SYNTHESIS, PROPERTIES AND ANALYSIS OF POLYDADMAC FOR WATER PURIFICATION

by

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Dissertation presented for the degree of Doctor of Philosophy
at the University of Stellenbosch

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March 2008

DECLARATION

I, the undersigned, hereby declare that the work contained in this dissertation is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

Signature:…… Date:……

ABSTRACT

This study concerns the synthesis, properties and quantitative analysis of polydiallyldimethylammonium chloride (polyDADMAC), a water-soluble polymer used world-wide for potable water purification. The special interest in this polymer is the result of its widespread use and the current lack of adequate analytical methods for it. This is especially important for water treatment organisations.

A novel gel permeation chromatography (GPC) method was developed and evaluated for polymer analysis. The scope was extended to determine the presence of unreacted monomer (DADMAC) as well as the percentage active polymer.

polyDADMAC was first prepared using a known synthesis method. The product was purified and characterized by GPC and ^{13}C-NMR spectroscopy. New and conclusive evidence of the existence of a five-member pyrrolidine ring system was obtained. A proposed mechanism of polymerization was determined. The activity of the synthesized polyDADMAC was evaluated and it was found to perform effectively as a coagulant.

The physical and chemical properties of polyDADMAC were then studied under simulated water treatment conditions. The polymer reaction with chlorine revealed the formation of trihalogenated methane compounds (THMs). Gas chromatography–mass spectrometry (GC–MS) was used to conclusively identify the formation of chloroform.

The polymer stability under different conditions of heat exposure, UV radiation and pH variations was studied. GPC results indicated that polyDADMAC is a very stable polymer and undergoes structural change only when subjected to extremes of pH, temperature and UV conditions. Results of a short study on microbial degradation indicated growth of the cultures, and subsequent polymer degradation. Reactions of polyDADMAC were concluded with a study of the impact of ozone on polyDADMAC. GPC results indicated a significant change in the ozonated polymer peak profile.

Analytical methods to determine polyDADMAC residues in water were reviewed and critically evaluated. Methods based on complex formation/spectroscopy suffered from severe limitations and produced no meaningful results, contrary to claims made by previous researchers. Colloid titration based on an established method was promising but required extensive modification for quantitative analysis. Finally four novel methods were developed, including: solid phase extraction, membrane filtration-GPC, the HACH complexation method, and a GPC method with indirect UV detection.

The study is concluded with a chemical risk assessment that indicated minimal human health risks associated with the production and use of polyDADMAC.

OPSOMMING

Hierdie studie behels die sintese, eienskappe en kwantitatiewe analise van polidiallielmetielammoniumchloried (polyDADMAC), 'n wateroplosbare polimeer wat wêreldwyd vir drinkwatersuiwering gebruik word. Die belangstelling in hierdie spesifieke polimeer is as gevolg van die wydverspreide gebruik daarvan en die feit dat daar tans onvoldoende eenvoudige analitiese metodes daarvoor bestaan. Dit is veral belangrik vir waterbehandelingsorganisasies.

'n Nuwe gelpermeasiechromatografie (GPC) metode is ontwikkel en geevalueer vir die analise van hierdie polimeer. Die omvang van die studie is later uitgebrei om die teenwoordigheid van ongereageerde monomeer (DADMAC) asook die persentasie aktiewe polimeer te bepaal.

polyDADMAC is eers volgens 'n bekende sintesemetode berei. Die produk is gesuiwer en gekarakteriseer m.b.v. GPC en ^{13}C-KMR. Nuwe bewyse vir die bestaan van 'n vyflid pirollidoonringsisteem is verkry. 'n Meganisme vir hierdie polimerisasie metode is vasgestel. Die aktiwiteit van die bereide polyDADMAC is geevalueer en daar is bevind dat dit effektief as koaguleermiddel optree.

Daarna is die chemiese en fisiese eienskappe van polyDADMAC onder gesimuleerde waterbehandelingskondisies bepaal. polyDADMAC het met chloor gerageer om trihalogeneerde metaanverbindings (THMs) te vorm. Gaschromatografie–massa-spektrometrie (GC–MS) is gebruik om die ontstaan van chloroform te bevestig.

Daarna is die stabiliteit van die polimeer onder verskei reaksiekondisies bepaal: hitte, UV-bestraling, en pH. GPC-resultate het aangeduiui dat polyDADMAC baie stabiel is en ondergaan strukturele veranderings slegs onder uiterste kondisies van pH, temperatuur en UV. 'n Kort studie van die effek van mikro-organismes op polyDADMAC het egtermikrobiese kultuurgroei met die gevolglike afbreek van die polimeer getoon. Resultate van 'n studie van die impak van osoon op polyDADMAC het getoon dat daar 'n groot verandering in die GPC-profiel van die ge-osoneerde vorm van die polimeer was.

Verdere analitiese metodes wat al gebruik is om polyDADMAC residue in water te bepaal, is uitgevoer en krities geevalueer. Metodes gebasseer op kompleksvorming/ spektroskopie het erge beperkings gehad en het nie betekenisvolle resultate gelewer nie. Dit was in teenstelling met wat voorheen deur ander navorsers bevind is. 'n Kolloiedtitrasie gebasseer op 'n bestaande metode het goeie resultate gelewer maar het omvattende veranderings benodig om kwantitatiewe resultate te lewer. Ten slotte is vier nuwe metodes ontwikkel: soliede fase-ekstraksie, membraanfiltrasie-GPC, die HACH-komplekseringsmetode, en 'n GPC-metode met indirekte UV-waarneming..

Die studie is afgesluit met 'n bepaling van die chemiese risiko wat poly DADMAC vir die gesondheid van die mens inhou. Daar is tot die gevolgtrekking gekom dat die produksie en gebruik van poly DADMAC slegs‘n minimum gesondheidsrisiko inhou.

PREFACE

This dissertation presents an account of research carried out by the author, and has not been submitted in part or in whole to any other institution for a degree. Where use has been made of the work of others, it has been duly acknowledged and referenced in the text. The research was conducted in the Laboratory Services Department of Umgeni Water at the head office buildings based in Pietermaritzburg, under the close supervision of Professor Christopher Buckley. Parts of the research were presented at WISA 2002, UNESCO/IUPAC Conference on Macromolecules 2002 and WISA 2006. Four manuscripts have been prepared for publication and will be submitted to the South African Journal of Chemistry. The potential for a further two papers also exists.

ACKNOWLEDGEMENTS

The author wishes to place on record his grateful thanks to Umgeni Water for their full financial support, the use of their laboratory facilities for conducting this research and the generous amount of study leave offered for the required preparation of this dissertation.

Special thanks go to the many people who supplied materials, equipment, information and their valuable time in assisting with this work. Mr Raj Somaru from the Department of Chemistry, UKZN, is acknowledged for his willingness to provide the much needed chemicals and equipment at very short notice; Dr Collin Southway also from the Department of Chemistry, UKZN, for the IR and ^{13}C-NMR analyses; Mr Vijay Bandu from the Electron Microscopy Unit, UKZN, for the TEM images; Mr Lloyd Donnelly of Umgeni Water for assistance with the microbiological studies, and Mrs Kathy Milford for her assistance with the toxicity testing of polyDADMAC.

My sincere appreciation goes to Professor Christopher Buckley of the School of Chemical Engineering, UKZN, for his supervision, constructive criticism and maintaining a close interest in the project; Dr Nicola Rodda of the School of Biology and Conservation Sciences, UKZN, for her close guidance in the preparation of the final chapter on the risk analysis of polyDADMAC; Professor Ronald Sanderson and Dr Margie Hurndall for their valuable comments, constructive criticism, editing, proof reading, and coordinating and facilitating all aspects of the project as required by the University of Stellenbosch.

Thanks also go to all close friends and colleagues, Mr Lloyd Donnelly, Dr Abie Khan, Mr Swaswa Nthloro, Mr Ashogan Sundrum, Mrs Kamish Mahabeer, Mrs Jean Haldane, Mrs Debbie Trollip, Mr Sundra Thaver, Mr Rachi Rajagopaul, Mr Wessel Moolman, Mr Petro Bezuidenhout and Mr Paul Gaydon for their moral support and encouragement throughout the preparation of this dissertation.

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LIST OF ABBREVIATIONS

AMPAM	Aminomethylated polyacrylamide
ANSI	American National Standards Institute
AUFS	Absorbance units full scale
AVE	Average
AWWA	American Water Works Association
AWWARF	American Water Works Association Research Foundation
BOD	Biochemical oxygen demand
^{13}C-NMR	^{13}Carbon nuclear magnetic resonance
C_{18}	Octadecylsilane
CAS	Chemical abstracts registry
COD	Chemical oxygen demand
CR	Concentration ratio
DADMAC	Diallyldimethylammonium chloride
DDPM	1,5-dimethyl-1,5-diazaundecamethylene polymethobromide
DEPT	Distortionless enhancement by polarization transfer
DHBA	3,5-Dihydroxy-benzoic acid
DHWTW	Durban Heights Water Treatment Works
DPD	N,N-Diethyl-p-phenylenediamine
ECD	Electron capture detector
EDTA	Ethylenediaminetetraacetic acid
EPI-DMA	Epichlorhydrin-dimethylamine
EPI-DMA-EDA	Epichlorhydrin-dimethylamine-ethylenediamine
EPI-MMA	Epichlorhydrin-monomethylamine
EPI-PA	Epichlorhydrin-polyamine
FID	Flame ionization detector
GC	Gas chromatograph/gas chromatography
GC ECD	Gas chromatography with electron capture detection
GC FID	Gas chromatography with flame ionization detection
GC MS	Gas chromatography mass spectrometry
GPC	Gel permeation chromatography
^{1}H-NMR	Proton nuclear magnetic resonance
HPLC	High-pressure liquid chromatography
LC_{50}	Lethal concentration to 50% of the test population
LD_{50}	Lethal dose to 50% of the test population
LOAEL	Lowest observed adverse effects level
MAL	Maximum allowable limit
MCL	Maximum contaminant level
mDADMAC	Diallyldimethylammonium chloride monomer
MO	Methyl orange
MP	Mobile phase
MSD	Mass selective detector
MSDS	Material safety data sheet
MWD	Molecular weight distribution
NOAEL	No observed adverse effects level

NSF	National Sanitation Foundation
NTU	Nephelometric turbidity unit
OECD	Organization for Economic Cooperation and Development
OSHA	Occupational Safety and Health Administration
PAM	Polyacrylamide
PAM-PAA	Acrylamide-acrylic acid co-polymer
PDA	Photodiode array
PEG	Polyethylene glycol
PEI	Polyethyleneimine
PEO	Polyethylene oxide
PEPA	Polyethylene polyamine
polyDADMAC	Diallyldimethylammonium chloride homopolymer
Poly(DMAEMA)	Polydimethylaminoethyl methacrylate
PPE	Personal protective equipment
PVC	Polyvinyl chloride
PVSK	Potassium polyvinyl sulfate
QAC	Quaternary ammonium compound
QC	Quality control
RI	Refractive index
RP HPLC	Reversed phase high pressure liquid chromatography
RSD	Relative standard deviation
SPE	Solid-phase extraction
STDEV	Standard deviation
SVOC	Semi-volatile organic compound
TA	Tannic acid
TBO	Toluidine blue-O
tC_{18}	Trifunctional octadecylsilane
TDS	Total dissolved solids
TEA	Triethanolamine
TEM	Transmission electron micrograph
THM	Trihalomethane
THMFP	Trihalomethane formation potential
TKN	Total Kjeldahl nitrogen
TOC	Total organic carbon
UF	Ultrafiltration
UV	Ultraviolet
VIS	Visible
VOC	Volatile organic compound
WHO	World Health Organization
WRC	Water Research Commission of South Africa

CHAPTER ONE

INTRODUCTION AND OBJECTIVES

1.1 The Origins of Polymers for Water Purification

The development of water soluble synthetic organic polymers in the 1950s was one of the most significant breakthroughs in water and wastewater treatment technology. Their use may seem to be a relatively new development in the field of coagulation but the process is in fact at least 4000 years old. Sanskrit literature dating back to 4000 BC suggested the use of a number of naturally occurring polymers such as starch, cellulose polysaccharide gums and other proteinaceous materials as a means of clarifying water [Cohen et al., 1958]. In spite of their varied sources these compounds are collectively called bio-colloids and carry electrical charges or ionizable groups.

The production of high molecular weight organic polymers is analogous in terms of general properties to the bio-colloids. All these compounds contain recurring units of small molecular weights and each unit carries one or more electrical charge or ionizable group. Because these compounds have properties of both polymers and electrolytes they have been called polymeric electrolytes or, more frequently, polyelectrolytes.

1.2 Classification of Polymers

When classifying synthetic organic polymers considerations are given to the charge, product form and molecular weight. The polymer may carry a negative charge (anionic), a positive charge (cationic) or no charge (nonionic). It could exist as a dry powder, an aqueous solution or as an emulsion. Classification in terms of the molecular weight can vary: low molecular weight (10^4 to 10^5 g mol^{-1}), medium (10^5 to 10^6 g mol^{-1}), high (10^6 to 5 x 10^6 g mol^{-1}) and very high (greater than 5 x 10^6 g mol^{-1}) [Mangravite, 1983].

Although there are over 1500 registered products they can be grouped into essentially 15 to 20 chemicals. The most commonly used polymers registered for drinking water are shown in Table 1.1 [Letterman and Pero, 1990]. The acrylamide polymers are the so-called coagulant aid polymers. All polymers in this group have acrylamide as the monomer or co-monomer, they are characterized by high molecular weight, and are essentially non-ionic or anionic. The Mannich reaction modified polyacrylamide and the polydimethylaminoethyl methacrylate (polyDMAEMA) are made to have positively charged sites by decreasing the solution pH or by including the quaternization reaction in the manufacturing process. These two polymers are also high molecular weight compounds used for sludge conditioning. The remaining polymers in Table 1.1 are the primary coagulants with low to moderate molecular weights. PolyDADMAC and EPI-DMA polymers are known to be the most widely used polymers worldwide, and reports suggest that they form 80% of polymers sold to the drinking water industry in the USA.

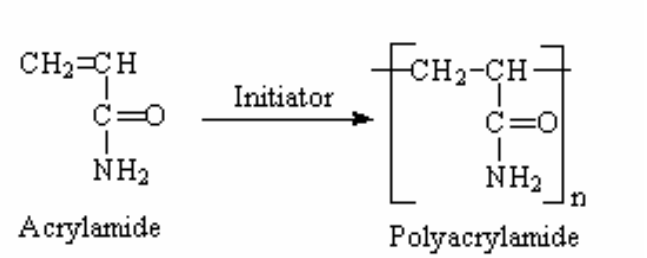
Table 1.1: Polymers registered for use in drinking water treatment [Letterman and Pero, 1990].

Common Name	Abbreviation	Chemical Name
Polyacrylamide	PAM	2-Propenamide homopolymer $(C_3H_5NO)_n$
Acrylamide-Acrylic Acid Co-polymer	PAM-PAA	2-Propenoic acid with 2-propenamide copolymer $(C_3H_5NO.C_3H_4O_2)_n$
Diallyldimethylammonium Chloride Homopolymer	polyDADMAC	N,N-Dimethyl-N-2-propenyl-2-propen-1-aminium chloride homopolymer $(C_8H_{16}N.Cl)_n$
Epichlorhydrin-Dimethylamine	EPI-DMA	N-Methyl methanamine with chloromethyl oxirane copolymer $(C_2H_7N.C_3H_5ClO)_n$
Epichlorhydrin-Dimethylamine-Ethylenediamine	EPI-DMA-EDA	1,2-Ethanediamine with N-methyl methanamine and chloromethyl oxirane copolymer $(C_2H_5N.C_3H_5ClO.C_2H_7N)_n$
Epichlorhydrin-Monomethylamine	EPI-MMA	Methanamine with chloromethyl oxirane copolymer $(CH_5N.C_3H_5ClO)_n$
Epichlorhydrin-Polyamine	EPI-PA	N,N-Dimethyl-1,3-propanediamine with chloromethyl oxirane copolymer $(C_5H_{14}N_2.C_3H_5ClO)_n$
Polyethyleneimine	PEI	Ethanimine homopolymer $(C_2H_5N)_n$
Polyethylene Polyamine	PEPA	1,2-Dicloroethane with ammonia $(C_2H_4Cl_2.H_3N)_n$ or 1,2-Ethanediamine with 1,2-dichloroethane $(C_2H_8N_2.C_2H_4Cl)_n$
Melamine Formaldehyde		1,3,5-Triazine-2,4,6-triamino polymer with formaldehyde $(C_3H_6N_6.CH_2O)_n$
Mannich Reaction Modified Polyacrylamide	AMPAM	N,N-[Dimethyl(amino)methyl]-2-propenamide homopolymer $(C_6H_{12}N_2O)_n$
Dimethylaminoethyl Methacrylate	Poly(DMAEMA)	N,N-Dimethylaminoethyl-2-methyl-2-propenoic acid homopolymer $(C_8H_{15}NO_2)_n$

1.3 Synthesis and Properties of Water Treatment Polymers

1.3.1 Acrylamide Homopolymer

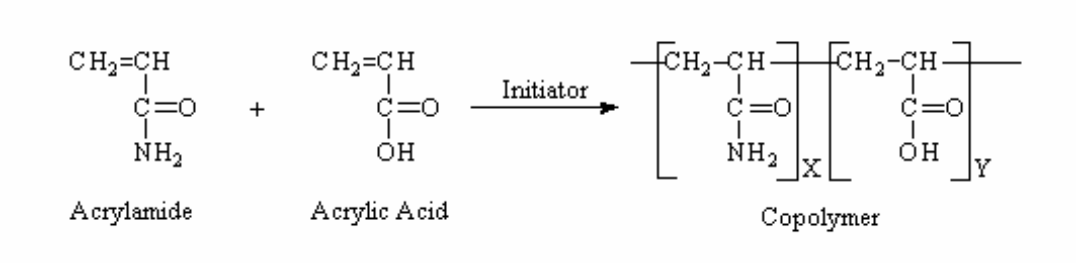
All very high or high molecular weight acrylamide homopolymers are based on homo-polymerization or co-polymerization of acrylamide. To make high molecular weight nonionic polymers, acrylamide is homo-polymerized as shown in Scheme 1.1 [Mangravite, 1983; Mortimer, 1991].



Scheme 1.1: Homo-polymerization of acrylamide to polyacrylamide.

1.3.2 Acrylamide Acrylic Acid Copolymer

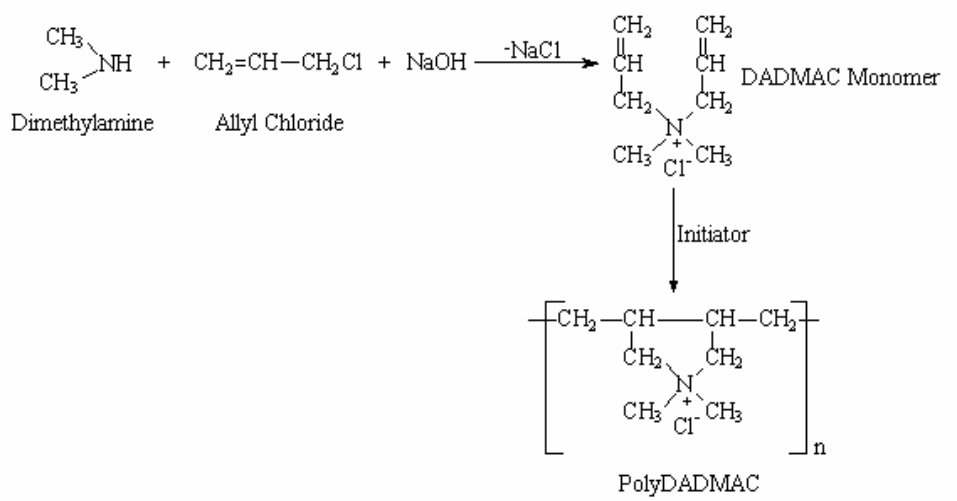
To make high molecular weight anionic polymers, acrylamide is co-polymerized with acrylic acid as shown in Scheme 1.2 to form acrylamide acrylic acid copolymers [Mangravite, 1983; Mortimer, 1991].



Scheme 1.2: Co-polymerization of acrylamide with acrylic acid.

1.3.3 PolyDADMAC

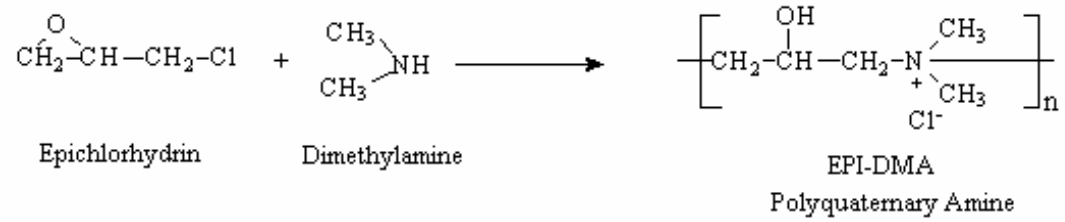
Diallyldimethylammonium chloride monomer (mDADMAC) is prepared from the reaction of allyl chloride with dimethylamine. The monomer is then polymerized to form polyDADMAC (Scheme 1.3) by free-radical initiated addition polymerization [Mangravite, 1983; Mortimer, 1991]. The resulting polymer is cationic in nature, with repeating pyrrolidine rings. The polyDADMAC polymers are the most widely used polymers for potable water purification. They are well known to be the most chlorine resistant and operate over a wide pH range.



Scheme 1.3: Synthesis of mDADMAC and subsequent polymerization to polyDADMAC.

1.3.4 EPI-DMA

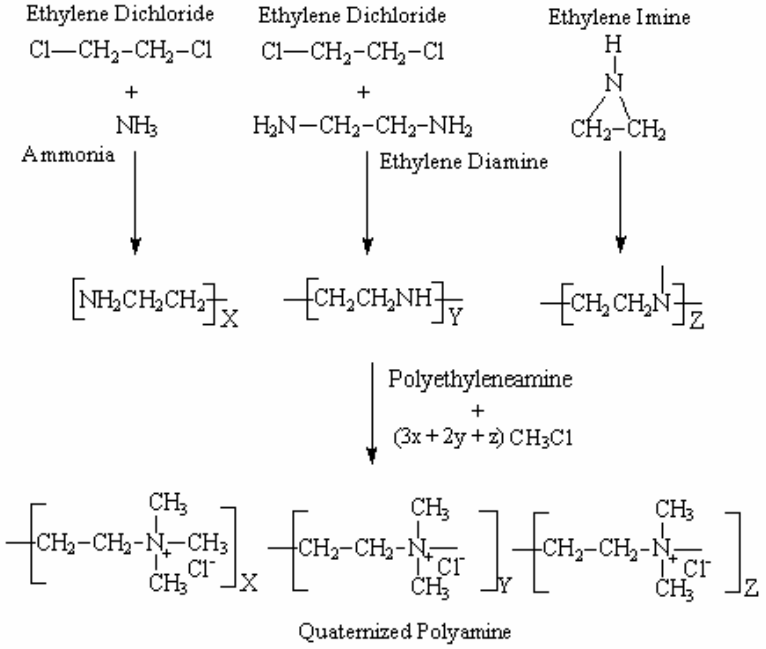
The EPI-DMA group of polymers is commonly referred to as the polyamines. They are synthesized by condensation polymerization of epichlorhydrin and dimethylamine to form low molecular weight products according to Scheme 1.4 [Mangravite, 1983; Mortimer, 1991].



Scheme 1.4: Condensation polymerization of EPI with DMA.

1.3.5 Polyethyleneamine

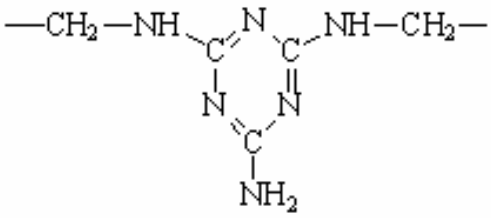
The polyethyleneamine polymers are prepared by condensation polymerization (Scheme 1.5). They are branched polymers and can be quaternized or partially quaternized. For this reason their charge is pH dependent and they are chlorine sensitive polymers [Mangravite, 1983].



Scheme 1.5: Synthesis of polyethyleneamine polymers [Mangravite, 1983].

1.3.6 Melamine Formaldehyde

Melamine formaldehyde polymers are generally acidic (pH 1.6 to 2.1). They are prepared in very dilute form (6 to 10%) in order to prevent gel formation as a result of further polymerization. The structure is branched and very complex. The repeating units can be described as the structure in Scheme 1.6 [Mangravite, 1983].



Scheme 1.6: Structure of melamine-formaldehyde polymer.

They are often used together with polyDADMAC or EPI-DMA polymers and are known to produce low turbidity water.

1.4 The Use of Polymers in Coagulation and Flocculation

Turbidity in water is caused by suspended colloidal material ranging in size from 10^{-3} to 10^{2} μm. Calculations have shown that some of these particles in the middle of this size range can take up to a decade to settle a mere 0.3 m, while the smallest particles will not settle at all due to Brownian motion [Kasper and Reichenberger, 1983]. Effective removal of these extremely slow settling colloids is only possible through efficient coagulation and flocculation. In the coagulation process the electrical charge that keeps the particles in suspension is broken by one of three mechanisms and

results in particle destabilization. Once destabilized, the particles are brought into intimate contact with one another and form flocs or aggregates, resulting in sedimentation. Coagulation and flocculation are critical operations in water treatment. Ineffective coagulation affects sedimentation, filtration, and disinfection, and results in poor quality treated water, increased chemical usage and ultimately higher production costs.

Since the development of synthetic organic polymers in the 1950s and subsequent approval of their use, they have gained enormous popularity for water and wastewater treatment. In many cases, their use has totally or partially replaced that of the inorganic coagulants of iron or aluminum. Umgeni Water is no exception, having changed over to using polymers in the mid-1980s [Nozaic et al., 2001].

1.4.1 Advantages of Polymers in Water Purification

The advantages of synthetic organic polymers for water treatment are numerous and account for their overwhelming popularity over the inorganic salts, so much so that, worldwide, only Japan and Switzerland do not permit their use. When used either as primary coagulants or coagulant aids, polymers can improve finished water quality, increase process stability (especially for raw water of varying quality), reduce chemical handling and reduce production costs. Improved quality of treated water occurs as a result of the formation of large, more settlable floc, or a stronger, more filterable floc [Mangravite, 1983]. Polymers do not produce a significant amount of dissolved solids as is often the case with inorganic coagulants. When used as aids together with inorganic coagulants, polymers are able to broaden the range over which the coagulant is effective. When polymers are used alone as primary coagulants, chemical usage on a volume basis is reduced significantly. Polymer dosages of 2 to 10% of the quantity of inorganic coagulants are required to achieve equivalent coagulation. The reduced chemical usage reduces chemical handling and storage and, more importantly, reduces production costs. Furthermore, there is a reduction in sludge volume, with higher settling rates, which results in an increase in plant capacity. There is also less sedimentation carryover, enabling longer filter runs and reduced backwashing. The reduced sludge volume also reduces the frequency of de-sludging of the clarifiers. Unlike the metal hydroxide sludge that is heavily hydrated, polymer sludge tends to be readily dewatered to produce a high solids content sludge [Nozaic et al., 2001].

Polymers are generally not sensitive to pH while inorganic coagulants, operate optimally within a fairly wide pH range. Therefore, in practice, no post pH correction is necessary. It only requires adjustment once, at the head of the works. The fact that the polymer does not affect the pH of the treated water to any significant extent is an added benefit as variations in dosage have no effect on the pH of the final treated water.

There have been serious concerns regarding the use of alum due to the link between aluminum and Alzheimer's disease. Although this has not been proven conclusively, there is some comfort that this is totally eliminated with the use of polymers, or greatly reduced when the polymers are in some cases blended with small amounts of polyaluminum chloride (PAC).

With polymers, there is an overall reduction in treatment costs. Savings originate from reduced chemical costs, use of smaller dosing pumps, reduced power consumption, and lower maintenance costs due to the mild nature of the polymers (the inorganic coagulants are aggressive and highly corrosive chemicals). Finally, polymers make possible the use of automatic dosage control, with the use of the streaming current detector and feed back control loop.

1.4.2 Disadvantages of Polymers in Water Purification

The use of synthetic organic polymers for water treatment is not totally without problems. However, these problems have been outweighed by the advantages, and polymers therefore continue to remain the products of choice for most water and wastewater treatment applications.

Polymers have a very narrow optimum dosage range and therefore poor control of this has a marked effect on the turbidity. Underdosing causes incomplete particle destabilization and overdosing causes re-stabilization, and both conditions result in high turbidity. In contrast, the dosage of inorganic coagulants can vary widely without significantly affecting the turbidity. Turbidity removal efficiency of polymers is generally less than that of the inorganic salts. From historical data produced at Umgeni Water, the inorganic salts produced turbidities of less than 0.1 NTU and the polymers from 0.2 to 0.3 NTU.

It has been found that polymers are not suitable for enhanced coagulation, the process of optimizing dosage for organics removal rather than turbidity removal. The optimum dose was found to be 3 to 7 times that required for optimum turbidity removal [Nozaic et al., 2001].

Some polymers have shown that they are susceptible to degradation in the presence of chlorine or ozone and in some cases form byproducts that are more harmful than the initial starting materials [Mallevialle et al., 1984]. As far as the polyamines are concerned, very little has been published on their toxicity. It has been assumed that the polymer is not absorbed in the gastrointestinal tract and therefore has negligible toxicity. Epichlorhydrin (EPI), however, present in the EPI-DMA polymers, is known to be both mutagenic and carcinogenic to laboratory animals. It also has been shown to cause sterility in male rats and mice. PolyDADMAC polymers are not considered to be toxic (based on studies conducted on laboratory animals) but overexposure by ingestion may irritate the gastrointestinal tract. Eye contact and skin contact may cause burns, and inhalation may cause irritation of the respiratory tract. Acrylamide is one of the monomers from which polyacrylamide is prepared and hence, due to its relatively high toxicity to humans, the monomer content is restricted to less than 0.25 $\mu g\ L^{-1}$ in drinking water. Research conducted on the ozonation of acrylamide containing polymers [Mallevialle et al., 1984; Soponkanaporn and Gehr, 1989] has however indicated almost total removal of acrylamide.

The reactions of chlorine with water treatment polymers were studied by Kaiser and Lawrence (1977), Amy and Chadik (1984), and Soponkanaporn and Gehr (1989). They all observed the formation of chloroform at levels that were proportional to the polymer concentration. Apart from the chemical reactions that may occur during the treatment process, the polymer may be contaminated with monomer or other

potentially harmful materials from the manufacturing process and will eventually enter into the treated water.

Other process related problems resulting from the use of polymers are sludge removal from clarifier hoppers. This was especially noticeable in the old clarifiers that were designed for alum sludge. Polymer sludge is more viscous and tends to stick to the side walls and is also prone to the formation of rat-holes. Finally, overdosing can lead to filter mudballing, especially where simultaneous air/water backwashing is not practiced [Nozaic et al., 2001].

1.5 Polymer Selection for Water Treatment

Polymers are characterized by charge (anionic, cationic, non ionic), charge density, composition, molecular weight and delivery form (liquid or dry powder). The coagulation mechanism requires that the polymer is in a dissolved form and is allowed to adsorb to the colloid surface to bring about aggregation. Additional factors that affect coagulation are the colloid surface chemistry, colloid concentration, and the organic (e.g. humic substances) and inorganic constituents (e.g. clay, silt) of the water.

No single polymer produces the best results for all water types since each type of natural water is unique. Certain polymers (e.g. EPI-DMA) are suited to highly colored waters containing large quantities of humic substances that form very fragile flocs. Waters containing large amounts of inorganic clays and silt are usually best treated with the polyDADMAC polymers and the polyamines tend to work best for waters containing algae and bacteria. Coagulant aids are generally required to treat low turbidity waters. Temperature, pH and chlorine are also factors that affect polymer performance (some are more affected than others).

Since the development and use of polymers after the 1950s, very few relationships between polymer type and water type have been identified and although procedure manuals for polymer selection are available [Dentel et al., 1986; Dentel et al., 1987], polymer selection still remains more of an art rather than a science. Even up to the present day, polymer selection is made by trial and error, by personal experience using a specific polymer, from experience of others treating the same or similar water, and recommendation from suppliers. Even so, extensive jar tests are required and floc settling rate and sludge volume are carefully monitored. Once the best candidate is found or a short list prepared, this is further tested in plant trials. It must also be noted that once the polymer is identified based on technical data from jar tests or plant trials, the selected polymer must be compared with others on a cost basis.

1.6 Plant Design Requirements for Polymeric Flocculants

The importance of full-scale plant trials cannot be overemphasized. The coagulation process may be obscured by improper design of the physical features required for coagulation and flocculation, chemical addition sequence, and the sludge handling system. One vital feature in the design of the rapid mix unit is that of achieving instantaneous and uniform dispersion of the polymer.

The flocculation system must be designed with the appropriate input energy requirements to achieve effective floc size. For color removal, the flocs are usually very fragile and require less energy but longer agitation times than for turbidity removal. For direct filtration, flocs must be strong and dense, and hence high-energy, short detention time flocculation is most effective.

As a rule, the chemical addition sequence requires that the coagulant aid follows the primary coagulant addition by a few minutes. The exception is if inorganic clay is used as the aid, in which case it is added prior to the primary coagulants, since its purpose is to provide the floc nuclei.

1.7 Polymers in Sludge Conditioning

Extensive studies on the use of polymers for waterworks sludge [Novak and O'Brien, 1975] have shown that polymer selection depends on the sludge pH, the polymer molecular weight, polymer charge type and charge density. In general, molecular weights exceeding 10^6 Da are the first requirement to achieve inter-particle bridging. The polymers are usually less effective at pH values greater than 8.5. Polymers improve the rate at which water is removed by increasing the size of the sludge particles.

1.8 Polymer Mode of Action

Polymers accomplish particle destabilization by one of three mechanisms, as described by:

- The charge neutralization model
- The inter-particle bridging model
- The charge neutralization-precipitation model.

The polymer effectiveness depends on the polymer, the particle and the solution chemistry.

1.8.1 The Charge Neutralization Model

The first charge neutralization model developed [Black et al., 1965], and later the second [Mangravite, 1983], states that destabilization of colloidal particles is accomplished by adsorption of oppositely charged polymers on the particle surface thereby neutralizing the particle surface charge. At the optimum dose of polymer the charge is reduced to near zero. A refinement of the model was made in 1971 [Kasper, 1971] and later in 1973 [Gregory, 1973], and called the charge patch model. The polymer molecules adsorb in a flat planar configuration as shown in Figure 1.1. There is an uneven distribution of adsorbed polymer resulting in patches of positive charge.

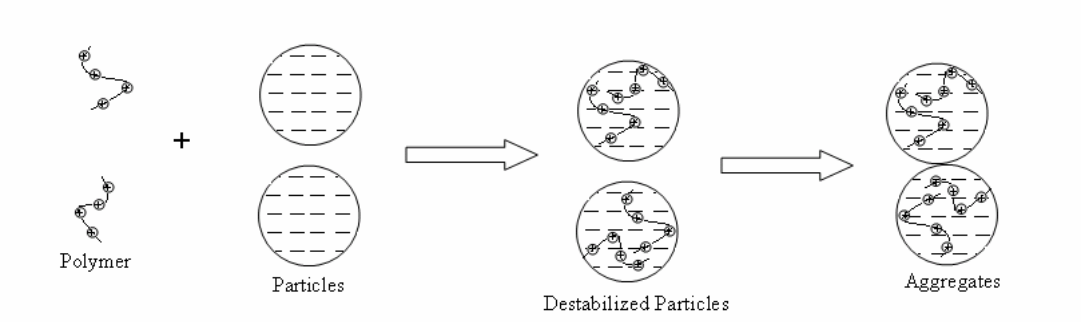


Figure 1.1: Schematic diagram of the charge patch model for the destabilization of negatively charged colloidal particles by cationic polymers.

Flocs are formed by strong electrostatic attraction between patches of positive charge and bare negatively charged areas on the particle. This eventually results in sedimentation.

1.8.2 The Inter-particle Bridging Model

Non-ionic and anionic polymers are used as coagulant aids to form large floc particles after the addition of alum or iron. These polymers usually have high molecular weights (greater than 10^6 Da) and achieve particle destabilization by first adsorbing at one or more sites on the particle surface, while the other end is sufficiently long to extend into the solution for distances larger than the double layer thickness and attach to other particles (Figure 1.2).

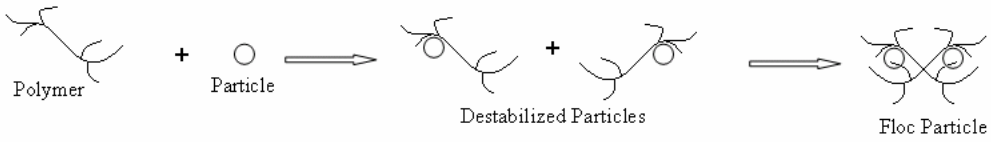


Figure 1.2: Schematic diagram of the bridging model for the destabilization of negatively charged colloidal particles by cationic polymers.

Adsorption may be due to electrostatic forces (polymer and particle are of opposite charge), hydrophobic interactions of the $-CH_2$ groups and H-bonding with the surface hydroxyl groups of particles.

Researchers [La Mer and Healey, 1963; Kane et al., 1977] recognized and accepted that colloid destabilization could not be explained by the double layer model and so developed and used the bridging theory. In its simplest form this theory proposes that a polymer molecule can attach itself to the surface of a colloidal particle at one or more adsorption sites, with the remainder of the molecule extending into the solution (Figure 1.2).

These extended segments can then interact with vacant sites on another particle. If another particle is not found, the extended segment can adsorb at other sites on the original particle. Each polymer can have many groups or segments that can be adsorbed. The extent of adsorption increases with increasing molecular weight. It is

important to note that adsorption of anionic polymers on negatively charged surfaces is common. Kane et al. [1977] and La Mer and Healey [1963] observed that with increasing hydrolysis and negative charge of polyacrylamide, improved flocculation of negatively charged silica particles was achieved. Other important findings were:

- Optimum destabilization occurred when only a portion of the available adsorption sites was covered.
- Polymer dosages that saturated the available surface of the dispersed phase resulted in a re-stabilized colloidal system.
- Under conditions of extended agitation a stabilized system can become destabilized due to the breaking of polymer-particle surface bonds.
- A direct relationship exists between the available surface area of the colloidal system and the amount of polymer required.

Chemical bridging between particles is also possible even if the polymer and particle surface are of opposite charge. Depending on the forces responsible for polymer adsorption, the prediction in surface potential may be either the principle destabilization mechanism or subsidiary to bridge formation. This accounts for the observation that optimum aggregation does not necessarily coincide with a zeta potential of zero.

1.8.3 The Charge Neutralization-Precipitation Model

The charge neutralization model shows that soluble humic substances can be coagulated by cationic polymers. The cationic polymer is used to neutralize the charge of the humic matter. Precipitates of the humic matter and cationic polymer are formed by cross-linking between the negatively charged macromolecule and the cationic polymer as shown in Figure 1.3.

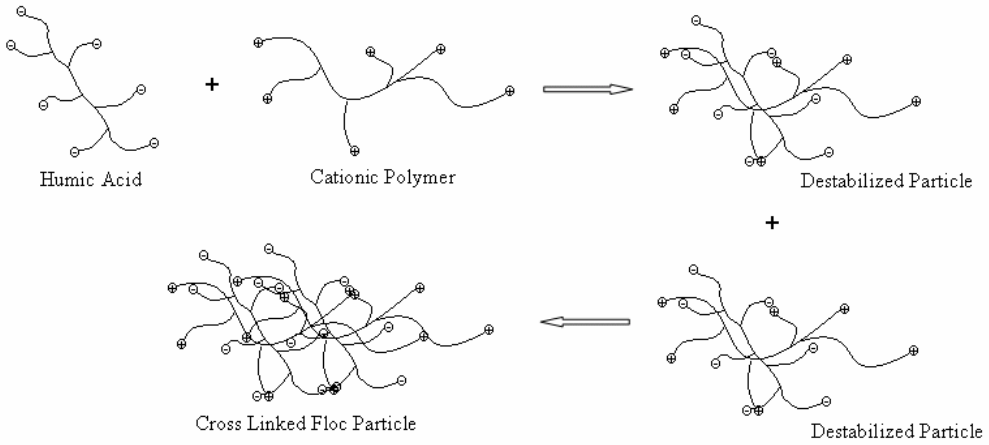


Figure 1.3: Schematic diagram of the charge neutralization-precipitation model for the coagulation of soluble humic matter.

1.9 Colloid Surface Charge and Zeta Potential

Particles that occur in natural waters are almost always negatively charged. This charge may arise from various sources, such as the adsorption of ions from solution, dissolution of ions from the solid into water, or ionization of the molecule at the surface of the solid. This results in particles exhibiting various electrokinetic

phenomena, such as electrophoretic mobility and inter-particle repulsions. Stern [1924] postulated that the ions in solution cluster around the charged particle to form an electrical double layer, as shown in Figure 1.4.

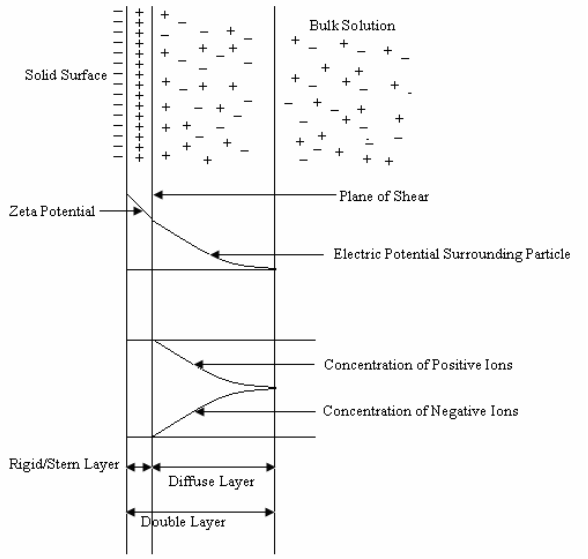


Figure 1.4: Schematic diagram of the Stern model of the electrical double layer.

An inner layer of opposite charge is attached firmly to the colloidal particle surface (rigid layer) and is surrounded by an outer, more diffuse layer. This is the so called double layer model proposed by Stern. Under dynamic conditions, when ions are attracted to the charged surface, the effective charge carried by the ions corresponds to that at the plane of shear and is called the zeta potential. Neutralization of the surface charge will permit the particles to come into contact and hence form aggregates. This is achieved readily by the addition of cationic polymers or the traditionally used inorganic metal salts. This results in coagulation and flocculation. Researchers who first proposed a distinction between the two phenomena [La Mer and Healey, 1966] described coagulation as a phenomenon whereby fine particles adhere directly to each other and flocculation as a process involving the formation of much more open aggregates.

1.10 Legislation

At present no legislation exists in South Africa to control the use of drinking water treatment chemicals. However, after 1994 the National Department of Health launched an initiative calling for the opinions of all interested and affected parties in an attempt to prepare a guideline document for the establishment of a registration system for these chemicals. The system was implemented and chemical suppliers were issued with certificates [Freese et al., 2004]. However, the situation is still uncertain as the Department of Health is currently working on a new national system for the purpose of regulating these chemicals. As an interim measure the larger suppliers are following international trends and obtaining accreditation of their products from the National Sanitation Foundation (NSF, USA). The NSF is a non-profit, non-government organization that is dedicated to research, education and public health service. It serves as a neutral third party in the development of products, equipment and services related to public health and the environment. The NSF also

provides product certification services to manufacturers of products meeting its standards. The NSF developed standards 60 [NSF/ANSI 60, 2005] and 61 [NSF/ANSI 61, 2005] for the protection of public health, and considers both the level of product as well as the contaminants that a product contributes to the water. The standard uses the maximum allowable limit (MAL), with MAL=10% the maximum contaminant level (MCL) [McClelland et al., 1989]. In Europe, the WHO guidelines are adopted, which place clear emphasis on the protection of public health. The guidelines are used as a basis for the development of national standards that will ensure the safety of drinking water supplies [Short Report, 1994].

Although product accreditation is desirable for the protection of public health, Umgeni Water has not enforced this as a procurement requirement. However, this may change in the near future, as all treatment works operated by Umgeni Water are certified in terms of the ISO 9001 code of practice. Furthermore, the Umgeni Water Laboratory Services Department is fully accredited by the South African National Accreditation System (SANAS) in terms of the ISO/IEC 17025 system. It therefore follows that to ensure that only the best quality water is produced, all treatment chemicals must undergo very stringent quality control processes. Umgeni Water is proactive in this field and is currently undertaking research on a project (K5/1600) for the Water Research Commission of South Africa (WRC) to develop a set of standards for all water treatment chemicals used in South Africa. This work is being conducted by a research team with the author of this dissertation as the project leader. The main objectives are to make testing of treatment chemicals mandatory for the protection of public health by providing a complete and comprehensive set of standards for all water treatment chemicals, including polymers for water purification.

1.11 PolyDADMAC Toxicity

PolyDADMAC is synthesized by the polymerization of the DADMAC monomer. The best available technology can drive the monomer content down to 1% on an active polymer basis. The general level of the monomer is however in the range 1 to 5%. Hazards associated with the polymer have been identified as slippery surfaces caused by accidental spills, and the possibility of long term adverse effects in an aquatic environment. It is not considered to be toxic based on studies conducted on laboratory animals but over exposure by ingestion may irritate the gastro intestinal tract. Other potential health effects as a result of over exposure can be summarized as follows:

- Eye contact: may cause irritation or burn eyes
- Skin contact: may cause irritation or burns
- Inhalation: may cause irritation of the respiratory tract.

The contribution of the DADMAC monomer to toxicity was calculated using the worst case scenario of 10 mg L^{-1} permitted polymer dosage and 5% monomer content (Appendix 1). The worst case daily dosage under normal water treatment conditions was found to be 1000 μg monomer. This translated to 875 times less than the no observed adverse effects level (NOAEL). Therefore the risk associated with exposure to the DADMAC monomer through drinking water can be deemed to be insignificant.

Based on the toxicity studies conducted on both the monomer and polymer, there appears to be a negligible amount of risk associated with the production and use of polyDADMAC. This is very reassuring to water authorities but more importantly to the general public. However, risks may arise as a result of overdosing, spills into drinking water or accidental dumping.

1.12 PolyDADMAC Methods of Analysis

Analytical methods that have been used to date for polymer analysis includes polarography [Parazak et al., 1987], chromatography [Wee, 1984], potentiometry [Christopoulos et al., 1982], two-phased titration [Tsubouchi et al., 1981], colloid titrations [Wang and Shuster, 1975], extraction spectroscopy [Kawase and Yamanaka, 1979] and flow injection analysis [Toei and Zaitzu, 1985]. A review of these methods revealed that they were old and outdated methods that suffered from severe limitations in terms of achieving good precision, accuracy, linearity and detection limits. These methods are also complicated, expensive, long and laborious, and often produced false positives due to matrix effects. Since there was little international work on the subject, there was a dire need for new methods of polymer analysis. Methods published by AWWA [ANSI/AWWA B451-92, 1993] focus on monomer and polymer assays using very labor intensive methods, but there is no mention of the analysis of polymer residues in drinking water.

