Cranfield University

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Natural Organic Matter Character and Reactivity: Assessing Seasonal Variation in a Moorland Water

School of Water Sciences

EngD

Cranfield University

School of Water Sciences

Eng. D. Thesis

Academic year 2002-2003

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Natural Organic Matter Character and Reactivity: Assessing Seasonal Variation in a Moorland Water

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October 2003

This thesis is submitted in partial fulfilment of the requirements for the degree of Doctor of Engineering

ABSTRACT

Natural organic matter (NOM) is described as an intricate mixture of organic compounds that occurs universally in ground and surface waters. After treatment for potable use, there is NOM remaining in the water that reacts with the chlorine used for disinfection to form disinfection by-products (DBPs). Some of the DBPs, trihalomethanes (THMs) are regulated. Several water treatment works (WTWs) in the Yorkshire Water and United Utilities (previously North West Water) region in England have recently experienced difficulty in meeting THM limits (100 μg L^{-1}) in their finished drinking water at certain times of the year.

An investigation into how NOM changes seasonally, pragmatic methods of NOM analysis and its reactivity with chlorine was undertaken. By separating the NOM using adsorbent resins into fractions, it was possible to gain an insight into the seasonality of NOM. It was observed that a particular, difficult to remove fraction was always more reactive with respect to THM formation in autumn.

Some of the methods proposed in the literature were used here with varying successes. It was found that High Performance Size Exclusion Chromatographic methods were most useful to the WTW operators for optimising treatment processes.

It is known that the formation of DBPs is very complex. An attempt was made to predict the reactivity of a raw water in terms of THM-FP by looking at the NOM make-up. However, it was found that the fluorescence spectra combined with the fluorescence index of raw water and chlorinated samples gave more insight into the reactivity of the raw water at a particular time than knowing the fraction distribution. The use of fluorescence as a tool for understanding chlorine-NOM reactions is promising.

ACKNOWLEDGEMENTS

I would like to extend my thanks and appreciation to the UK Engineering and Physical Sciences Research Council, Yorkshire Water and United Utilities for their financial support of this project.

I would like to thank Dr Simon Parsons for his endless enthusiasm for the project and valuable advice throughout.

I'd like to thank everyone in Water Sciences, particularly the fellow NOM people, PhD Room 3, the technicians, the other EngD students and the coffee and crossword people.

Finally I would like to thank Andrew for being there and my mum for listening. I would also like to acknowledge the support of my brothers, dad, Colin, Lottie and Trevor.

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ABBREVIATIONS AND NOTATION

α	THM yield coefficient	(μg THM mg^{-1} Cl_2)
AEM	Average electrophoretic mobility	(cm^2 V^{-1} s^{-1})
AMW	Apparent molecular weight	(Daltons)
BV	Bed volume	
CE	Capillary electrophoresis	
CER	Cation exchange resin	
C_f	Final concentration of sample	(mg L^{-1})
Char	Hydrophilic charged	
^{13}C-NMR	Carbon 13 nuclear magnetic resonance	
C_0	Initial chlorine concentration	(mg L^{-1})
C_p	Permeate concentration	(mg L^{-1})
C_{r0}	Initial concentration of sample	(mg L^{-1})
CRW	Colorado River water	
DAF	Dissolved air flotation	
DAM	Dynamic adsorption experiments	
DBP	Disinfection by-product	(μg L^{-1})
DCF	Discounted cash flow	
DOC	Dissolved organic carbon	(mg L^{-1}C)
DOM	Dissolved organic matter	(mg L^{-1}C)
DWI	Drinking water inspectorate	
ε	molar absorptivity	(M^{-1} cm^{-1})
EEM	Excitation emission matrix	
EPG	Electropherogram	
f	fraction of chlorine demand attributed to rapid reactions	
F	Fraction of original sample filtered	
FA	Fulvic acid	
FAF	Fulvic acid fraction	
FCS	Fluorescence correlation spectroscopy	
FFFF	Flow field-flow fractionation	
FI	Fluorescence index	

F^p	Fraction filtered to the power of the permeate coefficient	
FT-IR	Fourier transform-infra red	
GC-MS	Gas chromatography-mass spectrometry	
GPC	Gel permeation chromatography	
HA	Humic acid	
HAA	Haloacetic acid	($\mu g\ L^{-1}$)
HAF	Humic acid fraction	
HAN	Halo acetonitrile	($\mu g\ L^{-1}$)
HPIA	Hydrophilic acid fraction	
HPI-A	Hydrophilic acid	
HPI-B	Hydrophobic base	
HPI-N	Hydrophobic neutral	
HPINA	Hydrophilic non-acid fraction	
HPLC	High performance liquid chromatography	
HPO-A	Hydrophobic acid	
HPO-B	Hydrophobic base	
HPO-N	Hydrophobic neutral	
HPSEC	High performance liquid chromatography	
IC	Inorganic carbon	($mg\ L^{-1}$ C)
I_F	Intensity of fluorescence	(arbitrary units)
k’	Column distribution coefficient	
kDa	Kilo Dalton	
k_R	1^{st} order rate constant for rapid reactions	(h^{-1})
k_S	1^{st} order rate constant for slow reactions	(h^{-1})
L	Specific conductance at 25 ºC	($mS\ cm^{-1}$)
MALLS	Multi-angle laser light scattering	
Meq	Milli equivalent	
MF	Microfiltration	
MIEX®	Magnetic ion exchange resin	
MLD	Megalitres per day	
MW	Molecular weight	(Daltons)
MWCO	Molecular weight cut off	

$N(d_i)$	Probability that a deviation of $<d_i$ will occur in a standard normal distribution	
Neut	Hydrophilic neutral	
NF	Nanofiltration	
NOM	Natural organic matter	(mg L^{-1}C)
NPV	Net present value	
OFWAT	Office of water services	
p	permeate coefficient	
P	Current value of underlying stock	(£)
POM	Particulate organic matter	
PSS	Polystyrene sulphonate	
R	Riskless rate of interest	(%)
RO	Reverse osmosis	
σ^2	Variance of rate of return on stock	(%)
SEC	Size exclusion chromatography	
SEM	Scanning electron microscopy	
SFA	Soil fulvic acid	
SFS	Spectral fluorescent signatures	
SHA	Slightly hydrophobic acid	
SUVA	Specific ultraviolet absorbance	(m^{-1}.L mg^{-1} C)
t	time	(seconds, minutes)
t	time	(days, hours, years)
TC	Total carbon	(mg L^{-1} C)
TEM	Transmission electron microscopy	
THM	Trihalomethane	(µg L^{-1})
THM-FP	Trihalomethane formation potential	(µg mg^{-1} C)
TOC	Total organic carbon	(mg L^{-1} C)
TPHA	Transphilic acid	
U_x	x^{th} percentile UV absorbance	(m^{-1})
UF	Ultrafiltration	
U_{goal}	Clarified UV absorbance goal	(m^{-1})
uHPIA	Ultra-hydrophilic acid	

URI_{95}	UV robustness index using the 95th percentile	
USEPA	United States Environmental Protection Agency	
UV	Ultraviolet	
UV_{254}	Ultraviolet absorbance at 254 nm	(m^{-1})
V	Current value of call option	(£)
V_0	Void volume	(L)
V_E	Breakthrough volume of a solute in resin	(L)
VHA	Very hydrophobic acid	
WTW	Water treatment works	
X	Strike or exercise price of option	(£)

CHAPTER 1 INTRODUCTION

1.1 Background

Natural organic matter (NOM) is described as an intricate mixture of organic compounds that occurs universally in ground and surface waters. Whilst NOM itself is not problematic, it can cause major problems as it is converted into disinfection by-products (DBPs) when chlorine is used during water treatment (Krasner *et al.* 1989). These by-products can take the form of trihalomethanes (THMs), haloacetic acids (HAAs) and a host of other halogenated DBPs, a number of which have been shown to cause cancer in laboratory animals (Singer 1999, Rodriguez *et al.* 2000). Recent legislation has reduced the THM standard in the US from 100 to 80 μg L^{-1} (Crozes *et al.* 1995, Lin *et al.* 1999) and a similar standard of 100 μg L^{-1} is in force in the UK. The European Commission has proposed tighter standards for THMs including levels of chloroform (40 μg L^{-1}) and bromodichloromethane (15 μg L^{-1}), (Drinking Water Inspectorate UK 1998, www.dwi.gov.uk).

1.2 Motivation for work

Several water treatment works (WTWs) in the Yorkshire Water and United Utilities (previously North West Water) region in England have recently experienced difficulty in meeting THM limits (100 μg L^{-1}) in their finished drinking water. This is unexpected given that the colour of the raw water has been removed down to two hazen units or less. As the colour is being removed, it appears that residual organics are labile precursors to THMs. It is known that there are periods of the year where many treatment plants (typically those with low alkalinity, highly coloured waters) are

monitoring periods of high organic loading linked to heavy rainfall or snow melt waters. It is during these periods of elevated dissolved organic carbon (DOC) that current treatment processes are failing and water quality is rapidly deteriorating with respect to DOC, colour and THM formation potential.

Over the past 10 years, the colour content of the water at Albert WTW reservoir in the Yorkshire Water region which feeds Albert WTW has increased. As a result of the increasing colour, the coagulant dose required to treat the water has also increased (figure 1.1). This increases costs associated with increased sludge production. As well as increasing year on year, the increase in colour is also seasonal with peaks occurring in autumn. This generally occurs after heavy rainfall preceded by a dry spell, but can happen any time between October and December.

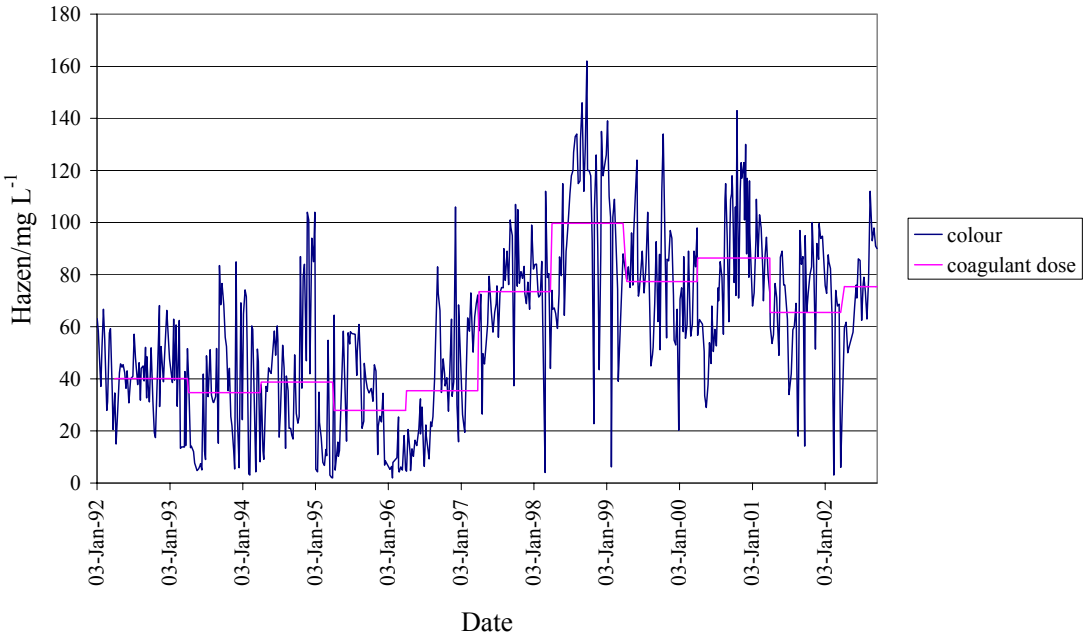


Figure 1.1 Increase in colour and coagulant dose over 10 years at Albert WTW reservoir

NOM is usually quantified by measuring the concentration of colour or DOC of the bulk water. The colour concentration will indicate the amount of coloured NOM present and

the DOC concentration will indicate how much total NOM is present in a water. However both of the measurements give little information about the nature of the organics in terms of treatability or reactivity with chlorine. By measuring bulk water parameters, such as colour and DOC, the information obtained is not detailed enough to determine how the NOM is changing seasonally. To investigate seasonal changes in more detail it was deemed necessary to separate the NOM into its component parts to assess its character.

1.3 Scope of study

This study focuses on Albert WTW in the Yorkshire Water region. Albert WTW treats reservoir water that is typical of many moorland waters. This thesis aims to investigate the character of NOM at Albert WTW and its variability throughout the year. Methods of NOM analysis will be investigated and assessed in terms of usefulness to the WTW operators. A link between character and reactivity of the NOM was investigated. This thesis aims to provide a guide on how to best assess NOM for the purposes of optimising treatment and meeting THM regulations.

1.4 Thesis plan and publications

Initially a review of the literature was carried out (Chapter 2) to determine methods of analysing water containing NOM and the information that the methods provided. As bulk water parameters were not deemed to give detailed enough information on the changing character of the water, it was separated into defined fractions using adsorbent resins. Findings of the work were presented as a poster at an international conference

(Goslan, E.H., Fearing, D.A., Banks, J., Wilson, D., Hillis, P., Campbell, A.T., and Parsons, S.A. (2001) Assessing Seasonal Variations in the Disinfection By-Product Precursor Profile of a Reservoir Water. In: American Water Works Association Water Quality Technology Conference, 11-15 November, Nashville, TN, USA.) and later published: Goslan, E.H., Fearing, D.A., Banks, J., Wilson, D., Hillis, P., Campbell, A.T., and Parsons, S.A. (2002) Seasonal Variations in the Disinfection By-Product Precursor Profile of a Reservoir Water. *Journal of Water Supply: Research and Technology – AQUA*, **51** (8), 475-482. The paper highlighted the information that could be gained by fractionating the NOM into defined parts rather than considering it as a whole. The limitations of the methodology used were also discussed. The samples produced by fractionation of the bulk water were subjected to staged treatment by coagulation to determine their treatability. The work carried out was published: Fearing, D.A., Goslan, E.H., Banks, J., Wilson, D., Hillis, P., Campbell, A.T., and Parsons, S.A. (in press) Staged Coagulation for the Treatment of Refractory Organics. *Journal of Environmental Engineering-ASCE.*

Further samples were taken and fractionated in the same way to determine if the relationships found for the original water samples still held. All characterisation information is reported in the Results and Discussion chapter (Chapter 5, Section 5.2).

The information obtained by fractionating the NOM was useful in understanding the character of the water but was a time-consuming process (> 1 month per sample). By the time the water had been fully 'characterised', it was too late for the information gleaned to be of use to the WTW operators. Therefore it is key to be able to determine

the character of the water more quickly. An excel macro was developed using information obtained from measuring the fluorescence spectra of the reservoir water and its corresponding fractions. This allowed the character of the water to be determined in less than an hour. The development of the model has been published (Goslan, E.H., Voros, S., Banks, J., Wilson, D., Hillis, P., Campbell, A.T., and Parsons, S.A. (in press) A Model for Predicting Dissolved Organic Carbon Distribution in a Reservoir Water using Fluorescence Spectroscopy. *Water Research*) and is discussed in the thesis (Chapter 5, Section 5.4).

Once the model had been tested, it was applied to samples from Albert WTW that were taken fortnightly over a period of six months. The model identified two samples that had very different characters. An investigation into the reactivity of these samples with chlorine was carried out. The fluorescence spectra of the two samples was studied before and after chlorination in order to investigate a link between character and reactivity. The work carried out was presented at an international conference as a paper (International Humic Substances Society, 9th Chapter, 19th –21st May 2003, Sundsvall, Sweden) and was submitted for publication: Goslan, E. H. and Parsons, S. A. (submitted) The use of fluorescence spectroscopy for investigating the seasonal variation in make-up and reactivity of natural organic matter. *Aquatic Sciences.* The analysis and findings are discussed in the thesis (Chapter 5, Section 5.5).

It was clear from the research carried out that investment will be required to be made at Albert WTW in order to continue to meet consents in the future. An economic assessment has been carried out to determine what investment should be made and also

when (Chapter 6).

The conclusions drawn from the work were discussed (Chapter 7) and suggestions for further work made (Chapter 8).

CHAPTER 2 LITERATURE REVIEW

2.1 Introduction

Natural organic matter (NOM) is described as an intricate mix of organic compounds that occur universally in ground and surface waters. NOM can cause major problems as it is converted into disinfection by-products (DBPs) upon chlorination (Krasner *et al*. 1989). These by-products consist of trihalomethanes (THMs), haloacetic acids (HAAs), halo-acetonitriles (HANs) and other halogenated DBPs. A number of the DBPs have been shown to cause cancer in laboratory animals (Singer 1999). The removal of NOM and hence reduction in DBPs is a major goal in the treatment of any water source. Due to the complex nature of the organic compounds, the concentration of NOM is expressed as dissolved organic carbon content (DOC) measured in milligrams per litre (mg L^{-1}) (Suffet and MacCarthy 1989).

Although many of the studies into the effects of DBPs on health have proved inconclusive, the United States Environmental Protection Agency (USEPA) recently proposed a two stage Disinfectants/Disinfection by-products (D/DBP) rule. During stage I the consent level for the total of 4 THMs (TTHMs: chloroform, dibromochloromethane, dichlorobromomethane, and bromoform) was reduced from 100 μg L^{-1} to 80 μg L^{-1} and the consent on the total of 5 haloacetic acids (HAA5: monochloro-, dichloro-, trichloro-, monobromo-, and dibromo-acetic acids) was set at 60 μg L^{-1}. Stage II is expected to reduce the consents to 40 and 30 μg L^{-1} for TTHMs and HAA5 respectively (Kitis *et al*. 2001a). The standard for THMs in the UK changes

from a three-month average of 100 μg L^{-1} to a limit of 100 μg L^{-1} for a single sample in December 2003 (New Drinking Water Regulations in the UK, 1998, www.dwi.gov.uk). There are currently no regulations regarding regulation of HAAs in European or UK legislation.

Dissolved organic matter is said to be (1) microbially derived (autochthonous), resulting from such processes as extracellular release and leachate of algae and bacteria and (2) terrestrially derived (allochthonous), originating from decomposition and leaching of plant and soil organic matter (McKnight *et al.* 2001). NOM consists of humic (non-polar) and non-humic (polar) substances, generally of terrestrial and biological origin respectively (Hwang *et al*. 2001). Non-polar NOM has been well characterised and is readily removed by coagulation. In contrast, polar NOM has been studied less extensively and is not readily removed by coagulation.

NOM has been characterised by a wide range of analytical techniques some of which will be discussed in this review. Characterisation is typically undertaken on either bulk NOM material or fractionated NOM material. The advantage of bulk water studies is that 100% of the NOM is present in an unaltered state. Results from such studies are directly applicable for the specific reaction conditions and water. The disadvantage is that the factors that effect the reactions of specific types of NOM cannot be identified or understood. In some waters, the influence of the inorganic matrix may impede understanding of the behaviour of NOM. The study of isolated NOM fractions can provide operationally defined samples for examination of NOM behaviour although synergistic effects are lost (Hwang *et al*. 2001). Concentrated samples produced by

fractionation can be further analysed by techniques such as elemental analysis, pyrolysis - gas chromatography/mass spectrometry (GC/MS), carbon-13 nuclear magnetic resonance (^{13}C-NMR) and capillary electrophoresis to elucidate structure. It would not be possible to carry out such analyses on bulk water samples due to the high concentrations of DOC required. A combination of bulk and isolation studies is needed to retain the advantages of both approaches while minimising their disadvantages. This review will focus on the information that can be obtained by different characterisation techniques and how the information is used.

2.2 Bulk characterisation of NOM

A review of the literature has identified a number of relationships between water quality parameters and the character and reactivity of NOM. Parameters measured include DOC (mg L^{-1}), ultraviolet absorbance at 254 nm (UV_{254} (m^{-1})) and THMs formed (μg L^{-1}). THM data is often reported as THM formation potential (THM-FP) where the THMs formed are divided by the DOC of the sample to give the μg of THMs formed per mg of carbon in the sample (μg THM mg^{-1} C).

The key relationships are:

- The higher the DOC, the higher the UV_{254}
- The higher the DOC, the higher the concentration of THMs produced
- The higher the SUVA, the higher the THM-FP (SUVA, is defined as the UV absorbance of a given sample determined at 254 nm and divided by the DOC concentration of the solution (expressed in m^{-1}. L mg^{-1} C)).

Data from 88 water sources from 10 references (Allgeier and Summers 1995, Amy *et al*. 1987, Collins *et al*. 1986, Croué *et al.* 1993a, Nokes *et al*. 1999, Ratnaweera *et al*. 1999, Siddiqui *et al*. 2000, Vilge-Ritter *et al*. 1999, Volk *et al*. 2000 and White *et al*. 1997) was collated and a good linear relationship between DOC and UV_{254} was observed with an R^2 value of 0.93 (figure 2.1).

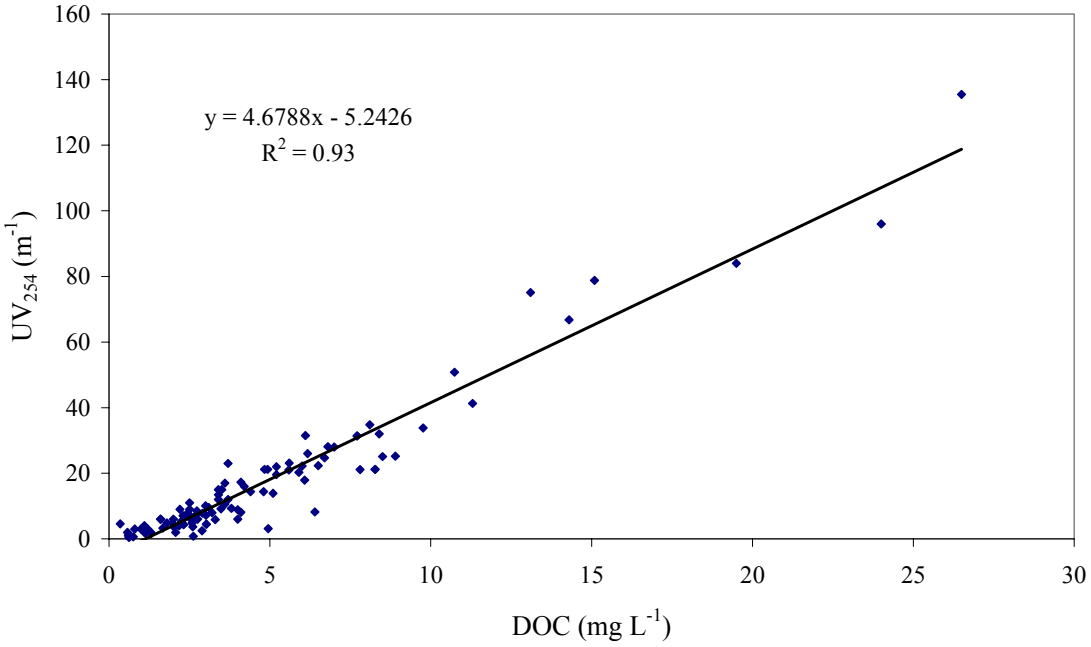


Figure 2.1 Relationship between DOC and UV_{254} for a range of 88 source waters

To investigate the relationship between DOC and THMs formed, data from 55 different source waters and 8 references (Allgeier and Summers 1995, Amy *et al*. 1987, Afcharian *et al*. 1997, Collins *et al*. 1986, Kitis *et al*. 2001a, Nokes *et al*. 1999, Pomes *et al*. 1999 and Singer *et al*. 1995) was plotted and it shows that as DOC increases so do the THMs formed (figure 2.2). The correlation found (R^2 = 0.55) was weak. The relationship between DOC and THMs formed is dependent on the water having a high humic component (Owen *et al.* 1993). The waters reported here are from a range of

sources and do not all have a high humic content. Excellent correlations have been reported between DOC and THM-FP for a single water but when waters from different sources are included, the correlations are not as good. This is because waters from different sources tend to have different specific THM yields as determined by their particular watershed characteristics (Reckhow and Singer 1990).

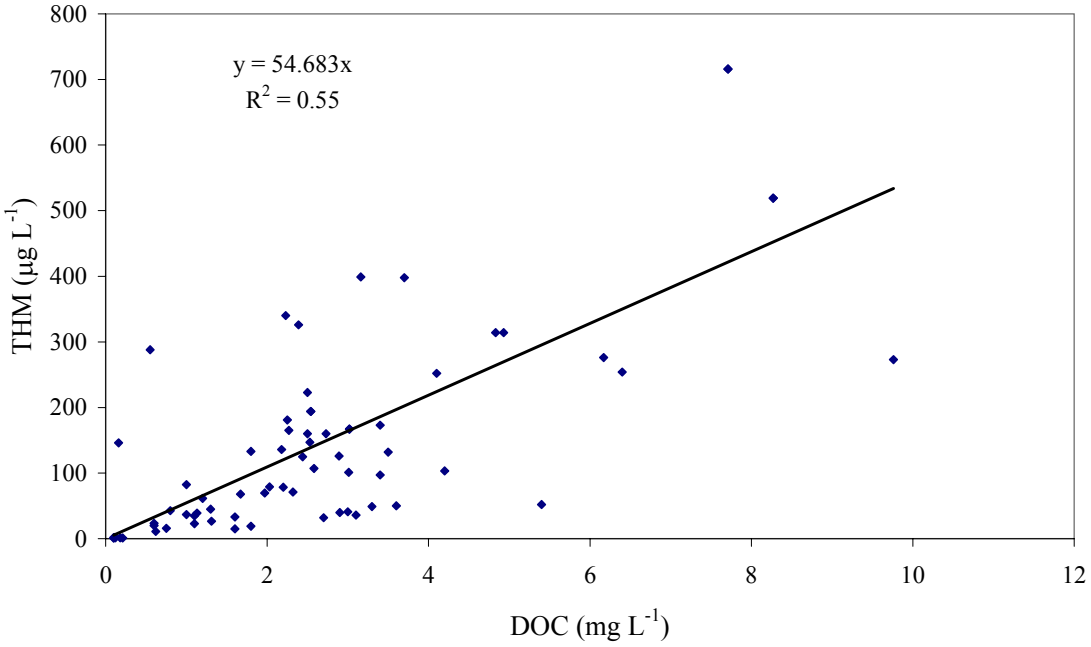


Figure 2.2 Relationship between DOC and THM for a range of 55 source waters

A useful indicator of NOM character is specific UV absorbance (SUVA). SUVA, is defined as the UV absorbance of a given sample determined at 254 nm and divided by the DOC concentration of the solution (expressed in m^{-1}. L mg^{-1} C). Edzwald and Tobiason (1999) first defined guidelines for SUVA and showed a good correlation between the value and the nature of the organic material (table 2.1).

SUVA has also been shown to correlate well with the aromatic content of NOM (Krasner *et al.* 1996, Croué *et al*. 1999a). The more aromatic the NOM the more hydrophobic it will be leading to a water with a higher SUVA.

Table 2.1 Guidelines for the nature of NOM and expected DOC removal by coagulation (Edzwald and Tobiason 1999)

SUVA	Composition	Coagulation	DOC Removal
>4	Mostly aquatic humics. High hydrophobicity, High MW	NOM Controls. Good DOC removal	>50% for Alum >50% for Ferric
2-4	Mixture of aquatic humics and other NOM. Mixture of hydrophobic and hydrophilic NOM, mixture of MWs	NOM influences. DOC removals OK	25-50% for Alum Little greater for Ferric
<2	Mostly Non-Humics. Low hydrophobicity. Low MW	NOM has little influence. Poor DOC removal	<25% for Alum Little greater for Ferric

A plot of THM-FP against SUVA data from the literature (40 bulk waters from 6 references, Allgeier and Summers 1995, Amy *et al*. 1987, Afcharian *et al*. 1997, Collins *et al*. 1986, Kitis *et al*. 2001) was prepared (figure 2.3) and the correlation found was poor (R^2 = 0.47). This is in contrast with Reckhow *et al*. (1990) who reported an R^2 value of 0.78. However the reported value was for separated NOM fractions at one site, not bulk waters.

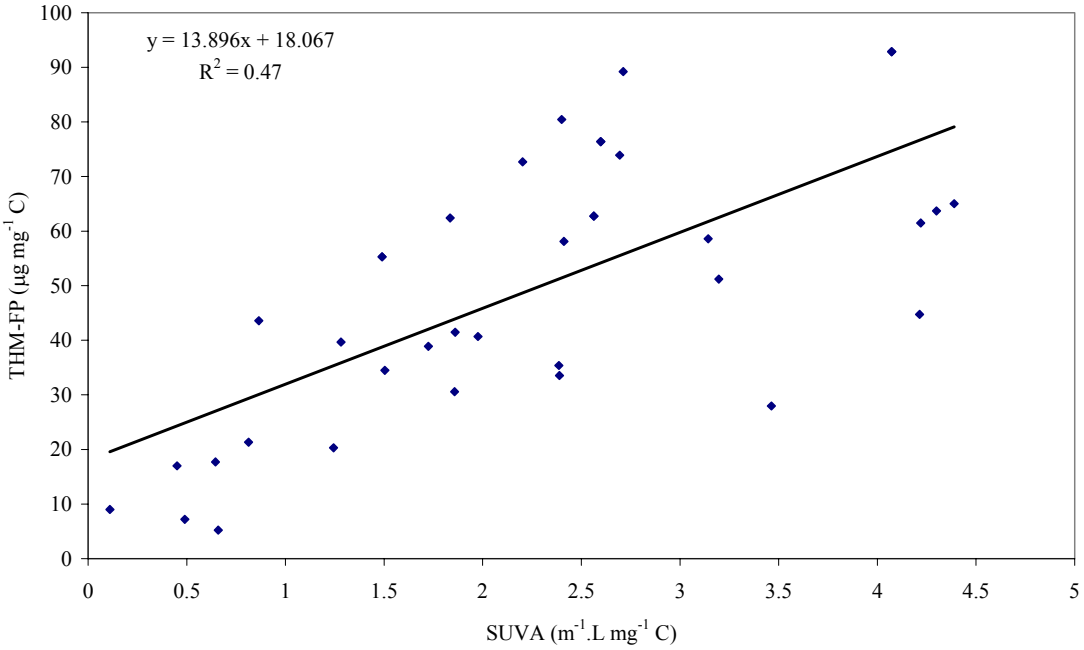


Figure 2.3 Relationship between SUVA and THM-FP for a range of 40 waters

The relationships shown here indicate that it is possible to gain information on the character of a water but not its specific reactivity by measuring bulk parameters. However a fuller understanding of the behaviour of a water will require operationally defined separation of the NOM.

2.3 Fractionation of NOM

The literature on NOM characterisation nearly always focuses on dissolved organic matter as in practice, particulate organic matter (POM) is separated from the aqueous phase by filtration through 0.45 μm pore filters. Once the POM has been removed, the choice of which separation method to use for isolation/concentration/fractionation will depend on the objectives of the study, the equipment available and the DOC concentration of the water.

Typically the bulk organic matter can be separated on the basis of either molecular charge or size and a range of options for concentration, isolation and fractionation are outlined in figure 2.4. The options for separating the NOM on the basis of adsorption/charge are resin fractionation, chromatography and less often, mineral adsorption. To separate the NOM on the basis of the size of the molecules, the options include membrane fractionation, flow field-flow fractionation (FFFF) and again chromatography. Chromatography is included in both categories as size exclusion chromatography columns can be used to separate organic matter on the basis of molecular size whilst normal chromatographic columns can be used to separate the organic matter on the basis of polarity.

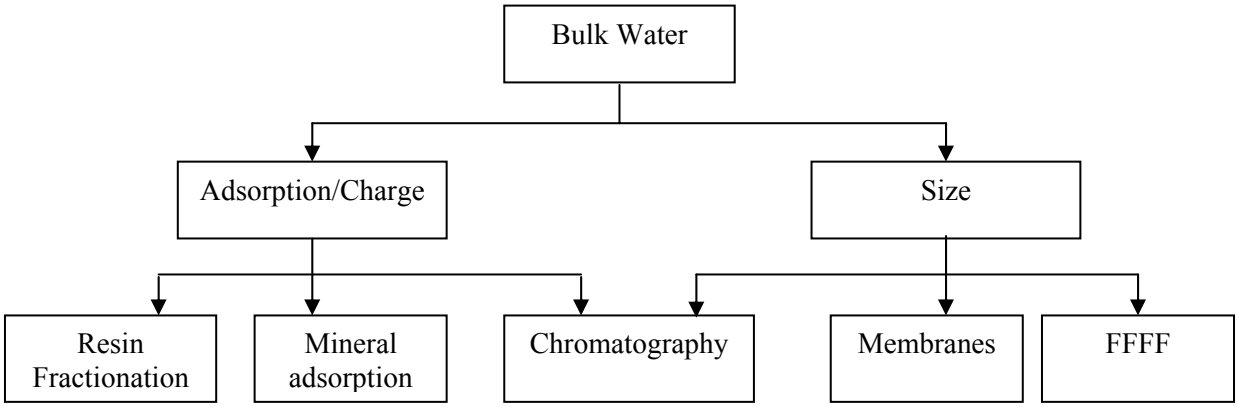


Figure 2.4 Options for isolation, concentration and fractionation of NOM

The study of the chemistry and characteristics of aquatic NOM is dependent on the limitations imposed on the sample by the methods used for isolation of the organic material. The isolated materials are operationally defined based on the concentration/fractionation procedure. For example, in the environment, NOM exists at relatively low concentrations and interacts with other dissolved species, both organic and inorganic. As part of the process of isolating these materials, many interactions are disrupted and changes in the chemical structure of the compounds themselves may result (Aiken 1988). It is important that results of analyses on extracted material are interpreted with the drawbacks of the extraction procedure in mind.

2.3.1 Fractionation by adsorption

2.3.1.1 Resin fractionation

A review of the use of resins for fractionation of organic matter has been carried out. Amberlite XAD non-ionic resins have been used extensively as adsorbents for organic solutes from water over the last 30 years. These resins have a large surface area and sorption of organic acids is determined by the solute's aqueous solubility and the solution pH (Cheng 1977). At low pH, weak acids are protonated and absorbed on the resin. At high pH, weak acids are ionised and desorption is favoured. Differences in resin pore size, surface area and chemical composition result in different capacity factors for the same solute on each resin. The properties of the XAD resins are shown (table 2.2).

Table 2.2 Resin Properties (adapted from Aiken *et al*. 1979)

XAD Resin	Pore Size (Å)	Surface Area ($m^2 g^{-1}$)	Polymer Composition	Polarity
1	200	100	Styrene divinylbenzene	None
2	90	330	Styrene divinylbenzene	None
4	50	750	Styrene divinylbenzene	None
7	80	450	Acrylic ester	Slight
8	250	140	Acrylic ester	Slight

Ion-exchange resins are often used in conjunction with XAD resins in order to further separate the organic matter and also to hydrogen saturate the isolated organic matter. These resins include Duolite A-7 (weak base, macroporous anionic exchange resin which is a phenol-formaldehyde condensation product), DEAE cellulose (a weakly basic anion exchange resin) and Bio-Rad AG-MP-50 (a non-macroporous cation exchange resin).

The use of these resins was explored and validated throughout the late 1970s to the 1990s (Kim *et al.* 1976, Van Rossum and Webb 1978, Leenheer 1981, Thurman and Malcolm 1981, Blok *et al.* 1983, Kunte and Pfeiffer 1985) and reviews carried out (Aiken 1985, 1988, Giabbai *et al.* 1987, Ben-Poorat *et al.* 1987, Le Cloirec *et al.* 1990).

Aiken (1988) concluded that the use of XAD resins for the isolation of humic substances from water are the most efficient and allow processing of large amounts of water. His critical review investigated all parts of the isolation procedure including initial filtration of the sample, flow characteristics of the column, sample preservation and the advantages and disadvantages of the use of nonionic macroporous sorbents. The evidence gathered in the reviews culminated in a study outlining the factors to be considered when isolating and characterising aquatic humic substances using Amberlite resins (Malcolm 1991). The main points are summarised below:

- Proper and complete cleaning of resins is vital to ensure that there are no impurities in the resin that may contaminate the isolated organic material.
- Resin columns do not have an infinite capacity for solute sorption and the sample volume and resin volume should reflect this.
- The amount of concentrated material that can be produced depends on the money and time available.
- With the correct precautions when handling and storing resins, quality results can be produced.
- Excessive resin bleed precluded the use of XAD-7 for concentration of humic substances from water.
- Testing of XAD-1 discontinued as its manufacture was stopped.

- XAD-8 exhibited the highest sorptive capacity for humic solutes and yielded 100 percent recovery of sorbed humic substances compared to XAD-2 and XAD-4 where the recovery of humic solutes ranged from 75 - 85 percent.

Once Amberlite XAD-8 was established as the most suitable resin for humic substance absorption and the validity of the fractions had been accepted, two defined methods were developed based on the methods of Leenheer (1981) and Thurman and Malcolm (1981). The first quantitatively evaluated the use of Amberlite XAD-8 and XAD-4 resins in tandem to separate hydrophobic acids and neutrals and hydrophilic acid (Malcolm and MacCarthy 1992). The second also used the XAD-8/XAD-4 two-column array to separate the organic matter into its hydrophobic and hydrophilic components (Aiken *et al.* 1992). Due to its small pore size and large surface area compared to XAD-8, XAD-4 was deemed more suitable for adsorbing non-humic organic substances (i.e. hydrophilic organic matter with a smaller molecular weight).

As previously mentioned, the isolated materials are operationally defined based on the fractionation procedure. Operational definitions of the fractions obtained using the XAD-8/XAD-4 two column are as follows (Aiken *et al.* 1992):

Hydrophobic acid fraction - that portion of the DOC that sorbs on a column of XAD-8 at pH 2 and are eluted at pH 13. This fraction can contain aliphatic carboxylic acids of 5-9 carbons, one- and two- ring aromatic carboxylic acids, one- and two- ring phenols and aquatic humic substances.

Hydrophilic acid fraction - that portion of the DOC contained in the XAD-8 effluent at pH 2 that sorbs on a column of XAD-4 resin and are eluted at pH 13. This fraction can contain polyfunctional organic acids and aliphatic acids of five or fewer carbons.

From these definitions it can be seen that the hydrophobic acids are large complex organic molecules that are aromatic in character whereas the hydrophilic acids are much smaller and simpler aliphatic molecules.

Although Amberlite XAD-8 resin had been used extensively to isolate and concentrate hydrophobic organic matter, further justification was sought for the validation of the humic substances isolated by XAD-8 and its suitability for concentrating humic substances (table 2.3). It was found that the use of XAD-8 as a concentration technique was unsuitable for determining relative polarity (Owen *et al.* 1995). However, the fractions produced using XAD-8 were found to be valid (Hautala *et al*. 1998, Tadanier *et al*. 1999). One study (Frimmel and Abbt-Braun 1999) emphasised that a well-defined sample treatment and isolation procedure were required for comparable results.

Table 2.3 Validation of Amberlite XAD-8 Resin for humic substance concentration

Application	NOM source	Outcome	Recovery of DOC	Reference
Separate the NOM into humics and non-humic substances for further analysis.	A wide range of different waters from aquifers, rivers and reservoirs.	Resin adsorption as a concentration technique was unsuitable for determining relative polarity as concentration creates analytical problems.	-	Owen *et al.* 1995
After being desorbed from the resin hydrophobic acids were separated into humic and fulvic acids.	Lake water.	When comparing XAD-8 resin against DEAE cellulose resin (carried out at neutral pH), the humic solutes isolated by the XAD technique are real rather than accidental products of the isolation procedure.	67%	Hautala *et al.* 1998
Hydrophobic acids were isolated and separated into humic and fulvic acid for further analysis.	A range of waters from soil seepage, groundwater and secondary effluent.	The combination of a constant source, a well-defined sample treatment and isolation procedure can develop the basis for comparable experimental results.	20 – 55%	Frimmel and Abbt-Braun 1999
Used alongside CERs to separate the NOM into hydrophobic and hydrophilic acid, bases and neutrals.	River and lake surface water.	Limitations of the method were highlighted. Use of chemically fractionated NOM in reactivity studies was warranted.	96 – 101%	Tadanier *et al.* 1999

CER – cation exchange resin

The use of XAD-8/XAD-4 in tandem has also been studied extensively. Often a third resin is used after the Amberlite resins to further separate the hydrophilic organic matter although this step may be omitted depending on the degree of separation required by the analyst. Throughout the fractionation of NOM using resins there has been no set procedure. Each analyst has sought to improve the basic methods set down by Leenheer (1981) and Thurman and Malcolm (1981) and later by Aiken *et al.* (1992) and Malcolm and MacCarthy (1992). This has led to many different names for each isolated fraction as well as different definitions. A summary of the different fractions obtained from varying methods of fractionation is presented (table 2.4).

Table 2.4 Fractions produced (abbreviations and definitions)

Fraction	Abbreviation	Definition	Reference
Fulvic acid Humic acid	FA HA	Absorbed on XAD-8 at pH 2 and desorbed at pH 13. Acidified to pH 1 where HA precipitates and FA remains soluble	Thurman and Malcolm 1981
Non-humic hydrophobic acids (low molecular weight)	None given	Non-adsorbed effluent separated from humics by chromatography on Encrazyl gel at pH 13	
Hydrophobic base	HPO-B	Adsorbed onto XAD-8 at neutral pH and desorbed at pH 2	Leenheer 1981
Hydrophobic acid	HPO-A	Adsorbed onto XAD-8 at pH 2 and desorbed at pH 13	
Hydrophobic neutral	HPO-N	Desorbed from XAD-8 resin by soxhlet extraction with methanol	
Hydrophilic base	HPI-B	XAD-8 effluent adsorbed onto AG-MP-50 and desorbed with NH_4OH	
Hydrophilic acid	HPI-A	AG-MP-50 effluent absorbed onto Duolite A-7 and desorbed with NH_4OH	
Hydrophilic neutral	HPI-N	Non-absorbed effluent from all columns	
Fulvic acid Humic acid Hydrophobic neutrals XAD-4 acids	FA HA HPO-N XAD-4 acids	As Thurman and Malcolm (1981) As Thurman and Malcolm (1981) As Leenheer (1981) XAD-8 effluent adsorbed onto XAD-4 at pH 2 and desorbed at pH 13	Malcolm and MacCarthy 1992
Fulvic acid Hydrophilic acid	FA HPI-A	As Thurman and Malcolm (1981) As XAD-4 acids	Aiken *et al.* 1992
Very hydrophobic acid	VHA	Adsorbed onto Supelite DAX – 8 at pH 2 and desorbed at pH 13	Bolto *et al.* 1999
Slightly hydrophobic acid	SHA	Adsorbed onto Amberlite XAD-4 at pH 2 and desorbed at pH 13	
Hydrophilic charged	Char	Anionic material absorbed onto Amberlite IRA-958	
Hydrophilic neutral	Neut	Not adsorbed on DAX-8, XAD-4 or IRA-958	
Fulvic acid Humic acid Transphilic acid Hydrophilic	FA HA TPHA HPI	As Thurman and Malcolm (1981) As Thurman and Malcolm (1981) As XAD-4 acids Adsorbed onto XAD-4 on second exposure but not first	Croué 1999
Ultra-hydrophilic acid	uHPIA	Not adsorbed onto XAD-8 or XAD-4	

Note: DAX-8 has been used as a substitute for XAD-8 as its manufacture has been discontinued.

There are other abbreviations and definitions for fractions but the above methods have been presented as they are used most frequently. Other methods generally involve making the same fractions but different names have been given depending on the preference of the author.

When most analysts have used their own method for fractionating organic matter, subsequent analyses of the fractions are difficult to compare against other fractions that have been produced by a different method. The International Humic Substances Society (IHSS) used a previously developed method (Thurman and Malcolm 1981) to produce standard humic and fulvic acids from a variety of sources for commercial use.

By 1999, organisations and universities from the USA, Canada, the UK and New Zealand were involved in the standardisation of humic substances as part of the IHSS. The next major collaboration to study organic matter was the HUMEX project (1988). This was an EC project where there was input from several universities. The project involved splitting a lake in Norway into two parts and one side was acidified in order to determine the effect of acid rain on organic matter. Another more recent project is ROSIG (refractory organic acids in aquatic systems), a German interdisciplinary research project (http://ebiwat24.ciw.uni-karlsruhe.de/index.en.html). The separation of the organic matter for ROSIG is the same as the IHSS method with humic acid, fulvic acid and non-humic substances (NHS) being isolated for analysis.

These projects are useful if an analyst is interested in only humic and fulvic acid or in the effects of acid rain on organic matter. There are many other reasons for studying organic matter. Tests have shown that the structure and characteristics of organic matter are dependent on its source (Owen *et al.* 1995, Krasner *et al.* 1996). Therefore, solving a source related problem would not be possible using organic matter from a different source no matter how standardised the method of preparation may be. Where there is a particular problem, for example with organic matter acting as a disinfection by-product

(DBP) precursor, studying the organic matter from its source is the only way to glean information that will be useful in controlling the formation of DBPs.

The use of XAD-8/XAD-4 resins in tandem has been thoroughly researched and validated by comparison with other techniques. Each of the studies outlined (table 2.5) emphasises the importance of isolating hydrophilic organic matter as it can be just as problematic and reactive with chlorine as hydrophobic organic matter. Further tests on isolated fractions can reveal information about their character. This will be further explored in section 2.4.

Table 2.5 Characterisation of NOM using XAD-8 and XAD-4 Amberlite Resins in Tandem

NOM Source	Fractions Separated	What was found?	Recovery	Ref
Artificially acidified and control lake water	HPO-A (FA and HA) HPO-N XAD-4 acids	Both sides of lake had almost identical fractions	>85%	Malcolm and MacCarthy 1992
Surface water and contaminated groundwater	HPO-A HPI-A	Importance of isolating HPI-A highlighted	83% maximum	Aiken *et al.* 1992
River and lake water	HPO-A (FA and HA) HPI-A HPI-B	Isolated fractions were similar to IHSS fractions	65 – 80%	Andrews and Huck 1993
Reservoir water	HPO-A (FA and HA) HPO-N HPI-A HPI-N	Hydrophilic acids are less aromatic than FA although they show a similar reactivity	75%	Croué *et al.* 1993a
River and reservoir water	HPO-A HPI-A HPI-N	HPO-A more abundant in reservoir waters. HPI-A more abundant in river waters	55 – 88%	Martin-Mousset *et al.* 1997
Various surface waters	HPO-A (FA and HA) HPO-N HPI-A	HPO/HPI split was 50:50. Structure of HPI-A was less aromatic than HPO-A	75%	Krasner *et al.* 1996
Artificially acidified and control lake water	HPO-A (FA and HA) HPO-N XAD-4 acids	Differences in aromaticity between both sides observed	80-85%	Knulst *et al.* 1998
Canal water	HPO-B HPO-A HPI-A HPI-N	HPI compounds are as reactive as HPO compounds	100%	Liu and Tao 1998
Artificially acidified and control lake water as well as reservoir water	HPO-A (FA and HA) TPHA HPI UHPIA	THPA organic matter has a lower molecular weight compared to HPO-A	-	Croué 1999

It should be remembered that the fraction definitions are not absolute. In the hydrophobic fraction there will be some hydrophilic organic matter such as carbohydrates and polysaccharides. One study has proposed that the basis of separation of the DOC should be between carbohydrates and non-carbohydrates (Boult *et al.* 2001). The use of a periodic acid-Schiff reagent is suggested as an index of

carbohydrate content of natural waters. This is a new approach to separating natural organic matter and it remains to be seen if it will endure.

2.3.1.2 Mineral adsorption

Mineral adsorption has been little used to fractionate NOM. The studies of minerals tend to focus on the general adsorption of NOM by minerals. Meier *et al.* (1999) used goethite (α-FeOOH) and kaolinite ($Al_2Si_2O_5(OH)_4$) to fractionate aquatic NOM. They demonstrated that the molecular weight (MW) of solution NOM decreases in the presence of absorbing clay minerals, suggesting that larger molecular weight and more aromatic moieties are preferentially sorbed to goethite and kaolinite. The results suggest that NOM sorption to clay minerals may influence the distribution, size, and reactivity of NOM components in natural aquatic systems. However, there was no desorption or further analysis of the sorbed material, only investigation into the size of the unadsorbed material. A later study (Zhou *et al.* 2001) investigated the size fractionation on adsorption of fulvic acid onto goethite. At low pH, high MW fractions were adsorbed. At a high pH, intermediate MW fractions were adsorbed.

2.3.2 Fractionation by size

The determination of molecular weight can be used to help identify and understand the basic chemistry of unknown organic compounds. Knowledge of molecular weights is important for several reasons (Wershaw and Aiken 1985):

1. To establish approximate molecular formulas in conjunction with data provided by other methods of characterisation.
2. To help establish stoichiometric relationships between humic substances and other chemical species through the conversion of weight to molar concentrations.

