Development of methods for the separation and characterization of natural organic matter in dam water.

Submitted in fulfillment of the requirements of the degree of Master of Technology: Chemistry in the Faculty of Applied Sciences at the Durban University of Technology

Pinkie Sobantu

MTech: Chemistry

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DECLARATION

I, Pinkie Sobantu declare that the dissertation submitted for the degree of Master of Technology (MTech): Chemistry at the Durban University of Technology is to my best knowledge my original work and no part of it has been published or accepted at any other institution.

_____ ____/_____/_____

Signature of the student Date

_____ ____/_____/_____

Signature of promoter Date

_____ ____/_____/_____

Signature of the HOD Date

DEDICATION

This is dedicated to my son, Lwethu. He keeps me going.

ACKNOWLEDGEMENTS

To God be the glory for making this happen.

I'm grateful to my family and my friends for all the support and love. They really made me feel like I could do this and here I am today. I would like to express my gratitude to my supervisor Prof KG Moodley and my co-supervisor Mr. DK Chetty for their academic support, encouragement and patience throughout this study.

I thank my Head of Department, Prof GG Redhi for encouraging me to finish this work in time. I acknowledge and thank Dr. Pienaar for his help at crucial times during the project. Special thanks are accorded to Mr. Gerhard Gericke and Mr. Theo Jongwana of Eskom R T & D Laboratories for their valuable input, collection of samples and for TOC and DOC measurements and to Mrs. Devrani Naicker (Avy) of the Chemistry Department (DUT) for technical help.

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ABSTRACT

This project arose out the need for a simple method to analyse NOM on a routine basis. Water samples were obtained from the Vaal dam, which is one of the dams used by a hydroelectric power station. Analysis was preceded by separation of NOM into the humic and non-humic portions. The humic portion was separated into two fractions by employing a non-ionic resin (DAX-8) to separate humic acid from fulvic acid. High performance size exclusion chromatography (HPSEC), equipped with an Ultraviolet(UV) detector and an Evaporative Light Scattering (ELS) detector connected in series, was used to obtain molecular weight distribution information and the concentration levels of the two acids. Mixed standards of polyethylene oxide/glycol were employed to calibrate the selected column. Suwanee River humic acid standard was used as a certified reference material.

The molecular weight distributions (MWDs) of the isolated fractions of humic and fulvic acids were determined with ELSD detection as weight-average (Mw), number-average (Mn) and polydispersity (ρ) of individual NOM fractions. The Mw/Mn ratio was found to be less than 1.5 in all the fractions, indicating that they have a low and narrow size fraction. An increase in Mn and Mw values, with increasing wavelength for all three humic substances (HS) examined was observed. The HS, isolated from the dam water, was found to be about the same molecular weight as the International Humic Acid Standard (IIHSS). For the fulvic acid standard, the molecular weight was estimated to be around 7500 Da.

Characterization of NOM was done to assist in the identification of the species present in the water. FTIR-ATR was used to as a characterization tool to identify the functional groups in the structure of the humic and fulvic acid respectively present in the Vaal Dam. Analysis of the infrared (IR) spectra indicated that the humic acids of the

Vaal dam have phenolic hydroxyl groups, hydroxyl groups, conjugated double bond of aromatic family (C=C), and free carboxyl groups.

The isolation method has proved to be applicable and reliable for dam water samples and showed to successfully separate the humic substances from water and further separate the humic substances into its hydrophobic acids, namely, humic and fulvic acids. It can be concluded that the Eskom Vaal dam composes of humic substance which shows that the technique alone gives a very good indication of the characteristics of water. The HPSEC method used, equipped with UV and ELSD was able to identify the molecular weight range of NOM present in source water as it confirmed that the Eskom Vaal dam contains humic substances as humic acid and fulvic acid and these pose a health concern as they can form disinfectant byproducts in the course of water treatment with chemicals. FTIR characterization was successful as important functional groups were clearly assigned. Lastly, the use of the TOC and DOC values to calculate SUVA was also a good tool to indicate the organic content in water. It is recommended to use larger amounts of water must be processed to obtain useful quantities of the humic and fulvic acid fractions.

LIST OF ABBREVIATIONS/ACRONYMS

AHS- aquatic humic substances

BDOC – biodegradable dissolved organic carbon

DBP – disinfection by product

DI- deionized

DOC – dissolved organic carbon

DOM – dissolved organic matter

FA – fulvic acid

FTIR – Fourier transform infrared spectroscopy

HA – humic acid

HPIA – hydrophilic acid

HPIB – hydrophilic base

HPIN – hydrophilic neutral

HPOA – hydrophobic acid

HPOB – hydrophobic base

HPON – hydrophobic neutral

HS – humic substances

IHSS- international humic substances society

IR – infrared spectroscopy

MF – microfiltration

MS – mass spectrometry

NF – nano filtration

NHS – non humic substances

NMR – nuclear magnetic resonance

NOM – natural organic matter

SUVA – specific UV absorbance

THMs – trihalomethanes

UF – ultrafiltration

UV – ultraviolet

LOD- limit of detection

UV-VIS- ultra violet visible

SEC- size exclusion chromatography

HPSEC- high performance Size exclusion chromatography

m/z- mass to charge ratio

NHS- non humic substances

PEOs- polyethylene oxides

PEG- polyethylene glycol

STD- standard

ELSD- evaporative light scattering detector

SRHA- suwanee river humic acid

SAHA- sigma Aldrich humic acid

SAFA- sigma Aldrich fulvic acid

UV254- ultraviolet detection at 254 nm

UV280- ultraviolet detection at 280 nm

AMW- average molecular weight

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CHAPTER 1

INTRODUCTION

1.1 Background information- Research problems, objectives and aims

Eskom, the Power Utility Company in South Africa, needs treated water for its power generation plant. For this purpose, it treats raw water drawn from various dams in the Mpumalanga region, including the Vaal dam. In a personal communication with Mr .Gericke of Eskom laboratories, he highlighted that the concentration of natural organic matter is higher in the Vaal dam than in the other dams from which they draw water for generation of electricity in the power stations. One of the steps involved in the treatment of dam water involves the removal of NOM. The removal methods would be based on knowing the structural composition of the NOM and the levels in which they are present. Methods developed for this have to be suitable for routine use.

In this current study, the untreated water from the Vaal dam is first separated into humic and non humic substances. From the humic substances, it is then fractionated into humic and fulvic acid from which molecular weight distribution information and concentration is predicted. HPSEC is used to get information on the molecular weight distribution of these, assisted by information on specific UV absorbance and finally, FTIR is used to get qualitative information of these fractions.

The water used in the steam water circuit of power plants at Eskom must be very pure to prevent scaling and corrosion from taking place. To achieve this desired state of purity, the water must be subjected to several water treatment processes for the removal of the suspended and dissolved inorganic and organic carbon. Several steps are followed in water treatment for the removal of suspended and dissolved contaminants before it is suitable for use in the power plant cycle from natural sources.

These include pre-treatment, filtration, membrane separation, ion exchange techniques, reverse osmosis and ozonation.

The varying structure and composition and sources of NOM imply that high NOM removal efficiencies are barely attained at conventional water treatment works (WTW) [1]. Poor removal of NOM can result in biofilm re-growth in distribution systems and the formation of potentially carcinogenic disinfectant by-products (DBP) such as trihalomethanes (THM) and haloacetic acids (HAA), formed when residual NOM reacts with disinfectants such as chlorine.

The characterization and analysis of NOM is generally very difficult for the following reasons:

- NOM are very large molecules which can disintegrate to give smaller species or aggregate to give larger species.
- NOM comprises humic and non-humic types of compounds.
- Standards and certified reference material are very difficult to obtain and they are expensive.

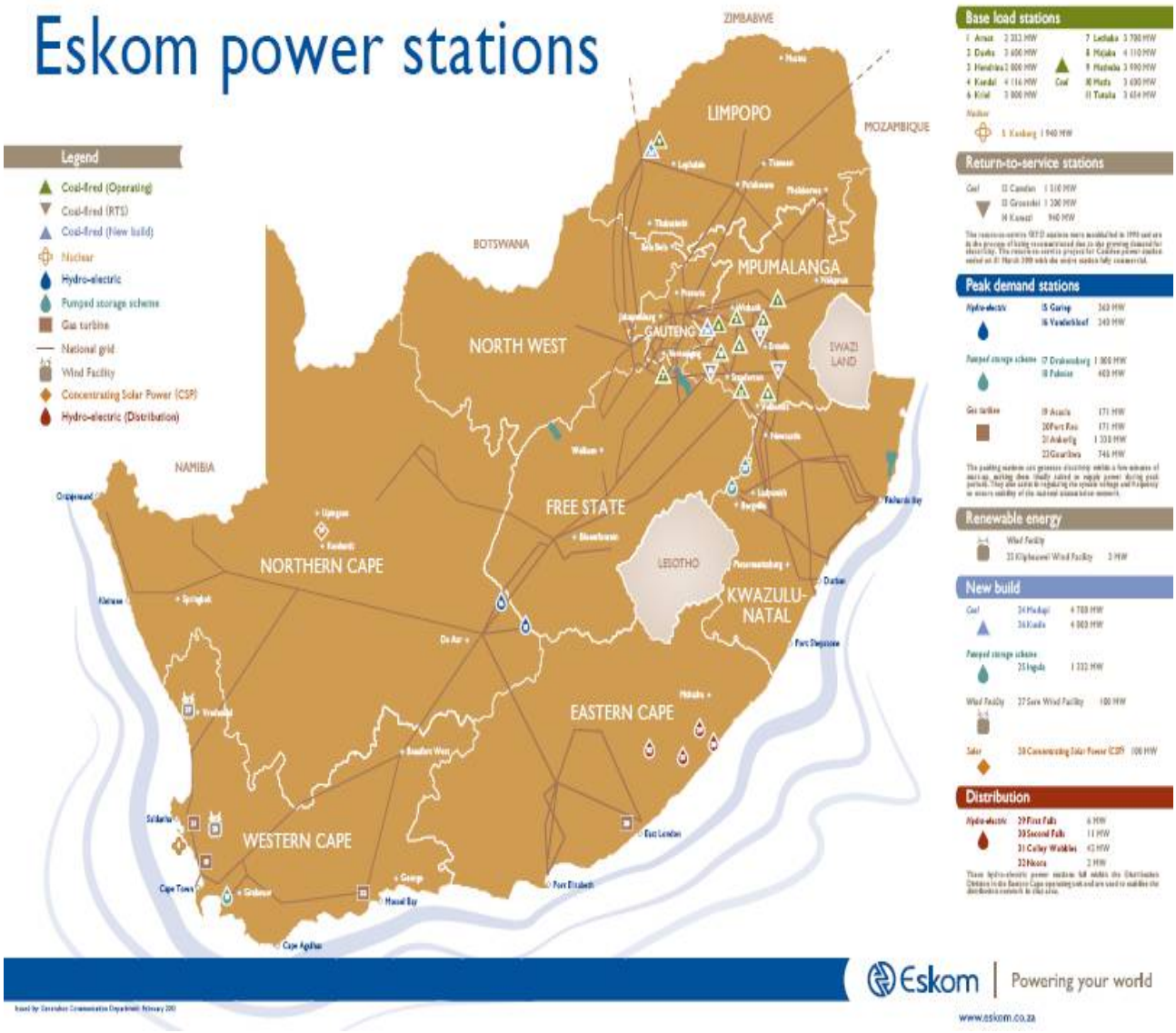

Fig. 1.1: Map of South Africa showing the location of power stations

1.2 **Origins and classes of organic matter in water**

Natural organic matter (NOM) is a complex mixture of many groups of high molecular weight organic compounds that enter the water source from animal and plant remains, domestic and industrial wastes. It has been shown that NOM is a likely precursor material for disinfection by products (DBPs) [2, 3, 4, 5 6]. NOM occurs in all marine sources when animal and plant material disintegrates [3, 6, and 7]. However, major problems occur when NOM reacts in various ways in the treatment processes [11]. The general reaction of NOM with chlorine is shown in equation 1 below [7].

NOM+ free chlorine→THMs + HAAs + cyanogen halides + other DBPs …… (1)

Most of the DBPs produced either cause cancer or are mutagenic, for example, haloacetic acids (HAAs), are considered harmful to human health and have toxicological effects, like reproductive and developmental effects. In addition, NOM is known to cause problems of membrane fouling and aesthetically displeasing flavor. NOM has also been found to cause corrosion of turbines and engineering systems [10]. An understanding of NOM in water and its removal is therefore important for human health and in industrial processes [6, 8, and 9].

Natural organic matter can also be divided into humic and non humic substances. Dissolved organic compounds are composed mainly of humic substances [11, 12]. These can be divided into six groups, hydrophobic acids, bases and neutrals and hydrophilic acids, bases and neutrals. The humic fraction is more hydrophobic and comprises humic acids and fulvic acids [12, 13]. The part of humic substances that do not dissolve in water at a pH lower than 2, are known as humic acids. The fraction of humic substances that does dissolve under all pH conditions is referred to as fulvic acids [14].

1.3 **Humic substances**

Humic substances (HS) are the main components of the natural organic matter in soil and water as well as in geological sources such as lake sediments, peats, brown coals and shales. They make up most of the brown color of decaying plant and they are the cause of the brown or black color in surface soils. They are the major components of NOM in surface waters and, at higher concentrations, can impart a dark color, especially in brown fresh water ponds, lakes, and streams. In leaf litter or composts, the color may be yellowish-brown to black, depending on the degree of decay and concentration [2].

A resin column separation is one of the tools employed to separate FA from the non-humic materials (amino acids, peptides, sugars, etc.) extracted from soils. At low pH the FA adsorbs on the resin, but non-humic materials pass through the column [15]. Non –humic materials are important because they form most of the source of non-living organic material that nature knows [16]. Humic substancesare also known to have a great impart in soil fertility, and are considered to have relevance for the stabilization of soil aggregates. They can be divided into three components according to their solubility: humic acids, fulvic acids and humin. Humic acids are the most explored group of humic substances [16]. Humic and fulvic acids are the hydrophobic fractions whereas the hydrophilic fractions comprise low molecular weight carbohydrates, proteins and amino acids. Fractionation allows for the evaluation of the extent with which each organic fraction present in the water sample can be effectively removed [17].

Humic substances		
Fulvic acid	**Humic acid**	**Humin**
Light yellow yellow brown	**Dark brown grey blue**	**Black**

_____increase in intensity of color…….

_____increase in degree of polymerization……..

2 000_____increase in molecular weight…….300 000?

45%_____increase in carbon content…….62%

48%_____decrease in oxygen content……30%

1 400_____decrease in exchange acidity……500

_____decrease in degree of solubility……..

Chemical properties of humic substances.(Stevenson 1982)

Fig. 1.2: Chemical properties of humic substances. Stevenson, 1982

The relationships between those are shown in Fig. 1.3 above, which clearly shows that the content of carbon and oxygen acidity and extent of polymerization change with an increase in molecular weight. The low - molecular weight fulvic acids have higher oxygen but lower carbon contents than the high - molecular weight humic acids. Fulvic acids contain more functional groups of an acidic nature, particularly COOH. The total acidities of fulvic acids (900 - 1400 meq/100g) are considerably higher than for humic acids (400 - 870 meq/100g) [19].

1.3.1 **Humic acids**

Humic acids do not dissolve in water in acidic media (pH less than 2) but are soluble at higher pH values; they are the main extractable component of humic substances in soil and they are dark brown to black in color [16, 18]. Humic acids a have a smaller portion of phenolic functional groups, which can be identified by different chemical methods. They are derived from peptide, lipid and carbohydrate precursors [19]. Chemically, they all have different compositions and molecular weights. Humic acids found in land tend to have an aromatic nature (containing benzene- and phenol-like structures) while those found in water tend to be more aliphatic in nature. There are aliphatic (grease, oil, fat) like components in terrestrial humic acids, as there are aromatic components in aquatic humic acids [19].

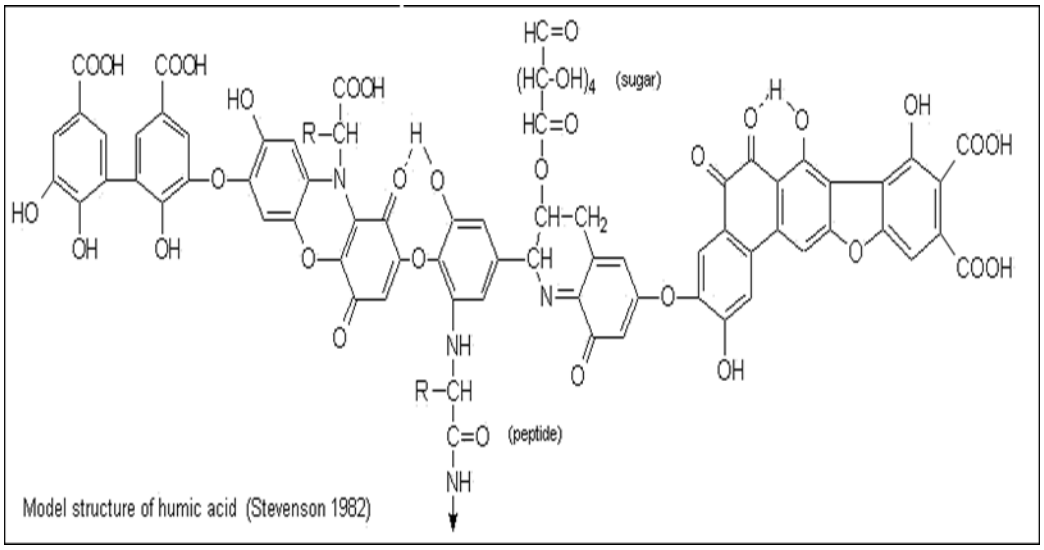

Fig. 1.3: Model structure of humic acid. Stevenson, 1982

1.3.2 **Fulvic acids**

Fulvic acids dissolve in water at any pH conditions. They remain in solution after removal of humic acid by adding acids [18]. These are light yellow to yellow-brown in color [18]. The low - molecular weight FAs have a higher content of oxygen but lower carbon contents compared to the high - molecular weight HAs. However, FAs have more acidic functional groups, particularly carboxylic groups. The total acidities of FAs (900 - 1400 meq/100g) are considerably higher than for humic acids (400 - 870 meq/100g). Their composition and shape varies. The size of fulvic acids (FAs) is smaller than humic acids (HAs); with molecular weights which range from approximately 1,000 to 10,000 and they have oxygen content twice that of humic acids (HAs). [18].

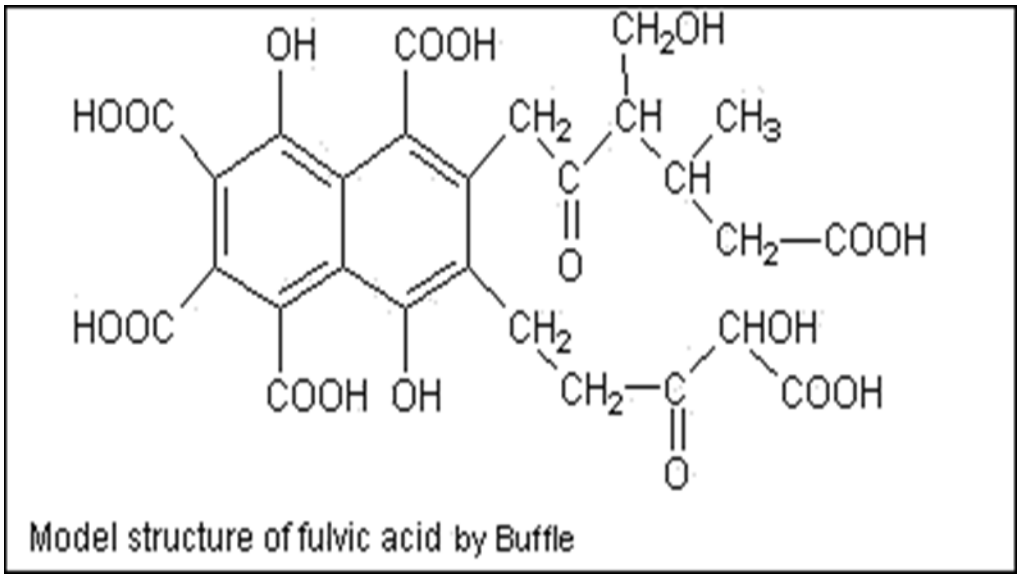

Fig. 1.4: Model structure of fulvic acid. Buffle- 1977 in Peuravuori 1992

1.4 Dam and drinking water treatment operations and natural organic matter removal

Some drinking water treatment processes can be optimized such that NOM is removed before it reacts with chlorine to form DBPs [9]. Coagulation-based processes, including conventional treatment, direct filtration, and dissolved air flotation, use metal salts to form NOM molecules that conglomerate so that they can be removed using clarification or filtration. Processes such as ozonation change NOM into smaller species that are not highly reactive. High pressure membrane filtration processes can remove NOM molecules through size exclusion. Adsorption processes that use activated carbon or various resins can also be employed to remove some types of NOM before disinfection [9, 20].

Dam water purification processes have a common goal of removing all the harmful species present in the water and then purify the water for domestic uses. There are clarifiers that add some chemicals to the water that remove solids and contaminants that pose a hazard to the users of the dam [21]. In addition, in the system, chemicals have to be added to make the pH such that it is perfect for home use [21]. Most of the processes used in drinking water treatment are affected by natural organic matter in the water. In South Australian water supplies where NOM levels are generally high, the impacts can be quite noticeable [22]. However, the same effects can be readily seen in waters with lower NOM levels; even in relatively clean groundwater sources [23].

For practical and hygienic reasons, some of these being that chlorination by-products are formed as mentioned above, which are carcinogenic substances, the presence of natural organic matter in drinking water, is not wanted [9]. Different technologies have been proposed for removal of NOM with varying degrees of success [9]. The properties and amount of NOM, however, can significantly affect the efficiency of the process. In order to improve and optimize these processes, the characterization and quantification of NOM at different purification and treatment processes stages is important. It is also important to be able to understand and predict the reactivity of NOM

or its fractions in different steps of the treatment [9]. The optimum selection of treatment processes to remove organics depends on the character of the organics present and the quality of water required. However, there is always a portion of the organic matter that cannot be removed by some processes and will require additional treatment if more NOM removal is required [24].

It is vital to understand the chemistry of NOM to have a clear understanding of the mechanism of NOM removal from water. This should lead to solutions best suited to treat contaminated water [3]. NOM can be removed from water by applying several techniques: conventional coagulation by deposition or flotation, filtration through different media, membrane filtration, and removal on ion-exchange resin, oxidation, absorption and biological processes. Depending on the nature of the problem, most of the NOM removal techniques are based on coagulation as the principal step. Besides the conventional technique and its variations with possible superstructures, coagulation is used to remove humic substances by membrane filtration [25, 26]. NOM is a precursor of disinfection by-products and the chlorination of NOM-rich waters produces carcinogenic compounds [27]. For this reason, the removal of NOM prior to disinfection is important. If conventional water treatment process such as sand filtration are ineffective in removing such contaminants and coagulants are often inefficient in removal of organics, membrane processes present an important technology [27].

1.4.1 **Coagulation**

Coagulation is the most important process in the drinking water treatment industry. It has been, over the years used in the treatment of water in an attempt to decrease turbidity levels [28, 29]**.** The current conventional method of natural organic matter removal is coagulation and flocculation. Coagulation results from the addition of positively charged coagulants, e.g., aluminium sulphate (alum) or a ferric salt, such as

ferric chloride, are the two most commonly used coagulants [12, 30]. However, the effectiveness of this type pf treatment in removing natural organic matter is also dependent on many factors, one of which is the type and amount of coagulant used, pH, temperature, particle, some properties of NOM, such as hydrophobicity and size [9]. The NOM remaining after coagulation can be removed using activated carbon, filtration, biologically activated carbon filtration and membrane filtration [31, 32]. A portion of the coagulant that has been used is not removed during the treatment and is left as residues in the treated water [32].

1.4.2 **Activated carbon filtration**

Activated carbon filtration is an effective adsorbent used in the removal of both man-made and natural pollutants such as pesticides, industrial chemicals, tastes, odors and algal toxins. It can be considered as an additional treatment process for NOM. Different fractions of NOM can adsorb on activated carbon to different extents. Although activated carbon filtration is effective in reducing DBP precursor compounds, the techniques has been subject to numerous regenerations, a degradation of AC has been found to occur. [9].

1.4.3 **Ion exchange- resin filtration**

Ion exchange is an effective method for removing NOM. The polymeric anion exchange resins and a magnetic ion exchange resin have previously been assessed to check for their effectiveness in the removal of dissolved organic carbon and in controlling the formation of DBPs [33].

1.4.4 **Ozonation**

The ozonation of natural organic matter in water remains unclear as NOM consists of a wide range of compounds that are different in chemical properties. Using ozone to oxidize NOM normally results in the formation of several by-products such as aldehydes and COOHs. These have large contributions in the amount of biodegradable organic carbon [9, 35]. Even though ozone is used to disinfect, it does lead to the oxidation of natural organic matter and hence forming low molecular weight organic compounds. This type of treatment converts non-biodegradable organic carbon into biodegradable organic carbon by breaking the NOM structure and enhancing the transformation of high molecular weight compounds into low molecular weight compounds such as carboxylic acids [35].

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CHAPTER 2

LITERATURE REVIEW

Overview

A range of analytical techniques are used to get information on the properties of NOM. The relevance of each technique is very much dependent on the chosen application [1]. Table 2.1 lists some of the properties and the techniques used to assess the properties of NOM. This will be followed by a review of aspects contributions of other researchers on aspects of NOM studies relevant to this project.

Table 2.1: Analytical techniques for NOM characterization [1]

Parameter	**Analytical tool**
Color	Visible Spectrophotometry Visual Comparators
Aromaticity (UV absorbance)	UV Spectrophotometry
Total Organic Carbon (TOC)	DOC Analyzer
Dissolved Organic Carbon(DOC)	DOC Analyzer
Biodegradable Organic Carbon (BDOC)	DOC Analyzer
Assimilable Organic Carbon Organic Carbon (AOC) Bacterial	Bacterial Regrowth Potential (BRP)
Molecular weight distribution	High Performance Size Exclusion Chromatography (HPSEC)
Hydrophobicity/Hydrophilicity	Rapid Fractionation (RF)
Trihalomethane Formation Potential (THMFP)	Gas chromatography (GC)
Functional Groups (Aliphatic, Aromatic, Nitrogen Containing)	Gas Chromatography-Mass Spectroscopy (GC-MS) Chemical tests -Infrared Spectroscopy (FTIR) Nuclear Magnetic Resonance (NMR) LC-MS

2.1 **Isolation, concentration and fractionation of NOM.**

The terms fractionation and isolation are often used interchangeably; however, there are significant differences between them. Fractionation refers to chemical or physical processes that separate components of the environmental samples into more homogenous groupings based on chemical or physical properties like acidity, polarity, molecular size etc. [2]. Fractionation procedures are a part of isolation procedures but isolation extends beyond fractionation [2]. Usually, isolation and concentration of aquatic NOM begin by separating the sample into dissolved and undissolved fractions. A widely accepted method is the filtration through a 0.45 micron membrane filter. To investigate the complex nature of natural organic matter, isolation or fractionation is often necessary to get more uniform material to further characterize. This is also useful in providing a more clear understanding between the properties of NOM and its behavior during treatment [3]. There are various ways of isolating NOM from water and some of them are reviewed below. The method of isolation and the conditions applied establish exactly the extent of separation of humic from non humic substances [4]. It also plays an important role in identifying the properties of the sample.

