of certain components of NOM.

Similar to the proportionality of FA and HA, water bodies have different fractional compositions. Hydrophobic components are listed as the most dominate fraction in most water bodies [53, 91, 318], citing numbers as high as 66% in the work by Golea et al. [102]. Of course, there exists deviations from the established rule [337], such as in the work carried out by Kim, Yu, and Han [167] who noted a 55-70% hydrophilic composition of the Han River

located in South Korea. In addition to the information provided in Table 2.2, hydrophobic components tend to be resistant to microbial degradation, rich in extended conjugation (i.e., phenols, tanins, lignins), and have a relatively high molecular weight (HMW) relative to their hydrophilic counterparts [188, 219]. Their low biodegradability due to their insolubility makes them difficult to degrade naturally, thereby making the cellulose polymers - a mainly HPO fraction - the main contributor to color in water [326]. Hydrophilics on the other hand, are readily biodegradable low molecular weight (LMW) components, including protein-like and complex polysaccharides such as hemicellulose [219, 255]. The functionality, size-distribution, and hydrophilicity all have an important impact on the fate of these components in engineered systems. In terms of DBP formation, the literature is very much mixed on this topic, with the dominant fraction between hydrophobic acids (HPOA) [19, 48, 57, 72, 108, 114, 130, 193, 204, 209, 288, 293, 324, 347, 350], and hydrophilic acids (HPIA) [29, 70, 106, 173, 205, 231, 239, 292]. As outlined in Table 2.2, HPO are synonymous with HS [203, 204, 283], therefore the potential for both to form THMs is well established. In the recent work of Kim et al. [168], organic acids were cited as being responsible for a higher trihalomethane formation potential (THMfp) and haloacetic acid formation potential (HAAfp). Zhao et al. [351] additionally cited that hydrophilic neutrals (HPIN) were a significant factor as well; at least for the water tested which was retrieved from the Songua River, a water noted by the authors as having relatively high dissolved organic carbon (DOC) (4.1 mg mL^{-1}) typical of Chinese waters. A similar finding was made by Chu et al. [61] who noted HPN were traced to the formation of the DBP dichloroacetamide.

Fractionation has had a significant impact on the way in which treatment methods have been used to target NOM fractions. In order to predict the effect of NOM fractions, treatments such as ozonation have been applied to characteristically reshape the nature of NOM itself. The literature which suggests HPI groups contribute most to THM and HAA formation attribute this relation to the presence of carboxylic groups listed in Table 2.2 [29]. Ozone has been applied to convert 50% of the proportion of NOM to 20% non-humic NOM, and

TABLE 2.2: Component analysis of various water constituents based on hydrophilic and acidity [1, 51, 84, 164, 193, 239, 246, 258, 267, 330].

Fraction	Components	Functional Groups	Molecular Weight (Da)
Hydrophobic Base	Proteins, Amino Acids	Aromatic amines, high MW alkyl amines	100-70,000
Hydrophobic Acid	Humic and Fulvic Acids	Aromatic acids, high MW alkyl mono-, di- carboxylic acids	450-1,000
Hydrophobic Neutral	Hydrocarbons	Aldehydes, high MW methyl ketones, alkyl alcohols, ethers, furans	100-70,000
Hydrophobic Base	Proteins, Amino Acids	Purines, pyrimidines, low MW alkyl amines	100-1,000
Hydrophilic Acid	Fatty Acids	Hydroxyl acids, sugars, sulfonics, high MW alkyl mono-, di- carboxylic acids	250-850
Hydrophilic Neutrals	Polysaccharides	Polysaccharides, aldehydes, ketones, low MW alkyl alcohols	120-900

have found the formation of non-humic acids, carbohydrates, lipids, and amino acids all had lower HAAfp and THMfp [162]. When Świetlik and Sikorska [330] applied ozone, they investigated the resulting excitation-emission matrices (EEM) and found HPIN and HPOA were the main precursors to carboxylic groups; while the HPIA fraction – similar to the findings of Zhao et al. [351] and Chu et al. [61] – was the main precursor for dichloroacetamide formation. Electro-coagulation has been found to specifically target aromatics, and HMW components of NOM; thereby discriminating HPO NOM that contribute most to DBP formation [47, 82, 287, 295]. The work of Wang et al. [316] found a similar relation with HPO NOM with their implementation of enhanced coagulation. They did however note their LMW HPI NOM had a higher HAAfp, as compared to their HPO fraction which had a higher THMfp [316]. The presence of HPO can also impact other treatment units, as displayed by Cheng et al. [52] who applied ozone to reduce the impact of the HPO fraction on irreversible membrane fouling. The HPO fraction – notably, humics and alginates – have been demonstrated as the main contributor to all forms of membrane fouling [152]. Research in membrane fouling has noted that LMW hydrophilic neutral (HPIN) was the major component of nanofiltration (NF) fouling [41]. The discrepancy between these conclusions can be attributed to different mechanisms of membrane fouling [127], which is due to the initial conditions of the source water used. Research has also suggested that the removal of DOC – a predictor of THM formation - can be impacted by the HPO fraction of NOM. As investigated in the work of Mergen et al. [224], DOC removal by ion-exchange (IX) was affected by the concentration of HPO in the 3 water sources studied. The authors noted the large MW of HPO may have impeded the adsorption mechanism of IX through steric effects. It was also suggested that HPI NOM were poorly removed by IX, as HPI components such as carbohydrates have poor electron density. This mechanism can also affect the sorption of HPO, as Egeberg and Alberts [87] suggested, as the hydrophobicity of NOM is pH dependent - at least for the range of pH levels tested in their work (4.7-7.0). The impact of IX on the removal of NOM with other treatment methods is further discussed in Section 2.4.

2.2 Absorbances Indices

Absorbance has been extensively used as a parameter for determining various components that exist in water bodies. The simply direct correlation between the concentration of a compound and the amount of light it absorbs, lends to the reality that the study of spectroscopy is one of the foundations of analytical chemical analysis. This allows for spectroscopy to act as a practical tool in the analysis of water and wastewater, to monitor an assortment of components that can be presumed to exist. The difficulty in measuring environmental samples stems from in the inability to account for molar extinction coefficients (ϵ) of multiple water components, which relates to the Beer-Lambert Law, $A = \epsilon l C$. Where A is the absorbance; ϵ is the molar extinction coefficient; C is the concentration; and l is the path length. Weighted linear regressive methods, or inverse least-squares methods have been used to resolve multiple peaks; however, these methods usually require the use of reference standards [36]. The work by Francisco et al. [99] traced the concentration of apramycin and vancomycin bioassays during kinetic studies, and utilized the inverse least-squares approach to resolve overlapping peaks in absorbance measurements. These methods require the use of initial calibrations, which therefore make them impractical when the aim is to distinguish components in environmental samples. Environmental samples, for the most part, contain components that are unfamiliar to researchers; therefore the use surrogate compounds - each with a characteristic ϵ - are difficult to select competently . Researchers have however developed practical approaches through the use of surrogate absorbance indices, which usually relate to a group of components collectively, or to a predictive outcome. One of the most common indices used is UVA_{254} , used to predict aromaticity of a source sample. This wavelength is selected because three unique $\pi \rightarrow \pi^*$ electronic excitations occurs for benzene, ethylenic, benzenoid and konjugierte bands occurring at 180-210, 250-295, and 275-330 nm respectively [120, 161]. The predictive nature of UV_{254} holds true for an assortment of tested waters (See Fig. 2.3), regardless of the fact that benzene does not exist as the sole chromophore in environmental waters. In the case of benzene, the value for ϵ decreases drastically (hypochromic)

with increasing wavelength, with values of 65,000, 8,000 and 240 at wavelengths of 180, 200, and 254 nm respectively. It has been observed in the work by Her et al. [121] that a large shift in ϵ occurs at 230 nm for all 4 surrogate NOM samples tested; which included FA, HA, albumin, and asparagine standards. While the reasoning wasn't clear in the work by Her et al. [121], this may be due to bathochromic (i.e. shift to a longer wavelength) shifts in the ϵ values between 180-200 nm. We readily observe this in environmental water samples, as increased levels of conjugation - due to alkene or polyaromatic substitution - leads to bathochromic shifts of these absorption bands; thereby allowing them to be more readily observed by UV spectrophotometers (>200 nm). This has led to a wide-field of research particularly interested in the use of these UV indices to observe or predict water source characteristics. A summary of these indices can be found in Table 2.3.

Researchers have particularly made use of these indices by monitoring these parameters following various pre-treatment methods. An example of this is the use of differential absorbance, defined as the change in UV absorbance at 272 nm, with the formation of DBP. This work was initially popularized by Korshin, Li, and Benjamin [178], and has since then been carried out by researchers at the University of Washington, U.S. and the University of Catalonia, Italy. The University of Washington has published a series of papers over the years, correlating this parameter with DBP formation following chlorination [90, 175, 176, 179, 201, 340]. The University of Catalonia have followed suit with collaborations with the University of Washington, by testing these parameters as well others such as differential fluorescence on various source waters, such as that of the Ancipa reservoir in Sicily, Italy [268–271]. A more recent implementation of this research is in the work carried out by Beauchamp et al. [22] in adapting onto the work by Marcoux et al. [216]. The authors noted a strong linear correlation with values for UV absorbance, taken at 254 and 272 nm, and DBP concentrations for THM4 (CHCl_3 , CHBr_3 , CHCl_2Br , CHClBr_2) and HAA5 (CHCl_2COOH , CHCl_3COOH , CHClCOOH , CHBrCOOH , CHBr_2COOH) (0.62-0.99 R^2). The original work carried out by Marcoux et al. [216] noted limitations in their analysis, namely, limiting their

TABLE 2.3: Summary of UVA indices used to predict NOM properties

Parameter		Ref.
A_{210}	Nitrate concentration, microbial activity, non-conjugated carboxylic groups	[68, 109, 325, 329]
A_{230}	Largest change in ϵ	[121]
A_{254}	Aromatics	[68, 83, 92, 107, 109, 161, 174, 175, 245, 248, 305, 336, 353]
A_{280}	Peptides and proteins	[226]
A_{280}	Molecular weight and percent aromaticity	[54, 246]
A_{270}	Colored organic matter	[300]
A_{220}	Carboxylic and aromatics	[175]
$A_{300}, A_{340}, A_{410}$	DOC concentration	[17]
$A_{254}, A_{272}, A_{280}$	DOC aromatics	[17, 160]
$SUVA_{254}$	Increase in DOM MW fractions, ratio of FA to HA, DOM composition	[145, 146, 237]
$SUVA_{254}$	Hydrophobics and aromatics, DBPfp	[54, 70, 85, 287, 299, 325]
$SUVA_{254}/SUVA_{280}$	Decrease in lignin derivatives and aromatics	[194, 355]
$A_{254}/204$	FA adsorption by fractionation, increasing HA content, SUVA	[146, 184]
$A_{253}/203$	DBP formation, phenolic content	[147, 166, 177]
$A_{254}/210$	Relative proportion of fulvic acids to biopolymers	[121, 175, 197, 296]
$A_{250}/365$	Aromaticity and M_n	[246]
$A_{280}/350$	Aromaticity	[70]
$A_{280}/350, A_{203}/254$	Activation of phenolic groups	[175, 177, 197]
$A_{254}/410, A_{250}/365$	Relative size of DOM and aromaticity	[17, 117]
$A_{251-256}/A_{202-205}$	FA adsorption by fractionation	[119, 177]

case-study to one season; however, an interesting take-away was that the majority of DBP monitored (THM, HAA, haloacetonitriles (HAN), haloketones (HK)) were formed within the treatment train, and not in the reservoir or distribution system. This was due to the 2 sites of chlorination that were implemented within the treatment train, and the long (24 hour) residence time of plant operation [216]. The work of Golea et al. [102] is another example of a long-term investigation built upon examining UV indices, where 30 treatment works were investigated over a 30-month period in Scotland. Investigations lead to the conclusion that UVA_{254} was the best overall indicator in predicting DBPfp (for HAA and THM) for both untreated and treated waters, with DOC following closely behind in terms of surrogate measurement. Fractionation for HPO and HPI components was done concurrently, with the results providing little benefit to discern trends in DBPfp. This work helps to illustrate that UVA_{254} still remains a practical tool for water treatment operators, and not all surrogate measurements have equal effectiveness or practicality. An example of this is the use of UVA_{300} as an extensive indicator for chromophoric DOM in oceanography studies [181, 253, 297]. Absorbance parameters outside of UV, such as $A_{254/436}$ has been used to trace terrestrial vs aquatic sources of NOM [20, 150]. With this in mind, researchers have employed the use of multiple surrogate measurements to aid in the characterization of NOM, with the use of a parameter called Specific Ultraviolet Absorbance (SUVA).

2.2.1 Specific Ultraviolet Absorbance and Excitation-Emission Matrices

The difficulty in the use surrogate of parameters to model NOM behaviour is the inability to correlate multiple physical and/or electronic effects. One of such tools used is specific ultraviolet absorbance ($SUVA_{254}$) - which relates the UVA_{254} to DOC concentration – which has been used extensively to predict DBP formation [125, 135]. Others have found little to no correlation, meaning these parameters are not fully effective in encompassing the full

complexity and reactivity of NOM [58, 98]. These parameters on their own have found considerable use in predicting water characteristics - including DOC which for all intents and purposes, has acted as the best surrogate indicator for NOM applied in various applications [257, 280]. SUVA aims to act as an indicator for electron rich moieties, which is predicated on the presence of electron rich aromatics [166]. UVA₂₅₄ is important as a preliminary water quality parameter, because it provides an initial benchmark for hydrophilicity [87]. In terms of DBP formation, high SUVA₂₅₄ is associated with HAA and THM formation [30, 60, 73, 124, 202, 260] while trihalonitromethanes (TRNM) - an N-DBP - form at low SUVA₂₅₄ values [125, 131].

Others have explored excitation-emission matrices (EEM) in order to map the changing behaviour of fluorophores of NOM following pre-treatment [6, 16, 40, 200, 282, 319]. It has been adopted to target HA and FA moieties by monitoring specific regions at $\lambda_{ex} = 270 - 440$ nm, $\lambda_{em} = 380 - 550$ nm [184], and trace the binding of NOM to various metals [212]. It has also been adopted to monitor soluble microbial products (SMP), as well as protein products [200]. The work of Huguet et al. [141] used EEM on water sources of varying salinity, and was successfully able to classify estuarine waters based on rate of humification indices and autotrophic productivity. Phetrak, Lohwacharin, and Takizawa [248] used total fluorescent intensity for selected regions, separated into tyrosine, tryptophan-like, FA, HA, and SMP. A cumulative EEM spectra was used by Yang et al. [342] in order to identify aromatics and HA, then correlate that with dichloroacetic acid (DCAA), chloroform, dichloroacetonitrile (DCAN) and TOX formation. EEM continues to be a productive tool used to by researchers, as they can be coupled with separation methods such as high-performance liquid chromatography (HPLC) [113, 248].

2.3 Instrumental Analysis of NOM

Given the complexity of NOM, it comes to reason that researchers have developed powerful tools to help characterize it. Section helped to describe many spectroscopic methods that are used, with a selection of indices listed in Table 2.3, in addition to the use of EEM matrices; however, the presence of chromophores only provide a small outlook into the properties of NOM. Functional groups of NOM can be better understood through the use of Fourier-transformation infrared spectroscopy (FTIR), which examines the vibrations and stretches of chemical bonds [121]. Nuclear Magnetic Resonance (NMR) imaging is also a useful tool at researcher's disposal, providing information on the electron environment of carbon, hydrogen, and nitrogen atoms via monitoring of ^{13}C , ^1H , and ^{15}N isotopes, respectively [167, 214, 215]. New developments have also been made in the use of chromatography methods, including size-exclusion chromatography (SEC) and flow-field fractionation (FFF) [5, 15, 154, 343]; as these methods provide a means of physical separation by passing samples through a separation column. This chapter provides an overview of one of the more promising column separation methods, high-performance size exclusion chromatography (HSPEC).

2.3.1 High Performance Size Exclusion Chromatography

HPSEC is a useful separation technique that has found uses in NOM weight fractionation, and operates similar to other separation techniques such as vapor pressure osmometry (VPO) or asymmetrical flow field-flow fractionation (AF4) [235, 242, 252]. HPSEC operates by allowing the operator to pass a source sample through the column – also known as the mobile phase - which contains a bed of porous beads in the 1-3 μm range. As the source sample passes through the porous beads, LMW components will more readily enter the pores while HMW ones will be excluded. Provided the conditions are met, a detector will be able to identify the HMW components eluting first and the LMW components eluting later. This provides a size distribution of your target sample, provided a detector is available to measure

your analyte of interest. HPSEC has become a widely used method for NOM characterization due to its size separation, speed of analysis, simple sample preparation, and the availability of HPLC systems already present in various research laboratories. HPSEC has also shown to be more effective than FFF, due to its ability to cover a wider distribution of MW [74, 300]. The use of HPSEC coupled with UVA [164, 189, 198, 217, 319] and/or TOC detection [6, 140, 164, 191, 192, 322] has shown promise in further NOM characterization. Researchers have used HPSEC-UVA to analyse the effect of pre-treatment applications for the purpose of comparison between water quality following membrane filtration [78], coagulation [58, 92, 175, 189, 336], ozonation [323], ion-exchange (IX) [8] and in analysing other general NOM characteristics [121, 197, 199, 341]. HPSEC research has more recently been expanded through the use of more advanced detection methods such as: photodiode arrays [277, 341], mass spectrometry [26, 149, 163, 187, 198], and fluorescence detection [6, 8, 17, 18, 118, 209, 240].

An example of the application of HPSEC in the work carried out by Lai, Chou, and Yeh [189] who examined the effects of coagulation on pre-treatment control for an UF membrane. By using peak-identification techniques the authors were able to identify that HMW components were readily removed first, followed by humic substances, followed with LMW acids [189]. This was to be expected as coagulation does preferably remove HMW components [92, 112, 284], which did, in practice, correlate to an improvement in normalized flux. However, a better understanding of NOM can help supplement the conclusions researchers and practitioners can make; as components such as hydrophilic NOM has been tied to membrane fouling [208]. When HPLC was used to characterize two surface waters as performed by Alberts, Takács, and Egeberg [6], SEC was used to characterize HS from the source water. HS was found to heavily correlate to UVA_{254} and characteristic fluorescence peaks at λ_{ex} and λ_{em} of 225 and 425 respectively [6]. The effects of heated aluminium oxide particles (AOP) on NOM-induced fouling was investigated using HPSEC in the work of Wang and Benjamin [320]. This work was of particular interest as AOP were found to remove aromatic

groups of NOM, and over a wide-distribution of MW ranges. This allows HSPEC to act as an investigator into the nature of NOM components, while additionally serving to provide information on the size distribution of these components; thereby providing a practical way of removing components that would have otherwise contributed to membrane fouling. HPSEC has also been expanded in the analysis of IX treatments, and other processes which isolate species based on charge affinity as oppose to physical separation [8]. A type of IX resin, known as MIEX (magnetic ion-exchange) have been implemented for the purpose of organics removal due to it's affinity for negatively charged NOM, therefore facilitating it's removal in various water bodies [144, 156, 158, 224, 289]. The work of Kaewsuk and Seo [158] utilized MIEX technology by looking at the major components of NOM treated using MIEX in examining fouling potential on NF systems [158]. Research using HPSEC led to the discovery that MIEX (and IX in general) is not effective in removing hydrophobic groups in NOM, which also have high corresponding values for SUVA. It was noted that both polyhydroxyl aromatics and lignin were not retained via adsorption, and this led to induced flux decline [158]. An earlier study carried out by Fearing et al. [96] was aware of this limitation, and noted a 70% removal of chromophoric groups (measured as UVA_{254}) and 60% removal of DOC following MIEX; but following a minimal dose of ferric coagulant (5 mgL^{-1}) values improved to 75% and 100% for the removal of DOC and UVA_{254} respectively. Investigators also looked at TTHMfp, which improved from an initial value of $200 \text{ } \mu\text{gramL}^{-1}$ following MIEX; to $71 \text{ } \mu\text{gL}^{-1}$ following coagulation; followed by $24 \text{ } \mu\text{gL}^{-1}$ following coagulation and MIEX in combination [96]. This outlines an interesting application of SEC, that is, treatments can be tested in tandem in order to provide additional information on the resulting water quality parameters affected. A more wide spread implementation of SEC was carried out in the work of Kim et al. [168], which traced various fractions of NOM and the effect of ozone and bank filtration on THM formation. The removal of organic acids - associated with humic acids and the HPO, aswell as transphilic (TPI) fraction, were noted to follow in a lower HAAfp per DOC carbon. Another interesting take-away from their work was that the use of a weak anion-exchange resin to remove weak acids, noted by

the authors as the HPO fraction, introduced a significant reduction in THMfp, HAAfp and SUVA [168]. In the work of Xing et al. [336], HPSEC was used to test the effectiveness of coagulation treatments on the elimination of chromatogram peaks compared using peak fitting. The authors found that the elimination of select peaks were tied to the means of treatment, including: conventional coagulation, enhanced coagulation; or were recalcitrant [336]. The main limitation to this work is that the waters tested, which included a series of Chinese waters, all included low SUVA values, therefore the limitation of their analysis was specific to only these water types. A newer application of HPSEC was carried out recently in the work of Chon, Chon, and Cho [56], that can potential establish a new field of research in NOM characterization. The researchers provided a means to reduce the heterogeneity of NOM using HPSEC, while allowing further characterization after separation. Many HPSEC applications are destructive methods, requiring a phosphate mobile phase to separate properly, while the work of Chon, Chon, and Cho [56] was able to do so with de-ionized (DI) water and with a 97% retrieval rate by carbon mass. The work was also able to relate the size of NOM with the binding affinity to form NOM complexes; with polyhydroxyl aromatics, with medium MW and high hydrophobic character, having the greatest propensity to form NOM-metal complexes [56]. This conclusion could have helped to explain more about the fate of metallic species in contact with NOM, as was investigated in the work of Li et al. [198]. The work attempted to demonstrate the impact of NOM on the sorption of As(III) and As(V) on a modified granular natural siderite. Using HPSEC, the researchers were able to demonstrate that As(V) complexed with HMW components of NOM – more specifically, the HA fraction - were thereby removed via ternary complex formation via adsorption onto the sorbent. HA and FA were found to have different impacts on complex formation, which is to be expected as they fundamentally have different structures as demonstrated in Table 2.1. This conclusion was based off of a series of kinetic isotherms and HPSEC chromatograms; while a more thorough analysis using pyrolysis-gas chromatography-mass spectrometer (Py-GC-MS) or FTIR could have been carried out if samples were preserved following HPSEC analysis. The series of work summarized previously provides an overview of the main uses

of HPSEC in a potable water setting - the benefits of which are two-fold: (i) HPSEC can trace the elimination of select MW ranges to a treatment method, in which various treatment methods have preferential removal for certain ranges due to steric effects; and (ii) combining HPSEC with fractionation and/or absorbance detection can show removal of select NOM fractions, and correlate those with an predictive outcome such as DBP formation. For these reasons, HPSEC can act as a practical tool for researchers to further unravel the heterogeneity of NOM.

2.3.2 Apparent Molecular Weight Determination

One of the advantages of HPSEC is the ability to trace removal of water components by apparent molecular weight (AMW). The determination is very much *apparent* as the separation by weight is specific to the SEC column conditions and apparatus for which it is measured, and it is not meant to be applicable to the results of others. This however does not mean that we cannot draw useful conclusions from the results of HPSEC. For example, researchers in a wide variety of disciplines have classified the main components of water bodies into AMW ranges. Since researchers have utilized different column conditions, for the aforementioned reasons these ranges have some variance. Figure 2.1 lists some of the main designations given to AMW ranges for biopolymers, building blocks (hydrolysates of humics), LMW acids/neutrals, and humics [287].