3. To aid in the comparison of humic substances extracted from various environments.

In 1989, the methods being used to study the molecular weight and size of NOM included ultracentrifugation, viscosity measurements, colligative property measurements, scattering techniques, gel chromatography, electrophoresis and electron microscopy (Hayes *et al.* 1989). These are briefly described below:

- Ultracentrifugation – an ultracentrifuge will commonly develop a centrifugal field in the range 100 000 – 600 000 Da. In such a field, molecules in solution undergo sedimentation and under suitable conditions the velocity of the sedimentation can be used to determine molecular size and shape.
- Viscosity measurements – viscosity is a measurement of the frictional resistance that a flowing liquid offers to an applied shearing force. Viscosity measurements on dissolved humic and fulvic acids can provide useful information about their particle size, shape, weight, polyelectrolytic behaviour and interaction with other macromolecules.
- Colligative property – a thermodynamic property that depends on the number of particles in solution and not on the nature of the particles. The colligative property measurements used are vapour pressure lowering and freezing point depression to determine number average molecular weights.
- Scattering techniques – light scattering and X-ray scattering have been used to measure the size and shape of particles of humic substances. Elastic scattered radiation is measured and extrapolated to give information on the particle size and shape.

- Gel chromatography – a separation technique in which solute molecules are separated according to their molecular dimensions. The separation is performed in columns by elution of a sample through a bed of porous beads (molecular sieves) that are generally composed of gel forming polymers.
- Electrophoresis – in addition to the use of electrophoresis for separation and fractionation, it can also be used to obtain information on the size and charge characteristics of the molecules or ions being separated.
- Electron microscopy – both transmission (TEM) and scanning electron microscopy (SEM) have been used to determine the morphological conformation of humic and fulvic acid molecular aggregates and their interactions. However, changes in molecular conformation are likely to occur as a result of sample drying.

More recently, methods that have been used to fractionate NOM on the basis of molecular size include membranes, flow field flow fractionation (FFFF) and chromatographic methods (HPSEC, GPC), as well as novel techniques such as fluorescence correlation spectroscopy (FCS), multi-angle laser light scattering (MALLS) and dynamic adsorption experiments (DAM) employed by Egeberg *et al*. (2002). These novel methods are described briefly for information:

- FCS – a laser light is focused into the sample of interest using confocal optics. A small, illuminated volume element called the confocal volume is created. At any point in time, the confocal volume is occupied by one or a few fluorescent molecules. Temporal fluctuations in the measured fluorescent intensity are used to derive an autocorrelation curve. This curve is related to the translational diffusion of the fluorophore across the confocal volume. Therefore diffusion times of the

fluorescent molecules through the confocal volume can be determined. The size of the molecule can be calculated if the diffusion time and confocal volume are known using a mathematical equation (Lead *et al.* 1999).

- MALLS – a technique used for measuring the classical, time-averaged angular dependence of the scattering of light by molecules in solution. It differs from SEC, UF, viscometry and ultracentrifugation in that it does not require a *priori* assumption about molecular conformation. MALLS gives information on molar mass and r.m.s. (root mean square) radii (Wagoner *et al.* 1997).
- DAM – NOM isotherms on activated carbon and alumina are determined. From these, diffusion coefficients are derived using software and from the diffusion coefficients, size information can be extrapolated (Fettig 1999).

2.3.2.1 Fractionation by membranes

Membranes can be used for either removal of NOM, concentration of NOM or characterisation of NOM. Microfiltration (Suzuki *et al*. 1998, Schäfer *et al.* 2000, 2001), ultrafiltration (Adham *et al.* 1991, 1993, Jacangelo *et al.* 1995, 1995a, Maartens *et al.* 1998, Cho *et al.* 1999, Amy and Cho 1999, Bian *et al.* 1999, Lin *et al.* 1999, 2001, Maartens *et al.* 1999, 1999a, Thorsen 1999, Ødegaard *et al.* 1999, Cho *et al*. 2000, Siddiqui *et al.* 2000, Aoustin *et al*. 2001, Schäfer *et al.* 2001), and nanofiltration (Fu *et al.* 1994, Allgeier and Summers 1995, Jacangelo *et al.* 1995, Nilson and DiGiano 1996, Bian *et al.* 1999, Cho *et al.* 1999, Siddiqui *et al.* 2000, Schäfer *et al.* 2001) have all been used with pre-treatment for removal of NOM. The major findings are that MF, UF and NF can be effective for removing NOM if suitable treatment is used up stream of the MF and UF, although is not effective without significant pre-treatment. Reverse

Osmosis (RO) membranes can be used to concentrate NOM for further analysis (Münster 1999). This has the advantage of utilising ambient conditions to minimise the possibility of destructive chemical reactions. In addition, large volumes of water can be processed easily but there is the disadvantage that all solutes, not just NOM, are concentrated (Aiken 1985). RO concentration is designed to concentrate a broad spectrum of organic matter rather than separating the NOM for further analysis (Maurice *et al.* 2002). Therefore the focus, in this section, will be on UF membranes for fractionation and characterisation of NOM.

2.3.2.2 Ultrafiltration

Ultrafiltration is a method of separating macromolecules according to molecular size, by filtration under an applied hydrostatic pressure through a membrane. Ultrafiltration separates particles in the range of 10 times the size of the solvent molecules up to approximately 0.5 μm. Many different types of membrane are commercially available with a wide range of nominal molecular weight cut-offs (500 to 10^6 daltons). Although membranes are classified by the manufacturer according to molecular weight cut-off, it should be emphasised that solute molecules are separated according to molecular size in ultrafiltration. The molecular size will be dependent on molecular charge and configuration (Wershaw and Aiken 1985). The effect of the membrane material will also have an effect on the separation achieved (Laîné *et al.* 1989).

Table 2.6 Characterisation of NOM using ultrafiltration (UF) membranes

Purpose	Membrane material	MWCO (Daltons)	Outcome	Reference
Determine effect of character of organic matter on removal by investigating size distribution and reactivity	Cellulose acetate Regenerated cellulose	500 1000 5000 10 000 30 000	Comparison with GPC showed GPC indicated higher MW than UF. THM yield increased as a function of molecular weight	Collins *et al.* 1986
Investigate reactivity of and effect of pH on UF fractions	Not reported	500 1000 5000 10 000 30 000	High MW fractions were more reactive with chlorine. No difference was observed in the carbon content of the size fractions when the pH was elevated from 7 to 9	Amy *et al.* 1987
Determine size distribution of DOM and effect of membrane rejection on size distribution	Cellulose acetate Regenerated cellulose	500 1000 5000 10 000 30 000	Precise estimates of MW using UF require delineation of membrane rejection properties. A model was developed to adjust experimental data to more accurately reflect MW.	Logan and Jiang 1990
Determine size distribution to gain an insight into treatment process selection and applicability	Cellulose acetate Regenerated cellulose	500 1000 5000 10 000 30 000	Raw water MW distribution provided insight into process applicability and treated water distribution demonstrated the hypothesised effects of treatment	Amy *et al.* 1992
Fractionation of fulvic acid using UF to investigate relationship between MW distribution and acidity	Cellulose acetate Regenerated cellulose	500 1000 5000 10 000	A higher fraction of the total acidity in the fulvic acid is associated with the higher MW fractions	Ephraim *et al.* 1996
Development of a non-adsorptive method for fractionation of NOM	Cellulose acetate Regenerated cellulose	500 1000 3000	Method was found to be viable although removal of inorganic ions gave rise to loss of low molecular size organic acids	Crum *et al.* 1996
Determination of NOM MW distribution with characterisation by measurement of sulphur containing compounds (SCC)	Regenerated cellulose	1000 10 000	UF was successful in determining the content of SCC as well as DOC and their changes from river water infiltrate towards a production well	Ludwig *et al.* 1997
Characterisation of size fractions by colour, charge and structure	Cellulose acetate Regenerated cellulose	500 3000 10 000 30 000	High MW fractions were highly coloured, highly branched with carbohydrate structures. Low MW fractions were lower in carbohydrate and colour content with a prevalence of long chain aliphatic carbon.	Newcombe *et al.* 1997

Testing a model developed to improve the accuracy of size distribution measurements using UF	Regenerated cellulose	3000 10 000	Concentrations of DOC obtained using UF for size distribution analysis need to be adjusted because of the rejection of molecules smaller than the MWCO	Cai 1999
Investigate rejection and flux decline characteristics of different membranes with filtration of NOM	Regenerated cellulose Cross-linked polyamide TFC Polyether-sulfone	3000 8000 10 000	The effective MWCO for a charged membrane with a charged solute (NOM) will be different than what is obtained with neutral solutes	Cho *et al.* 1999

Key: TFC – thin film composite

Caption: Description of membrane materials (American Water Works Association *et al*. 1996)

Cellulosic ultrafiltration membranes are hydrophilic polymers, have low cost and have low tendencies for adsorption.

Polyethersulfone ultrafiltration membranes are not hydrophilic and have a relatively high adsorption tendency but they have very good chemical, mechanical and thermal stability.

Polyamide ultrafiltration membranes are hydrophilic with good thermal, chemical and hydrolytic stability than cellulose membranes. The amide group, however, has a great sensitivity to oxydative degradation and cannot tolerate exposure to even traces of chlorine.

UF has been used as a tool for separation of NOM into size fractions with comparison against other separation techniques (e.g. Gel Permeation Chromatography (GPC)). Throughout the 1990s, the validity of the fractions has been further tested and proven with mathematical models being used to better estimate the molecular weight. The major findings are that the high MW fractions are more coloured and more reactive than low MW fractions.

Laîné *et al.* (1989) carried out trials of different UF membrane materials. These were a hydrophobic polysulfone membrane, a hydrophobic acrylic copolymer membrane and a hydrophilic regenerated cellulose membrane. They found that the most important quality of the membrane material was the hydrophilicity. Relatively hydrophilic membranes were found to perform much better than relatively hydrophobic membranes

with regard to separating aquatic NOM. The rejection properties of the membrane material should also be considered. These need to be taken into account for precise estimates of the molecular weight. If the rejection properties are not considered, the amount of the low MW fraction will be underestimated (Laîné *et al.* 1989).

The studies outlined above (table 2.6) were carried out using sequential ultrafiltration. This involves the same solution being filtered through a series of membranes of decreasing pore size, the filtrate of one step being filtered on the following membrane. Because the reproducibility of membrane filtration is not better than 5 to 10%, the accumulated error can become extremely large when more than ~5 filtering steps are used (Buffle *et al.* 1992). When compared with parallel filtration, where aliquots of the same initial sample are filtered through several membranes of different pore sizes, sequential filtration is preferred because it minimises the coagulation and aggregation processes that occur when colloid samples are stored for more than a few hours. Indeed the rate of aggregation decreases when the colloid concentration and chemical heterogeneity decreases. Therefore the ultrafiltration fractions are increasingly stable with respect to coagulation with decreasing pore size of the filters (Buffle *et al.* 1992).

Overall, ultrafiltration is a useful method for the fractionation of organic matter in water samples. Large volumes of water can be fractionated in a short time without extreme conditions and use of solvents leading to artefacts. However limitations such as the uniformity of membrane pore size and loss of organic material due to adsorption must be taken into account.

2.3.3 Fractionation by chromatography

2.3.3.1 GPC/HPLC/HPSEC

Gel Permeation Chromatography (GPC) is a method of measuring molecular size that has been extensively applied to the determination of molecular sizes of humic and fulvic acids. These studies mostly use Sephadex gels for this purpose. The separation is performed in columns by elution of the sample through porous beads. Molecular weight data can only be estimated by calibrating the gel with appropriate standards. Sephadex is a cross-linked dextran polysaccharide available in several different grades depending on the degree of cross-linking. The different grades allow fractionation over a wide range of molecular sizes (Wershaw and Aiken 1985).

Sephadex gel interacts via hydrogen-bonding mechanisms with weakly basic aromatic amines and weakly acidic polyphenols to fractionate these materials. The fractionation of aquatic substances on Sephadex gels in the absence of ion pair buffers is primarily attributed to adsorptive interactions (Leenheer 1985). Since humic acid is a polyelectrolyte, its molecular volume in aquatic solution varies with pH and ionic strength. Kim *et al.* (1990) used a column with Sephadex gel 100 – 120, calibrated with globular proteins, that showed a linear relationship between MW and hydrodynamic size. A calibration curve resulted, in which the molecular size (Dalton) and hydrodynamic diameter (nm) were given as a function of the elution volume. The elution profile tends to show a large peak whose maximum represents a logarithmic average of the size distribution. The GPC fractions can be further characterised by techniques such as IR or NMR spectroscopy. This study used previously separated humic and fulvic acids.

There are, however, many disadvantages when using GPC to fractionate aquatic natural organic matter. These include poor resolution and long analysis times (Becher *et al.* 1985). In order to measure accurate molecular sizes by GPC, the only interaction between the analyte molecule and gel must be a size-dependent interaction. All other physical and chemical interactions must be absent or very weak. Yet humic acids do interact with gels such as Sephadex, both electrostatically and by adsorption. Adsorption of humic molecules occurs at a low pH when they are associated and the electrostatic interactions are greatest in solutions of low ionic strength. It is recommended that measurements be made in a basic buffer of relatively high ionic strength to achieve a continuous fractionation on the basis of molecular weight (Wershaw and Aiken 1985).

Another factor that causes humic acids to act in a non-ideal fashion in a gel permeation column is that humic acids form aggregates in solution. The degree of aggregation is a function of both pH and concentration. If aggregation occurs, false results will ensue.

With the advances in High Performance Liquid Chromatography (HPLC) in the early 1980s, it was considered as an alternative to GPC that would produce higher resolution chromatograms and have shorter analysis times (Becher *et al.* 1985). HPLC, like GPC, uses a column for separation of species. A solvent (mobile phase) is pumped through the column (that is packed with the stationary phase) and the column effluent passes through a detector, often UV, where the eluted species are detected. HPLC methods can be based on adsorption or partitioning as well as ion-exchange. The mobile phase is

pumped through the column at high pressure with pumps. HPLC is sometimes distinguished by the relative polarity of the mobile and stationary phases. Normal-phase chromatography is when the mobile phase is a non-polar solvent and the stationary phase is a highly polar material. With reverse-phase chromatography, the mobile phase is a relatively polar solvent and the stationary phase is non-polar (Sawyer *et al.* 1994).

Reverse-phase HPLC of aquatic humic substances has not produced well resolved component chromatograms. Broad trailing peaks indicative of solute-solute or solute-sorbent, direct phase interactions are produced by reverse-phase chromatography. Normal-phase chromatography with an aqueous mobile phase has been found to fractionate aquatic humic substances according to the nature of their polar functional group content (Leenheer 1985). An attempt to find the structure of aquatic fulvic acid using reverse-phase HPLC was made but only 70% of the sample was recovered from the column (Saleh *et al.* 1989). This indicates that some of the fulvic acid was adsorbed onto the column stationary phase and was not removed by the mobile phase flowing through the column. Some structural information was obtained by subjecting the column effluent to NMR (nuclear magnetic resonance) and FT-IR (fourier transform – infra red) spectroscopy but the results were not conclusive.

However, with the introduction of rigid, aqueous compatible Size Exclusion Chromatography (SEC) materials, that can be silica- or polymer-based, it became possible to characterise the molecular size of humic substances. The separation by SEC is based on differential permeation of molecules of various size into a porous matrix.

As the sample traverses the column, the small compounds permeate the matrix pores to a greater degree than the larger components and are retained longer. The elution therefore depends on molecular size, with the larger materials eluting first and the smallest last (Hongve *et al.* 1996).

There are some important variables with HPSEC used for separation of NOM – the mobile phase, column packing (stationary phase) and use of standards for molecular size calibration.

The recommended mobile phase is distilled water for separation of non-ionic compounds. This does not work for humic substances that are repelled by ionic sites on the stationary phase because of their negative charge in aqueous solutions. Fulvic acid will elute in the void volume of the column and the fraction of stationary phase available for penetration will be zero. The ionic repulsion can be reduced by increasing the ionic strength of the mobile phase by the addition of neutral salts or a buffer (Hongve *et al.* 1996).

Many types of column packing have been tried and tested but the most popular are TSK gels that are silica based (Peuravuori and Pihlaja 1997a). Another popular column material is Protein-pak that is also silica based (Zhou *et al.* 2000).

The standards are also important in order that the size of the NOM can be determined. Some studies do not use standards but use HPSEC entirely comparatively (Vuorio *et al.* 1998, Frimmel and Abbt-Braun 1999, Hesse *et al.* 1999, Specht *et al.* 2000,

Makharadze *et al.* 2000). Other standards used are polystyrene sodium sulphonates, dextrans and globular proteins. The standards should have a similar structure to the NOM to obtain as close results to the actual size of NOM as possible.

Studies carried out using HPSEC for the analysis of aquatic NOM are summarised below:

Table 2.7 High Performance/Pressure Size Exclusion Chromatography (HPSEC) and Gel Permeation Chromatography (GPC) of NOM

Aim of Study	Column used	Mobile phase	Standards	What was found	Reference
Comparison of MW distribution before and after chlorination	TSK-G 3000SW	0.02M KH_2PO_4 buffer at pH6.5	Dextrans and globular proteins	Chlorination resulted in degradation of high MW humic substances	Becher *et al.* 1985
Investigate different SEC columns and optimise mobile phase conditions	TSK-G 3000SW TSK-G 3000PWXL, Shodex OHPak B-800P	0.02M KH_2PO4 buffer at pH6.5 0.02M neutral salt solution	Polystyrene sulfonates, polyethylene glycols	Results for columns gave good agreement with other independent methods. Nominal MW results achieved	Hongve *et al.* 1996
To measure molecular size distributions in whole water samples and compare fractions isolated using UF	TSK-G 3000SW	1. 0.01M sodium acetate 2. 0.02% sodium azide 3. various phosphate buffers	Polystyrene sulfonates, polyethylene glycols	Molecular weight ranges obtained using HPSEC agree with those using UF concentrates. But HPSEC of UF filtrates were greater than the membrane cut-off	Peuravuori and Pihlaja 1997a
HPSEC used to assess relative changes of NOM size distribution throughout a water treatment works	TSK-G 3000SW	0.01M Sodium acetate	No standards used	HPSEC was proved to be a reproducible method in determining NOM size distribution. Results were derived using UV detection only	Vuorio *et al.* 1998
Comparison of NOM isolates from resin and UF fractionation using GPC and spectroscopic measurements	TSK–HW–40 (S)	0.025M phosphate buffer at pH 6.8	No standards used	Alongside spectroscopic methods, GPC is a technique that requires no sample concentration and yields useful information for the characterisation of NOM.	Frimmel and Abbt-Braun 1999
Comparison of HPSEC with FFFF and investigation of solute-gel interactions	Protein-Pak 125	0.02M phosphate buffer at pH 6.8	Polystyrene sulfonates	HPSEC is not immune to solute-gel interactions. Good agreement was obtained between HPSEC and FFFF with regard to UF fractions	Pelekani *et al.* 1999
Characterisation of refractory organic substances in water treatment using GPC with UV	TSK–HW–40 (S)	0.028M phosphate buffer at pH 6.6	No standards used	Oxidative processes result in a decrease in molecular weight (MW) and specific UV absorbance (SUVA), whereas	Hesse *et al.* 1999

and DOC detection				biochemical and adsorption processes result in an increase in MW and SUVA.	
Assess and compare the performance of both columns with regard to NOM	TSK-G 3000SW, Biosep S2000	0.05M sodium nitrate	polysaccharides	Standards used for column calibration can only approximate the hydrodynamic radius and provide only nominal MW values. Both columns were suitable for monitoring changes in molecular size. Values were not absolute.	Conte and Piccolo 1999
Investigate Nitrogen distribution in NOM size classes	TSK-G 3000SW	0.02M sodium perchlorate at pH 6.5	Polystyrene sulphonates	Larger NOM molecules contain more nitrogen than smaller molecules The MW of the nitrogen is source dependent.	Egeberg *et al*. 1999
Characterisation of molecular size of nine reference samples from Norway. MALLS/HPSEC was employed.	Progel-TSK	0.02M KH_2PO_4 buffer at pH6.5	Bovine serum albumin	The use of MALLS has not yet been perfected. It uses concentrated samples that are prone to aggregation. Modern computerised molecular modelling may be useful in resolving the relationship between radius and molecular mass.	Wagoner and Christman 1999
Characterisation of molecular size of Latahco humic acid using HPSEC and MALS.	TSKTM G5000 PW	0.1M phosphate buffer at pH 6.8	Globular protein and Dextran	Method was effective for this humic acid and could be extended to other humic acids.	Von Wandruszka *et al.* 1999
Examination of specific analytical aspects of HPSEC: baseline correction, standard calibration, MW cut-off and UV/Vis detection wavelength.	Protein-pak 125	0.002M phosphate buffer at pH 6.8	Sodium polystyrene sulphonates (PSS)	The use of salicylic acid and acetone in addition to PSS is recommended to improve accuracy. A wavelength between 230 and 280 nm is suitable (254 nm is most used). Low MW cut-off is recommended at 50 daltons.	Zhou *et al.* 2000
Introduce a new detector for continuous detection of DOC.	TSK–HW–40 (S)	0.028M phosphate buffer at pH 6.65	No standards reported	The DOC detector was accurate to 0.2 mg/L but its mode of operation could be improved.	Specht *et al.* 2000
Molecular size distribution NOM and its carbon and nitrogen content.	TSK-G 3000SW	0.02M phosphate buffer at pH 6.5	Polystyrene sulphonate sodium salts	C:N ratio of fractions indicated that the lower molecular size NOM is enhanced in nitrogen relative to the higher fraction.	Andersen *et al.* 2000
Application of HPLC for	Separon C18	2-propanol, butanol,	No standards	Results suggest that, in aqueous	Makharadze *et al.*

studying fulvic acids.		0.020M tetra-butylammonium hydroxide	reported	solutions, fulvic acid exists in the form of an equilibrium system of high-molecular associates of various molecular weights. If disturbed, a MW re-distribution will occur, resulting in a new state of equilibrium.	2000
Applicability of different characterisation methods for distinction between humic acid and fulvic acid infiltrated via groundwater discharge and those originating from sedimentary organic carbon.	TSK–HW–40 (S)	Mixture of 0.05M Na_2HPO_4, 0.001M EDTA, 0.1M NaCl and 10% volume methanol	Globular proteins	GPC along with spectroscopic methods are useful tools for providing information about the origin, , mobility and long-term stability of humic substances.	Artinger *et al.* 2000
Effect of detector wavelength on molecular weight determination of humic substances by HPSEC.	Protein-Pak 125	Mixture of 0.1M NaCl, 0.002M KH_2PO_4 and 0.002 M Na_2HPO_4 at pH 6.8	Polystyrene sulphonate sodium salts	MW of humic substances increases as the operating wavelength increases. The magnitude of the increase in MW is not substantial enough to prevent meaningful comparisons between 220 and 280- nm.	O'Loughlin and Chin 2001
Comparison of molecular size distribution (MSD) between raw water and treated drinking water to investigate removal of NOM by treatment.	TSK-G 3000SW	Sodium acetate at pH 7	Globular proteins	There were clear differences in the MSD of different waters. Water treatment processes removed the largest fractions almost completely. UV detection was used.	Nissinen *et al.* 2001
Determination of MW by HPSEC using UV, DOC and fluorescence detection.	TSK-50S	Phosphate buffer at pH 6.8	Potassium hydrogen phthalate	The enhanced HPSEC system can be effectively used for analysing NOM in water with easiness, speed and low-cost.	Her *et al.* 2001
Comparison of HPSEC with seven other methods of determining molecular size.	TSK-G 3000SW	0.02M sodium perchlorate at pH 6.5	Polystyrene sulphonates	MW determined by HPSEC are of the same order of magnitude as molecular weights derived by diffusion coefficients.	Egeberg *et al.* 2002

The use of HPSEC is well researched and has been validated by many researchers. Its limitations are well known and can be taken into account when analysing NOM. It should be remembered that HPSEC is best for comparison of molecular weights of waters analysed on the same system rather than for obtaining absolute molecular weight values.

2.3.4 Flow field-flow fractionation (FFFF)

Field-flow fractionation was first proposed in 1966 by Giddings as an analytical separation method for macromolecules. It is a versatile technique, capable of separating and characterising materials in the macromolecular (0.001 – 1 μm) and colloidal range (> 1 μm and beyond) (Amarasiriwardena *et al.* 2000).

FFFF is an elution technique not unlike chromatography except that the separation in a FFFF channel applies only physical forces to the sample molecules rather than relying on chemical interactions. Fractionation is carried out in thin ribbon-like open channels with a membrane at the end. The sample is injected along with the channel flow. Just after sample injection, the channel flow is stopped temporarily. An external flow field applied perpendicular to the separation axis induces separation, and field-flow subsequently causes the sample molecules to migrate to the accumulation wall where they form an equilibrium cloud whose mean thickness depends on the fluid velocity and the concentration gradient induced flux away from the wall which is determined by the diffusion co-efficient (and hence molecular weight) of the sample molecules. Once equilibrium has been reached, the channel flow is started again and the separated macromolecules emerge from the channel at characteristic emergence times (Beckett *et al.* 1992).

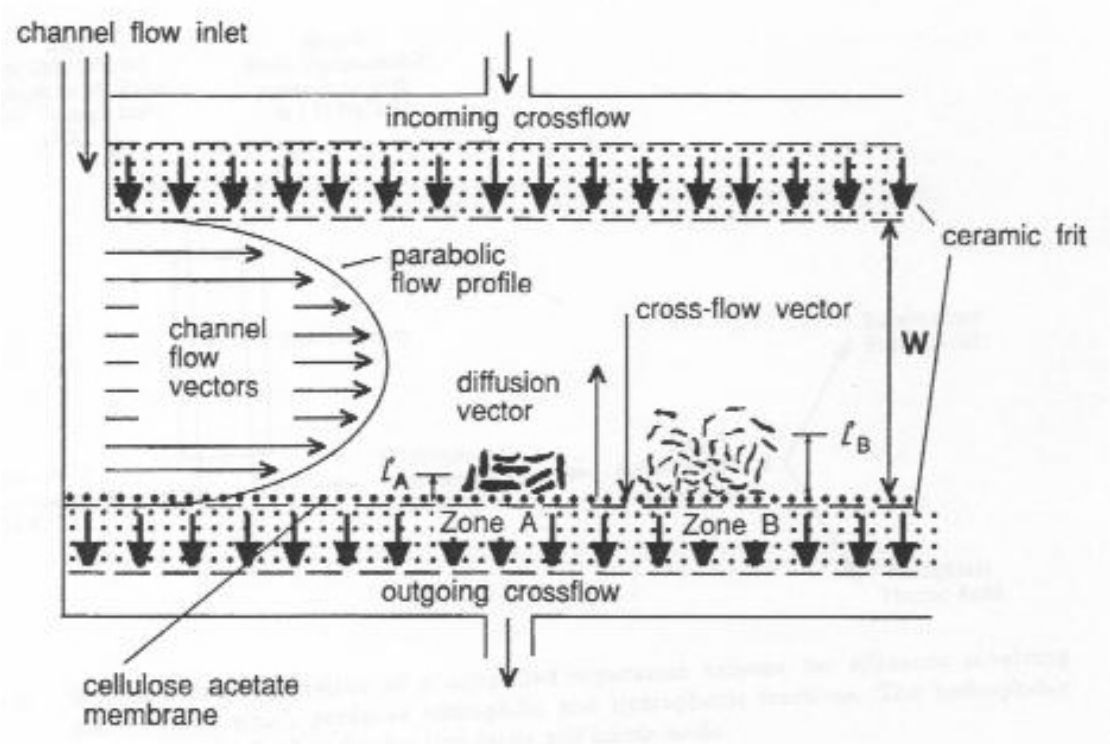


Figure 2.5 Illustration of Flow-Field Flow Fractionation (Beckett *et al.* 1992)

Beckett *et al.* (1992) used membranes to characterise organic compounds in pulp and paper mill effluent as an alternative to UF and HPSEC. The molecular weight distribution of the effluent could be successfully determined by FFFF using methods developed previously for study of natural humic substances (Beckett 1987, Beckett *et al*. 1987, 1988).

Newcombe *et al.* (1997), used FFFF to analyse fractions prepared by UF (see table 2.6). The measured molecular weights (by FFFF) were much lower than the nominal molecular weight cut-offs for the three highest molecular weight membranes. This was attributed to several factors, including charge rejection from the membrane pores and differences in molecular conformation between the NOM and the globular proteins used to calibrate the higher molecular weight membranes.

The use of FFFF for analysis has continued and been improved through optimisation studies (Lyvén *et al.* 1997, Thang *et al.* 2001, Zanardi-Lamardo *et al.* 2001). It is a useful technique for comparison with other size determination techniques as the interference inherent in the method can be minimised.

2.3.5 Comparison of fractions prepared using different methods

There has been much comparison of fractions prepared using resins, membranes and chromatography. The comparisons have been made in order to validate the methods and prove that the fractions produced are valid and not innate products of the procedure used to prepare them.

Amy *et al.* (1987) compared the molecular weight data obtained using GPC and UF. The GPC method indicated a higher molecular weight than the UF method for a given source. The GPC method was found to be more significantly affected by pH conditions. They emphasised that such size techniques are best applied to looking at relative differences between various water sources or between an untreated and corresponding treated water rather than using the methods to gain absolute size values.

GPC has also been used to analyse fractions prepared using Amberlite XAD-8 resin (adsorption) and fractions prepared using DEAE-cellulose (ion-exchange) (Pettersson and Rahm 1996). The yield from each resin was compared for a brackish water. This is perhaps an unfair comparison as the resins react differently to organic matter. It has been well documented that XAD-8 resin only adsorbs hydrophobic organic matter

(section 2.3.3.1). The yield or recovery of organic matter on the XAD-8 resin will depend on the hydrophobicity of the organic matter in the water and is not a reflection of the efficiency of the resin. Similarly, the ion-exchange resin will only exchange the organic matter that is ionised and this may be minimal at a neutral pH at which this study was carried out. GPC was used to give a measure of polydispersity of the humic matter but only for the DEAE-cellulose fractions.

Later, an investigation was launched into the validity of fractions produced using XAD resins (Peuravuori and Pihlaja 1997). They questioned whether the humic and fulvic acids isolated using XAD-8 resin were originally present in the dissolved organic matter or created by chemical alterations at strongly acidic conditions. In addition to XAD isolation, tangential UF was used for isolation of size fractions. The fractions were analysed for elemental composition, DOC, size by HPSEC and for iron and aluminium. Fractions that had been prepared in very different ways exhibited great similarities and it was concluded that isolated humic substances are definite entities in the original NOM. However, it was later concluded (Peuravuori and Pihlaja 1998) after looking at structural techniques (e.g. nuclear magnetic resonance – NMR) that the separation of organic matter into humic acid and fulvic acid at pH 1 has certain risks. Considerable compositional and structural alterations take place, in addition to loss of organic matter, during the acid precipitation. This is most important if the structure of the NOM is being investigated.

FFFF has been used as an independent method for comparison with HPSEC as a method of characterisation (Pelekani *et al.* 1999). Despite solute-gel interactions being

identified with the HPSEC, the technique was reported to provide useful and reliable molecular weight distributions of NOM in drinking water supplies.

Fluorescence spectroscopy was used by Belin *et al.* (1993) to compare tangenital (cross-flow) ultrafiltration (UF) separated organic matter with XAD8/4 resin isolates. The fractions produced in both cases were found to have very similar fluorescence characteristics. This is discussed in more detail in section 2.4.2.

2.3.6 Section conclusions

The main techniques discussed are all valid for the fractionation of NOM. The procedure used will depend on the information required. All the procedures have limitations but these can be minimised if due care is taken. The limitations should also be taken into account when analysing data obtained from fractionation.

2.4 NOM fraction characterisation

Once fractions have been prepared, analyses can be carried out to characterise them.

All fractions discussed in this section have been separated using XAD resins.

2.4.1 DOC, SUVA and THM-FP

Typical analyses carried out on fractions include measurement of DOC, UV and THM-FP. SUVA is calculated from DOC and UV measurements. Examples of published results have been collated in table 2.8.

Table 2.8 Analysis of NOM fractions by DOC, SUVA and THM-FP

Fraction	DOC	SUVA	THM-FP	Reference
Reservoir Water				Croué *et al*. 1993
FA	4.9	3.1	Not carried out	
HA	0.3	4.6		
HPO-N	0.6	2.0		
HPI-A (eluted and non-eluted)	eluted: 2.1 non-eluted: 0.7	total: 2.0		
HPI (non-adsorbed)	2.9			
Peat FA	4.8	4.4	65	Amy *et al*. 1987
UF fractionated FA				
<30k Da	4.1	4.2	62	
<10 kDa	2.4	3.2	51	
<5 kDa	2.0	1.7	39	
< 1 kDa	1.7	2	41	
0.5 kDa	1.1	1.5	35	
River HPO (treated - t)	4.4	Nr	114	Collins *et al*. 1986
(untreated – ut)	1.4	Nr	34	
HPI (t)	3.3	Nr	65	
(ut)	1.2	Nr	50	
Aquifer HPO (t)	4.8	Nr	75	
(ut)	3.2	Nr	42	
HPI (t)	3.5	Nr	45	
(ut)	3.1	Nr	39	
Reservoir HPO (t)	1.2	Nr	95	
(ut)	1.1	Nr	76	
HPI (t)	1.3	Nr	58	
(ut)	1.1	Nr	48	
River HPO (t)	1.1	Nr	70	
(ut)	1.0	Nr	55	

HPI (t)	2.0	Nr	48	
(ut)	1.9	Nr	40	
River Water				Marhaba
HPO-A	0.4	Nr	22	*et al*. 2000
HPO-B	0.2	Nr	34	
HPO-N	0.6	Nr	27	
HPI-A	1.7	Nr	33	
HPI-B	0.1	Nr	17	
HPI-N	0.7	Nr	6	
Lake Water				Gallard
HA1	1.3	4.1	149	and von
HA2	1.3	4.1	197	Gunten
HA3	1.3	3.9	207	2002a
HA4	1.2	1.8	118	
Lake HA	0.2	Nr	913	Pomes *et*
Lake FA	2.2	Nr	152	*al.* 1999
Lake HA	0.6	Nr	524	
Lake FA	3.2	Nr	126	
River HA	0.3	Nr	15	Pomes *et*
River FA	1.9	Nr	17	*al.* 2000
Lake HA	0.1	Nr	9	
Lake FA	1.2	Nr	18	
River Water	2.6	1.5	20	Hwang *et*
HPO	1.1	1.8	10	*al.* 2001
TPI	0.4	1.4	13	
HPIA+N	0.3	1.1	29	
Salt River Project non-humic substances	2.3	Nr	105	Owen *et al*. 1995
River Water				Croué *et*
FA	4.9	3.4	132	*al*. 1993a
HA	5.0	4.8	230	
HPIA	4.3	2.3	116	

DOC –dissolved organic carbon concentration in mg L^{-1}
SUVA- specific UV absorbance is defined as the UV absorbance of a given sample determined at 254 nm and divided by the DOC concentration of the solution (expressed in m^{-1}.L mg^{-1} C).
THM-FP – Trihalomethane formation potential: sample is chlorinated and incubated for seven days, after which the THM's are measured in µg L^{-1} and divided by the DOC of the sample to give THM-FP in µg mg^{-1} C.

As previously mentioned, SUVA can give a good indication of THM-FP for a single water sample and its corresponding fractions (Reckhow *et al*. 1990, Edzwald *et al*. 1985, Kitis *et al*. 2001a, Kitis *et al*. 2002). Investigation of the THM-FP of the fractions within a water sample can give some indication of the type of NOM that is forming the

largest amount of THMs. In some studies, it has been shown that the hydrophobic organic compounds such as humic acid are the primary contributors of THM precursors in natural waters (Collins *et al.* 1986, Croué 1999, Reckhow and Singer 1990, Kitis *et al*. 2002). For most waters the THM reactivity of the humic fraction was significantly higher than corresponding hydrophilic fraction.

Reckhow and Singer (1990) showed that the humic fraction produced 56 μg THM mg^{-1} DOC compared to 42 μg THM mg^{-1} DOC for the hydrophilic fraction. Krasner *et al.* (1996) showed that the humic fraction produced 51 μg THM mg^{-1} DOC compared to 21 μg THM mg^{-1} DOC for the hydrophilic acid fraction.

In a review by Croué (1999) it was noted that:

- The higher the SUVA, the higher the reactivity with oxidants such as chlorine and ozone
- Reaction of aromatic compounds (hydrophobic fraction) with ozone increased the concentration of hydrophilic material
- The compounds in the hydrophilic fraction are strong DBP precursors
- Conventional water treatment favours the removal of the hydrophobic fraction of NOM

In contrast, the impact of the hydrophilic fraction of the water on its THM-FP has been shown for a low DOC (non-coloured) water, Colorado River Water (CRW). It was found that 65% of the TOC and 56% of the THM-FP of CRW was contributed by hydrophilic compounds (Collins *et al.* 1986). A number of treatment plants along the river have reported problems reducing THM-FP (Amy *et al.* 1985). Collins *et al*.

(1986) reported a 23% reduction in THM-FP after treatment and only an 18% reduction in the THM-FP related to the hydrophilic fraction. This % reduction in THM-FP is very low when compared to the average of 54% for 8 surface waters (Collins *et al.* 1986, Croué *et al.* 1993). Further investigation of the works (Hwang *et al.* 1999) identified the relationship between specific fractions and THM-FP (table 2.9). They identified that for CRW the hydrophilic fractions were exerting the greatest chlorine demand (hydrophilic base 2.4 mg mg^{-1} vs hydrophobic 0.32 mg mg^{-1}) and produced a higher concentration of THMs. This suggests that the importance of the transphilic and hydrophilic fractions varies depending on the nature of the DOC in the water.

Table 2.9 Summary of DBP yields for Colorado River Water (Hwang *et al.* 1999)

DBP	Lowest				Highest
Cl_2 Demand	Colloids	Hydrophobic	Transphilic	Hydrophilic Acid & Neutral	Hydrophilic Base
THM's	Colloids	Hydrophobic	Transphilic	Hydrophilic Base	Hydrophilic Acid & Neutral

Bolto *et al*. (2002) also found that high THM-FPs do not necessarily result from waters with the highest proportion of hydrophobic compounds in their NOM. They studied a range of waters from Taiwan, the USA and Australia. The waters of high colour had among the lowest THM-FP of all the waters studied.

Kitis *et al*. (2002) recently confirmed that for waters with a large hydrophilic content it would be difficult to reduce total DBP formation as hydrophilic material is difficult to remove using conventional treatment processes. By carrying out fractionation it is possible to determine the types of organic compounds that are contributing most to the final THM-FP. This information can then be used to develop a treatment strategy that targets the NOM that forms the greatest amount of THMs.

Bolto *et al*. (2002) concluded that NOM is variable with location and time: no universal statement can be made that a certain NOM fraction is the main THM precursor for all waters.

2.4.2 Fluorescence

Humic molecules are thought to be largely responsible for the fluorescence of natural waters. As a result the energy (related to the wavelength, λ) and intensity of light absorption and/or emission can be used to infer structural information about the NOM molecules. Fluorescence spectra are usually obtained by analysing the intensity of emitted light as a function of its wavelength, in which case they are called emission spectra, or by analysing the intensity of light emitted at a fixed wavelength while scanning the wavelength of excitation. In this case, excitation spectra are produced. When both the excitation and emission wavelength are scanned but the difference between them is kept constant, the result is a synchronous spectrum.

NOM fluorescence is strongly affected by the molecular weight (MW) of the molecules, their conformation and the extent of their complexation with metallic ions and with other organic molecules (Croué *et al.* 2000). Relationships between the fluorescence and the average MW of NOM are among the strongest and most consistent correlation obtained for all spectral properties of NOM. This effect may be attributed to the increased probability of radiationless transitions and quenching of fluorophores in the larger molecules.

Croué *et al.* (2000) found a linear correlation between aromaticity (estimated using ^{13}C-NMR) and the wavelength of maximum emission intensity. The correlation had an R^2 value of 0.61.

A comprehensive study of the literature was carried out by Smith and Kramer (1999) to produce an excitation-emission figure that showed regions where selected model compounds fluoresce (figure 2.6). The regions indicated by the boxes represent the

seven classes of fluorophores observed in the study. The black line corresponded to the region where NOM was observed to fluoresce from a study of the literature.

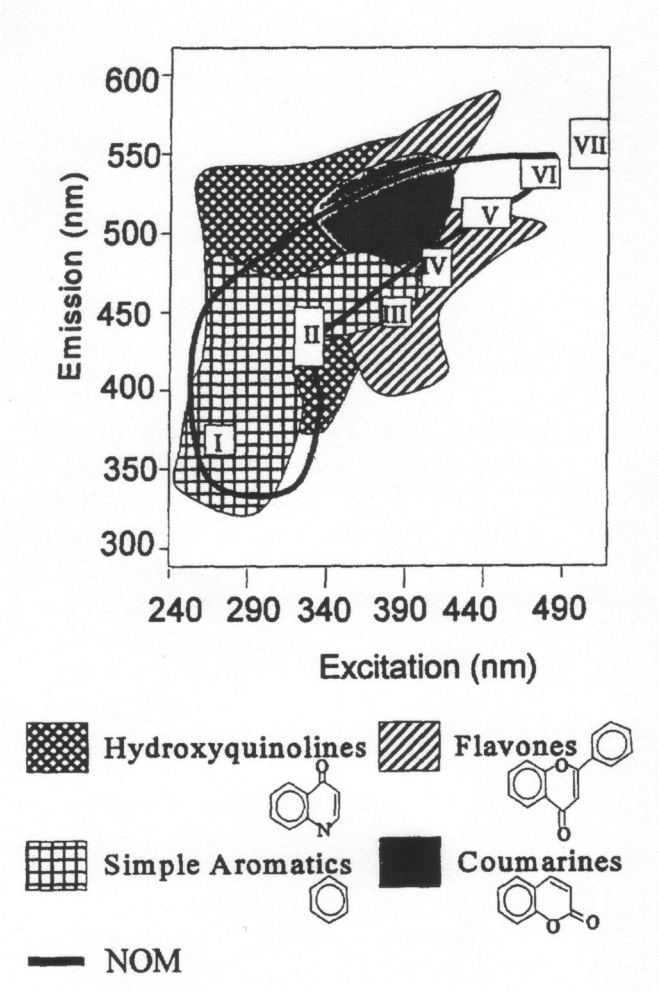


Figure 2.6 Regions where selected model compounds fluoresce (Smith and Kramer 1999)

It should be noted that the figure is only intended to give ideas of possible fluorophores and should not be interpreted too literally. It is intended to give a broad idea of possible fluorophores in NOM and where they might fluoresce.

Smith and Kramer (1999) also looked at aluminium-binding to NOM and its effect on fluorescence. With added aluminium the fluorescence intensity of the samples was observed to increase. They were also able to determine differences between NOM samples and yield some qualitative information about the nature of aluminium binding sites. Results were dependent on the isolation method used – reverse osmosis or low pressure evaporation.

The region a molecule will fluoresce will depend on its structure. A study of the literature revealed that NOM fluorescence typically exhibits two main fluorophores that are attributed to humic-like organic matter (Alberts *et al*. 2002). The position of the excitation and emission maxima will change seasonally (Baker and Lamont-Black 2001) and depending on source (Coble 1996).

Table 2.10 Examples of Fraction excitation and emission wavelength maxima

Reference	Sample identification	Excitation-Emission wavelength pairs (nm)	
		1st pair	2nd pair
Coble 1996	Freshwater humic acids and Fulvic acid	310, 428 310,423 310, 419	Disregarded
Miano and Alberts 1999	Suwannee River Water	352,453 355,448	252, 450 245,442
Alberts *et al.* 2000	Norwegian Lake Water	330, 437 335, 438	225, 428 225, 426
Marhaba *et al.* 2000	River Water		
	HPO-A	225-237, 345-357	Not reported
	HPO-B	225-237, 357-369	
	HPO-N	225, 609-621	
	HPI-A	237-249, 417-429	
	HPI-B	225-237, 369-381	
	HPI-N	225-237, 309-321	
Baker and Lamont-Black 2001	Borehole Water		
	Fulvic like centre	320, 407	None reported
	Protein like centre	278, 347	
McKnight *et al.* 2001	Lake Fulvic Acid	320, 406	230, 412
Her *et al.* 2001	Suwannee River		
	HA	325, 452	261, 457
	FA	320, 443	245, 445

HPO-A, HPO-B, HPO-N – Hydrophobic acid, base and neutral, HPI-A, HPI-B, HPI-N – Hydrophilic acid, base and neutral, HA – Humic acid, FA – Fulvic acid

From table 2.10, it can be seen that excitation and emission maxima for NOM fractions are not constant but vary with sample composition and source. Model compounds that fluoresce in the region of the first peak (approximately 335/440 ex/em (nm)) include chlorotetracycline, 4-hydroxycinnamic acid, coumestrol and coumestrol (Alberts *et al*. 2002).

Fluorescence spectroscopy was used by Belin *et al.* (1993) to compare tangential (cross-flow) ultrafiltration (UF) separated organic matter with XAD8/4 isolates. They found one-to-one correspondence between the UF fractions and the resin isolates. The matching pairs of resin isolates and UF fraction are shown below in table 2.11.

Table 2.11 Resin isolates and matching UF fractions (Belin *et al.* 1993)

Resin isolate	UF fraction MW (kDaltons)
HAF	>1500
FAF	500 – 1500
HPIA	200 – 500
HPINA	<200

The fluorescence spectra were very similar for each pair identified. The fluorescence maxima for each pair were almost identical as were the fluorescence quantum efficiencies (ϕ_E). Fluorescence quantum efficiency is the relative fluorescence of a sample compared with the fluorescence of quinine sulphate. Some conclusions were made regarding the molecular weight (MW) of each isolate/fraction:

- The larger the MW, the more shifted is the maximum of the fluorescence spectrum to long wavelengths.

- Fluorescence quantum efficiency varies in the opposite direction – it increases when the MW decreases.
- The contribution to fluorescence of the HPIA and HPINA is not negligible and must be taken into consideration in the interpretation of the total fluorescence of natural waters.

This agrees with Croue *et al*. (2000) who stated that NOM fluorescence is strongly affected by the molecular weight of the molecules. As the average molecular weight increases, the position of the maximum in the emission spectra shifts to longer wavelengths (red shift).

The properties of organic matter from the Amazon basin were differentiated using cross-flow ultrafiltration (UF) and UV-Spectrofluorescence (Mounier *et al.* 1999). The cross-flow UF was used to produce particulate, colloidal and fine colloidal organic material. It was deduced that the functional groups responsible for complexation with copper were not the same as those responsible for fluorescence and conductance. Mounier *et al.* (1999) concluded that the fluorescent sensitivity of total organic carbon (TOC) was independent of its size fraction. This is opposed to the relationship between molecular size and fluorescence reported by Belin *et al.* (1993).

Excitation-emission spectroscopy was used by Coble (1996) to characterise dissolved organic matter (DOM) in concentrated and unconcentrated water samples from a wide variety of sources (marine, coastal and freshwater). Several types of fluorescent signals were observed including humic-like, tyrosine-like and tryptophan-like (tyrosine and

tryptophan are amino acids). The humic-like fluorescence consisted of two peaks, one simulated by UV excitation and one by visible excitation. Differences in the mean position of the spectra maxima suggested that the humic material in marine surface waters is chemically different from humic material in the other environments sampled (freshwater and coastal). The study provided a means of distinguishing between water mass sources in the ocean.

An optical model was developed by Mittenzwey *et al.* (1996) to rapidly detect the presence of dissolved humic substances in eutrophic waters (waters that have a high nutrient content). The model was used as an alternative to colour analysis due to the high colour content in eutrophic waters caused by the presence of phytoplankton.

The studies by Coble (1996) and Mittenzwey *et al.* (1996) have defined objectives that are not applicable to the characterisation of natural organic matter in surface inland waters for potable use but they do show the flexibility and applications of fluorescence spectroscopy as a rapid analysis technique

Lombardi and Jardim (1999) used fluorescence spectroscopy to investigate HPLC fractionated marine DOM and soil fulvic acid (SFA). The method of separation was a tc18 Sep-Pak column that did not retain hydrophilic material, which was responsible for 20% of the fluorescence. However, there was no shift in wavelength maxima before or after the HPLC fractionation. Using XAD-8 resin instead of tc18 Sep-Pak to concentrate marine DOM, Coble (1996) also observed no shift in wavelength maxima after the extraction procedure. The higher intensity of fluorescence (I_F) exhibited by

SFA in relation to marine DOM was attributed to polar compounds since the compounds of low polarity fluoresce similarly in both categories of organic material.

It is also known that hydrophilic (XAD-4) acids have a higher I_F than hydrophobic (XAD-8) acids. The higher I_F for XAD-4 acids is almost certainly related to its lower molecular weight (compared to XAD-8) which decreases the rate of radiationless losses of excitation (Croué *et al*. 2000). That is, the energy transfer in small molecules is more efficient than in large molecules and small molecules will fluoresce more strongly per mg of carbon.

A model to estimate humus content in natural waters using spectroscopy, not fractionation, was developed by Hautala *et al.* (2000). A combination of absorbance and fluorescence measurement proved to be most reliable. It was found that fulvic acids fluoresce most and humic acids absorb most. Both have opposite trends in molecular weight. The study confirmed the analytical difficulty of determining aquatic humus content without fractionation. In comparison with the colour equivalent method developed by Hutchinson in 1957 – 1 mg L^{-1} humus = 6.6 mg Pt L^{-1} – this model was statistically more accurate. An estimate could also be made for the humic acid/fulvic acid ratio.