As much as natural organic matter molecules are known to be unique, they however, have many common properties. One of the main analytical challenges in characterizing NOM is lacking a good, suitable fractionation method which will produce pure components that can then be characterized by standard methods. One of the ways of overcoming this difficulty is to separate NOM into components that are different in character and to study the chemical structures of its components, that is, fractionation. The most commonly used chemical fractionation techniques are precipitation, solvent extraction, and adsorption chromatography [5].

The advantage of sorption-based isolation techniques is their ability to simultaneously concentrate, isolate and fractionate NOM making it the most frequently

used method for the isolation of aquatic humic substances. It applies the use of a non-ionic polymeric resin through column chromatography because of their ability to adsorb onto non-ionic macro porous resins. Non-ionic macro porous copolymers used together with ion exchange resins under optimized conditions classify organic solutes in a water sample into different fractions. An analytical procedure developed by Leenheer [6] quantitatively classifies organic solutes into hydrophobic fractions [7]. Sorption is used both in small scales system to process NOM for subsequent analysis, and in large scale for drinking water treatment. As water containing NOM is passed through a column packed with adsorbent particles, some of the NOM molecules adsorb onto the packed media. The sorption efficiency depends on the characteristics of the NOM molecules, the media and other aspects of the solution composition [8-10].

Ibrahim *et al* [12] isolated aquatic humic solutes using DAX-8 and diethylaminoethyl cellulose resins. The FA concentration range of the water samples was found to be between 1.36 and 4.67 mg C L^{-1}, while the level of HA was between 0.72 and 3.04 mg C L^{-1}. Makela *et al* [13] also used a non-polymeric resin for the isolation of humic and fulvic acid from ground water. This procedure was able to isolate 40% of the DOC as humic solutes. This method was also reported by Gray *et al* [14]. They used adsorption onto a synthetic resin to produce strongly hydrophobic acids, weakly hydrophobic acid, charged hydrophilic and neutral hydrophobic fractions of NOM.

Most of these methods are able to give information on the fractions of natural organic matter by isolation based on polarity and charge into the following six fractions: hydrophobic acid (HPOA), hydrophobic base (HPOB), hydrophobic neutral (HPON), hydrophilic acid (HPIA), hydrophilic base (HPIB), and hydrophilic neutral (HPIN) [3]. There are several reports on the use of NOM fractions instead of NOM. Rapid Fractionation explains natural organic matter as a mixture of four organic fractions, very hydrophobic acids, slightly hydrophobic acids, hydrophilic charged and hydrophilic neutral, by using adsorption on different resins[1] . This current study is focused on

separating NOM into the strong hydrophobic fractions, humic and fulvic acid, providing unique information on each fraction by characterization.

This was achieved in a few steps here namely; the NOM was separated from the water sources. It was further separated into humic and non humic substances, of which the non humic substance was of no interest as far as this study is concerned. The humic substances were further separated into the hydrophobic acids, namely, the humic and fulvic acids. One of the challenges in the study of NOM has always been isolating it whereby a sufficient amount of NOM, free of inorganic species can be found. Although no single technique can be used to quantitatively (approaching 100% recovery) and efficiently isolate and separate NOM from inorganics, a very high amount of dissolved organic carbon was separated using this method. This is particularly good for Eskom since they are looking for simple methods that can be used on a routine basis to characterize and estimate the molecular sizes of the dissolved organic matter in their water.

There are several reports on the use of three or more resins to achieve good separation of NOM [10, 15, 16, 17, and 18]. These include Nkambule *et al* [10], who used three types of resins, namely; XAD-7HP, Diaino-WA-10 and DOWEX 88. NOM was divided into six fractions, the hydrophobic neutral, hydrophobic base, and hydrophobic acid, hydrophilic acid, hydrophilic base and hydrophilic neutral. The DOC (Dissolved Organic Carbon) of each NOM sample was measured with a total organic carbon analyzer and UV/visible spectrometer. Results showed that the non-biodegradable organic carbon and the biodegradable organic carbon are 70% and 30% respectively. Marhaba *et al* [15] isolated NOM from locations within two surface water treatment plants, which used the same source water. Resin adsorption methods were used to isolate and enrich six fractions. The DAX-8 resin was used after purification by soxhlet extraction. The above six fractions were isolated on the basis of their chemical characteristics. The hydrophobic base fraction was found to be in the range of 0-6% of DOC, the hydrophobic acid 8-12% of DOC; the hydrophobic neutral 13-22% as

percentages of DOC, the fractions were found to be in the ranges shown. The hydrophilic base ranged between 4-6%, hydrophilic acid 44-55%.

Esteves *et al* [16] isolated dissolved organic matter from water using a two column array of XAD-8 and XAD-4 resins arranged in series. The hydrophobic organic acids composed of humic and fulvic acids and were isolated from the sample on XAD-8 followed by the isolation of more hydrophilic acids on XAD-4. Tero e*t al* [17] isolated samples with two different chromatographic methods: (1) non-ionic sorbing solid (standard XAD technique) with pre-acidification of a water sample to pH 2; and (2) weakly basic anion exchanger (DEAE, diethylaminoethyl cellulose) without any pre-acidification. The structural analyses and statistical calculations on the different samples support the postulation that the structural composition of the DEAE isolate corresponds to a certain average of the four different acidic XAD fractions.

Ma *et al* [18] used the XAD-8 resin together with the cation exchange resin technique and concentrated dissolved organic matter from natural waters. There was a larger amount of fulvic acid fractions in natural waters which accounted for fulvic acid (FA) and hydrophilic (HyI) fractions were isolated and purified by 54 ± 68% of the total amount of dissolved organic carbon (DOC).

2.1.1 **Evaluation of isolation methods**

Although these methods were used successfully by many researchers, there are disadvantages associated with the methods as noted by Orr [19] as follows:

1. There exists no universal fractionation protocol.

3. It is not suitable for waters with a TOC less than 5 ppm.

4. Extremely low and high pH conditions are required.

Metilainen [5] also mentioned that these methods do not isolate 100% of the dissolved organic matter and they expose the NOM to extreme pH levels and how these changes in pH during isolation and fractionation may change the structure and reactivity of natural organic matter. It's also been reported that although these methods of great use, they contain hard labor and are time consuming in the resin clean up and fractionation procedures [3].

2.2 **DOC and TOC analysis**

Total organic carbon (TOC) is the amount of carbon bound in an organic compound and is often used as a non-specific indicator of water quality. Organic molecules are fed into the water from the source water and from purification and distribution system materials. Total organic carbon can also be useful in controlling processes that monitor the performance of purification units. Dissolved organic carbon (DOC) is composed primarily of two categories of substances: (i) non-humic substances, a class of compounds that includes carbohydrates, proteins, peptides, fats, pigments and other low molecular weight compounds, and (ii) humic substances which contain most of the organic matter in waters, and consist of colored hydrophilic and acidic complexes ranging in molecular weight from the hundreds to thousands.

Most analytical methods that are used to determine the organic matter in water actually establish the carbon content. Carbon, being the essential element of organic compounds, is also found in the environment as a component of inorganic species. When determining the carbon content then, it is important to differentiate organic and inorganic forms. TOC and DOC are the most convenient parameters for use in the overall study of treatment processes and effects on NOM removal [5]. Organic carbon is oxidized to produce carbon dioxide by UV promoted or heat catalyzed chemical oxidation with a per sulphate solution. The carrier gas delivers the combustion products to the cell of a non-

dispersive gas analyzer, where carbon dioxide is detected. Inorganic carbon is removed by acidification and purging [19].

Nkambule *et al* [10] used dissolved organic carbon (DOC) as an indicator of content of organics in the water samples. The DOC of each NOM fraction was measured with a total organic carbon (TOC) analyzer. Measurements were carried out in triplicate and more replicates performed where need arose. Harhoff *et al* [21] tracked the removal and transformation of NOM during full scale treatment using DOC measurements. DOC varied with the type of water from approximately 0.5mg/L for colored water samples to over 30 mg/L for swamps. This was one of the first studies to combine particulate organic matter with dissolved organic matter fractionation into a total organic matter fractionation and characterization [22].

DOC concentrations of water samples (filtered through 0.45 μm) were determined using a total carbon analyzer and indirectly by measuring the UV absorbance at 254 nm using a UV/VIS spectrophotometer [23]. In the present study, dissolved organic carbon was measured to assist in the calculation of SUVA, which is specific UV absorbance. The latter gives an indication of the quality of water by estimating the aromatic content in the water.

2.3 **Molecular weight determination**

Many analytical methods have been used to measure the molecular size distribution of humic substances. These include High Performance Size Exclusion Chromatography (HPSEC) and Ultra membrane filtration (UFF). Disadvantages of these techniques are;

- While the MW can be determined, statistical averages cannot be calculated. Also, the overall shape of the MW distribution remains unknown [66].

- The molecular sizes of humic and fulvic acids as measured by these methods were between 500 and 1000 000 g/mol. In aquatic environments, the molecular sizes of humic acids are less than 10 000 g/mol [24].

Understanding the disinfection by-products characteristics and molecular weight (MW) distribution of natural organic matter (NOM) assists in the optimization of water treatment processes. This also assists to minimize the formation of DBPs as the molecular sizes of these substances are directly related to the formation of DBPs. The fractionation of NOM by SEC has been used as early as the 1960s [25]. Polydispersity of humic substances and the proportions of hydrophobic and hydrophilic fractions are important parameters to determine the solubility of humic acids and, as a result, their migration down the soil profile [26]. While many DBP precursors can be eliminated or reduced with treatment, very high molecular weight humic acids and lower molecular weight material (< 500 Da) may be more difficult to remove. Certain molecular weight fractions are preferentially removed using particular treatment processes. This points to the importance of characterizing NOM by its molecular weight distribution [27].

The molecular size distribution of NOM has also been found to affect the adsorption of trace micro pollutants such as pesticides and taste and odor compounds. Several studies have revealed that the low molecular weight NOM fractions show the greatest level of competition for adsorption sites because they can access the same pores as the contaminant [27]. Molecular weight distributions of NOM have been measured using size exclusion chromatography (SEC) and other analytical techniques. The present study also applied this technique to measure the molecular size of NOM. However, the difference is that UV and ELSD were used in series.

2.3.1 **High performance size exclusion chromatography methods**

High performance liquid chromatography is non-destructive, relatively fast and requires no sample pre-treatment. In general, this technique involves passing a liquid sample over a solid adsorbent material packed into a column using a liquid solvent flow. Each analyte in the sample interacts slightly differently with the adsorbent material, thus retarding the flow of the analytes. If the interaction is weak, the analytes flow off the column in a short amount of time, and if the interaction is strong, then the elution time is long.

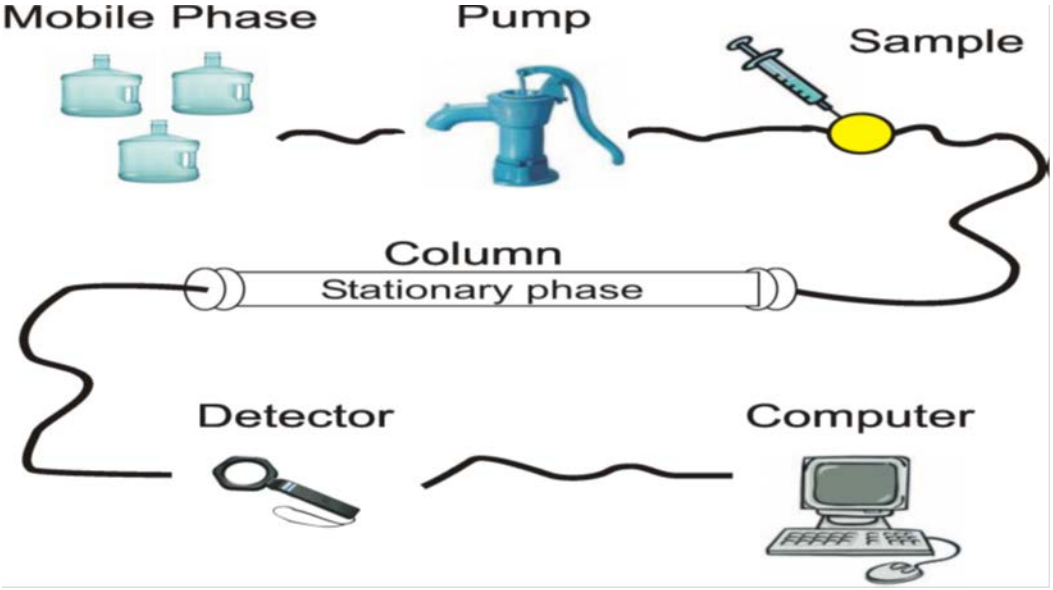

Fig. 2.1 Schematic diagram of an HLPC

The parts of an HPLC set up are: sampler, pumps and a detector. The sampler brings the sample mixture into the mobile phase stream which carries it into the column. The pump delivers the desired flow and composition of the mobile phase through the column. The detector generates a signal proportional to the amount of sample component

emerging from the column, hence allowing for quantitative analysis of the sample components. A digital microprocessor and user software control the HPLC instrument and provide data analysis. There are various types of high performance liquid chromatography, namely; partition chromatography, normal phase chromatography, high performance size exclusion chromatography (HPSEC).

High performance size exclusion chromatography (HPSEC) is used to obtain the apparent molecular weight distribution (AMWD) of natural organic matter (NOM). The size exclusion chromatography theory (SEC) is based on differences in molecular size. When a sample is applied to the SEC column, the components are eluted in order of decreasing molecular sizes [5, 25, and 28]. Separation is achieved by the differential exclusion from the pores of the packing material of the sample molecules as they pass through a bed of porous particles. HPSEC is commonly used to determine the molecular weight distribution (MWD) of NOM from a variety of aquatic and terrestrial environments. Molecular weight (Mw) is the weight of the molecule to which the 'average' atom belongs, whereas number average molecular weight (Mn) is the weight of the 'average' molecule in a mixture. For a pure substance with a single molecular weight, Mn is equal to Mw. For a mixture of molecules, Mn <Mw and p> 1 (polydispersity) [29]. Literature values indicate that the molecular weight of NOM can vary from a few hundred to greater than 100 000 Da and is highly polydisperse in nature. However, recent data show that molecular weights of less than 10 000 are more likely [27].

The advantages of HPSEC over other analytical techniques comes from small sample volumes, a minimal amount of pre-treatment, ease and speed of analysis and the ability of application of several detectors, i.e. UV-Vis, fluorescence and dissolved organic carbon (DOC) on-line analyzers [5, 30, 31and 32]. This technique is simple and informative [1]. This method can be used, not only to determine the MWD, but also, Mw and Mn. This is an advantage as compared to other methods. Another advantage is that it is possible to carry out other analyses without further isolation of DOM. Furthermore, its

relatively ease of operation, availability of equipment, better reproducibility, high analysis speed and small sample requirement make suitable for applications [33]. The present study used HPSEC to characterize the hydrophobic acids (humic and fulvic acids) according to their size using the evaporative light scattering detector and UV detection.

2.4 **Detectors used in HPSEC**

Several detectors have been used with HPSEC including multi-angle light scattering detector (MALS), refractive index (RI), evaporative light scattering detector (ELSD) and online DOC analyzers. Variable wavelength UV-VIS detector is amongst the most commonly used in HPSEC measurements. They are simple, rapid and widely available [5].

2.4.1 **HPSEC with UV-VIS detection**

UV absorption is commonly used to monitor the quality of water because humic substances have aromatic structure that is active in the UV light. The disadvantage is that, UV-VIS detectors are selective since they only detect analytes that absorb at the wavelength at which they are operating. Structures of humic substances contain a range of chromophores with different molar absorptivities, MW calculated for HPSEC chromatograms may be biased by the wavelength setting of the detector [31]. Due to the unequal molar absorptivities of organic components, it is inaccurate to evaluate the concentration of NOM by UV absorbance. The estimation of the concentration of humic substances is more reliable with larger molecular size fractions containing more aromatic structures than small fractions; smaller molecular size fractions make quantitation less reliable because of the weak UV 254 response caused by the scarcity of the aromatic structures.

Zhou *et al* [35] investigated the effect of increasing UV detector wavelength on the detection response for humic substances. They found that the Mn and Mw values increased with increasing wavelength, making MW determinations by this method unreliable [36]. Carson [27] noted that SEC-UV is not quantitative due to the varying absorbance of the different materials present as NOM in each water sample. However, on account of the nature of absorbance spectrophotometry and the diverse composition of natural organic matter in water, the UV method cannot be used for quantitative determination but may be used as a qualitative tool. This is why, in this study, this detector was simply used as a qualitative measure. It is for all these reasons that UV detection cannot be used solely, but can be used in line with another detector, that is to the coupling of UV with modified online detectors.

2.4.2 **HPSEC with UV-VIS/ DOC detection**

An enhanced HPSEC-UVA system employing a modified commercially available DOC detector yields data which leads to a better understanding of the qualitative and quantitative properties of natural organic matter (NOM) in water samples. This is so because it detects aromatic and non-aromatic fractions of NOM as a function of molecular weight (MW). The detection of DOC using the HPSEC system coupled with the UVA and the DOC detection has been well documented [31]. The advantage of these methods is that the detector signal is directly proportional to the concentration of organic carbon in the eluent and, irrespective of functionality; any type of organic species can be detected [24]. This method can be used to establish, not only the molecular weight distribution but also Mw and Mn, which gives an added advantage compared to other techniques. Another added is being able to carry out multi analyses without further isolation of DOM [31].

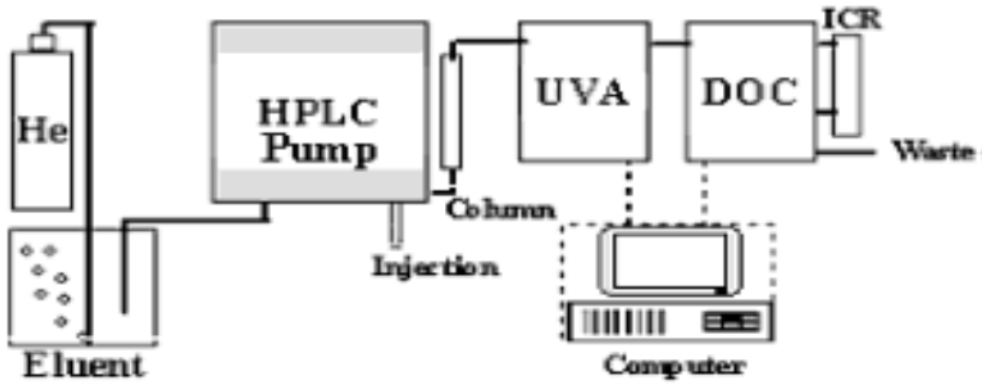

Fig. 2.2 Schematic of HPLC-UVA/DOC [40]

The only disadvantage of this method is that it assumes that the extinction coefficient is constant over the course of elution; such an assumption is likely to introduce some error in the analysis, so that Mw determinations are better viewed as relative rather than absolute. Swietlik *et al* [30] seemed to have used the same method for NOM characterization, that is, HP-SEC with UV-detection. They found that all NOM fractions were composed of molecules with relatively small molecular sizes. Thus, the second wavelength (220 nm) was chosen to observe the MWD of low molecular weight NOM fractions due to their relatively higher absorptivity at 220 nm.

2.4.3 **HPSEC with fluorescence detection**

Fluorescence in humic substances which contain aromatic carboxyl and phenolic groups is very sensitive. Using fluorescence in water analysis is becoming increasingly widespread due to it being able to interpret dissolved organic carbon fluorescence properties and high instrument sensitivity. Fluorescence spectrometry permits high optical resolution and the determination of excitation-emission matrices (EEMs). The fluorescence in different spectral regions is associated with different types of functional groups [35]. The UV detector coupled with the fluorescence detector can give much

more detailed information on the properties of NOM. UV and fluorescence detection can monitor UV-absorbing organic materials such as humic substances after separation on a SEC column. HPSEC with fluorescence detection is well suited to site-specific speciation of organic materials because it can resolve different fluorophores within organic materials.

Fluorescence detection has the additional advantage of being an order of magnitude more sensitive than UV detection [38, 39, and 40]. Lanxiu *et al* [41] used an HPSEC system that consisted of two detectors connected in series; a UV-VIS variable wavelength detector set at 254 nm and a fluorescence detector. Fluorescence detection results showed that there was no peak at the exclusion limit indicating that high molecular weight DOM did not give off fluorescence. One of the disadvantages is that high molecular weight NOM may not be detected by fluorescence.

2.4.4 **HPSEC with refractive index detection**

Some studies show that that the RI detector might be more usable in HS detection compared to UV, as the RI has been found to be more sensitive in detecting HA [39] . Luste *et al* [42] used a combination of the UV and RI detectors .The findings were that the effect of the chromatograms when UV254 was used as a detector are more explicit compared to chromatograms from RI measurements. There were also some problems with peak integration in RI measurements. Toshiyuki *et al* [43] used HPSEC with refractive index, and compared the results with that from MALDI-TOF-MS. Findings were that the application of HPSEC to humic substances often yields a wide variation in measurements for the same sample under different analytical conditions [44].

2.4.5 **HPSEC with evaporative light scattering detection**

In combination with HPSEC, a light scattering detector can provide information on the size and mass of macromolecules, which includes distribution of molecular weights and mean square radii.

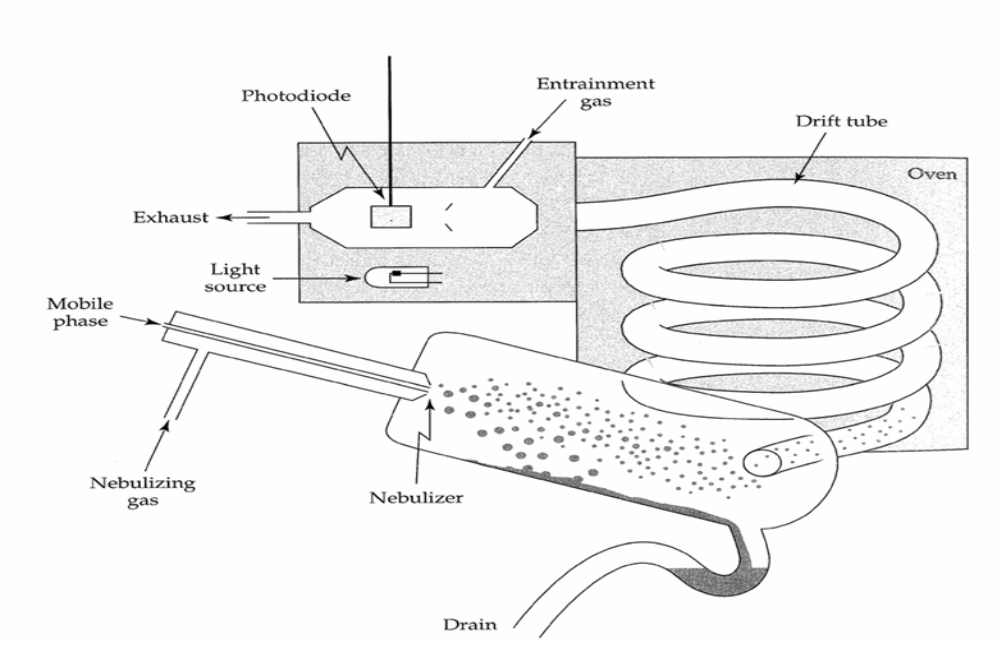

Figure 2.3: A schematic diagram of an ELS detector

Evaporative light scattering detectors measure the amount of light scattered by the particles which remain after the sample has been dried through evaporation. Analysis using the ELSD involves three stages: nebulization of the sample, evaporation of the mobile phase and detection of the light scattered by the dried particles. Any analyte less volatile than the mobile phase will be detected. A search of available databases brought up one report on the use of the ELSD to determine NOM. This involved the use of ELSD to estimate AMW.

Below are some of the advantages that the ELS detector provides:

- Universal, it responds to all compounds in the mobile phase
- Not dependent on spectroscopic properties of analyte
- Not susceptible to baseline drift during gradient elution, temperature or solvent pump fluctuations
- ELSD is compatible with a much wider range of solvents compared to the refractive index detector
- No interference from solvent front peaks (enables fast analysis)
- Flow rates up to 5ml/min can be achieved with no effect on baseline stability
- Ideal for high throughput screening and quantification [45]

The evaporative light scattering detector, which is a universal detector, provides high sensitivity and ease of operation since it has no base-line drift and has rapid equilibration and compatible with gradient elution [46]. Carson [27] used the HPSEC system equipped with ELS detection and discovered that it works more like the DOC detection except it is considered a universal detector since it detects anything in the sample which in not more volatile than the mobile phase.

2.5 **Color**

NOM is the major contributor of the brownish yellow color in water. Measurement of color, therefore, can give some indication of the amount of NOM in water [5]. Although much research has been performed on the nature of organic color in water during the past 60 years, important chemical information on these color bodies is still lacking. The dissolved organic coloring material in water consists almost totally of humic substances [47]. Humic acids are dark brown to black in color while fulvic acids are light yellow to yellow-brown [48]. Humic waters are yellow-brown and a raw

correlation exists between the darkness of water and its humus content. Thus, measuring the color of water is largely accepted as an easy way to estimate the humus content in natural waters. Molecular weight and aggregation of humic matter are positively correlated with color.

Color can be measured with a comparator, i.e., by comparing the color of water visually to the color of hexachloroplatinate and cobalt ions in solution or to calibrated colored glass discs. Results of color measurements are given as cobalt-platinum units (mg Pt/l) which are equal to Hazen-units [49, 50] .The visual method is not particularly precise because of the many associated disadvantages. This is the reason why absorbance measurements by spectrophotometry have been introduced instead [49]. Color measurements are the most common, and provide a simple index for assessing the concentration of humic substances in natural waters. Traditionally, the color of natural waters has been measured by visual comparisons between the water and various colored solutions. Europeans used the methyl orange and the Forel-Ule methods, whereas most North Americans used the Hazen method, which incorporates the use of platinum-cobalt standard solutions (mg/L), and records the results as Pt units [49, 50].

A study conducted by Hautala *et al* [49], where two methods were used for measurement of color: comparator and color measurements by spectrophotometry showed a good correlation. Measurements were performed at five selected wavelengths: 400, 410, 456, 465 and 490 nm and at one wavelength out of the visible range (350 nm). Concentrations of isolated/fractionated humic materials for absorbance measurements were between 17.2 and 21.2 mg/l. Also, in another study, [48], color was measured in water using a method that is based on physical observation using platinum-cobalt solutions which produce a yellowish brown color in water similar to that of humic substances. However, this method has the disadvantage of subjectivity. At the low color values of many natural waters, visual comparison is almost impossible, hence an alternative method of calibrating spectrophotometric readings was used [51, 52].

Although visual color techniques are still used, spectrophotometric methods are now more commonly utilized.

2.6 Fourier transform infrared (FTIR) spectroscopy and attenuated total reflectance methods

FTIR has been widely used for the characterization of NOM. Samples exposed to infrared light absorb energy corresponding to the vibrational energy of atomi-atom bonds. The resulting absorption spectrum is a unique fingerprint of compounds, allowing the identification of both inorganic and organic functional groups. The interpretation of the analysis may be difficult, however, because of severe overlapping of characteristic spectral features due to the complexity and poly functionality of NOM [5, 11]. IR is used to identify the functional groups in organic compounds based on the characteristic frequency of bond vibrations caused by stretching and bending motions. When a compound is subjected to radiation at a frequency that is equal to the bond vibration frequency, the molecule absorbs energy and exhibits an absorption band in the infrared spectrum. Each functional group has its own specific absorption bands which are indicated by a wavenumber [53].

