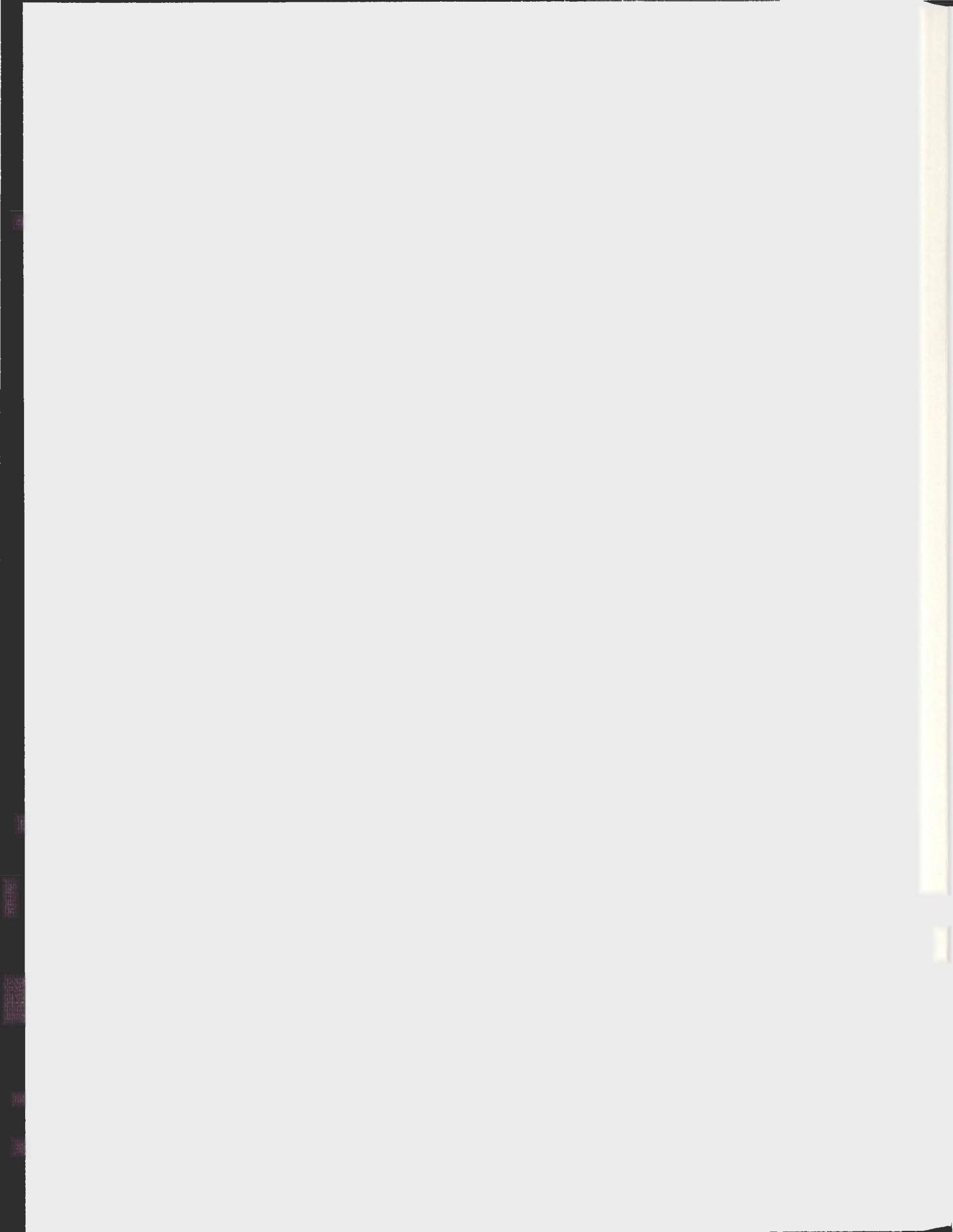


POLYSULFIDES - SYNTHESIS AND ANALYSIS

A. HARRISON IGENE



POLYSULFIDES - SYNTHESIS AND ANALYSIS

by

© Igene A. Harrison

A thesis submitted to the School of Graduate Studies in partial fulfillment of the
requirements for the degree of Master of Science

Department of Chemistry

Memorial University of Newfoundland

February 2013

St. John's

Newfoundland

ABSTRACT

Polysulfides of the general form $^{-}SS_nS^{-}$ occur naturally in sulfur rich waters and can be formed by the reaction of sulfide with elemental sulfur to yield water soluble polysulfides. Inorganic polysulfides have been found to form organic polysulfanes in the environment, and in water, oxidation of polysulfides can produce thiosalts and sulfuric acid, resulting in acidification of aquatic environments. Polysulfides are unstable and readily undergo changes in speciation, analysis is challenging and complicated by lack of standards. A series of polysulfides were synthesized to be used as standards. They were stabilized by methylation to form dimethylpolysulfanes and analyzed by gas chromatography-mass spectrometry (GC-MS), high performance liquid chromatography (HPLC) with UV detection, and matrix assisted laser desorption MS (MALDI-MS). The various dimethylpolysulfanes were fractionated using semi-preparatory HPLC; the fractions containing individual dimethylpolysulfanes were analyzed for sulfur content by inductively coupled plasma-optical emission spectrophotometry (ICP-OES). Stability studies were also carried out to confirm that the stabilization approach was effective.

ACKNOWLEDGEMENTS

I would like to thank my supervisor, Dr. Christina Bottaro and all members of the Bottaro group. Members of my supervisory committee, Dr. Kelly and Dr. Erika I say a very big thank you, for your help in the cause of this work and for accepting to supervise me.

Thanks also go to Linda Winsor, who helped me with instrumentation.

TABLE OF CONTENTS

ABSTRACT	ii
ACKNOWLEDGEMENTS.....	iii
TABLE OF CONTENTS	iv
LIST OF FIGURES	viii
LIST OF TABLES.....	x
LIST OF ABBREVIATIONS AND SYMBOLS	xi
Chapter 1 - Introduction and Literature Review.....	1
1.1 Introduction to Polysulfides: Origin and Environmental Impact	1
1.2 Types of Polysulfides	2
1.2.1 Inorganic polysulfides	3
1.2.2 Organic Polysulfides.....	4
1.2.3 Metal Polysulfide Complexes.....	5
1.3 Role of Polysulfides in the Environment.....	5
1.4 Analysis of Polysulfide ions	7

1.4.1	Total Polysulfides by Differential Pulse Polarography	8
1.4.2	Potentiometric Titration for the Determination of Sulfide, Thiosulfate and Polysulfides in Synthetic Polysulfide Solutions and Industrial Effluent.....	9
1.4.3	Capillary Electrophoretic Separation of Inorganic Sulfur- Sulfide, Polysulfides and Sulfur –Oxygen Species.....	11
1.5	Improvement in the Analysis of Polysulfides through Derivatization to form Alkylpolysulfanes – Formation, Stability and Analysis	12
1.5.1	In-house Preparation of Reference Materials for Determination of Inorganic Polysulfides	15
1.6	Matrix Assisted Laser Desorption Ionization (MALDI)	16
1.7	Project Objectives.....	19
1.8	References.....	21
Chapter 2 -	Synthesis and Analysis of Polysulfides	27
2.1	Introduction.....	27
2.2	Materials and Methods	29
2.2.1	Materials	29
2.2.2	Instruments and Settings.....	29
2.2.3	Synthesis and Derivatization	30
2.2.4	HPLC Analysis.....	31

2.2.5	ICP – OES Analysis	32
2.3	Results and Discussion	33
2.3.1	HPLC	33
2.3.2	ICP – OES	41
2.3.3	Stability Studies.....	44
2.4	Conclusions	47
2.5	References.....	48
Chapter 3 -	Synthesis and Analysis of Dimethylpolysulfanes using HPLC and GC-MS	50
3.1	Introduction.....	50
3.2	Materials and methods.....	52
3.2.1	Materials	52
3.2.2	Instruments and Settings.....	52
3.2.3	Synthesis and Derivatization	53
3.3	Analysis	53
3.3.1	HPLC Analysis	54
3.3.2	GC-MS Analysis.....	56
3.3.3	MALDI - TOF Analysis	57

3.4 Results and Discussion	57
3.4.1 HPLC	57
3.4.2 GC-MS.....	59
3.4.3 MALDI-TOF.....	65
3.5 Conclusions	66
3.6 References.....	68
Chapter 4 - Conclusions and future work	70

LIST OF FIGURES

Figure 2.1 HPLC chromatogram of the mixture of dimethylpolysulfane using reversed phase C18, analytical column.....	34
Figure 2.2 Plot of retention time against number of sulfur atoms, using linear regression with ($R^2 = 0.9804$) and both peaks at ~ 21.0 min and ~ 22.7 min having eight number of sulfur atoms each.....	35
Figure 2.3 Plot of retention time against the number of sulfur atoms (without the peaks at ~ 21.0 min and those after ~ 22.7 min).....	37
Figure 2.4 Analytical chromatogram of fraction 1 containing 1, 2-dimethyldisulfane ($\text{CH}_3\text{S}_2\text{CH}_3$) after isolation	38
Figure 2.5 Analytical chromatogram of fraction 2 containing 1, 3-dimethyltrisulfane ($\text{CH}_3\text{S}_3\text{CH}_3$) after isolation	38
Figure 2.6 Analytical chromatogram of fraction 3 containing 1, 4-dimethyltetrasulfane ($\text{CH}_3\text{S}_4\text{CH}_3$), after isolation	39
Figure 2.7 Analytical chromatogram of fraction 4 containing 1, 5-dimethylpentasulfane ($\text{CH}_3\text{S}_5\text{CH}_3$) after isolation	39
Figure 2.9 Plot of UV-vis detector response (area / mass) against the number of sulfur atoms, with X representing the peak which appeared at ~ 21.0 min, which is thought to be elemental sulfur (S_8^0).....	43
Figure 2.10 Change in speciation of an isolated fraction of dimethylpolysulfane	46

Figure 3.1 HPLC chromatogram of the mixture of dimethylpolysulfane using reversed phase C18, analytical column.	58
Figure 3.2 Chromatogram of fraction 1 containing 1, 2-dimethyldisulfane ($\text{CH}_3\text{S}_2\text{CH}_3$) after isolation	58
Figure 3.3 Chromatogram of fraction 2 containing 1, 3-dimethyltrisulfane ($\text{CH}_3\text{S}_3\text{CH}_3$) after isolation	59
Figure 3.4 Total ion chromatogram of the synthesized dimethylpolysulfanes.....	60
Figure 3.5 Mass spectrum of the peak that was eluted at 6.0 minutes from the GC with .	61
Figure 3.6 Mass spectrum of the peak that was eluted at 13.2 minutes from the GC chromatogram with m/z 126 characteristic of $\text{CH}_3\text{S}_3\text{CH}_3$ (dimethyltrisulfane).....	62
Figure 3.7 Mass spectrum of the peak that was eluted at 20.1 minutes from the GC with m/z 158 characteristic of $\text{CH}_3\text{S}_4\text{CH}_3$ (dimethyltetrasulfane).....	63
Figure 3.8 Mass spectrum of the peak that was eluted at 24.7 minutes from the GC with m/z 190 characteristic of $\text{CH}_3\text{S}_5\text{CH}_3$ (dimethylpentapolysulfane)	64
Figure 3.9 MALDI–TOF spectrum of the dimethylpolysulfanes mixture; insert is the isotope pattern of $\text{CH}_3\text{S}_9^{*+}$	65

LIST OF TABLES

Table 2.1 HPLC Gradient run used for the separation of dimethylpolysulfane on the reversed phase C18 analytical column	31
Table 2.2 Gradient used with the C18 reversed phase semi- preparative HPLC column in the isolation of individual dimethylpolysulfanes.....	32
Table 2.3 Calibration values for the ICP-OES used for the analysis.....	41
Table 2.4 Calibration table showing number of sulfur atoms, their retention times on the analytical chromatographic column, the chromatographic area of each peak, concentration of the various polysulfane as sulfur and the value of the detector response.	42
Table 2.5 Changes in speciation of sulfur in dimethylpolysulfane with time, where the second analysis is of the same samples but after 2 weeks in dichloromethane	45
Table 3.1 HPLC Gradient run used for the separation of dimethylpolysulfane on the reversed phase C18 analytical column	54
Table 3.2 Gradient used with the C18 reversed phase semi- preparative HPLC column in the isolation of individual dimethylpolysulfanes.....	55
Table 3.3 GC temperature used for the analysis of the mixture of dimethylpolysulfanes	56

LIST OF ABBREVIATIONS AND SYMBOLS

BGE	background electrolyte
CE	capillary electrophoresis
DCTB	<i>trans</i> -2-[3-(4- <i>t</i> -butylphenyl)-2-methyl-2-propenylidene] malononitrile
DAD	diode array detector
DPP	differential pulse polarography
EI	electron ionization
EOF	electroosmotic flow
ESI-MS	electrospray ionization mass spectrometry
GC	gas chromatography
HPLC	high performance liquid chromatography
ICP-AES	inductively coupled plasma – atomic emission spectrophotometry
ICP-OES	inductively coupled plasma – optical emission spectrophotometry
ID	internal diameter
KE	kinetic energy
MALDI-TOF	matrix assisted laser desorption ionization time of flight
MS	mass spectrometry
m/z	mass to charge ratio

PEC	photoelectrochemical
PDMS	polydimethylsiloxane
RP	reversed phase
S/C	sulfur to carbon ratio
SCP	sampled current polarography
SIM	selected ion monitoring
SPME	solid phase micro extraction
UV	ultra violet
UV-Vis	ultra violet-visible
VOSC	volatile organic sulfur compound

Chapter 1 - Introduction and Literature Review

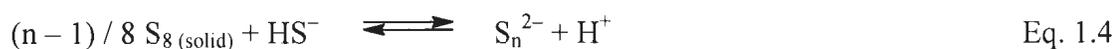
1.1 Introduction to Polysulfides: Origin and Environmental Impact

Polysulfides are chains of sulfur atoms containing two or more sulfur atoms linked together; sulfur is the building block of polysulfide. Understanding the reactions of sulfur from ring opening to charge distribution gives an insight into polysulfide chemistry [1]. Reactions of elemental sulfur are usually initiated by ring opening as seen in the nucleophilic attack demonstrated in equations 1.1 – 1.3. This leads to inorganic polysulfide formation and potentially a series of additional reactions [2].



Though there may be slight deviation from these characteristics as a result of differences in allotropy and purity. Sulfur is insoluble in water but highly soluble in carbon disulfide [3-5]. Polysulfides are linear compounds comprised of chains of zero valent sulfur atoms bracketed by sulfur ions in the -1 oxidation state, where the general form can be expressed in the following way: $^-\text{SS}_n\text{S}^-$ [6]. Polysulfides are present in both anoxic and oxic aquatic environments where they play a very important role in transition metal complexation, pyrite formation and thiosalt formation. Polysulfides result from the

desulfurization of fossil fuel, mining and processing of sulfidic minerals, and oxidation of hydrogen sulfide in alkaline waters [7]. Polysulfides partake in various redox reactions because of their nucleophilicity and they are also present in low concentrations in ground water [8]. The temperature of the environment in which polysulfides are found also plays a role in the chain length of the polysulfides; as the temperature of the environment increases polysulfide chain length and the formation constant also increases [9]. The effect of temperature, low concentrations, chemical interferences and pH make quantification of naturally occurring polysulfides in aqueous solution very difficult.



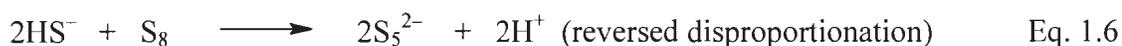
The following review of polysulfides, their reactions and analysis is comprehensive. Though it may seem to lack depth, this is because very little has been published on this subject.

1.2 Types of Polysulfides

Though their central structure does not deviate from the description above, polysulfides can occur in various forms: these include the native inorganic forms of the dianions or the salt of the dianions, metal-polysulfide complexes and organically-derivatized polysulfides called polysulfanes [10].

1.2.1 Inorganic polysulfides

Inorganic polysulfides are [3-5] ionic substances containing chains of zero valent sulfur atoms between terminal charged sulfur ions of -1 oxidation state (with the smallest form being the disulfide comprised of two charged sulfur atoms covalently linked). They are reactive due to the fact that they break down easily into different disproportionate species, and also due to their terminal charges. Inorganic polysulfides are reactive in aquatic environments [10].

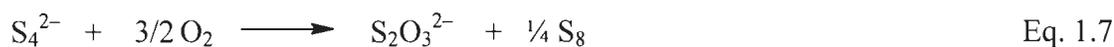


Equations 1.5 and 1.6 show the formation of inorganic polysulfides by oxidation and from elemental sulfur respectively [13]. Inorganic polysulfides are the most likely source of the organosulfur body burdens in aquatic organisms. They are also very important in industrial processes, like the pulp industry, where they are effective in improving pulp quality [11].

In the Kraft pulping industry, the goal is to get rid of the lignin (delignification) and reduce carbohydrate (cellulose and hemicellulose) loss; delignification occurs by modifying the structure of the lignin in two different ways [11]. The first is to degrade the lignin into smaller units by cleaving inter-unit linkages, while the second is to introduce hydrophilic groups into the lignin and cleaved fragments. This makes the lignin more soluble in the cooking liquor. The liquor is made up of sodium sulfide (Na_2S) and sodium hydroxide (NaOH). Attacks on the lignin by the hydrosulfide and hydroxide anions in the

liquor result in degradation of the lignin. This occurs at about 170°C and the reaction runs for 90 minutes. The fragments produced from the degradation are soluble in the liquor. This leads to a much higher quality pulp.