Le Coupannec *et al.* (2000) looked at DOM in landfill leachate using excitation-emission matrix (EEM) fluorescence. They noted that the fluorescence of minor compounds may be spectrally overlapped by dominant fluorophores in a matrix. They found no specific relationship between molecular size and spectroscopic characteristics

(which is again opposed to the relationship between molecular size and fluorescence reported by Belin *et al.* 1993) although they did find that the DOM in landfill leachates share some spectral features with the DOM present in natural waters.

Recently, fluorescence spectroscopy has also been used to identify and quantify dissolved organic matter (DOM) fractions in water using their spectral fluorescence signatures (SFS) (Marhaba *et al*. 2000). SFS is described as the total sum of emission spectra of a sample at different excitation wavelengths, recorded as a matrix of fluorescent intensity in co-ordinates of excitation and emission wavelengths, in a definite spectral window. The technique involved the spectroscopic analysis of fractions at a range of concentrations. A combination of spectral characteristics was used to identify each fraction. The aim of the SFS database was to rapidly determine the concentration of each dissolved organic matter fraction in a particular water sample without fractionation. With regard to determining the applicability to other water sources it was found that there are some sacrifices in the accuracy for some DOM fractions in return for the rapidity (a few minutes compared to weeks/months for fractionation) and convenience (minimal sample preparation) of the SFS method. It should be noted that the method is in its early stages and needs refinement as well as more fraction data.

It has recently been reported that it is possible to determine the origin of the fulvic acid in a sample containing natural organic matter by measuring the fluorescence index (FI). FI is defined as the ratio of emission intensity at 450 nm to the emission intensity at 500 nm both at an excitation wavelength of 370 nm (McKnight *et al*. 2001). If the FI > 1.6, the fulvic acid present in the sample is said to be microbially/algally derived. If the FI <

1.6, the fulvic acid present in the sample is said to be terrestially derived (Nguyen *et al*. 2002).

Fluorescence spectroscopy has a variety of applications with regards to natural organic matter but the fluorescence of NOM is not yet fully understood.

2.4.3 Capillary Electrophoresis

Capillary Electrophoresis (CE) is an automated analytical technique that separates species by applying voltage across buffer filled capillaries and is generally used for separating ionic species depending on their size and charge. The ions move at different speeds when the voltage is applied depending on their charge-to-size ratio.

The solutes (that can be dissociated molecules as well as ions) are seen as peaks as they pass through a detector and the area of each peak is proportional to their concentration which allows quantitative determination. Acidic species, such as humic and fulvic acids dissociate at a high pH and produce negatively charged anions that can be separated. Borate, which has a natural pH of 9.4, is the standard buffer (Altria 2000).

2.4.3.1 Capillary Electrophoresis for analysis of aquatic NOM

Due to the ionic nature of humic substances, it is possible to study them using electrophoretic techniques. Humic acids were initially analysed by CE to see if it was possible to separate them using the technique (Rigol *et al.* 1994). They showed that separation into two fractions could be achieved.

Garrison *et al.* (1995) went on to use CE to characterise humic substances. They found that aquatic humic substances exhibited a characteristic hump and also that soil fulvics exhibited a consistent and characteristic set of sharp peaks extending from a humic hump. The characteristic humic 'hump' was also seen by Dunkelog *et al.* in 1997 who used a similar borate buffer for analysis of lakewater humic acid isolated using XAD resins. Figure 2.7 shows a typical humpogram of soil humic acid analysed with borate buffer at various pHs (Garrison *et al.* 1995).

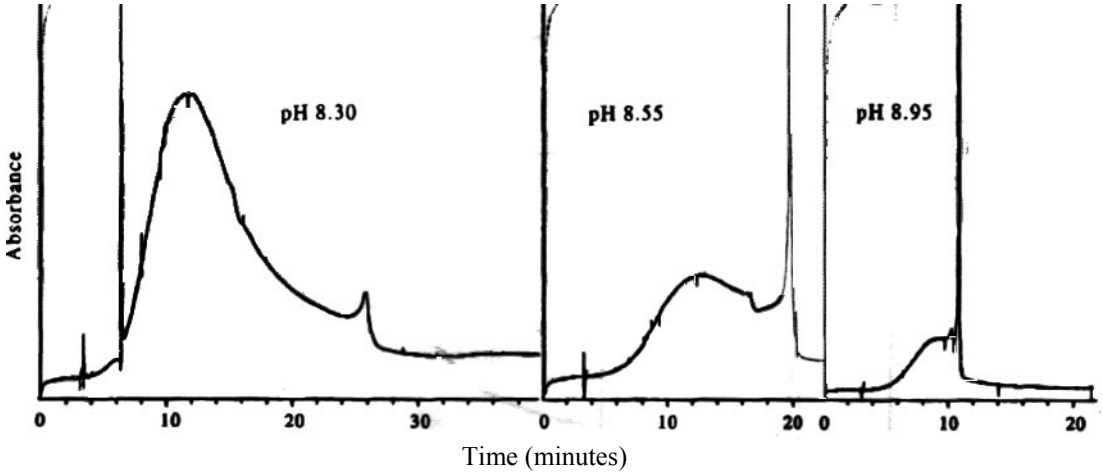


Figure 2.7 Typical 'humpograms' of soil humic acids analysed in borate buffer at various pHs (Garrison *et al.* 1995).

In 1996, Pompe *et al.* aimed to use CE to develop a 'finger-print' catalogue of humic and fulvic acids based on their functionality. They investigated five different commercially available humic acids and two commercially available fulvic acids all at 100 – 400 mg/L. Each electropherogram (EPG) showed an individual pattern. The substances also showed differences in their migration behaviour with different sized and shaped peaks. This was due to different charge-to-size ratios and various structural properties of the individual components of fulvic and humic acids caused by their different origins.

Schmitt *et al.* (1996) reported that the longer migration time of fulvic acids over humic acids indicates a larger charge-to-mass ratio for humic acids, i.e. a higher total acidity and a smaller molecular mass. The study went on to examine the effect of copper and

iron coagulants on the average electrophoretic mobility (AEM). No significant changes in the AEM were observed during addition of Fe(III) to humic acids. However the addition of Cu(II) to humic acid causes an initial decrease in the AEM (lower migration times). They concluded that flocculation occurred over all the acidity range of the humic acids, showing non-specificity of Fe(III)-humic interactions as compared to Cu(II). With increasing iron concentration, the AEM of the fulvic acids decreased (lower migration times) due to changes in the charge-to-mass ratio of the soluble acids because of the interaction of Fe(III) ions with the carboxyl groups. With 10 meq iron added the fulvic hump was still present indicating incomplete removal.

The addition of organic solvents to the borate buffer was investigated (Nordén and Debak-Zlotorynska 1997) and resulted in a clear difference in electrophoretic behaviour of the studied humic substances. With UV detection and a borate buffer, previously reported differences between fulvic acids and humic acids were observed (Garrison *et al.* 1995 and Schmitt *et al.* 1996). It was found that the results obtained with the addition of organic solvents were not reproducible with UV detection. The solvents acted as denaturising agents, resulting in uncoiling of the humic substances which affected the migration behaviour and additional fractions were observed. EPGs with fluorescence detection and the addition of 2-propanol to the borate buffer were reproducible. The fulvic and humic acids showed a weak fluorescing humic hump with larger peak broadening than with UV detection. This indicated that both fulvic and humic acids contain compounds with higher electrophoretic mobility (higher charge-to-mass ratio) that are not UV detectable. The humic acids exhibited sugnificantly more fluorescence compounds than fulvic acids.

The analysis of HA and FA samples is typically reported in terms of average electrophoretic mobility (AEM). This can be calculated by measuring the electrophoretic velocity v_e (cm/s) and the applied electric field strength E (V/cm). A plot of UV absorbance at 254 nm against electrophoretic mobility at different buffer pH gives a Guassian-like distribution as shown in the inner curve in figure 2.8. The calculated AEMs are always lower with HAs than with FAs indicating lower charge densities for HAs that are determined by the degree of ionisation at the separation buffer pH and the average molecular size. Schmitt-Kopplin *et al.* (1998) showed that fulvic acids exhibit higher polydispersities (wider peaks) than humic acids. Wider peaks indicate a larger range of molecular weight compounds. Several separated sharp peaks, corresponding to lower-molecular-mass compounds (with low charge-to-mass ratios) were found in the fulvic acid fractions but never in the humic acid fractions.

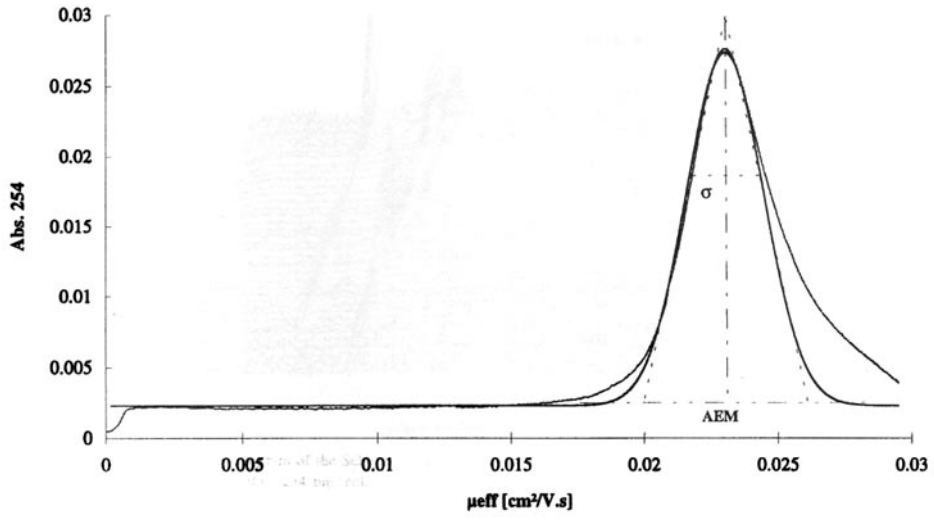


Figure 2.8 Gaussian distribution of electrophoretic mobilities of Humic acid (Schmitt-Kopplin *et al.* 1998).

Analysis of NOM isolates gives an insight into the effect of the preparation of the fractions on their structure (Schmitt-Kopplin *et al.* 1999). With CE analysis of the NOM isolates, prepared by Reverse Osmosis (RO) and evaporation, neither showed sharp peaks exhibited by fulvic acids (indicating phenolic acids) which suggests that phenolic peaks may be due to hydrolysis reactions occurring during acid/base fractionation. Special attention should also be given to the interpretation of the EPGs because they can be significantly influenced by the buffer used. Due to the complexation of borate ions with cis-diol groups within humic acid mixtures, borate buffers should only be used as a fingerprint of the NOM, showing the intensity and the amount of borate reacting fraction present in the NOM mixtures.

2.5 Disinfection

Public health concern over the disinfection process was first raised by Rook in 1974 with the identification of chloroform and other trihalomethanes (THMs) as disinfection by-products (DBPs) in drinking water (Liang and Singer 2001). On disinfection with chlorine, it is known that any NOM remaining in the water will react with the chlorine to create disinfection by-products.

2.5.1 The relationship between chlorine and NOM

The presence of THMs in finished drinking water has been attributed to reactions between chlorine and humic substances (precursors). THMs have been shown to account for only ~25 % of the DBPs (Singer and Chang 1989). This estimate was later increased to 30 – 60 % (Singer *et al*. 1995). These humic substances were found to be readily removed with alum coagulation (Reckhow and Singer 1990). However NOM consists of humic (non-polar) substances and non-humic (polar) substances. The non-polar NOM has been well characterised and is more readily removed by coagulation than polar NOM (Hwang *et al*. 2001). The reaction of chlorine with polar NOM has not been studied extensively. The rates of DBP formation change as the reactions proceed, reflecting the complex nature of the reactions involved.

2.5.2 Limitations of existing models

As a result of the complex nature of the DBP precursor compounds, models for quantification of DBPs have been largely developed using empirical approaches. Several statistical equations have been used to model THM formation and have the following general equation (Westerhoff *et al*. 2000, Singer and Chang 1989, Amy *et al*. 1987, El-Shafy and Grünwald 2000):

$$TTHM = K \cdot (DOC)^a \cdot (pH - b)^c \cdot T^d (Cl_2)^e \cdot (UVA)_{254}{}^f \cdot (Br)^g \cdot t^h$$

where K, a, b, c, d, e, f, g and h are fitting constants and DOC, pH, T, Cl_2, UVA, Br and t represent dissolved organic carbon, pH of the sample, temperature, chlorine dose, ultraviolet adsorption at 254 nm, bromide concentration and reaction time respectively. There is no theoretical basis to justify these equations and the number of fitting parameters ensures more than enough degrees of freedom to fit any data set (Gang *et al.* 2002). Another major limitation of existing mathematical models is that the equations are based on raw water parameters (Gang *et al*. 2002a). It is known that all NOM fractions separated by resin techniques contribute to DBP formation and chlorine demand (Andrews and Huck 1993). By only looking at certain raw water parameters, an indication of THM-FP is unlikely to be accurate as it is the treated water that is chlorinated and the treatment of the raw water may be varied to optimise removal of NOM resulting in varied treated water quality.

2.5.3 Focus of section

This section will not consider the empirical equations described above for predicting THM formation but will look at other techniques that can give insight into the reactions occurring. These will include mechanistic models based on chlorine demand and chlorination kinetics (Gang *et al.* 2002, Adin *et al*. 1991, Haag and Hoigné 1983, Gallard and von Gunten 2002), the use of UV spectroscopy for characterising the reaction between NOM and chlorine (Li *et al*. 2000) and the use of SUVA as a predictor for DBP formation (Kitis *et al.* 2002, Croué *et al.* 2000). The focus of the review will

be mainly on treated water or polar NOM disinfection (Chellam and Krasner 2001 and Hwang *et al.* 2001). The review will also concentrate on models that can be developed for specific sites and not models that are designed to work for any waters. The review will not discuss the effect of pH on THM formation as samples taken for analysis by the DWI are buffered to pH 7. It is known that THM production increases with increasing pH (Adin *et al*. 1991). A precursor for THMs has been proposed by Reckhow and Singer (1985). The precursor structure was (R-CO-CX_3) where R represents a hydrogen atom or a carbon-hydrogen group and X represents a halogen. The formation of the THM species is determined by the nature of the R-group and pH. Under alkaline conditions, base-catalysed hydrolysis prevails yielding more THMs. In the absence of oxidative cleavage (if R is not readily oxidisable) hydrolysis might still prevail, resulting in THMs. When R is a methyl group, THMs will be formed. Similarly, the effect of temperature will not be investigated as the samples taken will all be stored at 25 °C during formation of THMs. At higher temperatures, more THMs are formed than at lower temperatures (Krasner 1999).

2.5.4 Kinetic and mechanistic models

It is generally accepted that THM formation rate is highly dependent on the chlorine concentration. That is, the THM level rises with increasing chlorine dose (Adin *et al*. 1991). It is also possible that the order of reaction changes during the course of the reaction. Generally DBP formation occurs at a fast initial rate followed by a slower rate of formation that often continues for days in the presence of residual chlorine (Chowdhury and Amy 1999).

Early kinetic models focused on expressing THM formation as a function of humic substances present in the raw water (Haag and Hoigné 1983, Adin *et al*. 1991). More recently, THM formation is being expressed as a function of the NOM present in the treated water or 'polar NOM' (Hwang *et al*. 2001, Gang *et al*. 2002).

Most empirical equations for predicting THM formation are based on measuring organic carbon and UV absorbance. As the DOC is a measure of the amount of the precursor material in the water and UV absorbance at 254 nm can be considered a measure of the chemical reactivity of the organic carbon, this seems a plausible idea (Gang *et al*. 2002). However, such models based on DOC and UV have been found to underestimate THM formation by up to 30% (Singer 1999).

One approach around this is to investigate the changes in chlorine demand as a surrogate for predicting THM formation (Gang *et al*. 2002). A number of assumptions were made when developing the chlorine decay model. These were:

1. Two distinct types of reactive functionalities exist in NOM resulting in two parallel reactions forming halogenated organic by-products. One NOM functionality, possibly attributed to aldehyde and phenolic hydroxyl groups, results in a rapid rate of chlorine consumption. The other NOM functionality is less reactive, such as that

expected for activated double bonds and methyl groups and results in a slow, long-term chlorine demand.

2. The rate of reaction for each chlorine-consuming reaction is first-order in chlorine concentration.
3. Chlorine demand is solely attributed to reaction with NOM. The applied chlorine dose is selected to give a chlorine residual of ~1 mg/L after five days of reaction.
4. In the absence of inorganic demand, all chlorine consumption results in a proportional formation of DBP species.

The resulting equation for predicting THM formation takes the form:

$$TTHM = \alpha\, C_0\left(1 - fe^{-k_R t} - (1-f)e^{-k_S t}\right)$$

where α is the TTHM yield coefficient (defined as the ratio of the concentration of THM formed (μg L^{-1}) to the concentration of chlorine consumed (mg L^{-1})). C_0 is the initial chlorine concentration (mg L^{-1}). f is the fraction of chlorine demand attributed to rapid reactions. k_R is the first order rate constant for rapid reactions (h^{-1}) and k_S the first order rate constant for slow reactions (h^{-1}). t is time (h).

Again, this is a model for prediction of THM formation but it is based on the kinetics of the reaction rather than environmental factors such as pH and time and was found to be more accurate than the empirical equations described above. The data for the model was obtained by testing source water and water treated with alum coagulant. An insight into the reactions occurring can be obtained by comparing the reactivity of the source and treated water. There was no correlation found between TOC removal by alum and overall THM-FP reduction. From this it was construed that the reduction of THM-FP is

dependent on the qualitative nature rather than the quantitative amount of the NOM. By looking at the chlorine decay, it was found that coagulation by alum increases the f value by removing DOC with slower reaction sites (high molecular weight DOC). It was concluded that DOC with slower reaction sites or high molecular weights have a higher THM yield coefficient, α. This model only applies to surface water and not groundwater as THM formation in groundwater was found to not be a function of chlorine demand (Gang *et al*. 2002).

A different study also investigated the kinetics of THM formation (Gallard and von Gunten 2002). This study agreed that THMs were formed rapidly initially and later more slowly. The slowly reacting THM precursors followed 2nd order model kinetics, first order in chlorine concentration and first order in reactive sites. For surface waters, the second order rate constants were in the range 0.01 – 0.03 M^{-1} s^{-1} for the pH range 7-9. The initial THM-FP was found to correlate well with UV absorbance at 254 nm. A seasonal effect was observed with a higher chlorine demand in summer compared to water sampled in spring and winter. The corresponding rate constants were not significantly different from each other. However, the THMs formed finally were on average significantly higher in summer and winter (97.7 μg mg^{-1} C and 100.5 μg mg^{-1} C respectively) than in spring (66.7 μg mg^{-1} C). The initial THMs formed consisted 17 – 28% of the final THMs formed. This is in disagreement with Singer (1999) who reported that the formation of THMs increases with increasing temperature, giving higher levels of THMs in the warmer months of the year.

2.5.5 The use of UV spectroscopy for characterising the reaction between NOM and chlorine

When water containing NOM is chlorinated, the UV absorbance of the solution decreases at all wavelengths. This relationship can provide an approach for obtaining data that can be used to interpret the kinetics, stoichiometry and the mechanism of the reactions. By analysing the relationships, it was found that the functional groups that are the major precursors for DBPs may be highly activated aromatic rings (Li *et al*. 2000). However, these groups have fundamental differences from highly activated rings in pure compounds. The evidence for this is that the UV absorbance of the pure compounds increases upon chlorination which is in contrast to the reduction in UV absorbance seen for water containing NOM.

The rate of chlorine incorporation into organic molecules is initially rapid and decreases steadily thereafter. Chlorine reduction (or, equivalently, NOM oxidation) is negligible initially and then increases over time. The effect of these parallel processes is that the amount of chlorine that becomes incorporated into organic molecules as a fraction of the amount of HOCl and OCl^- consumed decreases from 100% initially to ~20% over the course of the reaction. It should be noted that, throughout this study, the reaction time reached a maximum of 20 minutes.

2.5.6 The use of SUVA as a predictor for DBP formation

When studying correlations between SUVA and DBP yields of UF and XAD-8 fractions, Kitis *et al*. (2002) confirmed the trend of increasing DBP formation with increasing SUVA. In the context of DBP reactivity, Kitis *et al*. (2002) also found the chemical properties (i.e. aromaticity) of the NOM mixtures were more important than

their physical size. Strong and simple linear trends (R^2 = 0.85 to 0.92) were observed between SUVA and THM-FP. From this, they confirmed that SUVA is a distributed parameter. That is, when SUVA is measured for a water sample it is a composite value representing contributions from different components. In the future, finding the SUVA distribution of NOM, which reflects the distribution of the unsaturated bonds, may become an important method of characterisation of NOM.

Presently, SUVA is a reasonable surrogate measurement to predict THM-FP. However it has been shown that NOM fractions isolated from various water sources did not have the same reactivity even if the SUVA values were similar (Croué *et al.* 1999a).

2.5.7 Treated water and polar NOM disinfection

It has been reported that humic acid isolates had higher THM-FPs than fulvic acid isolates (Oliver and Thurman 1983, Reckhow *et al.* 1990). But there have been few studies on the THM-FP of more hydrophilic NOM compounds. It has been hypothesised that the relative abundance of activated and non-activated aromatic rings is a major parameter that governs the reactivity of NOM with chlorine (Reckhow *et al.* 1990). The difference in aromatic sites between hydrophobic and hydrophilic NOM isolates is said to stem from the differing origins of the NOM (Hwang *et al.* 2001). The same study showed a 'loose' linear relationship (R^2 = 0.66) between chlorine demand and THM yield for hydrophilic base fractions of four waters. The study also reported a stronger linear relationship (R^2 = 0.75) between C/N ratio and chlorine demand for hydrophobic NOM. The hydrophobic NOM chlorine demands were higher at lower C/N ratios. No relationship was observed between chlorine demand and C/N ratio for the more hydrophilic fractions.

Chellam and Krasner (2001) sampled waters with a range of SUVA values and subjected them to nanofiltration (NF). This had the effect of reducing the SUVA value of each water. It was found that reductions in SUVA by NF did not significantly decrease the DBP yield. They also found linear relationships (R^2 = 0.88, 0.93) between THM concentration (μM) and the NF feed water and permeate DOC (mM) respectively. The reaction was stopped after 24 hours. Despite the high R^2 values, the data showed a lot of scatter but this was reduced when each water source was considered separately. Chlorine consumption was found to be a good predictor of the THM formation of NF feed waters ($R^2 > 0.9$) but not of permeate waters ($R^2 \sim 0.5$). It is thought that the majority of the waters studied here had a high inorganic content and thus a high inorganic chlorine demand (ICD). However, in the absence of ICD, chlorine consumption in NF permeates was found to be a good predictor of DBP formation.

To conclude, there are some relationships described above that can help to predict DBP formation for a particular water source. These relationships are strongest when waters are considered individually. The reactions that are occurring between chlorine and NOM are complex and difficult to understand. It has been shown that NOM does not react with chlorine in the same way as model compounds react with chlorine (Li *et al.* 2000). UV spectroscopy is a popular technique for measuring the 'reactive sites' in NOM. The SUVA distribution sought by Kitis *et al.* (2002) may enable further understanding of the reactions taking place between chlorine and NOM.

2.6 Summary of findings

- Bulk water analysis gives a limited understanding of the character of NOM
- Fractionation of NOM gives an insight into the fate through a treatment works and variability in source
- In NOM analysis there is a trade-off between bulk analysis where the NOM is unaltered and separation of NOM where the NOM is altered and synergistic effects are lost
- There is a range of analytical methods available although none are ideally suited to NOM analysis
- Fluorescence spectroscopy and HPSEC have potential as rapid assessment methods for NOM
- DBP formation is affected by fraction make-up (NOM quality) rather than quantity

CHAPTER 3 OBJECTIVES

The aim of the project was to investigate the change in character of reservoir water containing elevated levels of NOM.

This was achieved by carrying out the following objectives:

1. Examine and develop pragmatic methods of assessing NOM
2. Investigate seasonal variation in NOM character
3. Investigate the link between NOM character and chlorine reactivity

CHAPTER 4 MATERIALS AND METHODS

4.1 Summary of treatment works

This section describes the two water treatment works (WTW) from which the samples were taken. The sampling points at each works are denoted by a *. Albert WTW was chosen as it has recently experienced problems reducing the level of organics in the water. It is an example of a highly coloured water. Rivington WTW was chosen as an example of a less coloured water.

4.1.2 Albert WTW (Yorkshire Water)

Albert WTW is a three-stage plant (producing 33 – 55 megalitres per day (MLD)) on the western side of Halifax utilising clarification, primary filtration and manganese removal. Clarification is via 6 dissolved air flotation units (DAFs); primary filtration is via 6 rapid gravity filters and manganese removal is through 8 pressure filters. A basic process schematic is shown in figure 4.1.

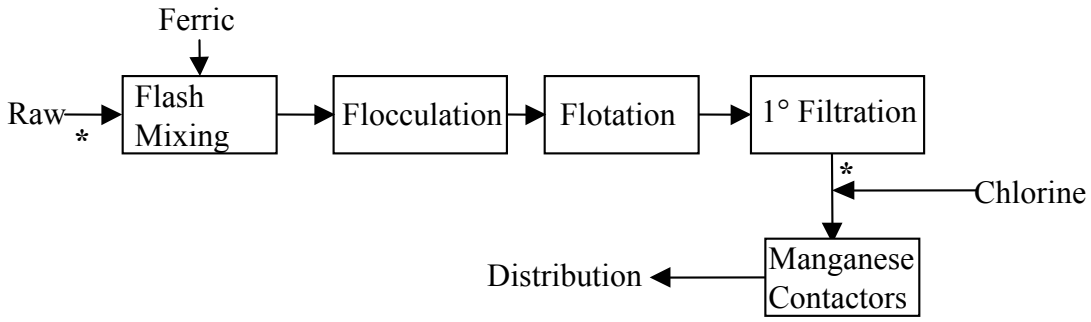


Figure 4.1 Process schematic of Albert WTW (Yorkshire Water)

4.1.3 Rivington WTW (United Utilities)

Rivington WTW is a three-stage plant situated to the north-west of Bolton treating 80 MLD utilising clarification, primary filtration and secondary filtration. Clarification is via DAF; primary filtration is via rapid gravity filters and secondary filtration is through pressure filters. A basic process schematic is shown in figure 4.2.

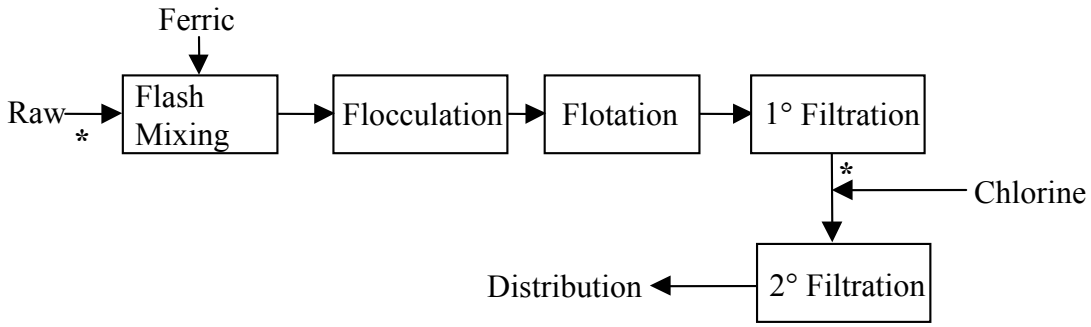


Figure 4.2 Process schematic of Rivington WTW (United Utilities)

4.2 Fractionation

4.2.1 Resin fractionation

Bulk waters were fractionated by XAD resin adsorption techniques into their hydrophobic acid (HPO-A) and hydrophilic acid (HPI-A) fractions using a method adapted from Malcolm & McCarthy (1992). A schematic of the procedure is shown in figure 4.3. The resins used were Amberlite XAD-7HP resin and Amberlite XAD-4 resin (Rohm & Haas, PA, USA). Amberlite XAD-7HP is an acrylic ester polymer. It has been used in place of XAD-8 resin as its manufacture has been discontinued. Amberlite XAD-4 is a styrene divinylbenzene polymer. Bio-Rad AG-MP-50 resin (Bio-

Rad Laboratories Ltd., Herts, UK), a non-macroporous cation exchange resin, was used to hydrogen saturate the fractions produced.

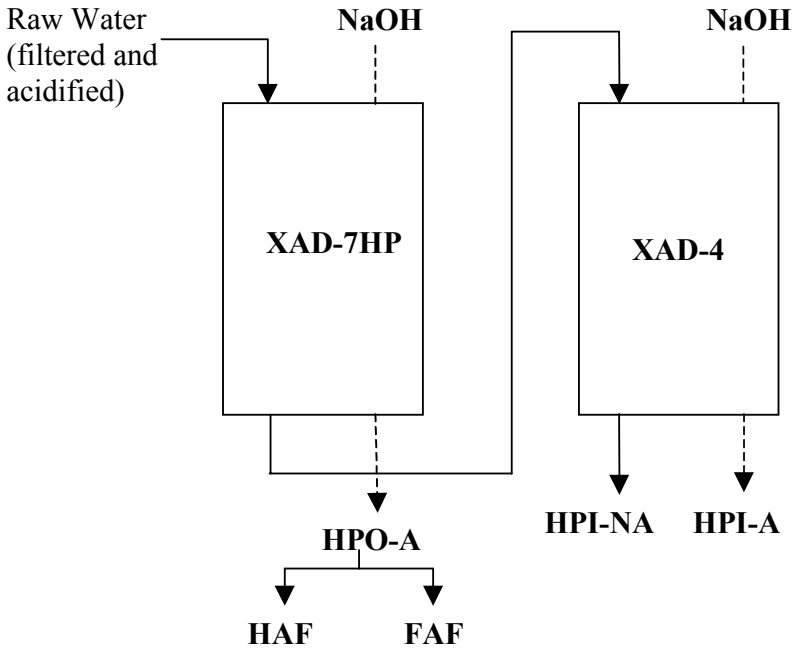


Figure 4.3 Schematic of Fractionation Process

4.2.1.1 Resin preparation

Amberlite XAD-7HP Resin - Amberlite XAD-7HP resin (1.5 L) was slurried with NaOH (0.1 M, 1.5 – 2 L) and the fines decanted off, this was then repeated. The resin was stored in Methanol (1.5 – 2 L) for 24 hours. The resin was sequentially Soxhlet extracted for 48 hours each with Methanol, Acetonitrile and Methanol again (~ 1.8 L). The resin was packed into the column and rinsed with reverse osmosis (RO) water until the column effluent TOC was < 2 mg/l.

The column was rinsed with 2.5 bed volumes (BV) NaOH (0.1 M) followed by 2.5 BV HCl (0.1 M) to remove impurities.

Amberlite XAD-4 Resin - As Amberlite XAD-7HP Resin preparation.

Bio-Rad AG-MP-50 Resin - The resin was Soxhlet extracted for 24 hours with Methanol. The resin was then slurried with RO water and packed into the column to be used. NH_4OH (3 M) was pumped through the column until breakthrough of ammonia was observed. The resin was hydrogen saturated by pumping four bed volumes of HCl (2 M) through the column. The resin was rinsed with RO water until the specific conductance of the column effluent was the same as the influent water. The resin was stored in Methanol.

Each column was wrapped in aluminium foil to prevent algal growth.

4.2.1.2 Column capacity calculation – XAD resins

Adsorption or elution of organic solutes on XAD-8 resin determines the hydrophobic-hydrophilic separation of the DOC fractionation. This arbitrary hydrophobic-hydrophilic designation is controlled by the polarity of the solute and by the ratio of the resin quantity to the volume passing through the resin bed. As most organic solutes (including hydrophilic solutes) show some affinity for XAD-8, the hydrophobic-hydrophilic break is not clear-cut but is an operationally defined separation in which the crossover of hydrophilic solutes into the hydrophobic fraction can be mathematically defined. For the DOC fractionation, hydrophobic solutes are defined as those solutes that are greater than 50% retained on XAD-8 at a given ratio of resin to water passed

through the column, and hydrophilic solutes are defined as those solutes that are greater than 50% eluted at the same ratio of resin to water eluent (Leenheer 1981).

To design a DOC fractionation, it is useful to refer to the column distribution coefficient, $k'_{0.5r}$, of a hypothetical solute that is 50% retained and 50% eluted at the hydrophobic-hydrophilic break. This $k'_{0.5r}$ is determined by the following calculations.

The breakthrough (elution) volume V_E of a solute from an XAD-8 resin column can be described by equation 4.1:

$$V_E = V_0(1 + k') \qquad \text{Equation 4.1}$$

Where V_0 = void volume

And k' = (mass of solute sorbed on XAD-8) ÷ (mass of solute dissolved in water)

Because the breakthrough volume V_E, where effluent concentration is 50% of influent concentration, does not correspond to the effluent volume of 50% retention, $V_{0.5r}$ is defined as (equation 4.2):

$$V_{0.5r} = 2V_E \qquad \text{Equation 4.2}$$

which can also be expressed as (equation 4.3):

$$V_{0.5r} = 2V_0(1 + k'_{0.5r}) \qquad \text{Equation 4.3}$$

It is assumed that the column distribution coefficient, $k'_{0.5r}$, is 50.

Example:

Sample is 1 L. ($V_{0.5r}$ = 1000 mL)

$k'_{0.5r}$, is 50.

Solving for the final equation gives V_0 = 9.8 mL

As the void volume of XAD-8 resin is ~65% of its bulk column volume, a 9.8 mL ÷ 0.65 = 15 mL column of XAD-8 resin is required.

4.2.1.3 Column capacity calculation – cation exchange resin

The exchange capacity of the Biorad AG-MP-50 resin has an exchange capacity of 4.9 meq g-1. The meq of cations in the sample can be estimated using equation 4.4.

$$meq\ of\ salt\ L^{-1} = 12.5L \qquad \text{Equation 4.4}$$

L is the specific conductance in mS cm^{-1} at 25 ºC.

The amount of resin required for a sample is calculated using equation 4.5.

$$g = V \times \frac{(meq\ salt\ L^{-1})}{exchange\ capacity} \qquad \text{Equation 4.5}$$

where g is the amount of resin required in grams and V is the sample volume in litres.

4.2.1.4 Fractionation procedure

The following resin quantities and sample volumes are based on the fractionation of natural reservoir water.

Raw inlet water (75 L) from Albert Reservoir was passed through a 1 µm pre-filter capsule and a 0.45 µm filter capsule (both from Whatman plc., Maidstone, Kent, UK) and acidified to pH 2 using HCl. All of the acidified filtered water was put through the

XAD-7HP/XAD-4 column pair (resin volume was 1200 ml in each column). The effluent from both columns contained the non-acid hydrophilic fraction (**HPINA**).

The XAD-8 column was back eluted with NaOH (0.1 M, 1800 ml). The eluate was acidified to pH 2 and passed through a 60 ml XAD-8 column. This was the hydrophobic acid fraction (HPOA). The XAD-4 column was back eluted with NaOH (0.1 M, 1800 ml). The eluate was acidified to pH 2 and passed through a 60 ml XAD-4 column. This was the hydrophilic acid fraction (**HPIA**).

The pH of the HPOA was adjusted to 1 (± 0.2) by adding concentrated HCl (12.1 M), and left to settle for 24 hours and centrifuged. The supernatant (fulvic acid fraction – **FAF**) was decanted. The residue (humic acid fraction – **HAF**) was dissolved in the minimum required volume of NaOH (0.1 M, ~50 ml). The HAF was hydrogen saturated by passing it through a 5 ml column of Bio-Rad AG-MP-50 resin and rinsed with RO water (5 ml). The FAF was passed through a 5 ml column of Bio-Rad AG-MP-50 resin and rinsed with RO water (5 ml).

The HPI-A was pumped through a 5 ml column of Bio-Rad AG-MP-50 resin and rinsed with RO water (5 ml).

The fractions produced consisted of FAF, HAF, HPIA and HPINA. The recovery of the DOC was quantified by measuring the influent DOC of the water and the DOC and volume of the fractions produced. Unextracted material was assumed to be made up of the hydrophobic neutral fraction (HPON) as well as unrecovered material. This was not quantified.

4.2.2 Ultrafiltration (UF) fractionation

Ultrafiltrations were performed under constant nitrogen pressure (1.4 bar) in a stirred cell reactor (model 8200, Millipore, Massachusetts, USA). Membranes with molecular weight cut off (MWCO) values of 3000, 1000 and 500 Daltons were used (YM1 - YM3, Millipore, Massachusetts, USA, YC05, manufactured by Millipore, supplied by Adelab Scientific, Thebarton, Australia). The YM membranes were made of regenerated cellulose and the YC membrane, cellulose acetate. The YM membranes were flushed with NaOH (0.1M), NaCl (0.5M) and deionised water to remove the wetting agents before use. The YC05 membrane was flushed with NaCl (1.0M) and deionised water. The ultrafiltration was carried out using a method described by Belin *et al*. (1993). It was carried out in three steps by successively using three membranes of decreasing MWCOs. The precautions and assumption made by Belin *et al*. are repeated here.

In order to have values that reflect precisely the central part of the molecular weight (MW) distribution in the fractions, we have arbitrarily adopted as cut-off values, the values given by the manufacturer corresponding to the MWs of model compounds retained at 70%. Four fractions were isolated: R3 (MW > 3000 Daltons) using the YM3 membrane, R1 (3000 > MW > 1000 Daltons) using the YM1 membrane and R05 (1000 > MW > 500 Daltons) and the fraction corresponding to the last effluent, F05 (MW < 500 Daltons) using the YC05 membrane. A schematic diagram of the process is shown in figure 4.4.

In the first step, an initial volume of 320 mL of bulk water previously filtered to 0.45 μm, was introduced into the ultrafiltration cell. The ultrafiltration process was stopped when the ratio of effluent volume over the initial volume was 8 to 9 leading to a

concentration increase in the retained fractions by a factor of 9. The ratio was chosen according to the following considerations:

- It must be high enough to allow the maximum amount of small molecules to pass through the pore membrane.
- It must not be too high to prevent possible formation of aggregates and to avoid an increasing amount of molecules of a larger size than the mean pore dimensions passing through the membrane.

Once the first step had been completed, the R3 fraction was put aside and a 15 mL aliquot of the ultrafiltrate, F3, was kept for further analysis. The rest of F3 was then introduced into the ultrafiltration cell fitted with the YM1 membrane. The process was repeated. The process was once again repeated with the YC05 membrane.

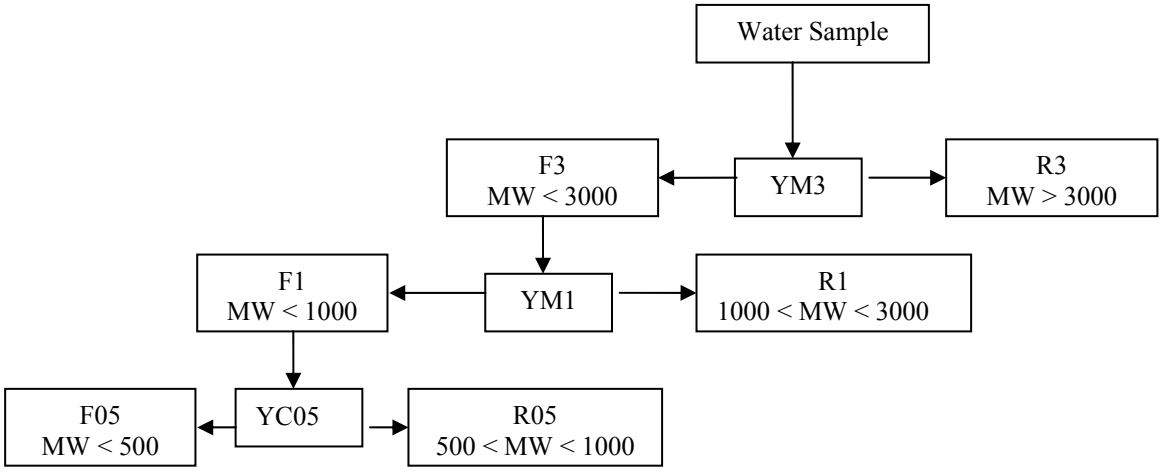


Figure 4.4 Schematised procedure for ultrafiltration fractionation

Key: R3, R1 and R05 are fractions retained on the membranes YM3, YM1 and YC05 respectively. F3, F1 and F05 are the fractions that pass respectively through these membranes.

Measurements of DOC, UV, THM-FP, HPSEC and fluorescence spectra were carried out on the four isolated fractions (R3, R1, R05 and F05).

The membranes used by Belin *et al*. had MWCOs of 1500, 500 and 200 Daltons. It was not possible to obtain the membranes with MWCOs of 1500 and 200 Daltons as their manufacture has been discontinued.

4.3 Analytical methods

4.3.1 Dissolved organic carbon

Dissolved organic carbon (DOC) was measured using a Shimadzu TOC-5000A analyser (Shimadzu, Milton Keynes, UK). TOC was calculated by measuring the total carbon (TC) and the inorganic carbon (IC) and subtracting the IC from the TC. The TC standard was made by dissolving 2.125 g potassium hydrogen phthalate in 1 L RO water. The IC standard was made by dissolving 1.750 g sodium hydrogen carbonate in 500 mL RO water and adding it to 2.205 g sodium carbonate in 500 mL RO water. Each standard had a concentration of 1000 mg L^{-1} and working standards were diluted to the appropriate concentration with RO water. The machine was calibrated regularly. The analyser took up to five replicates and reported an average of three given that the coefficient of variance was not greater than 2%. The analyser was recalibrated if the value of the standards were not within 10% of the expected value.

4.3.2 Ultraviolet absorbance

Ultraviolet (UV) absorbance at 254 nm was measured using a Jenway 6505 UV/VIS spectrophotometer (Patterson Scientific Ltd., Luton, UK). The machine was calibrated using RO water as a blank.

4.3.3 Specific ultraviolet absorbance

Specific UV absorbance (SUVA) (m^{-1} .L mg^{-1} C) was calculated as the ratio of UV absorbance at 254 nm (m^{-1}) to DOC (mg C L^{-1}).

4.3.4 Trihalomethane formation potential

Trihalomethane formation potential (THM-FP) was carried out using an adaptation of procedure 5710 in 'Standard Methods for the Examination of Water and Wastewater' (American Public Health Association 1992) and is described below.

4.3.4.1 Reagents

Determination of strength of hypochlorite (HOCl) solution – sodium hypochlorite (13%, 0.4 mL) solution was diluted to 25 mL in a volumetric flask with reverse osmosis (RO) water and mixed well. The diluted solution was placed in a conical flask containing acetic acid (5 mL) and potassium iodide (~ 1 g). The contents of the flask were mixed and titrated with aqueous sodium thiosulphate (0.1 M) prepared with RO water until the yellow colour of the liberated iodine was almost discharged. Iodine indicator powder (~ 1 g) was added and the titration continued until the blue/black colour was discharged. The volume was recorded. The chlorine concentration of the sodium hypochlorite was calculated using equation 4.6.

$$Hypochlorite\ concentration\ (mg\ mL^{-1}\ Cl_2) = \frac{(M \times 35.45 \times titrant\ volume\ (mL)}{hypochlorite\ added\ (mL)}$$

Equation 4.6

where M is the molarity of the titrant (sodium thiosulphate).

The titration should require as least 10 mL titrant. If this is not the case then 0.8 mL of the hypochlorite solution should be used. The strength of the hypochlorite solution was measured each time a dosing solution was made. The sodium hypochlorite solution was discarded when the concentration fell below 20 mg L^{-1} Cl_2.

Chlorine dosing solution (1000 mg L^{-1} Cl_2) – The volume of hypochlorite solution required was calculated using equation 4.7.

$$Hypochlorite\ required\ (mL) = \frac{1250}{hypochlorite\ concentration\ (mg\ mL^{-1}\ Cl_2)} \div 5$$

Equation 4.7

The calculated volume was diluted to 250 ml in a volumetric flask to the mark with RO water. It was mixed and transferred to an amber bottle with a PTFE-lined screw cap and refrigerated. The free chlorine concentration was measured by DPD powder pillow photometric method using a HACH DR/2010 spectrophotometer (Camlab Ltd, Cambridge, UK). This solution was discarded after 1 week.

Phosphate buffer – 68.1 g potassium dihydrogen phosphate (KH_2PO_4) and 11.7 g sodium hydroxide (NaOH) were dissolved in 1 L RO water. The buffer was refrigerated and discarded after 1 week.

Sodium sulphite solution – 10 g sodium sulphite was dissolved in 100 ml RO water. It was used for dechlorination. 0.1 ml destroyed about 5 mg residual chlorine. This solution was discarded after 2 weeks.

Reverse osmosis water – This water was processed in the laboratory by a reverse osmosis membrane filtration unit (USF Elga, High Wycombe, UK).

DHBA solution – anhydrous 3,5-dihydroxybenzoic acid, DHBA, (0.078 g) was dissolved in 2 L RO water. This solution needed to be made fresh before each use.

Hydrochloric acid (concentrated, 1 M and 0.1 M)

4.3.4.2 Method

Sample chlorination – all bulk waters and fractions were diluted to approximately 1 mg L^{-1} DOC before analysis. The actual concentration was determined by DOC analysis. The appropriate volume of chlorine dosing solution was put in a 250 mL bottle with 5 mL phosphate buffer and filled completely with sample. This bottle was stored in an incubator at 25 ± 2 ºC for seven days.

Reagent blank – 1 mL chlorine dosing solution was placed in a 50 mL volumetric and made up to the mark with phosphate buffer. A 22 mL PTFE-lined screw cap vial was completely filled with the mixture and stored with the sample at 25 ± 2 ºC for seven days.

Quality control samples – 1 mL chlorine dosing solution was diluted to 1 L with RO water. 5 mL phosphate buffer was added to each of two 250 mL bottles. 1 mL DHBA solution was added to one bottle and each bottle completely filled with the diluted chlorine dosing solution and capped with PTFE-lined screw caps. These were also stored with the sample at 25 ± 2 ºC for seven days.

Sample analysis – after seven days storage 0.88 mL sodium sulphite solution was placed in a 22 mL vial and gently and completely filled with sample. If the sample was not being analysed immediately, the pH was reduced to <2 by adding 5 drops of concentrated acid. The vial was sealed with a PTFE-lined screw cap. The sample was refrigerated but brought to room temperature before analysis by Gas Chromatography. The total THM (trichloromethane ($CHCl_3$), dichlorobromomethane ($CHBrCl_2$), dibromochloromethane ($CHClBr_2$), and bromoform ($CHBr_3$)) concentration was measured using a SRI 9300A Gas Chromatograph (California, USA) with the results in $\mu g\ L^{-1}$.

Blank analysis – after seven days storage 1 mL of sulphite reducing solution was added to a 250 mL bottle and 5 mL of the reagent mixture added without mixing. The bottle was immediately completely filled with RO water and capped with a PTFE-lined screw cap. A portion was analysed for THMs using the same method as for the sample. The sum of all THMs should be $<5\ \mu g\ L^{-1}\ CHCl_3$

Quality control sample analysis – after holding in the dark for seven days 1 mL of sulphite reducing solution was added to each of two 250 mL bottles and 5 mL of the

reagent mixture added without mixing. The bottles were immediately completely filled with RO water and capped with a PTFE-lined screw cap. A portion of each was analysed for THMs using the same method as for the sample. The THM concentration of the solution containing the added DHBA minus the concentration of the solution without the DHBA (the true blank) should equal 119 μg L^{-1} THM as $CHCl_3$. If the THM concentration of the true blank exceeds 20 μg L^{-1}, a more pure reagent water is required.

4.3.5 High performance size exclusion chromatography (HPSEC)

Bulk waters were analysed at their natural DOC concentration and filtered to 0.45 μm before analysis. Fractions were diluted to 1 mg L^{-1}. HPSEC was carried using an HPLC (Shimadzu VP Series, Shimadzu, Milton Keynes, UK) with UV detection set to 254 nm. The mobile phase was 0.01 M sodium acetate at a flow rate of 1 ml min^{-1}. The column was a TSK – gel G3000SW 7.5 mm (ID) × 30 cm and the guard column was TSK gel 7.5 mm (ID) × 7.5 cm (Tosoh Biosep GmbH, Stuttgart, Germany). For each sample a chromatogram of UV absorbance (arbritrary units) against time (minutes) was produced. Part way through the study a new column and guard column were purchased. This column was a BIOSEP-SEC-S3000 7.8 mm (ID) × 30 cm and the guard column was a 'Security Guard' fitted with a GFC-3000 disc 4.0 mm (ID) × 3.0 mm (Phenomenex UK, Cheshire, UK). The column packing in all columns was identical. Due mainly to the smaller guard column dimensions from Phenomenex compared with Tosoh Biosep GmbH, the retention times produced were different for each column. However the mechanism of separation and number of peaks produced by each column were the same.