Aqueous samples contain hydrogen bonds between hydroxyl groups. These bonds have a broad spectral response that masks the response of the dissolved organic components in FTIR measurements. To overcome this, several researchers have used methods that concentrate the water sample into a solid for analysis. This involves freeze drying or air-drying the water sample to remove water and making pellets using spectral grade potassium bromide [53]. Xing, [53] did DOC measurements using synchrotron light with FTIR. Results showed that the high sensitivity synchrotron beam light caused significant noise in the spectral response; therefore sample measurements were better conducted with lower sensitivity conventional light source. Dongjin *et al* [54] characterized NOM in river water using FTIR. No record of sample preparation is

documented but results showed that the NOM in Yeongsan River consisted mainly of hydrophilic substances. The fact that the O-H stretch is shown to be highest and widest may mean that it shows hydrophilic acid compounds. On the other hand, C=O stretches occurring at frequency range 1640~1585cm^{-1} were ketones and quinines that were aromatic or alicyclic and were indicative of hydrophobic compounds. However, the main disadvantage of this method is that detailed interpretation of infrared spectra with respect to NOM structural features is challenging due to significant overlapping of individual absorptions at different vibrational modes by different functional groups.

2.7 **Aromaticity**

Aromaticity is a chemical property describing the way in which a conjugated ring of unsaturated bonds, lone pairs, or empty orbitals exhibit stabilization stronger than would be expected by the stabilization of conjugation alone. Visible and ultraviolet absorbance has been widely used to characterize raw waters in general. Humic substances are generally known to show strong absorbance in the UV-Visible range because of the presence of aromatic chromophores [11]. This is usually measured at 254 nm and an increase in absorbance indicates an increase in NOM aromaticity and unsaturated carbon bonds. On account of the good correlation with dissolved organic carbon, color and UV-absorption (UV-abs) are also used as surrogate parameters to DOC [55]. It is commonly accepted that some NOM compounds, notably those with light absorbing chemical structures such as aromatic rings, are the most likely to react to form DBPs. These structures are known to absorb UV light at specific wavelengths, including 254 nm. Therefore, it is also common to quantify NOM by measuring the amount of UV light it absorbs (UV254). UV254 can be normalized to DOC to yield a specific UV absorbance (SUVA) value, which can be used to predict the aromaticity and treatability of the NOM being measured.

SUVA is described by Nkambule [10] as a tool that gives an indication of the amount of humic substances against non-humic substances in the natural organic matter and that it can also be used to indicate the treatability of water. He used this tool to determine if there were any humic substances in the water and the finding was that after treatment, SUVA values in all samples decreased. In another study of aromaticity, NOM samples were separated into six fractions using resin fractionation. The DOC of each fraction was measured at 254 nm. Results showed that UV 254 was lower in the treated water samples than in the raw water samples from each community [54]. According to Swietlik *et al* [30], specific ultraviolet absorbance (SUVA) at 254 nm provides a quantitative measure of aromatic content per unit concentration of carbon hence in this study, this served as a very useful quantitative tool especially since there was no calibration done to estimate the concentration of these. However, one of the disadvantages of SUVA is that in general, UV spectroscopy has little value for studying functionality in DOC and, unlike IR and NMR spectroscopy, cannot be used for the direct determination of functional groups in these materials.

2.8 **Nuclear magnetic resonance (NMR) spectroscopy**

The NMR phenomenon is based on the fact that nuclei of atoms have magnetic properties that can be utilized to yield chemical information. Magnetic nuclei in a magnetic field absorb and re-emit electromagnetic radiation. This energy is at a specific resonance frequency which depends on the strength of the magnetic field and the magnetic properties of the isotope of the atoms; in practical applications, the frequency is similar to VHF and UHF television broadcasts (60–1000 MHz). NMR allows the observation of specific quantum mechanical magnetic properties of the atomic nucleus. Many scientific techniques exploit NMR phenomena to study molecular physics, crystals, and non-crystalline materials through NMR spectroscopy. NMR is also routinely used in advanced medical imaging techniques, such as in magnetic resonance imaging (MRI) [56].

The heterogeneity and complexity of NOM typically limit the level of detail available from ^{13}CNMR to broad functional group classes such as aromatics or aliphatics. However, there is potential to improve this level of detail toward definitive identification of specific signature compounds by adsorptive fractionation. In particular, selective removal of hydrophobic NOM isolates the narrower class of hydrophilic compounds that dominate residual NOM after drinking water treatment [31]. Nuclear magnetic resonance (NMR) spectroscopy has been used for decades to study the functional groups in NOM. The NMR technique is especially useful in combination with elemental composition data, apparent molecular weight data, or IR spectroscopy data of fractionated NOM. NMR spectroscopy of NOM can be done on both solid and aqueous-phase samples. Solution state NMR is applicable to soluble samples like humic and fulvic acids; however, the solid sample enables various ^{13}C NMR experiments, including spectral editing of strongly overlapping bands, because of higher NOM concentration in the solid sample. In addition to ^{1}H and ^{13}C NMR techniques, ^{15}N NMR has also been used in NOM research [2].

Leenheer *et al* [57], Wong *et al* [31] and many other researchers, used NMR to characterize DOM fractions from a lake. Mao et al [58] also used solid state NMR. One challenge that was faced in this study was the problem of overlap between aromatic and alkyl carbon resonances around 90-120 ppm in 13C NMR spectra which was solved by a ^{13}C chemical shift anisotropy (CSA) filter technique.

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CHAPTER 3

EXPERIMENTAL

3.1 Sampling

3.1.1 **Preamble**: **Seasonal variations of NOM**

Natural organic matter (NOM) shows different compositions and distributions by region, season and river basin [1, 2]. Water quality is known to be worse in terms of having a high organic content, in the wet season compared to the dry season. Except in November, the discharge and water color correlation in summer and autumn seasons seems stronger and positive. The production of HS in the previous month also affects the concentration and its correlation with flow of the present month. (For example, generally the May's temperature is comparable with September [1].

Dongjin *et al* [2] conducted a study in spring and found out that all investigated sites were shown to be more hydrophilic than hydrophobic. Overall hydrophilic and transphilic characteristics in the river were stronger than its hydrophobic characteristics in August. Schumacher et al [3], after sampling in spring and fall seasons, concluded that there was no significant difference in the chemical composition or carbon isotope signature of the DOM sampled in spring and fall seasons [3]. Thapa *et al*[4] analyzed natural organic matter (NOM) in four different seasons of a year by several NOM characterization methods including GPC and pyrolysis GC/MS. Except for one area, the change in NOM characteristics in the lake water was found to be more pronounced in different seasons than at different locations [4]. In this current study, sampling was done in October; which is also in spring. Conclusions about the comparison of the molecular weight of these, with seasons and with work done by other researchers are made on the conclusions and recommendations.

3.1.2 Sampling and Sample storage:

Water samples were obtained from the Vaal dam in Mpumalanga. The Vaal dam is one of the dams in which the power utility Eskom draws water for its power stations. These were then divided into two categories: original filtered and unfiltered water samples. Sample pre-treatment was the same for both. They were collected using plastic as its non-stick and non-contaminating characteristics are a great advantage for water and sediment sampling. Original samples were filtered immediately after collection through a 0.45 µm membrane filter to remove particulate matter. Samples were stored in a refrigerator at 4°C for not more than 48 hours before analysis. Samples were stored in dark to prevent interferences.

3.2 Methods

3.2.1 Separation and fractionation of the NOM fractions

3.2.1.1 Resin wetting

This procedure was adapted from the supplier's recommendation, (Sigma Aldrich). The dry resin was transferred into a 500 ml beaker. Sufficient methanol was added to cover the resin (2-5 cm depth). The resin was stirred gently for a minute to ensure complete mixing. It was then allowed to stand for 15 minutes. Most of the methanol was carefully decanted and replaced with deionized water. The mixture was stirred and allowed to stand for 5-10 minutes.

3.2.1.2 Resin clean-up

A water line was attached to the bottom of the column. A slow upward flow of deionized water was introduced. The flow was increased until the entire resin was suspended. The flow was maintained until all the air bubbles were dislodged. Resin fines

passed out of the top of the column. The flow was stopped and resin was allowed to settle. The water level was then adjusted to 2.5 cm above the resin bed. This was adapted from the supplier's recommendation.

3.2.1.3 Column preparation and equilibration

The cleaned resin was slurry packed into a 1 cm x 30 cm column resulting in an 8 cm sorbent bed. The packed column was rinsed with purite ultra-pure water until it was completely free of methanol.

3.2.1.4 Sorption

50 ml of the pre-filtered/unfiltered water sample was adjusted to pH 2 and was passed through the column at a flow rate of 2ml/min. The column effluent was collected and acidified to pH 2 and labelled as a portion of non-humic substances (NHS). The CO_2 of this effluent was removed by purging with nitrogen for 10 minutes. The adsorbed humic substances were eluted with 30 ml 0.1 M NaOH at a flow rate of 1ml/min. The eluate was acidified to pH 2 and labelled as humic substances (HS). Similarly; the CO_2 of this eluate was removed. This eluate was re-concentrated on a smaller XAD-8 column and eluted with NaOH and acidified [5].

3.2.1.5 Fractionation of Humic substances into Humic acid and Fulvic acid fractions.

A portion of the eluate of humic substances eluate sample was acidified to pH 1 and left in dark for 24 hours to precipitate humic acid. The precipitated humic acid and the supernatant fulvic acid solution were separated by filtration through 0.45µm membrane filter. The CO_2 from the supernatant was removed as described above and the remaining solution was referred to as FA (Fulvic acid) [5]. Procedural blanks and

standard reference materials (Suwanee river humic acid) were analyzed with each batch of sample. The separated fractions were analyzed by HPSEC (ELSD/UV detectors).

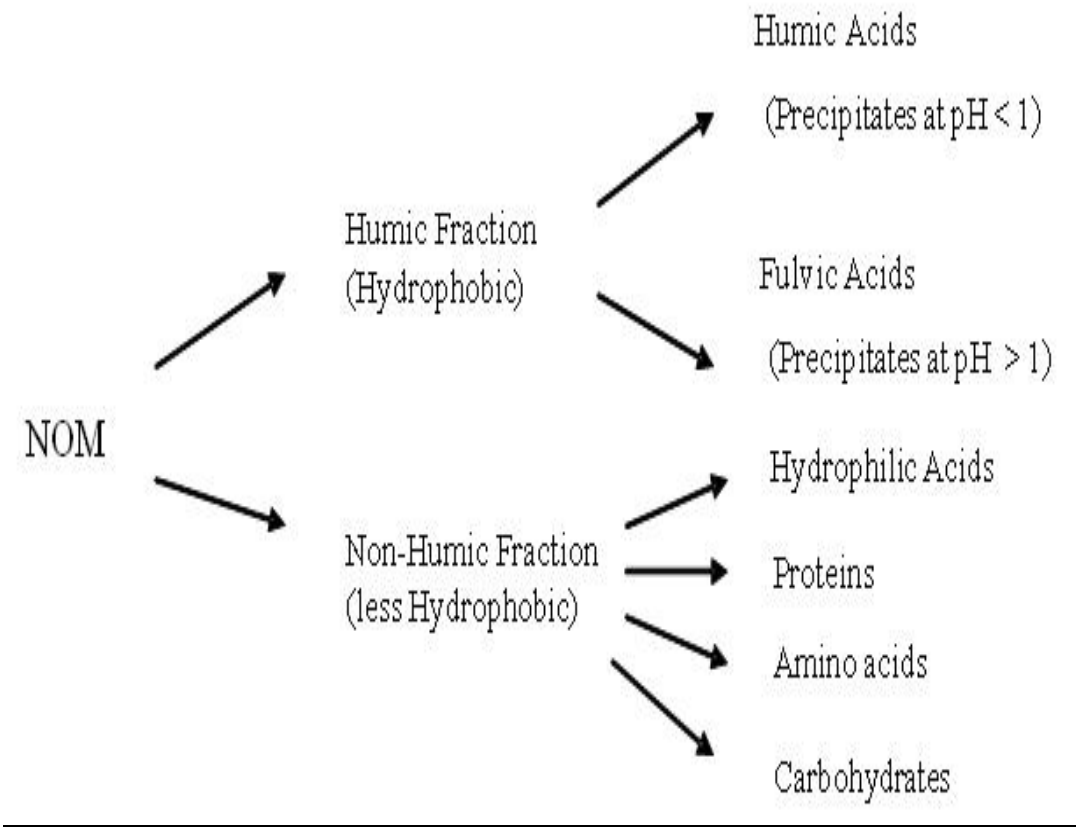

Figure 3.1: Schematic representation of the fractionation of NOM

3.3 Equipment, Chemicals and materials

3.3.1 HPSEC equipment and materials

High performance size exclusion chromatography (HPSEC) was used in this study. The HPLC instrument was equipped with a Binary LC pump 250 (Perkin Elmer Massachusetts USA), a UV detector (LC 235 Diode array, Perkin Elmer Massachusetts USA), evaporating light scattering detector (Sedex 75, Sedex France). The device used for injecting standards and samples comprised the Rheodyne 7010 injector equipped

with a 20 micro liter (µl) sample loop (California, USA). The column used was the Polysep-GFC-P linear column (Sigma Aldrich, 300x780 mm Canada, USA) and Polysep-GFC-P guard column, sigma Aldrich, 35x7.8 mm Canada, USA). Purite Ultra-pure Water System at 18 MΩ water was used. A glass column (30 cm lenght, 1cm internal diameter) was used for the separation, adsorption and fractionation of the water samples. A degassing and filtration apparatus used was purchased from Membrane solutions (Ohio, USA). A snijders hotplate magnetic stirrer (34532) was used for heating up solutions.

3.3.2 **HPSEC chemicals**

The following chemicals and material were used in the HPSEC technique: A polymeric Superlite DAX-8 resin purchased from Supelco: sodium hydroxide pellets from Fluka: 65% hydrochloric acid (Fluka): methanol (Fluka): ethanol (Fluka), analytical reagent (AR) grade of disodium hydrogen phosphate was used as a buffer: methanol (LiChrosolv, Merck, Darmstadt, Germany) was also used: polyethylene glycol/polyethylene oxide ready calibration standards (Fluka Analytical) were used for column calibration: suwanee river humic acid ll (2S101H) from the International Humic Substances Society: technical grade humic acid and technical grade, fulvic acids were both purchased from Sigma Aldrich: nylon membrane filters (0.45 and 0.2 µ) were from Membrane solutions. Syringes (2 mL) and disposable micropipette tips were obtained from Waters.

3.4 **Instrumentation**

3.4.1 **UV/VISIBLE spectrophotometer materials and chemicals**

Ultraviolet/visible spectrophotometer (Varian Cary 50 Conc model) was used for measuring the concentration of the humic acid and fulvic acid fractions. Hellma (100

QS) quartz cells (with 10 mm id) were used. Suwanee river humic acid ll (2S101H) from the International Humic substances Society was also analyzed as a reference standard. Technical grade humic and fulvic acids were used as standards for calibration.

3.4.2 **FTIR-ATR materials and chemicals**

A Varian 800 FTIR system (Scimitar series) equipped with attenuated total reflectance (ATR-PIKE Miracle TM 17633) from Pike Technologies was used for identification of functional groups. A Nuaire Glacier bio freezer was used to keep samples at -86 ° C. These were then then freeze-dried using the LTE Lyotrap freeze dryer; 15 ml plastic centrifuge tubes were used during centrifuge.

3.5 **Preparation of standards**

3.5.1 **Preparation of the Suwanee river Humic Acid Stock Solutions-192 ppm)**

The Suwanee river humic acid stock solution was prepared by introducing 28.3 mg of the dry Suwannee River humic acid powder (Standard II, International Humic Substances Society) into 52 mL of deionized water and stirring the solution for 2 hours. The solution was then filtered through 0.22 μm cellulose acetate membranes under vacuum. The pH of the solution was raised to 5.5 by adding NaOH, and the solution was subsequently stored in the dark at 4 °C [6].

3.6 Analysis- High performance size exclusion chromatography

3.6.1 Molecular weight determination by High performance size exclusion chromatography (HPSEC)

3.6.1.1 Column efficiency

100 ppm ethylene glycol standard was prepared by weighing 0.1 g of the standard into a 10 ml volumetric flask. This standard was prepared according to the column certificate of quality assurance. It was then injected into an HPLC under the following conditions;

Mobile phase: Water

Flow rate: 1ml/min

Detector: RI

Pressure: 136 psi

Amount (loop): 20 µl

3.6.1.2 LODs and LOQs

For the determination of the limit of detection and limit of quantification, for the humic and the fulvic acid fractions, serial dilutions were made from the stock solution of 100 ppm humic acid and 100 ppm fulvic acid standards (prepared according to the certificate of analysis provided by Sigma and Aldrich). From these, chromatograms of a 0.01 ppm standard and 1 ppm standards were used to determine the LODs and LOQs for humic acid and fulvic acid, respectively. The same was also done for the polyethylene oxide/polyethylene glycol standards that were to be used for calibration at concentrations of 0.001, 0.01, 0.1, 1, 10 and 100 ppm (PEO standards) were prepared. A chromatogram for 1 ppm standard was used to calculate the LOD and LOQ.

3.6.1.3 Column calibration with Polyethylene glycol/oxide standards

The PEO/PEG standards were prepared according to the Sigma Aldrich certificate of analysis. The Polyethylene glycol/Polyethylene oxide is supplied as a mixed standard in several configurations. Three or four standards are lyophilized in a 1.5 mL vial (black, blue and yellow); for 1 complete calibration, one of each of the black, blue and yellow vials was used. One set contains 30 vials for 10 complete calibrations. The certificate of analysis outlines the three (and four in the case of the yellow cap vial) standards included in each color coded vial.

Three standards per vial (four in the yellow capped vial) and three color coded vials (black, blue, and yellow) giving 10 total polymer standards. Each sample contains one high molecular weight one medium molecular weight and one low molecular weight standard so that the standards can be adequately resolved for one vial.

Table 3.1 PEO/PEG Mixed Standard 1 (Mp, Mw and Mn values)

Mp(Da)	Mw(Da)	Mn(Da)	Mass(mg)
1 015000	941 000	879 000	1.125
18 600	17 900	14 900	2.25
599	601	560	2.25

Table 3.2 PEO/PEG Mixed Standard 2 (Mp, Mw and Mn values)

Mp(Da)	Mw(Da)	Mn(Da)	Mass(mg)
478 000	496 000	339 000	2.25
86 200	87 800	75 800	2.25
6 690	6 550	6 170	2.25

Table 3.3 PEO/PEG Mixed Standard 3 (Mp, Mw and Mn values)

Mp(Da)	Mw(Da)	Mn(Da)	Mass(mg)
222 000	220 000	197 000	2.25
42 700	40 100	30 700	2.25
1 960	2 010	1 840	2.25
232	232	232	2.25

1500 ppm of each standard was prepared by adding 1.5 ml of water into each vial. These were then injected into an HPLC operated the following parameters.

3.6.1.4 ELSD conditions

Mobile Phase: 100% water

Column: polysep-GFC-P linear column 300x780 mm, polysep-GFC-P guard column

Injection Volume: 20µL

Flow Rate: 0.5 mL/min.

Gain: 6

Temperature: 40°C

Pressure: 3.2 psi

3.6.1.5 Sample analysis

After column calibration, samples prepared according to the procedure explained in 3.1.2 were analyzed by high performance size exclusion chromatography. The set up on the instrument was such that the two detectors, namely; the UV detector and the evaporative light scattering detector were reconnected in series. For all the samples,

measurements were made on both the detectors. For the UV detector, two wavelengths were used simultaneously. Measurements were made at 254 nm and at 280 nm to observe the differences in the absorption at the two wavelengths.

The following were the ELSD conditions:

Attenuation:	64
Temperature:	40 ° C
Gain:	9
Pressure:	3.3 bar
Signal :	10
Mobile phase:	100% Water
Flow rate:	0.5 ml/min
Column:	polysep-GFC-P linear column 300x780 mm, polysep-GFC-P guard column
Injection Volume:	20µL

The UV detector conditions were as follows:

Wavelength:	254 nm; 280 nm
Carrier gas:	Nitrogen
Mobile phase:	100% Water
Column:	polysep-GFC-P linear column 300x780 mm, polysep-GFC-P guard column

Injection Volume: 20µL

Flow rate: 0.5 ml/min

3.7 Ultraviolet/Visible (UV/VIS) Analysis

3.7.1 Preparation of standards

100 ppm Sigma Aldrich humic acid

The humic acid standard was prepared by dissolving 25.44 mg of the humic acid into 52 ml of water according to the Sigma Aldrich certificate of analysis. From this, serial dilutions were used to prepare a 10 ppm standard. From the 10 ppm standards, further dilutions were done to prepare standards for a calibration range. The working standards were 0.2, 0.4, 0.6, 0.8 and 1 ppm. The lowest and highest standards were run to determine wavelength of maximum absorption. A calibration curve was prepared with data from the above standards. The wavelength of the spectrophotometer was set at 254 nm.

1000 ppm Sigma Aldrich fulvic acid

The fulvic acid standard was prepared by dissolving 1.0526 g of the acid and making it up to 1L with deionised water. This solution gives a concentration of 100 ppm. The extra 0.0526 g compensates for the 5% impurities present in the fulvic acid. From this, serial dilutions were done to prepare a range of working standards with the following concentrations; 1.0, 1.2, 1.4, 1.6, 1.8 and 2 ppm.

3.7.2 Sample analysis

The lowest and highest standards were run to determine wavelength of maximum absorption. A calibration curve was prepared with data from the above standards, using deionized water as a reference. Samples prepared according to the procedure explained

in 3.1.2 were analyzed on a Varian UV/VIS spectrophotometer with a 10 cm length quartz cell. Absorbance values of the standards were used to determine the concentrations of the humic and fulvic acid in the samples. Similarly, an IHSS Suwanee river humic acid standard was run. Samples were analyzed within 48 hours of collection.

3.8 FTIR-ATR Analysis

3.8.1 Sample analysis

The Sigma Aldrich humic and fulvic acid standards, the Suwanee river humic acid and the sample fractions were centrifuged at 11 000 rpm for 30 minutes. These were then freeze-dried at 86 °C employing a Nuaire bio freezer. An infrared background was collected from the clean ATR crystal, using air. ATR-FTIR spectra of the humic acid fraction, the fulvic acid fraction, and the fulvic acid standard were collected. Similarly, a spectrum of Sigma aldrich humic acid standards, sigma Aldrich fulvic acid standard and the Suwanee river humic acid standard was collected in the transmission mode. The varian 800 FTIR system (Scimitar series) was set up to scan from 4000-400cm^{-1} and 15 scans were averaged at 1.0 $cm^{-1.}$ Infrared peaks were presented in the transmittance mode [7].

3.9 Total organic carbon and dissolved organic carbon analysis (TOC and DOC)

This was done in collaboration with Eskom RT and D laboratory. The data are given in appendix B1.

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Chapter 4

Results and discussion

4.1 Preamble: Separation and isolation of the NOM fractions

Humic substances were separated from the water source by the non-ionic macro porous DAX-8 resin which is a substitute for the most popular sorbent, the XAD-8 (poly methyl methacrylate) resin. This resin can be used to separate and fractionate natural organic matter into the six fractions: the hydrophobic neutral, hydrophilic acids, hydrophobic acids, hydrophobic base, and hydrophilic base and hydrophilic neutral. In this study, this resin was used primarily to separate the NOM into the humic and non humic substances, and to further fractionate it into the two acids of interest, namely the humic and fulvic acid. A sample of raw water was taken and filtered using a 0.45 μm membrane filter, to get dissolved organic carbon (DOC) which is defined as the organic matter that is able to pass through such a filter. The study focused more on the NOM that remains even after filtration of the water samples. 50 ml of the filtered water sample was adjusted to pH 2. This is done to protonate acid groups, prior to passage of the sample through the resin [1].

The sample was then passed through the column at a flow rate of 2ml/min. Adsorption columns generally perform well at lower flow rates, ensuring complete adsorption of the analytes. The column effluent was collected and acidified to pH 2 and labeled as a portion of non-humic substances (NHS). The CO_2 of the effluent was removed by purging with nitrogen for 10 minutes. This portion, which comprises mainly amino acids, peptides and sugars, was later discarded of as the interest of the study was not on the non-humic portion of NOM but the humic portion. At this pH, the humic substances are said to be adsorbed on the column. Since humic substances are defined as

the organic matter extracted from soil by 0.1 N NaOH, and also, in dilute sodium hyoxide, humic substances undergo dissociation of their acid functional groups, these were then eluted with 30 ml 0.1 M NaOH at a flow rate of 1ml/min.

Basic elution of the resin removes a mixture of hydrophobic acids (humic and fulvic acids) of relatively lower molecular weights [2]. It has been reported that by using sodium hydroxide to elute, more than 70% of the dissolved organic carbon can be eluted [3]. This eluate was acidified to pH 2 and labelled as humic substances (HS). A portion of the humic substances eluate sample was acidified to pH 1 and left in dark for 24 hours to precipitate humic acid. Humic acids are known to be the part of the hydrophobic fraction that precipitates at lower pH values, leaving the fulvic acids in solution. This was used as the operational distinction between humic and fulvic acids. Procedural blanks and standard reference materials (Suwanee river humic acid) were analyzed with each batch of sample. The separated fractions were analyzed by HPSEC (ELSD/UV detectors). As much as this resin fractionation has been found to be the most widely used technique to isolate and characterize natural organic matter (NOM) based on its hydrophobicity and hydrophilicity, it is also very time consuming [4].

Non humic substances can be bound covalently to humic substance and making it difficult to separate these two groups. However, these were separated on the basis of their solubility in aqueous acids and bases. The precipitate of the soluble fraction was treated to low pH and humic acid precipitated while fulvic acid remained in solution. The difficulty in separating NOM into identifiable components is a reflection of the complexity of NOM. Some of the drawbacks of this method were that there are high possibilities of contamination of the isolated fractions if the resin is not washed and cleaned thoroughly. However, there are advantages to using this type of fractionation, these include the fact that it can be done over a very wide range of pH and the fractionation method is simple.

4.2 **Analysis of the fractions**

The isolated fractions (humic and fulvic acid) were subjected to the following tests and determinations:

- Molecular weight determination
- Characterization by ATR-FTIR
- Characterization by SUVA(specific UV absorbance)
- Total and dissolved organic carbon determinations (TOC and DOC)

All of these are discussed below.

4.2.1 **Molecular weight determination**

Molecular weight (MW) is one of the fundamental properties that need to be known in order to understand the physical and chemical characteristics and chemical reactivity of humic substances (HS). Determination of the MW of humic substances is a difficult task because HS are a complex mixture of natural, heterogeneous organic materials with different structures and a broad molecular weight distribution (MWD) [5].

4.2.1.1 **Column efficiency**

The HPSEC (polysep-GFC-P linear) column was tested prior to use for efficiency (represented by the number of theoretical plates), using a 100 ppm ethylene glycol standard as shown in Fig. 4.1. The chromatogram below shows a good response consistent with one on the certificate of quality assurance. Column efficiency was calculated as shown in equation 2 below.

$$\mathbf{N = 16\ (t_R/W)^2 = 16\ (10.4/0.5)^2 = 6922.24\ldots..\ (2)}$$

In general, the bigger the number of theoretical plates, the better the efficiency of the column.