Carbohydrates present in the pulp also undergo similar reactions. For the carbohydrate a “peeling” reaction occurs, which is the removal of the reducing end sites from the carbohydrate by the hydrosulfide and hydroxide groups. This reaction produces a new reducing end which can undergo further “peeling” reactions. The carbohydrate material lost during this “peeling” is converted to different hydroxy acids that consume the alkali liquor. The carbohydrate “peeling” stops when there is a carboxyl group at the reducing end of the carbohydrate. These reactions typically occur at about 100°C, but the addition of polysulfides to the liquor allows for oxidation of these relatively unreactive aldehyde groups to carboxyl groups to occur with only a slight increase in temperature to 110°C [12]. Polysulfides play a very important role in desulfurization plants. Here they are oxidized directly by transition metal ions such as V^V, Fe^{III} and Cu^{II} to elemental sulfur which is removed from industrial plants when crystallized [13]. Inorganic polysulfides are oxidized in the presence of oxygen to form thiosulfate and potentially other thiosalts as seen in equation 1.7.



1.2.2 Organic Polysulfides

Inorganic polysulfides can undergo reactions to form organic substituted dialkylpolysulfanes. These are more stable than native polysulfides. One derivatizing

agent used to convert inorganic polysulfide to a simple organic polysulfide is methyl iodide, which gives rise to dimethylpolysulfane, with a methyl group on each end of the polysulfide chain]. Organic polysulfides are known for their bad odor and hence are unacceptable environmentally [14]. 1,3-Dimethyltrisulfane ($\text{CH}_3\text{-S-S-S-CH}_3$) is an example of a dialkylpolysulfane.

1.2.3 Metal Polysulfide Complexes

Polysulfides can also act as ligands; they can form complexes with a variety of metals, mostly transition and main group metals. Both inorganic and organic polysulfides can give rise to such complexes. These complexes are sometimes used in industrial processes, where they act as catalysts. The polysulfide ligands have different sizes, though typically they range from S^{2-} to S_9^{2-} and can participate in different coordination environments; they show a wide range of structural diversity. An example of such metal-polysulfido complexes is titanium polysulfido complex, which can be synthesized using different sulfur sources. These products are not only a function of the starting materials, but also the reaction conditions such as temperature, solvents used and reaction time [15].

1.3 Role of Polysulfides in the Environment

Alon *et al.* [16] showed that polysulfide anions played a vital role in the incorporation of sulfur into organic molecules in marine environment, being important source of sulfur. The authors reacted ammonium polysulfides with α - β -unsaturated aldehydes through a nucleophilic addition (Figure 1.1) to form polysulfide cross-linked oligo-polymers in artificial seawater purged with nitrogen, at pHs of 8-9 and 12-13, with

temperatures of 25°C and 0°C; noting that these are conditions outside what would be considered typical of natural environments. To understand the products formed at various intervals (20, 40, 60 and 80 min), the authors isolated the products by extraction with dichloromethane followed by solvent evaporation. The composition was assessed by elemental analysis and GC-MS analysis. It was found that there was no difference in the products formed at 25°C and 0°C, leading to the conclusion that these reactions can occur in colder marine environments. The study also showed that pH is a controlling factor in the incorporation of sulfur into organic molecules in marine water. At pH 12-13, the sulfur to carbon ratio is lower, suggesting that the sulfur linkages are shorter and therefore it is suggest that under these conditions S_2^{2-} and S_4^{2-} dominate, while at pH 8-9 the ions S_6^{2-} , S_5^{2-} and S_4^{2-} are more abundant. This may be important in our work as it is clear that the speciation may be influenced by pH, as temperature as we have observed. It is also useful to note that the polysulfides can act as nucleophiles, which is exploited in the derivatization procedure used in this thesis. The authors also found that the cation in the polysulfide salt also played an important role in the reaction, as it was found that with ammonium polysulfide the end product has more sulfur incorporated then when sodium polysulfide was used [16].

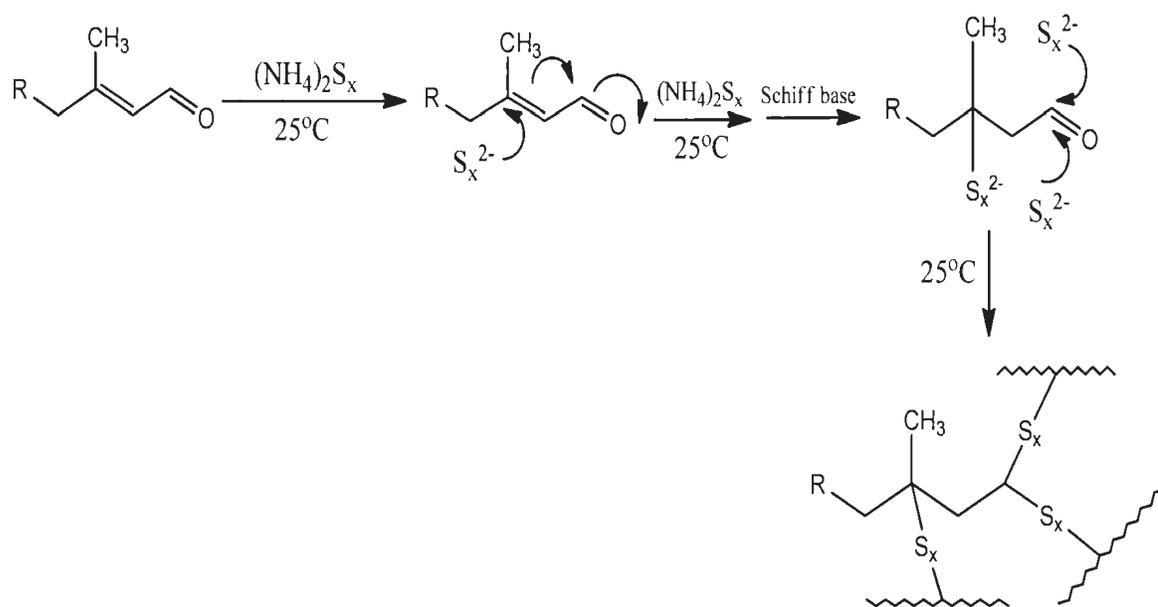


Figure 1.1 Incorporation of sulfur into α, β -unsaturated aldehyde (organic matter) by ammonium polysulfide, adapted from [16]

1.4 Analysis of Polysulfide ions

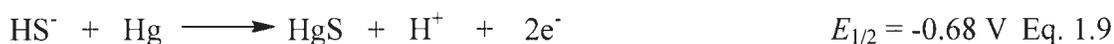
Analysis of polysulfides has been carried out with and without derivatization (methylation). When both results were compared, the effect of derivatization (methylation) had on the analysis of polysulfides can be clearly seen. Some analyses carried out directly on polysulfide ions without derivatization (methylation) are discussed below and their shortcomings indicated.

1.4.1 Total Polysulfides by Differential Pulse Polarography

In 2001, Kariuki *et al.* [17] used differential pulse polarography (DPP) with a dropping mercury electrode to measure total ionic polysulfides in aqueous solution in the presence of different sulfur species, such as polythionates and sulfide. The solution was buffered using carbonate at pH 9.5. They found that at -1000 mV relative to a silver/silver chloride reference electrode the current was at a minimum. This minimum changed in magnitude, and the changes were found to be proportional to the amount of zero valent sulfur (S^0) in the polysulfides. The reasons for this particular phenomenon are complex and are partially related to the adsorption of the polysulfides to the mercury surface, however it was found to give meaningful analytical results. They saw linearity for concentration of zero valent in the polysulfides from 10^{-5} - 10^{-3} M sulfur, and the found that most interferences were not significant [16]. These analyses were carried out on polysulfide they synthesized, which had Raman spectra that agreed with the results of Junz *et al.* who characterized polysulfides using Raman spectroscopy [18]. The disadvantages when using DPP as a method of analysis for polysulfides, are that speciation cannot be accurately determined and a non-linear response for concentrations greater than 10^{-3} M, hence, under these conditions this technique does not yield satisfactory results.

Jordan *et al* 1989 [19] also measured the concentrations of sulfur ion or sulfide with a charge of -2 (S^{2-}) and zero valent sulfur in polysulfides (S^0), using a dropping mercury electrode with sampled current polarography (SCP). The measurements were carried out at potentials below -0.68 V as can be seen from the three equations below.

Equation 1.7 shows the reaction responsible for the anodic limiting current related to S^{2-} in the polysulfide chain length and equation 1.9 shows the anodic sulfidic sulfur (any form of HS^-) concentrations, respectively. Equation 1.8 shows the cathodic limiting current as a measure of (S^0) concentration.

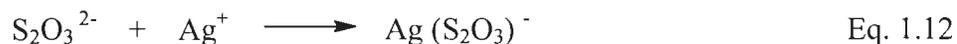
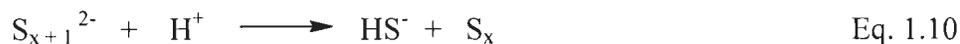


From the above equations, it can be seen that sulfidic sulfur adds to the anodic current [19]. However, interferences from other sulfur species were found to be the shortcoming of using SCP as a means of measuring polysulfides.

1.4.2 Potentiometric Titration for the Determination of Sulfide, Thiosulfate and Polysulfides in Synthetic Polysulfide Solutions and Industrial Effluent

In 1980, Hiromu *et al.* [20] used potentiometric titration with silver-silver sulfide, silver-silver iodide and silver-ion selective electrodes to determine sulfide, thiosulfate and polysulfide ions in synthetic polysulfide solution and lixiviation water containing all three ions [20]. This method relied on the difference between one terminal sulfur atom as been divalently charged and the other sulfur atoms as been zero valent in a polysulfide chain length. Here two aliquots were used. In one aliquot, sulfide (as free sulfide and polysulfide) and thiosulfate were determined. Prior to titration with silver nitrate ($AgNO_3$), the sample is treated with calcium nitrate and adjusted to pH 4 by the addition of acetate buffer or acetic acid. This first part of the titration is for the analysis of total

sulfide (S^{2-}) and thiosulfate ($S_2O_3^{2-}$). The endpoint is detected using the silver-silver sulfide indicator electrode, after which the electrode is replaced by a silver-silver iodide electrode indicator to detect silver. The titration with $AgNO_3$ is continued for determination of thiosulfate. To the second aliquot, sodium sulfite and oxygen-free water are added and the solution is heated to $50^\circ C$, left to stand for 3 minutes while cooling to $15^\circ C$, and then ammonia and calcium nitrate are added. This is then titrated for S^{2-} with silver nitrate solution and the endpoint is detected with the silver-silver sulfide indicator electrode. At the first endpoint, pH is adjusted to 1.5 – 3 with sulfuric acid and the titration continued till the second endpoint is reached, which is for both $S_2O_3^{2-}$ and S_x , where x is the number of zero valent sulfur atoms in the polysulfide chain length. The amount of S_x , which is a measure of the estimated concentration of polysulfides, is found by subtracting the value of the final endpoint of the first aliquot titration from the final endpoint value of the second aliquot titration (equations 1.10 – 1.12 show these reactions). The solution was protected from hydrogen sulfide evolution by covering it with liquid-paraffin layer.

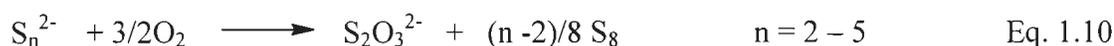


(Where each polysulfide ion is denoted as sulfide sulfur (S^{2-}) and S_x is the polysulfide sulfur or dissolved sulfur)

Results show that good reproducibility and accuracy can only be achieved for a limited concentration range of $10^{-4} - 5 \times 10^{-4}$ M, for S^{2-} and $S_2O_3^{2-}$. A number of problems arose in this method. Firstly, the speciation of the polysulfide could not be determined. Secondly, the assumption that only one sulfur ion is divalently charged at the terminal end of the polysulfide chain length does not agree with the Lewis structure of polysulfides. Thirdly, no method was used to account for the concentration of the terminal sulfur ion that is the part of the polysulfide which was added to the free sulfide ions concentration. And finally, the effectiveness using a layer of liquid paraffin to prevent the escape of hydrogen sulfide was not verified.

1.4.3 Capillary Electrophoretic Separation of Inorganic Sulfur- Sulfide, Polysulfides and Sulfur –Oxygen Species

Studies were carried out in 2005 by Catalin *et al.* [22] on the application of capillary electrophoresis for the separation, quantification and identification of inorganic sulfur, sulfide, polysulfides and sulfur oxyanions. This study came after Steudel *et al.* [21] in 1986 used ion-pair chromatography to separate polysulfide as S_x^{2-} , from HS^- , $S_2O_3^{2-}$ and SO_3^{2-} solution.



From equation 1.10, oxidation converts the polysulfides into thiosulfate and in equation 1.11 the polysulfides undergo fast equilibrium dissociation making speciation and quantification impossible; hence, analysis by ion-pair chromatography is not feasible.

For the CE studies carried out by Catalin *et al* in 2005 [22], polysulfides were prepared with sodium sulfide nonahydrate ($\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$) and elemental sulfur (S) in 0.1 M borate buffer which was adjusted to four different pH values: 8.2, 9.5, 10.5 and 12.2 with NaOH. It should be noted that distilled water, deoxygenated by bubbling with nitrogen, was used in this work. Detection was by UV-Vis absorbance using a diode array detector DAD at three different wavelengths; these were: 214 nm (bandwidth 10 nm), 214 nm (bandwidth 10 nm) with reference at 372 nm (bandwidth 20 nm) and 230 nm (bandwidth 10 nm) with reference at 372 nm (bandwidth 20 nm). The separation was carried out in negative mode (negative voltage) and the background electrolyte (BGE) was chromate electrolyte (from Na_2CrO_4) prepared with distilled water; hexamethonium bromide was the EOF modifier. This study showed that separation and detection of sulfate, thiosulfate, sulfite and tetrathionate were possible. There was evidence of only two peaks, which may be attributed to the polysulfide species S_4^{2-} and S_3^{2-} but this could not be confirmed. Hence the CE could not be used to develop a protocol for separation, quantification and identification of inorganic polysulfide.

1.5 Improvement in the Analysis of Polysulfides through Derivatization to form Alkylpolysulfanes – Formation, Stability and Analysis

Development of a method to derivatize polysulfides and then analyze derivatized polysulfides was carried out after results from direct analysis showed that speciation was a problem. Analysis of the inorganic polysulfides has shown a high degree of uncertainty

due to spontaneous redistribution of species. Large changes in concentrations would occur and oxidation to various sulfur oxyanions could not be eliminated. Derivatization was found to surmount some of the shortcomings of direct analysis, though long-term stability continues to be an issue.

Dan *et al.* 2003 [14], produced standards as reference material for the determination of inorganic polysulfides in water. Aside from producing and isolating the polysulfides for use as standards, they used methyl iodide for the derivatization of the inorganic polysulfides to impart stability. Methyl iodide was chosen because it is fast and its reaction is stoichiometric [14]. Alexey *et al.* 2004. [26] described the use of the methyl trifluoromethane sulfonate for methylating polysulfides, they were able to determine speciation of polysulfide S_3^{2-} to S_7^{2-} and S_2^{2-} could not be ascertained [24,26]. Questions were asked as to whether there was disproportionation of the polysulfides during the methylation process, and also if the methylating agent underwent hydrolysis. Hence studies were carried out to address this concern by Alexey *et al.* 2007 [25]. They carried out kinetic analysis to determine the disproportionation rate of inorganic polysulfide in methanol/water solution during methylation [25]. They found that the disproportionation rate was small. They also used isotope dilution to study methylation by looking at the mixing rate of isotopically labeled polysulfide and non-isotopically labeled polysulfide in which a solution of ^{34}S labeled Na_2S_4 was mixed with an unlabeled solution of the Na_2S_4 in a 1:1 ratio. These solutions were kept for different times to allow for the total isotope mixing of the different batches before methylation. The resulting methylated polysulfides were analyzed by GC/EI-MS in selected ion monitoring mode (SIM). Their results show

that disproportionation during methylation was very small, which was also what was seen in the kinetic test they carried out. The rate of methyl trifluoromethane sulfonate hydrolysis in water-methanol was also studied spectrophotometrically at 275 nm absorbance. The pH of the solutions was varied from 8.2 to 11.7; the results showed that the change in pH during hydrolysis was irrelevant, and because pH is the driving force for hydrolysis, it was concluded that the hydrolysis rate does not play any role in the methylation of inorganic polysulfides.

Once the alkylated polysulfides are synthesized, there are a number of options for analysis including HPLC-UV-vis and GC-MS. A modification of the traditional means of analysis for polysulfides with headspace solid phase microextraction (SPME) with GC-MS was reported by Ina *et al.* in 2010 [26]. Furthermore, Ina *et al.* did not use methyl trifluoromethane sulfonate as [26] the method is labor intensive with multiple liquid-liquid extractions and difficult sample preparation conditions. Derivatization by either methyl trifluoromethane sulfonate and methyl iodide yields the same major product (dimethylpolysulfanes) but different side products which have no effect on the reaction because of the high pH in which the reaction was carried out. Instead Ina *et al.* used methyl iodide as the methylating agent for the inorganic polysulfides [8]. The resulting dimethylpolysulfanes from the analysis carried out by Ina *et al.* were analyzed using headspace SPME with a 100 μm polydimethylsiloxane (PDMS) fiber and GC-MS analysis was carried out. Selected ion monitoring (SIM) mode was used for the MS. This method was tested for reliability, linearity, precision and sensitivity and found to be good

for only the smallest of the sulfides, that is disulfide and trisulfide, but longer chain lengths could not be analyzed possibly due to thermal instability or as a result of fibre selectivity in the course of carrying out HS SPME.