Variance exists between groups and within definitions due to the nature of the application. For example, in the work of Liu et al. [210], a distinction is made between LMW acids and LMW neutrals; while in other studies these compounds are grouped together. It is also the case that the HPSEC chromatograms produced in Liu et al. [210] did not include MW standards, and therefore relied on attributing the retention time of peaks to the work of others [139, 275]. Other work such as that carried out by Li et al. [200] included EEM matrices to assign peak to humics and humic-like components in reverse-phase HPLC. As noted by

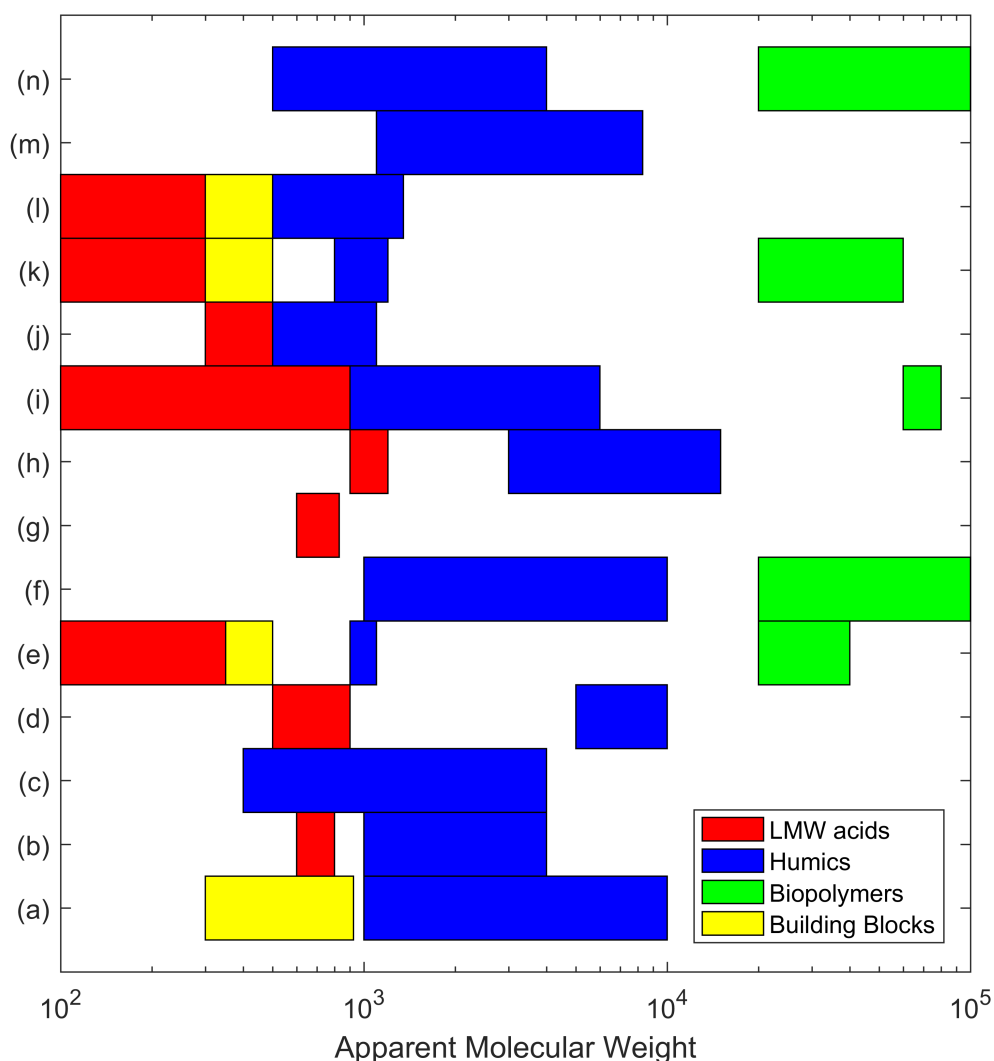


FIGURE 2.1: Summary of AMW ranges used by researchers investigating potable/wastewater treatment using HPSEC, (a) [43, 195], (b) [144, 248], (c) [249], (d) [9, 220], (e) [132, 140], (f) [120, 158, 285], (g) [1, 2, 54], (h) [23], (i) [189], (j) [44, 174, 194], (k) [122], (l) [24], (m) [51, 219], (n) [199]

the authors, reverse-phase HPLC separates based on hydrophilic as opposed to size; thereby treating hydrophobic humics differently depending on the mobile phase used [200]. This of course can have an impact on the AMW determined for your sample if the relative elution of peaks varies from one condition to the next. It was also mentioned prior that FFF and HPSEC are two methodologies applied in this regard, and it has been shown in literature that the AMW determined between these two methods varies when applied on the same source

water [149, 235]. However, consistent with the bulk of literature outlined in Figure 2.1, HMW components consist of polysaccharides and proteins (biopolymers), while humics and aromatics are concentrated in the LMW range of the distribution [120, 158, 207, 285]. The work of Lewis et al. [195] outlined HMW components as those that are greater than 1k Da, most of which are derivations of lignin-like material. Lignin materials have been heavily tied to fouling in membrane filtration units, so HMW components identified by HPSEC can be used to monitor this effect [4]. As these MW distributions constitute complex groups of organic constituents, researchers have developed strategies to target them. The impact on these AMW distributions by conventional and advanced treatment methods will be explored in this section.

The effect of conventional potable treatment methods on the AMW distribution is well established in the literature. HMW components in water are readily removed in conventional treatment plants, defined as those having coagulation coupled with clarification [51, 79, 220, 256]. This is due, in part, to the nature of floc formation in coagulation treatment, with HMW components settling better than LMW components. When activated carbon (AC) was used to address this shortfall in the work of Matilainen et al. [220], it did not readily remove the LMW fraction. In theory, LMW components would have additional surface area available for sorption within the porous structure of AC [222]; however, LMW components are known to have high charge density, thereby making it more difficult for them to overcome the zeta potential of the sorbent surface [62]. It has however been concluded by others that HMW components in fact, with a larger aromatic character, have the highest charge density [193, 286]. The discrepancy may be due to the use of terminology, as aromatics rich in electron density do not necessarily have a high charge density, which is more of a product of functionalization. It was noted in the work of Kreller, Schlautman, and McGunigale [184] that aromatics are preferentially removed by adsorption on α -aluminum oxide (α -Al₂O₃), with greatest removals of components < 1k Da. It was also confirmed that over several treated bed-volumes, 5-10k Da displaced the LMW components - leading to the conclusion that

LMW components do take precedence in sorption applications, at least for the Suwannee river fulvic acid (SRFA) standard tested [184]. This conclusion had been echoed by others [184]; as well as predicted in a model developed in the work of Vandenberg et al. [310] who modelled the kinetics of HMW and LMW components of NOM.

The exact MW range targeted by coagulation is up for debate, but waters tested with alum have been shown to leave AMW components in the range of 150 - 3k Da [58, 195]. In the work of Lewis et al. [194], coagulation was found to target components within 3–8k Da, with a noticeably peak at approx. 1700 Da that was found to belong to humification by-products that contribute to color development. Oxidation using UV has also been examined using HPSEC, with a similar preference for HMW components of NOM [24]. Oxidation also has the added effect of increasing the portion of carboxylic acidity in the treated water [304], thereby changing the hydrophilic nature of the water distribution. This is important, as not only is MW a consideration for identifying a treatment specific for the source water, but so is choosing a treatment that accounts for the hydrophilic nature of the water. Investigation into MW fractions using IR spectroscopy was carried out by Piccolo, Rausa, and Celano [250], who observed HMW components are less hydrophilic than LMW fractions; thereby concluding that HMW components may aggregate due to their HPO nature [251]. Other accounts of this phenomena have been confirmed by Xing et al. [336] and Wang et al. [318], who noted LMW components were hydrophilic in nature - and were identified as so by their low SUVA. It was established in Section 2.1.3 that the formation of DBP can follow from identification of hydrophilicity, which leads to a more focused area of research for this purpose. As mixed as this relation may be, the additional information provided by HPSEC aims to close some of the gaps in the literature.

Investigations led by Liu et al. [207] noted that wastewater organic biodegradation by-products – known as soluble microbial products (SMP) – were responsible for the bulk of NOM in potable water treatment. The LMW components (< 1k Da) were responsible for 85% of the organic carbon pool and 65% of all DBP precursors [207]. This focus on LMW

components was also identified in a series of work by Hua and Reckhow [134] in a series of papers published in 2007 and 2015; as well as by others [12]. This series of work is mainly focused on the impact of chlorination conditions (more widely, disinfection in general) on the formation of DBP. The earlier work identified LMW compounds (< 500 Da) with the greatest formation of HAA, THMs and other identified DBP [133]. While HPO NOM have been heavily tied to the formation of known DBPs, the work identified HPO as being responsible for 80% of all unknown DBP (associated as total TOX). This follows from the statement made earlier (See Section 2.1.2) that very few DBP are classified and regulated to begin with. In the later work of Hua, Reckhow, and Abusallout [135] similar findings were made, with the addition of relationships between SUVA values and the formation of select DBPs. The findings of these publications were consistent when tested on a series of Chinese waters in the work of Lin et al. [204]. Researchers identified NOM with MW $< 1k$ Da as being the prime contributors to carbonaceous DBP (C-DBP) and THMs. When the investigation was expanded to include nitrogenous-DBP (N-DBP), the findings were mixed and inconclusive. The only other significant finding was that hydrophilics were still responsible for the bulk of iodated-DBP (I-DBP) formation, with no trend as a function of MW. Given what these selected studies tell us - the formation of DBP with different halogens, with nitrogen, and with alternative disinfectants is very much a multi-faceted environment, with no one mechanism expected to be solely responsible. With this in mind, treatments that can target the bulk MW distribution of NOM, or at the very least the HPI portion, would find practical use in preventing DBP formation.

IX is one of the treatment strategies that comes to mind that have been known to target a wide variety of NOM components. A good example of this is the work carried out by Allpike et al. [8] who investigated enhanced coagulation in tandem with MIEX treatment. HPSEC performed indicated that enhanced coagulation preferentially removed HMW components of aromaticity and humic character, while MIEX was able to removed a more evenly distribution of NOM with less discrimination. This was determined on a MW basis and confirmed

using EEM matrices, but in other work this has been confirmed using absorbance detectors [79, 256]. A further investigation by Phetrak et al. [249] confirmed that the MW cutoff effectiveness for IX was in the region of 1.6k Da, with aromatic DOM less than 1k Da still remaining. The removal of components >1k Da translated to removal rates for THMfp and HAAfp in the region of 52-77% and 47-89%, respectively [249]. This observation suggests that humics do in fact exist in the 1.5k Da range as suggested in the bulk of literature summarized in Figure 2.1. Other accounts for IX put the effectiveness of MIEX at 1k Da due to a combination of effects, including: entropy assisted sorption due to hydrophobic moieties, and size exclusionary effects [70, 302]. When MIEX was operated in a fluidized bed, the work of Kaewsuk and Seo [158] put the MW cut-off of IX closer to 500 Da. It is uncertain whether or not this lower approximation is due to the nature of the MIEX resin itself, or whether it is due to the conditions of the source water used; as other investigations by Humbert et al. [143] came to a similar conclusion when using MIEX. In a thorough analysis carried out by Phetrak, Lohwacharin, and Takizawa [248], MIEX was found to be responsible for the removal of MW components greater than 1k Da, equated as the HPO and HPI components of NOM, with accompanying removal rates of 89-92% and 86-90% for humic-like and fluvic-like components respectively. This also led to the observation that the HPI non-acid <700 Da was the remaining fraction that was not effectively removed [144, 248], which is a finding confirmed by other similar accounts [79, 247]

2.4 Ion-Exchange Resins and Applications

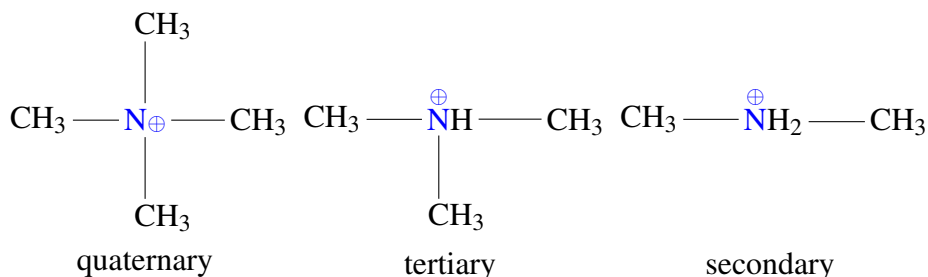
Magnetic Ion-Exchange (MIEX) are a subset of ion-exchange resins that utilize a magnetic property which allows them to act as weak magnets, promoting fast aggregation and rapid settling [33, 142]. This technology was initially developed Orica Watercare, and has since been adapted for a wide assortment of applications including: heavy metal removal [115,

165, 308], wastewater effluent [236], nitrate reduction [223]; including the removal of phosphate [76], mercury [232], pharmaceuticals [132, 153, 211, 319, 321], cyanobacterial toxins [77], and NOM removal [77, 233]. IX research all have a few common aspects in their implementation, the reasons are two-fold, which is that they (1) manipulate the physical and electronic properties of the exchange site to match the application; and (2) modify the backbone/counter-ion to change the selectivity of the resin towards different species. This chapter hopes to summarize the existing literature on the newest and most recent advancements of the implementation of the IX technology, with a particular emphasis on the industry-leading technology MIEX.

2.4.1 Resin Properties

Manipulation of the resin properties has become a field of research in water technology in of itself. Bolto et al. [28] investigated a wide selection of IX resins and the relative effects of crosslinking and water content on effective NOM removal. As a general rule, low degrees of crosslinking and high-water contents improved resin performance [28, 344]. This is due to the fact that decreased cross-linking leads to an increase in the fraction of total porosity contributed by mesopores [344]. This conclusion has been echoed by others, such as in the work by Zhang and Huang [349] who found an increase in pore volume and mesoporosity followed a decrease in microporosity volume. The reason for this phenomenon is that crosslinking opposes osmotic forces caused by the exchange with ions with large hydrated radii; thereby facilitating the exchange of components with smaller radii that are more tightly bound to the resin. [69]. This allows for an indirect mechanism by which the selectivity of compounds can be affected simply through steric and physical effects. The degree of crosslinking has also showed to have an effect on the acidity of IX groups in ethoxylated polyethyleneimines [45, 46]. The presence of acidic and basic groups in these tertiary amine groups were noted in Chanda, Sarkar, and Modak [46] to facilitate the diffusion path for adsorption. The structure of the backbone group has also been identified

FIGURE 2.2: Ammonium group typically found in strong and weak anionic exchange resins. Increased methyl substitution increases the basicity of the ion.



as a consideration when determining application – the two most common forms include polystyrene or polyacrylic. Styrene forms have been identified as having preferential removal for aromatic NOM versus their counterparts [110, 144, 249]; and has even shown preferential removal for brominated groups over other halogens [249]. A quick application that comes to mind would a treatment system that has difficulty meeting effluent goals for brominated DBPs. Counter ions are another consideration, as their removal from the adsorbent must be thermodynamically favorable to exchange with the adsorbate of interest. Walker and Boyer [314] noted bicarbonate counter-ion resins have a greater bromide removal than chloride form resins. Counter-ions have limited application, as the removal rates for DOC, UVA₂₅₄, and sulfate were identical for both counter-ions tested. As noted earlier, the degree of substitution on the IX resin is what provides the greatest degree of selectivity, and is one of the main considerations when deciding which resin is appropriate for which application. Fig. 2.2 provides the series of strong-base ammonium groups and their degree of functionalization. This represents the nature of the adsorbent, and not include consideration for the backbone, counter-ion, or cross-linking effects mentioned earlier.

With an increase in substitution the amine group becomes more strongly basic, and thus can dissociate in acidic as well as alkaline environments. Their strong basicity allows them to exchange with the weakest of acids (i.e. H₂CO₃, H₄SiO₄), as well as inorganic and neutral salts (NaCl, HCl). This is true in practice, as substitution of hydrogens on quaternary amines has been shown to coordinate with the selectivity of sulfate and chloride, by influencing the

basicity of the resin [25, 123, 278, 352]. This can be useful in applications where chloride, or other water components (NaCl, MgCl₂), are a substantial part of the water table, such as in seawater desalination [46]. Primary amines have been shown to be effective in the removal of water carbonic acid CO₂ [241]; tertiary amines in the removal of water salinity [46]; and quaternary amines in the removal of perchlorate [75]. As further demonstrated by Darracq, Baron, and Joyeux [75] - resins with varying densities, polymer structures, capacities, and functional groups all have different specificity for the adsorbent of interest relative to other water ions.

Strong base resins are typically employed to remove even the weakest acids in NOM, as many of these acid groups are responsible for various water quality woes as outlined in Sec. 2.1. In the work carried out by Bolto et al. [28] the authors noted a comparatively even removal of hydrophilic NOM groups between strong and weak base resins, while strong base resins have more total sites available at neutral pHs. A similar conclusion was given in the work by Cornelissen et al. [67], where the best performing resins were ones with the highest water contents. It was also suggested in the work that at neutral pHs physical adsorption comes into play [70], and hydrogen bonding of the acidic species to the unprotonated amino groups in the resin backbone was suggested as the mechanism [28]. In the work by Cornelissen et al. [67], a decrease in resin size was found to improve resin performance, although the driving mechanism for which was inconclusive. A larger surface area (i.e. higher capacity) was found to be the worst performing resins tested by Cornelissen et al. [67]; and Boyer [33] noted MIEX resins - with smaller particle sizes (4-5 fold smaller) and smaller capacities (3 fold smaller) than SBA resins - were the best performing for NOM removal. The reason for which may be attributed to the selectivity of medium AMW NOM over competing ions such as carbonates, nitrates, bromides, and chlorides; while general SBA resins are more effective for inorganic ions such as bromide [35].

2.4.2 IX Applications

A wide spread field of research has been conducted on the implementation of MIEX to supersede, or to accommodate existing water treatment trains. This means improving treatments such as coagulation through enhanced optimization with MIEX [144, 229, 338] and in the implementation of pilot scale operations [33, 290, 291]. All these studies have a common goal in mind: improving the removal rate of NOM – measured as DOC – and identify the underlying mechanisms for NOM removal. This also includes accommodating for the associated removal of water constituents which are removed in competition with NOM, such as alkalinity, chloride and bromide [76, 129]; and even the presence of components which may enhance removal of NOM such as calcium [11]. The work performed by Xu et al. [338] found that a moderate MIEX dose (undefined) was sufficient to improve the removal of humic acids (measured as DOC) and sulfamethozole, and reduce the chemical consumption of aluminum sulfate ($\text{Al}_2(\text{SO}_4)_3$). MIEX was noted as being an effective in combination with $\text{Al}_2(\text{SO}_4)_3$ for the removal of HMW NOM components, and an effective combination for the reduction in chemical demand [338]. When anionic exchange is combined with cation exchange in a mixed vessel, an effective removal of NOM and hardness can both be achieved as demonstrated in the work of Comstock and Boyer [63]. In a fluidized bed operation, Cornelissen et al. [66] utilized anionic and cationic resins for NOM and calcium/magnesium removal respectively. In this configuration, they were able to reduce the formation of calcium-NOM complexes by targeting both components separately. It was also noted that removing one component, in removing calcium using cationic exchange, actually promoted irreversible fouling on an UF membrane [66]. Therefore, ion-exchange is very much a multi-faceted treatment that requires proper implementation necessary for its application.

IX in DBP removal

In terms of THM formation, when put under full-scale conditions, MIEX did not show improvement over coagulation in the work carried out by Bond et al. [29]. The work tested 8 surrogate groups of NOM, and based this conclusion on relative removals of groups based on hydrophilicity prepared using octanol-water coefficients [29]. When source water from nine different water utilities was compared to coagulation with alum, MIEX showed improvement for all parameters including TOC, UVA₂₅₄, and both HAA and THM formation [289]. The enhanced performance of MIEX was also described by Kingsbury and Singer [172], but was compared to a semi-batch treatment with ozone [172]. This conclusion was further reinforced two years later in the work performed by Fearing et al. [96], who tested a unique water source in Halifax, England over a one-year period. The authors noted the advantage of a serial operation of a MIEX/Coagulation train, with the coagulation process alone targeting the hydrophilic groups that contributed most to trihalomethane formation potential (TTHMfp) [96]. The contrast with the conclusions of Bond et al. [29] can be attributed to the methodology by which the group established their baseline DBPfp. The performance of MIEX was based off of the relative removal of NOM surrogates which each had their associated contributions to DBPfp; the compound resorcinol, a hydrophobic neutral, had only a 9% removal by MIEX but had the highest DBPfp (1588 mg mg C⁻¹). The removal of tannic acid, a transphilic acid, had the best rate of removal (56-92%) but a very low DBPfp (5 mg mg C⁻¹ for chloroform, and 3 mg mg C⁻¹ for DCAA). This lack of agreement with other literature may be due to the inability for a surrogate parameter to be assumed as represented of a larger group, and the lack of consensus on the most reactive form of NOM [132, 173, 205, 316, 351]. The fact that hydrophilic groups are more readily removed by MIEX is not unfamiliar in the literature [224, 248] but has been at odds with others [79]. The work of Phetrak, Lohwacharin, and Takizawa [248] noted hydrophobic acids were the dominant fraction in their surface waters, and have the largest contribution to THMfp; however, the hydrophilic acids had the highest specific THMfp [248]. The work also used surrogates for NOM which

noted that the removal of hydrophilic groups was mainly driven by IX mechanisms, while hydrophobic groups can be partially removed through physical sorption in the MIEX porous structure [248]. The inner porous structure has size exclusionary effects as the rate of free liquid diffusivity in the pores is proportional to the inverse of MW [21, 86]. This interaction has shown to be important in applications where the inner porous structure of IX is available for exchange with for pesticides such as atrazine and isoproturon, while the removal of HMW NOM readies the availability of the sites [143]. Hu et al. [132], performed a similar analysis of the sorption of atrazine and caffeine in the inner porosity of an activated carbon resin, but utilized a MIEX resin as the pre-treatment. Similar to the conclusions of Humbert et al. [143], MIEX removed the bulk of the HMW humic substances thereby facilitation the diffusion of LMW pesticides [132].

IX in Membranes

The long-term performance and mitigation of membrane fouling is of prime consideration for those seeking to manage a variety of source waters. Therefore, the ability for MIEX to target components of NOM that contribute to irreversible membrane fouling is paramount. The effectiveness of MIEX to remove color and DOC has been shown to operate effectively over a series of resin doses and a range of UF membranes with different MW cut-offs [156]. When MIEX performance was analyzed over a long-period (August 2005 – June 2007) - as was carried out by Drikas, Dixon, and Morran [79], Fabris et al. [93], and Huang et al. [138] - DOC and SUVA₂₅₄ was consistently removed over a variety of source water compositions. The work also showed better effectiveness in MIEX combined with NF or coagulation. The work carried out by Jutaporn et al. [155] attempted to correlate the improvement in MF membrane flux following MIEX, by monitoring parameters of SUVA, UVA₂₅₄ and DOC. While none of these parameters were directly tied to normalized membrane flux over time, MIEX pre-treatment did reduce membrane fouling potential in four natural and two synthetic waters with a wide range in composition (DOC: 1.8-11 mgL⁻¹, UVA₂₅₄: 0.056-0.208

cm^{-1}). Humbert, Gallard, and Croué [142] used an IX/NF coupled operation following coagulation in a high DOC source water (DOC: 7.5-9.8 mgL^{-1} , UVA_{254} : 0.2-0.35 cm^{-1}), and found a reversibility in fouling as a consequence of NOM removal (final DOC residual of 1.6 mgL^{-1}). In the work of Cornelissen et al. [66], the removal of NOM in addition to calcium is an effective means to reduce irreversible fouling due to the elimination of calcium-NOM complexes [66]. Biopolymers have been linked as key contributors to fouling, therefore investigation into their removal has been carried out [171, 303]. Kimura and Oki [170] found that MIEX combined with coagulation, in that order, performed better than MIEX/PAC, and even coagulation followed by MIEX, for normalized flux decline in MF and UF membranes. The authors also noted a better relationship between the concentration of biopolymers and fouling potential, than with DOC and fouling potential [170].

The presence of biopolymers - involving LMW carbohydrates and proteins in water - are considered hydrophilic constituents, and which have also shown to promote irreversible fouling when compared to fouling caused by solely hydrophobic groups [158, 339]. This has been proven in combined MIEX-NF systems which show the main components in membrane permeate are carboxylic, phenolic, and fatty acids [158]. MIEX was noted to remove the majority of phenolic components and aliphatic hydrocarbons, which are components with relatively low SUVA values. A similar conclusion was found by Huang et al. [136] where fouling potential did not follow from DOC removal by MIEX, but the author noted this was most likely due to the composition of the membrane itself [136]. The same work by Yamamura et al. [339] noted the effect due to the membrane was hydrogen bonding between the hydroxyl groups of the biopolymers and the fluorine groups of the polyvinylidene fluoride membrane [339]. Other researchers have examined the size composition of the water itself rather than using any surrogate form, and have found promising results. Fabris et al. [93] looked at the effects of high molecular weight ($< 50,000$ Daltons) peaks corresponding to colloidal fractions on MF membrane fouling. HPSEC analysis found that the HMW component contributed most to MF fouling, and similar to the conclusions of Huang et al. [136] and

Kimura and Oki [170], DOC was not indicative of any trend in fouling. When the averaged AMW for DOC was removed using a combination of biological activated carbon combined with MIEX, NF membrane saw an increase steady flux over time [12]. A more recent study by Gibert et al. [101] reinforced this notion, where the LMW fractions of NOM (corresponding to humic and fulvic substances) were more readily removed by MIEX (DOC removal of 32-46%), as confirmed by a combination of HPSEC and Excitation-Emission Matrices (EEM) [101]. NF was found to have a preferential removal of HMW components (5% removal of LMW components) – due to size exclusion – which would indicate an ability to foul more readily when the concentration of the HMW components are higher.

3 Materials and Methods

3.1 High-performance Chromatography

3.1.1 Instrumental

HPSEC was performed using a Waters e2696 HPLC module (Waters Associates, Milford, MA) with a temperature control module and column heater, coupled with a Waters 2998 photodiode array detector capable of operating within the 190 to 400 nm range. Sample injections were set at 100 μL and an isocratic (constant) flow of 1.0 mL min^{-1} was set for the eluent rate. System components and data acquisition was controlled using Empower3 software. The analysis involved a BioSep-SEC-S2000 silica column, 300 x 7.8 mm (Phenomenex, Terrance CA). The column was selected based on its exclusion range (1-75k Da), and it has also been noted the relative standard deviation for several columns, including the BioSep-S2000, only differs by 5% [65]. The hydrophilic bonded silica stationary phase of the BioSep-S2000 will minimize hydrophobic interaction with the mobile phase. This effect was shown to have a significant effect on minimizing peak elution and spread as shown by Ruhl and Jekel [276]. Mobile phase included a 100 mM phosphate buffer (0.0016 M Na_2HPO_4 and 0.0024 M NaH_2PO_4 to yield a pH of 6.8). 75 mM KCl was added to yield a final ionic strength of 0.1. Both the pH and ionic strength have a large effect on the change in elution volume [246]. This change in elution volume is indicative of the presence of repulsive forces between the solute and the stationary phase. Repulsion forces will cause a higher apparent molecular mass than the actual molecular mass; while attraction forces

cause the opposite, in that compounds will elute later than expected [149, 235, 242]. A high ionic-strength (> 0.04 M) has shown to be optimal when used alongside PSS; however, gross errors can occur even with slight changes in these parameters [234].