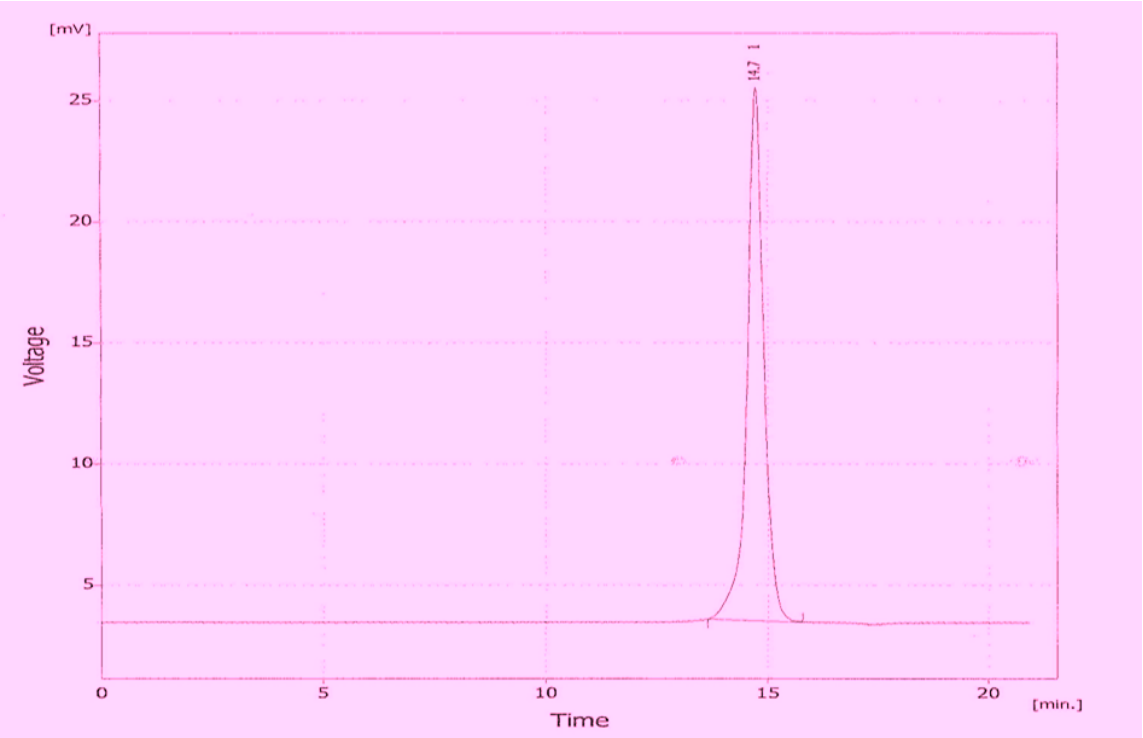

Fig. 4.1: SEC Chromatogram of 100 ppm ethylene glycol

4.2.1.2 **Column calibration**

The relationship between average molecular weight (AMW) and elution time for HPSEC results must be determined by calibration using compounds of known MW. Column calibration is necessary to establish if the analytes will fall in the range for determination. Fulvic and humic acids have low molecular weights and it is difficult to find standards for a suitable range. The molecular weights of the standards are either too high or too low. Hence a set of standards containing low, middle and high molecular weights had to be used. The ploysep- gfc column was calibrated to evaluate whether linear and consistent calibration results could be obtained with the polyethylene oxide/polyethylene glycol standards. Mixed PEO/PEG standards of molecular weights as shown in Table 4.1 were used for the investigation of the molecular size distribution of the isolated humic substances. The concentration of the standards was 1500 ppm.

Three mixed standards were used and each one of them contained different molecular sizes. Standard 1 had the following molecular weights, 10 15000 Da, 18600 Da and 599 Da. Standard 2 had these molecular weights, 47 8000 Da, 86200 Da and 6 690 Da. The last standard, standard 3 contained 222000 Da, 42700 Da, 1 960 Da and 232 Da. The results for each of these mixed standards were plotted on the PEO/PEG - calibration curve to determine whether good agreement could be attained. Several factors, especially the type of the calibration standard, have been reported to influence the MW determinations by HPSEC; however, log (MW) vs. retention time yielded an equation of the straight line as shown in Fig. 4.5 below. An excellent correlation was obtained with the PEO/PEG standards. The order of elution of components of the mixed standards is shown in Fig. 4.2.

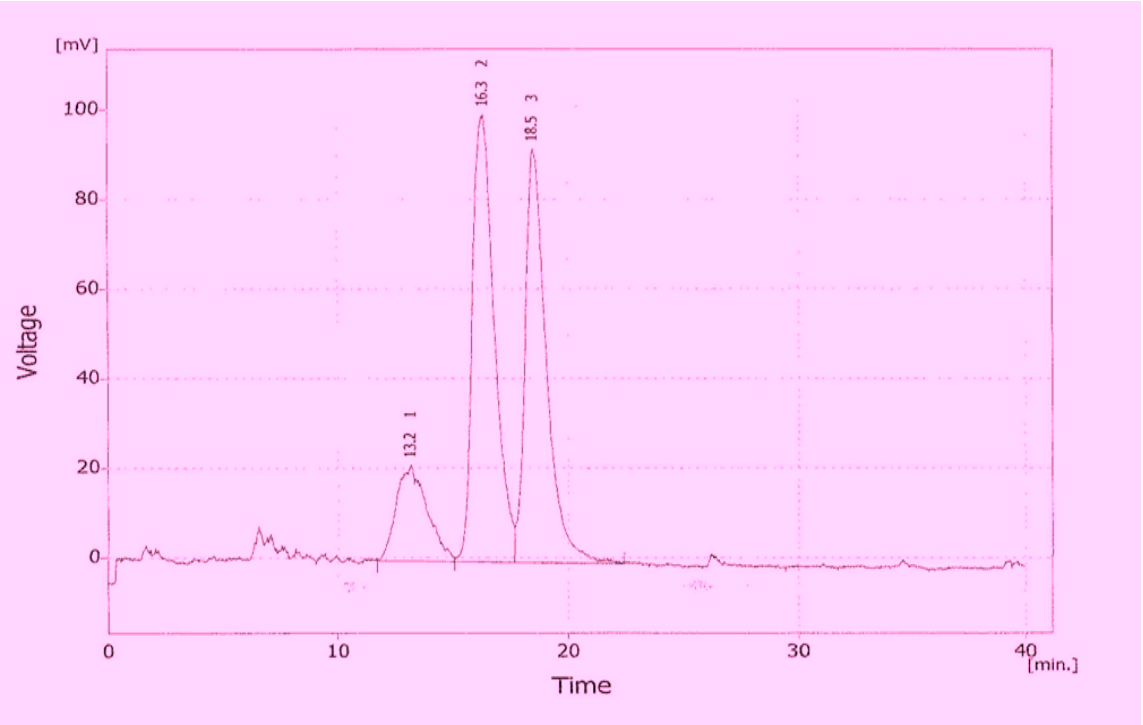

Fig. 4.2: HPSEC Chromatogram of PEO/PEG Mw Stds: Order of elution of peaks with molecular sizes-ELSD (Mixed standard 1: Mp values: 1 015 000 Da, 186 00 Da and 599 Da)

The order of elution of components of the mixed standards is shown in Fig. 4.3.

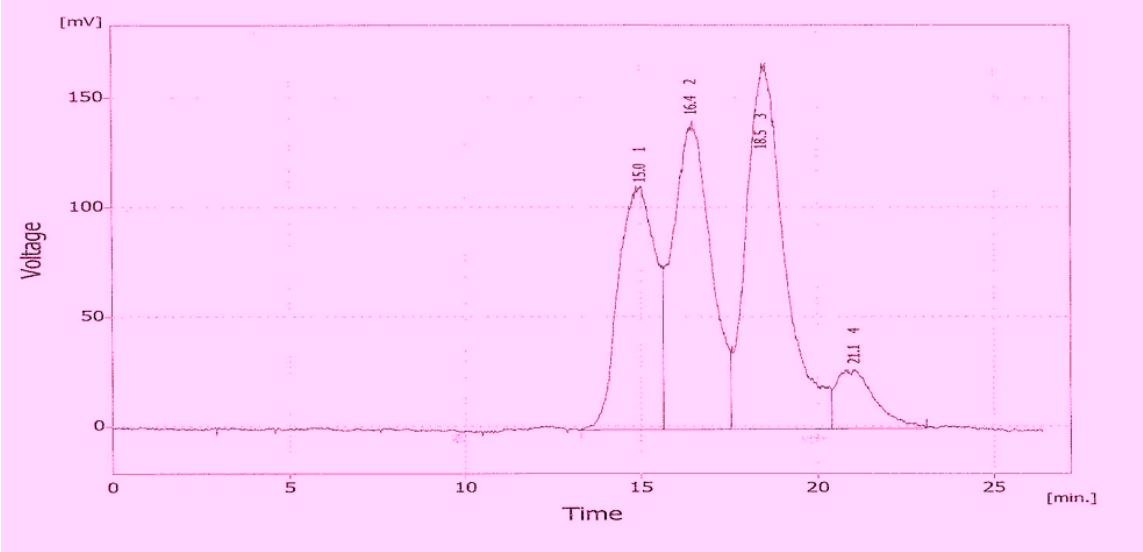

Fig. 4.3: HPSEC Chromatogram of PEO/PEG Mw stds: Order of elution of peaks with molecular sizes -ELSD detector (Mixed standard 2: Mp values: 478 000 Da, 86 200 Da and 6690 Da)

The order of elution of components of the mixed standards is shown in Fig. 4.4.

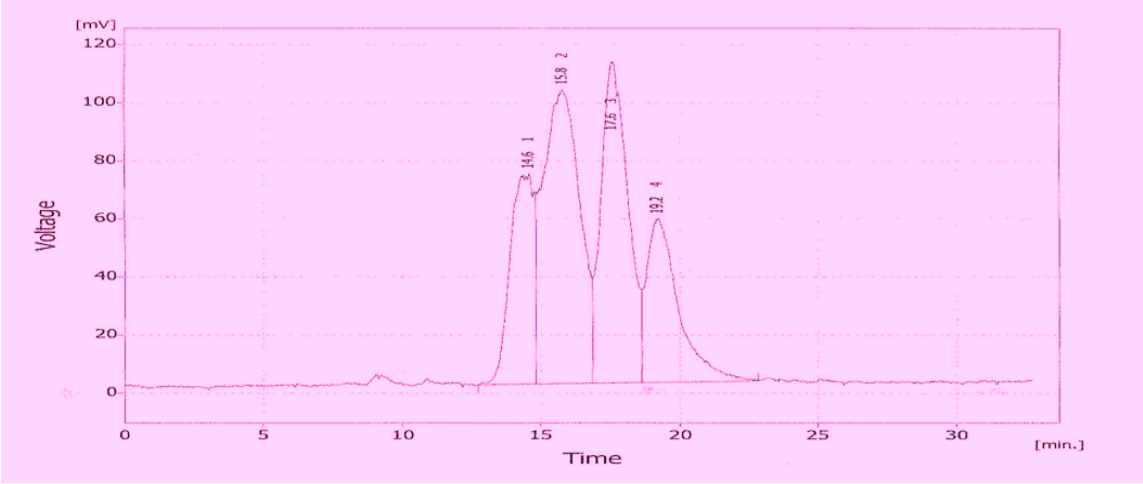

Fig 4.4: HPSEC Chromatogram of PEO/PEG Mw stds: Order of elution of peaks with molecular sizes -ELSD detector (Mixed standard 3: Mp values: 222 000 Da, 47 200 Da 1960 Da and 232 Da)

Table 4.1 Mp values, log Mw and retention times PEO/PEG calibration via ELSD

Mp(Da)	**Log Mw**	**Retention time(min)**
232	2.37	19.7
599	2.78	18.3
1960	3.29	18
6690	3.82	17.1
18 600	4.27	16.4
42 700	4.63	15.9
86 200	4.94	15
222 000	5.35	14.3
478 000	5.68	13.6
1 015 000	6.01	13

The principle of size exclusion chromatography is that the analytes are separated on the basis of size. Smaller molecules experience a more complex pathway to exit the particle than do larger molecules. The calibration graph of the PEO/PEG standards is shown in Fig. 4.5 below.

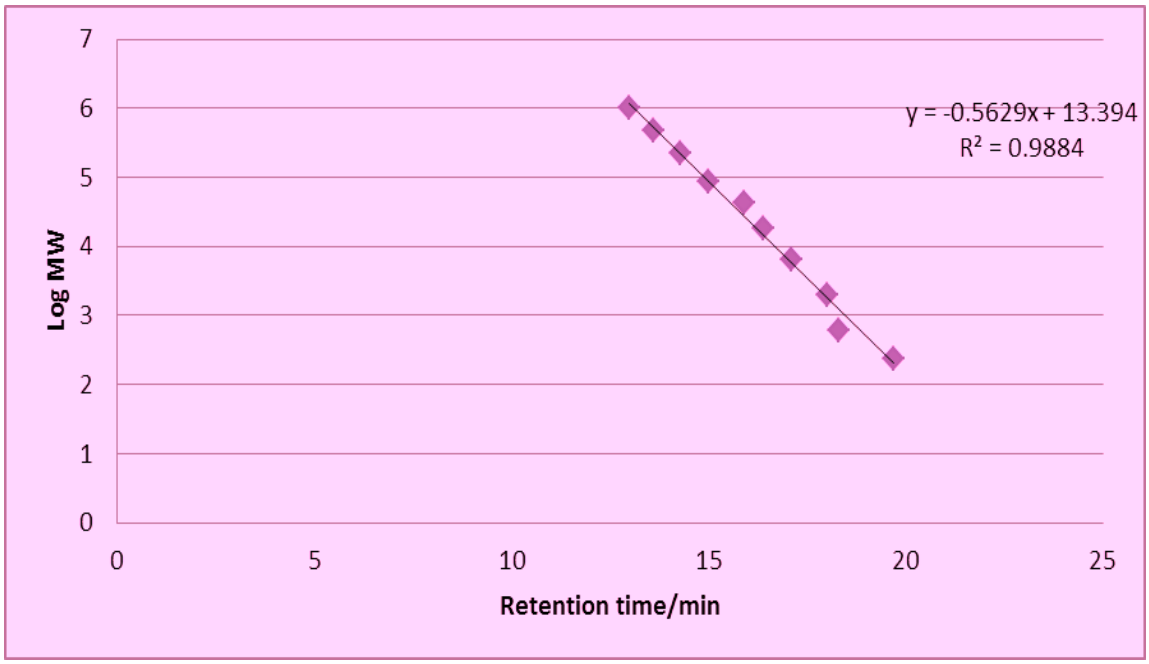

Fig. 4.5: Column calibration with PEO/PEG stds: Graph of Log molecular weight vs. retention time-ELSD

From the calibration graphs using the ELS detector in Fig. 4.5, the equation of the straight line y = mx + c, where m is the gradient and c is the "y-cut-off". i.e., the y co-ordinate of the point at which the line cuts the y-axis, was used to determine the molecular weights of the standards, CRM and the unknown samples as shown in equation 3 below.

Example: Sigma Aldrich fulvic acid standard

Retention time = 14.9 min

y = mx + c…. (3)

y = -0.5629 x + 13.394

y = -0.5629(14.9) + 13.394 = 5.01

In this case, y is the log molecular weight, x is the retention times of each of the standards and c is where the graph cuts the y-axis. Inverse log 5.01 = 102 329 Da (molecular weight of the sigma Aldrich fulvic acid). The accuracy and precision of using this method is reflected by the value of the correlation co-efficient (R^2). Correlation measures the dependability of the relationship between the y and the x-variable. It is a measure of how well one variable can predict the other, and determines the precision which can be assigned to a relationship. This value has to be as close to 1 as possible. The same method was used for the calculation of log molecular weights of all the standards and samples molecular weights. Even though there was no significant difference, different graphs were used to estimate the molecular weights of the standards and samples.

The correlation co-efficient was found to be 0.9884 and 0.9605 for the UV and ELS detector, respectively. A plot of log of ELSD response with log concentration was used to determine the quantities of the humic substances in dam water. Although ELSD does not respond linearly over its dynamic range, the convenience of a linear response can be achieved. One disadvantage of the ELSD is the decreased sensitivity for low-concentration analytes. However, because its response is independent of the light absorbing properties of molecules, it can reveal sample components that UV detectors cannot detect and provide a more accurate profile of relative component abundance than is possible with a spectroscopic detector. The structures of the two analytes, humic and fulvic acids contain a fair amount of non-aromatics, which the UV detector wouldn't be able to detect. This study, over and above characterizing these two fractions, focuses more on determining the molecular weights of these fractions using the ELS detector, for the reasons mentioned above.

4.2.1.3 Molecular weight ranges

As discussed above, the size exclusion technique is based on molecules in solution being separated by their size, and in some cases molecular sizes. The principle of SEC is that particles of different sizes will elute through a stationary phase at different rates. This phenomenon was observed with the PEO/PEG standards and is shown in Fig. 4.1, 4.2 and 4.3 above. The first peak represents the standard with largest Mp value (1 015 000 Da), while the last one represents the standard with the lowest Mp value (232 Da). According to the separation mechanism of SEC, the first eluting peaks are for compounds with a higher molecular weight. This can be clearly seen in Fig. 4.3, with the increasing retention times the molecular weight is decreasing. There is a limited range of molecular weights that can be separated by each column and therefore the size of the pores for the packing should be chosen according to the range of molecular weight of analytes to be separated.

The exclusion limit defines the molecular weight at the upper end of the column 'working' range and is where molecules are too large to be trapped in the stationary phase. The lower end of the range is defined by the permeation limit, which defines the molecular weight of molecules that are small enough to penetrate all pores of the stationary phase. All molecules below this molecular mass are so small that they elute as a single band. The exclusion limit of the polysep-gfc column is 10 000 000 Da. None of the peaks appeared near the exclusion limit or near the permeation limit. All the molecular weights were distributed throughout the curve. The graph shows that these standards fall on one calibration curve. The same was observed for mixed standards 2 and 3. Some of the factors that would affect calibration are solvent, temperature changes, flow rate, none of these were varied during the calibrations. For the ELSD PEO-PEG calibration in Fig.4.5, the correlation co-efficient was not very good. This was in agreement with the report by Carson [6].

4.3 Results from analysis for humic acid

This study employed an analysis system comprising a UV detector connected in series with the ELS detector. The UV detector was set at two wavelengths, 254 nm and 280 nm. This was done to accommodate relatively small molecular sizes and also to detect the MWD of low molecular weight NOM fractions due to their relatively higher absorptivity at a lower wavelength. This is consistent with the observation of Swietlik et al [5]. According to Matilainen [7], the use of the UV 254 nm method was a limitation in HPSEC, known to underestimate the amounts of low molar mass organic matter with less UV absorptive chromophores. SEC chromatograms of the humic acid fraction by the ELS detector, UV detector at 254 nm and 280nm have been shown in Figs. 4.6, 4.7 and 4.8 respectively**.** The molecular weight distributions of these fractions are presented in Table 4.2.

Table 4.2 Calculations of Mn, Mw and Polydispersity

Fraction	**Detector**	**Wavelenght(nm)**	**Molecular weight (Da)**
Sigma Aldrich HA	ELS		2754
Sigma Aldrich HA	UV	254	1366
Sigma Aldrich HA	UV	280	575
Suwanee river HA	ELS		12882
Suwanee river HA	UV	254	3019
Suwanee river HA	UV	280	3019
HA sample	ELS		1258
HA sample	UV	254	991
HA sample	UV	280	991

The range of distribution is between 7000 Da and 13 000 Da. However, the present study's results were not easy to rationalize because the column covers a very wide range of molecular weights that are not necessarily the lower range size of molecular weights as one would expect for humic acids. Comparing the SEC chromatograms of these is not a good idea for the fact that the UV detector is known to only detect conjugated double bonds and chromophores as stated above. This was done here because the operating conditions (mobile phase and flow rate) were not too different since these were connected in series. The same scenario that was observed with the sigma Aldrich humic acid standards is observed here, the high absorption seen at lower wavelengths and higher peaks heights noted with the ELS detector compared to the UV detector.