1.5.1 In-house Preparation of Reference Materials for Determination of Inorganic Polysulfides

As mentioned previously, Dan *et al.* [14] gave a detailed account of methods to produce and analyze a range of polysulfides that could be used as reference compounds; a key point in the study is that speciation is relevant to environmental studies and in understanding many industrial processes. Moreover, standards are not commercially available. Their method involves the reaction of elemental sulfur with hydrazine sulfate and sodium hydroxide to produce a range of inorganic polysulfides. The mixture was then methylated using methyl iodide. It is notable that the methanol was evaporated under vacuum without a problematic loss of key products. The dimethylpolysulfanes were purified by extraction from the aqueous solution into dichloromethane. The resulting mixture was analyzed using reversed phase HPLC-UV-vis and HPLC using an inductively coupled plasma-atomic emission spectrophotometer (ICP-AES) for detection. The chromatogram from the HPLC-UV-vis was analyzed and then the logarithm of the retention time ($\log R_t$) was plotted against the number of sulfur atoms, yielding a straight line plot showing that the produced dimethylpolysulfanes belong to a homologous series [27]. Preparatory scale separation was carried out using open column (C-18 bulk phase) with gravity elution to fractionate the dimethylpolysulfanes. Since the eluent was

methanol/water, the products were extracted into carbon tetrachloride to facilitate concentration [14]. The total sulfur content of each fraction was determined after oxidation of the sulfur with perchloric acid using ICP-AES.

1.6 Matrix Assisted Laser Desorption Ionization (MALDI)

Matrix-assisted laser desorption ionization mass spectrometry (MALDI) was developed by Karas and Hillenkamp [28] and Tanaka *et al.* [29] in 1988 as variation of laser desorption/ ionization to yield a softer ionization method suitable for the formation of a variety of ion types, which are then introduced into a mass spectrometer. A MALDI-MS instrument basically is a pulsed instrument in which molecules are desorbed and then ionized before passing into the mass spectrometer. MALDI is primarily used for polymers, biomolecules, organic and inorganic molecules, to confirm their molecular weight and also for their characterization. MALDI is usually connected to time of flight (TOF) or ion trap mass analyzers [30]. The advantages of MALDI-TOF MS are that it is simple, fast, typically forms singly-charged protonated molecules or molecular ions, is sensitive and has high resolution and mass accuracy. A time of flight mass analyzer can be linear or reflectron. Linear time of flight analyzer works by acceleration of the produced ions to the detector and here all the ions have the same energy but different masses. The low mass ions get to the detector first followed by those of heavier masses, the low mass ions have greater speed compare to the heavier mass ions [31]. The mass, kinetic energy (KE) and charge of the ions are the determining factors of the arrival time of the ions to the detector while the reflectron time of flight analyzer has a reflectron

attached to it, which is an electrostatic mirror and it serves to minimize differences in temporal/spatial dispersion due to differences in kinetic energy of the ions. It does this by refocusing the kinetic energy distribution at the detector resulting in increased resolution for low mass ions, usually less than m/z 10,000.

The MALDI process involves the use of a laser for desorption/ ionization of the matrix and the analyte. Traditionally, most suitable matrices are soluble in the solvent used and the analyte should also be soluble in the same solvent as the matrix [32]. The matrix functions by absorbing laser energy and transferring it to the analyte leading to vaporization of the analyte. It can also donate and receive protons, hence causing the ionization of the analyte in both positive and negative modes [33]. Different matrices have been used for sample preparation and the choice depends on the nature of the analyte; matrices can be either organic or inorganic matrices. Tanaka *et al* favored inorganic matrices, while Karas and Hillenkamp used organic matrices [32]. Normally, the matrix (in excess) and analyte are then mixed together and spotted on a plate, which in most cases is stainless steel. Recently gold surfaces have been used. Characteristics of ions produced by MALDI are: mainly singly charged ions (positive or negative) depending on the polarity that is chosen. Radical molecular ions are possible with many UV matrices. Similar mass spectra are obtained even if different regimes are used. Little fragmentation occurs because MALDI generally functions as a soft ionization technique [34]. UV-MALDI has the advantage of producing stable ions with better sensitivity compared to other methods of desorption and is clearly seen when this method is applied to large proteins [35]. Laser intensity also plays a major role in the desorption/ionization

process where a minimum threshold energy is required. However, higher fluxes can cause more fragmentation, perhaps due to thermal decomposition [36]. The matrix plays three primary roles in MALDI process, these are, (i) to absorb the laser energy and transfer it to the sample to cause desorption while protecting the analytes from the energy of the laser, (ii) to enhance ionization of the analyte and (iii) to help to prevent aggregation of the analyte molecules, reducing undesired intermolecular reactions [37].

Analysis by MALDI can be complicated by the formation of metal adduct peaks, which appear in the spectrum. These metal adducts sometimes arise from salt impurities that can come from glassware, solvents, the matrix and at times even the sample [38]. Metal salts are sometimes added to the matrix to cause ionization of synthetic polymeric materials via cationization [39]. Ionization of analytes by MALDI which is soft, occurs either by cationization or protonation, or by both processes, mediated by an excited gas phase matrix plume that forms above the solid matrix upon irradiation by a laser [40]. In a 2004 study by Juan *et al.* [41], it was found that suppression of the cationized matrix signal correlated with the gas-phase cation basicities of the matrix. Consequently gas-phase cationization of the target analyte was also observed, rather than the expected protonation, but this is not unusual in MALDI-mass spectrometry [41].

Though MALDI-TOF MS has been used in the analysis of many inorganic species, including metal-ligand complexes, one of the few reports of analysis of sulfur-containing inorganic compounds was from Julien *et al.* in 2010 [42]. They used MALDI-TOF to analyze polythiophene, using *trans*-2-[3-(4-*t*-butyl-phenyl)-2-methyl-2-

propenylidene] malononitrile (DCTB), terthiophene and dithranol as matrices.

Chloroform was used as solvent for all matrices and analytes. One μL of each matrix solution was applied to the stainless steel plate and air dried. One μL of each aliquot of analyte was then applied to the spot that contains the matrix crystals and then air dried. A Nitrogen laser was applied at 337 nm in reflectron mode. The results showed that DCTB is a better matrix than dithranol and terthiophene for MALDI-TOF characterization of polythiophenes [43]. DCTB has also been demonstrated to be a softer matrix, working by charge exchange to form protonated molecules.

1.7 Project Objectives

Many attempts have been made to quantify polysulfides due to the negative role they play in the environment, but they have achieved limited success. There are many reasons for the difficulties in developing suitable analytical approaches including lack of standards, instability of the polysulfide species and too many unreliable assumptions which have inspired this project. It is important to know how different polysulfide chain lengths interact with different components of the environment such as oxygen, water, hydrogen, metal and the overall role polysulfides play in the environment. Hence the goal of this work is to produce polysulfide standards which will help in quantifying the different polysulfide chain lengths in the analysis of real sample.

Characterization of the polysulfides produced, method development and implementation of the developed method of analysis, form the basis of this project. The analytical work was carried out using high performance liquid chromatography (HPLC)

with analytical and semi preparative columns for separation. Matrix assisted laser desorption ionization- time of flight mass spectrometer (MALDI-TOF MS) and gas chromatography mass spectrometry (GC-MS) were used for characterization. Inductively coupled plasma optical emission spectrophotometer (ICP-OES) was used for quantification of the isolated polysulfide fractions from the semi preparative column.

Since issues with stability of polysulfides are well known, another aim of this research was to carry out stability studies on the polysulfides produced and the isolated fractions of different dialkylpolysulfane chain lengths in order to establish acceptable conditions for reliable analysis and for storage of samples and standards. These results will be used to construct calibration curves and from the calibration curves, quantification of polysulfides in real samples will be done. For example, polysulfides in waste from tailings, in the hydrometallurgical processes, and waste from the oil industry can be quantified. This will be very important in optimizing industrial processes and mitigating the effects of industrial waste residues. It may also be useful for water supply operators and managers, who will need to assess the efficacy of treatment methods to rid influent waters of polysulfides prior to distribution.

1.8 References

1. Nickless, G.; *Inorganic Sulfur Chemistry*, 1968, pp. 1-21
2. Karchmer, J.; *The analytical Chemistry of Sulfur and its Compound*, 1970, 29, pp. 1-11
3. Draganjac, M.; Rauchfuss, B.; Transition Metal Polysulfides: Co-ordination Compounds with Purely Inorganic Chelate Ligands, *Angewandte Chemie International Edition*, 1985, 24, pp. 742-757
4. Vairavamurthy, A.M.; Zhou, W.; Characterization of a Transient +2 Sulfur Oxidation State Intermediate from the Oxidation of Aqueous Sulfide, *ACS Symposium Series Volume*, April 1995, pp.1-8
5. Brown, T. L.; Lemay, H. E.; Bruce, E.; Catherine, J.; Patrick, M.; *Chemistry The Central Science*, 2012, 12th, pp. 305-309
6. Steudel, R.; Mechanism for the Formation of Elemental Sulfur from Aqueous Sulfide in Chemical and Microbiological Desulfurization Process, *Industrial and Engineering Chemical Research*, 1996, 35, pp 1417-1423
7. Chen, Y.; Morris, J.; Kinetics of Oxidation of Aqueous Sulfide by Oxygen, *Environmental Science Technology*, 1972, 6, pp. 529-537
8. Kristiana, I.; Heitz, A.; Joll, C.; Sathasivan, A.; Analysis of Polysulfide in Drinking Systems Using Headspace Solid-Phase Microextraction and Gas Chromatography-Mass Spectrometry, *Journal of Chromatography A*, 2007, 1217, pp. 5995-6001
9. Kamyshny, A.; Zilberbrand, M.; Ekelchik, I.; Voitsekovski, T.; Gun, J.; Lev, O.; Speciation of Polysulfide and Zerovalent Sulfur in Sulfide-Rich Water Wells in Southern and Central Israel, *Aquatic Geochemistry*. 2008, 14, pp.171-175

10. Ralf, S.; Inorganic Polysulfide S_n^{2-} and Radical Anions $S_n^{\cdot-}$, *Topics in Current Chemistry*. 2003, 231, pp. 128-131
11. Smook, G.A.; Carbohydrate Complex from Spruce. A paper from the institute of paper science and technology, *Wood and Science Technology*. 1985, 19, 3 pp. 243–251
12. Luthe, C.; Berry, R.; Li, J.; Polysulfide for Yield Enhancement in Sawdust Pulping. Does it Work? *Pulp & Paper Canada*, 2004, 105(1):pp. 32-37
13. Steudel, R.; Mechanism for Formation of Elemental Sulfur from Aqueous Sulfide in Chemical and Microbiological Desulfurization Process. *Industrial and engineering chemistry research*, 1996, 35, pp. 1417-1423
14. Dan, R.; Lev, O.; Jenny, G.; Boris, A.; Ilya, K.; Development of in-House Reference Materials for The Determination of Inorganic Polysulfide in Water, *Accreditation and Quality Assurance*, 2004 , 9, pp. 399-403
15. Nobuhiro, T.; Norihiro, T.; Renji, O.; Polysulfido Complexes of Main Group and Transition Metals, *Topics in Current Chemistry*. 2003, 231, pp. 153-202
16. Alon, A.; Zeev, A.; Reactions of Polysulfide Anions with α , β Unsaturated Isoprenoid Aldehydes in Aquatic Media: Simulation of Oceanic Conditions, *Organic Geochemistry*, 2004, 35, pp. 909-921
17. Kariuki, S.; Morra, J.; Umiker, K.; Cheng, I.; Determination of Total Ionic Polysulfides by Differential Pulse Polarography, *Analytica Chimica Acta*, 2001, 442, pp.277-285

18. Janz, G.; Downey Jr, J.; Roduner, E.; Wasilczyk, G.; Goutts, J.; Eluard, A.; *Inorganic Chemistry*, 1976, 15, 1759
19. Jordan, J.; Talbott, J.; Yakupkovic, J.; *Analytical Letter*, 1989, 22, 1537
20. Hiromu, S.; Takeo, H.; Ikeda, S.; The Rapid Determination of Sulfide, Thiosulfate and Polysulfide in the Lixiviation Water of Blast- Furnace Slag by means of Argentimetric Potentiometric Titration, *The Chemical Society of Japan*, 1981, 54, pp. 1968-1971
21. Steudel, R.; Holdt, G.; Gobel, T.; *Journal of Chromatography*, 1989, 475, pp. 442-446
22. Catalin, F.; Faical, L.; Capillary Electrophoretic Separation of inorganic Sulfur-Sulfide, Polysulfides and Sulfur- Oxygen species, *Journal of Separation Science*, 2006, 29, pp 144- 152
23. Takeshige, N.; Jitsuo, T.; Takuzo, Y.; Organic Polysulfides. IV, Synthesis of Bis (triphenylmethyl) *Journal of Organic Chemistry*, 1964, 29, pp.1236-1238
24. Ganesh, K.; Kishore, K.; Studies on Model Oligosulfides to Understand Polysulfide Polymers, *Colloid and Polymer Science*, 1995, 7, pp.648-652
25. Kamyshny, A.; Golfman, A.; Jenny, G.; Dan, R.; Lev, O.; Equilibrium Distribution of Polysulfide Ions in Aqueous Solutions at 250⁰C:A new Approach for the study of Polysulfides Equilibria, *Environmental Science Technology*, 2004, 38, pp. 6633-6644
26. Kamyshny, A.; Ekeltchik, I.; Gun, J.; Lev, O.; Method for the Determination of Inorganic Polysulfide Distribution in Aquatic Systems, *Analytical Chemistry*, 2006, 78, pp. 2631-2639

27. Singer, E.; Mockel, J.; RPLC Retention of 1, ω -di(alkoxy)-polysulfides, *Chromatographia*, 1989, 27, pp. 27-30
28. Karas, M.; Hillenkamp, F.; Laser Desorption Ionization of Proteins with Molecular Masses Exceeding 10,000 Daltons, *Analytical chemistry* 1988, 60, pp. 2299-2301
29. Tanka, K.; Wuli, H.O.; Ido, Y.; Akita, S.; Yoshida, Y.; Protein and Polymer Analysis Up to m/z 100,000 by Laser Ionization Time of Flight Mass Spectrometry, *Rapid communication Mass Spectrometry*, 1988, 2, pp. 151-153
30. Abersold, R.; Mann, M.; Mass Spectrometry –Based Proteomics, *Nature*, 2003, 422, pp 198-207
31. Kathleen Lewis, J.; W., Gary, S., Matrix Assisted Laser Desorption / Ionization Mass Spectrometry in Peptide and Protein Analysis, *Encyclopedia of Analytical Chemistry*, R.A. Meyers (Ed) 2000, pp. 5880-5894
32. Michael, J.; Richard, F.; Wei-Hong, G.; Arwah, J.; Jeffrey, J.; Charles W.; *Applied Spectroscopy Reviews*, 2002, 37, pp. 275-303
33. Hillenkamp, F.; Karas, M.; Holtkemp, K.; P, Klusener; Energy Deposition in Ultraviolet Laser Desorption Mass Spectrometry of Biomolecules, *International Journal of Mass Spectrometry Ion Physics*, 1986, 69, pp. 265- 276
34. Yao, O.; Scott, R.; Young, K.; Wilkins, L; Importance of Matrix : Analyte Ratio for Buffer Tolerance using 2,5-Dihydroxybenzoic Acids as a Matrix in Matrix-Assisted Laser Desorption/Ionization Fourier Transform Mass Spectrometry and Matrix-Assisted Laser Desorption/Ionization–Time of Flight, *Journal of America Society of Mass Spectrometry*, 1998, 9, pp. 805-813

35. Karas, M.; Hillenkamp, F.; *Analytical chemistry* 1988, 60, pp. 2299
36. Pasch, H.; Schrepp, W.; MALDI-TOF Mass Spectrometry of Synthetic Polymer, 2003, pp 1-84
37. Michael, K.; Ralf, K.; Ion Formation in Maldi, the Cluster Ionization Mechanism, *Chemical Review*, 2003, 103, pp. 427-439
38. North, S.; Okafo, G.; Birrell, H.; Hasking, N.; Camilleri, P.; Matrix Assisted Ultraviolet Laser Desorption of Nonvolatile Compounds, *Rapid Communication Mass Spectrometry*, 1999, 11, pp. 1635-1642
39. Fujil, T.; Alkali-Metal Ion Molecule Association Reaction and their Application to Mass Spectrometry, *Mass Spectrometry Review*, 2000, 19, pp. 111- 138
40. Richard, C.; Electrospray and Maldi Mass Spectrometry, Fundamentals, Instrumentation, Practicalities and Biological Application, Second Edition, 2010, pp. 149-175
41. Juan, Z.; Renoto, Z.; Matrix-Dependent Cationization in MALDI-Mass Spectrometry, *Journal of Mass Spectrometry*, 2004, 39, pp. 808-816
42. Julien, De W.; Gaelle, D.; Florian, B.; Oliver, C.; Philippe, D.; Pascal, G.; MALDI-TOF Analysis of Polythiophene: Use of Trans-2-[3-(4-t-butyl-phenyl)-2-Methyl-2-Propenylidene] Malononitrile - DCTB, *Journal of Mass Spectrometry* 2011, 46, pp.237-246
43. Ulmer, L.; Mattay, J.; Torres-Garcia, G.; Luftmann, H.; The Use of Trans-2-[3-(4-t-butyl-phenyl)-2-methyl-2-propenylidene] malononitrile, as a Matrix for Matrix-

Assisted Laser Desorption / Ionization Mass Spectrometry, *European Journal of Mass Spectrometry*, 2000, 6, pp.49-52

Chapter 2 - Synthesis and Analysis of Polysulfides

2.1 Introduction

Inorganic polysulfides which are chains of sulfur atoms with a negative charge on each of the two terminal sulfur atoms [1-3], originate from the oxidation of hydrogen sulfide in water bodies [4, 5]. They tend to be unstable and break down to form thiosalts in the presence of dissolved oxygen in water [6]. Polysulfides can also be converted to organic polysulfides (*e.g.*, dimethylpolysulfane) in these water bodies and are considered unacceptable due to their unpleasant odor [4, 5]. This instability, makes analysis challenging. For example, it has been shown that the rate of transformation from S_4^{2-} to S_5^{2-} is in the order of seconds at room temperature. With spontaneous, rapid changes in speciation, preserving sample composition for analysis of inorganic polysulfides by chromatography and electrophoretic separation are impossible [7]. Alternate methods have been developed for the analysis of inorganic polysulfides directly and more quickly such as potentiometric titration and differential pulse polarography. These methods have not been successful due to the limited range of concentrations that can be measured, lack of information on speciation (total inorganic sulfur is measured), and inaccuracy of the measurements because of potentially invalid assumptions [8, 9]. Electrospray ionization mass spectrometry has also been carried out to determine inorganic polysulfide distribution in aqueous solutions, but lack of standards make meaningful quantification impossible. Adding to the problems with ESI-MS was the presence of high mass

polysulfides, which authors suggest may originate in gas-phase processes occurring in the ionization source or in the mass spectrometer.