With an ionic strength of 0.1, polystyrene sulphonate (PSS) standards have been shown to have similar coiled shapes as humic substances [23, 54, 55, 121]. An increased ionic strength of 0.1 has been shown to minimize Fuoss theory effects - where in the presence of salts, cations are attracted to the carboxylate anions of organic acids and causes them to coil. In low salt solutions macromolecules tend to stretch, which leads to an increased intramolecular coulombic interaction in the macromolecule chain [151]. This has the effect of increasing hydrated radii, therefore altering their shape [111]. An adequate ionic strength also allows for the penetration of polyelectrolytes into the porous stationary phase, thereby allowing for a separation that is based solely on size [246].

Samples were pre-filtered with a $0.45\ \mu\text{m}$ polyvinylidene fluoride (PVDF) syringe filter (Merck Millipore, Billerica MA) and adjusted to a similar pH and ionic strength conditions as the carrier solvent using 50% NaOH and Na_2HPO_4 respectively. Appendix A(b) provides a comparison between samples with differing ionic strengths that the mobile phase, showing negative absorbances throughout the profiles that are not 0.1 M. All samples were run following overnight degasification with N_2 to prevent bubble formation during HPLC separation.

Various molecular weight calibrants for SEC analysis of humic substances have been employed by researchers, most commonly: polysulfonated salts (PSS) [54, 55, 58, 175, 210, 235, 244, 248, 336, 353] and polyethylene glycol (PEG) [121, 200, 348]. In this work PSS standards at 1.7K, 5.2K, 7.5K, and 16K Da (Scientific Polymer Products Inc., Ontario NY) were used and prepared at concentrations of $1\ \text{g L}^{-1}$. The 1.7K PSS standard displayed peak splitting, therefore a 200 apparent molecular weight (AMW) PEG (Alfa Aesar, Haverhill MS) standard was used as a replacement for the low molecular weight standard. The calibration curve in Figure 3.1 with the inclusion of the polyethylene glycol standard yielded an R^2

of 0.9999, and was used to relate retention times as a function of the logarithmic of apparent molecular weight [81]. HPSEC work was performed within the day of calibration as the column conditions and instrument drift can after the regression. Standards were detected at 230 nm corresponding to their maximum peak absorbances compared to values obtained at 215 and 254 nm (See Appendix A Figure A.2) [234].

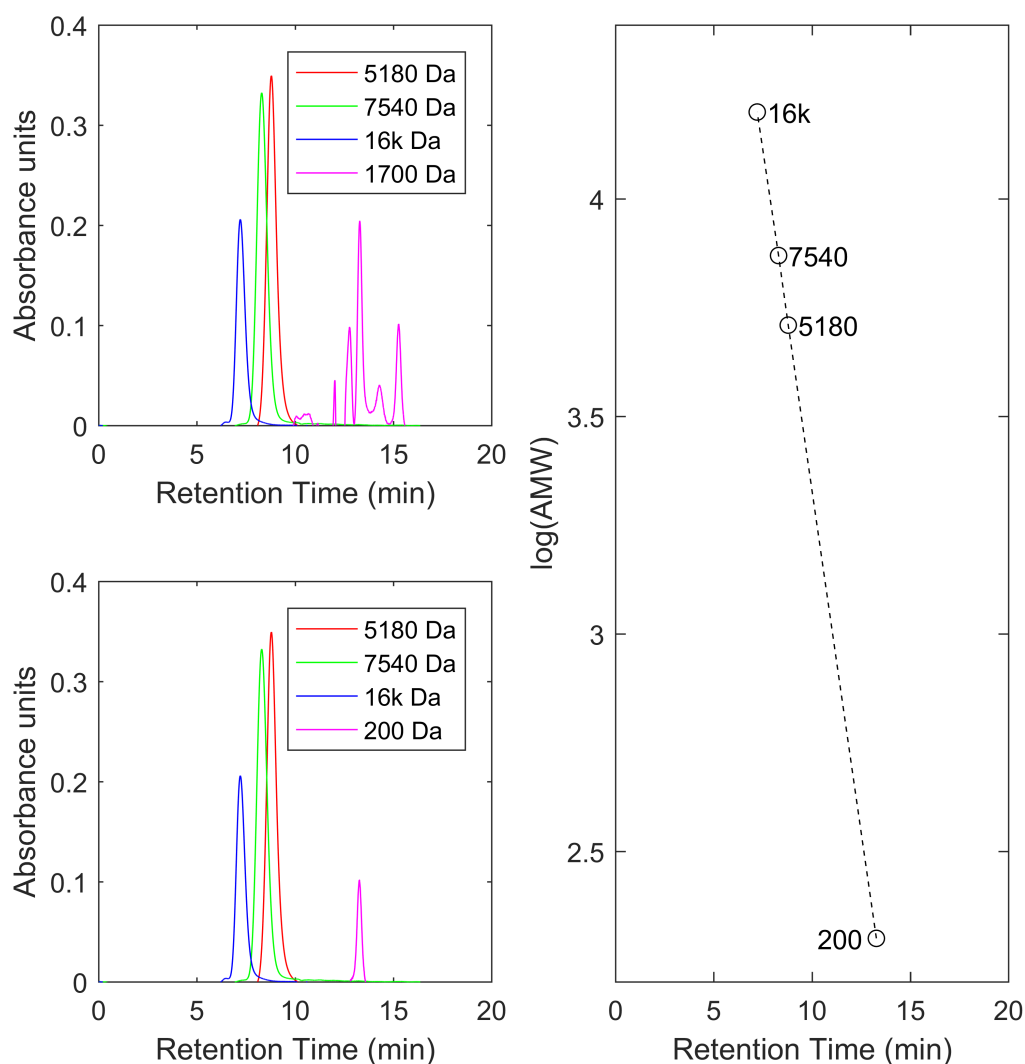


FIGURE 3.1: Calibration curve of the PSS and PEG standards for AMW profiling of NOM

3.1.2 Source Water and Resin Preparation

Synthetic water was prepared using the Suwannee River NOM (SRNOM) (IHSS, St. Paul MN) standard prepared at concentrations of 100 mg L^{-1} in deionized water. Source waters were obtained from several (7) locations within Manitoba (See Appendix C), from either the raw intake feeding into the plant or the raw water clear well. The samples were collected from DuckBay (DB), Glenella (GL), Letellier (LET), Morris (MOR), Pine Creek First Nation (PC), Rainy River (RR), and Sanford (SAN) - all representing source waters from locations drawn along the Assiniboine River, Red River, and Lake Winnipegosis. This selection of source waters will add some variance to initial water quality in order to take into account a variety of NOM compositions. Samples were stored at 4°C in a dark, cold room for no more than two weeks of time. Total organic carbon (TOC) for the raw water was determined to deviate no more than $\pm 1.4 \text{ mg L}^{-1}$ for an extended period of 2 months.

TABLE 3.1: Properties of strong base ion-exchange resins

Resin	Structure	Capacity (meq mL^{-1})	Water Content % (m/m)
Dowex TAN-1	Polystyrene, Quaternary Amine	0.7^α	70-82(65-77)
Purolite A502P	Polybenzene, Quaternary Amine	0.85^β	66-72(63-69)
Amberlite PWA9	Polyacrylic, Quaternary Amine	0.8^α	66-72(61-68)
Purolite A860	Polyacrylic, Quaternary Amine	0.8^α	52-72(52-69)

$^\alpha$ Chloride – form, $^\beta$ Hydroxide – form.

A series of strong-base anionic exchange resins (summarized in Table 3.1) were initially

preconditioned and rinsed with 1 M NaCl and deionized water solution respectively. The resins contain quaternary ammonium groups (NH_3^+) which have a strong base functionality; thereby allowing for the sorption of even the weakest acids contained within NOM. They are also specifically advertised as being used to control increased levels of NOM to meet water treatment objectives. Samples were dosed volumetrically using wet doses converted from dry doses as per the resin manufacturer by allowing the wet resin to settle in a pre-measured micropipette tip. Samples were mixed according to work performed by others [63, 64, 97]. This corresponded to a weighted dose of 3 and 5 mg L^{-1} ; calculated from densities listed in Table 3.1. Samples came to equilibrium in 4 oz. amber glass bottles using an orbital shaker (Labnics Equipment, Fremont CA) set at 100 rpm for 24 hours.

3.1.3 Signal Analysis

Computation and numerical methods were carried out in the MATLAB R2016a (MathWorks Inc., Massachusetts) environment. HPSEC absorbances were imported into the Matlab workspace as a structured array, and sequenced for signal analysis. Each peak was detrended from the baseline in order to remove the contribution due to instrumental noise, and to distinguish statistically significant peaks from noise and impurities. This was done by computing the limit of detection (LOD) for the absorbance values, calculated according to Eq. 3.1.

$$LOD = x_{blk} + 1.96s_{blk} \quad (3.1)$$

Where x_{blk} and s_{blk} is the mean and standard deviation of the baseline noise, respectively. The LOD calculated assures a 95% confidence in selected values, which corresponds to 1.96 standard deviations from the mean. This value was used to identify the initial and final indices of significant peaks, and establish the conditions for peak detrending. A linear regression was then established up until the first significant peak index, which was used to interpolate under the signal peak Figure 3.2.

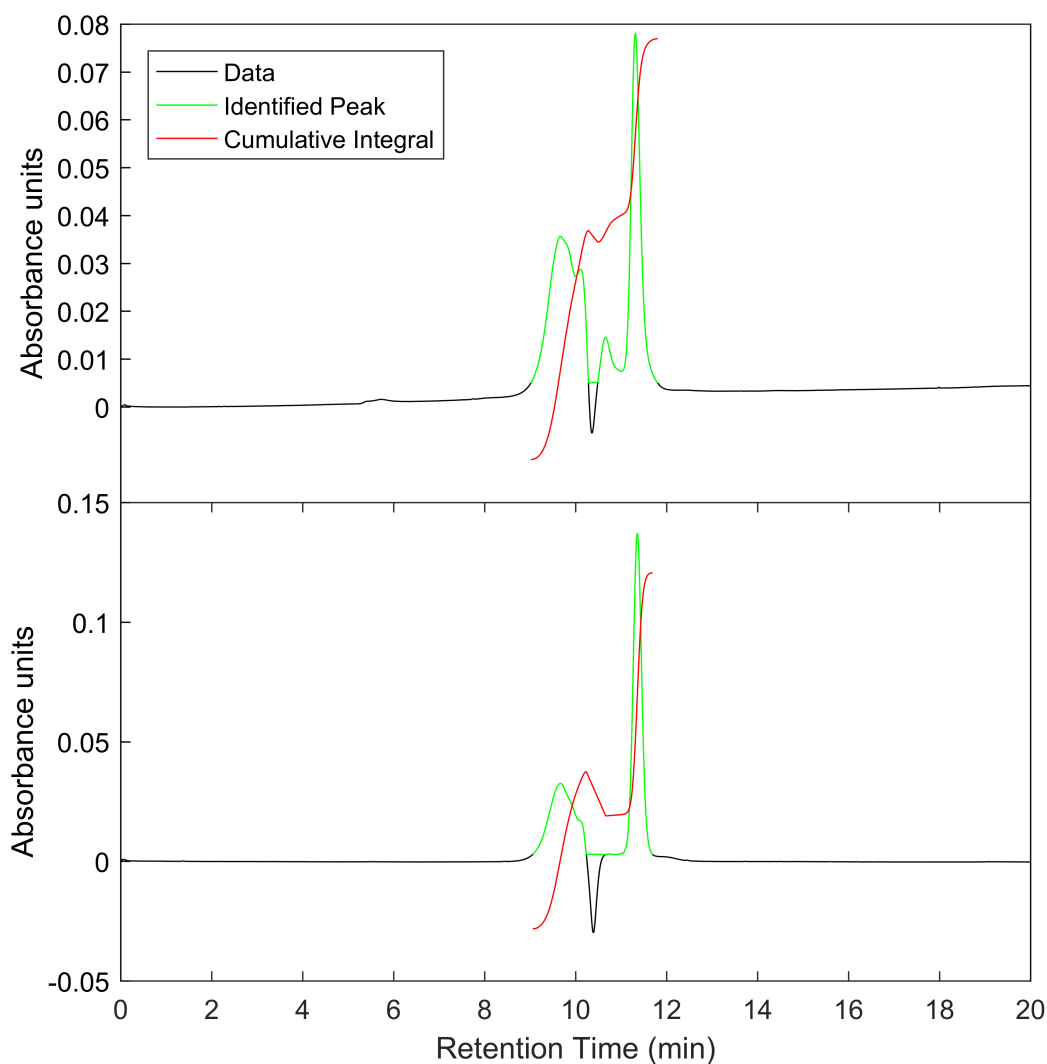


FIGURE 3.2: Detrending of an Absorbance Peak

The green segment in Figure 3.2 indicates the indices that were identified as statically significant according to Eq. 3.1. Figure 3.2 also displays an example of a peak exhibiting negative absorbance values. This was found to occur due to the salting-out effect of the phosphate buffer with the sample matrix, which leads to the appearance of a significant negative peak once the mobile phase reaches the PDA. This was confirmed to be case and not due to impurities as this occurred consistently between multiple runs at a similar retention time (10.4 min) (See Appendix A, Table A.3). The effects were minimized by using wavelengths greater

than 220 nm where the effect was minimized and/or eliminated entirely. Peaks were then integrated over the indices identified as significant, and evaluated according to the following:

$$\int_{W_m}^{W_n} A(W) dW \quad (3.2)$$

Which can also be simplified as:

$$\simeq \frac{W_n - W_m}{2N} \sum_{n=1}^N (f(x_n) + f(x_{n+1}) \dots f(x_N)) \quad (3.3)$$

Where W_n and W_m are the final and initial AMW for the significant peak respectively; $A(W)$ is the value of absorbance as a function of AMW (W); $f(x_n)$ is the function of x_n evaluated at index n , up until the final point x_N ; and N is the total number of points. The axis in Figure 3.2 is time, but when calibrated using 3.1 it becomes W in Equations 3.2 and 3.3. Eq. 3.3 is the approximate form of Eq. 3.2, where $\frac{W_n - W_m}{2N}$ is the distance between consecutive points of W . Each value for $f(x_n)$ evaluates to be negative due to the nature of the SEC separation. Larger compounds elute earlier, therefore with each dW the $\log(\text{AMW})$ (determined from calibration in Figure 3.1) decreases, and therefore the integral evaluates as a sum of negatives. This was accounted for when determining the value for the detrended integral shown alongside the peaks in Figure 3.2. Any occurrence for negative peaks due to the salting-out effect were accounted for when evaluating the integral, as shown by the downward dip in the plot of the detrended integral.

The peaks were sequentially integrated over each range obtained by the photodiode array (PDA), that is greater is 220nm (220-400nm). Once each value was stored, the integrand was calculated over different wavelength ranges according to set defined wavelengths of interest obtained in literature. The wavelength ranges chosen were 220-240 nm (R2040), 240-272 nm (R4072), and 272-365 nm (R7265). The expression defining the evaluated integrand over surface space is shown in Eq. 3.4

$$\int_{\lambda_j}^{\lambda_i} \int_{W_m}^{W_n} A(W, \lambda) dW d\lambda \quad (3.4)$$

Where λ_j and λ_i are the final and initial bounds for λ respectively. $d\lambda$ was automatically determined from the vector storing values for λ , which for the PDA was set at a resolution of 2.4 nm. Evaluating the integrand over certain ranges will provide additional information on the nature of the chromophores present in the sample. A graphical display of the nature of the partitioning of the SEC profile is shown in Figure 3.3(a).

In addition to the wavelength ranges obtained from literature, a sequentially scanning algorithm was used to evaluate the integrand over different wavelength ranges of the SEC profile. This was done by setting the initial indices λ_i and λ_j within a narrow range at a low wavelength (e.g. $\lambda_i = 220$ nm, $\lambda_j = 221$ nm), evaluating the integrand, then incrementing λ_j . Once the surface is scanned in its entirety, the index for λ_i is incremented by 1 and the process continues until exhausted. This produces an array of evaluated integrands that represents each slice of the chromatogram (See Eq. 3.5), as well as every iteration of wavelength ranges between 220 nm and 400 nm.

$$A(\lambda, W) = \begin{bmatrix} a_{i,j} & a_{i,j+1} & \cdots & a_{i, \text{len}(\lambda)} \\ 0 & a_{i+1,j+2} & \cdots & a_{i+1, \text{len}(\lambda)} \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \cdots & a_{\text{len}(\lambda)-1, \text{len}(\lambda)} \end{bmatrix} \quad (3.5)$$

Where $\text{len}(\lambda)$ is the length of the vector storing values for λ ; and $a_{i,j}$ is the absorbance retrieved from wavelength index i to index j . A unique array was produced for every sample, and stored in a 3D-array for further statistical analysis.

Additionally, the peaks were further partitioned based on ranges in apparent molecular weight.

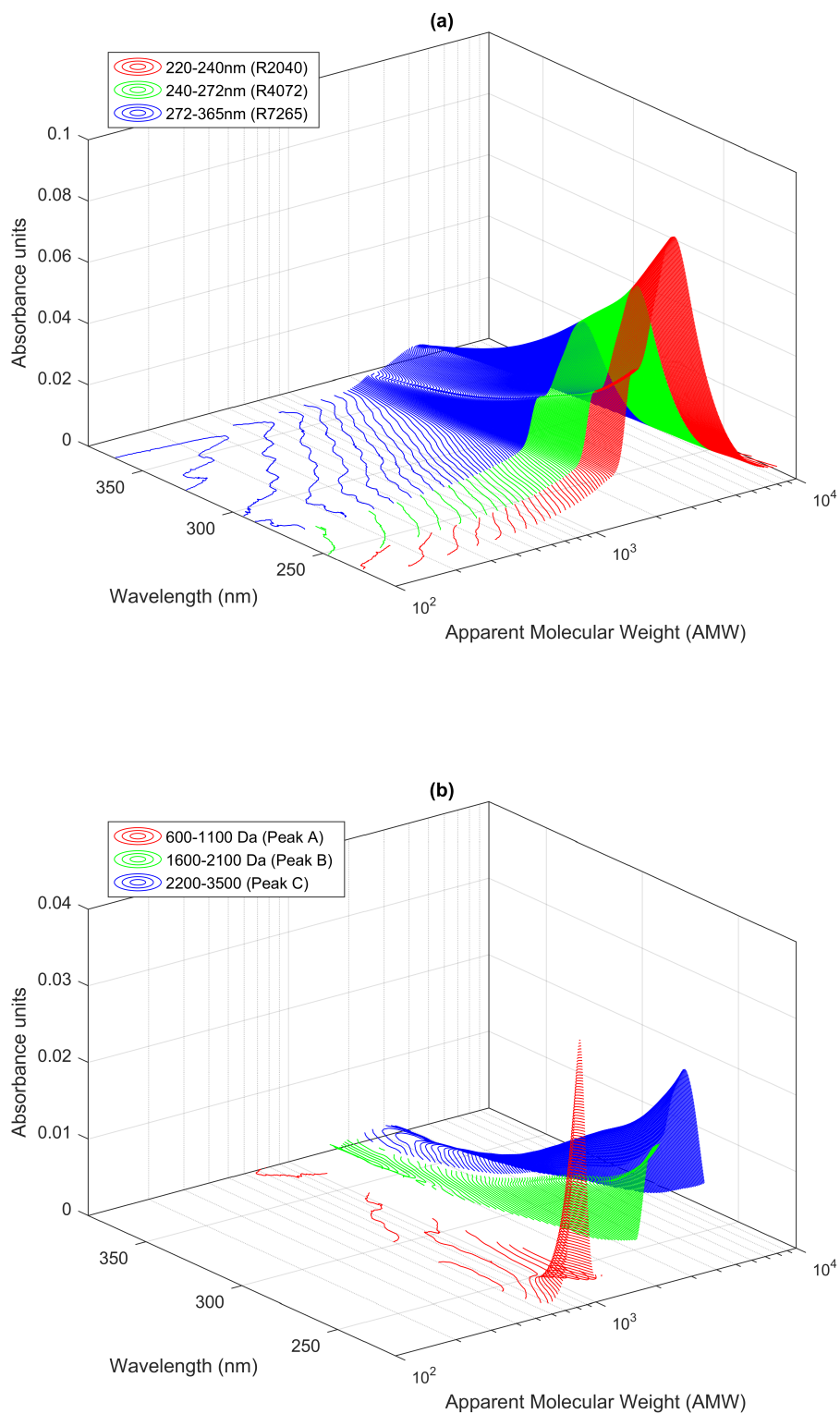


FIGURE 3.3: The subdivision of a 3D chromatogram by (a) wavelength range (R2040/R24072/R7265) for SRNOM, and (b) apparent molecular weight (Peak A/B/C) for SRAW.

As previously discussed in Section 2.3.2 and summarized in 2.1, researchers have identified and characterized natural organic matter constituents using a combination of HPSEC and FFF approaches. Samples from this work were plotted together in order to identify significant peaks of interest within the range of chromophoric components. These peaks are shown in Figure 3.4.

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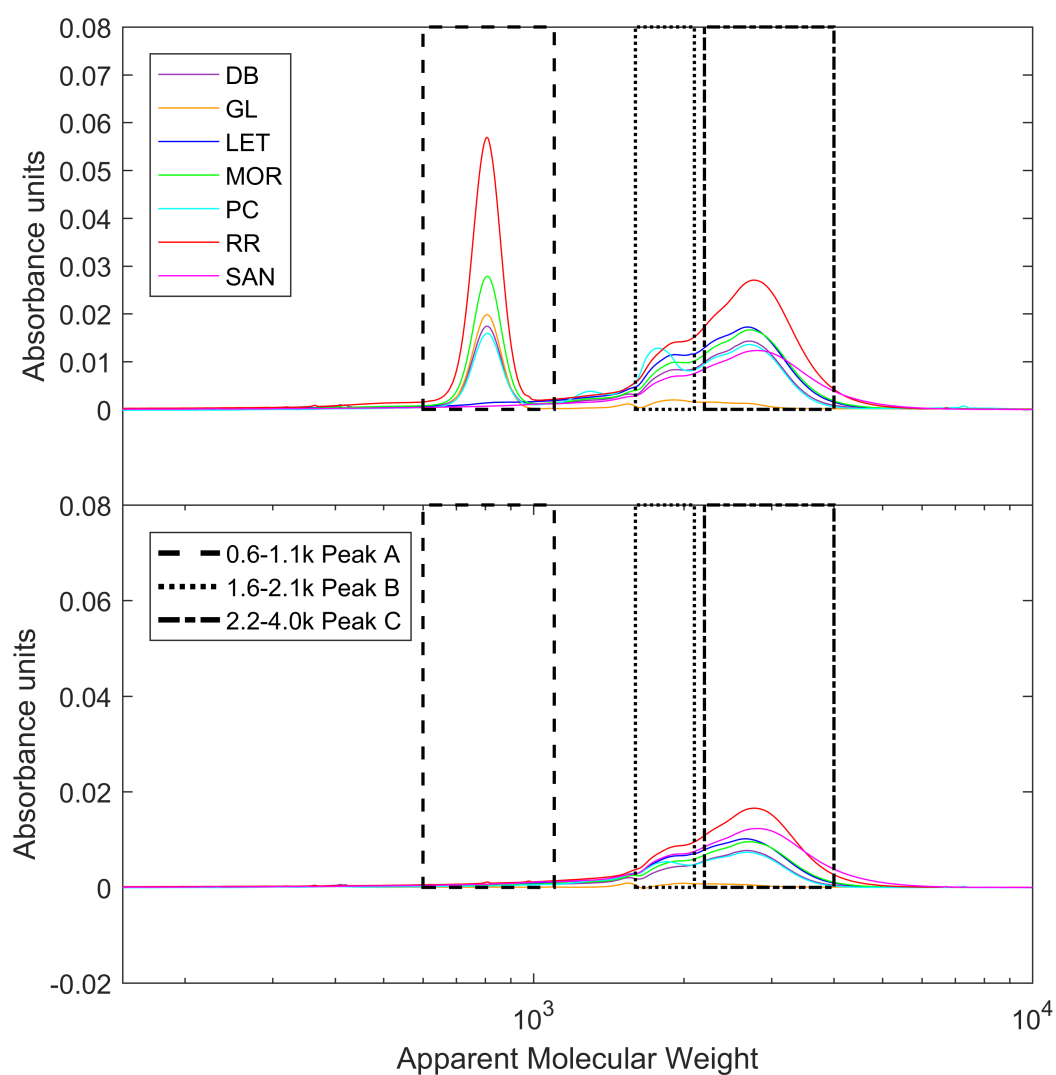


FIGURE 3.4: Identified AMW peaks as significant contributors to chromophoric organic matter, with peaks of interest identified at (a) 254 nm and (b) 280 nm.

