FRACTIONATION OF NATURAL ORGANIC MATTER (NOM) IN WATER USING PREPARED POROUS SILICA BASED MATERIALS AS SIZE EXCLUSION (SEC)/GEL PERMEATION CHROMATOGRAPHY (GPC) STATIONARY PHASES

By

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I declare that the above dissertation/thesis is my own work and that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references.

_____ _____

SIGNATURE DATE

DEDICATION

This entire project is dedicated to my God, family and leaders. My mother R.N.K Madiba, my late father L.S Bopape, sisters; Puseletso, Thabi, Mmakgoshi, my brother; Lethabo, and my daughter; Phenyo. This work is dedicated to you for always supporting me and showing me an unending love.

"Romans 8:28 All things work together for good for those who love the Lord…"

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ABSTRACT

Natural organic matter (NOM) is a diverse blend of decomposed animal and plant material found in different natural water sources. Due to its large and complex structure, NOM is difficult to both remove and characterize in water. Therefore, there is a need to separate NOM into its components before it can be characterized. The aim of this project was to fractionate NOM through a novel size exclusion chromatography (SEC) composite (poly (styrene-divinyl benzene) (PS-DVB) and Polysilsesquioxane (PSQ)) packed column. Raw and final water samples from Mid-Vaal (MV), Olifantspoort (LO), Mtwalume (MT) and Preekstoel (P) were investigated. Poly (styrene-divinyl benzene) (PS-DVB) and polysilsesquioxane were both synthesized and optimized at various temperatures, compositions and time periods. An end-capping material such as hexamethyldisilizane (HMDS) was added on the PSQ to prevent active silanol groups on the polysilsesquioxane (PSQ) from reacting with active sites of NOM (our analyte). The E-PSQ (end-capped PSQ) and PS-DVB materials were packed in eight different SPE cartridges first, before the materials could be packed in the SEC column. This packing was done to check for the best mass composition of the E-PSQ and PS-DVB. From the obtained SPE results, both the E-PSQ and PS-DVB were packed in one SEC/GPC column at a ratio of 1:1 in order to form the composite hybrid material. The packed SEC column was connected to an HPLC instrument and various column efficiency tests were evaluated. The results for the test of interactions with acidic compounds implied that the column can be used for the acidic analytes such as those forming NOM composition (humic acids, fulvic acids) and the column had minimum silanol groups. For hydrophobic interactions the stationary phase strength was different to that of the commercial columns and it could selectively elute molecules based on their different masses. The steric selectivity test showed that the stationary phase could separate and distinguish between molecules with similar hydrophobicity and structure but different shapes (o-terphenyl and triphenylene). The Hydrogen bonding capacity (HBC) test showed that the column had minimum silanol groups and the end-capping was successful on the E-PSQ.

After fractionation of all the water samples, the MT raw showed NOM peaks around 1.8 mins, 3.4 mins and 5.3, and the final showed NOM peaks around 1.8 mins and 5.5 mins. The Mid-Vaal (MV) raw and final samples shows NOM peaks at around 1.8 mins and 6

mins. The Preekstoel (P) final water had one NOM peak at around 1.8 mins and raw samples had two NOM peaks around 1.8 mins and 6 mins.

KEY TERMS:

Natural organic matter, size exclusion chromatography, poly (styrene-divinyl benzene), polysilsesquioxane, column packing

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LIST OF ABBREVIATIONS

AC	activated carbon
MW_{ave}	average molecular weight
BET	Brunauer–Emmett–Teller
k’	capacity factor
ClO_2	chlorine dioxide
n	column efficiency
DBPs	disinfection by-products
DOC	dissolved organic carbon
DVB	divinyl benzene
EPS	extracellular polymeric substances
FTIR	Fourier transform infra-red spectroscopy
FEEM	Fluorescence excitation-emission matrices
FA	fulvic acid
EDS	Energy Dispersive spectrometer
GC	gas chromatography
GAC/PAC	granular or powdered activated carbon
GFC	gel filtration chromatography
GPC	gel permeation chromatography
HAAs	haloacetic acids
HKs	haloketones
HNMs	halonitromethanes
HMDS	hexamethyldisilazane (HMDS)
HPSEC	high performance size exclusion chromatography

HA	humic acid
HSs	humic substances (HSs)
HBC	Hydrogen bonding capacity (HBC)
HPI	hydrophilic
HpiA	hydrophilic acid
HpiB	hydrophilic base
HpiN	hydrophilic neutral
HPO	hydrophobic
HpoA	hydrophobic acid
HpoB	hydrophobic base
HpoN	hydrophobic neutral
HR	Hydrophobic retention
HS	Hydrophobic selectivity
IEC	ion exchange chromatography
LC	liquid chromatography
LC-OCD	liquid chromatography-organic-carbon detection
LMW	low molecular weight
MW	molecular weight
MWD	molecular weight distribution
NOM	natural organic matter
OCD	organic carbon detection
OND	organic nitrogen detector
O_3	ozone
PC	paper chromatography

POC	particulate organic carbon
PRAM	polarity rapid assessment method
PSQ	polysilsesquioxane
PS-DVB	poly (styrene-divinyl benzene)
RP-HPLC	reversed phase high performance liquid chromatography
SEM	scanning electron microscopy
SEC/GPC	size exclusion/gel permeation chromatography
α	selectivity factor
SPE	solid-phase extraction
SUVA	specific ultraviolet absorbance
SS	Steric selectivity
S	Styrene
TGA	thermogravimetric analysis
TLC	thin layer chromatography
TPI	transphilic
THMs	trihalomethanes
TOC	total organic carbon
Uv-Vis	Ultra violet-visible
UVD	ultraviolet Detection
XRD	X-ray diffractometer

CHAPTER 1:

INTRODUCTION

1.1. BACKGROUND

Lack of clean and safe drinking water has for many years been a pressing issue especially in rural and economically disadvantaged communities of South Africa. Individuals living in such places, consume and/or use both treated and untreated water from various natural sources (rivers, dams, ponds etc.) for their daily activities. However, drinking untreated and/or contaminated water result in many life threatening water-borne diseases including gastrointestinal infections and drinking half-treated water may result in the consumption of carcinogenic disinfection by-products (DBPs), of which the most common are trihalomethanes (THMs) and haloacetic acids (HAAs). [1–5]

Many diseases emanate from the occurrence of various contaminants such as pathogens, bacteria, protozoans, and natural organic matter (NOM) (a major precursor for the formation of the DBPs) in natural water.[1,5–7] NOM is regarded as one of the most problematic pollutant that affect raw water, water undergoing treatment and water in the distribution systems.[8–12]

1.1.1. NATURAL ORGANIC MATTER (NOM)

NOM is derived from a composite mixture of animal and plant remains[1,3–15] which are chemically or physically bound in natural water sources and thus possesses a variety of chemical properties.[11,16,17] Since NOM is recorded to be found in all natural water sources (e.g. rivers, dams, and ponds).[13,17–19] NOM in natural sources can be a resultant of external sources (animals and plants) and internal sources.[14] However, the main source of aquatic NOM was reported to be terrestrial plants and aquatic soils.[14] The animal and plant matter are transported into the source by means of rain, floods and runoff. In fact, factors such as rainfall, flood, snowmelt runoff, biological activity, soil, seasonal changes, vegetation, mixture of lake water with river water, human activity, drought seasons and variation in geographical area all influence the quantity (amount) and quality (structural morphology) of NOM in natural water sources.[4-9]

NOM is also a source of food for heterotrophic bacteria, therefore, it promotes bacterial growth in both raw and water undergoing treatment.[21,22]

1.1.2. EFFECTS OF NOM IN WATER

1.1.2.1. Water quality effects

When NOM is present in natural water, it decreases the water quality. NOM changes natural water to a dark brownish colour and gives natural water a foul smell and a foul taste.[12,14,21,23–25] It is important to note that the foul taste, foul smell and dark brownish colour that result from NOM contaminated water is dependent on the quantity of NOM in the natural water source. Since the NOM quantity change with changing season,[21,26,27] it is also important to note that its quality also changes.[21,24,26] It is well reported that the turbidity, total organic carbon (TOC), dissolved organic carbon (DOC) and pH of natural water is much more in winter as compared to summer.[20] The transphilic (TPI), hydrophobic (HPO) and hydrophilic (HPI) fraction of NOM cannot be detected in summer but can be detected in winter.[20]

NOM is large and complex and therefore difficult to remove in water. This is due to the different fractions which are present in NOM. These fractions were reported to be present with different molecular weight and composition uniquely from one treatment plant to another and this property affect both the removal of NOM and their reactivity with coagulants or other chemicals in water.[28]

1.1.2.2. Water treatment and distribution system effects

NOM causes problems in almost all water treatment processes. This is due to its large structure, complexity, high aromaticity and its ability to co-exist in different polarities.[9,29] When water contaminated with NOM passes through the coagulation/flocculation process, it demands a large amount of both oxidants and coagulants.[19,30]. NOM promotes bio-growth, since it is known to be a food source for heterotrophic bacteria, this characteristic of NOM affect the efficiency of the granular activated carbon during water treatment processes.[30–32] During the filtration process, NOM blocks the pores of the membranes, therefore NOM is classified as one of the major membrane foulants.[9,19,30] At the last stage of the water purification process, which is the disinfection process, NOM reacts with the chlorine to form carcinogenic and mutagenic DBPs.[2,33–37] It is important to note that NOM does not only interrupt the water purification processes, it bonds with other contaminants

such as metals and hydrophobic organic species and interrupt their removal from water.[4,34] It is also reported that humic substances (HSs) combine with inorganic colloidal silica to yield double fouling of membranes.[39] NOM is also problematic in distribution systems as it corrodes the distribution tunnels.[9] NOM is therefore problematic throughout the different stages of the drinking water treatment. The presence of NOM in a water treatment processes forces a change in the design, operation and maintenance of the process [30]. It also disturbs the process towards the removal of inorganic particles and block the pores of granular or powdered activated carbon (GAC/PAC).[30]

1.1.2.3. Disinfection by-products (DBPs)

Natural water i.e. water from dams, rivers, ponds or even ground water undergo treatment such that it is safe for drinking. This water treatment began around the 20th century, this process was made possible by using chlorine as a disinfectant in drinking water treatment processes.[37] Chlorine was found to be good in destroying pathogens that caused typhoid, cholera and dysentery.[37]

When NOM is found in effluents of water treatment plants, it tends to react with disinfectants such as chlorine and therefore, weakens the power of the disinfectant which in turn forces a high demand of disinfectants.[30,40] This reaction produces disinfection by-products (DBPs). DBPs are reported to be either mutagenic or carcinogenic. There are different types of DBPs reported thus far, DBPs such as haloketones (HKs), trihalomethanes (THMs), haloacetic acids (HAAs) and halonitromethanes (HNMs) and amongst these trihalomethanes (THMs) and haloacetic acids (HAAs) were found to be the most common DBPs.[9,37]

Many researchers suggest that NOM present in natural water (raw water) should be removed or reduced before it undergoes treatment. This will reduce the quantity of DBPs that will result after disinfection by chlorine (i.e. chlorination). Since most of the chlorine reacts with NOM during chlorination, higher dosage of chlorine is required to compensate the chlorine that is consumed by NOM. This additional dosage of chlorine increases the costs of water treatment.

1.1.2.4. Environmental effects

When NOM reacts with coagulants during the coagulation and flocculation process of water treatment plant, flocs (i.e. sludge) are formed (**Fig. 1.1**). When is exposed to the environment, sludge causes environmental pollution. Since NOM has the ability to bind with metals and other contaminants, this complex mixture of metal bound NOM can affect humans when exposed to the environment.[41] It is important to note that the complexes that are formed have increased mobility in nature.[30]

Figure 1.1: Sludge from the coagulation and flocculation process [42]

1.1.2.5 Removal impacts

Water treatment practises are able to remove most of the NOM.[43] It was found that the HSs can be removed by the coagulation and the nano-filtration process.[14,43,44] Such processes, however, fail drastically to remove the hydrophilic part of NOM as well as NOM that has high carboxylic group functionality and charge density.[27,44] Although a process such as the granular activated carbon (GAC) is popularly utilized to remove NOM from water, this process also fail to remove NOM to parts per billion levels.[24] The enhanced coagulation, which is a modification of coagulation whereby excess amounts of coagulants are added, has also failed to remove the hydrophilic NOM.[45] Although hydrogels have demonstrated high removal rates towards hydrophobic NOM, they have a low removal efficiency towards hydrophilic NOM.[45] Other methods like ozonation are used to degrade NOM into smaller sizes such that NOM can be easily removed from water.[24]

1.1.3. THE PHYSICAL AND CHEMICAL PROPERTIES OF NOM

Since the nature and characteristics of NOM are influenced by its source of origin, seasonal changes and industrial or agricultural activities around or near the water source.[11,46]. NOM is a diverse mixture of organic materials, which include larger molecular weight humic substances (HSs) and smaller molecular weight substances (acids, lipids, proteins, carbohydrates carboxylic acids, hydrocarbons and amino acids).[19] NOM in natural water system can further be divided into the dissolved organic carbon (DOC) and the particulate organic carbon (POC).[14] DOC is described as the organic carbon that can diffuse through a 0.45 μm filter.[9,14,20] Most of the research relating to NOM has focussed largely on the DOC. DOC can be further divided into the hydrophilic acids, bases and neutrals, also known as non-humic substances (non-HSs), and hydrophobic acids, bases and neutrals which are also called humic substances (HSs).[19,4717] The HSs and non-HSs are also called the hydrophobic and hydrophilic part of NOM, respectively. The hydrophobic component constitutes the major part of NOM, which is very rich in aromatic compounds. The hydrophilic fraction of NOM is made up of proteins, sugars and carbohydrates.[17,48,49] The humic and fulvic acids (humic substances) make up over 60% of the total dissolved organic carbon (DOC) and their concentration can be directly estimated from the DOC values.[21,50] HSs have higher specific ultraviolet absorbance (SUVA) values as compared to non-HSs.[24]

HSs are negatively charged species, which are dominated by carboxylic functional groups this (group constitute above 80% of the molecule) and the phenol groups.[51] Humic substances (HSs) have an ability to bind heavy pollutants and other contaminants in water such that they can be simultaneously removed with the other contaminants (i.e. in the form of metal-bound HS complex) from water.[41]. The HSs are black or brown in colour, amorphous in nature, and have a variety of molecular weight (MW) ranging from 100-10 000 g/mol.[41] It is difficult to chemically remove and to separate NOM into the HSs and non-HSs parts using conventional methods of NOM characterization because these components are covalently bound to each other.[14]

Therefore, the large structure of NOM, its complexity, covalently bound fractions, as well as its variation from one location to the other necessitates the need to separate the fractions of NOM in water before its characterization (identifying its properties) in different water sources.[9,11,21,52,29] It is important to note that the different fractions of NOM differ in both

size and polarity.[17,35,54] The separation of each individual fraction of NOM is necessary, such that, the removal can target specific fraction of NOM.

1.2. PROBLEM STATEMENT

Unlike other pollutants, NOM is complex that when fractionated using the conventional methods such as the polarity rapid assessment method (PRAM) it yields three fractions. These fractions are known as the transphilic, hydrophilic and hydrophobic fractions, and they are also called large, medium and small NOM fractions, respectively.[9] The fractions have various molecular weights, sizes and polarity. Therefore, the other fractions of NOM which are not polar in nature, cannot be fractionated (separated) using this method. This problem is also found in other NOM separation methods; that some fractions/parts of NOM are excluded during the separation.

As such, a method that can effectively separate all the fractions of NOM according to both their sizes and molecular weight is worth investigating. Size exclusion/gel permeation chromatography (SEC/GPC) is a physical separation method that allows molecules in solution to be separated according to size and molecular weight.[55] The SEC/GPC method is therefore ideal for NOM separation (fractionation) since NOM fractions co-exist in different sizes and molecular weights.

It has been reported that during the separation of macromolecules (i.e. separation of polymers by the SEC/GPC method) there are possibilities that the macromolecules will be reduced to unevenly sized fragments. This is due to the inability of the macromolecules to fit in the small pores of the stationary phase of the column. This phenomenon is known as shear degradation.[56] NOM is reported to be a large molecule and it is complex in nature, therefore, it is subject to shear degradation. Careful selection of the pore sizes of the stationary phases is thus required for this study.

Shear degradation also occurs when a normal SEC/GPC is replaced with a high performance size exclusion chromatography (HPSEC).[57] Most SEC packing materials are composed of small particles in order to achieve higher column efficiencies. However, this increases the shear degradation of macromolecules.[56] The particle size of silica based material of the SEC/GPC stationary phase should be bigger than 2 μm, to prevent shear degradation of macromolecules from occurring.[58] The challenge with commercially

available columns is that they possess stationary phases that contain small particles (less than 2 μm). These particles promote shear degradation of NOM and other macromolecules. Therefore, a need of a SEC/GPC stationary phase that is compatible with the NOM large structure and complexity is a necessity.

1.3. JUSTIFICATION

The fractionation of NOM into different molecular weight (MW) fractions is important to drinking water treatment plants, since NOM has been reported to be problematic in all treatment processes. By fractionating NOM before water treatment, this will minimize NOM from causing problems during the different stages/processes of water treatment processes. During the water treatment stages for drinking water, the humic substances of NOM give the water the dark colour, promote bio-growth and also transport pesticides (hydrophobic pollutants) as well as produce disinfection by-products (DBPs) (during the disinfection process).[31,59] The negatively charged hydrophilic fraction of NOM add more contaminants in the water by transporting heavy metal cations.[31,59] The higher MW NOM can be removed by coagulation while lower MWs are removed by activated carbon (AC) adsorption during water treatment, and they therefore block the adsorption sites of the AC and this prevents other contaminants from being removed by AC.[60]

The SEC/GPC fractions of NOM reported in the literature vary with the source and the stationary phase of the SEC/GPC column. It is important to note that the stationary phases of SEC/GPC developed cannot account for the polydispersity and different chemical composition of NOM. Therefore, during the application of SEC/GPC technique in NOM fractionation, careful selection of the stationary phase should be taken into consideration. The likelihood of shear degradation of NOM to occur in SEC/GPC is very high because NOM is very large in size and complex (in terms of MW).[58]

It is important to take note of other factors that increase shear degradation of NOM. These factors include: NOM structural complexity, the relativity that exist between the size of NOM and the spaces between the particles (NOM is bigger and the spaces are slim) and the ability of other smaller functional groups found on the NOM compound to be trapped in the small pores of the porous stationary phase.[41] Shear degradation is a mechanical

process and not a chemical process. Compounds do not dissociate due to chemical reactions that exist in the column but it is due to the pressure that is applied to push the mobile phase (containing different compounds) through a stationary phase. The compounds disassociate while trying to fit through the small spaces available.

To minimize or eliminate shear degradation in SEC/GPC suitable stationary phases are required. The spaces between the stationary phases particles should be bigger and their sizes should be greater than 2 μm.[58] The stationary phases must tolerate a wide range of pH (since NOM consists of a variety of pH values), must be cross-linked (to trap the smaller particles), porous and must not dislocate from their place on the column (rigid and sessile). They must also be polymeric and abide to the principles of SEC/GPC.[55] Hence, the selection of polysilsesquioxane and poly (styrene-divinyl benzene) as the stationary phases for the study.

1.4. AIM AND OBJECTIVES

1.4.1. AIM

The aim of this study is to synthesize porous poly (styrene-divinyl benzene) (PS-DVB) and polysilsesquioxane (PSQ) and to assess them as SEC/GPC stationary phases for the fractionation of NOM.

1.4.2. OBJECTIVES

The objectives of this study were:

- To synthesize PSQ and PS-DVB and their characterization by FTIR, TGA, XRD, Raman and SEM
- To fractionate NOM via the packed SEC/GPC column and to characterize the fractions using UV 254 detector from the HPLC

1.5. RESEARCH QUESTIONS

1.5.1. How can we better optimize the SEC/GPC method such that it can separate NOM into its different fractions?

1.5.2. Which fractions of NOM can be separated by the developed SEC/GPC column?

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1.7. DISSERTATION OUTLINE

The structure of this dissertation is as follows:

Chapter 2: Literature review

This chapter gives the general description of NOM, its effects, characterizations, removal and fractionation techniques. It gives theories corresponding to chromatography, size exclusion/ gel permeation chromatography (SEC/GPC) and the advantages as well as the disadvantages of the poly(styrene-divinyl benzene) (PS-DVB) and polysilsesquioxane (PSQ). This chapter concludes by identifying the PSQ and the PS-DVB as suitable stationary phases to fractionate NOM.

Chapter 3: Experimental methodology

This chapter explains all the experimental methods which were followed to accomplish both the aim and the objectives of this study.

Chapter 4: Characterization of poly (styrene-divinyl benzene) and polysilsesquioxane

This chapter gives the results of the PS-DVB and PSQ as confirmed by various structural and morphological techniques.

Chapter 5: Application of materials as solid–phase extraction (SPE) and size exclusion/gel permeation (SEC/GPC) stationary phases

This chapter gives the overall column performance tests and the fractions of NOM from both standards and real samples.

Chapter 6: Conclusion and recommendations

This chapter gives an overall conclusion of the study and the recommendation towards furthering the study.

CHAPTER 2:

LITERATURE REVIEW

2.1. INTRODUCTION

This chapter reviews the literature on the analytical methods that have been reported for the characterization and fractionation of NOM. The limitations and attractive features of the reported methods of NOM are also covered.

2.2. CHARACTERIZATION OF NOM

2.2.1. Introduction

When quantifying NOM, it is important to note that its concentration can be measured in two ways. Firstly, by measuring the total organic matter (TOC), which is the organic matter that did not undergo filtration with a 0.45 μm filter, Secondly, the concentration of NOM can be measured via the dissolved organic matter (DOC), which is an organic matter that was filtered through a 0.45μm filter.[1] The concentration of NOM in water can be measured by both the TOC and DOC analysis on the TOC instrument.

2.2.2. Methods of NOM Characterization

2.2.2.1. Direct measuring methods

Direct methods such as pH, conductivity and turbidity influence the nature and characteristics of NOM in water. It is reported that the pH, TOC and turbidity of water are higher in winter compared to summer.[2] The measure of TOC and DOC has helped researchers to predict the concentration of NOM in water and to study the change in TOC and DOC concentration in every season.[3–5]

2.2.2.2. Ultraviolet-Visible (UV-Vis) spectroscopy

The UV–Vis absorption at 254 nm measures the organic carbon rich in aromatic rings, which correspond to the humic substances.[6] This is due to the presence of the UV chromophores.[7] Other wavelengths such as: 214 nm, 272 nm and 300 nm indicate the presence of nitrites and nitrates, trihalomethanes and DOC, respectively.[7]

2.2.2.3. Specific Ultra-Violet Absorbance (SUVA)

Specific ultraviolet absorbance (SUVA) must be calculated to assess the concentration of the non-humic versus the humic substances and it is calculated using the equation 2.1 below.[8–10]

$$SUVA = \frac{UV_{254}}{DOC} \times 100 \quad \text{(2.1)}$$

SUVA ($cm^{-1}/mg.L^{-1}$), where UV_{254} is the UV-Vis absorbance at 254 nm (cm^{-1}) and DOC is the quantity of dissolved organic carbon in mg/L.

SUVA at 254 nm gives the nature of the aromatic compounds of NOM by classifying the aromatic NOM samples.[11–14] The SUVA value can also be used to evaluate the reduction or removal of the content of HSs.[12] SUVA can also indicate the potential of natural organic matter to produce trihalomethanes (THMs) and haloaceticacids (HAAs).[14] Low values of SUVA indicate that the NOM content is rich with the hydrophilic (HPI) fraction and therefore has lower aromaticity.[11] If the SUVA value obtained is close to 5 and above then the organic matter fraction is known to be the hydrophobic (HPO) fraction that is rich with aromatics; a SUVA value that is lower than 2 and between 2 to 4 corresponds to the HPI and TPI fractions, respectively.[11,15]

2.2.2.4. Fluorescence excitation-emission matrices (FEEM)

The NOM that have fluorescent chromophores can be measured by both the intensity peaks from the FEEM contour plot as well as the excitation and emission wavelength pairs at which NOM occur.[16,17] FEEM has high sensitivity as it is able to analyse fractions of NOM at concentrations lesser than 0.1 mg/L.[9] FEEM can be used to monitor and measure a variety of processes that occurs during the water treatment. Additionally, FEEM can be used to monitor reprocessed water systems, to evaluate the TOC removal, characterize NOM from surface water, trace the removal of TOC using clarification process from surface water treatment and distinguish between terrestrial and aquatic DOC using fluorescence index (FI).[9,16–20]

2.3. FRACTIONATION OF NOM FROM WATER

2.3.1. Introduction

The first step to NOM characterization, is through its fractionation (separation) into its different components. This is due to the complex and large structure of NOM.[6,21,22]

2.3.2. Liquid chromatography-organic carbon detection (LC-OCD)

The LC-OCD is a method that can measure the quantity of both total organic carbon (TOC) and dissolved organic carbon (DOC) in water samples at concentration range of 10 ppb - 5 ppm.[23] The technique was first developed by DOC-Labor of Karlsruhe, Germany. The LC-OCD is a type of liquid chromatography, which utilizes a size exclusion chromatography (SEC)-column to separate and fractionate the TOC from water samples and uses the ultraviolet detector (UVD), organic carbon detector (OCD), organic nitrogen detector (OND) and the UV 254 (absorbance) to detect the organic compound.[11] This method separates NOM into five fractions according to both their chemical properties and: biopolymers, humic substances, building blocks, low MW (LMW) acids and LMW neutrals.[11]

2.3.2.1. Principles of LC-OCD

The mobile phase (phosphate buffer) is pumped through the instrument using an HPLC pump (1.1 mL/min flow rate), into an auto sampler (injection volume of 1 µL), and the column of polymethacrylate source (weak cation exchanger). The 0.45 mm polyether sulfone (PES) filter was used to filter the samples before analysis. Upon separation, the sample goes to the 254 nm UV-Vis detector (UVD), and thereafter the organic carbon detector (OCD). Carbonates are converted to carbonic acid at a 0.2 mL/min flow rate, right at the inlet of the OCD detector. The DOC at dead volume time are measured by passing the mobile phase at 0.1 mL/min.

2.3.2.2. Fractions of NOM reported from LC-OCD

The LC-OCD method have been reported to fractionate NOM into the following fractions: fraction A, which corresponds to the biopolymers; B (humic substances (HSs)); C (building blocks); D (low molecular-weight acids); and fraction E (low molecular-weight neutrals)[23].

2.3.2.2.1. Fraction A: Biopolymers

This fraction was able to elute near the SEC column (exclusion volume). The resulting peak indicated that there was no hydrophobic interaction and diffusion through the pores of the stationary phase of the column. The position of the peak described the fraction to be hydrophilic with high molecular weight. The mass of this fraction was taken to be greater than or equal to 10 kDa, since the column that was used can separate materials from 0.1 kDa, to 10 kDa.[23] This fraction did not respond to the ultraviolet detector (UVD) but it responded to the organic nitrogen detector (OND). This means the biopolymer fraction were having nitrogen containing proteins or amino sugars which are found in polysaccharides.[23]

The extracellular polymeric substances (EPS) have polysaccharides as dominant materials in their structure along with matter that is mainly composed of proteins. EPS is a general word that describes biopolymers.[23]

2.3.2.2.2. Fraction B: Humic substances (HSs)

The humic substances (HSs) peak from this studies eluted around 45 mins for both organic carbon detector (OCD) and UVD. [23] The retention time, shape of the peaks and the ratios of the detector all corresponded to the HS fraction. The retention times of maximum peak for OCD and UVD were different, this difference proved that the UVD is not properly suitable for both the quantification and characterization of HS. For commercial fulvic and humic acid (FA and HA) the chromatogram appeared are 43.4 min (HA) and 46.7 (FA) min which proved the reality of the literature which depicted that HA is always eluted before FA in a SEC column.[23] Therefore, OCD for SEC can be used detect the MW of HSs in both real water samples and the prepared water samples but this can only be achieved if the HS method of fitting, molecular mass of HSs and the retention time of HSs are available.

2.3.2.2.3 Fraction C: Building blocks

The building block fraction is eluted after the HS-fraction. This fraction shows a high response from the UVD detector and the fraction describes the lower molecular weight HSs-like materials.[23]

2.3.2.2.4 Fraction D: Low molecular-weight acids

The right slope of the building blocks fraction defines the left boundary of the low molecular-weight acids. This peak is formed when the buffer (mobile phase) interact with the weak cation exchange resin (stationary phase and samples (unbuffered), through the column, the whole process is called ionic chromatography effect.[23]

2.3.2.2.5 Fraction E: Low molecular-weight (LMW) neutrals

This fraction was designated as LMW-neutrals. This material is known to be hydrophilic to amphiphilic since it can be eluted near/after to the column permeation volume. The fraction show minimum or no response by the UVD detector.[23] These LMW correspond to sugars, carbohydrates, proteins and amino acids.

2.3.2.3 Advantages of LC-OCD

The SEC-OCD (LC-OCD) can be utilized to study the character of NOM in drinking water, waste waters, marine waters and membrane fouling studies where biopolymers can be used as quality control membrane foulants to test for NOM-free water.[23]

2.3.2.4 Disadvantages of LC-OCD

This method uses three detectors (UVD, OCD and OND) in order to view all five fractions of NOM since one detector cannot reveal all fractions. The fractions are not specific to individual compounds such as fulvic acid or humic acid but they correspond to a collective group, i.e. humic substances.

2.3.3 Ion exchange resins

This is an ion-exchange fractionation, which uses the same principle as ion exchange chromatography, whereby, resins with the aid of the eluent (solvent that carries the samples) exchange ions with a certain analyte (usually oppositely charged). The resin retains different charged ions and elutes similar charged ions. [24–26] The resin is also called an ion exchanger and it is hydrophobic in nature .[26] There are two types ion exchange processes, the cation and anion exchange; the name of the process is dependent on the type of the ion exchanger present in the resin, if resin has positively charged ions, it will be called the cation exchange resin .[26–28] The physical structure of the ion exchanger is described as resins composed of great amount of a positive or negative charge sites

alongside with oppositely charged ions (ion-exchange capacity).[28] The ion-exchange resin ions move freely to allow direct replacement by same charge ions from a sample being eluted on the ion-exchange column.[26]

During drinking water treatment, the anion exchange method is used as a substitute for the conventional water treatment method for raw water with high NOM content greater than 2–10 ppm concentration. This method was reported to be more effective than coagulation. The anion exchange method is limited to the removal of about 10% to 40% of NOM, while the remaining NOM is not removed by anion exchange method due to the uncharged species in NOM.[26]

2.3.3.1. Ion exchange principle

This method uses different resins such as XAD-7HP, Dowex-88 and Diaion WA10 and solvents like HCl, MeOH and NaOH (see **Fig 2.1**). The six fractions which are eluted from the resins are divided into three of the original humic substances (HSs) (hydrophobic fractions) and three of non-humic substances (non-HSs) (hydrophilic fractions). Individual fraction possess different physical and chemical properties.[6]

Humic and fulvic acid can be detected from all the hydrophobic fractions and the low molecular weights (LMW) can be found in all three hydrophilic fractions.[29] Each independent fraction can be identified by their individual properties (see **Table 2.1**).