Methylation has been proposed as one method to stabilize inorganic polysulfides by converting them to dimethylpolysulfanes. Dimethylpolysulfanes can be determined by high performance liquid chromatography (HPLC) [11] and the dimethylpolysulfanes produced are similar in distribution to that of the inorganic polysulfides [12]. In this report, fractionation of the series of dimethylpolysulfanes was carried out by semi-preparative reversed phase HPLC [13]. Quantification of the amount of sulfur in each fraction (each corresponding to one dimethylpolysulfane) was done by quantitatively converting the dimethylpolysulfane to sulfate by the use of hydrogen peroxide, which is quantitative, followed by the use of ICP-OES.

The main aim of this work has been to develop a fast and robust method of analysis for inorganic polysulfides in real samples (example, from tailings and hydrometallurgical processes), however the lack of commercially-available standards impede this work.. The aim of this work which is the production of standards for polysulfide analysis can be divided into three steps; (I) synthesis and methylation for stabilization, (II) fractionation of the species and (III) quantification of the various derivatized sulfanes [14].

2.2 Materials and Methods

2.2.1 Materials

The reagents used for the synthesis of dimethylpolysulfane were of analytical grade purity or better. Elemental sulfur (sulfur flakes) and sodium phosphate dibasic dodecahydrate (98.1-101) % were both from Sigma–Aldrich Missouri, USA. Hydrazine sulfate (99+ %) was obtained from Alfa-Aesar Lancaster, USA. Methyl iodide (99.5%) copper stabilized was obtained from Alfa- Aesar Massachusetts, USA. Sodium hydroxide in pellets was purchased from EMD chemicals USA. Methanol (99.9%) was purchased from EMD chemicals New Jersey, USA. Dichloromethane and hydrogen peroxide (29.0 - 32.0) % both were obtained from ACS chemicals Montreal, Canada. Distilled, deionized water used was also produced in-house.

2.2.2 Instruments and Settings

All analytical separations were carried out on an Agilent 1100 series high performance liquid chromatograph (HPLC) equipped with UV- vis diode array detector with a Synergi 4- μm , C18 reversed-phase analytical column (150x4.60 mm). Semi-preparatory scale separations were carried out using an HP 1050 series HPLC with a 200- μL sample loop, Luna 5- μm C18 reversed phase column of 250x10.0 mm. An Optima 5300 DV inductively coupled plasma optical emission spectrometry (ICP-OES) from Perkin Elmer was used to quantify sulfur content in samples from the semi-prep purification of the dimethylpolysulfanes; plasma flow rate was 15 Lmin^{-1} , auxiliary flow

rate was 0.5 Lmin^{-1} , sample flow was 1.20 mLmin^{-1} , sample flush time was 17 seconds, wash rate was 2 mLmin^{-1} and wash time was 30 s.

2.2.3 Synthesis and Derivatization

Inorganic polysulfides were first synthesized and then derivatized to dimethylpolysulfane. This was carried out in fume hood by reacting 10 g elemental sulfur, 3 g hydrazine sulfate and 8 g sodium hydroxide and dissolve in 60 mL of deionized water. The reaction was carried out in a three-neck round bottom flask while undergoing magnetic stirring; one neck of the flask served as an inlet for nitrogen gas, the second had a thermometer and the third was for escape of any gas given off during the reaction process. The flask was immersed in a water bath and placed on top of a hot plate for heating; the temperature of the reagents in the flask was controlled between 30°C and 33°C and the contents heated for 3 hours. After heating the resulting solution of inorganic polysulfides was allowed to cool down under a flow of nitrogen gas. The contents of the flask were then poured into a fresh solution consisting of methanol, 40 mL, methyl iodide, 20 mL and 2 g sodium phosphate dodecahydrate. This was allowed to react for 30 minutes at room temperature. The methanol was evaporated under a stream of nitrogen gas leaving the desired dimethylpolysulfane solution.

The dimethylpolysulfane solution thereafter was poured into a separatory funnel containing 40 mL each of distilled water and dichloromethane. The dimethylpolysulfanes were extracted into the dichloromethane and then dried over anhydrous sodium sulfate. The dimethylpolysulfane solution in dichloromethane was stored at -7°C . After 24 hours

the precipitated yellow crystals of sulfur were isolated by decanting the oily supernatant containing the dimethylpolysulfanes. (The procedure used was adapted from the method used *Dan et al.* 2004 and modified by me as I did not evaporate under vacuum, to reduce the loss of dimethylpolysulfane).

2.2.4 HPLC Analysis

HPLC reversed phase C18 analytical and semi-preparative scale columns were used for separation and analysis of the oily solution of dimethylpolysulfanes. It was intended that the analytical scale separation would be used for quantitative analysis but it was also used to investigate the scale up to the semi-preparative separation necessary for preparation of the standards. Separations on the analytical scale was carried out by gradient elution using methanol and water starting with 40 % and increasing to 100 % methanol. The injection volume was 5 μL , with a concentration of dimethylpolysulfane mixture of $\sim 7600 \text{ mg L}^{-1}$ and a flow rate of 1.5 mL min^{-1} ; the gradient used is as shown in Table 2.1.

Table 2.1 HPLC Gradient run used for the separation of dimethylpolysulfane on the reversed phase C18 analytical column

Time (minutes)	% A (H ₂ O)	%B (MeOH)
0	40	60
10	30	70
20	20	80
30	10	90
45	0	100

Isolation of individual dimethylpolysulfane from the mixture was accomplished using the semi-preparative C-18 column, with an injection volume of 200- μ L and concentration of $\sim 20 \times 10^4 \text{ mg L}^{-1}$, flow rate of 3.3 mL min^{-1} , and total run times of ~ 140 min. Gradient elution was with methanol and water starting with 40 % and increasing to 100 % methanol, . The gradient is summarized in Table 2.2. Following exclusion of the void for the column, fractions were collected in 10 mL increments. Each 10-mL fraction collected was divided in half, giving two 5 mL samples. Analytical scale HPLC analysis was carried out on one of the samples using the method standardized for the mixtures.

Table 2.2 Gradient used with the C18 reversed phase semi- preparative HPLC column in the isolation of individual dimethylpolysulfanes

Time (minutes)	% A (H ₂ O)	%B (MeOH)
0	40	60
30	30	70
60	20	80
90	10	90
140	0	100

2.2.5 ICP – OES Analysis

To each reserved 5 mL portions containing the fractionated dimethylpolysulfanes, hydrogen peroxide was added to quantitatively oxidize the sulfur in the dimethylpolysulfanes to sulfate. In the oxidation procedure, NaOH pellets $\sim 0.5 \text{ g}$ were

added to each 5 mL portion containing a single dimethylpolysulfane to raise the pH to ~ 10, followed by addition of 5 mL of 30% (v/v) H₂O₂ and 2 mL of 14 µg mL⁻¹ iron(III) chloride, which acts as a catalyst to ensure complete oxidation. Heat was applied to the resulting solution for 3 minutes at 70 °C; the reaction was carried out in a beaker and then transferred to a measuring cylinder where distilled water was used to make to the 10 mL mark. This solution was analyzed for sulfur content by ICP–OES. Analysis was completed by Dr. Geert Van Biesen and the reported results used to calculate the concentration of the dimethylpolysulfane in each fraction. (Adapted from the FMC Corporation, chemical products group Philadelphia, *The use of Hydrogen Peroxide for the Oxidation of Sulfur Chemical Wastes*, <http://www.fmcchemicals.com>). I applied hydrogen peroxide for the complete oxidation of inorganic polysulfides based on the information gotten in the above publication which gives evidence to show that oxidation of inorganic polysulfides by hydrogen peroxide is quantitative).

2.3 Results and Discussion

2.3.1 HPLC

It has been demonstrated that there is a predictable relationship for the chromatographic retention time of analytes in a homologous series, where for RP columns, without gradient elution, the spacing between peaks increases as chain length increases [14]. A similar trend would be expected for sulfur compounds like the ones under study here. The use of gradient elution gives more regular spacing for a homologous series improving the analysis time and peak shape. As can be seen in the

chromatogram from the analysis of the dimethylpolysulfanes on the analytical column, Figure 2.1, a first analyte peak shows at ~2.7 min, with increasing distance between peaks up to 26.0 min. as the sulfur chain length increased. The trend does seem to be all that regular with the exception of a peak that appears at ~21.0 min.. Since there are no standards for polysulfides or their alkylated analogues, it required some investigation to both establish the identity of the dimethylpolysulfanes and this outlier.

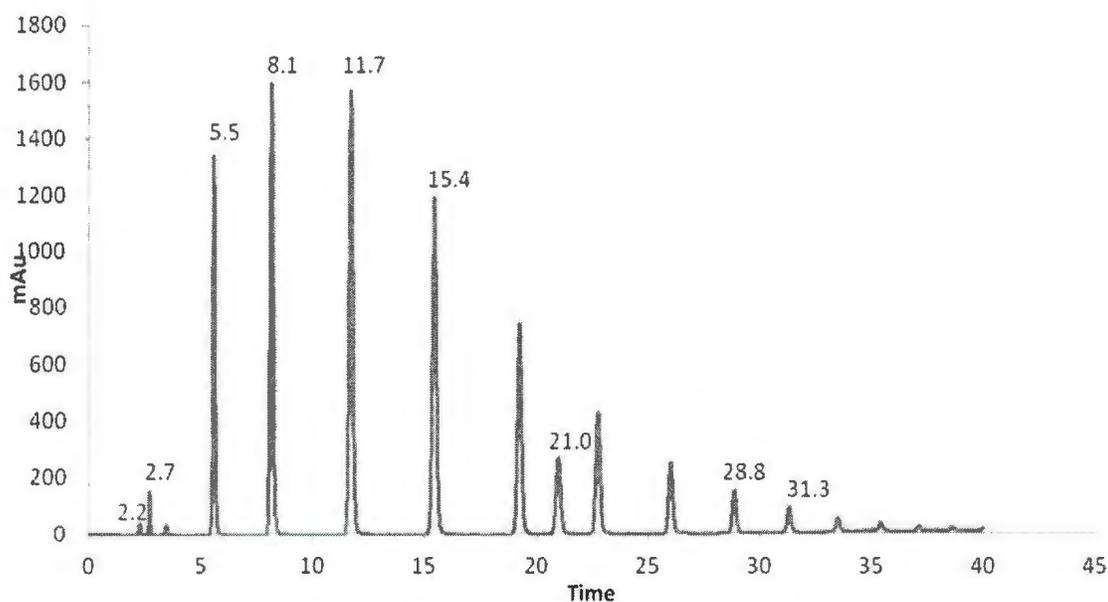


Figure 2.1 HPLC chromatogram of the mixture of dimethylpolysulfane using reversed phase C18, analytical column

In investigating the relationship between the synthesized dimethylpolysulfane homologous series and sulfur chain length, a plot of the retention time against the number of sulfur atoms in the compound associated with each peak was drawn, assuming the second peak in Figure 2.1 which appeared at ~2.7 min is the first chain length for the synthesized dimethylpolysulfane homologous series (dimethyldisulfane), with the first

peak which appeared at ~2.2 min the system peak and that each subsequent peak is for a molecule increased by one sulfur atom. We are also going to assume that the peak which appeared at ~21.0 min and ~22.7 min each contain eight number of sulfur atoms. While this is not exactly the approach normally used in this sort of analysis, it is clear that there is regularity in the graph, corresponding to a nearly straight line ($R^2 = 0.9804$ from linear regression analysis) as seen in Figure 2.2. The plot is not perfectly linear with lower than expected retention times for longer chain lengths which can be attributed to the increasing strength of the organic modifier used as the run progresses, which ends with the organic modifier at 100%.

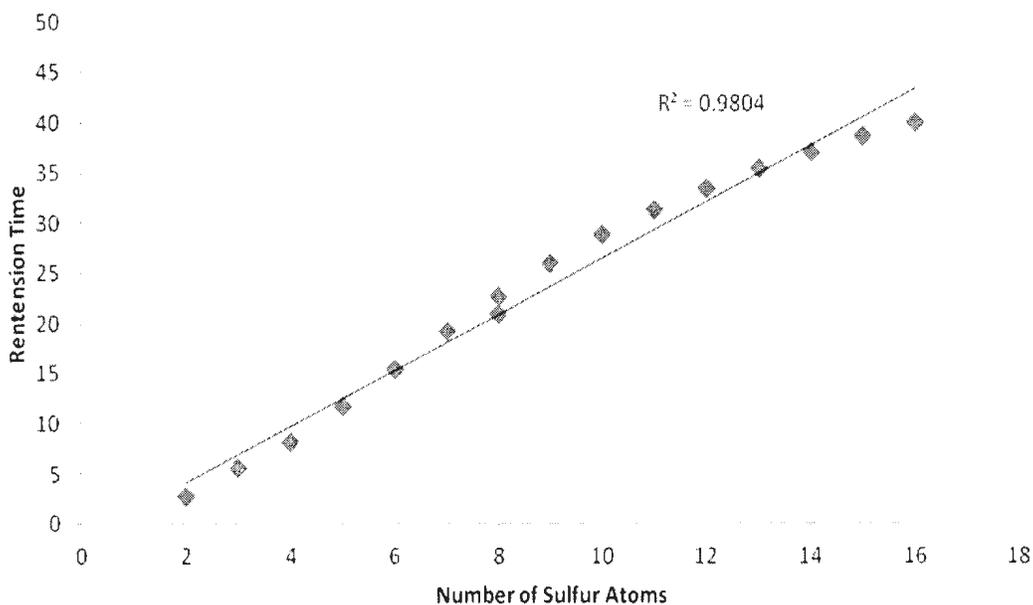


Figure 2.2 Plot of retention time against number of sulfur atoms, using linear regression with ($R^2 = 0.9804$) and both peaks at ~21.0 min and ~22.7 min having eight number of sulfur atoms each.

A second plot was also made, limiting the number of sulfur atoms to between 2 and 8. The peaks appearing at ~21.0 min and those after ~22.7min were not included in the plot and the linear regression line gave a value of $R^2 = 0.9956$ as seen from Figure 2.3. If all conditions are held constant (ideal conditions), the value of R^2 should be equal to 1. This will confirm that homologous series has been synthesized. The value of 0.9956 is close to 1 and method error associated with the use of HPLC may be attributed to the value of R^2 been 0.9956 and not equal to 1. Studies have shown that uncertainty associated with the use of HPLC can be as high as 5% *Barwick et al. 2001* [15]. This also shows that the peak at ~21.0 min is an outlier. The most likely explanation as to the occurrence of the peak at ~21.0 min is that the peak is elemental sulfur (S_8^0) that did not undergo reaction, [1] which is ring opening during the synthesis of inorganic polysulfides.

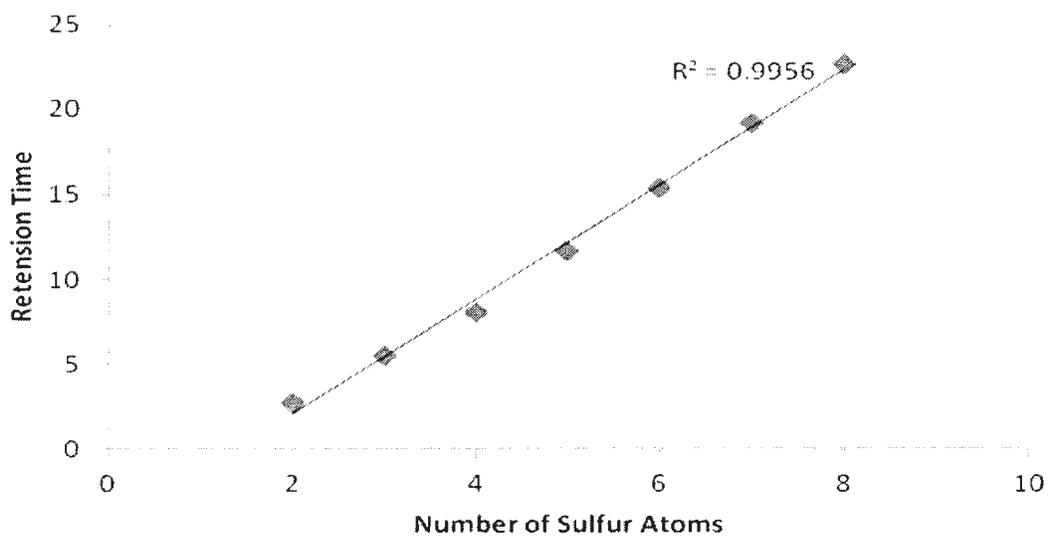


Figure 2.3 Plot of retention time against the number of sulfur atoms (without the peaks at ~21.0 min and those after ~ 22.7 min)

From Figures 2.4–2.7, the isolated fractions from the semi- preparative column were run on the HPLC using the analytical column and the elution time of each fraction corresponded to the time of the same peak in the synthesized dimethylpolysulfanes mixture in Figure 2.1, when the mixture was run using the analytical column and all the same gradient elusion scheme.