Figure 3.4 identified three regions of interest, Peak A (0.6-1.1k Da), Peak B (1.6-2.1k Da), and Peak C (2.2-4.0k Da). Peak A can be identified as LMW fulvic components, while Peak B and C correspond to the wide band encompassing humic substances and humic substance-like components outlined in Figure 2.1. Peak A components have a high absorbance that falls off quickly with increasing wavelength, while Peak B and C persist at longer wavelengths. It is not clear whether or not Peak B is a unique indicator in the spectral profile, or if it is a feature of the wider peak describing humic substances; however, one sample identified as Pine Creek (PC) did show a unique profile in this range, and all samples did indicate a shoulder peak is present. A graphical representation of the peaks selected in the 3D chromatograms are shown in Figure 3.3b

3.2 Laboratory Analysis

Total trihalomethanes (TTHM) were determined according to Standard Methods 5710B [262]. TTHM analysis was performed as the measurement of THM4 which comprises of the species, chloroform (CHCl_3), bromoform (CHBr_3), chlorodibromomethane (CHClBr_2), and bromodichloromethane (CHCl_2Br). This definition is consistent with those outlined in the Canadian Guidelines for Drinking Water Quality [39] and World Health Organization (WHO) [238]. Gas chromatography (GC) for THM analysis was carried out using an Agilent 7820A system (Agilent, CA) equipped with electron capture detection (ECD) and a CombiPal CTC Analytics auto-sampler (CTC Analytics, Zwingen). Separation was performed with an Agilent DB-5 column, with dimensions: 30 m x 0.32 mm x 0.24 μm . Detection limits (DL) for THM4 analysis were determined according to Eq. 3.1 with a s_{blk} of 3.3 - equating to a 95% confidence interval - to be 10 μg . TOC analysis was performed using a FormacsHT Series High-Temperature TOC Analyzer (Skalar Inc., Georgia) coupled with FormacsHT/TN Total Carbon (TC)/Total inorganic carbon (TIC) detection (ND25) - DL was determined to be 0.4 mg L^{-1} . pH measurements were performed using a Fisher Scientific Accumet 50 pH meter (Fisher Scientific, NJ). UVA_{254} was carried out using an Ultrospec 2100 UV/Vis spectrophotometer (Biochrom, Cambridge) with a quartz cuvette. Specific Ultraviolet Absorbance at 254nm (SUVA_{254}) was determined according to USEPA Method 415.3 [261] and the propagation of error calculated according to the following:

$$\Delta\text{SUVA}_{254} = \left| \frac{A_{254}}{\text{DOC}} \right| \sqrt{\left(\frac{\Delta A_{254}}{A_{254}} \right)^2 + \left(\frac{\Delta \text{DOC}}{\text{DOC}} \right)^2} \quad (3.6)$$

Where ΔA_{254} and ΔTOC are the uncertainty of the associated variables A_{254} and DOC variables, respectively.

3.3 Statistical and Correlation Parameters

3.3.1 Molecular Weight Distribution

Molecular weight distributions and polydispersity indices were used to provide information on the nature of the distribution of NOM moieties determined from HPSEC peak responses [353]. Weight averaged molecule weight M_w , number averaged molecule weight M_n , and polydispersity ρ were determined from Eq. 3.7 - 3.9 adopted from Striegel et al. [301], and used by others [38, 54, 58, 137, 307, 313, 328, 336, 353]:

$$M_w = \frac{\sum_i h_i}{\sum_i \frac{h_i}{M_i}} \quad (3.7)$$

$$M_n = \frac{\sum_i h_i M_i}{\sum_i h_i} \quad (3.8)$$

$$\rho = \frac{M_w}{M_n} \quad (3.9)$$

Where h_i corresponds to the baseline height in the chromatogram at retention time i ; and M_i corresponds to the equivalent calculated molecular weight of a compound at retention time i . h_i is a function of the M_i of a compound and its frequency of occurrence as described by Zhou, Cabaniss, and Maurice [353]. Values for ρ indicate whether or not a heterogeneous sample such as NOM has a particularly high or low MW dispersivity - where a value of 1 indicates a uniform sample, while a number that deviates from 1 indicates dispersivity [180]. As discussed in Section 2.3.2, select treatment methods have shown to impact the calculated M_n and M_w from the SEC profile. IX has been shown to remove an evenly weighted distribution of absorbing moieties in source waters, therefore this information was recorded in this work to confirm these findings. These values were also recorded to provide additional

information on potential relationships between the weighted average of the sample and other water parameters.

3.3.2 Correlation Coefficient

In order to test the correlation between HPSEC peak characteristics and other water quality parameters, a series of statistical tools were utilized. The Pearson correlation coefficient was one of such tools used to provide a relation between an X and a Y variable given they are either on an interval or a ratio scale. Eq. 3.10 provides the mathematical formulation for the Pearson correlation coefficient.

$$r = \frac{\sum_{i=1}^N (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_{i=1}^N (x_i - \bar{x})^2 \sum_{i=1}^N (y_i - \bar{y})^2}} \quad (3.10)$$

Where \bar{x} and \bar{y} are the mean values for variable x and y respectively; x_i and y_i are the values for variables x and y at sample number i , respectively; and N is the total number of samples. Pearson coefficient values can range from 1 to -1, where 1 is a perfect positive correlation, -1 is a perfect negative correlation, and 0 is no linear correlation. Two necessary conditions exist for the Pearson correlation to gauge the relationship between variables properly, that is: (i) the relationship between x and y must be linear, and (ii) one of the variables must follow a normal distribution. Linearity of the data was determined according to adjusted R^2 listed as Eq. 3.11.

$$R_{adj}^2 = 1 - \left[\frac{(1 - R^2)(n - 1)}{n - k - 1} \right] \quad (3.11)$$

Where R^2 is the coefficient of determination; n is the number of sample points; and k is the number of variables used in the model. Adjusted R^2 penalizes for independent variables which do not contribute to the dependent variable, and prevents cases where the addition of

more variables contribute to a higher R^2 but do not necessarily fit the data well. Similar to coefficient of determination, values ranging from 0 to 1.0 indicate the percentage of variation explained by the independent variables on the dependent variable. The second condition for the Pearson coefficient - a normal distribution - was also accounted for by computing the skewness of the data according to Eq. 3.12.

$$\text{Sk}_2 = \frac{6(\bar{x} - Md)}{s} \quad (3.12)$$

Where Md and s are the median and standard deviation of the sample, respectively. While the Pearson coefficient can confidently predict relationships for linearized data, another measure is required in order to account for data that is either logarithmic or semi-logarithmic - or is not sufficiently normalized to satisfy the conditions for the Pearson coefficient. An additional parameter was calculated known as the Spearman coefficient, and is summarized in Eq. 3.13.

$$\rho = \frac{3\sum d_i^2}{n(n^2 - 1)} \quad (3.13)$$

Where n is the number of samples points; and d_i^2 is the difference between ranked variables in the data set, squared. The Spearman coefficient takes into account the effect of one monotonic variable which either increases or decreases as another variables increases. This is important as many interactions in the environment are not linearly correlated, and may have any variety of correlations present.

p values were determined in order to test no correlation versus the alternative null hypothesis. The p test for the Pearsons correlation was determined using a student's t distribution, and the Spearmans coefficient was calculated using a permutation distribution. A calculated value of p less than 0.05 is deemed to statistically significant and the null hypothesis can be rejected.

3.3.3 Cluster Analysis

Clustering is a technique in which objects are clustered into groups based on their Euclidean distance, or alternative distance method, from one-another. Euclidean distance is a 3-dimensional distance determination derived from the 2-dimensional Pythagorean theorem. It can be used to track trends in groups of samples, and draw distinctions and make predictions based off of those trends. In order to compare distances of variables that are of different orders of magnitude from one another, sample data was normalized. Computing the Euclidean distance is calculated according to Eq. 3.14.

$$d_{x,y} = \sqrt{\sum_{j=1}^J (x_j - y_j)^2} \quad (3.14)$$

Where x_j and y_j are the x and y variables and j is the vector index; and j and J are the initial and final indices respectively. Values for $d_{x,y}$ are normalized according to Eq. 3.15, and referred to as the squared Euclidean distance.

$$x_j = \frac{x - \mu}{\sigma} \quad (3.15)$$

Where μ and σ are the mean and standard deviation of the vector variables respectively. In doing so, values are all set with the same variance of 1, and large values no longer over-compensate in the calculation of $d_{x,y}$ in Eq. 3.14. Clustering was performed using the *pdist* function in the Statistics and Machine Learning Toolbox developed in the MATLAB 2016a environment. Raw samples were subject to the analysis to find trends within Manitoba waters based on source water used.

4 Results and Discussion

4.1 2-D HPSEC Chromatograms

4.1.1 pH Effects

A selection of resins summarized in Table 3.1 were analyzed to test their associated effects on a sample of SRNOM standard. This will provide a general overview of the effectiveness of various IX resins to target UV absorbing moieties; as well as the conditions - such as initial pH - that would help or hinder the adsorption process. Fig. 4.1 displays the chromatogram following removal by an excess of strong base anion exchange (SBA) resin. Initial observations indicate that the bulk removal of NOM absorbing moieties are targeted by the SBA resins tested, at the two chosen wavelengths of 215 and 254 nm. As noted in Section 2.3 Table 2.3, $UV_{210-215}$ is a function of non-conjugated carboxylic groups, while UV_{254} is characteristic of conjugated aromatic moieties. These results demonstrate something that is well established in research: which is that SBA is effective at removing aromatic moieties present in NOM [121]. This was further evident in the measured UVA_{254} values shown after SBA treatment, listed in Table C.1 in Appendix B. Figure 4.1 also demonstrates two other anomalies, namely, the presence of a peak (502P) that exceeds the absorption of the SRNOM sample; and the negative absorption seen in the chromatogram displayed at 215 nm. The latter was discussed at length in Section 3.1.3 as being due to the salting-out effect, while the former can be traced to the presence of artifacts most likely due to insufficient preliminary

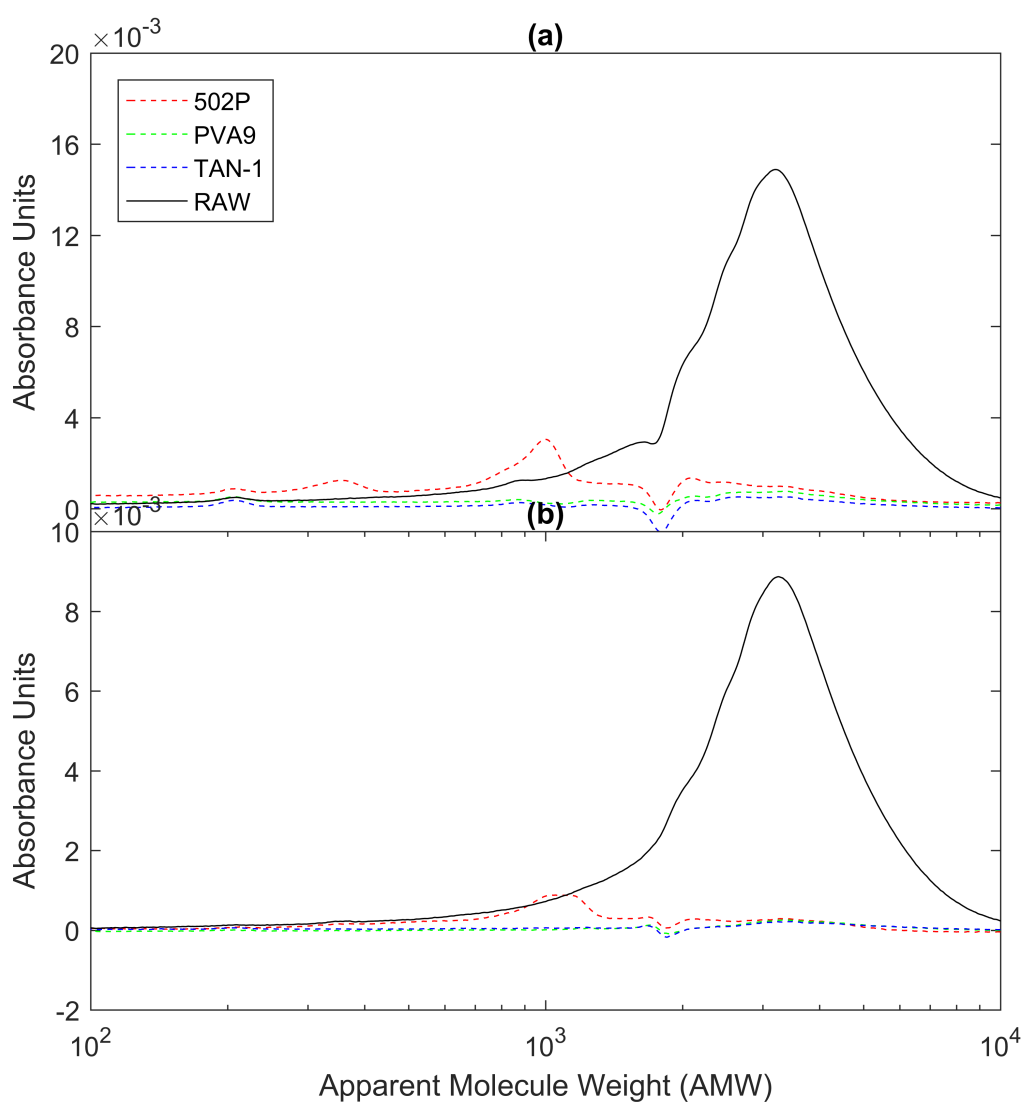


FIGURE 4.1: Removal of SRNOM standard using a series of SBA resins. (a) Removal at 215 nm, and (b) removal at 254 nm. SBA peak signals were smoothed using a Savitzky-Golay lowpass filter with a filter span of 11.

washing of the resin. To eliminate the salting-out effect, all further analysis was carried out at wavelengths exceeding 220 nm.

The effect of initial water pH was also investigated at a pH range typical of environmental surface waters. Figure 4.2 observes the effects of initial pH on the resulting chromatogram following contact with an excess of DOW TAN SBA resin. Between the three pH values tested, the DOW TAN resin was able to adequately remove the entirety of UV absorbing

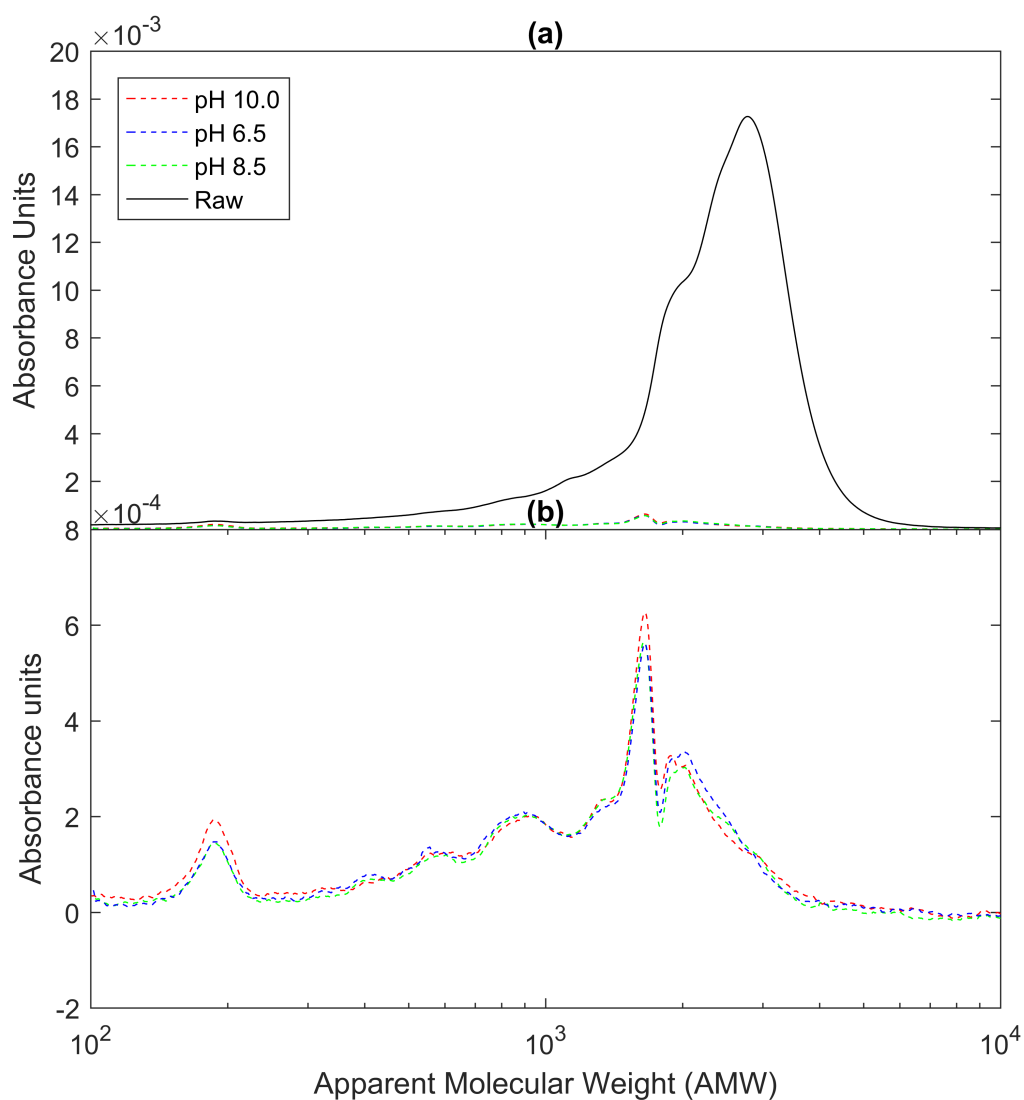


FIGURE 4.2: Removal of SRNOM standard at a variety of initial pH levels, using DOW TAN SBA resin. (a) Including the original SRNOM standard, and (b) excluding the SRNOM standard for better resolution. SBA peak signals were smoothed using a lowpass filter with a filter span of 41.

moieties present in the SRNOM standard. Any discrepancy between pH values is not statistically significant, measured according to a signal-to-noise ratio (S/N) - a simple ratio between the signal produced, divided by the signal of the noise - which was evaluated to be 1-2 for these peaks. These values translate to a % relative standard deviation (RSD), calculated as $50/(S/N)$, of 25-50%; thereby proving to be a poor level of precision and accuracy for repeated injections.

The effects of initial pH aimed to account for the acid dissociation constants of the compounds of NOM present. As outlined in Table 2.1 Section 2.1, the functional group composition is dominated by carboxyl and phenolic groups for FA and HA alike. If a surrogate compound of benzoic acid (C_6H_5COOH) and phenol (C_6H_5OH) were assumed as representative compounds for carboxyl and phenolic functionality - this would translate to pK_a values of 4.2 and 9.9 respectively. At the lower bound of the pH range tested at 6.5, it can be assumed that all phenolic groups are fully protonated, and therefore are unavailable for IX. This assumption is based on the modified Henderson-Hasselbalch equation, $pH - pK_a = \log \left(\frac{[A^-]}{[HA]} \right)$ where at pH values of 6.5, the ratio of the conjugate base to its acid form exceeds 1:1000. At pH values this low, for all intents and purposes, the conjugated base does not exist and is unavailable in its charged form. The removal of SRNOM is still pronounced at low pH levels of 6.5, indicating either carboxyl groups dominate IX to the resin site, or, other non-IX mechanisms are at play. Seeing as how NOM compounds are HMW, multi-functionalized species, it may be the case that only a few sites need to be readily available on the NOM surface in order to diffuse onto the resin surface. This trend was seen for all IX resins tested, indicating that the strong base nature of their NH_4^+ groups are adequate for exchanging with the acidic groups of NOM at pH levels as low as 6.5 (i.e. relevant for most applications). Research related to the availability of carboxylic acidic groups as a function of pH indicates this may be the case [37, 169, 267, 294]. Potentiometric titrations and/or indirect acidity measurements (via baryta adsorption or Ca-acetate methods) could be used to confirm these speculations [294].

4.1.2 Basic Water Quality Parameters and Associations

Water quality analysis was carried out for the aforementioned 7 raw water sources in Manitoba (listed in Section 3.1.2), followed by analysis of the waters treated with 2 doses of SBA of DOW TAN, at 3 mgL^{-1} and 5 mgL^{-1} . A summary of these parameters are on display in Figure 4.3 and compounded in Appendix B (Table C.1 and B.3). Initial observations of the

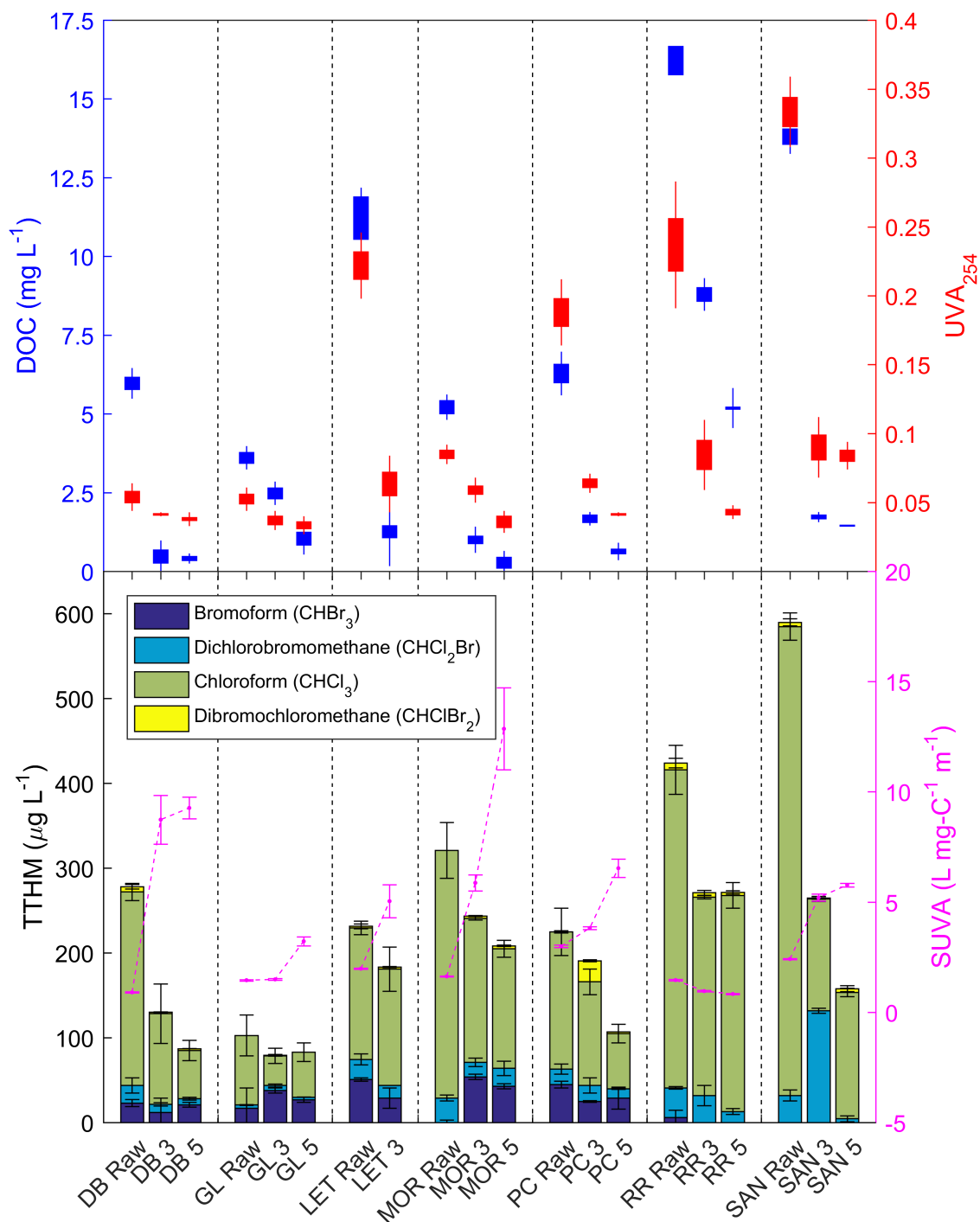


FIGURE 4.3: Water quality trends for 7 source waters treated with 3 mgL^{-1} and 5 mgL^{-1} doses of DOW TAN resin. Candlestick charts indicate the highest and lowest recorded value in the upper and lower shadow respectively - and the top 75th and bottom 25th percentile in the real body. Raw values are summarized in Appendix B Table C.1 - B.3.

graph indicate the steady removal of DOC and UVA₂₅₄ with increasing SBA dose. This is to be expected as more sites - in terms of equivalents per resin weight - are available for IX and other sorption mechanisms. A similar trend is seen for TTHM4, where an increase in resin dose corresponds to a removal in THM precursors. The nature of the precursors removed can be traced to the two surrogate measurements - UVA₂₅₄ and SUVA₂₅₄. As discussed at length in Section 2.3 and 2.2.1, UVA₂₅₄ is used to indicate aromaticity, while SUVA₂₅₄ is used to provide a gauge of electron rich moieties. In all but one source water treated (RR), SUVA₂₅₄ increased following increased resin dose. This indicates the preferential removal of non-conjugated components of NOM - which tend to be more hydrophilic - over conjugated components which tend to be more hydrophobic. Hydrophobic NOM tend to have higher SUVA₂₅₄ values, therefore it follows that on a per mg-C basis a higher SUVA₂₅₄ is indicative of a lower proportion of hydrophilic NOM [86]. This finding is consistent with the work carried out by others who identify the hydrophilic components of NOM are more readily removed by IX than their hydrophobic counterparts [225, 248]. The RR source water was the only water that did not follow this trend, which may be due to several reasons. RR was recorded as having the highest initial inorganic carbon (IC) concentration at $76.6 \pm 0.8 \text{ mgL}^{-1}$ (See Appendix B, Table C.1), which may have complicated the IX process. IC species at a pH range of 6-8 typical compose of carbonate (CO_3^{2-}) and bicarbonate (HCO_3^-) forms, which compete for SBA sites available on the resin site. This is further supported by the fact that dosing the RR sample at 5 mgL^{-1} did not reduce TTHM to any significant degree from the 3 mgL^{-1} dose (271 ± 17 vs. $272 \pm 21 \text{ }\mu\text{g mL}^{-1}$). This is also indicated by the relatively high DOC value following the 5 mgL^{-1} dose - at $5.2 \pm 0.6 \text{ mgL}^{-1}$ - than any other sample water tested. It follows that IC may have impacted the IX process, given the several indicators provided by the other parameters tested.