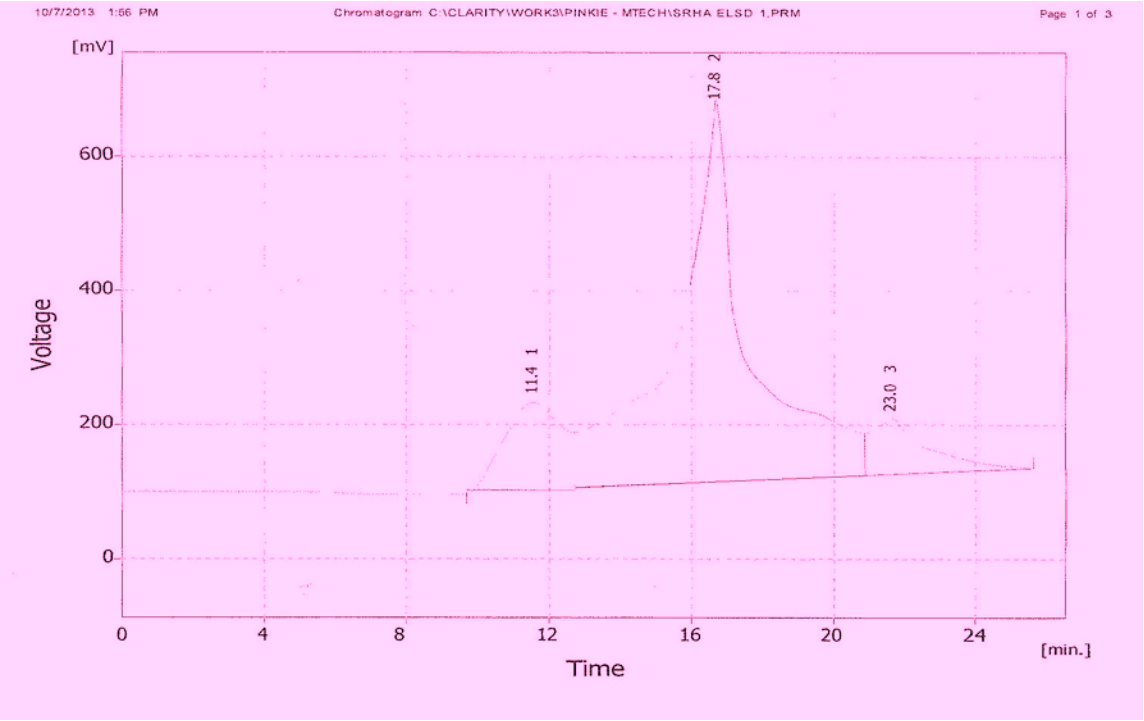

Fig. 4.6: HPSEC chromatogram of 1 ppm humic acid standard via ELSD

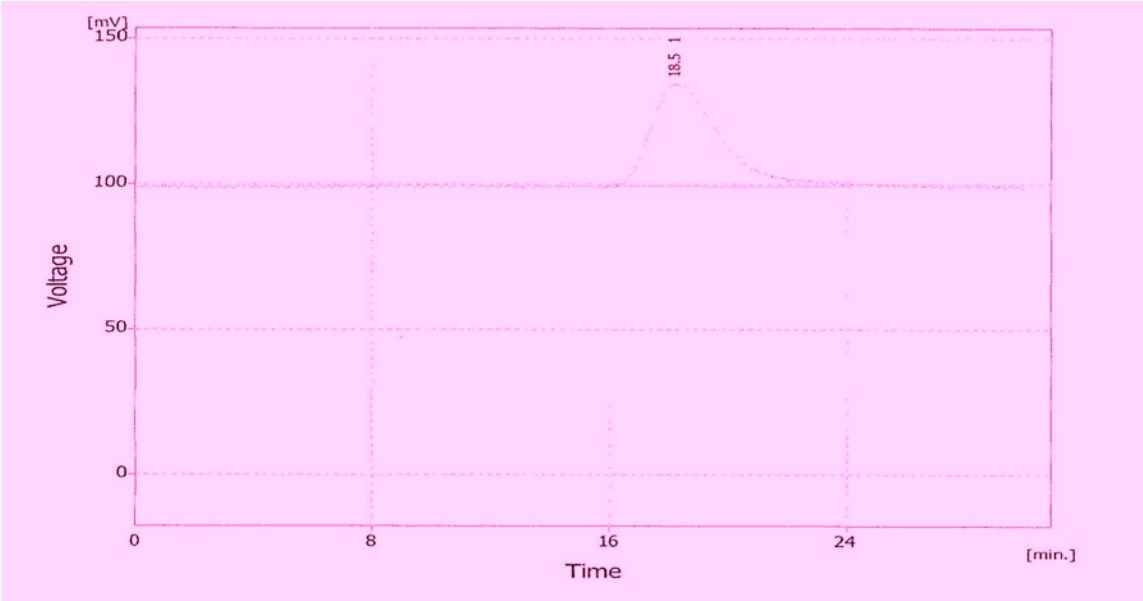

Fig. 4.7 HPSEC chromatogram humic acid sample fraction via UV 254 nm

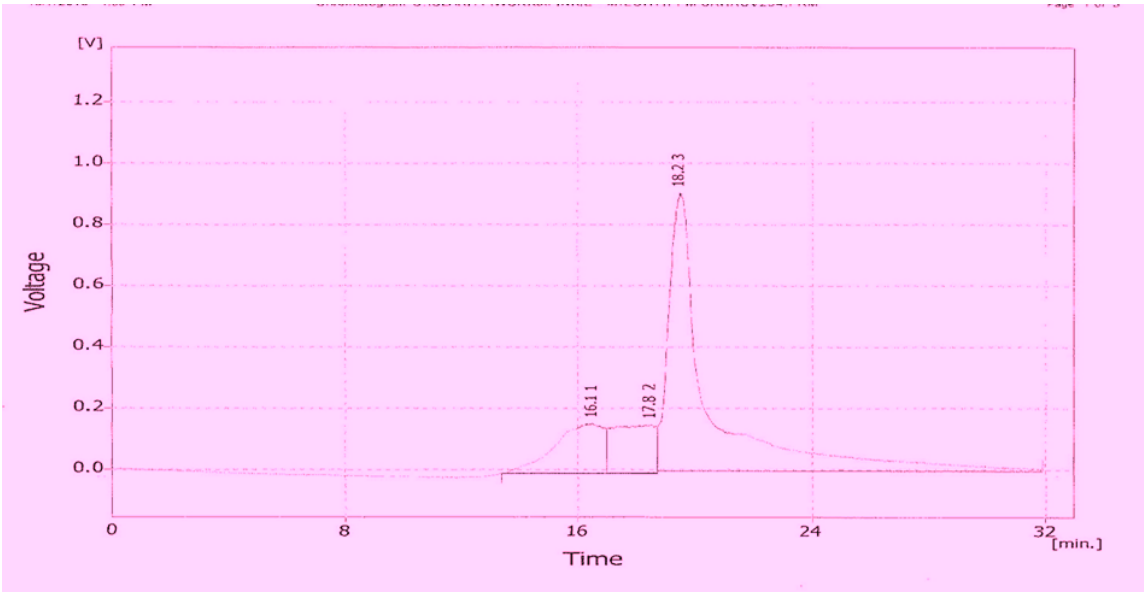

Fig. 4.8: HPSEC chromatogram of 1 ppm humic acid standard via UV 280 nm

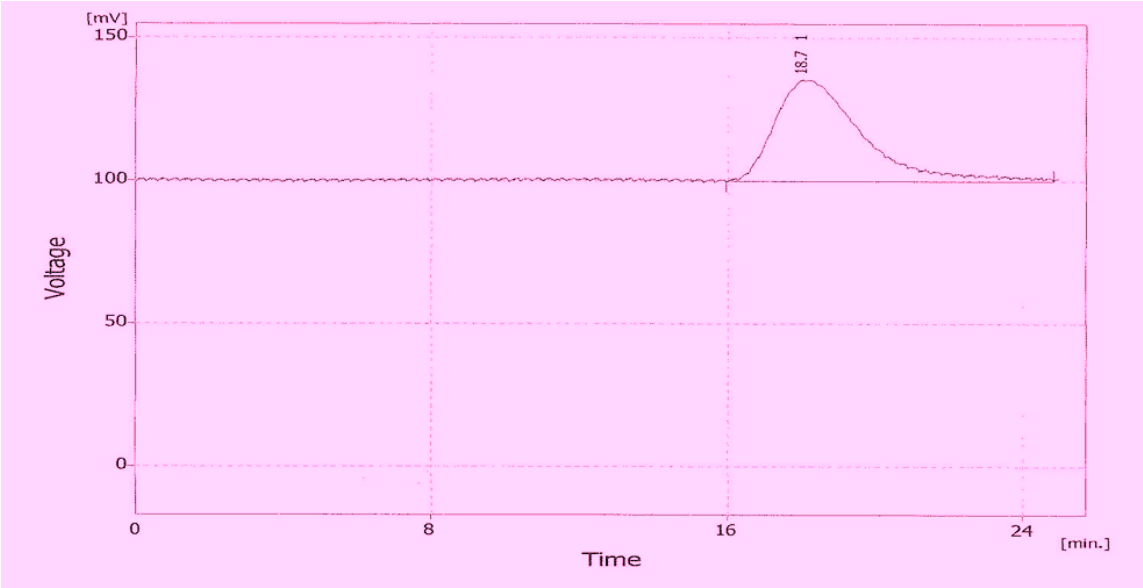

Fig. 4.9 HPSEC chromatogram humic acid sample fraction via UV 254 nm

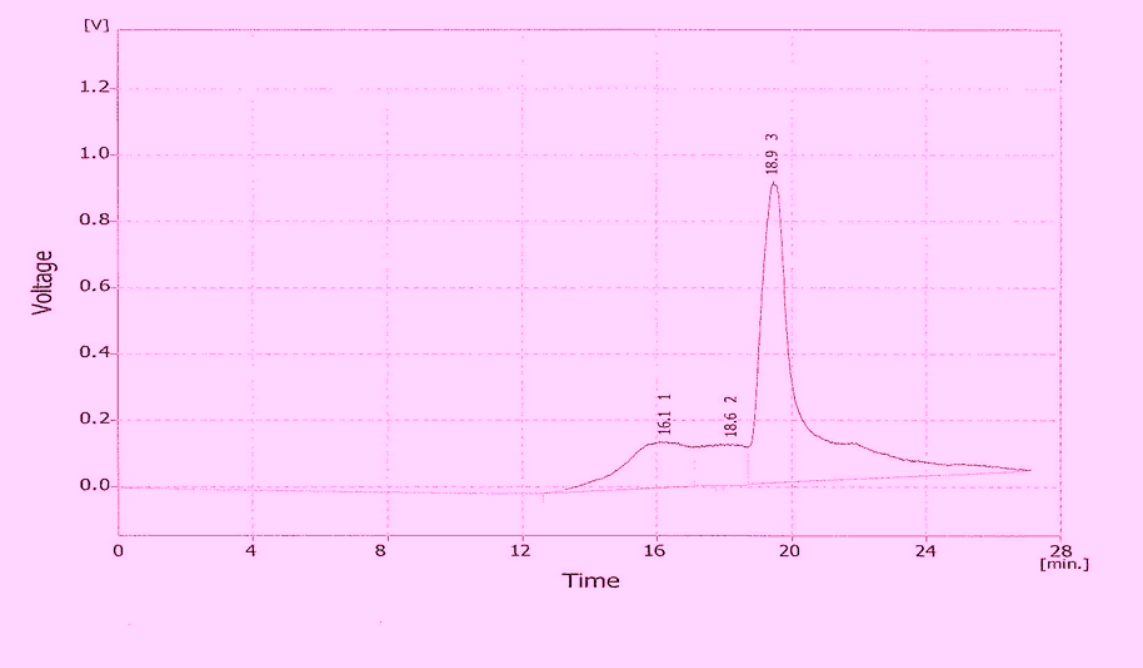

Fig. 4.10: HPSEC chromatogram of 1 ppm humic acid standard via UV 280 nm

As shown by the chromatograms (Figure 4.7-4.12), there are differences noted as follows:

- For the 1 ppm humic acid standard, the retention time is 17.8 min via ELSD, 18.5 min via UV 254 nm and 18.9 via UV 280 nm.
- It is also noted that the peak height recorded using the ELSD is slightly higher than that of the UV detector. This is expected in the light of the fact that the ELSD is nearly a universal detector and that it detects analytes which remain after the solvent has evaporated, [6]. The UV detector will only pick up UV active species (conjugated double bonds and aromatic structures). Hence there are molecular weight fractions that will be undetected by the UV detector.
- Also, there is a difference in the heights of the two UV chromatograms, the 254 nm and the 280 nm. The 254 nm is showing a greater peak height, showing more absorption at a lower wavelength, compared to the UV 280 nm. This is consistent with Beer's law and the observation of Bertilsson *et al* [9]. The molecular weights of these were determined from the calibration graph with PEO/PEG standards. The molecular weights were determined in the same way as for the UV detection, that is, calculations done from the calibration plot of log molecular weight vs retention time. The molecular weights are displayed in Table 4.2 below are 2754 Da, 1366 Da and 575 Da for the ELS detector, the UV 254 and UV 280 respectively. The big differences can be accounted for by the changes in the retention times. Although the change is not too big, it makes a slightly bigger difference in the molecular weight calculation.

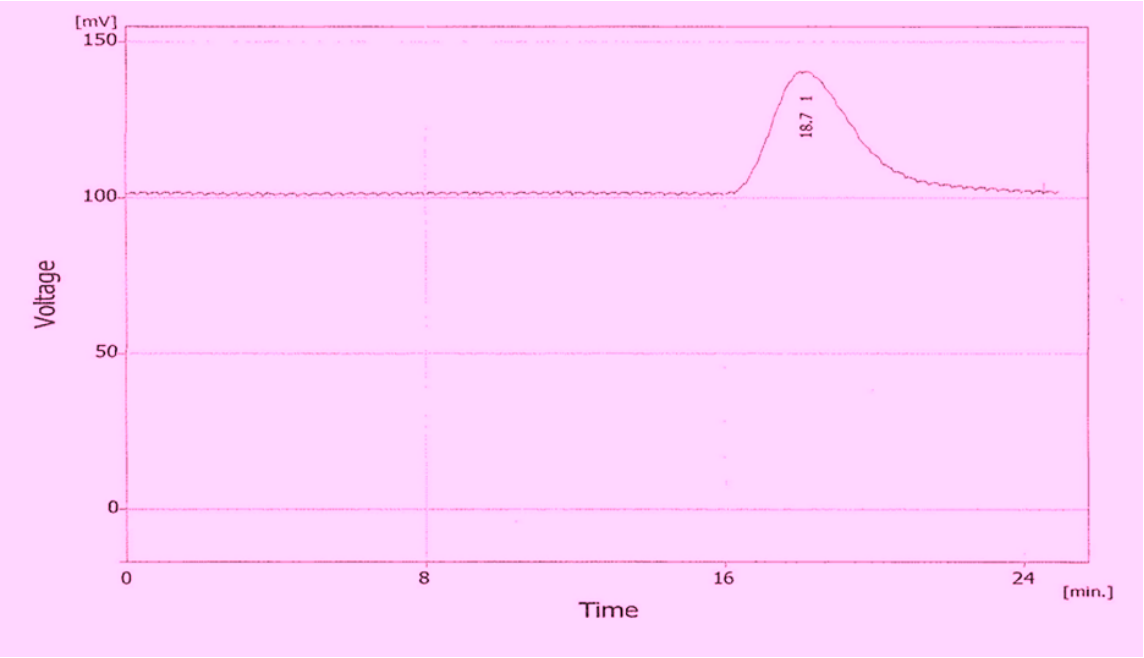

Fig. 4.11: HPSEC chromatogram humic acid sample fraction via UV 280 nm

Chromatograms of the Suwanee river humic acid reference material characterized by HPSEC via the UV detector at 254 nm and UV 280 nm are displayed in Figs. 4.12 and 4.13 respectively. All chromatograms share similar characteristics in terms of retention time, all appearing between 16 and 18 minutes. The chromatograms obtained here were slightly similar to those obtained by Her et al [10]. The molecular weight values obtained for these are consistent with most of the values reported before, even though humic substance are known to have an extremely variable range of molecular weights, from hundreds to millions of daltons [6]. These calculated values are shown in table 4.3. The peaks of these are also different in that, in some cases, more than one peak was detected, but this was in agreement with the report of Carson [6]. The challenge with this reference material was getting a HPSEC chromatogram from the Humic substances society to match with.

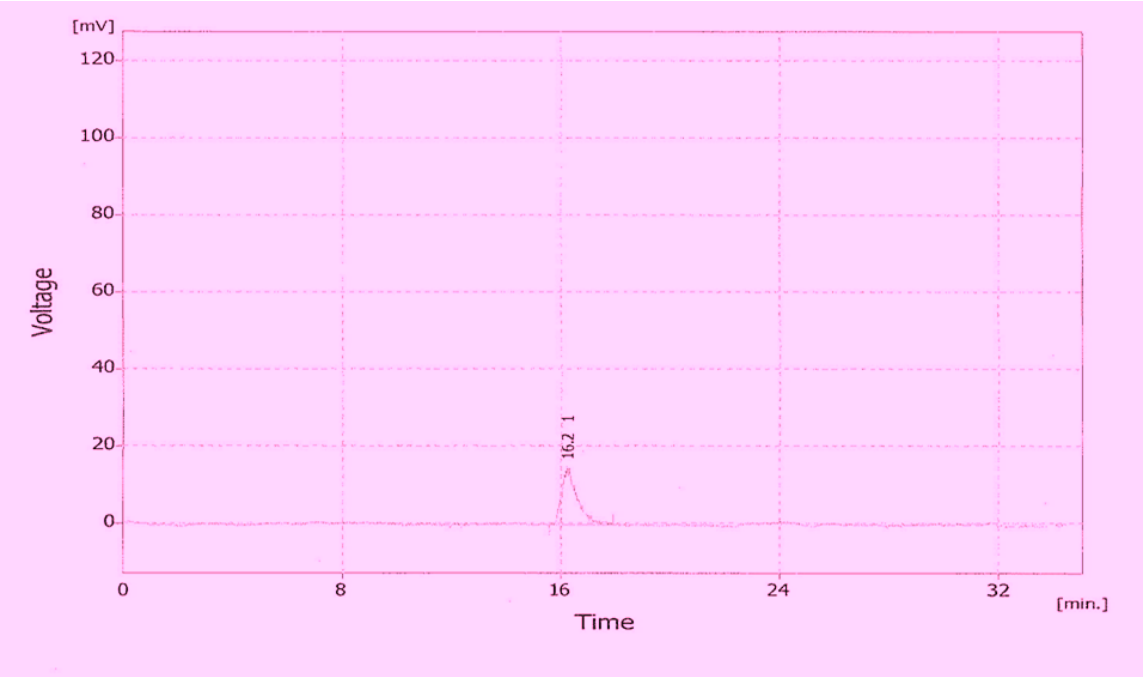

Fig. 4.12: HPSEC chromatogram of a 1 ppm Suwanee river humic acid standard via UV 254 nm

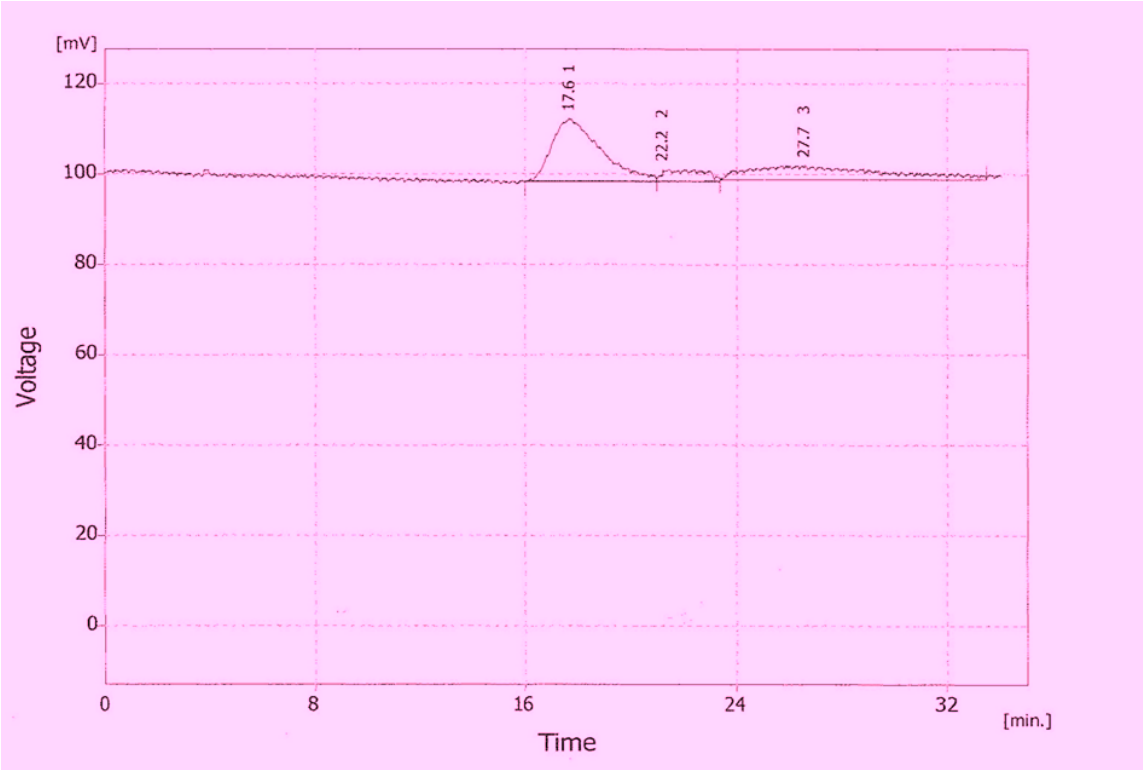

Fig.4.13: HPSEC chromatogram of a 1 ppm Suwanee river humic acid standard via UV 280 nm

SEC chromatograms of the humic acid fraction by the UV detector at 254 nm and 280 nm, and of the ELS detector have been displayed above in Figs 4.7 and 4.11 above. The molecular weight distributions of these fractions are presented in Table 4.3 below. The distribution is between 7000 Da and 13 000 Da. The same results were reported by Carson [6].

Table 4.3 PEO/PEG Standards (Mp, Mw and Mn values)

Mp(Da)	Mw(Da)	Mn(Da)	Polydispersity
1 015000	941 000	879 000	1.07
18 600	17 900	14 900	1.20
599	601	560	1.07
478 000	496 000	339 000	1.46
86 200	87 800	75 800	1.16
6 690	6 550	6 170	1.06
222 000	220 000	197 000	1.12
42 700	40 100	30 700	1.31
1 960	2 010	1 840	1.09
232	232	232	1.00

The MW distribution of a mixture of polymers is defined by its polydispersity; the weight-average to number-average MW ratio (i.e., Mw/Mn). A pure substance will have Mn = Mw. However, a mixture of molecules will have Mw > Mn and p = Mw/Mn. The standards above have Mw values less than Mn values. The weight average to number average ratio is very close to 1 for each set of mixed standards. Mn and Mw values were calculated using the equations from Yan *et al* [12] and the data is presented in Table 4.4 above. In principle the distinction of broad and narrow standards is based on the Polydispersity Index, PDI =Mw/Mn. The PDI of a broad range of standards is usually >1.5.

4.4. **Results from analysis for fulvic acid**

Comparing the SEC chromatograms of the 1 ppm fulvic acid standards with the UV 254 nm, UV 280 nm and the ELSD, the peak with the ELS detector eluted slightly later than those of the UV detector and is noticeably having a bigger height compared to the UV detector one. Another notable difference is the lower peak height in the UV 280 nm chromatogram compared to the 254 nm, showing less UV absorption at higher wavelengths as was seen in the humic acid standards and sample fractions. The molecular weights of the Sigma Aldrich FA by ELSD, UV 254 nm and UV 280 nm are respectively 199 Da, 2041 Da and 2041 Da respectively. These are presented in Table 4.4. The distribution range that has been reported is from a few thousand to about 20 000.These results are similar to those obtained by Carson in that the molecular weights of fulvic acids are known to be slightly lower than those of humic acids, and this was observed here.

There was not much of a difference in the retention times obtained for these fractions by the ELS and the UV detectors. The same differences that were noted with the humic acid fractions were observed here. The consistency of the fulvic acids molecular weights being generally lower compared to the humic acids was also observed here. PEO standards generally have MW values above those of humic substances. Even though the calibration graph can be used to estimate the molecular sizes of humic and fulvic acid, the distribution is not the best one can get. Standards in the molecular weight region of these humic substances could improve the calibration and hence give a better understanding of their size distribution. In this study, whilst most of the molecular weights were within the calibration range, there were some that were below the molecular weight range of the calibration standards. The results for the fulvic acid fractions presented in table 4.4 below.