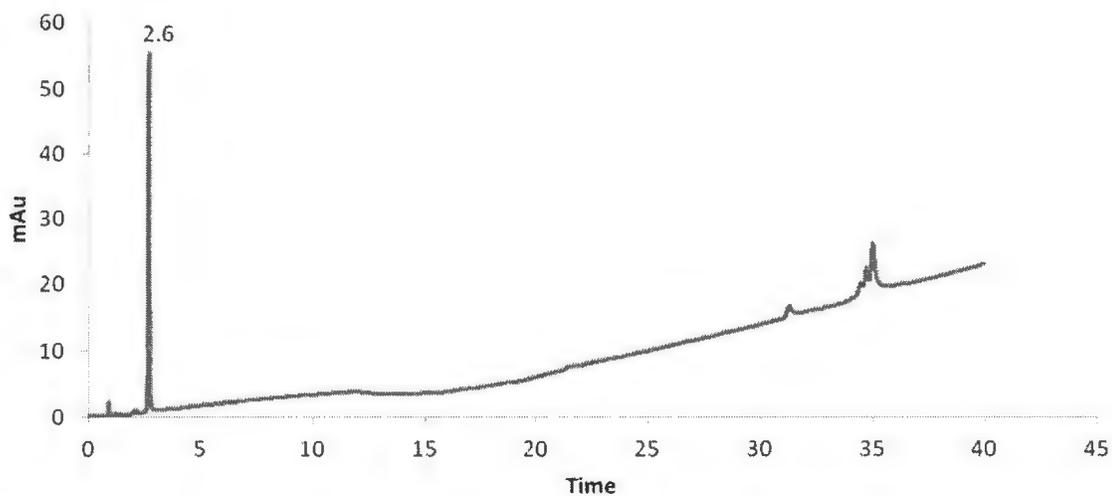


Figure 2.4 Analytical chromatogram of fraction 1 containing 1, 2-dimethyldisulfane ($\text{CH}_3\text{S}_2\text{CH}_3$) after isolation

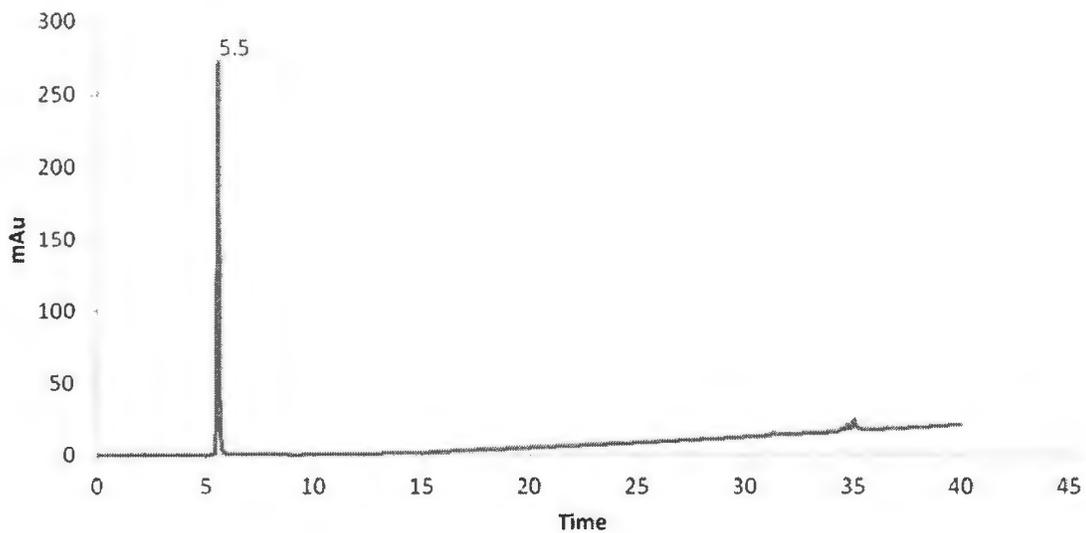


Figure 2.5 Analytical chromatogram of fraction 2 containing 1, 3-dimethyltrisulfane ($\text{CH}_3\text{S}_3\text{CH}_3$) after isolation

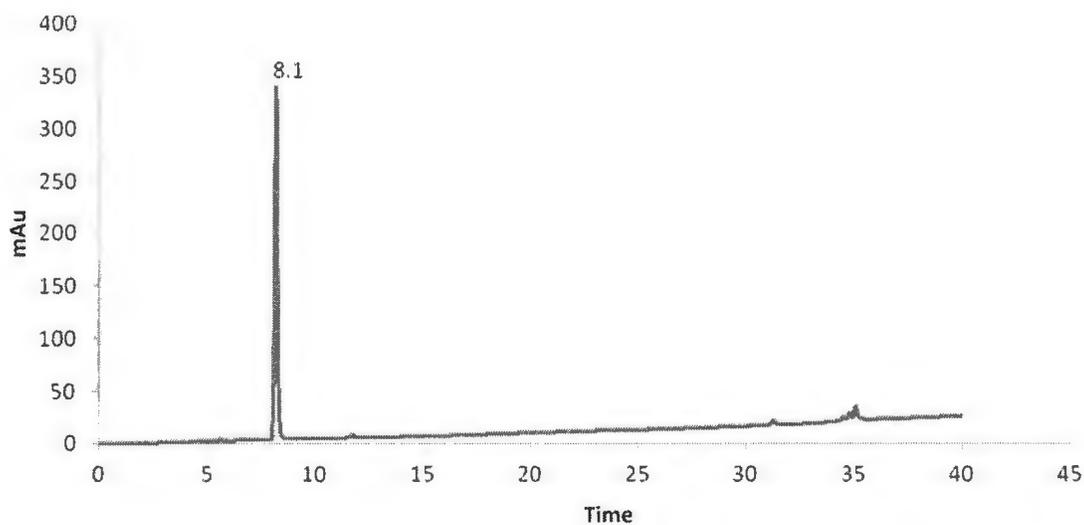


Figure 2.6 Analytical chromatogram of fraction 3 containing 1, 4-dimethyltetrasulfane ($\text{CH}_3\text{S}_4\text{CH}_3$), after isolation

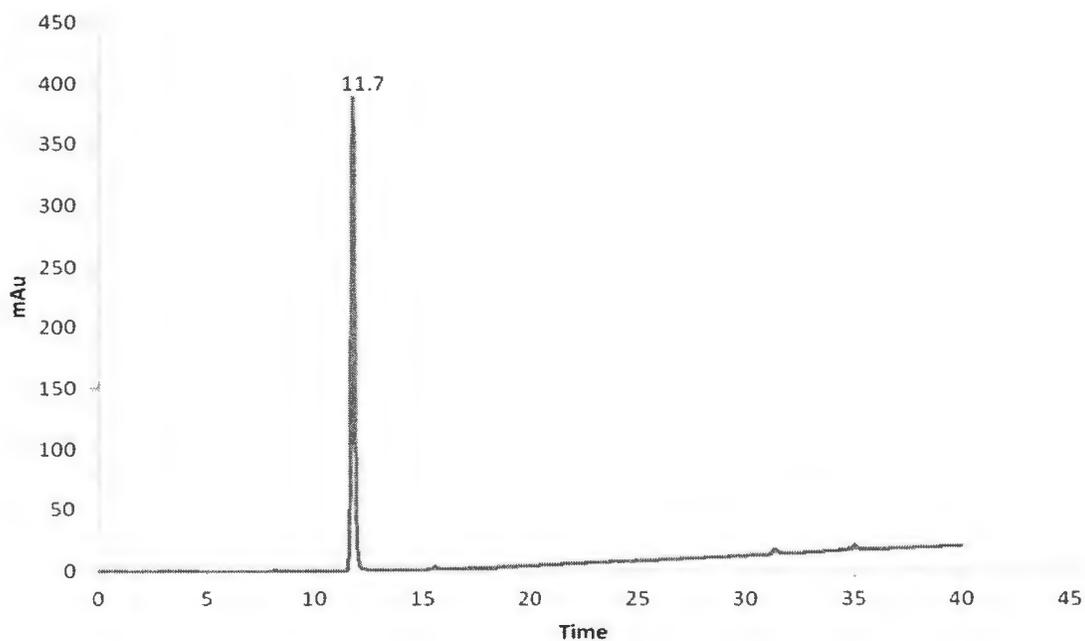


Figure 2.7 Analytical chromatogram of fraction 4 containing 1, 5-dimethylpentasulfane ($\text{CH}_3\text{S}_5\text{CH}_3$) after isolation

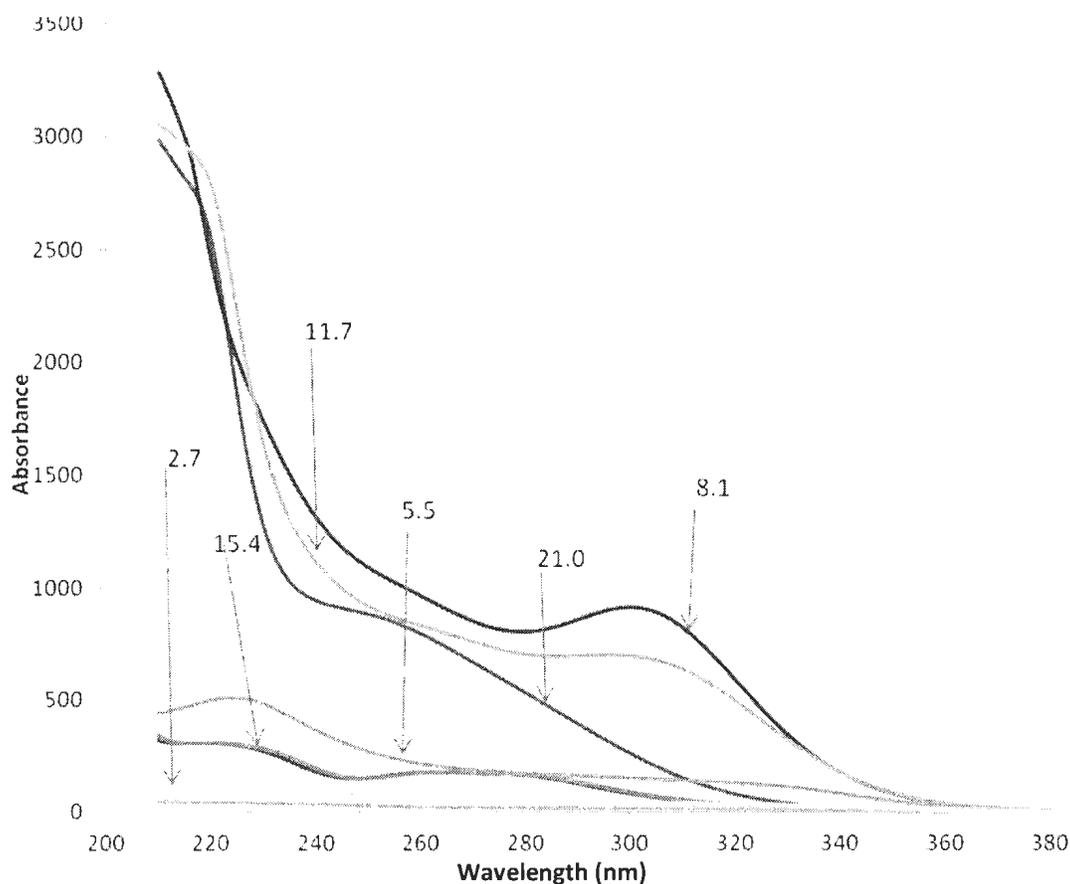


Figure 2.8 UV spectra of the dimethylpolysulfanes with the numbers showing the peak retention times, where spectra for the compound eluting at 21.0 minutes is markedly different with respect to slopes and maxima and is proposed to be S⁰8

Adding support to the observation that the peak at ~21 min is an outlier is the analysis of the UV-vis spectrum of the dimethylpolysulfanes, which shows consistency in the spectral features in all cases except for the peak at ~21.0 min (Figure 2.8). Based on

this evidence, it has will be concluded that a homologous series was synthesized and the peak at~ 21.0 min which is most likely elemental sulfur (S_8^0) should excluded in the fractionation and isolation protocol.

2.3.2 ICP – OES

The ICP – OES was first calibrated before analysis was by preparing known concentrations of sulfide and then oxidizing them to sulfate, whose final concentration was read as sulfur. See Table 2.3 below.

Table 2.3 Calibration values for the ICP-OES used for the analysis

Concentration ($mg L^{-1}$) of sulfide before conversion to sulfate	Concentration ($mg L^{-1}$) of sulfur after conversion to sulfate measured at λ 180.669 nm by ICP–OES
4166	4255
41.25	41.96
27.59	33.56
6.9	10.15

The values of the concentration of sulfur from the different dimethylpolysulfane fractions were used to construct a calibration table as seen in Table 2.4, in order to serve as standards for polysulfide analysis, as measured by ICP–OES, at 180.669 nm wavelength.

Table 2.4 Calibration table showing number of sulfur atoms, their retention times on the analytical chromatographic column, the chromatographic area of each peak, concentration of the various polysulfane as sulfur and the value of the detector response.

Number of S/mole in methylated polysulfides	t_r (min)	Area (mAu s) 5 μ L injection volume	Concentration of sulfur (mg L ⁻¹)	Detector (R) = Area/ mass (μ g)
2*	2.70	2.45×10^2	1	—
3	5.50	1.97×10^3	184	2.14×10^3
4	8.10	3.13×10^3	282	2.22×10^3
5	11.1	4.46×10^3	176	2.80×10^3
6	15.4	3.32×10^3	116	5.72×10^3
7	19.2	2.78×10^3	93.4	5.95×10^3
8 ^{0*}	21.0	9.79×10^3	57.4	34.1×10^3
8*	22.7	2.87×10^3	30.2	19.0×10^3
9*	26.0	3.37×10^2	23.0	29.3×10^3
10	28.8	8.30×10^2	20.7	8.01×10^3
11	31.3	1.44×10^2	3.20	9.04×10^3
12	33.4	2.72×10^2	5.50	9.89×10^3

* represent the sulfur number in the dimethylpolysulfane whose concentration does not follow the trend shown by other components, all sulfur concentration determined at $\lambda = 180.669$ nm

Relative uncertainty for this ICP-OES method is 2%; due to insufficient sample numbers, time and cost of analysis, the statistics were not determined for each of the polysulfides.

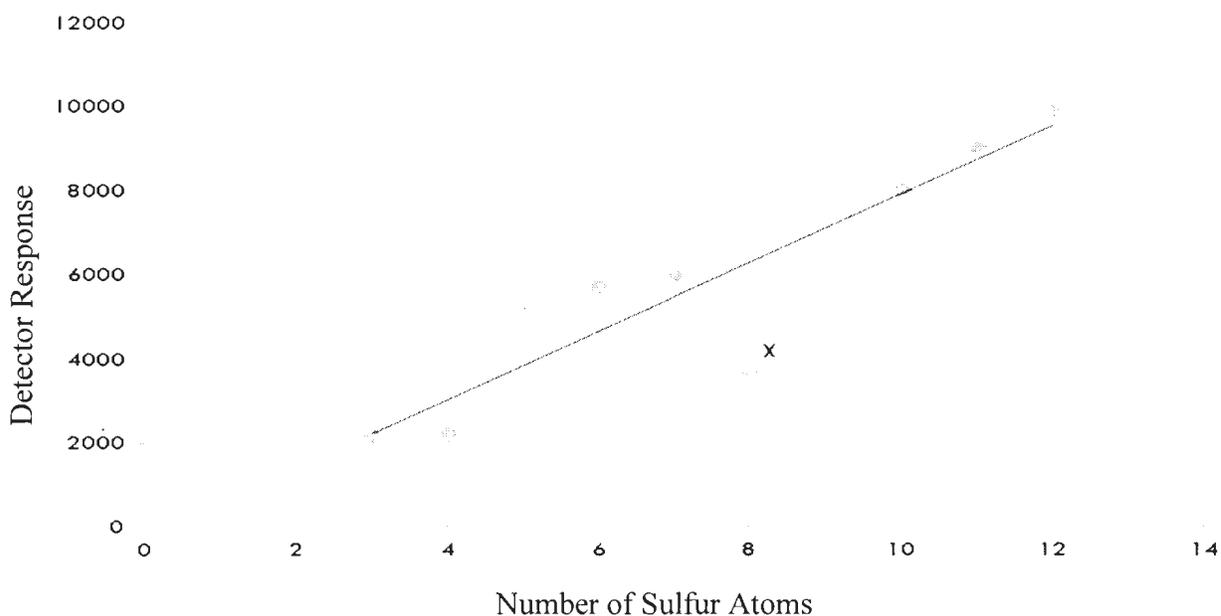


Figure 2.9 Plot of UV-vis detector response (area / mass) against the number of sulfur atoms, with X representing the peak which appeared at ~21.0 min, which is thought to be elemental sulfur (S_8^0)

The plot of the UV-vis detector response against number of sulfur atoms is non-linear. This is may be attributed to the uncertainty in the data from the ICP-OES, as during the oxidation of the polysulfides to sulfate losses occur due to incomplete oxidation or losses in manipulations for the samples. The relative standard deviation from the chromatogram peak is $\pm 7.2\%$ based on 5- μL injection volume, which is reasonable as the uncertainty typically associated with HPLC, according to *Barwick et al. 2001* [15], is $\pm 5\%$. In addition to measurement uncertainty, trends in absorptivity may be perturbed by special electronic and conformational features for particular analogues, examples S_4 and S_8 which stand out as being the most abundant allotropes. The detector response is

related to increase sensitivity (molar absorptivity) due to more delocalization of electrons with sulfur chains.