Figure 4.4 provides a better description of the associated removal of basic water quality parameters. A paired plot better describes the trends in removal between parameters, as well as to help visualize and distinguish trends between Raw and IX treated waters. The plot

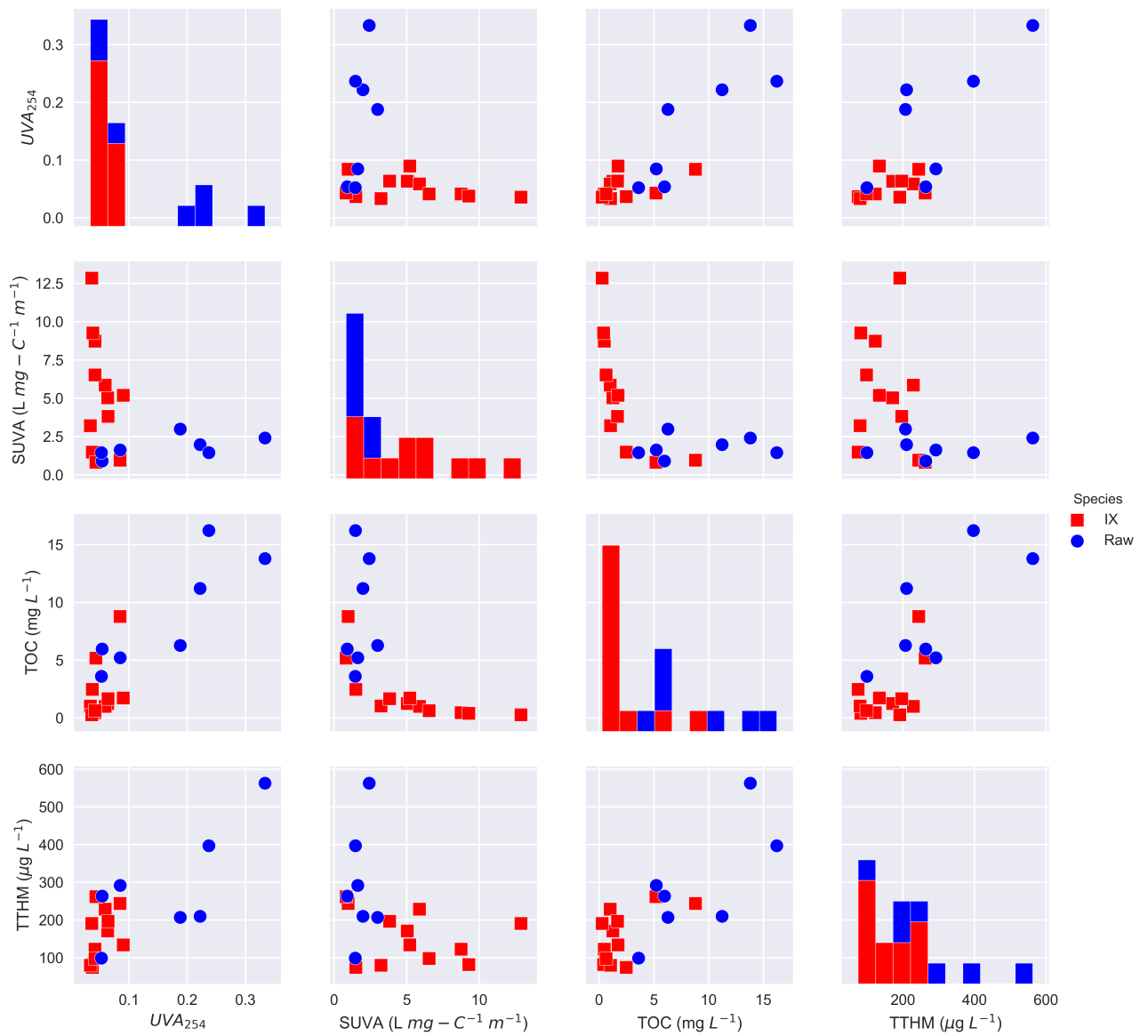


FIGURE 4.4: Paired plot for the removal of basic water quality parameters, as well as their value distributions for both Raw and IX treated waters

displays the trend between each parameter (x-axis) and a corresponding parameter (y-axis); with the histogram plots in the diagonal showing the distribution (x-axis) and frequency (y-axis) of each sample value for each parameter. As a general rule, IX results in the decrease in a parameter; seen in trends for UVA_{254} , TOC, and TTHM, as well as every intersection between these parameters. Conventially, parameters of TOC and UVA_{254} are used as surrogate measurements for TTHM; therefore we see this to be the case in the linearized trends between these parameters [316]. The clear outlier in this trend is seen for the L-shaped plots for SUVA with all other parameters, i.e. treatment with IX increases values for SUVA. We can also see this is the case in the SUVA histogram plots which display IX treated values concentrated on the low-end of the distribution. This just further demonstrates the ability for IX to target hydrophilic groups, thereby leading to increased values for SUVA.

4.1.3 Molecular Weight Determination

The effect of SBA treatment on the molecular weight distribution of the source water was analysed. A script was developed to compute the weight-averaged and number-averaged molecular weights of the samples according to Equations 3.7-3.9. A summary of the results are on display in Table 4.1.

Findings display a general trend of increasing M_n and M_m with increasing resin dose. This signifies the ability for IX to target the LMW range of the AMW distribution, thereby pushing the averaged AMW to a higher value; consistent with the findings of Kaewsuk and Seo [158] who noted a selective removal of LMW aromatics and humics. It is also exemplified in the literature that the removal of LMW components, which mainly constitute hydrophilic components, are preferable targeted by IX and will lead to an increase in AMW as demonstrated for the bulk of the source waters tested [250, 251, 318, 336]. Additionally, the findings shows a shift in ρ for 5 out of 7 of the source waters tested; with MOR and RR as the notable exceptions at the 5 mgL⁻¹ dose. This exception was taken to be due to the mislabeling of

TABLE 4.1: Weight-averaged, number-averaged and polydispersity index for 7 water sources contacted with 3 and 5 mgL⁻¹ doses of DOW TAN resin. Values were taken from the median of values retrieved between 220-300 nm.

	M _w	M _n	ρ
DB RAW	3726	3735	0.9976
DB 3	4634	4706	0.9963
DB 5	5521	5778	0.9565
GL RAW	3985	4347	0.9676
GL 3	4523	4696	0.9644
GL 5	4736	5078	0.9335
LET RAW	3763	3775	0.9969
LET 3	4817	5016	0.9622
MOR RAW	3785	3799	0.9962
MOR 3	4648	4692	0.9898
MOR 5	4680	4784	1.0017
PC RAW	3760	4097	0.9569
PC 3	4096	4131	0.9915
PC 5	4109	4553	0.9621
RR RAW	5400	5591	0.9632
RR 5	3706	3721	0.9964
RR 3	4663	4977	0.9419
SAN RAW	3837	3857	0.9947
SAN 3	4014	4064	0.9876

the significant peak in the AMW profile, as the trend was not seen in the 3 mgL^{-1} dose. The values for polydispersivity on the most part increased with respect to resin dose, leading one to conclude that the value for M_n increased as the parameter is heavily weighted by the LMW end of the distribution. However, the effectiveness of these parameters amongst researchers and over time is very much ambiguous. Chin and Gschwend [55] noted the consistency of these parameters with other research; however, these conclusions are not without flaw. It was noted in the work of Xing et al. [336] that the use of AMW determinations is problematic due to irregularities in the distribution, more specifically, the change in response as a function of wavelength used. The work of Xing et al. [336] determined AMW from the distribution at 254 nm, which is not necessarily representative of the sample as a whole. An illustration of this problem is shown in Figure 4.5.

As shown in Figure 4.5, as a function of wavelength M_n varies ~ 80 Da over the wavelength range. Even within the sample range with an appreciable absorbance peak present ($\lambda = 220\text{-}300$), M_n varies ~ 60 Da (or 1.6%). Therefore it is important to be aware of the sensitivity of this method to bias certain values depending on the wavelength used. Table 4.1 was compiled with this in mind, and was calculated as the median of the values obtained between wavelength values of 220 and 300 nm. The tendency for the wavelength to bias the dataset is shown in Appendix A Figure A.4. Figure A.4 helps to display the trends in positive/negative bias attributed to values of M_n , M_m and ρ . As a general rule, M_n and M_m values are positive bias - as much as 17% for some samples - while ρ is less impacted at a negative bias of $\sim 1\%$. It is important to note that large slopes attributed as bias may be due to outliers in the upper or lower range of the wavelength profile, which therefore shifts the regression towards negative or positive extremes. Proper adjustments can be made in order to account for this discrepancy and shift the deviation more in line with the bulk of the samples measured, which was recorded to be 0.7-1.4%, 0.2-2% and -0.1-1% for M_n , M_m and ρ respectively.

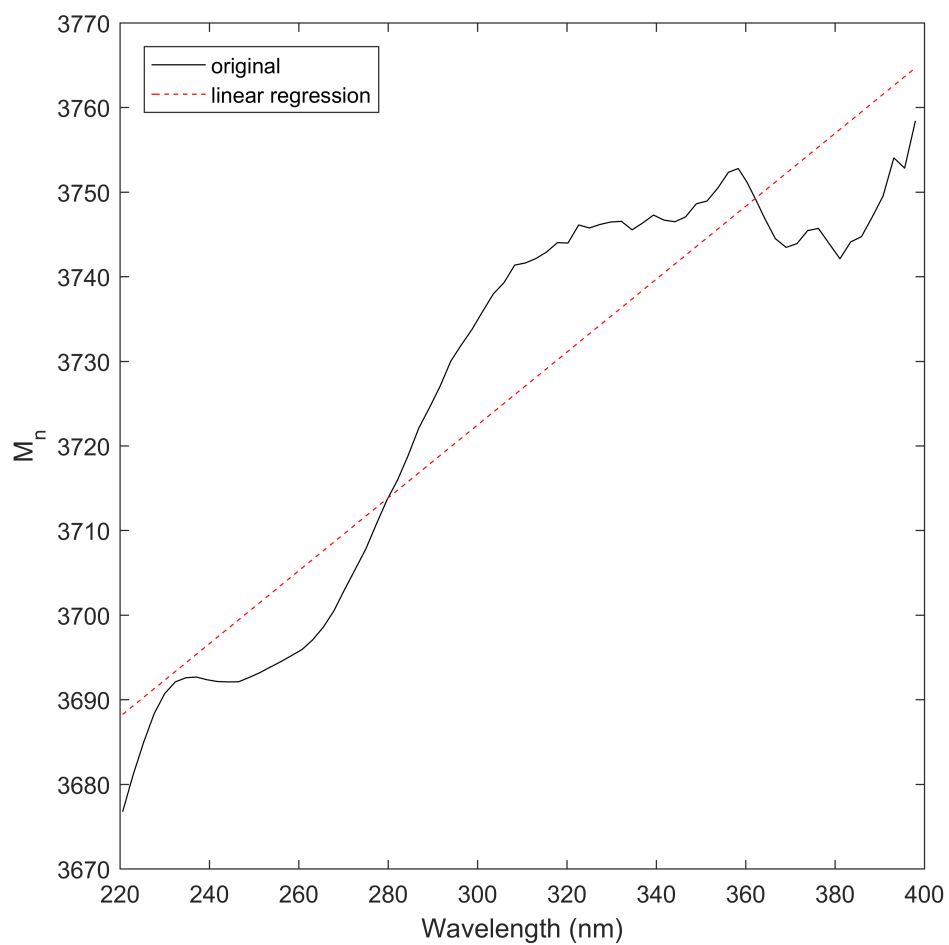


FIGURE 4.5: Number-averaged molecular weight of a sample of DB Raw as a function of wavelength. Sample plot indicates the calculated number-averaged molecular weight, as well as the linear regression of the dataset.

4.2 3-D HPSEC Chromatograms

4.2.1 Contour Plots

HPSEC chromatograms are used to better appreciate the multi-wavelength, AMW distribution of the samples measured. HPSEC profiles also provide researchers with a tool in NOM profiling, that is simply unattainable using simple parameters such as UVA_{254} , DOC and $SUVA_{254}$. Figure 4.6 displays the resulting chromatographs following SBA treatment. The figure helps to illustrate the species components specifically targeted by two different doses of SBA resin.

Common features can be seen between samples from DB, MOR, and LT which all show large removals of moieties between the AMW range of 2400-3100. The removal of these components, as well as the removal of the LMW moieties, would explain the shift in M_n and M_w towards higher values indicated in Table 4.1. These large bulk peaks would be identified as those labeled as Peak C representing AMW in the range of 2.2-4k, noted in Figure 3.4. Additionally, components attributed as Peak C would compromise the bulk of NOM - including FA and HA - as they mirror the SRNOM standard found within the same range (Figure 3.4). All samples with the exception of GL saw a reduction in a small narrow peak identified as Peak A (0.6-1.1k Da) in Figure 3.4, which may be associated with LMW acids or building blocks (See Figure 2.1). This association was also made by Liu et al. [210] who investigated a similar removal trend in the Peaks A's AMW range following TiO_2 photocatalytic treatment. GL is seen as a noticeable exception as the water source was found to have a peak C that was far lower than the other source waters (Figure 3.4); therefore the effective removal of the peak would not be apparent to begin with. The water quality for GL summarized in Figure 4.3 lists GL as having the lowest starting TOC, UVA_{254} , and TTHM4 amongst the source waters; which can lead one to propose there may be a connection between the size of Peak C and these parameters. This proposition will be tested and confirmed in the following section. Other peaks of interest include the pronounced removal of Peak B

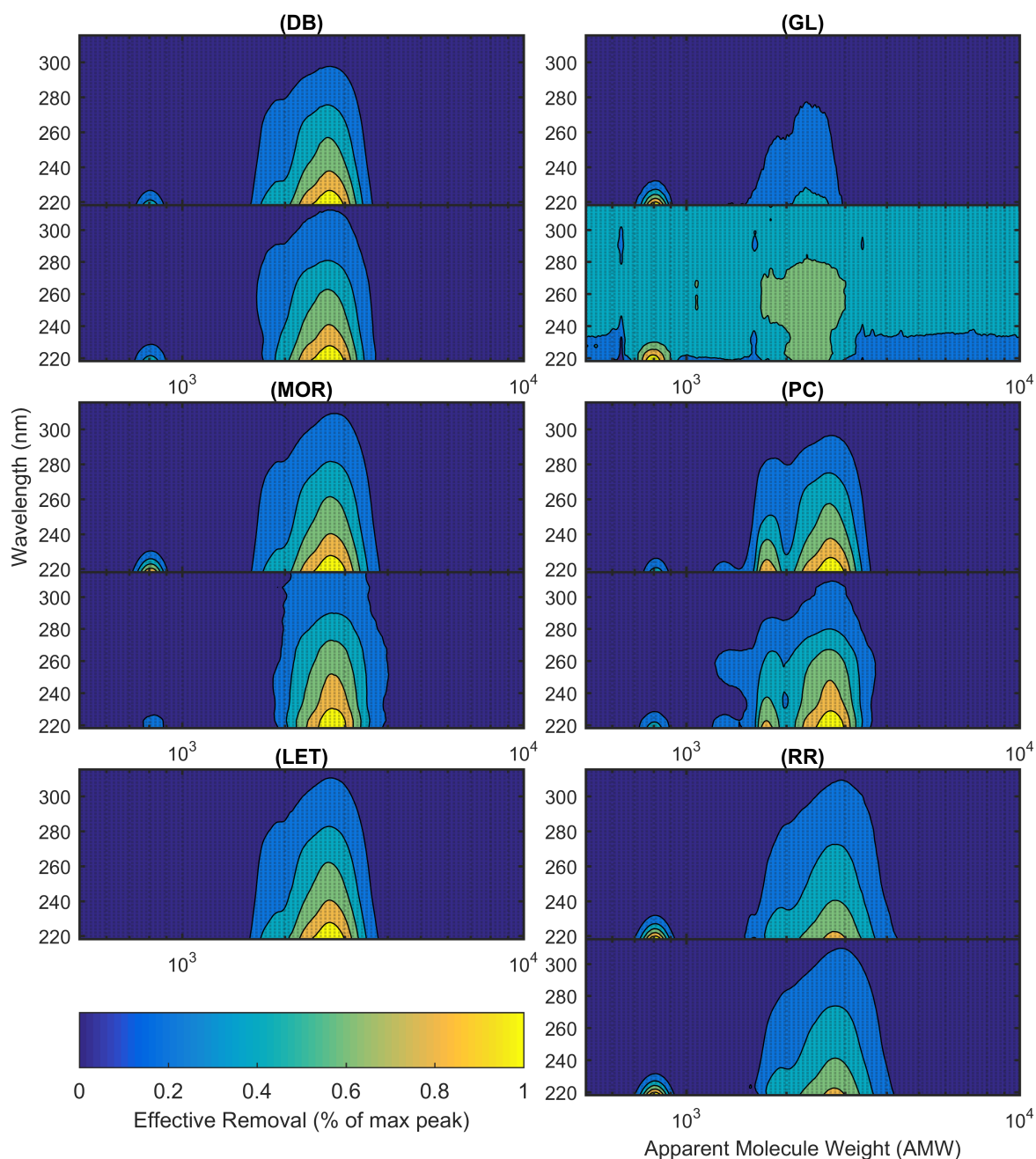


FIGURE 4.6: Effective removal for water sources treated with DOW TAN doses of 3 mgL^{-1} (top subplot) and 5 mgL^{-1} (bot subplot). Effective removal was determined by the subtraction of raw peak absorbance by the associated treated water absorbance. Colorbar is based on % of max peak for simplification.

(1.6k-2.1k Da), found in the resulting chromatogram for the PC treated sample. The impact of this peak is unknown upon initial observations, and further statistical analysis will confirm or deny the impact of this peak. SAN water source was excluded from the contour analysis as it exhibited excessive peak oscillations that could not be effectively detrended.

4.2.2 Apparent Molecular Weight and Wavelength Intervals

Basic Water Quality Trends

Correlations between variables can be found given an adequate dataset is available and a trend exists between the variables selected. The Pearson and Spearman correlation coefficients were described in Section 3.3.2 and were applied to the dataset as a whole, as well as a separate dataset excluding raw samples. Excluding raw samples allows us to determine whether or not the associations are treatment specific, as improvements with the addition of the raw samples will only strengthen the case that the associations are universal. Figure 4.7 and 4.8 provides a summary of Spearman and Pearson coefficients respectively for the profiles defined in Section 3.1.3 and displayed in Figure 3.3. As a rule of thumb, coefficients exceeding ± 0.5 and approaching a perfect correlation of ± 1 are considered to have a large strength of association. Correlations with coefficients between $\pm 0.1 - \pm 0.3$ and $\pm 0.3 - \pm 0.5$ are considered to have small and medium strengths of association, respectively. Labelled values for *Abs* are synonymous with the measurement UVA₂₅₄.

Among the AMW and wavelength peaks profiled, the strongest Pearson correlation for the basic water quality parameters was found to exist between the prevalence of Peak B and TTHM formation with a coefficient of 0.76 ($p = 0.00$). A strong correlation was also found to exist between Peak B and TOC (0.55, $p = 0.01$), more so than Peak C and TOC (0.12, $p = 0.60$), leading one to conclude that components within the range of Peak B (1.6-2.1k Da) have a higher organic carbon character than components of Peak C (2.2-4k Da). This may be the case as Peak B was the only range that exhibited a medium/strong correlation

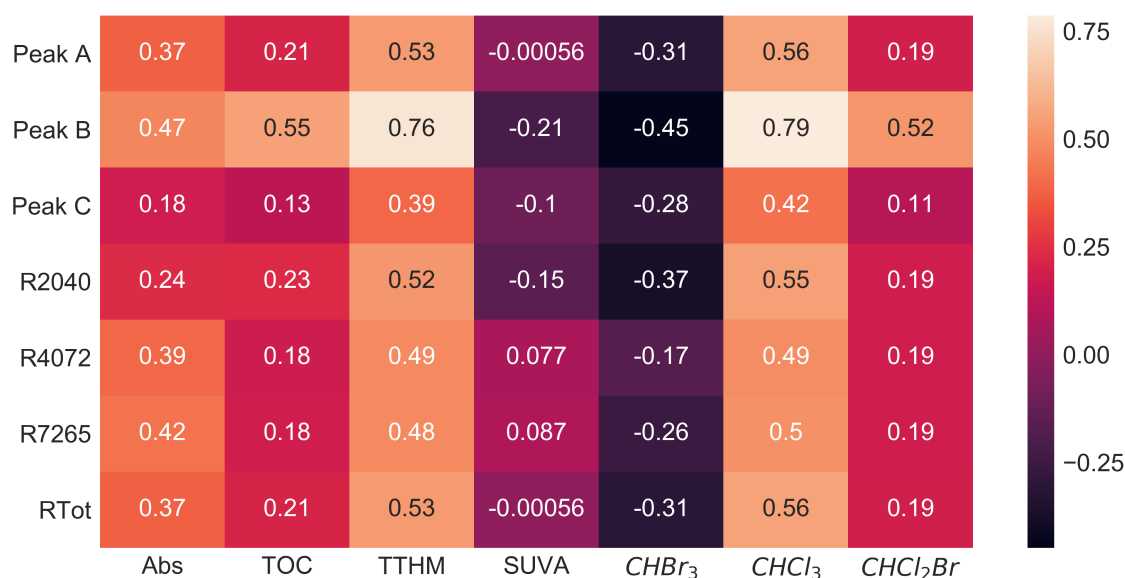


FIGURE 4.7: Pearson correlation parameters for a series of integrated peak trends with common water quality and THM species measurements ($n = 19$).

with TOC. In the previous section, Peak C was found to be heavily targeted by IX treatment; however, it does not describe what the nature of that removal is on the bulk of NOM. It may be the case that Peak B may have properties that are more consistent with those attributed to FA, while Peak C - with a higher molar absorptivity coefficient and higher M_n and M_w [54] - would be consistent with properties attributed to HA. Medium associations were found between TTHM and Peak A (0.53, $p = 0.01$), R2040 (0.52, $p = 0.02$), and RTot (0.53, $p = 0.01$); with the latter two cases showing better association due to the inclusion of Peak A which is only present at low wavelengths (See Figure 4.6). All other correlations either had low association - as is the case with TOC - or very poor correlation, such as with SUVA and CHBr₃. Since the calculation of SUVA₂₅₄ includes the measurement of UVA₂₅₄, one would not expect a correlation to exist as a function of wavelength. The strongest negative correlation for SUVA₂₅₄ was found with Peak B (-0.2, $p = 0.38$), which consequently follows from the strength of association with Peak B and TOC (0.55, $p = 0.01$). Since SUVA₂₅₄ calculation includes the inverse of TOC, it is to be expected that SUVA₂₅₄ would have a

corresponding association in the negative direction (i.e. the inverse of a positive correlation is a negative correlation). The Spearman correlation shown in Figure 4.8, for the most part, did not improve the association between parameters. Most correlations with TOC values improved to a small degree, while some associations worsened, such as with Peak B and TTHM. Improvements were made for ranges that were not Peak B, such is the case for CHCl_3 , CHCl_2Br , TTHM, TOC and Abs.

Since the Spearman coefficient accounts for non-linear associations, the improved values for TOC could be indicative of a potential monotonic relation between absorbing moieties and carbon content. The one exception would be the strong association mentioned earlier between Peak B and TOC (0.55, $p = 0.01$), which worsened to 0.45 ($p = 0.18$) when evaluated using the Spearman coefficient. Since Peak B was the only one effected, it strengthens the case that Peak B and TOC are highly correlated with one another linearly. Additionally, adjusted correlation coefficients ($adj.R^2$) were determined for every iteration of interval and water parameter; and are listed in Table B.1 in Appendix B. For TTHM, the only interval which exhibited a somewhat strong $adj.R^2$ was Peak B at 0.58. It is important to note that $adj.R^2$ is a more stringent coefficient than the predicted correlation coefficient ($pred.R^2$), because it penalizes additional measurements which do not conform to the model. Therefore, an $adj.R^2$ of 0.58 is considered a strong relationship between Peak B and TTHM on all fronts. A strong $adj.R^2$ was also found for Peak B and TOC (0.3), and a weaker relation was determined between TTHM and all 4 wavelength intervals: 0.27, 0.23, 0.23, and 0.28 for R2040, R4072, R7265, and RTot respectively. These values echo the values of the Spearman coefficient, as they are both tests for linearity in sample sets. The Spearman coefficient, however, is also predicated on the normalized distribution of the sample set. Table B.1 in Appendix B lists the Pearson correlation of skewness for each AMW and Wavelength interval. Many sample sets have poor distributions (i.e. 0 is perfectly normalized) as they were retrieved from many sources, and the sample set was restricted to 19. This skewness can also be seen between basic water quality parameters in Figure 4.4, where the distribution

of values is not normally distributed.