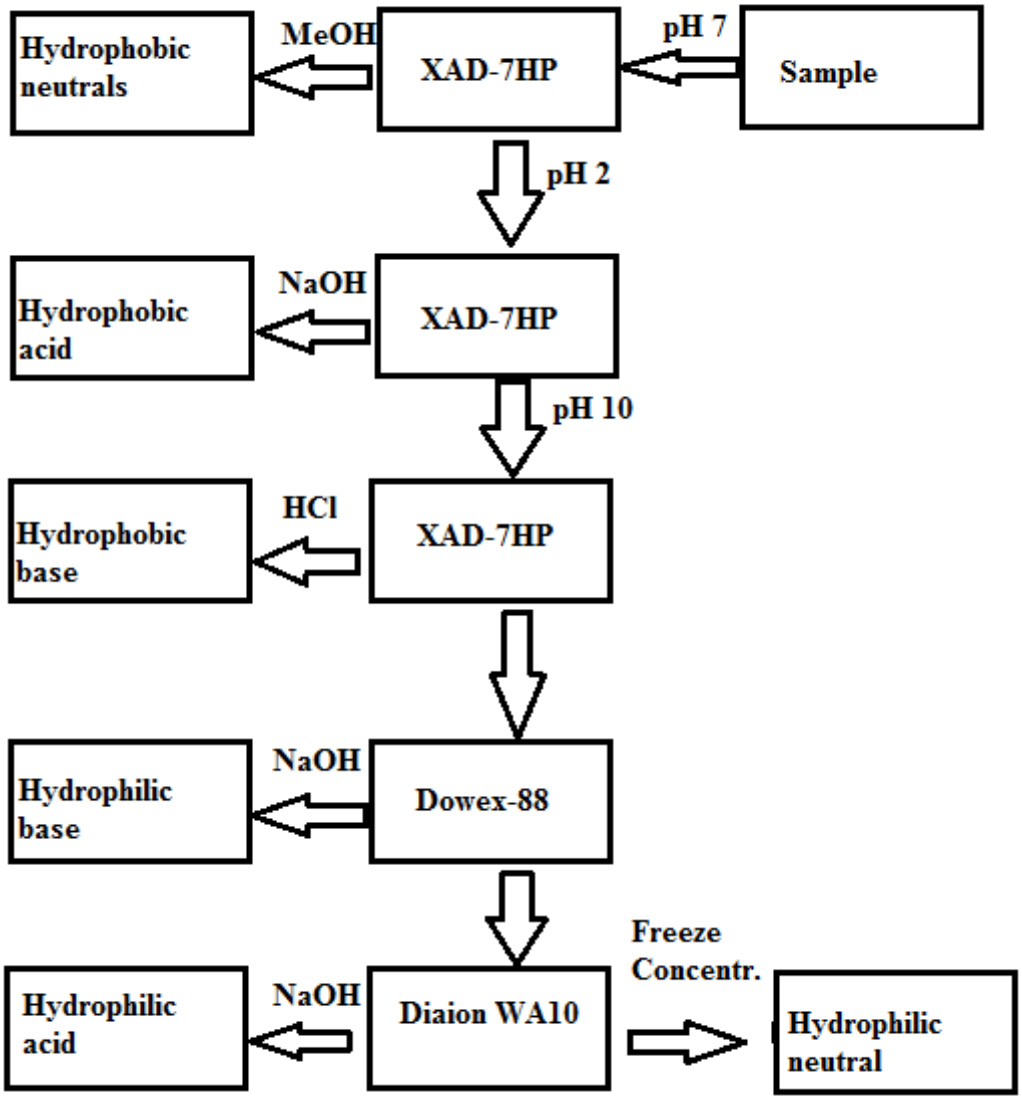

Figure 2.1: Fractionation procedure of NOM by ion exchange[6]

2.3.3.2. Fractions of NOM reported

Six fractions of NOM from the different resins: hydrophobic acid (HpoA), hydrophobic base (HpoB), hydrophobic neutral (HpoN), hydrophilic acid (HpiA), hydrophilic base (HpiB) and hydrophilic neutral (HpiN) all possess different properties (**Table 2.1**)

Table 2.1: Properties of NOM fractions[29]

Fraction	Abbreviation	Class and Properties
Humic acid	HA	A part of HSs that precipitates at pH=1
Hydrophobic acid	HpoA	Soil fulvic acids, containing 5-9 straight chain COOH, 1-2- aromatic ring COOH and 1- 2-ring C_6H_5OH
Hydrophobic base	HpoB	A part of HSs that is trapped by the XAD-8 resin (pH=7)
Hydrophobic neutral	HpoN	It is washed by hydrochloric acid, has; 1- 2 aromatic ring aromatic amines A mixture of greater thanC_5 straight chain–COH, -$CONH_2$, -COOR, -COR, -CHO; >C_9 straight chain c –COOH and and–$CONH_2$; >3- aromatic ring aromatic –COOH and –$CONH_2$.
Hydrophilic acid	HpiA	Greater than C_5 straight chain–COOH, acids, multi-functionalized –COOH with numerous H^+ acids
Hydrophilic base	HpiB	Proteinaceous compounds with the amino groups, peptides and proteins; straight chains of greater then C_9 $COONH_2$; pyridine
Hydrophilic neutral	HpiN	Shorter straight chain COH, -$CONH_2$, -COOR, -COR, -CH <C_5 aliphatic multi-functionalized COH, -$CONH_2$, -COOR, -COR, -CH and polysaccharides

2.3.3.3. Advantage of ion exchange

This method give six fractions of NOM instead of five fractions that come out of the LC-OCD fractionation method. The fractions of NOM can be further separated to individual compounds such as humic acid (HA).

2.3.3.4. Disadvantage of ion exchange

This method is expensive and too long to accomplish, as it uses different solvents and resins in order to isolate all six fractions of NOM.

2.3.4. The polarity rapid assessment method (PRAM)

The polarity rapid assessment method (PRAM) aids in rapidly monitoring the variations in polarity of NOM from natural sources of water, by using different solid-phase extraction (SPE) cartridges (see **Table 2.2**). The overall monitoring of NOM by PRAM doesn't affect the conditions or nature of NOM in the aqueous medium.[30]

PRAM was first explored by Bree Carlson by investigating the seven different sorbent cartridges (C18, C8, CN, NH_2, Diol, Phenol, and SAX) in 3mL syringe cartridges then monitored by UV-Vis absorbance at 254 nm and 272 nm from the SPE effluents.[31] However, Fernando Rosario-Ortiz focused his PhD by exploring the PRAM technique and publishing academic papers on PRAM in peer reviewed journals.[31]

Table 2.2 Solid phase extraction (SPE) cartridges which are utilized for polarity assessment of NOM

Cartridge	Polarity
C_{18}	Non-polar
C_2	not polar (moderate)
C-N	Polar (moderate)
Silica	Polar
Diol	Polar
NH_2	Anion exchange (weak)
SAX	Anion exchange (strong)

2.3.4.1 Principle of PRAM

The principle of PRAM of NOM is centred on selective adsorption of certain fractions of NOM onto solid-phase extraction (SPE) cartridges based on the fraction polarity.[32]

The different SPE cartridges are placed in parallel positions where only one detector is used; this set-up permits a multidimensional NOM polarity assessment.[32] The samples from cartridges are analysed in a sequential form (one after the other). The breakthrough curve is measured by the UV_{254} and DOC.[7,30–32]

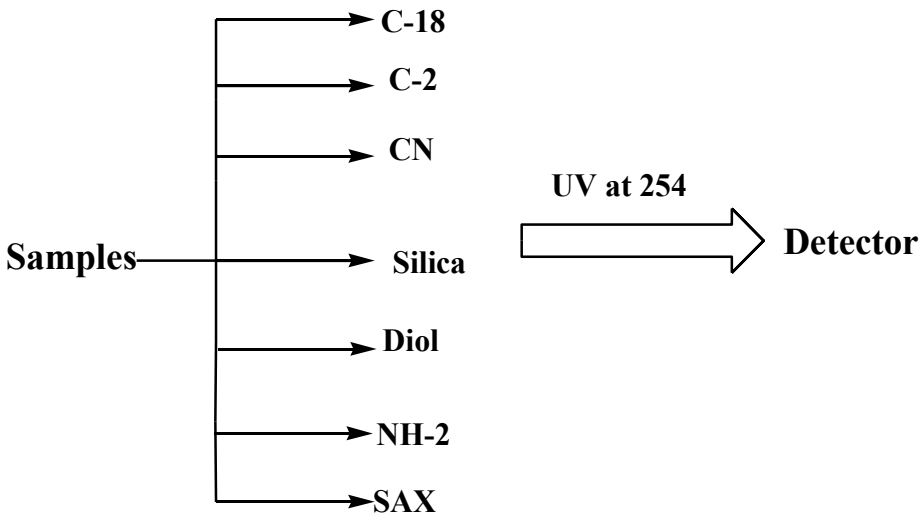

Figure 2.2: Experimental set-up of PRAM

2.3.4.2 Fractions of NOM reported

Three fractions of NOM are reported from this characterization method. The hydrophobic fraction (HPO) generated by the C18 cartridge, the hydrophilic (HPI) generated by the CN SPE cartridge and the transphilic fraction (TPI) generated by the NH_2 SPE cartridge.

2.3.4.3 Advantage of PRAM

It is a rapid method for NOM characterization and it is applied in water treatment plant to assess NOM at a larger scale.

2.3.4.4 Disadvantage of PRAM

This method gives few fractions of NOM. It requires continuous replacement of SPE cartridges, which is an added expense to the plant. And also it doesn't account for the non-polar part of NOM.

2.3.5 The size exclusion chromatography (SEC)/high pressure size exclusion chromatography (HPSEC)

2.3.5.1 Principle of SEC/HPSEC

SEC separates components with respect to their molecular weight (MW) and shape.[27] Separation is due to ionic exclusion and hydrophobic attraction.[27] The identification of NOM fractionation that eluted from the normal SEC method, is limited when ultraviolet

detection (UVD) is used on its own. The UVD detects only the humic substances. Therefore, coupling the UVD with the organic carbon detection (OCD) allows the detection of other fractions of NOM.[27,29]

2.3.5.2 Fractions of NOM reported

2.3.5.2.1 HPSEC Fractions

The HPSEC is a popular method that has been utilized to detect molecular weight distribution (MWD) of small to large fractions of NOM which results from the oxidation process of drinking water treatment. The resulting chromatograms were reported at 254 and 220 nm.[29] The wavelength of 220 nm was utilized to monitor the variations in molecular weight distributions of the fractions of NOM with lowest molecular weight this accounted for NOM fractions which are degraded by oxidation and other processes.[29]

The HPSEC molecular weight sizes of NOM from the literature was recorded to be around (2500–300 Da). NOM fractions with the highest MW were found to react mostly with both ozone (O_3) and chlorine dioxide (ClO_2) and this necessitated a decline in the UV_{254}-absorbance for each fraction of NOM. The decrease showed a degradation of aromatic moieties as higher molecular weights react with both O_3 and ClO_2.[29]

The UV_{254} nm measures the intensity of the humic substance chromophores at 254 nm.[7] These chromophores include double bonds. When the UV_{254} absorbance decrease, it means there is a breakage of the double bonds from the humic substances chromophores as it degrades to smaller molecular sizes, this decrease of the UV_{254}.

Oxidation of NOM with O_3 give various changes in the MWD of the HpoN, HpoA and HA and slight changes in the MWD of HpiA and HpiB, and no changes for HpiN.[29] The molecular weight of NOM fractions in the study was between 2500–300 Da.

2.3.5.2.2 SEC Fractions

In literature, the peaks around 35 minutes were recognized to belong to anthropogenic organic matter (have lower SUVA).[27] The SEC fractions were divided into three distinctive SEC fractions. The SEC fraction I eluting at 22 min < t_R<34 min were allocated to large molecules such as polysaccharides and humic substances, these materials dominate the brown water and aqueous soil samples.[27] The SEC fraction II eluting at 34 min < t_R < 39

min were allocated to building blocks of refractory organic substances and the SEC fraction III eluting at t_R>39 min were attributed to the low-MW molecules such as carbohydrates, aldehydes, ketones, or alcohols.

2.3.5.3 Advantages and disadvantages of SEC/HPSEC

This technique gives the NOM fractions as well as their corresponding molecular weights. The fractions are grouped into three representative fractions, which are the higher, medium and lower molecular weights. More specific molecular weights cannot be detected.

2.4. TECHNIQUES FOR THE SEPARATION OF NOM

2.4.1. Introduction

These are techniques which have substantial ability to separate a mixture of compounds into its individual components which may or may not comprise of pure components. These techniques are aimed at successfully separating a complex mixture of solutes/solvents in order to obtain pure ones.

2.4.2. Distillation

This techniques is used for the separation of volatile components.[33] The separation occurs when the sample (liquid) is heated to vapour phase and individual components turn into vapour at different and unique temperatures.[34–36] The vapour for the individual components is condensed and thereafter collected.[37] The condensed liquid is called a distillate and the non-distilled liquid is called the reflux.[33]

2.4.3. Electrophoresis

The separation occurs when there is movement of charged particles in a potential gradient.[38,39] There are two types of electrophoresis, zonal electrophoresis (carried out on a support such as paper) and frontal electrophoresis (carried out on a supported solution).[39]

2.4.4. Dialysis

This is a separation technique whereby two immiscible liquids that are separated by a membrane.[40] The membrane controls the selectivity of the compounds as it allows certain solutes to pass through.[41] Molecular weight cut-off (shear degradation) of NOM by the dialysis method is a possibility but there are vague reports in literature about this

possibility.[41] The literature reports that the fluorescent properties of NOM after dialysis were less than fluorescent properties of NOM before dialysis.[41]

2.4.5. Chromatography

2.4.5.1. Introduction

Chromatography is composed of variety of methods that separate more similar/different components in complex mixtures.[42–44] This is a powerful technique used for purification of solvents (qualitative) and obtaining a desired component from a mixture (quantitative).[42,44]

There are various kinds of chromatographic techniques which are used: i.e. paper chromatography (PC); thin layer chromatography (TLC); gas chromatography (GC); liquid chromatography (LC); high performance liquid chromatography (HPLC); ion exchange chromatography (IEC); and gel permeation chromatography (GPC) or gel filtration chromatography (GFC). The straightforward principle of chromatography is that all these methods function with the stationary phase (sessile) and the mobile phase (moving) in order to achieve separation. For separation to be successful each chromatographic technique use the following principles to achieve separation: adsorption; partition; ion exchange; or molecular exclusion.

The first principle is adsorption chromatography, this was the first principle adapted. It requires a stationary phase (solid) and a mobile phase (liquid/gas). The adsorption chromatography separates solutes (analytes) based on their ability to achieve different equilibrium between adsorption on the stationary phase and their solubility in the mobile phase. The best adsorbed are eluted last while the least adsorbed are eluted first.[44]

Partition chromatography is different, the stationary phase is not a solid but a non-volatile liquid. This liquid is placed on a solid surface as a thin layer (or film). The mobile phase is either a gas or a liquid. The solutes (analytes) separate when the mobile phase get in contact with the stationary phase. The more soluble analytes (with the mobile phase) are eluted first while the non/less soluble analytes are elutes last. A good example of this technique is paper chromatography.[45]

Ion exchange chromatography uses an ionic resin (rich with anions or cations, depends on its nature) as a stationary phase. The ions are covalently bonded to the stationary phase, while ions with an opposite charge of that of the stationary phase are bound to the resin

with electrostatic forces. Separation occurs when the mobile phase (liquid) passes through the stationary phase to replace the electrostatically bound ions (from stationary phase) with ions of the same charge (from mobile phase).[44]

In size exclusion there is no equilibrium state that is achieved by the analyte and the stationary phase. The mobile phases diffuse through the pores of the stationary phase (gel). The pore size of the stationary phase is aimed at allowing only the small solutes (analytes) to diffuse through stationary phase while excluding the large solutes. As the small particles diffuse (permeate) through the gel they are retained in the column such that they take longer time to elute through the column, thus allow the larger particles to elute first.

In liquid/gas chromatography; separation is achieved by allowing a sample to be dissolved in a solvent or gas (mobile phase) then diffused through a stationary phase (fixed on a column or on a solid surface in the column).[42,43] In paper and thin-layer chromatography the mobile phase is the solvent and the is the strip or piece of paper (paper chromatography) and a thin-layer cell (thin-layer chromatography,) which is dipped in the mobile phase containing our components interest.[43] Both these kinds of chromatography practise capillary action to move the solvent through the stationary phase because the movement is against gravity.[42,43]

2.4.5.2. Liquid chromatography

Liquid chromatography uses a liquid (either polar or non-polar) as a mobile and a solid stationary phase.[46]

Before separation with this technique, the stationary phase must be eluted with a solvent (ideally the same as the mobile phase) to prevent air from interrupting the smooth flow during separation.[44] A ceramic wool is placed near the tap at the bottom of the column to prevent any clogging of the tap by the stationary phase. Separation is achieved by gravity.

A concentrated solution of the sample is added on the column and then eluted with a solvent (mobile phase). It is easy to collect the individual analytes from the sample mixture when they are coloured. If they are not coloured of small measurements of the eluate (liquid) will be collected after certain time of elution.[42,44]

The analytes can be retained from the eluate by heating and evaporating the solvent. Further identification of the analytes will be done using TLC. High performance liquid chromatography (HPLC) is a more advanced LC method.[42,44]

2.4.5.3. High performance liquid chromatography (HPLC)

HPLC is a kind of liquid chromatography, whereby accurate separation is achieved by pumping the sample of analytes with a constant pressure. The size of the analytes is important, as separation increases with smaller analytes. This allows rapid balance between the solid (stationary phase) and the solvent (mobile phase).[44] The stationary phase (silica particles diameter of 10^{-6} m) of the HPLC system must be uniform, porous (10^{-8}–10^{-9} m) and must be bonded to a non-volatile liquid (held by covalent bonds), which permit interactions of analytes with various polarities.[44]

The HPLC stationary phase particles are placed on glass fibres and packed inside the HPLC column.[44] A constant flow of solvent not only allow efficient separation but it also allow reproducibility of the results.[44] The HPLC system requires flow rates of 0.5–5 mL/min with sample injection volumes of 0.5–20 μL.[44]

HPLC can take diverse samples, has high sensitivity, can do rapid, precise automated analysis (1-60 mins, Precision < 1% RSD), and has quantitative sample recovery (preparative technique from μg-kg quantities).[42]

Separation of the analytes are achieved between mobile phase (liquid) and a stationary phase (solid, column packed porous particles) through differential interactions (repetitive sorption/desorption, partitioning, ionic interactions, size, with the (porous) support and different mobile phase nature.[42] Mobile phase may be constant (isocratic) or may change with elution time (gradient). The system has an on-line detector, which reports the concentration of analytes as they are separated on the system then gives a report (chromatogram).[42]

2.4.6. HPLC COLUMN SEPARATION PARAMETERS

The HPLC column has important separation parameters, which when governed can assist with the analysis of the separation. These parameters are the selectivity factor (α), resolution (Rs), and capacity factor (k') and column efficiency (n).

2.4.6.1. Selectivity factor (α)

Selectivity factor refers ability of a HPLC column to be able to separate different analytes by means of eluting one analyte first and retaining the other (see **Fig. 2.4**).[42,47] Both the stationary phase and the selected mobile phase play a role on the differential retention of two or more analytes by the column. Better separation is achieved when the selectivity factor of the analytes is greater than 1, because the selectivity factor of 1 indicates that the two analytes are co-eluting.[42] The selectivity factor of two analytes can be expressed by using equation 2.2.

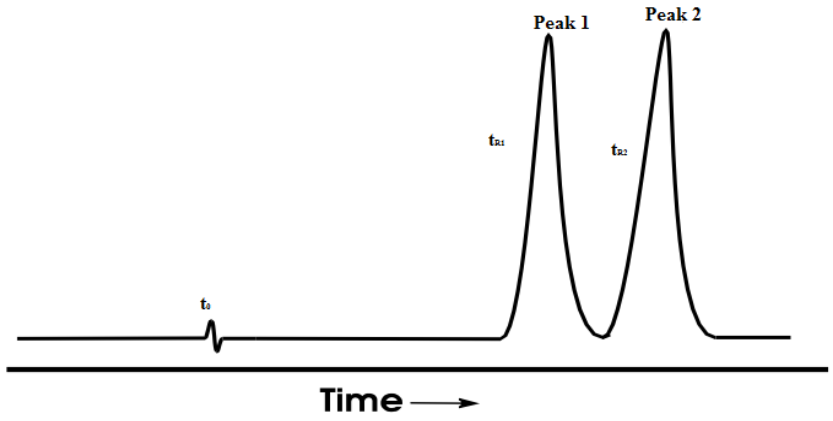

Figure 2.3: An example of a Selectivity factor of two analytes

$$\alpha = \frac{(t_{R2} - t_0)}{(t_{R1} - t_0)} \quad \text{..... (2.2)}$$

Where t_{R2} = retention time for peak 2; tR_1= retention time for peak 1; and t_0= void volume retention time

2.4.6.2 Resolution (Rs)

Resolution measures a degree of separation between two successive peaks (see **Fig.2.5**). High resolution can be attained by increasing the theoretical number plates increasing the length the column and decreasing the size of the stationary phase particles. When the resolution (Rs) equals to zero it means no separation was achieved. When the resolution is equal to or is greater than 1 then partial separation or complete separation was achieved, respectively.[42,44] The Rs can be expressed mathematically using equation 2.3

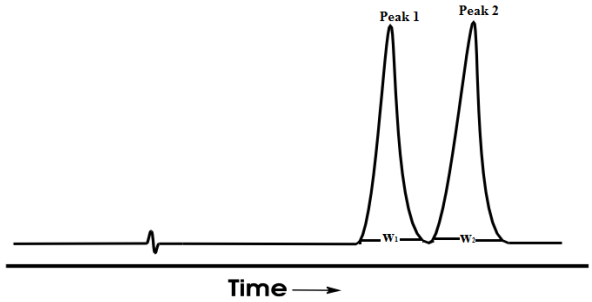

Figure 2.4: Demonstration of Resolution (Rs) between two successive analytes[42]

$$Rs = \frac{2\Delta t_R}{W_1 + W_2} \quad \text{..... (2.3)}$$

Where Δt_R = the difference of retention time between peak 1 and 2; W_1 = base widths for peak 1; W_2 = base widths for peak 2

2.4.6.3 Capacity factor (k`)

Capacity factor measures the retention of analytes as they are separated through the column. It measures the number of times a peak is retained ($t_R - t_0$) against the non-retained peak (t_0) of the analyte on the column (see **Fig. 2.6**).[25,44,47] The solvent strength can be altered such that it contains low composition of the solvent in order to achieve a high retention factor (since the analyte strongly interacts with the stationary phase) or high composition of organic solvent to achieve a retention factor (less interaction between analytes and stationary phase). In order to achieve good separation, the retention factor

should be kept between 1 and 10 to avoid the quality reduction of the separation. The mathematical expression of the capacity factor is expressed in equation 2.4.

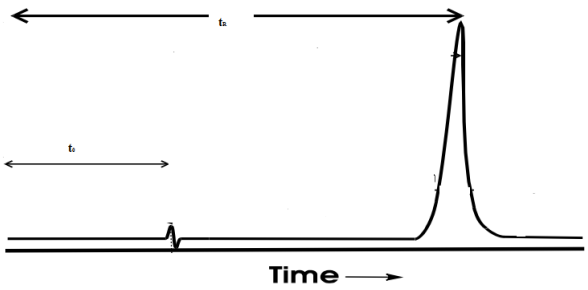

Figure 2.5: Demonstration of Capacity Factor (k`)[42]

$$k' = \frac{(t_R - t_0)}{t_0} \quad \text{..... (2.4)}$$

Where t_R =retention time of a retained peak; t_0 = void volume retention time

2.4.6.4 Column efficiency (n)

The column efficiency (n) can be measured based on theoretical plate's height (HETP or H) or the number of theoretical plates (N).[48,49] The efficiency of chromatographic peak is measured from the resulting theoretical plate's height (HETP or H) or the number of theoretical plates (N). The efficiency of the column depends strongly on the packing materials (quality), the particle size (analyte) and column diameter, length and film thickness. The longer the column the better separation is achieved (increased peak efficiency). Equation 2.5 expresses both the HEPT and the N.[50,51]

$$HETP = \frac{L}{N} \quad \text{.....(2.5)}$$

Where L = the length of the column.

Both H and N can be calculated by using the equation 2.6-2.8 to express the high column efficiency.

$$H = \frac{LW_b^{\,2}}{16(t_R)^2} \quad \text{.....(2.6)}$$

$$N = 16\left(\frac{t_R}{W_b}\right)^2 \quad \text{.....(2.7)}$$

$$N = 5.54\left(\frac{t_R}{W_h}\right)^2 \quad \text{.....(2.8)}$$

Where t_R = the time of retention for the retained peak; Wb = the base width of the triangle; W_h =the base width of the triangle at half peak height

2.5. SIZE EXCLUSION (SEC)/GEL PERMEATION (GPC) CHROMATOGRAPHY

Other names such as gel permeation, gel filtration, steric exclusion, or gel chromatography can be used to describe the size exclusion chromatography, which is a well-known technique for separating both synthetic polymers and biopolymers.[52,53] Separation in the SEC/GPC column involves entrapment of the smaller compounds or molecules into the small pores of the stationary phase (see **Fig. 2.7**) making their path way of elution to be longer (long retention time) while bigger molecules which cannot fit into the small pores of the stationary phase elute first (shorter retention time).[53] The SEC/GPC technique is controlled by entropy and the size of the analytes with relative to size of the pores for the stationary phase.[52–55] This method can measure average molecular weights, molecular weight distribution (MWD), absolute molecular weight, molecular conformation, branching on the long-chain of analytes, fractionation of polymers and for separating small analytes from larger ones, all these measurements achievable with the aid of on-line light-scattering detector (without column calibration)/ on-line viscometer with universal calibration.[52,54] SEC separations, highly depend on the hydrodynamic volume than the molecular mass.[53] Therefore, it is impossible to use SEC to separate analytes with the same hydrodynamic volume but different chemical compositions or chain alignments.[53] Separation of these analytes can be achieved with reversed phase high performance liquid chromatography (RP-HPLC).[53] The term SEC can be substituted for gel filtration

chromatography (GFC), which is mainly used in the biochemistry, pharmaceuticals and polymer science.[56]

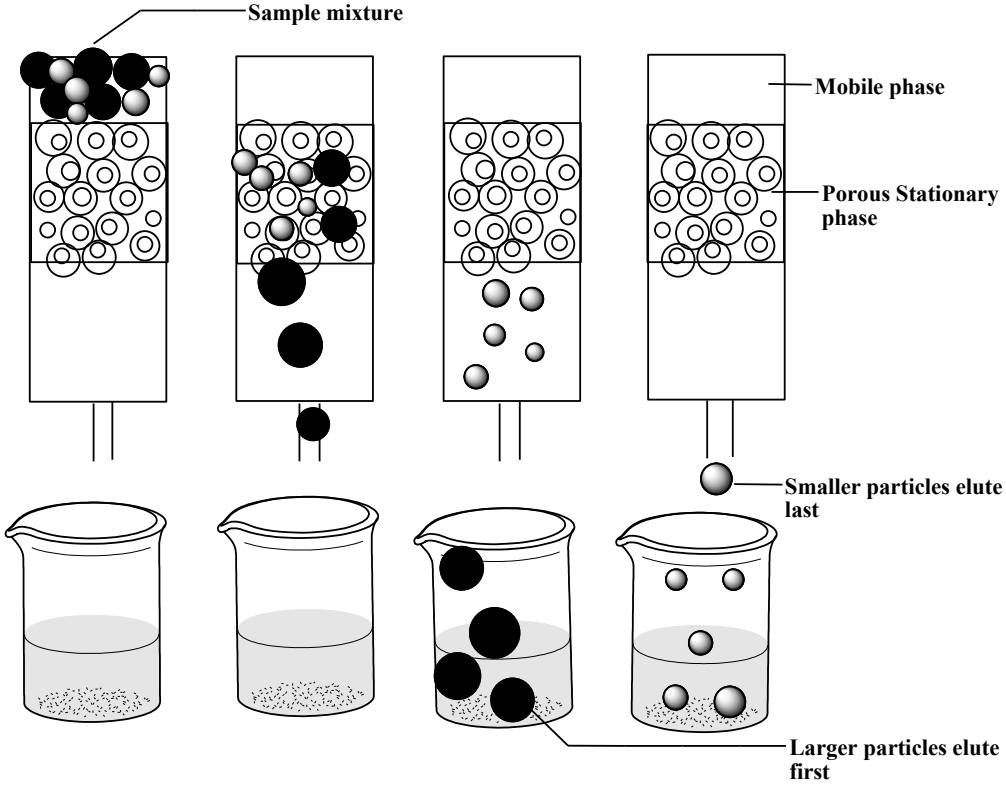

Figure 2.6: Demonstration of Size exclusion chromatography (SEC)

Various methods for separation of macromolecules in water can be applied in separation of macromolecular such as NOM. Conventional methods such as solid-phase extraction, liquid-liquid extraction, XAD resins and anion exchange methods have been used to separate certain fractions of NOM. These methods favor NOM fractions with specific affinities only. The chromatography method can be used to separate all parts of NOM in a solution according to their sizes, not affinities.[55] In order to separate both the hydrophilic and hydrophobic part of NOM, a suitable chromatographic technique must be used. There exist a diversity of chromatographic methods that are useful in the separation (fractionation) of NOM in water. However, amongst the vast number of chromatographic techniques used in the separation of components in water, SEC/GPC has been found to be the most important chromatographic technique. SEC/GPC can separate different fractions of NOM in the removal of the hydrophobic fraction of NOM according to their corresponding molecular weight distribution.[55] Since the 1960, the method have been employed to characterize both soil and aquatic human substances.[22,55] Furthermore,

SEC/GPC is used to fractionate NOM, since NOM components have both molecular weight and size distribution as two important bulk properties.[57] However in previous studies, NOM was recorded to have molecular weight of few hundred to greater than 100000 Da but more common molecular weights of less than 10 000 are more likely are reported.[57]. For the application of SEC/GPC technique in NOM fractionation, careful selection of the stationary phase (particle size), and the pore sized and the nature should be taken into consideration, because smaller particle sizes can result in shear degradation of macromolecules. The likelihood of shear degradation of NOM to occur in SEC/GPC is very high because NOM is very large and complex (in terms of molecular weight) [48] It is important to take note of other factors that increase shear degradation of NOM. These factors include: NOM structural complexity, the relativity that exist between the size of NOM and the spaces between the particles (NOM is bigger and the spaces are slim) and the ability of other functional groups found on the NOM compound to be trapped in the small pores of the porous stationary phase [55]. Shear degradation is not a chemical but a mechanical process. Compounds do not dissociate due to chemical reactions that exist in the column but it is due to the pressure that is applied to push the mobile phase (containing different compounds) through the small spaces found in the stationary phase. However, most of the NOM fractions reported in literature were eluted from SEC/GPC stationary phases, which were not tailor-made to meet the polydispersity, complexity and the various chemical nature of NOM.[7,11,57] This is because the SEC/GPC technique was not designed to accommodate the complex nature of NOM. It is rare to purchase a SEC/GPC column that can meet the requirements of NOM.

To minimize shear degradation of NOM in SEC/GPC suitable stationary phases are required. The spaces between the stationary phases particles should be bigger and their sizes should be greater than 2 μm.[48] The stationary phases should be able to tolerate wide range of pH (since NOM consists of a variety of pH values), must be cross-linked (to trap the smaller particles), porous and must not dislocate from their place on the column (rigid and sessile). They must also be polymeric and abide to the principles of SEC/GPC [52].

2.6. CONCLUSION

In literature the NOM characterization techniques such as TOC, SUVA, UV_{254} and more advanced methods such as FEEM allow the quantitative analysis of NOM, these techniques cannot separate and characterize the different fractions of NOM. Therefore,

there is a need for the separation (i.e. fractionation) and full characterization of the different fractions of NOM.

When NOM was fractionated by different techniques such as PRAM, ion exchange resins, LC-OCD, SEC and HPLC, different fractions of NOM were detected but these fractions cannot be grouped into one category. Instead, these fractions were not fully defined, some were a generic group) (e.g. humic substances) and some were charged (e.g. hydrophilic acids). These undefined fractions are a result of the type of the stationary phase used in the column/cartridge of PRAM, ion exchange resins, LC-OCD, SEC and HPLC. The stationary phases of these techniques phases were not designed to suit the complex chemical properties of NOM. Instead these techniques compromised some chemical structures of NOM fractions, hence there were ionic fractions (e.g. hydrophobic acids). Other disadvantages are: slower response time, very difficult to maintain, expensive and uneasy to use. This literature review has revealed a need to design a cheap and simple technique with a stationary phase designed to accommodate the complex nature of NOM. The desired method should elute NOM according to their molecular weight (in order to prevent exclusion of NOM fractions due to their chemical properties).

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CHAPTER 3:

EXPERIMENTAL METHODOLOGY

3.1. INTRODUCTION

This chapter gives an overview of the methodology that was followed for the synthesis and characterisation of the stationary phase materials using polysilsesquioxane (PSQ) and poly (styrene-divinyl benzene).The experimental studies to determine the ability of the selected materials to separate NOM is also discussed in full detail.

3.2. SAMPLE COLLECTION

All samples were collected using either a 1L glass or plastic bottle. Bottles were filled to the top in order to prevent NOM pollution with trapped oxygen or air. Water was either collected with a plastic container (hooked to a stick or a rope) or was collected from a tap, depending on the water treatment process. After collection, the samples were stored in a cooler box filled with ice and thereafter taken to the laboratory for analysis. The samples were stored in a refrigerator at 4 °C in order to prevent NOM from degrading before analysis. [1]

3.3. SAMPLING SITES

Samples were taken from four different drinking water treatment plants located at four different provinces: Limpopo Province, Olifantspoort (LO); Western Cape Province, Preekstoel (P); Kwa-Zulu Natal Province, Mtwalume (MT) and Gauteng Province, Mid-Vaal (MV). Each of the sample locations is shown in **Fig. 3.1**. The choice of the sampling sites was motivated by the difference of NOM character and quality and also the different types of the water sources and designs of each treatment plant.