1.13 Motivation for this Research

To date little research has been conducted on residual polymer analysis in water internationally. The AWWA standard for polyDADMAC [ANSI/AWWA B451-87, 1993] focuses on the analysis of the active polymer content by gravimetric means and the monomer content by titration. Both methods are long and also very labor intensive, and neither method can be applied to the analysis of residues in treated water. It has therefore been assumed that the process of coagulation and flocculation results in 100% precipitation and removal of the added product, as the monitoring of residues in final treated water is not mandatory [SANS 241, 2005]. However, there is still much uncertainty regarding this. Furthermore, it is well known that there are cases of incomplete adsorption as well as the inevitable cases of overdosing and accidental spills that may result in significant concentrations of polymer residues in treated water. One of the main challenges that water utilities are faced with is the ability to detect polymer residues in treated drinking water. The problem is particularly prominent with the use of polyDADMAC polymers as they are non-UV absorbing and difficult to detect at low levels.

Furthermore, there are several other potential problems associated with their use, including the following:

- Degradation by micro-organisms
- Degradation by ageing
- Reactions with other water treatment chemicals
- Contamination by the monomer and other starting materials used in their preparation
- Toxicity
- Batch-to-batch consistency

- Percentage of available active polymer.

As a result of the extensive use of polymers since the mid 1980s, Umgeni Water spends millions of rands annually in polymer procurement for water treatment. It is therefore of paramount importance that the products meet all the relevant manufacturing specifications. Therefore some sort of an independent audit of the products was deemed necessary. The importance of polymers in the business of water purification warranted further study and resulted in the initiation of this project.

1.14 Objectives

From the literature review conducted on polymers used for water purification it was noted that polyDADMAC was the most frequently used, but least researched, largely due to the lack of adequate methods of analysis. The polymer molecule is UV inactive, making detection extremely difficult. The latter is one of the main challenges that water utilities currently face. The objectives of the study therefore focused primarily on addressing this need as well as acquiring an intimate knowledge of the production of polyDADMAC, legislation governing its use, factors affecting polymerization, mechanism of polymerization, structure, chemical reactions, degradation and toxicity. The following were the new objectives:

- Develop and optimize a gel permeation chromatography method for polyDADMAC analysis.
- Apply the method to screen samples of treated water to establish the presence or absence of polyDADMAC residues.
- Monitor batches of polymer to ensure product quality, with specific focus on molecular weight consistency, the amount of unreacted monomer and the amount of active polymer in the product.
- Acquire a good working knowledge of the synthesis of polyDADMAC and determine the various parameters that impact on the quality of the product.
- Verify the identity of the synthesized product by structure elucidation and study the mechanism by which polymerization occurs.
- Determine the molecular weight distribution of the polyDADMAC.
- Evaluate the performance of the polymer in water purification.
- Study the stability of polyDADMAC under various water treatment and environmental conditions, and monitor any formation of degradation products.
- Review existing polyDADMAC methods of analysis, adapt and improve the methods, and research and develop novel methods for low level detection.
- Assess the risks associated with the production and use of polyDADMAC for water purification.

It is important to note that there are no national or international standards for polyDADMAC in drinking water, and currently, no reports were found on research undertaken to measure polyDADMAC residues in drinking water.

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CHAPTER TWO

GEL PERMEATION CHROMATOGRAPHY

OF POLYDADMAC

2.1 Introduction

From an extensive literature review undertaken in this study, little information was found to be available on gel permeation chromatography (GPC) of polyDADMAC. This research therefore commenced with the development of an in-house method specifically for the study of this and related polymers used in water clarification. This can be considered a first but valuable step in categorizing the polymer to better understand its structure-property relationships. A systematic approach was used starting with instrument familiarization, column selection, mobile phase (MP) testing and column bank optimization to achieve good polymer separation. The objectives were to use this method in the determination of polymer residues in treated water. The selection of GPC as the method of choice was due to the fact that no single method is available for polymer analysis that gives as much information about the physical and processing characteristics of a polymer as GPC. A commercially available polyDADMAC polymer, Z464N, was used as the test compound. As a starting point, the Ultrahydrogel 2000 column having the largest pore size (exclusion limit of 20 MDa) in the Ultrahydrogel range of columns for aqueous GPC was tested.

2.2 The Basic Principles of GPC

In theory, GPC is the simplest mode of HPLC. Separation is based purely on the hydrodynamic radius of each solute molecule in solution and is independent of the chemical or electronic nature of the substance, or the MP carrier. This independence is also associated with the fact that the column packing, usually composed of porous particles of controlled pore size, is relatively inert.

Separation is therefore achieved on a mechanical basis according to effective size, conveniently represented by molecular weight or chain length, relative to the pore diameter and volume of the porous stationery phase of the column. A schematic representation of the size separation process is shown in Figure 2.1. For a packing with a given mean pore diameter, molecules larger than a particular size, called the exclusion limit (V_0), are excluded from entering the pores. Their passage through the column is hindered only by the tortuosity of the route between and around the stationery phase particles, with the result that they elute first. Molecules that are too large do not elute at all since the packing acts as a filter. Conversely, molecules of a size below a specific value, called the permeation limit (V_t), enter a pore and diffuse into the smallest confinements within the pore.

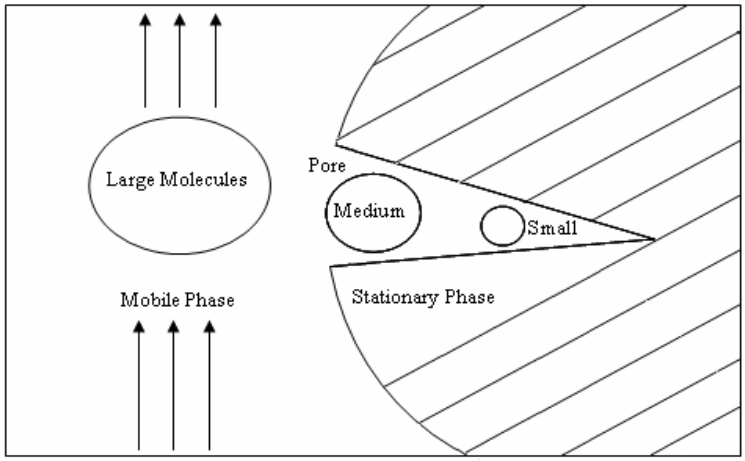


Figure 2.1: Schematic representation of the exclusion-permeation process of GPC.

These molecules elute last and cannot be separated by this mechanism. For molecules with sizes between these two limits, separation proceeds by a combination of size exclusion and diffusion permeation, with the large molecules spending less time within the pores and eluting ahead of the small molecules. A hypothetical plot of the separable molecular weights against elution volume is shown in Figure 2.2.

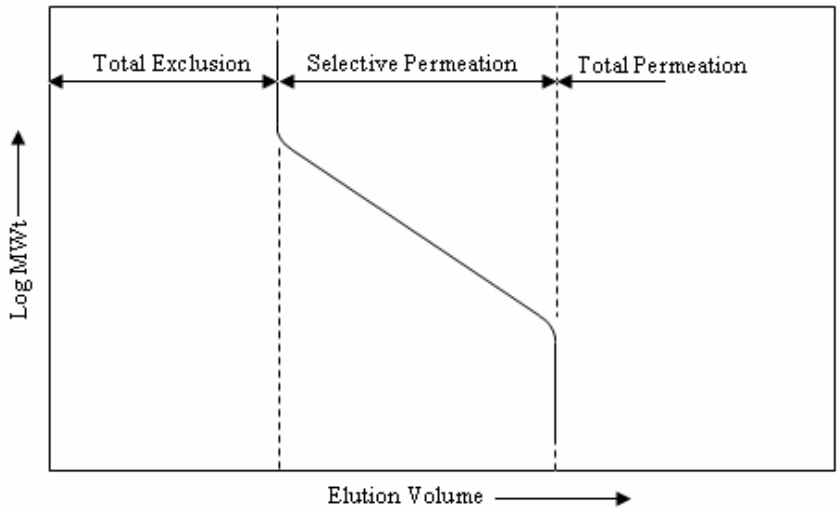


Figure 2.2: Hypothetical plot of elution volume as a function of molecular weight.

Although GPC is theoretically the simplest separation technique, in practice several problems are experienced, particularly with aqueous GPC. The Ultrahydrogel columns are made up of hydroxylated methacrylate gel. The latter has residual carboxylate groups, resulting in the column having an overall anionic character. This can result in electrostatic interaction between the column and positively charged species present in test samples. To minimize this interaction, a careful choice of MP is required. Some of the main problems associated with aqueous GPC include non size-exclusion effects such as adsorption, ionic effects and viscosity effects.

2.3 Non Size-Exclusion Effects in GPC

2.3.1 Adsorption

Adsorption can arise from hydrogen bonding, hydrophobic interactions and ionic interactions. It is more pronounced for high molecular weight compounds such as polymers because multiple contacts are possible. Many studies indicate that MP strengths of 0.2 M are sufficient to eliminate these interactions [Crone, 1974; Schmidt et al., 1980]. The MP pH can also be used to eliminate these interactions but this can be complicated in that the characteristics of the column stationary phase as well as the sample can be affected. Urea has been used to eliminate H-bonding in protein and sugar analysis. Hydrophobic interactions can be removed by the use of sodium dodecyl sulfate or by the addition of an organic modifier such as alcohol or glycol to the MP.

2.3.2 Ionic Effects

2.3.2.1 Ion-exchange

Charged sites may exist on column packing materials. Silica columns are prone to ionization of the silanol groups and the residual carboxylate groups present in methacrylate gels form negatively charged active sites. Counter cations attached to these sites can be exchanged for cationic analytes such as cationic polymers and results in the latter eluting late or not at all. Low pH MPs are recommended to suppress ionization [Snyder and Kirkland, 1979]. Alternatively, a small amount of electrolyte (0.05 M) is added to the MP to compete for the active sites and reduce the ion-exchange properties [Mizutani and Mizutani, 1977]. Cationic modifiers are also known to be used in the MP to reduce the effect [Buytenhuys and van der Maeden, 1978].

2.3.2.2 Ion-exclusion

If a surface of a packing material has a net charge, then solutes of a similar charge will be excluded from the pores because of electrostatic repulsions. This results in the polymer eluting early as a result of ion-exclusion. This is usually overcome by the addition of a small amount of electrolyte to the MP [Neddermeyer and Rogers, 1969].

2.3.2.3 Ion-inclusion

The ion-inclusion effect is based on Donnan membrane equilibrium and is responsible for spurious permeated peaks in aqueous GPC of polyelectrolytes. Ion-inclusion occurs if an electrolyte and polyelectrolyte are placed on one side of a semi-permeable membrane that is permeable only to the electrolyte. The charge of the electrolyte on the one side of the membrane is compensated for by a reduced concentration of permeable ions of like charge and by an increased concentration of permeable ions of opposite charge [Tanford, 1961]. In GPC a similar phenomenon occurs in which the column packing acts as a semi-permeable membrane [Stenlund, 1976]. Since the excluded polyelectrolytes cannot pass through the “membrane” the activity of the excluded species is increased and causes ions of the same charge as the polyelectrolyte to be forced into pores, giving rise to permeated peaks. This process

is called the Donnan equilibrium [Neddermyer and Rogers, 1969; Lindstrom et al., 1977; Rochas et al., 1980]. If the MP does not contain any electrolyte the retention time of permeable polyelectrolytes will be increased. This effect is reduced by reducing the charge on the polyelectrolyte and is achieved in practice by a pH adjustment.

2.3.2.4 Intra-molecular Electrostatic Effects

Most polyelectrolytes have the interesting property of being salt sensitive. Small amounts of added electrolyte can drastically reduce the viscosity of a polyelectrolyte solution. This occurs as a result of the shielding effect of the added electrolyte on ionic groups and reduces electrostatic repulsive forces, decreases the counter ion layer and diminishes intramolecular osmotic forces. This reduces the hydrodynamic volume of the polymer and causes greater permeation into the pores [Stenlund, 1976; Buytenhuys and van der Maeden, 1978; Cooper and Matzinger, 1979]

2.3.3 Viscosity Effects

Solution viscosity affects chromatographic behavior and therefore only dilute solutions should be used. As the concentration increases so do peak elution volumes and peak distortions occur. The effects can be explained in terms of two phenomena, macromolecular crowding and viscous fingering. At high polymer concentrations individual polymer chains become crowded and this reduces the hydrodynamic volume. If the MP viscosity is significantly lower than that of the polymer sample, the MP will tend to push through the sample, creating fingers of sample that results in distorted peaks.

2.4 Refractive Index Detection

For substances such as polyDADMAC that are UV inactive, refractive index (RI) detection is used. The RI detector is the only universal detector in HPLC and, as such, it suffers from low sensitivity. The detection principal involves measuring the change in refractive index of the column effluent passing through the detector flow cell. It is a pure differential instrument and requires the use of a two-path flow cell in which the sample containing side is constantly compared to the reference side. The greater the difference in RI between the test polymer and the mobile phase the greater is the detector response that is produced. In cases where the RI of the test sample closely matches that of the mobile phase it becomes invisible to the detector. Polymers and monomers can have a wide range of RI values but many do have similar values. Provided that the RI values are different to that of the mobile phase, and that they are adequately separated by the chromatographic system, RI detection will be possible.

2.5 Experimental

2.5.1 Instrumentation

GPC analyses were conducted on a Waters Alliance 2695 interfaced to a Waters 410 RI detector. Separation was achieved using Ultrahydrogel GPC columns (hydroxylated polymethacrylate based gel) supplied by Waters. The following five

columns were used in this study. They were assessed individually and by connecting them in series.

- Ultrahydrogel 120, dimensions 7.8 x 300 mm, 120 Å pores, 6 µm particles
- Ultrahydrogel 500, dimensions 7.8 x 300 mm, 500 Å pores, 8 µm particles
- Ultrahydrogel 1000, dimensions 7.8 x 300 mm, 1000 Å pores, 10 µm particles
- Ultrahydrogel 2000, dimensions 7.8 x 300 mm, 2000 Å pores, 13 µm particles
- Ultrahydrogel Guard Column, dimensions 7.8 x 50 mm, mixed bed.

The system was driven by Millenium^{32} software with the GPC option enabled. The flow rate was set at 0.5 mL min^{-1} and the column maintained at 40 ^{o}C using a column heating module. The detector cell internal temperature was set at 40 ^{o}C to prevent the effects of ambient temperature fluctuations on the detector stability.

2.5.2 Reagents

Milli-Q Water: Milli-Q water (2 L) was filtered and degassed using a 0.45 µm membrane type filter and MP filtration unit, both supplied by Millipore. Milli-Q water refers to water that has been passed through a Milli-Q water purification system that consists of a series of ion-exchange and organic removal resins.

Triethanolamine: Triethanolamine (Merk) was diluted to 0.1% (v/v) in Milli-Q water, filtered and degassed as described previously.

KH_2PO_4, 0.01 M: A phosphate buffer was prepared by transferring *ca* 2.72 g of KH_2PO_4 (BDH, Poole, UK) into a 2 L volumetric flask and diluting to volume with Milli-Q water. The buffer pH was adjusted to 2.3 with 10% (v/v) phosphoric acid. It was filtered and degassed prior to use.

KH_2PO_4, 0.25 M: A higher ionic strength phosphate buffer was prepared by transferring 68 g of KH_2PO_4 into a 2 L volumetric flask and diluting to volume with Milli-Q water. The buffer pH was adjusted to 2.3 with 10% (v/v) phosphoric acid. It was filtered and degassed prior to use.

Polyethylene Glycol Standards: Polyethylene glycol (PEG) narrow standards with a mass range from 106 to 23 000 Da were purchased from the Waters division of Millipore. Individual solutions at a concentration of 1000 mg L^{-1} (0.01 g in 10 mL Milli-Q water) were prepared and filtered through 0.45 µm syringe type filters prior to GPC analysis.

PolyDADMAC Test Sample: The test sample used in the development and optimization of the analytical method was Z464N, a commercially available polymer supplied by Zetachem (Durban, South Africa). The polymer concentrations used ranged from 0.1 to 0.5%. They were diluted with Milli-Q water or the appropriate MP.

mDADMAC Analysis: The External Standard Method

Stock Standard, 10000 mg L^{-1}: A 65% (m/v) diallyldimethylammonium chloride monomer (mDADMAC) solution (1.538 g, Sigma) was diluted to 100 mL in KH_2PO_4 MP.

Calibration Standards: The calibration standards of mDADMAC were prepared in KH_2PO_4 as per Table 2.1.

Table 2.1: Preparation of the calibration standards for mDADMAC analysis.

Sample ID	Volume Stock (mL)	Total Volume (mL)	mDADMAC (mg L^{-1})
Std 1	1	100	100
Std 2	5	100	500
Std 3	10	100	1000
Std 4	20	100	2000

The Samples: Two samples were prepared from Z464N polymer using 0.2323 g and 0.2692 g per 50 mL volumes of KH_2PO_4 MP. The samples were filtered through 0.45 μm filters for GPC analysis.

mDADMAC Analysis: The Method of Standard Addition

The Calibration Standards: The calibration standards were prepared by diluting the 10 000 mg L^{-1} stock standard of mDADMAC as per Table 2.2.

Table 2.2: Preparation of the calibration standards of mDADMAC for the internal standard method.

Sample ID	Volume Stock (mL)	Total Volume (mL)	mDADMAC (mg L^{-1})
Std 1	0	100	unknown
Std 2	1	100	100
Std 3	5	100	500
Std 4	10	100	1000
Std 5	20	100	2000

The Sample: The sample was prepared from Z464N polymer using 2.005 g in 500 mL of KH_2PO_4 MP. The samples were filtered through 0.45 μm filters for GPC analysis.

2.5.3 Amount of Active Polymer

A 0.1% (m/v) Z464N polymer solution was diluted in KH_2PO_4 MP (100 mL) for GPC analysis.

2.5.4 Precision

Data for the polymer and monomer were determined from 10 replicate injections of Z464N (0.1% m/v).

2.5.5 Detection Limits

The GPC detection limit for the Z464N polymer was determined by injecting increasing volumes (1 to 100 µL) of a 5000 mg L^{-1} polymer solution until the polymer peak could be clearly distinguished from the baseline.

2.6 Results and Discussion

2.6.1 Mobile Phase Selection

2.6.1.1 Milli-Q Water

Since separation of molecules occurs essentially on a mechanical basis or due to molecular sieving through the pores of the GPC column the only solvent requirements are that it should be compatible with the column packing material and the test sample must be soluble in the MP. Water was therefore evaluated as a MP for the analysis of Z464N, a polymer used extensively as a primary coagulant in water purification.

The overlay GPC chromatogram (Figure 2.3) shows five replicate injections of Z464N. Some degree of baseline instability can be noted in terms of drift and noise. This is usually noticed if the GPC system is not sufficiently equilibrated. Careful inspection of the chromatogram shows that this may not necessarily be the case as the y-axis scale is highly magnified.

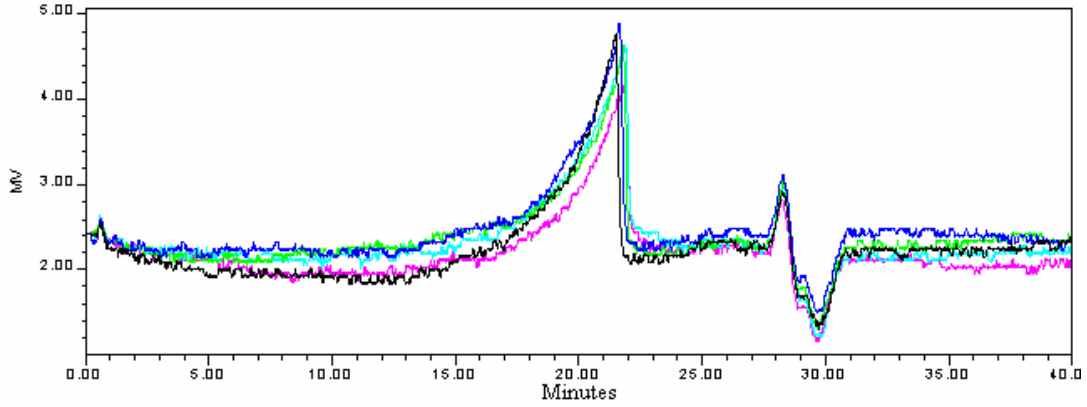


Figure 2.3: GPC chromatograms of five replicate injections of Z464N, using water as the MP.

The broad peak eluting in the region 13 to 23 min was reproducible for all injections and was a typical profile of a high molecular weight polymeric species. Since no other large molecular weight species was present, this peak was assumed to be that of polyDADMAC. Low molecular weight species appeared at 28 min. The GPC profile of the polymer is non-symmetrical, indicating a statistical non-uniform molecular weight distribution that is biased to the high molecular weight species. No further details are revealed in the chromatogram.

2.6.1.2 Triethanolamine

The second solvent tested as MP was 0.1% (v/v) triethanolamine (TEA). The overlay chromatogram of five replicate injections of Z464N (Figure 2.4) assumes a profile that is different to that of Figure 2.3.

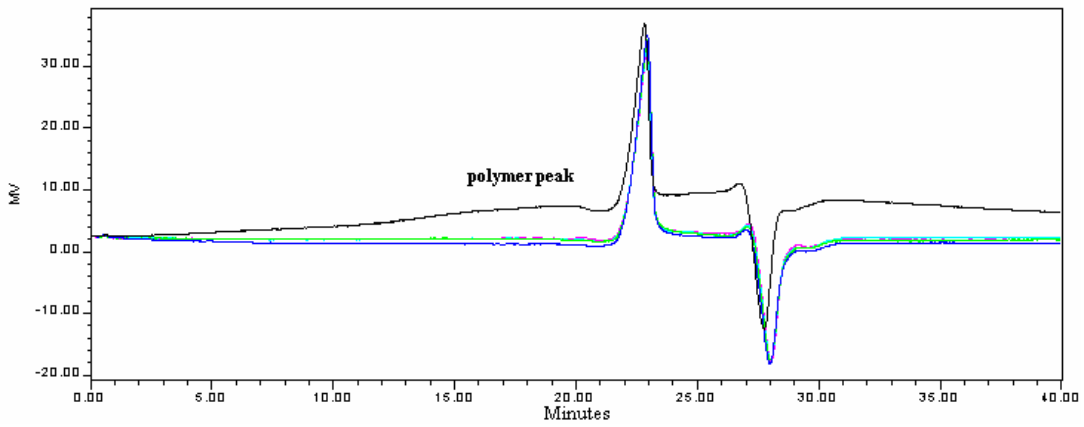


Figure 2.4: GPC chromatograms of five replicate injections of Z464N, using TEA as the MP.

The polymer was assumed to be the broad peak stretching from 10 to 21 min. It is separated from another relatively lower molecular weight species occurring at 22.5 min. The apparent improvement in efficiency and resolution of the GPC system indicates that non-size exclusion mechanisms are minimized when TEA is used as the MP. However the polymer peak appears to be adversely affected; it gives a very broad flat peak that is not clearly distinguished from the baseline.

2.6.1.3 KH_2PO_4, 0.01 M

With KH_2PO_4 as MP, a new and different GPC profile was obtained (compared to those shown in Figures 2.3 and 2.4). Several replicate injections indicated good reproducibility. Analyses done one day later (Figure 2.5) indicated that apart from a slight drift in the baseline on the first injection the other injections are almost perfectly superimposed on each other.

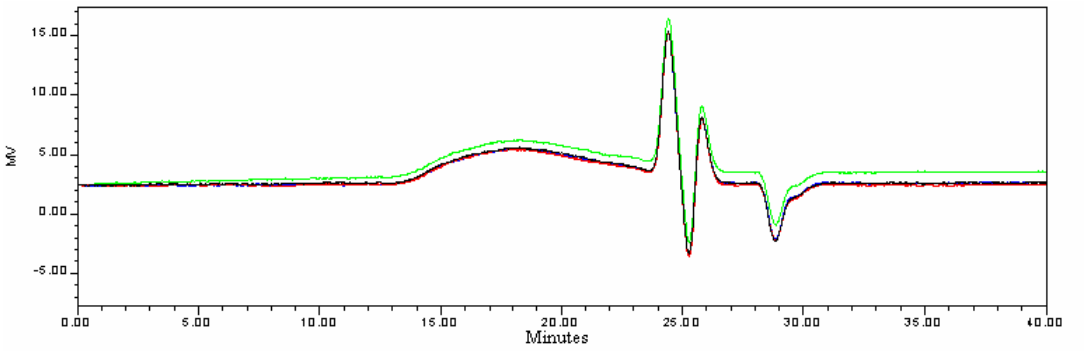


Figure 2.5: GPC chromatograms of Z464N, using 0.01 M KH_2PO_4 as the MP.

The polymer peak is well defined, with reasonable sensitivity. The low molecular weight components appear as two fully resolved peaks. There is however a slight dip in the baseline after the first low molecular weight peak. This is usually quite normal in GPC with RI detection and arises from the differences in the refractive indices of the MP and the sample dilution solvent.

2.6.1.4 KH_2PO_4, 0.25 M

For aqueous GPC, a MP having a relatively high ionic strength is recommended as it aids in the suppression of non-size exclusion mechanisms. For this reason a high concentration of KH_2PO_4 was tested as the MP. The chromatogram (Figure 2.6) shows an increase in the baseline noise and drift. The peak shape of the low molecular weight species shows some degree of improvement in resolution with no baseline dip occurring.

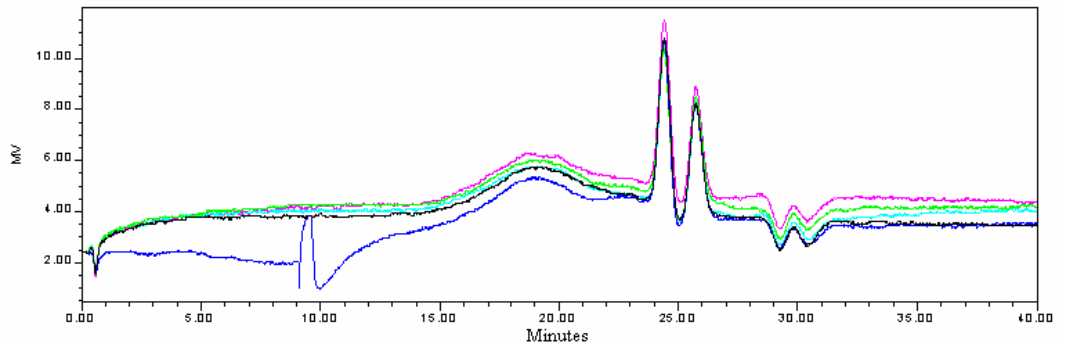


Figure 2.6: GPC chromatograms of Z464N using 0.25 M KH_2PO_4 as the MP.

The increase in MP ionic strength does however not appear to impact on the elution volume of the polymer species. This shows that the hydrodynamic volume of the polymer has not decreased as a result of the increase in the MP ionic strength. Theoretically, the polymer volume should have decreased, resulting in a greater penetration into the pores of the GPC column and hence a higher retention time. It may well be that this phenomenon occurred but was not clearly observed due to the fact that a large pore size GPC column (2000 Å) was used. Figure 2.7 is a repeat run of five injections, after allowing the column to stabilize further.

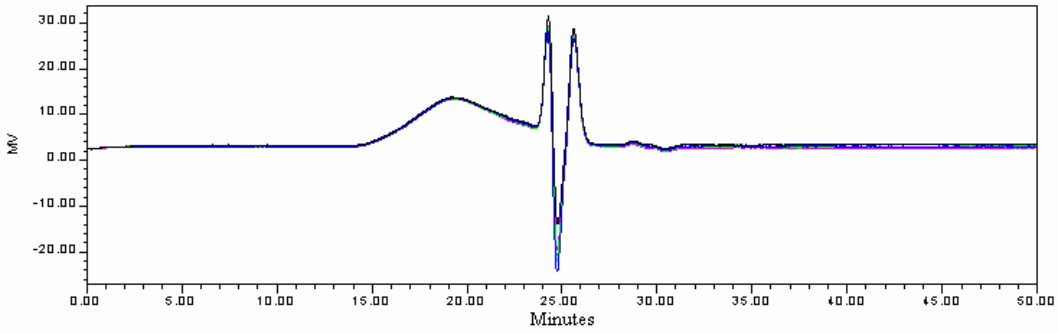


Figure 2.7: GPC chromatogram of Z464N using 0.25 M KH_2PO_4 as the MP and increased instrument stabilization time.

The polymer profiles are perfectly superimposed on each other, showing relatively good sensitivity, and baseline noise was minimized. Hence this MP was selected as the one best suited for the GPC of polyDADMAC polymers. The polymer peak width was approximately 10 min. The dip in the baseline at 25 min is not unique and is a common phenomenon observed with the RI mode of detection. It is attributed to slight differences in refractive indices of the mobile phase and sample dilution solvent. Since this baseline dip elutes well after the polymer peak, it does not place any limitations on the application of the method.

2.6.2 Column Selection and Optimization

The use of the correct column or column combination is essential for obtaining optimum GPC separations. Columns should be chosen so that their calibration curves are linear over the polymer molecular weight distribution range. As there is little knowledge of the molecular weight composition of the polymer Z464N, the column selection was done randomly. The Ultrahydrogel 2000 column with a broad molecular weight range (50 000 to 20 000 000 Da) was first selected. During the development of the GPC method no reference standards were available for the calibration of the GPC 2000 column that was used for the GPC analysis of the polymer samples. The suitability of the column was tested by inspection of the changes in the resolving power of the column during the analysis of Z464N. With the Ultrahydrogel 2000 column, the polymer peak appeared reasonably well resolved from the low molecular weight species, as shown in Figure 2.7. However, the polymer peak itself appears as a single component.

Attempts were made to improve the resolving power of the system in order to determine the number of peaks present in the polymer region of the chromatogram. This was done by coupling the Ultrahydrogel 500 column (molecular weight range 5000 to 400 000, PEO) to the system. Using this column combination, the test polymer was re-injected into the chromatograph. The overlay chromatogram of five replicate injections is shown in Figure 2.8. This system exhibited a considerable amount of baseline drift and noise.

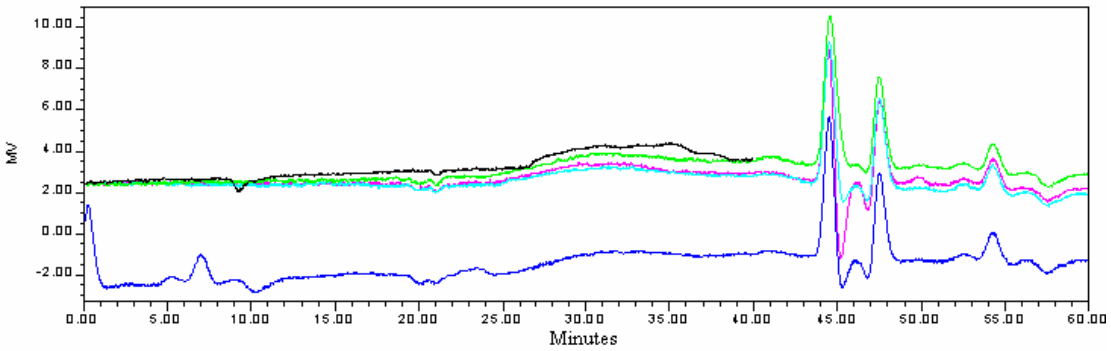


Figure 2.8: GPC chromatograms of Z464N using the Ultrahydrogel 2000 column coupled to the Ultrahydrogel 500 column.

Methanol was injected into the system and the resulting peak (Figure 2.9) was used as a marker of total permeation (V_t) of the system. It appears after the two low molecular weight species in the chromatogram. This is significant, and indicates that the columns have resolving power that extends beyond the two low molecular weight species. Furthermore, the fact that no other peaks are present is evidence that all components of the polymer were eluted from the column.

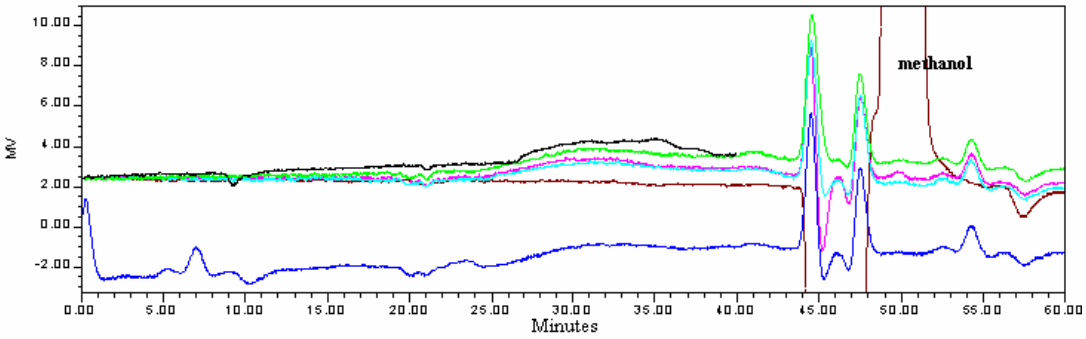


Figure 2.9: Overlay GPC chromatogram of replicate injections of Z464N and methanol using the Ultrahydrogel 2000 column coupled to the Ultrahydrogel 500 column.

More importantly, two new features result from the added column. There is an improvement in the resolution of the low molecular weight species and the polymer peak appears with low intensity as a broad distribution, with a peak width of 20 min. Furthermore, there was an increase in the total analysis time from 35 to 60 min. Even so, no additional components are visible in the polymer peak. It remains as a single broad distribution.

To confirm that no other species were present, a third column, the Ultrahydrogel 120, was included in the column bank. There was a further increase in resolution of the low molecular weight species (Figure 2.10) in the region 58 to 70 min.

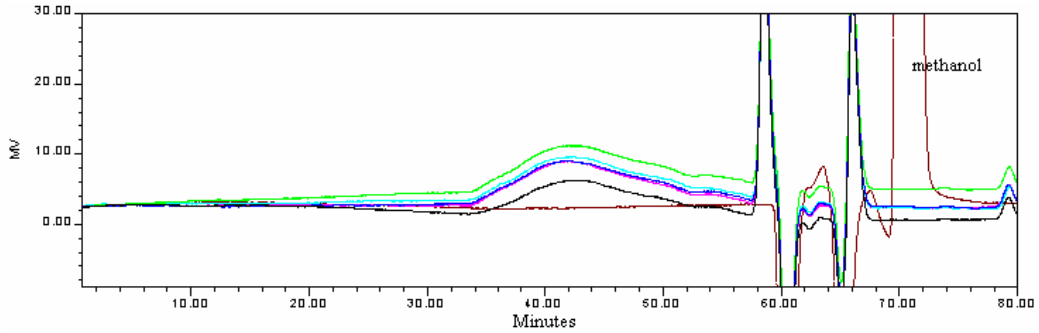


Figure 2.10: GPC chromatograms of Z464N using the Ultrahydrogel 2000 column coupled to the Ultrahydrogel 500 and 120 columns.

Once more, methanol was used as the marker for V_t, and it now shows a shift in value from 50 to 72 min. Further improvements in the resolution of the low molecular weight species is noted but there are no changes in the features of the polymer peak except for a further broadening of the peak width from 20 to 30 min.

Changing from the Ultrahydrogel 2000 column to the combination of columns, the polymer peak width increases from 10 min (Figure 2.7) to 20 min (Figure 2.8) and finally to 24 min (Figure 2.10). The addition of the further columns however shows no positive effect on the resolution of the polymer peak and in fact adversely affects the profile of the polymer peak. The polymer peak becomes significantly stretched out and the total analysis time increases drastically. As predicted by theory, the addition of the small pore size columns impacts only on the low molecular weight

species in terms of resolution. The polymer peak of interest however, shows no important additional features of interest except for the negative aspects of the increases in polymer peak width and the increase in total analysis time. Hence the Ultrahydrogel 2000 column alone appeared to be the best choice for the analysis of Z464N, as shown in Figure 2.7. Further work was carried out to determine the effect of removing the Ultrahydrogel 2000 and 120 columns on the polymer peak profile. When using only the Ultrahydrogel 500 column the polymer peak appears earlier in the chromatogram (Figure 2.11) than when using the Ultrahydrogel 2000 column.

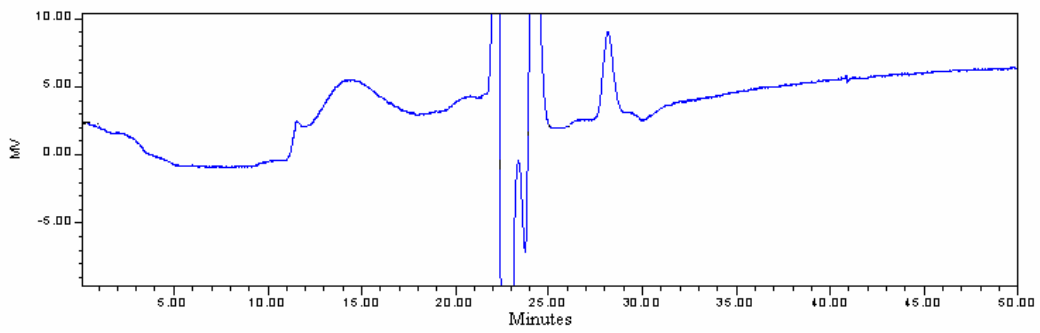


Figure 2.11: GPC chromatogram of Z464N using the Ultrahydrogel 500 column.

Another interesting point to note is that the polymer peak appears with better resolution and a shoulder is observed on the main peak that was previously not visible. Such features give important analytical information on the product performance, which would otherwise be totally lost with improper selection of columns. In addition, the low molecular weight species are slightly better separated from the polymer. Hence the Ultrahydrogel 500 column is clearly a better alternative than the Ultrahydrogel 2000 column. Attempts were made to go one step further, by using the Ultrahydrogel 120 column (molecular weight range 200 to 5000 Da), to move the low molecular weight species and the negative baseline dip further away from the polymer peak. This is desirable for good quantification of the components of Z464N.

The GPC chromatogram obtained for Z464N using the Ultrahydrogel 120 column (Figure 2.12), shows a very different profile to that obtained with the other columns.

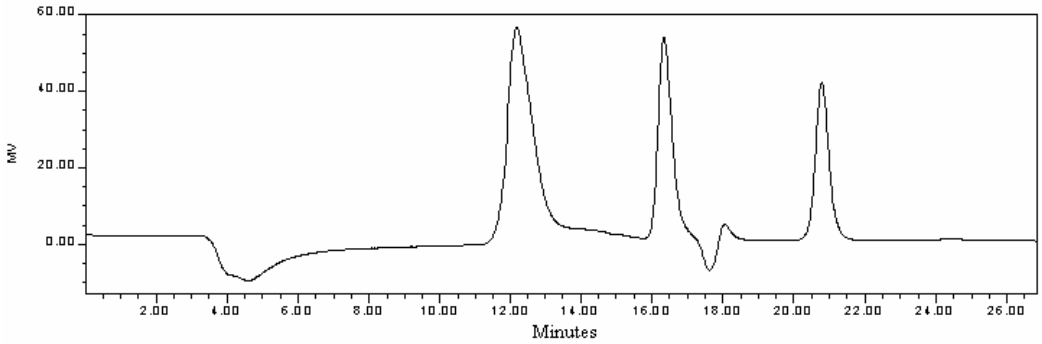


Figure 2.12: GPC chromatogram of Z464N using the Ultrahydrogel 120 column.

The symmetrical and sharp polymer peak shows that it is to a large extent excluded from entering into the small pore sizes of this column. This results in little size separation occurring and therefore all polymer molecular weights are not adequately

resolved from each other. This column is therefore unsuitable for GPC work as all molecular weight distribution (MWD) data are lost due to inadequate mass resolution.

To confirm that the polymer was outside the separating limit of the column, attempts were made to calibrate the column using a set of PEG standards with molecular weights ranging from 106 to 23 000 Da. A series of 10 standards were prepared, chromatographed individually, and then the chromatograms superimposed on each other (Figure 2.13).

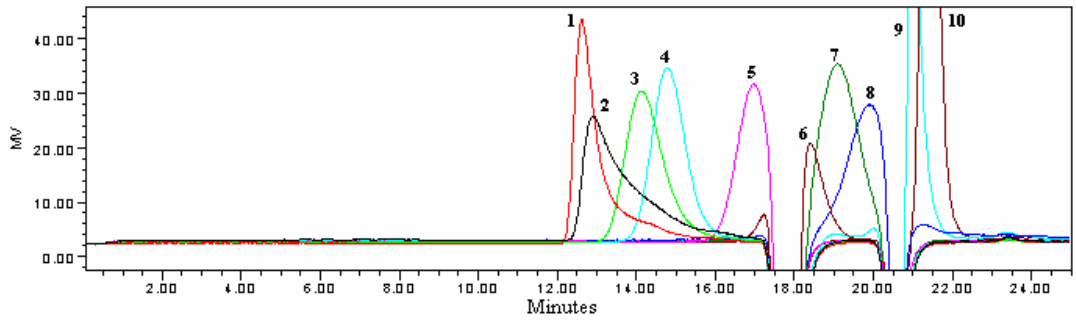


Figure 2.13: Overlay of GPC chromatograms of PEG standards using the Ultrahydrogel 120 column.

From the chromatograms it is clear that standards 1 and 2 co-elute with each other and hence this can be regarded as the exclusion limit, V_0, of the column. When the polymer was superimposed on the standards (Figure 2.14), it elutes together with standards 1 and 2, with only a slight degree of resolution.

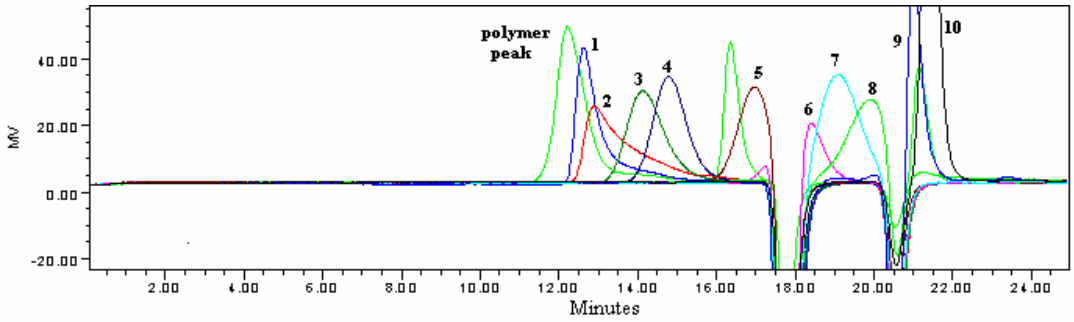


Figure 2.14: Overlay of GPC chromatograms of Z464N and the PEG standards using the Ultrahydrogel 120 column.

The major part of the mass distribution lies outside the exclusion limit of the column. No higher molecular weight standards were available to demonstrate this exclusion limit further. When total exclusion occurs, all component peaks will co-elute and be fully superimposed on each other.

From the PEG data a GPC calibration curve was plotted for the Ultrahydrogel 120 column (Figure 2.15). The highest and lowest standards were used as markers of V_0 and V_t respectively. The region of selective permeation is the linear region of the curve that occurs between 12.5 and 21 min.

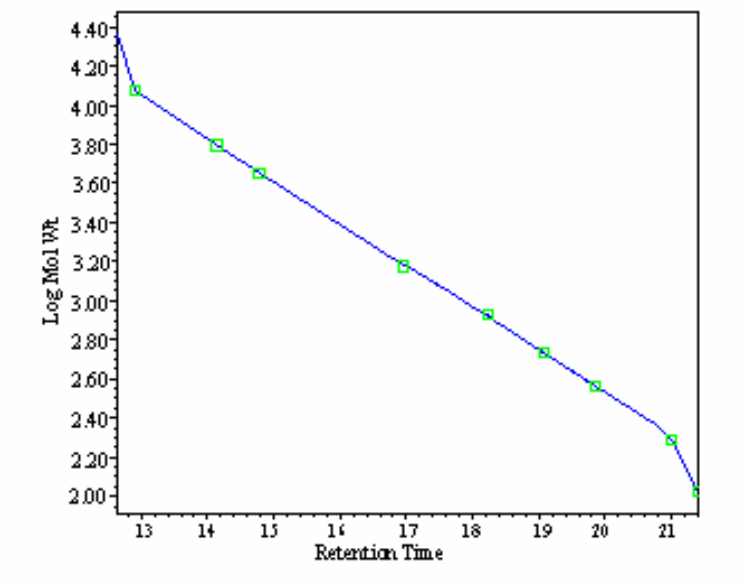


Figure 2.15: GPC calibration curve for the PEG narrow calibration standards.

It is clear that the major part of the polymer peak lies outside this range and no size separation takes place since the polymer is excluded from the column pores. The Ultrahydrogel 120 column is designed with an effective molecular weight separation range of 200 to 5000 Da. The additional separating power to approximately 23 000 Da is due to the use of the Ultrahydrogel guard column in series with the analytical column. The guard column comprises essentially a short length (50 mm) of a linear or mixed bed column that has a wide effective mass separation range.

2.6.3 The Composition of Z464N

Although it was identified that the Ultrahydrogel 120 column installed was unsuitable for the GPC analysis of the polymer the method was identified to have potential for other applications. It was decided to test the GPC method purely for the purposes of identification and quantification of the matrix components of the polymer. The reasons for this study were that good sensitivity, good efficiency and good component resolution were achieved with the method. The low molecular weight species were well removed from the negative peak arising from the dip in the baseline and this promotes good quantification.

One usually expects to find residues of monomer present in a polymer, the amount of which depends on the completeness of the polymerization reaction. The test polymer Z464N was therefore tested for the presence of the monomer. To establish the monomer peak a 0.1% mDADMAC solution was prepared and analyzed by GPC (Figure 2.16).

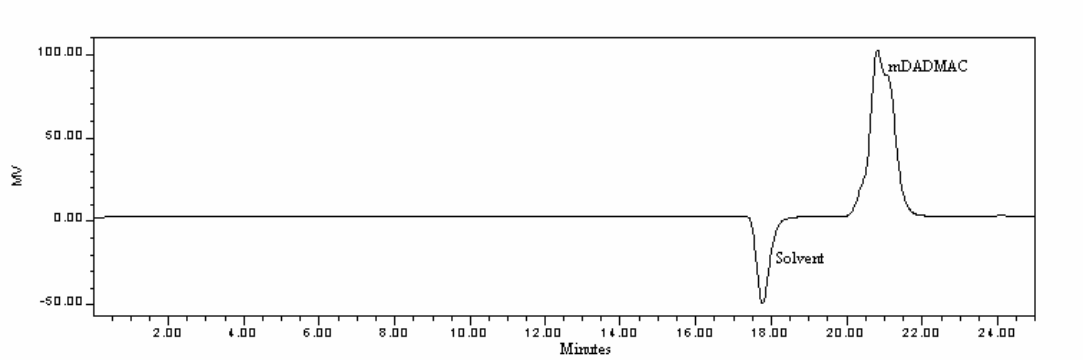


Figure 2.16: GPC chromatogram of mDADMAC using the GPC method developed for polyDADMAC analysis.