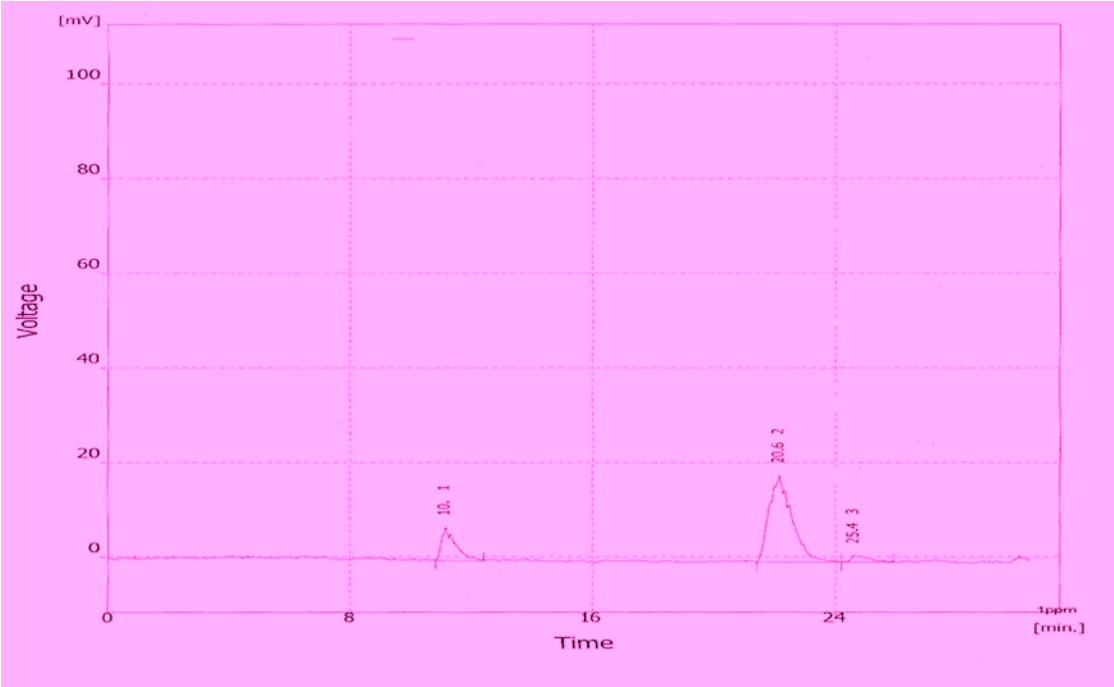

Fig. 4.14: HPSEC chromatogram of 1 ppm fulvic acid standard via ELSD

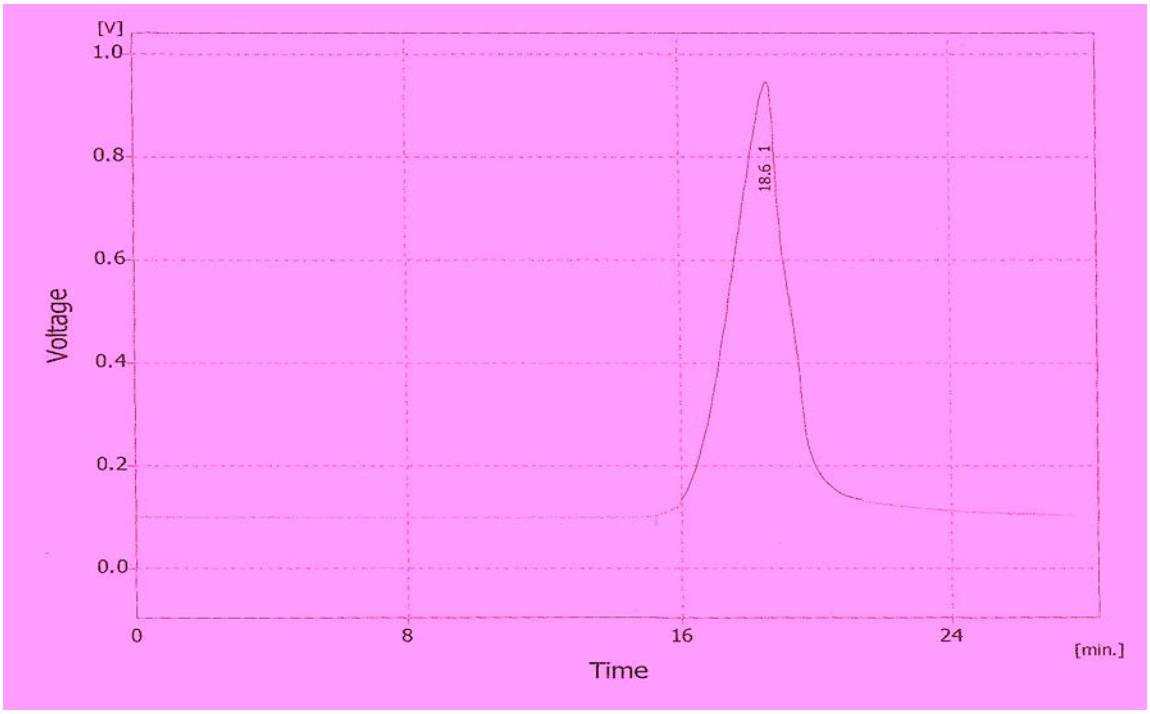

Fig. 4.15: HPSEC chromatogram of fulvic acid sample fraction via ELSD

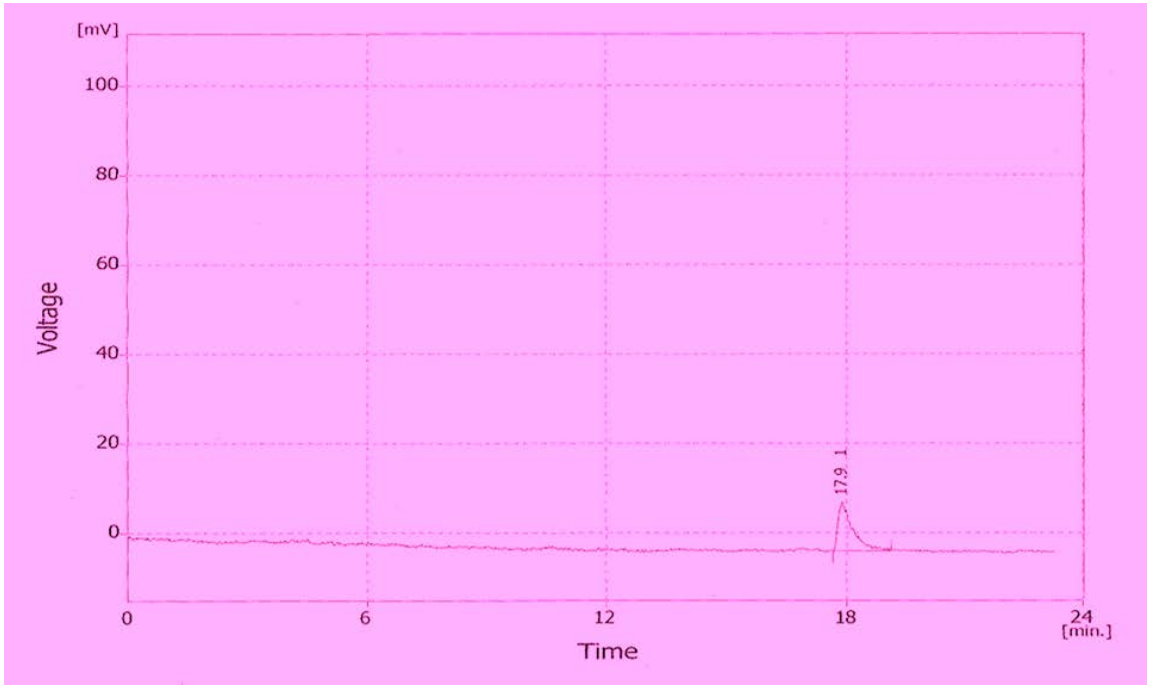

Fig.4.16: HPSEC chromatogram of 1 ppm fulvic acid standard via UV 254 nm

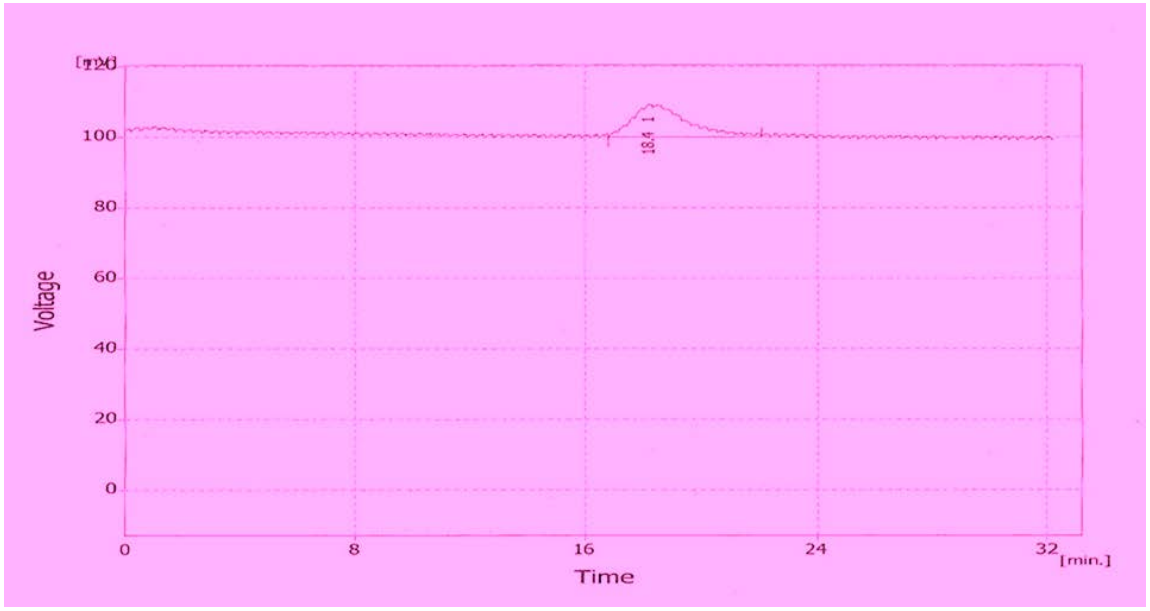

Fig. 4.17: HPSEC chromatogram of fulvic acid sample fraction via 254 nm

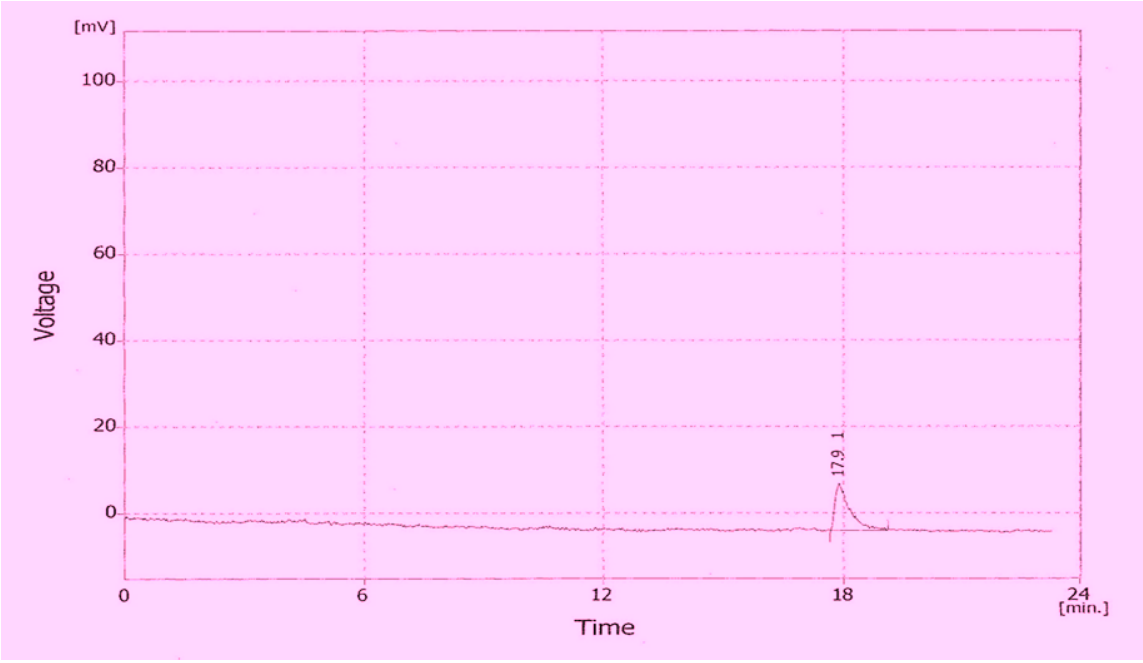

Fig. 4.18: HPSEC chromatogram of 1 ppm fulvic acid standard via UV 280 nm

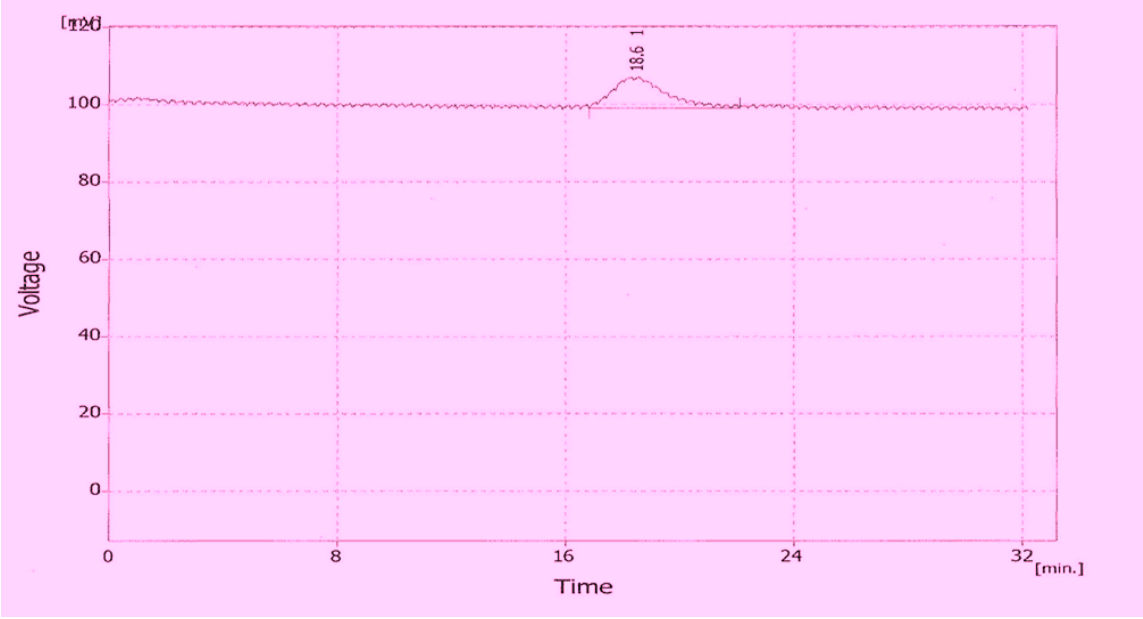

Fig. 4.19: HPSEC chromatogram of fulvic acid sample fraction via 280 nm

Table 4.4: Molecular weight values of the fulvic acid standard and fraction

Fraction	**Detector**	**Wavelength(nm)**	**Molecular weight(KDa)**
Sigma Aldrich FA	ELS		199
Sigma Aldrich FA	UV	254	2041
Sigma Aldrich FA	UV	280	2041
FA sample fraction	ELS		1258
FA sample fraction	UV	254	1074
FA sample fraction	UV	280	952

Table 4.5 PEO/PEG Molecular weight vs Elution volume

Elution volume	**Log Molecular weight**
34,3498	2.37
32,0890	2.78
2,3998	3.29
27,2622	3.82
25,6390	4.27
24,2998	4.63
22,9788	4.94
21,2748	5.35
19,9538	5.68
18,9723	6.01

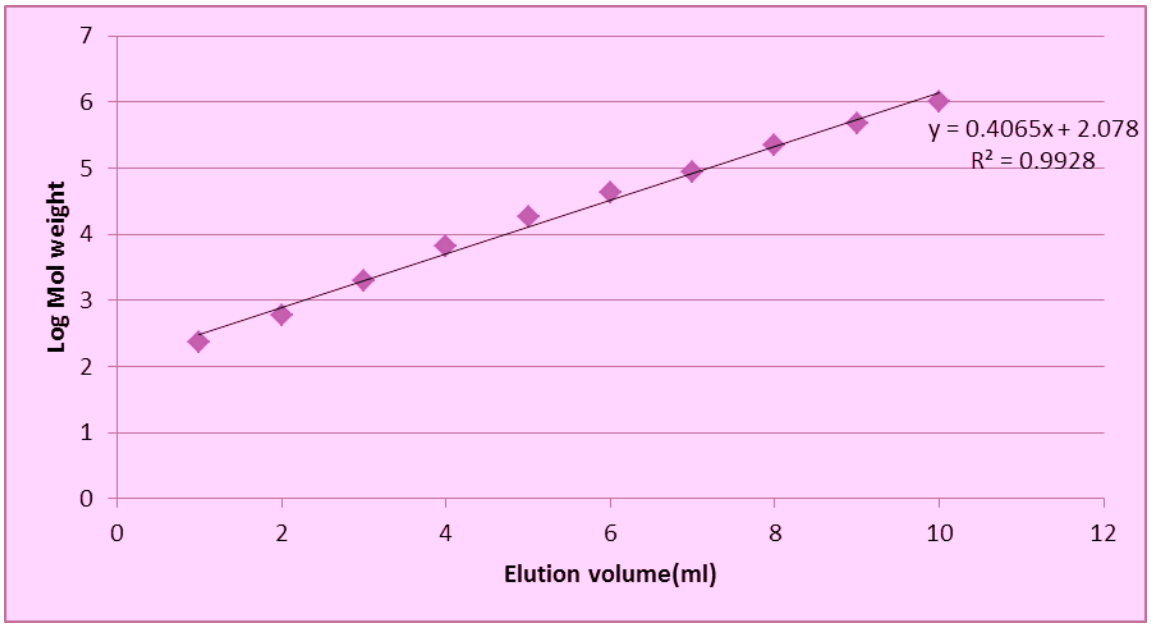

Fig. 4.20 Graph of PEO/PEG Molecular weight vs Elution volume

4.5 **Limits of detection and quantification**

The limit of detection is the point at which the analysis is just feasible while the limit of quantification is the concentration at which results can be reported with a high degree of confidence. These were calculated using the signal to noise method where a signal to noise ratio of 3:1 is acceptable for the LOQ and 10:1 for the LOD. These were determined for both humic and fulvics acid using the 1ppm fulciv acid standard and 0.01 ppm humic acid standards in Figure 4.23 and 4.24 respectively. Table 4.7 shows the calculated results of the LODs and LOQs.

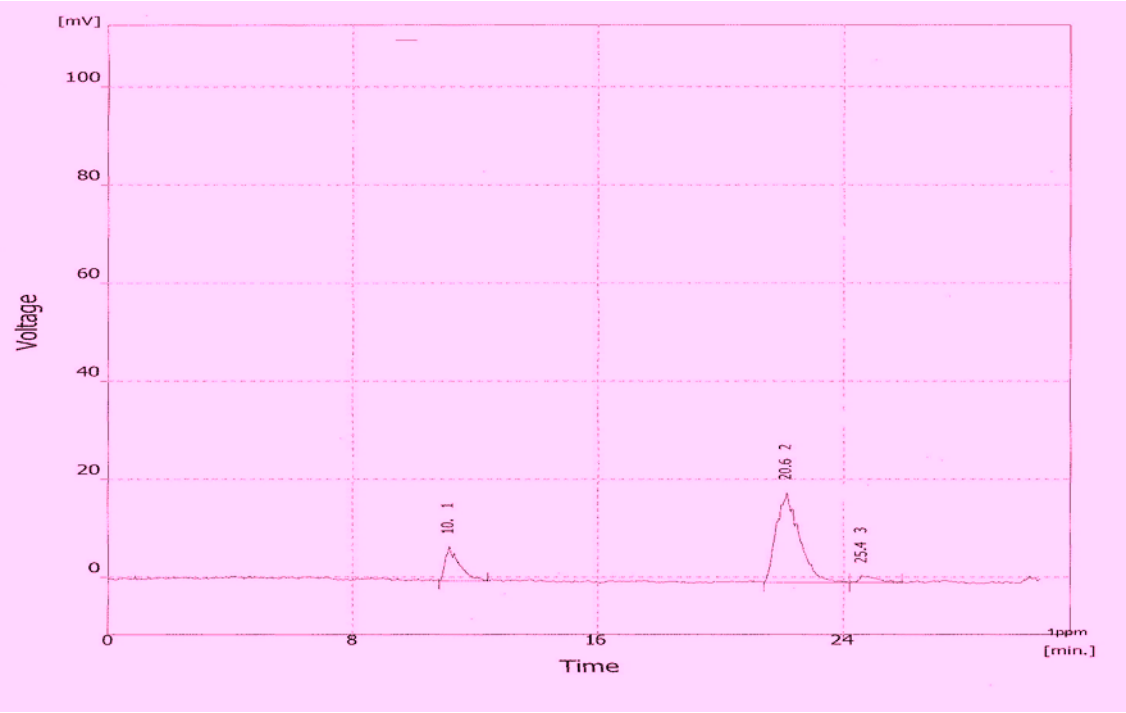

Fig. 4.21 SEC chromatogram 1 ppm Sigma Aldrich fulvic acid via ELSD

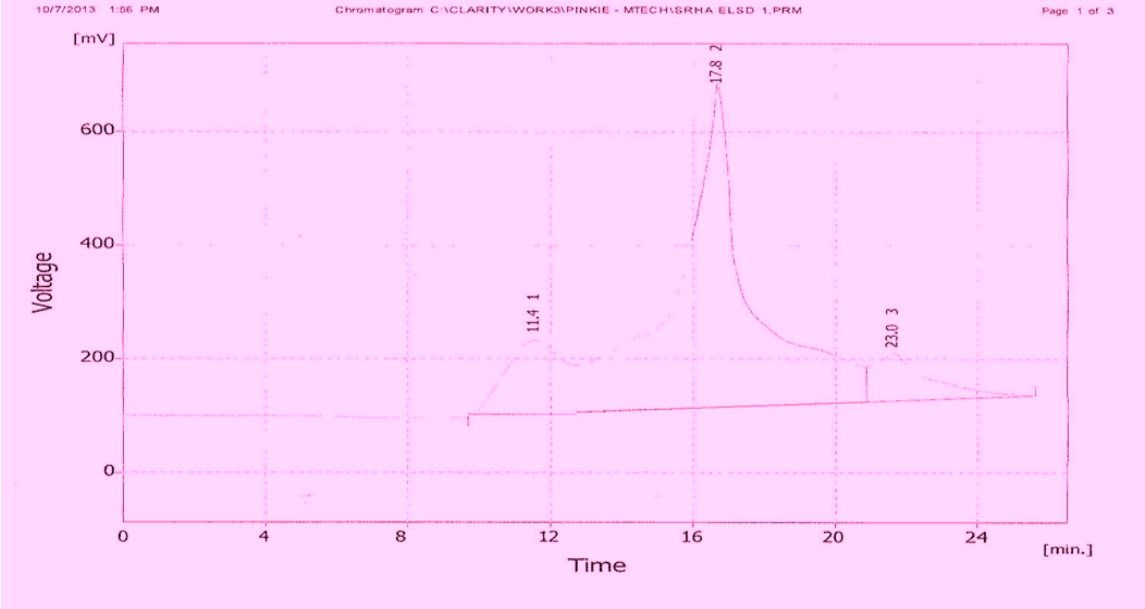

Fig. 4.22 SEC chromatogram 0.01 ppm humic acid via ELSD

Table 4.6 List of limit of detection and quantification for humic and fulvic acid acid calculated from a 1ppm standard fulvic acid and 0.01 ppm humic acid

	LOD/ppm	**LOQ/ppm**
Humic acid	0.002	0.008
Fulvic acid	0.8	2.7

4.6 Significance of the use of the ELS detector in determining molecular weights of humic substances

One of the objectives of this study was to develop a size exclusion method that employs the ELS detector. One of the reasons being that the Eskom utility that this project is undertaken for has this type of a detector, another reason being that this type of a detector is more like a universal detector. Basically all compounds that are less volatile than the mobile phase can be detected. Detection is based on a universal property of all analytes and does not require the presence of any chromophoric group or electroactive group [13]. Unlike the UV detector that will detect only UV active species, it will detect molecules with almost all functional groups.

Although the aromatic portion may appear to be dominant in the humic substances structures, the non-aromatics may form a significant portion of regulated disinfection byproducts and contribute to poor water quality. Hence it is important to utilize a detector that will not only detect aromatic double bonds or chromophores. All results show that increased resolution was obtained when using the evaporative light scattering detector, as opposed to the UV detector. In all the sample fractions, the peak height obtained by the ELS detector being significantly higher that peaks obtained by the UV detector. This denoting a large number of functional groups is being detected.

4.7 Aromaticity

Organic matter, especially aromatic type organic matter, readily absorbs UV light because of the strong absorption properties of the double bonded carbon atoms present in aromatic organics. Because natural organic matter (NOM) generally contains fairly high aromatic content, measuring the amount of UV light absorbed or transmitted through the water indicates the amount of natural organic matter (NOM) in the water [11]. Aromaticity is used in this study to measure the organic content in the dam water which would consequently give a measure of how much of humic substances are in the water. The analysis is done on the fractions that were previously separated from water and fractionated into the hydrophobic fraction of NOM, namely, fulvic and humic acid. The absorbance of the these two fractions is measured by a UV spectrometer, using Sigma Aldrich humic and fulvic acis standards to generate a calibration curve from which the concentration of each can be calculated.

The main limitation of UV absorbance techniques in organic matter characterization in water treatment includes the interference from UV absorbing compounds present in water (turbidity, inorganic substances like nitrate nitrogen) [14]. Humic substances generally show strong absorbance in the UV–Vis range (from 190 to 800 nm), particularly in the UV region, because of the presence of aromatic chromophores and/or other organic compounds. All measurements were at done at 254 nm. The UV-Vis absorption spectra of humic substances is usually featureless with absorption increasing at lower wavelengths due to the overlapping absorption spectra of the many functional groups in NOM [14] in view of this, no definite curve with a clear maximum wavelenghth could be generated to determine wavelength of maximum absorbance (lambda max). All measurements were made at 254 nm.

Specific UV absorbance (SUVA) is calculated here because it gives an indication of the amount of humic substances relative to the amount of non humic substances in the NOM. For this calculation, measurements of total organic carbon and absorbance values of the analytes are needed. Hence the UV measurements of the two fractions were done. Absorbance values of the humic acid standards (0.2, 0.4, 0.6, 0.8 and 1 ppm) and fulvic acid standards (1.0, 1.2, 1.4, 1.6, 1.8 and 2 ppm) were used to determine the concentration of humic and fulvic acid in the samples and are listed in Table 4.7 and 4.8 respectively. An excellent correlation was obtained with the standards. From the calibration graphs, the absorbance values of humic and fulvic acid fractions were determined. All samples were analyzed in triplicate for statistical purposes.

Table 4. 7: Tabulated UV HA results

Standard concentration/ppm	**Absorbance at 254 nm**
0	0.0002
0.2	0.1468
0.4	0.3127
0.6	0.4890
0.8	0.6101
1.0	0.8346
10 ppm SRHA	8.8321
HA fraction 1	0.2361
HA fraction 2	0.2227
HA fraction 3	0.2346

Table 4. 8: Tabulated UV FA RESULTS

Standard concentration/ppm	Absorbance at 254 nm
0	0.0114
1	2.414
1.2	2.946
1.4	3.467
1.6	3.920
1.8	4.458
2	5.012
FA fraction 1	0.3065
FA fraction 2	0.2952
FA fraction 3	0.2997

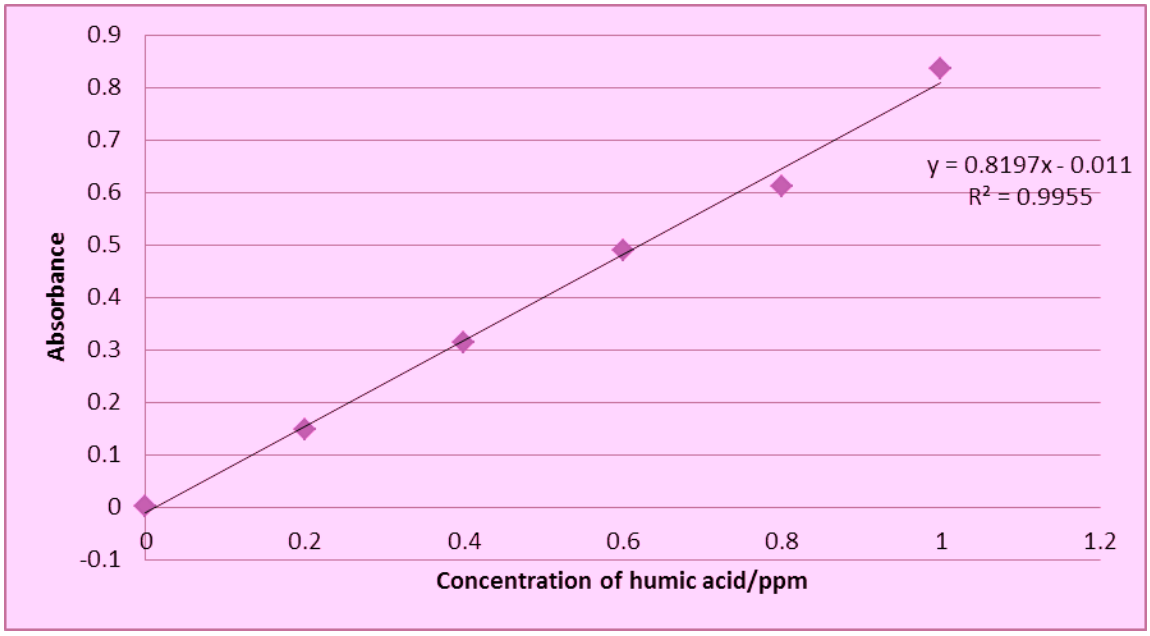

Fig. 4.23 Graph of absorbance versus concentration of humic acid

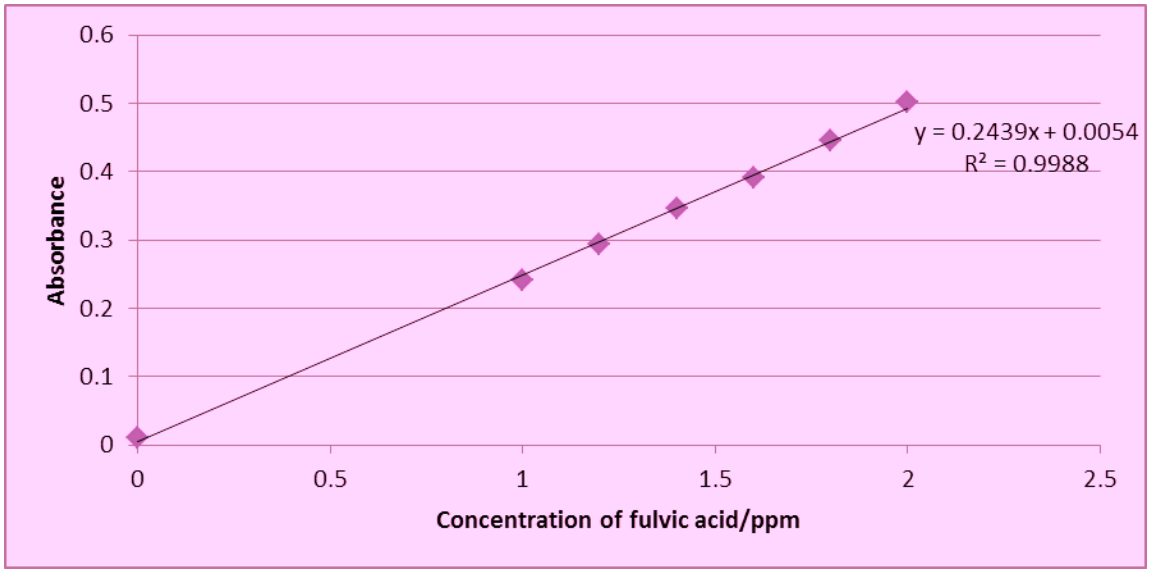

Fig. 4.24 Graph of absorbance versus concentration of fulvic acid

4.8 TOC and DOC analysis

There are various types of detection techniques utilized by TOC analyzers. However the detection method used by ESKOM is the membrane/conductivity detector. Traditionally the specification limit for organic impurities determined indirectly by acid conductivity was < 0.2 μS/ cm. This value gives allowance for the carbon dioxide ingression. A significant elevation of cation conductivity by carbon dioxide can be observed on power plants that have neither de-aerators nor condensate polishers [14].TOC is used to monitor removals during treatment. DOC was the most important one in this study as it measures the organic carbon concentration after filtration using a 0.45μ filters.

This part of the work was done at Eskom laboratories in Johannesburg and is indicated in Appendix A-1.Table 4.9 shows the results of the TOC and DOC measurements of the water samples as provided by Eskom. The amount of humic substances in the dam water at Vaal seems to be high and this has been confirmed by

converting this to specific absorbance using absorption at 254 nm. The samples showed high absorbance indicating a higher concentration of organics in the water .The strong absorbance shown by these humic substances in the UV region is due to the presence of aromatic chromophores and/or other organic compounds [14] .These results did confirm the presence of humic substances in the Vaal dam.

SUVA (Specific Ultraviolet Absorbance) is calculated by dividing the UV absorbance of a sample (cm^{-1}) at 254 by the DOC value (mg/L) and then multiplying by 100 mg/M [15]. It's an indication of the aromaticity of the humic fraction. Any values above 4 are said to represent a high aromatic content (humic substances), while values below 2 have a low aromatic content (non humic substances) [15]. Dissolved organic carbon (DOC) and total organic carbon (TOC) were 5.38 mg/l and 6.23 mg/l respectively. Results obtained from the UV analysis together with the DOC and TOC results were then used to calculate SUVA according to the formula in 1 below. The

UV254 absorbance and SUVA254 values of the two examined NOM fractions (Table 4.25) are all above 4 mg/C/L confirming the high content of aromatics .

$$\mathbf{SUVA = UV\ absorbance}\frac{(\mathbf{cm-1})\mathbf{x}\frac{\mathbf{100cm}}{\mathbf{M}}}{\mathbf{DOC}} = \frac{\mathbf{L}}{\mathbf{mg}} - \mathbf{M} \text{.....(18)}$$

Table 4.9 Specific UV Absorbance

Sample Fraction	**DOC (mg/L)**	**TOC (mg/L)**	**UVA**	**SUVA/ L/mg-M**
Humic acid	5.38	6.23	0.2361	4.4
Fulvic acid	5.38	6.23	0.3005	5.6

High SUVA values indicate the presence of humic substances, while low SUVA values indicate the presence of organic matter which is measurable by DOC and TOC but does not impact color or absorb UV light [5]. The SUVA values obtained here are in agreement with the Swietlik's report [5].

4.9 ATR-FTIR characterization

Infrared spectroscopy has been widely used for gross characterization of humic substances and can provide valuable information on the structural and functional properties of NOM molecules [14]. It is a very powerful tool because direct information about the presence of functional groups is easily provided [14]. Even though it is difficult to characterize humic substances using FTIR because of the generation of many absorption bands, NOM fractions make the interpretation easier because only the strongest bands can be identified and associated with the predominant structures [14].

The technique of Attenuated Total Reflectance (ATR) has, in recent years, revolutionized solid and liquid sample analyses because it combats the most challenging aspects of infrared analyses, namely sample preparation and spectral reproducibility. Though carboxyl group contents in NOM fractions may be determined easily, detailed mechanistic models of many of the above processes are difficult to construct. This is because the chemical behavior of carboxyl groups is also dependent on their structural environment. In this study, the main objective of using FTIR was to identify the functional groups of the hydrophobic fraction of NOM. Information on the functional groups of these is critical for understanding their reactivity with organic and inorganic contaminants. It was also used to differentiate between the two fractions, the humic and the fulvic acid. To determine whether the expected differences between the two acids will be confirmed in the IR spectra. Suwanee river humic acid reference material was also measured. For each of the fractions, a comparison with the Sigma aldrich standards was done and is discussed below.

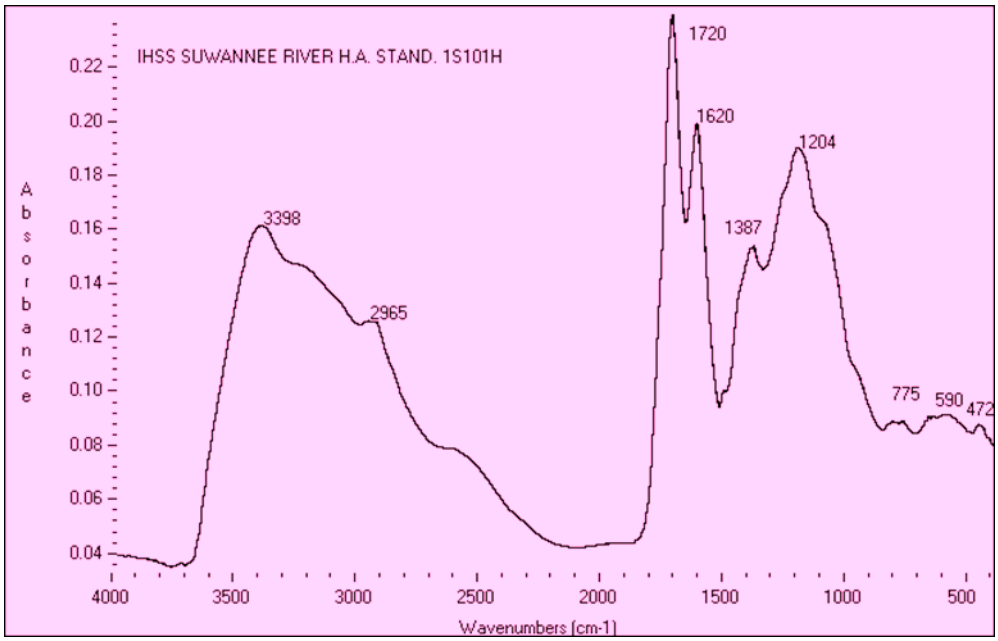

Fig. 4.25 IHSS FTIR spectra of a Suwanee river humic acid standard

The spectrum in Fig 4.25 is an FTIR spectrum of IHSS Suwanee river humic acid standard. It shows an intense signal at 3398 cm^{-1} which is assigned to the O–H stretching vibrations, confirms the presence of alcohols/phenols. The peak at n 2965 cm^{-1} and 2852 cm-1 is indicative of the C–H stretching vibration of alkyls .The band at 1620 shows the C=O stretching., and the shoulder at 1720 cm^{-1} can be assigned to the stretching vibration of carbonyl groups of carboxylic acids and ketones. The bending vibrations of methyl and methylene groups are at 1387 cm^{-1} and the stretching vibration of the C–O bond in alcohols, phenols and ethers are at 1204cm^{-1}.