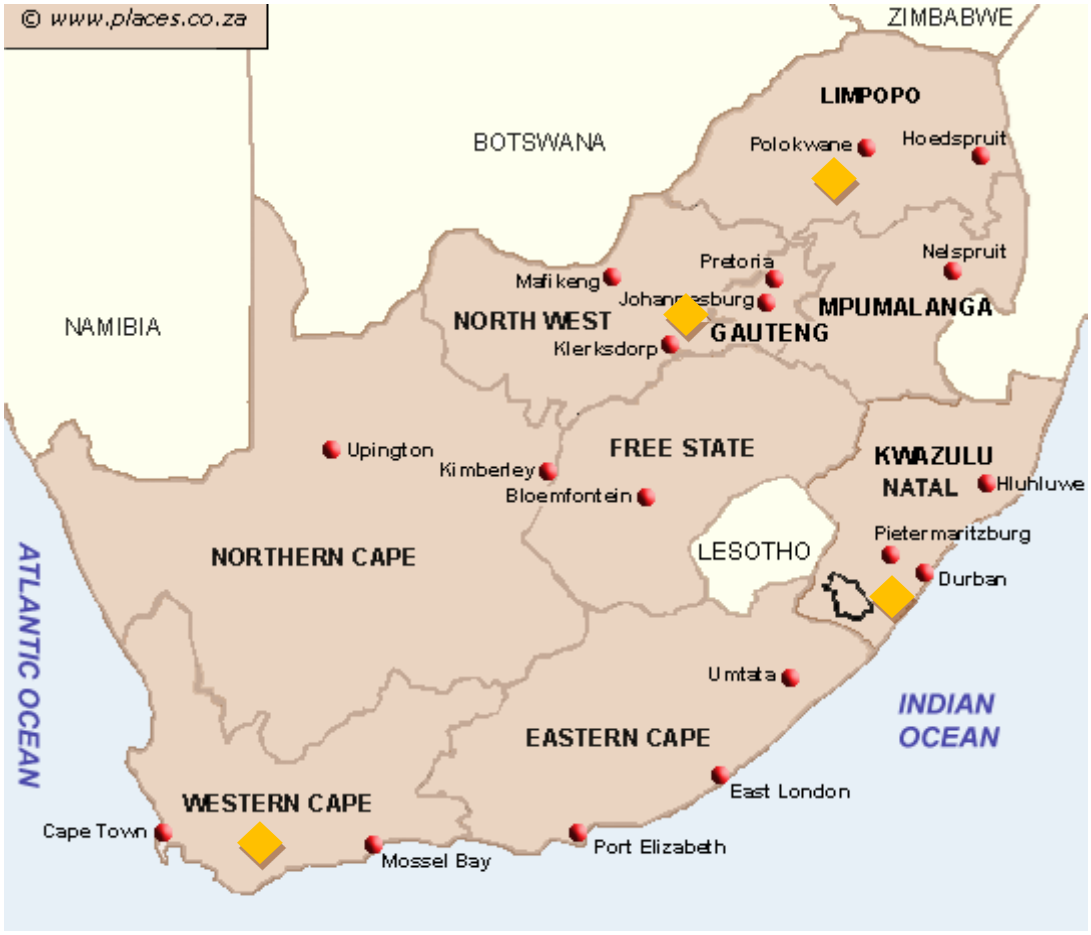


Figure 3.1: The sampling sites and their locations

3.4. REAGENTS AND SOLVENTS

Most chemicals were purchased from Sigma-Aldrich (Johannesburg, South Africa) without further purification: 1, 2-Bis (triethoxysilyl) ethane (97%), 1, 4-bis (trimethoxysilylethyl) benzene (96%), Sodium hydroxide (NaOH) (≥98%, Reagent Grade), Hydrochloric acid (HCl) (32%), cetyl trimethyl ammonium chloride CTAC (25 wt. % in H_2O), Ethanol (EtOH) (≥99.8% (GC), absolute, HPLC Grade), benzoyl peroxide (40 wt. % in dibutyl phthalate), dibutyl phthalate (99%, impurities ≤0.005% Phthalic acid),sodium dodecyl sulphate (SDS) (≥99.0% (GC), dust-free pellets),styrene (≥99%), divinyl benzene (80%, Technical Grade), toluene (99.9%, HPLC Grade), polyvinyl alcohol (PVA) (average MW 3,000, crystalline, 75% hydrolysed),methanol (99.9%, HPLC Grade), tetrahydrofuran THF (≥99.9%, inhibitor-free, HPLC Grade), hexamethyldisilazane (HMDS) (99%, Reagent

Grade), acetone (99.8%, HPLC Grade), and humic acid (HA) (80%, Technical Grade). Fulvic acid FA (≥95%, HPLC Grade) was bought from Enzo and was supplied by BIOCOM Biotech. The de-ionized water that was used was obtained from a MilliQ integral 10 deionized water purification system. The measured conductivity of this water was found to be 18.4 micro-Ohm.

3.5. PREPARATION AND SYNTHETIC METHODS

3.5.1. SYNTHESIS OF POLYSILSESQUIOXANE

Polysilsesquioxane was prepared by following the protocol reported by Burleigh et al. (**Fig. 3.2**).[2] Cetyl trimethyl ammonium chloride (CTAC) was added to a solution of deionized water and NaOH (25 % solution) in a ratio of (1:16.67:0.35) and the blend was stirred gradually such that a thick and clear substance was formed. To the blend: 1, 4-bis-(trimethoxysilylethyl) benzene and 1, 2-Bis (triethoxysilyl) ethane were added at ratios (1:1 and 1:1.25 v/v). The mixture from the above step was then stirred for 2 hours until a gel was formed. The gel was then heated at constant temperature of 70 °C for 48 hours and to the resulting product excess acidified ethanol (350 mL/g) was added. Refluxing for 6 hours generated a product, which was collected by filtration with acidified ethanol wash. The final precipitate of polysilsesquioxane was dried under vacuum at 40-60 °C.

3.5.1.1. END-CAPPING OF POLYSILSESQUIOXANE

End-capping of the polysilsesquioxane was carried out by a modified method from literature (see **Fig. 3.3**)[3] Polysilsesquioxane and hexamethyldisilazane (HMDS) were mixed and heated at 70 °C for 24 hours. The product of the previous step was then washed successively with toluene, ethanol, ethanol/deionized water (1:1), acetone and deionized water) to remove any remaining reagents. The final product was then dried overnight at 80 °C.

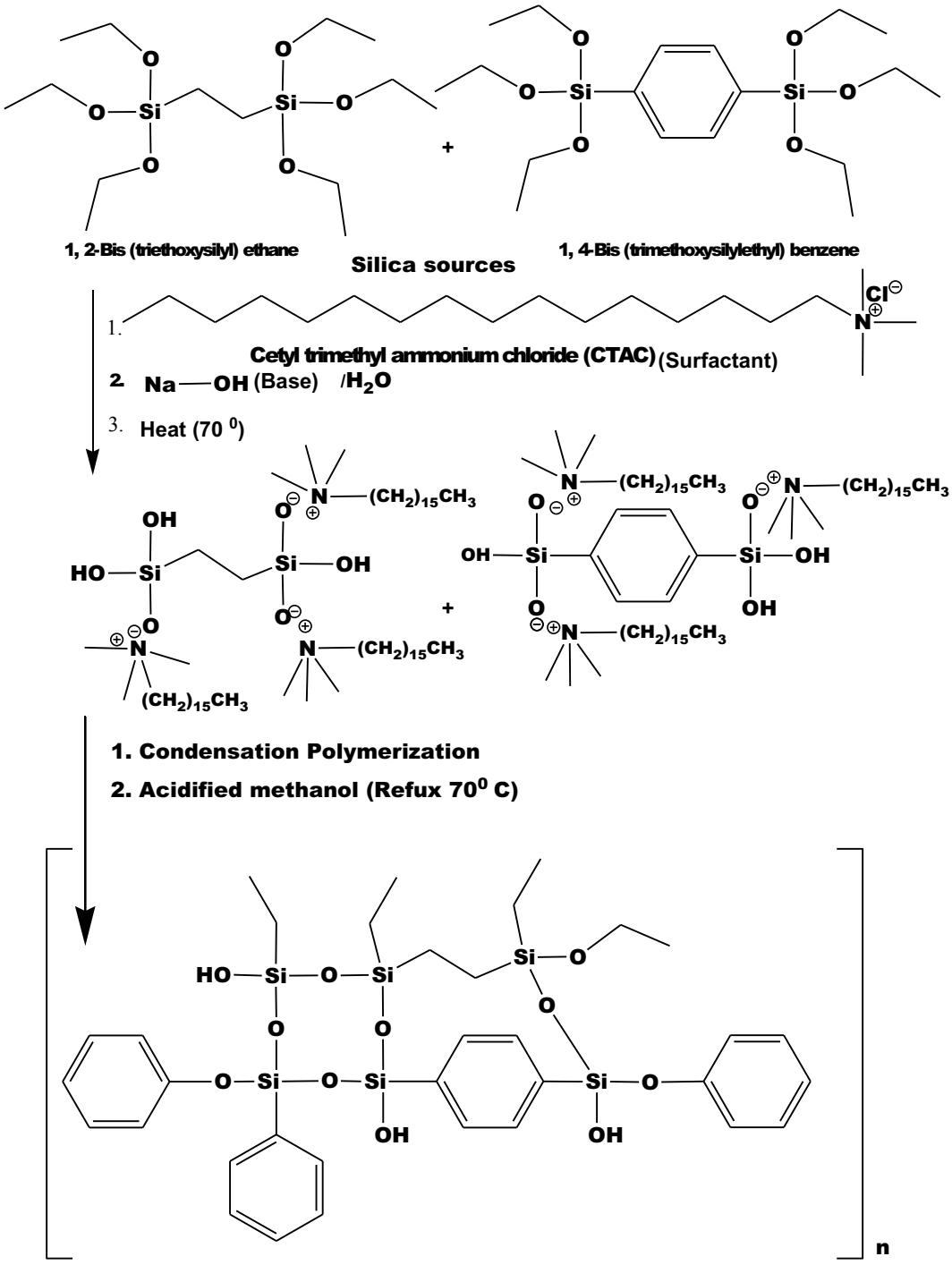


Figure 3.2: Chemical reaction of Polysilsesquioxane

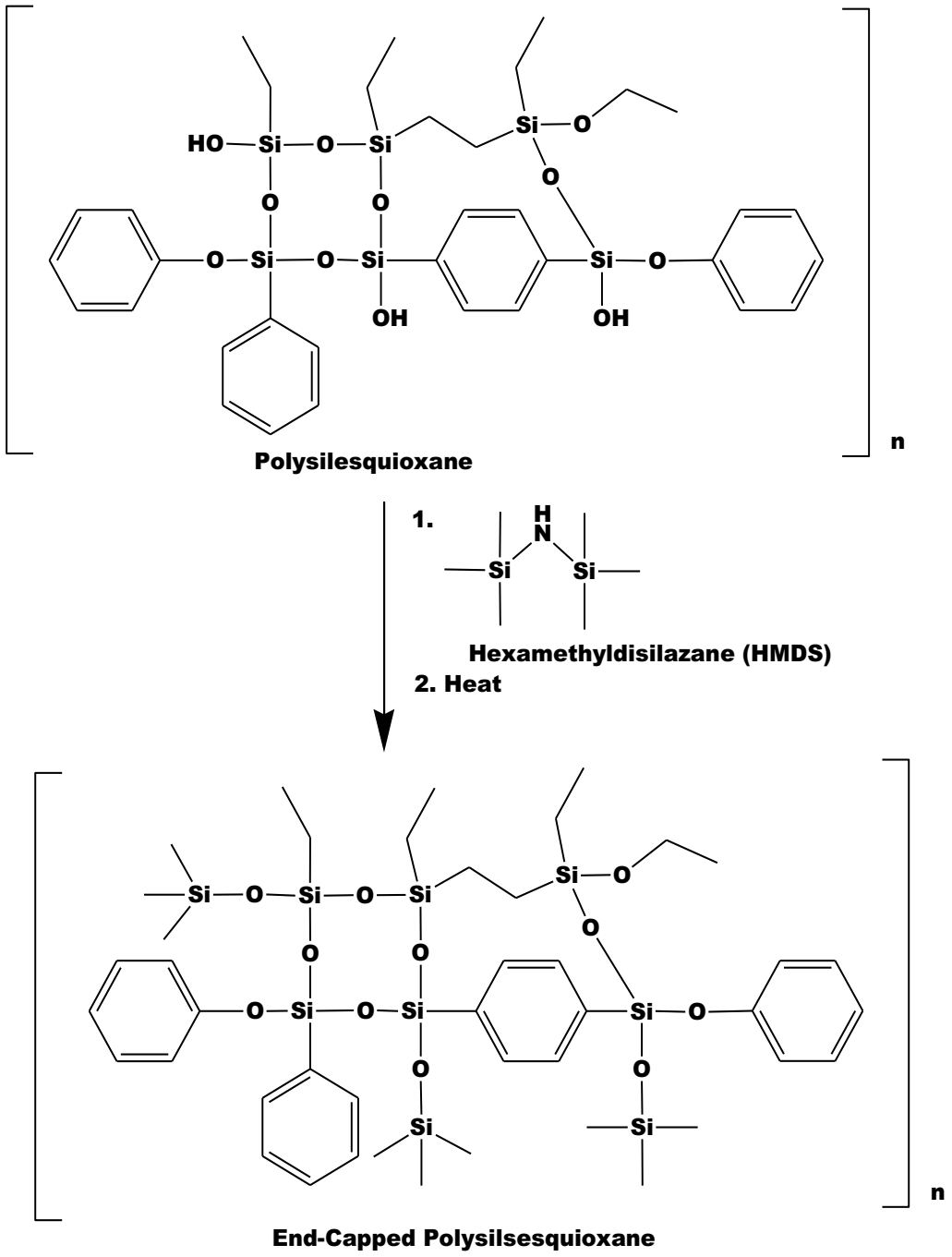


Figure 3.3: End-capping of Polysilsesquioxane

3.5.2. SYNTHESIS OF POLY (STYRENE-DIVINYL BENZENE)

The synthesis of poly(styrene-divinyl benzene) was carried out following the protocol reported by Hosoya & Frechet (see **Fig. 3.3**).[4] A blend of benzoyl peroxide and dibutyl phthalate was added to deionized water at a ratio of (1:10.67:23.3). Following the addition

of sodium dodecyl sulphate (SDS) solution at ratio of (1:0.5) and a 30 minute sonication of the reaction mixture, the styrene and divinyl benzene along with a mixture of toluene, deionized water and of polyvinyl alcohol (PVA) at a ratio of (1:1.5:0.7:0.2) were added to initiate the polymerization step. The mixture was stirred continuously at 100 ºC for a further 10 hours. In the final step, the resulting product was washed successively with methanol (about 2× 400 ml) and THF (2 × 100 ml) (see **Fig. 3.4**)

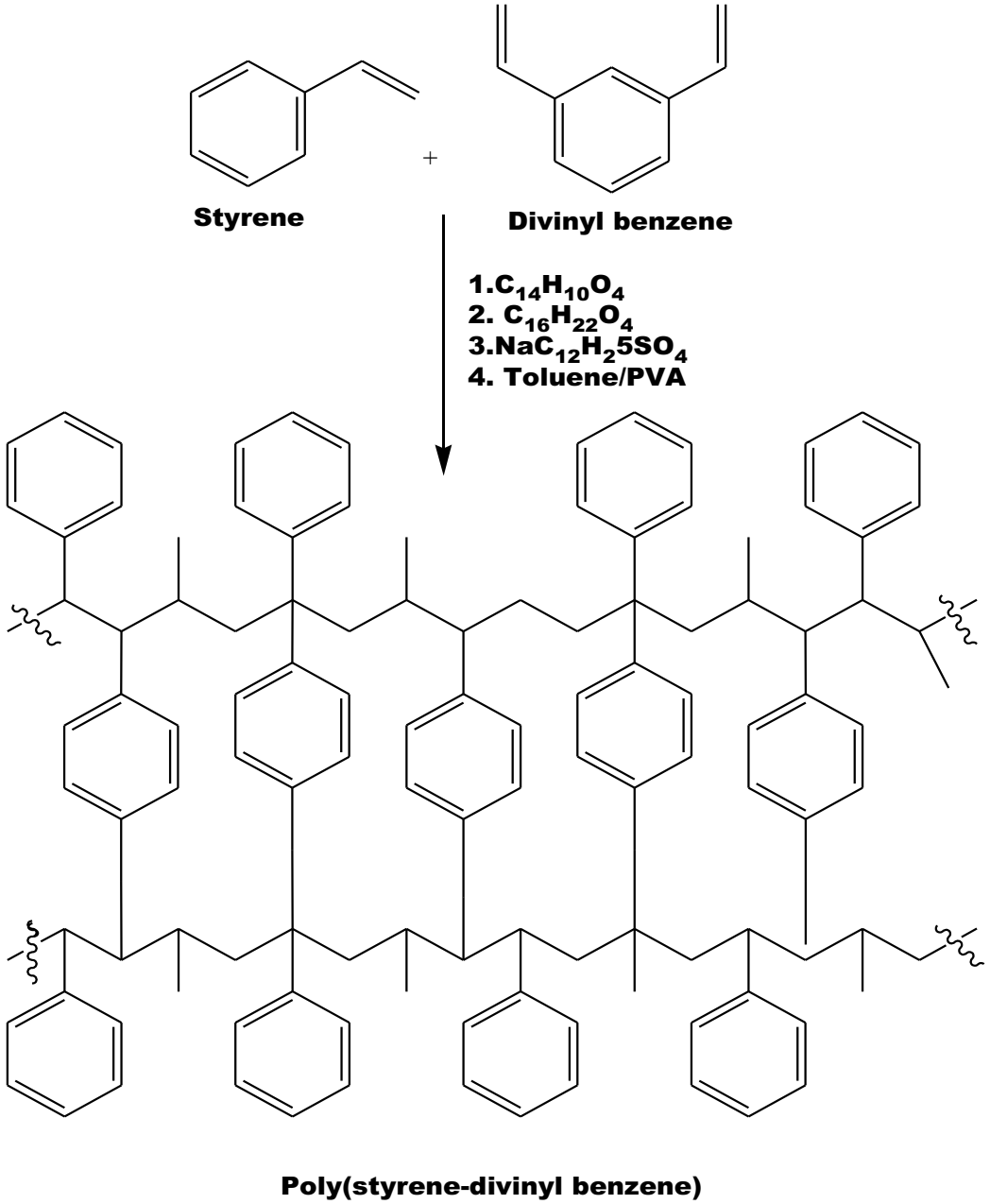


Figure 3.4: Chemical reaction of Poly (styrene-divinyl benzene)

3.6. MATERIAL CHARACTERIZATION

The morphological, structural, and physical properties of the poly(styrene-divinyl benzene) and the polysilsesquioxane were studied using various techniques such as: Fourier transform infra-red spectroscopy (FTIR), scanning electron microscopy (SEM)/Energy Dispersive spectrometer (EDS), X-ray diffractometer (XRD), Raman spectroscopy, Thermogravimetric analysis (TGA) and Brunauer–Emmett–Teller (BET). The conditions and parameters of these techniques are discussed in detail in the following sections.

3.6.1. Fourier transform infra-red (FTIR) spectroscopy

The Fourier Transform Infrared Spectrometer (FTIR), Frontier Perkin Elmer (USA) was used to elucidate the existence of functional groups for the as-synthesized polymers for this study (PSQ, E-PSQ and PS-DVB). Samples were scanned at 520-4000 cm^{-1}. No sample preparation was required for this method as all samples were in a powder form. A background check, before the actual analysis was performed. The background check aids in the identification of peaks of the actual sample.

Approximately 0.2 mg of sample was used for the analysis. In this work, the FTIR results of reactants and products were used to identify complete polymerisation and capping of the polymers.

3.6.2. Scanning electron microscopy (SEM)

The Scanning Electron Microscope/Energy Dispersive spectrometer (SEM/EDS), Jeol JSM-IT300 model, USA, was used to determine the morphology of the synthesized polymeric compounds.

The samples were first ground, then a small measure of fine samples were mounted on a double-sided tape then placed on the steel cylindrical rod-shaped sample holder. The carbon and gold coated samples were placed on the stage of the JEOL, JSM IT300 model electron microscope and view at about 20 mm length between the stage at the SEM focal lens. The necessary adjustments of both the magnification and focus of the SEM were

made such that the particle shape and pores of the polysilsesquioxane and poly (styrene-divinyl benzene) were clearly visible.

3.6.2.1. EDS

EDS in conjunction with SEM was utilised to study the elemental composition of the carbon coated polymeric samples. The SEM and EDS are hyphenated with the aid of giving more qualitative results. An X-ray light from the EDS was scanned through the polymeric samples. From the polymeric samples, both the qualitative and quantitative results of the elements of interest and their composition were measured by the EDS spectrum.

3.6.3. X-ray diffractometer (XRD) analysis

XRD analysis was performed on Rigaku X-ray diffractometer, Smartlab, USA. The XRD was used to elucidate the sample nature and the success of end-capping. About 1 g of polymeric samples were placed in an XRD sample holder and thereafter manually placed in the X-ray sample compartment. The X-ray light was refracted on the samples at different angles (10-80 °) to give the plane of symmetry of synthesized material.

3.6.4. Raman spectroscopy analysis

Raman analysis were performed on HORIBA Jobin-Yvon T64000 Raman spectrometer, USA with excitation wavelength of 514.5 nm. The Raman spectroscopy was used to measure the D-, G-band and to explore the type of carbon found in the samples. No specific sample preparation was done. The instrument had specialized samples holders specific for the powder samples (polymeric materials). The polymeric samples were directly placed on sample holders of the instrument. The polymeric materials were excited by the (Ar^+) laser at the wavelength of 514.5 nanometres. The instrument operated at 600 lines per grating and single spectrograph mode. A magnification of 20 x was attained on the sample by adjusting the lens of the laser on the sample. Nitrogen was used to cool the charge couple detector (CCD).

3.6.5. Thermogravimetric analysis (TGA)

The TGA analysis of the polymeric materials were carried out using the SDTQ600 differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA), Germany. These technique were used to measure the stability of materials at high temperatures. No specific sample preparation was done. The instrument had a small sample holder which accommodated about 1 mg of the powdered polymeric samples. The polymeric samples were directly placed on sample holders of the instrument then inserted onto a sample compartment of the instrument. Sample quantities of about 1 mg were heated from room temperature to temperatures higher than 100 ºC. The analysis was accomplished with a heating rate of 5 ºC per minute under air. Degradation of polymeric materials i.e. weight loss due to the increase in temperature was recorded by the instrument.

3.6.6. Brunauer–Emmett–Teller (BET)

BET measurements were undertaken with Micromeritics TRISTAR 3000 analyser, USA for studying of the pore size, pore volume, surface area of the polymeric materials and the distribution of the pore size of the synthesized stationary phases. These studies were carried out by using the nitrogen adsorption-desorption measurements where materials (0.2 g) were placed in vacuum (degassed) in temperatures lower than its decomposition. Nitrogen gas was pumped through the polymeric material. Samples were outgassed at the temperature of 150°C and pressure of 0.147 bar simultaneously for 6 hours before measurements. Nitrogen adsorption isotherms were found at -195. 75 °C, which is the liquid-nitrogen temperature.

3.6.7. Laboratory analysis of fractionated HA and real NOM samples

3.6.7.1. Total organic carbon (TOC) and dissolved organic carbon (DOC) analysis

SPE eluents were transferred to TOC vials (40 mL). The TOC and DOC measurements were carried out with the total organic carbon (TOC) analyser (Teledyne Tekmar TOC Fusion, USA).

No sample preparations were required for all TOC measurements. For DOC measurements, 0.45 syringe filter membranes were used to filter deionized water samples prior to analysis with the TOC instrument.[5]

3.6.7.2. Ultra violet-visible (UV–Vis) spectrophotometric analysis

For UV-Vis measurements, a Perkin Elmer spectrophotometer 650 S (USA) instrument was used at a UV-Vis range of 200-800 cm^{-1}. The filtered and unfiltered NOM samples (same samples as those used to measure TOC and DOC) were inserted in a UV-Vis cuvette. The cuvette was then placed in the UV-Vis instrument. A full scan from 800-200 cm^{-1} scan of the samples was undertaken to verify absorption of light in the regions of 214 cm^{-1} (specific absorption of NO_3- and NO_2-), 254 cm^{-1} (specific absorption of humic and fulvic acid along with other aromatic compounds), 272 cm^{-1} (specific absorption of trihalomethane (THM) and 300 cm^{-1} (specific absorption of DOC).

3.6.7.3. Specific ultra-violet absorbance (SUVA)

The SUVA values were attained by using equation 2.1, which is a quotient value from UV-254 divided by DOC and a product of the quotient value and a value of one.[6] The SUVA values for the samples gave a description of the NOM nature i.e. a difference between the highly aromatic NOM and the highly aliphatic NOM.[5] SUVA value of 2 or below indicates the hydrophilic NOM, SUVA values of 4 and above indicate the hydrophobic NOM, and SUVA values between 2 and 4 indicate the transphilic NOM.

3.6.8. Fluorescence excitation emission matrices (FEEM) analysis

Both prepared and real deionized water samples were analysed using the HORIBA Aqualog FEEM, USA. This technique was targeted at the analysis of humic substances of different regions and morphology from all deionized water samples.

No sample preparations were needed, thus all prepared and real deionized water samples were analysed without further modifications/purifications.

The FEEM instrument allows the differentiation of various humic substances from real samples and synthetic samples as well as the extent of their removal or fractionation by the SPE cartridges.[7] Blanks for this method consisted of a mixture of ultra-pure deionized water and a known concentration of DOC. A water sample containing NOM was transferred to a clear cuvette, which was then inserted into the FEEM instrument. The FEEM provided the fluorescence light from a xenon source of light to the samples containing NOM. The xenon light is transmitted through the sample (water containing

NOM), then the sample will absorb and emit some of the light at different wavelengths. This result in excitation and emission behaviours of humic substances in the sample. The excitation and emission bands are set such that only the band of 5 nm will be allowed to pass through the excitation and emission slits.[7,8] The wavelength range of interest for the analysis is 200 nm to 600 nm. The humic substances (fulvic acid, humin and humic acid) in the sample absorbed and emitted the fluorescence light at different wavelengths. The Raleigh scattering were corrected by subtracting the fluorescence spectra of the blank from the spectra of the analyte.

3.7. MATERIAL APPLICATION

3.7.1. Solid phase extraction (SPE) empty cartridge packing

The SPE analyses were carried out using the SPE manifold (see **Fig. 3.5**); an external pump was used to extract the mobile phases of all samples.

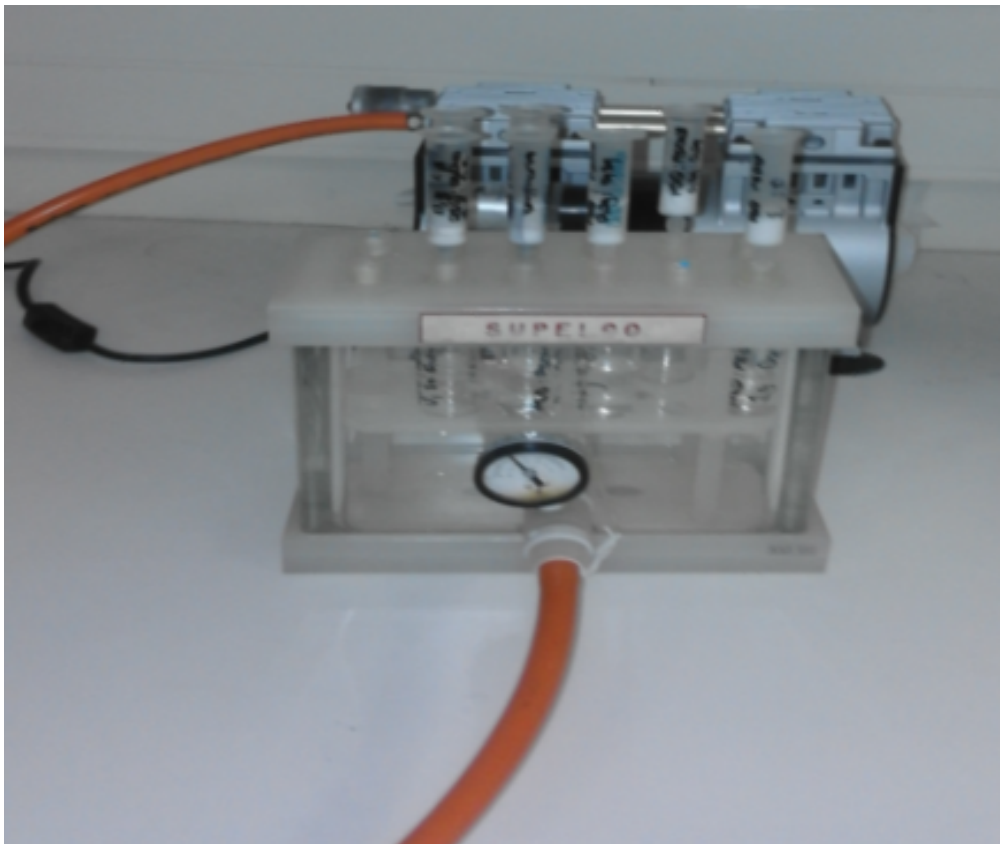


Figure 3.5: Solid-phase extraction (SPE) set-up

3.7.1.1. Preparation of poly (styrene-divinyl benzene) (PS-DVB) and polysilsesquioxane (PSQ) as SPE packing materials

About 1-2 g of polymeric samples were mixed with deionized water (collected from a MilliQ integral 10 deionized water purification system) to form a slurry. The two polymers, namely poly (styrene-divinyl benzene) (PS-DVB) and polysilsesquioxane (PSQ), were hand packed manually in empty cartridges following reported literature procedures.[3,9,10]

3.7.1.2. Packing the empty SPE columns with PS-DVB and PSQ

The PS-DVB and PSQ materials were hand-packed in the empty SPE cartridges and dried by sucking solvents with a SPE vacuum pump. SPE slits were put in between the packed materials to prevent materials from eluting with solvents. Eight different mass quantities (g) of materials were studied and compared (see **Table 3.1**).

Table 3.1: Quantities of poly (styrene-divinyl benzene) (PS-DVB) and polysilsesquioxane (PSQ) packed in empty SPE cartridges

Cartridge number	Mass of PS-DVB (g)	Mass of PSQ (g)	Packing style	Total mass of cartridge (g)
1	0.8	0.2	PS-DVB:PSQ	1
2	0.2	0.8	PS-DVB:PSQ	1
3	0.2	0.8	PSQ:PS-DVB	1
4	0.8	0.2	PSQ:PS-DVB	1
5	0.5	0.5	PS-DVB:PSQ	1
6	0.5	0.5	PSQ:PS-DVB	1
7	0	1	PSQ	1
8	1	0	PS-DVB	1

3.7.2. SIZE EXCLUSION/GEL PERMEATION CHROMATOGRAPHY (SEC/GPC) EMPTY COLUMN PACKING

For all column packing procedures, an Ultra High Pressure Pack in a Box (Dual Piston Pump) Column Packing Machine from Scientific Systems Incorporated, USA shown in **Fig. 3.6** was used.

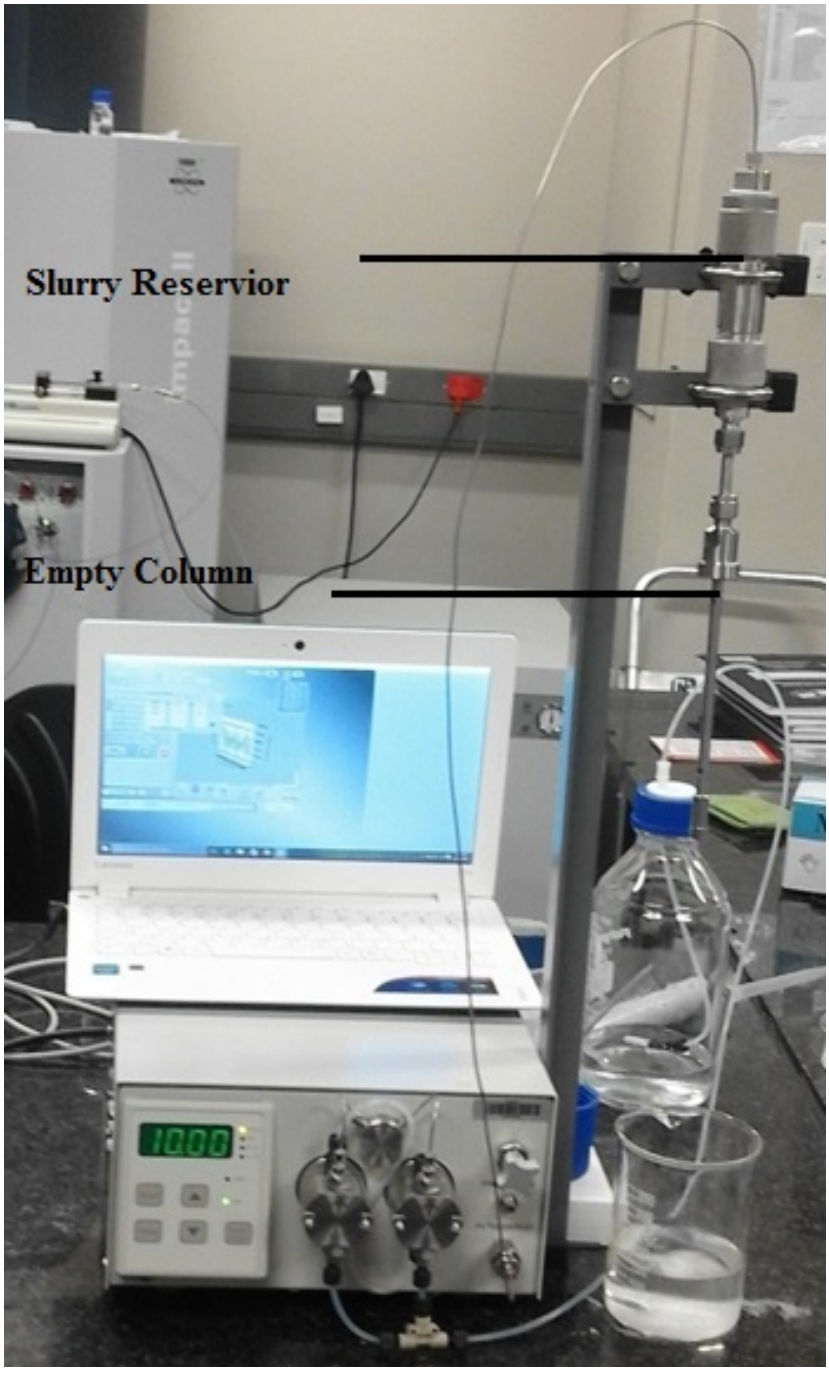


Figure 3.6: Scientific Systems Incorporated Ultra High Pressure Pack in a Box (Dual Piston Pump) Column packing machine

3.7.2.1 Fractionation procedure

The SEC/GPC packed column was placed in an HPLC system and thereafter eluted with a mixture of deionized water and methanol (80:20) for 30 minutes and phosphate buffer: methanol (70:30) for about 15 minutes. The column temperature was kept at 40 °C and the following conditions were adopted: mobile phase of phosphate buffer: methanol (70:30 v/v), wavelength of 254 cm^{-1}, flow rate of 0.25 ml/min and elution time of 10-30 minutes.