Only a single peak was observed in the chromatogram, and was tentatively assigned as the monomer peak. An overlay of the chromatogram with that of Z464N, (Figure 2.17) revealed clearly the elution time of the monomer peak at 21 min.

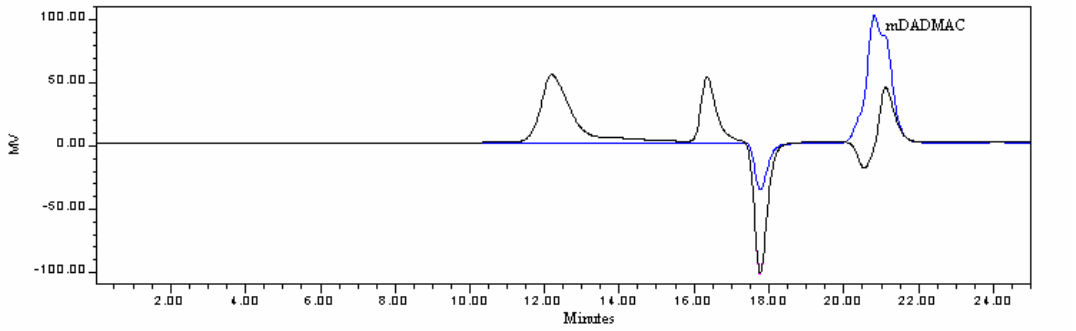


Figure 2.17: Overlay of GPC chromatograms of Z464N with the mDADMAC monomer.

The identity of the monomer was based on a comparison of the retention times. To establish the position of the solvent peak, Milli-Q water was chromatographed and found to give the negative baseline dip at *ca* 17.8 min. The monomer content of all polyelectrolytes used for potable water purification is a very important parameter to measure. This is one of the main requirements for accreditation by the National Sanitation Foundation (NSF). The recommended standard for mDADMAC is 5%. The monomer level gives an indication of the completeness of the polymerization reaction. The potential adverse health effects associated with the monomer is of great significance and therefore requires very careful monitoring. The findings from the GPC analysis with the Ultrahydrogel 120 column when coupled to the guard column were very significant, and showed potential use for monomer analysis.

2.6.4 mDADMAC Quantification: External Standard Calibration

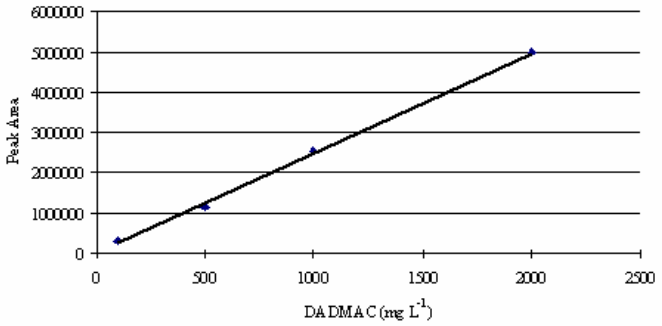
The standard method for the determination of the DADMAC content in polymer samples involves titrimetric analysis. Other methods commonly used involve paired ion chromatography [ANSI/AWWA 451-92, 1993]. Both two techniques are extremely long and require very labor intensive sample preparation procedures. Hence the newly developed GPC method showed good potential as a rapid method for

monomer analysis. The peak appeared to be well resolved from other matrix components and it was symmetrical, with good sensitivity. The method was simple and only required dilution and filtration, followed by sample analysis. Two techniques were used for the quantification of mDADMAC, including the external standard calibration method and the method of standard addition. Using the former method, a series of DADMAC standards and tests samples were chromatographed and peak areas determined (Table 2.3).

Table 2.3: Peak area data for the external calibration method for mDADMAC analysis.

Sample ID	mDADMAC (mg L^{-1})	Peak Area
Std 1	100	322761
Std 2	500	1132064
Std 3	1000	2514277
Std 4	2000	4977888
Sample 1	From Calibration	2317218
Sample 2	From Calibration	2478356

The data were used in regression analysis (Figure 2.18). The regression line shows excellent linearity of the calibration with a correlation coefficient $R^2 = 0.9996$.



Regression Statistics		
Multiple R	1.00	
R Square	0.999	
Adjusted R Square	0.998	
Standard Error	96497	
Observations	4	STDEV
Intercept ± STDEV	1865	77858
Slope ± STDEV	2483	68
y = 2483x + 1864		

Figure 2.18: The external standard calibration for mDADMAC.

The sample was analyzed in duplicate and the concentrations calculated from the regression equation to give 932 and 997 mg L^{-1} for the two samples. The percentage of mDADMAC was calculated using equations 2.1 and 2.2.

$$\%\, mDADMAC_1 = mg\, L^{-1}\, Result \times \frac{Volume_{Sample}}{1000} \times \frac{1}{Mass_{Sample}} \times 100 \qquad 2.1$$

$$= 932 \times \frac{50}{1000} \times \frac{1}{232.3} \times 100$$

$$= 20\,\%$$

$$\%\, mDADMAC_2 = mg\, L^{-1}\, Result \times \frac{Volume_{Sample}}{1000} \times \frac{1}{Mass_{Sample}} \times 100 \qquad 2.2$$

$$= 997 \times \frac{50}{1000} \times \frac{1}{269.2} \times 100$$

$$= 19\,\%$$

The monomer results were relatively high and exceeded the guideline standard for this polymer. This was cause for concern and indicated that there was a serious problem with method interference.

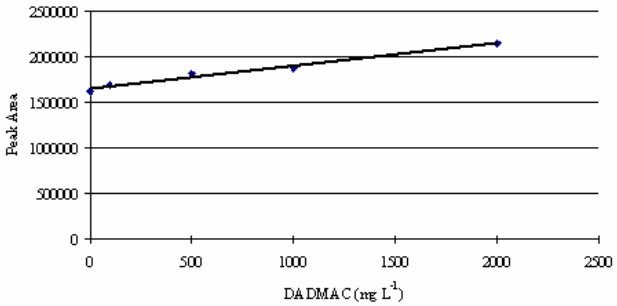
2.6.5 mDADMAC Quantification: The Method of Standard Addition

The polymer sample was then analysed by the method of standard addition. The peak area data are shown in Table 2.4.

Table 2.4: Peak area data for the mDADMAC analysis by standard addition.

Sample ID	Addition (mg L^{-1})	Peak Area
Sample 1, Aliquot 1	0	1617021
Sample 1, Aliquot 2	100	1682845
Sample 1, Aliquot 3	500	1811269
Sample 1, Aliquot 4	1000	1878826
Sample 1, Aliquot 5	2000	2142983

The data were used in a regression analysis (Figure 2.19). The regression statistics indicate a poorer degree of linearity with a correlation coefficient $R^2 = 0.983$.



Regression Statistics		
Multiple R	0.991	
R Square	0.983	
Adjusted R Square	0.977	
Standard Error	31080	
Observations	5	STDEV
Intercept ± STDEV	1647671	19516
Slope ± STDEV	248	19
y = 248.5x + 2E+06		

Figure 2.19: The standard addition calibration line for mDADMAC.

The mDADMAC concentration was calculated from the linear regression equation and found to be 123 mg L^{-1}. The percentage of mDADMAC was calculated using equation 2.3.

$$\%\, mDADMAC = mg\, L^{-1}\, Result \times \frac{Volume_{Sample}}{1000} \times \frac{1}{Mass_{Sample}} \times 100 \qquad 2.3$$

$$= 123 \times \frac{500}{1000} \times \frac{1}{2005.5} \times 100$$

$$= 3\%$$

From the large difference in results of the two methods it is clear that there is a severe interference problem with the method, which was not apparent from a visual inspection of the chromatograms. This means that the monomer peak was co-eluting with other low molecular weight species and contributing to the unusually high result. The result obtained by standard addition is a more realistic result as the standard for polyDADMAC [ANSI/AWWA B451-92, 1993] specifies a value ≤ 5% for monomer. This is a requirement that polymer manufacturers need to adhere to, for their product to be suitable for drinking water purification.

2.6.6 Determination of the Percentage Active Polymer

The standard procedure used by AWWA to determine the amount of active polyDADMAC in a sample of product involves the determination of the total solids content of the sample (gravimetry) minus the amount of mDADMAC (titration) and the sodium chloride content (titration) [ANSI/AWWA B451-92, 1993]. This is a lengthy process.

The GPC method developed was potentially an easier method of polymer analysis. The requirement to determine the percentage active polymer is that there must be adequate resolution of the polymer peak from other matrix components (monomer and NaCl). This method proposes using the peak area data to calculate the active polymer content, using equation 2.4.

$$\%\, Acive\, Polymer = \frac{Area_{Polymer}}{\sum Area} \times 100 \qquad 2.4$$

Hence a simple analysis of the sample with the generation of an area percent value will give the percentage active polymer. Using this method, a polymer content of 50% was determined, and verified by gravimetry. The typical amount of polymer specified by the AWWA standard is in the range 10 to 50% [ANSI/AWWA B451-92, 1993], indicating that the method produces satisfactory results. Even so, it is being tested rigorously on new batches of polymer for possible interferences by comparing the GPC results with results obtained with the standard method. Usually between 10 and 30 sets of data are required to fully validate the performance of the new GPC method for monomer and percentage active polymer analysis, and users of the method are therefore encouraged to show an initial demonstration of capability by performing comparative studies on several batches of polymer.

2.6.7 Precision of the GPC Method

The area precision for the polymer and monomer peak was determined by performing 10 replicate injections of a 0.1% (m/v) solution of Z464N. The precision was found to be 1 and 3% RSD for the polymer and monomer respectively (Table 2.5).

Table 2.5: Precision of the GPC method for polymer and monomer analysis.

	Peak Area			
	Run 1		Run 2	
Replicate	pDADMAC	mDADMAC	pDADMAC	mDADMAC
1	2573076	1498303	2617995	1393639
2	2585515	1451314	2611585	1443097
3	2602899	1443689	2594582	1373844
4	2604198	1400512	2575187	1440099
5	2615404	1386856	2613867	1403243
6	2613867	1403243	2615404	1386856
7	2575187	1440099	2604198	1400512
8	2594582	1373844	2602899	1443689
9	2611585	1443097	2585515	1451314
10	2617995	1393639	2573076	1498303
AVE	2599431	1423460	2599431	1423460
STDEV	16634	38116	16634	38116
%RSD	1	3	1	3

2.6.8 Detection Limits

The method detection limit was estimated by injecting decreasing amounts (50 to 5 µg) of polymer into the system. Figure 2.20 is a GPC chromatogram of the polymer near the detection limit of 5 µg polymer. Calculations show that this limit of detection translates to a value of 5 µg per 100 µL injected, giving a result of 50 mg L^{-1}, or 0.005% polymer.

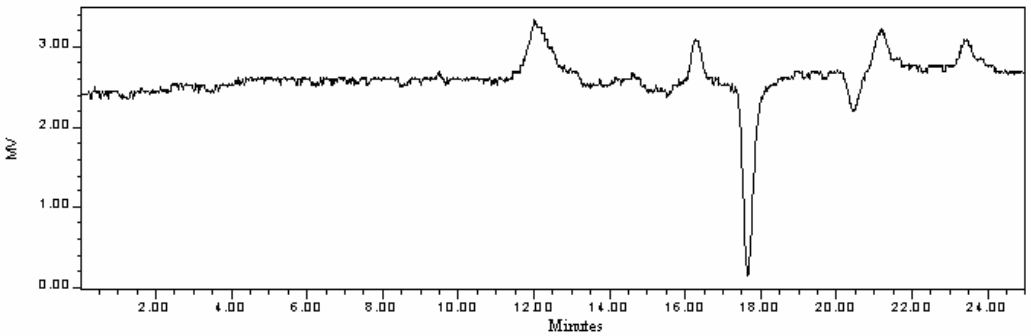


Figure 2.20: GPC chromatogram of Z464N near the method detection limit.

Improved detection limits are predicted with the use of light scattering methods of detection. However, these methods require the use of volatile mobile phases containing little or no salts and were not investigated for polymer analysis. The method developed for this study made use of a high salt containing buffer (0.25 M KH_2PO_4) that was not compatible with the light scattering detector. Future studies

ought to focus on developing a volatile mobile phase for polymer analysis by GPC with light scattering detection.

2.6.9 Application of the GPC Method

During the course of this work the GPC method that was developed was used in the analysis of samples taken at the Durban Heights Water Treatment Works. A layer of scum was observed floating on the surface water of the sand filters as well as the canal leading to the pulsators. This occurred after pre-chlorination and polyelectrolyte dosing, and was therefore suspected as being caused by the coagulation and flocculation process. It was decided to apply the GPC method to determine the presence or absence of polymers. Knowledge of this is very significant from a production cost point of view as well as a public health point of view. Little is known on the health effects of polymers used in water purification and the possibility existed that if the polymer was detected in either of the two samples, there would be a danger of it passing through the sand filters and entering into the final treated water.

The investigation commenced with the collection of samples of the sludge from the canal dead end and the scum sample from the surface of the sand filters. Initial screening of the samples was done by GPC, using only the guard column to reduce the total analysis time. Four samples were chromatographed: a blank (Milli-Q water), the reference standard (0.1% Z553D) and the two samples. The overlay chromatogram (Figure 2.21) indicates that all components elute in the region less than 4 min.

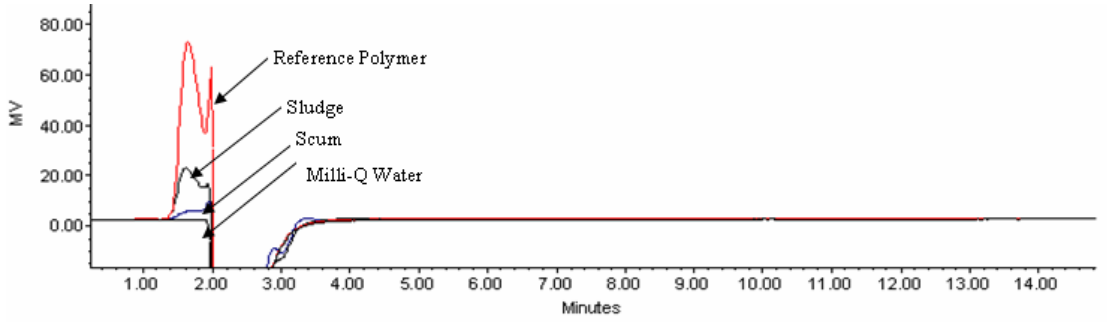


Figure 2.21: Overlay of GPC chromatograms of the Z553D with the sludge, scum and Milli-Q water. Separation was achieved using only the guard column.

Both the sludge and scum samples show the presence of a high molecular weight species in the region 1.9 to 2.0 min. This is followed by a negative peak arising from water. The reference standard shows a similar profile to the samples. The relative amount of polymer is higher in the canal sludge than in the filter sample. Since these results showed the possible presence of polymer it was decided to confirm the results using both the guard column and the analytical column, connected in series. The overlay chromatogram obtained for the four solutions (Figure 2.22) shows a significant improvement in resolution and a corresponding increase in the run time, as expected.

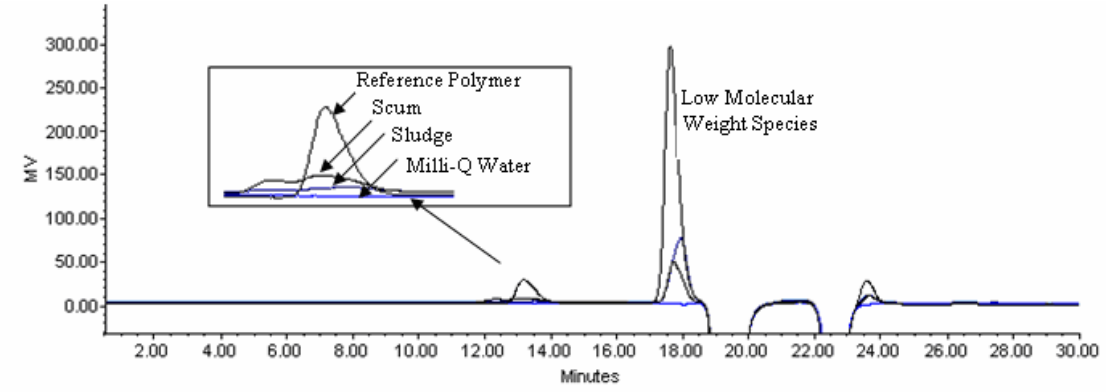


Figure 2.22: Overlay of GPC chromatograms of the Z553D with the sludge, scum and Milli-Q water. Separation was achieved with the guard and analytical columns connected in series.

The high molecular weight species appears in the region 11 to 15 min. The reference polymer solution profile shows some degree of difference in the peak profile to that observed in the two test samples under investigation but elutes at approximately the same retention time. The samples have a shoulder preceding the main peak that is more pronounced in the scum than in the sludge. This indicates the presence of relatively more of a high molecular weight species, slightly exceeding that of Z553D. The origin of this may be attributed to the presence of large molecular weight humic substances present in the sample from coagulation and flocculation. Low molecular weight species appear with improved resolution in the region greater than 17 min.

Since the GPC chromatogram clearly indicated the presence of polymer in the samples, additional work was carried out to investigate the final treated water from DHWTW for polymer presence. The final water was first analyzed for polymers and subsequently spiked with 100 µL of a Z553D solution (0.1%) and chromatographed. The overlay GPC chromatogram of the spiked and un-spiked final water (Figure 2.23) shows an absence of polymer in the region 11 to 15 min of the un-spiked sample.

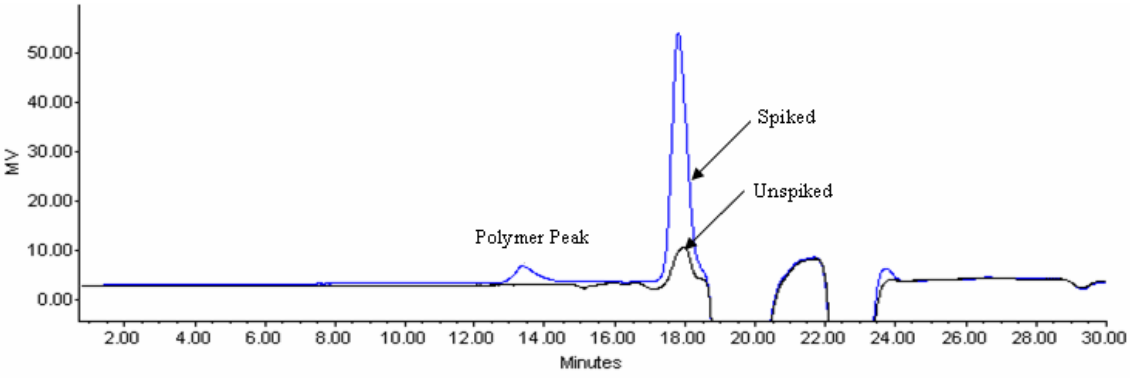


Figure 2.23: Overlay of GPC chromatograms of spiked and unspiked samples of DHWTW final. Separation was achieved with the guard and analytical columns connected in series.

However, the small molecules in the region greater than 17 min approximate the profile of the two test samples under investigation. The spiked sample shows the

position at which the polymer was expected to elute if present in the sample. The results indicated that the polymers remained bound to the floc (sludge and scum) particles and were not entering the treated water system.

2.7 Conclusions

A GPC method for polyDADMAC analysis and characterization was successfully developed. The best mass resolution was obtained using the Ultrahydrogel 500 column and a MP of 0.25 M KH_2PO_4. No further mass resolution of the polymer could be achieved despite the addition of a further two columns (Ultrahydrogel 2000 and 120) in series with each other. The additional columns impacted adversely on the separation. There was too much resolution of the low molecular weight species and the polymer peak became poorly defined; the peak width increased from 10 to 24 min. The total analysis time was also affected negatively; it increased from 35 min to 80 min.

During the course of the investigation it was found that the Ultrahydrogel 120 column had potential for the analysis of the low molecular weight species and the monomer content could be determined by the method of standard addition with a 3% precision. Co-elution problems were experienced and limited the use of the external standard calibration method. The method could be used to quantify the amount of polymer present in a test sample in a similar way to the HPLC method up to a limit of 50 mg L^{-1} polymer and a 1% precision. The method was applied successfully in the analysis of a sludge and scum sample from the DHWTW. Analysis of the final treated water showed an absence of polymer or a level that was below the limit of detection of the GPC method. This finding is reassuring, especially to Umgeni Water, it being a bulk water service provider to a population in excess of 7 million people. Apart from the other applications demonstrated, the GPC method is currently being used in setting up a database of polymer profiles for each new batch of product procured. This meets one of the objectives of the study, namely monitoring the quality of polyDADMAC by overlaying GPC chromatograms with previously acquired ones. Differences in the MWDs of polyDADMAC are obtained by visual observation of the GPC curves and a good product can be readily distinguished from an inferior product.

2.8 References

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CHAPTER THREE

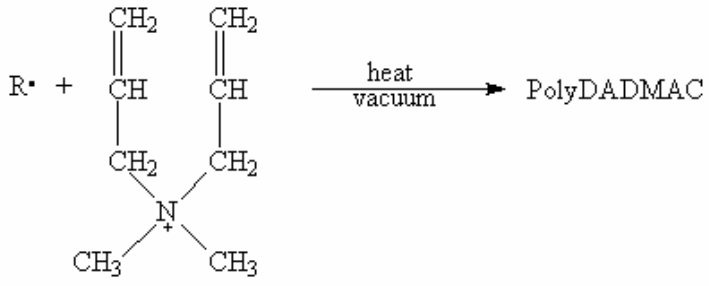
SYNTHESIS AND STRUCTURE OF POLYDADMAC

3.1 Introduction

As the synthesis procedure of polyDADMAC for commercial use is highly confidential, little information on the production of the polymer is available from manufacturers. To acquire a good working knowledge of polyDADMAC synthesis and the various parameters that impact on the degree of polymerization and product quality, polymer synthesis was deemed to be a vital part of this study. Additionally, in the event that polyDADMAC produced adverse effects or controversial results during simulated water treatment studies, the use of a laboratory-synthesized polymer would be necessary, to avoid any legal complications, and possible lawsuits from manufacturers. PolyDADMAC was subsequently synthesized by free-radical addition polymerization of mDADMAC using a persulfate initiator. In this study, the exothermic reaction was conducted under carefully controlled conditions of temperature, pressure and monomer concentration. Poor control of the above parameters resulted in the formation of a highly cross-linked product early in the reaction, which was of no use for the purposes of water clarification. Under better controlled conditions the polymer was synthesized successfully and isolated from the aqueous solution by precipitation. The product was characterized in terms of its molecular weight distribution using the GPC method developed in Chapter 2 and the chemical structure was elucidated by ^{13}C-NMR spectroscopy. It was conclusively determined that the reaction mechanism favored the formation of the pyrrolidine ring system. The synthesized product was tested in a water treatment application. Results showed that a dose of 6 to 7 mg was able to clarify a raw water sample (800 mL), reducing the turbidity from 21 to less than 0.5 NTU.

3.2 Theoretical Considerations and Background

PolyDADMAC is a cationic polymer used extensively for the purpose of potable water purification. It is synthesized by the free-radical initiated addition polymerization of mDADMAC according to Scheme 3.1.



Scheme 3.1: The synthesis of polyDADMAC by free-radical initiated addition polymerization of mDADMAC.

Prior to 1949 it was understood that, when polymerized, non conjugated dienes produced cross-linked and therefore water insoluble nonlinear polymers or

copolymers [Staudinger and Heuer, 1934]. It was only between 1949 and 1957 that exceptions to this were observed. Work conducted on the polymerization of allyl and substituted allyl quaternary ammonium compounds showed the formation of water insoluble polymers when monomers with three or more allyl groups were used. Monomers containing two allyl groups produced non-cross-linked polymers that were completely soluble in water, while monomers containing one allyl group did not polymerize at all [Butler and Bunch, 1949; Butler and Ingley, 1951; Butler and Goette, 1952; Butler et al., 1952; Butler and Goette, 1954; Butler and Johnson, 1954; Butler and Angelo, 1956; Butler and Angelo, 1957]. These findings contradicted the widely accepted view that monomers with one double bond resulted in the formation of linear polymers with some degree of unsaturation while monomers with two or more double bonds formed cross-linked polymers possessing little or no solubility.

The aims of this part of the study were to investigate the polymerization of the DADMAC monomer containing two allyl groups and the mechanism by which it forms a water soluble product, to establish its molecular weight distribution (MWD), to determine the chemical structure and test the performance of the product in water clarification. The synthesis was conducted on a laboratory scale with a 1 L volume reactor designed specifically for this work. The synthesis procedure [Hunter and Siedler, 1979] was relatively difficult; it required the reaction to be conducted under vacuum, with carefully controlled conditions of temperature, monomer concentration and the absence of oxygen.

3.3 Experimental

3.3.1 Apparatus

A reactor for the synthesis was constructed from a 1 L glass vessel attached to a four-neck manifold by an o-ring gasket and clamp assembly (Figure 3.1).

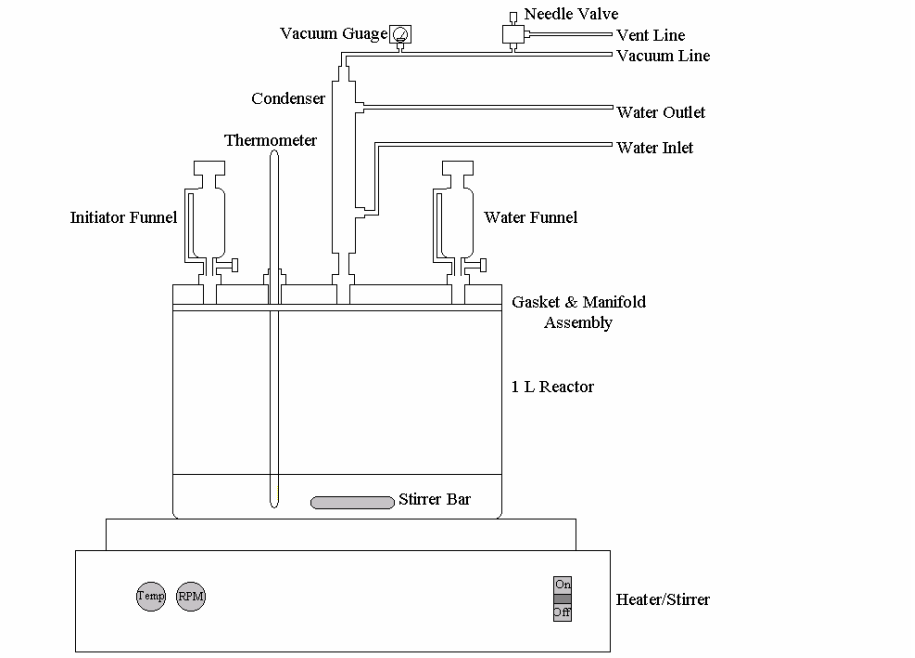


Figure 3.1: Schematic diagram of the reactor used for the synthesis of polyDADMAC.

Two pressure equalizing dropping funnels were used, one for the addition of the free radical initiator and the other to replenish water loss as a result of evaporation. Vacuum was applied through the condenser outlet and the pressure controlled by a needle valve installed to the main vacuum line using a PVC T-connector. The vacuum was monitored by the use of a vacuum guage, also fitted in the main vacuum line. PVC tubing was used for the water inlet, outlet and vacuum lines. The heating and stirring of the reaction mixture was achieved using a standard laboratory heater-stirrer unit.

3.3.2 Reagents

Ammonium persulfate, $(NH_4)_2S_2O_8$ crystals (Merck).

***mDADMAC*,** 65% (m/v) solution (Aldrich).

***Polyethylene Oxide*,** (PEO) narrow standards (Polymer Standards Service) from 25 300 to 850 000 Da, each in a concentration of 0.1% (m/v), diluted with Milli-Q water.

***Potassium Dihydrogen Phosphate*,** 0.25 M KH_2PO_4 (BDH) prepared in Milli-Q water and adjusted to pH 2.3 with 10 % phosphoric acid.

***Methanol*,** pesticide grade or equivalent (BDH).

***EDTA*,** $C_{10}H_{14}N_2Na_2O_8$ crystals (BDH).

***Milli-Q Water*,** refers to water that has been passed through the Millipore Gradient A10 water purification system.

3.3.3 PolyDADMAC Synthesis

The reactor was charged with sufficient monomer and EDTA as described by Hunter and Siedler [1979]. The mixture was first purged with nitrogen for 20 min and then evacuated. This was followed by heating to the recommended reaction temperature, after which slow addition of the initiator commenced. The mixture was stirred gently throughout the exothermic reaction.

3.3.4 Gel Permeation Chromatography

The MP (0.25 M KH_2PO_4) was filtered and degassed using a 0.45 µm membrane filter and a MP vacuum filtration unit. Column calibration was achieved using the PEO narrow standards. All samples and standards were filtered through 0.45 µm cellulose acetate syringe type filters. Data acquisition and analysis was accomplished using Millenium[32] software (Waters) with the GPC option enabled.

3.3.5 Application of the Polymer for Water Treatment

The synthesized product was used in a laboratory-scale water treatment application.

Apparatus: Coagulation and flocculation experiments (jar test) were conducted using a Sedimentation Jar Stirrer (Phips and Bird). Glass beakers (1 L) were used to contain the sample and polymer mixture. A HACH Turbidity Meter was used for the measurement of the supernatant turbidity.

Procedure: A 0.1% (m/v) solution of the synthesized polyDADMAC product was used as the primary coagulant in flocculation experiments. The test sample to be clarified was a sample of raw water with a turbidity of 21 NTU. Aliquots of 800 mL were transferred to each of six 1 L beakers and dosed with increasing amounts (3 to 11 mg) of polymer. Using the sedimentation jar stirrer apparatus, the samples were mixed at 300 rpm for 2 min followed by 40 rpm for 15 min. The supernatant turbidity at a depth of 40 mm was measured after 15 min of settling.

3.3.6 PolyDADMAC Purification

The polymer was added drop-wise using a glass pasteur pipette into a 250 mL beaker containing 100 mL rapidly stirred ethanol [Mathias and Viswanathan, 1987]. The polymer precipitate was observed almost immediately after polymer addition. The polymer was then filtered through a Whatman 1 filter paper and dried for at least 1 h prior to use.

3.3.7 ^{13}C-NMR Spectroscopy

The polymer was purified by precipitation from ethanol [Mathias and Viswanathan, 1987], re-dissolved in D_2O and submitted to the Department of Chemistry, University of KwaZulu-Natal, Pietermaritzburg, for analysis. A Varian Unity Inova 500 spectrometer with a radio frequency source of 500 MHz was used for the analysis.

3.3.8 Molecular Weight Distribution

Polyethylene oxide (PEO) standards with a mass range from 23 500 to 850 000 Da were used for the purpose of the GPC column calibration. Individual solutions at a concentration of 1000 mg L^{-1} (0.01 g in 10 mL Milli-Q water) were prepared and filtered through 0.45 µm syringe type filters for GPC analysis.

3.4 Results and Discussion

The synthesis of polyDADMAC involved a complicated procedure that required careful control of parameters such as temperature, pressure and reaction rate. The reactor had to be fitted with several ports for inert gas purging, for the addition of reagents and dilution water. The reaction mixture also required constant and gentle stirring for the entire reaction time of six hours and, lastly, monitoring devices for temperature and pressure control was required.

The first of a series of experiments was plagued with problems of poor temperature and pressure control and resulted in a rapid increase of the reaction temperature, well

in excess of 140 ^{o}C. The problems were associated with flaws in the reactor design. It did not have an efficient cooling system and resulted in poor heat dissipation. In addition, there was poor heat distribution of the reaction mixture with the use of a magnetic stirrer. During the polymerization process there was an increase in the solution viscosity and the use of a magnetic stirrer was found to be not sufficiently powerful to achieve efficient mixing. Heat transfer problems were aggravated by this inefficiency and impacted heavily on the reaction. The reaction proceeded rapidly without control and in less than 5 min a waxy solid material that was insoluble in water formed.

In general, the rates of reactions are affected by the composition and temperature of the reaction mixture. Therefore it followed that for better control of the reaction rate attention had to be focused on the above mentioned parameters. The reaction for polyDADMAC synthesis represented by Scheme 3.1 indicates that the rate law for the polymerization reaction may be given by equation 3.1.

$$\upsilon = k[R][M] \qquad 3.1$$

$$\begin{aligned} \upsilon &= \textit{reaction rate} \\ k &= \textit{rate constant} \\ [R] &= \textit{initiator concentration} \\ [M] &= \textit{monomer concentration} \end{aligned}$$

The equation shows that the reaction rate may be affected by the initiator concentration, the monomer concentration, or both. To test the impact of this concept, the monomer was diluted from 65% to 50%. Similarly, the initiator concentration was reduced by adding minute quantities into the reaction mixture. This was achieved by drawing the tip of the initiator dropping funnel into a fine capillary for dispensing drops of greatly reduced size. The second parameter, namely temperature, was controlled by heating the mixture to the recommended value and switching off the heating unit. The temperature was maintained at a constant level by the heat of the exothermic reaction and the amount of initiator increased or decreased to maintain the recommended reaction temperature.

The reaction proceeded for 11.5 min, after which the viscosity of the mixture increased steadily and caused the stirrer to fail. Initiator additions were stopped and after 15 min froth formation was observed. The mixture formed a gel that subsequently solidified. Improved control of the reaction was achieved with a reduction in the concentration of the reactants and the regulation of the temperature. However, a third parameter that required attention was the increase in viscosity of the reaction mixture during polymerization. One important observation was that although water removed from the mixture condensed on cooling, the droplets formed were in the form of a very fine mist that adhered to the walls of the reactor. This was thought to contribute to the increased viscosity of the mixture that was still in the very early stages of polymerization. The water loss was estimated and replenished by incorporating a second pressure equalizing dropping funnel to the reactor. When all the factors affecting the polymerization were identified and brought under control, a successful synthesis was achieved. The reaction proceeded for three hours without

any precipitation of product. After the first hour, the heat produced by the reaction alone was not sufficient to maintain the reaction temperature and there was a need to apply or remove heat as required. The mixture was allowed to react for a total of six hours. The resulting product was a clear liquid with a viscosity of 82 mPas.

3.4.1 Gel Permeation Chromatography

The GPC profile and MWD of the synthesized product was determined to confirm the polymerization of mDADMAC to polyDADMAC. The GPC method developed as described in Chapter 2 was used for the analysis. Selection of the appropriate column involved testing a series of Ultrahydrogel columns with pore sizes ranging from 120 Å to 2000 Å. The best column combination was found to be the Ultrahydrogel 500 column connected in series with the Ultrahydrogel guard column. Under these operating conditions and a MP of 0.25 M KH_2PO_4, a test sample of the product was chromatographed (Figure 3.2).

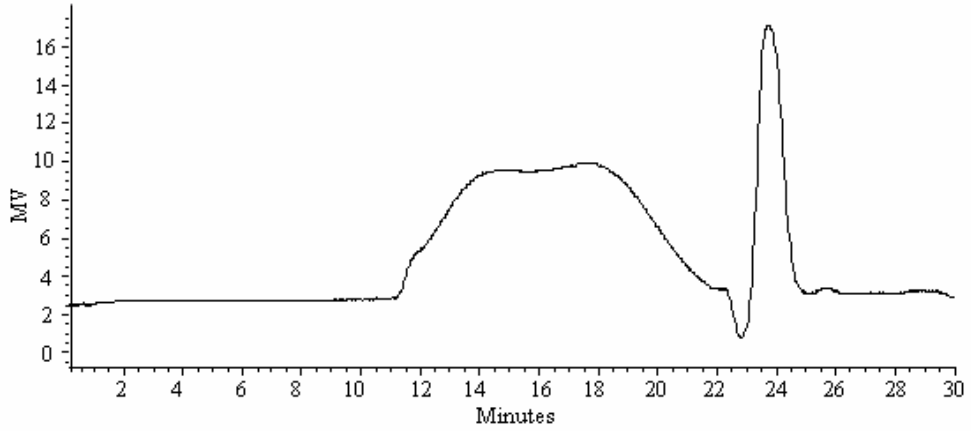


Figure 3.2: GPC profile of the synthesized polyDADMAC.

The broad peak eluting in the range 11 to 22 min is a typical profile of a high molecular weight polymeric species while the remaining peak is that of the low molecular weight species present in the reaction mixture. From this peak profile alone there was no doubt that the synthesis was successful and that polymerization had occurred.

3.4.2 Molecular Weight Distribution

For an accurate and reliable assessment of the MWD of a polymer it is vital that the structure of the reference calibration standards resemble as close as possible the structure of the test compound. For polyDADMAC the most suitable material commercially available is the polyvinylpyridine calibration kit. However, the cost of these standards was prohibitive and a range of polyethylene oxide (PEO) standards was acquired instead. The GPC calibration procedure involved the analysis of all of the reference PEO standards in the range 23 500 to 850 000 Da and the preparation of a GPC calibration curve (Figure 3.3). The data points indicated a relatively linear response and that the range of standards used was within the region of selective permeation of the GPC separating column. Since no additional standards were supplied in the PEO standards kit, the highest and lowest masses were taken as

indicators of the column exclusion limit, V_0, and the total permeation limit, V_t, respectively.

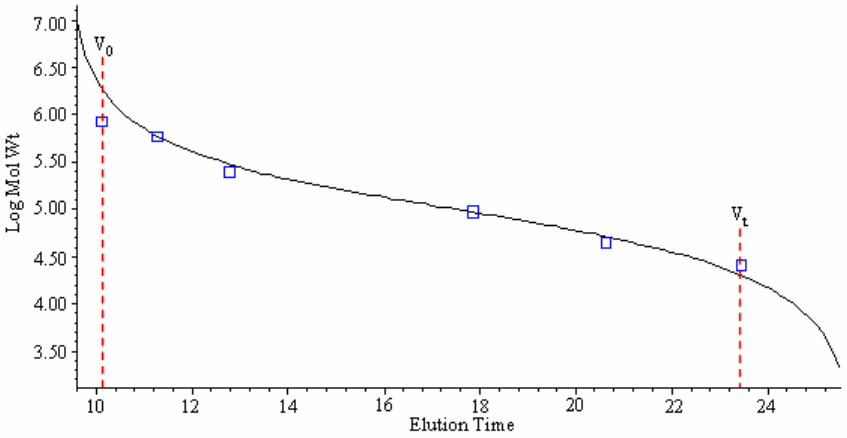


Figure 3.3: GPC calibration curve using PEO narrow standards.

More importantly, the polymer was found to elute within the linear region of the calibration as can be noted by the retention time window of the polymer peak (Figure 3.2) and the retention time window of the region of selective permeation in Figure 3.3. The V_0 and V_t data values were required for the software to obtain a GPC calibration curve that is linear through the range of standards and then asymptotically approach V_0 and V_t. This feature has advantages over polynomial fits if extrapolation is required beyond the calibration range. The MWD was calculated by Millenium[32] software with the GPC option enabled, and was as follows:

Mn = 32977	Mw = 382940
MP = 62098	Mz = 14498670
Mz + 1 = 45367052	Polydispersity = 11

Figure 3.4 shows the calculated MWD presented as a cumulative MWD and a differential MWD of the product. The differential plot shows the amount of material (differential weight fraction) present in any molecular weight interval and the cumulative plot gives for each molecular weight x the percentage of material having a molecular weight less than or equal to x. Based on the peak molecular weight, the product can be categorized as a low to medium molecular weight polymer, which is typical for charged polymers used as primary coagulants in water purification.

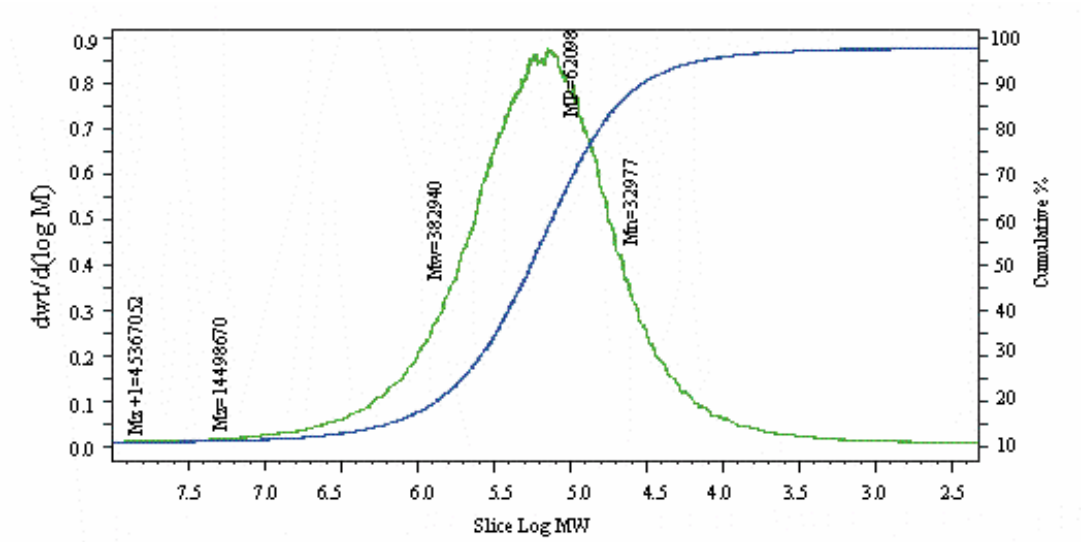
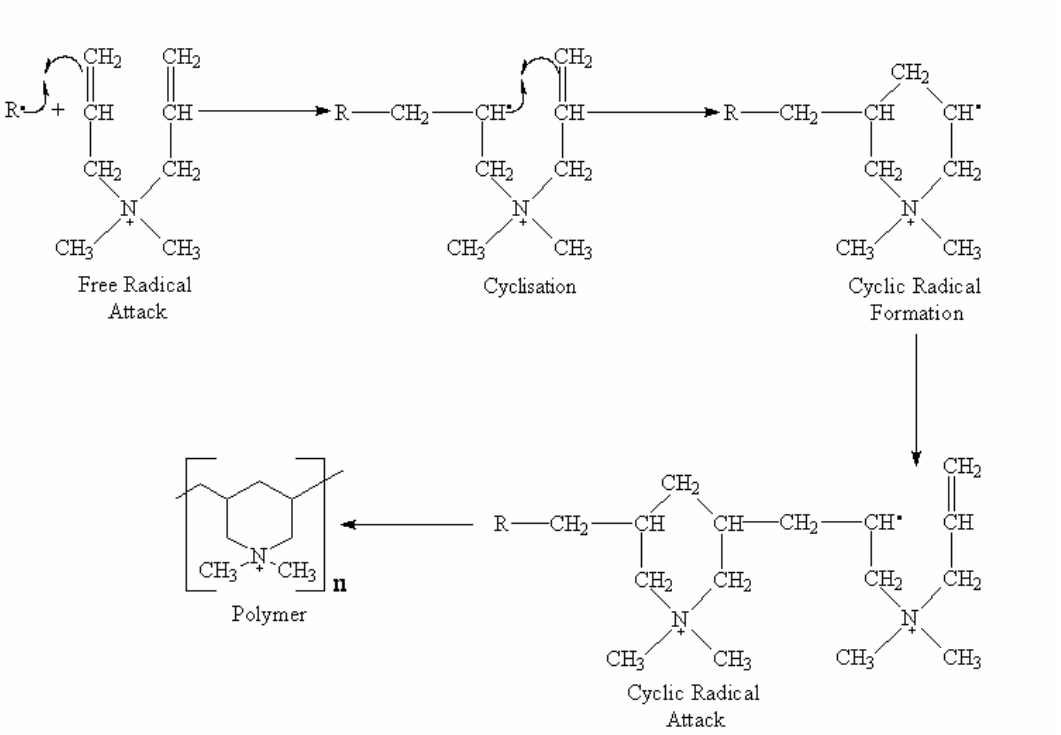


Figure 3.4: The molecular weight fractions of polyDADMAC as a distribution and a cumulative mass fraction.

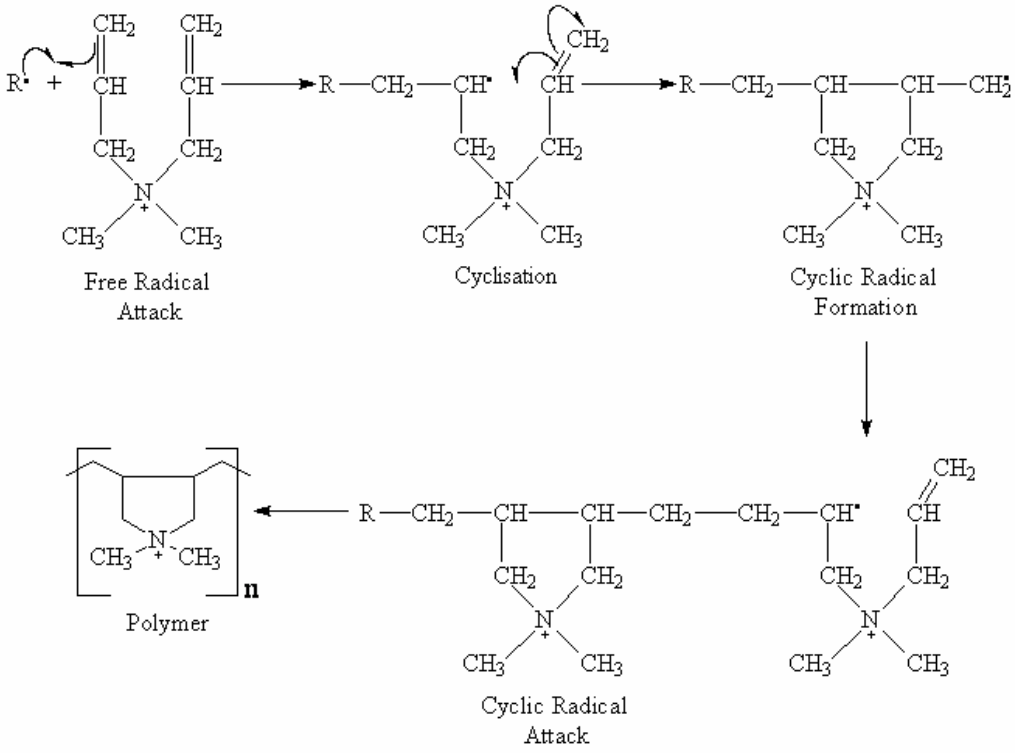
3.4.3 The Chemical Structure of PolyDADMAC

The generally accepted hypothesis proposed for the radical initiated cyclopolymerization of 1,6-diene is the formation of six-member structures. However, later studies have shown that there are numerous cases where cyclopolymerization does not adhere to this hypothesis and cyclic structures are derived by propagation through the less stable intermediate. In this case the cyclopolymerization is said to be under kinetic rather than thermodynamic control [Butler, 1985].