4.3.6 Fluorescence spectrophotometry

Fluorescence measurements were carried out using a Varian Cary Eclipse Spectrophotometer (Middelburg, The Netherlands). Bulk waters were filtered to 0.45 μm before analysis and analysed at their natural DOC concentration as well as being filtered to 0.45 μm before analysis at 1 mg L^{-1} DOC. Fractions were diluted to 1 mg L^{-1}. The pH of each sample was adjusted to 7. A synchronous scan was carried out on each sample. This involved exciting each sample from 225 to 525 nm. At each excitation level, emission was recorded from (excitation + 24 nm) to 633 nm. An optimal stepwise increment of 12 nm was used for both excitation and emission measurements. For each sample an excitation-emission matrix (EEM) was produced. Good spectroscopic practice was exercised throughout the study.

Absorption correction was not applied to the fluorescence spectra. It was not deemed necessary at the low concentrations of samples analysed (~ 1 mg L^{-1}). It would be necessary at high concentrations to eliminate inner-filter effects. The sensitivity of the instrument was tested by finding the Raman maximum of deionised water and calculating the S/N ratio. This was >750:1 at Ex = 350 nm and >500:1 at Ex = 500 nm. The wavelength accuracy was validated by scanning two peaks in the Xenon lamp spectrum at 541.92 nm (±0.5 nm) and 260.54 nm (±1.0 nm). The wavelength reproducibility was tested by repetitively measuring the wavelength of the 541.92 nm Xenon lamp peak for 10 scans and calculating the difference between the largest and smallest measured wavelength. The difference had to be <0.2 nm for the test to be passed. When measuring the wavelength accuracy of the emission monochromator, a ground silica screen diffuser was placed in the sample cell. No sample was placed in

the cell when measuring the accuracy of the excitation monochromator as it was the signal from the reference photomultiplier tube (PMT) that was being monitored.

Fluorescence Index (FI) is defined as the ratio of emission intensity at 450 nm to the emission intensity at 500 nm both at an excitation wavelength of 370 nm. This measurement was taken from the EEMs.

4.3.7 Capillary electrophoresis

Resin separated samples of raw and filtered fractions of Albert Reservoir Water were analysed by capillary electrophoresis (CE) with UV detection by 'Express Separations' in Leeds, UK. The parameters are reported in table 4.1

Table 4.1 CE Parameters

Parameter	Value
Electrolyte Buffer	90 mM Borate Buffer at pH 8.3
Temperature	Ambient
Applied Voltage	20 kV
UV wavelength	254 nm
Capillary material and dimensions	Fused silica, 60 cm × 50 μm internal diameter
Sample volume	5.9 nL
Sample concentration	50 – 100 mg/L

For each sample an electropherogram of UV absorbance (arbritrary units) against time (minutes) was produced.

4.3.8 Pyrolysis – gas chromatography – mass spectrometry (Py-GC-MS)

Sample preparation – resin separated fractions were freeze dried (Thermo Savant ModulyoD freeze drier, Thermo Electron Corporation, Massachusetts, USA) before Py-GC-MS analysis.

Sample analysis - Awaiting details from Finland.

CHAPTER 5 RESULTS AND DISCUSSION

5.1 Water treatment works performance indicators

At Albert WTW it is known that the influent (reservoir) water quality is variable throughout the year in terms of colour and this is impacting on the ability of the WTW to treat the water sufficiently in order to meet THM regulations. Initial experiments were undertaken to identify links between bulk water parameters and treatment performance (Banks and Wilson 2002). A surrogate parameter for THM precursors was sought. UV absorbance at 254 nm (UV_{254}) was chosen as the surrogate as it could be measured on-line and the level in the treated water was above the limit of detection. A target UV_{254} value was set that would ensure THM compliance. By comparing the measured UV_{254} with the target value, the performance of the works was measured in terms of 'robustness'.

5.1.1 Robustness

Water companies seek to provide their customers with high-quality drinking water at all times. This is especially challenging in the north of England, where the water companies are experiencing difficulty in meeting regulations due to the widely varying reservoir water quality. It is important that drinking water systems are as robust as possible to ensure continued compliance with regulations and wholesome water for the customers. Huck and Coffey (2002) defined a robust system as one that provides excellent performance under normal conditions and deviates minimally from this during periods of upset or challenge. This is demonstrated in figure 5.1. Here the clarifier performance is the percentage removal of turbidity from the water by the clarifier.

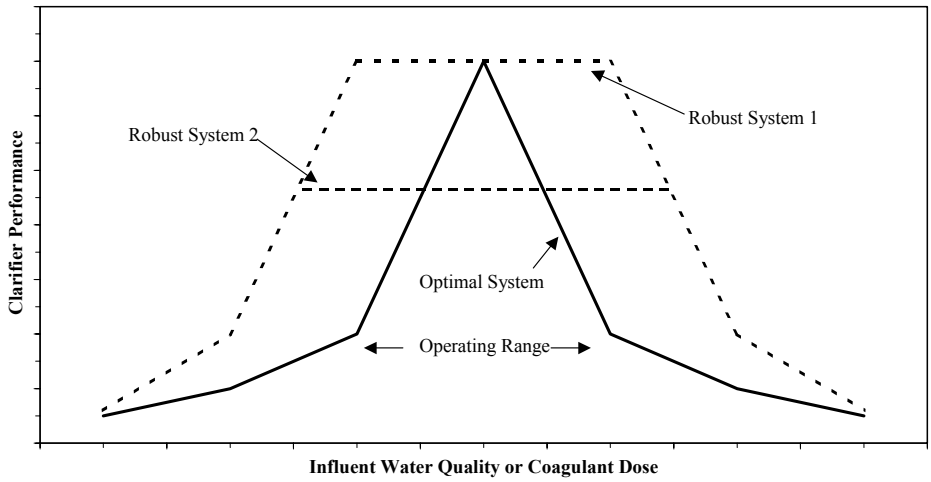


Figure 5.1 Representation of the robustness concept (Huck and Coffey 2002)

Robustness can be described in terms of an index that incorporates both average quality and deviation from stable operation (Huck and Coffey 2002). The robustness index is calculated using equation 5.1. Generally turbidity is used as the key water quality parameter to determine the performance at a WTW but here we have used UV_{254} as the performance indicator.

$$URI_{95} = \frac{1}{2}\left[\frac{U_{95}}{U_{50}} + \frac{U_{50}}{U_{goal}}\right] \qquad \text{Equation 5.1}$$

where URI_{95} = UV robustness index using the 95th percentile, U_{50}, U_{95} = 50th and 95th percentile UV absorbance and U_{goal} = clarified UV absorbance goal.

The index was calculated for Albert WTW in 2001 and is shown in figure 5.2. There was no data available for February 2001. The value used for U_{goal} was 3.5 m^{-1} for this WTW (Banks and Wilson 2002). This value was set to ensure THM compliance as well as sufficient removal of turbidity, colour and metals. It is known that the influent water quality is decreasing over time at Albert WTW and also that the water is more reactive

with respect to THM formation at certain times of the year. With this in mind, upper and lower limits for U_{goal} were set at 2 m^{-1} and 5 m^{-1} to determine the robustness of the system should the reactivity of the water increase and to investigate whether values of unity or less could be achieved with a higher U_{goal} value.

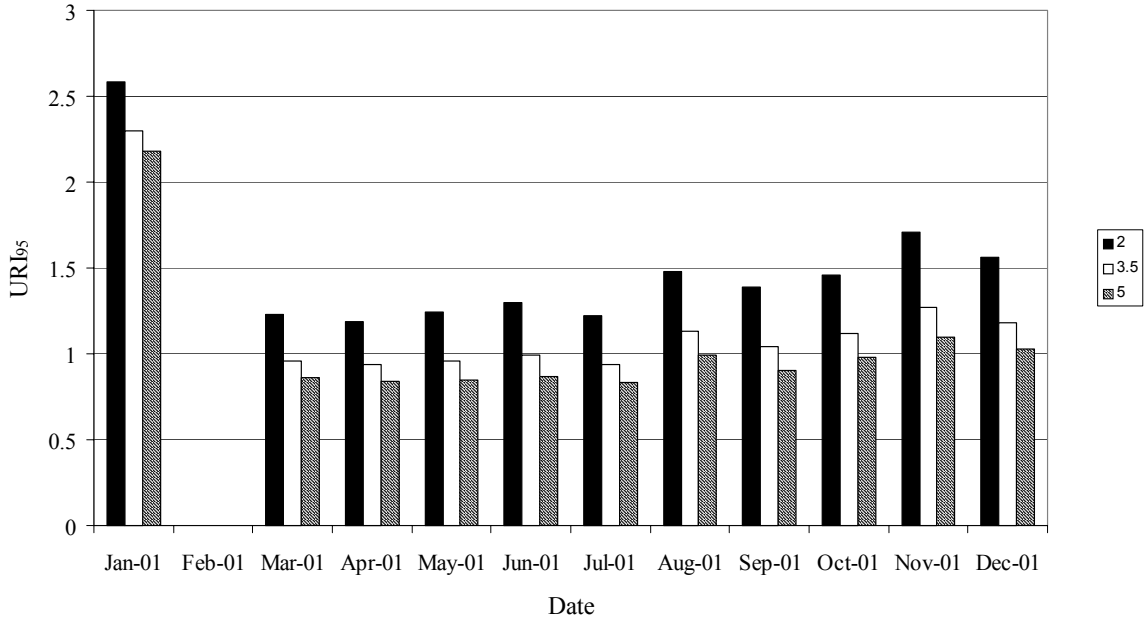


Figure 5.2 Robustness index against time

Using equation 5.1, a value lower than unity for the robustness index indicates a treatment process that is meeting the water quality goal with relatively low variation. If the UV absorbance met the goal of 3.5 m^{-1} exactly, the robustness index would be equal to 1.0. A higher robustness value indicates that the treatment is not meeting the goal (on average) or the variability is high.

In January 2001 the URI_{95} is greater than 2. During this month, the variability of the UV_{254} of the treated water was high and frequently, the goal of 3.5 m^{-1} was not being met. From March through to July 2001, the URI_{95} was consistently less than 1.0 indicating that the WTW was consistently meeting the goal. This could be because the

influent water quality was good and the treatment was sufficient. From August through to September, the URI_{95} values were greater than 1.0 reaching a peak in November at 1.27. These values are higher than unity and indicate that the deteriorating influent water quality is affecting the WTW performance during the autumn/winter period.

With the U_{goal} lower limit set at 2 m^{-1}, the URI_{95} values are consistently greater than 1.2. This indicates that THM consents may not be met if the reactivity of the water increased in terms of THM-FP per unit UV. With a further decrease in water quality, the treatment processes may need to be changed in order to continue to meet consents. Conversely, with the U_{goal} upper limit set at 5 m^{-1}, the URI_{95} values are less than 1.0 with the exception of January (2.2) and November (1.1). With a higher U_{goal} the current treatment process seems to be sufficient. By determining the robustness at different U_{goal} limits, the importance of deciding on the U_{goal} that should be implemented is highlighted.

By monitoring the URI_{95}, it is possible to determine when the WTW is experiencing problems. The problems could be due to the influent water quality falling or the treatment processes not working optimally.

5.1.2 Section conclusions

It was reported in the introduction (Chapter 1) that the colour in the reservoir at Albert WTW varies seasonally and becomes more difficult to treat at certain times of the year. The increase in colour is echoed in the robustness index calculated here with values

greater than unity being observed from August to December 2001. It is possible to use the robustness index as a performance indicator. It is suggested that U_{goal} values are reviewed regularly as their value can determine the validity of the robustness index. When the URI_{95} value is greater than 1.0, enhanced coagulation (Edzwald and Tobiason 1999) can be implemented in order to improve NOM removal and bring the URI_{95} value to less than 1.0. Although enhanced coagulation is effective in that it optimally removes NOM and colour from the reservoir water, it may not the best approach to meeting consents. It uses more coagulant, produces more sludge and leads to increased operating costs (Crozes *et al*. 1995).

By measuring the 'robustness' of a WTW, it is possible to determine when the treatment processes are failing to work optimally. It will not, however, give any information on the relationship between the influent water character and its reactivity. Further analysis of the NOM rich water is required to understand more about how it changes throughout the year.

5.2 Analysis of bulk and fractionated NOM

In this section, the make-up and reactivity of the bulk waters was investigated by separation of the NOM in the water into fractions. Bulk water samples were separated by adsorption and by size. The resulting separated fractions and the original bulk waters were subjected to laboratory tests. A comparison of the different fractions was made based on the method of separation and seasonal variation.

5.2.1 Resin fractionation

5.2.1.1 Sampling (2000)

Water samples were collected from the reservoir inlet (75 L) and after the rapid gravity filters (300 L) at Albert WTW in the Yorkshire Water region in January, June and November 2000. Samples were taken at these points to determine the 'character' of the reservoir and treated water and to determine the effect of treatment on the water.

5.2.1.2 Bulk water analysis

Samples of raw and filtered water collected in January, June and November 2000 were analysed for pH, DOC, UV and THM-FP. The chlorine dose was 5 mg Cl_2 mg^{-1} C. Throughout this section, the THM-FP of each sample was measured at least twice with the average finding reported. These results are summarised with calculated SUVA values in table 5.1.

Table 5.1 Comparison of raw and filtered water from Albert WTW (2000)

Sample	DOC ($mg\ L^{-1}$)	pH	UV (m^{-1})	SUVA (m^{-1} .L mg^{-1} C)	THM ($\mu g\ L^{-1}$)	THM-FP ($\mu g\ mg^{-1}$ C)
January Raw Water	7.5	6.0	34.0	4.53	632.2	84.3
January Filtered Water	2.6	7.4	4.4	1.70	77.1	29.7
June Raw Water	8.1	4.9	38.0	4.70	468.3	57.8
June Filtered Water	2.6	6.8	4.5	1.73	29.6	11.4
November Raw Water	10.2	5.0	60.2	5.90	907.5	89.0
November Filtered Water	2.1	5.6	4.6	2.19	66.6	31.7

SUVA is a useful parameter when assessing NOM as it allows classification of water in terms of organic content (table 5.2, Edzwald and Tobiason 1999).

Table 5.2 Guidelines for the nature of NOM (Edzwald and Tobiason 1999)

SUVA (m^{-1}.L mg^{-1} C)	Composition
>4	Mostly aquatic humics. High hydrophobicity, High MW
2-4	Mixture of aquatic humics and other NOM. Mixture of hydrophobic and hydrophilic NOM, mixture of MWs
<2	Mostly Non-Humics. Low hydrophobicity. Low MW

The SUVA values tell us that the raw water is rich in humic material whilst the filtered water is more hydrophilic in nature and contains mainly low molecular weight organic compounds. Over the year there are some variations in raw water DOC and UV observed but little variation in the filtered water samples.

The results show a variation in SUVA in both the raw and filtered water and show that there is significantly more hydrophobic material in November than in January or June.

There is also a corresponding increase in reactivity of both the raw and filtered water with chlorine in November in terms of THM-FP compared to June but the THM-FP in November and January are very similar. The November raw water has a THM-FP of 89 μg THM mg^{-1} C compared to 58 μg THM mg^{-1} C in June whilst the filtered water THM-FP increases from 11 μg THM mg^{-1} C in June to 32 μg THM mg^{-1} C in November. The THM-FP in January is also very similar to that in November – raw water 84 μg THM mg^{-1} C in January compared with 89 μg THM mg^{-1} C in November, filtered water 30 μg THM mg^{-1} C in January compared with 32 μg THM mg^{-1} C in November. To investigate what is causing the increased reactivity in January and November, the nature of individual organic fractions has been assessed.

5.2.1.3 Fractionated water analysis

Both raw and filtered water samples were fractionated using XAD resins to allow the content and fate of the individual fractions of DOC to be investigated (table 5.3). The removal percentage of each fraction is shown (table 5.4).

Table 5.3 Fraction distribution (Albert, 2000)

	January	June		November	
Fraction (%)	Raw	Raw	Filtered	Raw	Filtered
HAF	8.0	19.3	5.0	18.0	2.0
FAF	56.3	42.0	14.0	61.0	33.0
HPIA	1.1	8.0	1.0	8.0	11.0
HPINA	34.5	30.7	80.0	13.0	54.0

Table 5.4 Fraction removal by treatment process (Albert, 2000)

	Removal (%)	
Fraction	June	November
HAF	91.6	97.7
FAF	89.3	88.9
HPIA	95.6	71.7
HPINA	17.2	14.5

It should be noted that the fractions will have been denatured. That is, structural changes will have resulted due to the extreme pH conditions used in the fractionation procedure. However, research has been carried out to validate the fractions produced by XAD resins, to prove that the fractions are not innate products of the procedure used to prepare them (Peuravuori and Pihlaja 1997). It has been found that the separation of organic matter at pH 1 has certain risks (Peuravuori and Pihlaja 1998). Considerable compositional and structural alterations take place, in addition to loss of organic matter, during the acid precipitation. This would be an important consideration if the structure of the NOM was being investigated.

The results show, as expected, that the raw water contains mainly hydrophobic material (here fractionated into humic and fulvic acids) whilst the filtered water contains mainly hydrophilic material. Seasonal changes can be observed in the distribution of the fractions. There was an increase from ~65% to ~80% in hydrophobic material from the water collected in November compared to the water collected in January and June, mainly due to an increase in the FAF. The filtered water also showed a significant increase in the FAF content in November.

Analysis of the SUVA values for each fraction gives further information on the types of organics found in each fraction (tables 5.5 and 5.6).

Table 5.5 Summary of raw water fraction data (2000)

	January		June		November	
Fraction	SUVA (m^{-1} .L mg^{-1} C)	THM-FP (µg mg^{-1} C)	SUVA (m^{-1} .L mg^{-1} C)	THM-FP (µg mg^{-1} C)	SUVA (m^{-1} .L mg^{-1} C)	THM-FP (µg mg^{-1} C)
HAF	5.8	32.4	6.1	64.0	4.9	118.9
FAF	3.9	40.8	5.3	26.7	6.1	186.5
HPIA	1.1	17.7	2.3	37.4	3.7	171.3
HPINA	1.4	8.8	0.6	2.0	1.6	85.4

Table 5.6 Summary of filtered water fraction data (2000)

	June		November	
Fraction	SUVA (m^{-1} .L mg^{-1} C)	THM-FP (µg mg^{-1} C)	SUVA (m^{-1} .L mg^{-1} C)	THM-FP (µg mg^{-1} C)
HAF	4.2	11.7	6.5	154.0
FAF	2.5	84.3	2.9	92.0
HPIA	2.1	11.9	2.0	43.9
HPINA	0.6	4.0	1.3	70.2

In January and June, the HAF SUVA value is higher than the FAF SUVA value. In November it is the FAF SUVA value that is higher. It is possible that this is a characteristic of the autumnal FAF and that the FAF in November has a higher MW and is more hydrophobic than in January and June. The SUVA of the raw HPIA in November was particularly high compared with analysis of fractions taken in June indicating material that has a higher MW and is more hydrophobic than previously observed. Comparison of the raw and filtered SUVA values shows that typically, higher molecular weight and hydrophobic molecules are being removed through treatment. This is true with the exception of the November HAF where the raw SUVA is less than the filtered SUVA. The reactivity of the fractions also varied considerably through the year although for all samples, the hydrophobic acid fractions generally had the highest THM-FP.

Typically the hydrophilic fraction is less reactive than the hydrophobic fraction with chlorine in raw water samples (Collins *et al.* 1986, Krasner *et al.* 1996 and Croué *et al.* 2000). At Albert WTW this trend reported in the literature was not observed and the reactivity of the hydrophobic and hydrophilic fraction was the same. The values for the hydrophobic fraction have been taken as a weighted average of the HAF and FAF but the values for the hydrophilic fraction have been taken as the value for the HPIA. This has been done to give a reasonable comparison with the literature values. In June, the hydrophobic acid fraction formed 38 μg mg^{-1} C THM compared to 37 μg mg^{-1} C THM for the hydrophilic acid fraction. This continued in November where the hydrophobic and hydrophilic acid fractions formed 171 and 171 μg mg^{-1} C THM respectively.

All of the fraction SUVA and THM-FP data was plotted for each month individually and R^2 values of 0.61, 0.19 and 0.67 were observed in January, June and November 2000 respectively (figure 5.3). There is a linear relationship observed between the fraction THM-FP and SUVA in January and November but the relationship does not hold for the fractions from June.

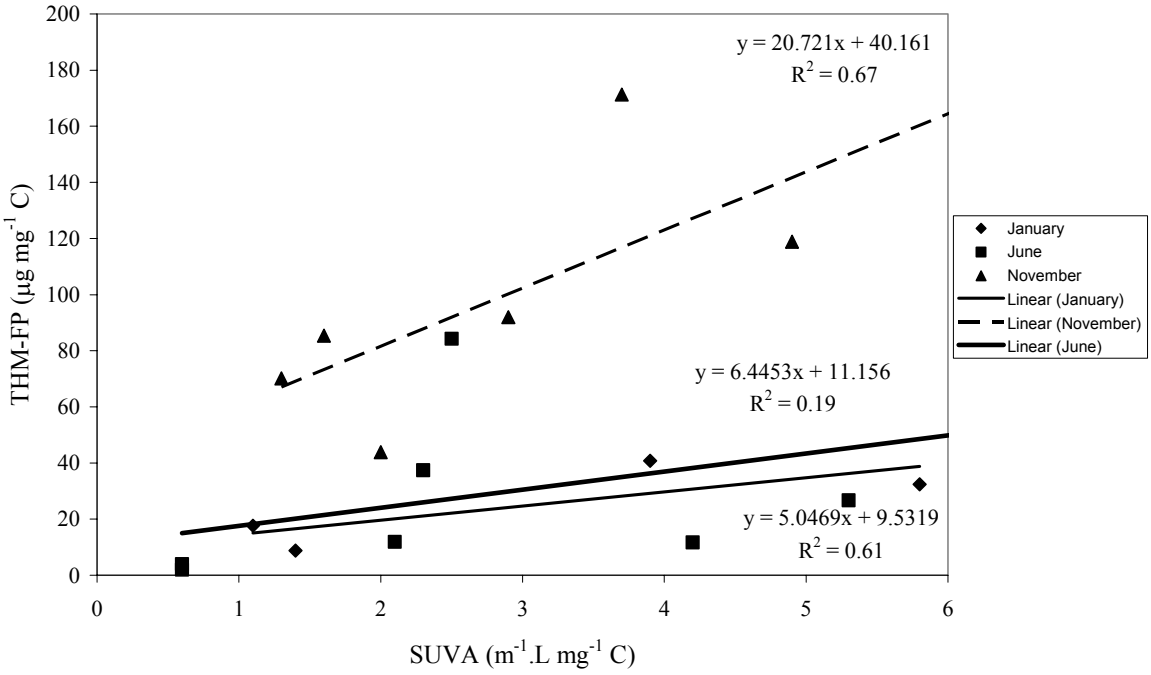


Figure 5.3 Plot of THM-FP vs SUVA for Albert fractions (January, June and November 2000)

It can be seen that the gradient and y-intercept of the lines observed in January and June are very similar. In November, the gradient and the y-intercept are both approximately four times higher than those seen earlier in the year. It was thought that a different linear equation could be used at different times of the year by using measured SUVA values to determine the likely THM-FP of the bulk water. The SUVA values of the bulk waters were entered into the corresponding equations to determine the predicted THM-FP. The values obtained were compared against the actual values measured (table 5.7). The 95% confidence level of each data point was calculated which gave a range for each data point. The range for each THM-FP value is given in brackets in table 5.7. Although some values were in the range calculated, it was found it was not

possible to use the correlation found between the fraction THM-FP and SUVA to reliably determine the bulk water THM-FP.

Table 5.7 Comparison of predicted THM-FP with actual THM-FP (2000)

Sample	Actual THM-FP (μg mg^{-1} C)	Predicted THM-FP (μg mg^{-1} C)
January Raw Water	84.3	32.4 (8.1 – 56.7)
January Filtered Water	29.7	18.1 (-2.1 – 38.3)
June Raw Water	57.8	41.5 (16.1 – 66.8)
June Filtered Water	11.4	22.3 (2.6 – 42.0)
November Raw Water	89.0	162.4 (134.5 – 190.4)
November Filtered Water	31.7	85.5 (63.8 – 107.3)

The values predicted in November were overestimated by the equation but were mainly underestimated in January and June with the exception of June filtered water where the THM-FP value was overestimated. For this water in this year SUVA is not a good indicator of THM-FP.

5.2.1.4 Sampling (2001)

Water samples were collected from the reservoir inlet (2 L in November) and after the rapid gravity filters (1 L in November) at Albert WTW in 2001.

5.2.1.5 Bulk water analysis

The raw and filtered water characteristics are presented in table 5.8.

Table 5.8 Raw and filtered water characteristics (2001)

Sample	DOC (mg L^{-1})	pH	UV (m^{-1})	SUVA (m^{-1} .L mg^{-1} C)	THM (µg L^{-1})	THM-FP (µg mg^{-1} C)
November Raw Water	9.9	5.2	48.0	4.9	563.8	56.9
November Filtered Water	2.7	Nm	4.0	1.5	63.2	23.4

Nm – not measured

According to the guidelines (Edzwald and Tobiason 1999), the SUVA value for the raw water sample indicates the water is rich in humic material, has high hydrophobicity and contains material with a high molecular weight. The SUVA value was within the range observed in 2000 but was significantly lower than that observed at the same time of the year. The November 2001 raw water had a THM-FP of 57 µg THM mg^{-1} C compared to 89 µg THM mg^{-1} C in November 2000 which corresponds to the lower SUVA value observed.

5.2.1.6 Fractionated water analysis

A small sample (2 L) of the raw water was fractionated and the DOC recovery was 100%. The fraction distribution was HAF – 24%, FAF – 42%, HPIA – 17% and HPINA – 17%.

The distribution of fractions produced has a higher proportion of HPIA (17 %) than the range observed in 2000 (1 – 8 %). The proportion of the other fractions was within in the range found in 2000. The November 2001 raw water has a similar SUVA to the January and June 2000 raw water and also has a similar hydrophobic:hydrophilic ratio. The split was 66:34 in November 2001 compared with 65:35 and 61:39 for January and June 2000 respectively. No further analysis was carried out on the fractions as the

fractionation was carried out purely to determine the fraction distribution for comparison with the distribution determined using fluorescence spectroscopy (see section 5.4).

5.2.1.7 Sampling (2002)

Water samples were collected from the reservoir inlet (500 L in April and 50 L in October) and after the rapid gravity filters (700 L in April) at Albert WTW in 2002.

5.2.1.8 Bulk Water Analysis

The raw and filtered water characteristics are presented in table 5.9. The dose used here was 10 mg Cl_2 mg^{-1} C. This dose was used to ensure that there was some chlorine remaining and that the reaction was allowed to continue to completion. As different doses were used in 2002 compared with 2000, it was not possible to compare the results between years but only within years. This is because the amount of THMs formed will be dose dependent (Chowdhury and Amy 1999). The original dose of 5 mg Cl_2 mg^{-1} C was used in 2000 as this was the dose used at Yorkshire Water laboratories to determine THM-FP.

Table 5.9 Raw and filtered water characteristics (2002)

Sample	DOC ($mg\ L^{-1}$)	pH	UV (m^{-1})	SUVA (m^{-1} .L mg^{-1} C)	THM ($\mu g\ L^{-1}$)	THM-FP ($\mu g\ mg^{-1}$ C)
April Raw Water	7.5	5.9	38.1	5.1	602.3	80.3
April Filtered Water	1.4	6.5	4.6	3.3	11.8	8.4
October Raw Water	10.4	5.8	52.3	5.0	511.7	49.2

According to the guidelines (Edzwald and Tobiason 1999), the SUVA values for both raw water samples indicate the waters are rich in humic material, have high hydrophobicity and contain material with a high molecular weight. The filtered water SUVA is higher than has been previously seen for this water. The UV absorbance is the same as that seen in November 2002 but the lower value for DOC results in a higher SUVA value. The guidelines indicate that the filtered water is a mixture of hydrophobic and hydrophilic material. It should be remembered that the SUVA values are a ratio and fractionation will be required to determine the amount and proportion of hydrophobic and hydrophilic material. The SUVA of the April and October 2002 raw waters is almost identical (5.1 compared to 5.0). Previously an increase in THM-FP has been observed in autumn (November 2000) compared with summer (June 2000) that corresponded with an increase in SUVA. Here, samples with very similar SUVA values exhibit different THM-FP values: the April raw water has a THM-FP of 80 μg THM mg^{-1} C compared to 49 μg THM mg^{-1} C in October. The difference in THM-FP despite similar SUVA values was investigated by assessing the nature of the individual fractions.

5.2.1.9 Fractionated water analysis

The raw and filtered water samples were fractionated and the DOC recoveries for the raw and filtered water in April were 80% and 115% respectively. The recovery of DOC in the October raw water was 90%. The fraction distributions are reported (table 5.10). The removal percentage of each fraction is shown for April (table 5.11).

Table 5.10 Fraction distribution (Albert, 2002)

	April		October
Fraction (%)	Raw	Filtered	Raw
HAF	19.9	0.5	23.0
FAF	51.7	21.0	51.5
HPIA	11.9	19.6	9.5
HPINA	16.4	59.0	16.0

Table 5.11 Fraction removal by treatment process (Albert, April 2002)

Fraction	Removal (%)
HAF	99.3
FAF	92.5
HPIA	69.7
HPINA	32.5

Both the raw water samples have almost identical SUVA values. They also have almost identical fraction distributions. The filtered water has slightly more hydrophilic material (59.5%) than hydrophobic material (40.5%).

The SUVA and THM-FP of each of the fractions was measured. The results are presented (table 5.12).

Table 5.12 Summary of fraction data (2002)

Fraction	Raw (April)		Filtered (April)		Raw (October)	
	SUVA (m^{-1} .L mg^{-1} C)	THM-FP (μg mg^{-1} C)	SUVA (m^{-1} .L mg^{-1} C)	THM-FP (μg mg^{-1} C)	SUVA (m^{-1} .L mg^{-1} C)	THM-FP (μg mg^{-1} C)
HAF	4.59	32.0	5.82	12.1	5.57	126.3
FAF	4.70	82.0	4.25	14.2	6.25	65.5
HPIA	1.46	24.3	1.04	11.4	3.21	21.9
HPINA	0.77	9.0	0.72	3.6	0.86	31.1

The values of SUVA in the both the raw waters FAF are higher than the HAF. This was also seen in November 2000 indicating that the organic material in the FAF has a higher

MW and is more hydrophobic than the corresponding HAF. The SUVA value for the HPIA in October 2002 is high compared with that observed in April 2002. A high SUVA value for this fraction was also observed for November 2000 raw water where it was attributed to a higher concentration of hydrophobic material than observed in June 2000. By comparing the SUVA of the raw and filtered April 2002 fractions, it appears that high molecular weight and hydrophobic molecules are being removed as the SUVA is reduced. The exception to this is the HAF where the SUVA is observed to increase following treatment. The reactivity expressed as THM-FP again varied seasonally. Typically the hydrophilic fraction is less reactive than the hydrophobic fraction with chlorine in raw water samples (Collins *et al.* 1986, Krasner *et al.* 1996 and Croué *et al.* 2000). In 2002, this trend reported in the literature was observed and the reactivity of the hydrophobic fraction was greater than the hydrophilic fraction. In April, the hydrophobic acid fraction formed 68 μg mg^{-1} C THM compared to 24 μg mg^{-1} C THM for the hydrophilic acid fraction. This continued in November where the hydrophobic and hydrophilic acid fractions formed 84 and 22 μg mg^{-1} C THM respectively. The values for the hydrophobic fraction have been taken as a weighted average of the HAF and FAF but the values for the hydrophilic fraction have been taken as the value for the HPIA. Again, this has been done to give a reasonable comparison with the literature values. As with the samples from 2000, the autumnal water was found to have higher reactivity in terms of THM-FP.

When comparing the THM-FP of the raw HPINA fraction in April and October 2002, the reactivity is more than three times greater in October. This is a significant finding as this is the least removed fraction through the treatment process.

All of the fraction SUVA and THM-FP data was plotted (figure 5.4) and a R^2 value of 0.27 was observed in April 2002 meaning that there is no linear relationship between SUVA and THM-FP for these fractions. The data for October SUVA and THM-FP was also plotted with an R^2 value of 0.47 indicating that if there is a linear relationship between SUVA and THM-FP, it is not very strong.

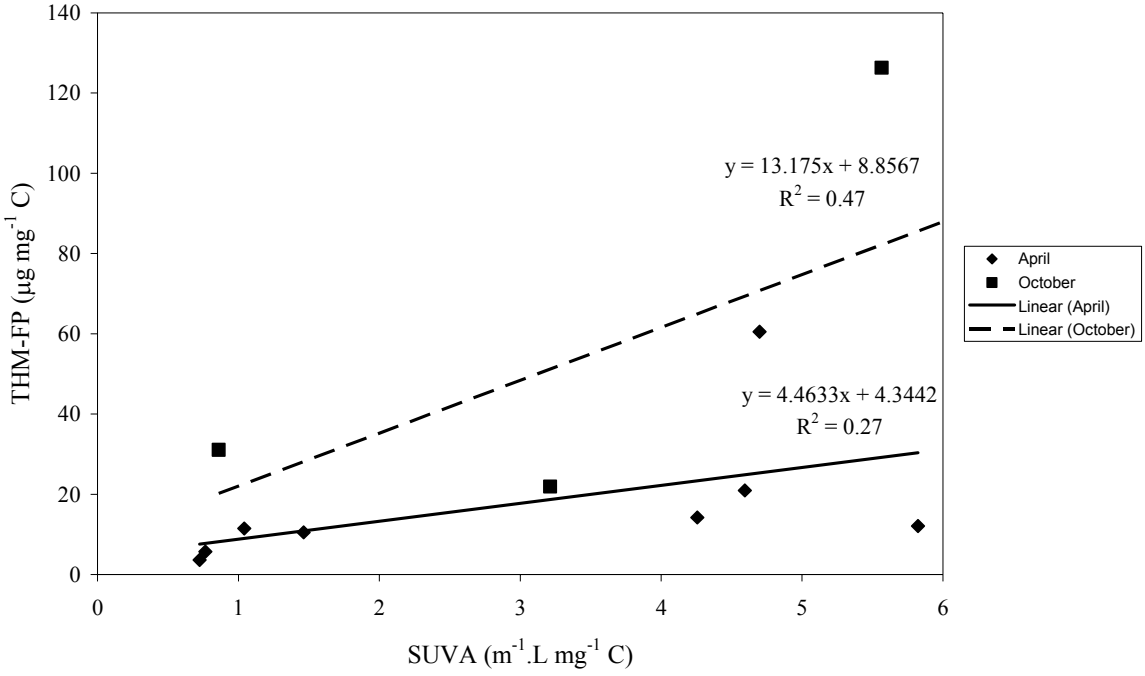


Figure 5.4 Plot of THM-FP vs SUVA for Albert fractions (April and October 2002)

It can be seen that the gradient and y-intercept of the line observed in April and October do not correspond with the equations of the lines observed in 2000 although higher values were exhibited in October than in April. Again the SUVA values of the bulk waters were entered into the corresponding equations to determine the predicted THM-FP. The values obtained were compared against the actual values measured (table 5.13). Again, the confidence limits and range for each data point were calculated. The range for each THM-FP value is given in brackets in table 5.13. Although two of the

three values fall within the range, it was believed it was not possible to use the correlation found between the fraction THM-FP and SUVA to determine the bulk water THM-FP with any degree of accuracy.

Table 5.13 Comparison of predicted THM-FP with actual THM-FP (2002)

Sample	Actual THM-FP (μg mg^{-1} C)	Predicted THM-FP (μg mg^{-1} C)
April Raw Water	80.3	27.1 (12.4 – 41.8)
April Filtered Water	8.4	12.1 (4.8 – 33.3)
October Raw Water	49.2	74.7 (-2.1 – 151.6)

5.2.1.10 Sampling (Rivington - 2001)

Water samples were collected the WTW inlet (75 L) and after the filters (300 L) at Rivington WTW in February 2001.

5.2.1.11 Bulk water analysis

Samples of raw and filtered water collected in February 2001 were analysed for pH, DOC, UV and THM-FP. Here the chlorine dose was 5 mg Cl_2 mg^{-1} C. The results are summarised with calculated SUVA values in table 5.14.

Table 5.14 Raw and filtered water characteristics (Rivington, 2001)

Sample	DOC (mg L^{-1})	pH	UV (m^{-1})	SUVA (m^{-1} .L mg^{-1} C)	THM (μg L^{-1})	THM-FP (μg mg^{-1} C)
February Raw Water	6.1	7.1	24.6	3.7	143.5	23.5
February Filtered Water	1.5	nm	2.8	1.9	38.7	26.0

Nm – not measured

The SUVA guidelines indicate that this water is less hydrophobic than the water from Albert WTW. It contains a mixture of hydrophobic and hydrophilic NOM with a mixture of high and low molecular weights. The filtered water SUVA indicates that the higher molecular weight, more hydrophobic material has been removed by the water treatment process leaving low molecular organic material. The SUVA values for the raw and treated water are different but the THM-FP values are very similar: the raw water has a THM-FP of 24 μg THM mg^{-1} C compared to 26 μg THM mg^{-1} C for the filtered water. It is possible that the NOM forming THMs was not removed by the water treatment process. The difference in THM-FP coupled with similar SUVA values was investigated by assessing the nature of the individual fractions.

5.2.1.12 Fractionated water analysis

The raw and filtered water samples were fractionated and the DOC recoveries for the raw and filtered water were 65% and 62% respectively. The fraction distributions are shown in table 5.15. The removal percentage of each fraction is shown (table 5.16).

Table 5.15 Fraction distribution (Rivington, 2001)

	February	
Fraction (%)	Raw	Filtered
HAF	42.6	1.2
FAF	23.0	30.1
HPIA	6.6	5.6
HPINA	27.9	63.1

Table 5.16 Fraction removal by treatment process (Rivington, February 2001)

Fraction	Removal (%)
HAF	99.2
FAF	68.5
HPIA	81.0
HPINA	44.0

The raw and filtered water samples have different SUVA values but almost identical THM-FP values. The raw water fraction distribution shows that there is more hydrophobic material (65%) than hydrophilic material (35%). The filtered water is predominantly hydrophilic (69%) but still contains a significant amount of hydrophobic material (31%).

The HAF and HPIA have been preferentially removed with removal percentages of 99.2% and 81.0% respectively. The FAF is 68.5% removed and the HPINA shows the least removal of 44.0%. It was expected that the HPINA would be least removed by the treatment process although the removal of this fraction is much higher than observed at Albert WTW (17.2% removal in June and 14.5% in November 2000).

The SUVA and THM-FP of each of the fractions was measured. The results are presented (table 5.17).

Table 5.17 Summary of fraction data (Rivington, 2001)

Fraction	Raw		Filtered	
	SUVA (m^{-1} .L mg^{-1} C)	THM-FP (µg mg^{-1} C)	SUVA (m^{-1} .L mg^{-1} C)	THM-FP (µg mg^{-1} C)
HAF	3.75	97.6	5.18	136.4
FAF	5.03	52.7	5.17	59.6
HPIA	1.66	8.0	0.63	15.7
HPINA	1.73	17.3	2.24	7.3

Again, the values of SUVA in the raw water FAF are higher than the HAF. This was also seen for some Albert raw waters but is not typically reported in the literature (Croué *et al*. 1999a). In the filtered water, the HAF SUVA is almost the same as the FAF SUVA (5.18 compared to 5.17). Generally, the SUVA values reported for Albert WTW for the HPIA are higher than for the HPINA. However, in both the raw and filtered water at Rivington, the SUVA values for the HPINA are higher. The fact that

the SUVA values are higher than expected and the removal of this fraction is also higher than expected indicate that the material in the HPI-NA at Rivington is different from the material in the HPI-NA at Albert. Although both fractions have been designated the same name, it does not mean the material will be the same as the definition is operational. The SUVA of all the fractions was observed to increase after treatment except the HPIA. For the raw and filtered water samples, the hydrophobic fractions had much higher THM-FP values than the hydrophilic fractions. The hydrophobic fraction reactivity ranged from 53 –136 μg THM mg^{-1} C compared to 7 - 17 μg THM mg^{-1} C for the hydrophilic fractions. The THM-FP of the raw water fraction were similar to the THM-FP of the filtered water fractions with the exception of the HAF which increased in reactivity after treatment.

All of the fraction SUVA and THM-FP data was plotted and a correlation of 0.58 was observed with THM-FP rising with SUVA.

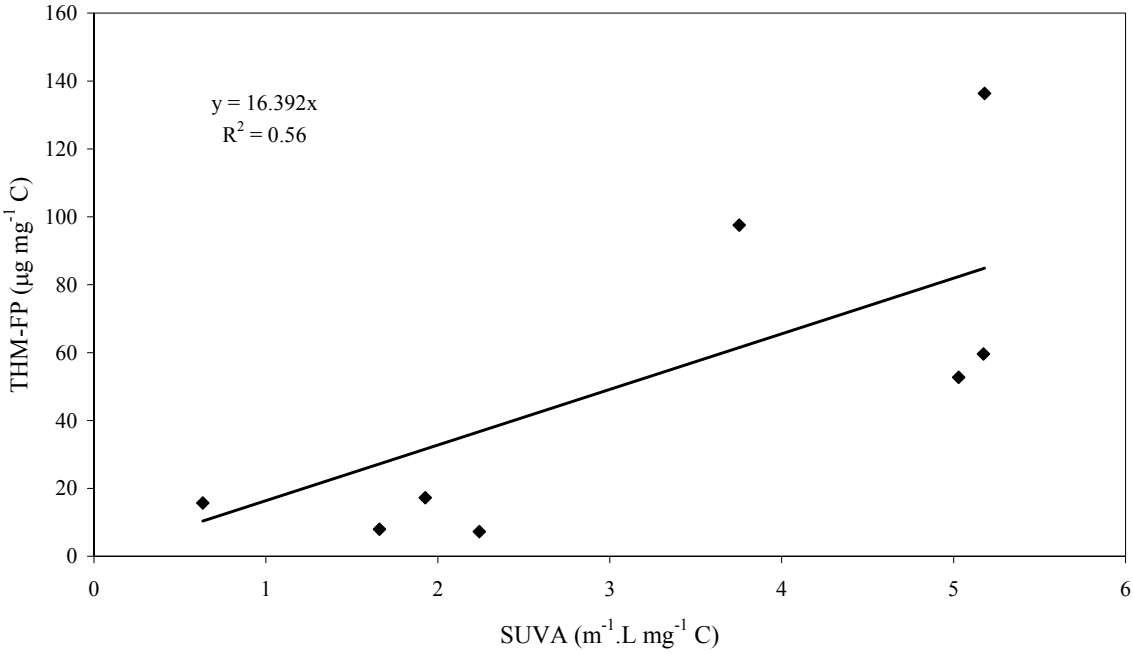


Figure 5.5 Plot of THM-FP vs SUVA for Rivington fractions (February 2001)

The y-intercept was found to be negative. As it is not possible to form negative THMs, the y-intercept was set at zero. This changed the R^2 value to 0.56 (figure 5.5). It can be seen from figure 5.5 that the gradient is within the range seen at Albert WTW. The low y-intercept value indicates that the fractions from Rivington form less THMs per unit of SUVA than the fractions obtained from Albert WTW. As with the samples from Albert WTW, the equation obtained from the Rivington fractions was used to attempt to predict the bulk water THM-FP from SUVA values, again with the range reported (table 5.18).

Table 5.18 Comparison of predicted THM-FP with actual THM-FP (2001)

Sample	Actual THM-FP (μg mg^{-1} C)	Predicted THM-FP (μg mg^{-1} C)
February Raw Water	23.5	60.7 (32.5 – 86.0)
February Filtered Water	26.0	31.1 (-3.31 – 50.3)

The values predicted for the raw and filtered water were overestimated by the equation but the value for filtered water was within the calculated range. Again SUVA was not found to be a good indicator of THM-FP. This can clearly be seen in figure 5.5 where almost identical SUVA values are showing vastly different THM-FP values.

5.2.1.13 Resin fractionation findings

The fractionation data can provide considerable information on how the treatment processes are performing when compared to bulk water parameters. It is well known that traditional coagulation/flocculation processes are excellent at removing humic/fulvic acid material and an optimised process can successfully remove greater than 90% of the HAF and FAF (Crozes *et al.* 1995). It is clear from the results presented that Albert water treatment works is performing well and removing the majority of the hydrophobic fractions at the time points that were studied. During November 2000 the treatment processes are struggling to deal with the higher organic load and primarily the FAF. Although almost 90% removal of the FAF is being achieved, there is still a significant amount of FAF remaining due to the high initial organic load. It is also clear that the treatment processes are struggling to remove the hydrophilic non-acid fraction (HPI-NA) which makes up 80% of the June 2000 filtered water, 54% of the November 2000 filtered water and 59% of the April 2002 filtered

water. In January 2000, June 2000 and April 2002 the THM-FP of this fraction was <10 μg mg^{-1} C THM. However in November 2000, the reactivity of the raw and filtered HPINA was 85 and 70 μg mg^{-1} C THM respectively and in October 2002, the reactivity of the raw HPINA was also elevated (31 μg mg^{-1} C).

The removal of the hydrophilic fractions was more variable than the hydrophobic fractions. The range of the HPIA removal was 69.7 – 95.6% at Albert WTW and the range of HPINA removal was 14.5 – 32.5%.

At Rivington WTW, the HAF and HPIA have been preferentially removed with removal percentages of 99.2% and 81.0% respectively. The FAF is 68.5% removed and the HPI-NA shows the least removal of 44.0%. It was expected that the HPINA would be least removed by the treatment process although the removal of this fraction is higher than observed at Albert WTW. The increased removal of the HPINA is due to the material being more amenable to coagulation at Rivington than at Albert WTW. Although the fraction label is the same, the fraction definitions are operational. This means that the material will not be the same at a different site. In water treatment, the coagulation process is used to increase the rate of kinetics at which particles aggregate, i.e. to transform a stable suspension into an unstable one (American Water Works Association 1990). It is possible that HPINA at Rivington is less stable than the HPINA at Albert. The removal of the FAF is lower than previously observed at Albert WTW (89.3 – 92.5%). The most reactive fraction (HAF) shows the greatest removal. Both hydrophilic fractions have low reactivity in the raw and filtered water.

The fractionation of raw and filtered water from Albert WTW showed that there was an increase in the amount and proportion of hydrophobic material in autumn compared

with winter and summer in 2000. This corresponded with an increase in the THM-FP of both hydrophobic and hydrophilic fractions. This was not only due to the amount of each fraction but also a significant change in THM per mg of carbon for each fraction. In 2002, the difference between the water in April and October was less pronounced with similar reactivities for the hydrophobic acid (weighted average of HAF and FAF) and hydrophilic acid. However the increase in reactivity of the HPINA in autumn was as found in 2000.

With regard to SUVA as an indicator of reactivity, the plots of SUVA vs THM-FP show a higher gradient observed in autumn (October and November) than at other times of the year. This indicates a higher reactivity in autumn waters in terms of THM-FP. However the equations produced from the fraction data were not found to be able to predict bulk water THM-FP with any degree of accuracy.

The information gained from fractionating the water was far more in depth than the information obtained by just using bulk water parameters alone. The fractionation gives an insight into the seasonal effect on NOM and there were indications that a water with a high FAF content will be more reactive and the water will be more difficult to treat.

5.2.2 Membrane fractionation

A sample of Albert raw water from October 2002 was separated using UF membranes into four size fractions. These were R3 (>3 kDa), R1(1- 3 kDa), R05 (0.5 - 1 kDa) and F05 (<0.5 kDa). The recovery of DOC from the fractionation was measured and was 97%. The DOC and UV distribution is shown in figure 5.6. The majority of the DOC (91.7%) and UV (78.5%) is in the >3 kDa fraction. In the smaller fractions, the UV is higher than the corresponding DOC indicating that the degree of unsaturation is highest in the smaller fractions. That is, the amount of carbon to carbon double or triple bonds will be higher in the smaller fractions.