3.7.2.2. Data analysis

All data were exported from the software with no further alterations.

3.8. PERFORMANCE TESTS FOR THE PS-DVB-PSQ PACKED SEC/GPC COLUMN

The following performance tests were employed to characterize the performance and efficiency of the synthesized stationary phases:

3.8.1. Interactions with acidic compounds

(a) Activity toward acids

The capacity factor and tailing factor of 4-chlorocinnamic acid (**Fig. 3.7**) was measured using a mobile phase of ratio 30:70 methanol/ aqueous 0.02 M phosphate buffer of pH 2.7.[11] This was done to test for the applicability of the stationary phase to acidic analytes. A good peak shape indicates a high degree of inertness toward acidic compounds.

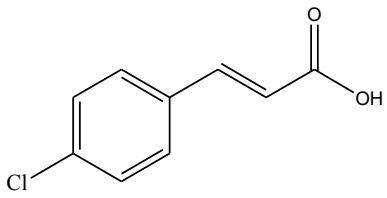
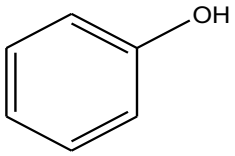


Figure 3.7: 4-chlorocinnamic acid

(b) Tanaka test

The retention factor of protonated silanol (SiO^-) were estimated by the selectivity factor between phenol (**Fig. 3.8**) and benzyl amine (**Fig. 3.9**). The compounds were eluted in the column by using a mobile phase of ration 30:70 methanol/aqueous 0.02 M phosphate at pH

2.7.[11] The retention of benzyl amine on the column indicates that the surface of the stationary phase of the silica is essentially free of acidic silanol groups.



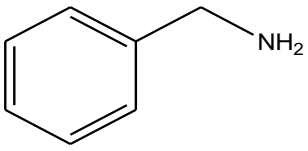


Figure 3.8: Phenol Figure 3.9: Benzyl amine

3.8.2. Hydrophobic Interactions.

(a) Hydrophobic retention (HR)

This parameter was evaluated by eluting pentyl benzene (**Fig. 3.10**) in a column packed with the prepared stationary phase. Deionized water/methanol (20; 80 v/v) was used as a mobile phase [12,13] The elution profile of pentyl benzene gave an indication of the amount of carbon load on stationary phase, and thus provided a measure of the strength of the hydrophobicity of the stationary phase.

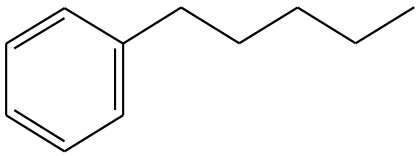


Figure 3.10: Pentyl benzene

(b) Hydrophobic selectivity (HS)

The hydrophobic selectivity of the synthesized stationary phases was ascertained by comparing the retention measures of pentyl benzene (**Fig. 3.10**) and butyl benzene (**Fig. 3.11**). The pentyl benzene and butyl benzene were eluted through the column with 80:20 (v/v) methanol/deionized water mobile phase.[11] The result provided information about the surface coverage of the stationary phase because the selectivity of the two compounds is dependent on the density of the stationary phase.

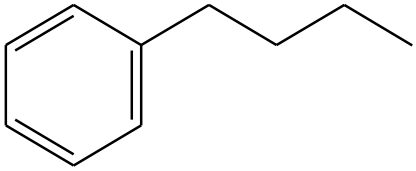


Figure 3.11: Butyl benzene

(c) Steric selectivity (SS)

Steric selectivity measured the ability of the stationary phase to distinguish between molecules with similar hydrophobicity and structure but different shapes. The steric selectivity was investigated by eluting a mixture of o-terphenyl (**Fig. 3.12**) and triphenylene (**Fig. 3.13**). These compounds have very similar chemistry. The two compounds were eluted through the column using a mobile phase with 80:20 (v/v) methanol/deionized water.[11]

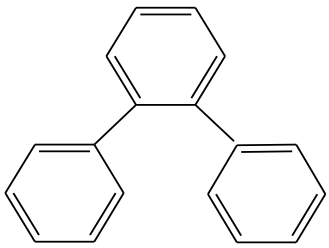


Figure 3.12: O-terphenyl

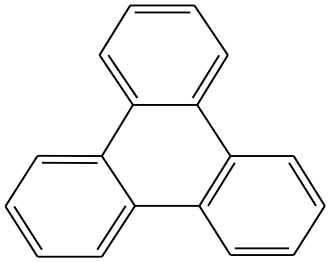


Figure 3.13: Triphenylene

(d) Hydrogen bonding capacity (HBC)

This technique is used to measure the number of free silanol groups and the degree of end capping. The test was done by comparing the relative retention of caffeine (**Fig. 3.14**) with respect to phenol (**Fig. 3.8**). A mixture of methanol and deionized water (30:70 v/v) was used as mobile phase.[11] A low or high value of the retention of caffeine indicated that the stationary phase had a low or high level of silanols available for hydrogen bonding. This therefore, indicated successful or unsuccessful the end capping.

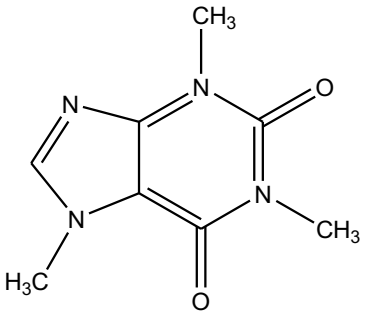


Figure 3.14: Caffeine

3.8.3. Stability at high pHs

To test the stability of the stationary phase at basic pH ranges, amitriptyline (**Fig 3.15**) was eluted with a mobile phase of 30:70 methanol/aqueous 0.02 M phosphate at pH 7.6.[11] The values of the capacity factor and tailing factor were evaluated. Those factors provided information on the overall performance of the column at pH levels above neutral.

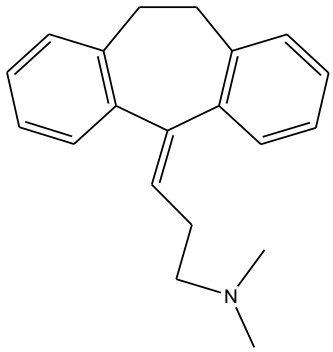


Figure 3.15: Amitriptyline

3.8.4. Ion exchange capacity

This parameter was investigated by eluting a mixture of benzyl amine (**Fig. 3.9**) and phenol (**Fig. 3.8**). The two compounds were eluted with a mobile phase of 70:30 aqueous 0.02 M phosphate/methanol at pH of 7. 6.[11] The magnitude of the selectivity factor between benzyl amine and phenol were used to get information about the measure of the total silanol activity on the surface of the prepared stationary phase.

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CHAPTER 4:
CHARACTERIZATION OF POLYSILSESQUIOXANE (PSQ) AND POLY (STYRENE-DIVINYL BENZENE) (PS-DVB)

This Chapter presents the theory, results and discussions of the synthesized polymeric materials (i.e. Polysilsesquioxane (PSQ) and poly (styrene-divinyl benzene) (PS-DVB).

The results from Fourier transform infra-red spectroscopy (FTIR), scanning electron microscopy (SEM)/energy dispersive spectrometer (EDS), X-ray diffractometer (XRD), transmission electron microscope (TEM), Raman spectroscopy, thermogravimetric analysis (TGA) and Brunauer–Emmett–Teller (BET) are also presented**.**

4.1. Stationary phases for SEC/GPC

The first polymers which were used as packing materials for SEC/GPC stationary phases were porous polymers. These polymers were introduced in the late 1950's when ion-exchange resins were developed.[1] The most effective SEC/GPC polymers are composed of particles as opposed to gel stationary phases. Other stationary phases which are common in HPLC are chemically modified silica hydride; these stationary phases have unique selectivity, flexibility and can separation of both polar and non-polar in one run.[2] Gel packing materials are poorly cross-linked, require a good solvent, become soft, fragile and compressible.[3] These packing materials are weaker than porous packing material. They can swell in protic solvents and have very low reactivity. SEC/GPC with soft gels as stationary phases, do not use solvent delivery pump. This means there is no constant flow of mobile phase and inefficient separation of molecules through the column.[4] This study seeks to address the SEC/GPC packing material that can separate NOM with minimum or no shear degradation.

Polymeric porous materials are used in catalysis, enzymes movement restrictors, templates for synthesis and as molecular sieves.[3,5,6] Polymeric packing materials are well known for their ability to tolerate wider pH range as compared to gel silica packing materials, which are effective between pH 2-8. [7] Polymeric silica based materials are highly cross-linked hence the high rigidity. They require both best and poor solvents and they cannot swell in protic solvents.[4] The advantages mentioned above renders them the best in pore formation, since their pores are formed during preparation (wet) and are able to persevere even in a

dry state.[4] Their applications extend to separation processes including chromatographic separations [8] and large demineralization columns in water treatment plants.[1] The polymers available commercially have limited range of particle sizes below 2 μm which influence shear degradation.[4] There is therefore a need for synthesizing a new series of silica based porous stationary phase materials to be applied for the fractionation of NOM. Amongst the vast number of chromatographic techniques used in the separation of components in water, SEC/GPC has been found to be the most effective chromatographic technique that can separate all fractions of NOM without compromising the structure or excluding other fractions. SEC/GPC technique is a common method that is used to separate macromolecules and micro molecules from a complex mixture of molecules according to their different sizes. The very same technique is utilized to fractionate/separate NOM into different fractions. It is important to note that, in order to separate both the hydrophilic and hydrophobic part of NOM, a suitable GPC/SEC stationary phase must be used.

4.1.1. Selected polymers for the study

4.1.1.1. Poly (styrene-divinyl benzene)

Poly (styrene-divinyl benzene) (PS-DVB) is known for its ability to endure very wide eluent polarity and pH.[9] It is stable against most bases, acids and oxidizing agents.[10] PS-DVB is suitable for HPLC, GPC and ion exchange chromatography.[9,10] PS-DVB is faster and more effective than large particle styragel® columns. It takes 30 minutes and only one column to analyse cellulose with SEC/GPC and it takes 3-4 hours with 8-10 columns of large particle styragel® to perform one single cellulose analysis.[9] It has shown great results in the removal of heavy metals in ion exchange chromatography.[10] PS-DVB has low chemical energy group, hence hydrophobic and their contact angle can be varied from 100-110° by altering the crosslinking degree between the monomers.[5] PS–DVB is less rigid and therefore less efficient in separation as compared to inorganic polymeric stationary phases e.g. polysilsesquioxane.[11]

4.1.1.2. Polysilsesquioxane

Polysilsesquioxane (PSQ) is a polymer that can exist as both inorganic and organic compound. It contains a flexible quantity of siloxane groups. This property allows the polysilsesquioxane to be a very good adsorbent.[12]

Polysilsesquioxane SEC/GPC stationary phases that comprises of macro-, meso- pores and Si-C bond have been found to escalate alkaline resistance.[13] They can be easily synthesized via sol-gel techniques and they are applied in different processes such as separation, catalysis, electrochemistry and photochemistry.[14] Polysilsesquioxane is flexible enough to contain various organic groups bridged on them. This allows the stationary phase to have both organic and inorganic properties.[12] They have accomplished wide applications in analysis and separation. They possess outstanding properties in adsorption due to their ideal pore structure, and their ability to contain a variety of functional group attached.[15] Various functional groups can be introduced on the silica material such as acetyl, O-carboxybenzoyl, benzoyl, and hydroxymethyl functionalities to selectively target specific analytes.[15] PSQ has been commonly used as coupling agent, gases/chemicals separation agent.[16] Silica-based packings such as polysilsesquioxane have a low stability under great pH conditions.[11]

4.1.3. The PSQ-PS-DVB hybrid material

The two polymeric materials (PSQ and PS-DVB) possess various advantages as SEC/GPC stationary phases, however separately they meet various challenges as PS-DVB has limited rigidity and PSQ has low stability against extreme pH conditions.[11] To compensate the shortfalls of each polymeric material, a hybrid (silica-based) inorganic-organic stationary phase should be prepared using the PSQ and PS-DVB.

Previous studies report that the silica-based hybrid materials are very advantageous as HPLC stationary phases, because of their thin skeleton, well displaced pores (various ranges) and ability to alter their hydrophobic and hydrophilic nature by substituting the alkoxysilane with different organic groups.[17]

The hybrid PSQ-PS-DVB stationary phase material has more advantage towards the fractionation of NOM because it is a mixture of hydrophobic-hydrophilic nature similarly to NOM.[17–20]

4.1.4. Particle and pore sizes of the Polymers

4.1.4.1 Pore sizes of stationary phases

The pore sizes of polymeric materials used in various applications vary in sizes, which include the macro (>50nm), meso (2–50nm) and micro (<2nm) sizes.[6] However, because of the molecular sizes of NOM, the pore sizes of the stationary phases should be in a macro range. Macroporous polymers were accidentally discovered in the late 1950s after a long search of a mechanically resistant polymer with enhanced exchange kinetics ion-exchange resin and were useful as ion-exchange resins, catalysts in syntheses, adsorbents, templates, carriers, and chromatographic stationary phases.[1] Macroporous PS-DVB were reported to be highly crosslinked and have permanent multifaceted pores possess (prepared by adding a porogen during the polymerization process).[3] The macro porous PS-DVB were also reported to be chemically and mechanically stable.[21] The macro porous silica materials have been widely used as catalyst supports, stationary phases in separation, materials used in batteries, and thermal insulators.[22]

4.1.4.2. Particle sizes of stationary phases

Mono-sized (smaller) particles of a particular polymer material have an advantage of achieving an increased column efficiency, a shorter separation time and a very low back pressures.[7,23–25] Particles should be similar sized from 2 µm upwards (3 to 20 µm), equally dispersed, monolith and comprise of pore sizes with sizes from 0.005-100 µm [1,7,26]. This will be done to avoid shear degradation of NOM, column inaccuracy and to ensure better separation of NOM molecules with different sizes and molecular weight. For better separation of the NOM, the pore sizes of the stationary phases of the SEC/GPC column must have different sizes.

4.2. FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY MEASUREMENTS FOR E-PSQ AND PS-DVB

4.2.1. Blending of styrene and divinyl benzene

The percentage yield of PS-DVB ranged from 80-98 % as the conditions of the synthesis were altered. The percentage yield from literature was 95 %, which means the method was successfully modified.[7] The percentage yield for PSQ ranged from 70-90 % with respect to

the changes applied on the synthesis conditions. No specific percentage yield was reported on literature.[12]

Poly (styrene-divinylbenzene) (PS-DVB) is a product of co-polymerization of styrene(S) and divinylbenzene (DVB) monomer. [27–29] In this study the PS-DVB was synthesized as described in section 3.5.2. and P and S were purchased and analysed without further purification. Their FTIR spectra were obtained as described in section 3.6.1., whose general theory is also described in section 3.6.1. The FTIR spectra of S and DVB were studied first, in order to observe the success of polymerization from the three PS-DVB polymers (1:1, 1.5:1 and 10:1). The FTIR spectra (**Fig. 4.1**) reports the functional group present in both the monomers and the polymer materials. The summary in **Table 4.1** further explains the functional group present in each monomer and polymer.

The FTIR spectra of styrene (S) and (DVB) show similar peaks but intensity of the DVB peaks is lower than the S peaks. There are medium to weak multiple peaks appearing at 1400-1600 cm^{-1}, which indicate the existence of the C=C aromatic (from the aromatic ring) on the S and DVB compound. At around 3000-3100 cm^{-1} there exist a medium peak indicating a C-H aromatic from the aromatic ring. There are strong peaks from 3010-3100 cm^{-1} which indicate the =C-H aliphatic bending bands. At around 1620-1680 cm^{-1} there are variable peaks which indicate the presence of the C=C stretch (aliphatic) from S and DVB.

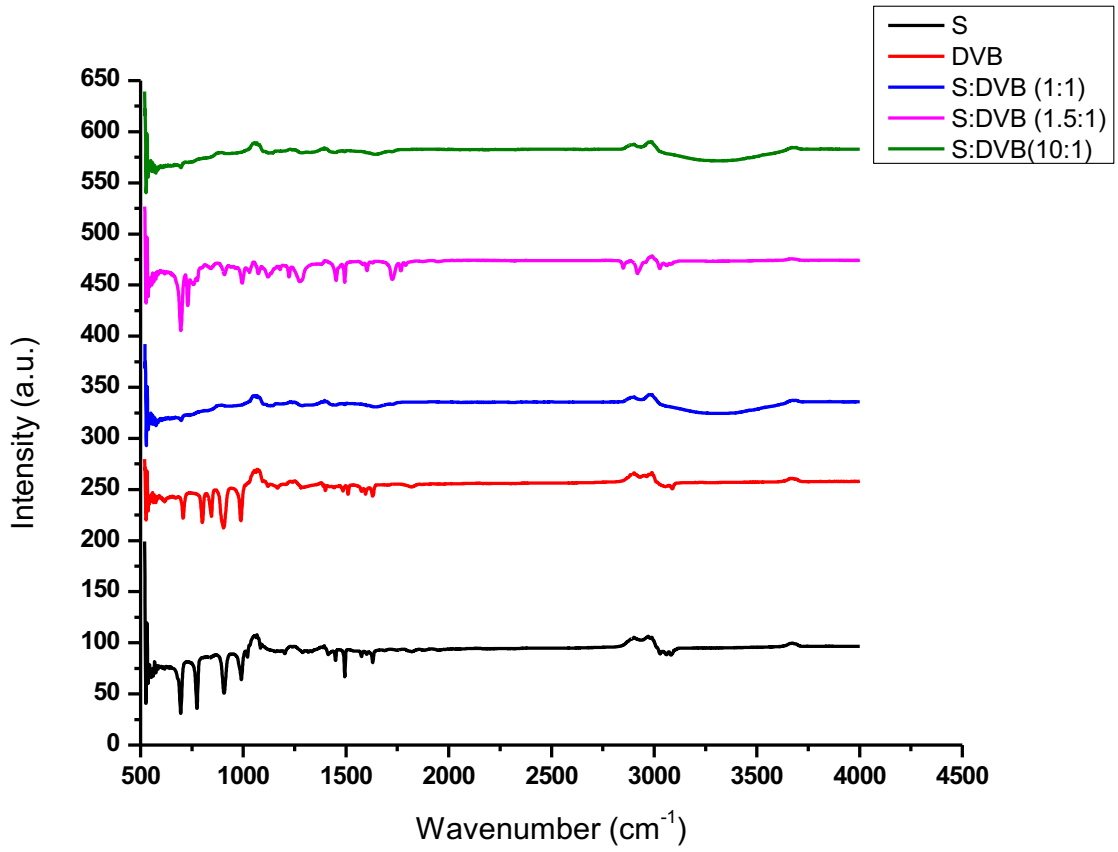


Figure 4.1: FTIR Spectra of styrene and divinyl benzene compounds

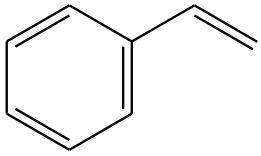
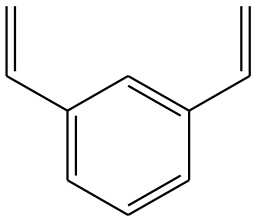
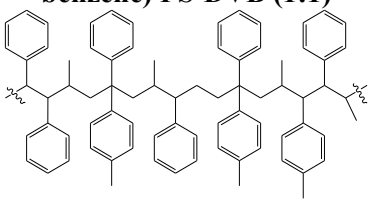
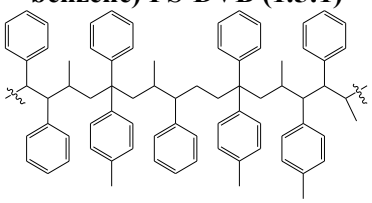
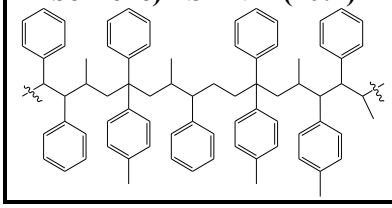
The FTIR spectra of DVB and S indicate flexural vibrations from benzene rings (δC−H) of the divinylbenzene [30] around 600-800 cm^{-1} . Medium to weak multiple peaks that appear at 1400-1600 cm^{-1} indicate the existence of the C=C aromatic of the S and DVB.[31] Peaks at 1400- 1700 cm^{-1} are due to benzene ring vibrations (νC−C) of S and DVB [30]. At around 2800-3200 cm^{-1} , there exist a medium peak indicating a C-H aromatic from the aromatic ring.[30,32] There are strong peaks from 694-988 cm^{-1}, which indicate the =C-H bending, from the two vinyl groups attached on the benzene ring (DVB), and one vinyl group (S). At around 1620-1680 cm^{-1}, there are variable peaks which indicate there presence of the C=C stretch (aliphatic) from divinyl benzene.

The FTIR spectra of PS-DVB (S: DVB, 1:1, v/v), (S: DVB, 1.5:1) and (S: DVB, 10:1) showed weak peak as compared to the peaks that are observed from the two monomers (S

and DVB). There were multiple peaks around 3010-3100 cm^{-1} (=C-H) from S and DVB, peaks at 600-800 cm^{-1} δC−H) of the polymers (PS-DVB). [30,33]. At around 1400- 1700 cm^{-1} there are multiple peaks (νC−C) of the polymeric material PS-DVB (benzene rings) [30]. At around 2800-3200 cm^{-1} there exist multiple peaks(C-H aromatic) from the aromatic rings on the S and DVB [30,32,34]. At around 1620-1680 cm^{-1} multiple weak peaks indicating the C=C stretch (aliphatic) the monomers. At around 1400-1600cm^{-1} there are multiple peaks indicating the vibrations of C=C stretching from benzene rings which are present in both the S and DVB. [34]. Peaks observes from PS-DVB 1.5:1 (S: DVB 1.5:1, v/v) are stronger than the peaks for PS-DVB 1:1 and 10:1.

There exist a strong and broad O-H peak at around 3000-3500 cm^{-1} from the FTIR spectra of PS-DVB 1:1 and 10:1 indicating the presence of O-H group in the samples. The O-H group was a residue from solvents such as, ethanol and methanol as the two alcohols during the synthesis of the polymers. The decrease of peaks on all three polymeric materials proved that polymerization was successful, this is evidenced by the FTIR results of the monomers. The decrease in peak intensity between styrene and divinyl benzene as the peaks decrease with an introduction of vinyl groups. The PS-DVB peaks on the FTIR spectra corresponds to the peaks which were previously reported in literature, therefore, this means the synthesis of PS-DVB was successful.[10]

Table 4.1: Functional groups observed from the FTIR spectra of compounds

Compound	Functional groups	Adsorption (cm^{-1})	Peak(s) type
Styrene (S) 	C=C (aromatic)	1415-1577	medium-weak
	C-H (aromatic)	3030-3057	Medium
	=C-H (aromatic)	694-1084	strong (multiple)
	C=C (aliphatic)	1415-1627	Medium
	=C-H (aliphatic)	2957-3116	Medium
Divinylbenzene (DVB) 	δC−H (aromatic)	617 and 802	medium-weak
	C=C (aromatic)	1402-1593	medium (multiple)
	νC−C (aromatic)	1402,1593 and 1631	Strong
	C-H (aromatic)	3048-3090	Medium
	=C-H (aliphatic)	694-988	Strong
	C=C (aliphatic)	1415-1627	medium (multiple)
	=C-H stretch (aliphatic)	2933-3110	Medium
Poly(styrene-divinyl benzene) PS-DVB (1:1) 	=C-H (aliphatic)	695-1073	weak-multiple
	δC−H (aromatic)	695 and 748	Weak
	νC−C (aromatic)	1455, 1582, and 1602	Weak
	C-H aromatic	3030-3085	Weak
	C=C (aliphatic)	1602-1719	weak (multiple)
	C=C (aromatic)	1402–1631	Weak
	O-H	3320	Broad
Poly(styrene-divinyl benzene) PS-DVB (1.5:1) 	=C-H (aliphatic)	697-1119	weak-multiple
	δC−H (aromatic)	2925, 2851-2925	Weak
	νC−C (aromatic)	697, 1428 and 1628	Weak
	C-H aromatic	3303	Weak
	C=C (aliphatic)	1658	weak (multiple)
	C=C (aromatic)	1428-1628	Weak
Poly(styrene-divinyl benzene) PS-DVB (10:1) 	=C-H (aliphatic)	693-1118	weak-multiple
	δC−H (aromatic)	693,729 and 753	weak
	νC−C (aromatic)	1450 and 1601	weak
	C-H aromatic	3025-3086	weak
	C=C (aliphatic)	1601-1764	weak (multiple)
	C=C (aromatic)	1314–1658	weak
	O-H	3303	broad

4.2.2. Synthesis of end-capped polysilsesquioxane (E-PSQ)

The FTIR analysis of both there Polysilsesquioxane (PSQ) and end-capped Polysilsesquioxane (E-PSQ) was done and in **Fig. 4.2**, there exist one broad peak (800-1200 cm^{-1}) characteristic absorbance of Si–O–Si stretching. [35–37] This peak is known to be a characteristic peak for silica functional groups. There is also a peak at 895 cm^{-1} which showed existence of Si–C bonds in both PSQ and E-PSQ.[36] The Si-C and Si-O-Si peaks are known to be a characteristic peaks for silica functional groups.

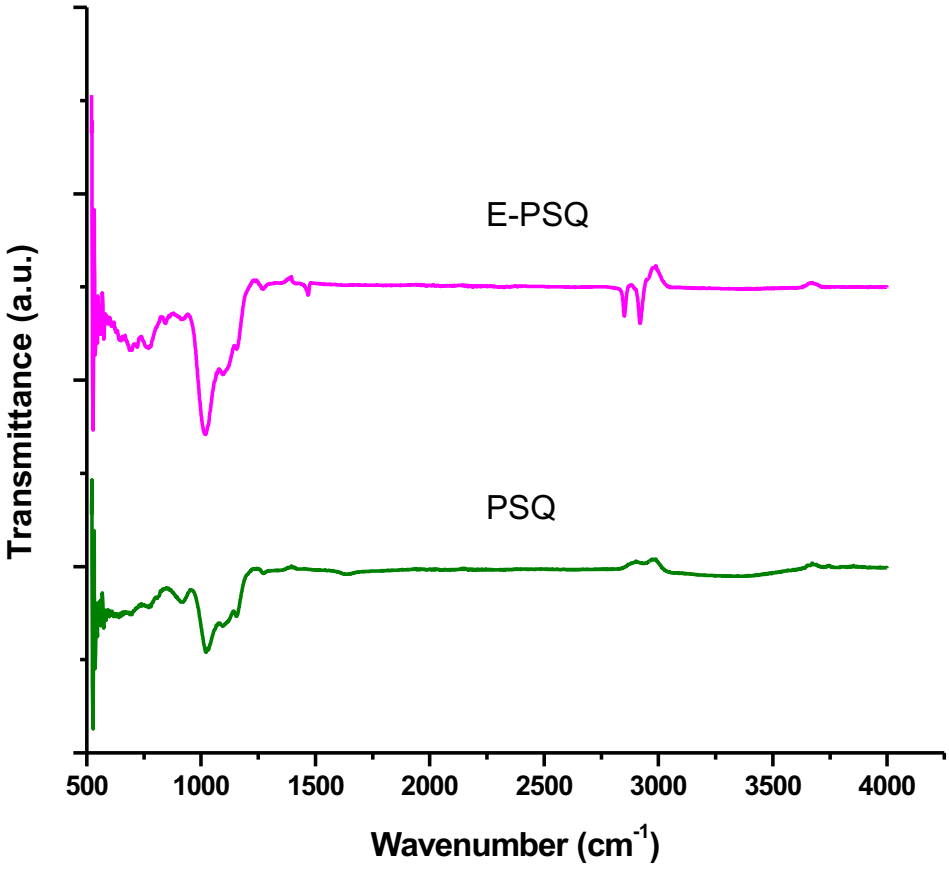


Figure 4.2: FTIR Spectra of PSQ and E-PSQ

The end-capped spectrum of PSQ shows the existence of strong peaks appearing at 2845-3000 cm^{-1}, which signifies the C-H stretching of the added alkyl group.[38,39] At 1282-1467 cm^{-1}, weak peaks exist that indicates the –C-H bending of the added alkyl group. The existence of the C-H stretching peaks indicates that a reaction had occurred between HMDS and silanol groups on the active sites of the synthesized silica materials. Therefore

the end-capping of the silanols from PSQ proved to be a success [38,39]. And the synthesis of PSQ was successful.

4.3. SCANNING ELECTRON MICROSCOPY (SEM) AND ENERGY DISPERSIVE SPECTROMETER (EDS)

The PS-DVB, PSQ and E-PSQ SEM and EDS spectra were obtained as described in section 3.6.2 and 3.6.2.1, respectively, whose theory is also described in the sections above.

4.3.1. SEM and EDS analysis of PS-DVB

The SEM image of PS-DVB (1:1) **Fig. 4.3a** show a spherical particle with pores of various size and shape. The corresponding EDS graph **Fig 4.3b** shows the presence of both carbon and oxygen as main elements in the sample of poly(styrene-divinyl benzene) (PS-DVB). The information confirms the elemental composition of the resulting polymer as PS-DVB.[40] The pores from PS-DVB are not well-defined as compared to PS-DVB (1.5:1) but has visible pores as compared to PS: DVB (10:1).