To explain the formation of soluble non-crosslinked polymers such as polyDADMAC from a monomer containing two double bonds (DADMAC), a chain propagation mechanism is proposed that is similar to that provided for quaternary ammonium bromides [Butler and Angelo, 1957]. It involves alternating intra-molecular, followed by inter-molecular, growth steps. Hence two mechanisms are possible. Scheme 3.2 results in the formation of the six-member piperidine ring system and Scheme 3.3 results in the five-member pyrrolidine ring system.



Scheme 3.2: Reaction mechanism for the polymerization of mDADMAC to form the six-member piperidine ring system [Butler and Angelo, 1957].



Scheme 3.3: Proposed reaction mechanism for the polymerization of mDADMAC to form the five-member pyrrolidine ring system.

3.4.4 ^{13}C-NMR Analysis of PolyDADMAC

To establish the preferred mechanism, the reaction product was analyzed by NMR spectroscopy. A sample of the polymer was isolated and purified by precipitation from ethanol [Mathias and Viswanathan, 1987]. The solid polymeric material was filtered through glass fiber filter paper, dried and desiccated for *ca* 3 h. The sample was re-dissolved in D_2O and submitted to the University of KwaZulu-Natal for analysis. The ^{13}C-NMR spectrum of the polymer (Figure 3.5) shows both a set of strong and weak lines for each signal. This may be attributed to the formation of isomers which are cis and trans with respect to the inter-connecting rings.

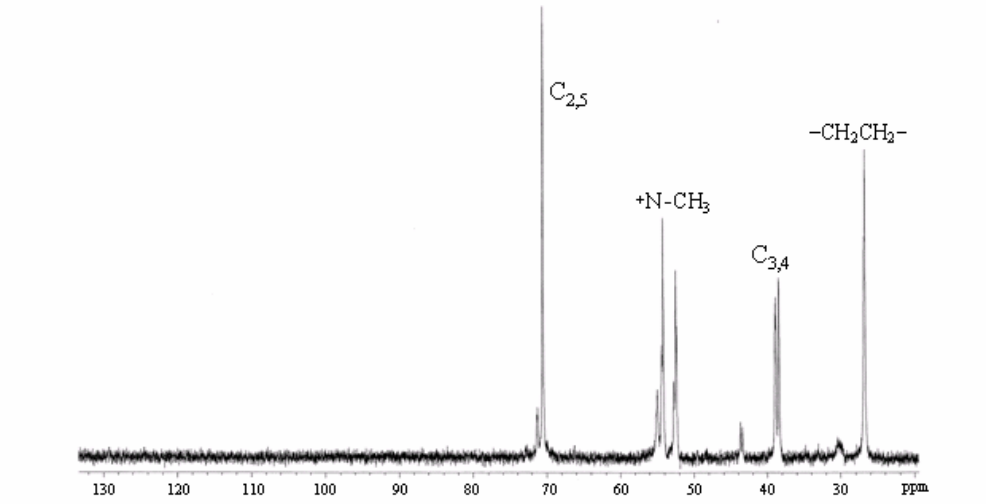


Figure 3.5: ^{13}C-NMR spectrum of the synthesized polyDADMAC dissolved in D_2O**.**

A careful study of the spectrum strongly suggested that the product was consistent with the pyrrolidine ring structure (Figure 3.6).

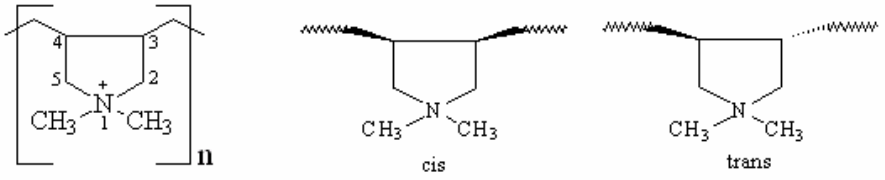


Figure 3.6: Structure of the five-member pyrrolidine ring system.

The N^+-methyl groups are non-equivalent in the cis isomer resulting in the formation of two signals in the region 52 to 54 ppm. These signals are further split due to N^+-C coupling. The N^+-methyl groups of the trans isomer are equivalent and therefore give rise to a single weak signal at 54.9 ppm. The splitting of the equivalent $C_{3,4}$ signal in both isomers into signals of equal intensity at 39 and 43 ppm may be due to steric configurational differences between the inter-connecting rings. The $C_{2,5}$ are also equivalent in both isomers and give rise to single peaks in both instances. Similarly, the -CH_2CH_2- carbons are equivalent in both isomers and give rise to a single peak in both cases. The absence of any further peaks in the spectrum to account for one

additional carbon indicates that the product is not likely to be a six-member ring system. A summary of the peak assignments is shown in Table 3.1.

Table 3.1: Summary of the ^{13}C-NMR chemical shift data of polyDADMAC.

Carbon No.	Cis Isomer		Trans Isomer	
	δ Value (ppm)	Comment	δ Value (ppm)	Comment
2,5	70.5	Equivalent C	71.2	Equivalent C
3,4	38.8	Equivalent C	43.4	Equivalent C
	38.9	Split Peak	43.7	Split Peak
$-CH_2-CH_2-$	26.7	Equivalent C	30.8	Equivalent C
N^+-CH_3	52.4	Non-Equiv. C	54.9	Equivalent C
	54.2	2nd Peak		

The distortionless enhancement by polarization transfer (DEPT) spectrum (Figure 3.7) was used to verify the peak assignments presented in Table 3.1. The relative positions of the CH_3, CH_2 and CH carbons are clearly shown. From Figure 3.7A and C, the positions of the CH_3 and CH groups were deduced, with the CH_3 signals in the region 52 to 54 ppm and the CH lines in the region of 39 ppm. Spectrum B shows only two CH_2 signals, at 70 ppm and 26 ppm. This confirmed the absence of a third CH_2 group, thus totally eliminating the possibility of the six-member piperidine ring structure. Finally, spectrum C shows one CH signal split into two peaks of almost equal intensity. This has been explained as being due to the steric configurational differences of the inter-connecting rings.

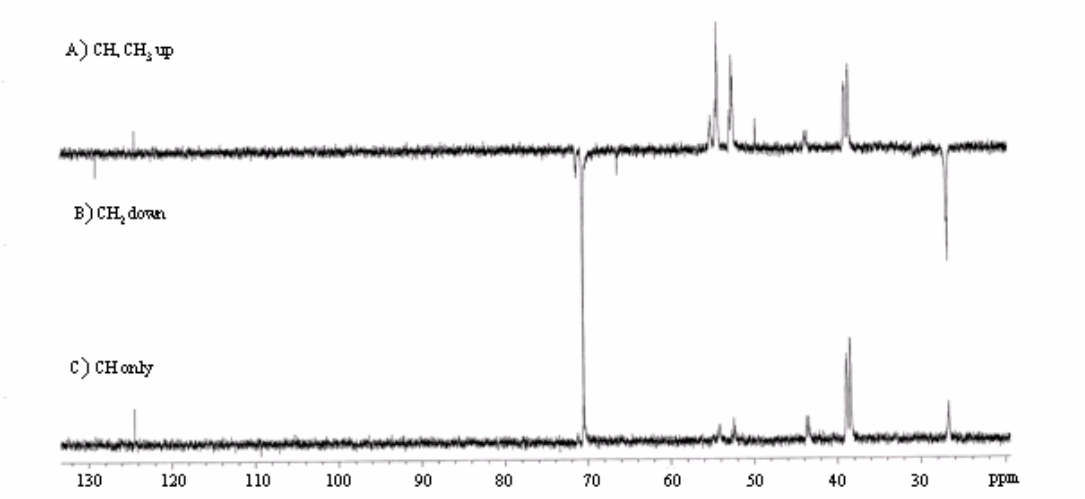


Figure 3.7: The DEPT spectrum of the synthesized polyDADMAC.

Other small signals in the spectrum were attributed to residual magnetization and therefore ignored. The synthesis of polyDADMAC was therefore confirmed and its structure was found to comprise pyrrolidine rather than piperidine rings. These findings were consistent, and are supported by the findings of other researchers [Brace, 1970; Hawthorne et al., 1975; Lancaster and Panzer, 1976]

3.4.5 Application of the Synthesized PolyDADMAC for Water Treatment

The polymer activity was tested in a process related application. A raw water sample having a turbidity of 21 NTU was subjected to the standard jar test by dosing with the product. Increasing amounts of polymer (3 to 11 mg) were added to each of six 1000 mL beakers containing 800 mL of the test sample. The stirrer apparatus for the test was adjusted to 300 rpm for 2 min, followed by 40 rpm for 15 min. The supernatant turbidity was measured after 15 min of settling. The supernatant turbidity results plotted as a function of polymer dose (Figure 3.8) show three distinct regions, representing the underdose, the optimum dose and overdose conditions.

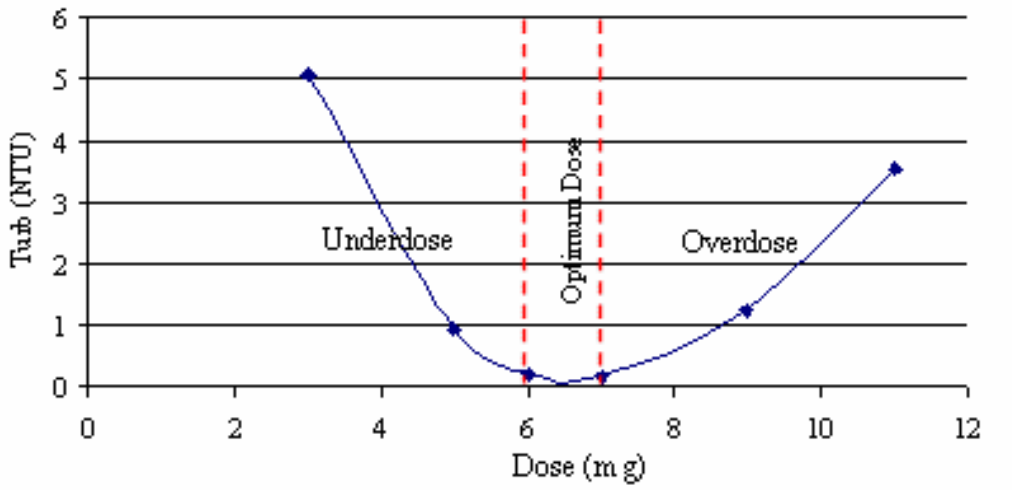


Figure 3.8: Plot of supernatant turbidity versus coagulant dose.

The initial decrease in turbidity can be explained by the charge neutralization model. According to this model, destabilization of colloidal particles that usually carry a negative charge in natural water is accomplished by adsorption of oppositely charged polymers on particle surfaces. This results in the neutralization of the particle surface charge and the formation of flocs or aggregates that settle out of solution. At the optimum dose (6 to 7 mg), the surface charge is reduced to near zero. Overdose is caused by excess polymer adsorbing on particles, thereby producing positively charged re-stabilized colloidal particles. The experiment was repeated using a commercial polymer and the optimum dose was found to be *ca* 8 mg.

3.5 Conclusions

PolyDADMAC was successfully synthesized by free-radical initiated addition polymerization of the DADMAC monomer under very carefully controlled conditions of temperature, pressure, monomer concentration and a gentle reaction rate. Poor control of the above resulted in the formation of a heavily cross-linked, water insoluble product that was of no use as a flocculant in water clarification. The MWD of the product was determined by GPC and confirmed the success of the polymerization reaction. The ^{13}C-NMR and, in particular, the DEPT spectra showed conclusively that the pyrrolidine ring structure formed in preference to the piperidine rings. This indicated that the predictions made by Butler and Angelo [1956] of forming water soluble as well as cyclic polymers with monomers containing two allyl groups are not necessarily true. Very stringent synthesis conditions are required for

the preparation of the desired water soluble polyDADMAC product. The DADMAC monomer containing two allyl groups is capable of forming both water soluble and cross-linked, water insoluble polymers. From this work, a good knowledge of the preparation, chemistry, structure and properties of polyDADMAC was developed.

The synthesized product performed effectively as a coagulant in a water clarification application, reducing turbidity from 21 to less than 0.5 NTU at an optimum dosage of between 6 to 7 mg. The dosage required was marginally less than that of the commercial product (*ca* 8 mg) required to treat the same water.

3.6 References

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CHAPTER FOUR

POLYDADMAC REACTIONS WITH CHLORINE

4.1 Introduction

Although polymers such as polyDADMAC are known to remove naturally occurring trihalogenated methane (THM) precursors from water, preliminary studies undertaken by the author have shown that the polymer itself contributes to the formation of the THM, chloroform. This is contrary to the claims that polyDADMAC polymers are the most chlorine resistant of commercially available water treatment flocculants [Mangravite, 1983]. To test the validity of this an assessment of the stability of the polymer after exposure to varying concentrations of chlorine was carried out. The chlorine dose ranged from the typical dose of 1 and 2 mg L^{-1} to extremes of 10 to 20 mg L^{-1}. Chemical change to the polymer was monitored by the GPC method developed as described in Section 2.6.1.4. The results obtained provide conclusive evidence of polymer degradation. Analytical techniques including GPC and purge and trap gas chromatography mass spectrometry (GC MS) were used to identify the reaction product chloroform. A detailed and systematic study was conducted to determine the extent of THM formation using a standard method. It involved dosing a 2 mg L^{-1} solution of the test polymer with a standardized solution of NaOCl. The appropriate chlorine dose was determined by first establishing the chlorine demand of the polymer solution using a high chlorine concentration and a reaction time of four hours. Residual free chlorine was measured by iodometric titrations to a starch-iodide end point. The THM formation potential was determined over a seven day reaction time and a set of data (n=30) was collected over a two month period. The average THM formation potential was found to be 12.5 µg L^{-1}. This is well below the guideline standard of 100 µg L^{-1}. However, several other parameters must be considered that can adversely affect the production of THMs.

4.2 Background to THM Formation

It is known that naturally occurring substances such as humic acids and fulvic acids present in raw water react with chlorine to form THMs [Trussel and Umphres, 1978]. The primary THMs of concern are chloroform ($CHCl_3$), bromodichloromethane ($CHBrCl_2$), dibromochloromethane ($CHBr_2Cl$) and bromoform ($CHBr_3$). Of the four compounds, chloroform is known to be the most toxic, with serious carcinogenic properties. Predictive models for estimating THMs can be used, however, as with most modeling studies, often the results cannot be determined precisely. The THM formation potential (THMFP) is defined as the difference in the final total THM concentration and the initial concentration of a test sample after subjecting the sample to a set of standard conditions [Eaton et al., 1995]. THMFP can be experimentally determined with good accuracy and precision. Careful control of parameters such as temperature, reaction time, pH, chlorine dose and chlorine residual are required. THM formation is enhanced at elevated temperatures, alkaline pH, and increasing levels of free chlorine, and tends to level off at residuals greater than or equal to 3 mg L^{-1} [Stevens and Symons, 1977; Symons et al., 1981]. The method involves chlorinating the polymer test sample with an excess of aqueous NaOCl solution, and incubation for seven days at 25 ^{o}C, to allow the reaction to continue to completion.

The THMs are then determined by gas chromatography with electron capture detection (GC ECD).

Studies conducted by previous researchers have demonstrated that the use of polymers as primary coagulants (cationic) or coagulant aids (nonionic or anionic) can enhance the removal of THM precursors [Amy and Chadik, 1983]. They used four different polymers, including a low molecular weight quaternary ammonium polymer, a medium molecular weight quaternary ammonium polymer, a low molecular weight polyalkyl polyamine, and an ultra low molecular weight quaternary polyamine. The objectives were to demonstrate the removal of THMFP.

In as much as the removal of THMs was noted, polymer residues resulting from incomplete adsorption as well as the inevitable cases of overdosing can act as THM precursors themselves [Keiser and Lawrence, 1977]. Their investigation involved testing of a nonionic polyacrylamide as well as polyDADMAC using thermal and UV light activation conditions. This study addresses the determination of the THMFP of polyDADMAC as a result of its use in water clarification, under a set of standard conditions [Eaton et al., 1995]. A commercial solution of NaOCl containing approximately 5% active chlorine was used for chlorination and the levels of residual free chlorine determined by iodometry [Bassett et al., 1978].

4.3 Experimental

4.3.1 Instrumentation

GPC: A Waters Alliance 2695 fitted with an Ultrahydrogel 500 column was used. The optimized conditions were as described in Chapter 2, Section 2.5.1.4.

GC MS: An HP 6890 gas chromatograph (GC) interfaced to an HP 5973 mass selective detector (MSD) was used for the identification of chlorination byproducts. Separation was achieved on a DB 5 capillary column (J&W Scientific) of dimensions 30 m x 0.25 mm x 0.25 μm. The system was driven by the Chemstation software with advanced data acquisition and analysis features supplied by HP.

MS Parameters:	Quadrupole Temperature	150 ^{o}C
	Source Temperature	230 ^{o}C
	Acquisition Mode	Scan
	MS Mode	Electron Impact (EI)
	Mass Range	35 to 250 amu
	Solvent Delay	0 min
GC Parameters:	Inlet Temperature	280 ^{o}C
	Column Flow	1 mL min^{-1}
	Initial Column Temperature	50 ^{o}C
	Initial Time	1 min
	Rate	15 ^{o}C min^{-1}
	Final Temperature	280 ^{o}C
	Final Time	5 min

Purge and Trap: Sample introduction was achieved with the CDS 6000 purge and trap unit fitted with a tenax trap and a cryo-focusing module with liquid nitrogen as the cryogen. The sample was purged using a 25 mL fritted disc sparger.

Operating Parameters:		
	Purge Time	11 min
	Trap Temperature	40 ^{o}C (initial)
	Dry Purge	2 min
	Temperature	50 ^{o}C
	Desorb Temperature (Trap)	220 ^{o}C (final)
	Desorb Time	5 min
	Cryo-focus Temperature	-50 ^{o}C (initial)
	Cryo-focus Temperature	250 ^{o}C (final)

GC ECD: Routine testing for THMs was conducted on a Hewlett Packard (HP) 6890 gas chromatograph fitted with an electron capture detector (ECD). Separation was achieved on a DB 624 capillary column (J&W Scientific) of dimensions 30 m x 0.32 mm x 1.8 μm.

Operating Parameters:		
	Inlet Temperature	80 ^{o}C
	Column Flow	1.8 mL min^{-1}
	Column Temperature	80 ^{o}C
	Initial Time	1 min
	Ramp Rate	2 ^{o}C min^{-1}
	Final Temperature	100 ^{o}C
	Final Time	3 min
	Injection Mode	Split, 5:1

4.3.2 Reagents

Starch Indicator Solution: The indicator was prepared by adding a few drops of cold water to 5 g of starch and mixed to form a thin paste. The paste was poured into 100 mL of boiling Milli-Q water with constant stirring. The solution was left to stand overnight and the clear supernatant liquid was decanted. KI (2 g) was added and the solution stored at 4 ^{o}C.

Standard Potassium Iodate, 0.1 N: KIO_3 (5 g), the primary standard, was dried in an oven at 105 ^{o}C for 180 min. The crystals were removed from the oven and desiccated for 60 min.

The mass required was calculated using equations 4.1 and 4.2.

From the half reaction for IO_3^- *:*

$$IO_3^- + 6H^+ + 6e^- \rightleftharpoons I^- + 3H_2O$$

1 mol IO_3^- *produces 6 molar equivalents of electrons*

$$equivalent\ mass\ KIO_3 = \frac{molecular\ mass}{6} \qquad 4.1$$
$$= \frac{214}{6}$$
$$= 35.667\ g$$

$$N_{KIO_3} = \frac{Mass_{KIO_3}}{equivalent\ Mass_{KIO_3}} \times \frac{1000}{Volume} \qquad 4.2$$
$$Mass_{KIO_3} = \frac{0.1 \times 35.667 \times 1000}{1000}$$
$$= 3.567\ g$$

An accurate mass of 3.567 g was weighed, transferred into a volumetric flask, and diluted to 1000 mL with chlorine demand free water.

Sodium Thiosulfate, ca. 0.1 N: $Na_2S_2O_3.5H_2O$ (25 g) was dissolved in boiled Milli-Q water and diluted to 1000 mL.

Standardization of $Na_2S_2O_3$: To 80 mL Milli-Q water was added 1 mL of conc. H_2SO_4, 25 mL of 0.1 N KIO_3 primary standard and 1 g of KI. The solution was titrated with ca 0.1 N $Na_2S_2O_3$ until a pale yellow color was observed. At this point, a few drops of starch indicator were added and the titration continued until the solution changed from blue to colorless.

The titration results for the standardization (Table 4.1) were used in the calculation of the $Na_2S_2O_3$ concentration.

Table 4.1: Titration of standard 0.1 N KIO_3 with *ca* 0.1 N $Na_2S_2O_3$.

	Volume $Na_2S_2O_3$ (mL)		
Solution ID	**Initial**	**Final**	**Titer**
1	0.10	29.70	29.60
2	0.40	30.00	29.60
3	1.00	30.70	29.70
		Mean	29.63

The oxidation-reduction reaction for the standardization is as follows:

In step 1 of the standardization, potassium iodate oxidizes KI to iodine according to the reaction

$$IO_3^- + 5\,I^- + 6H^+ \rightleftharpoons 3\,I_2 + 3H_2O$$

In step 2 the iodine produced is reduced by thiosulfate according to the reaction

$$2\,S_2O_3^{2-} + I_2 \rightleftharpoons S_4O_6^{2-} + 2\,I^-$$

At the equivalence point, the number of equivalents of I_2 produced corresponds to the number of equivalents of IO_3 consumed in the reaction. The thiosulfate concentration was calculated using equations 4.3 and 4.4.

During Oxidation

$$\begin{aligned} equivalents\ IO_3^- &= equivalents\ I_2 \\ &= N_{IO_3^-} \times V_{IO_3^-} \\ &= 0.1 \times 25 \\ &= 2.5\ equivalents \end{aligned} \qquad 4.3$$

During Reduction

$$\begin{aligned} equivalents\ I_2 &= equivalents\ S_2O_3^{2-} \\ &= N_{S_2O_3^{2-}} \times V_{S_2O_3^{2-}} \\ N_{S_2O_3^{2-}} &= \frac{2.5}{29.63} = 0.084\ N \end{aligned} \qquad 4.4$$

The thiosulfate concentration was confirmed by titration with standard potassium dichromate. The number of equivalents of KI oxidized to iodine corresponds to the number of equivalents of $Cr_2O_7^{2-}$ consumed. The iodine is then reduced by the $S_2O_3^{2-}$ and the concentration is determined by calculation. The equations for the above sequence of reactions are as follows:

$$Cr_2O_7^{2-} + 14H^+ + 6e^- \rightleftharpoons 2Cr^{3+} + 7H_2O \quad \text{Reduction}$$

$$6I^- \rightleftharpoons 3I_2 + 6e^- \quad \text{Oxidation}$$

$$Cr_2O_7^{2-} + 14H^+ + 6I^- \rightleftharpoons 2Cr^{3+} + 7H_2O + 3I_2$$

The I_2 produced is then titrated with thiosulfate according to the reaction:

$$2S_2O_3^{2-} + I_2 \rightleftharpoons S_4O_6^{2-} + 2I^-$$

The titration was done in triplicate, and the results from Table 4.2 together with equations 4.5 and 4.6 were used in the calculation of the $Na_2S_2O_3$ concentration.

Table 4.2: Titration of 0.01N $K_2Cr_2O_7$ with *ca* 0.01 N $Na_2S_2O_3$.

	Volume $Na_2S_2O_3$ (mL)		
Solution ID	**Initial**	**Final**	**Titer**
1	0.00	12.00	12.00
2	0.00	11.97	11.97
3	0.00	11.98	11.98
Blank	0.00	0.08	0.08
		Mean	11.90

At equivalence point:

$$\begin{aligned} equivalents\ I_2 &= equivalents\ Cr_2O_7^{2-} \\ &= N_{Cr_2O_7^{2-}} \times V_{Cr_2O_7^{2-}} \\ &= 0.01 \times 10 \\ &= 0.1\ \ equivalent \end{aligned} \qquad 4.5$$

$$\begin{aligned} equivalents\ I_2 &= equivalents\ S_2O_3^{2-} \\ &= N_{S_2O_3^{2-}} \times V_{S_2O_3^{2-}} \\ N_{S_2O_3^{2-}} &= \frac{0.1}{11.9} \\ &= 0.0084\ N \end{aligned} \qquad 4.6$$

The concentration of $Na_2S_2O_3$ obtained with the KIO_3 primary standard was therefore confirmed when $K_2Cr_2O_7$ was used as the primary standard, keeping in mind that both the primary standard and thiosulfate solutions were diluted to 0.01 N prior to titration.

Stock NaOCl: All hypochlorite solutions were prepared from a 5% aqueous NaOCl solution, commercially available as Jik Original.

Standardization of Stock NaOCl: To a conical flask containing 25 mL Milli-Q water was added 1 mL NaOCl, 1 mL conc. H_2SO_4 and 2 g KI. The solution was titrated with previously standardized $Na_2S_2O_3$ (Table 4.3) and the NaOCl concentration determined using equation 4.7.

Table 4.3: Titration of Jik Original (NaOCl) with standard 0.084 N $Na_2S_2O_3$.

	Volume $Na_2S_2O_3$ (mL)		
Solution ID	**Initial**	**Final**	**Titer**
1	0.30	9.20	8.90
2	9.20	18.00	8.80
3	18.00	26.85	8.85
Mean			8.85

$$\begin{aligned} [Cl_2] &= \frac{N_{S_2O_3^{2-}} \times 35.45 \times V_{S_2O_3^{2-}}}{V_{NaOCl}} \\ &= \frac{0.084 \times 35.45 \times 8.85}{1} \\ &= 26.47\ mg\ mL^{-1} \end{aligned} \qquad 4.7$$

Chlorine Dosing Solution, 5 mg mL^{-1}: The dosing solution (250 mL) was prepared by diluting the stock solution with chlorine demand free water using equation 4.8.

$$mL\ required = \frac{Molarity_{dosing} \times Volume_{dosing}}{Molarity_{stock}} = \frac{250 \times 5}{Molarity_{stock}} \quad 4.8$$

Polymer Chlorination: Polymer solutions (0.5% m/v) were dosed with the chlorine dosing solution to give a range of 0 to 20 mg chlorine per 100 mL polymer solution. The samples were allowed to remain in contact with the polymer overnight for the initial investigation.

Phosphate Buffer: Anhydrous potassium dihydrogen phosphate (68.1 g) and sodium hydroxide (11.7 g) were dissolved in 1000 mL of Milli-Q water. The buffer was stored at 4 ^{o}C.

Hydrochloric Acid, 0.1 N: Concentrated HCl (9 mL of 37% solution) was added to 500 mL of Milli-Q water and diluted to 1000 mL.

Sodium Hydroxide, 0.1 N: NaOH (4 g) was dissolved in Milli-Q water and diluted to 1000 mL.

Chlorine Demand Free Water: Milli-Q water (2000 mL) was treated with 500 µL of stock NaOCl and stored for two days. The residual free chlorine was measured using the DPD colorimetric method. A value of > 2 mg L^{-1} was desired. The solution was then irradiated with UV radiation overnight using a Spectroline Model EA-160/FE UV lamp. Finally the solution was purged with high purity, instrument grade nitrogen (99.999%) for 60 min. Once more the DPD method was used to confirm the total removal of free and combined chlorine. This water was used for the preparations of all dilutions of samples, standards and blanks for the experiments.

DHBA Solution: Anhydrous 3,5-dihydroxy-benzoic acid (DHBA, 0.078 g) was dissolved in 2000 mL of chlorine demand free water. This solution was freshly prepared for each experiment as it is known to be unstable.

Sample Preparation: Polymer samples were prepared using a commercial polyDADMAC dosing solution sampled directly from the storage tank at the Wiggins Water Treatment Works, Durban, South Africa. A concentration of 2 mg L^{-1} diluted in chlorine demand free water was used in all experiments. Prior to chlorine dosing, the diluted polymer solution was adjusted to pH 7. Chlorine demand free water was used as the blank and the DHBA solution for quality control. The polymer solutions were dosed with 1 mL of the chlorine dosing solution and incubated for 7 days at 25 ^{o}C. After seven days the free chlorine content was measured using the DPD Colorimetric Method for chlorine analysis [Eaton et al., 1995]. A second aliquot was used for THM analysis. The aqueous sample was poured into a 100 mL volumetric flask containing 0.5 g of $Na_2S_2O_3$ crystals to remove residual free chlorine from the sample. Internal standard (1 mL 1,2-dibromoethane) was added to the sample after making up to volume, to give a concentration of 20 µg L^{-1}. The samples were then analyzed by GC ECD using a method developed at Umgeni Water for routine

monitoring of THMs. A series of THM standards (10 to 50 µg L^{-1}) containing the internal standard was used for the purposes of calibration and quantification.

Determination of Chlorine Demand: Chlorine dosing solution (5 mL) was added to chlorine demand free water and diluted to 250 mL. The solution was capped, shaken well, and a 100 mL aliquot removed for titration with standard $Na_2S_2O_3$. Similarly, a solution of the test polymer was prepared at a concentration of 2 mg L^{-1} in chlorine demand free water and added to a 250 mL bottle containing 5 mL of the chlorine dosing solution and 5 mL of phosphate buffer. The sample was stored in the dark at 25 ^{o}C. After 4 h a 100 mL aliquot test sample was titrated with standard $Na_2S_2O_3$.

4.4 Results and Discussion

GPC chromatograms indicated no apparent chemical changes of the polymer at chlorine doses <10 mg L^{-1} as the polymer peak profiles remain essentially unchanged compared to the control. However, at high doses (>10 mg L^{-1}), there is a significant change in the polymer peak profile, and the MWD (Figure 4.1a/b).

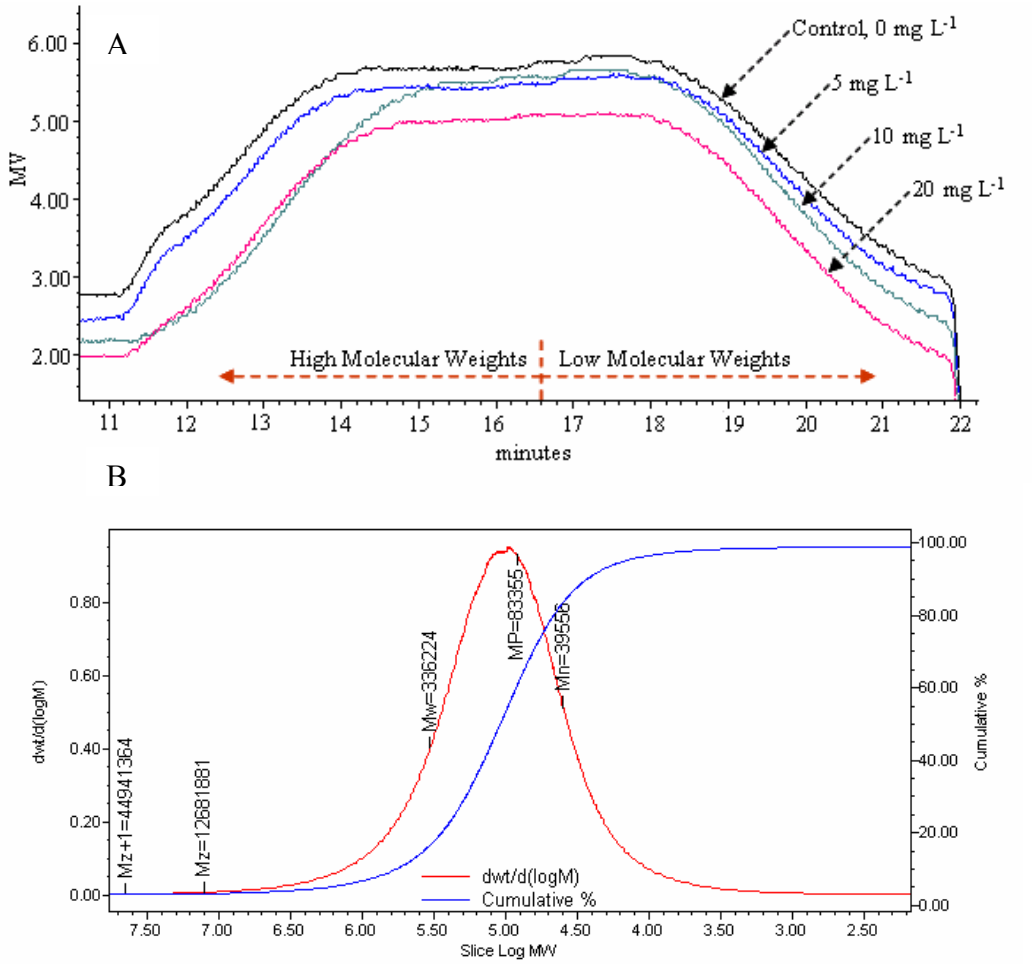

Figure 4.1: (a) GPC profiles of polyDADMAC after dosing with NaOCl and (b) the MWD at a 20 mg L^{-1} chlorine dose.

The polymer peak profile at 5 mg L^{-1} appears reduced in size when compared to the control. On close inspection, it can be clearly seen that all features of the peak are the same as the control. The apparent reduction in peak area can be attributed to a slight

shift in the baseline and can be easily misinterpreted as degradation. In contrast, at high chlorine doses there is a distinct change in the polymer peak profile, both in terms of size and shape. There is a general reduction in the overall peak area, the extent of which is greater at the higher chlorine dose. The profile also shows that the high molecular weight region appears to be affected the most. The polymer peak shape changes from a more symmetrical to a skewed distribution, and consequently impacts on the molecular weight averages.

Since the RI detector used for GPC is a non specific detector it suffers from low sensitivity, and hence it is not expected to detect low levels of degradation products. Furthermore, GPC itself is a low resolution method and is often not capable of producing discreet peaks for molecules having molecular weights less than or equal to one order of magnitude from each other. GPC provided conclusive evidence of polyDADMAC degradation under heavy chlorine doses. Polymer degradation at low chlorine doses was not detected by GPC due to sensitivity limitations of the RI detector. It was therefore of interest to determine the nature of any compounds formed. High-resolution capillary gas chromatography mass spectrometry (GC MS) was used for the determination of the polymer degradation products. An aliquot of the sample containing 10 mg L^{-1} chlorine was analyzed together with the control using a purge and trap unit for sample introduction. The overlay chromatogram of the test sample and control (Figure 4.2) shows the appearance of one additional and very prominent peak occurring at 2.984 min in the test sample, which is absent in the control.

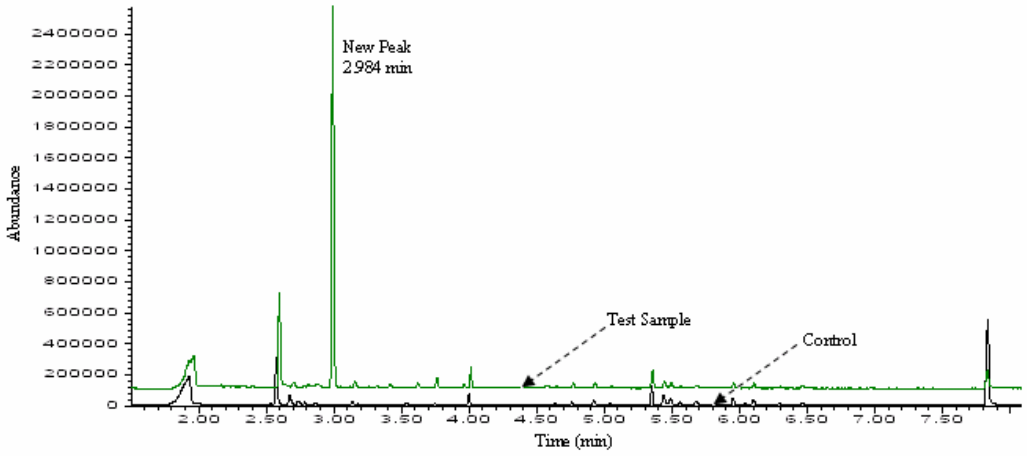

Figure 4.2: Overlay of GC MS chromatograms of the control and chlorinated test sample off-set by 20%.

Based on the mass spectrum of the new peak (Figure 4.3), the compound was identified using the Wiley275 spectral library (Hewlett Packard) as being that of chloroform. This compound is well known to the water sector as it is one of the main byproducts of chlorination and the most potent of the four THMs.

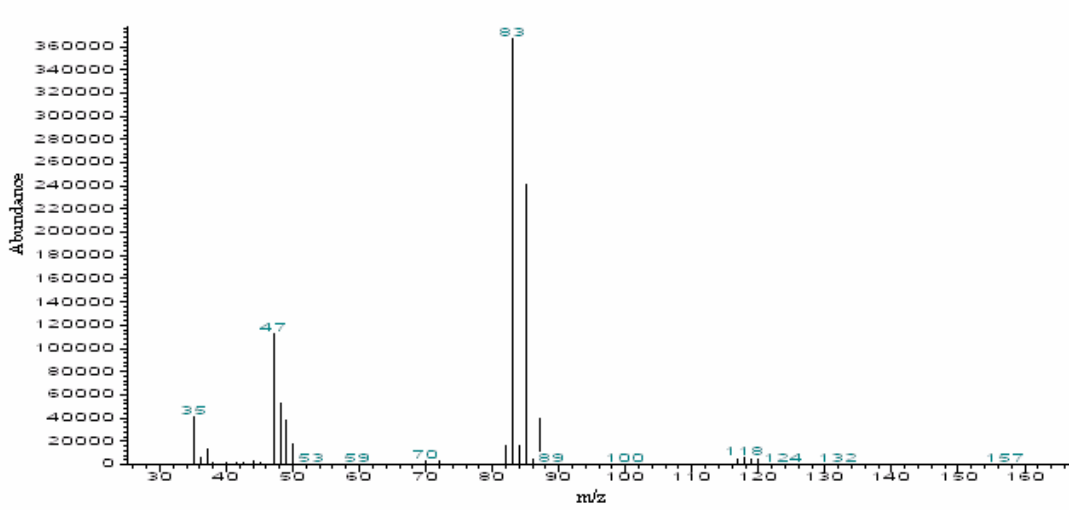

Figure 4.3: Mass spectrum of the new peak at 2.984 min in the GC MS chromatogram, showing the formation of chloroform.

Polymer degradation occurred under laboratory conditions only at relatively high chlorine doses ($\geq$ 10 mg L^{-1}). Even so, this warranted further study as the products of degradation are of special concern to water utilities. To determine the extent of the problem a process of formally establishing the total THMFP was carried out, using aliquots of a 2 mg L^{-1} polymer solution. The appropriate chlorine dose was determined by establishing the chlorine demand of the 2 mg L^{-1} polymer test solution.

Chlorine concentrations were determined by iodometry. Several steps were required prior to commencing with the chlorine dosing experiments. These included the following:

- Preparation and standardization of $Na_2S_2O_3$ with standard KIO_3
- Confirmation of the $Na_2S_2O_3$ concentration by standardization with standard $K_2Cr_2O_7$
- Standardization of the stock NaOCl solution for use in chlorination
- Dilution of the stock NaOCl solution for use as the dosing solution.

Sodium thiosulfate ($Na_2S_2O_3.5H_2O$) is a readily available chemical in a state of high purity, but there is some uncertainty as to the exact water content of the salt. It therefore cannot be used as a primary standard. KIO_3 on the other hand has a purity of at least 99.9% and is well suited for use as a primary standard. It was therefore used to standardize the thiosulfate solution. The concentration was found to be 0.084 N (equations 4.3 and 4.4), and confirmed by standardization with potassium dichromate (equations 4.5 and 4.6).

The seven-day chlorine demand of the test polymer was estimated experimentally, by determining the difference in the chlorine content of a solution of chlorine demand free water and the polymer test sample, each dosed with equal concentrations of chlorine. The average titer volume of the chlorine demand free water was 3.50 mL and the initial chlorine concentration was calculated using equation 4.9.

$$Initial\ Chlorine\ Concentration, Cl2_{initial} = \frac{N_{S_2O_3^{2-}} \times 35.45 \times V_{S_2O_3^{2-}}}{V_{water}} \quad 4.9$$
$$= \frac{0.08437 \times 35.45 \times 3.50}{100}$$
$$= 105\ mg\ L^{-1}$$

The average titer volume of the polymer test sample was 2.85 mL, and the final chlorine concentration and chlorine demand were calculated using equation 4.10 and equation 4.11, respectively.

$$Final\ Chlorine\ Concentration, Cl2_{final} = \frac{N_{S_2O_3^{2-}} \times 35.45 \times V_{S_2O_3^{2-}}}{V_{polymer}} \quad 4.10$$
$$= \frac{0.084 \times 35.45 \times 2.85}{100}$$
$$= 85\ mg\ L^{-1}$$

$$Chlorine Demand, Cl2_{demand} = Cl2_{initial} - Cl2_{final} \quad 4.11$$
$$= 105 - 85$$
$$= 20\ mg\ L^{-1}$$

The sample chlorine dose V_D was determined using equation 4.12 [Eaton et al., 1995].

$$V_D = \frac{Cl_{demand} + 3}{5} \times \frac{V_S}{1000} \quad 4.12$$
$$= \frac{20 + 3}{5} \times \frac{250}{1000}$$
$$= 1.15\ mL$$

V_S = *sample volume*

Samples prepared as described in Section 4.3.2 were dosed with 1 mL dosing solution for practical reasons (calculated volume, 1.15 mL) and incubated for a seven day reaction time at 25 ^{o}C. Several sets of samples were prepared initially to optimize the level of residual free chlorine after the incubation period. For good accuracy and precision, a free chlorine residual of between 3 to 5 mg L^{-1} was required. Polymer test samples were prepared at a concentration of 2 mg L^{-1}, a dose typically used at water treatment works with relatively good quality raw water.

Due to the expectation that the THM levels would be low as a result of the low doses of chemicals (polymer and NaOCl) used, the analytical technique of choice was capillary GC ECD. This detection method is very selective to halogenated organic compounds and therefore extremely sensitive. The method involved direct injections of aqueous samples and required the use of a multipoint calibration, with the composite standards $CHCl_3$, $CHBrCl_2$, $CHBr_2Cl$ and $CHBr_3$ (Figure 4.4) for

quantifying the THMs in the test samples. The quantification of the THMs was achieved using the internal standard method of calibration with 1,2-dibromoethane as the internal standard. All calibrations were tested for linearity using the regression statistics and a quality control (QC) sample having a true value of 20 µg L^{-1}.

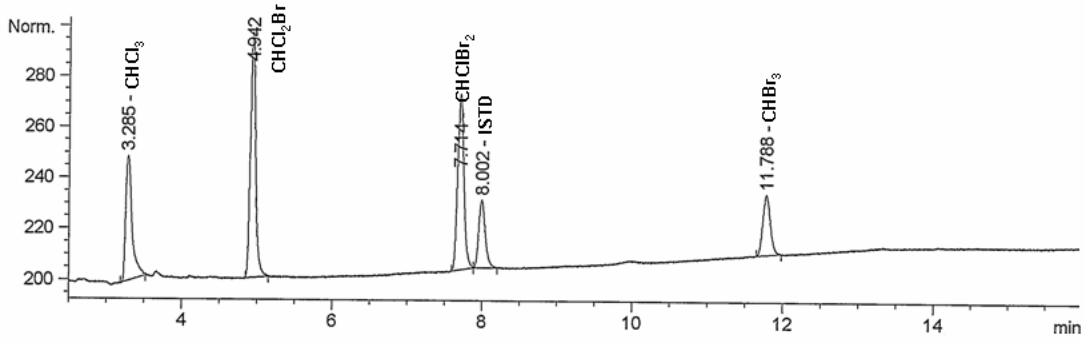

Figure 4.4: GC ECD chromatogram of THM reference standards.

The QC sample was prepared from stock solutions obtained from a different source to that of the calibration standards. QC values in the range 18 to 22 µg L^{-1} were deemed to be within acceptable limits (mean ±2σ) of the test method. DHBA was used as a QC test for monitoring the THMFP over the seven-day reaction time. An empirical value of 119 µg L^{-1} was specified as the expected value. The obtained results were within 30% of this value and indicated that all the standard conditions specified by the method were being met. A typical chromatogram of the test sample (Figure 4.5) shows the presence of chloroform, the internal standard and an unidentified broad peak. It is suspected that this peak may due to either the presence of residual DADMAC monomer or DADMAC monomer resulting from polymer degradation. The peak profile suggests strong interaction with the chromatographic separating column, which is consistent with the ionic nature of the monomer.

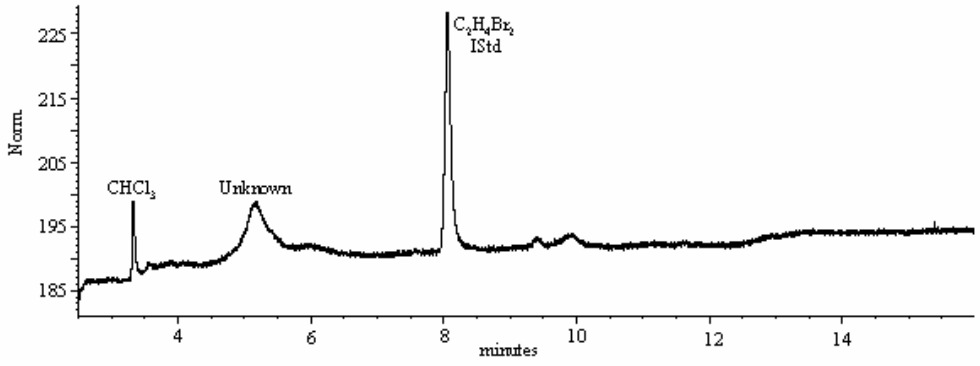

Figure 4.5: GC ECD chromatogram of a polyDADMAC test sample after a seven-day reaction time at 25 °C with NaOCl**.**

Apart from chloroform, no other detectable levels of THMs were visible in the chromatograms. The data collected over a period of two months (n=30) are shown in Figure 4.6. There is a random distribution of the chloroform data about the mean. All results were within 2 standard deviations of the mean value of 12.5 µg L^{-1}. This value for THMs is well below the guideline standard of 100 µg L^{-1} [SANS 242, 2005] and hence appears to pose no real problems to the consumer.