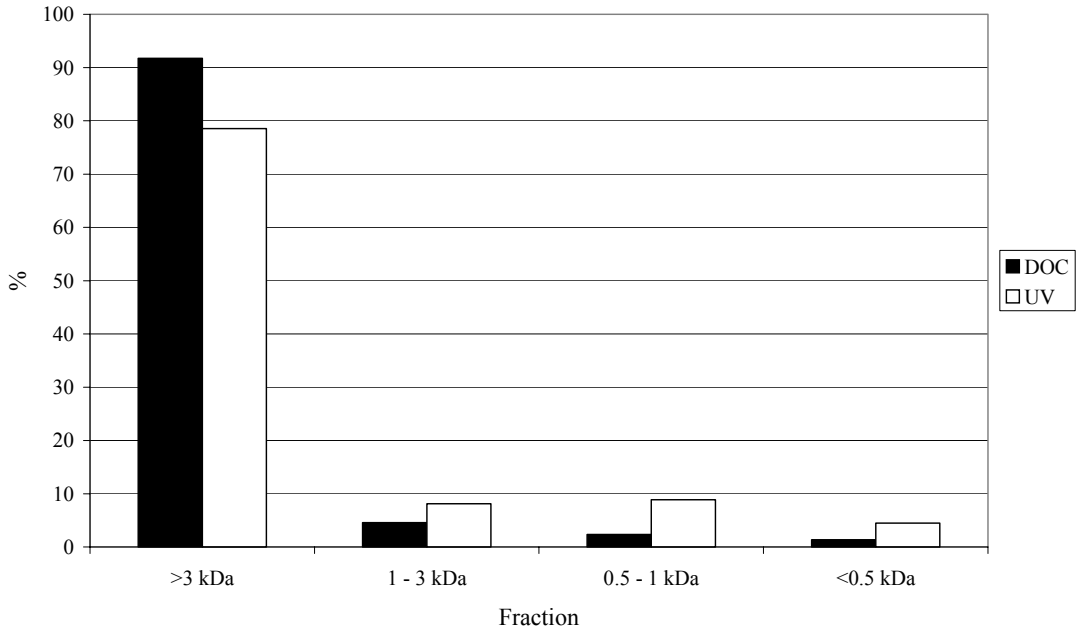


Figure 5.6 UF fraction distribution

A study by Collins *et al*. (1986) separated waters rich in humic substances using UF membranes into 6 fractions. These were <0.5 kDa, <1.0 kDa, <5.0 kDa, <10.0 kDa, <30.0 kDa and <0.45 µm. Generally for the smaller fractions (<0.5 kDa, <1.0 kDa and <5.0 kDa), the proportion of DOC was higher than the proportion of UV. For the

remaining larger fractions, the opposite was found. This is opposed to what was observed for Albert reservoir water. There was one exception where the <0.5 kDa fraction had a higher proportion of UV than DOC. It may be that the proportion of UV compared with the proportion of DOC is source specific and that the findings are characteristic of Albert reservoir October water.

As with the resin separated fractions, the membrane separated fractions were diluted to ~1 mg L^{-1}, chlorinated at 10 mg Cl_2 mg^{-1} C for 7 days and the THM concentration measured. The chlorine remaining after 7 days was also measured. The results are shown (table 5.19).

Table 5.19 THM-FP and remaining chlorine of UF fractions.

Sample	THM-FP (µg $mg^{-1}C$)	Cl_2 remaining (mg L^{-1})
R3 (>3 kDa)	19.0	0.02
R1 (1 – 3 kDa)	49.6	3.40
R05 (0.5 – 1 kDa)	144.7	5.68
F05 (<0.5 kDa)	154.2	4.92

The largest fraction formed the least THMs per mg C but used the most chlorine. As the fractions got smaller, the amount of THMs formed increased. This is opposed to the relationship found by Amy *et al*. (1987) who reported that high MW fractions produced more THMs per mg C. It is known that DBPs other than THMs are formed when chlorine reacts with NOM (Singer 1999). It is assumed that here, DBPs other than THMs have been formed by the larger MW fractions which would explain the higher chlorine demand observed for these fractions.

Another study investigated the THM-FP of UF separated organic matter (Collins *et al*. 1985). Their results are presented (table 5.20).

Table 5.20 THM-FP of UF fractions from various waters (taken from Collins *et al*. 1985)

	THM-FP (μg mg^{-1}C)				
Water	< 0.5 kDa	< 1.0 kDa	< 5.0 kDa	< 10.0 kDa	< 30.0 kDa
Springfield	55.4	91.3	72.0	81.0	78.6
Canton	56.5	68.2	75.5	93.0	92.8
Daytona	54.1	55.2	59.4	62.1	62.4
Las Vegas	55.7	58.3	56.7	56.6	55.6

They found that THM-FP generally increased with increasing MW although there were several departures from this. The observed trend was more pronounced for a water such as Canton as opposed to Las Vegas where the reactivity was the same for each fraction regardless of size. Another noticeable departure from the observed trend was the <1.0 kDa sample from Springfield that had the highest reactivity of all the size fractions from that area. The chlorine dose was 3 mg Cl_2 mg^{-1} C compared with 10 mg Cl_2 mg^{-1} C used in this work so it is not possible to directly compare the THM-FP values, only the observed trends.

It has been hypothesised (Luong *et al*. 1982) that when chlorinating humic substances, some chlorine is initially expended in 'activation' of the humic structure through oxidation reactions to produce active sites followed by substitution reactions with chlorine. Therefore subjecting a <0.5 kDa sample to the same chlorine dose as a <30 kDa sample may in actuality be providing a higher driving force for organo-chlorine formation since less chlorine is required for partial oxidation of the molecules. The

relationship between THM-FP and SUVA is shown (figure 5.7) and is the opposite of that reported in the literature. It is also different to the relationship seen between SUVA and THM-FP for resin separated fractions from Albert WTW. With UF separated fractions, the THM-FP is observed to fall with increasing SUVA. The phenomenon described above by Luong *et al.* (1982) could account for the high THM-FP values seen for the low MW fractions. That is, less chlorine is required for partial oxidation of the molecules so more chlorine is available to form THMs. A higher chlorine dose will result in more THMs formed (Singer 1999) therefore more THMs will be formed by the smaller molecules.

It has been reported that various organic compounds can cause chloroform on chlorination (Croué *et al*. 1999a). These compounds possess varying efficiencies in the formation of THMs as electron-rich moieties are extremely vulnerable to electrophilic attack. This attack may be the first in a series of oxidation and substitution reactions that eventually produce smaller DBPs such as THMs and HAAs. Here the larger MW fractions may be less efficient in the formation of THMs than the smaller MW fractions.

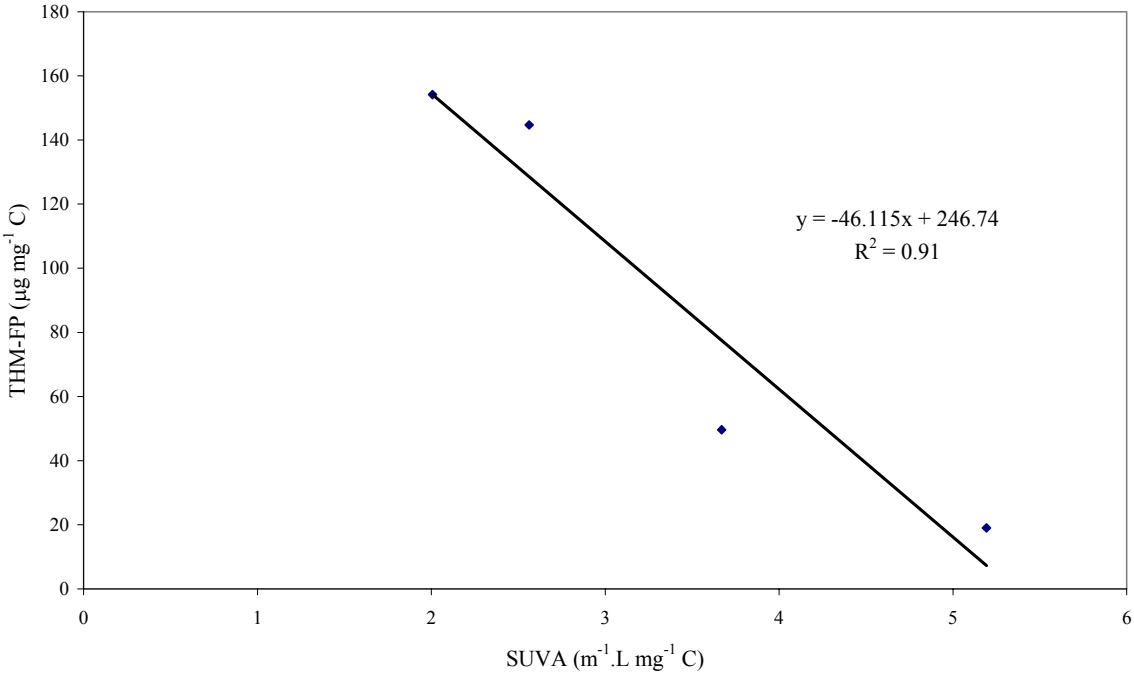


Figure 5.7 THM-FP vs SUVA for UF fractions

5.2.2.1 Membrane fractionation findings

The fractions produced had unexpected behaviour with regard to THM-FP. A strong relationship was seen between SUVA and THM-FP (R^2 = 0.91) that is the opposite of that reported in the literature. With only four data points, the error range will be large. It is thought that more information could have been obtained with a range of membranes with different MWCOs that split the NOM more evenly.

5.2.3 Section conclusions

Fractionation and subsequent analysis can indicate which fractions in a water are the most reactive. However, it is a labour intensive and time-consuming process. Traditional SUVA-THM-FP relationships reported in the literature apply to Albert WTW resin separated fractions on occasion but are not a reliable indicator of reactivity.

Further analysis of the fractions to determine their make-up might give more information as to why the waters are more reactive at certain times of the year.

5.3 Methods of Analysis

In the last section, the make-up and reactivity of the bulk waters was investigated by separation of water samples into fractions. Simple laboratory tests on the bulk water samples and fractions produced were carried out using standard equipment. In this section, further methods of analysis will be investigated to find out more about the differences between the individual fractions within a bulk water. Where possible the analyses were carried out on the bulk waters as well as the fractions. All analyses were carried out on samples from Albert WTW.

5.3.1 Capillary Electrophoresis

5.3.1.1 Resin Separated Fractions

Selected Albert Reservoir fractions from November 2000 were analysed. These were the raw HAF, FAF and HPIA and the filtered FAF and HPIA. The resulting electropherograms (EPGs) were compared against the literature and each other. The raw water HAF EPG (figure 5.8) shows a characteristic 'humpogram' which has been shown to be exhibited by commercially available humic acids in borate buffer at pH 8-9 (Garrison *et al.* 1995, Pompe *et al.* 1996 and Nordén and Dabek-Zlotorzynska 1997).

All following EPGs have time in minutes along the X-axis and UV absorbance at 254 nm along the Y-axis.

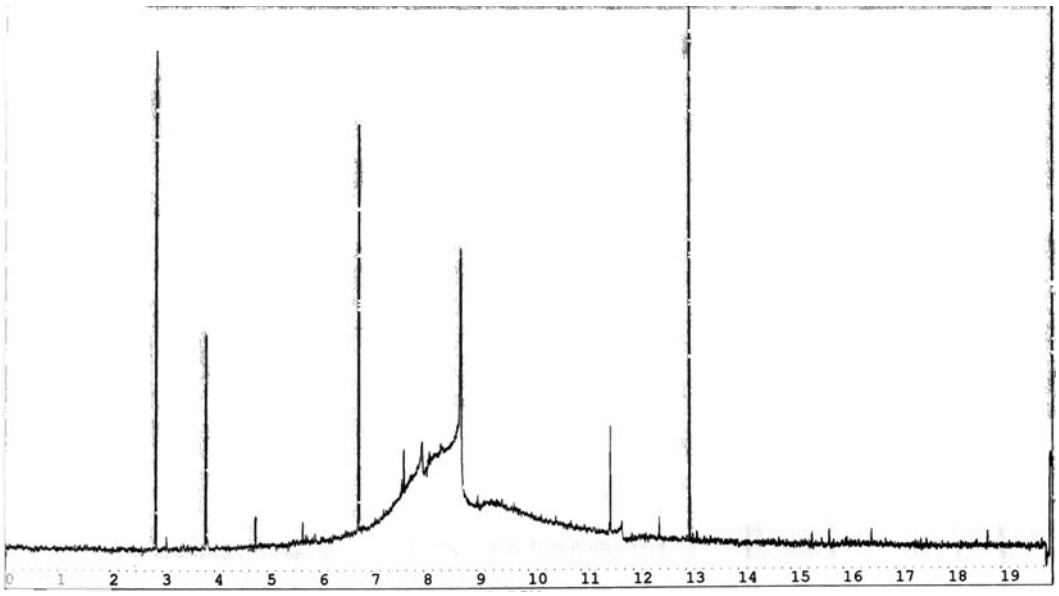


Figure 5.8 Albert Raw Water HAF EPG

A comparison of the raw water FAF EPG with the filtered water FAF EPG shows that although greatly reduced, the FAF hump remains after going through the water treatment process (figures 5.9 and 5.10). The shape of the EPG of the raw water FAF shows a less resolved peak than that reported by Garrison *et al.* (1995) under the same experimental conditions. The migration time of the raw water FAF is longer than that for the raw water HAF indicating a larger charge-to-mass ratio for the FAF, i.e. a higher total acidity and a smaller molecular mass as described by Schmitt *et al.* (1996). The sharp peaks in the raw water FAF remain in the filtered water FAF showing their non-reactivity with the coagulant, ferric chloride. The peaks may correspond to low-molecular weight phenols as reported by Schmitt *et al.* (1996).

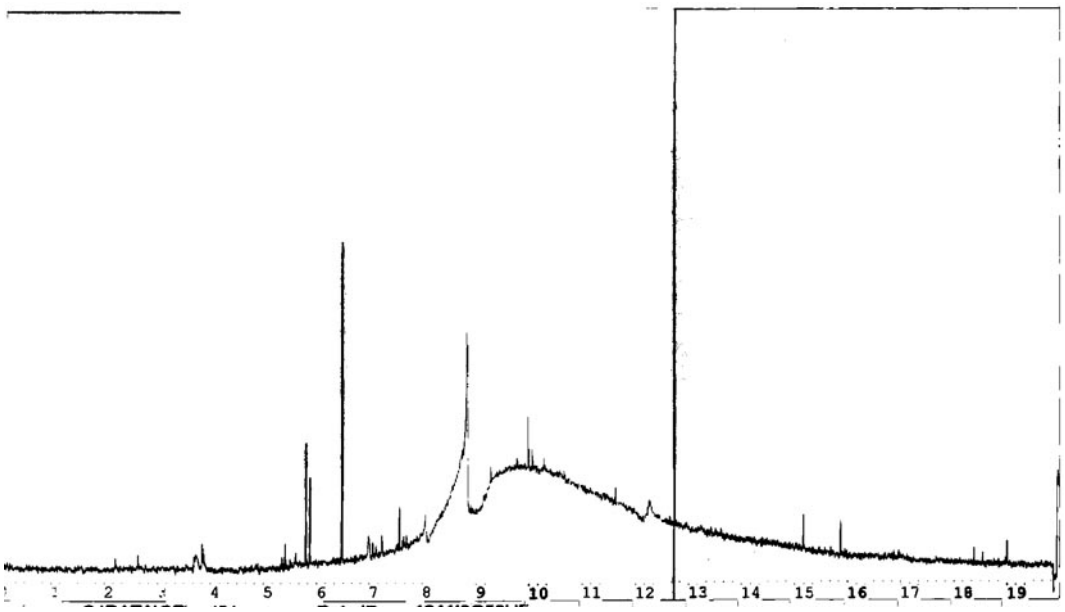


Figure 5.9 Albert Raw Water FAF EPG

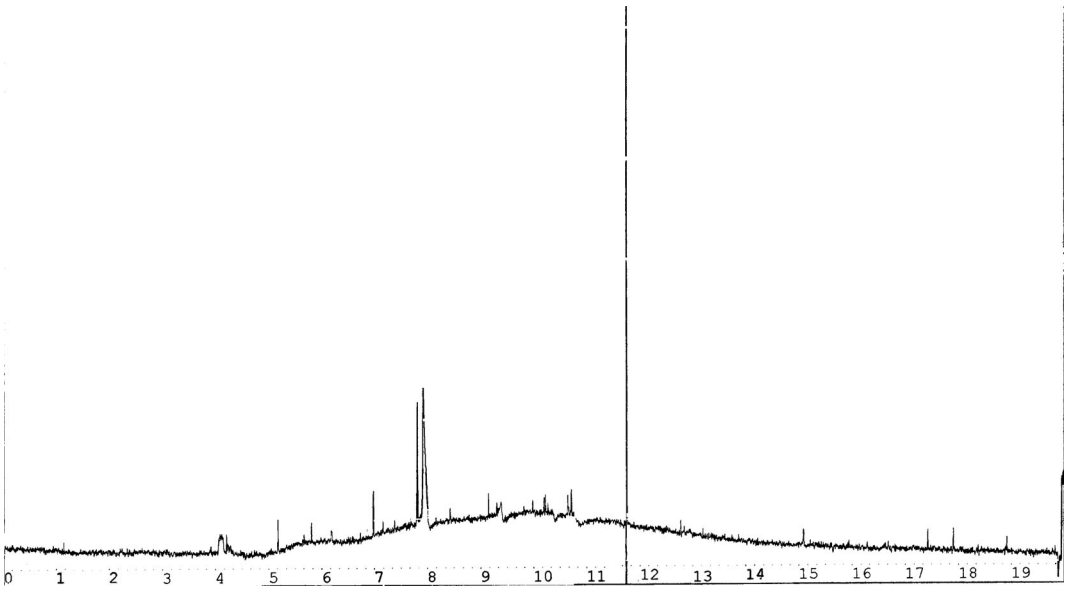


Figure 5.10 Albert Filtered Water FAF EPG

The raw water HPIA EPG exhibits a very similar shape to the filtered water HPIA EPG (figures 5.11 and 5.12). Although the EPGs are on the same scale, variations in UV absorption in the EPGs may be caused by:

a) variation in the individual aromatic carbon content

b) the presence of a different number of UV-active groups

c) variation in the concentrations of the individual fractions

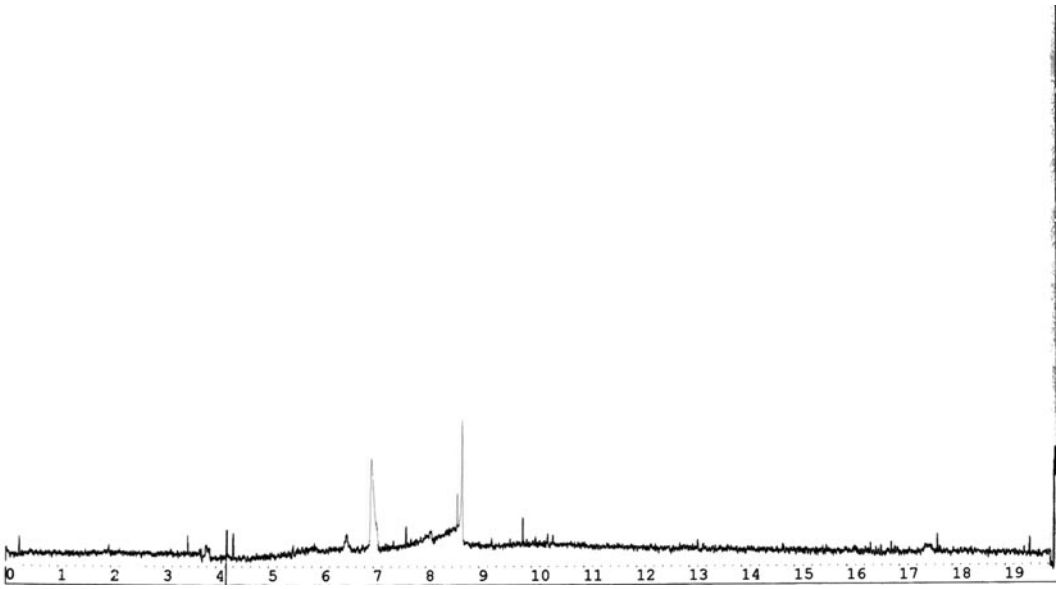


Figure 5.11 Albert Raw Water HPIA EPG

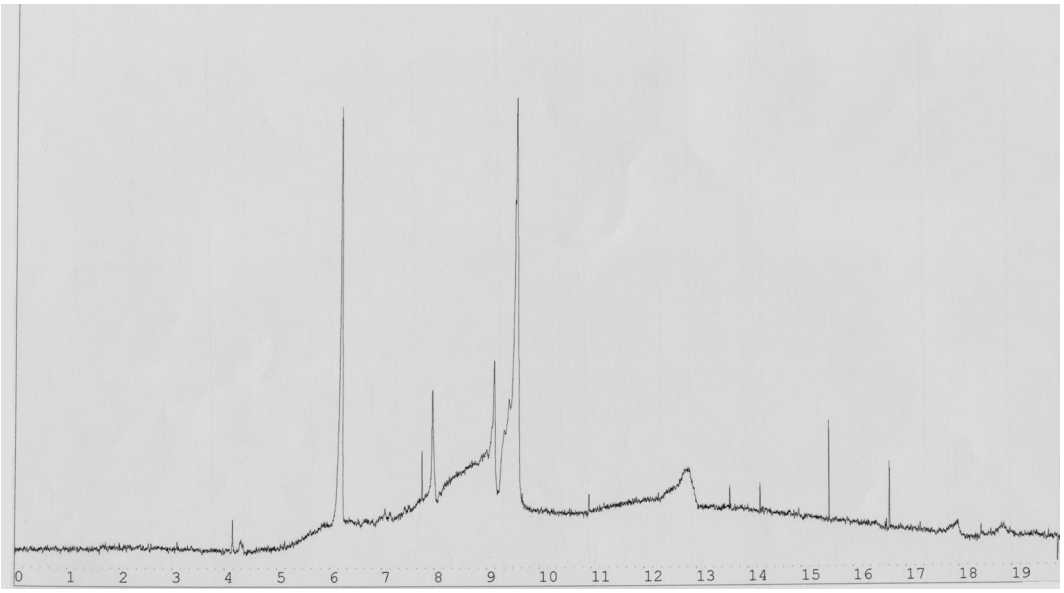


Figure 5.12 Albert Filtered Water HPIA EPG

5.3.1.2 Capillary Electrophoresis conclusions

The EPGs produced using capillary electrophoresis have broad peaks that are unresolved. The sample analysed must have a concentration of 50 – 100 mg L^{-1} C in order to see a signal above the background noise. Due to the sample preparation required, the cost of analysis and the small amount of information obtained by CE, it was decided not to continue using this method to analyse more samples.

5.3.2 Fluorescence

5.3.2.1 Resin separated fractions

Fluorescence scans (emission and excitation) were performed on raw, filtered and fractionated samples from Albert WTW (2000). Samples were taken from June and November to allow investigation of seasonal variations. For the emission spectra, the excitation wavelength was 313 nm and the emission was recorded from 350 – 600 nm. This excitation wavelength was used previously by Belin *et al*. (1993) for the analysis of XAD-4/8 resin separated fracions.

The effect of environmental factors was minimised by taking the following precautions. Each sample was diluted to 1 mg L^{-1} DOC to avoid inner-filter effects, quenching and ionic strength effects (Senesi 1990). The pH of each sample was adjusted to 7 and the samples were at room temperature before analysis to avoid any effects from pH or temperature. Effects that were unable to be minimised included the effect of dissolved molecular oxygen that can act as a fluorescence quencher. Although this could easily be eliminated by bubbling nitrogen through the solution to flush out the oxygen, there were no facilities available to do this. Another effect that was not minimised was long-range energy transfer reactions that can occur without collisional interactions at lower quencher concentrations than collisional quenching. This effect is difficult to eliminate by dilution (Senesi 1990).

Comparison of raw and filtered water samples using Ex = 313 nm (figure 5.13) shows that the maximum emission wavelength (Em_{max}) is typically higher for those organics found in the raw water compared to those in the filtered water. Although the peaks are

broad and featureless, it is reported that the higher Em_{max}, the higher the average molecular weight (MW) (Croué *et al*. 2000). Therefore it can be concluded that the filtered water has a smaller average MW than the raw water, implying that large MW organic material has been removed by the WTW. This is known to be true from the SUVA values of the raw and filtered water.

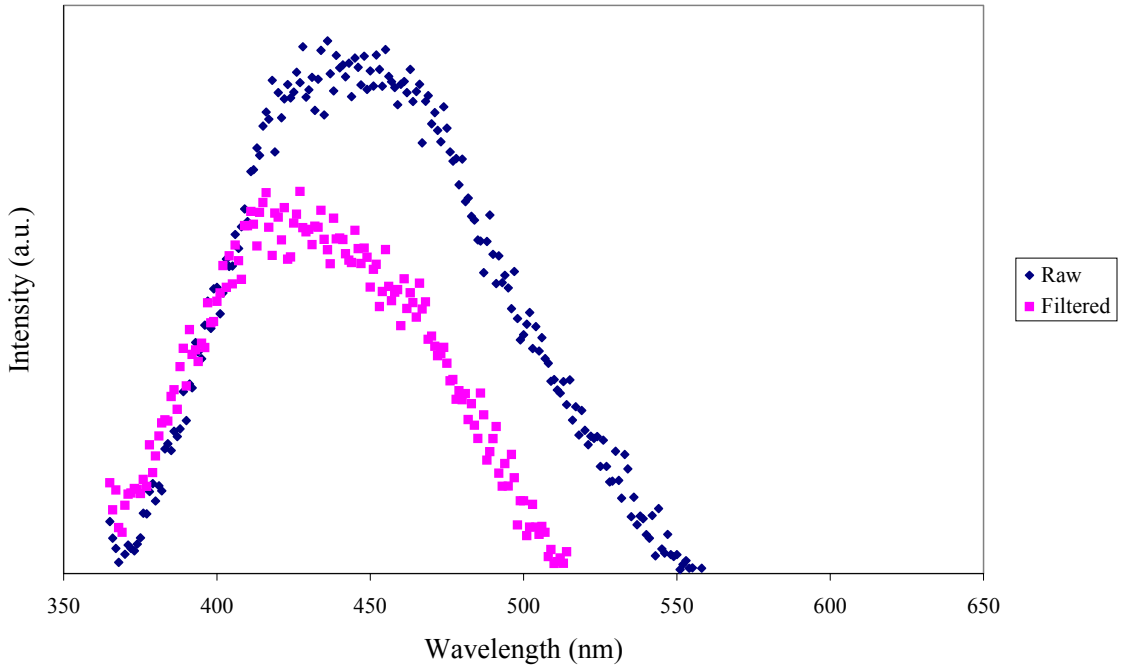


Figure 5.13 Emission spectra of raw and filtered water from Albert WTW (November 2000)

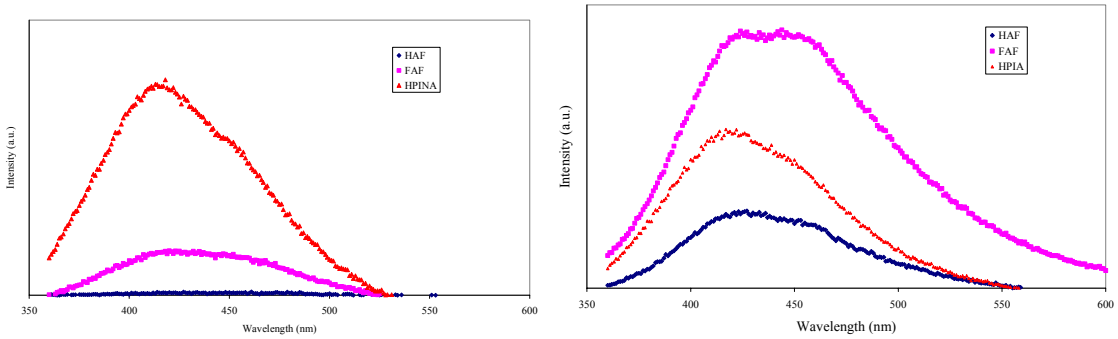
The emission spectra for the fractionated NOM from June and November 2000 are shown in figures 5.14 and 5.15 respectively. As with the bulk water samples the spectra exhibit a broad brand profile but the values for Em_{max} compare well with those seen previously at Apremont Reservoir (table 5.21, Croué *et al*. 1993a). Here it is observed that the higher the MW of the fraction, the greater is the value of Em_{max}. For

the June samples the maximum emission wavelength decreases in the order HAF>FAF>HPIA indicating that the MW of the HAF is greater than the FAF which in turn has a greater MW than the HPIA.

In November the maximum emission wavelength of the fractions decreases in the order HPIA>FAF>HAF>HPINA indicating that the MW of the HPIA is highest decreasing to the HPINA which has the lowest MW. However, the difference between the emission maxima is only a few nanometers as shown in table 5.21. The filtered water fractions follows a similar trend as reported above for June in that the maximum emission wavelength decreases in the order HAF>FAF>HPI-A>HPI-NA.

The intensity of fluorescence can also give some information on the type of material present in the fractions although the relationship between intensity and structure is less clear than the emission maxima – molecular weight relationship. It is known that hydrophilic acids exhibit a higher intensity of fluorescence than hydrophobic acids. The higher intensity of fluorescence observed for hydrophilic acids is attributed to their lower molecular weight (compared to hydrophobic acids) which decreases the rate of radiationless losses of excitation (Croué *et al*. 2000). Here, the relative intensity of the raw fractions is in the order FAF>HPIA>HAF>HPINA in November 2000.

NOM samples that exhibit a long wavelength emission maxima combined with low fluorescence intensity indicates the presence of condensed aromatic ring and other unsaturated bond systems with a high degree of conjugation and electron withdrawing groups such as carbonyl and carboxyl groups (Chen *et al*. 2002).



A B

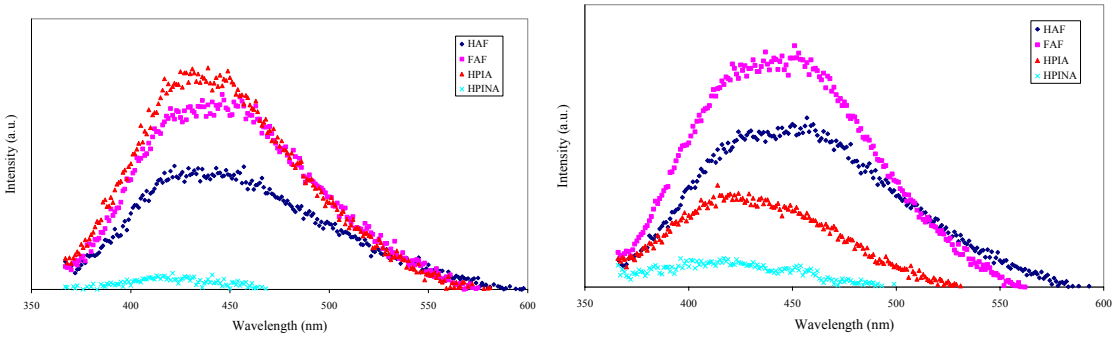
Figure 5.14 Emission spectra of NOM fractions from raw (A) and filtered (B) water (June 2000)

A long emission wavelength and low intensity of fluorescence is observed for the raw HAF (June 2000). NOM samples that exhibit a short emission maxima wavelength combined with a high fluorescence intensity are said to have low aromatic content, low molecular weight components and electron donating groups such as hydroxyls and methoxyls as has been commonly observed for humic and fulvic acids (Chen *et al*. 2002). In November 2000, the fractions that have high fluorescence intensity also have long emission wavelengths. None of these fractions fit into the descriptions given by Chen *et al*. (2002).

Table 5.21 Comparison of emission maxima for Albert Reservoir and Apremont Reservoir

Fraction	June ARW	June AFW	Nov. ARW	Nov. AFW	Apremont
Bulk Water	nm	nm	450	411	nm
HAF	427	461	425	458	456
FAF	424	420	430	424	437
HPIA	417	413	431	419	426
HPINA	Nm	nm	424	413	414

KEY – ARW – Albert Raw Water, AFW – Albert Filtered Water



A B

Figure 5.15 Emission spectra of NOM fractions from raw (A) and filtered (B) water (November 2000)

The effect of treatment on the FAF on the emission spectra is shown in figure 5.16. The emission maxima is reduced from 430 nm to 424 nm indicating that large molecular weight material is being removed. The intensity of the filtered water FAF fluorescence is greater than that of the raw water FAF. This indicates that the filtered FAF material has more smaller molecules than the raw FAF.

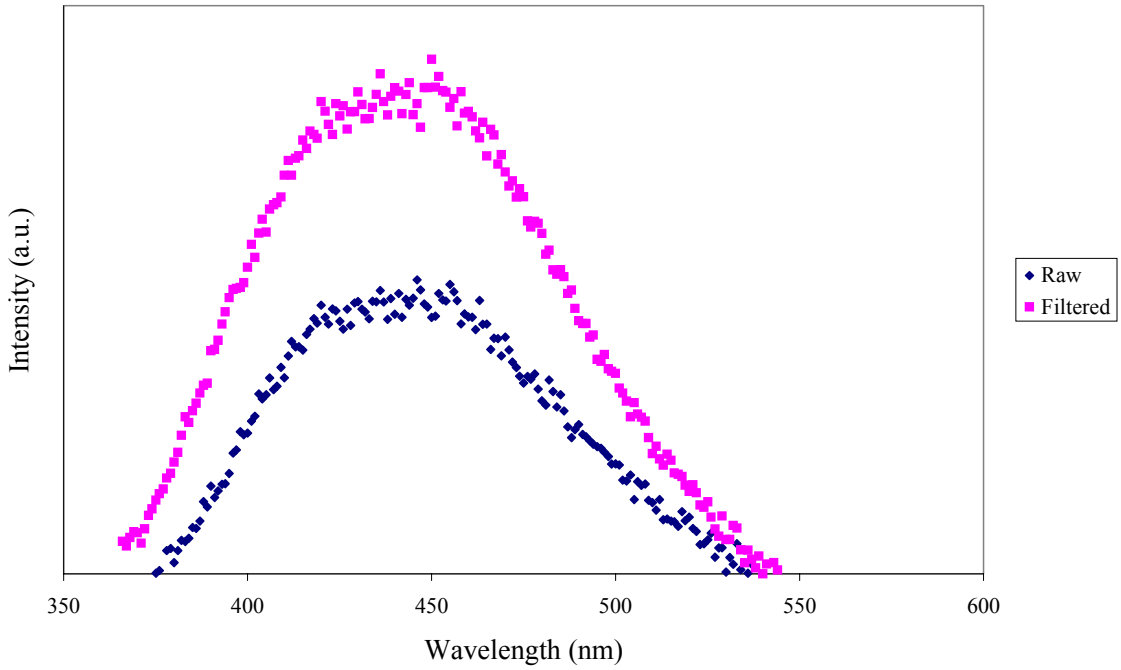


Figure 5.16 Comparison of Emission Spectra for the raw and filtered FAF (November 2000)

The information obtained from the single emission spectra should be treated cautiously as the emission maxima obtained will be dependent on the wavelength of excitation that was used. When both the excitation and emission wavelengths are scanned simultaneously but the difference between them is kept constant, the resulting spectrum is a synchronous spectrum (Croué *et al*. 2000). The synchronous scan can be represented as a contour plot that comprises a 3-D plot of excitation vs emission vs fluorescence intensity that is referred to as an excitation emission matrix (EEM). A synchronous scan offers several major advantages over single-scan methodologies. It provides a new piece of information regarding fluorescent organic matter of a sample: the wavelength-independent fluorescence maxima (Ex_{max}/Em_{max}). The Ex_{max}/Em_{max} is not dependent on the wavelength at which fluorescence was stimulated or which

emission was observed because it represents the one combination of emission and excitation wavelengths that results in maximum fluorescence (Coble 1996).

Further analysis of Albert bulk waters and fractions by synchronous spectroscopy was carried out to avoid obtaining wavelength dependent Em_{max} values. Synchronous scans were performed on raw water fractions from November 2000 (figures 5.17 – 5.20).

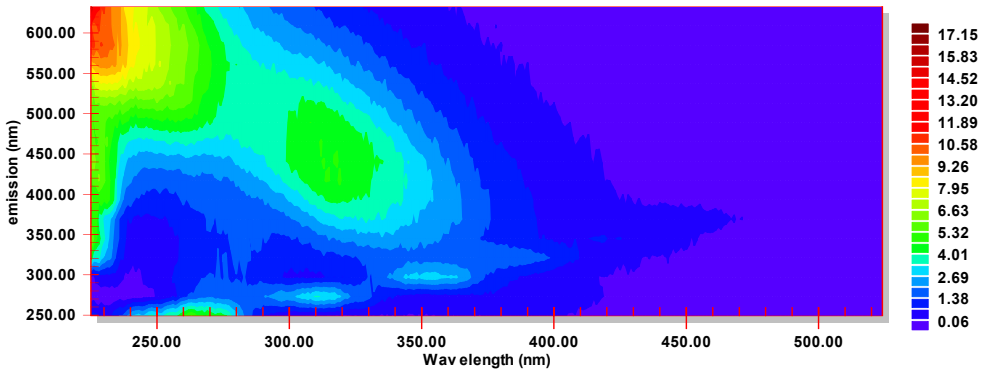


Figure 5.17 Raw water FAF EEM (November 2000)

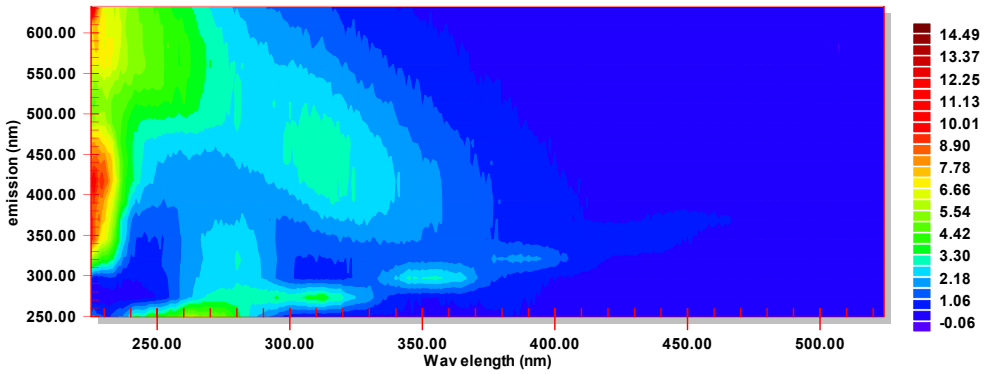


Figure 5.18 Raw water HAF EEM (November 2000)

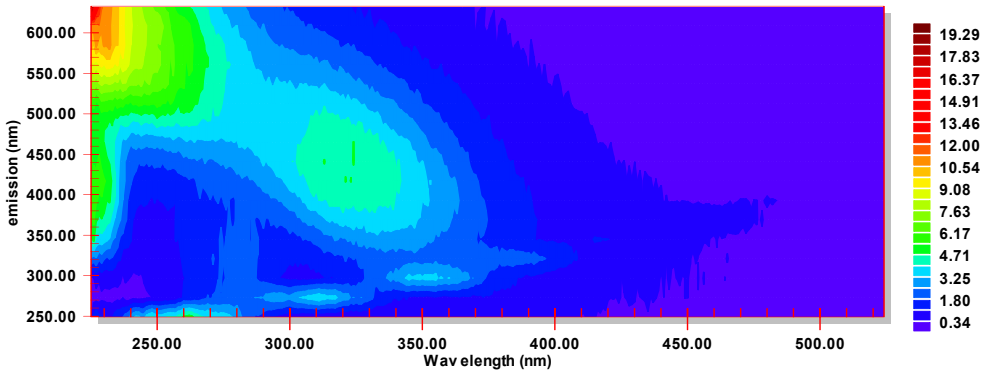


Figure 5.19 Raw water HPIA EEM (November 2000)

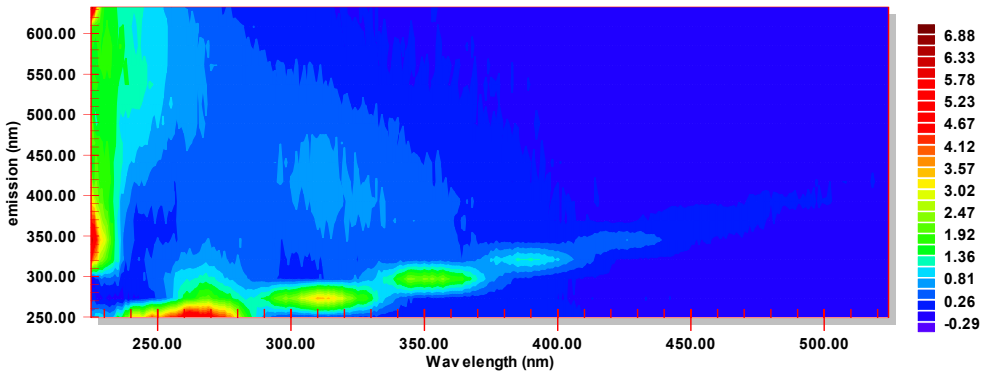


Figure 5.20 Raw water HPINA EEM (November 2000)

The FAF and HPIA contour plots showed a bimodal peak distribution indicating the presence of two main fluorophores in the macromolecular structure of the dissolved NOM. The HAF contour had four distinct peaks. The HPINA contour plot showed a trimodal distribution with much lower intensity of fluorescence being exhibited when compared with the other fractions. Wavelength pair values for the two major peaks exhibited by each fraction are reported in table 5.22 alongside literature values.

Analysis of the fractions by high pressure size exclusion chromatography (HPSEC) has shown that the FAF contains smaller molecules than the HAF (see section 5.3.3.1). The stronger intensity of fluorescence exhibited by the FAF (figure 5.17), when compared to

the HAF (figure 5.18) (in the excitation area 300 - 350 nm), indicated that smaller sized molecules fluoresce more strongly per mass of carbon. This could be as a result of more efficient energy transfer through internal conversions in the larger molecules (Alberts *et al.* 2000). The presence of a variety of fluorescent structures has been reported as being indigenous in the fulvic macromolecule. These include condensed aromatic moieties bearing various functional groups and unsaturated aliphatic chains. The lower fluorescence emission wavelength maxima reported for FAF when compared with HAF indicates a lower average MW for the FAF as well as the presence in FAF of simpler structural components i.e. a low degree of aromatic substitution and polycondensation and low levels of conjugated chromophores (Senesi 1990).

In the literature, wavelength pairs at 278/353 (excitation/emission (nm)) have been reported for protein-like substances and at 337/423 (ex/em (nm)) for fulvic-like substances (Her *et al.* 2001). A faint peak is observed in the protein-like region in the HAF contour plot at 280/325 (ex/em (nm)) (figure 5.18). A fulvic-like fluorescence peak has also been reported in the range 290-340/380-430 (ex/em (nm)) (Baker and Genty 1999). Wavelength pairs have been reported for humic-like substances at 310/423 (ex/em (nm)) (Coble 1996). These reported values agree with those observed for the Albert Reservoir fractions (table 5.22).

It has been reported by Baker and Lamont-Black (2001) that after the first flush of the hydrological year in the UK (in autumn), fluorescent dissolved organic matter (DOM) had a lower emission wavelength than the annual mean. Furthermore, a second (late winter) organic matter flush comprised fluorescent DOM that had a lower excitation wavelength than the annual mean. The fact that the samples investigated here were taken in November (autumn/winter) could explain the blue shift (shorter wavelengths)

in the excitation wavelengths observed for the fulvic- and humic-like fluorescence when compared with other values found in the literature (Alberts *et al.* 2000, Miano and Alberts 1999).

Only Marhaba *et al.* (2000) have reported wavelength pairs for hydrophilic acids in the literature (Table 5.22). These reported pairs do not correspond with the region of fluorescence for the Albert Reservoir water HPIA. The first wavelength pair in the HPIA contour plot at 318/424 (ex/em (nm)) (figure 5.19) is in the region of fulvic-like fluorescence but the intensity of fluorescence is much lower than the FAF perhaps indicating less aromaticity and less unsaturation in the HPIA.

The wavelengths in the first pair shift towards shorter wavelengths for the HPINA fraction signifying a smaller nominal molecular size when compared with the other fractions. The lower intensity of fluorescence indicates a lower degree of aromaticity for the HPINA fraction when compared to the other fractions. A peak for carboxylic acids at 310/400 (ex/em (nm)) was identified in the literature (Alberts *et al.* 2000). A peak is observed in this region in the HPINA fraction contour plot (figure 5.20). This peak at 310/400 (ex/em (nm)) is also said to be representative of small nitrogen containing compounds (Alberts *et al.* 2000).

In conclusion, the position of the peaks for each fraction can vary seasonally and with the sample source. There is no wavelength pair that can be definitively identified with any DOC fraction although a wavelength range could be identified.

Table 5.22 Comparison of luminescence data of Albert November Raw Water (2000) fractions with literature values

Reference	Sample identification	Excitation-Emission wavelength pairs (nm)	
		1st pair	2nd pair
Coble (1996)	Freshwater HA	310, 428	Disregarded
	Freshwater HA	310,423	Disregarded
	FA	310, 419	Disregarded
Miano and Alberts (1999)	Suwannee River Water	352,453	252, 450
	Suwannee River Water	355,448	245,442
Alberts *et al*. (2000)	Norwegian Lake Water	330, 437	225, 428
	Norwegian Lake Water	335, 438	225, 426
Marhaba *et al*. (2000)	River Water		
	HPO-A	225-237, 345-357	Not reported
	HPO-B	225-237, 357-369	Not reported
	HPO-N	225, 609-621	Not reported
	HPI-A	237-249, 417-429	Not reported
	HPI-B	225-237, 369-381	Not reported
	HPI-N	225-237, 309-321	Not reported
Baker and Lamont-Black (2001)	Borehole Water		
	Fulvic like centre	320, 407	None reported
	Protein like centre	278, 347	None reported
McKnight *et al*. (2001)	Lake FA	320, 406	230, 412
Her *et al*. (2001)	Suwannee River		
	HA	325, 452	261, 457
	FA	320, 443	245, 445
This work	FAF	314, 423	226, 593
	HAF	305, 457	227, 425
	HPIA	318, 424	226, 625
	HPINA	307, 390	225, 351

Key - HPO-A, HPO-B, HPO-N – Hydrophobic acid, base and neutral, HPI-A, HPI-B, HPI-N – Hydrophilic acid, base and neutral, HA – Humic acid, FA – Fulvic acid, FAF - Fulvic acid fraction, HAF - Humic acid fraction, HPIA - Hydrophilic acid fraction, HPINA – Hydrophilic non-acid fraction

Returning to the information obtained by the single emission scans, it was found that the fractions that exhibited long emission wavelengths (relative to the mean for these

fractions – 427.5 nm) also exhibited high fluorescence intensity and that the range of emission wavelengths was very narrow (424 – 431 nm). With the synchronous scan, the FAF and HPIA have low emission maxima (compared to the HAF – 457 nm) and high intensities of fluorescence meaning that these fractions have low MW components and electron donating groups (Chen *et al*. 2002). The HAF has a long wavelength emission maxima coupled with a lower intensity of fluorescence (compared to the FAF and the HPIA) indicating the presence of condensed aromatic ring and other unsaturated bond systems, a high degree of conjugation and electron withdrawing species (Chen *et al*. 2002). The HPINA fraction has a low intensity of fluorescence and a short wavelength emission maxima. A study by Chen *et al*. (2003) stated that, in general, the peak emission wavelengths of NOM shift from shorter to longer wavelengths with increased molecular size and aromatic content. This indicates that the HPINA contains low molecular weight species that are aliphatic in nature. Although smaller molecules fluoresce more strongly than large molecules per unit carbon, due to more efficient energy transfer, very small molecules are unlikely to fluoresce because the excitation energy will dissipate too quickly for fluorescence to occur.

5.3.2.2 Membrane separated fractions

A sample of Albert raw water from October 2002 was separated using UF membranes into four size fractions. These were R3 (>3 kDa), R1 (1- 3 kDa), R05 (0.5 - 1 kDa) and F05 (<0.5 kDa). A synchronous scan on the fluorescence spectrophotometer produced an EEM for each fraction. Each EEM is shown (figures 5.21– 5.24). The position and intensity of the fulvic-like peak in each matrix was determined and compared against the other UF fractions (table 5.23).