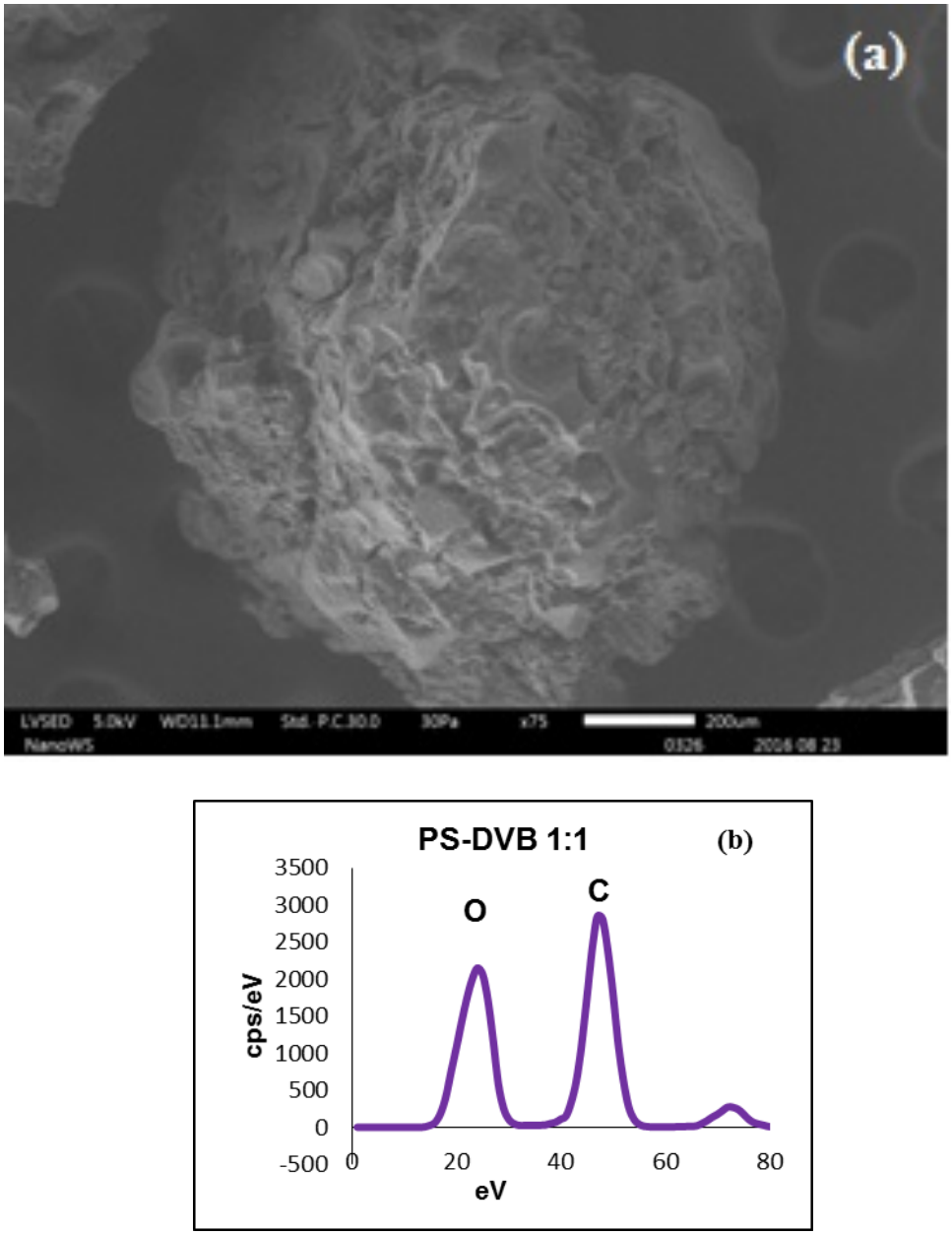


Figure 4.3: SEM (a) and EDS (b) results of PS-DVB (1:1 v/v)

The SEM image of PS-DVB with a ratio (S:DVB,1.5:1, v/v) **Fig. 4.4a** show a particles with pores of various sizes and shapes and the corresponding EDS graph **Fig 4.4b** shows carbon and oxygen as being the main elements in the sample of PS-DVB. The SEM and EDS results confirm the structural morphology, porosity and elemental composition of the resulting polymer as PS-DVB 1.5:1. Further studies regarding the pore sizes and pore volumes were undertaken using other instruments e.g. BET and will be discussed further in the following sections.

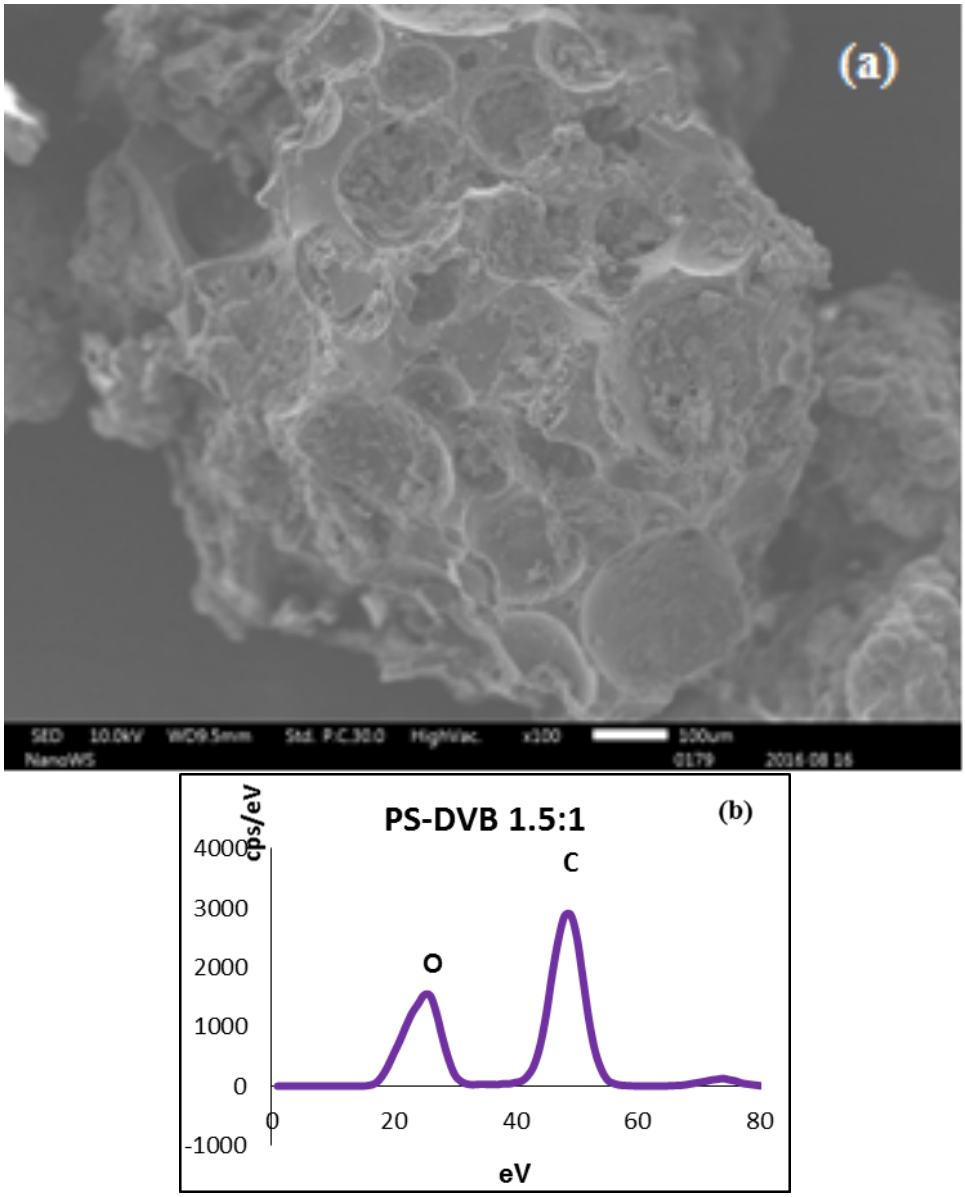


Figure 4.4: SEM (a) and EDS (b) results of PS-DVB (1.5:1 v/v)

The SEM image of PS-DVB (10:1) (see **Fig. 4.5a**) show a spherical particle with no visible pores. The more the composition of styrene relative to the divinyl benzene. The lesser the pores. This means the styrene competes with toluene (porogen) during the polymerization hence preventing pores from forming. According to the EDS results (see **Fig. 4.5b**), both carbon and oxygen are present as main elements and thus confirming the elemental composition of the PS-DVB polymer. The pores of the PS-DVB(1:1) and PS-DVB(1.5:1) materials resembled those found in previous works but the pores of PS-DVB (10:1) did not resemble any previous work.[6]

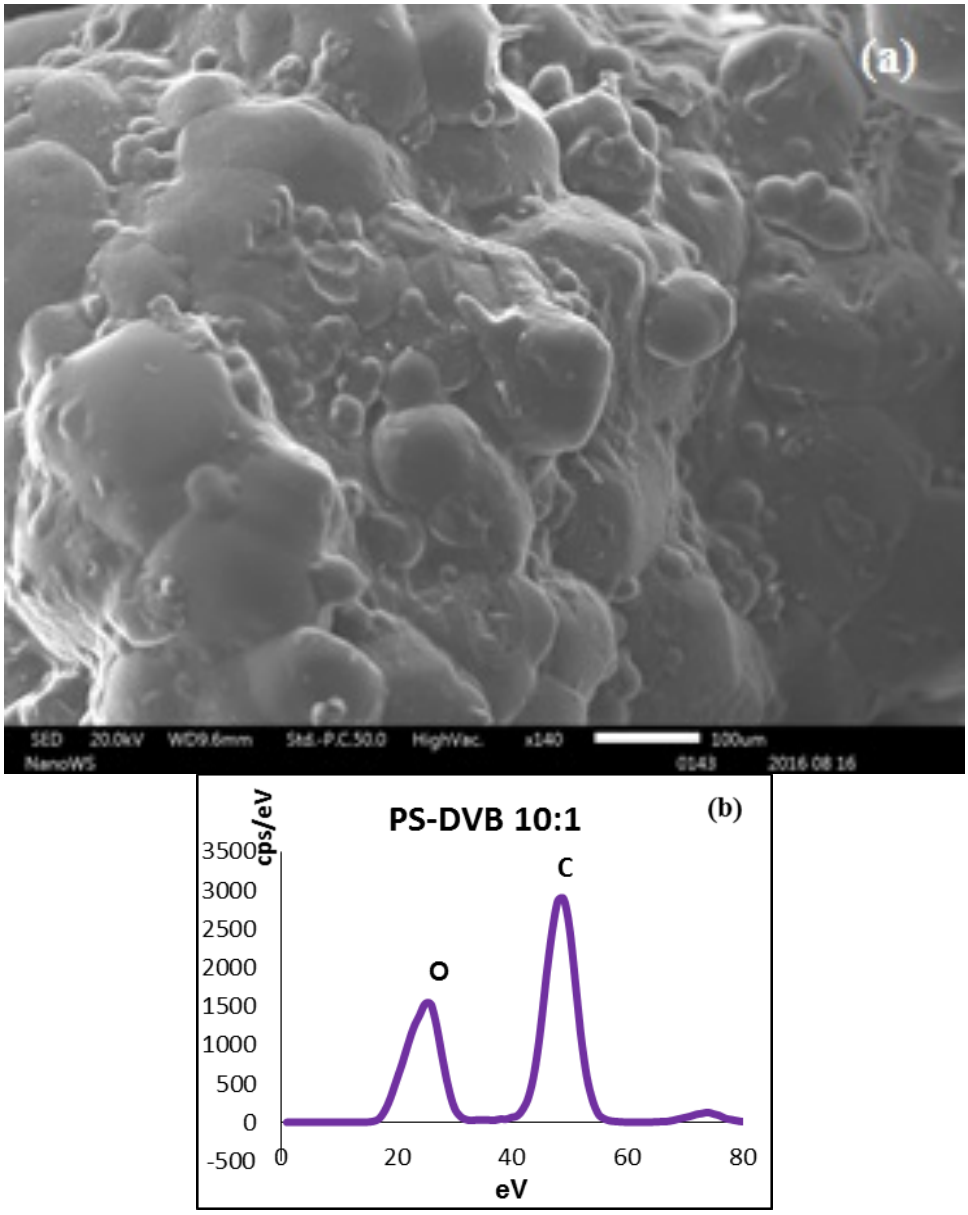


Figure 4.5: SEM (a) and EDS (b) results of PS-DVB (10:1 v/v)

4.3.2. SEM and EDS analysis of PSQ and E-PSQ

The SEM image of PSQ shown in **Fig. 4.6a** show particle with pores of various shape and sizes which are smaller than the PS-DVB (1:1 and 1.5:1) but more visible pores than the PS-DVB (10:1). The EDS results (see **Fig. 4.6b**) graph show the presence of both carbon, oxygen and silicon as main elements in the sample of PSQ as well as remains of sodium emanating from the salts that were used during the synthesis. The EDS results confirms the elemental composition of the resulting polymer as PSQ.

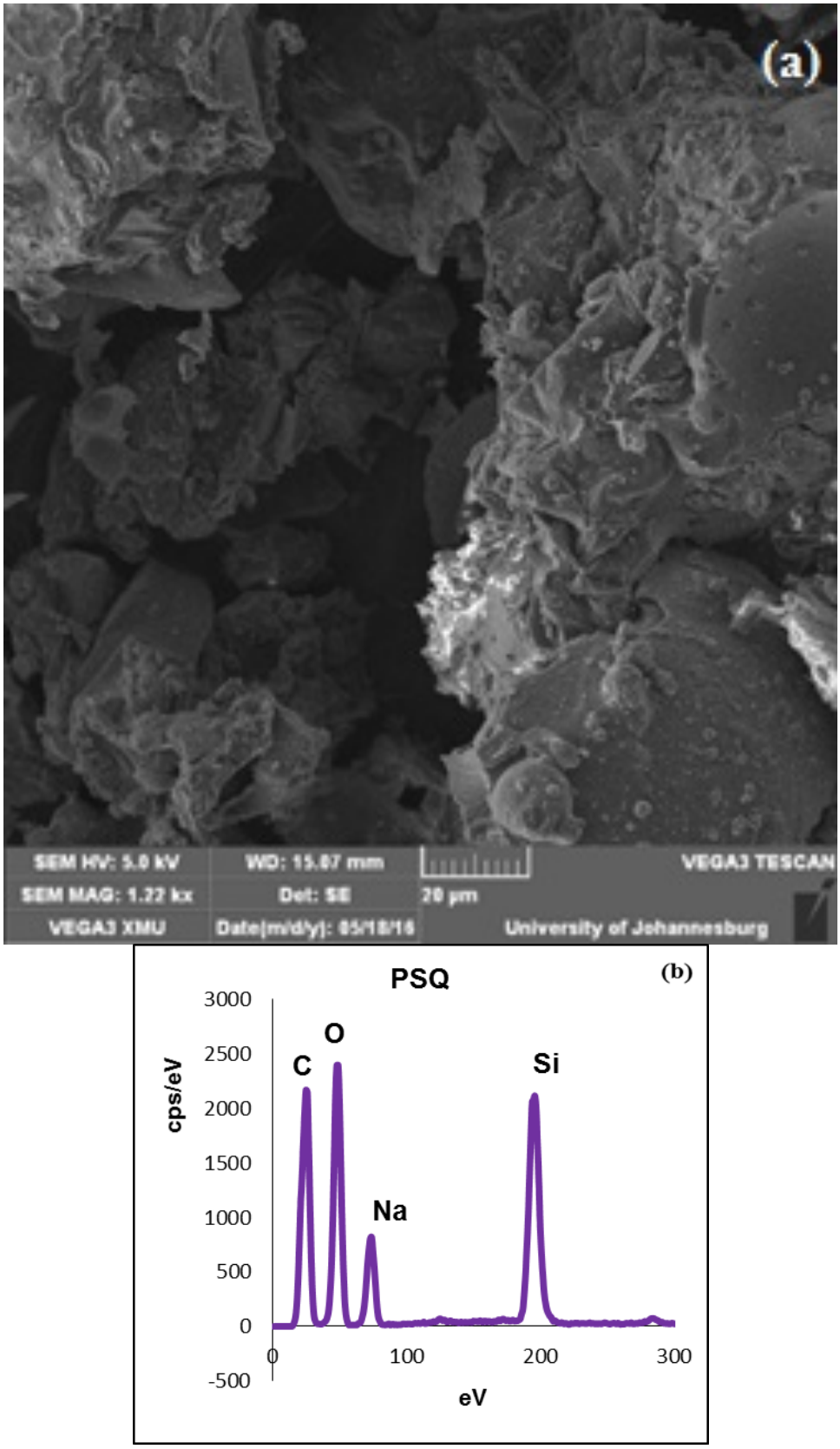
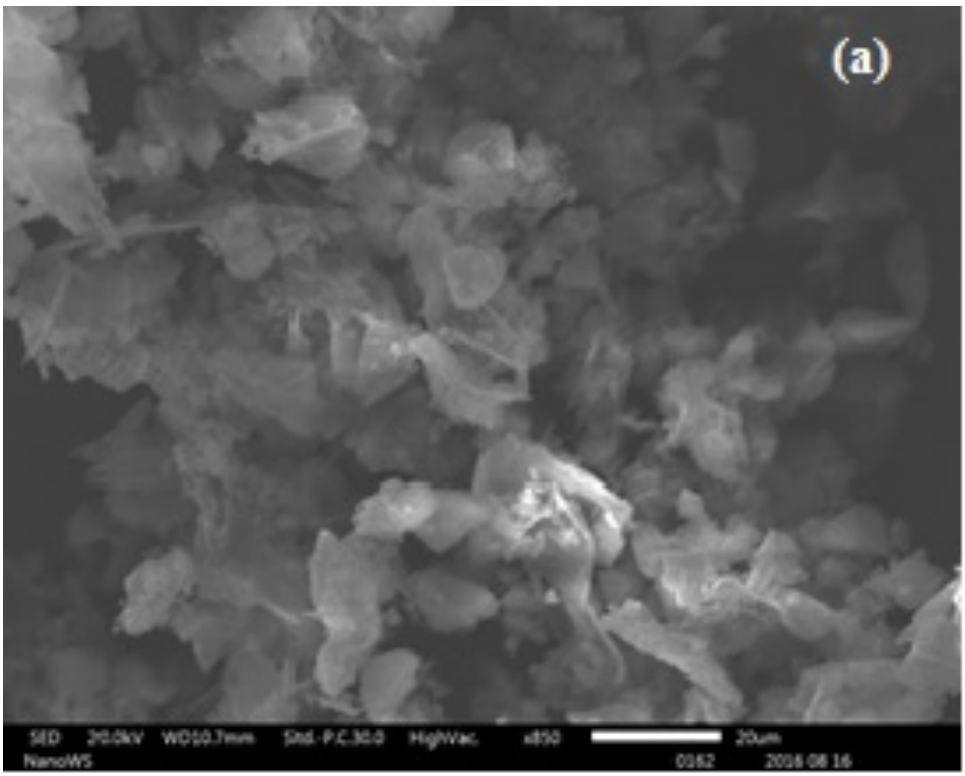


Figure 4.6: SEM (a) and EDS (b) results of PSQ

The SEM image of E-PSQ **Fig. 4.7a** show particle with pores of various shape and sizes. EDS results according to **Fig. 4.7b** show the presence of both carbon, oxygen and silicon as main elements in the sample of PSQ as well as remains of sodium from the salts which were used during the synthesis. The pores of the polymer have decreased and the quantity of silicon is smaller which confirms the addition of alkyl groups that resulted from end-capping. The E-PSQ has the smaller pores as compared to the PSQ, and PS_DVB (1:1 and 1.5).The information confirms the elemental composition of the resulting polymer as PSQ.



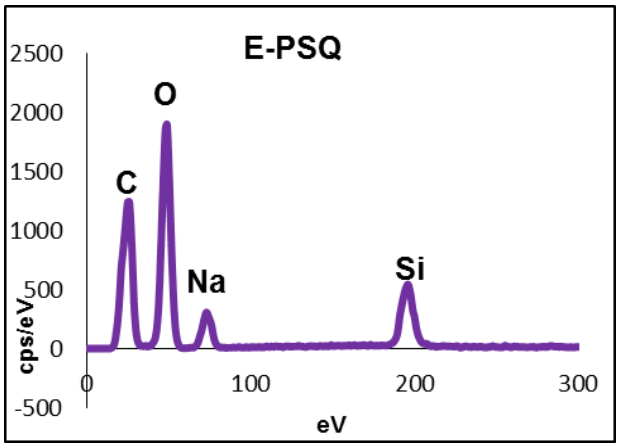


Figure 4.7: SEM (a) and EDS (b) results of E-PSQ

4.4. X-RAY DIFFRACTION (XRD)

The PS-DVB, PSQ and E-PSQ XRD results were obtained as described in section 3.6.3, whose theory is also described in section 3.6.3.

4.4.1. XRD analysis of PS-DVB

The two PS-DVB materials PS-DVB (10:1) **Fig. 4.8b** and (1:1) **Fig. 4.8a** have no peaks appearing on the theta angle and the X-ray light is non-directional and non-continuous.[33] No visible oscillation and deep minima was observed, therefore the polymeric materials were found to be amorphous and this also show polydispersity of the particles. As shown in **Fig. 4.8c**, the PS-DVB (1.5:1) polymeric materials show two visible peaks at 19.78° and 41.11°, these peaks are not sharp and do not form a continuous pattern, instead they are broad. This might be due to the impurities in the PS-DVB (1.5:1) polymer. These results suggest the texture of the product to be amorphous and the particles of PS-DVB are polydipersed.

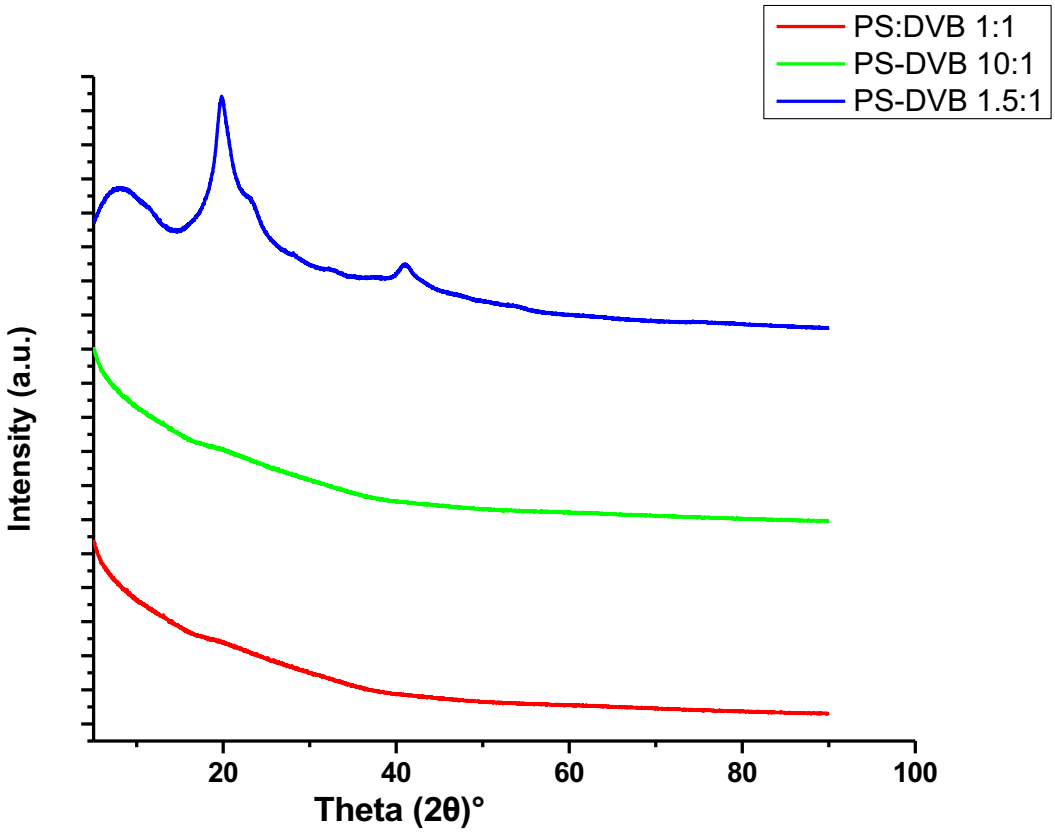


Figure 4.8: XRD spectra of PS-DVD 1.5:1, 10:1 and 1:1

4.4.2. XRD analysis of PSQ and E-PSQ

The XRD depicts the nature of the polymeric materials to either be amorphous or crystalline, monodisperse or polydisperse.[20,40] Below are the XRD results from the two polymeric materials (Poly (styrene-divinyl benzene) and Polysilsesquioxane) and their modifications. In **Fig. 4.9**, there are no 3D arrangement of atoms for PSQ (**Fig. 4.9a**), which means that the diffraction of the X-ray light is non-directional and non-continuous.[12] There is no visible oscillation and deep minima, therefore the sample is amorphous and the particles are polydispersed. The theta angle of E-PSQ (**Fig. 4.9b**) is showing a peak intensity at around 20 ° (corresponding to the end-capping material) which depicts the nature of the polymer to be amorphous and the particles to be polydipersed as no visible oscillation and deep minima is observed. The broad peak at around 20 ° is caused by intramolecular siloxane structure.[39]

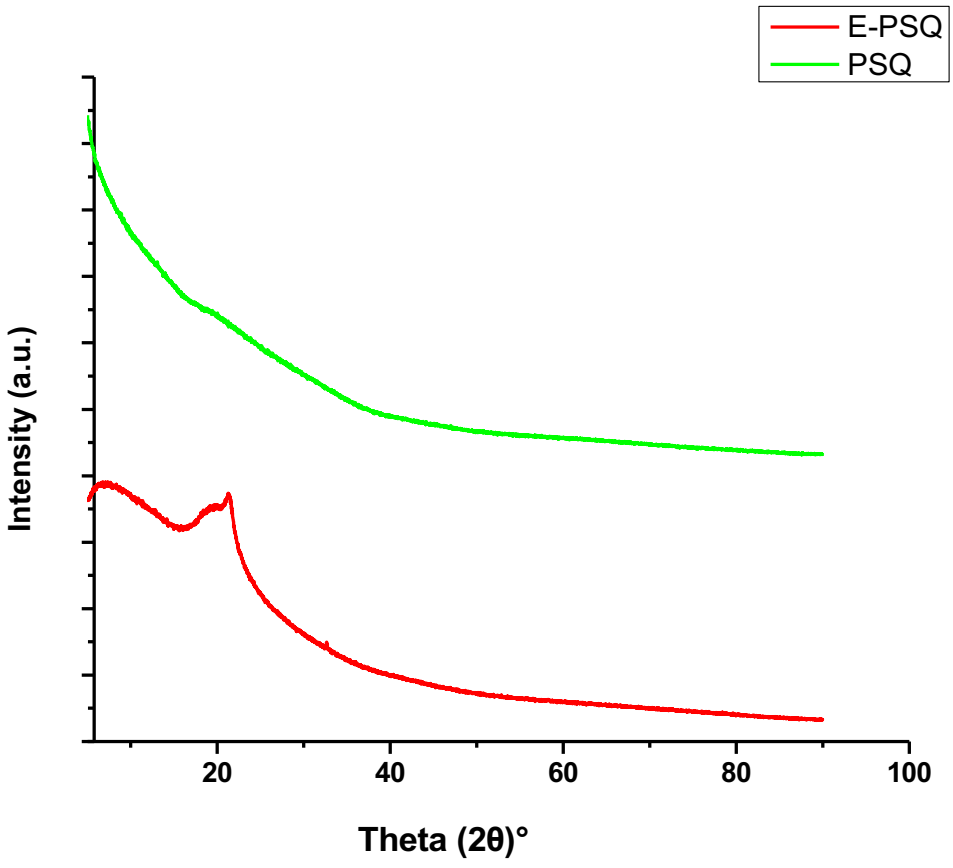


Figure 4.9: XRD spectra of PSQ and E-PSQ

4.5. RAMAN SPECTROSCOPY

The PS-DVB, PSQ and E-PSQ Raman spectra were obtained as described in section 3.6.4, whose theory is also described in the section.

4.5.1. Raman analysis of PS-DVB

The Raman spectra revealed the presence of the additional groups that were not detected by FTIR. It also revealed the nature of carbon in the polymeric materials. The Raman spectroscopy for PS-DVB (see **Fig. 4.10a-c**) were studied extensively and the vibrational and structural properties of the sp-sp^2 for amorphous carbon have been characterized by G and D bands. Raman spectroscopy was used to investigate the structural characteristics of the PS-DVB and PSQ materials. As shown in **Fig. 4.10**, the three PS-DVB polymers revealed three broad peaks at ~1340 cm^{-1}, 1585 cm^{-1}, ~2675 cm^{-1} and specific peaks corresponding to the polymer structure. The peak at ~1346-1347 cm^{-1} corresponds to the D-band (represents the defects of C-C bond), and the G-band at ~1570-1586 cm^{-1} corresponds to the sp^2 graphitic carbon (C=C)[11,41]. In addition, the spectra also revealed another D-band peak at ~2685 cm^{-1} characteristic of the 2D-band. In general, the intensity ratio of D-band over G-band (I_D/I_G) it is used to understand the degree of disorder of the materials, all summarized in **Table 4.1**.[11] The calculated I_D/I_G ratio was found to be 0.84, 0.86 and 0.78 for PS-DVB 1:1, PS-DVB 1:1.5 and PS-DVB 1:10, respectively. Furthermore, it was found that the I_D/I_G values increased from 0.84 to 0.86 when the amount of styrene (S) increased within the PS-DVB polymer from 1:1 to 1.5:1 ratio relative to divinyl benzene (DVB), respectively. This increase may be due to the transformation of the sp^2- C-C domain of polymers to sp^3-domain, which result from the strong covalent bonding interaction between the S and DVB polymer networks at low loading of S, which then lead to rise or shift to the D-band peak. However, further increase of the amount of S to a PS-DVB ratio of up to 10:1 resulted in the decrease in the I_D/I_G ratio of about 0.74. The reason for this decrease is that 1wt % amount of DVB polymer was able to chemically interact with specific content of S polymer to a certain extent, hence at high loading of S the effect is reduced.

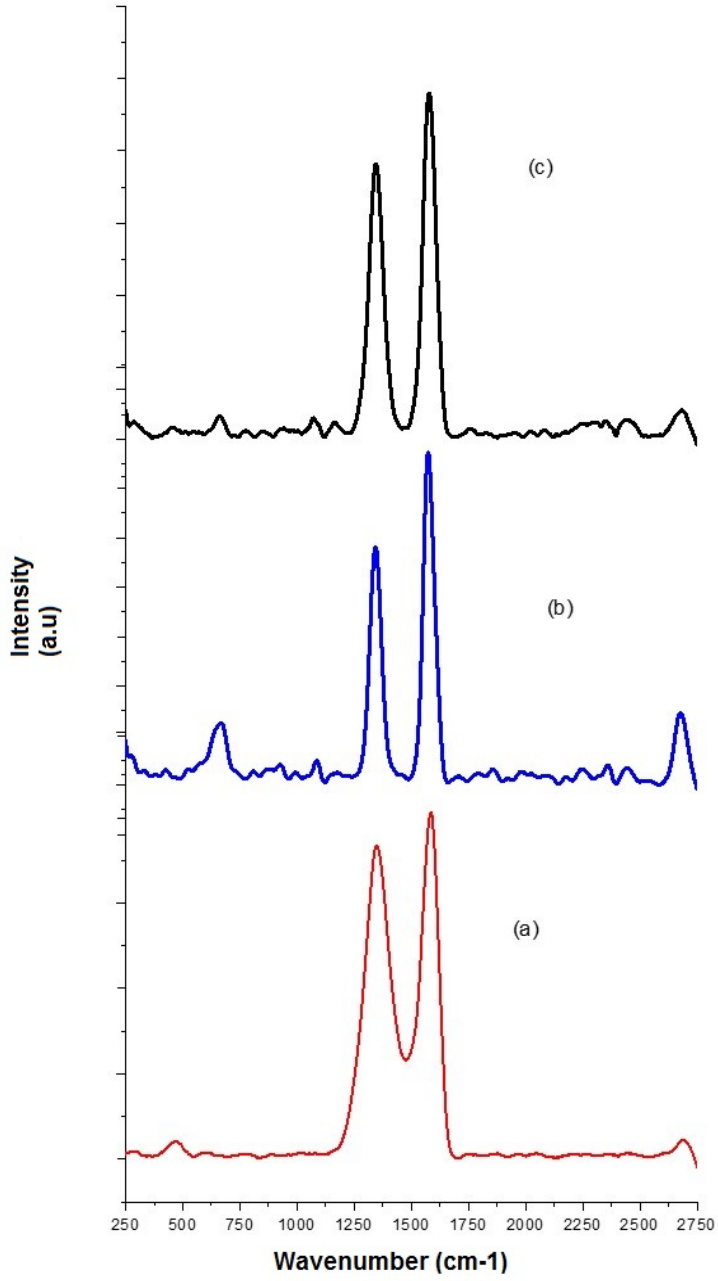


Figure 4.10: Raman spectra for PS-DVB (a) 1:1, (b) 1.5:1 and (c) 10:1

Table 4.2: The intensity ratio of the D and G bands for polymeric materials

Sample	I_D	I_G	I_{2D}	I_D/I_G
PS-DVB 1:1	1346	1586	-	0.84
PS-DVB 1:1.5	1344	1570	2676	0.86
PS-DVB 1:10	1347	1580	2686	0.78
PSQ	1356	1586	-	0.85
E-PSQ	1351	1585	-	0.85

4.5.2. Raman analysis of PSQ

The results of a Raman analysis of PSQ and E-PSQ are indicated in **Figure 4.11 (a and b).** The intensity ratio of the D-band to G-band for both PSQ and E-PSQ polymers were found to be 0.85 for both polymeric materials. The peak at 560 cm^{-1} serves as an indication that during the end-capping of PSQ with hexamethyldisilazane (HMDS), the HMDS was able to react with the OH–(hydroxyl group) from Si-OH. The reaction resulted in the formation of methyl siloxy (Si-C) group, which can produce SiO_2 at high temperatures. [35,42]. However, the structure of the PSQ was not transformed or destroyed by incorporation of the E polymer, hence there is no change in the sp^3 hybridized carbon and the I_D/I_G intensity ratio.