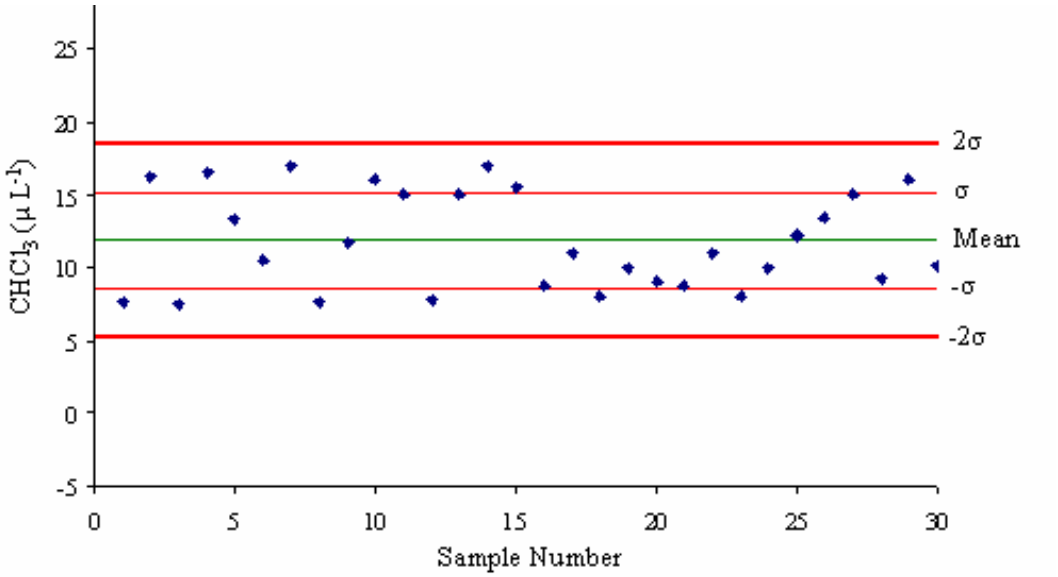

Figure 4.6: THMFP data collected over a two month period.

Similar THM concentrations were determined by other researchers in recent studies [Bolto, 2005]. Consideration must however be given to factors such as temperature effects, UV radiation effects and retention time or chlorine contact time. The THMFP was determined under controlled laboratory conditions to determine specifically the potential of the coagulant as a precursor to THM formation. Although the method used is based on a standard method it is not intended to simulate water treatment processes; it is designed to estimate the concentration of THM precursors in general. Water is exposed to various conditions of sunlight (temperature and UV radiation), pH, and the chlorinated water may be stored in a reservoir for lengthy periods of time. These are all factors that may affect the production of THMs. Furthermore, the polymer dose can vary from 1 to 10 mg L^{-1} depending on the raw water quality, and may significantly increase the THMFP.

4.5 Conclusions

This study shows unambiguously that although there are claims that polyDADMAC is one of the most chlorine resistant polymers it can degrade in the presence of chlorine to form the undesirable byproduct chloroform. However, when treated under standard conditions (3 to 5 mg L^{-1} chlorine residual) and at low polymer concentrations (2 mg L^{-1}) the amount of chloroform is well within the guideline standard recommended by the South African National Standards [SANS 241, 2005] for THMs in water. Therefore, it must be considered that although studies have shown that polyDADMAC coagulants are capable of removing THM precursors such as humic and fulvic acids, polyDADMAC in itself can be regarded as a precursor to some extent. There is every possibility that the THMFP under real water treatment conditions may be enhanced or reduced.

4.6 References

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CHAPTER FIVE

POLYDADMAC STABILITY STUDIES

5.1 Introduction

The study conducted on the chlorination of polyDADMAC focused only on the formation and determination of volatile organic compounds (VOCs). There is however also a real possibility of the potential formation of semi-volatiles, non-volatiles and inorganic species that warranted further investigation. As a continuation of the study on polymer reactions and degradation, the polymer stability was tested under different experimental conditions of exposure to temperature, pH variations, UV radiation and ozone. In many cases, extremes unlikely to be encountered in real water treatment applications were used to determine the limits and stability of the product, and for purposes of reaction product identification. The final part of the study focused on the polymer degradation by microorganisms. Various techniques, including GPC, GC ECD, GC FID and HPLC, were used to determine the products of degradation.

5.2 Background and Literature Review

Studies conducted on polymer degradation have shown that, in general, the rate of degradation increases with decreasing polymer concentration, increasing pH, and an increase in temperature. GPC measurements conducted on the acrylamide based polymers [Soponkanaporn and Gehr, 1989] revealed that complete degradation occurrs only during biodegradation, to form CO_2 and other inorganic compounds. Ozonation and chlorination increased degradation rates, but also produced refractory compounds. Acrylamide, one of the starting monomers of the acrylamide dimethyl-aminoethyl acrylate copolymer, was not produced; in fact it decreased in all the degradation studies that were undertaken. On the other hand, chloroform was a product of chlorination, especially at high pH (pH 9).

Degradation of polymers may occur due to natural processes such as ageing [Gehr and Kalluri, 1983] and biodegradation [Suzuki et al., 1978], or due to interaction with chemicals such as chlorine [Kaiser and Lawrence, 1977; Amy and Chadik, 1983] or ozone [Suzuki et al., 1979a/b]. Aizawa and co-workers, investigated the effects of the reaction of chlorine with acrylamide and polyacrylamide [Aizawa et al., 1990]. The polymer decreased in molecular weight and formed organic halides as by-products of chlorination. The monomer that was an impurity of the polymer formed chloroform and 2,3-dichloropropionic acid. These findings were in agreement with those of Mallevialle et al., who showed that chlorination of polyacrylamide resulted in the formation of Total Organic Halogens (TOX) and trihalogenated methanes (THMs) [Mallevialle et al., 1984]. In a more recent study, the effect of radiation on polyDADMAC was investigated [Zhang et al., 2003]. The irradiated samples were evaluated in terms of changes in viscosity and electric conductivity. The results suggested cross-linking at high concentrations (above 13 g L^{-1}) and chain scission at low concentrations.

5.3 Experimental

5.3.1 Instrumentation

GPC: GPC was conducted on a Waters Alliance 2695, using optimized operating conditions as described in Section 2.5.1.

HPLC: Reversed Phase High Performance Liquid Chromatography (RP HPLC) was conducted on a Waters Alliance 2690 system fitted with a Waters 996 photodiode array (PDA) UV detector. A Waters µBondapak C_{18} column of dimensions 7.8 x 300 mm was used. The mobile phase was methanol:water (50:50) at a flow rate of 1 mL min^{-1}. Samples were filtered through 0.45 µm syringe type filters prior to analysis.

GC-FID: Gas chromatography was conducted on a Hewlett Packard (HP) 6890 gas chromatograph fitted with a flame ionization detector (FID). Separation was achieved on a DB 5 capillary column (J&W Scientific) of dimensions 30 m x 0.25 mm x 0.25 µm. Data acquisition and analysis were conducted using the Chemstation software.

GC-FID Parameters:		
	Inlet Temperature	250 ^{o}C
	Column Flow	1 mL min^{-1}
	Column Temperature	50 ^{o}C
	Initial Time	1 min
	Ramp Rate	15 ^{o}C min^{-1}
	Final Temperature	280 ^{o}C
	Final Time	5 min
	Injection Mode	Splitless, purge at 1 min.
	Detector Temperature	280 ^{o}C

GC-ECD: Gas chromatography was conducted on a Hewlett Packard (HP) 6890 gas chromatograph fitted with an electron capture detector (ECD). Separation was achieved on a DB 624 capillary column (J&W Scientific) of dimensions 30 m x 0.32 mm x 1.8 µm. Data acquisition and analysis were conducted using the Chemstation software.

GC-ECD Parameters:		
	Inlet Temperature	80 ^{o}C
	Column Flow	1.8 mL min^{-1}
	Column Temperature	80 ^{o}C
	Initial Time	1 min
	Ramp Rate	2 ^{o}C min^{-1}
	Final Temperature	100 ^{o}C
	Final Time	3 min
	Injection Mode	Split, 5:1
	Detector Temperature	250 ^{o}C

5.3.2 C_{18} Solid Phase Extraction

C_{18} solid phase extraction cartridges (Waters) were activated with methanol (10 mL) followed by Milli-Q water (10 mL). The chlorinated polymer test sample (100 mL) prepared as described in Section 4.3.2 was then passed through the cartridge using a syringe. The cartridge was purged with several syringe volumes of air to remove traces of water. Organic materials trapped in the cartridge were eluted in 1 mL methanol into a glass vial for GC and HPLC analyses.

5.3.3 The Effect of Temperature

Aliquots of a 0.5% (m/v) polymer solution were subjected to temperatures ranging from ambient temperature to 80 ^{o}C. The samples were heated for 30 min at each temperature, with constant stirring. After cooling to ambient temperature the samples were filtered through 0.45 μm syringe type filters for GPC, GC ECD, GC FID and HPLC analyses.

5.3.4 The Effect of pH

Aliquots of polymer solution (0.5% m/v) were collected in beakers and the pH adjusted using phosphoric acid (10% v/v) and sodium hydroxide (0.1 N) to yield solutions in the pH range 2 to 12. The samples were allowed to stand for 1 h prior to filtration and GPC, GC ECD, GC FID and HPLC analyses.

5.3.5 The Effect of Radiation

A 0.5% (m/v) polymer solution was prepared and exposed to long wavelength UV radiation (365 nm), using a 9 A UV lamp (Spectroline Model EA-160/FE, Spectronix Corporation, USA) for 24 h. A sample of the product was filtered and analyzed by GPC, GC ECD, GC FID and HPLC.

5.3.6 DADMAC Monomer Analysis

Monomer analysis was conducted using a paired ion chromatography method as described in the ANSI/AWWA Standard [ANSI/AWWA 60, 2005]. An alternate titrimetric method is available in the AWWA Standard for polyDADMAC [ANSI/AWWA B451-92] but was found to be very labor intensive.

5.3.7 Polymer Degradation by Microorganisms

Polymer Stock, 20 000 mg L^{-1}: A polymer stock solution was prepared by dissolving 0.20 g polymer crystals (Z553D precipitated from ethanol, Section 3.3.6) in 10 mL Milli-Q water.

Working Solution, 200 mg L^{-1}: The stock solution was diluted to 100 mL with Milli-Q water.

Sample Preparation: A 25 mL aliquot was inoculated with 200 μL of *Candida Albicaris* (Yeast) and a second aliquot with *Bacillus Subtilis* (Bacteria). The samples

were incubated at 30 ^{o}C and agar plates were set up to monitor growth of the cultures [Eaton et al., 1995] over a period of 6 days at two-day intervals.

5.4 Results and Discussion

5.4.1 Solid Phase Extraction

The presence of any degradation products as a result of subjecting the polymer to varying levels of chlorine, temperature, pH, ozone and UV radiation is expected to occur in trace and ultra trace quantities and hence sample pre-concentration is necessary to obtain any meaningful results. Furthermore, apart from HPLC, all the remaining GC techniques used required the sample to be dissolved in an organic solvent. Sample pre-treatment was necessary and solid phase extraction (SPE) was adopted for this purpose. This technique involves passing the sample through a bed of activated C_{18} solid phase housed in a cartridge. Any organic degradation products will adsorb and concentrate onto the solid phase by non-polar (Van der Waals) forces of interaction. SPE theory predicts that polar species such as the polymer dilution solvent (water), inorganic salts, and ionic species such as polyDADMAC will pass through the SPE cartridge with no retention. This was verified experimentally by passing a polymer solution through the cartridge and analyzing the filtrate together with a control sample by GPC (Figure 5.1). Both the filtrate and the control sample had similar polymer peak areas, indicating that it was not retained by the C_{18}. The slight reduction in area in the case of the C_{18} effluent may be due to the action of the solid phase acting as a simple filter or some degree of non-polar interaction that was deemed to be insignificant.

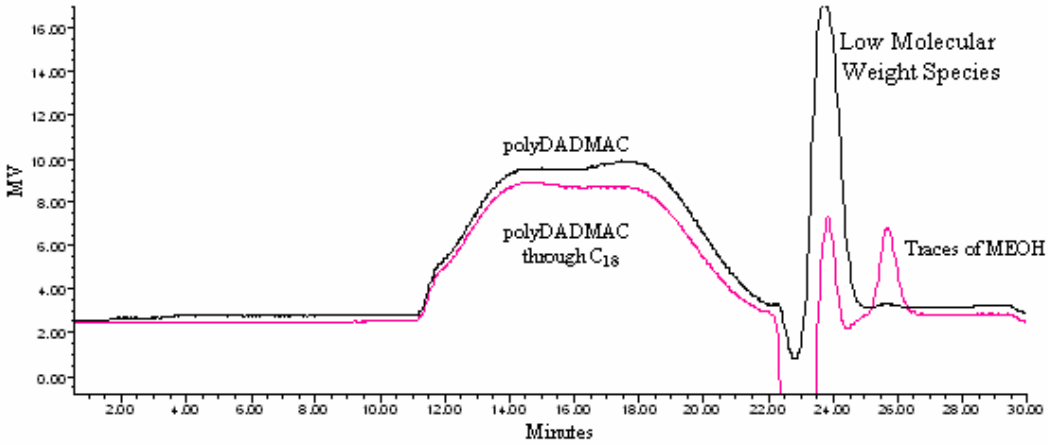


Figure 5.1: Overlay of GPC chromatograms of a polyDADMAC reference sample and the effluent after passing through a C_{18} SPE cartridge.

There is a significant reduction in the area of the small molecular weight species caused by the action of the solid phase and the presence of an additional peak arising from the solid phase activation solvent, methanol. The reduction in the low molecular weight species demonstrates clearly the action of the C_{18} phase in removing organics from an aqueous matrix. The organic components that accumulate in the extraction cartridge are then eluted in a small volume (typically 1 mL) of methanol and analyzed by both GC (semi-volatiles) and HPLC (non-volatiles and thermally labile compounds).

5.4.2 GC ECD Analysis of the Chlorinated Polymer

The GC ECD technique is a very selective and therefore sensitive technique for the analysis of halogenated compounds and compounds that have an affinity for electrons.

Using this method the C_{18} extract of the chlorinated polymer was therefore analyzed for the formation of other semi-volatile organic compounds (SVOCs) that may have been missed by the purge and trap GC MS method because of low sensitivity problems or due to low purging efficiency. The overlay chromatograms of the sample and control (Figure 5.2) show no evidence of the formation of any new chlorinated compounds.

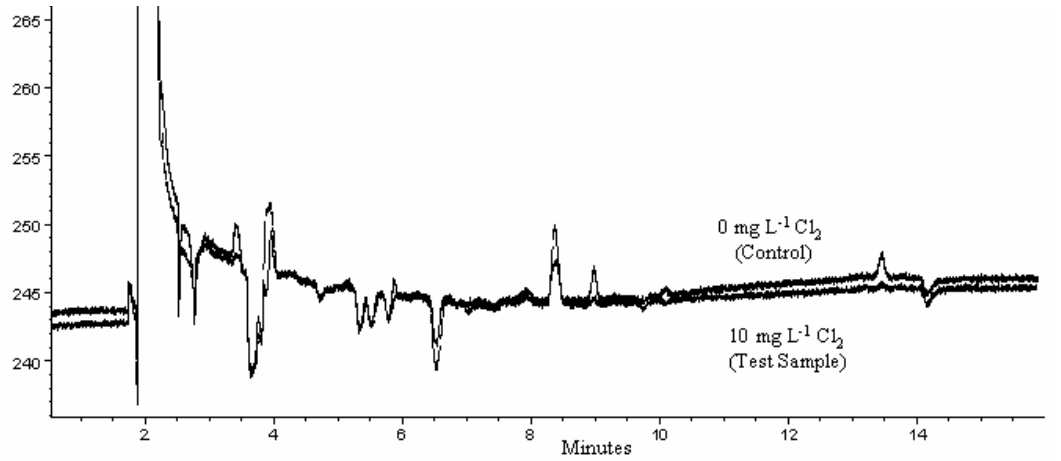


Figure 5.2: Overlay of GC ECD chromatograms of C_{18} extracts of a chlorinated polymer test sample and a control.

The test sample and control have identical GC ECD profiles. Since this is commonly known to be one of the most sensitive techniques for the analysis of chlorinated compounds [Eaton et. al., 1995], there was no need to investigate further for the presence of these compounds.

5.4.3 GC FID Analysis of the Chlorinated Polymer

The GC FID is a non-selective detector and can be used for monitoring the most volatile and semi-volatile organic compounds. The C_{18} extract of the chlorinated polymer was therefore analyzed for the formation of other semi-volatile organic species. The FID chromatograms of the extracts of the sample and control (Figure 5.3) show more peaks in the control than in the sample.

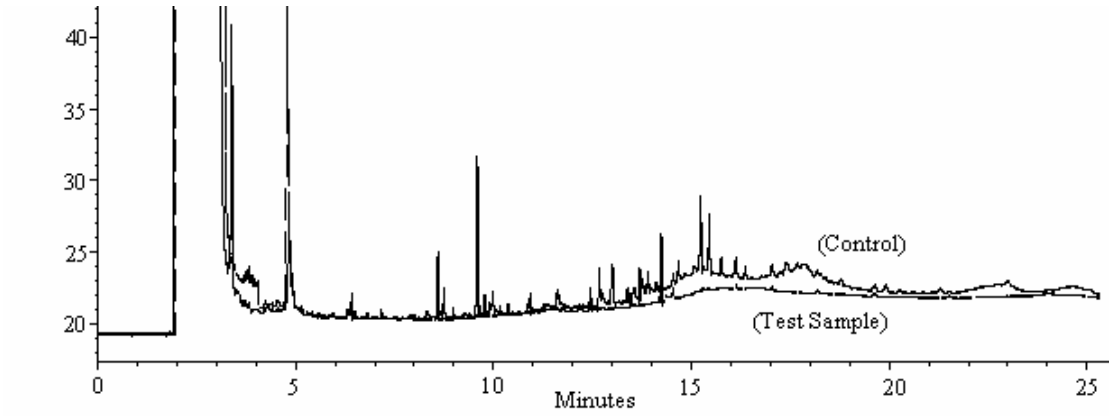


Figure 5.3: Overlay GC FID chromatograms of C_{18} extracts of a chlorinated polymer test sample and a control.

This was attributed to contamination originating from several potential sources, including:

- The C_{18} solid phase
- Poor quality methanol activating/eluting solvent
- Dirty or contaminated glassware
- Contaminated control
- Dirty chromatographic system
- Sample labeling error.

The problem was systematically investigated and identified as a contaminated control. Careful inspection of the chromatographic profile of the test sample revealed the absence of any prominent peaks and as such it was taken that no SVOCs were formed.

5.4.4 HPLC Analysis of the Chlorinated PolyDADMAC

The C_{18} extracts were finally analyzed for the possible formation of non-volatiles and thermally labile compounds during the chlorination experiment by RP HPLC with photodiode array (PDA) UV detection. The preliminary results (Figure 5.4) of the extracts show that there are no new compounds formed during chlorination.

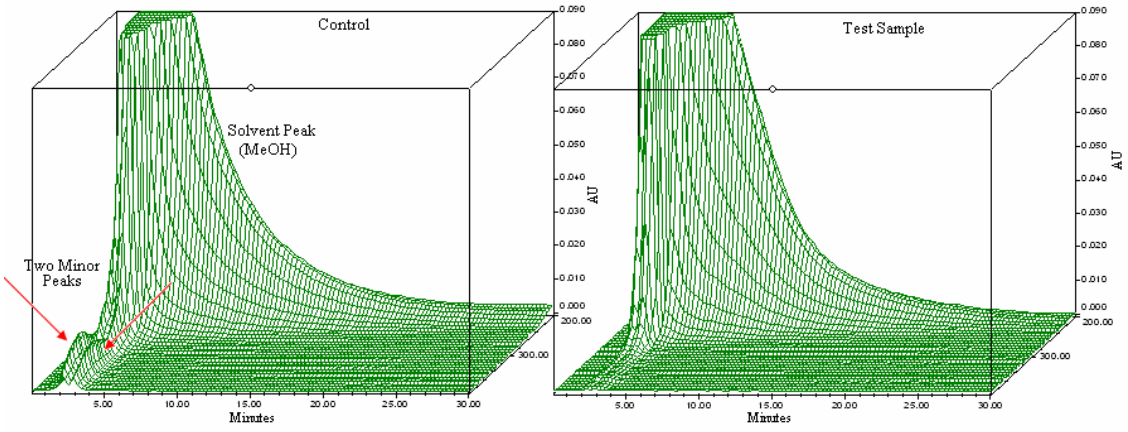


Figure 5.4: 3D HPLC PDA chromatogram of C_{18} extracts of the control and test sample.

Monitoring was done over the UV wavelength range of 400 to 200 nm, using an analysis time of 30 min. Two minor peaks at *ca* 2 min and a λ_{max} of 360 nm can be seen in the control but they were not present in the test sample. The significance of this was not fully understood but appears to be consistent with the trend observed with the GC FID results, namely the presence of more peaks in the control than in the test sample. No other species were observed over the entire UV region.

5.4.5 The Effect of Temperature on PolyDADMAC

Polymers may be subjected to different conditions of temperature during transportation, storage and use. An experiment was therefore conducted to establish whether temperature had any adverse effects on the product that could affect its performance during water purification.

Aliquots of a 0.5% (m/v) polymer solution prepared in Milli-Q water were subjected to increasing temperatures, from ambient up to an extreme of 80 ^{o}C. Samples were held at predetermined temperatures for 30 min, followed by cooling to ambient temperature. Thereafter a 10 mL aliquot was removed and filtered for GPC analysis. The polymer sample at room temperature was used as the control or reference against which changes were assessed.

The overlay of GPC chromatograms of the polymer at the various temperatures (Figure 5.5a) show little or no change from ambient temperature (25 ^{o}C) up to and including 70 ^{o}C. The three samples at 80 ^{o}C show clear evidence of a structural change occurring in the polymer.

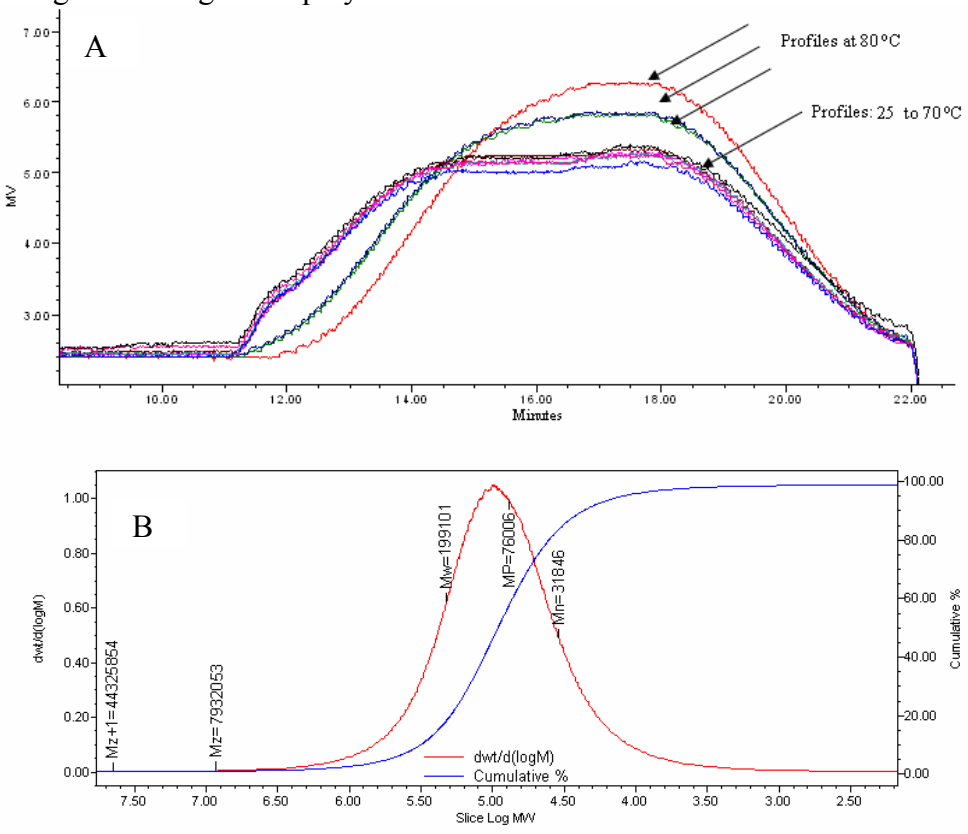


Figure 5.5: (a) GPC chromatograms showing the effect of temperature on polyDADMAC and (b) the MWD at 80 ^{o}C.

This is depicted by a change in the peak shape from a more symmetrical distribution to one that is biased towards the low molecular weight region. The corresponding MWD at 80 ^{o}C is shown in Figure 5.5b. Details of the change in the molecular weight distribution as a function of temperature are tabulated in Table 5.1.

Table 5.1: Changes in MWD data of polyDADMAC as a function of temperature.

Temperature (^{o}C)	Mn	Mw	MP	Mz	Mz + 1	Polydispersity
25	37315	450966	81547	15651673	45405714	12
30	39134	460528	76365	15716909	45497405	11
40	38282	450141	82811	15500108	45283818	11
50	36261	443465	68789	15425754	45316135	12
60	36414	439671	86397	15516987	45559208	12
70	32219	423423	63859	15148049	45207923	13
80	35704	294433	94647	11848584	44921226	8
80	34165	302953	80459	12356136	45165263	8
80	31846	199101	76006	7932053	44325854	6

Mn = number average molecular weight
Mw = weight average molecular weight
MP = peak average molecular weight
Mz = z-average molecular weight

The only significant change is noted for the weight average molecular weight (Mw) and Z-average molecular weight (Mz). Both in turn impact on the polydispersity at 80 ^{o}C. The reduction in polydispersity indicates a narrower distribution of molecular weight averages, which is consistent with the polymer peak profile (Figure 5.5). The elevated temperature appears to cause scission of long polymer chains to shorter ones, which may have a negative impact on polymer performance in coagulation and flocculation. In general, polymers with high charge densities are recommended for water clarification while high molecular weight polymers are widely used for the purpose of sludge conditioning [Mangravite, 1983].

5.4.6 GC FID Analysis of the Heat Exposed PolyDADMAC

To investigate the formation of low molecular weight species, a similar approach was used as discussed in the chlorination experiments. The samples were extracted with activated C_{18} solid phase and eluted into 1 mL of methanol. Since no peaks were observed in the chlorination experiment (Figure 5.2), it was deduced that no halogen or ECD sensitive compounds formed. Therefore only GC FID and RP HPLC work was conducted. The overlay of chromatograms of the extracts at room temperature and at 80 ^{o}C (Figure 5.6) shows no new peaks. Another important observation is the appearance of a homologous series of compounds eluting in the control (15 to 25 min) which is not present in the test sample.

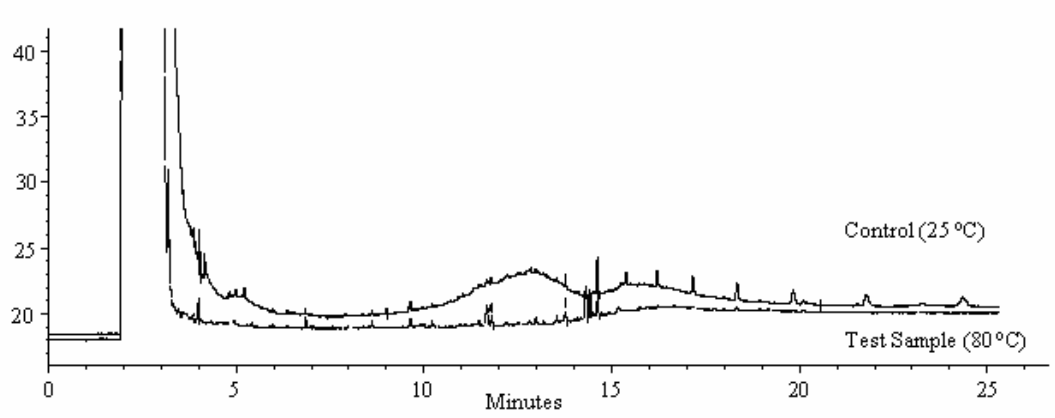


Figure 5.6: The GC FID overlay chromatogram of C_{18} extracts of the polymer test sample and control.

This profile is typical of the n-alkanes that originate from petroleum or petrochemical products and was therefore attributed to carryover from "dirty" samples previously analyzed on the GC. The problem was subsequently eliminated by thermal cleaning of the chromatographic system. There were no prominent peaks present in the test sample that could be attributed to degradation by heat exposure.

5.4.7 HPLC Analysis of the Heat Exposed PolyDADMAC

The HPLC chromatograms of the C_{18} extracts of the reference and test sample after exposure to a temperature of 80 ^{o}C for 90 min (Figure 5.7) show no evidence of any new product formation.

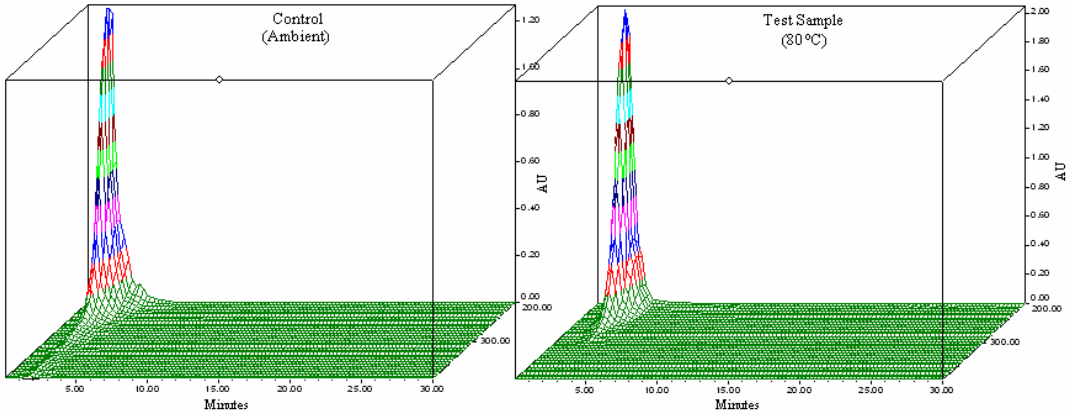


Figure 5.7: The HPLC chromatograms of C_{18} extracts of the control and test sample after heating.

The 3-D plot feature of the PDA detector was found to be extremely useful in the search for new and unknown compound formation. Compounds may absorb strongly at one wavelength but very weakly or not at all in another region. By collecting the data in three dimensions, the PDA scans the entire wavelength range throughout the analysis and the wavelength or wavelengths of maximum absorption can be determined by inspection of the peak intensities. There was therefore little chance of missing any newly formed compounds unless they were non-UV absorbing. This does not imply that the heat exposed polymer is as good as the normal unexposed polymer. The GPC results clearly show some degree of structural change at 80 ^{o}C.

5.4.8 The Effect of pH Variations on PolyDADMAC

As mentioned earlier, polyDADMAC polymers are reported to have good chlorine resistance and be largely unaffected by pH [Mangravite, 1983]. This forms the basis for their popularity in the water sector. To confirm this claim, aliquots of a 0.5% (m/v) polymer solution prepared in Milli-Q water were treated with NaOH and HCl to give solutions in the pH range 2 to 12. After a 2-hour exposure time, the samples were filtered for GPC analysis.

The overlay of chromatograms of polyDADMAC samples at pH values in the range 2 to 12 and the MWD at pH 12 (Figure 5.8a/b) confirms that the polymer is relatively resistant to pH changes. At pH 12 however, there is a decrease in the polymer peak area but the peak shape and MWD remain essentially unchanged when compared to the control.

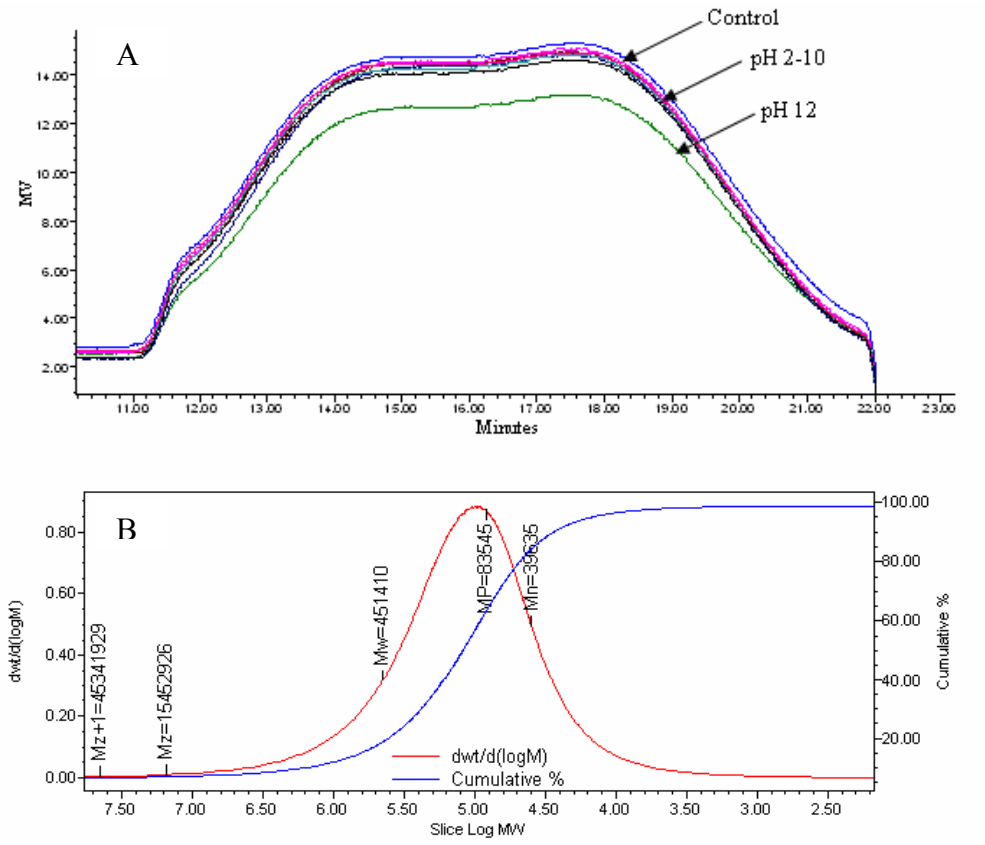
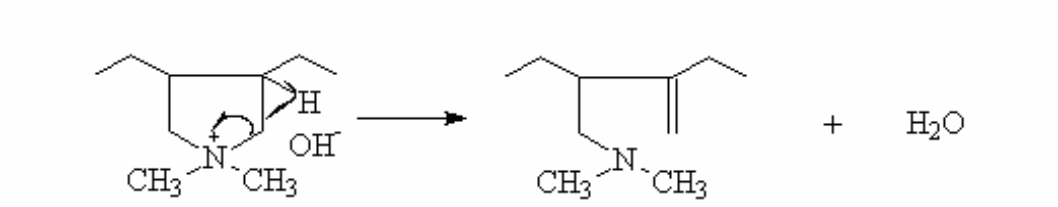


Figure 5.8: (a) Overlay of GPC chromatograms of polyDADMAC test and control samples at different pHs and (b) the MWD at pH 12.

In general, quaternary ammonium compounds are known to be relatively stable to pH changes. This is because they have no protons to give and they are not affected by the OH^- ions. However, at very high pH extremes, the quaternary ion may form a hydroxide that can undergo a Hoffman elimination reaction, as shown in Scheme 5.1 [Morrison and Boyd, 1987].



Scheme 5.1: Proposed Hoffman elimination reaction of polyDADMAC at basic pH.

It still remains unclear whether this reaction can account for the overall reduction in the polymer peak area at pH 12. As this extreme pH condition is unlikely to prevail during water purification, further investigation of this was not pursued. Good pH stability is a very important property of this polymer as it can then be used with a wide range of raw water types without the need for pH adjustment. PolyDADMAC does not change the pH of the treated water and hence eliminates the need for post pH correction.

5.4.9 GC FID Analysis of pH Degradation Products of polyDADMAC

The GC FID overlay chromatogram (Figure 5.9) of polyDADMAC samples in the pH range 2 to 12 shows a number of new peaks occurring at pH 12. This is especially evident in the region 3 to 5 min. In the region 14 to 15 min there is a cluster of three peaks, the area of which increases as the pH increases. The identity of these components was unknown. Attempts to identify them by GC MS were unsuccessful.

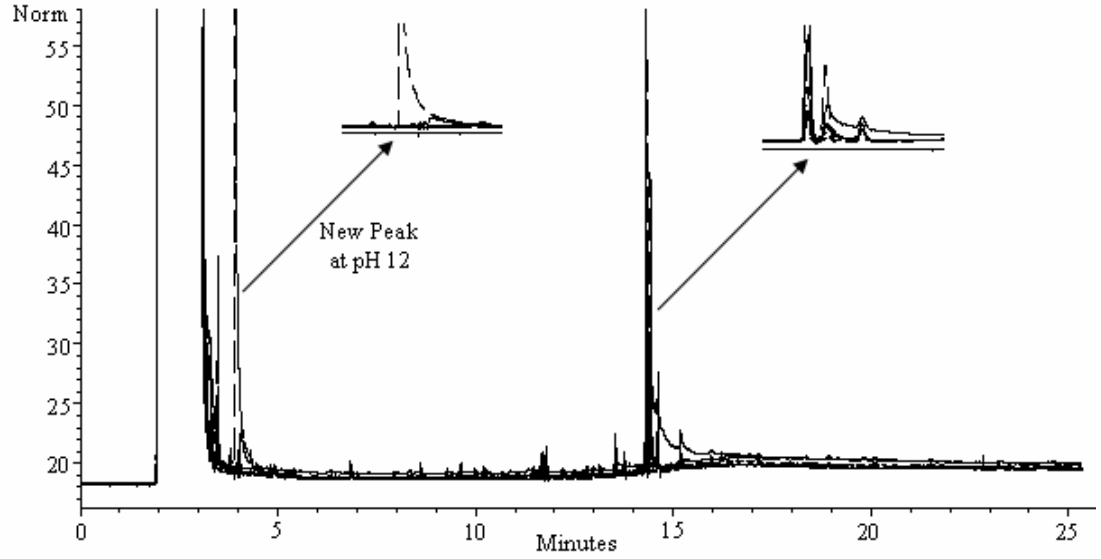


Figure 5.9: GC FID overlay chromatogram of polyDADMAC samples in the pH range 2 to 12.

The difficulty with component identification was attributed to the low levels of the pH degradation products formed and also to the fact that the degradation products may have eluted within the solvent delay time of the GC MS. This time is usually set at 3 to 5 min (when the detector is off) to protect the mass spectrometer from the large volume of solvent entering the system.

5.4.10 RP HPLC Analysis of pH Degradation Products of PolyDADMAC

3-D plots of the test samples in the pH range 2 to 12 were acquired. The plot at pH 2 (Figure 5.10) shows that there are three or four poorly resolved peaks eluting in the region 0 to 10 min. Similar peaks were found in the other chromatograms and no important differences were noted.

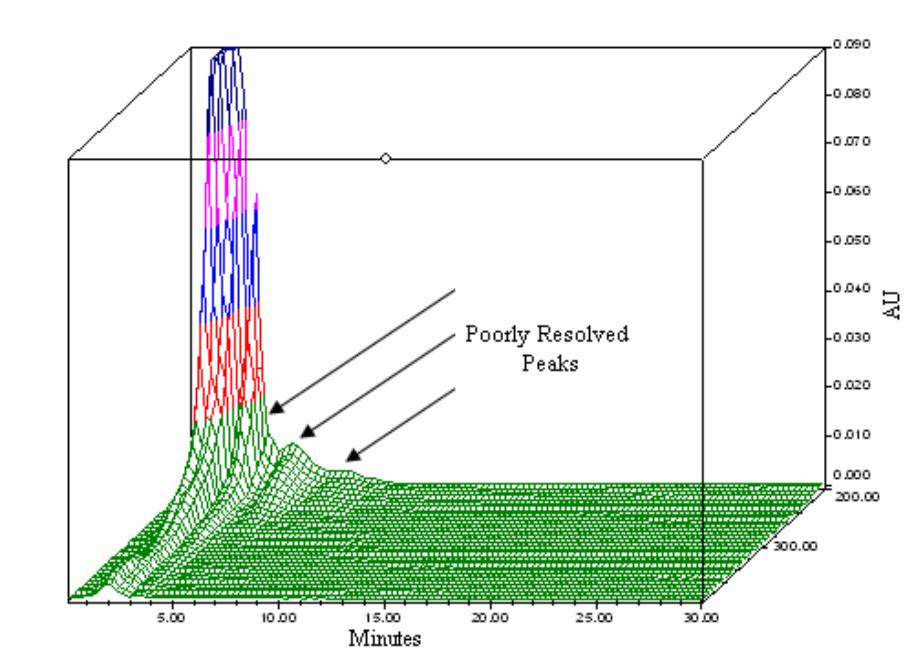


Figure 5.10: 3-D HPLC chromatogram of a polyDADMAC test sample at pH 2.

5.4.11 The Effect of Ultraviolet Radiation on PolyDADMAC

Water treatment polymers are exposed to varying levels of UV radiation during storage and use. The stability of the products under such conditions is therefore of great importance to users. The aims of this experiment were to subject polyDADMAC to intense UV radiation and observe the short term effects. Results, if positive, could then be used to design further experiments using conditions similar to those in a water treatment facility, or they could be used to determine the possible formation of undesirable products during water treatment. A 0.5% (m/v) polymer solution was prepared and exposed to short wavelength UV radiation for 12 h. The sample was then filtered and analyzed by GPC. The overlay chromatogram of the exposed and unexposed sample (Figure 5.11a) shows that there is a distinct difference in the polymer peak shape occurring in the test sample, especially in the high molecular weight region. This is clear evidence of polymer degradation. The profile shows unambiguously that the high molecular weight region of the distribution is affected. The corresponding MWD is shown in Figure 5.11b. There is every possibility that polymer chain scission was occurring. This can be concluded by noting the reduction in the peak area of the high molecular weight region and a corresponding increase in area of the low molecular weight region of the distribution.

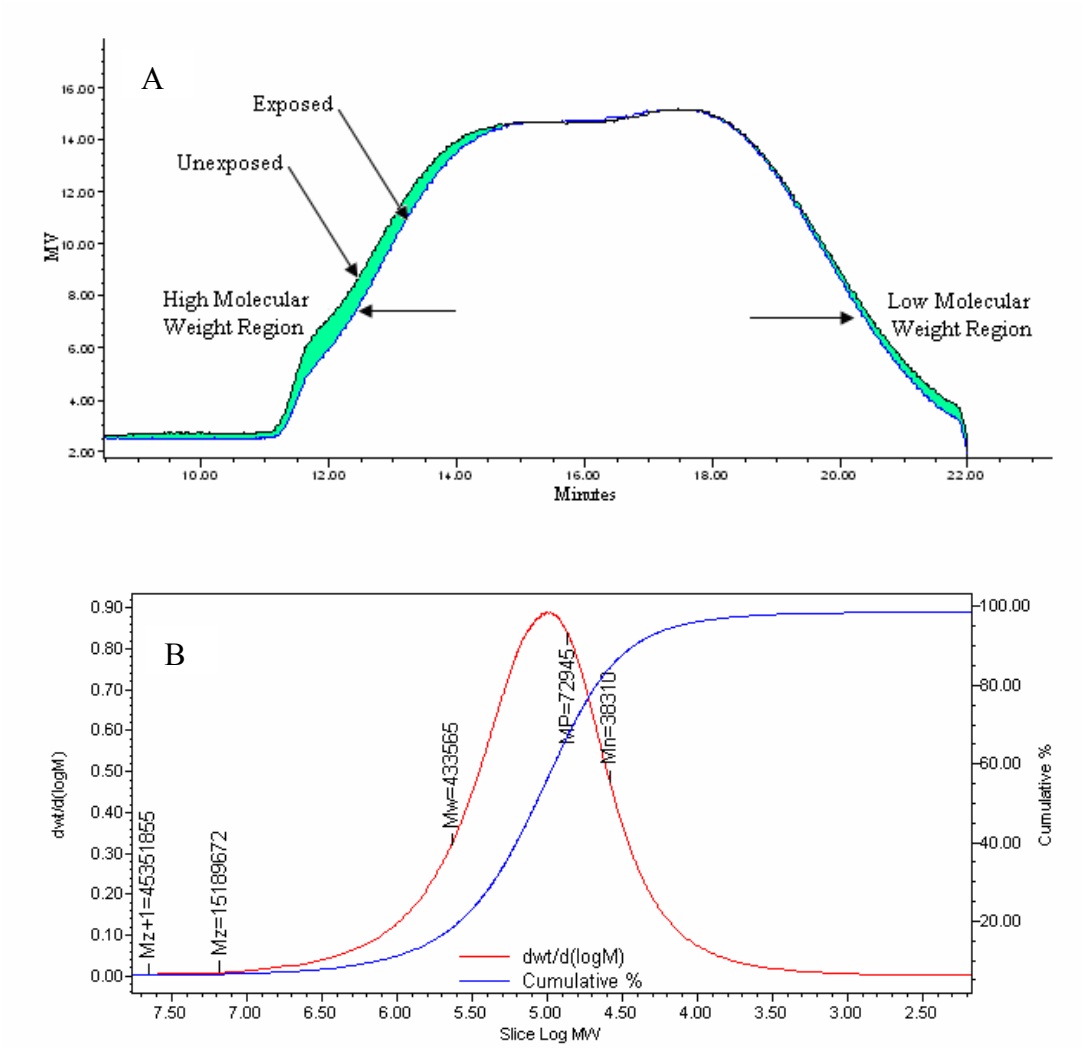


Figure 5.11: (a) GPC profiles of the UV exposed and the unexposed polymer and (b) the MWD of the UV exposed polymer.

5.4.12 GC FID Analysis of the UV Exposed PolyDADMAC

The test sample and control was pre-treated by C_{18} solid-phase extraction prior to analysis by GC FID. Figure 5.12 shows a number of new peaks occurring in the UV exposed test sample that do not appear in the unexposed reference sample. It must be noted that the chromatogram is highly magnified and may be in the region of the limits of detection of the detector. Hence these peaks may be spurious peaks and therefore carry no meaningful information. Considering the fact that the polymer is composed primarily of one monomer (mDADMAC), there is little chance of the formation of so many different species from one compound.

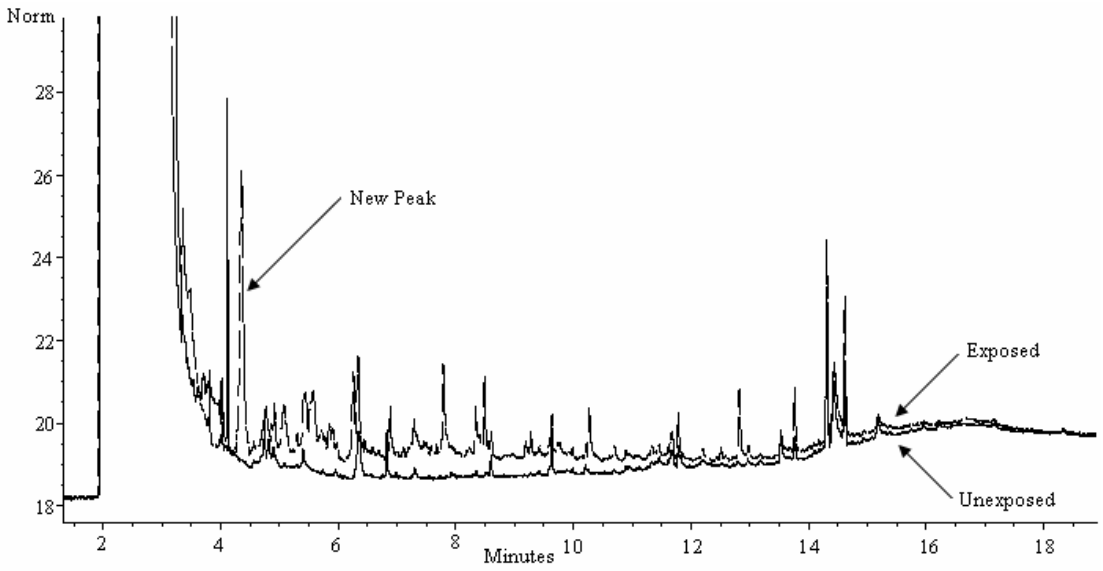


Figure 5.12: Overlay GC FID chromatogram of the UV exposed and unexposed polyDADMAC samples.