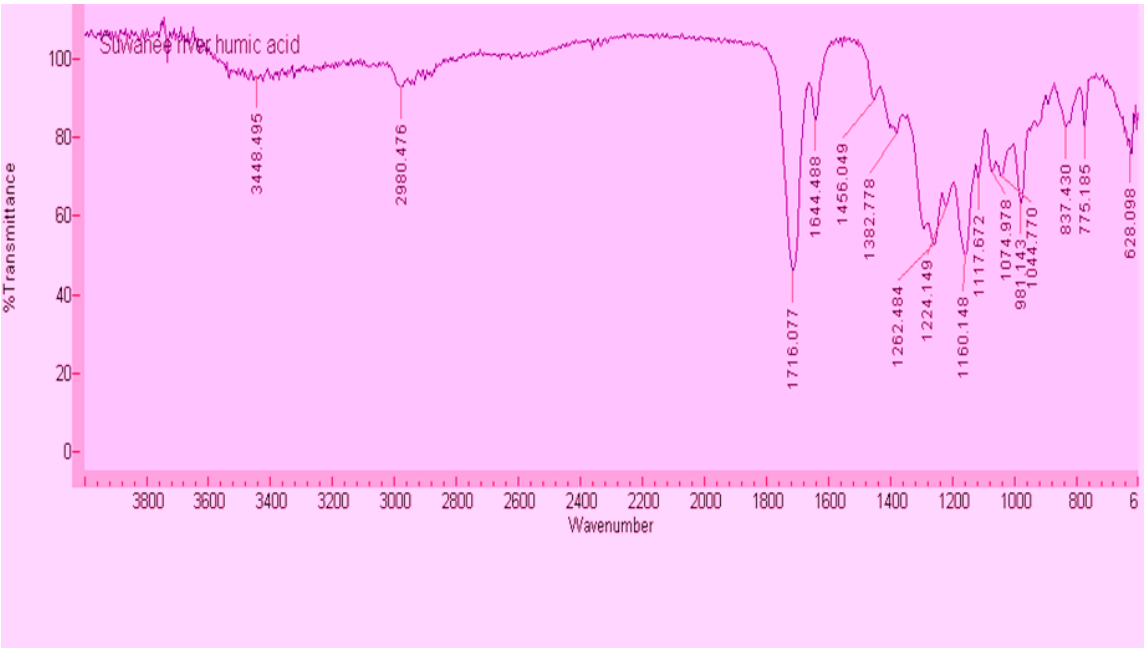

Fig. 4.26 ATR/FTIR spectra of a Suwanee river humic acid standard

Fig. 4.26 shows the functional group content of the humic acid in comparison to the IHSS spectra in Fig. 4.26 .The spectrum of the humic acid has fewer functional groups than the IHSS SRHA. All of the expected absorption frequencies were also observed in this study's FTIR spectra of the SRHA, except for the following; no broad bad representing the O–H stretching vibrations was observed. It has shown very little to no amount of phenols or alcohols and carboxylic acids which are very important.

Fig. 4.25 above shows type 1 spectrum of SRHA while the study used a type 2. This could explain why type 2 in Fig. 2.26 is showing fewer functional groups than type 1. The C-H stretching vibration of the alky groups was observed at 2980cm^{-1}. The aromatic C=C and C=O of conjugated ketones is observed at 1716 cm^{-1}.The bending vibrations of methyl and methylene groups are at 1382 cm^{-1}. The stretching vibration of the C–O bond in alcohols, phenols and ethers was observed at 1224cm^{-1}.

Table 4.10 FTIR bands of sigma Aldrich fulvic acid standard and sigma Aldrich humic acid reference standards from references and analysis

	Sigma Aldrich FA ref std(cm^{-1})	Sigma Aldrich HA ref std(cm^{-1})	Sigma Aldrich HA standard (results)	Sigma Aldrich FA standard(results)
OH stretching	3420	3410	3427	-
H-bonded OH stretching	2685- broad	2680 broad	3427	-
Aliphatic C-H stretching(symmetric and asymmetric stretching of CH_3, CH_2)	2940-sharp	2920-sharp	2935	2924
Aliphatic CH_2 and CCH3 bending	1435 weak	1435 weak	1433	1455
C==O stretching of COOH and ketones	1720	1718	-	1721
CO stretching and OH deformation of COOH and phenolic groups	1205 very strong	1205 very strong	1218	1220
COO asymmetric stretching(and stretching of aromatic C=C)	1630 strong	1630 strong	-	-
COO asymmetric stretching(and CH deformation CO stretching of phenolic OH	1388	1377	1380	-
Aromatic ring stretching	1560 weak	1560 weak	1522	-
COH bending, CO stretching of alcohols and ethers	1100	1095	1069	-

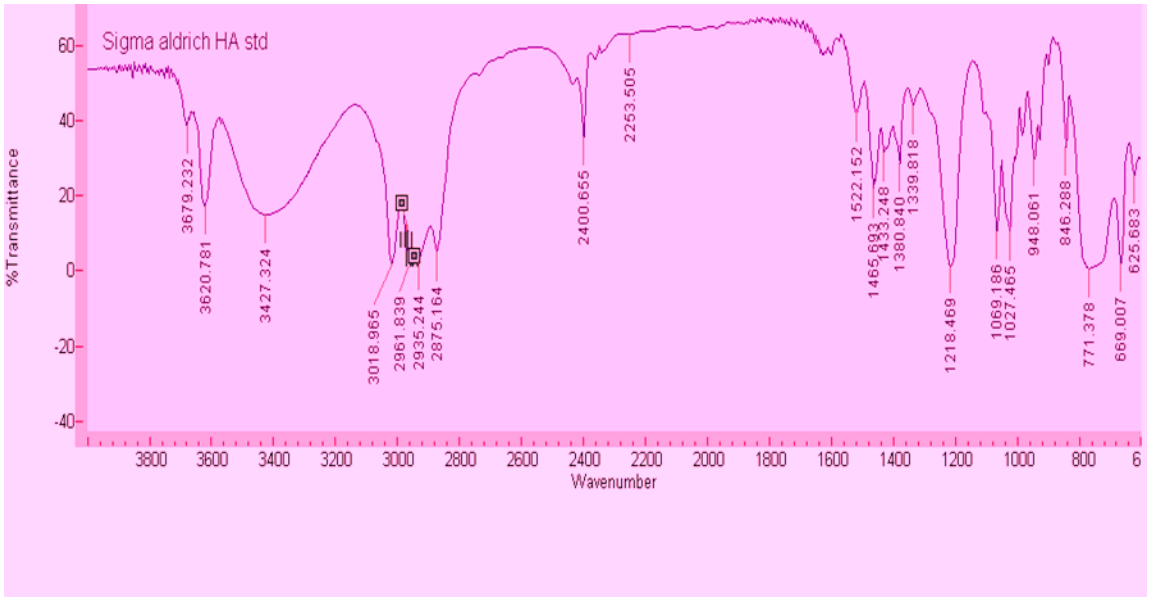

Fig. 4.27: ATR/FTIR spectra of a Sigma aldrich humic acid standard

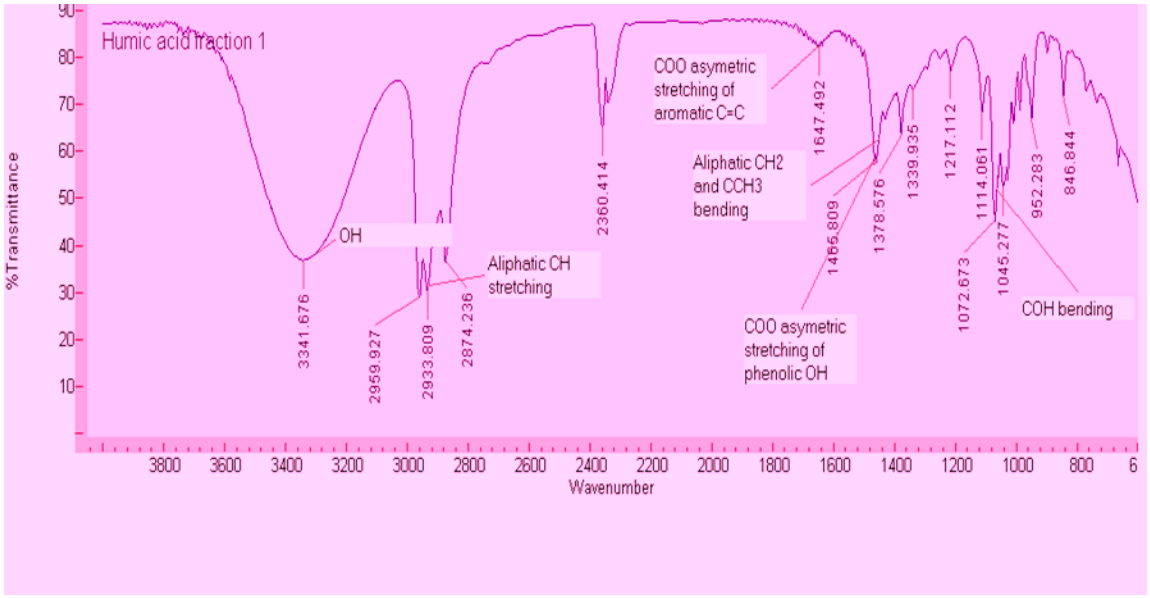

Fig. 4.28: ATR/FTIR spectra of a humic acid sample fraction

The infrared spectrum of the humic acid sample fraction confirmed the structural composition of the humic acids present in the Vaal dam. These results indicated that the humic acids of the Vaal dam contains phenolic hydroxyl groups, conjugated double bond of aromatic family (C=C), and free carboxyl groups. In this characterization, the main functional groups that were expected for the humic acid fraction were as follows; an O-H stretch, which is usually broad and strong in the 3400-2500 cm^{-1} range. Aliphatic groups including C-H, C-H_2, and C-H_3 stretches which are strong between 2900 cm^{-1}, C=O stretches occurring 1715cm^{-1} could be that of ketones and quinines that are aromatic or alicyclic . These are evident in Fig.4.26 and this is consistent with Chen *et al* [14]. The humic acid sample showed broad bands at about 3341 cm^{-1} which can be attributed to hydrogen bonded OH groups while the 1217 cm^{-1} peak is due to the C-O stretching of phenols, carboxylic acids, ethers and COOH groups.

Comparing the spectra for the Sigma Aldrich standard and the sample fractions, there are similarities and some differences which can be rationalized. In both standard and the samples, there is evidence of the CH- vibrational stretching of the alkyl groups at about 2900 cm^{-1}. No strong peak at around 1700 cm^{-1} due to the C═O stretch from a carboxyl functional group is seen in either. Sharp bands were seen in the region and 1072 cm^{-1}, which can be assigned to the bending vibration of aliphatic C−H groups and the OH bending deformation of carboxyl groups, respectively. The biggest difference was the band observed in the sample, for OH/phenols, which is not seen ion the spectrum of the standard. Also, the Aldrich humic acid standard possesses a higher content of aliphatic structural elements indicated by the higher intensities of the absorption bands at 2920 cm^{-1} and 2850 cm^{-1}. The band at 1114 cm^{-1} indicates the presence of secondary alcohols. This was not necessarily seen in the SAHA. The bands at 1465 cm^{-1} and 1339 cm^{-1} in the humic acid fraction confirm the presence of aliphatic chains.

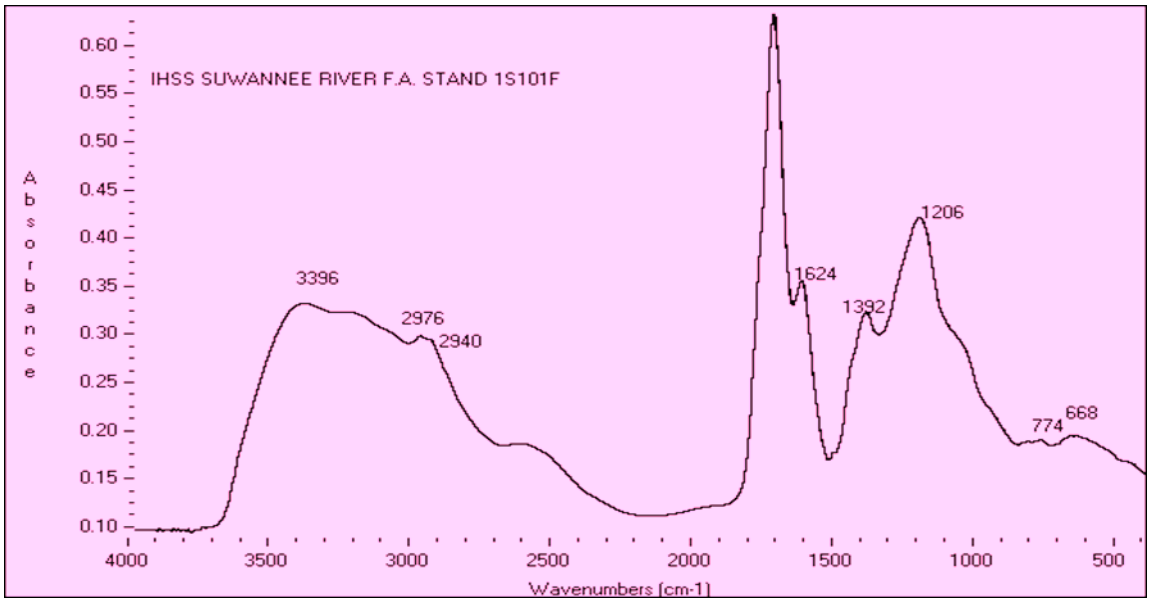

Fig. 4.29: IHSS FTIR spectra of a Suwanee river fulvic acid standard

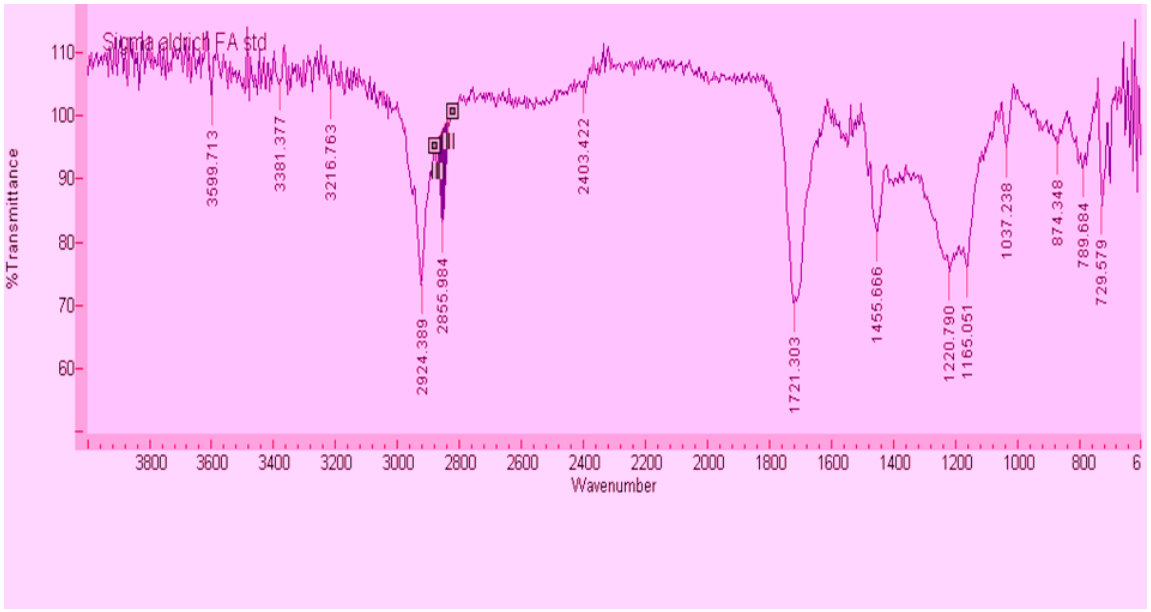

Fig. 4.30: ATR/FTIR spectra of a sigma aldrich fulvic acid standard

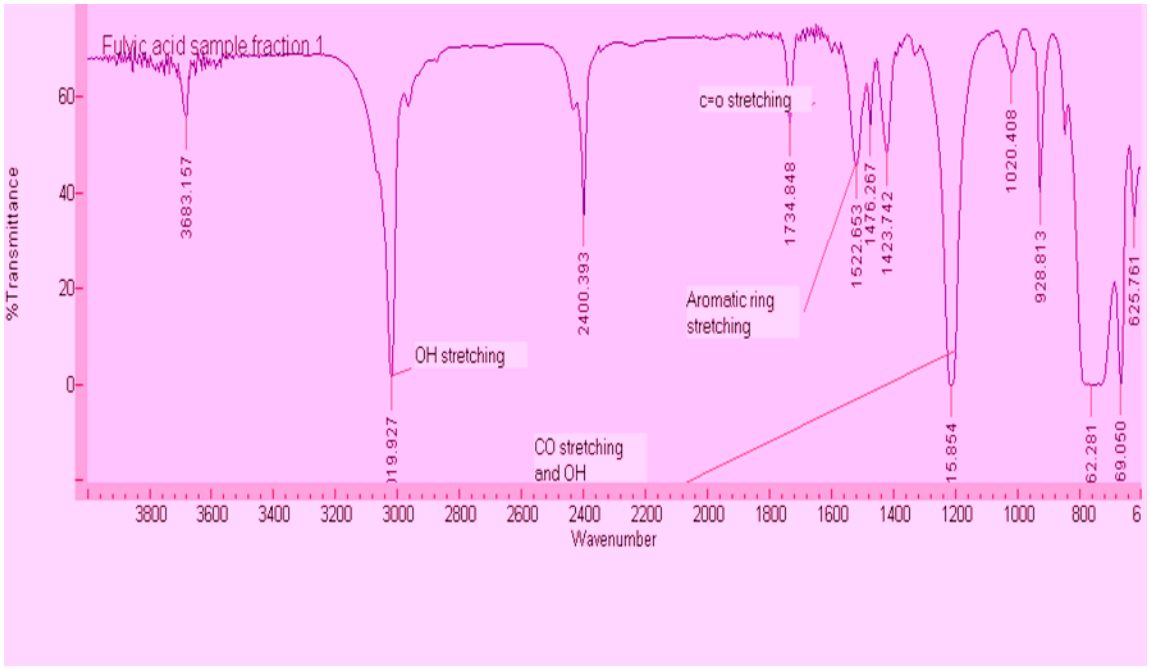

Fig. 4.31: ATR/FTIR spectra of a fulvic acid sample fraction

With reference to the FTIR spectrum of the fulvic acid sample fraction in Fig. 4.31, the functional groups shown in Table 4.12 above were expected. OH stretching was observed at 3341cm^{-1}. The C=O stretching of COOH and ketones were not observed. The strong peak assigned to the CO stretching and OH deformation of COOH and phenolic groups was observed at 1378 cm^{-1}. COO asymmetric stretching (and stretching of aromatic C=C) was observed at about 1647 cm^{-1} and this was consistent with results reported by Jayaganesh *et al* [16] and Yanan [17].

4.9.1 **Differences in the IR spectra of humic and fulvic acids**

Comparing fulvic and humic acid fractions is not easy because they are both hydrophobic fractions of NOM. Although FTIR is not recommended for use in quantitative analysis, it is known that fulvic acids represent the low molecular weight fraction of the whole group of substances that are water soluble at all pHs. On the other hand, humic acids are insoluble at lower pH values with fewer acidic functional groups

than fulvic acids but a more significant degree of aromatic character. And these can be used to identify the differences between the two hydrophobic acids. The main advantage in using infrared spectroscopy are, easy handling, enhanced signal to noise ratios, high reproducibility and the fact that even small quantities of a substance can be measured.

Firstly, the carboxyl C=O stretching absorption peak (around 1734 cm^{-1}) observed in the fulvic acid fraction is not seen in the humic acid fraction which is consistent with the report produced by Giovanela *et al* [18], even though it was observed to be slightly stronger in the FA than in the humic acid. This shows a larger concentration of carboxyl groups, which is expected. The humic acid fraction is usually more aromatic, owing to the C=O of ketones.This phenomenon was not observed. This is expected as extraction procedures are bound to alter some of the chemical linkages present in soil humic substances. Fulvic acids show little to no evidence of $–CH_3$ groups, and are less aromatic compared to humic acids of most literature studies [16, 19 and 20]. These do confirm the effectiveness the separation of the humic substances from water and the fractionation procedure into humic and fulvic acids. The characterized humic and fulvic acids, had overall a fewer functional groups than their corresponding standards. However, this is in agreement with a report by Pompe *et al* [21], where other natural humic acids have low amounts of functional groups and comparable low amounts of carboxylic groups.

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Chapter 5

Conclusions and recommendations

5.1 Conclusions

5.1.1 Fractionation method

The isolation method based on a non-ionic polymeric sorbent (DAX-8) has proven to be applicable and reliable for dam water samples. The developed method has shown to successfully separate the humic substances from water and further separate the humic substances into its hydrophobic acids, namely, humic and fulvic acids. This is a good indication considering that these species are difficult to separate. The ease of isolation of the humic substances from the non-humic substances in water also shows the high retaining capacity of the DAX- resin used. The methods developed are especially suitable for the South African dam water.

Most methods used to isolate NOM were complicated and had long isolation procedures. These were substituted by much simpler spectrophotometric methods. Both of these were done in this study. NOM was separated, isolated and fractionated, and spectrometric methods were used to confirm their character. From the fractionation technique applied it can be concluded that the Eskom Vaal dam composes of humic substance which shows that the technique alone gives a very good indication of the characteristics of water. Knowing the character of South African or local NOM is very important as it leads to informed decisions of what types of treatment processes should be employed.

5.1.2 HPSEC UV and ELS Detection methods

By using the HPSEC column with water as an eluant, MWD values were obtained. HPSEC is a good technique for MWD determination; especially one equipped with UV-VIS and ELSD detection for characterization. Molecular weight distribution of these compounds is a powerful indication of how much DOM is in the dam water. HPSEC with UV and ELSD methods were able to identify the molecular weight distribution of NOM present in source water. Humic and fulvic acids are the natural high-molecular-weight hydrophobic acids which were separated into two fractions. Despite the similarity in terms of MWD in the two NOM fractions, HA showed a higher number of molecules with the highest molecular weights and aromaticity. Some of the samples showed sub-peaks in their chromatograms which could be the impurities originating from the isolation and fractionation procedure. The Eskom Vaal dam contains humic substances in the form of humic acid and fulvic acid and these pose a health concern as they can form disinfectant byproducts in the course of water treatment with chemicals.

Even though the UV method was useful in characterizing these substances, the recommended analysis system would be one that uses the ELS detector as no fractions remain undetected. These results can be used to measure how effective water treatments are by doing analysis of treated water samples and comparing the results before and after treatment. The difference in the results between the UV and ELS detector mean that there are materials which are undetected by the UV detector, as expected. The objective of this study, which was to develop simple methods that can be used to characterize the natural organic matter in water, was successful in that, a method that uses both the UV and ELSD in series was developed successfully.

5.1.3 ATR-FTIR

The characterized humic and fulvic acids, had overall a fewer functional groups than their corresponding standards. However, this is in agreement with a report by Pompe et al [1], where other natural humic acids have low amounts of functional groups and comparable low amounts of carboxylic groups. Characterization using this technique is mainly qualitative and, in general, only some specific bands can be clearly assigned. Progress towards increasing resolution can be made using the FTIR DRIFT method.

5.2 Recommendations

5.2.1 Fractionation method

Most methods have had to use large volumes of water to obtain good TOC and DOC recoveries, up t0 90%. This is necessitated by the fact that NOM in water is generally present in low concentrations. Therefore large volumes of water must be processed to obtain useful quantities of the humic and fulvic acid fractions. Alternatively, pre-concentration methods can be used.

5.2.2 Molecular weight determination by HPSEC

Unfortunately, PEO standards generally have MW values greater than those of humic substances. Even though the calibration graph can be used to estimate the molecular sizes of humic acid and fulvic acid, the distribution is not the best one can get. Standards in the molecular weight region of these humic substances could improve the calibration and hence give a better understanding of their size distribution. Also, one disadvantage of the ELSD is the decreased sensitivity for low-concentration analytes. This is why pre-concentration is a crucial recommendation especially if large sample volumes cannot be used.

References

1. Pompe, S., Bubner, M., Heise, K.H., Schmeide, K., Bernhard, G. and Nitsche, H. 2000. Influence of humic acids on the migration behavior of radioactive and non-radioactive substances under conditions close to nature.