From the analysis of the experimental results, it is clear that a homologous series was synthesized and there is good correlation between the number of sulfur atoms and chromatographic retention time for $n = 2-8$. The isolated fractions from the semi-preparative column were run on the analytical column using the same gradient elution scheme used for analysis of the dimethylpolysulfanes mixtures. The retention time for each isolated dimethylpolysulfane fraction corresponded with the elution time of each peak in the dimethylpolysulfanes mixture (Figures 2.1-2.5), this shows that the fractionation method is satisfactory.

2.3.3 Stability Studies

The main purpose for derivatizing the inorganic polysulfides was to improve their stability for analysis, speciation determination and to allow for isolation of their standards. Ultimately, it is necessary to demonstrate that this derivatization would help to stabilize the polysulfides and maintain the integrity of the samples with respect to speciation. The first step in proving the suitability of this approach is to carry out stability studies on the synthesized dimethylpolysulfanes in dichloromethane and also on the isolated fractions in the water and methanol mixture. The results shows that the mixture of dimethylpolysulfanes in dichloromethane are stable for close to 2 weeks when stored at -7°C or colder, while the fractions in water/methanol with 2 to 4 sulfur atoms per dimethylpolysulfane chain length were stable for only 3 days when stored at -29.9°C .

Fractions with 5 to 13 sulfur atoms per dimethylpolysulfane chain are very unstable and break down within hours of their isolation in the water/methanol mixture. Table 2.5 shows the change in speciation of dimethylpolysulfane mixture in dichloromethane with time.

Table 2.5 Changes in speciation of sulfur in dimethylpolysulfane with time, where the second analysis is of the same samples but after 2 weeks in dichloromethane

Peak Identification	Peak Area* from HPLC on Analytical Column (mAu·s)	
	Analyzed on 2/ 29/ 2012	Analyzed on 3/13/2012
CH ₃ S ₂ CH ₃	0.35 x 10 ³	0.63 x 10 ³
CH ₃ S ₃ CH ₃	11.30 x 10 ³	8.83 x 10 ³
CH ₃ S ₄ CH ₃	17.46 x 10 ³	13.67 x 10 ³
CH ₃ S ₅ CH ₃	21.05 x 10 ³	17.07 x 10 ³
CH ₃ S ₆ CH ₃	17.10 x 10 ³	14.09 x 10 ³
CH ₃ S ₇ CH ₃	11.08 x 10 ³	9.52 x 10 ³
S ₈ ⁰	4.33 x 10 ³	3.20 x 10 ³
CH ₃ S ₈ CH ₃	6.46 x 10 ³	5.45 x 10 ³
CH ₃ S ₉ CH ₃	3.72 x 10 ³	3.34 x 10 ³
CH ₃ S ₁₀ CH ₃	2.19 x 10 ³	2.04 x 10 ³
CH ₃ S ₁₁ CH ₃	1.50 x 10 ³	1.99 x 10 ³
CH ₃ S ₁₂ CH ₃	0.79 x 10 ³	1.43 x 10 ³
CH ₃ S ₁₃ CH ₃	0.46 x 10 ³	1.91 x 10 ³

*The area of each peak is proportional to the concentration of each dimethylpolysulfane.

Based on peak area, the concentration of the dimethyldisulfane ($\text{CH}_3\text{S}_2\text{CH}_3$) increases after 2 weeks, while the concentrations of the other species, ($\text{CH}_3\text{S}_3\text{CH}_3$ – $\text{CH}_3\text{S}_{10}\text{CH}_3$) decreases. This shows that disproportionation has taken place, which has resulted in the increase of the chromatographic area hence concentration of dimethyldisulfane. The greatest change occurred in the dimethylpentasulfane ($\text{CH}_3\text{S}_5\text{CH}_3$) as seen in Table 2.5 above.

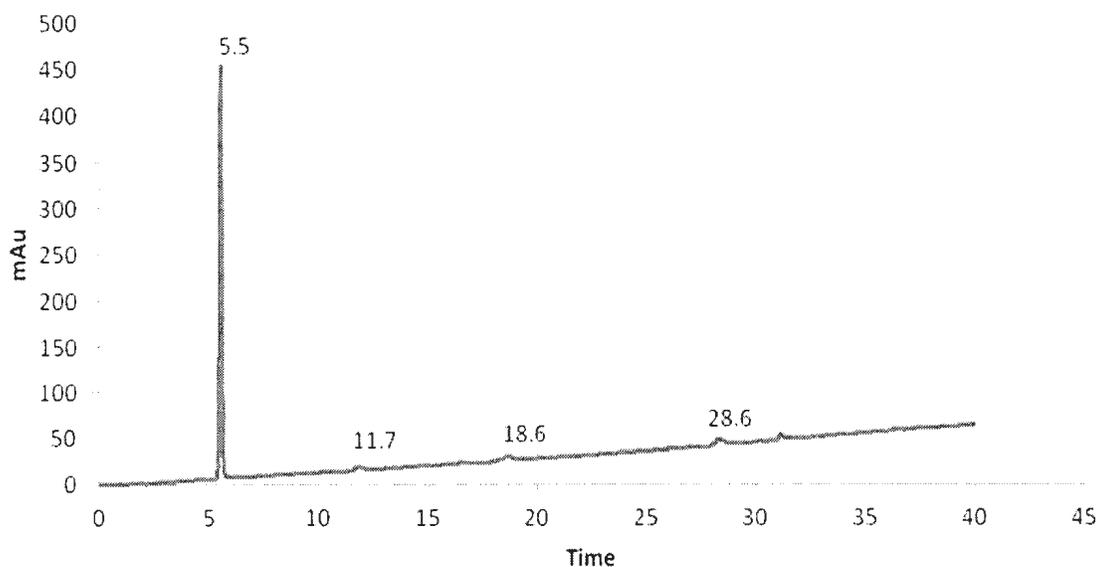


Figure 2.10 Change in speciation of an isolated fraction of dimethylpolysulfane

Dimethylpolysulfanes undergo change in speciation with time just like the inorganic polysulfides but at a much slower rate. This is seen from Figure 2.12 where

isolated 1,3-dimethyltrisulfane in water/methanol mixture undergo change in speciation after 3 days of isolation. It was stored at -29.9°C .

2.4 Conclusions

A robust analytical method has being developed for the determination and quantification of inorganic polysulfides. A calibration table has also been produced from which the concentrations of different inorganic polysulfide chain length can be calculated. This will be useful for real sample analysis from tailings and water bodies. Methylated polysulfides can be stored for up to 2 weeks in dichloromethane at temperatures of -7°C or lower, while the isolated fractions with 2-4 sulfur atoms per chain length in water/methanol mixture are stable for only 3 days at -29.9°C though higher number sulfurs chain lengths break down within hours of their isolation in the water/methanol mixture.

From the calibration of the ICP-OES, the accuracy, precision and sensitivity of the instrument shows that it is not sufficient, and the method should be investigated and improved for application to samples of the type analyzed here. Improvement in the design of the ICP-OES method will help improve its accuracy, precision and sensitivity for analysis of sulfur compounds associated with industrial processes. The percentage uncertainty associated with the ICP-OES data is 2.0%.

2.5 References

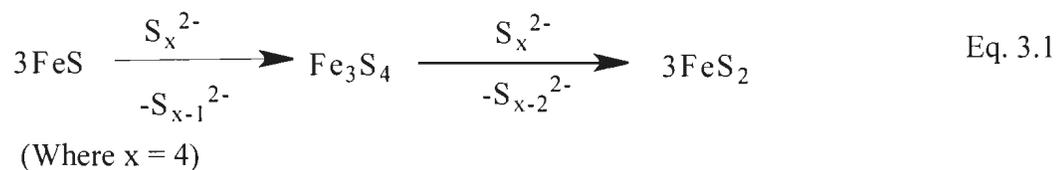
1. Theodore, I. B.; Lemay, H. E.; Bruce, E. B.; Murphy, J. C.; Woodward, P. M.; *Chemistry the central science*, 2012, 12th Edition, pp. 305 – 309
2. Mark, D.; Thomas, R.; Transition Metal Polysulfide Coordination Compounds with Purely Inorganic Chelate Ligands, *Angewandte Chemie International Edition, England*, 1985, 24, pp. 742 - 757
3. Murthy, A. V.; Zhou, W.; Characterization of a Transient +2 Sulfur Oxidation State Intermediate from the Oxidation of Aqueous Sulfide, *ACS Symposium Series Volume*, April 1995, pp.1-8
4. Catalin, F.; Faical, L.; Capillary Electrophoretic Separation of Inorganic Sulfur-Sulfide, Polysulfides and Sulfur-Oxygen Species, *Journal of Separation Science*, 2006, 29, pp 144- 152
5. Chen, Y.; Morris, J.; Kinetics of Oxidation of Aqueous Sulfide by Oxygen, *Environmental Science Technology*, 1972, 6, pp. 529-537
6. Steudel, R.; Holdt, G.; Nagorka, R.Z.; *Zeitschrift für Naturforschung B*, 1986, 41, 1519 – 1522
7. Kamyshny, A., Jr.; Golfman, A.A.; Rizkov, D.; Lev,O.; Kinetics of Disproportionation of Inorganic Polysulfides in Undersaturated Aqueous Solutions at Environmentally Relevant Conditions, *Aquatic Geochemistry*, 2003, 9, pp. 291 - 304
8. Kariuki, S.; Morra, J. M.; Umiker, J.K.; Cheng, I. f.; Determination of Total Ionic Polysulfides by Differential Pulse Polarography, *Analytical Chimica Acta*, 2001, 442, pp. 277–285

9. Hiromu, S.; Takeo, H.; Ikeda, S.; The Rapid Determination of Sulfide, Thiosulfate and Polysulfide in the Lixiviation Water of Blast-Furnace Slag by means of Argentimetric Potentiometric Titration, *The Chemical Society of Japan*, 1981, 54, pp. 1968-1971
10. Gun, J.; Modestov, A. D.; Kamyshny, A.; Ryzkov, D.; Gitis, V.; Grischek, T.; Worch, E.; ESI-MS Analysis of Aqueous Polysulfide Solution, *Microchimica Acta* 2004, 146, 229 – 237
11. Dan, R.; Lev, O.; Jenny, G.; Boris, A.; Ilya, K.; Development of In-house Reference Materials for The Determination of Inorganic Polysulfides in Water , *Accreditation and Quality Assurance*, 2004 , 9, pp. 399-403
12. Kamyshny, A.; Golfman, A.; Jenny, G.; Dan, R.; Lev, O.; Equilibrium Distribution of Polysulfide Ions in Aqueous Solutions at 250°C: A new Approach for the study of Polysulfides Equilibria, *Environmental Science Technology*, 2004, 38, pp. 6633-6644
13. The Use of Hydrogen Peroxide for The Oxidation of Sulfur Chemical Waste by FMC Corporation, Chemical Products Group, Environmental Business, Philadelphia, USA
14. Ute, M.; Mockel, N. J.; Nelsen, S. F.; Retention of 1,2-Bis(Dialkylamino)-Polysulfides and Related Compounds in Reverse-Phase HPLC, *Chromatographia*, 1989, 27, 2/3
15. Barwick, V. J.; Ellison, S. L. R.; Lucking, C. L.; Burn, M. J, *Journal of Chromatography A*, 2001, 918, pp. 267-276

Chapter 3 - Synthesis and Analysis of Dimethylpolysulfanes using HPLC and GC-MS

3.1 Introduction

Polysulfides are chains of zero valent sulfur atoms linked together to terminally (-1) charged sulfur atoms [1-4] Polysulfides take part in many environmental processes such as the formation of pyrite, which occurs by oxidation of iron monosulfide. This oxidation involves the zero valent sulfur atoms present in the polysulfide chain length as seen in equation 3.1 [5].



Sulfides and polysulfides are important because these compounds are both industrially and environmentally relevant. Aqueous polysulfides play a very important role in the area of photoelectrochemical solar cells. The photoelectrochemical solar cells where they serve as a source of energy in a cadmium chalcogenide/aqueous polysulfide photoelectrochemical solar thin-film cell (Cd/X/S_x²⁻PEC) [6]. Metal/molten sulfur batteries (containing aluminum) are also attractive for electrochemical energy storage. The ability of polysulfide to serve as high storage capacity battery is associated with the

zerovalent sulfur present in the polysulfide [7]. Polysulfides also present a problem for domestic and industrial wastewater treatment where metal sulfides present as deposits on the walls of pipes in drinking water distribution systems can form polysulfides. Studies have shown that polysulfides are as much as 79 mg kg^{-1} dry weight of these deposits and polysulfides react with necessary disinfectant residuals in treated drinking water [8]. Polysulfides also consume dissolved oxygen which impacts water quality [9]. Derivatives of polysulfides also play a vital role in the tire and rubber industry, where they are used as coupling and cross-linking agents [10]. For example, bis[3-(triethoxysilyl)propyl] tetrasulfide is used to chemically modify the surface of silica when mixed with rubber to improve dispersion and to chemically link the silica with the structure of the rubber. This leads to a highly stable crosslinked structure with the added mechanical stability associate with the silica.

When they occur in the environment, polysulfides are of interest because of the products they form. For example, a study carried out by Stuart Licht *et al.* in 1996 [11], shows that aqueous polysulfide solutions are unstable within the temperature range of 25°C - 85°C , where they break down to form thiosulfate ($\text{S}_2\text{O}_3^{2-}$), which is implicated in acidification of natural waters [11]. Polysulfides, when present in waters that contain organic compounds, react with these compounds to form odorous volatile organic sulfur compounds (VOSC), which can be unpleasant and potentially toxic.

Different analytical methods have being used to determine the presence of polysulfides in aquatic systems, but because they undergo changes in speciation, most

methods focus on the measurement of total sulfur, total sulfides or total polysulfides. Adding to the complexity of the problem is the lack of polysulfide standards, which makes generating accurate quantitative data for polysulfides difficult [12]. Analytical methods that have been used for polysulfide species include HPLC-UV analysis, where quantification is a problem without standards [13], and potentiometric detection of the titration endpoint in a method that measures sulfide, with the assumption that polysulfide is made up of two components, S^{2-} sulfide sulfur and S_x . Headspace solid-phase microextraction GC-MS analysis has been used for dimethylpolysulfanes analysis but it's labor intensive [14]. The work presented in this chapter introduces a method for analysis of dimethylpolysulfanes, using HPLC, GC-MS (without headspace or solid-phase microextraction) and also describes the efforts to characterize these compounds using MALDI-TOF MS.

3.2 Materials and methods

3.2.1 Materials

Details regarding these are given in Section 2.2.1.

3.2.2 Instruments and Settings

All analytical separations were carried out on an Agilent 1100 series HPLC equipped with UV- vis diode array detector with a Synergi 4- μ m, C18 reversed-phase analytical column (150x4.60 mm). Semi-preparatory scale separations were carried out using an HP 1050 series HPLC with a 200- μ L sample loop, Luna 5- μ m C18 reversed

phase column of 250x10.0 mm. An Applied Biosystems/MDS Sciex (Toronto, ON) 4800 Plus matrix-assisted laser desorption ionization- time of flight mass spectrometer (MALDI-TOF MS) was used in positive ion mode with reflector engaged when analyzing the sample mixtures. The separations by GC were done using helium as carrier gas with a flow velocity of 36.0 cm sec⁻¹, with (Agilent 6890) column 30 m x 0.25 mm ID, DB-5 and film thickness 0.25 µm. For MS, the compounds were ionized using electron ionization at 70 eV, and the mass analyzer was in full scan mode where spectra were collected from *m/z* 50- 550.

3.2.3 Synthesis and Derivatization

Inorganic polysulfides were synthesized and then derivatized to dimethylpolysulfane. The same procedure as was done in sections 2.2.3 was carried out here, for synthesis and methylation.

3.3 Analysis

After synthesis, the oily mixture of dimethylpolysulfanes was diluted with dichloromethane (1:25). HPLC was first used for the analysis of the mixture and isolated fractions of dimethylpolysulfanes. For analysis of the mixture, HPLC-UV was carried out, while the semi-preparative column was used for the separation and isolation of fractions of individual dimethylpolysulfanes. Both the mixture and isolated fractions of the dimethylpolysulfane mixture were analyzed using GC-MS, in which case a small amount of the diluted sample (mixture or fractions) was placed in a 1.0-mL vial before being placed in the sample holder for GC-MS analysis. The injector port temperature was

lowered to 25°C to prevent breakdown of the dimethylpolysulfanes. MALDI-TOF MS was also used for analysis of the dimethylpolysulfanes.