This leaves the one remaining relatively strong peak at 4.1 min. Even so this may be insignificant, leaving the degradation restricted to the formation of smaller polymer chains.

5.4.13 HPLC Analysis of UV Exposed and Unexposed PolyDADMAC

Figure 5.13 shows differences in the control and the test samples. The peaks in the UV exposed test sample are of higher intensities than the control. There is also a second absorption maxima at *ca* 350 nm which is absent in the control.

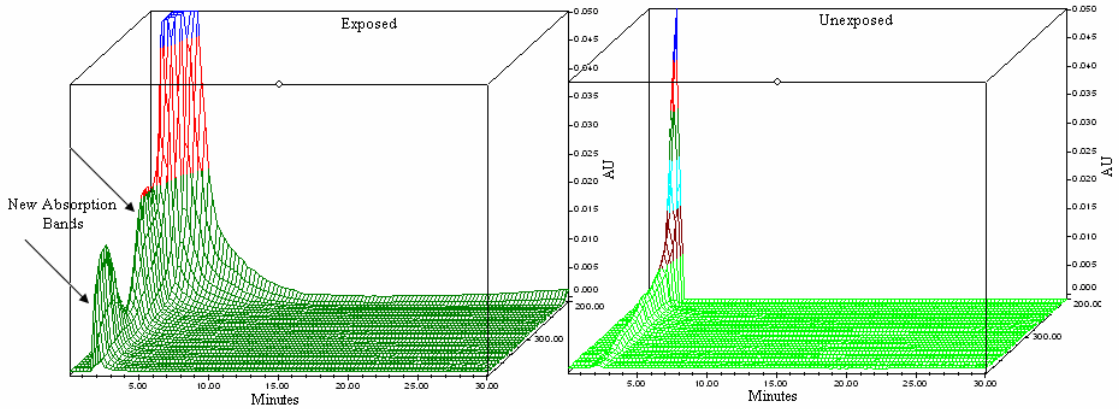


Figure 5.13: 3-D HPLC chromatograms of the UV exposed and unexposed polymer samples.

These differences are real and confirm the GPC results, namely that a positive change has occurred as a result of exposure to UV radiation. The HPLC method is however a rapid method that was not fully developed or optimized adequately to resolve the components, but it was sufficient for the purposes of this investigation.

5.4.14 Monomer Analysis

The levels of unreacted monomer present in the polymer after exposure to pH extremes, temperature changes, and UV radiation, can be used as a diagnostic tool in determining the nature of the changes occurring in the polymer. In the temperature and UV experiments, changes in both the peak profiles and peak areas were observed. It was thought that the former would occur as a result of further polymerization. Experiments were conducted to determine the monomer content after UV and heat treatment experiments were performed using a paired ion chromatography method. The overlay chromatogram of a reference polymer with that of the heat treated and UV exposed product (Figure 5.14) shows that the monomer peak area remained relatively unaffected by temperature and UV exposure. The %RSD was found to be 3%, confirming that the fluctuations in the area were in fact negligible and normal for a typical HPLC analysis.

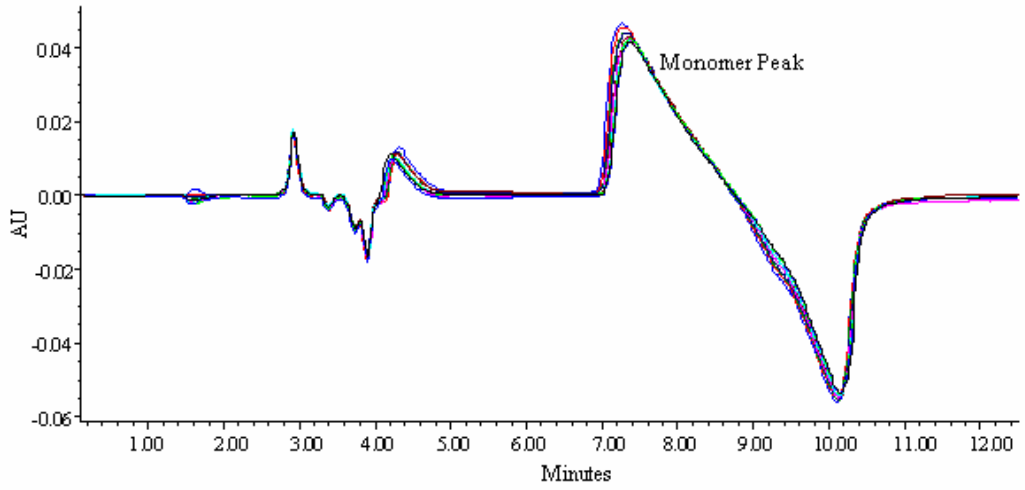


Figure 5.14: Overlay HPLC chromatogram of DADMAC monomer after heat and UV exposure.

5.4.15 Degradation of PolyDADMAC by Microorganisms

Although studies have been conducted on the degradation of polyamines and polyacrylamides, investigations on polyDADMAC polymers have always been hindered by the lack of sufficient quantitative analytical methods [Fielding et al., 1999]. The overall objective of this study was to obtain some insight into polymer degradation as a result of sludge disposal after the water purification process. To commence with the investigation, laboratory experiments were conducted under controlled conditions in an attempt to establish the nature and extent of degradation. An aliquot of a 0.02% polymer solution was inoculated with 200 µL of *Candida albicaris* (Yeast) and a second 25 mL aliquot with *Bacillus subtilis* (Bacteria). These organisms are partially representative of soil microbes. Tests conducted with *Ecoli* and *Coliforms* were unsuccessful due to the death of the cultures. Plate counts were done and used as an indicator of growth of the cultures, and the GPC profiles and polymer peak areas were used to monitor degradation. The counts are shown in Table 5.2. Incubation conditions were 30 ^{o}C for a minimum period of 48 h.

Table 5.2: Plate counts for microbial degradation of polymer solutions.

Date		Sample 1 *Bacillus*	Sample 2 *Candida*
Prepared	**Counted**	**Plate Counts (cells per 100 mL)**	
290304	310304	26300	280000
310304	020404	11580000	16200000
020404	060404	>11580000	>16200000
060404	080404	>11580000	>16200000

The plate counts indicate growth of both cultures (Figure 5.15). In fact, from a previous trial (data not reported here), the result obtained suggested that the sample possessed some degree of cell toxicity and caused death of cells and a corresponding reduction in the plate counts. The contradiction in the two sets of growth trends can be explained by the fact that *Candida* and *Bacillus* are more resilient than *Ecoli* and *Coliforms.*

Figure 5.15: Cultures of *Bacillus subtilis* and *Candida albicaris* from inoculated polymer solutions.

The results are in direct conflict with results previously obtained in which the simulated laboratory experiment made use of a cocktail of commercially available organisms supplied in the form of a capsule called the Bioseed. The bioseed experiment showed that the polymer used was toxic towards the organisms by the drastic reduction in the plate counts. An explanation for the two different growth trends observed may be due to the well known fact that the two microbes *Bacillus* and *Candida* are generally the more resilient strains and therefore able to withstand and thrive under harsh conditions. The other possibility that needs to be considered is that for the current study, purified polymer crystals were used, with little or preferably no monomer or other starting materials that is likely to exhibit the toxic effects observed. Therefore, the normal commercial polymer could not be used in this experiment due to the impact of the other matrix components on the microorganisms. There was also concern that degradation products may originate from materials other than the test polymer and render the results of little scientific use.

During the course of the degradation studies a sludge sample collected from DHWTW was also assessed for natural microbial activity. Plate counts were also done and the

results below indicate growth of the natural microbes present in the sludge. The results were as follows:

- prepared on 020406, counts = 180 000 cells per 100 mL
- re-counted on 080404, counts = 230 000 cells per 100 mL.

Preparation of a microscope slide revealed that the sample comprised predominantly a large amount of sand as well as living microbes. The GPC profiles were collected during the course of incubation of the samples and degradation assessed in terms of the polymer peak areas (Table 5.3).

Table 5.3: PolyDADMAC peak areas acquired at various stages of incubation.

Date Acquired	Polymer Peak Area		
	DHWTW Sludge+Natural Microbes	Z553D+Bacillus	Z553D+Candida
160404	ND	-	-
190404	ND	-	-
220404	ND	1014299	1044288
030504	ND	961625	916982

ND=not detected, polymer < detection limit

No polymer peak was detected in the DHWTW sample. This may be the result of low detection sensitivity of the system (*ca* 0.005%) or due to strong adsorption of the polymer onto the particles of floc, resulting in a low polymer content of the sludge extract. The inoculated samples showed a reduction in the peak areas for *Bacillus* and *Candida* of 5% and 12% respectively.

5.5 Conclusions

GPC analysis, as carried out under all experimental conditions of temperature, pH, UV and exposure to microorganisms described in this study, indicated some degree of change in the polymer. However, there were serious limitations with the technique particularly with the detection of low molecular species. The characteristic drop in the baseline at the retention time in the vicinity of the low molecular weight coupled with low sensitivity hindered the investigation. Techniques such as GC ECD, GC FID, Purge and Trap GC MS and RP HPLC proved to be more successful. Sample pre-treatment using SPE technology was introduced and a number of unidentified organic species were detected using these methods. The monomer content remained relatively unchanged during these experiments and suggested that the changes in the GPC profiles were not related to further polymerization. Analysis of the test samples and control of each experiment for total organic carbon (TOC) and total Kjeldahl nitrogen (TKN) were inconclusive. These tests were conducted to establish if the carbon skeleton or quaternary amine groups were transformed during the degradation experiments. A reduction in TOC and TKN would indicate loss of carbon (CO_2) and nitrogen (NO_2) respectively. GPC results indicate that polyDADMAC is a very stable polymer and undergoes change only when subjected to extremes of pH, temperature and UV conditions. As such conditions are unlikely to be experienced during the normal course of water treatment users can be reassured of its stability.

The unusual conditions were used solely to aid identification of the degradation products. Since GPC proved to be a useful technique to study the behavior of the polymer, further studies ought to be conducted using a more sensitive detector. The evaporative light scattering detector (ELSD) is in general more sensitive than RI detection by about two orders of magnitude and the more recently developed condensation nucleation evaporative light scattering detector (CN ELSD) is reported to be about three orders of magnitude more sensitive than RI detection. The only limitation of this detector is that volatile mobile phases are required for the GPC separation.

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CHAPTER SIX

OZONATION OF POLYDADMAC

6.1 Introduction

Ozonation is used in some water treatment works as a pre-treatment process for the removal of taste and odor causing chemicals such as iron, manganese, organic compounds, colloids (organic and inorganic) and biological contaminants (by eliminating the growth of algae and bacteria) from raw feed water.

Although ozonation with relatively small doses (0.1 to 1 g m^{-3}) in raw feed water is very effective, the potential for it to react with other water treatment chemicals such as polyDADMAC exists. Such reactions may yield undesirable end products that could be a serious threat to human health. This study was conducted to assess the fate of polyDADMAC, and the formation of byproducts during ozonation and to assess any potential effects on the consumer. It involved the generation of ozone, determination of the ozone output, sample dosing and determination of ozone dosage by iodometry. The chemistry of ozone in aqueous solution is complex and has been studied extensively [Hoigne and Bader, 1983]. Basically, ozone may react directly with solutes or decompose to form OH radicals. These radicals in turn initiate a series of chain reactions. The products from direct reaction with ozone and radical mediated reactions are usually different. The reaction path via radical attack is promoted by pH values above 7. Ozone effects on the polymer structure at neutral pH and under ambient temperature conditions were studied by GPC.

6.2 Background and Literature Review

Studies conducted on the ozonation of acrylamide based copolymers [Soponkanaporn and Gehr, 1989] indicated an increase in the rate of degradation, resulting in the production of refractory compounds. Researchers noted with interest that acrylamide, one of the starting monomers of the copolymer, was not produced during degradation and in fact also decreased in concentration. In more recent studies conducted by Fielding and co-workers revealed that ozonation of polymers led to the formation of low levels of aldehydes but generally very few low molecular weight byproducts were detected [Fielding et al., 1999]. Four commercially available polymers representing the major polymer types in the US were chosen as case studies. These included an anionic polyacrylamide (powder and emulsion form), a polyDADMAC and an EPI-DMA polymer. Oxidation reactions with EPI-DMA polymers with various oxidants were minimal. Work on polyDADMAC polymers was severely hindered by the lack of quantitative methods of analysis [Bolto, 2005].

The ozonation of copolymers of acrylamide and sodium acrylate resulted in rapid depolymerization to form shorter polymer chains [Mallevialle et al., 1984]. Monomers of acrylamide and acrylic acid were rapidly removed. Ozonation of polyacrylamide resulted in the breaking of polymer chains at pH 10 but no effects were observed at pH 2 [Suzuki et al., 1978]. Chain degradation was in direct proportion to the amount of ozone consumed. ^{13}C-NMR and UV spectroscopy were used to establish the formation of new functional groups of either aldehydes or ketones that reacted with the amide groups of polyacrylamide to form an unspecified

ring structure. Ozonation in the presence of UV radiation resulted in an increase in the rate of decomposition of the polymer to form formaldehyde. Under exhaustive ozonation, oxamic acid and oxamide were produced. Ozonation of polyacrylamide produced negligible amounts of CO_2, leading to the conclusion that it was not effective for complete degradation [Suzuki et al., 1979a/b]. This conclusion was consistent with the findings of other researchers [Malleviale et al., 1984]. A literature review revealed that little work has been done on investigating polyDADMAC and its reaction with ozone.

6.3 Experimental

6.3.1 Apparatus

The Ozonizer: For this study ozone was produced using a silent electrical discharge ozonizer (Sorbios Model GSG 001.2) operated at 220 V, and a feed of pure oxygen at a pressure of 50 kPa (Figure 6.1). The electrical discharge produces free energetic electrons that dissociate O_2 molecules into oxygen atoms.

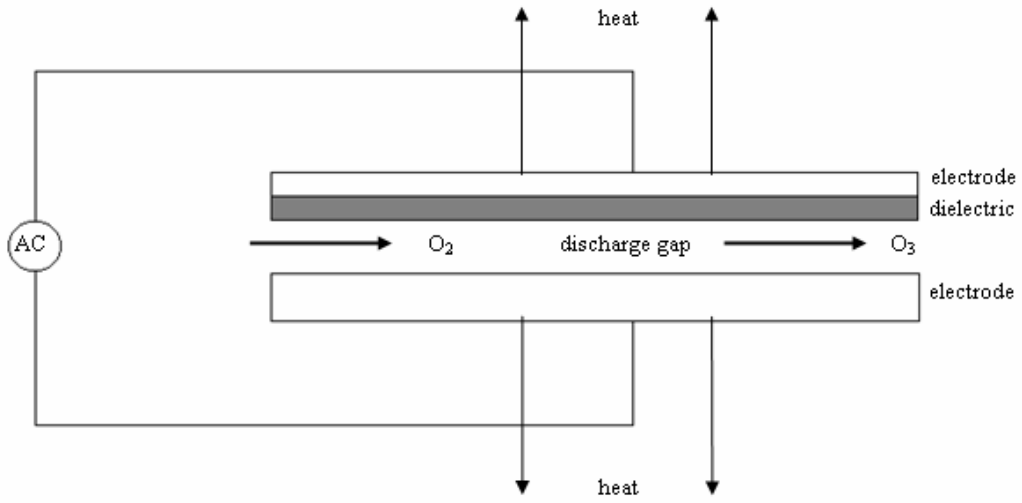


Figure 6.1: Schematic diagram of the dielectric discharge cell used for ozone production.

Oxygen atoms are the intermediates in the formation and destruction reaction of ozone. The dielectric discharge cell comprises two electrodes separated by a dielectric and gas-filled gap. An AC voltage (220V) is applied to the cell and this induces the formation of ionized gas in the gap.

The Ozone Unit: Sample ozonation was achieved in an ozone unit comprising the ozone generator with 1/8 inch stainless steel tubing transporting the ozone to the valve V_1 (Figure 6.2). The remaining tubing comprised 1/8 inch PTFE tubing.

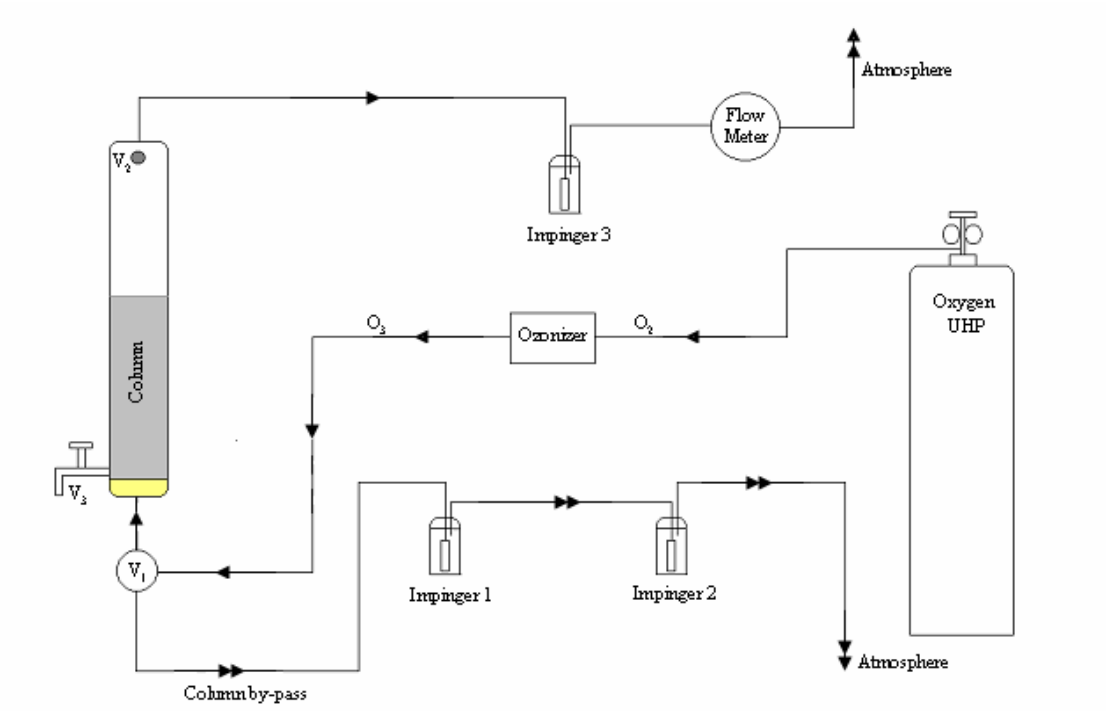


Figure 6.2: Schematic diagram of the ozone generation unit.

A cylindrical polyethylene column (20 L) was used to contain the sample during ozonation. Excess ozone from the column was directed into a 250 mL ozone scrubber (Impinger 3) containing a solution of 2% KI, connected in series with the column. Another two ozone scrubbers (Impingers 1 and 2) were used when the three-way valve was switched to the column by-pass mode during sample removal or loading.

GPC: Sample analysis was conducted on a Waters Alliance 2695 and under operating conditions as described in Section 2.5.1.

6.3.2 Reagents

Starch Indicator: Preparation of the starch indicator is described in Section 4.3.2.

Standard Potassium Iodate, 0.1 N: Preparation of the KIO_3 primary standard is described in Section 4.3.2.

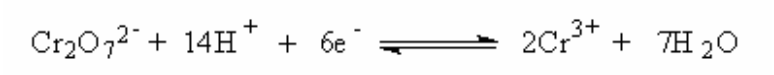
Sodium Thiosulfate, ca 0.1 N: The $Na_2S_2O_3.5H_2O$ was prepared as described in Section 4.3.2.

Standardization of $Na_2S_2O_3$: To 80 mL Milli-Q water was added 1 mL of conc. H_2SO_4, 10 mL of standard 0.1 N KIO_3 and 1 g of KI. The solution was titrated with *ca* 0.1 N $Na_2S_2O_3$ until a pale yellow color was observed. At this point, a few drops of starch indicator were added and the solution turned bluish black. Titrant was added until the solution became colorless.

Standard Potassium Dichromate, 0.01 N: The standardization of *ca* 0.01 N $Na_2S_2O_3$ was also carried out with 0.01 N $K_2Cr_2O_7$, (previously oven dried at 105 ^oC and

desiccated) to confirm the results and calculations obtained with KIO_3. The mass $K_2Cr_2O_7$ required for the preparation of the standard solution was calculated using equations 6.1 and 6.2. The $Na_2S_2O_3$ prepared previously (*ca* 0.1 N) was diluted 10 times and then standardized.

For $K_2Cr_2O_7$, the half reaction is



1 mol $K_2Cr_2O_7$ produces 6 molar equivalents of electrons

$$equivalent\ Mass_{K_2Cr_2O_7} = \frac{Molecular\ Mass}{6} \qquad 6.1$$
$$= \frac{294.191}{6}$$
$$= 49.430$$

$$N_{K_2Cr_2O_7} = \frac{Mass_{K_2Cr_2O_7}}{equivalent\ Mass_{K_2Cr_2O_7}} \times \frac{1000}{Volume} \qquad 6.2$$
$$Mass_{K_2Cr_2O_7} = \frac{0.01 \times 49.430 \times 1000}{1000}$$
$$= 0.494\ g$$

An accurate mass of 0.494 g $K_2Cr_2O_7$ was dissolved in ozone-demand-free water and diluted to 1000 mL.

Standardization: To 80 mL of Milli-Q water was added 1 mL conc. H_2SO_4, 10 mL of standard $K_2Cr_2O_7$ and 1 g KI. The reaction mixture was stored in a dark cupboard for 6 min and then titrated with $S_2O_3^{2-}$.

Sample Ozonation: A 4 L volume of 0.5% Z553D prepared in Milli-Q water, and neutral pH, was ozonated for exactly 40 min in the ozonation unit. Aliquots of 50 mL were removed for the titrimetric determination of the ozone dosage.

6.3.3 Procedure

For purposes of this study, only high concentrations of both polymer and oxidant were used in order to aid identification of reaction products. It must be noted that limitations of high reactant concentrations were placed on the ozone experiment owing to the fact that there are no reliable analytical techniques for the analysis of polyDADMAC and its degradation products. Additional ozone studies were intended to be investigated using simulated water treatment conditions (1 to 2 mg L^{-1}ozone) once all polymer-ozone reaction products were successfully determined and suitable analytical methods developed.

The detailed steps involved in sample ozonation, from calibration of the ozone generator, to standardization of $Na_2S_2O_3$, and to ozone dosage determination, are described in Appendix 2. Once the ozone dosage was determined satisfactorily, an

aliquot of the ozonated sample was removed for GPC analysis. For the purposes of comparison a reference solution (unozonated sample) was analyzed together with the test sample.

6.4 Results and Discussion

The GPC chromatogram (Figure 6.3) shows a very distinct change in the polymer peak profile.

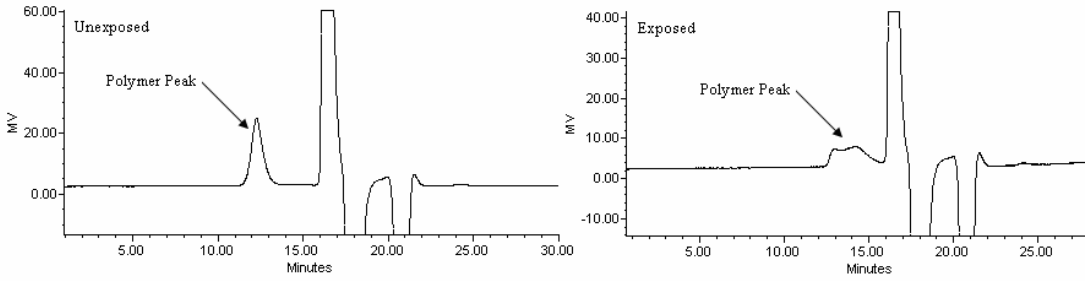


Figure 6.3: GPC RI of Z553D before and after ozone exposure for 40 min.

Ozone is known to be selective in the sense that it almost never attacks saturated centers [Hendrickson et al., 1970]. It is useful however in the cleavage of a molecule specifically at its double bond. PolyDADMAC has no sites of unsaturation that are likely to be attacked to form carbonyl compounds. These GPC results therefore present a challenge in explaining the observed phenomenon. Some important questions that arise include whether there was formation of any carbonyl compounds or whether the polymer itself was slightly altered. The GPC trace shows no evidence of the formation of any small molecular weight species. This however cannot be used to make any conclusions as the GPC method is a low sensitivity, low resolution method. The GPC evidence does however suggest that the polymer, almost in its entirety, is converted to a lower molecular weight product as there is a shift in the peak retention time from 12.5 min to a broad distribution from 12.5 to 15 min. This suggests that the long polymer chains were cleaved to form smaller chains. Attempts were therefore made to measure the difference in levels of total organic carbon (TOC) and total Kjeldahl nitrogen (TKN) of the ozonated and unozonated samples as part of the investigation of polymer degradation. A decrease in TOC would indicate loss of organic carbon (destruction of the carbon skeleton) by oxidation to CO_2 and a decrease in TKN would suggest a loss of NO_2 (destruction of the pyrrolidine ring). Unfortunately the results from the two tests produced similar results. Subsequent tests indicated that the polymer matrix was not included in the scope of the TOC and TKN methods. These methods were applicable exclusively to water and wastewater analysis.

The polymer degradation study was conducted at a high ozone dose of 76 mg L^{-1} (first trial of a series of experiments). Since such doses are unlikely to be used in water treatment this extreme was selected purely as an initial assessment. However it must be noted that low ozone dosages and long contact times may have an equal if not more prominent effect on the polymer. The ozone generation unit (Figure 6.2) was not available for further work but the following is recommended as a continuation of the investigation on ozonation.

- To test the ozonated product in water treatability experiments
- To use typical water treatment dosages, followed by GPC analysis
- To establish if N is lost as NO_2 during ozonation by modifying the TKN method
- To establish if CO_2 is lost during ozonation by modifying the TOC method
- To test for the formation of any carbonyl compounds by UV, measuring UV absorption at 254 nm.

Results of these studies would provide more information on the nature and extent of the polymer degradation observed by GPC.

6.5 Conclusions

This study indicated that polyDADMAC undergoes a significant molecular weight change during ozonation. Although a high ozone dosage was used on concentrated polymer solutions, the contact time was relatively short. This was done intentionally to allow for monitoring by GPC. There is a strong possibility that the effect of ozone on more dilute polymer solutions may be far more prominent. GPC evidence suggests that the polymer is degraded to a lower molecular weight product by cleavage of long polymer chains to form shorter ones. Loss of one link in a long chain was not able to be quantified by alternative means. This may imply that there would be a corresponding impact on the product performance in water treatment. Due to the unavailability of the equipment, the study remains incomplete. Although GPC provided adequate evidence of polymer transformation, supporting evidence as recommended is required for final remarks on ozonation.

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CHAPTER SEVEN

REVIEW OF ANALYTICAL METHODS

FOR POLYDADMAC ANALYSIS

7.1 Introduction

There are a number of published methods describing the quantitative determination of polymers. These include the techniques polarography [Parazak et al., 1987], chromatography [Wee, 1984], potentiometry [Christopoulos et al., 1982], two-phased titration [Tsubouchi et al., 1981], colloidal titration [Wang and Shuster, 1975], extraction-spectroscopy [Kawase and Yamanaka, 1979] and flow injection analysis [Toei and Zaitzu, 1985]. In this study, several of these methods were evaluated for the analysis of polyDADMAC and found to suffer from severe limitations in terms of achieving good linearity, accuracy, precision and detection limits. The methods were also complicated, very labor intensive, expensive, and at times produced false results due to matrix effects. Six analytical techniques were assessed:

- complex formation-spectroscopy
- colloid titration
- ultrafiltration-GPC
- indirect UV detection
- microscopy
- solid phase extraction.

These techniques covered a total of nine individual methods:

- the tannic acid method
- the methyl orange method
- the rose bengal method
- colloid titrations
- the direct binary complex (HACH) method
- indirect detection with acetone
- indirect detection with K_2CrO_4
- electron microscopy
- SPE with C_{18} and tC_{18}.

The methods were selected based on the availability of the required equipment, the reagents and the practicality of the method for routine analysis. Methods involving techniques that were deemed old and outdated were not included. At all times consideration was given to the fact that a detection method was required that could be easily applied and that produced reliable results. The more commonly used methods including complex formation-spectroscopy (the tannic acid method, the rose bengal method, the methyl orange method), and colloid titration (the PVSK method) were extensively evaluated. Two novel methods involving sample pre-concentration by membrane filtration GPC and solid phase extraction (SPE) were developed and tested. Novel methods involving complex formation (the HACH method) and GPC with indirect UV detection were also investigated. Some preliminary work was also

conducted on electron microscopy during the studies of polymer complex formation with anionic dyes.

7.2 Analysis of PolyDADMAC by Various Methods

7.2.1 The Tannic Acid Method

Tannic acid (TA) is a colloidal suspension of negatively charged particles. It is capable of flocculating positively charged polymers by forming ionic bonds and nonionic polymers by forming hydrogen bonds. During floc formation the percentage transmittance (%T) of the solution decreases with increasing amounts of polymer. This is measured spectrophotometrically at 554 or 830 nm [Hanasaki et al., 1985]. In this study attempts were made to set up a method for the quantitative determination of polyDADMAC in solution.

7.2.1.1 Instrumentation

Spectrophotometry: All UV/VIS analyses were followed spectrophotometrically using a Varian Cary 50 UV/VIS spectrophotometer. The instrument was operated in the visible region and absorbance readings were recorded at 553 nm. A glass cuvette with a 50 mm path length was used.

7.2.1.2 Reagents

Tannic Acid Solution: TA (0.1 g) was dissolved in 100 mL of Milli-Q water.

Stock Standard, 100 mg L^{-1}: A stock solution was prepared by diluting 10 mg polyDADMAC in 100 mL of Milli-Q water.

Calibration Standards: PolyDADMAC standards were prepared by diluting a 100 mg L^{-1} stock solution as per Table 7.1. Fortified samples of tap and Milli-Q water were prepared together with the standards.

Table 7.1: Preparation of standards and samples of polyDADMAC for assessment of the TA method.

Sample ID	Volume Stock (mL)	Total Volume (mL)	Polymer Conc. (mg L^{-1})
Std 1	1	100	1
Std 2	2	100	2
Std 3	5	100	5
Std 4	10	100	10
Blank	0	100	0
Tap Spike	2	100	2
Milli-Q Spike	2	100	2

7.2.1.3 Sample Pretreatment Procedure

To 50 mL of polymer standard was added 5 mL of TA reagent and the mixture stirred for 1 min on a magnetic stirrer. The solution was allowed to stand for 1 h and then its

absorbance measured by UV/VIS spectrometry at 550 and 830 nm. The solutions were also scanned to establish the wavelengths of optimum transmittance.

7.2.1.4 Results and Discussion

The TA complexation method was optimized. Good linearity of the calibration functions could however not be achieved and significant fluctuations in the accuracies of fortified tap water (137%) and Milli-Q water (104%) were noted (Appendix 2). The amount of TA added to the polymer samples had a major influence of the magnitude of the %T for each of the calibration solutions.

7.2.2 The Rose Bengal Method

Cationic polyelectrolytes such as polyDADMAC form a 1:1 complex with anionic dyes such as rose bengal. The resulting polymer-dye complex can be precipitated by centrifugation [Dey and Palit, 1968]. This phenomenon was utilized in an attempt to develop a method for the quantitative estimation of the polymer concentration in aqueous solutions.

7.2.2.1 Instrumentation

Spectrophotometry: All absorption measurements were recorded on a Varian UV/VIS spectrophotometer as described in Section 7.2.1.1. The instrument was operated in the visible region and absorbance readings were recorded at 640 nm. A glass cuvette with a 50 mm path length was used.

7.2.2.2 Reagents

Rose Bengal Solution: Rose bengal was prepared by adding a few crystals of the dye into Milli-Q water to give an absorbance of *ca* 1 AU. A pH 9 buffer pillow was added and the solution diluted to 100 mL with Milli-Q water. The absorption from 800 to 400 nm was measured.

Polymer-Dye Complex: To a 50 mL aliquot of the dye was added 1 mL of 1% (m/v) polymer solution. The resulting solution was scanned from 800 to 400 nm.

Polymer-Dye Complex, Centrifuged: To 1 mL of 1% (m/v) polymer in a centrifuge tube was added 10 mL of rose bengal. The solution was centrifuged at 300 rpm for 15 min. The supernatant was filtered through a 0.45 μm filter and then scanned from 800 to 400 nm.

The Control: To 1 mL of Milli-Q water in a centrifuge tube was added 10 mL of rose bengal. The solution was centrifuged for 15 min at 300 rpm. The supernatant was filtered through a 0.45 μm filter and then scanned from 800 to 400 nm.

Calibration Standards: **S**tandards of polyDADMAC ranging from 1 to 5 mg L^{-1} were prepared in Milli-Q water from a 100 mg L^{-1} stock solution. A Milli-Q blank and a fortified tap water sample was also prepared. The dilutions are shown in Table 7.2.

Table 7.2: Preparation of polyDADMAC calibration standards for testing of the rose bengal method.

Sample ID	Volume Stock (mL)	Final Volume (mL)	PolyDADMAC Conc. (mg L^{-1})
Std 1	1	100	1
Std 2	2	100	2
Std 3	3	100	3
Std 4	4	100	4
Std 5	5	100	5
Milli-Q spike	3.5	100	3.5

7.2.2.3 Sample Pretreatment Procedure

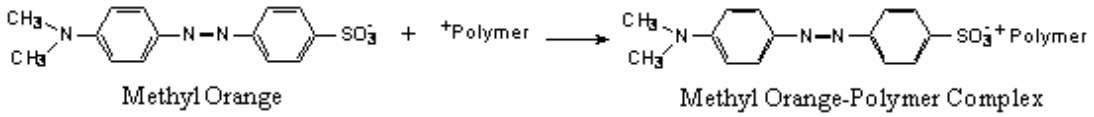
All samples, standards and control solutions (1 mL) were treated with 10 mL of rose bengal in a centrifuge tube. The solutions were centrifuged at 300 rpm for 15 min. The supernatant was filtered through a 0.45 μm filter prior to recording absorbance measurements.

7.2.2.4 Results and Discussion

The rose bengal method was not very successful (Appendix 4). Problems of poor linearity were experienced. Furthermore, the magnitude of difference in intensity of the calibration solutions was negligible and there was some doubt that the complex formation was occurring.

7.2.3 The Methyl Orange Method

The principle of this method is similar to that of the rose bengal and TA methods involving complexation. Methyl orange (MO) is a negatively charged dye that forms a 1:1 complex with cationic polymers according to the reaction in Scheme 7.1.



Scheme 7.1: Ion association reaction of MO with a cationic polymer.

This reaction was exploited in an attempt to quantitatively determine the amount of polymer in water samples. A decrease in the intensity of the MO solution was predicted from the above reaction as a result of complexation in the presence of polymers. Hence, in a solution of MO, there would be a decrease in the absorbance with a corresponding increase in the amount of polymer added.

7.2.3.1 Instrumentation

Spectrophotometry: The UV/VIS scans of each MO-polymer solution were acquired on a Varian UV/VIS spectrophotometer described in Section 7.2.1.1.

7.2.3.2 Reagents

Methyl Orange Stock, 500 mg L^{-1}: A stock solution of MO was prepared by dissolving 0.25 g of the sodium salt of MO and 7.6 mL of HCl (0.1 N) in 500 mL of Milli-Q water.

Calibration Standards: The stock solution was diluted as per Table 7.3 for the preparation of the calibration standards.

Table 7.3: Preparation of MO standards for absorption experiments using a 500 mg L^{-1} stock solution.

Standard ID	Volume Stock (mL)	Total Volume (mL)	MO Conc. (mg L^{-1})
Std 1	1	100	5
Std 2	2	100	10
Std 3	4	100	20
Std 4	8	100	40
Std 5	10	100	50

An intermediate range of MO standards was prepared by diluting the 20 mg L^{-1} standard as per Table 7.4.

Table 7.4: Preparation of an intermediate range of MO standards for absorption experiments using a 20 mg L^{-1} stock solution.

Standard ID	Volume Stock (mL)	Total Volume (mL)	MO Conc. (mg L^{-1})
Std 1	1	100	0.2
Std 2	2	100	0.4
Std 3	4	100	0.8
Std 4	8	100	1.6
Std 5	10	100	2.0

A low range of MO standards was prepared by diluting the 5 mg L^{-1} standard as per Table 7.5.

Table 7.5: Preparation of a low range MO standards for absorption experiments using a 5 mg L^{-1} stock solution.

Standard ID	Volume Stock (mL)	Total Volume (mL)	MO Conc. (mg L^{-1})
Std 1	1	100	0.05
Std 2	2	100	0.10
Std 3	4	100	0.20
Std 4	8	100	0.40
Std 5	10	100	0.50

MO-Polymer Standards: A 2 mg L^{-1} solution of MO was selected as the optimum concentration for the preparation of the polymer-MO standards. To 10 mL of MO in each of five test tubes, polyDADMAC (5000 mg L^{-1}) was spiked as per Table 7.6.

Table 7.6: Preparation of MO-polymer standards.

Sample ID	Volume Polymer (mL)	Total Volume (mL)	Polymer Conc. (mg L^{-1})
Std 1	1	20	250
Std 2	2	20	500
Std 3	3	20	750
Std 4	4	20	1000
Std 5	5	20	1250

The standards were filtered as described previously, followed by spectrophotometric analysis at 465 nm.

Conductivity Measurements: Four samples were prepared for conductivity experiments by dilution of stock polymer solutions as per Table 7.7. The 2 mg L^{-1} MO solution was used for the complexation.

Table 7.7: Preparation of MO-polymer samples for conductivity experiments.

Sample ID	Volume Stock (mL)	Volume MO (mL)	Final Volume (mL)	Polymer-MO Conc. (mg L^{-1})
Test*	25	10	50	500
Control*	25	0	50	500
Test**	25	10	50	5000
Control**	25	0	50	5000

**prepared from 1000 mg L^{-1} polymer stock*
***prepared from 10000 mg L^{-1} polymer stock*

UV/VIS Absorption Spectra: Two samples were prepared for the determination of the UV/VIS absorption spectral differences between the MO and the MO-polymer complex (Table 7.8). The 1000 mg L^{-1} stock solution was used.

Table 7.8: Preparation of MO-polymer samples for UV/VIS scans.

Sample ID	Volume Polymer (mL)	Volume MO (mL)	Final Volume (mL)	Polymer-MO Conc. (mg L^{-1})
Test	3	10	50	60
Control	0	10	50	0

7.2.3.3 Procedure

The concentration of MO was first optimized by measuring the absorbance profiles of the standards in Tables 7.3 to 7.5. This concentration of the dye was then used in all complexation studies with the test polymer. All samples were filtered through 0.45 µm filters.

7.2.3.4 Results and Discussion

Complexation of polyDADMAC with MO produced poor results and could not be used for quantitative analysis despite extensive method optimization (Appendix 5). After a lengthy investigation, the problem of poor linearity and low absorbance of the calibration solutions were found to persist. This resulted in calibration functions with nearly zero slopes. The method was extended to chromatographic analysis of the MO-polymer complex. Although there was evidence of complex formation, it was not in a sufficiently stable form for GPC. The possibility also existed that since MO has both a sulfate group and a tertiary amine group it could be quaternized by the sulfate or swap an H^+ with polyDADMAC. This would result in little change on complexation with polyDADMAC.

7.2.4 The Direct Binary Complex Method

The next method tested was supplied by HACH, USA and recommended for the analysis and quantification of quaternary ammonium compounds (QACs). There are however no reports available on whether it was or could be used for polymers. This is therefore a novel approach that was tested for the measurement of polyDADMAC polymers. It involved the spectrophotometric analysis of the QACs after treatment with two reagents (sodium citrate, HACH Reagent 1 and citric acid, HACH Reagent 2). In the presence of QACs a reaction takes place with an indicator, resulting in a color change from pale pink to vivid purple. Since the test polymer is in fact a QAC, it was hoped that the technique could be extended to include polymers. No further details of the reagents or the reactions were available from HACH and there were also no reports of the method being used for the analysis of polymers.

7.2.4.1. Instrumentation

Spectrophotometry: A Varian UV/VIS spectrophotometer as described in Section 7.2.1.1 was used for sample analysis.

7.2.4.2 Reagents

HACH Reagent 1: Sodium citrate was supplied by HACH in the form of powder pillows.

HACH Reagent 2: Citric acid was supplied by HACH in the form of powder pillows.

Polymer Stock Solution, 15 mg L^{-1}: A polymer stock solution was prepared by diluting 15 mg polymer in 1000 mL of Milli-Q water.

Calibration Standards: The calibration standards were prepared by diluting the stock solution as per Table 7.9.

Table 7.9: Preparation of polyDADMAC standards for a trial run using the HACH method.

Sample ID	Volume Stock (mL)	Final Volume (mL)	Polymer Conc. (mg L^{-1})
Std 1	0	25	0
Std 2	2	25	1.2
Std 3	10	25	6.00

A full set of calibration standards was prepared to obtain data for the preparation of a calibration function. Dilutions were made from the stock solution as per Table 7.10. A calibration check (Cal Chk) sample, fortified tap water, and a Milli-Q water blank were also used, together with the standards.

Table 7.10: Preparation of polyDADMAC calibration standards for use with the HACH method.

Sample ID	Volume Stock (mL)	Final Volume (mL)	Polymer Conc. (mg L^{-1})
1	1	25	0.60
2	2	25	1.20
3	4	25	2.40
4	8	25	4.80
5	10	25	6.00
Cal Chk	5	25	3.00
Tap Spike	10	25	6.00
Blank	0	25	0

7.2.4.3 Procedure

To each 25 mL volumetric flask containing the standard, Cal Chk, fortified test sample and blank was added a HACH Reagent 1 powder pillow. The flasks were swirled gently to dissolve the powder. This was followed by the addition of a HACH Reagent 2 powder pillow. The flasks were swirled gently to dissolve the reagent. The reagents were allowed to react for 2 min before absorbance measurements were taken at 575 nm.

7.2.4.4 Sampling and Sample Preparation

Sludge: A sludge sample was collected by scraping residues from the walls of the canal leading to the pulsator clarifier at the Durban Heights Water Treatment Works (DHWTW). The sludge was stored in a 500 mL plastic honey jar. A mass of 28 g was extracted with 100 mL of Milli-Q water by stirring on a magnetic stirrer for 15 min. The resulting sludge-water mixture was then filtered through glass wool, followed by a second stage of filtration through Whatman 41 glass fiber filter paper. The final filtration was carried out using 0.45 μm filters. This was followed by pH adjustment to pH 6 followed by titration using the PVSK method.

Scum: A scum sample was taken by sieving the floating masses from the surface water of the sand filters. A 100 mL aliquot was passed through glass wool, followed

by filtration through glass fiber filter paper. The final filtration was carried out using 0.45 μm filters.

Fortified Scum: A second 100 mL aliquot of the scum was filtered and spiked with 500 μg of polymer.

Quality Control: A quality control (QC) sample spiked with 500 μg of polymer was used.

Blank: Milli-Q water (100 mL) was used as the blank and analyzed by the PVSK method.

The Reference Polymer: A reference solution (0.1% Z553D in Milli-Q water) for the case study was prepared using polymer sampled from the storage tanks at the DHWTW.

7.2.4.5 Results and Discussion

The direct binary complex method, referred to as the HACH method, because of the use of special reagents supplied by HACH, is a novel method for polyDADMAC analysis. There are no reports of it being used for polymer analysis. The method was developed and validated successfully (Appendix 6). It was found to give excellent linearity, good precision, good accuracy, and the calibrations were valid for over two weeks. The method was rugged, easy to use, and was successfully applied for the analysis of samples from DHWTW.

7.2.5 Indirect UV Detection

The main challenge posed by the polymer under investigation is that it is non-UV absorbing and hence is only amenable to the RI mode detection. This being the case, the limitation of the method is that of low sensitivity, as the RI detector is known to be a non-selective general purpose detector. The approach employed to address this problem included sample pre-concentration (by membrane filtration and solid phase extraction) followed by GPC with RI detection and chemical modification followed by GPC and PDA UV detection.

The objective of this experiment was to develop a method based on indirect UV detection for polymer analysis. The motivation for this was because attempts to tag the polymer with a UV active agent (MO) were unsuccessful. A reverse strategy was therefore considered. The principle of operation of the technique requires a suitable chromophoric group which, when added to the mobile phase, provides a high background absorbance. When non absorbing species such as the test polymer pass through the detector, a reduction in the background absorbance occurs, which appear as an inverted or negative peak. By reversing the detector polarity this can be reflected on the positive axis as a normal chromatographic peak, and can be used for quantitative analysis.

7.2.5.1 Instrumentation

GPC: Sample analysis was conducted on the Alliance 2695 system as described in Section 2.4.1. The RI detector was replaced with a photodiode array UV detector (Waters PDA 996). The detector polarity was reversed and the scan range was from 400 to 200 nm.

7.2.5.2 Reagents

Mobile Phase 1: KH_2PO_4 (0.25 M) containing 0.1% (v/v) acetone was used as the mobile phase. The mobile phase was filtered and degassed as for previous GPC work.

Mobile Phase 2: To 2L KH_2PO_4 (0.25 M) mobile phase was added 10 mM Na_2CrO_4 (4.68 g). The mobile phase was filtered and degassed as for previous GPC work.

Polymer Reference: Z553D at a concentration of 0.5% (m/v) in Milli-Q water was used to test the sensitivity of the indirect UV detection method. The injection volumes ranged from 100 to 1 μL. A similar concentration of polymer was used for comparison by RI detection.