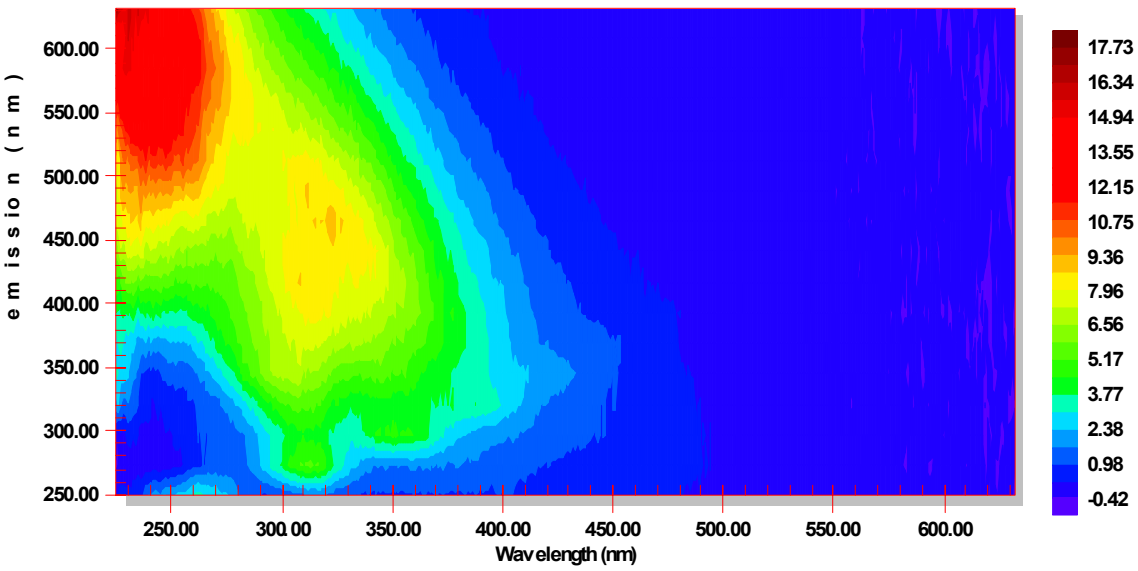


Figure 5.21 Raw water R3 EEM (October 2002)

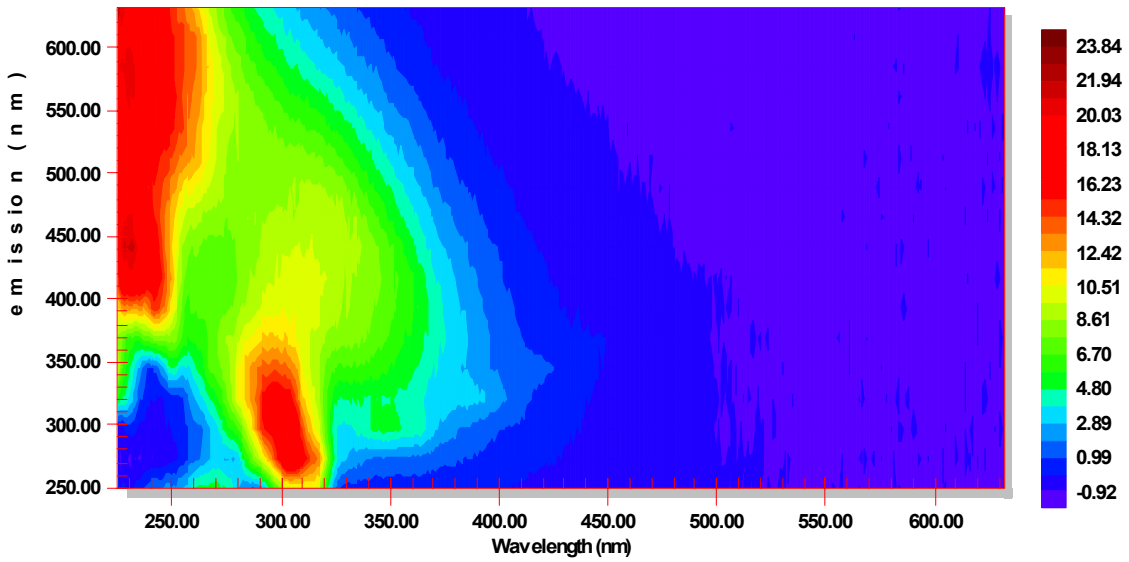


Figure 5.22 Raw water R1 EEM (October 2002)

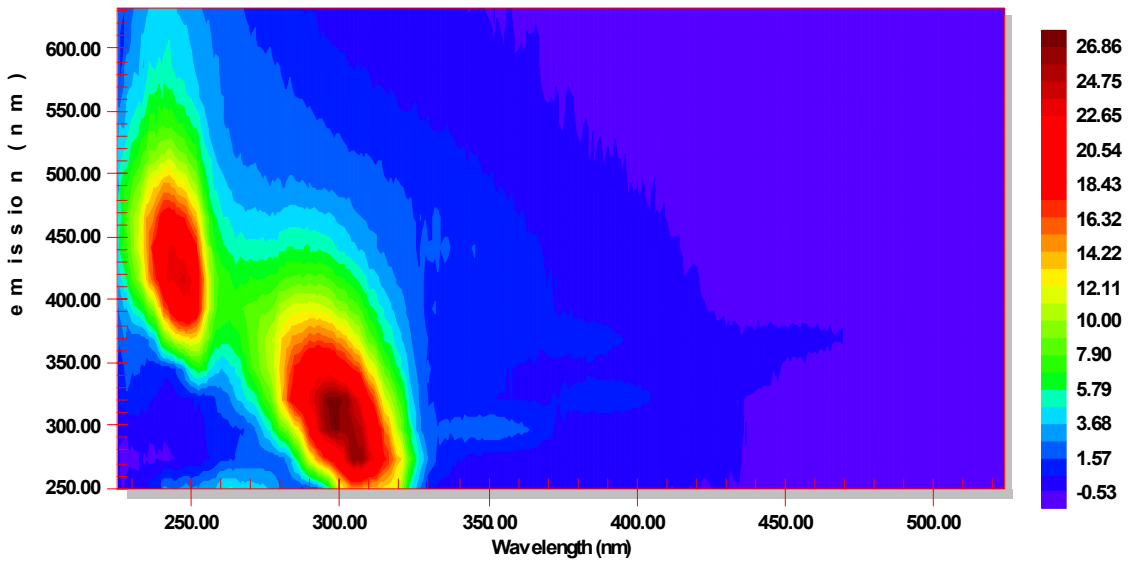


Figure 5.23 Raw water R05 EEM (October 2002)

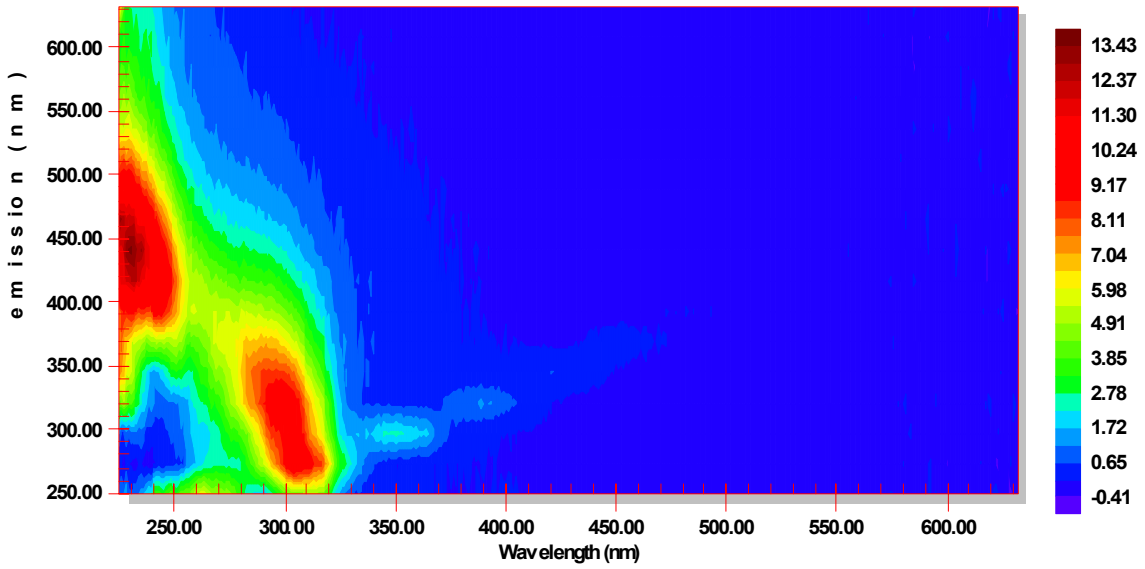


Figure 5.24 Raw water F05 EEM (October 2002)

Table 5.23 UF fraction contour plot peak characteristics

Sample	Peak position (ex, em (nm))	Peak intensity (arbitrary units)
R3	320, 446	9.1
R1	296, 336	17.9
R05	296, 325	27.5
F05	298, 323	10.4

It has been previously discussed that the position of the peak on the emission axis is directly proportional to molecular weight (MW). NOM fractions with low MW have high excitation and emission intensity and the position of the maxima in the emission spectrum shifts to lower wavelengths as the average MW decreases (Croué *et al*. 2000). This is seen here with the emission maxima decreasing as the MW decreases. The intensity of fluorescence is also seen to increase as the MW decreases until fraction F05 that exhibits a lower intensity of fluorescence than R1 and R05. It could be that most of the molecules in this fraction are too small to fluoresce or that the degree of unsaturation is less in this fraction.

Single emission scans were carried out on the size fractions and compared with the resin fractions. It was found that the fractions separated by Belin *et al*. (1993) using XAD resins and UF membranes had almost identical emission spectra. Here the spectra of the resin separated fractions were not found to be the same as the UF separated fractions.

5.3.2.3 Fluorescence conclusions

By only carrying out a single emission scan, the information obtained is incomplete and can be misleading. This is because the fluorescence observed in emission spectra will be dependent on the excitation wavelength used. An EEM gives a more complete picture as the fluorescence observed is independent of the excitation and emission wavelengths and allows comparison of fractions within a water sample. The information obtained from the contour plots of the resin separated fractions confirmed the information on the types of organics present and mean MW values that were shown from the SUVA values. The fluorescence of the UF separated fractions showed that the position of the maxima in the emission spectrum shifts to lower wavelengths as the MW decreases. This agrees with the literature and means that the position of the maxima in the emission spectra can be used to determine the relative MW when comparing resin separated NOM fractions. It was noticed that the mean MW of the three lowest MW UF separated fractions had lower mean MW than the resin separated fractions (table 5.22, table 5.23) including the HPINA indicating that the resin separated fractions contain molecules with a range of MWs.

5.3.3 High Performance Size Exclusion Chromatography (HPSEC)

5.3.3.1 Resin separated fractions

Samples from 2002 were analysed using HPSEC. With HPSEC, large molecules are eluted from the column first and smaller molecules later. Comparison of the raw and filtered water gives an indication of the molecular size of material being removed by the water treatment process. It should be remembered that only UV absorbing species will be detected by the UV detector that was set at 254 nm. By superimposing the filtered water chromatogram onto the raw water chromatogram (figure 5.25), it can be seen that the material being removed by the water treatment process has a higher MW.

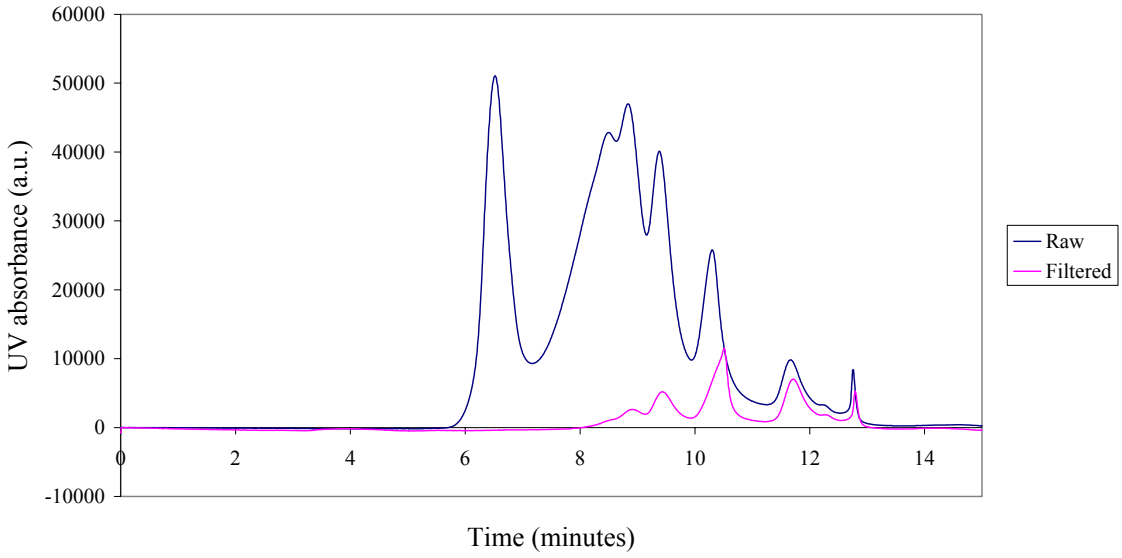


Figure 5.25 Raw and filtered water chromatograms (April 2002)

Raw water fraction chromatograms were also produced to determine the relative size of each fraction (figure 5.26).

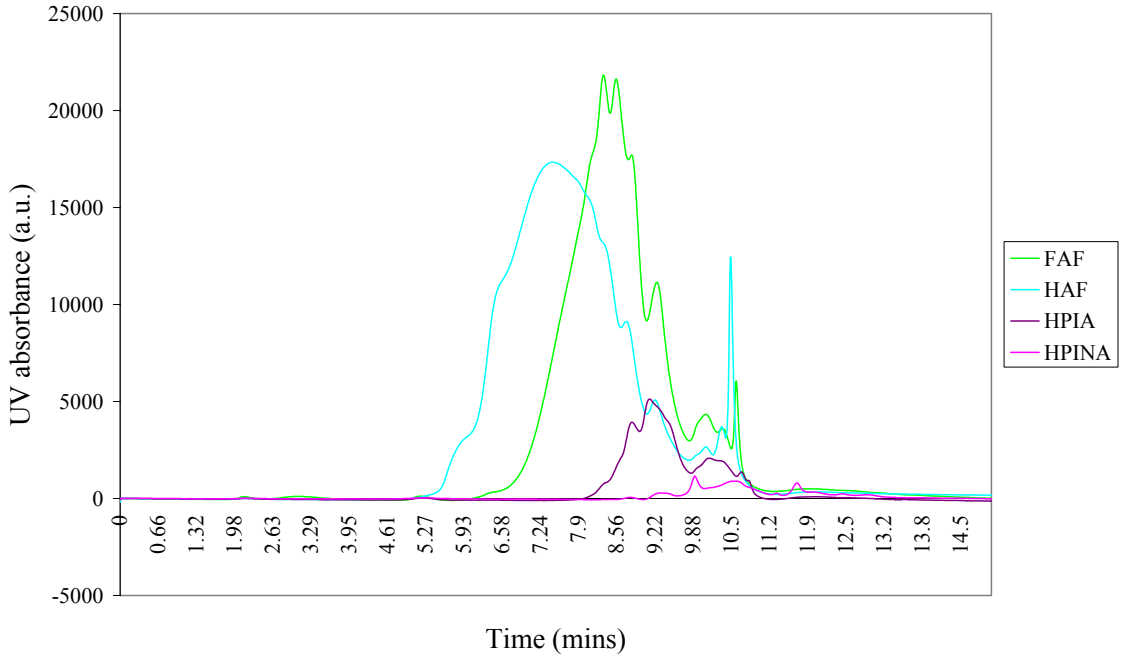


Figure 5.26 Raw water fractions (April 2002)

It can be seen that the molecular size of the fraction decreases in the order HAF>FAF>HPIA>HPINA. This is the similar to the MW assigned using the contour plots for the November 2000 raw fractions with the exception of the FAF and HPIA which were shown by fluorescence synchronous scans to have almost identical mean MWs. It has been pointed out that MWs measured with HPSEC with UV detection generated higher values than those measured with other methods (Her *et al*. 2002a). This was ascribed to higher MW fractions having a higher molar absorptivity (ε). Conversely, lower MW fractions with a lower ε appeared to be lower in concentration. A UV detector on the HPSEC quantifies the response intensity based on the ε. As a result, the MW determined with a UV detector at 254 nm is primarily the MW of only the high ε components such as humic and fulvic acids leading to inherent inaccuracy and over- or underestimation of MWs. Her *et al*. (2002) concluded that MW estimation with a UV detector is problematic for a hetero-mixture of NOM and that a UV detector

cannot be accurately used for quantitative measurements of NOM. With this in mind further samples were run on the HPSEC.

Filtered water fraction chromatograms were produced for the April 2002 samples (figure 5.27).

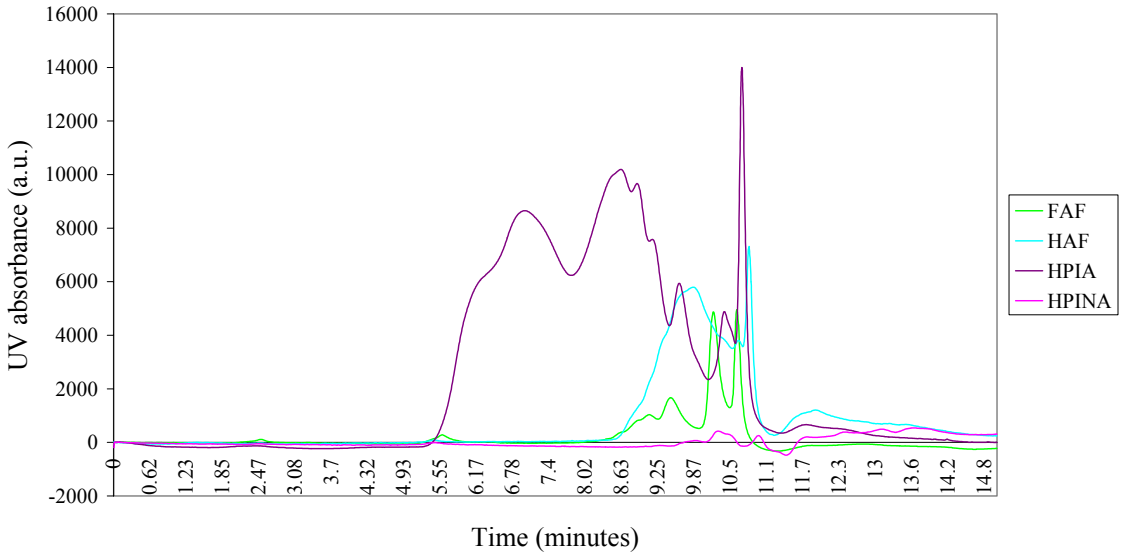


Figure 5.27 Filtered water fractions (April 2002)

After treatment the MW of the FAF and the HAF has been reduced. The MW of the HPIA appears to have increased. It is difficult to say whether the MW of the HPINA has been reduced by treatment. This is because it is the UV absorbing species in the fraction that are being detected and in this fraction the proportion of UV absorbing species is low. It appears to have increased but it possible that the amount of large MW material has been overestimated.

The October 2002 raw water fractions were run through the HPSEC and the resulting chromatograms are shown (figure 5.28). As with the April 2002 raw water fractions, the molecular size of the fractions decreases in the order HAF>FAF>HPIA>HPINA.

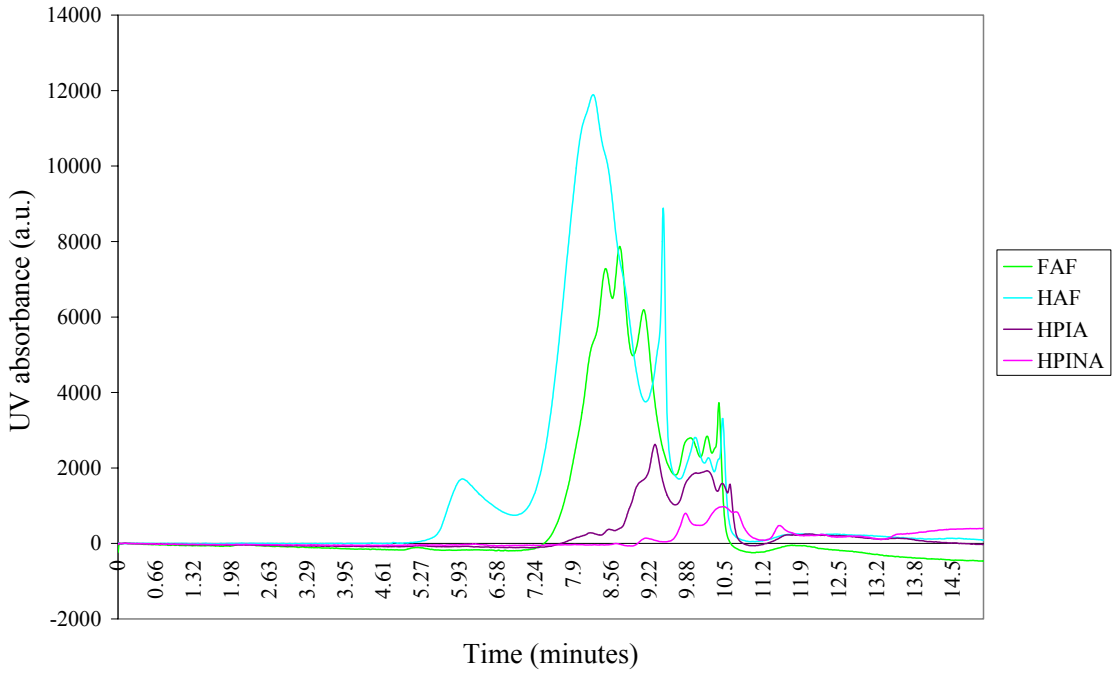


Figure 5.28 Raw water fractions (October 2002)

5.3.3.2 Comparison of April and October fractions

If the raw water chromatograms from April and October are superimposed on each other, it can be seen that the UV absorbance of the October water is greater than that of the April water (figure 5.29). This was also shown in the UV analysis of the raw waters that had an absorbance of 38.1 m^{-1} in April and 52.3 m^{-1} in October.

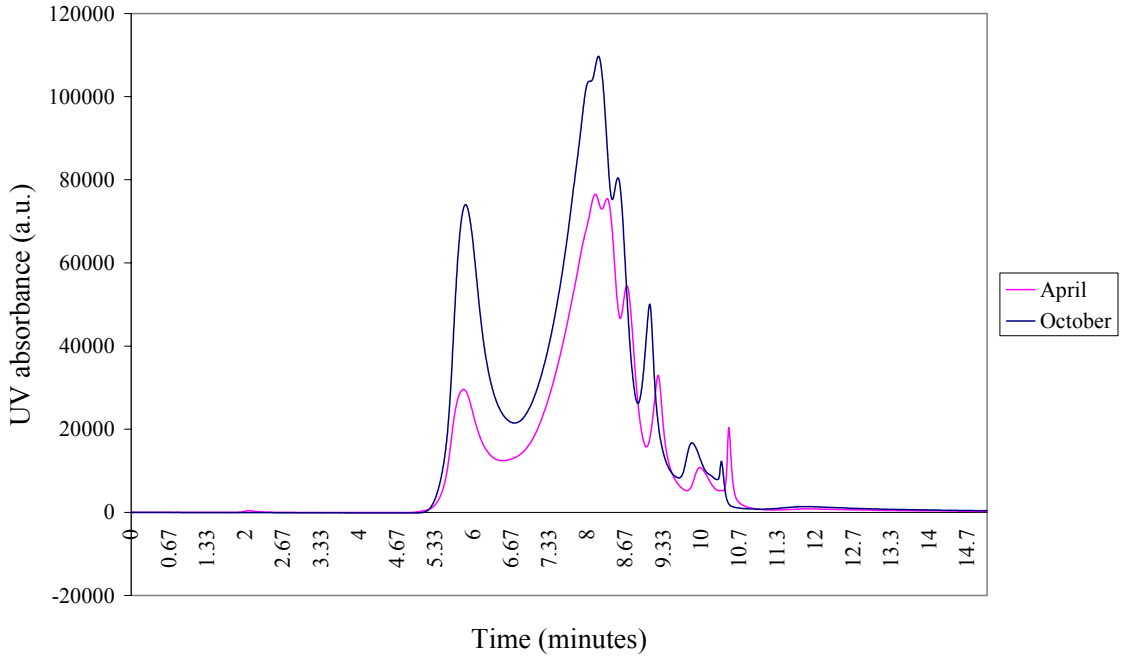


Figure 5.29 Comparison of 2002 raw water samples

The individual fractions were also compared (figures 5.30 – 5.33). It was only possible to compare the relative molecular size of each fraction as the analysis was carried out on samples that were approximately 1 mg L^{-1} C and not the natural concentration of the fractions.

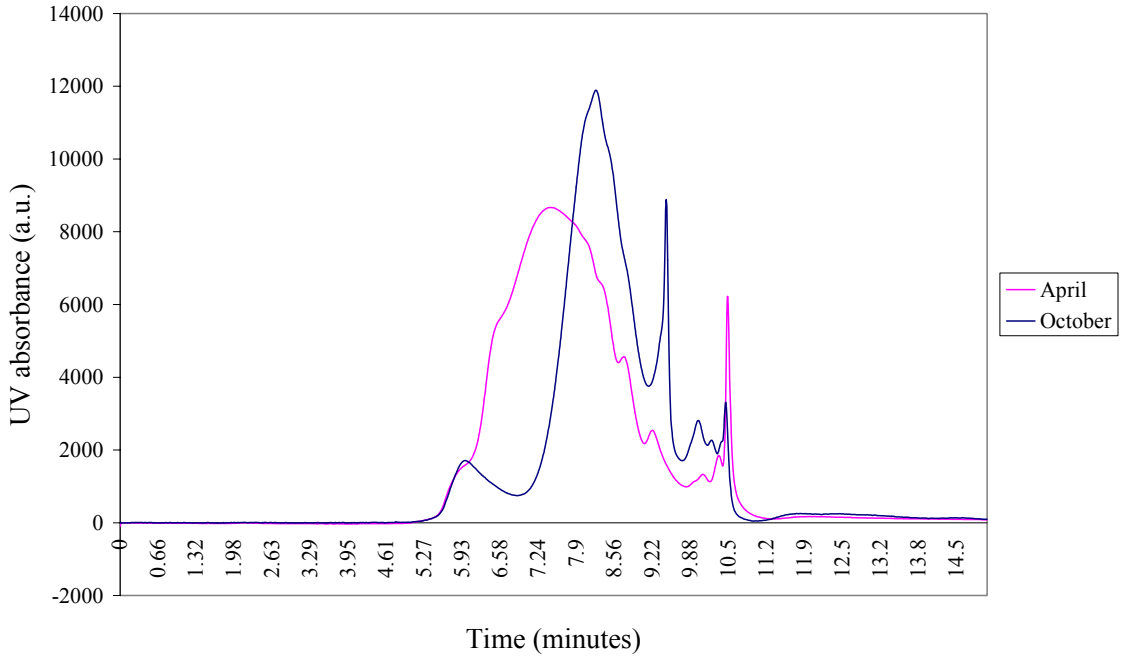


Figure 5.30 Comparison of 2002 HAF samples

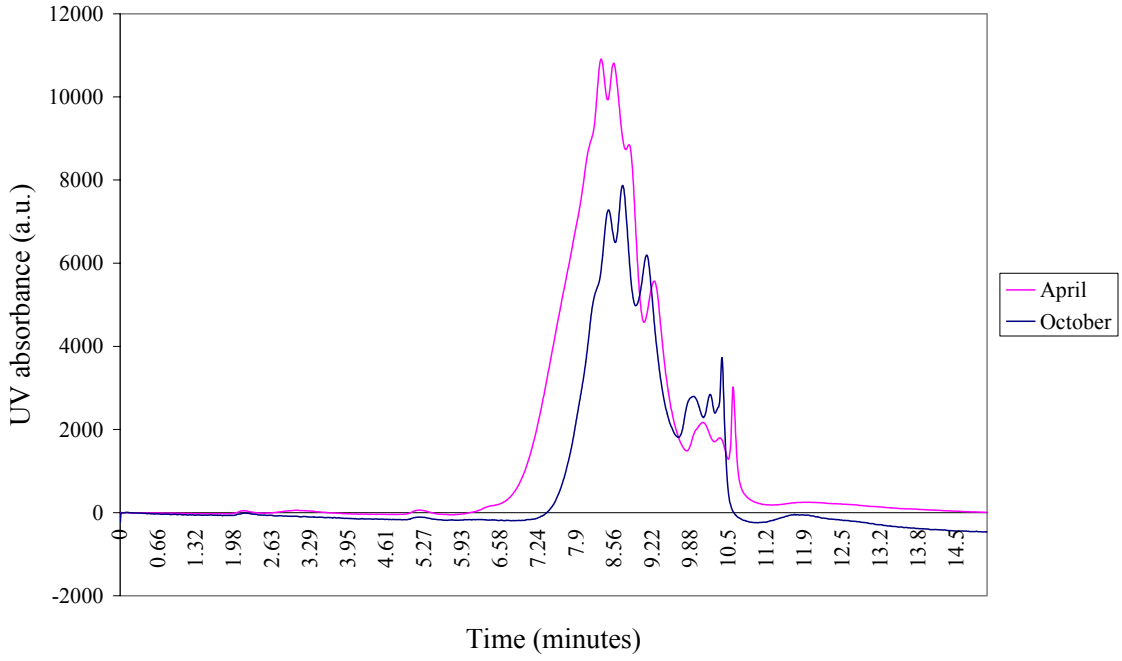


Figure 5.31 Comparison of 2002 FAF samples

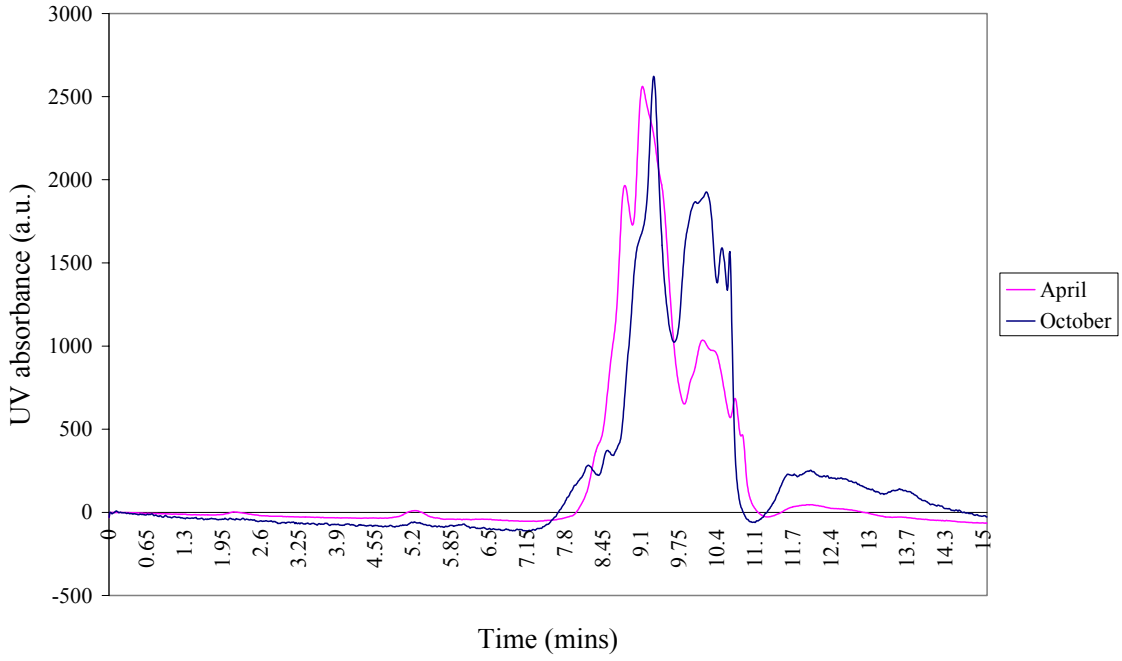


Figure 5.32 Comparison of 2002 HPIA samples

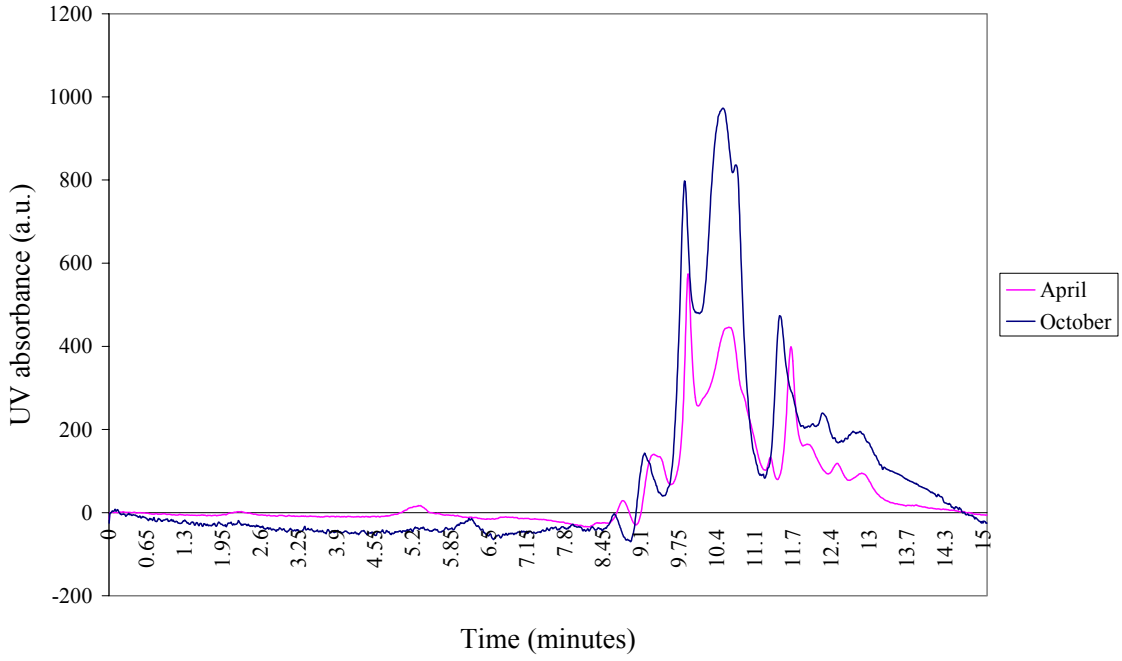


Figure 5.33 Comparison of 2002 HPINA samples

With all the fraction chromatograms, it should be noted that the signal to noise ratio is small due to the low concentration of the fractions (~ 1 mg L^{-1}). This has a

disadvantage in that the resolution of the chromatograms is poor. However, there is an advantage that there are no concentration effects and that agglomeration of the molecules is less likely. A comparison of the HAF samples shows that they have different profiles and that in April there is more high MW material present than in October. Both FAF chromatograms exhibit almost identical profiles but the MW range for April is slightly wider. A comparison of the HPIA samples shows different profiles and in October there is more small MW material but a similar amount of high MW compared with April. The HPINA chromatograms both have a similar profile with a similar range of MWs exhibited in April and October. Overall the FAF and the HPINA exhibit similarly sized material throughout the year whereas the size of the HAF and HPIA seems to vary throughout the year. Bearing in mind that the profile of the raw water samples is almost identical, it seems that fractionation has provided more information than bulk water analysis.

5.3.3.3 Membrane separated fractions

A sample of Albert raw water from October 2002 was separated using UF membranes into four size fractions. These were R3 (>3 kDa), R1(1- 3 kDa), R05 (0.5 - 1 kDa) and F05 (<0.5 kDa). HPSEC analysis of the fractions was undertaken. The results are shown (figure 5.34).

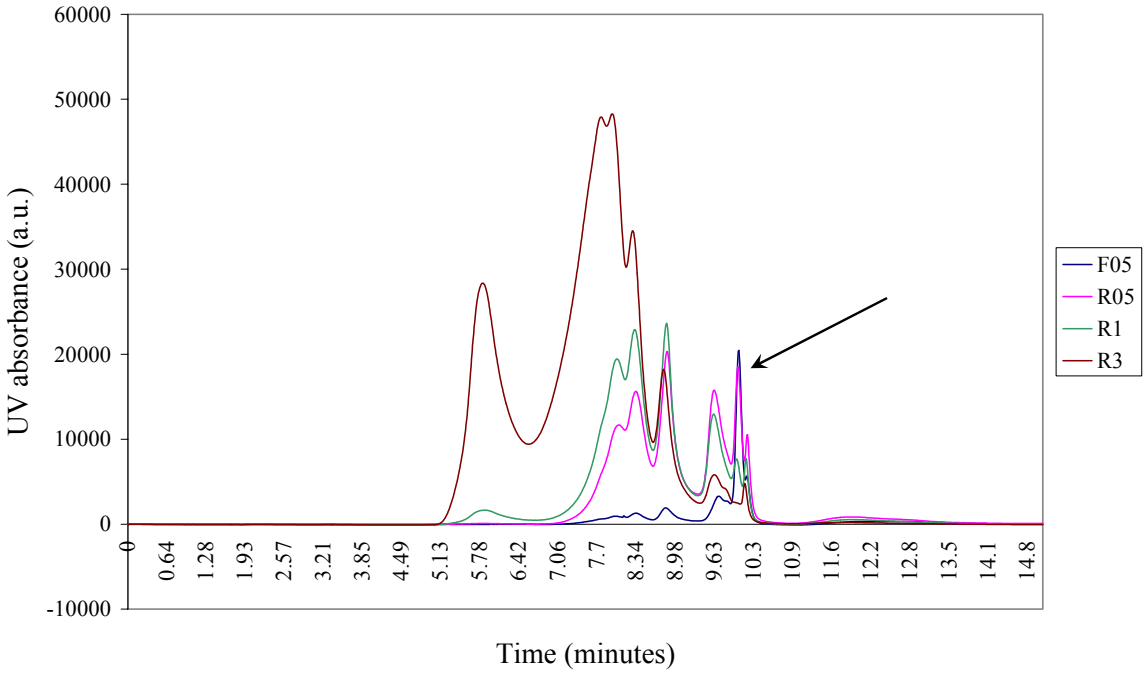


Figure 5.34 HPSEC analysis of UF fractions

The profile of the R3 fraction is very similar to the raw water chromatogram with seven distinct peaks being exhibited. The MW range of the other fractions is, as expected, lower than the R3 fraction. The chromatograms of the fractions R1, R05 and F05 all exhibit a new peak at ~10 minutes (indicated by arrow) that is not seen in the raw water chromatogram. It is possible that the new peak could have been caused by aggregation of the smaller molecules in solution resulting in an increase in UV absorbing species.

5.3.3.4 Practical application

Although differences in the fraction chromatograms can give some information on seasonality, the advantages of HSPEC is that the sample preparation is minimal, concentrated samples are not required and the analysis time is very short (< 20 minutes). Preparation of the fractions, however, is a time consuming, labour intensive process that negates the advantages of HPSEC mentioned above. HPSEC was found to be more

useful for determining the size of organic material removed during water treatment processes in order that removal could be optimised. Treatment by coagulation using ferric sulphate shows removal of the high MW fraction of the NOM (figure 5.35). Treatment using MIEX® (a magnetic ion-exchange resin) shows preferential removal of the lower MW fraction of the NOM. Here, the treatment has been optimised using a mixture of ferric chloride and MIEX® to remove almost all the UV absorbing species in the raw water.

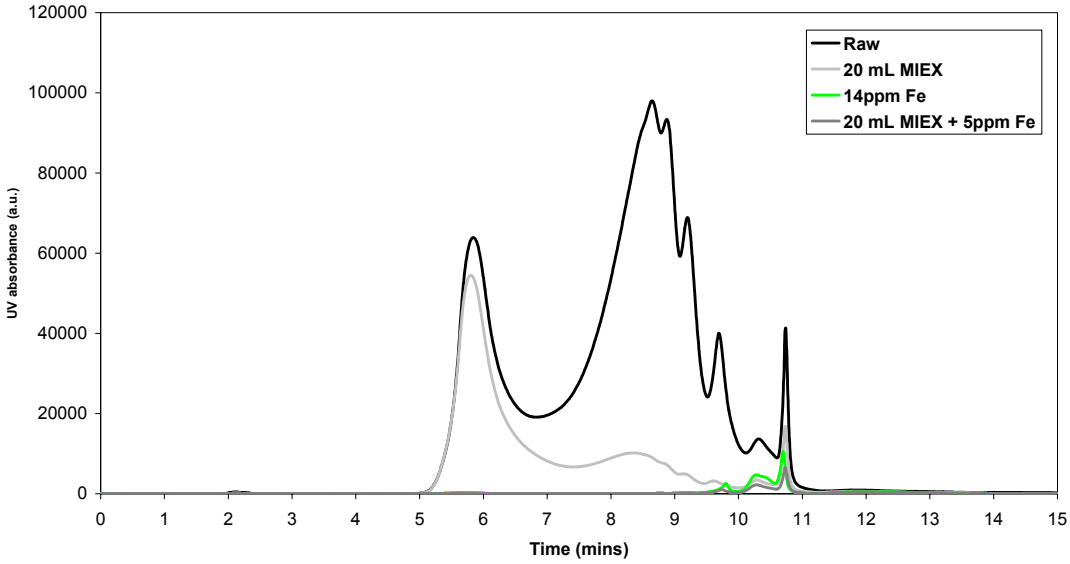


Figure 5.35 HPSEC chromatograms of raw water and treated waters

5.3.4 HPSEC column calibration

5.3.4.1 Introduction

With HPSEC it is known that large molecules are eluted from the column first and smaller molecules later (i.e. they have a greater retention time). However, it would be useful to know the apparent molecular weight (AMW) of each of the peaks. The sizes of dissolved organic substances are referred to as 'apparent molecular weights' since separations are calibrated with compounds of known molecular weights, not size. The AMW of each peak can be estimated using ultrafiltration (UF) membranes. The diffusive and advective transport of organics through UF membranes is influenced by a variety of factors (Logan and Jiang 1990). The most important of these factors is the quantification of the membrane rejection. The membrane rejection is the flux reduction through the membrane from concentration polarisation. A method presented by Logan and Jiang (1990) is used here to determine an experimental permeation coefficient that can be used to correct size distributions of dissolved organics by estimating the membrane rejection.

Before using UF fractions of Albert water, an attempt was made to calibrate the HPSEC column using Polystyrene Sulphonate (PSS) standards. A calibration curve was produced but it was not possible to infer any AMWs from the curve. It is believed that under or overestimation of the molecular weight (MW) occurred due to chemical interaction within the column. Some components can pass through the column more rapidly than expected due to ion exclusion or complex formation resulting in an overestimation of the component molecular weight (Knuutinen *et al*. 1988). Other

components can be delayed by adsorption or electrostatic interaction with column packing leading to an underestimation of molecular weight (Miles and Brezonik 1983).

The use of UF for estimating molecular weight fractions can result in erroneous data. Broad nominal molecular weight cut-offs and solute interactions with membrane surfaces make the analysis of UF data for these solutes very difficult (Aiken 1984).

5.3.4.2 Experimental conditions

A sample of raw water taken in April 2002 from Albert Reservoir was used for the calibration.

Ultrafiltrations were performed under nitrogen pressure (30 – 60 psi) in a stirred cell reactor (model 8200, Millipore, Massachusetts, USA). Membranes with MWCO values of 0.5, 1, 3, 5, 10 and 30 kDa were used (YM1 - YM30, Millipore, Massachusetts, USA, YC05, Australia). The YM membranes were made of regenerated cellulose and the YC membrane, cellulose acetate. The YM membranes were flushed with NaOH (0.1M), NaCl (0.5M) and deionised water to remove the wetting agents before use. The YC05 membrane was flushed with NaCl (1.0M) and deionised water.

110 mL samples of the raw water were filtered through each membrane at room temperature and five 20 mL samples of permeate collected (approximately 90% of the original sample). Each sample was analysed for DOC and UV absorbance at 254 nm as well as HPSEC analysis.

5.3.4.3 Molecular size distribution

It can be seen that the permeate concentrations of each sample increased in proportion to the volume of sample filtered (figure 5.36). By using the data in figure 5.36 and Equation 5.2 (Logan and Jiang 1990), the permeation coefficients were calculated (table 5.24).

$$\ln C_p = \ln(pC_{r0}) + (p-1)\ln F \qquad \text{Equation 5.2}$$

where C_p is the concentration of the permeate, p is the permeate coefficient, C_{r0} is the initial concentration of the sample (where $p > C_p/C_{r0}$) and F is the fraction of the original sample filtered. By plotting ln C_p against ln F, ln pC_{r0} is the y intercept and (p – 1) is the gradient (figure 5.37).

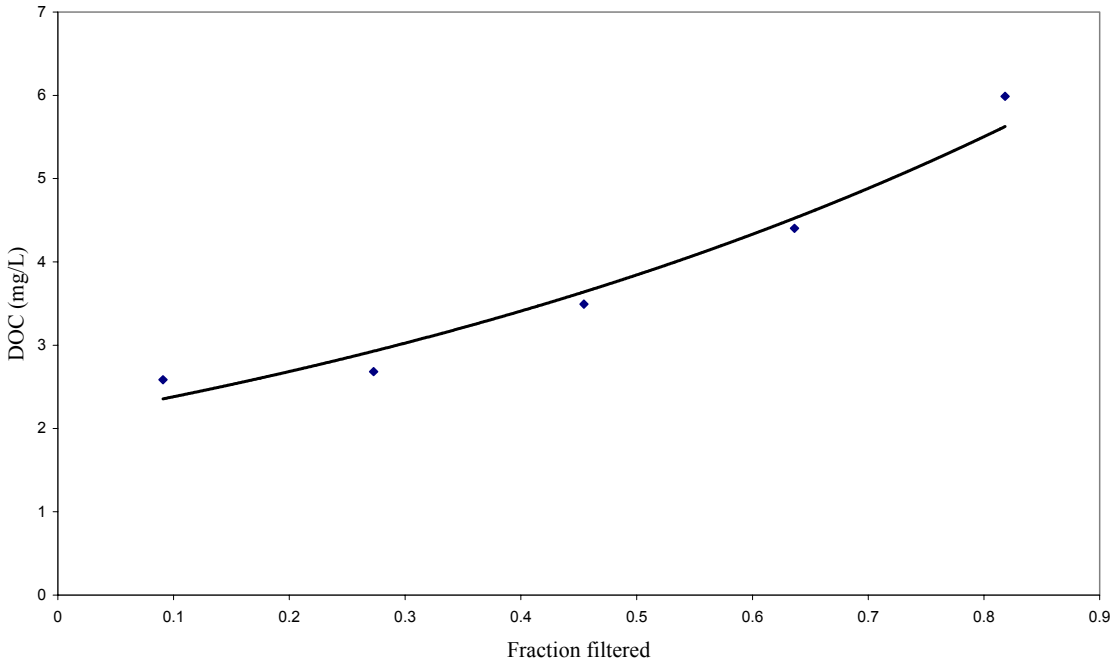


Figure 5.36 Permeate concentration as a function of fraction filtered

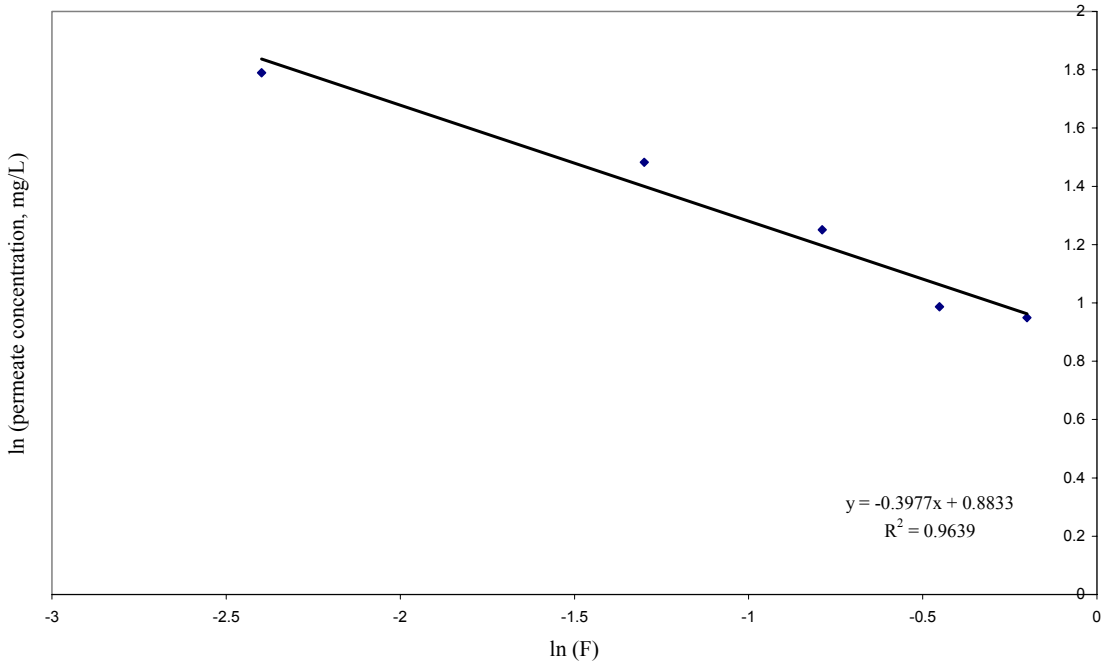


Figure 5.37 ln transformed data used to determine permeate coefficient

An average permeation coefficient, p, of 0.50 was calculated for all the membranes. This is similar to the expected value of p = 0.60 calculated from the manufacturer's rejection coefficient of 0.4.

Table 5.24 Permeate coefficients for each membrane for different natural water samples (adapted from Logan and Jiang 1990)

Membrane cut-off (kDa)	p reported (1)	p reported (2)	p calculated (3)
30	0.97	0.99	0.60
10	0.73	0.36	0.58
5	0.56	0.28	0.66
3	Nr	Nr	0.71
1	0.54	0.22	0.27
0.5	0.80	0.20	0.18

Nr – not reported
(1) – Orange County Water District (California groundwater)
(2) – Biscayne Aquifer (Florida groundwater)
(3) – Albert Raw Water April 2002

It is demonstrated that the average concentration of material measured after a batch filtration is a function of the volume collected as well as the permeation coefficient (equation 5.3). This is illustrated in figure 5.36.

$$C_f = C_{r0}\frac{(1-F^p)}{(1-F)} \qquad \text{Equation 5.3}$$

where C_f is the final concentration of the sample.

The extent that membrane rejection contributes to underestimation of material less than the membrane cut-off can be estimated using Equation 5.3. For example, when 90% of the sample has passed through the membrane and p = 0.60 (for the 30 kDa membrane), the collected filtrate concentration will be 61% of the true concentration of the material having a smaller size than the membrane cut-off.