Trihalomethanes

Similar to the previous section, Pearson and Spearman correlation parameters were recorded for the THM4 species, with the exception of Dibromochloromethane (CHClBr_2). Dibromochloromethane was recorded to have concentrations that were either below the instrumental detection limit, or had levels of variance that exceeded the recorded mean values (See Table B.3 in Appendix B). It follows that with the strong relation between TTHM peaks and certain peak intervals, THM4 species may be impacted differently as well. A summary of the correlation parameters are displayed in Figure 4.8.

The data based on individual THM4 species reveals some interesting things about their associations. When considering chloroform (CHCl_3) alone, Spearman coefficients improve for all intervals listed in Table 4.7. Consequently, a strong correlation between Peak B and TTHM 0.76 ($p = 0.00$) is due to the fact that CHCl_3 is inclusive in the measurement for TTHM. The strongest association is found between Peak B and CHCl_3 (0.79, $p = 0.00$, $\text{adj.}R^2 = 0.59$), with very strong coefficients ranging from 0.51-0.6 for the other intervals. Another strong correlation is found between CHCl_2Br and Peak B (0.52, $p = 0.02$), which is not found with any other sample interval, and disappears when evaluating the Spearman coefficient. This may show a clear linearized trend; however, it is not supported by the associated $\text{adj.}R^2$ of 0.22 (Table B.1).

The coefficients for CHBr_3 tells a different story, that is there is an inverse relationship between peak sizes and the formation of CHBr_3 . A possible explanation for this is can be found in the work of Boyer and Singer [34] and Phetrak, Lohwacharin, and Takizawa [248]. Phetrak, Lohwacharin, and Takizawa [248] noted that when NOM fractions are removed by SBA resin, bromide is removed as well. SBA resin does not have an equivalent exchange capacity for both NOM, bromide, and chloride; therefore, increasing resin dose may remove

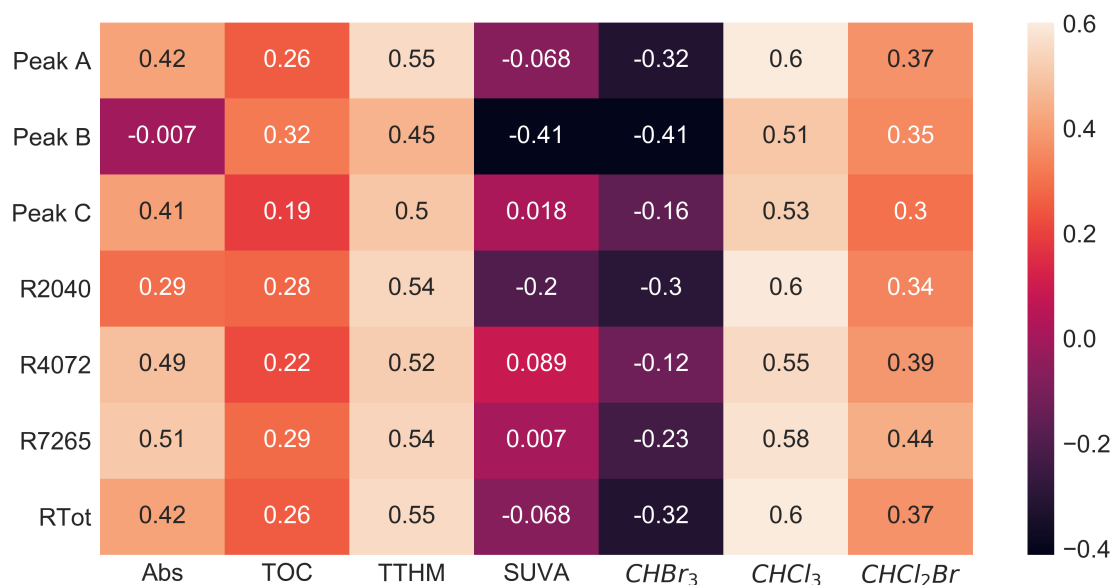


FIGURE 4.8: Spearman correlation parameters for a series of integrated peak trends with common water quality and THM species measurements ($n = 19$).

more bromide than NOM, thereby showing an inverse relationship in CHBr₃ formation. It could be the case that bromide is incorporated more into brominated-THM formation - as reinforced by Hong et al. [125] who noted this was the case at higher chlorine doses. The selectivity for chlorine and bromine by SBA exchange is not the same, therefore we see it impact brominated THM formation but not chlorinated THM formation. Boyer and Singer [34] provides a similar suggestion, as bromide concentration in the waters tested were impacted by the water alkalinity - moreso than chlorine and iodine in THM formation [158]. Another reason was suggested by the work of Rodrigues, Silva, and Antunes [272] who noted FA concentration was inversely proportional to brominated THM species, therefore with a greater proportion of FA remaining the recorded CHBr₃ will not follow suite. This is further in line with the conclusions made earlier where Peak B and Peak C were more characteristic of FA and HA respectively. Peak C was noted to be greater impacted by SBA treatment, leaving Peak B (FA) as a greater proportion of NOM remaining; and consequently, inversely correlated to CHBr₃ formation. Formation for CHCl₃ also improved using the Spearman's

coefficient, especially amongst values for R2040, R4072, R7265 and RTot: 0.6 ($p = 0.00$), 0.55 ($p = 0.01$), 0.58 ($p = 0.01$), and 0.6 ($p = 0.00$) respectively. Values for CHCl_2Br provided a middle ground between the formation of CHCl_3 and CHBr_3 , as the resulting values seem to be very specific to the halogen used in the formation of the specific THM species.

With the removal of NOM samples, select parameters were improved from their values listed in Figures 4.7 and 4.8. Since the improvements were few and far between, the tabulated values are listed in Tables B.4 and B.5 in Appendix B. Certain associations were seen to improve in the removal of the raw samples from the datasets. This was found to be the case in the improvements for Spearman's for R2040 and TTHM (0.53 to 0.63), Pearson's for R2040 and CHCl_3 (0.54 to 0.63), and Spearman's for R2040 and CHCl_3 (0.59 to 0.67). Since these improvements were all associated with the range of R2040, it comes to reason that IX may be specifically targeting components at this early wavelength range which results in the associated trend with the parameter. As the sole exceptions, it suggests that they act as better markers for treatment with IX, as opposed to being better indicators overall. One explanation may be due to the ability for SBA resins to preferentially remove HPI components [224, 248]; thereby allowing the remaining HPO components to be the sole contributors to CHCl_3 /TTHM formation. This effect however was not mirrored by any other AMW or wavelength interval. Since the peak prominence quickly disappears at higher wavelengths - including the elimination of Peak A - (See Figure 4.6), interval R2040 represents the bulk of UV absorbing moieties present in the water sample. This trend is not due to Peak A, as the Pearson and Spearman coefficients decreased for CHCl_3 (0.55 to 0.44 and 0.60 to 0.59 for Pearson and Spearman coefficients, respectively). Therefore, one can suppose that the HPO moieties in R2040 are highly reactive and would have high levels of specific CHCl_3 formation (i.e. conc. CHCl_3 per mg carbon). Since the vast majority of sample associations were not improved upon removing of the 7 water sources, the effect of their exclusion will not be discussed further. It does however help to illustrate that samples treated with SBA resin do not have chromatogram trends that are specific to the treatment used. Therefore,

the methodology and trends seen are universal, at least so far in application of SBA resins. This further supports the novelty of this work as it encompasses multiple source waters, and potentially, to more treatment methods.

4.2.3 Wavelength Interval Array

Basic Water Quality Trends

In Section 4.2.3 AMW and wavelength intervals were used to find correlations between some basic water quality parameters, as well as individual THM species. Wavelength intervals were established based on literature values, and additional information is required in order to parse more useful wavelengths from others. In Section 3.1.3, an array of values (Eq. 3.5) were extracted from each unique spectra and are summarized in Figures 4.9 and 4.10, corresponding to Pearson and Spearman coefficients respectively. Figure 4.9 provides a more descriptive representation of the trends seen over the whole chromatogram. Medium associations can be seen with Abs (or UVA₂₅₄) with levels approaching -0.42; and high associations with TTHM approaching values of -0.52. While the value for Abs at -0.42 seems significant, the range in Figure 4.9 spans from -0.4(-)0.42; therefore, little to no variance exists with respect to λ_{int} and λ_{fin} . The trend in Abs is not consistent with any prior intuitions, and a further explanation will be given in the investigation of the Spearman coefficients in Figure 4.10. Consistent with the findings in Section 3.1.3, TTHM is heavily influenced by UV absorbing moieties at earlier wavelengths, i.e. R2040; however, these plots do not provide information on any trends in AMW distribution. TOC and SUVA plots have relatively low degrees of associations - maxing out at -0.2 and -0.16 respectively - therefore they were excluded from further consideration.

Spearman coefficients provides a more impactful description between these parameters and their trends. The largest associations are again seen for Abs and TTHM, with the largest values exceeding -0.52 and -0.57 respectively. Abs has the largest coefficients at $\lambda_{int} =$

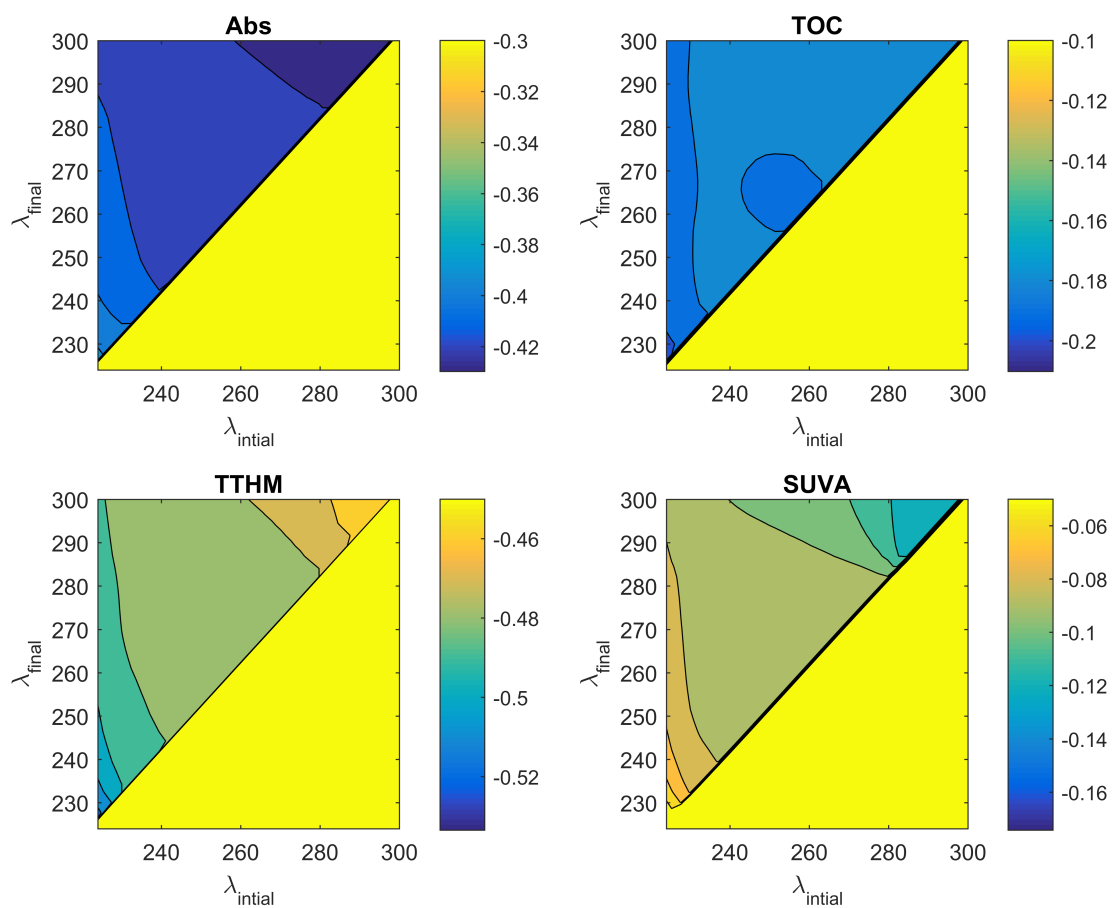


FIGURE 4.9: Pearson correlation coefficient evaluated for the integral array described in Eq. 3.5, for $N = 19$ samples

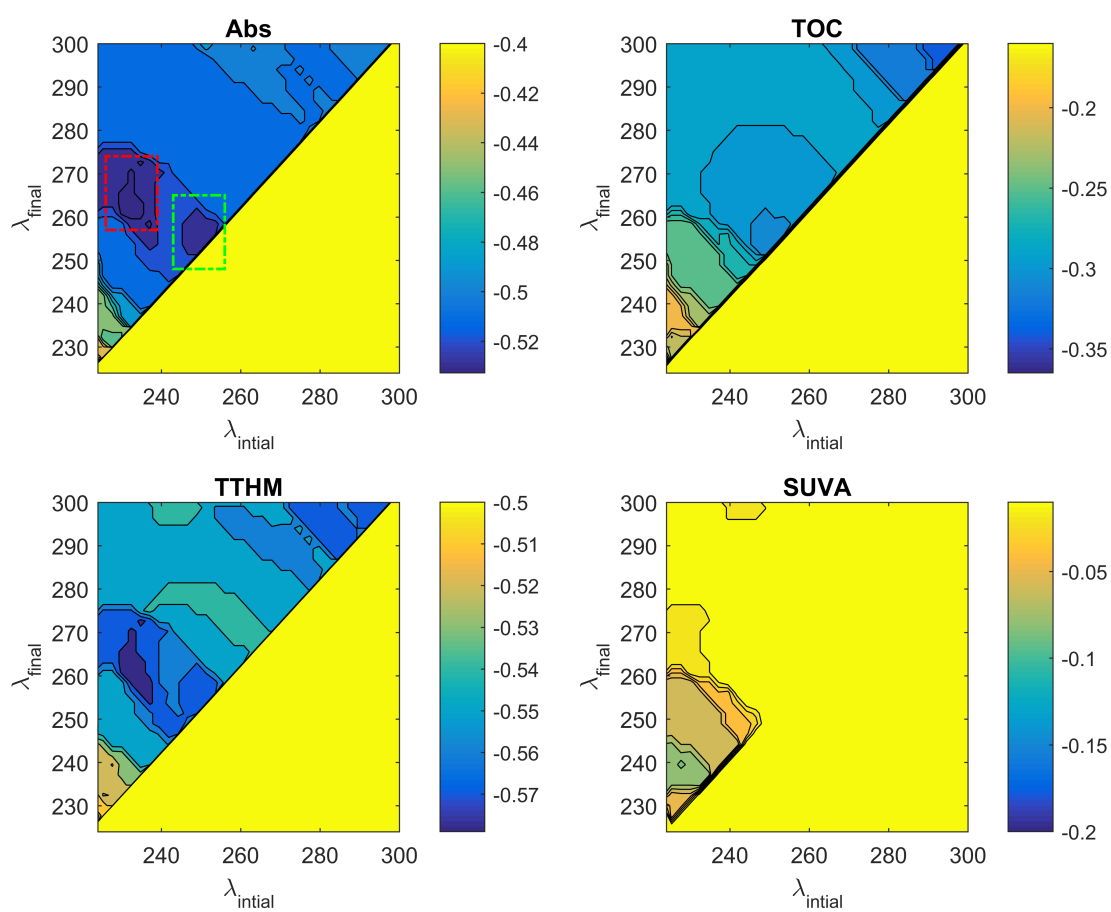


FIGURE 4.10: Pearson correlation coefficient evaluated for the integral array described in Eq. 3.5, for $N = 19$ samples. The red hashed box represents absorbance moieties at $\lambda_{\text{int}} = 226 - 239$ and $\lambda_{\text{fin}} = 257 - 273$ (S1), and the green hashed box for moieties at $\lambda_{\text{int}} = 243 - 256$ and $\lambda_{\text{fin}} = 248 - 265$ (S2).

226 – 239 and $\lambda_{fin} = 257 – 273$ (further identified as S1 for convenience), with a smaller patch appearing at $\lambda_{int} = 243 – 256$ and $\lambda_{fin} = 248 – 265$ (identified as S2). S1 and S2 both appear in regions that are typically associated with aromatic and aromatic-like components, which is consistent with prior intuitions as the measurement is taken at 254 nm. S1 is apparent in the plot of TTHM, which again is consistent with the widespread use of UVA₂₅₄ as a way of monitoring THM formation. It does however illustrate the degree to which UVA₂₅₄ approximates TTHM formation spectroscopically, but lacks the accuracy necessary to predict its formation in absolute terms. The plot for TOC shows an inverse relationship of Spearman's coefficient with increasing wavelength, with the greatest correlation at larger wavelengths. Since the Pearson and Spearman coefficient approach a maximum at -0.2 and -0.35 respectively, it can be suggested that the Spearman coefficient has a greater association with the TOC variable. At high wavelengths Peak A does not exist and Peak B and C exist to varying degrees - with Peak C existing to a greater extent (broader peak in Figure 4.6). The disappearance of Peak B with increasing wavelength seems to be impacting TOC, suggesting Peak B components contain greater proportions of organic carbon. This is in line with the conclusions made in Section , where Peak B and TOC had high associations of 0.55 and 0.45 for Pearson and Spearman coefficients, respectively. Since these arrays do not account for the AMW partitioning between Peak A, B and C, these conclusions can only be speculated. Trends for SUVA₂₅₄ were low to non-existent, as demonstrated in earlier examinations of SUVA₂₅₄, therefore they were removed from further consideration.

Trihalomethanes

In Figure 4.11 Pearson and Spearman coefficients are compared side by side for direct comparison. We see similar trends as for TTHM, that is, a high correlation at earlier wavelengths for CHCl₃ (-0.55); however, the trends for CHBr₃ and CHCl₂Br are both low, with max values of 0.2 and -0.18 respectively. The identified regions for the Spearman coefficient tell

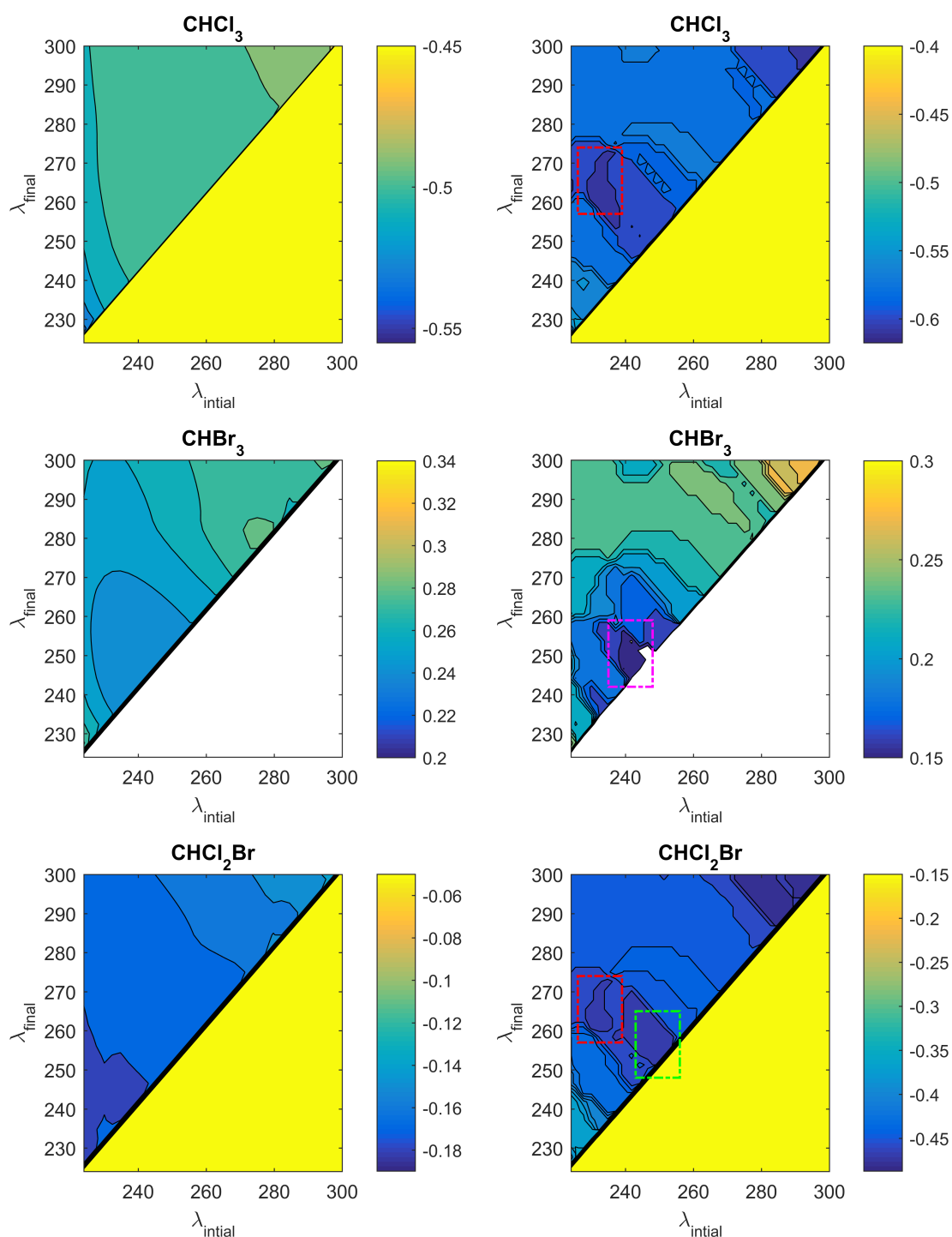


FIGURE 4.11: Pearson and Spearman correlation coefficient evaluated for the integral array described in Eq. 3.5, for $N = 19$. Plots on the right describe those evaluated using the Spearman coefficient, and those on the left are evaluated using the Pearson coefficient. The red hashed box represents absorbance moieties at $\lambda_{\text{int}} = 226 - 239$ and $\lambda_{\text{fin}} = 257 - 273$ (S1), the green hashed box for moieties at $\lambda_{\text{int}} = 243 - 256$ and $\lambda_{\text{fin}} = 248 - 265$ (S2), and the purple hashed box for moieties at $\lambda_{\text{int}} = 235 - 248$ and $\lambda_{\text{fin}} = 242 - 259$ (S3).

a different story. We see consistency in the region S1, identified in Figure 4.10, in the formation of CHCl_3 with a high coefficient approaching -0.6. We see the same region S1 in the contour for CHCl_2Br (-0.50) but absent from the contour of CHBr_3 . This region may be consistent with components of NOM that contribute to the formation of chlorinated by-products but not brominated by-products. We found in Section 4.2.3 Table 4.8 that the trends in CHCl_2Br were a middle ground between trends found in CHCl_3 and CHBr_3 . We see this in the presence of both regions S1 and S2 in CHCl_2Br , but each region is found individually in plots for CHCl_3 and CHBr_3 . Figure 4.11 provides a further explanation that was not apparent otherwise, that is, particular NOM precursors identified in the specified regions are sensitive to the halogen used to form the end product THM. The prevalence of region S1 and S2 in CHCl_2Br (-0.45) may lead one to consider that the measurement for UVA_{254} may aid in predicting the formation of all the THM4 species, but to varying degrees. The strong association for S1 in CHCl_3 and Abs indicates that this may result in the ability for UVA_{254} to predict CHCl_3 formation, as it is the main TTHM4 species (See Figure 4.3) in most water bodies generally. It is also the case that region S2 is found in CHCl_2Br , but the association is weaker and the concentration of CHCl_2Br is lower in water bodies. A smaller region of interest was identified in the formation of CHBr_3 , identified as S3; however, the region exhibited a low association of 0.15. The plot for CHBr_3 has correlations (positive) that are inverse to that of the other THM4 species (negative) which confirms the findings made in Section 4.2.2 Tables 4.7 and 4.8; namely, associations for CHBr_3 are monotonically inversely proportional to peak area. Since peak prominence quickly decreases at high wavelengths, all associations found at high λ_{int} and λ_{fin} are considered to be in the fingerprint region and their significance is negligible. Provided a larger sample size was carried out, further trends could then be extracted from the resulting chromatogram and a better appreciation for this method can be carried out. Identified regions of interest (i.e. S1 and S2) can be used to better select surrogate compounds used for the prediction of THM forming precursors. These arrays do not include the effect of AMW on these trends; so with a slight modification to the methodology, an array can be produced for each significant peak (A,B,C) and a further evaluation in the

trends can be carried out.

4.2.4 Cluster Analysis

Cluster Analysis provides a mean in which researchers can improve classification of their water sources based on water characteristics and parameters. Figure 4.12 provides the resulting dendrogram of the Squared Euclidean distance for the 7 water sources tested. Two plots are provided, one compounding all the common water characteristics, UVA₂₅₄, TOC, SUVA₂₅₄, and TTHM; and the other with all the individualized THM species, CHCl₃, CHBr₃, and CHCl₂Br. Figure C.1 in Appendix C provides a geographical view of the water sources tested in Manitoba. Source water from SAN, MOR, RR and LET locations all draw from different locations along the Red River; GL draws from the Assiniboine River which forks from the Red River; while PC and DB both draw from the water source Lake Winnipegosis. Forks identified in Figure 4.12 show water sources which are of proximal distance from one-another based on their Squared Euclidean distance. Lower forks show highly relateable characteristics, while larger forks corresponding to separable distinctions among clusters of water sources. In Figure 4.12(a) SAN, RR, and LET all form a cluster which is consistent with their common water source the Red River. The MOR water source finds itself clustered amongst water sources of Lake Winnipegosis, which is not consistent with initial inclinations. A cluster (identified by color for Euclidean distance values less than 2) is formed between GL, MOR, and DB - all of which draw from different source waters and only two are of proximal distance. PC forms a cluster amongst the smaller cluster at a distance value 2.75; however, it does not form a close cluster with its neighbor PC. The variance in the data could be due to a number of factors, such as the use of certain parameters. Values of TOC vary from one source to the next; however, values such as TOC are not on their own characteristic of the source water. If TOC was further categorized based on fractionation, or contribution from allochthonous and autochthonous sources, then one would be able to see better distinctions with these clusters between source waters.