3.3.1 HPLC Analysis

HPLC reversed phase C18 analytical and semi-preparative scale columns were used for separation and analysis of the oily solution of dimethylpolysulfane. It was intended that the analytical scale separation would be used for the quantitative analysis, but it was also used to investigate the scale up to the semi-preparative separation necessary for preparation of standard. Separations on the analytical scale were carried out by gradient elution using methanol and water starting with 40 % and increasing to 100 % methanol, the injection volume was 5 μL , the concentration of dimethylpolysulfane mixture was $\sim 7600 \text{ mg L}^{-1}$ and the flow rate was 1.5 mL min^{-1} . The gradient used is as shown in Table 3.1.

Table 3.1 HPLC Gradient run used for the separation of dimethylpolysulfane on the reversed phase C18 analytical column

Time (minutes)	% A (H ₂ O)	%B (MeOH)
0	40	60
10	30	70
20	20	80
30	10	90
45	0	100

Isolation of individual dimethylpolysulfanes from the mixture was accomplished using the semi-preparative C-18 column, with an injection volume of 200- μ L and concentration of $\sim 20 \times 10^4$ mg L⁻¹ of total polysulfides. The analytes were eluted using a gradient of methanol and water (Table 3.2) at a flow rate of 3.3 mL min⁻¹; where the gradient started with 40 % methanol and increased to 100 % methanol. Following exclusion of the void for the column, fractions were collected in 10-mL increments, with a total run time of \sim 140 min.

Table 3.2 Gradient used with the C18 reversed phase semi- preparative HPLC column in the isolation of individual dimethylpolysulfanes

Time (minutes)	% A (H ₂ O)	%B (MeOH)
0	40	60
30	30	70
60	20	80
90	10	90
140	0	100

Each 10-mL fraction collected was divided in half, given two 5-mL samples. Analytical scale HPLC analysis was carried out on one sample using the method standardized for the mixtures.

3.3.2 GC-MS Analysis

GC-MS was used because it was an effective way to carry out MS analysis of the dimethylpolysulfane species in a mixture. Below is the GC-MS temperature programme run used for the analysis of the mixture of dimethylpolysulfanes. The temperature program starts at nearly ambient conditions (i.e. 25⁰C) to allow for the detection of the smaller mass volatile species. It should also be noted that the larger dimethylpolysulfanes are thermally labile and thus cannot be analyzed by this GC-MS system, this confirms that what was synthesized was dimethylpolysulfanes and also its a homologous series.

Table 3.3 GC temperature used for the analysis of the mixture of dimethylpolysulfanes

	Temperature (°C)	Hold Time (min)
Initial Temperature	25°C	2
Temperature ramp #1	5°C/min	10
Final Temperature # 1	75°C	2
Temperature ramp # 2	10°C /min	6
Final Temperature # 2	135°C	2
Temperature ramp # 3	15°C /min	12
Final Temperature # 3	315°C	3

3.3.3 MALDI - TOF Analysis

The dimethylpolysulfanes produced were also analyzed by MALDI-TOF MS. The matrix used was anthracene in dichloromethane at a concentration of $793 \mu\text{g mL}^{-1}$ with a volume ratio of 1:5 for dimethylpolysulfane in dichloromethane to matrix. A $5 \mu\text{L}$ portion of the mixture was spotted on the stainless steel sample plate and allowed to air dry in the fumehood before being placed in the MALDI - TOF instrument.

3.4 Results and Discussion

3.4.1 HPLC

Analysis was carried out first by HPLC using an analytical column for the separation of the synthesized dimethylpolysulfanes. The chromatogram from the run is shown in Figure 3.1 below. The more or less regular increase in retention time for the eluted species shows that the peak at ~ 21.0 min which is an outlier. This indicates that a homologous series was synthesized. This was followed by isolation and fractionation of the dimethylpolysulfanes using the semi-preparative column and the two fractions are as shown in Figures 3.2 and 3.3 below.

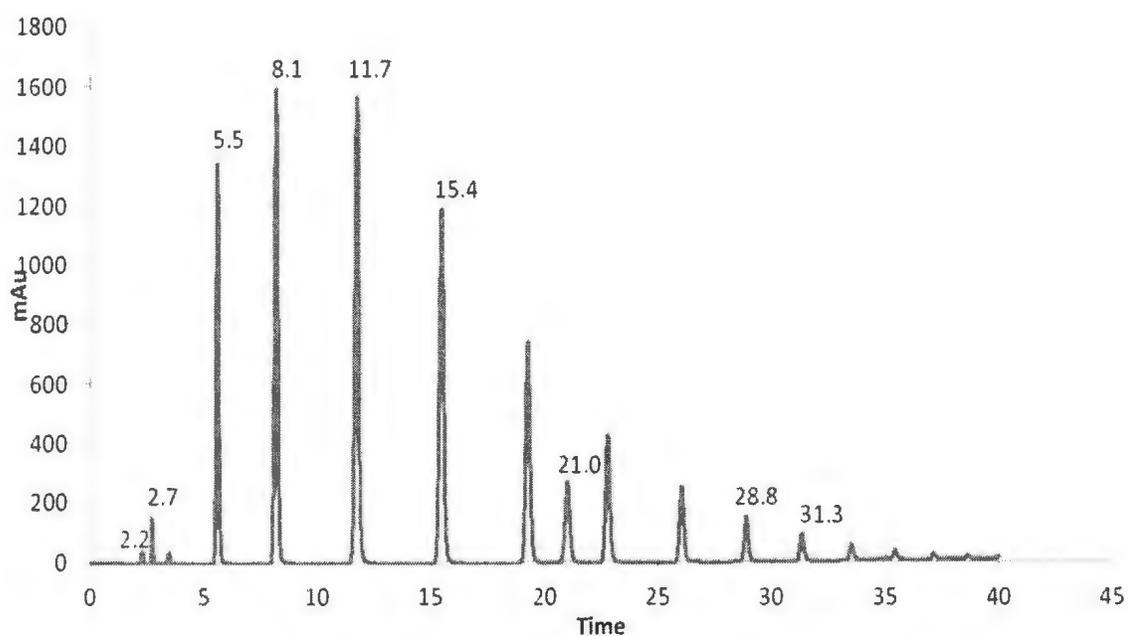


Figure 3.1 HPLC chromatogram of the mixture of dimethylpolysulfane using reversed phase C18, analytical column.

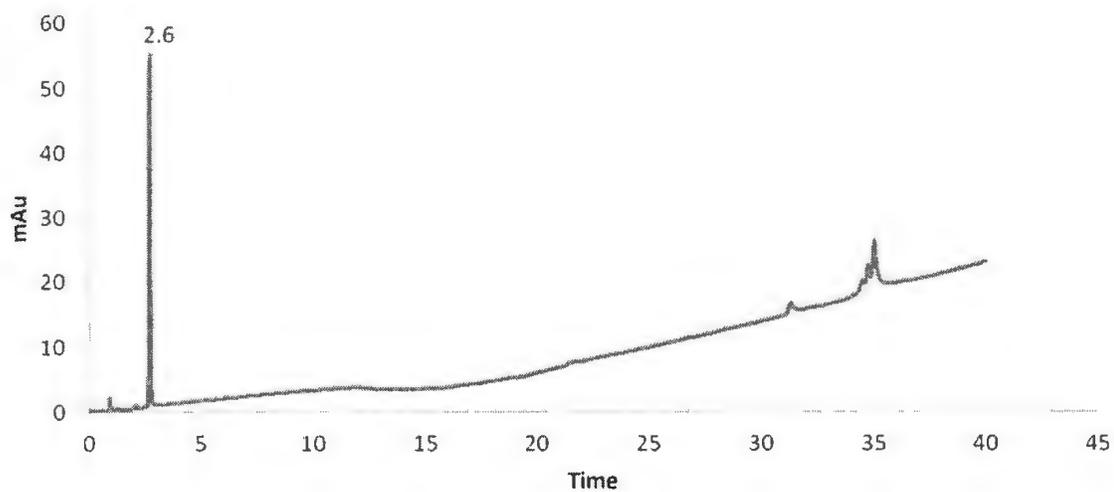


Figure 3.2 Chromatogram of fraction 1 containing 1, 2-dimethyldisulfane ($\text{CH}_3\text{S}_2\text{CH}_3$) after isolation

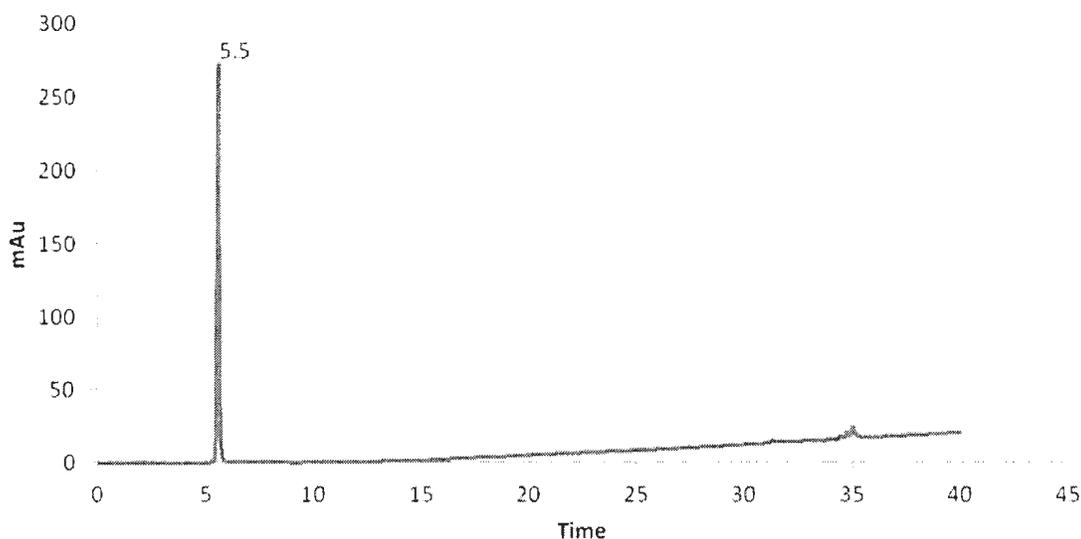


Figure 3.3 Chromatogram of fraction 2 containing 1, 3-dimethyltrisulfane ($\text{CH}_3\text{S}_3\text{CH}_3$) after isolation

3.4.2 GC-MS

Figure 3.4 below shows the chromatogram for the analysis of the mixture of dimethylpolysulfanes using the GC-MS; the conditions for the analysis were optimised to ensure reproducibility. This includes reducing the injector port temperature to as low as 5°C as the initial port temperature and having a heating rate of 3°C min^{-1} , ensured good separation of the dimethylpolysulfanes. The tailing of the peaks that were seen when the injector port temperature was 100°C and 60°C were eliminated by this optimized conditions. The first four dimethylpolysulfanes were separated and confirmed while the fifth chain length could not be ascertain with certainty as this could be the result of decomposition of other products when identified by the MS . The longer chain lengths are

thermally labile and probably breakdown in the injector. Isolated fractions of the dimethylpolysulfanes from using semi-preparative HPLC could not be analysed by the GC-MS.

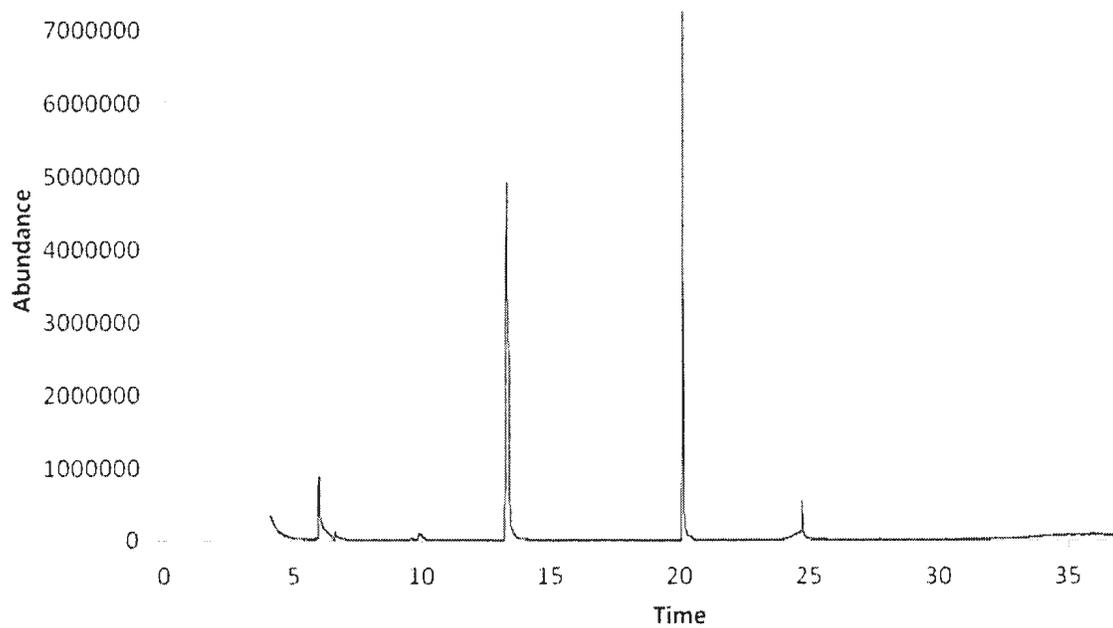


Figure 3.4 Total ion chromatogram of the synthesized dimethylpolysulfanes

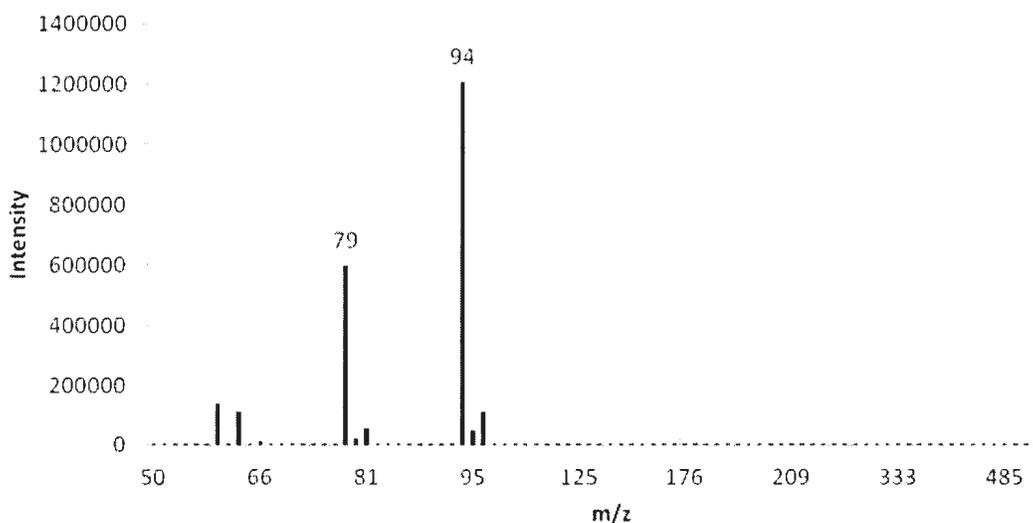


Figure 3.5 Mass spectrum of the peak that was eluted at 6.0 minutes from the GC with m/z 94 being characteristic of $\text{CH}_3\text{S}_2\text{CH}_3$ (dimethyldisulfide)

Each peak from the total ion chromatogram in Figure 3.4 was analyzed to confirm their m/z by mass spectrometry. Figure 3.5 above shows the mass spectrum of the first peak that was eluted at 6.0 min and which shows m/z 94 as the molecular ion or base peak and the m/z ratio of 94 corresponds to dimethyldisulfane ($\text{CH}_3\text{S}_2\text{CH}_3$). The fragmentation pattern goes to confirm this. The mass 79 represents the cationic radical $[\text{CH}_3\text{S}_2]^{+\bullet}$ which results from the break down or fragmentation of the molecular ion.

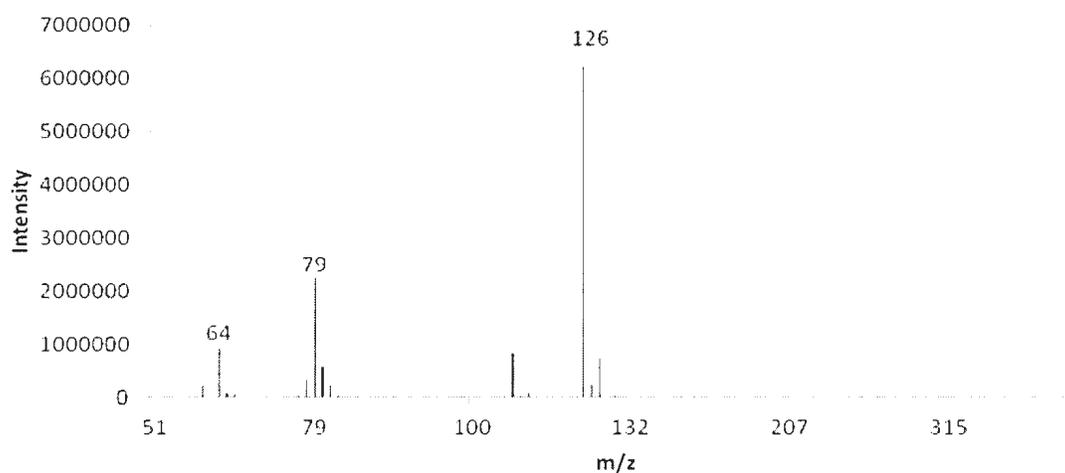


Figure 3.6 Mass spectrum of the peak that was eluted at 13.2 minutes from the GC chromatogram with m/z 126 characteristic of $\text{CH}_3\text{S}_3\text{CH}_3$ (dimethyltrisulfane)

The second peak which was eluted at 13.2 min from Figure 3.4 was also analyzed by the mass spectrometer to confirm its identity. The base peak at m/z 126 seen in the mass spectrum in Figure 3.6 corresponds to the mass of dimethyltrisulfane ($\text{CH}_3\text{S}_3\text{CH}_3$). The fragmentation pattern confirms this. Ions at m/z 79 and 64 represent the cationic radical $[\text{CH}_3\text{S}_2]^+$ and S_2^+ , which result from the fragmentation of the molecular ion.