In general errors in C_{r0} are reduced when p > 0.3. A low p indicates that much of the material is about the same size as the membrane cut-off or that the material is strongly rejected by the membrane due to charge repulsion or some other effect. If p <0.2 or

>0.9, the size distribution should not be adjusted (Logan and Jiang 1990). The unadjusted (white bars) and adjusted (black bars) molecular size distribution is shown in figure 5.38. This adjustment is based on the UV absorbance of each sample, not the DOC concentration.

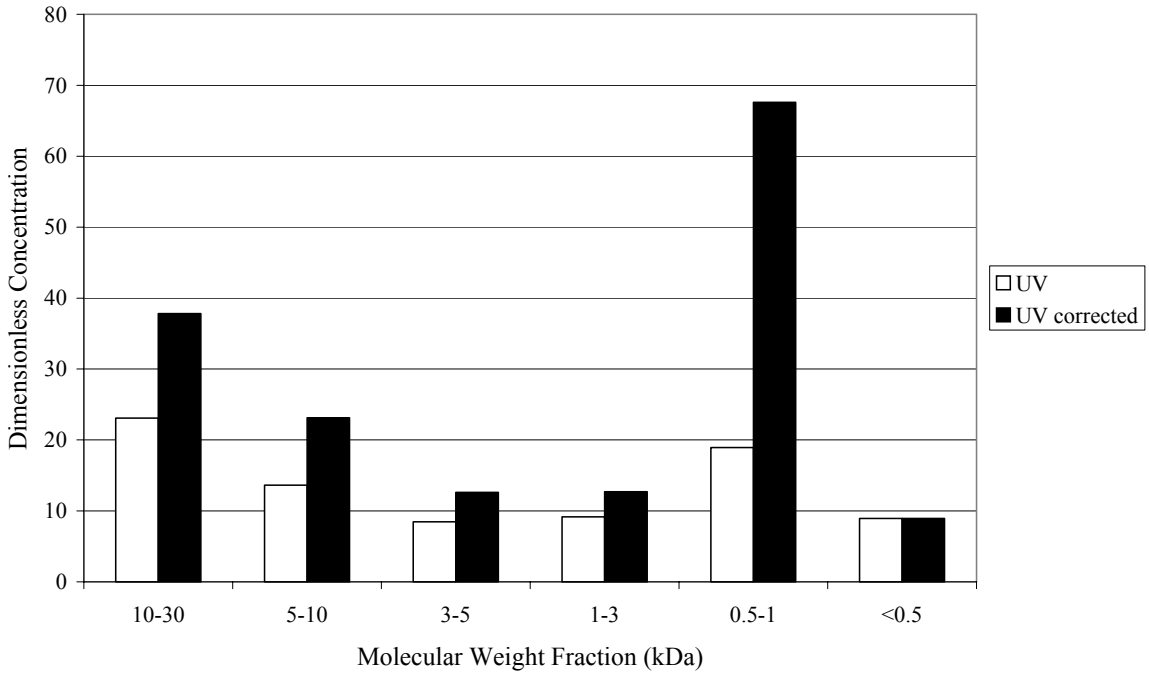


Figure 5.38 Molecular size distribution of Albert raw water (April 2002)

The UV absorbance measured is observed to fall as the MW falls. This has been reported in the literature (Collins *et al*. 1986). The exception to the trend is molecules with the size fraction 0.5 – 1.0 kDa which had a low p value compared with the higher MWCO membranes that were made of the same material. As stated above, a low p value indicates that the material is about the same size as the membrane cut-off or that the material is strongly rejected by the membrane. It seems unlikely that the material is strongly rejected by the membrane given that the membranes were all made from the

same material with the exception of the 0.5 kDa MWCO membrane. It is assumed here that adjustment is not required for the 1 kDa MWCO membrane.

5.3.4.4 Column calibration

Each sample collected after 90% of the sample had been ultrafiltered was run on the HPSEC. Each chromatogram exhibited five distinct peaks. The retention times for these are shown in table 5.25 alongside the assigned molecular weight range. These peaks corresponded to peaks observed in the raw water sample (figure 5.39). As the membrane pore size decreased, the height of the peaks decreased. The remainder of NOM measured as the reduction of the heights of the peaks was plotted against the log MWCO value of the membranes as given by the manufacturers (figure 5.40).

Table 5.25 Assigned molecular weight range for each peak

Peak	Retention Time (minutes)	AMW (kDa)
1	8.3 – 8.6	> 5
2	8.7 – 9.0	3.5 –5
3	9.1 – 9.7	2 – 3.5
4	9.7 – 10.4	1 – 2
5	10.5 – 10.8	0.5 – 1

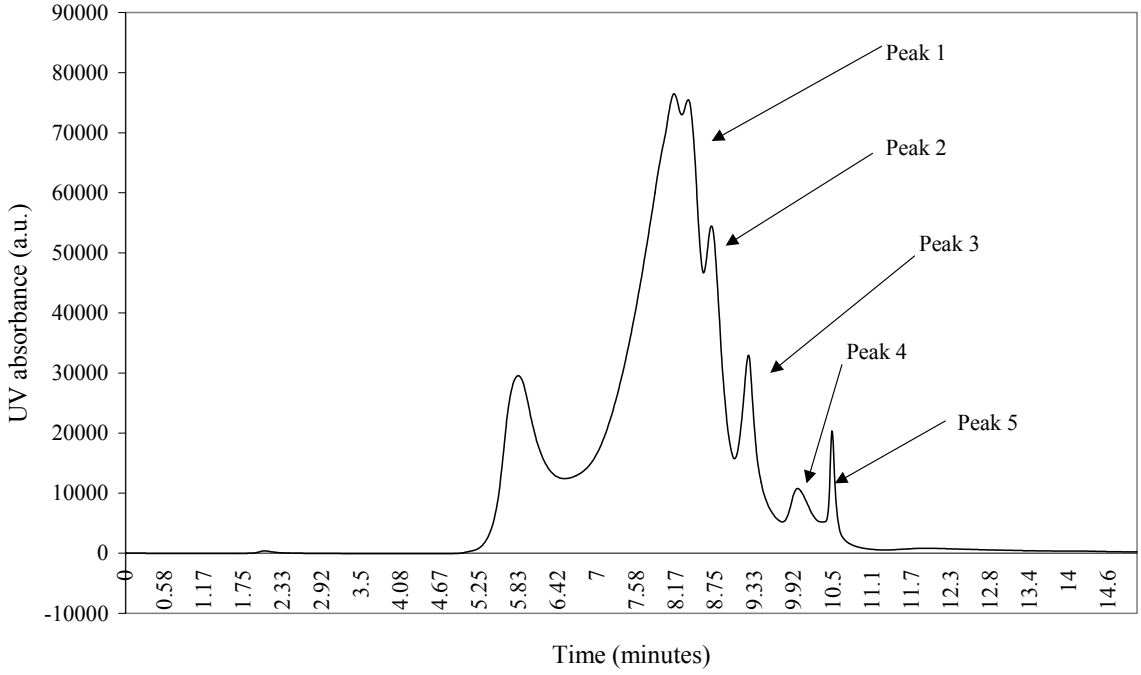


Figure 5.39 HPSEC chromatogram of Albert raw water with peaks assigned

The retention of the molecules depends on the pore size distribution of the membranes as well as the characteristics of the molecules, particularly the molecular volumes. Therefore, the retention of the molecules is not sharp. The 30 kDa membrane retains molecules from all size ranges as can be seen in figure 5.40. The membrane cut-off values are usually defined as the mass of a molecule whose retention is 90% on that membrane. When this retention value of 90% is applied to the peaks in an HPSEC chromatogram, the following AMW ranges can be assigned to each peak (table 5.25, figure 5.39). A range for each peak is given as each peak does not represent one specific molecular weight.

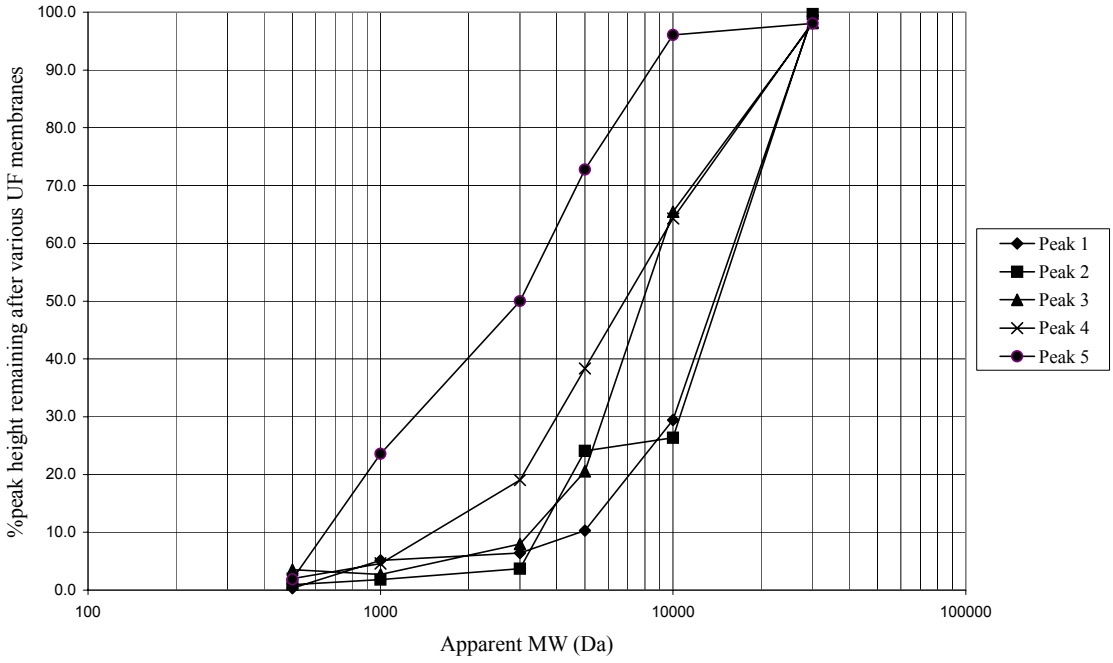


Figure 5.40 Remainder of NOM after various UF membranes measured as the reduction of peak heights in HPSEC traces

The shape of the raw water chromatogram is repeatable but the retention times for each peak can vary depending on the lengths of tubing used between the injector, the guard column and the column.

5.3.4.5 Sensitivity Analysis

It is possible that the TOC measurements made here could vary by ± 10%. The permeation coefficients were re-calculated from the TOC data with variance of ± 10%. The results are shown in table 5.26.

Table 5.26 Variance of permeation coefficients

Membrane cut-off (kDa)	p	p + 10%	p – 10%
30	0.60	0.71	0.53
10	0.58	0.69	0.51
5	0.66	0.83	0.65
3	0.71	0.81	0.62
1	0.27	0.23	0.05
0.5	0.18	0.27	0.08

Figure 5.50 was redrawn using the varied permeation coefficients and the new AMWs were assigned to each peak. The results are reported as the AMW where the line for each peak cross the 10% line of the '%peak height remaining' and are presented in table 5.27. The peak height values were not adjusted when the permeate coefficient values were less than 0.2.

Table 5.27 AMW values

Peak	Original values	+ 10%	- 10%
1	5000	3900	5000
2	3600	3400	3600
3	3250	3100	3400
4	1500	1500	1700
5	650	675	650

By varying the TOC values by ± 10%, the ranges given for peaks 2 to 4 (table 5.23) are not altered. A variance of +10% for the TOC gives a significantly lower value for peak 1 that does not fit into the range.

By carrying out the sensitivity analysis, the importance of assigning a range to each peak rather than an exact AMW value is illustrated. Again, it should be emphasised that the values for AMW calculated here should be taken as a rough guide.

5.3.4.6 Disadvantages of using UV detection

It should be noted that the analysis was carried out using an HPLC with UV detection at 254 nm. Such a system will not detect carbon single bonds. This may lead to an overestimation of the large molecule fractions. It has been pointed out that MWs measured with HPSEC with UV detection generated higher values than those measured with other methods such as ultracentrifugation and field flow fractionation (Her *et al*. 2002a). This was ascribed to higher MW fractions having a higher molar absorptivity (ε). Conversely, lower MW fractions with a lower ε appeared to be lower in concentration. A UV detector on an HPSEC quantifies the response intensity based on the ε. As a result, the MW determined with a UV detector at 254 nm is primarily the MW of only the high ε components such as humic and fulvic acids leading to inherent inaccuracy and over- or underestimation of MWs. Her *et al*. (2002a) concluded that MW estimation with a UV detector is problematic for a hetero-mixture of NOM and that a UV detector cannot be accurately used for quantitative measurements of NOM. With this in mind, the determination of AMWs made here should be used as a rough guide and not taken as exact values.

5.3.4.7 HPSEC calibration conclusions

UF cannot provide absolute values of molecular weight but can provide an 'index' of AMW that can be used for relative comparisons. The data obtained using the UF calibrated HPSEC should be interpreted by the observation of trends and not absolute values (Collins *et al*. 1986).

5.3.5 Reactivity with chlorine

The seasonal variation of the THM-FP of the water samples and their fractions has been studied. The relationship between SUVA and THM-FP reported in the literature was not found to hold for Albert water. So it was clear that the reaction between chlorine and NOM changed seasonally, which in itself could be a predictor of character. Therefore, it was thought that by investigating the THMs formed after 10 hours and 7 days as well as the changing fluorescence properties of the chlorinated samples, a greater understanding of the seasonal formation of THMs could be gained.

5.3.5.1 Chlorine decay

Raw water samples and fractions were chlorinated at a dose of 10 mg Cl_2 $mg^{-1}C$. All samples were diluted to 1 mg L^{-1} C before chlorination. After 10 hours and 7 days, the chlorine present in each sample was measured (figure 5.41). The THM-FP at these times was also measured (table 5.28).

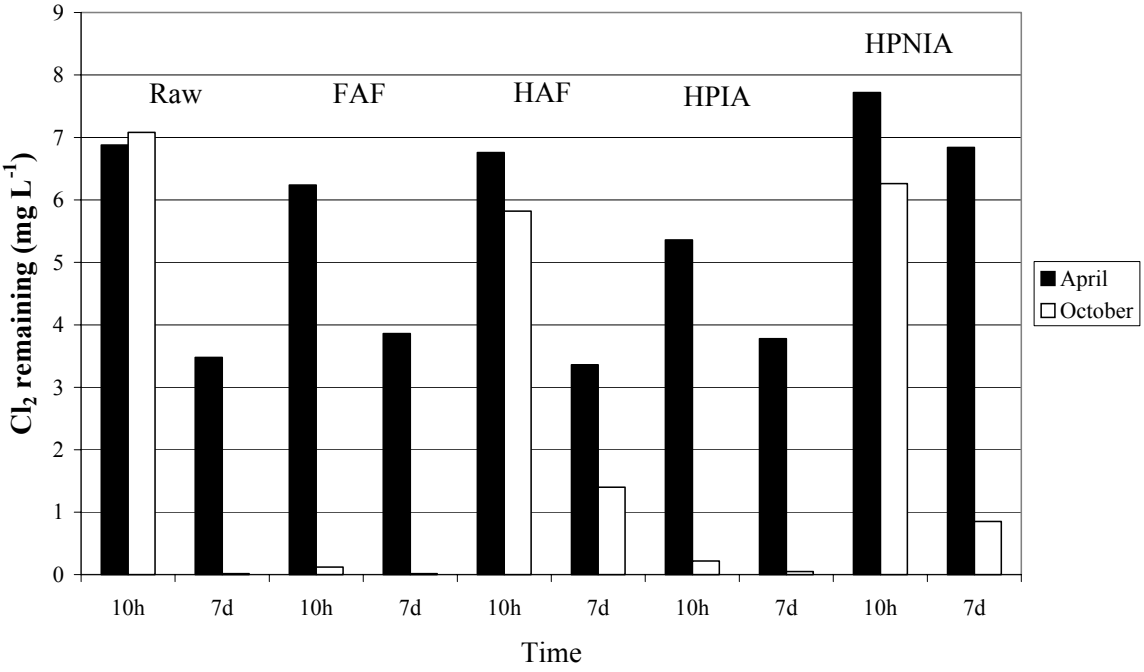


Figure 5.41 Chlorine remaining after 10 hours and 7 days

Generally DBP formation occurs at a fast initial rate followed by a slower rate of formation that often continues for days into the presence of residual chlorine (Chowdhury and Amy 1999). The initial chlorine consumption of the October samples was not very high for the raw water, HAF and HPINA (5.8 – 7.1 mg L^{-1} Cl_2 remaining) but for the FAF and HPIA, the demand was such that almost 100% of the chlorine had been used after 10 hours. The initial chlorine demand for the April fractions was slightly higher for the HPIA (5.4 mg L^{-1} Cl_2 remaining) and FAF (6.2 mg L^{-1} Cl_2 remaining) compared with the other samples that had a similar chlorine demand (6.8 to 7.7 mg L^{-1} Cl_2 remaining). The final chlorine demand was similar for all the April samples (3.4 – 3.9 mg L^{-1} Cl_2 remaining) with the exception of the HPINA that had 6.8 mg L^{-1} Cl_2 remaining. All the October samples had less than 0.5 mg L^{-1} Cl_2 remaining with the exception of the HAF which had 1.4 mg L^{-1} Cl_2 left. It is clear that the October

samples are exerting a higher chlorine demand overall than the April samples. It has been report that average chlorine demands for humic, fulvic and hydrophilic acids were 2.8, 2.0 and 1.7 mg Cl_2 mg^{-1} C respectively (Croué *et al*. 1999) after 3 days chlorination. After 7 days the order was HAF>HPIA>FAF for the April fractions with the fraction with the highest chlorine demand listed first. In October the order was FAF>HPIA>HAF.

Table 5.28 THM-FP (μg mg^{-1} C) of April and October Raw water and fractions

	April		October	
	10 hours	7 days	10 hours	7 days
Raw	32.8	80.3	73.5	49.2
HAF	36.3	32.0	62.2	126.3
FAF	25.7	82.0	51.4	65.5
HPIA	20.1	24.3	15.2	21.9
HPINA	3.7	9.0	9.7	31.1

The THM-FP was plotted against the chlorine remaining for each month but there was no correlation found (graph not shown). This agrees with Croué *et al*. (1999) who also found no correlation between chlorine demand and THM-FP.

The fraction distribution for the raw water from April and October 2002 is almost identical. The chlorine demand of the October raw water and fractions is much greater than for the corresponding April raw water and fractions. It is thought that the October raw water and fractions are more reactive with chlorine than in April. This is reflected in the THM-FP values for the HAF and HPINA that are higher for October than for April. The THM-FP values for the raw water, FAF and HPIA are all lower in October compared with April. It has been hypothesised that two distinct types of reactive functionalities exist in NOM resulting in two parallel reactions forming halogenated

organic by-products (Gang *et al*. 2002). One NOM functionality, possibly attributed to aldehyde and phenolic hydroxyl groups results in a rapid rate of chlorine consumption. The other NOM functionality is less reactive such as that expected for activated double bonds and methyl groups and results in a slow long-term chlorine demand. The different proportion of these functionalities present in the October and April samples could account for the differences in reactivities seen here.

To investigate the chlorine-NOM reaction further, the fluorescence spectra of the chlorinated samples were studied.

5.3.6 Section conclusions

By looking at different methods of analysis, it was possible to determine which methods would be of practical use to a WTW operator. Capillary Electrophoresis was not found to be useful as concentrated samples were required and the information gleaned was not of any practical use. Analysis of fractions by fluorescence showed that a synchronous scan gave useful information compared to a single emission scan. It was confirmed that for these samples, the position of the fulvic-like peak on the emission axis was linked to the MW of the molecules. HPSEC analysis was useful for determining the size of molecules that had been removed by the treatment process. It was thought that HPSEC would be the most useful technique for optimising removal of NOM at a WTW.

Further analysis of the fractions has allowed some insight into the seasonal variation of the bulk waters and fractions. These are single time points taken only up to three times a year that provide a ‘snapshot’ of the water character at each time point. It would be

useful to look at samples regularly over a period of months to determine a link between character and reactivity.

5.4 Tracking changes in character over time

In order to track the changes in the raw water quality in Albert Reservoir, samples were taken approximately fortnightly between 17th September 2002 and 2nd April 2003. This was undertaken to determine how the character of the water changed from month to month rather than taking a single 'snapshot' during each season.

5.4.1 SUVA

The DOC and UV of each sample was measured and SUVA calculated (figure 5.42). Over this time period, the SUVA varies between 3.97 and 6.04. According to the guidelines set out by Edzwald and Tobiason (1999) the water contains mostly aquatic humics, has high hydrophobicity and a high overall molecular weight. The guidelines dictate water character when SUVA is >4, 2 – 4 and <2 and are not detailed enough to determine any changes in character over this period.

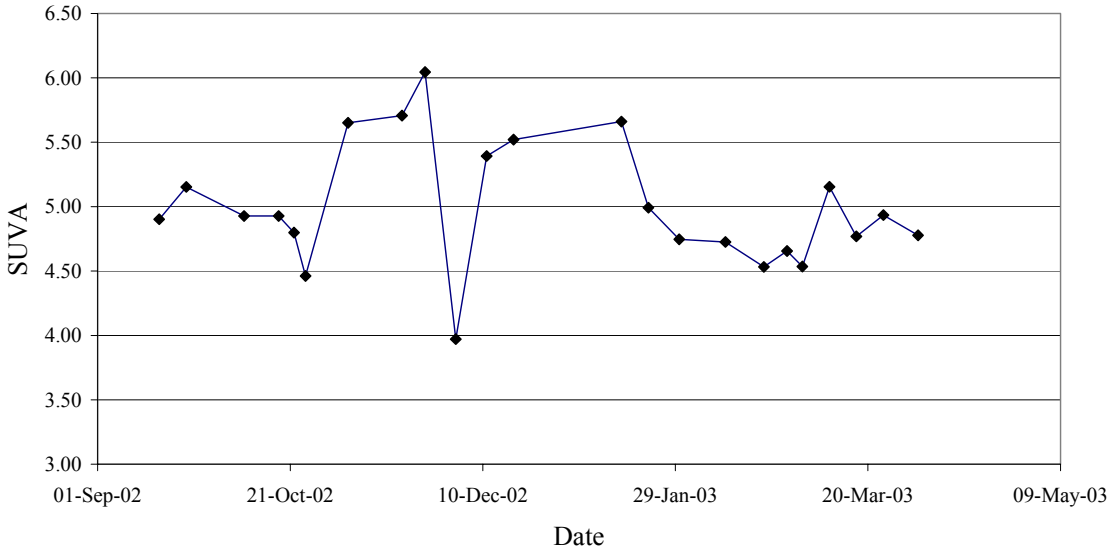


Figure 5.42 Change in SUVA (m^{-1} .L mg^{-1}) over time

5.4.2 Fluorescence Spectroscopy

Recently a method has been proposed by Marhaba *et al.* (2000) that can rapidly identify organic matter fraction concentrations in a water using Spectral Fluorescent Signatures. Here, a study was carried out to investigate the use of fluorescence spectroscopy as a technique for rapidly determining the concentration of fractions in raw water as an alternative to the traditional resin separation technique. The Spectral Fluorescent Signatures (SFS) technique (Marhaba *et al.* 2000) was investigated before developing the method described here.

5.4.2.1 Sampling and sample treatment

Water samples (raw water) were collected from the reservoir inlet at Albert WTW in the Yorkshire Water region in the UK in November 2000, November 2001 and April 2002. Water samples were collected from the reservoir inlet at Rivington WTW in the United Utilities Water region in the UK in February 2001. The parameters of each raw water are summarised (table 5.29).

Table 5.29 Raw Water Parameters

Sample	pH	DOC (mg L^{-1})	UV (m^{-1})	SUVA (m^{-1}.L mg^{-1} C)	THM (μg L^{-1})
Albert raw water November 2000	5.0	10.2	60.2	5.9	907.5
Albert raw water November 2001	5.2	9.9	48.0	4.9	563.8
Albert raw water April 2002	5.9	7.5	38.1	5.1	126.8
Rivington raw water February 2001	7.1	6.1	24.6	3.7	143.5

The chlorination conditions for Albert raw water November 2001 and 2002 and Rivington raw water 2001 were 5 mg Cl_2 mg^{-1} C and for Albert raw water April 2002 was 10 mg Cl_2 mg^{-1} C. The raw waters were fractionated using XAD resins into FAF, HAF, HPIA and HPINA using the methods described earlier (section 4.2).

The raw water fractions from Albert reservoir (November 2000) were diluted to concentrations of 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mg L^{-1} (as well as 0.5 and 0.7 mg L^{-1} for the FAF) using deionised water. The pH of each sample was adjusted to 7. A synchronous scan was carried out on each sample at each dilution using a fluorescence spectrophotometer.

5.4.2.2 Model Development

When studying fluorescence spectra, the position of peaks on the contour plot that exhibit maximum intensity values are investigated. Typically, a contour plot for a NOM sample will exhibit two main peaks. The position of these peaks gives information on the types of molecules present. Marhaba *et al.* (2000) found that the areas of maximum intensity of fluorescence were shown to be representative of the concentration of each fraction. The region of the contour plots investigated in the SFS technique was maximum intensity values in the 225 – 249 nm excitation region (figure 5.43). Figure 5.43 is a collation of maximum intensity values reported in the literature, as well as maximum intensity values found in this study, shown as a plot of emission wavelength against excitation wavelength.

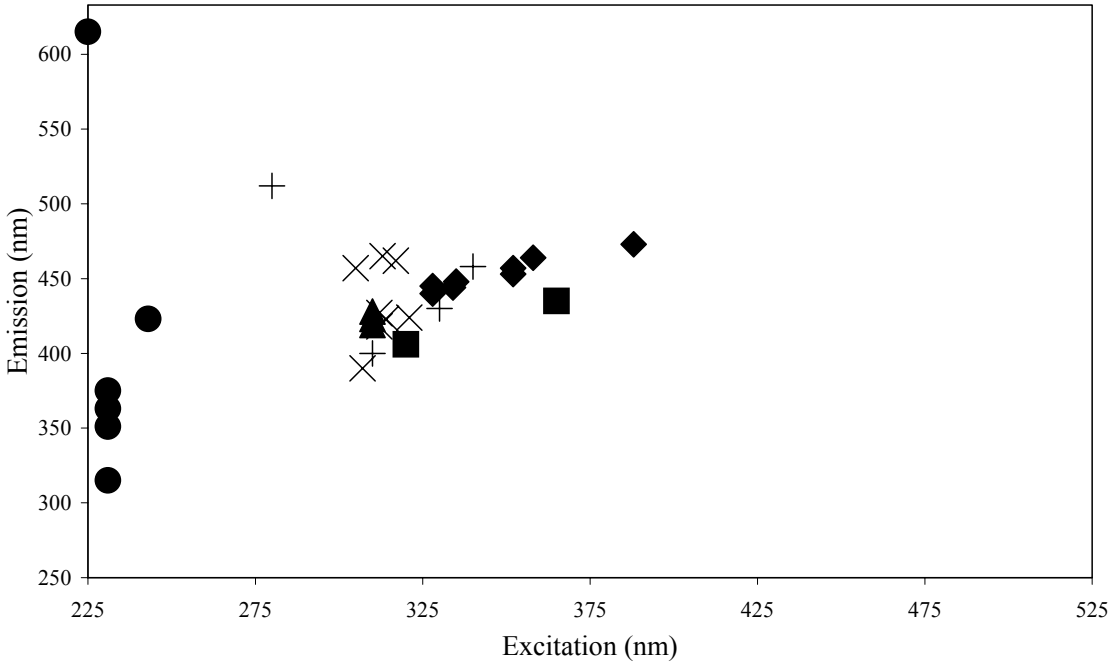


Figure 5.43 Fraction excitation and emission wavelength maxima

Key

Coble (1996) ▲, Marhaba *et al.* (2000) ●, Alberts *et al.* (2000) +, Miano and Alberts (1999) ◆, McKnight *et al.* (2001) ■, this work ×

Another study has reported two intensity maxima observed in each EEM (Coble 1996). One from excitation at the shortest wavelength used (in this case ~260 nm) and another from excitation in the region 300 – 370 nm. The author found it difficult to determine the actual position and shape of the first maxima as the observations only extended to 260 nm. This peak on the edge of the EEM was disregarded (Coble 1996).

It is this 'on the edge' peak at the limit of observation that Marhaba *et al.* (2000) proposed as being representative of the organic matter in the sample. In a different study the two peaks observed were referred to as fluorescent pairs that were shown on the EEM as a bimodal peak distribution (Alberts *et al.* 2000). In that study the second of the pairs was again at the edge of the observed excitation range. The authors

simplified the spectra by taking the second derivative of the entire spectra to create four peaks. The chemical identity of this peak has been reported as unknown but typical for aquatic NOM (Blaser *et al.* 1999). Another study attributes the region of fluorescence at 230-280/310-420 (excitation/emission (nm)) to a single fluorophore such as a protein although it is acknowledged that fluorescence at this excitation wavelength is poorly understood (Baker and Lamont-Black 2001). In the literature, the peaks observed at the limit of observation have been either disregarded or derived to create new data. Only Marhaba *et al.* (2000) have quantitatively used the data as it stands.

EEMs were produced for all the fractions at varying concentrations for the Albert Reservoir water taken in November 2000. The data from each synchronous scan was saved in a database. Each contour plot exhibited at least two major areas of fluorescence. As with the SFS technique (Marhaba *et al.* 2000), the region of highest intensity of fluorescence was taken as being representative of each fraction. However, it was found that the information contained in this area could not help to determine fraction concentration, as the peak was too close to the edge of the observations made.

The region of fulvic-like fluorescence (excitation 300-350 nm/emission 300-500 nm) was then taken as being representative of each fraction. It was found through trial and error that the emission spectrum of raw water at the excitation wavelength of 311 nm was approximately equal to the sum of the emission spectra of each of the fractions at 311 nm given that one fraction had a predominant concentration. 311 nm was the average excitation wavelength for the second maxima of all the fractions measured. The predominant fraction was determined by resin fractionation (table 5.30).

Table 5.30 Real vs predicted (pred.) DOC (mg L^{-1}) values of natural samples

	FAF		HAF		HPIA		HPINA	
Sample	Real	Pred.	Real	Pred.	Real	Pred.	Real	Pred.
Albert raw water November 2000	6.2	6.2	1.8	1.5	0.8	0.9	1.3	1.5
Albert raw water November 2001	4.2	4.4	2.4	2.4	1.7	1.1	1.7	2.1
Albert raw water April 2002	3.9	3.3	1.5	1.7	0.9	0.9	1.2	1.7
Rivington raw water February 2001	1.4	0.4	2.6	4.3	0.4	0.4	1.7	1.0

A Microsoft Excel macro was created to process the data. The steps followed by the macro are illustrated in figure 5.44.

Step 1: Once a synchronous scan of the raw water was carried out, the emission spectra at excitation = 311 nm was extracted.

Step 2: The predominant fraction spectra (FAF) at eight different concentrations was compared against figure 5.44 (a) until the concentration that matched the raw water spectra most closely was found (figure 5.44 (b)). The closest spectra was where the squared difference between the spectra was closest to zero.

Step 3: Now the concentration of the predominant fraction was established. To determine the concentration of the remaining three fractions, the spectra for the remaining fraction at each concentration were added to it in all possible combinations until a combination was found that was closest to the raw water spectra (figure 5.44 (c)).

As there are three remaining fractions at six different concentrations, there are 6^3 combinations.

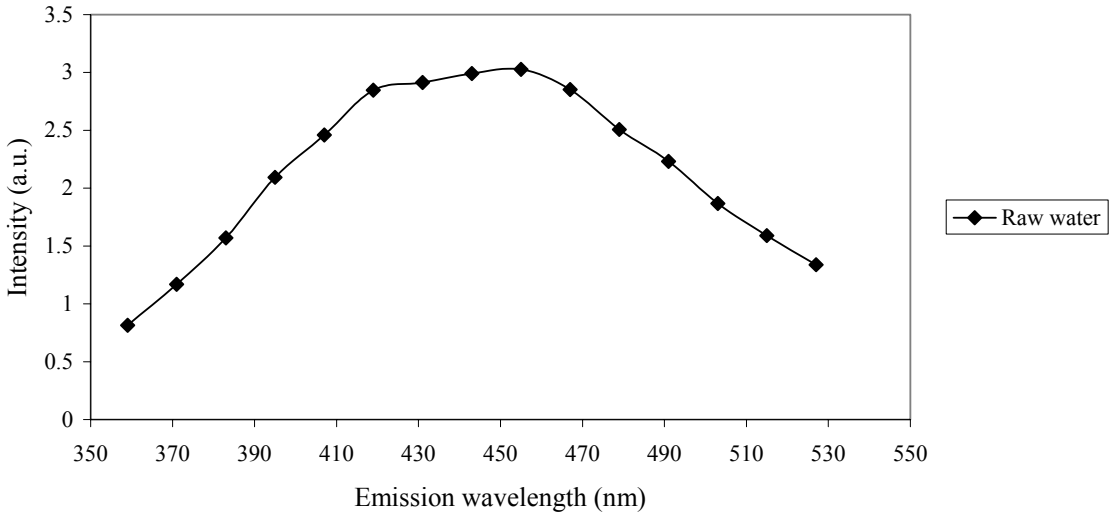


Figure 5.44 (a)Raw water emission spectra at excitation = 311 nm

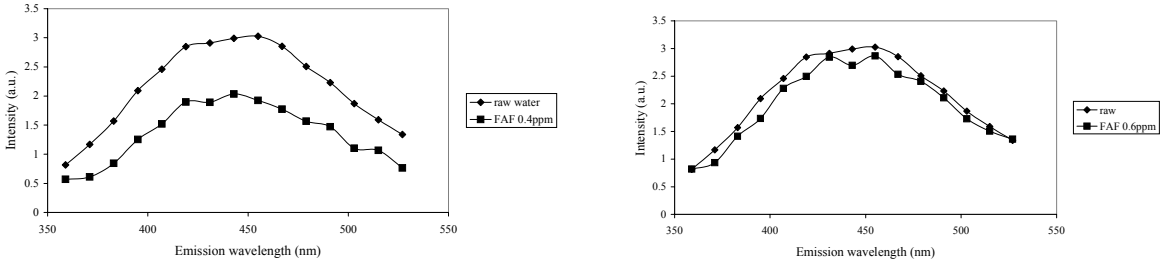


Figure 5.44 (b) Comparison of raw water emission spectra with predominant fraction emission spectra (excitation = 311 nm) at different concentrations

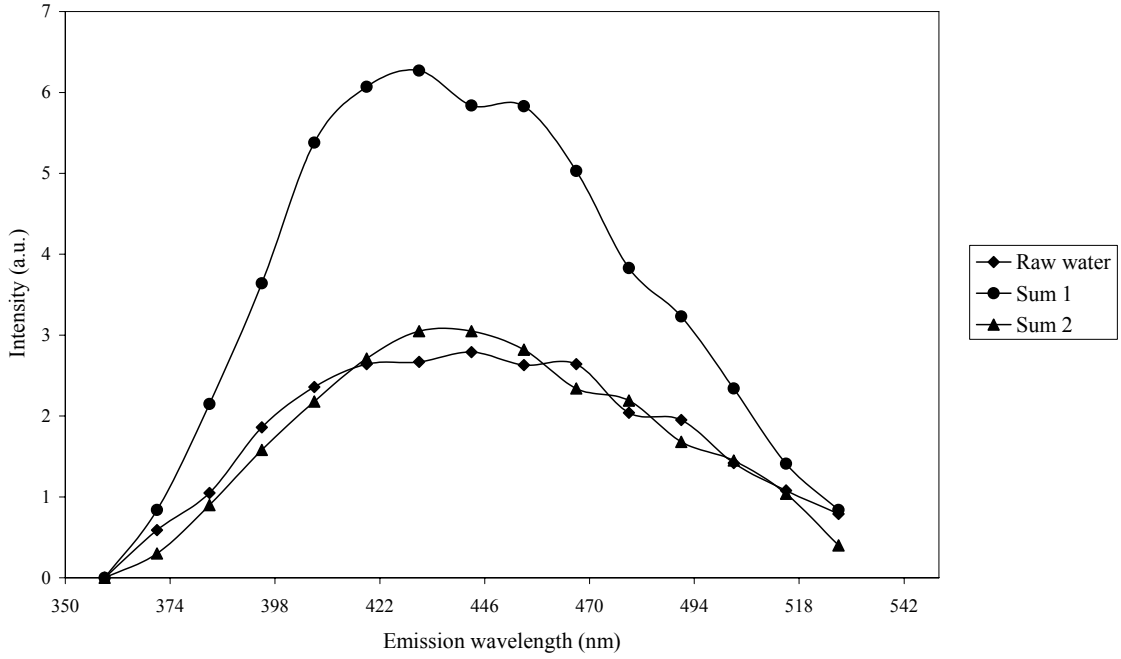


Figure 5.44 (c) Comparison of summed fraction emission spectra with raw water emission spectra (excitation = 311 nm)

It seems unlikely that the fluorescent behaviour of the individual fractions will be the same as the bulk water because NOM is known to associate with itself in aqueous solution (Leenheer *et al*. 1989). On separation of the NOM into its component fractions, the synergistic effects are lost and the NOM is altered. The localised electron

environment will be different for the bulk water compared to the isolated fractions. However, it has been reported that spectra of reference Suwannee River NOM and Suwannee River Fulvic Acid were found to be almost identical (Ahmad *et al*. 2002). In this case it was assumed that the Suwannee River NOM consisted largely of fulvic acid.

5.4.2.3 Initial Testing of Model on Synthetic Waters

The model was tested on Albert raw water sampled in November 2000. The results are shown in a table that compares the real values as determined from the resin fractionation with the values predicted using the model (table 5.31). A close match was expected as the fractions from this water were used to produce the fraction data for the database.

The model was also tested on samples A to C that were artificially created from solutions of the isolated fractions. Theses synthetic waters were made to test the model on HAF and FAF rich waters. Table 5.31 shows the real proportion of each fraction in the samples for analysis compared to the fraction concentrations predicted using the model.

Table 5.31 Real vs predicted proportions (%) of synthetic water samples

	FAF (%)		HAF (%)		HPIA (%)		HPINA (%)	
Sample	Real	Pred.	Real	Pred.	Real	Pred.	Real	Pred.
A	61	58	18	19	8	8	13	14
B	13	11	65	61	9	10	14	18
C	79	70	4	9	4	7	13	14

With sample A, the predicted results are within ± 3 % of the 'real values' for all fractions. The predicted results were expected to be very close to the real results as sample A was made with the same proportions as the November raw water (2000) that

was used to create the database. With sample B, the predicted results are within ± 4 % of the real values. With sample B, the predominant fraction is the HAF. This shows that even when the predominant fraction is changed, an accurate result is achieved. Sample C is accurate to within ± 9 %. With sample C, the FAF concentration was underestimated by the model thus leading to an overestimation of the other fraction concentrations.

Synthetic waters with a hydrophilic fraction as the principal fraction were not made as the water from Albert Reservoir has been shown to be mainly hydrophobic throughout the year (section 5.2). It is also thought that the prediction of the HPINA concentration may not be accurate due to its lower intensity of fluorescence when compared with the more fluorescent fractions (FAF, HAF and HPIA).

5.4.2.4 Testing of Model on Natural Waters

The model was tested on Albert raw water taken in November 2001. The results are shown in a table (table 5.30) that compares the real values as determined from the resin fractionation with the values predicted using the model.

The results from the model are very close to the results determined by resin fractionation for the FAF and HAF (within ± 2%) but are less accurate for the more hydrophilic fractions (within ± 6%). This could be attributed to the lower intensity of fluorescence exhibited by the HPINA. The model was tested further on Albert raw water, this time taken in April 2002. The results are shown (table 5.30). The results here are less accurate (within ± 8%) than observed with the water sampled in November. It has been reported that the position of the excitation and emission maxima will change seasonally (Baker and Lamont-Black 2001). This could account for the less

accurate results found when analysing water from April with a database set up using November water.

5.2.4.5 Use of the Model on Other Watersheds

The model was tested on raw water from Rivington WTW in the North West of England. The results are shown (table 5.30). With an error of >25% for the HAF, the results show that the model is unable to predict the concentration of fractions for a water that has not been used to create the fraction database. Therefore, for the model to work on a different water, a fraction database for that water would need to be set up. This would involve resin fractionation followed by a synchronous scan of each fraction at varying concentrations to produce the fraction database.

5.4.2.6 Determining Albert character over time

The model was used on raw water samples taken approximately fortnightly from Albert WTW from 17th September 2002 to 2nd April 2003. The findings are presented (figure 5.45). It can be seen that there are elevated levels of modelled FAF between the 18th December 2002 and the 3rd March 2003. It is thought that elevated levels of modelled FAF in the raw water will indicate an increase in the reactivity of the water with respect to THM-FP. It would be possible to determine the increase in FAF on-site at Albert WTW. Treatment could then be adapted to optimise removal of the organics based on the proportion of each fraction present rather than the bulk water characteristics.

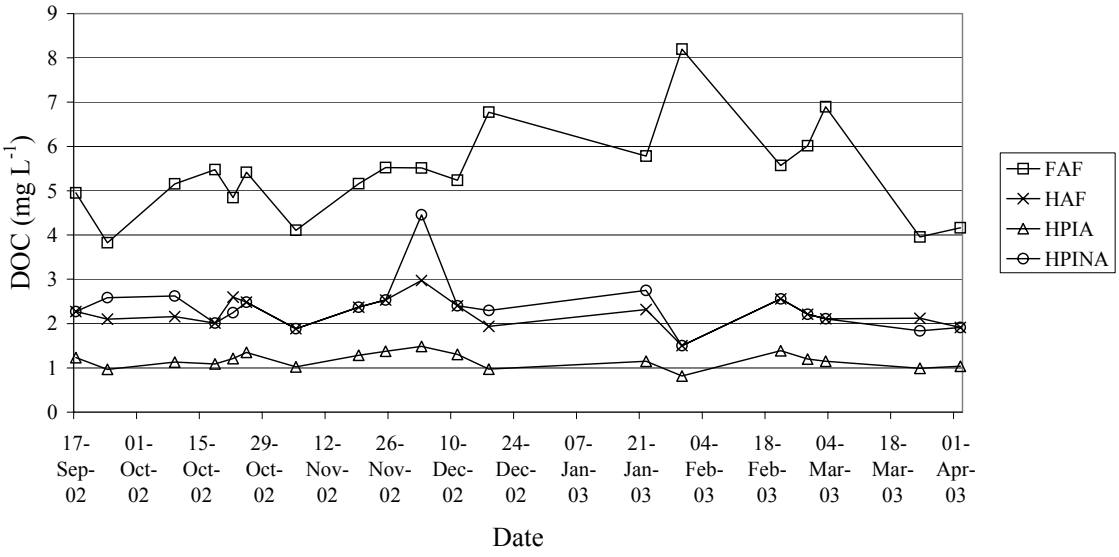


Figure 5.45 Change in modelled fraction distribution over time

5.4.2.7 Fluorescence model conclusions

- The model described here can be used for a specific site to rapidly determine changes in the DOC distribution in a raw water that has previously been fractionated and characterised.
- The model contains a database of fractions at different concentrations for comparison with a raw water sample.
- Compared with resin fractionation that can take many days to determine fraction distribution, this model can determine fraction distribution within an hour (once the model has been set up for a particular water).

5.4.3 Fluorescence Index (FI)

Dissolved organic matter is said to be (1) microbially derived (autochthonous), resulting from such processes as extracellular release and leachate of algae and bacteria and (2) terrestrially derived (allochthonous), originating from decomposition and leaching of

plant and soil organic matter (McKnight *et al*. 2001). It is possible to identify the origin of fulvic acid in a water by measuring the ratio of emission intensity at 450 nm to 500 nm given that the wavelength of excitation is 370 nm (McKnight *et al*. 2001). The change in FI of Albert raw water over time was determined (figure 5.46). The cutoff at FI = 1.5 was suggested by Nguyen *et al.* (2002).

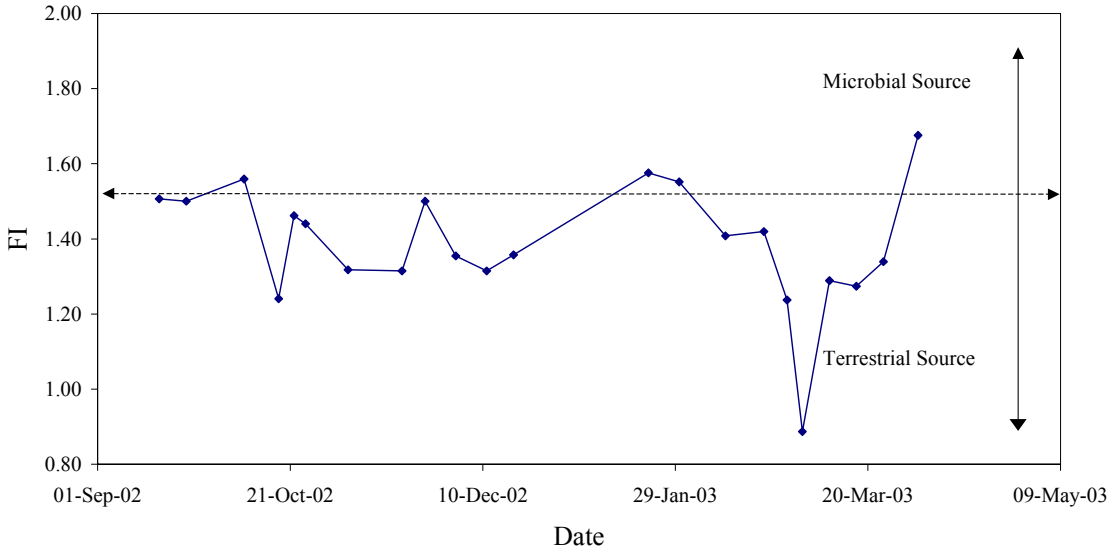


Figure 5.46 Change in FI over time

Over winter the FAF in the water is mainly terrestrially derived according to the FI but appears to become more microbially derived towards spring-time. This could be as a result of increased temperatures at that time of year.

5.4.4 HPSEC

The samples were run also through the HPSEC. The results are shown (figure 5.47). It was thought that samples that exhibited a higher UV absorbance may be more reactive with chlorine with respect to THM-FP. However, there was not enough THM-FP available data to determine a link between the peak characteristics (height and area) and the reactivity of the treated samples.

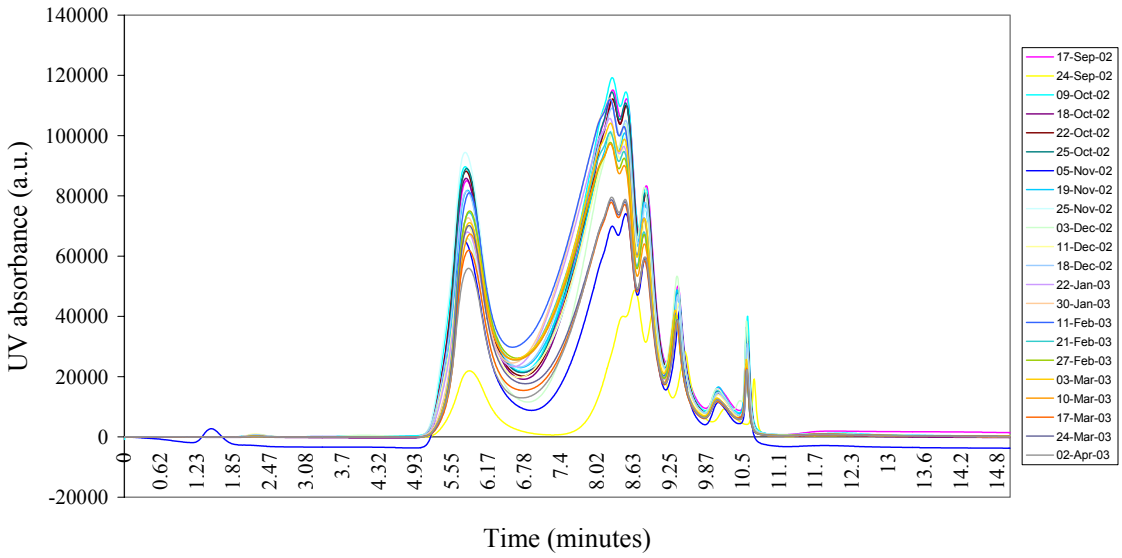


Figure 5.47 HPSEC analysis over time

5.4.5 Section conclusions

Although SUVA and HPSEC give limited information on the changing character of Albert raw water over time, the fluorescence model has shown the variation in fraction distribution over time. It was possible to model how the character of the water changed over time. The fraction distribution was more variable than first thought but it was modeled that FAF levels were elevated for a period of time during the winter. From the fluorescence model, samples that have a high FAF content were identified. It was

decided to investigate two samples that exhibited very different FAF contents to determine any link between character and reactivity. The analysis of the two samples is presented in the next section.