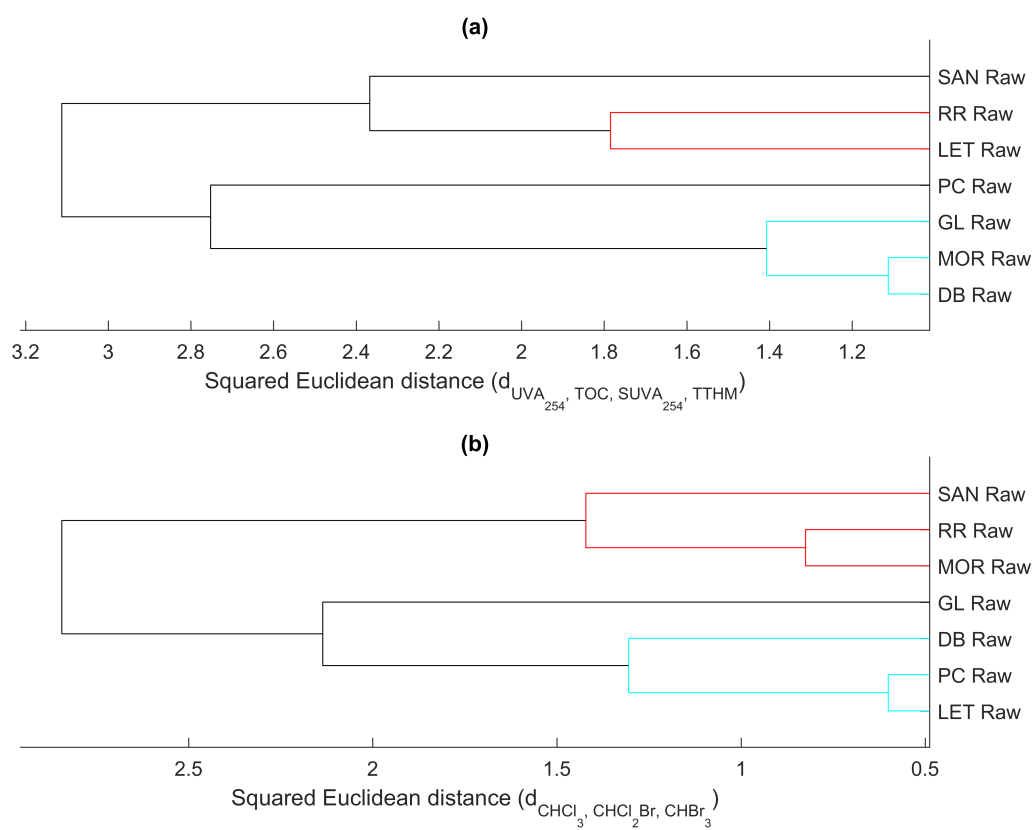


FIGURE 4.12: Cluster analysis based on squared Euclidean distance for a series of raw waters tested. (a) Euclidean distance based on UVA_{254} , TOC, $SUVA_{254}$, and TTHM. (b) Euclidean distance based on $CHCl_3$, $CHBr_3$, $CHCl_2Br$

Figure 4.12(b) provides an additional dendrogram based solely on THM species. Since THM values are highly correlated with select UVA₂₅₄ absorbing moieties, along with concentrations of other components such as bromide which lead to the proportional formation of a THM species, we would hope to expect greater distinctions and clustering with the source waters tested. Upon initial observation, water sources for SAN, RR, and MOR are all closely clustered together - consistent with their source water. A cluster for DB and PC are also both clustered more closely together as compared to Figure 4.12(b). A noticeable exception to the trend is the presence of LET water clustered together with one source water from Lake Winnipegosis. The reasons for this are unknown, but there are many factors which can be further implemented to improve the clustering of source waters. Use of this clustering technique by Xing et al. [336], was able to cluster source waters from 6 Chinese and 4 Australian source waters successfully from each other. Since water characteristics are highly correlated with source waters, one would expect greater clusters to form between waters from similar countries. It is however a tall order to attempt to cluster waters of a common watershed, but of different source waters - all of which are located in the same geographical region with similar soil types, vegetation, and climate. Given a further classification of NOM components, as well as other immutable variable types, clustering within smaller regions could be carried out successfully in the future.

5 Conclusions

5.1 Main Conclusions

The objective of this research was to develop a methodology that could reliably and effectively extract the peak prominence of UV moieties from a unique 3D spectra using HPSEC. This would in turn be used to test the correlation between peak prominence and water quality measurements for NOM and select DBPs. With this information researchers and investigators will be able to formulate a water *autopsy* that can be used to make recommendations on the best treatments and pre-treatments necessary to treat the particular water source for potable water consumption. Information related to molecular weight distribution and polydispersity can further highlight the weighted distributions of the source water components; thereby providing an indication of the steric effects for a particular pre-treatment method. This study focused on the universality of the methodology, so that the application may be widely used for multiple water sources and treatment methods which are currently outside of the scope of this work. This work limited its scope to 7 water sources in Manitoba and pre-treatment using strong base anionic exchange resins. The sub-objectives of the developed methodology can be summarized as the following:

- Develop a chromatographic method that could reliably and effectively partition 7 natural water samples from Manitoba, Canada, into a multi-spectral array as a function of AMW;

- Develop an automated script that could extract useful information from the 3D array, including: weight averaged, and number averaged molecular weight (M_n, M_m); polydispersity (ρ); absorbances of wavelength and AMW ranges proposed to be significant towards the reactivity of NOM; and absorbances of a multi-dimensional array consisting of every iteration of wavelength ranges possible between 220-365 nm;
- Carry out a parametric, and non-parametric statistical analysis to test the association between absorbances extracted from the 3-D chromatogram with common water parameters and THM species;
- Perform a cluster analysis to trace the proximity of the variables in Euclidean space in order to classify the 7 water samples with their source water neighbor.

As a preliminary work, SRNOM standard was contacted with 3 types of SBA resins to test the effectiveness of the resin backbone and counter-ion on NOM selectivity. All 4 SBA resins tested targeted the removal of the SRNOM, and was able to do so at environmentally relevant pH levels (6.5-10.5) of the original water. It was noted that phenolic and carboxylic groups - which are large contributors to NOM functionalization - were adequately deprotonated in sufficient quantities to facilitate adsorption of NOM onto the resin site. Treatment with a variety of SBA doses on 7 water sources showed the targeted removal of hydrophilic NOM - indicated by the increase in $SUVA_{254}$ following SBA application. Rainy River was a clear exception to this rule, and it was considered that a high TIC concentration may have impacted the IX process through exchange with carbonate alkalinity species (HCO_3^- , CO_3^{2-}).

A major objective of this work involved carrying out a bivariate analysis using the Spearman and Pearson coefficients to test monitor trends in peak prominence and water parameters. High correlations with TTHM and TOC values were recorded with UV absorbing moieties in the 1.6-2.1k Da range. This was more so the case than components between 2.2k-4k Da, which may be the distinction between FA and HA components of NOM as these two AMW ranges were present in SRNOM. TTHM was also closely tied to the LMW peak at

0.6-1.1k and absorbance at wavelengths between 220-240 nm. These two parameters are closely entwined as the LMW peak is only apparent at low wavelengths. When the bivariate analysis was extended to include the THM4 species (with the exception of CHClBr_2), the associations grew much stronger. Components between 2.2-4k Da were heavily associated with CHCl_2Br and CHCl_2 , with medium associations at other AMW and wavelength intervals. CHBr_3 was found to show an alternative trend than the chlorinated species, which was attributed to the greater incorporation of bromide in the total formation of THMs. When investigating the monotonic relationship using the Spearman coefficient, coefficients improved for all wavelength intervals used. This highlights a more exponential trend in THM formation as a function of UV absorbing moieties present. The inclusion of only the SBA treated samples did not improve the coefficients for the vast majority of relations tested; with the sole exceptions being correlations with UV absorbing moieties at $\lambda = 220 - 240$. This range is consistent with large changes in molar absorptivity, and may be a highlight specific only to SBA applications.

A secondary objective involved targeting specific wavelength intervals which can act as surrogates for synthetic water preparation. The array based on initial and final wavelength provides a means to pinpoint target UV moieties, with trends being seen amongst parameters and THM species. Associations based on the Spearman coefficient identified specific regions of interest which was consistent between UVA_{254} , TTHM, CHCl_3 , and CHCl_2Br at $\lambda_{int} = 226 - 239$ and $\lambda_{fin} = 257 - 273$. Another region of interest was specific to CHCl_2Br and UVA_{254} , which may lead one to believe these parameters are closely related. Using processes that can specifically target components in these ranges would aid in the removal of the chlorinated THM products. With further implementation, the methodology can be expanded to encompass more DBP and their surrogate components.

A tertiary objectivity involved determining the weight averaged, and number averaged molecular weight determinations for the 7 water sources following dosing with SBA resin. The

effect of SBA on the MW distribution, as well as the polydispersivity of the sample was investigated. Samples treated with SBA resin had a consistent increase in M_n and M_w , with the trend following suite at higher resin doses. Values for polydispersivity showed an increase as a function of resin dose, leading one to conclude that the value for M_n increased as the parameter biases the LMW end of the distribution. An additional investigation was carried out as higher wavelengths have been known to bias M_n and M_w towards higher recorded values. With the removal of a few exception cases, values for M_n , M_w and ρ only varied by 0.7-1.4%, 0.2-2% and -0.1-1%, respectively, among the data set. Investigators who determine the AMW distribution of their sample set should be wary of this effect, and corrections to their determinations should be made.

An additional investigation was carried out with utilized cluster analysis based on squared euclidian distance to cluster 7 Manitoba raw samples with the aim of categorizing them based on whether they draw from the same source water. Calculating the euclidean distance based on their simple water parameters was able to cluster waters from 2 Red River sources, but was not able to cluster off 4 of them present in the sample set. The two Lake Winnipegosis waters, PC and DB, were not clustered initially either - leading to the suggestion that parameters such as TOC are not source specific enough to be used. When the THM4 species (excluding CHClBr_2) were used, 3 out of 4 Red River sources were clustered, and the 2 Lake Winnipegosis waters were grouped as well. Since individual THM species are heavily impacted on the halogen concentration in the water body and other immutable characteristics, clustering was able to adequately distinguish 5 out of 7 of the water bodies. Provided more site specific variables were used (i.e. NOM fractions) the cluster method could potentially be able to cluster waters from different water sources but within a common watershed, as is the case for southern Manitoba waters.

5.2 Engineering Significance

Many of the developments carried out in this research have direct relevance to practical design analysis in an engineering setting. Unraveling the heterogeneity of NOM is one of the most pressing concerns in the design and implementation of systems to target NOM in potable water sources; therefore any work that helps to provide a solution to this problem is warranted. Providing better tools for researchers to describe NOM characteristics and behaviour will enable researchers to discover new ways to mitigate the harmful effects these components cause to water and wastewater systems. HPSEC analysis provides better outcomes than through the use of surrogate parameters such as TOC, UVA_{254} , and $SUVA_{254}$, because the complexity of NOM - and its subsequent fate in aquatic systems - requires a more developed approach. The design phase for water treatment projects should make use of whatever tools are at our disposal, so the application of HPSEC should not be limited to a research settings, but rather, should be incorporated in every treatment design decision.

The methodology in this work can very much be expanded into any useful cases involving testing the effects of a pre-treatment, or the effects of an alternative disinfection method. As the reactivity of NOM is heavily tied to the nature of its absorbing moieties, the multi-spectral analysis will always find use in investigations involving reaction mechanisms to form harmful, or yet-to-be-known harmful, by-products. A typical case study for its implementation would involve a client who is looking to construct a potable water treatment plant, and is looking for the optimal treatment train required to do so. The source water can be *autopsied* using HPSEC-PDA, with a breakdown of the UV absorbing contribution due to humic matter and LMW building blocks. Using metadata obtained from prior HPSEC analyses- including libraries containing information on treatment effects on UV absorbing moieties - a recommendation for treatment design can be made. The use of molecular weight distributions can be used to account for steric effects of the source water composition onto an adsorption type

media, or a membrane molecular weight cut-off. A library containing information with correlations between absorbing moieties and a wide variety of DBPs can be used to predict the expected formation of a DBP simply based on its HPSEC features. The algorithm developed can take into account other parameters of the water body (e.g. chloride, bromide or nitrogen) which would help in making predictions. In summary, the HPSEC based methodology developed in this work will improve the design of potable water systems in a way that is more encompassing and hollistic in its approach to using big data.

The use of big data to solve big problems should not be excluded to the field of water treatment, therefore this work hopes to act as a stepping stone towards a greater implementation of this methodology. With a dataset containing an plurality of source waters from many watesheds, the algorithm can be refined and the predictions it can make will be improved. The source code of this work is freely available online at the original author's [Github](#) under an Apache 2.0 Licence.

5.3 Future Work and Recommendations

Many of the developments and recommendations of this work involve improving the accuracy and efficiency of the scanning algorithm that extracts useful information from the 3-D chromatogram. Improvements can be made to the detection of significant peaks, and computing their peak areas using distribution functions. Ideally, peaks can be deconvoluted into Lorentzian, Gaussian, or a combination of the two, functions. This allows for a quicker way of determining peak area based on the remaining peak area as a percent of the original peak [13, 24, 146, 189, 317]. Figure 5.1 illustrates the work that has been done in this regard. As depicted, the first Lorentzian was not able to deconvolute the larger Peak that would be ascribed as Peak A (0.6-1.1k Da) and Peak B (1.6-2.1k Da) previously. Without seperating these peaks, the information is readily lost on the impact of the shoulder peak in determining trends of association.

Figure 5.1 displays the results of using a *findpeaks* function on the data peak. The shoulder was identified (depicted as the number 7), however it required a very low tolerance that labeled other non-prominent peaks in the data set.

Future work could also involve collecting samples from a wider variety of water sources, and being able to test the effects of an assortment of water treatment technologies. Since the methodology focuses on UV absorbing moieties, the water parameters that are most likely to impact disinfection by-product formation are those that currently act as surrogates for NOM (i.e. TOC, SUVA, UVA₂₅₄, etc.). Consideration of other parameters such as alkalinity, hardness, and assimilable organic carbon may impact the results - given it can indirectly impact DBP formation. The factors to be considered are specific to the pre-treatment tested using the method, and care should be given when determining which parameters to include in the analysis. This methodology can be used to further explore filtration-based or microbial-based technologies, in an attempt to stress test the methodology and its application to beyond usage with IX. The research was constrained to basic water quality parameters: TOC, UVA₂₅₄, SUVA₂₅₄; and the THM4 species - which limits its application greatly. An investigation that included haloacetic acid (HAA) species, nitrogenous-DBP (N-DBP), and haloacetonitriles would expand the relevancy of this work to beyond solely Manitoba waters and their local concerns.

The use of cluster methods was briefly elaborated on in this work. Provided a large enough sample set were to exist, the use of squared Euclidean distance can be expanded on via use of K-means clustering. K-means uses an iterative algorithm that clusters the Data in K number of clusters based on their relative distance to a centroid. The centroid is the point at which the sample distances to them are minimized, and the same is done for K clusters used. Figure 5.2 displays a 2 centroid cluster for the THM4 species excluding CHClBr₂.

Items centered around the centroid (depicted as a black circle) are closer to each other in proximity in Euclidean space. The use of the treated samples were used for demonstrative purposes. Given a set of variables that were independent of one another and characteristic

of the water source, clustered trends will emerge. Clustered trends will be more adaptable to select treatment methods, and more susceptible to things like by-product formation or photo-oxidation. The use of K-means clustering could be a novelty in the field of water treatment; however, clusters from a sufficient sample size is required in order to draw trends. This is the main limitation to this type of method, but it should nevertheless be a useful tool for researchers to investigate trends amongst water sources.

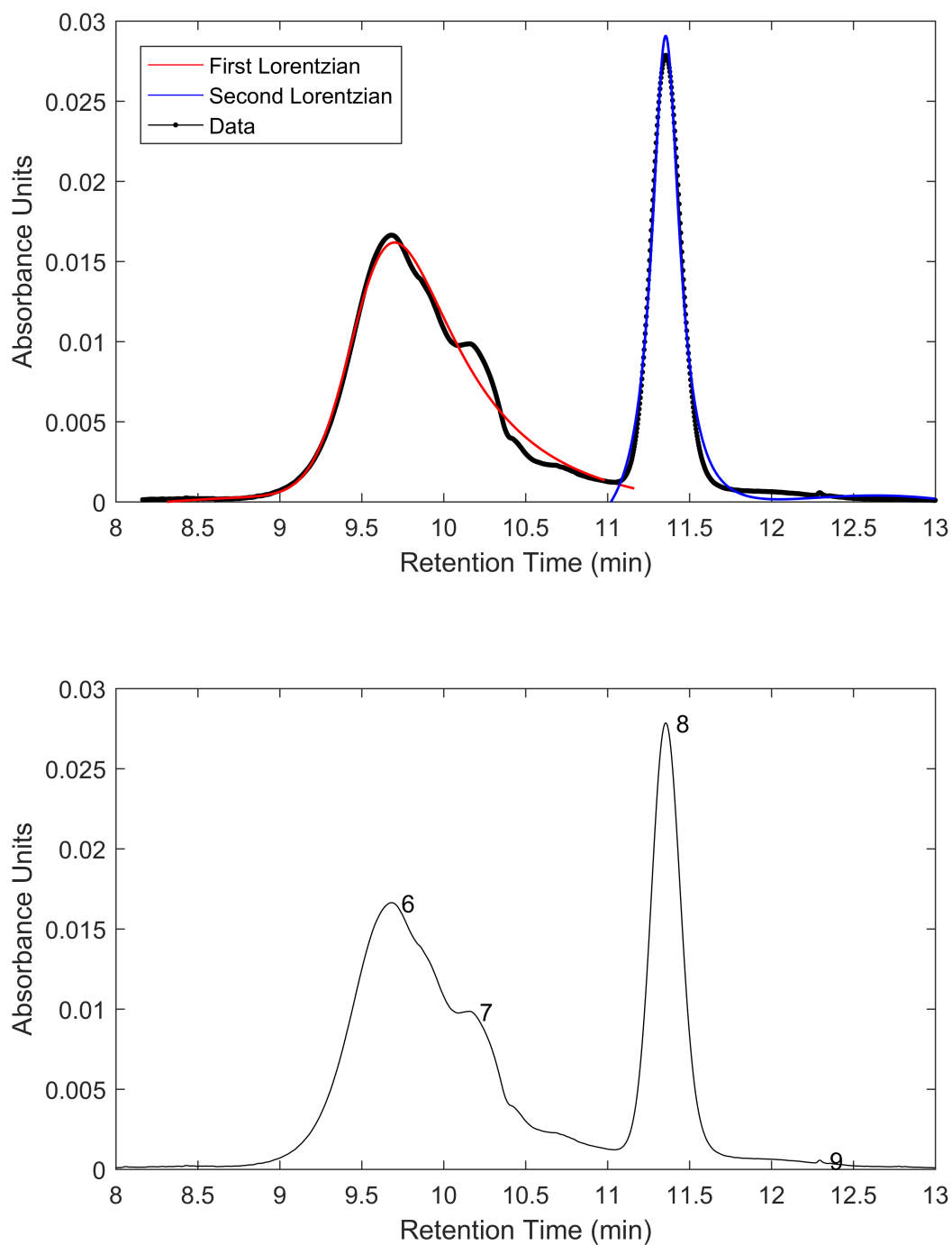
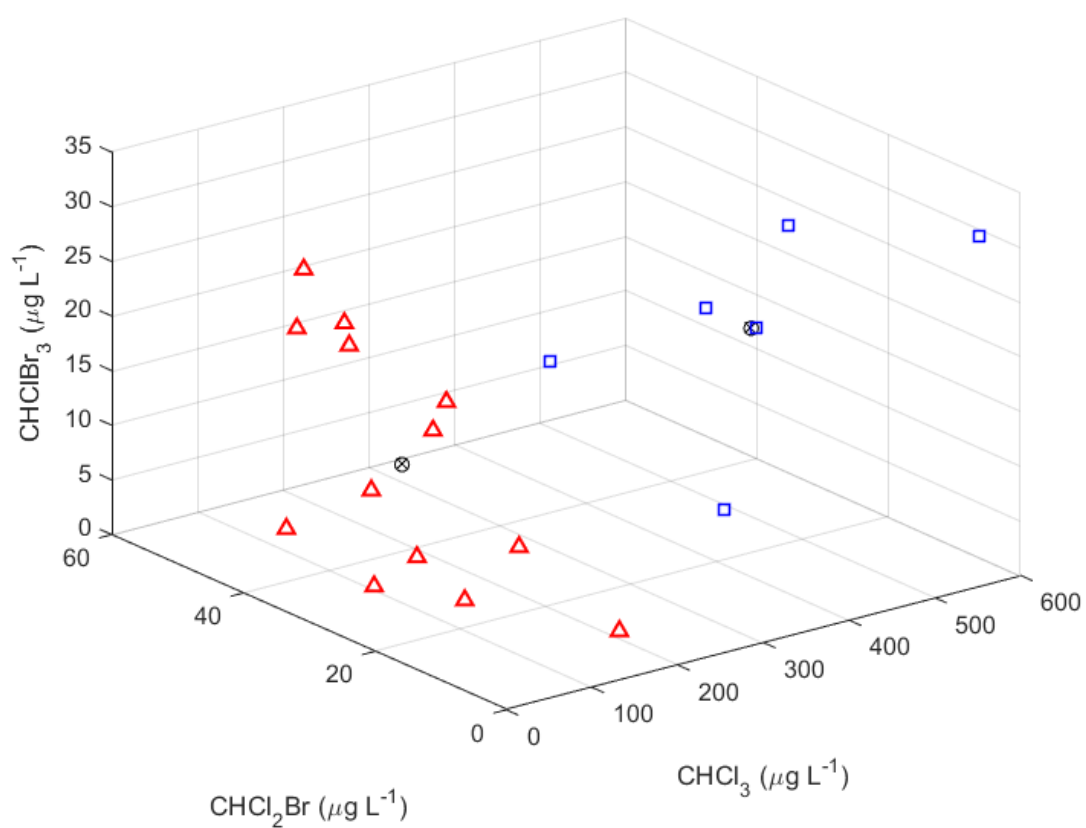


FIGURE 5.1: Illustration of the peak identification techniques used to deconvolute the HPSEC peaks. (Top) Peaks separated into two Lorentzian functions, (Bottom) Peaks identified using the *findpeaks* Matlab function with a 'Min-PeakProminence' factor set to (1E-4).