7.2.5.3 Results and Discussion

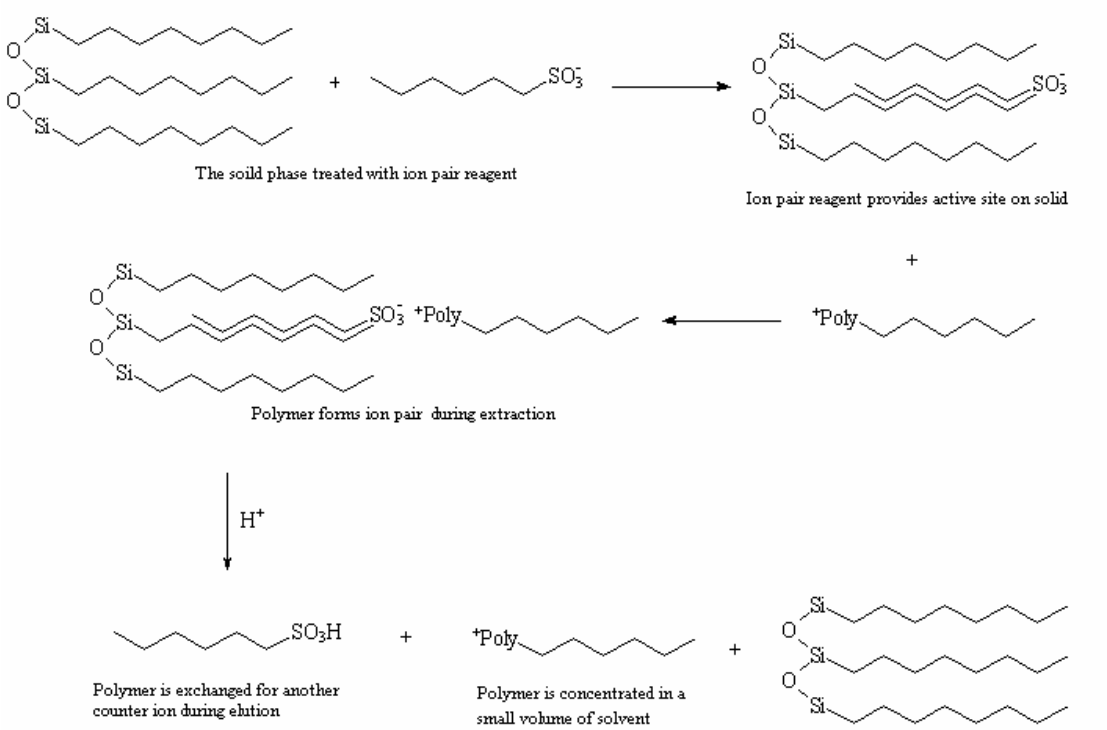
Indirect UV detection was the second novel method successfully developed for polymer analysis, although it proved to be of limited value for residual polymer analysis (Appendix 7). Acetone, as the added chromophore, was found to be unstable and produced erratic results. The second chromophore, Na_2CrO_4, was found to be a better alternative than acetone, but even so, the method of indirect UV detection was less sensitive than RI detection. The method had great potential for use in polymer analysis and could be the method of choice for polymer analysis. The search for a more sensitive chromophore is ongoing, and further experimentation ought to look into modifying the polymer to be either UV detectable; e.g. detectable by the HACH procedure, or by tagging with a fluorescent reagent such as fluorescein-5-isothiocyanate (FITC).

7.2.6 Solid Phase Extraction of PolyDADMAC

In the quest for a sensitive method for residual polymer analysis, numerous techniques were considered. Solid-phase extraction is a popular sample enrichment technique that has numerous advantages over the more traditional techniques such as liquid-liquid solvent extractions. The basic principle of the technique involves activating the solid phase material followed by passing a known volume of the sample to be extracted through the activated solid. Analytes interact with the solid and are retained in the activated solid while, simultaneously, the solvent and other non-target matrix species pass through unhindered. The trapped components are then eluted in a small volume of a suitable solvent. Typical sample enrichment factors of 1000 times are achieved.

The test polymer under study, namely polyDADMAC, belongs to the chemical class of quaternary ammonium compounds. It is well known that some ordinary QACs (biocides such as the dialkyl dimethyl ammonium chlorides) may be extracted from

aqueous samples by chemically modifying C_{18} or C_8 extraction media by a technique called paired ion chromatography [Hodgeson et al., 1992]. This is yet another novel technique that was investigated for the potential extraction of polymer from dilute aqueous solutions, according to the proposed mechanism in Scheme 7.2.



Scheme 7.2: Extraction mechanism of polymers from water by paired ion chromatography.

The scheme uses C_8 instead of C_{18} chains purely for simplicity of the drawings. The solid phase was treated as per EPA Method 549.1 prior to sample extraction [Hodgeson et al., 1992].

7.2.6.1 Instrumentation

GPC: Sample analysis was conducted on the Alliance 2696 system as described in Section 2.4.1.

7.2.6.2 Reagents

Extraction Material: The extraction material was a C_{18} column, commercially sold in a cartridge type configuration called a Sep Pak (Waters). Experiments were conducted on the standard mono-functional C_{18} as well as the higher capacity tri-functionally bonded tC_{18} phase for improving polymer recoveries.

Samples: Polymer samples (Z553D) for extraction were prepared in concentrations of 2, 5 and 50 mg L^{-1} in Milli-Q water.

7.2.6.3 Extraction Procedure

The solid phase was activated as per EPA Method 549.1 [Hodgeson et al., 1992]. This was followed by sample extraction, elution and analysis by GPC RI.

7.2.6.4 Results and Discussion

C_{18} and tC_{18} solid phase extraction of Z553D had limited success (Appendix 8). The mechanism of paired ion chromatography can be used for the extraction of polymers, however low or even negligible recoveries were obtained with normal C_{18} phase. Improved recoveries were achieved with the tC_{18} phase but even so they were unacceptably low for quantitative analysis. The method does have potential and it is recommended that future studies investigate the impact of other available ion pairing reagents for a stronger polymer-solid phase interaction. Replacing hexane sulfonic acid with C_{18} sulfonic acid may be a useful starting point. The latter has a longer carbon chain and would interact to a greater extent with the C_{18} or tC_{18} thereby minimizing breakthrough of components pairing with this reagent.

It must be noted that apart for the complexation of cationic polymers with TA, rose bengal and MO, no material or publications on the remaining work (Sections 7.2.3; 7.2.4; 7.2.5; 7.2.6) were available. They were novel ideas that were thought to be useful in polymer analysis and therefore the investigations were very time consuming with numerous problems being encountered in the process. The PVSK and membrane filtration methods mentioned in the introductory chapter are discussed in detail in Chapters 8 and 9 respectively.

7.2.7 Electron Microscopy

The objectives of this study were to:

- Determine the physical architecture and size of the polymer.
- Determine the effect of complexation agents on the polymer structure.
- Determine the charged centers and their interaction with oppositely charged species.
- Establish whether electron microscopy could be applied as a qualitative tool in the determination of polymer residues in final treated water.

7.2.7.1 Experimental

A commercially available polyDADMAC solution (Z553D) was submitted to the University of KwaZulu-Natal for analysis. Droplets of the polymer were placed on a Formvar copper grid for 30 s. The liquid was then drawn onto a Whatman filter paper and viewed with an electron microscope (Phillips CM120 Biotwin) operated at 80 kV. The samples were diluted at random to obtain the best transmission electron micrographs.

7.2.7.2 Results and Discussion

The TEM images of polyDADMAC at different levels of dilution are shown in Micrographs A to D below (Figure 7.1).

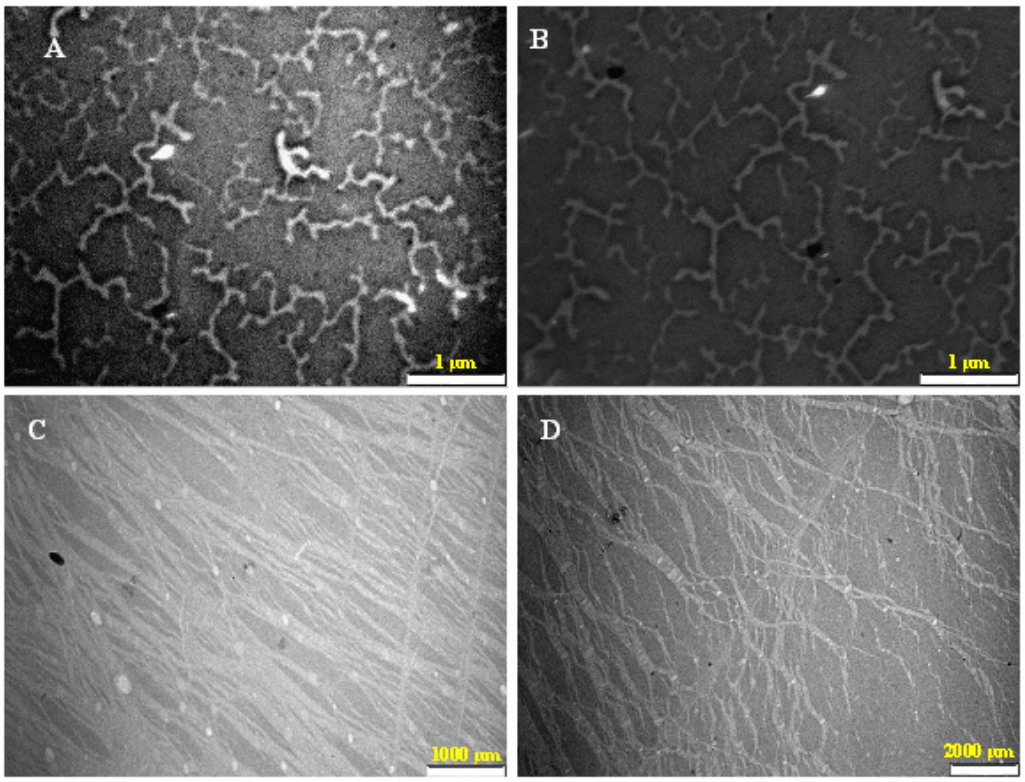


Figure 7.1: TEM images of Z553D at different levels of dilution.

Polymer complexes can be seen. Micrographs A and B are dilute solutions at high magnification (18 000 times) whilst C and D are more concentrated at slightly lower magnification (14 000 and 6000 times respectively). Magnified views of the images are shown in Appendix 9.

Using TEM images A to D, the chain width was measured to be 1.5 Å and the length from 100 to 300 Å in coiled form. The investigation into the nature of the polymer dye complex by TEM was not pursued any further than the initial study since complexation-spectroscopy methods of analysis (TA, rose bengal, MO) were unsuccessful for polyDADMAC determination.

7.3 Conclusions

A total of nine individual methods were investigated and extensively validated. The three methods involving complex formation (tannic acid, rose bengal, and methyl orange) were found to be totally unreliable and of little use in polyDADMAC analysis. The magnitude of the absorbance values of the calibration solutions were negligible and resulted in poor linearity. The HACH method was developed and validated successfully. Excellent linearity, good precision and good accuracy was achieved, and the calibrations were valid for over two weeks. The application of the method was demonstrated on real water samples. Indirect UV detection was

established as a method with great potential for polymer analysis but a chromophore with better sensitivity than Na_2CrO_4 is yet to be found. Similarly solid phase extraction was found to be partially successful and future studies ought to focus on the evaluation of long chain ion pair reagents such as C_{18} sulfonic acid. Finally, from appropriate dilutions and magnification of Z553D, good quality TEM images of the polymer were obtained. The images were used to measure the chain width and chain length of the polymer molecule. Furthermore, the molecular architecture of polyDADMAC in aqueous solution was established from the images.

7.4 References

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CHAPTER EIGHT

QUANTITATIVE DETERMINATION OF POLYDADMAC

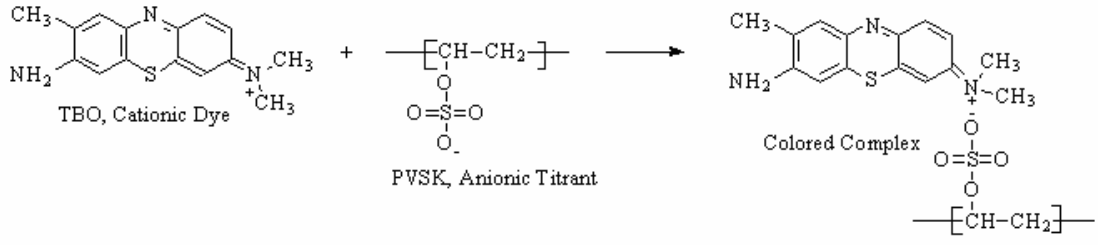
BY THE COLLOID TITRATION METHOD

8.1 Introduction

This chapter describes the development and application of the colloid titration method for the quantitative determination of residues of the water treatment chemical polydiallyldimethylammonium chloride (polyDADMAC) in water, sludge and scum samples. In contrast to the work of previous researchers [Wang and Shuster, 1975] this study focuses on the direct determination of the polyDADMAC content in samples by the use of an external standard calibration with reference standards prepared from the polyDADMAC dosing solution rather than quantification based on the cationic polymer 1,5-dimethyl-1,5-diazaundecamethylene polymethobromide (DDPM). Furthermore, the titration was followed by spectrophotometry and the end point determined by the Tangents Method as opposed to visual observation of a color change. The method showed accuracies of 85% and 95% for tap and Milli-Q water respectively, each fortified at a concentration of 1250 μg L^{-1}. Precision for fortified tap water was found to be 8% and the limit of detection estimated at 300 μg L^{-1} polyDADMAC, with the assumption that the dosing solution used as the reference standard was 100% (m/v). The method performance was excellent in Milli-Q water however some difficulty was experienced when applied to raw water, sludge and scum samples taken at the DHWTW. A discussion on the possible reporting of false results is presented and suggestions are made with respect to the interpretation of results. Some advantages and disadvantages of the method are highlighted and recommendations are made for the application of this method.

8.2 Background and Theory

The colloid titration method is one way of estimating the net charge density of polymers, surfaces and colloidal materials in aqueous mixtures. The capacity of the mixture to adsorb a polymer of opposite net charge is measured. With the test compound polyDADMAC, the mixture is first treated with a small amount of a cationic dye such as toluidine blue-O (TBO). The blue solution containing the cationic polyDADMAC is then titrated with an anionic polymer such as potassium polyvinyl sulfate (PVSK). The PVSK is preferentially adsorbed onto the polyDADMAC until all the reactive sites are occupied. The excess PVSK then complexes the cationic dye to form a purple-pink end point according to Scheme 8.1.



Scheme 8.1: The complex formation reaction of PVSK with TBO.

The color intensity of the free dye is measured spectrophotometrically at 640 nm. In the presence of polyDADMAC the concentration of the free dye remains unchanged with each successive addition of titrant (PVSK). The absorption of radiation at 640 nm therefore remains unchanged. Once all the polyDADMAC active sites have been exhausted, the TBO dye begins to form a complex with the titrant and a decrease in absorbance is noted as a result of the removal of the free dye from solution. This continues until all the free TBO is removed and an excess of titrant remains. The result is an inverted S-shaped titration curve. This study is an extension of the method first proposed by Wang and Shuster [1975] and evaluated by Ross [1976] as well as other researchers [Wang et al., 1976a/b; Wang and Pooler, 1976]. The method described here differs from that of the above researchers in several ways. Firstly, this method follows the course of the complexation reaction and the corresponding color changes spectrophotometrically rather than by visual methods. In addition, the actual polyDADMAC dosing solution from the water treatment works was used as the reference material for the preparation of a calibration curve whereas Wang et al. used DDPM as the reference polymer. Finally, the polymer titration curves obtained were manipulated in an attempt to find a relationship between the amount of polyDADMAC present and the amount of PVSK added for complete complexation. The results were used in linear regression analysis. The performance of the method was critically evaluated in the analysis of spiked Milli-Q water, raw water, tap water, a surface scum and sludge sampled at the DHWTW.

8.3 Experimental

8.3.1 Instrumentation

Spectrophotometry: All titrations were followed spectrophotometrically using a Varian spectrophotometer as described in Section 7.2.1.1. The instrument was operated in the visible region and absorbance readings were recorded at 640 nm. A glass cuvette with a 50 mm path length was used.

8.3.2 Reagents

Stock Standard, 5000 mg L^{-1}: PolyDADMAC reference material was obtained directly from the storage facility at the DHWTW. The polymer was supplied by Zetachem (Durban, South Africa) and marketed under the trade name Z553D. The stock solution was prepared by dissolving 0.25 g of the polymer in 50 mL of Milli-Q water.

Primary Dilution Standard, 50 mg L^{-1}: The above stock solution (5 mL) was diluted to 500 mL in Milli-Q water.

Toluidine Blue-O: The dye was prepared by weighing 0.01 g of toluidine blue-ortho (TBO, Aldrich) in 100 mL of Milli-Q water.

Acetic Acid: Acetic acid (Merck) for pH correction was prepared by diluting 1 mL of concentrated acetic acid to 100 mL in Milli-Q water.

Potassium Polyvinyl Sulfate, 10% (v/v): Potassium polyvinyl sulfate (PVSK, Wako Chemical Company) was prepared by diluting 5 mL of the solution to 50 mL in Mill-Q water.

Polymer Calibration Standards: To each of four 100 mL volumetric flasks was added approximately 50 mL Milli-Q water and 1 mL TBO. Varying amounts of polyDADMAC were added as per Table 8.1 to give standards in the range 0.05 to 0.20 mg of polyDADMAC. The pH was adjusted to 6 after diluting the solutions to volume with Milli-Q water.

Table 8.1: Preparation of polyDADMAC calibration standards and test samples.

Sample ID	Volume Polymer (mL)	Total Volume (mL)	Mass Polymer (mg)
Std 1	1	100	0.050
Std 2	2	100	0.100
Std 3	3	100	0.150
Std 4	4	100	0.200
QC	2.5	100	0.125
Tap Water	0	100	Unknown
Milli-Q Blank	0	100	Unknown

A quality control sample (QC) containing 0.125 mg polymer, a tap water sample and a Milli-Q water blank were prepared together with the calibration standards.

8.3.3 Sample Preparation

Sludge: A sludge sample was collected by scraping residues from the walls of the canal leading to the pulsator clarifier at the Durban Heights Water Treatment Works (DHWTW). The sludge was stored in a 500 mL plastic honey jar. A mass of 28 g was extracted with 100 mL of Milli-Q water by stirring on a magnetic stirrer for 15 min. The resulting sludge-water mixture was then filtered through glass wool followed by a second stage of filtration through Whatman 41 glass fiber filter paper. The final filtration was carried out using 0.45 μm filters. This was followed by pH adjustment to pH 6 and titration using the PVSK method.

Scum: A scum sample was taken by sieving the floating mass from the surface water of the sand filters. A 100 mL aliquot was passed through glass wool, followed by filtration through glass fiber filter paper. The final filtration was carried out using 0.45 μm filters.

Fortified Scum: A second 100 mL aliquot of the scum was filtered and spiked with 500 μg polyDADMAC.

Quality Control: A quality control (QC) sample spiked with 500 μg polyDADMAC was used.

Blank: Milli-Q water (100 mL) was used as the blank and analyzed by the PVSK method.

8.3.4 Analytical Procedure

A 100 mL of the calibration standard was transferred into a beaker and the pH adjusted to 6 with dilute acetic acid or 0.1 M NaOH. TBO (1 mL) was added and the solution stirred for 2 min. The solution was then titrated with additions of 100 µL PVSK and the absorbance values recorded at 640 nm after each addition. The titration was continued until there was a decrease in absorbance and a constant value was reached. A minimum of five readings of constant absorbance were collected. The procedure was repeated for all standards, samples and blank.

8.4 Results and Discussion

8.4.1 The Calibration Curve

The absorbance data of a series of reference standards (0.05 to 0.20 mg) were used to generate titration curves for each of the standards, test samples and blank. The curves in Figure 8.1 show the absorbance profiles of each standard as a function of PVSK addition.

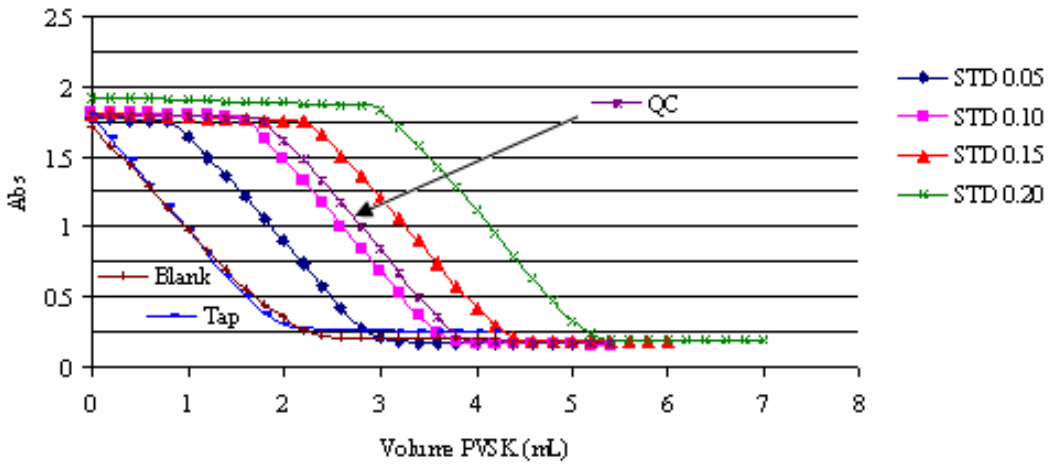
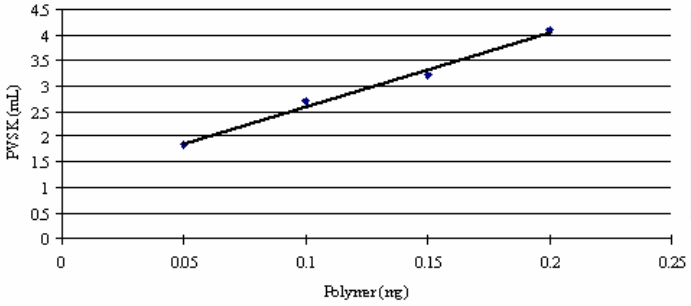


Figure 8.1: Titration curves obtained for polyDADMAC calibration standards, test samples and blank, using the colloid titration method.

There are three distinct absorbance regions visible in the titration curves. The upper plateau region of constant absorbance reflects that region in which the anionic polymer (PVSK titrant) reacts preferentially with the cationic polyDADMAC test polymer. Since the cationic dye (TBO) content remains unchanged at this stage of the reaction there are no changes in the intensity of the color of the solution, which is observed as a constant absorbance at 640 nm until such time that the test polymer has reacted to completion. Further additions of titrant result in the complexation of the TBO to produce the sloping region of the curve. The reduction in absorbance continues with each successive addition of titrant until all the TBO has been consumed. The third region is the lower region of constant absorbance and indicates that all the TBO dye has been complexed, with further titrant additions having no effect on the absorbance

The resulting titration curves were manipulated to determine the end points using the tangents method. This method involves drawing tangents to the upper and lower baselines followed by a diagonal line passing through the data points on the sloping section of the curve. The midpoint of the diagonal line was then established and the corresponding PVSK volume recorded as the end point. This operation was repeated for all standards and samples. The end point volumes were then plotted as a function of polymer mass and used in regression analysis for Calibration 1. The regression line and related statistics (Figure 8.2) show that there is a reasonable line fit with the calibration data.



Regression Statistics		
Multiple R	0.995	
R Square	0.989	
Adjusted R Square	0.984	
Standard Error	0.12	
Observations	4	STDEV
Intercept ± STDEV	1.14	0.15
Slope ± STDEV	14.56	1.08
y = 14.56x + 1.14		

Figure 8.2: Linear regression line and related statistics obtained for Calibration 1.

8.4.2 Method Accuracy

Together with the calibration standards, a quality control (QC) sample containing Milli-Q water fortified with 0.125 mg of polymer and a tap water test sample were analyzed to make an assessment of the method performance. The QC titration curve positioned in the center of the calibration standard curves gave an accuracy of 93%. The curve for tap water shows an immediate decrease in absorbance on addition of titrant. This indicates an absence of polymer residues in the sample and therefore the titrant complexes all free TBO dye present in the sample. The ideal set of curves should have equal starting and ending absorbance values. The only difference should be that the starting absorbance for each standard remains constant during titrant additions until all the polymer active sites have been occupied. Thereafter, there should be a decrease in absorbance by complexation of the free dye. Since no upper plateau region was present in the tap water sample the tangents method could not be applied, and the level of polymer was taken as being below the limit of detection.

Calibration 2 was done with freshly prepared reagents and standards together with a test sample of tap water and a QC sample of Milli-Q water, each fortified with 0.125 mg of polymer. The titration curves (Figure 8.3) show a significant improvement in shape and, in particular, the starting and end absorbance values are approximately equal in magnitude, and are in good agreement with the expected trend.

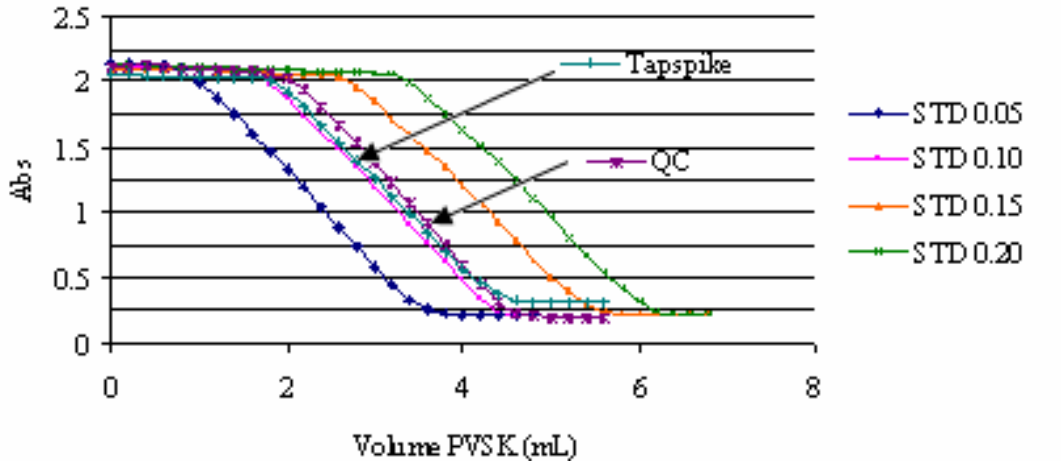
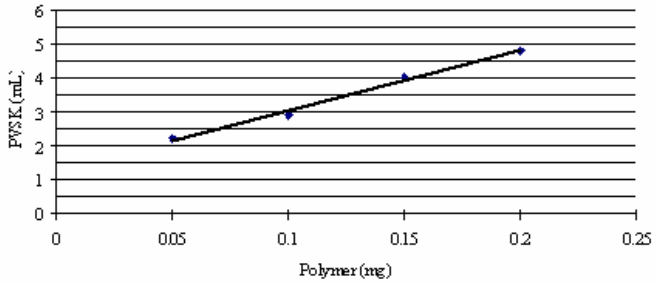


Figure 8.3: Titration curves obtained for the second batch of polyDADMAC calibration standards and test samples.

The result is a series of smooth and perfectly formed titration curves. The titration end points were determined with the tangents method and used in regression analysis. The regression line and related statistics (Figure 8.4) indicate a significant improvement in the fit of the regression line as shown by a marked decrease in the standard error and the reduction in the standard deviations of the slope and intercept when compared to Calibration 1.



Regression Statistics		
Multiple R	1.000	
R Square	0.999	
Adjusted R Square	0.999	
Standard Error	0.04	
Observations	4	STDEV
Intercept ± STDEV	1.25	0.05
Slope ± STDEV	17.40	0.35
y = 17.8x + 1.25		

Figure 8.4: Linear regression line and related statistics obtained for Calibration 2.

Excellent accuracy results of 85 and 94% respectively were achieved with the fortified tap water and QC samples.

One of the main problems experienced with the method was that the calibrations were not valid from run-to-run and poor accuracy results were obtained for the fortified, test and QC samples. Clearly this was expected, as can be noted from the difference in the slopes of Calibration 1 (slope=14.6) and Calibration 2 (slope=17.4). For good accuracy, the method required that all calibration standards and test samples be prepared and analyzed in one run. During the course of method development it was found that the absorbance values showed significant fluctuations from run to run and in some cases within the same run. This was attributed to one or more of the following possible causes:

- slight errors in the added dye content during sample pre-treatment
- instrument drift
- pH fluctuations

- PVSK degradation during dilution of the stock solution.

Information from suppliers revealed that PVSK is an unstable polymer, with a relatively short shelf life, and hence it was suspected to be the primary cause of the changes in slopes of the calibration functions. Unfortunately no new solutions could be sourced during this study to verify my suspicion.

Attempts were made to manipulate the data in order to obtain absorbance values that were more stable. A method that was tested involved the use of the difference in absorbance values (ΔAbs) instead of the absolute values of absorbance. The difference was calculated using equation 8.1.

$$\Delta Abs = Abs_x - Abs_y \qquad 8.1$$

$\Delta Abs = Difference\ in\ absorbance$

$Abs_x = Absorbance\ at\ zero\ PVSK\ addition$

$Abs_y = Absorbance\ after\ PVSK\ addition$

The ΔAbs data derived from the absorbance values from Calibration 2 were used to generate a new set of titration curves (Figure 8.5).

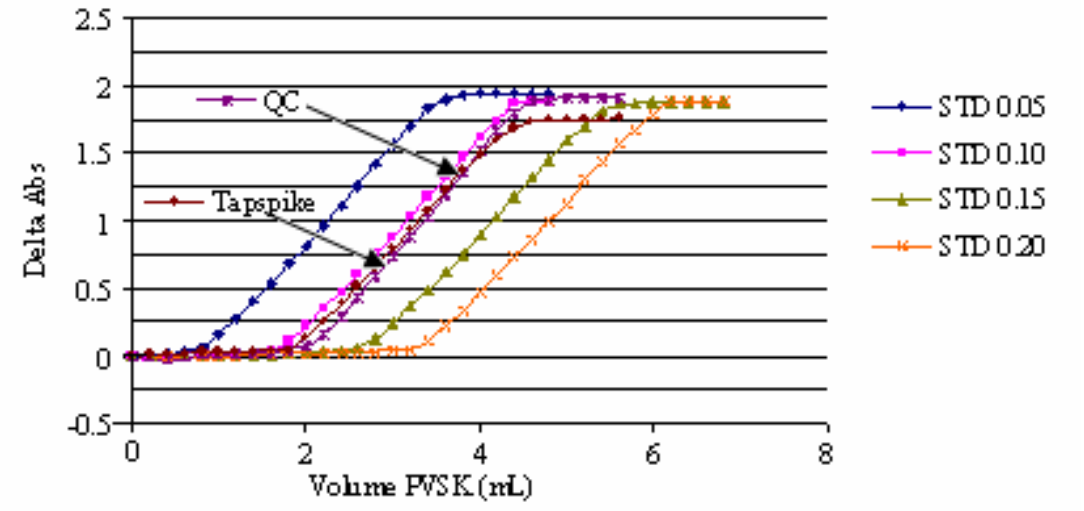
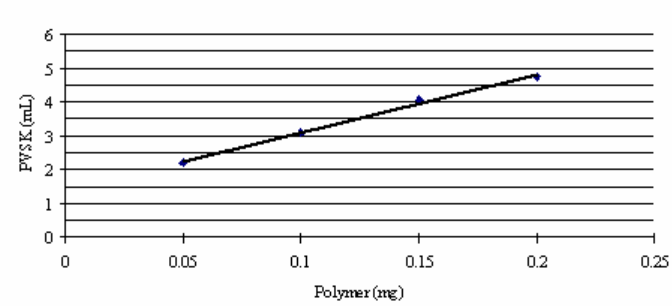


Figure 8.5: Titration curves obtained using the ΔAbs data.

The end points were determined using the tangents method as described previously and used in regression analysis for Calibration 3. The regression line fit shows excellent linearity, with the standard error and standard deviations of the slope and intercepts (Figure 8.6) marginally better than that of Calibration 2.



Regression Statistics		
Multiple R	1.000	
R Square	0.999	
Adjusted R Square	0.999	
Standard Error	0.03	
Observations	4	STDEV
Intercept ± STDEV	1.35	0.04
Slope ± STDEV	17.20	0.28
y = 17.02x + 1.39		

Figure 8.6: Linear regression line and related statistics obtained for Calibration 3 using the ΔAbs data.

The accuracy results for the fortified tap water and QC samples were 85% and 95% respectively. The differences in the final results of the two methods are therefore negligible. However, the ΔAbs mode of data analysis is recommended when the absorbance readings are erratic and short term instrument stability problems are experienced. The colloid titration method was found to give a linear response under very stringent experimental conditions. Good accuracy results were achieved from regression analysis and the best results were obtained when the samples and calibration standards were analyzed in the same run.

8.4.3 Method Precision

The precision of this method was assessed by determining the titration end points of six fortified samples of equal polymer concentration. Using the tangents method, the end points were found to be in the range 1.3 to 1.6 mL, giving an RSD = 8%.

8.4.4 Method Detection Limits

A systematic approach to determining detection limits was used to establish the minimum detectable levels of polymer in fortified tap water samples. The polymer spike concentrations ranged from 10 to 40 µg in 100 mL sample volumes. The titration curves (Figure 8.7) show that some degree of uncertainty exists in confirming the presence of polymer in the samples with 10 and 20 µg polymer. The curve for the 30 µg polymer spike appears to be the minimum level that can be detected with confidence, resulting in a limit of detection, LD = 300 µg L^{-1}. This value may appear somewhat high as the polyDAMAC reference standard was not in a pure form and therefore the actual dosing solution from the water treatment works was used. The concentration was assumed to be 100% (m/v). For the purpose of this study all concentrations and amounts of polyDADMAC used are expressed relative to this value. It may still be a difficult task to formalize the criteria for determining a positive result for polymers by pure inspection of the titration curve. It is proposed that the curve should display a minimum of three, but preferably four, points of equal absorbance to quantitatively establish the presence of polymer in the sample.

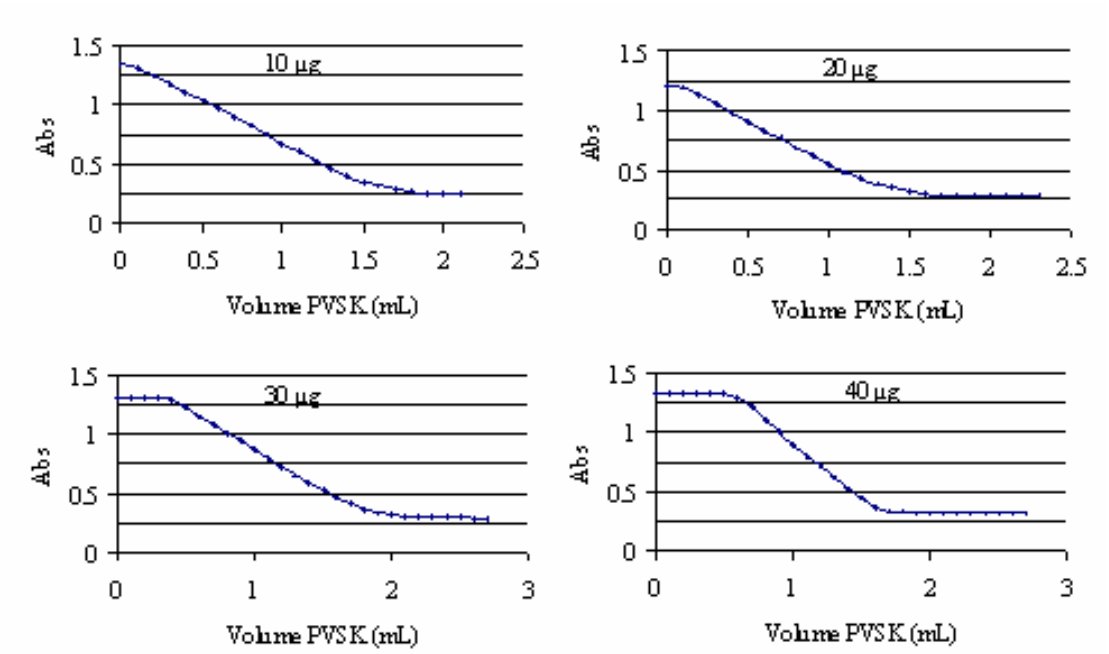


Figure 8.7: Detection limit titration curves for fortified tap water samples.

A lower polymer concentration may be used as the limit of detection for qualitative analysis. Since the upper region of constant absorbance is not distinctly visible at low spike concentrations, the proposed tangents method can no longer be applied and a study of the slopes of the test sample and blank must be made. Careful analysis of several titration curves with low polymer concentrations revealed that there were discreet differences in the profiles of the test sample and the blank. The slopes of the blanks were steeper than those of the test samples and the decrease in absorbance of the sample did not approximate a linear function as noted with the blanks. Even so, this presented a challenge with the interpretation of the titration curves and the production of conclusive results. A rapid method for making a qualitative assessment of polymer presence when the tangents method cannot be applied is proposed. Here it is referred to as the difference method, and is used as follows:

- Determine the difference between the 1st and 3rd absorbance readings of the test sample.
- Compare the above with the corresponding difference of the 1st and 3rd readings of a blank.
- If the difference of the test sample is less than that of the blank, then the result can be reported as positive for polymers.
- If the difference is more than or equal to the blank, then the result is reported as negative.

This is, in essence, a comparison of the gradients of the test samples with that of a blank. If the gradients of the test samples are steeper than that of the blank then the results are negative for the presence of polymers. This method was applied to several samples and found to produce satisfactory results.

8.5 Case Study

During the course of this work a case study was undertaken as a result of an unexplained occurrence at the DHWTW. A layer of scum was observed floating on the surface of the sand filters as well as a build up of sludge on the walls of the canal leading to the pulsators. This occurred after pre-chlorination and polymer dosing and was therefore suspected as being caused by coagulation and flocculation. It was decided to conduct tests to determine the presence or absence of polymers in the sludge and surface scum by application of the PVSK method. There was a real chance that if polymers were present, there would be a danger of them passing through the sand filters and entering the final treated water. Knowledge of this was considered very significant from a production cost point of view as well as from a public health point of view. Little is known on the health effects of polymers in water purification.

The investigation commenced with sample collection; the scum sample was sieved from the surface water of the sand filters and the sludge sample arising from a build-up of scum was scraped off the canal walls. The samples were used in the preparation of slides for microscopy. Due to poor light penetration, little was visible at 100 times magnification. Suspending the samples in water revealed a little more information. The samples comprised mainly particles of sand with a small amount of live microorganisms. The samples were pre-treated and analyzed using the PVSK method. The titration curves (Figure 8.8), were inspected. The blank indicated an absence of polymer, as expected. This is illustrated by an immediate decrease in the absorbance caused by the complexation of the free dye from solution during its reaction with PVSK.

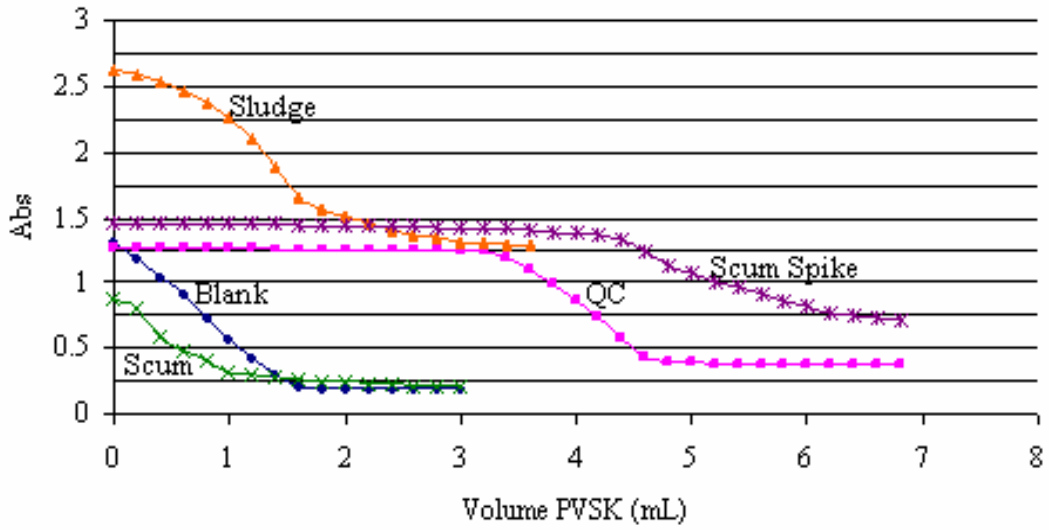


Figure 8.8: Titration curves of the blank, QC, sludge, scum and polyDADMAC fortified scum.

The fortified scum and QC sample on the other hand showed the typical profile of a positive polymer presence. The titration curves of the two test samples had characteristics of both the fortified sample and the blank. Although there was a decrease in absorbance with PVSK addition, the gradients of the curves of both test samples were gentler than that of the blank. Applying the proposed difference

method to the sludge, the absorbance of the test sample and blank was found to be 0.09 and 0.27 respectively. Based on the difference method, this indicated clearly a positive result for polymer presence in the sludge. However, there was a problem with quantitative analysis, and this was deemed the main limitation of this method. The titration curve for the fortified scum on the other hand showed unambiguously a high polymer concentration, as indicated by the well defined profile with the extended upper plateau region. The curve profiles at low spike concentrations were required to illustrate the matrix effects of the sample on the method.

To continue with the investigation, a new batch of samples was taken. This time a sample of the raw water was collected at the polymer dosing point and a sludge from the canal walls. The samples were treated as previously described with the raw water being filtered and the sludge extracted and then filtered. The sludge extract was separated into two 100 mL aliquots. One portion was spiked with 50 µg polymer (previously 500 µg). A new QC sample was also prepared containing 50 µg of polymer in Milli-Q water. The titration curve for the QC sample (Figure 8.9) shows a very distinct profile, with the upper and lower regions of constant absorbance indicating a positive presence of polymers.

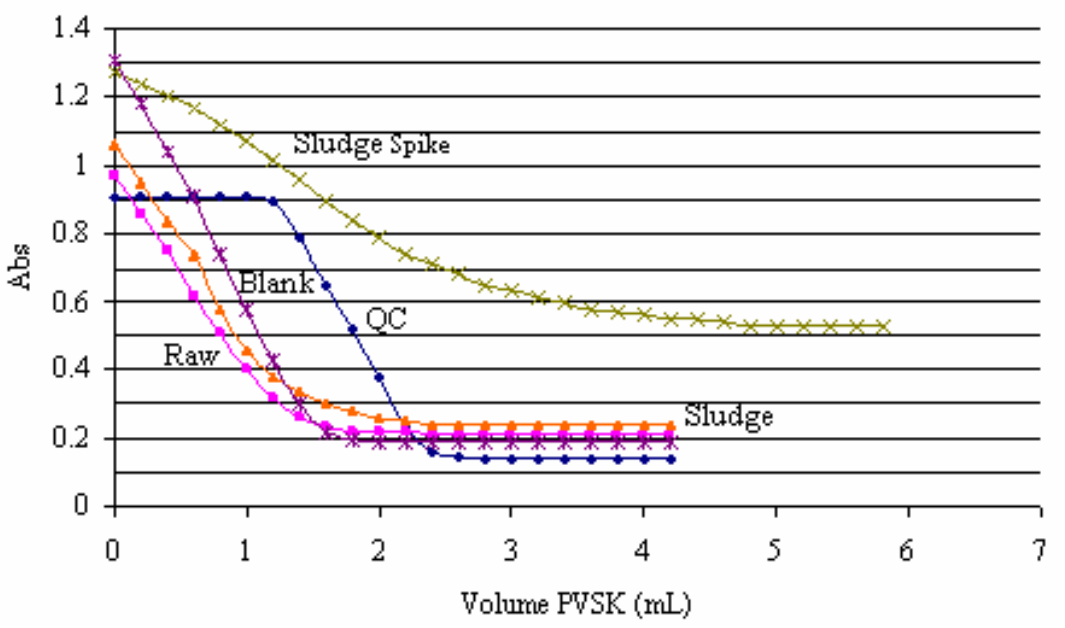


Figure 8.9: Titration curves of a new batch of samples comprising a blank, QC, raw water, sludge and polyDADMAC fortified sludge.

In contrast, the sludge fortified with exactly the same concentration of polyDADMAC as the QC, showed a poorly defined titration curve. This clearly indicated that the sample matrix had a very significant masking effect on the absorption profile and rendered the tangents method of little use for quantitative analysis. In addition to this, the poor detection limits in more complex sample matrices place limitations on the method, as can be noted from the titration curves of the raw and sludge samples. The method is far more complicated than originally anticipated, especially when working at low levels of polymer in the water, sludge and scum samples. There is every possibility that the method can produce false results when the three zones of the titration curve appear poorly defined. The method has severe limitations and

therefore is not the best method for polymer analysis. However, it may be useful in determining polymers quantitatively at relatively high concentrations. The titration curves do however require careful analysis and interpretation by a skilled analyst. By applying the difference method to the absorbance values, positive results were obtained for the raw, the sludge and the spiked sludge samples.

8.6 Conclusions

This research shows that the colloid titration method can be adapted using the tangents method for good quantitative analysis. In cases where well defined titration curves are obtained, accuracies of greater than 85% can be achieved and a precision of under 10%. Where erratic and unstable absorbance readings are obtained the difference in absorbance data should be used for good quantification. However, the colloid titration method is not without problems and must be used with great caution. It is very labor intensive and is prone to yielding false results, as observed here with the sludge and raw water samples. This indicated that the limits of detection in the raw water and sludge samples were much higher than in tap or Milli-Q water and that the sample matrix had a significant impact on the titration profile. It must be noted that all concentrations and amounts reported in this study are values relative to the dosing solution used at the water treatment facility. Full validation of the method was not the objective of the study, only the development of potential test methods for residual polymer analysis. For the determination of the true concentrations and true limits of detection, a polyDADMAC reference material of high purity is required. Pure polyDADMAC was prepared in a parallel study but was not available for use during the development of this method. Finally, users of this method must be able to recognize very discreet features of the titration curves. For curves that have characteristics of both a blank as well as that of a positive polymer presence, as observed here with the sludge, it is proposed that the difference method be applied for confirmation and that a qualitative result be reported.

8.7 References

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CHAPTER NINE

ANALYSIS OF LOW LEVELS OF POLYDADMAC IN WATER BY MEMBRANE FILTRATION GPC

9.1 Introduction

PolyDADMAC is UV inactive and is only amenable to the low sensitivity refractive index method of detection. This chapter describes a novel method for sample pre-treatment and the analysis of low levels of polymer in water. The sample was first subjected to membrane filtration using a laboratory-built ultrafiltration (UF) unit comprising an Amicon hollow-fibre polysulfone membrane operated in the recycle mode, followed by GPC. Separation was achieved using an Ultrahydrogel column, with a mobile phase of 0.25 M KH_2PO_4 adjusted to pH 2.3. Problems associated with the method, such as solute polarization and adsorption, are highlighted and steps to improve solute recovery are discussed. This study also focuses on innovative and creative ways to optimise the technique. Results showed an enhancement in sensitivity of more than four orders of magnitude, bringing the limit of detection of polyDADMAC down from 1000 mg L^{-1} to 0.01 mg L^{-1}. Finally, the practical application of the membrane filtration GPC method will be described in the analysis of real samples from various locations in the Umgeni Water operational area.

9.2 Background and Theory

In general, the various filtration systems that currently exist are categorized on the basis of particles that can be removed from a feed stream. The conventional microfiltration of suspended solids is accomplished by passing the feed solution through the filtration medium in a perpendicular direction. The entire feed solution passes through the medium, creating only one exit. Examples of such filtration devices include cartridge filters, bag filters, sand filters and multimedia filters. Microfiltration is limited to undissolved particles greater than 1 micron in size. For the removal of small particles and dissolved salts, membrane separation systems are utilized that use a different method to particle filtration. The technique is called cross-flow membrane filtration. This method uses a pressurized feed stream that flows parallel to the membrane surface (Figure 9.1).