5.5 Relationship between character and reactivity – a study of two samples

5.5.1 Selection of samples

In the period from 24/12/02 to 04/03/03 elevated levels of FAF were observed in the raw water. Previously, it has been shown that raw water with high levels of FAF are more reactive than raw water with low levels of FAF (Section 5.2). As the model is intended to give an idea of when the water becomes more reactive, specific samples were selected to test this (figure 5.48).

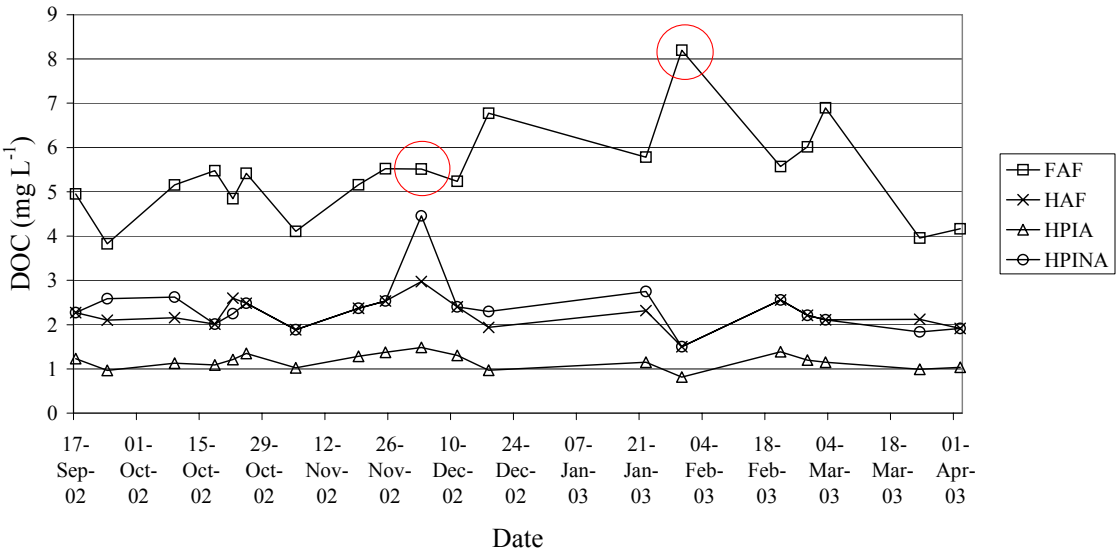


Figure 5.48 Change in fraction distribution over time with selected samples highlighted

The samples containing the lowest and highest proportion of FAF (3rd December 2002 and 30th January 2003) were selected. Their fraction distribution is reported (table 5.32).

Table 5.32 % of each fraction present in raw water according to model (mass C in mg L^{-1})

Sample	FAF	HAF	HPIA	HPINA
03/12/02	38.2 (5.5)	20.6 (3.0)	10.3 (1.5)	30.9 (4.5)
30/01/03	68.2 (8.2)	12.5 (1.5)	6.8 (0.8)	12.5 (1.5)

5.5.2 Reactivity of selected samples

The selected samples were diluted to ~1 mg L^{-1} chlorinated with a dose of 10 mg L^{-1} Cl_2 mg^{-1} C and their THM-FP measured (table 5.33). The chlorinated samples were quenched after 10 hours and 7 days and their fluorescence spectra were run to determine any differences from the unchlorinated samples.

Table 5.33 Sample DOC, SUVA and THM-FP

Sample	DOC (mg L^{-1})	SUVA (m^{-1} .L mg^{-1} C)	Time	THM-FP (μg mg^{-1} C)
03/12/02	14.4	4.0	10 hours	21.5
			7 days	142.5
30/01/03	12.0	4.7	10 hours	13.6
			7 days	60.6

It was expected that the January 2003 raw water would be more reactive than the December 2002 raw water. However, the opposite was found to be true. The THM-FP for the January 2003 raw water was greater than twice that of the December 2002 raw water (table 5.33).

5.5.3 Fluorescence spectra of chlorinated samples

The data obtained by running the fluorescence spectra of the raw water samples and the chlorinated raw water samples is shown (table 5.34 and figure 5.49).

Table 5.34 Data obtained from Fluorescence Spectra

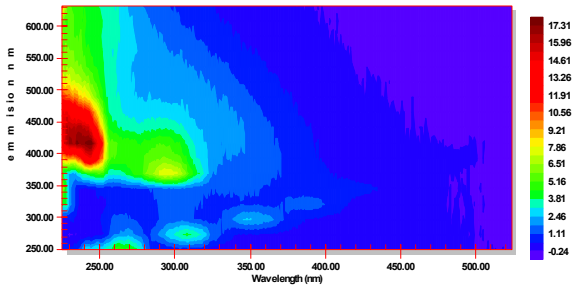
Sample	Time	Peak position (ex/em (nm))	Intensity
03/12/02	0 hours	293, 378	8.1
	10 hours	315, 393	1.5
	7 days	297, 349	1.1
30/01/03	0 hours	325, 448	4.6
	10 hours	316, 397	2.0
	7 days	311, 392	1.3

Two types of data were obtained: the most intense peak position in the fulvic or protein like region and the intensity of that peak. NOM fluorescence is strongly affected by the molecular weight of the molecules. As the average molecular weight decreases, the position of the maximum in the emission spectra shifts to lower wavelengths (blue shift) (Croué *et al*. 2000). The relationships regarding the intensity of fluorescence (I_F) and NOM are less straightforward. It is known that hydrophilic (XAD-4) acids have a higher I_F than hydrophobic (XAD-8) acids. The higher I_F for XAD-4 acids is almost certainly related to its lower molecular weight (compared to XAD-8) which decreases the rate of radiationless losses of excitation (Croué *et al*. 2000). That is, the energy transfer in small molecules is more efficient than in large molecules and small molecules will fluoresce more strongly per mg of carbon. This, however, will not be relevant for samples that have been altered by chlorine.

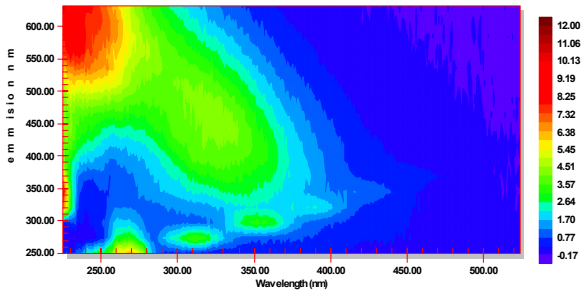
Before chlorination, it can be seen that the average MW is higher in the January sample compared with December. This is supported by the intensity of fluorescence (I_F) exhibited by the samples. The lower IF observed in January compared with December would be consistent with the smaller molecules in December fluorescing more efficiently than the larger molecules observed in January per mg carbon.

The differing size of the molecules in each sample could also explain the difference observed in chlorine demand. As previously mentioned, it has been hypothesised (Luong *et al*. 1982) that when chlorinating humic substances, some chlorine is initially expended in 'activation' of the humic structure through oxidation reactions to produce active sites followed by substitution reactions with chlorine. The sample from January has larger molecules that may require more chlorine for the partial oxidation of the molecules.

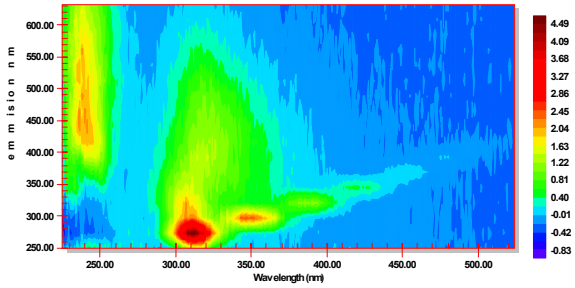
It is not possible to say whether the change in fluorescent behaviour upon chlorination of the NOM samples is due to a structural change in the NOM molecules or due to the change in the local environment as a result of the addition of chlorine.



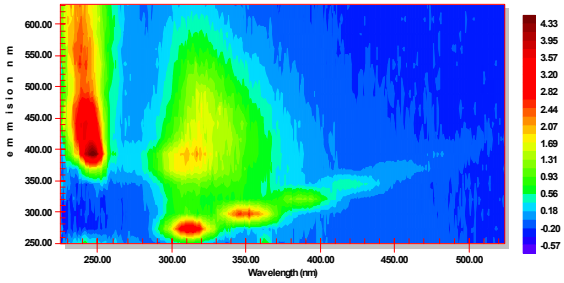
5.49 (a)



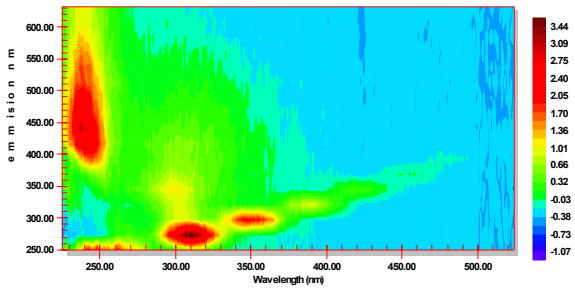
5.49 (b)



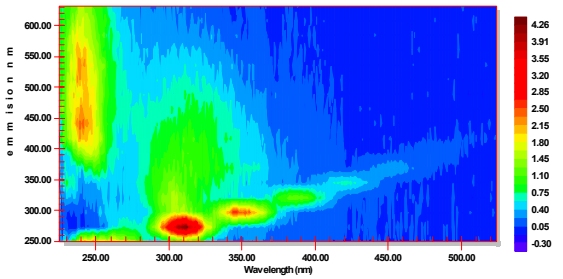
5.49 (c)



5.49 (d)



5.49 (e)



5.49 (f)

Figure 5.49 (a) December 2002 spectra (0 hours), (b) January 2003 spectra (0 hours), (c) December 2002 spectra (10 hours), (d) January 2003 spectra (10 hours), (e) December 2002 spectra (7 days), (f) January 2003 spectra (7 days)

5.5.4 Comparison with other 'unconventional waters'

Although the behaviour exhibited by these samples was unexpected based on previous analyses of this water, it is not unheard of. A study by Bolto *et al*. (2002) looked at a range of waters that were fractionated using resins to determine the hydrophobic/hydrophilic split and carried out THM-FP analyses on the individual fractions as well as the bulk water. They found that high THM-FPs did not necessarily result from waters with the highest proportion of hydrophobic compounds. They also found that some waters of high colour had amongst the lowest THM-FP and that eutrophic waters were very reactive with most of the THMs formed from the hydrophobic fractions. A eutrophic water is one with an abundant supply of nutrients and a high rate of formation of organic matter by photosynthesis. In the study, each water sample was analysed at one time point. There was no investigation into seasonality.

White *et al*. (2003) found that NOM contributing to DBPs were primarily phenolic compounds. Unlike other studies, no correlation was found between aliphatic compounds in the raw waters and DBP-FP. The study also explored the use of SUVA as a DBP-FP predictor. SUVA is a only a measure of the aromaticity of the NOM, so its success at predicting total THM depends strongly on the relative abundance of non-aromatic, chlorine-reactive and non-reactive components of NOM. Compounds such as transphilic acids and other hydrophilic acids are known to be chlorine reactive but would not be included in a UV_{254} measurement. The presence of non-reactive compounds in NOM were equally important for identifying the likelihood of NOM to be enriched in DBP precursors (White *et al*. 2003). Therefore, reactive and non-reactive species can have an effect on DBP-FP.

5.5.5 Section conclusions

It is known that the formation of DBPs is very complex. Here we have tried to predict the reactivity of a raw water in terms of THM-FP by looking at the DOC make-up. However, it was found that the THM-FP was independent of the DOC make-up but may be related to the relative size of the fulvic-like molecules determined by fluorescence. The use of fluorescence as a tool for understanding chlorine-NOM reactions requires more research to determine its applicability.

CHAPTER 6 REAL OPTIONS

6.1 Outline of problem

At Albert WTW the quality of the water in the reservoir has been deteriorating. The colour of the water has increased over the last 10 years making the water more difficult and more expensive to treat (figure 6.1).

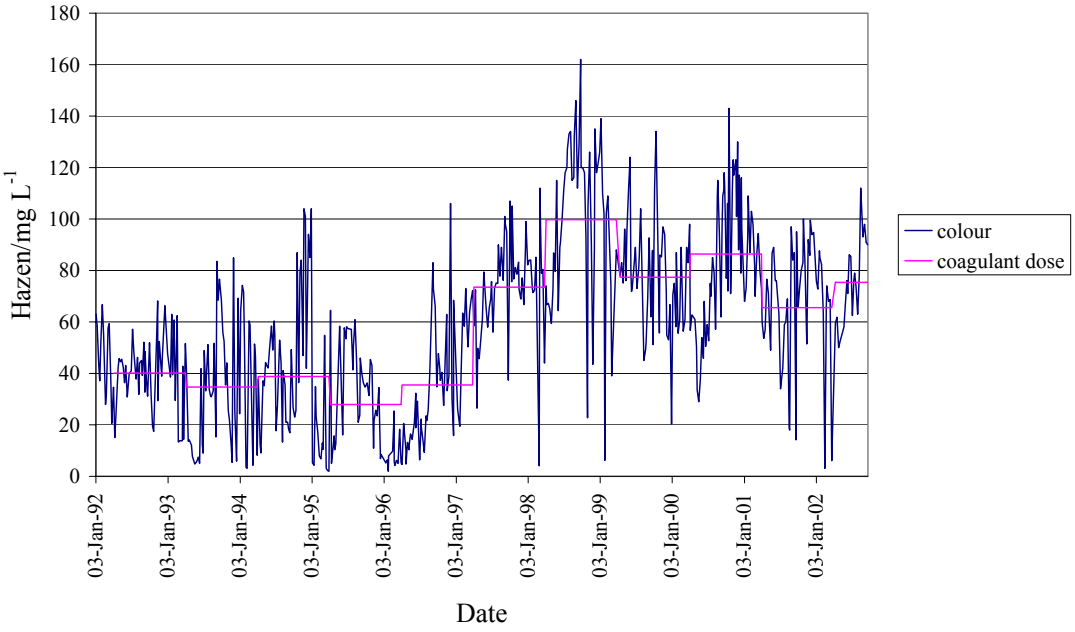


Figure 6.1 Increase in colour over 10 years

It is likely that this increase in colour and natural organic matter (NOM) in the water will continue. The majority of the NOM in the water is removed by treatment using an inorganic coagulant and in the case of Albert WTW, a ferric sulphate compound. When the water is chlorinated for disinfection before distribution, the remaining NOM in the water reacts with the chlorine to form disinfection by-products (DBPs). One type of DBP, trihalomethanes (THMs), are regulated. The standard for THMs in the UK

changes from a three-month average of 100 µg L^{-1} to a limit of 100 µg L^{-1} for a single sample in December 2003 (New Drinking Water Regulations in the UK, 1998, www.dwi.gov.uk). The levels of THMs in the finished drinking water is close to the limit.

As the organic level at Albert WTW has increased, the amount of coagulant required to treat the water has increased from 40 to ~80 mg L^{-1} of Ferripol XL (contains 13.75% ferric sulphate). This in turn has led to an increase in sludge production and sludge disposal costs. It is clear that investment will be required, at Albert WTW, at some point in the future to continue to meet the THM regulations and minimise treatment costs. 'Discounted cash flow' methods can be used to determine whether an investment should be made on a financial basis but here it is proposed that a 'Real Options' approach should be used as it can take into account the value of delaying an investment. The Real Options approach will be used to determine when and how to invest at Albert.

6.1.1 Investment possibilities

There are three options that will be considered here. Their costs and benefits are outlined (table 6.1).

Table 6.1 Investment options

Option	Action	Costs	Benefits
1	Do nothing	Consents not being met, enhanced coagulation costs	No investment costs
2	Invest in new plant to implement double coagulation	New plant, additional sludge costs	Consents being met
3	Invest in new plant to implement MIEX treatment	New plant, disposal of brine	Consents being met

Options 2 and 3 will be considered in 1 year and in 5 years to determine when they might best be implemented.

6.2 Real Options

6.2.1 Theory

An investment is said to be worth undertaking if it creates value for a company and enhances shareholder value. A net present value (NPV) calculation is carried out to determine the value an investment creates. NPV is the difference between the present value of future cash flows and the cost of the investment. This procedure is known as discounted cash flow (DCF). Generally, if the NPV of a project is positive, the investment will go ahead. If the NPV is negative, the investment will not be made. This implies that an investment will be made now or never (Coff and Laverty 2001). It should be noted that the decision to reject or accept a project on the basis of its NPV will be subject to a sensitivity analysis to reflect variability in future returns and capital/operating costs.

Many observers of managerial processes have come to the conclusion that DCF methods lead to a damaging neglect of long term and strategic investments (Laughton and Jacoby 1993). Real Options are used as an alternative to financial options because they involve real, rather than financial, assets.

6.2.2 Option Pricing

An option is defined as a contract that gives its holder the right to buy or sell an asset at some predetermined price within a specific period of time. The following example (Brigham and Ehrhardt 2002) explains the terms used in option pricing theory. Suppose you owned 100 shares of ABC corporation, which on the 1st of March 2002, sold for £50 per share. You could sell to someone the right to buy your shares at any time during the next four months at a price of, for example, £55 per share. The £55 is called the **strike** or **exercise price**. Such options exist and are traded on a number of exchanges. This type of option is defined as a **call option** because the buyer has a 'call' on 100 shares of stock. You can also buy an option that gives you the right to sell a stock at a specified price within some future period. This is called a **put option**.

A widely used option pricing formula is the Black-Scholes model. In the development of the model the following assumptions were made:

1. The stock underlying the call option provides no dividends or other distributions during the life of the option.
2. There are no transaction costs for buying or selling either the stock or the option.
3. The short-term, risk free interest rate is known and is constant during the life of the option.

4. Any purchaser of a security may borrow any fraction of the purchase price at the short-term risk free interest rate.
5. Short selling is permitted and the short seller will receive immediately the full cash proceeds of today's price for a security sold short.
6. The call option can be exercised only on its expiration date.
7. Trading in all securities takes place continuously and the stock price moves randomly.

The model can be used to value any asset that has the characteristics of an option and thus can be applied to real option situations provided the limitations of the model are realised and that these reduce the accuracy of the model when applied to non traded assets.

$$V = P[N(d_1)] - Xe^{-Rt}[N(d_2)] \qquad \text{Equation 6.1}$$

$$d_1 = \frac{\ln(P \div X) + [R + (\sigma^2 \div 2)]t}{\sigma\sqrt{t}} \qquad \text{Equation 6.2}$$

$$d_2 = d_1 - \sigma\sqrt{t} \qquad \text{Equation 6.3}$$

V = current value of the call option, P = current price of the underlying stock, $N(d_i)$ = probability that a deviation less than d_i will occur in a standard normal distribution, X = strike or exercise price of the option, e = ~2.7183, R = riskless rate of interest, t = time until the option expires, σ^2 = variance of rate of return on the stock.

The value of the option is a function of the variables discussed earlier:

1. P, the stock's price
2. t, the option's time to expiration
3. X, the strike price
4. σ^2, the variance of the underlying stock
5. R, the risk free rate

Using the Black-Scholes model, if the option price is different from the one found by equation 6.1, this would provide an opportunity for arbitrage profits which would force the option price back to the value indicated by the model. The first term of equation 6.1, $P[Nd_1]$, can be thought of as the expected present value of the terminal stock price given that P>X and the option will be exercised. The second term, $Xe^{-Rt}[Nd_2]$, can be thought of as the present value of the exercise price, given that the option will be exercised.

6.2.3 Identification of optionality

Real Option value exists in many commercial investment opportunities. To exploit this value, the source of the optionality must be identified. To use the Black-Scholes model to value Real Options there is a need to identify characteristics of an investment opportunity that equate to the model variables. These are identified in table 6.2.

Table 6.2 Comparing Real and Financial Option variables (taken from Luehrman 1998)

Real Option	Variable	Call Option
Present value of project assets to be acquired	P	Stock price
Investment cost	X	Exercise price
Length of option life	t	Time to expiration
Time value of money	R	Risk free rate of return
Riskiness of project assets	σ^2	Variance of stock's return

6.2.4 Types of real options

There are different types of real options. The first step in valuing projects in embedded options is to identify the options. Although no two projects are identical, several types of real options are often present. Types of Real Options include:

- **Investment timing options** - conventional NPV analysis implicitly assumes that a project will either be accepted or rejected, which implies they will be taken now or never. Sometimes a third choice is available, the option to delay until later when more information is available. It is the option to delay that distinguishes Real Option analysis from NPV analysis
- **Growth options** – this allows a company to increase its capacity if market conditions are better than expected. This is where the value of an investment is generated by future growth options rather than currently quantifiable cash flows.
- **Abandonment option** - where uncertainty is large enough that flexibility increases value.
- **Flexibility options** – these allow a company to alter operations depending on how conditions change during the life of the project.

This chapter will concentrate on 'Investment timing options'. This gives the option of undertaking an investment now, never or deciding later.

It is acknowledged that the timing of an option can be considered using NPV. There is a case where the conventional NPV of a project and the option value are identical (Luehrman 1998). This is when the investment decision can no longer be delayed and

the company's option has reached its expiration date (i.e. time = 0). It is the option to delay that distinguishes Real Options analysis from NPV analysis.

6.2.5 Options at Albert WTW

Investment timing options will be used here to determine when is the best time to invest in Albert WTW. The options considered are listed below along with the costs associated with each option and any assumptions made.

Option 1

Do not invest and continue to use enhanced coagulation. Assume that the THM standard is breached once a year for the next five years.

Costs incurred include increased sludge production and disposal, increased coagulant use, increased pH correction costs, loss of income from customers each day consents are not met.

Option 2

Adapt WTW to incorporate double coagulation in 1 year or after 5 years. Assume that consents are met after new plant has been implemented but that the THM standard is breached once a year for the next five years before the new plant is implemented.

Costs incurred include construction of new mixing tank, additional sludge production and disposal, increased coagulant dose, increased pH correction costs, costs of failing to meet consents.

Option 3

Adapt WTW to incorporate MIEX® dosing with coagulation in 1 year or after 5 years. Install settling tank, regeneration tank and fresh resin tank. Assume that standards are met once the new plant has been implemented. Costs include cost of contactor with mixing, settling tank with pump, regeneration tank, regenerant, fresh resin tank, brine disposal and MIEX® (one-off cost). There will be savings made with a decreased coagulant dose and less pH correction.

6.2.6 Validation of assumptions

Failure to meet consents - a model for predicting THMs at Albert WTW has been proposed (Banks and Wilson 2002). It takes the form THM ($\mu g\ L^{-1}$) = 6.22 UV + 2.25 Temp – 6.88. UV is the UV absorbance of the treated water at 254 nm with units, m^{-1}. Temp is the treated water temperature in ºC. The model was constructed with data from 2000 to set a limit on the UV absorbance of the treated water in order that standards would be met. The limits for UV were set between 2.3 – 5.7 m^{-1} with a target of 3.5 m^{-1}. It was tested on water from 2001. The model was shown to be effective for the majority of the year but there were some unexplained high values between September and November 2001. These high values were 1.375 times greater than those predicted. At one time point in 2001, the UV absorbance reached 10.1 m^{-1} in autumn. If this is fed into the model with an assumed temperature of 8 ºC, the predicted THM is 73.7 $\mu g\ L^{-1}$. If this value is underestimated by the same amount as other outliers, the resulting value would be 101.3 $\mu g\ L^{-1}$ which would breach standards. It is therefore assumed that consents are breached once a year for the next five years if the WTW remains as it is now.

The water industry is regulated and the DWI is responsible for assessing the quality of drinking water in England and Wales, taking enforcement action if standards are not being met, and appropriate action when water is unfit for human consumption. The following statements are taken from the DWI annual report from 2002 (ww.dwi.gov.uk).

Introduction

'Where there were failures to meet consents in 2002, none were considered harmful to consumer's health.'

Report on Yorkshire Water

'THMs are a group of compounds that can form during the disinfection process. The standard is very stringent and it is not unusual for water companies to fail to meet it occasionally.'

'There was a slight increase in the number of failures in 2002. The increase may be due to the deteriorating raw water quality. The Inspectorate is considering enforcement action for one zone.'

From the report excerpts, it can be seen that the regulator is aware of the deteriorating raw water quality. It is also aware that the standard is stringent and may be breached on occasion. It is not thought that the regulator will tolerate the standards being breached continually beyond a 5 year period without taking enforcement action providing that Yorkshire Water is researching other treatment options.

6.2.7 Financial Analysis

The NPV of each option was calculated (table 6.3). A description of the assumptions made and the calculations carried out are described at the end of the chapter.

Table 6.3 NPV of each option

Option	NPV (£000, investment at 1 year)	NPV (£000, investment at 5 years)
1*	-568.917	N/a
2	-907.456	-928.877
3	-2393.363	-2515.961

* - option 1 is the option not to invest so the time factor is not relevant for this option.

The discount factor used was 4.75%. This is the real cost of capital estimated by OFWAT (Cooper and Currie 1999). The cost of failing to meet consents is the loss of income from customers for the day that consents were not met. The average water bill in the Yorkshire Water region is £104 per year (2002 - 2003) (www.ofwat.gov.uk). As Albert WTW supplies 113,000 properties, the loss was calculated as £32,197.26 per day of failure (assuming there are 365 days in a year). The prices for water are set to increase by 2.9% for the period 2003 – 2004 (www.ofwat.gov.uk). The same price increase was assumed in subsequent years.

According to conventional NPV analysis, the most likely option would be 1 as this option makes the least loss. This option would not please the regulator as the water company is not seeking to optimise its efficiency and the option to do nothing will

expire eventually. It would seem unwise for a company to undertake a project with a negative NPV. But if a water company is maximising its efficiency then the price it is able to charge for water will increase (see section 6.3).

NPV analysis does not take into account the time factor. The option to delay investment is similar to a call option on a stock, hence the Black-Scholes model can be used. The model requires five inputs:

1. The risk free rate of return
2. The time until the option expires
3. The exercise price
4. The current price of the stock
5. The variance of the stock's rate of return

The risk free rate of return is taken to be the interest rate on a Government treasury bill. This rate was reported as 3.62% for a one-year bond and 4.34% for a 5 year bond (Financial Times, 30th September 2003). The time until the option expires will be 5 years for option 1 and 1 year and 5 years for options 2 and 3. The investment cost for options 1 will be £1 (the investment cost for option 1 is taken as £1 although no further investment is made. This is because it is not possible to calculate the natural log of zero). For option 2, the investment cost will be £414,773 in year 1 and £469,278 in year 5. For option 2, the investment cost will be £2,562,087 and £2,898,766 in year 5 (the calculation of these costs is explained in section 6.4.3). The investment costs will be used as the exercise price. The current price of the stock is taken as the NPV of each option. This is because the current price of stock is the present value of its expected

future cash flows. The variance of the project's expected return can be used to represent the variance of the stock's return in the Black-Scholes model. One approach to obtaining this value is to make an educated guess. It is recalled that a company is a portfolio of projects (or assets), with each project having its own risk. Since returns on the company's stock reflect the diversification gained by combining many projects, we might expect the variance of the stock's returns to be lower than the variance of one of its projects (Brigham and Ehrhardt 2002). The variance of the stock price of the parent company of Yorkshire Water is ~15% (www.keldagroup.com). It is assumed that the variance for an individual project is higher at 20%.

Once these values were set, the Black-Scholes model was used to determine V (the value of the call option) for options 1, 2 and 3 (table 6.4).

Table 6.4 Value of options

Option	Value (£000, investment at 1 year)	Value (£000, investment at 5 years)
1*	-568.372	N/a
2	-473.310	-346.971
3	-1830.748	-1823.957

Given that the value of option 2 with investment at 5 years is the least negative compared to options 1 and 3, Real Option analysis would suggest that this option is best. Conventional NPV analysis favoured option 1.

It can be seen that there is value in delaying investment for five years with a modest initial investment. This value is diminished with a larger investment such as that in option 3. In terms of the stock market, this is because there is more time and opportunity for the stock price to increase. At Albert WTW it may be wise to delay investment for a number of reasons:

- to further understand the changes in raw water quality
- to understand why there are high THM levels when the UV levels are within specified limits in the treated water
- the extensive research into NOM character and treatment world wide may provide a wider choice of solutions to treating the water at Albert WTW
- the DWI is currently sympathetic to companies that are not meeting THM standards
- more advanced solutions may be able to cope with the deterioration in raw water quality for longer than the solutions suggested thereby avoiding the need to re-invest in the near future

Using the Real Options approach it is possible to quantify the hidden value embedded in the project.

Although Real Option analysis is more sophisticated than conventional NPV analysis it fails to take any other considerations into account such as perceived company performance and the workings of the water industry with regard to the government appointed economic regulators. These ‘other considerations’ are discussed in more detail below.

6.3 Other considerations

The economic regulation of the private sector water providers is the responsibility of the Director General of Water Services who is supported by the staff of the Office of Water Services (OFWAT). Currently, the economic regulator has two statutory duties:

- To ensure that the private companies that he has licensed carry out their functions properly.
- To ensure that the companies can raise sufficient revenue to finance the proper carrying out of their functions but only just enough for that purpose.

Price limitation is a key feature of the arrangements. Historically in the UK, control of private utilities entailed the limitation of profits on the basis of a rate of return on capital. In privatising utilities in the 1980s, the UK Government opted for the limitation of prices over the medium term. The formula adopted seeks to take into account: inflation, the obligations to incur expenditure to improve the quality of service, safety of drinking water and protection of the environment, the likely path of relevant external factors (e.g. the likely cost to the company of raising the necessary capital on the private markets) and crucially the likely capacity of the company to become more efficient over the period for which the price is being set (Summerton 1998). The formula is shown (equation 6.4).

$$K = RPI - P_0 - X + Q \pm V \pm S \qquad \text{Equation 6.4}$$

where K is the permitted annual price increase, RPI is the general domestic price inflation annually, P_0 is the annual past performance of the regulator's efficiency

improvement estimate, X is the regulator's estimate of scope for future efficiency gains, Q is the requirements of improvements in quality of drinking water and the environment, V is the requirements of enhancements to the security of the supply, S is the requirements of enhanced levels of services.

The water companies could seek to maximise Q, V and S by claiming that improvements will be expensive or by claiming they are under pressure to improve. It may then be possible to influence the value of K by impacting on the regulator's perception of how the industry is likely to evolve over time and with it the level of prices the regulator will permit to ensure an acceptable return to shareholders.
The price limits set are reviewed every five years by the regulator to take into account any economic or environmental changes. Thus a company is not free from all risk and at the same time is left free to seek to outperform the regulator's judgement.

The price that can be charged for water is directly related to the current efficiency and the scope for improved efficiency of the water company. If the water company is failing to meet consents, the regulator may estimate that scope for future efficiency gains (X) is higher than a water company that is meeting all its consents. If the water company does nothing and continues to fail to meet consents for the next five or ten years, the estimated scope for improved efficiency will not be met and the price that can be charged for water will be reduced. This will have a direct impact on the profit of the water company. With reduced profits, benefits (dividends) to shareholders will be reduced and investor interest to raise capital will be reduced.

6.4 Calculations and assumptions

6.4.1 Base case scenario

There was data available for the cost of treating water for the last 10 years from 1992 to 2002. This was provided by Yorkshire Water and is shown in table 6.5.

Table 6.5 Albert WTW operating costs

Year	'92-93	'93-94	'94-95	'95-96	'96-97	'97-98	'98-99	'99-00	'00-01	'01-02
Annual average colour (Hazen)	40.1	34.7	38.8	27.9	35.5	73.5	99.7	77.4	86.4	65.5
Calculated ferric dose (mg L^{-1})	6.0	5.2	5.8	4.2	5.3	11.0	15.0	11.6	13.0	9.8
Ferripol XL dose (mg L^{-1})	43.8	37.9	42.3	30.4	38.7	80.2	108.8	84.4	94.3	71.5
Ferripol XL cost (£ ML^{-1})	1.8	1.5	1.7	1.2	1.6	3.2	4.4	3.4	3.8	2.9
Lime dose (mg L^{-1})	10.8	9.4	10.5	7.5	9.6	19.9	26.9	20.9	23.3	17.7
Lime cost (£ ML^{-1})	0.5	0.5	0.5	0.4	0.5	1.0	1.4	1.0	1.2	0.9
Sludge formed (mg L^{-1})	19.5	16.8	18.8	13.5	17.2	36.7	48.4	37.5	41.9	31.8
Sludge treatment cost (£ ML^{-1})	1.9	1.7	1.9	1.4	1.7	3.6	4.8	3.8	4.2	3.2
Colour treatment costs (£ ML^{-1})	4.2	3.7	4.1	3.0	3.8	7.8	10.5	8.2	9.1	6.9
Total treatment cost (£ ML^{-1})	18.7	18.1	18.5	17.4	18.2	22.2	25.0	22.6	23.6	21.4

Treatment costs are reported in table 6.6.

Table 6.6 Treatment costs

Treatment	Cost
Ferripol XL (£ tonne^{-1})	40
Lime (£ tonne^{-1})	50
Sludge treatment (£ dry tonne^{-1})	100
Chlorination (£ ML^{-1})	0.42
pH correction (£ ML^{-1})	0.5
Electricity and Maintenance (£ ML^{-1})	2.65
Manpower (£ ML^{-1})	5.96
Plumbosolvency (£ ML^{-1})	4.50
Sludge cost due to turbidity (£ ML^{-1})	0.40

Below are the assumptions made to calculate the treatment costs:

- Annual average colour was calculated from archive data
- The ferric dose was calculated by (Colour (Hazen) × 0.15)
- Ferripol XL has a ferric content of 13.75%
- The lime equivalent of ferric is (1.8 × ferric dose)
- Sludge formed is calculated by (0.2 × colour (Hazen) + 1.9 × ferric dose)
- pH correction was based on a dose of 10 mg L^{-1} lime
- Plumbosolvency cost was based on a dose of 1.5 mg L^{-1}
- Sludge cost due to turbidity was assumed to be 4 FTU on average producing 4 mg L^{-1} dry sludge

6.4.2 Operating costs

Option 1 - it was assumed that the deterioration of the raw water and increase in treatment costs continued as in the last 10 years. The cost of treatment for the predicted elevated levels of colour was calculated taking into account the increased coagulant dose, the increased lime dose, increased chemical costs and increased sludge production.

Option 2 - it was assumed that a higher dose of Ferripol XL was required to implement double coagulation. The dose was increased by 1.4 mg L^{-1} as ferric. The increase in lime required for pH adjustment was considered as well as the associated costs for sludge treatment and disposal.

Option 3 - the cost of implementing MIEX® is high but this is offset by lower operating costs. The dose of Ferripol XL is reduced to 7 mg L^{-1} from ~80 mg L^{-1}. This leads to decreased sludge production and costs. Around 10% of the MIEX® resin is regenerated after each use with a 10% brine solution. The organic rich brine solution was disposed of by being tankered off-site. The cost was £10 per tonne with 200 L being produced per ML water treated. The specific gravity of the brine solution was 1.3 kg m^{-3}. With a reduced Ferripol XL dose, the pH of the water will not require lime to adjust it. MIEX® resin will work at any pH. There is a cost associated with the loss of MIEX® resin during treatment. The lost resin will have to be replaced. At present there is no set price for MIEX® so it is not possible to quantify this cost.

6.4.3 Capital costs

Option 1 – there was no investment made in new plant. The investment costs were assumed to be £1 to enable calculations to be made.

Option 2 – Double coagulation was implemented in two stages. To incorporate the second stage, 6 new mixing tanks would need to be constructed. The cost of this would be ~£365,000 based on the size of each tank being 9.724 m^3 (Gerrard 2000). Using the UK Plant Cost Index for construction, the cost was set at £414,773 in 2003 (UK PREDICT indices, Process Engineering 2003, volume 84, issue 7, p4). With inflation assumed to be 2.5% for the next five years (target rate, www.bankofengland.co.uk), the cost was set at £469,278 in year 5.

Option 3 - MIEX® resin is a new product that is only available in Australia. Information on the cost of the MIEX® resin is not readily available. A capital cost estimate for installing MIEX® treatment at an existing WTW was reported in the literature as AU$15 million for a plant with a flow of 112.5 MLD (Slunjski *et al*. 2002). A capital cost for implementing MIEX® was calculated using the current rate of exchange (1 AU$ = £ 0.407 on 3rd September 2003) and by adjusting the flow to fit Albert WTW. The Australian price index for international plant in 2003 is 107.7 compared with 110.3 for the UK. This was taken into account when calculating the cost of investment which was set at £2,562,087. The economy of scale was not considered. Using an inflation rate of 2.5%, the cost was set at £2,898,766 in year 5.

6.4.4 NPV calculations

To calculate the increase in colour over the next 10 years, the linear TREND function in Microsoft Excel was used. The change in cash flow was calculated over 10 years using 4.75% as the discount factor. This was the cost of capital in the industry as estimated by OFWAT. The cost of failing to meet consents was taken as the loss of income from customers in the region. The implementation of double coagulation was calculated by increasing the dose by 1.4 mg L^{-1} as ferric. This increase in dose was shown to achieve optimal removal (Fearing *et al*. in press). The calculation of the increased lime required was included.

6.5 Conclusions

An advanced financial tool, such as Real Options analysis, can take into account factors that were not considered by traditional NPV analysis. However a more sophisticated tool is not ideal. It does not consider the way an industry is regulated or incentives to invest that may not be the best option on a purely financial basis. The results from the tools should always be interpreted in the context of the industry being considered. NPV analysis and Real Options analysis suggest that options 1 and 2 are the best options financially. This is due to the fact that no initial investment is required for option 1 and the investment in option 2 is considerably less than that made in option 3. I believe that option 3 is the best. This opinion is based on the fact that with the increased efficiency in treatment gained by choosing option 3 will enable a higher price to be charged for the water produced. This will in turn increase the profit margin, improve shareholder benefits and encourage investment in the company.

The value of a Real Options approach is as much in the process as in the result obtained. By identifying optionality in a project, aspects of the project are considered that are not possible with NPV analysis. With Real Options analysis, caution should be taken to avoid misapplying the framework to justify poor investment decisions on the grounds of 'strategic value'.

CHAPTER 7 CONCLUSIONS

7.1 Introduction

Natural organic matter (NOM) is described as an intricate mixture of organic compounds that occurs universally in ground and surface waters. An investigation into how NOM changes seasonally, how it might best be characterised and its reactivity with chlorine was carried out. It was apparent from the literature that there is no ideal way of analysing NOM and there is a debate about whether NOM is best analysed in bulk or by separation. Bulk water analysis gives a limited understanding of the character of NOM and fractionation of NOM gives an insight into the fate through a treatment works and variability in source. In NOM analysis there is a trade-off between bulk analysis where the NOM is unaltered and separation of NOM where the NOM is altered and synergistic effects are lost. Some of the methods proposed in the literature have been used here with varying successes. It was found that there is no direct link between bulk NOM character and reactivity with chlorine but that DBP formation is affected by fraction make-up rather than quantity.

7.2 Investigation into seasonal variation of NOM character

The fractionation of raw and filtered water from Albert WTW showed that there was an increase in the amount and proportion of hydrophobic material in autumn compared with winter and summer in 2000. This corresponded with an increase in the THM-FP of both hydrophobic and hydrophilic fractions. This was not only due to the amount of each fraction but also a significant change in THM per mg of carbon for each fraction. In 2002 the fraction distribution was almost the same in October (autumn) as it was in

April (spring). The difference in reactivity between the water in April and October was less pronounced with similar reactivities for the hydrophobic acid (weighted average of HAF and FAF) and hydrophilic acid. However the increase in reactivity of the HPINA in autumn was the same as that found in 2000.

By fractionating raw and filtered water, it was possible to determine the seasonal performance of the WTW in terms of fraction removal at Albert WTW. The hydrophobic fractions exhibited different behaviour from the hydrophilic fractions. The removal of the HAF was always > 90% and removal of the FAF was within a narrow range (88.9 – 92.5%) indicating that the nature of the material is the same throughout the year but it is known that the quantity is variable. The range of the HPIA removal was 69.7 – 95.6% with the highest removal observed in June 2000 and similar removals in November 2000 and April 2002. The range of HPINA removal was 14.5 – 32.5% with the lowest removal occurring in November 2000 followed by a similar removal in June 2000. In April 2002, the removal of the HPINA was twice that observed in 2000 indicating that the nature of the material in each year is quite different.

The fractionation gives an insight into the seasonal effect on NOM and it was shown that the HPINA was always more reactive in autumn regardless of the fraction distribution.

7.3 Comparison of fractions

A comparison of resin-separated fractions and size-separated fractions was made by comparing the SUVA values, HPSEC traces and EEM fluorescence spectroscopy contour plots. All analyses were from the October 2002 as this was the only water that had been subjected to both types of separation.

7.3.1 SUVA

The SUVA values of all samples from October 2002 are reported (table 7.1). The SUVA of the raw water was 5.03. SUVA is the ratio of UV absorbance of a sample measured at 254 nm to the DOC of that sample.

Table 7.1 SUVA values (Albert, October 2002)

Resin separated		Size separated	
Fraction	SUVA (m^{-1}.L mg^{-1} C)	Fraction	SUVA (m^{-1}.L mg^{-1} C)
HAF	5.57	R3	5.19
FAF	6.25	R1	3.67
HPIA	3.21	R05	2.56
HPINA	0.86	F05	2.01

Literature guidelines published state that the higher the SUVA of a sample, the higher the molecular weight and the more hydrophobic the sample (Edzwald and Tobiason 1999). The published guidelines fit both the resin and size separated fractions. The SUVA values of the more hydrophobic resin separated fractions are higher than the more hydrophilic fractions. The distinction between the HAF and FAF is less obvious with similar SUVA values being exhibited by both. With the size separated fractions, the higher the MW, the higher the SUVA.

The SUVA values for the largest UF fraction (R3) is less than the values found for the HAF and FAF. This could be attributed to the HAF and FAF having a higher degree of unsaturation than that in the UF fraction, R3. Conversely, the HPIA has a lower SUVA value than R1 and the HPINA has a lower SUVA value than R05 and F05. This indicates a higher degree of unsaturation in the UF separated fractions compared with the resin separated fractions.

7.3.2 Fluorescence

The position of the discernible peak with maximum intensity from the fluorescence EEMs of the resin separated and UF separated fractions is shown (table 7.2). The position of the raw water peak is 309, 425 (ex, em (nm)) with an intensity of 3.1.

Table 7.2 Fluorescence of fractions (Albert October, 2002)

Resin separated			Size separated		
Fraction	λ_{max} (ex,em (nm))	Intensity (a.u.)	Fraction	λ_{max} (ex,em (nm))	Intensity (a.u.)
HAF	296,328	7.2	R3	320,446	9.1
FAF	305,395	4.3	R1	296,336	17.9
HPIA	317,420	3.1	R05	296,325	27.5
HPINA	291,351	4.0	F05	298,323	10.4

Again, the size separated fractions follow the trend reported in the literature that the higher the emission maxima, the higher the MW. The trend seen for the resin separated fractions shows that MW decreases in the order HPIA>FAF>HPINA>HAF. The trend seen for the November raw water from 2001 was HAF>HPIA>FAF>HPINA. In October 2002, the HAF seems to be smaller than that seen in November 2001. The intensity value for the resin separated October 2002 HAF also indicates that it has a smaller mean MW than the other fractions as generally a higher intensity is seen with

smaller molecules. The SUVA values for the resin separated fractions suggest that the MW of the HAF is higher than that of the HPIA and HPINA. However SUVA is a measure of hydrophobicity as well as MW, so the HAF could consist of many unsaturated small molecules as well as some less unsaturated larger molecules. The emission maxima is an indication of the mean MW and does not represent all of the molecules present.

7.3.3 HPSEC analysis

The size distribution of the UF fractions is as wide as the resin fractions. This indicates that the MWCO of the membranes is not sharp. The extra peak seen for the UF fractions was attributed to aggregation of the smaller molecules in solution resulting in an increase in UV absorbing species.

7.3.4 Practical considerations

With resin fractionation, the sample volume was 75 L of raw water with the 1.2 L columns of resin. With the UF cell reactor, the maximum sample size was 200 mL. Although the time taken to fractionate by UF is shorter than that used to fractionate using resins, the amount of sample left after the separation by UF is limited. To increase the sample volume gained from UF, the analysis would have to be repeated many times or a larger cell reactor would have to be used.

7.4 Pragmatic methods of analysis

The methods of analysis used during this project were considered in terms of their usefulness to a WTW operator. Factors considered included the sample preparation required, the time taken and equipment required for the analysis as well as the information gained from the analysis (table 7.3).

Table 7.3 Methods of analysis

Method of analysis	Sample preparation	Time taken	Equipment required	Information gleaned	Useful to WTW operator?
DOC	None	30 minutes	TOC analyser	Sample TOC	No
UV_{254}	None	5 minutes	Spectrophotometer	Sample absorbance	Yes
SUVA	None	35 minutes	TOC analyser and Spectrophotometer	Indication of type of organics present	Yes
THM-FP	Chlorine dosing solution and buffer required	7 days	Air tight sample bottles, GC	Reactivity of sample with chlorine	No
Resin fractionation	Acidification to pH 2	> 1 month	Resin, soxhlet extractors, solvents, columns, peristaltic pumps, centrifuge	Fraction distribution + samples for analysis	No
UF fractionation	None	1 week	Stirred cell reactor, nitrogen gas, membranes	Molecular size distribution	No
CE	Concentrated fractions required	> 1 month + 1 hour	CE, buffered solutions, capillary column	Relative charge to size ratio of selected fractions	No
HPSEC	None	30 minutes	HPLC, SEC column, solvents	Molecular size distribution	Yes
Fluorescence spectroscopy model	Dilution to 1 mg L^{-1} once TOC has been measured	40 minutes (set-up time > 2 months)	Fluorescence spectrophotometer and TOC analyser	Fraction distribution	Yes

It is assumed that all samples are filtered prior to analysis. This is not included in the 'sample preparation'.

Methods of analysis that were not considered to be useful to the WTW operators took longer than half a day to execute. A WTW needs to be controlled on a day to day basis. Any information gained after that time would be too late to assist in the water treatment process. DOC was not considered to be particularly useful on its own as it gives no insight into the types of organics present in the water. It is required to calculate SUVA. Methods of analysis that have been deemed as useful to the WTW operators are: UV, SUVA, HPSEC and the fluorescence spectroscopy model. Measurement of the effluent UV_{254} is regularly used to assess the performance of the WTW and can be an early indication of failure of the treatment process. SUVA can be a useful parameter to measure as it generally rises with rising THM-FP of the water although the relationship for Albert water is not entirely consistent. HPSEC is very useful for optimising treatment processes particularly for removal of the smaller molecular weight organics that are not removed by conventional treatment. Once set up, the HPSEC analysis takes little time and little sample preparation. It would be even more useful if a DOC detector was used rather than just a UV detector. When treating raw water, it would be useful to know the fraction distribution in order to apply the best treatment. The fluorescence spectroscopy model can determine the fraction distribution within an hour. It is thought that it may not be used routinely at a WTW but may be used at the end of summer/beginning of autumn to determine the time when the water becomes more difficult to treat and needs to be treated differently.

7.5 Is there a link between character and reactivity?

Although SUVA and HPSEC give limited information on the changing character of Albert raw water over time, the fluorescence model has shown the variation in fraction

distribution over time. From the fluorescence model, samples that have a high FAF content were identified. An investigation into two samples, that were shown to have very different FAF contents, was made to determine any link between character and reactivity.

It is known that the formation of DBPs is very complex. Here we have tried to predict the reactivity of a raw water in terms of THM-FP by looking at the DOC make-up. However, it was found that the THM-FP was independent of the DOC make-up but may be related to the relative size of the fulvic-like molecules determined by fluorescence. The use of fluorescence as a tool for understanding chlorine-NOM reactions requires more research to determine its applicability.

CHAPTER 8 FURTHER WORK

It is widely accepted that NOM analysis is complex and there is no ideal way of characterising NOM. It has been reported that understanding the unknown structure of NOM is key to better drinking water treatment. Future work should be concentrated on structural/elemental analysis particularly on the hydrophilic, 'difficult to remove', fraction.

The scope of this work should include:

1. Dissolved organic nitrogen (DON) analysis – there is evidence that the nitrogen rich constituents represent an important class of the unremoved hydrophilic NOM fraction that is acting as a precursor to DBPs.
2. Fuller analysis of DBPs – although other DBPs are not yet regulated in the UK, they are clearly being produced upon chlorination of water containing NOM. By not quantifying them it is difficult to determine what is happening in the NOM-chlorine reaction.
3. DOC detection for HPSEC – with UV detection only, the UV absorbing species are detected but this limits the information obtained on the low MW less unsaturated NOM molecules. A DOC detector would enable a fuller analysis of these molecules.
4. Acid-base properties – the phenolic and carboxylic group concentration may be related to the DBP formation potential. The information could also be used in conjunction with the NOM source data as it is know that NOM from a microbial source has a high phenolic content.

5. Climatic effects – it was reported that there was an increase in the amount of organic substances entering the WTW after heavy rainfall. There is data available on the levels of soil moisture around the reservoir as well as rainfall data. It may be possible to link the nature of the organics to the climatic events.
6. Treated water analysis – it is the NOM in the treated water that is acting as a precursor to DBPs. A fuller understanding of the structure of the NOM in treated water may enable would allow the design of suitable water treatment processes.

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