A Appendix

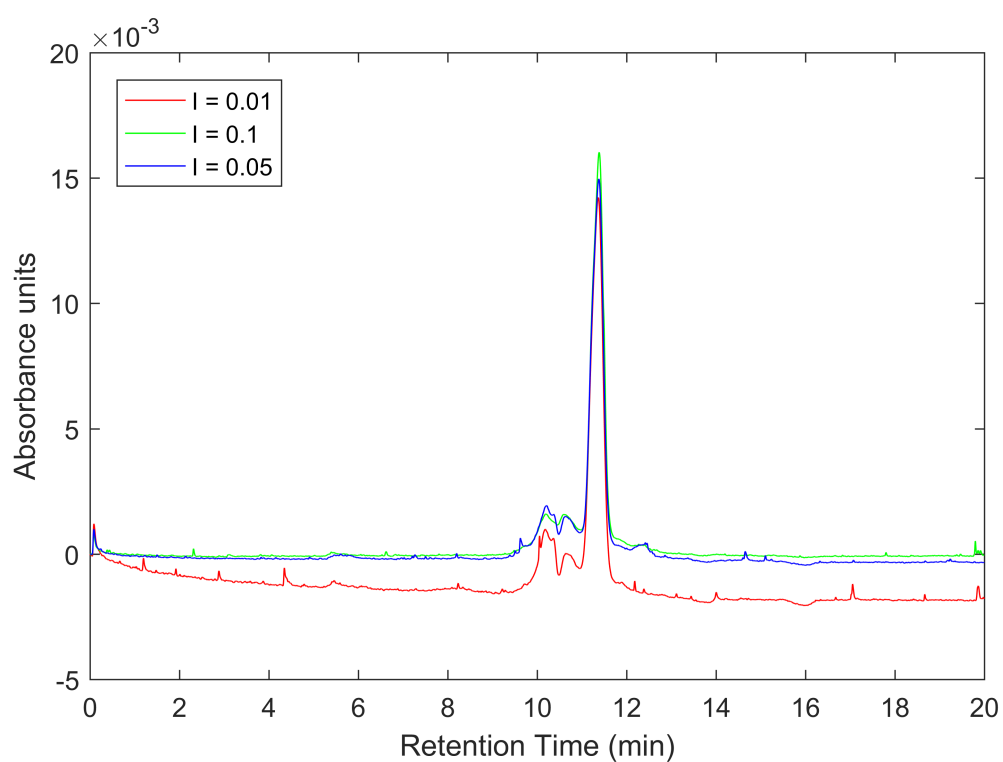


FIGURE A.1: HPSEC peak profile as a function of ionic strength of the sample. Mobile phase ionic strength was set at 0.1

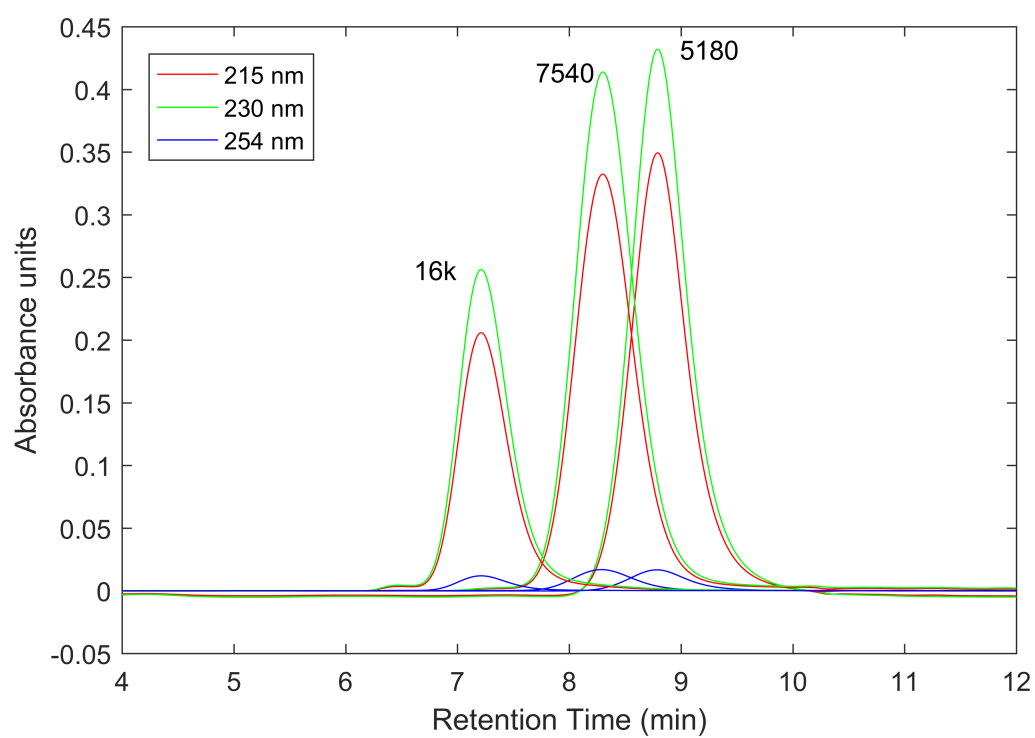


FIGURE A.2: HPSEC PSS standard profiles as a function of measured wavelength.

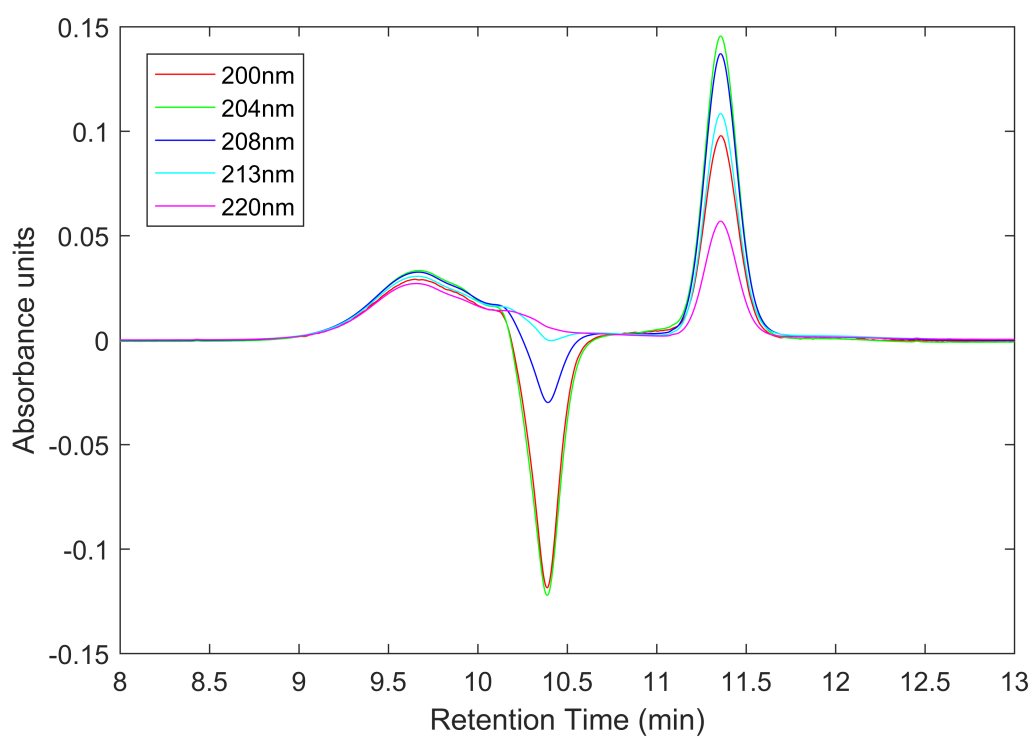


FIGURE A.3: HPSEC peak profile for SAN Raw as a function of measured wavelength. Low wavelengths exhibit negative peak absorbance due to the salting out effect.

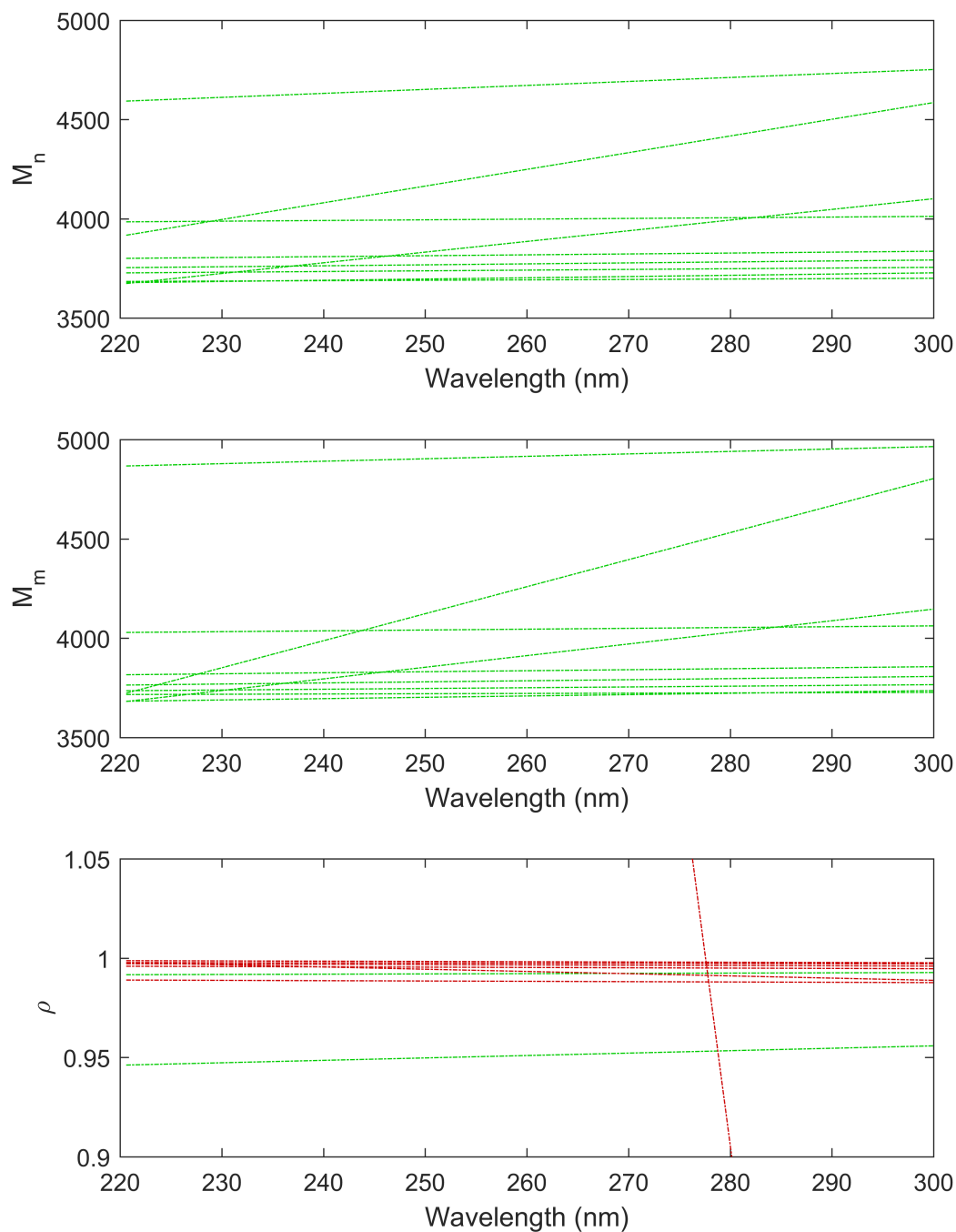


FIGURE A.4: Apparent molecular weight determinations, and polydispersity indices for all 19 samples treated with DOW TAN resin, displayed as a function of wavelength. All plots shown are linear regressions of the original data as carried out in Figure 4.5. Green plots indicate a positive slope, while a red plot indicates a negative one.

B Appendix

TABLE B.1: Adjusted correlation coefficients and Pearson coefficients of skewness for the values in Tables 4.7-4.8. Numbers in parenthesis represent values for the Spearman coefficient.

	Adjusted R ²						
	TOC	TTHM	SUVA	CHCl ₃	CHBr ₃	CHCl ₂ Br	Sk
S2040 (220-240 nm)	0.05(-0.00)	0.27(0.24)	0.02(-0.05)	0.25(0.34)	0.08(0.05)	-0.02(-0.09)	0.76(1.46)
S4072 (240-272 nm)	0.03(-0.09)	0.23(-0.01)	0.00(-0.05)	0.19(0.03)	-0.02(-0.06)	-0.02(-0.09)	1.90(1.74)
S7265 (272-365 nm)	0.03(-0.08)	0.23(-0.06)	0.00(-0.05)	0.20(-0.02)	0.01(-0.04)	-0.02(-0.09)	1.69(1.71)
STot (200-365 nm)	0.04(-0.09)	0.28(0.06)	0.00(-0.09)	0.26(0.15)	0.04(0.01)	-0.02(-0.09)	1.55(1.93)
Peak A (0.6-1.1 kDa)	0.04(-0.09)	0.28(0.06)	0.00 (-0.09)	0.26(0.15)	0.04(0.01)	-0.02(-0.09)	1.55(1.93)
Peak B (1.6-2.1 kDa)	0.30(0.11)	0.58(0.11)	0.04(-0.07)	0.59(0.12)	0.15(-0.09)	0.22(0.01)	0.37(0.34)
Peak C (2.2-3.5 kDa)	0.01(-0.06)	0.15(0.18)	0.01(-0.07)	0.12(0.29)	0.02(0.06)	-0.04(-0.1)	1.21(1.26)

^aPearson's coefficient of skewness was determined to be 1.32, 0.23, and 0.93 for TOC, TTHM, and SUVA respectively.

TABLE B.2: Measured basic water quality parameters for the raw and IX treated samples (N = 3). Units for TC, IC, TOC are in mg L^{-1} ; UV_{254} are AU; and SUVA is $\text{L mg}^{-1} \text{m}^{-1}$.

	TC	IC	TOC	UV_{254}	SUVA
DB RAW	43.4 ± 2.1	37.3 ± 0.4	5.9 ± 0.3	0.054 ± 0.006	0.9 ± 0.01
DB 3	37.6 ± 5.2	37.3 ± 0.8	0.4 ± 0.3	0.041 ± 0.001	8.7 ± 0.5
DB 5	37.5 ± 7.7	37.0 ± 0.8	0.4 ± 0.1	0.038 ± 0.004	9.3 ± 0.2
GL RAW	51.5 ± 6.5	50.7 ± 0.8	3.61 ± 0.2	0.052 ± 0.005	1.4 ± 0.01
GL 3	52.8 ± 5.6	50.4 ± 4.4	2.48 ± 0.2	0.037 ± 0.004	1.5 ± 0.02
GL 5	51.4 ± 6.1	50.6 ± 4.9	1.0 ± 0.3	0.033 ± 0.004	3.2 ± 0.1
LET RAW	72.1 ± 0.8	60.2 ± 0.8	11.2 ± 0.3	0.222 ± 0.014	1.9 ± 0.01
LET 3	59.9 ± 0.8	58.8 ± 7.6	1.3 ± 0.9	0.063 ± 0.012	5.0 ± 0.3
MOR RAW	71.9 ± 1.7	66.5 ± 3.1	5.2 ± 0.2	0.085 ± 0.004	1.6 ± 0.0
MOR 3	66.9 ± 1.9	66.0 ± 0.8	1.0 ± 0.3	0.059 ± 0.006	5.8 ± 0.1
MOR 5	66.0 ± 9.9	65.9 ± 8.4	0.28 ± 0.2	0.036 ± 0.004	12.8 ± 0.9
PC RAW	52.3 ± 7.4	45.7 ± 7.0	6.3 ± 0.4	0.188 ± 0.014	2.9 ± 0.03
PC 3	46.5 ± 5.3	45.8 ± 0.8	1.7 ± 0.1	0.064 ± 0.004	3.8 ± 0.03
PC 5	47.1 ± 0.8	45.3 ± 0.8	0.6 ± 0.2	0.041 ± 0.001	6.5 ± 0.2
RR Raw	84.5 ± 0.8	76.6 ± 0.8	16.2 ± 0	0.237 ± 0.027	1.4 ± 0.01
RR 3	66.4 ± 4.5	63.3 ± 0.8	8.8 ± 0.3	0.085 ± 0.015	0.9 ± 0.01
RR 5	62.0 ± 0.8	60.0 ± 0.8	5.2 ± 0.6	0.043 ± 0.003	0.8 ± 0.01
SAN RAW	38.9 ± 0.8	36.7 ± 0.8	13.8 ± 0.3	0.333 ± 0.015	2.4 ± 0.01
SAN 3	20.0 ± 2.2	19.9 ± 0.8	1.4 ± 0.2	0.08 ± 0.008	5.7 ± 0.1
SAN 5	23.5 ± 6.4	26.4 ± 0.8	1.7 ± 0.1	0.09 ± 0.013	5.2 ± 0.08

TABLE B.3: Measured Trihalomethane species for the raw and IX treated samples (N = 3). Units are in μgL^{-1} .

	CHCl_3	CHBr_3	CHCl_2Br	CHClBr_2	TTHM
DB RAW	228 ± 10	23 ± 4	21 ± 9	6 ± 3	278 ± 26
DB 3	107 ± 35	12 ± 17	9.5 ± 2	2 ± 1	130 ± 54
DB 5	57 ± 12	21 ± 3	7 ± 2	2 ± 0	87 ± 17
GL RAW	82 ± 24	17 ± 24	4 ± 0	0 ± 0	103 ± 48
GL 3	35 ± 9	38 ± 3	6 ± 2	1 ± 1	79 ± 15
GL 5	53 ± 11	27 ± 3	3 ± 0	0 ± 0	83 ± 14
LET RAW	155 ± 8	51 ± 2	24 ± 7	2 ± 3	232 ± 19
LET 3	137 ± 26	29 ± 12	15 ± 0	3 ± 1	184 ± 39
MOR RAW	292 ± 33	0 ± 3	29 ± 4	0 ± 0	321 ± 40
MOR 3	170 ± 2	54 ± 3	17 ± 5	3 ± 1	243 ± 11
MOR 5	141 ± 10	43 ± 3	21 ± 9	4 ± 1	209 ± 22
PC RAW	162 ± 28	45 ± 4	18 ± 6	0 ± 2	225 ± 39
PC 3	122 ± 15	25 ± 1	19 ± 9	25 ± 1	191 ± 26
PC 5	65 ± 11	29 ± 13	11 ± 1	2 ± 0	107 ± 25
RR Raw	375 ± 29	6 ± 8.5	35 ± 1	8 ± 6	424 ± 47
RR 3	234 ± 2	0 ± 0	32 ± 12	5 ± 3	271 ± 17
RR 5	255 ± 15	0 ± 0	14 ± 4	4 ± 2	272 ± 21
SAN RAW	553 ± 16	0 ± 0	32 ± 6	5 ± 4	590 ± 27
SAN 3	132 ± 3	0 ± 0	132 ± 3	1 ± 1	265 ± 7
SAN 5	149 ± 5	0 ± 0	5 ± 4	5 ± 4	158 ± 12

TABLE B.4: p values determined to test no correlation versus the alternative null hypothesis. Numbers in parenthesis represent p values for the Spearman's coefficient. Values are attributed to findings in Figure 4.7-4.8

	p					
	TOC	TTHM	SUVA	CHCl ₃	CHBr ₃	CHCl ₂ Br
R2040 (220-240 nm)	0.34(0.25)	0.02(0.01)	0.52(0.40)	0.01(0.00)	0.11(0.21)	0.44(0.14)
R4072 (240-272 nm)	0.47(0.36)	0.03(0.02)	0.75(0.71)	0.03(0.01)	0.49(0.61)	0.44(0.10)
R7265 (272-365 nm)	0.45(0.23)	0.03(0.01)	0.72(0.97)	0.02(0.01)	0.28(0.34)	0.44(0.05)
RTot (200-365 nm)	0.38(0.28)	0.01(0.01)	0.99(0.78)	0.01(0.00)	0.19(0.18)	0.43(0.11)
Peak A (0.6-1.1 kDa)	0.38(0.28)	0.01(0.01)	0.99(0.78)	0.01(0.00)	0.19(0.18)	0.43(0.11)
Peak B (1.6-2.1 kDa)	0.01(0.18)	0.00(0.05)	0.38(0.08)	0.00(0.02)	0.05(0.07)	0.02(0.13)
Peak C (2.2-3.5 kDa)	0.60(0.42)	0.09(0.03)	0.67(0.94)	0.07(0.02)	0.25(0.51)	0.65(0.21)

TABLE B.5: Pearson and Spearman correlation parameters for a series of integrated peak trends with common water quality measurements ($n = 19$). Values within brackets exclude raw water samples ($n = 12$).

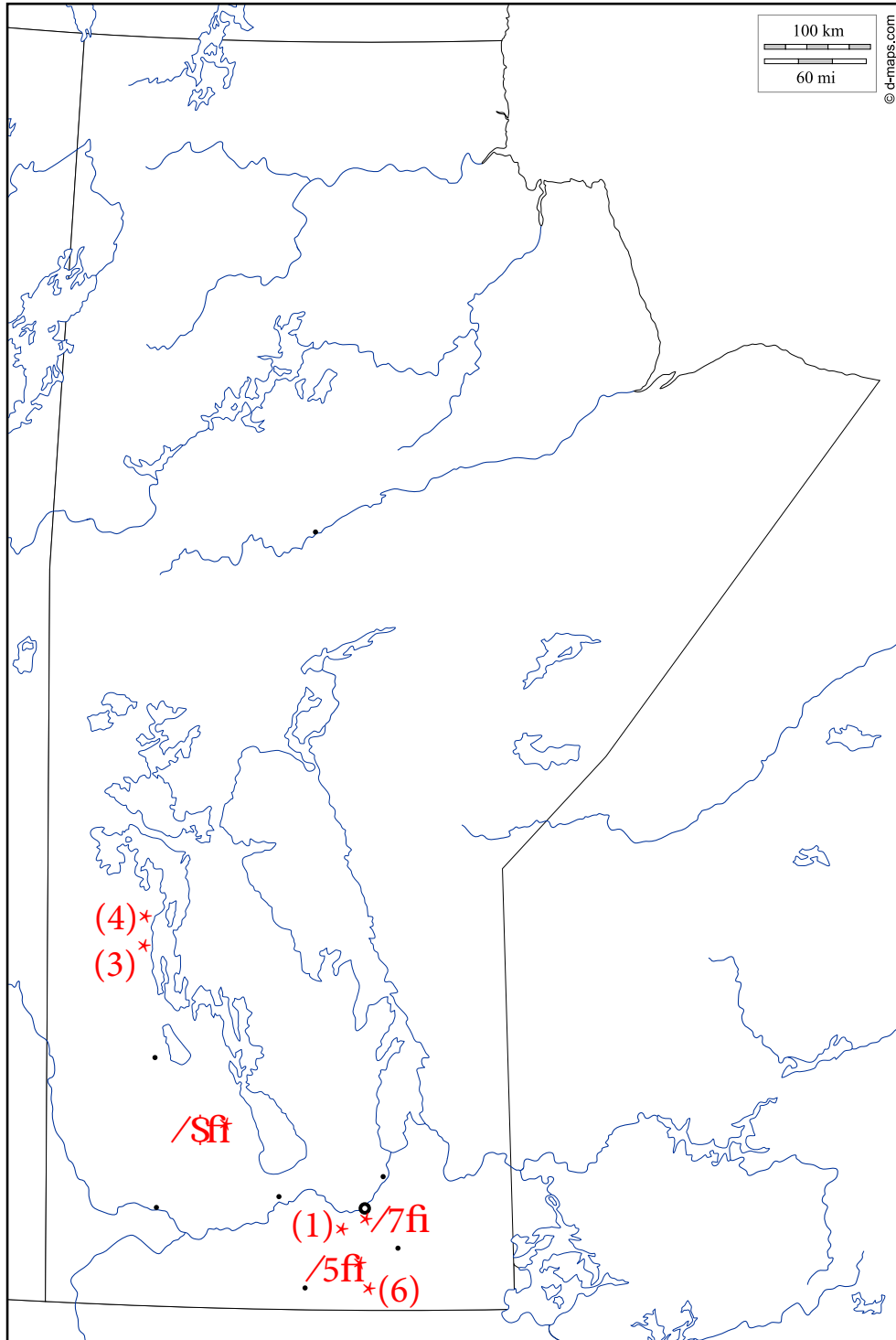
	Pearson				Spearman			
	TOC	TTHM	SUVA	TOC	TTHM	TOC	TTHM	SUVA
R2040 (220-240 nm)	0.23(0.28)	0.52(0.56)	-0.15(-0.20)	0.27(0.36)	0.53(0.63)	0.27(0.36)	0.53(0.63)	-0.20(-0.08)
R4072 (240-272 nm)	0.17(-0.08)	0.48(0.27)	0.07(0.20)	0.22(-0.08)	0.52(0.38)	0.22(-0.08)	0.52(0.38)	0.08(0.20)
R7265 (272-365 nm)	0.18(-0.09)	0.48(0.17)	0.08(0.20)	0.28(0.13)	0.54(0.46)	0.28(0.13)	0.54(0.46)	0.07(0.01)
RTot (200-365 nm)	0.21(0.06)	0.53(0.38)	-0.05(0.04)	0.25(0.22)	0.55(0.54)	0.25(0.22)	0.55(0.54)	-0.06(-0.11)
Peak A (0.6-1.1 kDa)	0.21(0.06)	0.53(0.38)	-0.05(0.04)	0.25(0.22)	0.55(0.54)	0.25(0.22)	0.55(0.54)	-0.06(-0.11)
Peak B (1.6-2.1 kDa)	0.55(0.44)	0.76(0.44)	-0.21(-0.15)	0.31(0.12)	0.45(0.26)	0.31(0.12)	0.45(0.26)	-0.40(-0.28)
Peak C (2.2-3.5 kDa)	0.12(0.18)	0.39(0.51)	-0.10(-0.14)	0.19(-0.03)	0.50(0.47)	0.19(-0.03)	0.50(0.47)	0.01(0.11)

TABLE B.6: Pearson and Spearman correlation parameters for a series of integrated peak trends with 3 THM species (n = 19).
Values within brackets exclude raw water samples (n = 12).

	Pearson				Spearman			
	CHCl ₃	CHBr ₃	CHCl ₂ Br	CHCl ₃	CHBr ₃	CHCl ₃	CHBr ₃	CHCl ₂ Br
R2040 (220-240 nm)	0.54(0.63)	-0.37(-0.37)	0.18(0.06)	0.59(0.67)	-0.29(-0.17)	0.34(0.36)		
R4072 (240-272 nm)	0.49(0.34)	-0.16(-0.17)	0.18(-0.01)	0.55(0.44)	-0.12(-0.03)	0.38(0.15)		
R7265 (272-365 nm)	0.50(0.25)	-0.25(-0.22)	0.18(-0.05)	0.57(0.52)	-0.23(-0.26)	0.44(0.27)		
RTot (200-365 nm)	0.55(0.47)	-0.31(-0.32)	0.19(-0.01)	0.60(0.59)	-0.32(-0.28)	0.37(0.25)		
Peak A (0.6-1.1 kDa)	0.55(0.47)	-0.33(-0.32)	0.19(-0.01)	0.60(0.59)	-0.32(-0.28)	0.37(0.25)		
Peak B (1.6-2.1 kDa)	0.78(0.44)	-0.44(-0.02)	0.52(0.32)	0.50(0.30)	-0.41(-0.01)	0.35(0.20)		
Peak C (2.2-3.5 kDa)	0.41(0.59)	-0.27(-0.38)	0.10(-0.00)	0.52(0.51)	-0.16(-0.20)	0.29(0.18)		

C Appendix

FIGURE C.1: Sample collection locations in Manitoba, Canada. (1) Sanford, (2) Glenella (3) Pine Creek First Nations, (4) Duck Bay, (5) Morris, (6) Letellier (7) Red River.



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