Appendices

Appendices- A1 – A9 (HPSEC chromatograms)

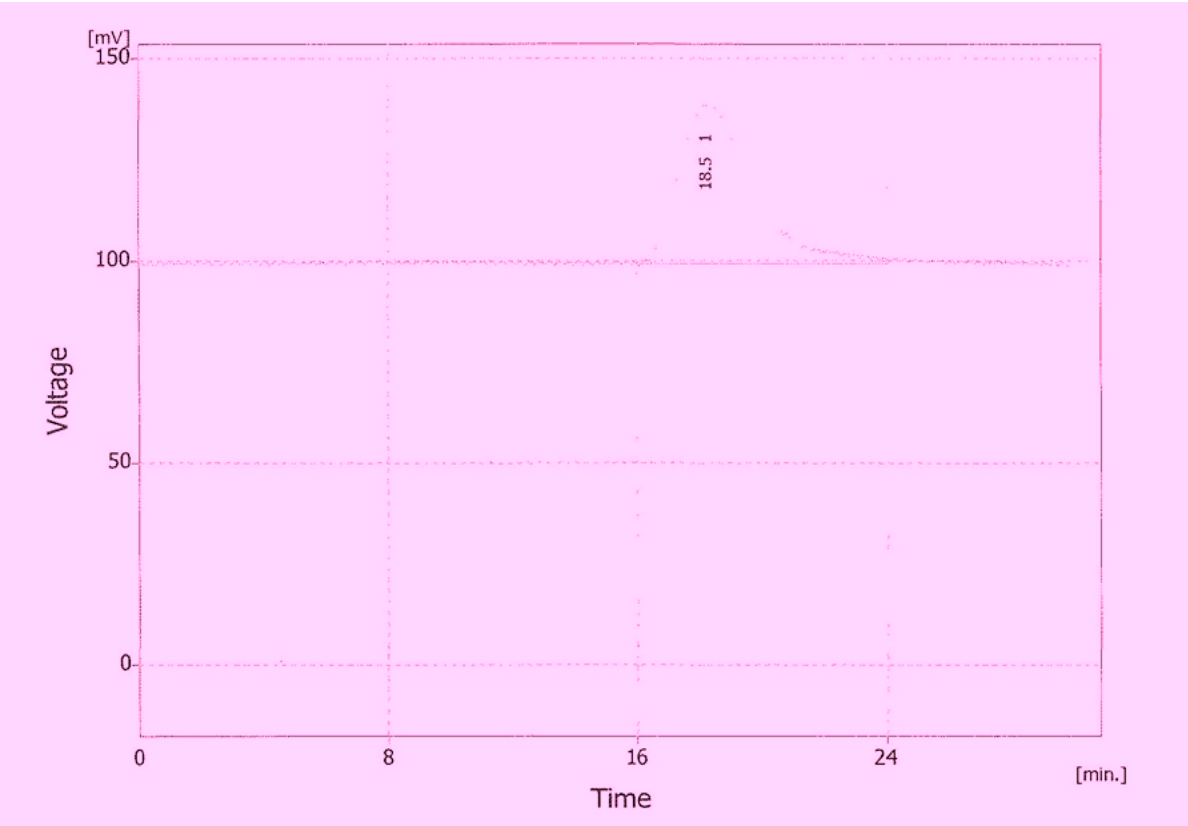

Figure A1: HPSEC chromatogram Humic acid sample fraction ELSD -replicate 1

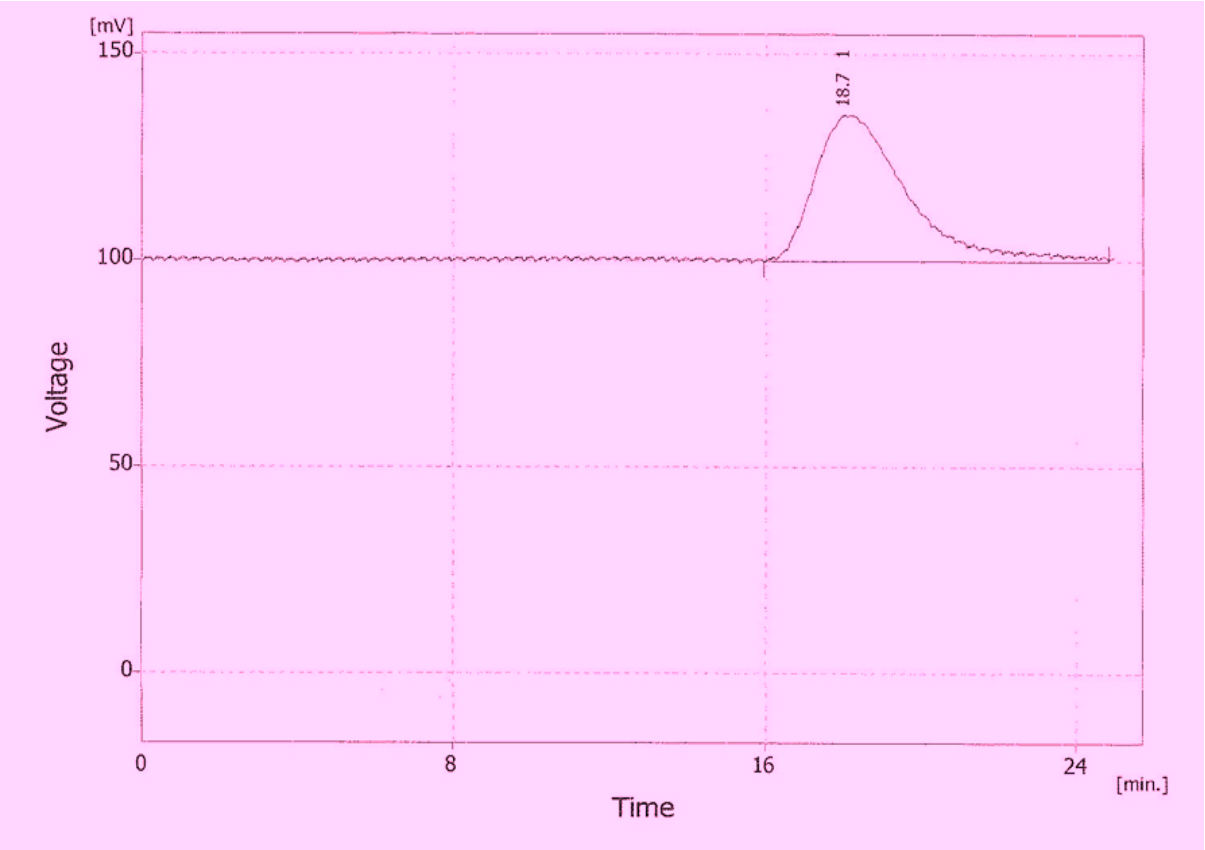

Figure A2: HPSEC chromatogram Humic acid sample fraction UV 254 nm -replicate 1

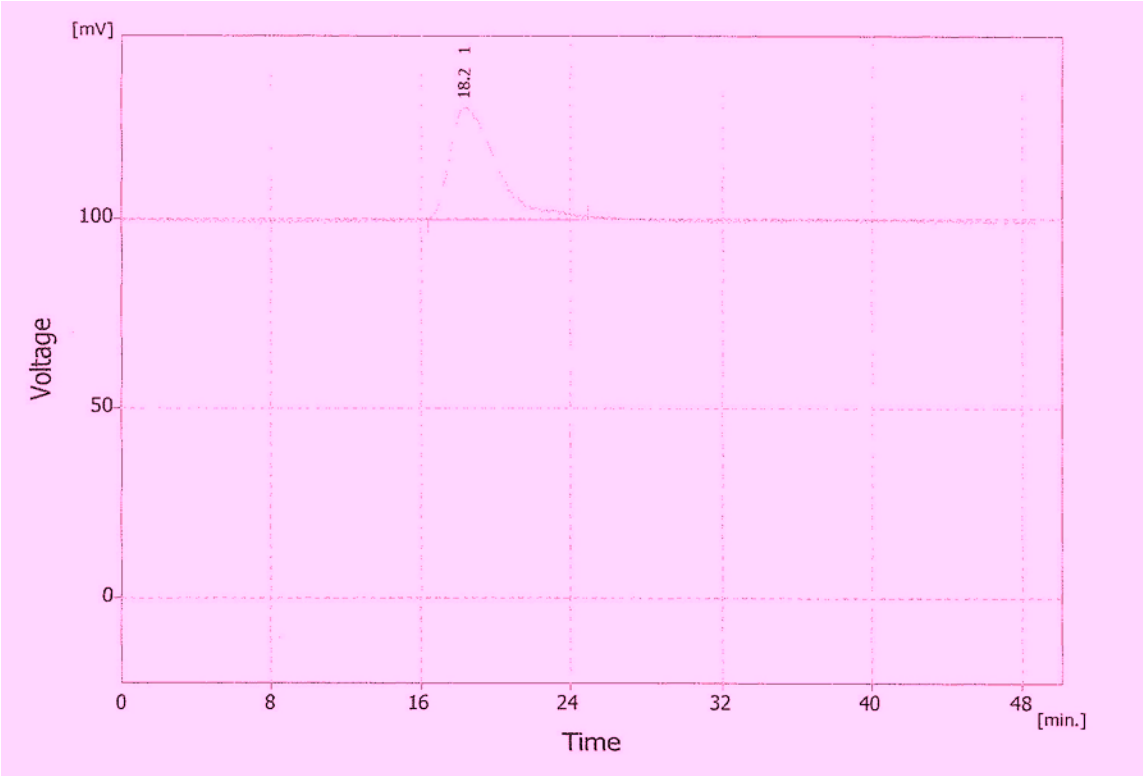

Figure A3: HPSEC chromatogram Humic acid sample fraction UV 254 nm -replicate 2

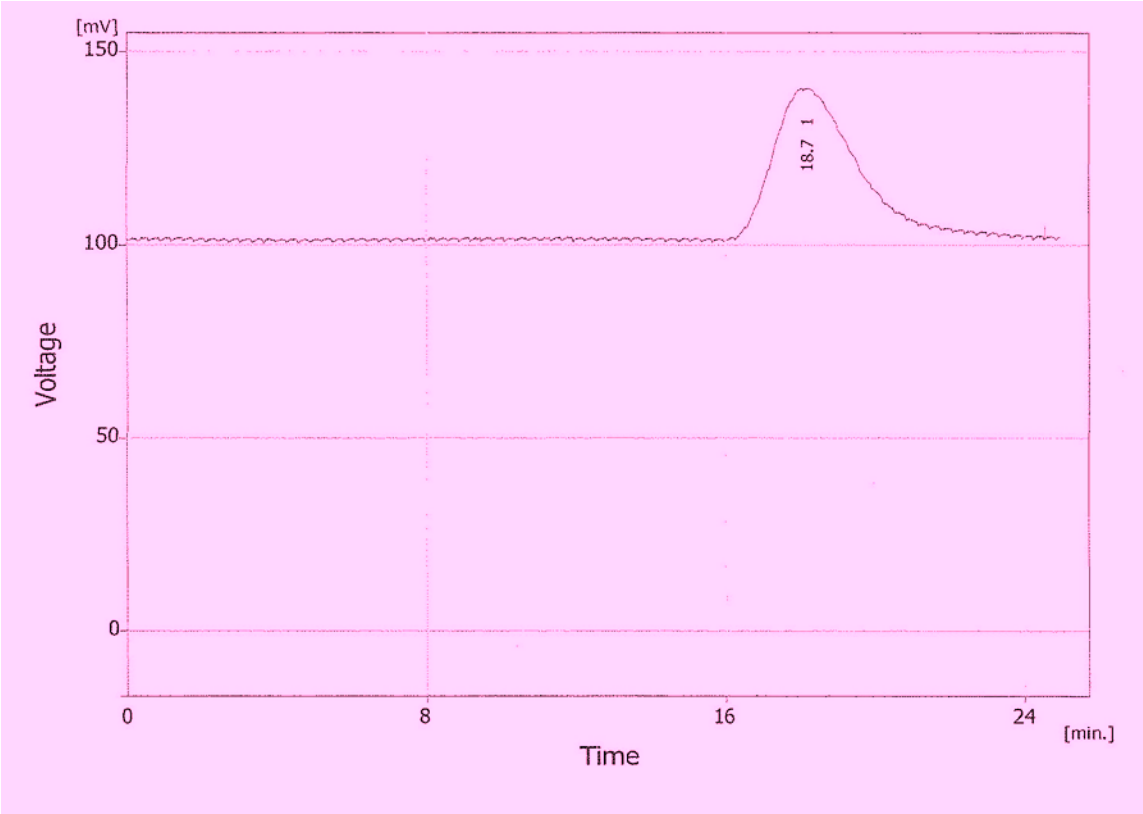

Figure A4: HPSEC chromatogram Humic acid sample fraction UV 280 nm -replicate 1

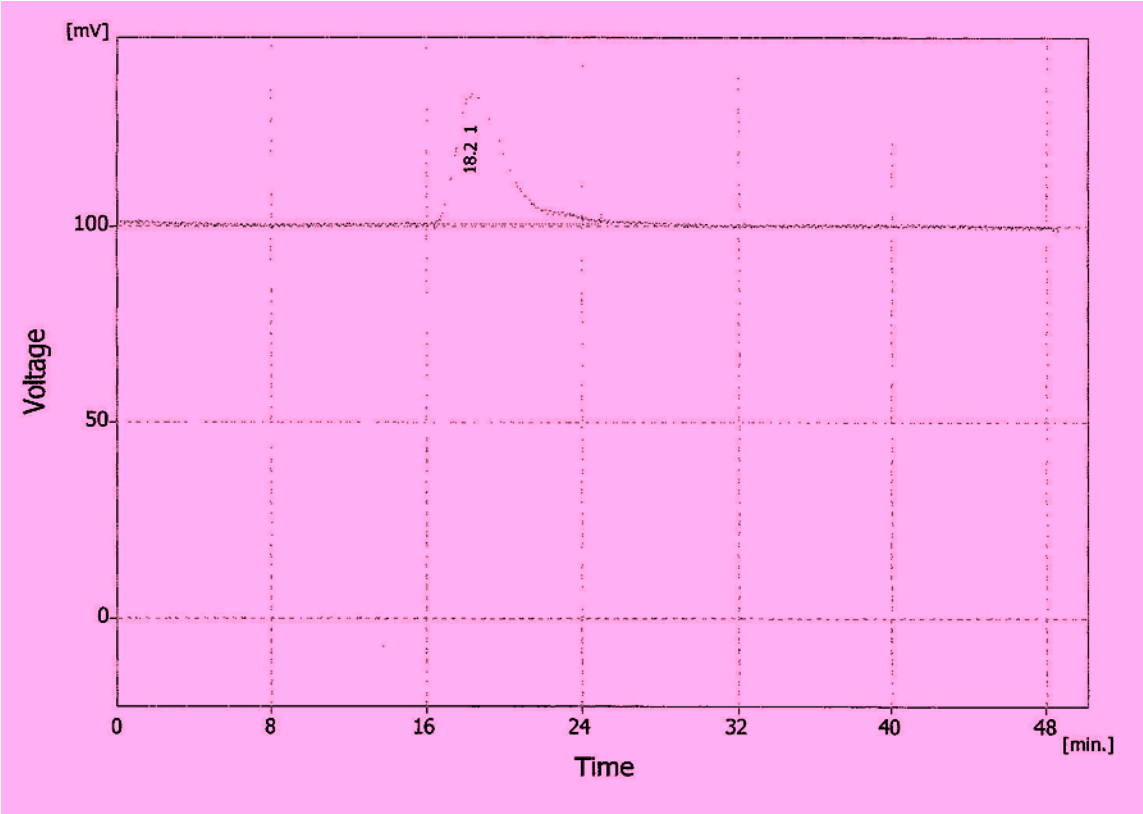

Figure A5: HPSEC chromatogram Humic acid sample fraction UV 280 nm -replicate 2

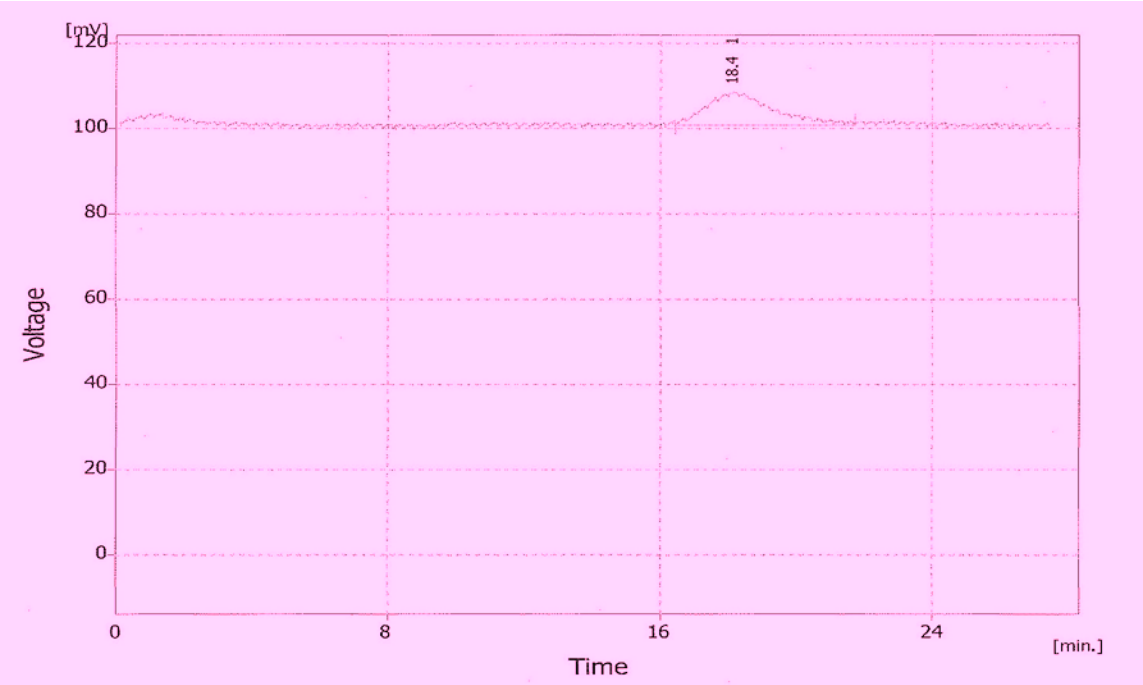

Figure A6: HPSEC chromatogram of fulvic acid sample fraction 254 nm- replicate 1

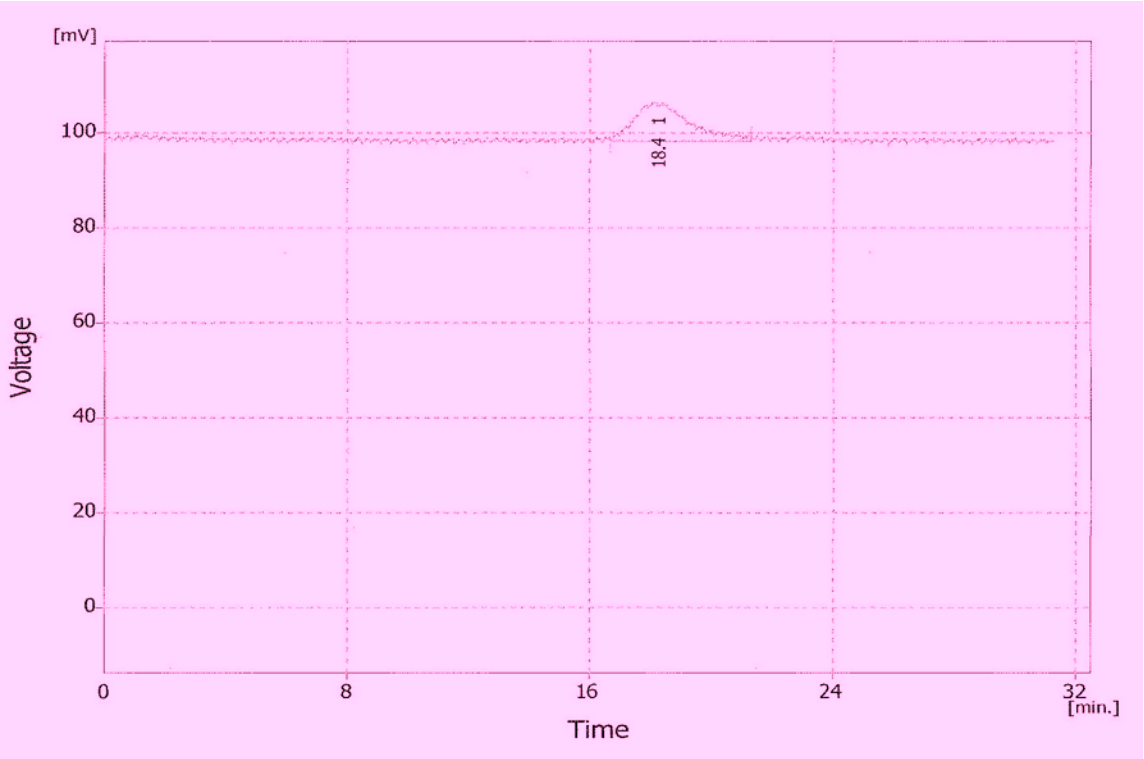

Figure A7: HPSEC chromatogram of fulvic acid sample fraction 254 nm- replicate 2

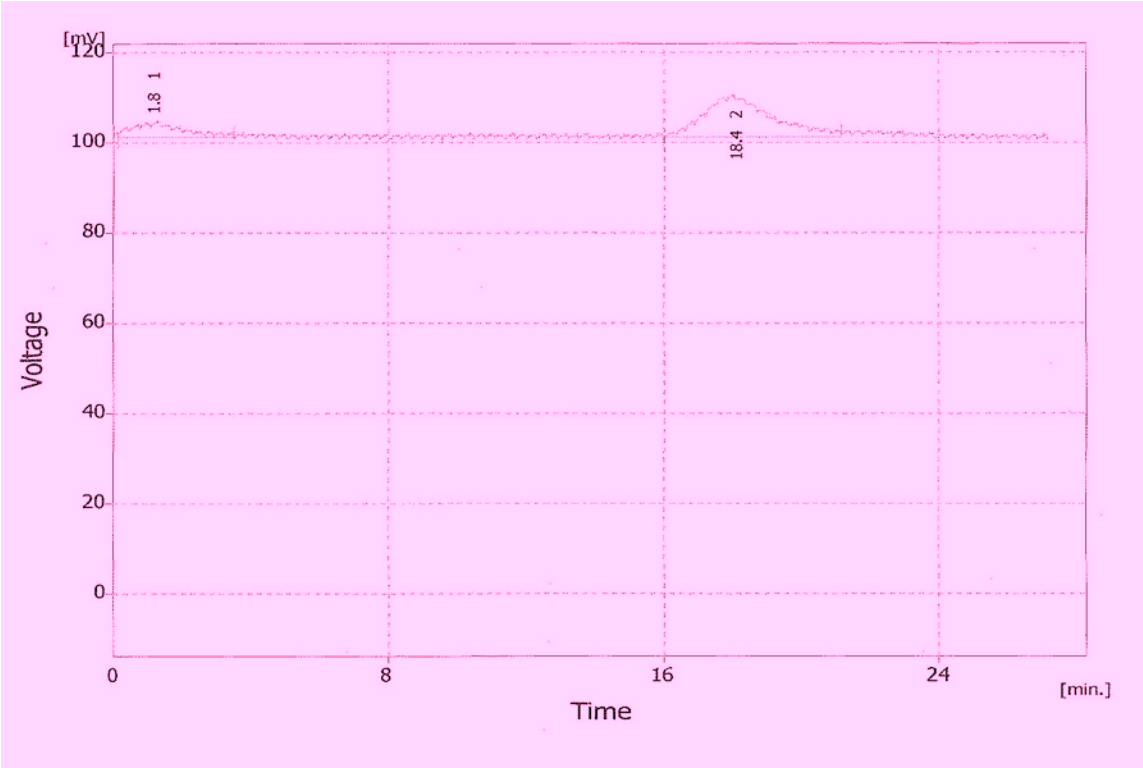

Figure A8 HPSEC chromatogram of fulvic acid sample fraction 280 nm -replicate 1

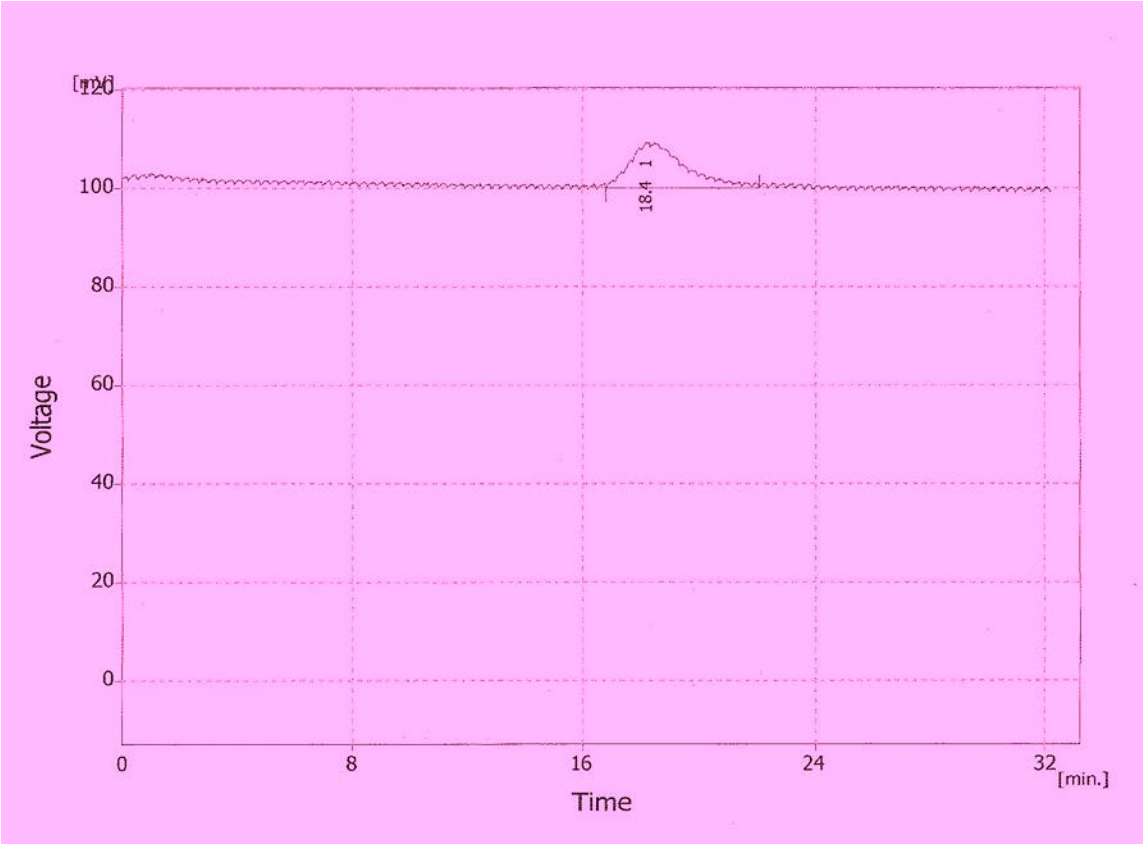

Figure A9: HPSEC chromatogram of fulvic acid sample fraction 280 nm -replicate 2

Appendix B- Eskom report (TOC and DOC analysis)

Eskom

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Laboratory Number T0065

Central Water Laboratory

Final Task Report

Report Reference
WL2012-010381

Attention	Gerhard Gericke
Client Name	WATER & APPLIED CHEMISTRY
Address	ESKOM HOLDINGS Private Bag 40175
Fax	011 629 5528
Telephone	011 629 5721

Date 2012/11/08
Tel. No. +27 11 629 5596
Fax. No. +27 11 629 5528

Report Title WMC
TEST RESULTS FOR THE ANALYSIS OF WATER SAMPLES

Number of Samples 1
Description of Samples ACCEPTABLE
Date Registered 07-November-2012
Date Reported 08-November-2012
Task Comments:

Approved By : _____

Cody Makhuba
Snr Technician
011 629 5596

Date : _____

Tests marked "Not SANAS accredited" in this report are not included in the SANAS Schedule of Accreditation for this laboratory.

Opinions and interpretations expressed herein are outside the scope of SANAS accredition,
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Lower Germiston Road Cleveland 2022 Private Bag 40175 Cleveland 2022 SA
Tel +27 11 629 5686 Fax +27 11 629 5628 www.eskom.co.za
Eskom Holdings Reg No 2002/015627/06

Eskom Central Water Laboratory Test Results

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Laboratory Number T0055

Sample ID 4220525	WMC-2012-11-7/49	WL2012-010381
Raw water		
Raw water		
Component	**Unit**	**Value**
High Level DOC Elementar	mg/l	5.38
TOC HIGH LEVELS	mg/l	6.23

The analyses were performed using the following methods:

DOC ELEMENTAR	**ESKOM METHOD NO 414**	**Not Accredited**
TOC ELEMENTAR	**ESKOM METHOD 414**	**Accredited**

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4. With the exception of all microbiological analyses, unless otherwise stated, sampling is not carried out by the laboratory.

5. All water samples are preserved according to procedure P511 unless otherwise stated.

6. Unless otherwise specified all analyses on water samples give the dissolved constituents.

End of the Report