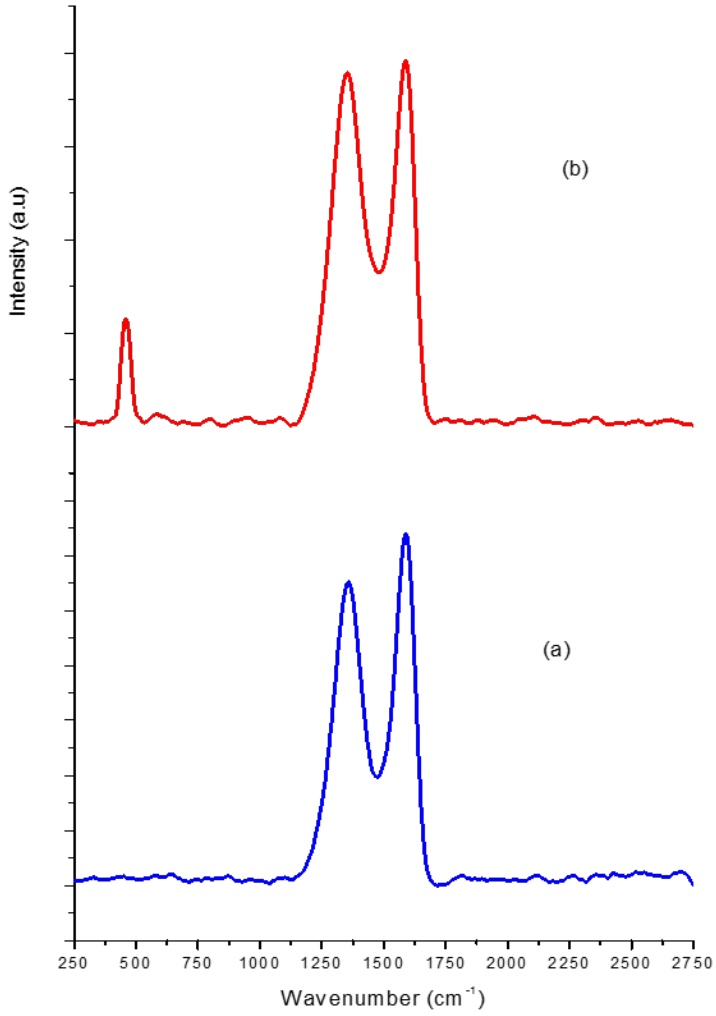


Figure 4.11: Raman spectra for (a) PSQ (b) E-PSQ

4.6. THERMOGRAVIMETRIC ANALYSIS (TGA)

The PS-DVB, PSQ and E-PSQ TGA were obtained as described in section 3.6.5, whose theory is also described in the section.

4.6.1. TGA analysis of PS-DVB

The TGA measurements of the polymeric materials were carried out to confirm the stability of the materials over high temperatures.[31,33,34,40] For the three PS-DVB materials, **Fig. 4.12b** shows that there was about 5 % mass loss due to evaporation at 0-100 °C for PS-DVB (1.5:1). About 20 % mass loss due to evaporation 0-200 °C was recorded for PS-

DVB (1:1) (**Fig. 4.12a**) and about 25 % mass loss due to evaporation at 0-150 °C for PS-DVB (10:1) was recorded (**Fig. 4.12c**). The weight loss of about 70 % was observed at 300-450 °C for the PS-DVB 1.5:1, about 60 weight loss from 150-450 °C for PS-DVB (1:1) and about 65 % weight loss from 100-450 °C for PS-DVB (1:1). The most stable PS-DVB was found to be the PS-

DVB (1.5:1). Since weight loss over evaporation was the lowest and the overall weight loss was observed from higher temperatures of 200 °C.

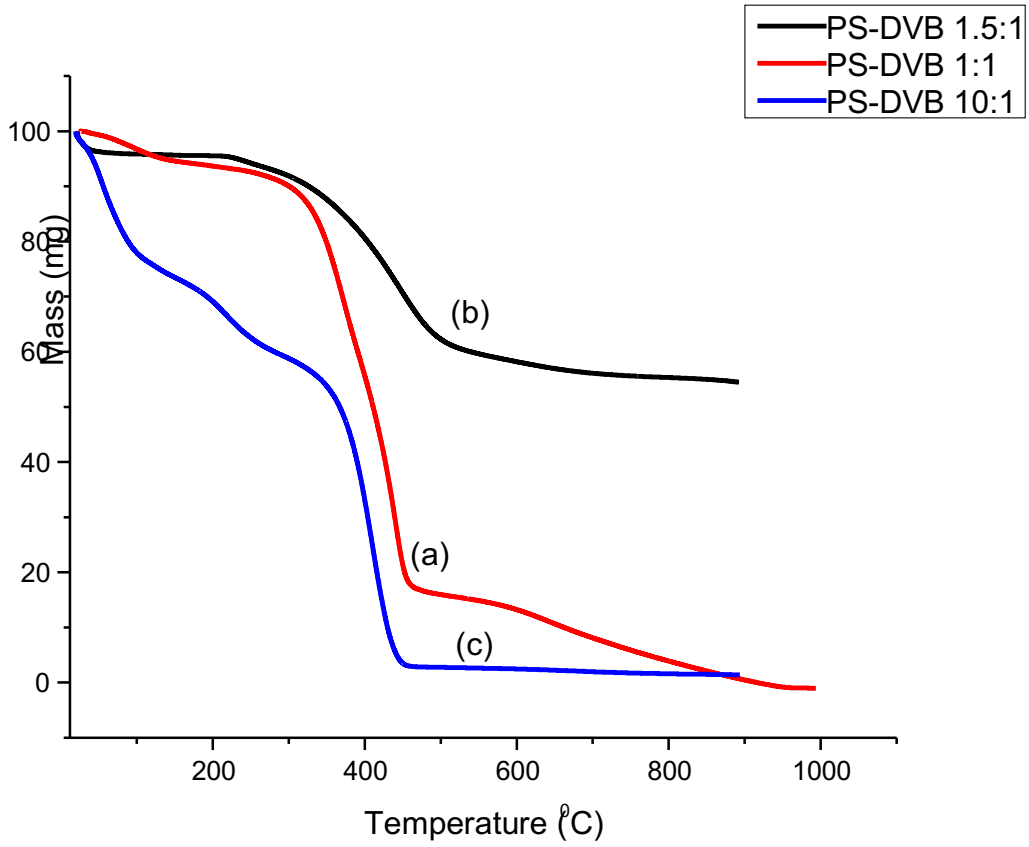


Figure 4.12: TGA graphs for PS-DVB (a) 1:1, (b) 1.5:1 and (c) 10:1

4.6.2. TGA analysis of PSQ and E-PSQ

The TGA measurements of the silica polymeric materials was undertaken to study the thermal stability properties of the materials.[31,40] A 5 % mass loss due to evaporation of moisture at 0-50 °C was observed for PSQ (1.5:1) (see **Fig. 4.13a**); a 10 % mass loss due

to evaporation 0-100 °C was observed for E-PSQ (**Fig. 4.13b**). The weight loss of about 40 % was observed at 50-500 °C for the PSQ and about 15 % weight loss from 100-250 °C for E-PSQ. The addition of the HMDS (end-capping) material decreased the stability of the material against high temperatures because the material showed a constant continuation of mass degradation from 250-900 °C.

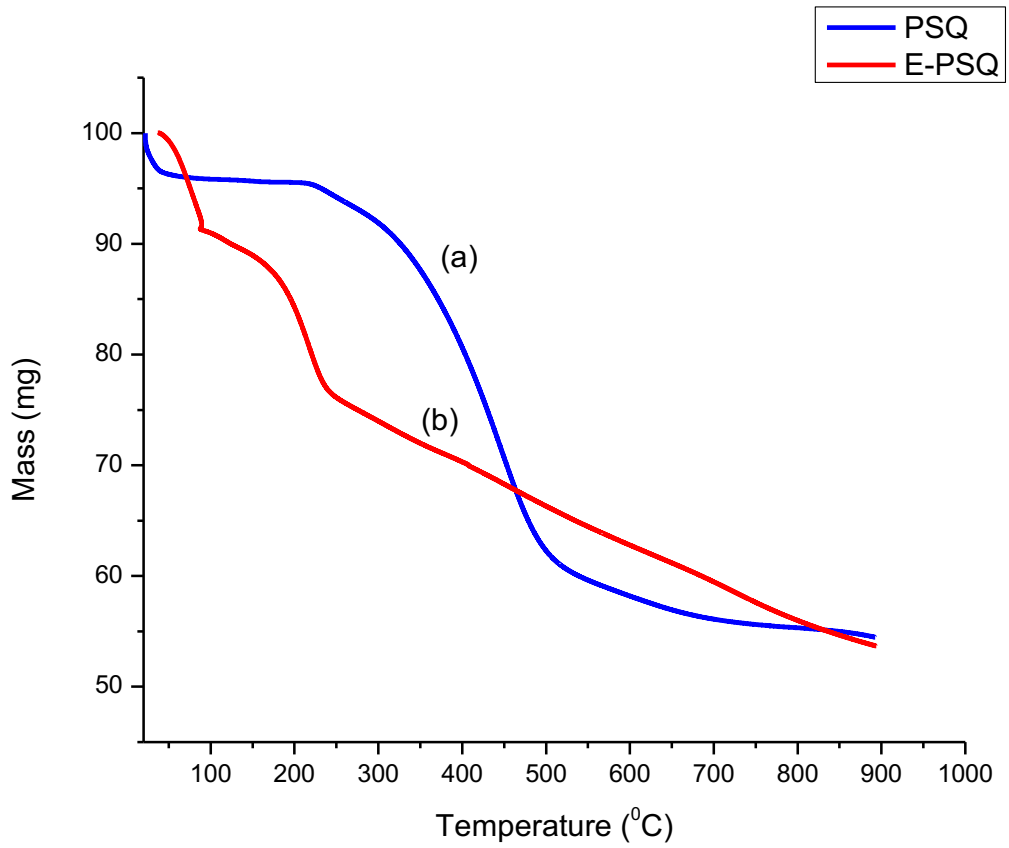


Figure 4.13: TGA graphs for PSQ (a) and E-PSQ (b)

4.7. BRUNAUER–EMMETT–TELLER (BET)

The PS-DVB, PSQ and E-PSQ BET results were obtained as described in section 3.6.6, whose theory is also described in the section.

The BET results from **Table 4.3**, showed that the average pore sizes and surface areas of PS-DVB particles were 18.7 and 2.9 nm, 0.035 and 84.67 m^2/g for 1.5: 1 and 1:1, respectively. The average pore volumes were 0.000391, 0.003609 and 0.062123 cm^3/g for PS-DVB 1.5:1, 1:1 and 10:1, respectively. No pore size and surface area was recorded for

PS-DVB 10:1 because it was not stable under instrument temperatures. The pore size, surface area and pore sizes of PS-DVB 1.5:1 were found to be better and more suitable for SEC/GPC as compared to PS-DVB 1:1 and 1:1. There is a decrease in the pore size from 6.69 to 2.9 nm as the PSQ material was end-capped. An increase in both the surface area and pore volume was observed as the PSQ is end-capped (E-PSQ). The pore volume increased from 0.001744 (PSQ) to 0.763882 cm^3/g (E-PSQ) and surface area increased from 1.0414 (PSQ) to 1038.02 m^2/g (E-PSQ). Due to the increased in pore volume and the pore surface area of the E-PSQ as compared to the PSQ, the end-capped PSQ (E-PSQ) material is more suitable for SEC/GPC as compared to the PSQ.

Table 4.3: BET results all polymeric materials

Sample name	Surface area(m^2/g)	Pore size (nm)	Pore Volume (cm^3/g)
PS-DVB (1.5:1)	0.0835	18.72692	0.000391
PSQ	1.0414	6.69712	0.001744
PS-DVB (10:1)	Not available	Not available	0.003609
PS-DVB 1:1	84.6761	2.93462	0.062123
E-PSQ	1038.0202	2.94361	0.763882

4.8. CONCLUSION

Two polymeric materials namely, polysilsequioxane (PSQ) and poly (styrene-divinyl benzene) (PS-DVB), were identified as potential stationary phases for the fractionation of NOM. Although less rigid than PSQ, PS-DVB tolerates wide ranges of pH and polarity. In contrast, PSQ can escalate alkaline resistance but cannot tolerate a wide range of pH. In this study, it was envisaged that the two polymeric materials (PSQ and PS-DVB) will be blended and subsequently used as a hybrid PSQ-PS-DVB material.

The hybrid material should ideally be monosized (>2 μm), macroporous (0.005-100 μm) and equally dispersed on a SEC column, which will be used for the fractionation of NOM.

Successful blending of the S and DVB monomer to PS-DVB as well as the successful end-capping of PSQ to E-PSQ was confirmed by the FTIR spectroscopy. SEM analysis of PS-

DVB (1:1, 1.5:1 and 10:1), PSQ and E-PSQ material showed the porous nature of all polymeric. The EDS of PS-DVB polymeric materials show dominant elements in all as carbon and oxygen. Both the XRD and Raman Spectroscopy proved that the PS-DVB, PSQ and E-PSQ are amorphous in nature, The TGA results showed that the PSQ and E-PSQ were more stable to high temperature than the all the PS-DVB. The materials decomposed at temperatures higher than 50 °C, which is higher than the oven temperature of the HPLC which will be used for this study. Pore sizes and volumes for PS-DVB were 2-18 nm and 0.0003-0.06 cm^3/g, respectively and for PSQ the pore sizes were 2-6 nm and pore volumes of 0.001-0.7 cm^3/g. The FTIR, SEM, XRD, Raman and BET show that the E-PSQ and PS-DVB (1.5:1) were the most stable and suitable polymeric materials to be chosen as SEC stationary phases.

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CHAPTER 5:
APPLICATION OF POLYSILSESQUIOXANE AND POLY (STYRENE-DIVINYL BENZENE). COMPOSITE MATERIAL AS SOLID –PHASE EXTRACTION (SPE) AND GEL PERAMEATION (GPC) STATIONARY PHASES

This chapter provides the details of the application of a composite of polysilsesquioxane (E-PSQ) and poly (styrene-divinyl benzene) (PS-DVB) as solid-phase extraction (SPE) and also as gel permeation chromatography (GPC) stationary phases. The optimal ratio of the two polymeric materials was obtained through optimizing experiments performed by packing the materials at various ratios (w/w) on empty SPE cartridges and eluting a known compound then measuring the efficiency in terms of recoveries. Thereafter the polymeric material with the optimized ratio was packed on an empty column of SEC/GPC. The experimental procedures followed in this sections were taken from Section 3.6-3.8 of the dissertation.

5.1. TESTING OF THE EFFICIENCY OF E-PSQ/PS-DVB USING COMPOSITE ONTO SOLID PHASE EXTRACTION (SPE) CARTRIDGES

5.1.1. Sorbent quantity optimization

To determine the optimal ratio of the two polymeric materials (PS-DVB and E-PSQ) as GPC stationary phases, empty SPE cartridges were used to pack the materials at different ratios. Samples of humic acid (HA) were prepared at concentrations of 1, 3, 5 and 10 mg/L to represent NOM. Humic acid forms part of the humic substances (HSs) of NOM, which are known to be a major part of NOM as HSs constitutes about 70% of the total organic carbon (TOC).[1,2] Since the concentration of NOM can be measured as the TOC, the TOC of HAs were investigated using the TOC (Teledyne Tekmar TOC Fusion, USA) before (see **Table 5.1)** and after elution from all the eight hand-packed SPE (see**Table 5.2)**.

5.1.2. Total organic carbon measurements

The results of TOC measurements before and after elution with SPE are presented in **Tables 5.1** and **5.2**, respectively.

Table 5.1: TOC values for all samples before SPE elution

Aliquot	TOC measured (mg/L)
HA-1	0.95
HA-2	3.04
HA-3	5.3
HA-4	10.36

Table 5.2: TOC values for samples after SPE elution

E-PSQ/PS-DVB ratio	HA-1 (TOC) at 1 mg/L	HA-2 (TOC) at 3 mg/L	HA-3 (TOC) at 5 mg/L	HA-4 (TOC) at 10 mg/L
1. PS-DVB:E-PSQ (4:1)	7.49	16.32	25.31	16.30
2. PS-DVB:E-PSQ (1:4)	22.76	13.58	21.51	21.90
3. E-PSQ:PS-DVB (1:4)	27.15	23.22	15.26	15.32
4. E-PSQ:PS-DVB (4:1)	11.82	7.35	18.06	26.85
5. PS-DVB:E-PSQ (1:1)	12.00	0.00	20.27	22.89
6. E-PSQ:PS-DVB (1:1)	0.00	18.80	16.30	24.01
7. E-PSQ	25.92	12.58	10.19	6.19
8. PS-DVB	6.19	24.40	17.18	14.48
9. Carbon	16.62	24.99	26.09	28.08
10. C 18	9.80	10.24	9.55	12.92

The **Table 5.1** above reports the TOC concentration of HA before elution through the packed SPE cartridges. From the results presented in **Tables 5.2**, there was either an increase or decrease of the TOC value in each cartridge. The composition of the SPE stationary phases (either PS-DVB:E-PSQ or E-PSQ:PS-DVB) were chosen as to observe

the best sorbent quantity for the fractionation of NOM. The E-PSQ: PS-DVB ratio of 4:1 showed an increase in the TOC values after fractionation as compared to the initial TOC values (**Table 5.1**). This was attributed to organic carbon leaching from the stationary phase materials. However, the 3 mg/L sample of HA showed the least increase TOC value of 7.35 mg/L as compared to other values, which were above 9 mg/L; this means the organic carbon leaching was minimum.

Both the 0.5:0.5 PS-DVB: E-PSQ and E-PSQ: PS-DVB ratio registered organic carbon leaching and retention by the stationary phases since there was a decrease in TOC up to 0.00 mg/L and an increase of TOC up to a concentration of 24 mg/L. When 3 mg/L of humic acid (HA) was eluted through the PS-DVB: E-PSQ ratio of 1:1 the stationary phase retained all the organic carbon such that no organic carbon was detected by the instrument (see **Table 5.2**). The same trend was observed with the 1 mg/L HA, which was retained by the E-PSQ: PS-DVB (1:1) stationary phase. The carbon cartridge showed the maximum leaching, while the C-18 cartridge overall had the least carbon leaching. The C-18 and carbon cartridges were commercially bought while all other cartridges were hand-packed. Organic carbon leaching from hand-packed cartridges is due to inconsistency of the hand-packing process. The organic carbon leaching from the carbon and C-18 cartridge is due to excess of organic carbon available as the stationary phase.

From data depicted in **Table 5.2**, it is clear that both the sorbent ratios, PS-DVB: E-PSQ ratio (1:1, w/w) and the E-PSQ: PS-DVB (1:1, w/w)) had the lowest TOC leaching as compared to other composition of PS-DVB: E-PSQ and E-PSQ: PS-DVB as well as commercial carbon and C18 SPE cartridges. However, the E-PSQ: PS-DVB (1:1, w/w) proved to have lesser TOC leaching than the PS-DVB: E-PSQ ratio (1:1, w/w) and was selected (as the best sorbent quantity) for the GPC column packings.

5.1.3. Fluorescence excitation emission matrices (FEEM) analysis

The eluents from the ratio selected was then further characterized using the Fluorescence excitation emission matrices (FEEM) in order to confirm the organic leaching and to further identify the NOM types remaining from humic acid (HA) eluents. The FEEM method normally provides information about the types of NOM present in different fractions.[3] This method classifies NOM by giving a unique absorption and excitation

pattern of NOM at a specific region.[4] The regions of interest in this sections are the fulvic-like (Ex 325 nm, Em 425 nm), humic-like (Ex 350 nm, Em 475 nm) and the tryptophan-like (225 nm ≤ Ex≤ 450 nm, Em 450) acids.[5]

The NOM that was selected is known to be a hydrophobic NOM.[3,6–9] The FEEM results shown below report the type of NOM to be humic-like (**Fig. 5.1a**) and tryptophan-like (**Fig. 5.1b-d**). The FEEM results below show the leaching of the TOC from the selected E-PSQ: PS-DVB (1:1, w/w), affected the nature and morphology of NOM. The hand-packing SPE procedure was inaccurate, since the pressure was not controlled and flow of solvents was not constant, during the fractionation/separation of HA. The vacuum pump had pressure adjustable knob, hence non-constant pressure.

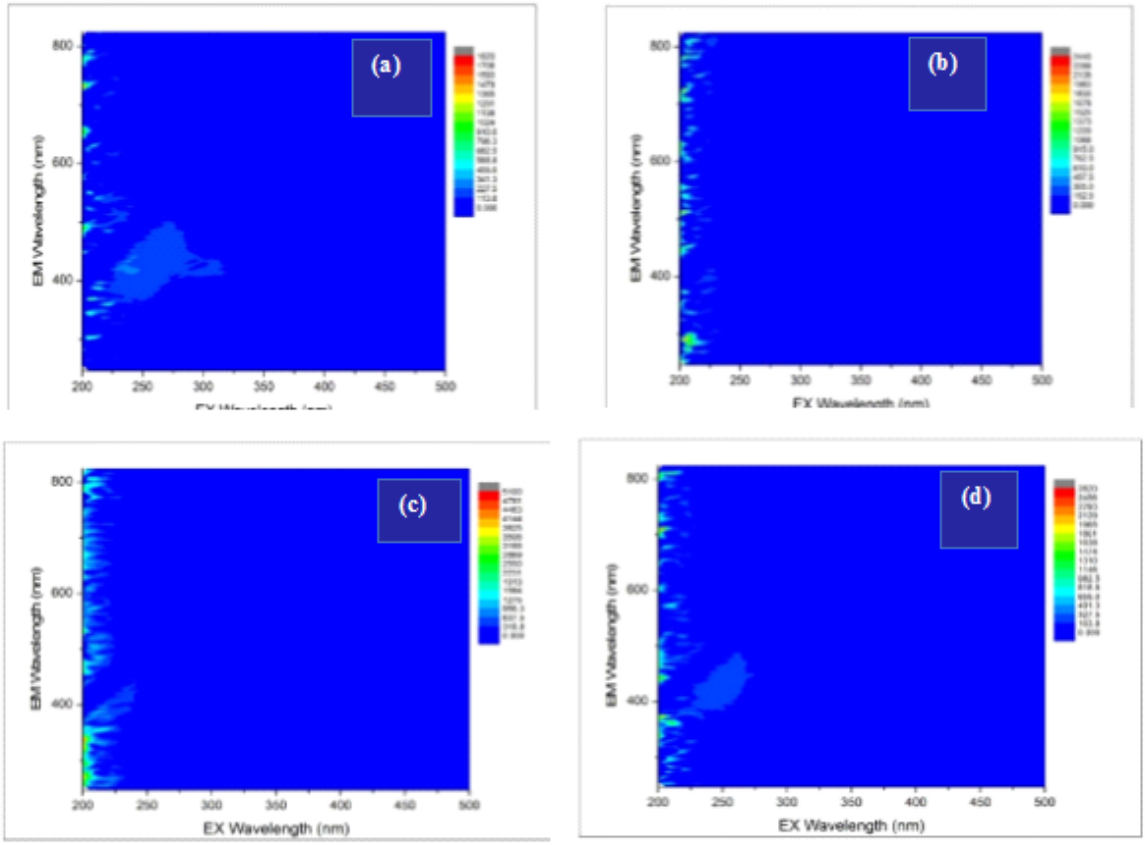


Figure 5.1: FEEM graphs for HA eluents from 1 (a), 3 (b), 5 (d) and 10 mg/L (c)

5.1.4. Ultraviolet-visible (UV-VIS) and specific ultraviolet absorbance (SUVA) analysis of samples

In order to identify the nature of NOM in each samples, both SUVA and UV analysis were carried out. UV_{254} absorbance measures the presence of humic substances, while SUVA values of >2, 2-4, >4 correspond to the hydrophilic, transphilic and hydrophobic part of NOM, respectively.

Table 5.3 summarizes the UV-Vis results obtained from HA eluents from cartridge 5 at different concentrations. The SUVA calculations were carried out using the equation 2.1 in order assess the concentrations of the hydrophilic, hydrophobic and transphilic fraction of NOM.[10]

Table 5.3: UV-VIS and SUVA values of HA eluents from cartridge 5

PSQ/PS-DVB ratio (1:1) 2 g	UV-Vis (cm^{-1})	SUVA $cm^{-1}/mg.L^{-1}$
Cartridge 5 at 1 mg/L	2.57	17
Cartridge 5 at 3 mg/L	0.148	0
Cartridge 5 at 5 mg/L	2.322	11
Cartridge 5 at 10 mg/L	2.886	12

The SUVA provides information on the specification of humic substances versus the non-humic substances of NOM.[11,12] SUVA can also be used as a tool to determine the nature of the organics found in NOM samples with regards to the aromaticity and conjugated C=C bonds.[13,14] The SUVA values obtained from **Table 5.3** are relatively high as compared to SUVA values obtained from the literature, thus indicating high aromaticity associated with the hydrophobic NOM fraction.[14,15] The information in **Table 5.3** indicate that the samples are rich in humic substances; these are actually the hydrophobic fraction of NOM since the SUVA value is above 4.[5,14]

5.2. THE EFFICIENCE OF E-PSQ/PS-DVB AS STATIONARY PHASE FOR GEL PERMEATION CHROMATOGRAPHY (SEC/GPC)

Packing the SEC/GPC column was achieved with constant pressure (0-500 sand flow-rate of 0.2 mL/min). To test for the efficiency of the column, various performance tests on the packed columns were performed.

5.2.1. Packed column performance tests

5.2.1.1. Interactions with acidic compounds

(a) Activity toward acids

The packed SEC/GPC column was tested against 4-chlorocinnamic acid (**Figure 5.2**). The column was able to elute a peak of the analyte at about 10 mins. This was achieved using a mobile phase of ratio 30:70 (methanol/ aqueous 0.02 M phosphate buffer of pH 2.7),[16] flow rate of 0.25 mL/min, sample volume of 5 µL, column temperature of 40 °C, elution time of 15 mins, and wavelength of 254 nm. In comparison with data from the literature,[17,18] the test results imply that it can be used for the acidic analytes such as those forming NOM composition (humic acids, fulvic acids). The band broadening observed in the results, is due to the Eddy diffusion, the longitudinal diffusion and the mass transfer which are all expressed in the Van Deemter Equation (5.1).[19–21] The packed SEC column consist of particles of different sizes and pore-sizes which lead to analytes taking different routes hence promoting brand broadening due to Eddy diffusion.[21,22] Although, the HPLC column was narrower, it is important to note that the flow rate which was selected (suitable for the packed-column) was very low(0.25 mL/min) this lead to the longitudinal diffusion.[21] As the analytes diffused through the pores of the stationary phase some analytes penetrated through the pores, some did not penetrate, some penetrated deeply than the others this lead to mass transfer of analytes.[21,22]

$$HETP = A + B/u + Cu \quad (5.1)$$

Where A= Eddy diffusion

B= Longitudinal diffusion

C= Mass transfer of analytes due to different pore sizes

It is important to note that similar band broadening effects are observed in all our results since the same packed column was used throughout the study.

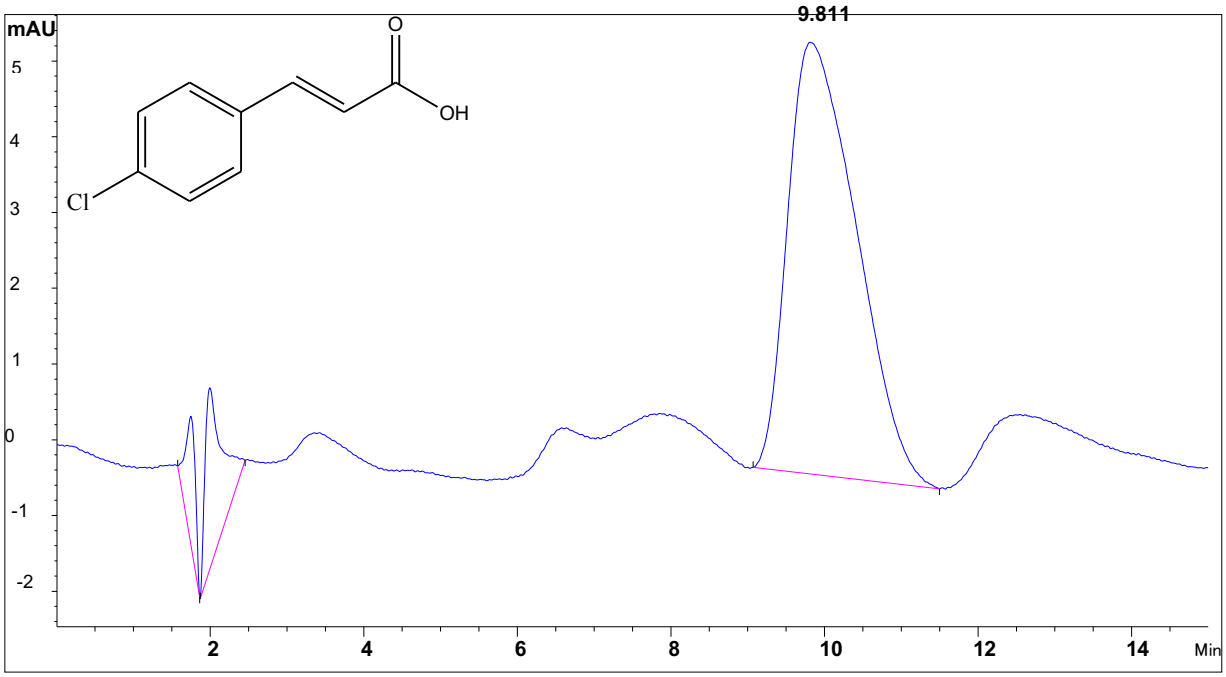


Figure 5.2: Chromatogram of 4-chlorocinnamic acid using E-PSQ/PS-DVB (0.5:0.5; w/w) as stationary phase

(b) Tanaka test (Acidic ion exchange capacity)

This test measured acidic (H^+) activity of the silanol groups present on the stationary phase.[23] The concentration used for the analytes was 5 mg/mL. The retention factor of protonated silanol (SiO^-) were estimated by the selectivity factor (equation 2.2) between phenol and benzyl amine. The benzyl amine was retained in the column and was eluted at around 7 mins while the phenol was eluted at around 2.5 mins (**Fig. 5.3**). The selectivity factor of the two analytes was found to be 9 (higher than C18 and lower than Zr-PBD), which is an acceptable value since the retention factors of commercial column such as Discovery C18, Discovery Zr-PBD, ACE phenyl, ACE AQ and Discovery F5 were reported to be 0.0672, 23.288, 0.14, 0.11 and 0.34 , respectively.[23,24] This high value of the selectivity factor of the two analytes indicates that the column has minimum silanol groups. The compounds were eluted in the column by using a mobile phase of ratio 30:70 (methanol/aqueous 0.02 M phosphate at pH 2.7),[16] with the flow rate of 0.25 ml/min, sample volume of 5 µL, column temperature of 40 °C, elution time of 15 min, and wavelength of 254 nm.

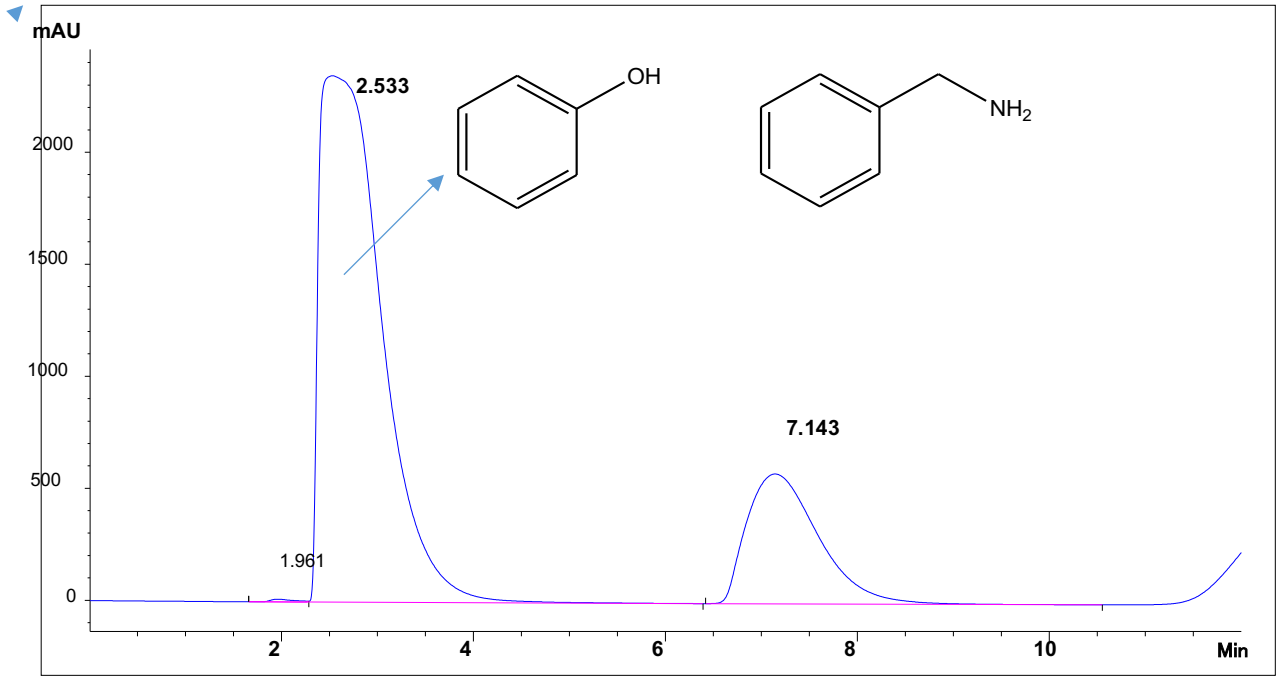


Figure 5.3: Chromatogram of phenol and benzyl amine using E-PSQ/PS-DVB (0.5:0.5; w/w) as stationary phase

5.2.1.2. Hydrophobic Interactions

(a) Hydrophobic retention (HR)

This test reveals the surface area and surface coverage of the stationary phase with the aid of calculating the retention factor pentyl benzene (K_{PB}).[23] This parameter was evaluated by eluting pentyl benzene in a column packed with the PS-DVB: E-PSQ. The K_{PB} value was calculated using the equation 2.2. The retention factor of analyte was found to be 0.87, different from the literature values for Discovery C18, Discovery Zr-PBD, ACE phenyl, ACE AQ and Discovery F5 are quoted as 3.19, 0.86, 1.20, 2.30 and 1.70, respectively.[23,24] The broad peak that resulted is due to all the variables of the Van Deemter equation. The results obtained show that the strength of the stationary phase is different to that of the commercial columns (**see Fig. 5.4**), this may be caused by the mixture of two stationary phases (E-PSQ: PS-DVB). The experimental conditions are as follows: Water/methanol (20; 80 v/v) was used as mobile phase;[25,26] flow rate of 0.25 mL/min; sample volume of 5 µL; column temperature of 40 °C; elution time of 10 mins; and wavelength of 254 nm.