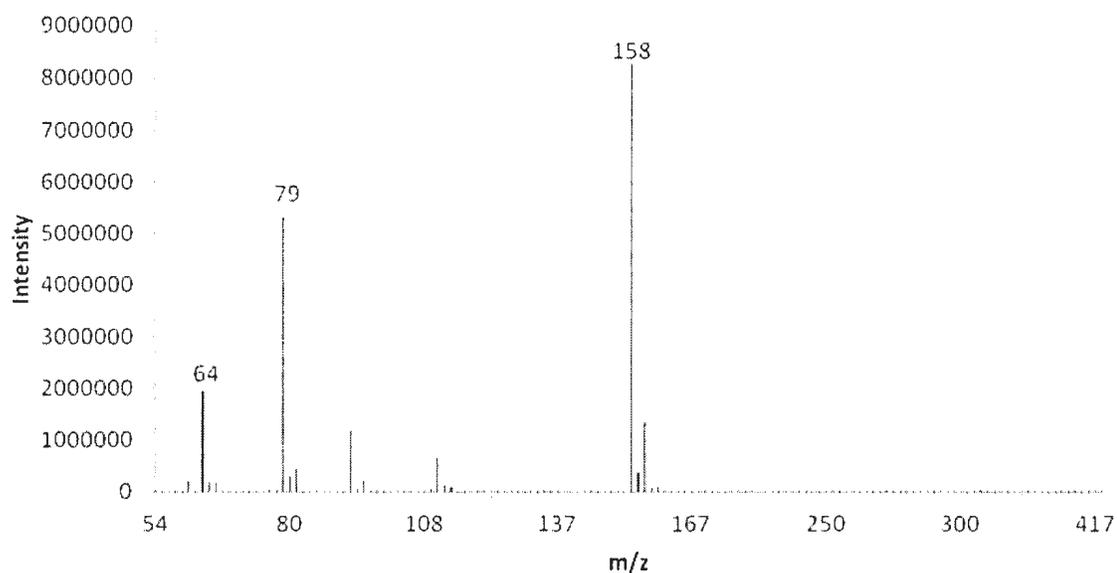


Figure 3.7 Mass spectrum of the peak that was eluted at 20.1 minutes from the GC with m/z 158 characteristic of $\text{CH}_3\text{S}_4\text{CH}_3$ (dimethyltetrasulfane)

The mass spectrum shown in Figure 3.7 of the peak appearing at 20.1 min (Figure 3.4) shows an ion at m/z 158, which is the mass of $\text{CH}_3\text{S}_4\text{CH}_3$ (dimethyltetrasulfane). The peaks from its fragmentation that have masses of m/z 79 and m/z 64 (the cationic radical $[\text{CH}_3\text{S}_2]^{+\cdot}$ and $\text{S}_2^{+\cdot}$) confirms that the molecule is $\text{CH}_3\text{S}_4\text{CH}_3$ (dimethyltetrasulfane).

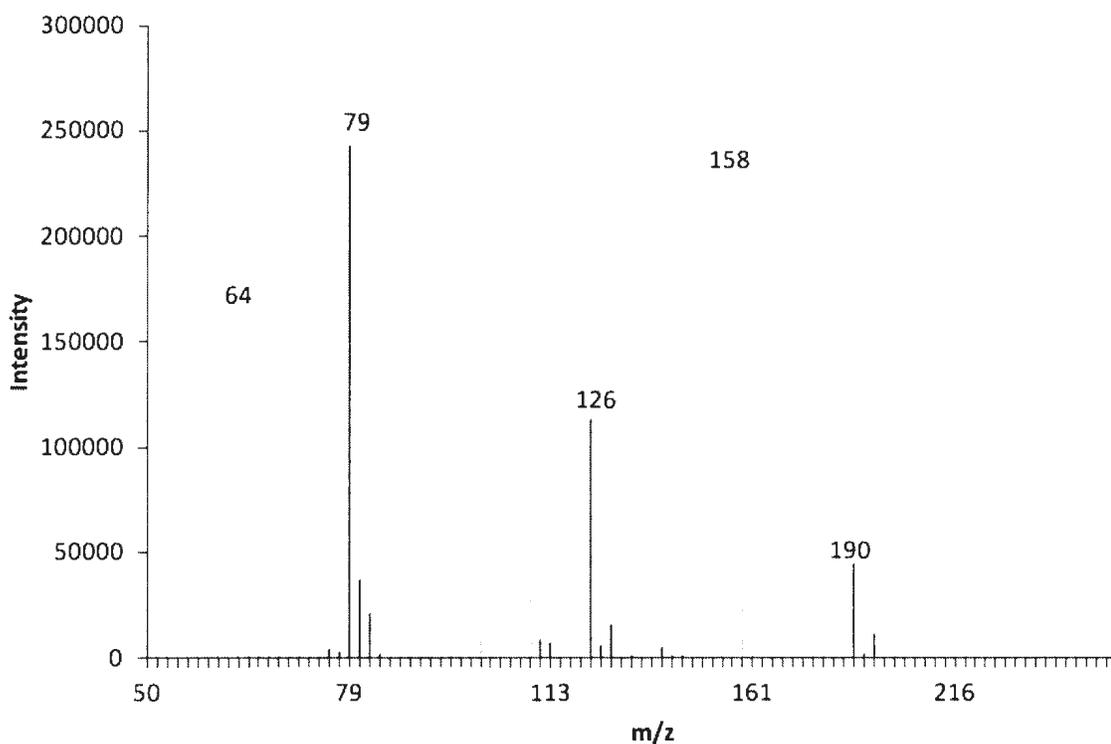


Figure 3.8 Mass spectrum of the peak that was eluted at 24.7 minutes from the GC with m/z 190 characteristic of $\text{CH}_3\text{S}_5\text{CH}_3$ (dimethylpentasulfane)

The peak which appeared at 24.7 min as seen in Figure 3.4 has a molecular ion at m/z 190, which is characteristic of $\text{CH}_3\text{S}_5\text{CH}_3$ (dimethylpentasulfane). The fragmentation pattern shows an ion at m/z 154 corresponding to $[\text{CH}_3\text{S}_4\text{CH}_3]^{+\cdot}$ (dimethyltetrasulfane), m/z 126 the mass of $[\text{CH}_3\text{S}_3\text{CH}_3]^{+\cdot}$ (dimethyltrisulfane) an ion at m/z 79 the mass of the cationic radical methyldisulfane $[\text{CH}_4\text{S}_2]^{+\cdot}$ and m/z 64 is the mass of sulfur radical cation $\text{S}_2^{+\cdot}$.

3.4.3 MALDI-TOF

MALDI-TOF was used to confirm the masses of the dimethylpolysulfanes synthesized. It was not possible to develop a satisfactory method for detection by electrospray ionization or atmospheric pressure chemical ionization MS. The MALDI-TOF spectrum is shown in Figure 3.9 as well as the isotopic pattern for $\text{CH}_3\text{S}_9^{++}$.

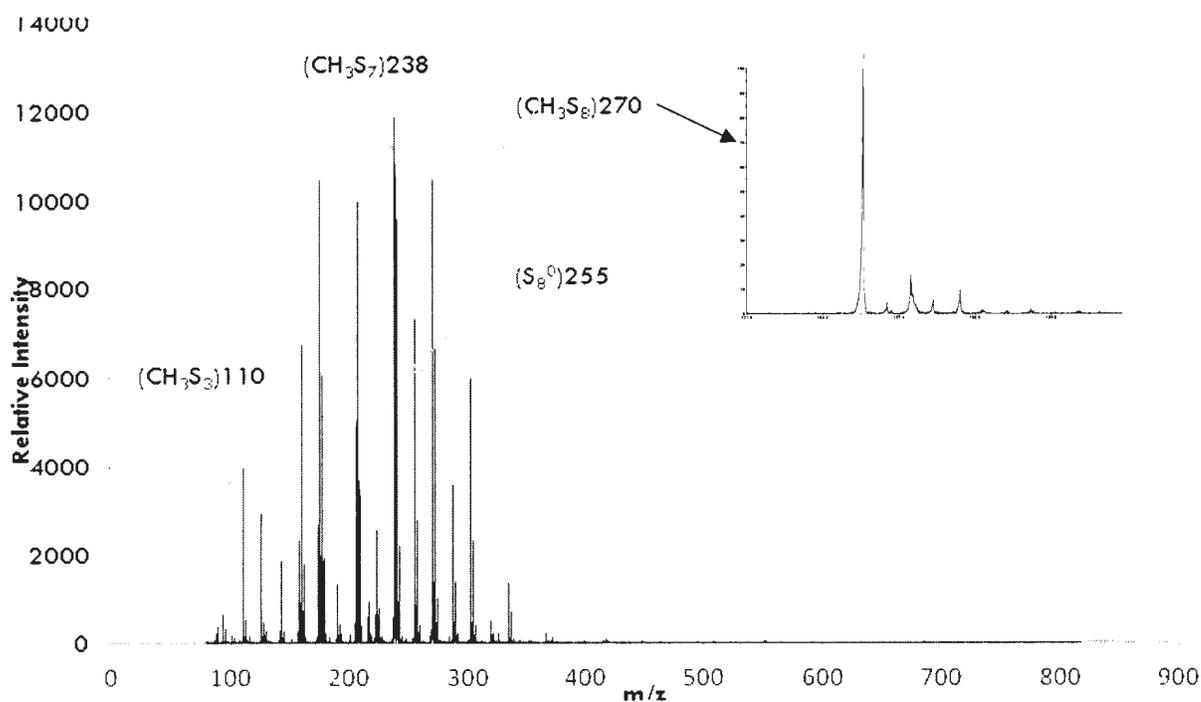


Figure 3.9 MALDI-TOF spectrum of the dimethylpolysulfanes mixture; insert is the isotope pattern of $\text{CH}_3\text{S}_9^{++}$

From the spectra it is clear that the result of the synthesis is a mixture of dimethylpolysulfanes with characteristic peaks appearing such as m/z 110 to $\text{CH}_3\text{S}_3^{++}$, m/z 238 to $\text{CH}_3\text{S}_7^{++}$, m/z 270 to $\text{CH}_3\text{S}_8^{++}$ and m/z 255 to S_8^{0+} . The MALDI spectra show that

this technique could be very useful as a method for rapid identification of the dimethylpolysulfane species; the signal to noise ratio is high as can be seen from the clear peaks, and there is little or no background ion interference.

3.5 Conclusions

An HPLC analytical column was used to separate the synthesized mixture of dimethylpolysulfanes and an HPLC semi-preparative column was used to isolate the various fractions of dimethylpolysulfanes for analysis. GC-MS was used to confirm the masses of the first four chain lengths of dimethylpolysulfanes in the synthesized mixture, while longer chain lengths could not be confirmed. The trend for the first four polysulfide analogues measured show that a homologous series was synthesized by this method, and that the reason longer chain lengths could not be confirmed is most likely due to the fact that the longer chain lengths are thermally unstable and may break down in the injector or perhaps on column. GC-MS could not be used to confirm the mass of the isolated fractions from the HPLC, this could be due to lack of inter molecular bonding. In the headspace solid- phase microextraction and GC-MS (HS-SPME/GC-MS) of polysulfides in drinking water distribution systems using carried out by Ina *et al.*, the longest chain length that could be determined was dimethyltrisulfide. In spite of the limitations regarding the higher order polysulfides, they found that the method had good sensitivity, linearity and precision, and was essentially free from interferences. MALDI-TOF MS was used to confirm the masses of the dimethylpolysulfanes and also to show the isotope

pattern distribution of the dimethylsulfanes, hence we are able to confirm the presence of the longer chain lengths. The reliability of GC-MS, HPLC and MALDI-TOF MS were very good, in terms of reproducibility. It was also found that as the optimized MALDI-TOF MS method may be suitable for high throughput screening as the dimethylsulfane ions were very obvious and the spectra were not substantially impacted by interferences from MALDI matrix ions.

3.6 References

1. Theodore, I. B.; Lemay, H. E.; Bruce, E. B.; Murphy, J. C.; Woodward, P. M.; Chemistry The Central Science, 2012, 12th edition, pp. 305–309
2. Mark, D.; Thomas, R.; Transition Metal Polysulfide Coordination Compounds with Purely Inorganic Chelate Ligands, *Angewandte Chemie International Edition*, 1985, 24, pp.742-757
3. Murthy, A. V.; Zhou, W.; Characterization of a Transient +2 Sulfur Oxidation State Intermediate from the Oxidation of Aqueous Sulfide, *ACS Symposium Series Volume*, April 1995, pp.1-8
4. Luther, G.W.; Pyrite Synthesis of Polysulfide Compounds *Geochimimca Cosmochimica Acta*, 1991, 55, pp. 2839–2849
5. Hunger, S.; Benning, L. G.; Greigite: A true Intermediate on the Polysulfide Pathway to Pyrite, *Geochemical Transaction*, 2007, 21, 8:1
6. Licht, S.; Combined Solution Effects Yield Stable Thin-Film Cadmium Selenide Telluride/Polysulfide Photoelectrochemical Solar Cells, *Journal of physical chemistry*, 1986, 90, pp.1096-1099
7. Licht, S.; An Energetic Medium for Electrochemical Storage Utilizing the High Aqueous Solubility of Potassium Polysulfide, *Journal of electrochemical society*, 1987, 134, 2137-2141
8. Franzmann, P. D.; Heitz, A.; Zappia, L.R.; Wajon, J. E.; Xanthis, K.; The Formation of Malodorous Dimethyl Oligosulphides in Treated Groundwater: The Role of Biofilms and Potential Precursors, *Water resources*, 2001, 35, pp. 1730-1738

9. Kamyshny, A.; Borkenstein, C. G.; Ferdelman, T. G.; Protocol for Quantitative Detection of Elemental Sulfur and Polysulfide Zero-Valent Sulfur Distribution in Natural Aquatic Samples, *Geostandards and geoanalytical research*, 2009,33, pp.415-435
10. Hashim, A.S.; Azahari, B.; Ikeda, Y.; Kohjiya, S. *Rubber Chemistry and Technology*: 1998, 71, pp. 289-299
11. Stuart, L.; Davis, J.; Disproportionation of Aqueous Sulfur and Sulfide: Kinetics of Polysulfide Decomposition, *Journal of physical chemistry B*, 1997, 101, pp. 2540-2545
12. Kristiana, I.; Heitz, A.; Joll, C.; Sathasivan, A.; Analysis of Polysulfides in Drinking Water Distribution System Using Headspace Solid-Phase Microextraction and Gas Chromatography-Mass Spectrometry, *Journal of chromatography A*, 2010, 1217, pp. 5995-6001
13. Kamyshny, A.; Ekeitchik, I.; Gun, J.; Ovadia, L.; Method for the Detection of Inorganic Polysulfide Distribution in Aquatic Systems, *Analytical chemistry*, 2006, 78, 2631-2639
14. Satake, H.; Hisano, T.; Ikeda, S.; The Rapid Determination of Sulfide, Thiosulfate and Polysulfide in the Lixiviation Water of Blast-Furnace Slag by Means of Argentimetric Potentiometric Titration, *The chemical society of Japan*, 1981, 54, pp. 1968-1971

Chapter 4 - Conclusions and future work

Polysulfides were synthesized and derivatized into dimethylpolysulfanes which are more stable than the native polysulfides. Analysis of the resulting mixtures by HPLC showed peaks that correspond to a homologous series; this was confirmed from the linear analysis of the HPLC chromatograms and also from GC-MS. The GC-MS was particularly important in further confirming that a homologous series has been synthesized in that it showed that the first four peaks had m/z values that corresponded to the first four chain lengths of the synthesized dimethylpolysulfanes.

Application of MALDI-TOF MS gave more data to confirm the identities of synthesized dimethylpolysulfanes, with m/z and their isotope pattern distribution consistent with the proposed structures. The concentration values for each dimethylpolysulfane semi-prep fractions were determined from the ICP-OES analysis, which gave total sulfur as sulfate, where all sulfur in each sample was oxidized quantitatively to sulfate prior to analysis. Hence a calibration curve was produced from the calculated values and the results can be used to quantify polysulfides in real samples (from tailings and also hydrometallurgical process). The relative percentage error from the ICP-OES is 2%.

Dimethylpolysulfane mixtures in dichloromethane are stable for up to 2 weeks when stored at -7°C or below, while isolated fractions from the semi-preparative column with 2-4 sulfur atoms per dimethylpolysulfane chain length are stable for 3 days in water/methanol mixture, when stored at -29.9°C . Fractions with 5-12 sulfur atoms per

chain length are unstable in water/methanol mixture, as they redistribute within hours of their isolation, longer and shorter polysulfides.

Further studies need to be carried out to fully understand the polysulfide chemistry. With special emphasis on dimethyldisulfane, which is highly labile, dimethyloctasulfane and dimethylnonasulfane, as they have been shown to be more difficult to resolve and there seemed to be some issues with their analysis by ICP-OES. The role polysulfides play in the generation of thiosalts should also be investigated. More work will also need to be done to refine the analytical approach reported here as the above method of analysis is currently only semi-quantitative. It will also be useful to find a direct approach to polysulfides analysis, with minimal sample handling. However, any method will require standards and it is quite clear that production of stable polysulfide standards will be a challenge.