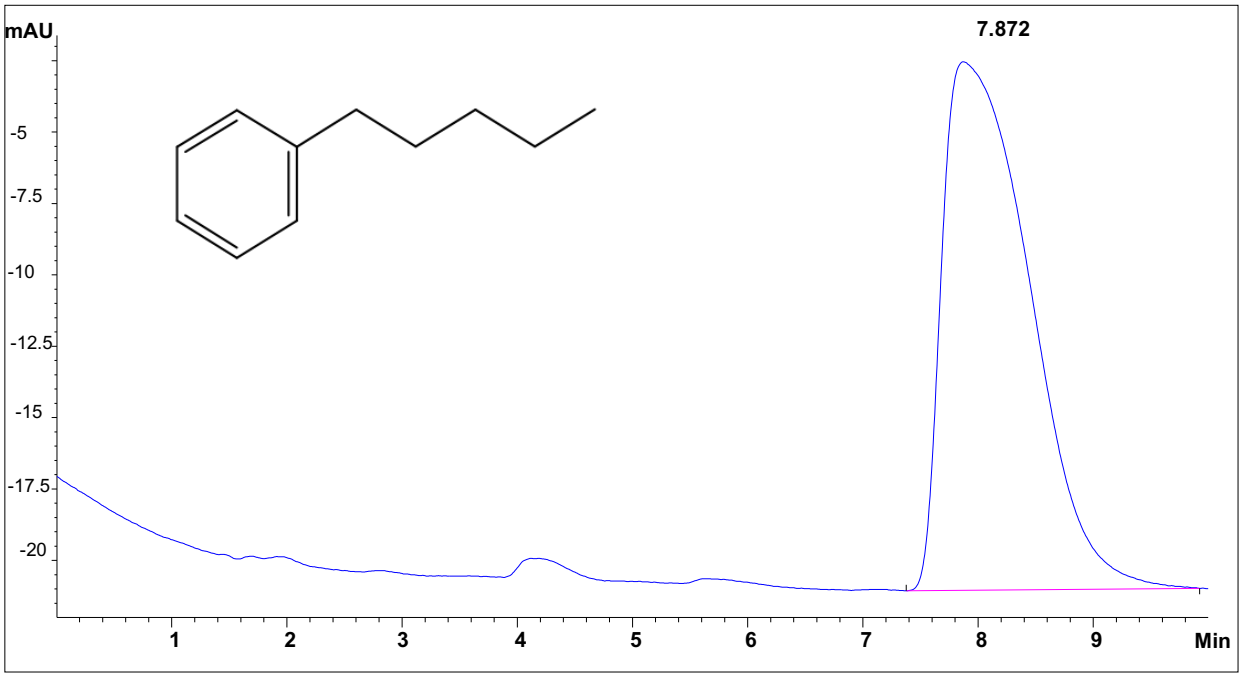


Figure 5.4: Chromatogram of pentyl benzene using E-PSQ/PS-DVB (0.5:0.5; w/w) as stationary phase

(b) Hydrophobic selectivity (HS)

This test measured the retention factor ratio between the pentylbenzene (PB) and butylbenzene(BB); the retention factor is calculated as : αCH2 = k_{PB}/k_{BB}.[23] This test measures the surface coverage of the stationary phase as it can separate benzenes attached to different chains of alkyl group.[23] The separation is shown in **Fig. 5.6**. The pentyl benzene and butyl benzene were eluted through the column with 80:20 (v/v) methanol/water mobile phase,[16] flow rate of 0.25 ml/min, sample volume of 5 µL, column temperature of 40 °C, elution time of 10 mins, and wavelength of 254 cm^{-1} and using the DAD detector. The first peak around 5.6 mins is associated with the pentyl benzene and the second peak around 7.9 mins concurs with butyl benzene (see **Fig. 5.5**). The retention factor was found to be 0.574. This value is lower while for Discovery C18, Discovery Zr-PBD, ACE phenyl, ACE AQ and Discovery F5 was found to be higher, 1.406, 1.423, 1.26, 1.35 and 1.26 respectively.[23,24] The elution sequence of the two molecules, as well as the retention factor ratio obtained (was is lower than most columns found in literature). The peaks on the chromatogram prove that the column can selectively elute molecules based on their different masses.

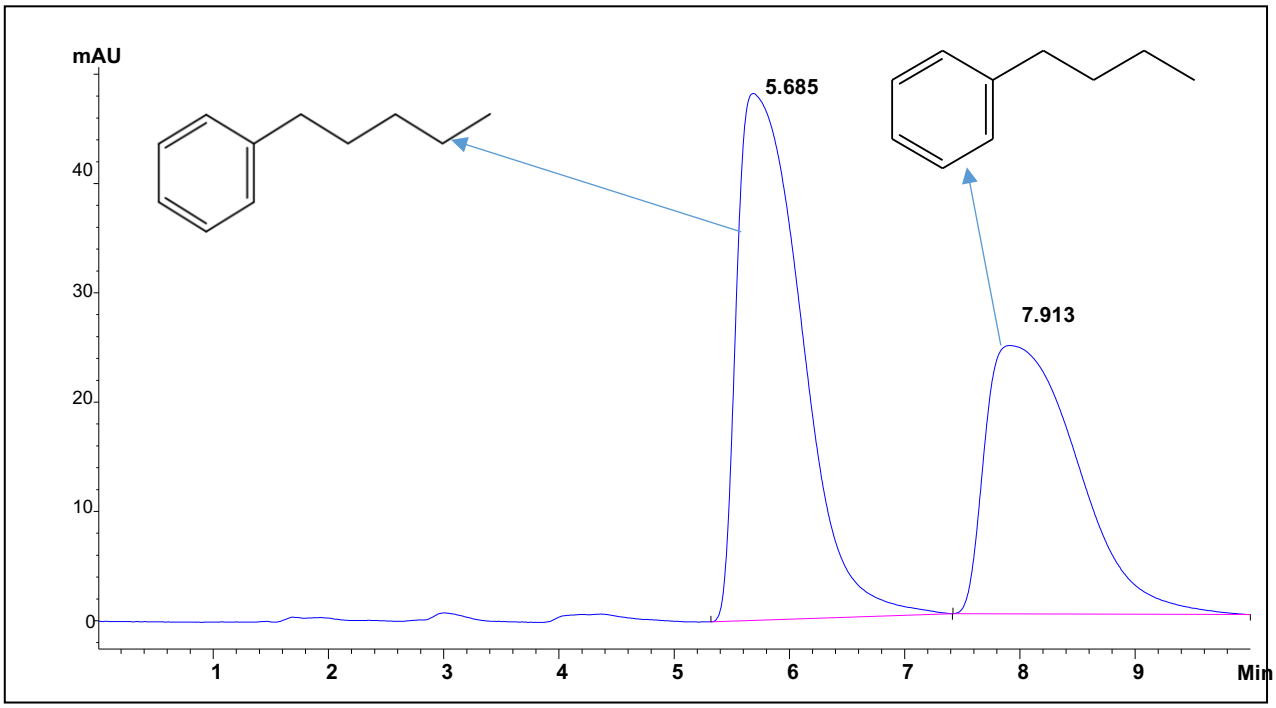


Figure 5.5: Chromatogram of pentyl benzene and butyl benzene

(c) Steric selectivity (SS)

This test measured retention factor ratio between triphenylene (T) and o-terphenyl (O), (α T/O = k_T/k_O).[23] Steric selectivity measures the ability of the stationary phase to distinguish between molecules with similar hydrophobicity and structure but different shapes. The steric selectivity was successful since the elution times of o-terphenyl and triphenylene were found to bet 6.6 mins and 10.1 mins, respectively (**Fig. 5.6**). The retention factor ratio value was found to be 0.61, which is below the literature values of 1.474, 1.634, 1.00, 1.22 and 2.55 for Discovery C18, Discovery Zr-PBD, ACE phenyl, ACE AQ and Discovery F5, respectively.[23,24] The two compounds were eluted through the column using a mobile phase with 80:20 (v/v) methanol/water,[16] flow rate of 0.25 ml/min, sample volume of 5 µL, column temperature of 40 °C, elution time of 10 mins, wavelength of 254 and the DAD detector. The chromatogram below (**Fig. 5.6) s**hows two peaks which correspond to the two analytes, therefore the packed GPC column can separate the two analytes.

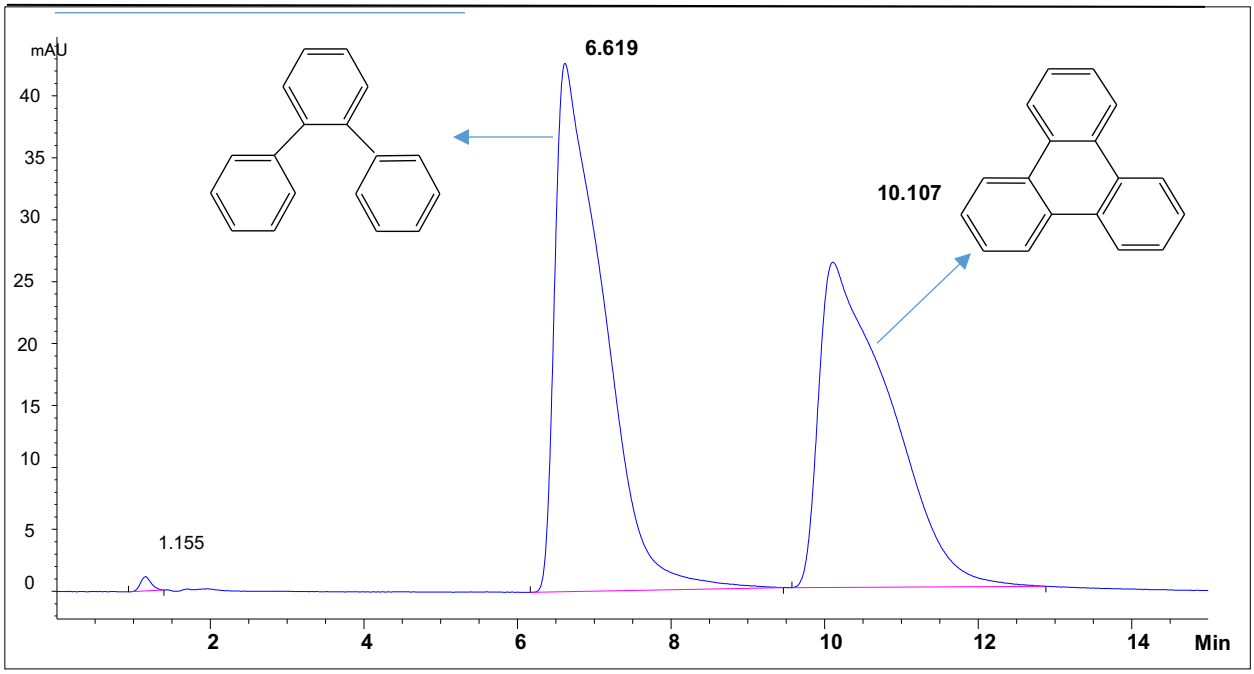


Figure 5.6: Chromatogram of O-terphenyl and Triphenylene

(d) Hydrogen bonding capacity (HBC)

This technique measured the number of free silanol groups and the degree of end capping by comparing the relative retention of caffeine with respect to phenol since the two analytes can easily donate a proton (H^+) through hydrogen bondings with other compounds and to calculate the retention factor ratio between caffeine (C) and phenol (P) ($\alpha C/P = k_C/k_P$) which will confirm hydrogen bonding capacity of the column.[23] The obtained retention factor ratio of 0.43 is comparable to the values of commercial columns such as Discovery C18, Discovery Zr-PBD, ACE phenyl, ACE AQ and Discovery F5 which are 0.615, 0.307, 1.14, 0.48 and 0.68, respectively.[23,24] The results in **Figure 5.7** show that there are minimum silanol groups and the end-capping was successful on the E-PSQ. Methanol and water (30:70 v/v) were used as mobile phase,[16] flow rate of 0.25 ml/min, sample volume of 5 µL, column temperature of 40 °C, elution time of 10 mins, wavelength of 254 and the DAD detector.

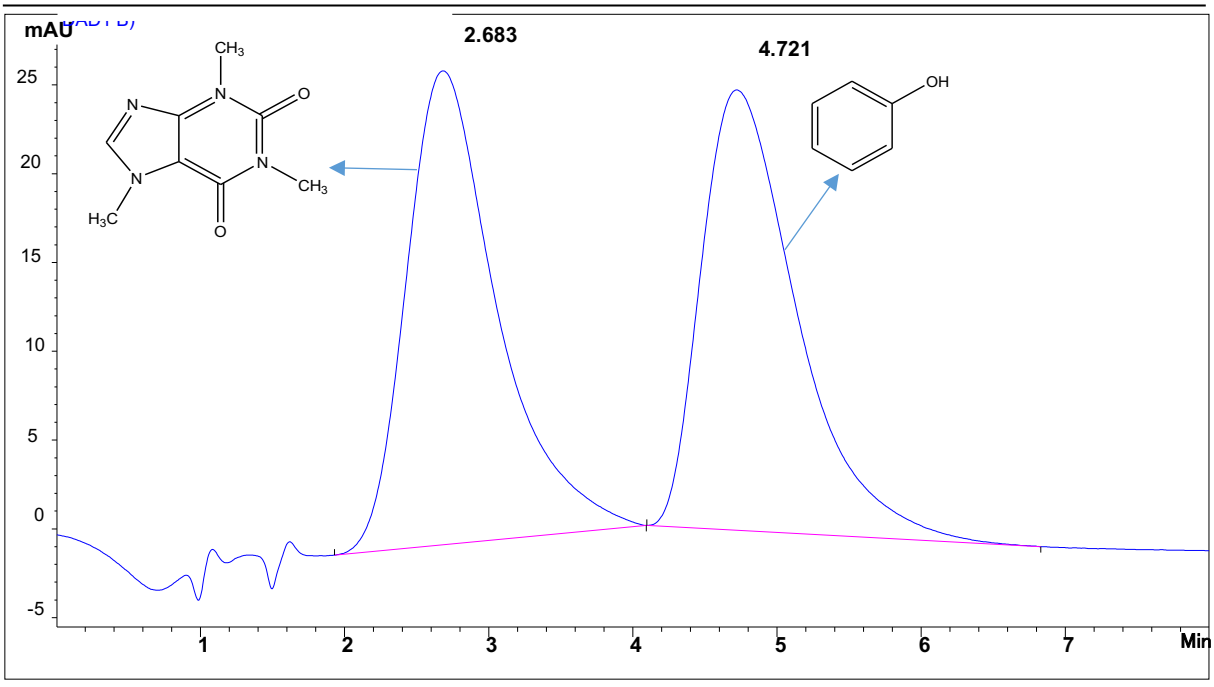


Figure 5.7: Chromatogram of caffeine and phenol

5.2.1.3. Stability at high pH values

The stability of the stationary phase at basic pH ranges using amitriptyline as an analyte (see **Fig. 5.8**) was eluted with a mobile phase of 30:70 methanol/aqueous 0.02 M phosphate at pH 7.6,[16] flow rate of 0.25 ml/min, sample volume of 5 µL, column

temperature of 40 °C, elution time of 10 mins, wavelength of 254 and the DAD detector. The values of the capacity factor (equation 2.4) and tailing factor (equation 5.2) were evaluated. The capacity and tailing factor of amitriptyline was measured to be 0.22 and 0.65 respectively, indicating that the column can tolerate high pH values.[17] The other peaks on the chromatogram were due impurities (other chemical species) which came with the amitriptyline as the compound was purchased with 100 % purity.

$$T_f = \frac{AB}{2AC} \quad \text{..... (5.2)}$$

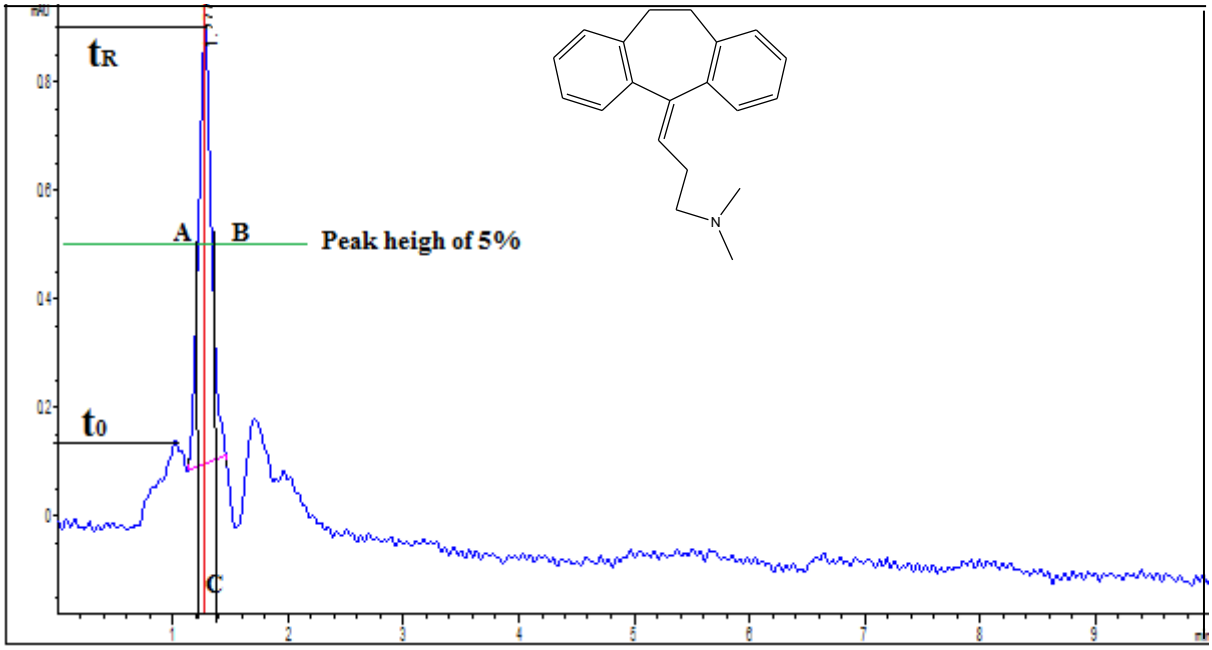


Figure 5.8: Chromatogram of Amitriptyline

5.2.1.4 Ion exchange capacity

This parameter was investigated by eluting a mixture of benzyl amine and phenol. This test estimates the total silanol activity by the retention factor of the two analytes at pH 5.6.[23] The two compounds were eluted with a mobile phase of 70:30 aqueous 0.02 M phosphate/methanol at pH 7. 6,[16] flow rate of 0.25 ml/min, sample volume of 5 µL, column temperature of 40 °C, elution time of 10 mins, wavelength of 254 and the DAD detector. The magnitude of the selectivity factor between benzyl amine and phenol was found to be 4 (see **Fig. 5.9**) when compared to Discovery C18, Discovery Zr-PBD, ACE phenyl, ACE

AQ and Discovery F5 value of 0.684, 24.309, 0.46, 0.32 and 0.85, respectively.[23,24] The results were very far from the commercial column, therefore, there might be some silanol activity on the surface of the prepared stationary phase.

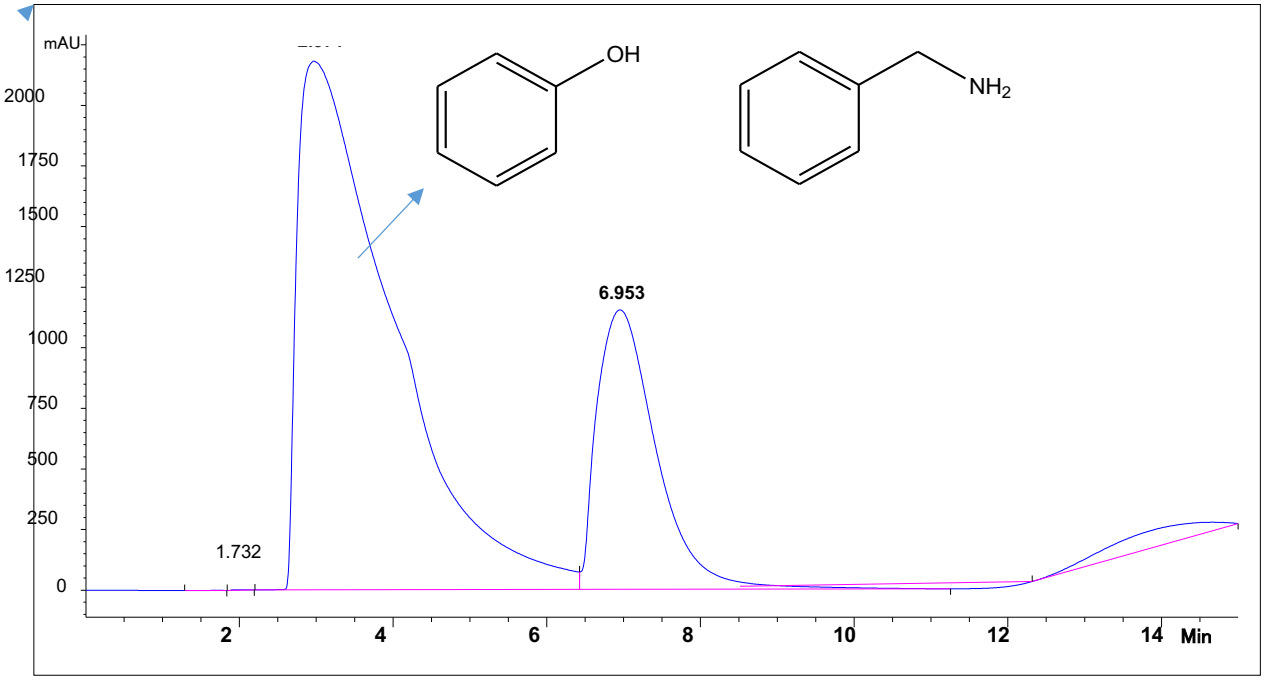


Figure 5.9: Chromatogram of benzyl amine and phenol

5.2.2. Fractionation of prepared and real NOM samples with the packed E-PSQ/PS-DVB GPC column

The packed column was then connected to a HPLC system to fractionate/separate the fulvic acid (FA), humic acid (HA) and real samples according to molecular weight. The SEC/GPC method is a popular method for separation of molecules according to different molecular weights.[13] The molecular weight separation of NOM was achieved by the elution of NOM through a porous stationary phase; smaller molecules took a longer time to elute because they passed through the pores of the stationary phase packed-particles. Large molecules cannot to diffuse through the pores so elute more quickly.[13] The pure standards of HA of concentrations 1, 3 and 5 mg/L and FA concentrations of 1 and 5 mg/L were eluted separately to test for the reproducibility of the elution time for both FA and HA.

The elution of FA at 1 mg/L (**Fig. 5.10a**) and FA at 3 mg/L (**Fig. 5.10b**) were found to be 1.286 mins and 1.281 mins, respectively; these elution times correspond to those of the

humic substances reported in literature [13,27,28]. It is important to note that the analytes (humic and fulvic acid) were not 100 % pure, they were purchased with impurities. Hence there is more than one peak for each analyte.

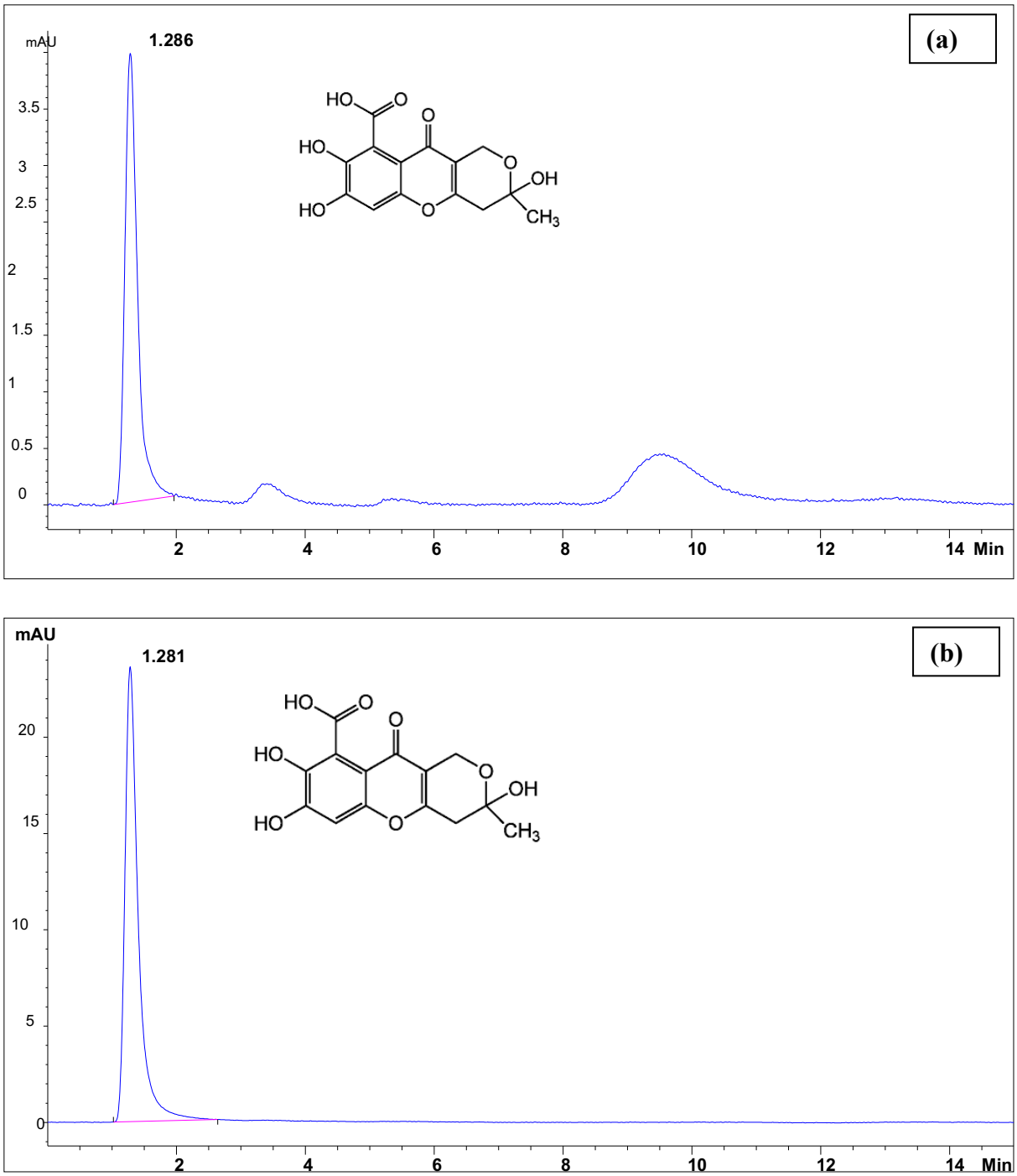
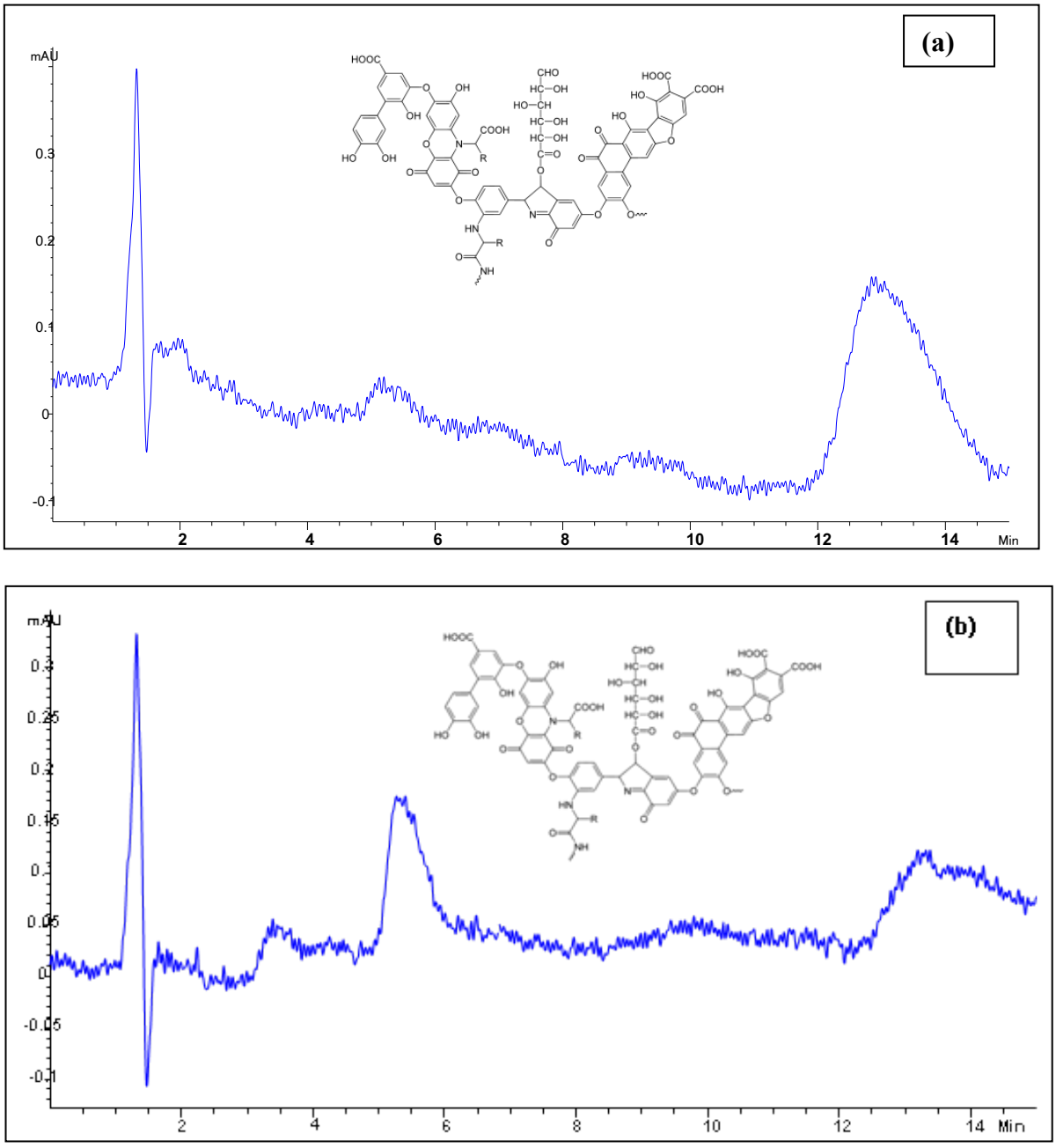


Figure 5.10: Chromatogram of FA at (a) 1 mg/L and (b) 5 mg/L

The separation of HA using the packed column showed a peak at around 1, 5 and 14 mins for all three concentrations at 1 mg/L (**Fig. 5.11a**), HA at 3 mg/L , (**Fig. 5.11b**) and HA at

5 mg/L (**Fig. 5.12c**). There are three peaks because the HA that was used contains 20% residues of ash, most HA peaks were reported to be around 4.91 and 10 mins depending on the eluent type and the source of the HA.[29,30] These three peaks will be taken as reference peaks for HA.



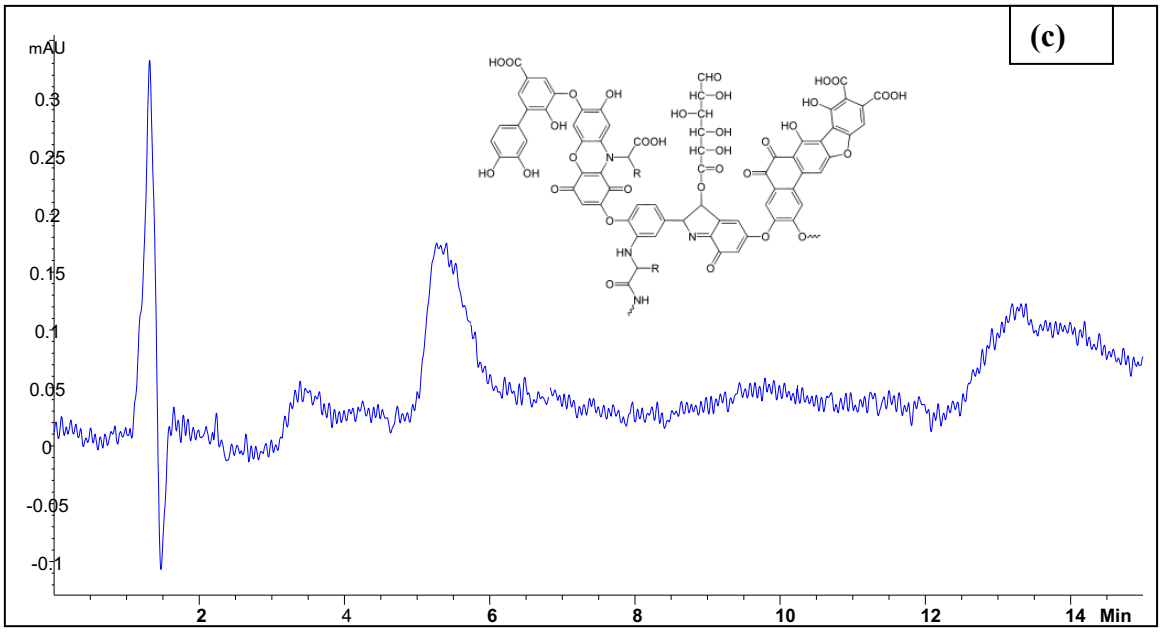


Figure 5.11: Chromatogram of HA samples at (a) 1 mg/L, (b) 3 mg/L and (c) 5 mg/L

All real samples were eluted with a mobile phase of phosphate buffer 70:30 methanol but all other conditions were the same for column packing tests. Raw and final water samples from the Olifantspoort in Limpopo province (LO) (rich with hydrophobic (HPO) part of NOM) in Limpopo Province (coordinates: 24° 21`1 6.308`` S, 29° 45` 33.66`` E) were fractionated using the prepared GPC column. It was found that from the final water (TOC: 2.52 mg/L, UV_{254}: 0.12 cm^{-1} and the SUVA value of 4.96 $cm^{-1}/mg.L^{-1}$) (**Fig. 5.12**). The raw water (TOC: 3.17 mg/L, UV_{254}: 0.16 cm^{-1} and the SUVA value of 5.32 $cm^{-1}/mg.L^{-1}$) (see **Fig. 5.13**) there are traces of HA and FA. The peak at around 1.6 mins correspond to the peak from the prepared FA standard sample and the peak at around 5.8 mins correspond to one of the HA peaks. The results indicate that the current water treatment procedure does not remediate NOM completely. This is evidenced by the increased in intensity of the HA and FA peak from raw to final water samples. However the baseline drifts on the LO raw and final samples implicate that the water is also concentrated with other chemical species which are not covered in the scope of the work.

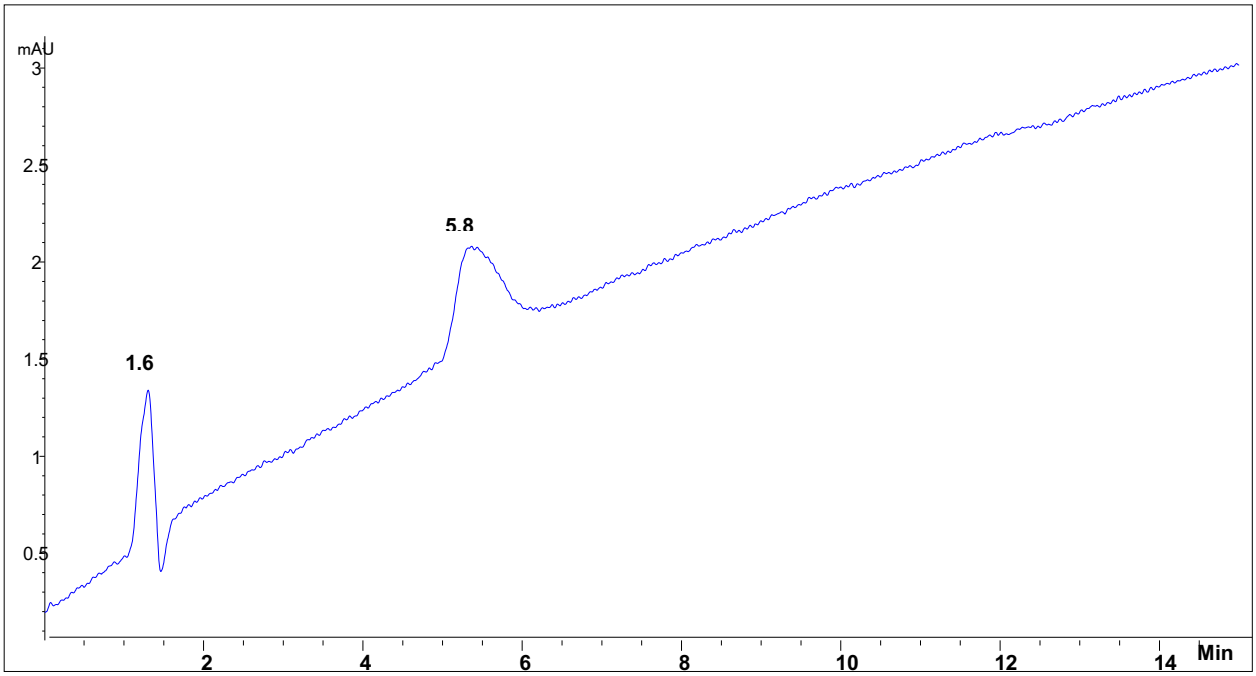


Figure 5.12: Chromatogram of LO final water sample

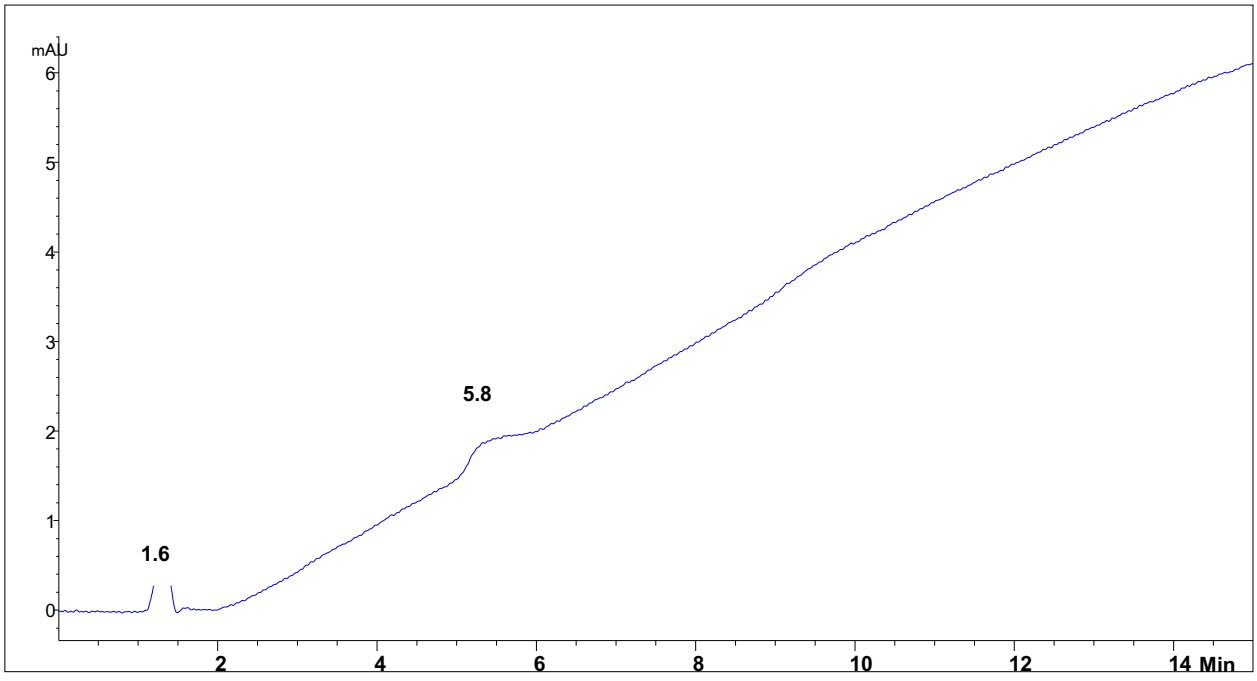


Figure 5.13: Chromatogram of LO raw water sample

The chromatograms obtained from Umgeni Mtwalume (MT) water treatment plant in Kwa-Zulu Natal (coordinates: 29.6033° S, 30.3847° E) indicate that there are traces of FA at around 1.6 mins and HA at around 5.8 mins for the final samples (TOC: 0.88 mg/L, UV_{254}: 0.12 cm^{-1} and SUVA value of 14.18 cm^{-1} /mg.L^{-1}) (**Fig. 5.14**). The raw water samples (TOC: 4.28 mg/L, UV_{254}: 0.24 cm^{-1} and SUVA value of 5.93cm^{-1} /mg.L^{-1}) (**Fig. 5.15**) indicate the presence of FA (1.6 mins), HA (5.32) mins and another peak around 3.4 mins. The disappearance of the third peak on the final water gives us an indication that the current NOM water treatment was partially successful. The high SUVA value from the final water indicate that there is deposition of the HPO fraction of NOM.[5]

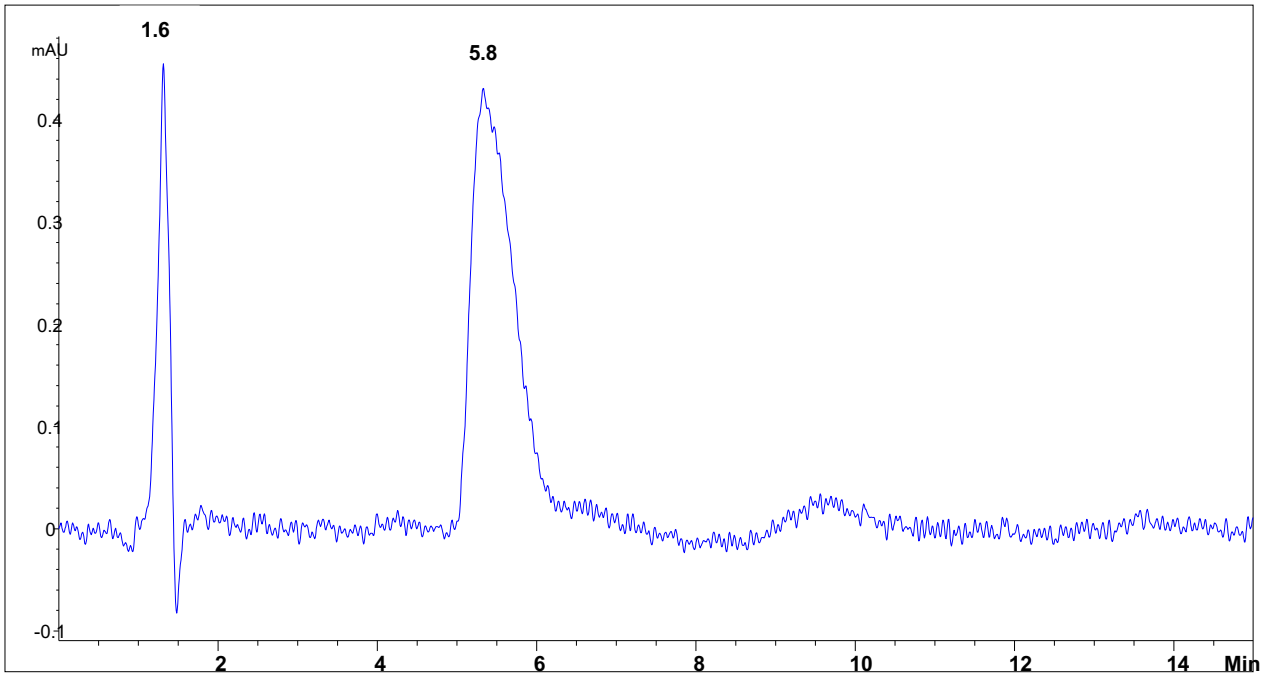


Figure 5.14: Chromatogram of MT final water sample

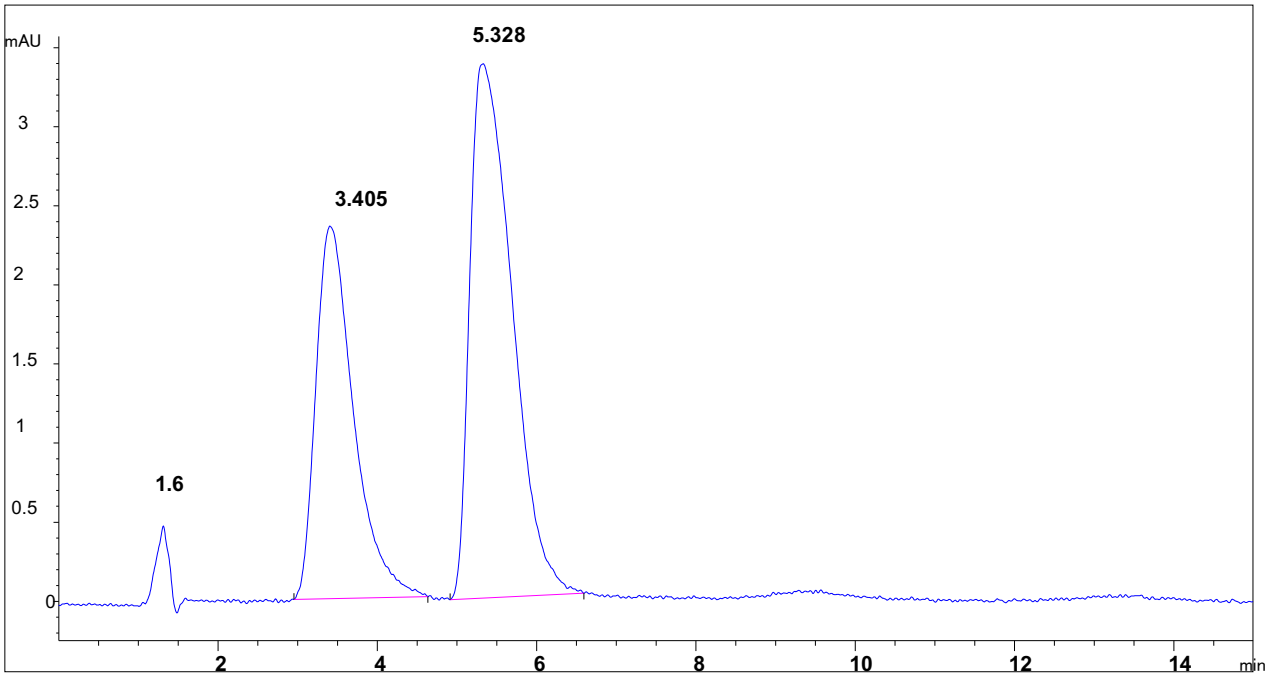


Figure 5.15: Chromatogram of MT raw water samples

The Midvaal (MV) water treatment plant in Gauteng Province (Coordinates: 24° 40`S 28°20`E) show both HA and FA. The MV raw (TOC: 7.84 mg/L, UV_{254}: 0.26 cm^{-1} and SUVA value of 3.42cm^{-1} /mg.L^{-1}) (**Fig. 5.17**). TheMV final (TOC: 3.47 mg/L, UV_{254}: 0.25 cm^{-1} and SUVA value of 7.33 cm^{-1} /mg.L^{-1}) (**Fig. 5.16**) was used for the study. The intensity of the peaks corresponding to FA and HA increased from MV raw to MV final, which means that NOM is accumulated throughout the water treatment process. The raw water is dominated by the transphilic (TPI) fraction of NOM while the final water is dominated by a HPI fraction of NOM.[5]

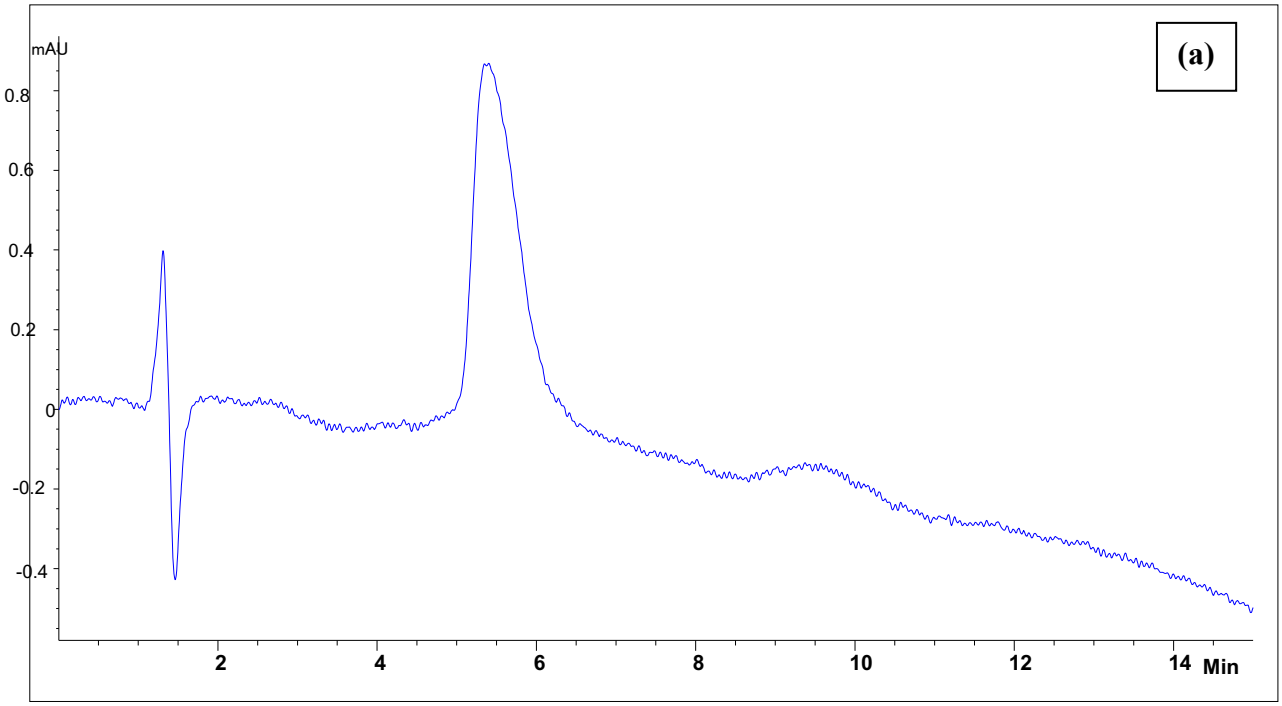


Figure 5.16: Chromatogram of MV final water samples

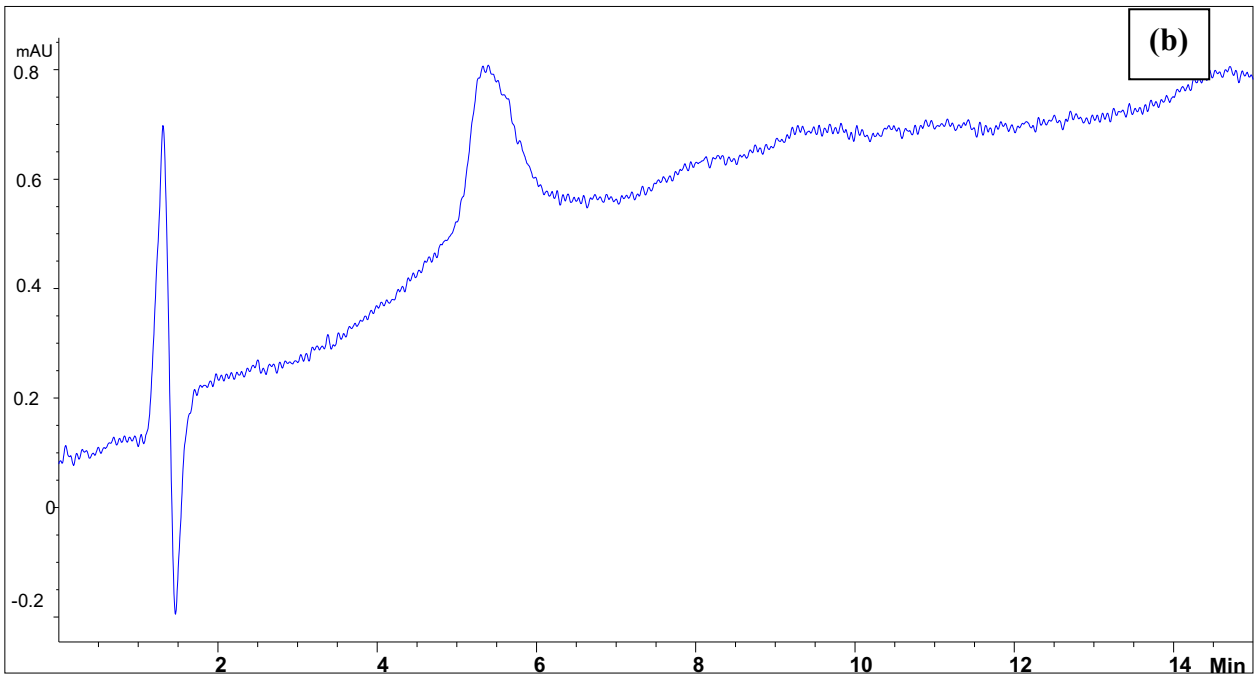


Figure 5.17: Chromatogram of MV raw water samples

The Preekstoel (P) water treatment plant situated in the Western Cape showed an effective treatment of HA and FA from the final water samples (TOC: 4.35 mg/L, UV_{254}: 0.22 cm^{-1} and SUVA value of 5.00cm^{-1} /$mg.L^{-1}$) (**Fig.5.18**) as compared to the raw water samples (TOC: 10.19 mg/L, UV_{254}: 0.52 cm^{-1} and SUVA value of 5.25 cm^{-1} /$mg.L^{-1}$). It shows weak peaks corresponding to HA and FA as compared to the strong peaks in the raw sample (see **Fig. 5.19**)

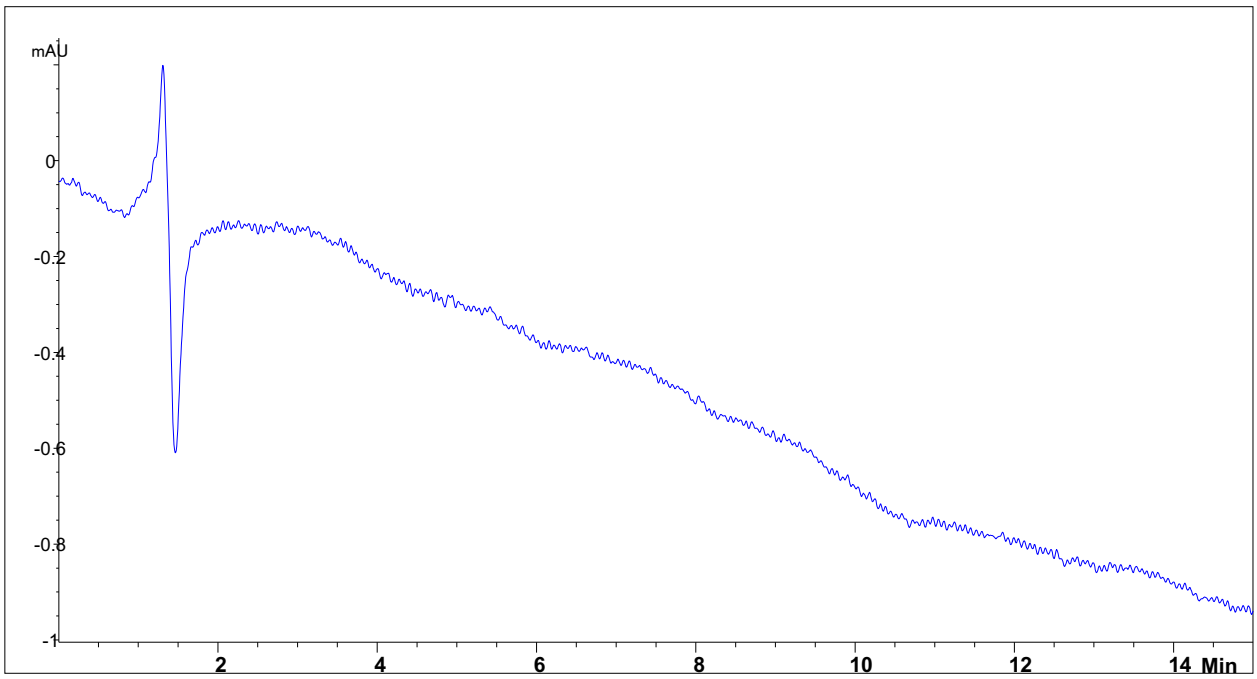


Figure 5.18: Chromatogram of P final water samples

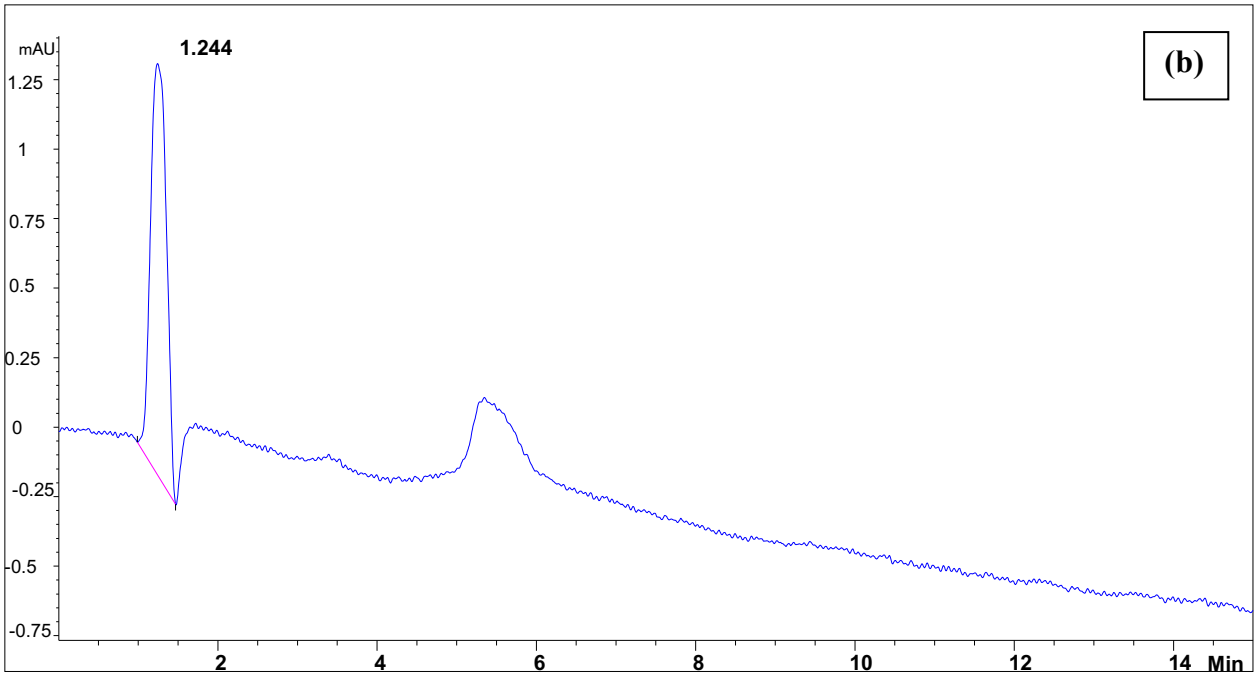


Figure 5.19: Chromatogram of P raw water samples

5.3. CONCLUSION

The results which were obtained from the test against activity of acid on the column showed that the SEC packed column can elute acidic compounds and other acidic NOM fractions like hydrophilic acid (HpiA) and hydrophobic acid (HpoA). The column packing tests were proficient. The tests confirmed further usage of the column for NOM fractionation

The column was able to fractionate the NOM fractions (FA and HA) samples. Successful fractionation of NOM from Olifantspoort (LO) raw and final water. Mtwalume (MT) raw and final water, Mid-Vaal (MV) raw and final, and the Preekstoel (P) raw and final water samples. The overall results show that the SEC packed column can possibly fractionate NOM into its different fractions. The E-PSQ: PS-DVB (1:1 w/w) packed SEC/GPC column was able to separate NOM in all samples according to its different MW fractions. Therefore, the E-PSQ-PS-DVB hybrid SEC/GPC column is indeed the best combination as described by literature.

5.4. REFERENCES

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CHAPTER 6:
CONCLUSIONS AND RECOMMENDATIONS

6.1. CONCLUSION

The PS-DVB and PSQ, the polymeric composite material were successfully synthesized and characterized using FTIR and Raman spectroscopy. In addition, the PSQ was successfully end-capped. The synthesized materials proved to be porous, as evidenced by SEM images and the BET analyses. An XRD analyses showed that the synthesized materials were all amorphous in nature. The TGA analyses proved that all synthesized materials have the virtue of withstanding high temperatures, the PS-DVB being the most stable material. Based on comparative characterization of all synthesized materials, the E-PSQ and PS-DVB (1.5 styrene: 1 divinyl benzene) were selected to be the best polymeric materials that could be applied as the stationary phases in this study.

Based on the packing of the selected polymeric materials on empty SPE cartridges, minimum TOC leaching was observed for the E-PSQ/PS-DVB composite with an optimum ratio of 1:1 The SUVA and FEEM results also showed minimum leaching for the 0.5:0.5 E-PSQ: PS-DVB combination. Consequently, the mass composition of E-PSQ: PS-DVB (50:50) ratio was considered for packing on empty SEC/GPC column.

Various column performance tests were undertaken on the packed SEC/GPC column and the following were found:

- For the test against acidic compounds was successful as the analyte (4-chlorocinnamic acid) showed a broad peak.
- The column also showed minimum silanol activity of the stationary phases when Tanaka test was performed using the phenol and benzyl amine.
- The hydrophobic retention (HR) was successful since the carbon load on the stationary phase gave the elution profile of pentyl benzene phase to be higher since the peak was broader and very intense.
- The test against hydrophobic selectivity (HS) proved that the column can selectively elute molecules based on their molecular weight. This test also proved

that the column can separate NOM according to its different molecular weight fractions.

- The test against steric selectivity (SS) showed that the column can separate compounds that have very similar chemistry. It is noteworthy that separation of the two compounds was achieved since other commercial columns cannot separate the two compounds.
- The hydrogen bonding capacity (HBC) reported a minimum retention of caffeine, therefore, there are minimum silanol groups and the end-capping of PSQ was successful.
- The stability of the stationary phase at basic pH ranges resulted in lower tailing and capacity factor. This suggests that the column can tolerate high pH values.
- The ion exchange showed high selectivity between benzyl amine and phenol therefore, less silanol activity on the surface of the prepared stationary phase. This proves that the end-capping of the material was successful.
- The column was able to elute synthetic solutions of commercial FA and HA, which were used to represent FA and HA in real water samples.

A comparative analysis of raw and final water samples from the Olifantspoort (LO) treatment plant of the Limpopo Province indicate the deposition of NOM on the treated effluent. This shows that there is still a need to address NOM contamination in this area as the current water treatment procedure does not remediate NOM completely.

The Mtwalume (MT) water treatment plant indicate the presence of traces of FA and HA and other unknown contaminants. The packed column was able to detect other fractions of NOM (non-HSs). The Mid-Vaal (MV) water treatment plant showed a decrease of HA and FA in the treated water samples as compared to the raw water. The Preekstoel (P) water treatment plant, showed an effective treatment of humic substances.

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6.2. RECOMMENDATIONS

This study recommends the following:

1. Functionalization of PSQ and PS-DVB with other groups (organic or inorganic) should be explored in order to explore the efficiency of the stationary phases.
2. A mixture of commercialized non-humic substances and humic substances should be explored in detail, such that more fractions of NOM should be detected.
3. The UVD and OCD detector as well as the SEC/GPC software for MW analysis should be explored in order to compare the MWD of the fraction of NOM from samples (both prepared and real) eluted from the packed SEC/GPC column.
4. Reproducibility studies should be undertaken more in order to test for the columns reproducibility and reliability of the findings.