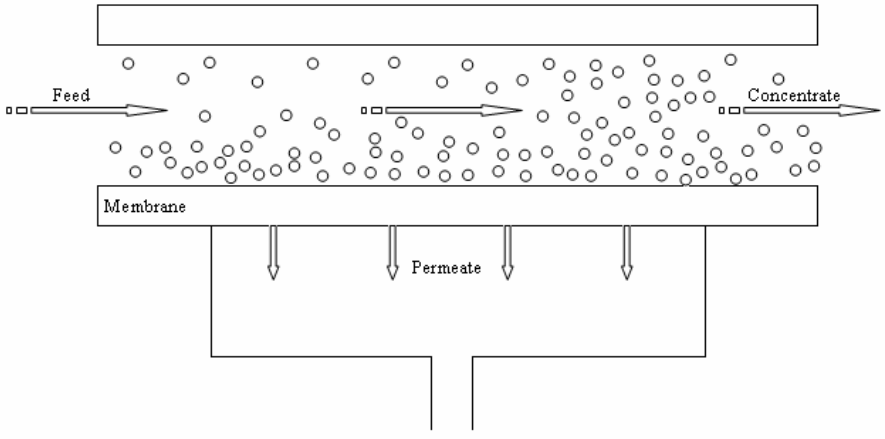

Figure 9.1: Schematic diagram of a cross-flow membrane filtration system.

A portion of this stream passes through the membrane, leaving in the feed stream a concentration of the rejected particles. Since there is a continuous flow across the membrane surface the rejected particles are swept away by the feed to form a concentrate stream, while the solution passing through the membrane forms the second stream.

There are four categories of cross-flow membrane filtration: microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO). The essential differences of each technique can be summarized as follows:

9.2.1 Microfiltration

- Particle sizes removed range from 0.1 to 1 μm.
- Applied for the separation of suspended particles, large colloids, bacteria and flocculated materials.
- Trans-membrane pressures are typically 10 psi.
- Macromolecules and dissolved solids pass through the membrane.

9.2.2 Ultrafiltration

- Macromolecules in the 20 to 1000 Å size range are separated.
- Applied for the separation of fine colloids, pigments, proteins, large organic molecules, and microorganisms.
- Osmotic pressure of these solutes is negligible and hence trans-membrane pressures are relatively low at 5 to 100 psi.
- Dissolved salts and small molecules pass through.

9.2.3 Nanofiltration

- Particles in the size range of 1 nm are separated.
- Applied for the separation of organics with molecular size 200 to 400 D, dissolved salts with mainly divalent anions, color removal, TOC and TDS.
- Trans-membrane pressures are typically 50 to 225 psi.

9.2.4 Reverse Osmosis

- RO is the finest level of filtration available.
- RO membranes act as a barrier to all dissolved salts, organic and inorganic molecules.
- Water passes through freely, creating a purified product stream.
- Rejection of dissolved salts is typically 95 to 99%.
- Solute osmotic pressure is relatively high and hence trans-membrane pressures of 500 to 2000 psi are required.

Considering the nature and size of the test polymer under study, UF was expected to be the most suitable technique for the separation of these macromolecules.

Separation between solute and solvent, or between different solutes in a multi-component system, is achieved by the use of a membrane with pore size

typically in the range 10 to 50 nm. The applied pressure gradient across the membrane causes solvent and small solute species to pass through the membrane and collect as the filtrate or permeate. Other large solute species are retained by the membrane as the concentrate or retentate. Poiseuille's law (equation 9.1) describes the solvent or product flux (J) through the membrane.

$$J = \frac{N \pi d_p^4 \Delta P}{128 \Delta x \mu} \qquad 9.1$$

J = product flux
N = number of pores per unit area of membrane
ΔP = applied hydraulic pressure
Δx = pore length
μ = solvent viscosity
d_p = pore diameter

The equation shows that product flux is directly proportional to the applied pressure, porosity, and the 4th power of pore diameter, and inversely proportional to the membrane thickness and solution viscosity.

The technique has been used in the production of potable water [Cheryan, 1986], as well as the clarification and sterilization of wine [Wang at al., 1989]. UF membranes have an asymmetric substructure (Figure 9.2) and are produced from polymeric materials such as cellulose acetate, polyacrylates and polysulphones by a phase inversion process [Aptel et al., 1985].

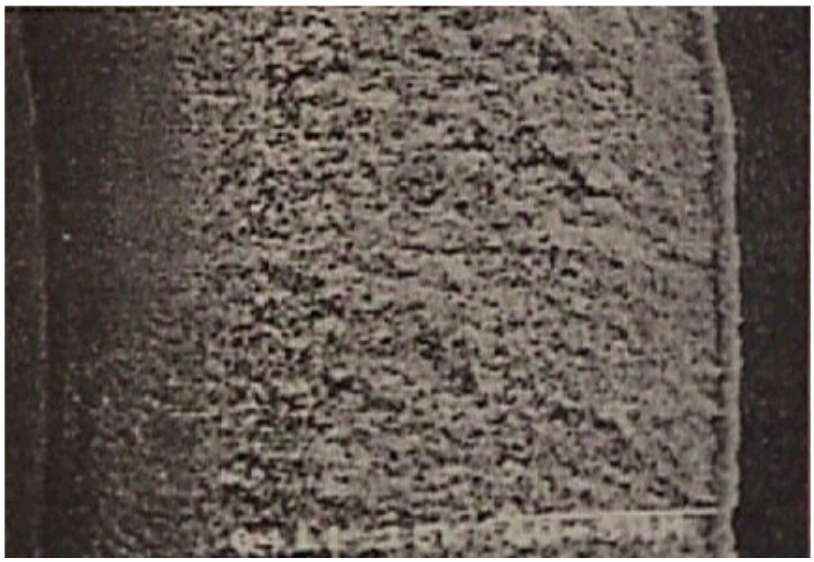

Figure 9.2: Scanning electron micrograph of the surface of an UF membrane obtained at 100 000 times magnification [Hanemaaijer et al., 1989].

The membranes are produced in tubular or flat sheets (Figure 9.3). These membranes have a relatively high surface to volume ratio that promotes high filtration rates.

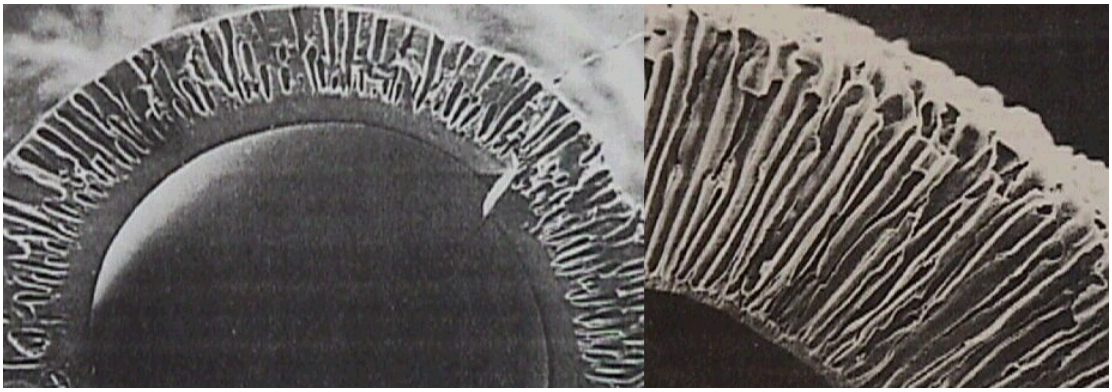

Figure 9.3: Electron micrograph of a cross-section of a hollow fiber membrane at 500 times magnification [Jacobs et al., 1997].

Capillary tubes are arranged in bundles and housed in an epoxy tube with an axial flow configuration (Figure 9.4).

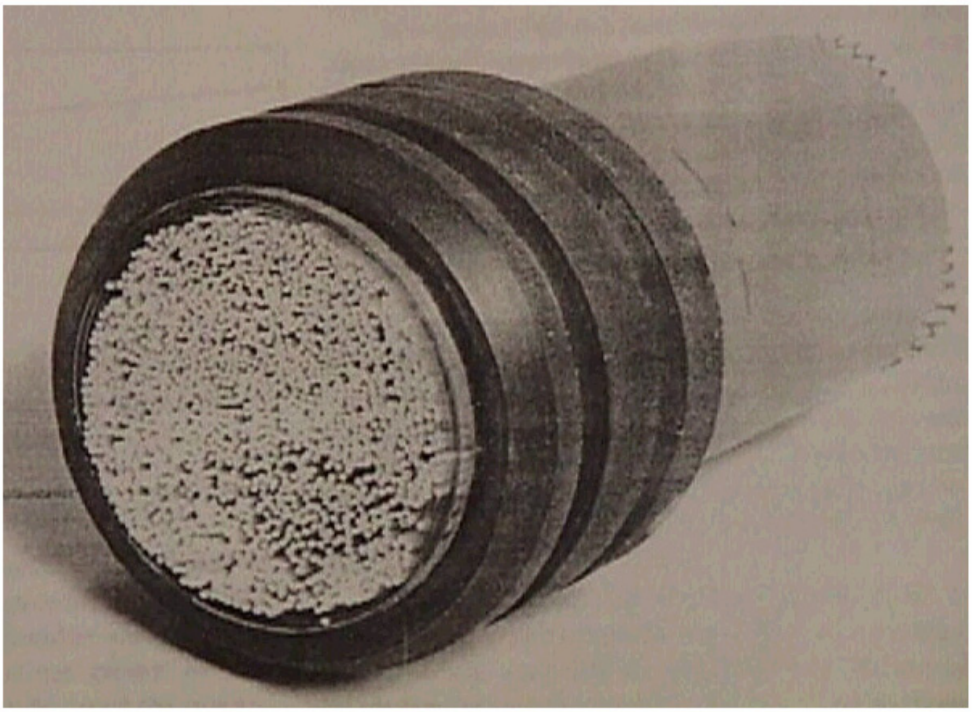

Figure 9.4: Hollow fiber capillary membrane module used for UF [Jacobs et al., 1997].

The main advantages of UF over other separation techniques are as follows:

- The process is athermal
- Involves no change in phase
- Can be conducted at low hydrostatic pressure
- The process is gentle and non-destructive of sensitive materials
- Does not require the use of chemical agents
- Can be conducted at low temperatures.

9.3 Experimental

9.3.1 Instrumentation

GPC: All samples were analyzed on an Alliance 2695 system as described in Section 2.4.1. The flow rate was set at 0.5 mL min^{-1} and the column maintained at 40 ^{o}C using a column heating module. The detector cell internal temperature was set at 40 ^{o}C. The Ultrahydrogel 500 and Ultrahydrogel 120 columns were used in this study.

Apparatus: A laboratory-built UF system (Figure 9.5) containing a cartridge type polysulfone hollow fiber membrane (Waters H1P10-20) with a nominal molecular mass cut-off of 10 000 Da was used. A second membrane, in the spiral wound configuration, with a nominal molecular weight cut-off of 5000 Da was also tested.

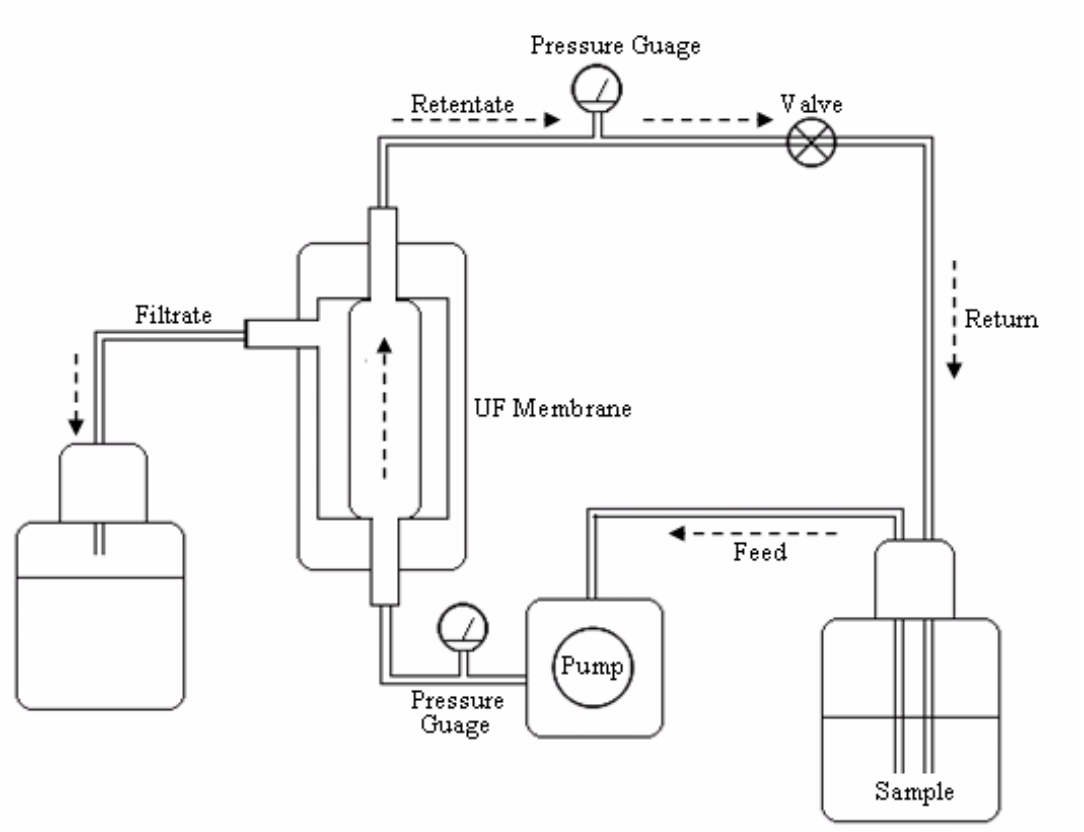

Figure 9.5: Schematic diagram of the UF system used for polyDADMAC separation.

Neoprene tubing of either ¼ inch or ½ inch outer diameter (Masterflex 6402-18) was used. The pump was a Watson Marlow 504S peristaltic pump with variable pump speeds. A backpressure valve was fitted to the return line to control the membrane backpressure and solvent flux. The inlet and backpressure was monitored with pressure gauges graduated from 0 to 166 kPa.

9.3.2 Reagents

The Mobile Phase: A 0.25 M phosphate buffer was prepared as described in Section 2.4.2.

PEG Standards: PEG narrow standards were prepared as described in Section 2.4.2.

PEO Standards: PEO narrow standards were prepared as described in Section 3.3.2.

Polymer Test Samples: Samples used in this study were obtained from the polyDADMAC synthesized as part of this study. A further two commercially available polymers (Z464N and Z553D) used in this study were obtained from Zetachem (Durban, South Africa). Appropriate masses of the polymers were used for the preparation of the test samples in Milli-Q water.

9.3.3 Filtration Procedure

The Single-Pass Mode: In this mode of filtration the entire sample (5 L) was pumped through the system in one cycle and 10 mL of retentate was collected.

Recycle Mode: In this mode, the test sample was returned to the sample container and the flux was controlled by adjusting the backpressure valve of the filtration unit. Three samples with decreasing polyDADMAC content (10 mg L^{-1}; 1 mg L^{-1} and 0.1 mg L^{-1}) were filtered and analyzed by GPC.

9.4 Results and Discussion

Commercially available polyDADMAC (Z464N) was used to develop a GPC method suitable for polymer analysis. Several columns were tested and the optimum column bank (Ultrahydrogel 500 and guard column) was selected to give good sensitivity and resolution of the polymer. The region of total exclusion (V_0) and total permeation (V_t) was established using PEO narrow standards with a molecular mass in the range 25 300 to 850 000 Da (Figure 9.6), as described in Chapter 2.

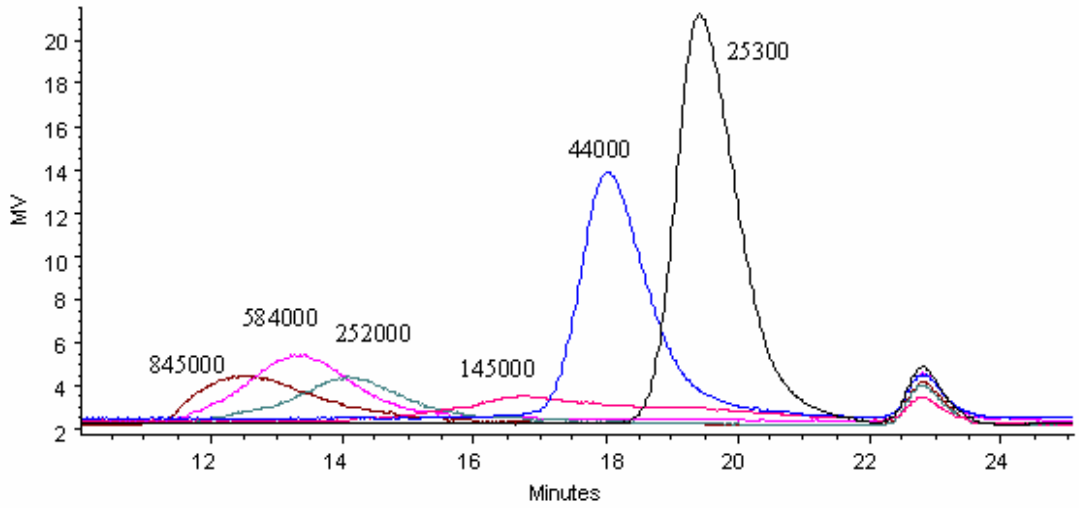

Figure 9.6: Determination of V_0 and V_t of the GPC column bank with PEO narrow standards.

This was followed by the analysis of a polymer synthesized previously (refer to Section 3.3.3), using the optimized operating conditions. The polymer was found to elute in the effective size separation region (selective permeation) of the column as shown in Figure 9.7.

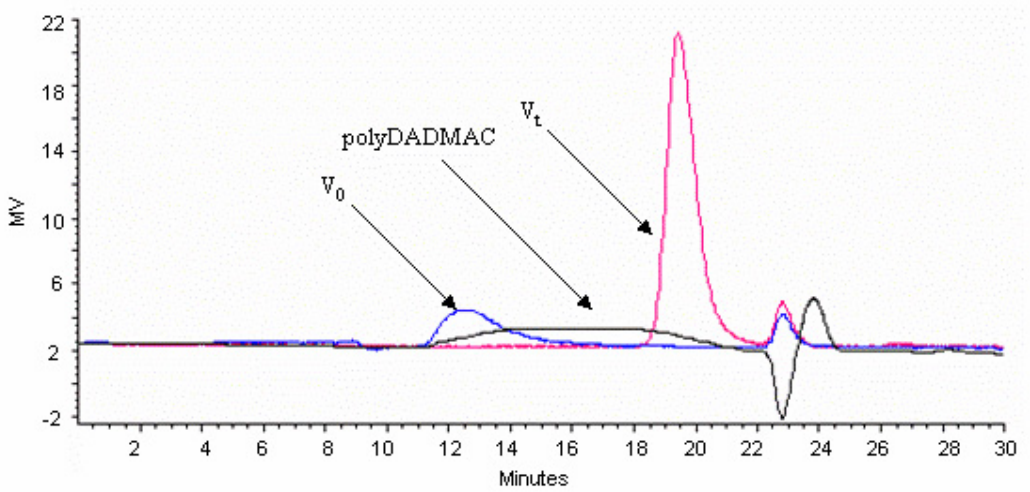

Figure 9.7: GPC profiles of the laboratory synthesized polyDADMAC relative to V_0 and V_t.

Since the polymer peak lies within the boundaries of V_0 and V_t, the operating conditions and especially the choice of the column were regarded as being the most suitable for the analysis and detection of polyDADMAC. These parameters were used to measure polymer levels in the UF experiments.

9.4.1 Ultrafiltration: The Single-Pass Mode

In the single-pass mode (Figure 9.8), the entire sample (feed) is pumped through the system in one cycle and the desired concentration ratio (CR) is obtained using a graduated cylinder and stopwatch. The CR is calculated from equation 9.2.

$$Concentration\ Ratio = \frac{Volume_{filtrate} + Volume_{retentate}}{Volume_{retentate}} \qquad 9.2$$

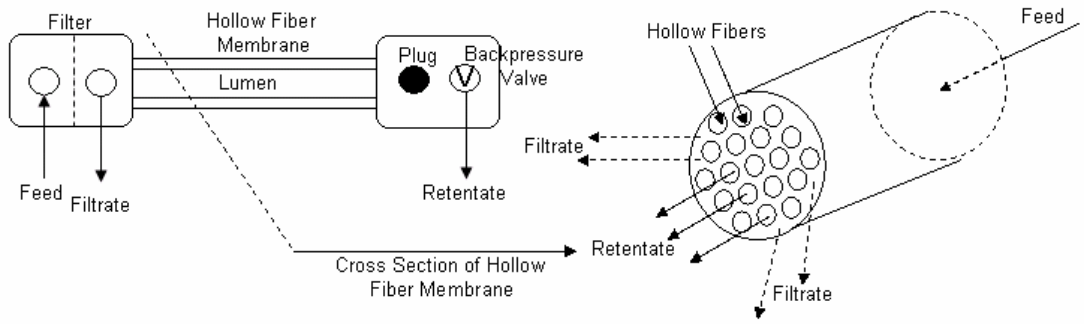

Figure 9.8: Schematic diagram of UF in the single-pass mode.

In practice, this concentration ratio was obtained by adjusting the backpressure valve (V) of the UF unit. Solvent molecules and other small molecular weight species are driven through the membrane pores (emerging as the filtrate) and go to waste. The polymer, due to its large molecular weight, is unable to pass through and remains behind in a small volume of solvent, and hence solute concentration is achieved. Using this technique, a 5 L sample of synthesized polyDADMAC feed solution (500 mg L^{-1}) was filtered through the system and the backpressure was adjusted to collect a small volume of retentate (10 mL) in one filtration cycle of sample through the membrane. The retentate and a 2 mL aliquot of the original sample were filtered through a 0.45 µm syringe type filter for GPC analysis. The GPC profiles of the retentate and original sample (Figure 9.9) indicate clearly that there was some degree of polymer enrichment in the retentate (when compared to the original sample).

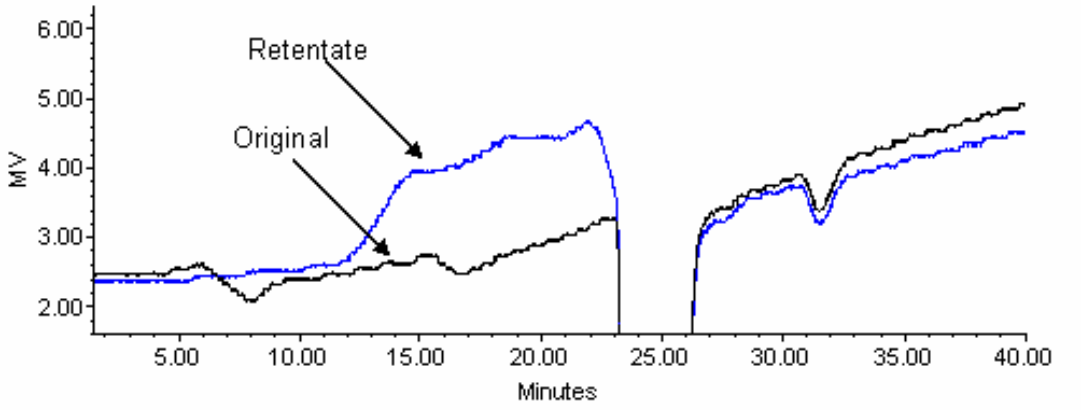

Figure 9.9: GPC profile of the retentate and original polymer sample after UF.

However, the profiles obtained show the typical broad polymer peak as well as the characteristic dipping baseline at 25 min, as was encountered previously during method development. These two factors presented a significant problem with regard to accurate quantification and polymer detection sensitivity. A rapid assessment of the polymer detection limit by the GPC system was conducted in order to establish the working range for future UF experiments. A range of polyDADMAC standards (50 to 1000 mg L^{-1}) was prepared and analyzed by GPC (Figure 9.10).

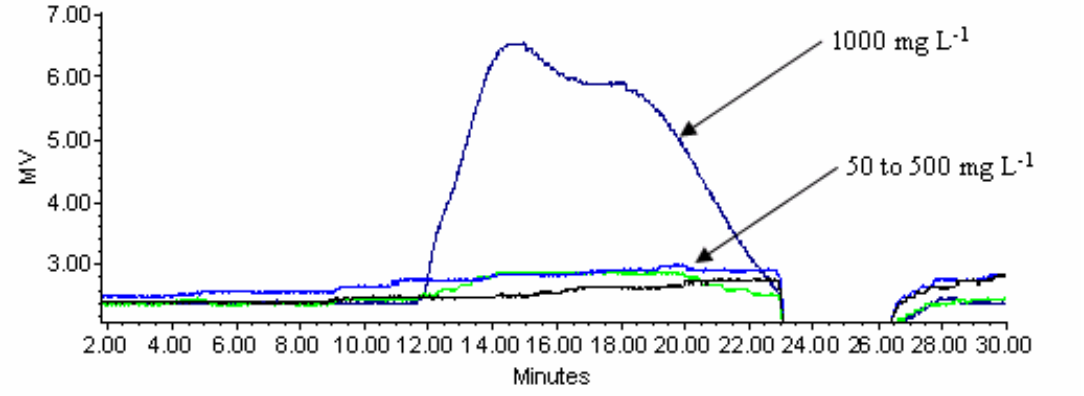

Figure 9.10: Overlay of GPC chromatograms of standards prepared from the synthesized polyDADMAC.

The GPC profiles show a series of poorly defined broad peaks for polyDADMAC concentrations 50 to 500 mg L^{-1}. By inspection, it was noted that from the range of standards tested, the peak at 1000 mg L^{-1} was the lowest concentration that could be detected with some degree of confidence. It was therefore immediately anticipated that severe problems would be experienced in future experiments in which polymer pre-concentration followed by quantification by GPC RI was to be done.

Consideration was given to sacrificing molecular weight distribution data, a property that was not the focus of this study, and to use an approach in which the polymer would be partially excluded from the GPC column but at the same time sufficiently resolved from other potentially interfering small molecular mass species present in the sample. This was achieved by the use of the Ultrahydrogel 120 GPC column. Analysis of the test polymer with this column gave a totally different profile (Figure 9.11) to that obtained with the Ultrahydrogel 500 column.

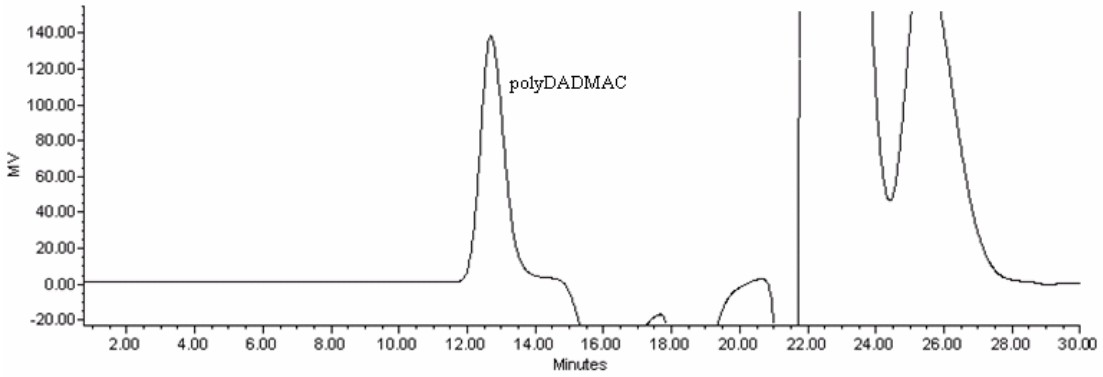

Figure 9.11: GPC of polyDADMAC using the Ultrahydrogel 120 column.

An important feature of this chromatogram is the narrow and symmetrical polymer peak eluting in the region 12 to 14 min as a result of being partially excluded from the GPC separating column. In the low molecular mass region (22 to 28 min) two new, high intensity peaks are observed. These can be explained by the additional separating power of the column, particularly for the small molecular mass species.

To establish the position of the polymer with respect to the exclusion limit of the column, a series of PEG standards in the range 106 to 23 000 Da was analyzed by GPC and superimposed with the GPC trace of a commercial polyDADMAC sample (Figure 9.12) taken from the DHWTW.

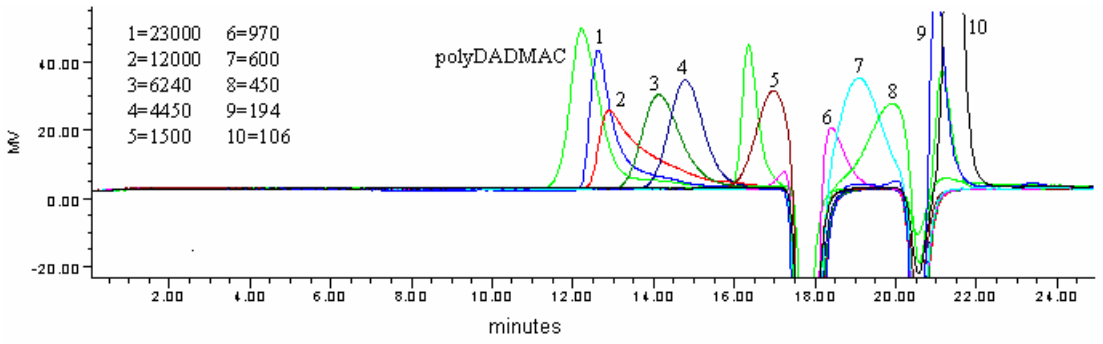

Figure 9.12: GPC chromatogram of PEG standards run on the Ultrahydrogel 120 column and superimposed on a polyDADMAC test sample.

From the overlay chromatogram of the polymer and the PEG standards it is clear that there is very little size separation between the polymer and the 23 000 Da standard. The column specification indicates that the column exclusion limit is 5000 Da. The additional separation power may be attributed to the action of the guard column (dimensions 7.8 x 50 mm of the mixed bed linear column) connected in series with the analytical column. The impact of this partial exclusion of the polymer from the GPC column and simultaneously its sufficient separation from the small molecular weight species had enormous implications for this research. It immediately indicated the potential of this method for the quantitative determination of polyDADMAC.

The polymer peak had good symmetry and appeared well resolved from other components as well as the baseline dip. This is desirable for good peak integration and quantitative work. Although the polymer peak eluted close to the exclusion limit of the column and resulted in a peak that resembled a typical reversed or normal phase HPLC chromatogram, with no molecular weight distribution data, technically there was no reason why this GPC method could not be used for the quantitative analysis of polymers. The polymer peak was symmetrical and exhibit improved sensitivity and resolution. It could therefore be predicted that this would prove beneficial for polymer analysis. The first benefit of the method of analysis was demonstrated by making an assessment of the limits of detection using the polymer standards prepared in Section 9.3.2 (Figure 9.13).

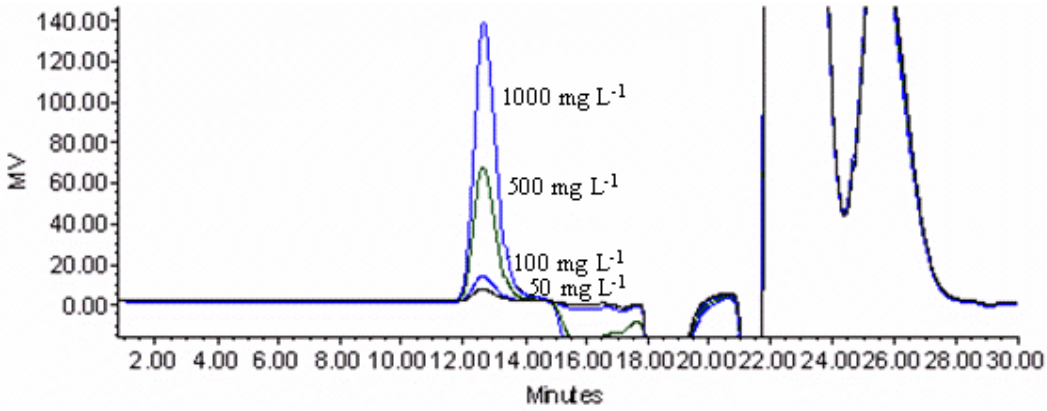

Figure 9.13: GPC chromatogram of the synthesized polyDADMAC reference standards prepared previously. Separation was achieved using the Ultrahydrogel 120 column.

The polymer peaks are symmetrical and well defined at all concentrations, including the lowest standard of 50 mg L^{-1}. The modified GPC method therefore had potential to be used as a reversed or normal phase HPLC method for quantitative analysis. The main advantage of this method is an immediate improvement in sensitivity, from 1000 mg L^{-1} to less than 50 mg L^{-1}. This rendered the modified GPC method far more amenable to the analysis of polyDADMAC.

A new UF experiment was conducted on a polymer test sample having a spike concentration of 0.1 mg L^{-1}. The UF system was operated once more in the single-pass mode of filtration and 10 mL of retentate was collected. GPC analysis of the concentrate together with a 50 mg L^{-1} polymer reference standard resulted in a recovery of 15%. Solute polarization (Figure 9.14) and in some cases adsorption (solute binding onto the membrane surface through hydrophobic interactions) are

known to contribute to low solute recovery in UF methods of analysis [Amicon, 1993].

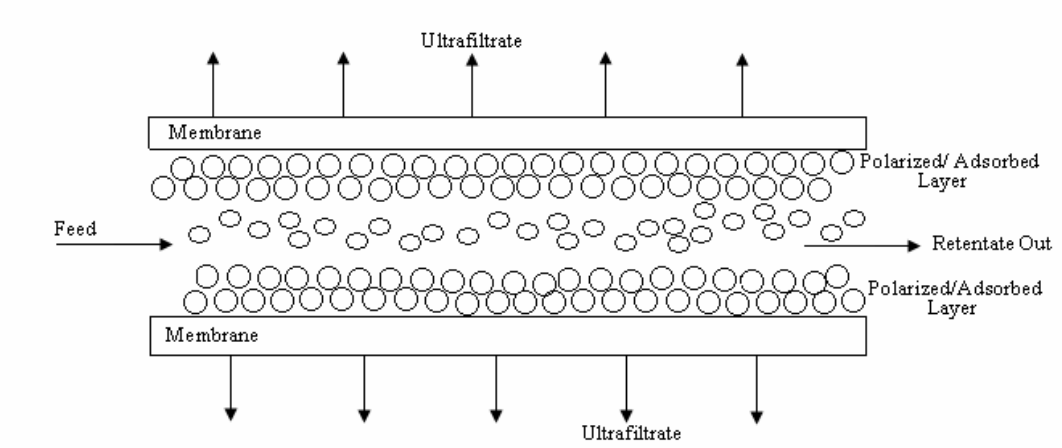

Figure 9.14: Schematic diagram showing solute polarization effects during UF.

The phenomenon of solute polarization occurs when a solution containing solute of colloidal material retained by an UF membrane is forced under pressure through the membrane. The rejected colloids tend to accumulate at the upstream surface of the membrane, thereby creating a gel layer at the membrane solution interface. Several factors interact to affect the extent of solute polarization. These include fluid shear, geometry of the membrane, macro-solute concentration and membrane molecular mass cut-off. Methods to minimize these problems include operating at a high inlet pressure and moderate backpressures. With adequate flow relative to the internal fiber diameter, sufficient shear is generated to reduce the polarized boundary layer at the fiber wall. In the event of the boundary layer forming, it can be removed by back-flushing the membrane. The second possible cause of the poor solute recovery may be attributed to solute losses by permeation of analyte through the membrane pores (molecular mass cut off 10 000 Da). The above factors were considered and the operating pressures adjusted to the maximum permitted pressures (inlet 100 kPa, backpressure 66 kPa) and the filtration conducted in the re-circulation mode.

9.4.2 Ultrafiltration: The Re-circulation Mode

To minimize solute losses by permeation through the UF membrane, the filtration was conducted in the re-circulation mode (Figure 9.15).

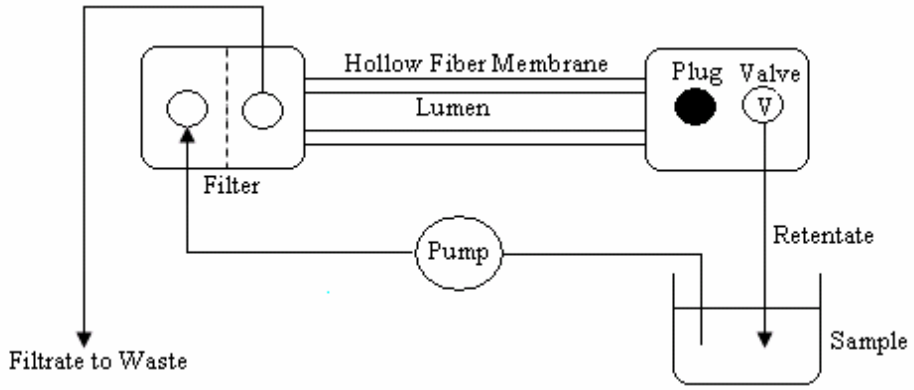

Figure 9.15: Schematic diagram of the re-circulation mode of UF.

In this mode, the test sample was returned to the sample container and the flux was controlled by adjusting the backpressure valve (V). UF was continued until a retentate volume of 50 mL was collected. Three samples with decreasing polyDADMAC content (10 mg L^{-1}; 1 mg L^{-1} and 0.1 mg L^{-1}) were filtered using the re-circulation mode and analyzed by GPC (Figure 9.16).

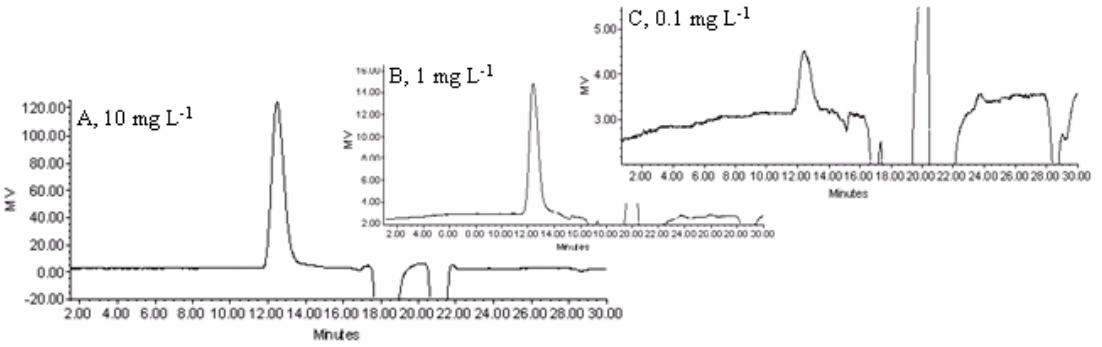

Figure 9.16: GPC of polyDADMAC at spike concentrations of (a) 10 mg L^{-1}, (b) 1 mg L^{-1}, and (c) 0.1 mg L^{-1}.

The recovery was calculated by comparison with the peak area of a reference polymer standard and was found to be in the range 37 to 49%. Using data from the filtration of six samples at 10 mg L^{-1} concentration, the precision was found to be 21%. Recovery losses and poor precision was attributed to the molecular mass cut-off (10 000 Da) of the membrane.

Attempts were made to acquire a second membrane with a lower molecular mass cut-off (5000 Da). However, it was discovered that Amicon had discontinued the entire range of the polysulfone hollow fiber membranes. As a consequence, another membrane was sourced from G.I.C Scientific (Florida North, South Africa). The new membrane was made of cellulose acetate and was in the spiral wound configuration, with a molecular mass cut-off of 5000 Da. The membrane was tested at length, and in each successive experiment slight adjustments were made to the filtration procedure, and the impact on solute recovery noted. The best filtration conditions were the following:

- The test sample (5 L) was pumped in the recycle mode to dryness.
- Milli-Q water (200 mL) was then pumped to remove polymer residues from the sample container and feed tubing.
- The pump flow direction was reversed, and the retentate collected by backflushing the membrane.
- This was followed by a second rinse with 150 mL Milli-Q water, and the retentate collected once more, by backflushing.
- Finally, the inlet feed line was disconnected, the liquid drained from the membrane housing, and combined with the retentate.
- The combined retentate was filtered through a 0.45 µm syringe type filter, and analyzed by GPC.

Using this procedure, the recoveries were 83 to 99% (n = 4) and a spike concentration of 20 mg L^{-1}. When the spike concentration was reduced by an order of magnitude to 2 mg L^{-1}, the recoveries were 64 and 69%. A further reduction in the spike

concentration to 0.2 mg L^{-1} resulted in the presence of a small polymer peak in the chromatogram. The theoretical concentration of the polymer was 5.3 mg L^{-1} after pre-concentration, and assuming 100% recovery. The method was used to screen several final water samples that were found to be free of any polymer residues. The method was subsequently applied successfully for identifying the presence of polymer in a surface scum sample collected from the DHWTW (Figure 9.17).

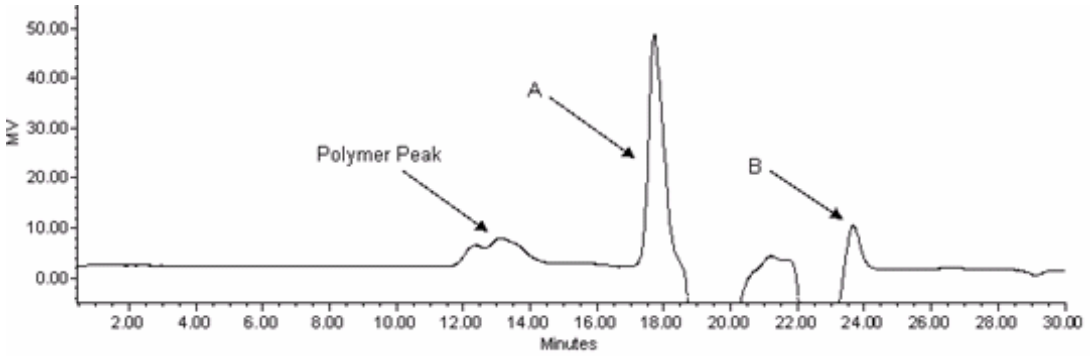

Figure 9.17: GPC RI chromatogram of a surface scum sample taken at the DHWTW.

In the case of the scum sample no pre-concentration was required, and the sample was filtered and analyzed directly by GPC RI. The presence of small molecular weight species are observed at 18 and 24 min (A, B), as a result of not subjecting the sample through the process of UF.

9.5 Conclusions

This research has shown that although GPC RI is a method that suffers from low detection sensitivity for polyDADMAC analysis, the method can be manipulated by careful choice of the GPC analytical column to allow the polymer to elute close to the exclusion limit of the column and at the same time provide sufficient size separation from other low molecular weight species. Features of the resulting polymer peak were good symmetry and, good efficiency, and there was a 20-fold increase in the limits of detection purely by this operation. UF using the Amicon H1P10-20 hollow fiber membrane was partially successful and can be recommended for semi-quantitative or qualitative screening of polyDADMAC. Recoveries of greater than 47% could not be achieved despite optimization of several filtration parameters. However, higher recoveries are predicted with the Amicon H1P3-20 hollow fiber membrane with a MW cut-off of 5000 Da but it is currently not commercially available.

A spiral wound equivalent to the H1P3-20 membrane produced superior polymer recoveries at 20 mg L^{-1} spike concentration, good recoveries at 2 mg L^{-1}, and the polymer could also be detected at 0.2 mg L^{-1}. When coupling the modified GPC method with UF there was a 5000-fold enhancement in sensitivity (1000 mg L^{-1} to 0.2 mg L^{-1}). A further improvement in sensitivity by one order of magnitude was achieved by reducing the final retentate volume, typically 100 or 200 mL, to 5 or 10 mL, by evaporative concentration. This was accomplished using a rotary evaporator under reduced pressure, and the result was a final detection limit of 0.01 to

0.02 mg L^{-1}. This is well below the WHO guideline limit of 0.05 mg L^{-1}. Hence this method is a simple and practical method for residual polymer analysis in water.

9.6 References

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CHAPTER TEN

CONCLUSIONS AND RECOMMENDATIONS

10.1 Conclusions

The current legislation governing the production and use of polymers for water treatment is severely lacking. There is an urgent need in South Africa for controls to be put in place to regulate their production and use, and this is currently being addressed by the author together with the Water Research Commission of South Africa, and various stakeholders (as part of a new WRC project (K5/1600)).

Synthetic organic polymers continue to be preferable to inorganic coagulants for potable water purification for reasons discussed in Section 1.4.1.

In this study significant progress was made in understanding the synthesis, structure, properties and mechanism of polymerization of polydiallyldimethylammonium chloride (polyDADMAC). The results of this research proved to be valuable to Umgeni Water.

In addressing the limitations of the methods of polyDADMAC analysis, a gel permeation chromatography (GPC) method was successfully developed, optimized and evaluated. The best component resolution was achieved with an Ultrahydrogel 500 analytical column heated to 40°C, and using a 0.25 M KH_2PO_4 mobile phase adjusted to pH 2.3. The scope of the GPC method was extended to include the analysis of the diallyldimethylammonium chloride monomer (mDADMAC), and determination of the percentage active polymer, and polymer residues in water, scum, and sludge samples. Currently, the method developed in this study is being applied to product quality control. A database of molecular weight distribution (MWD) profiles for each new batch of polyDADMAC is being created. This enables monitoring of batch-to-batch product quality and consistency, and variations in the MWD can be correlated with product performance. Ultimately the impact of product quality on water treatment costs will be established and hence fulfill a crucial research objective.

The chemistry of polyDADMAC was studied extensively, commencing with its successful synthesis in a laboratory-designed reactor. Good control of reaction temperature, pressure, monomer concentration, catalyst concentration and reaction rate is crucial for the successful polymerization of polyDADMAC. Uncontrolled conditions resulted in the production of a highly cross-linked product that was insoluble in water and of no use as a coagulant. The MWD of the synthesized polymer was determined using GPC. Based on the peak molecular weight, the product was characterized as a low to medium molecular weight polymer. The structure was elucidated using ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra. The product was identified as comprising the five-member pyrollidine ring system.

The activity of the synthesized polymer was demonstrated by clarifying raw water of turbidity 21 NTU to less than 0.5 NTU. An optimum dose was 6 to 7 mg, making the

dose approximately equivalent to the required dose of a commercial polyDADMAC polymer.

PolyDADMAC stability studies revealed that, despite reports of being a most chlorine resistant polymer, it contributed to the formation of the trihalogenated methane (THM), chloroform. However, under laboratory conditions, the levels of chloroform were well within the recommended WHO guidelines. PolyDADMAC was considered to be a resilient and stable compound and only displayed some degree of change when subjected to extremes of temperature, pH, and UV radiation. It is biodegradable, as noted by the reduction in the polymer peak areas of the test samples that were exposed to microorganisms.

The review of polyDADMAC methods of analysis revealed that complex formation with tannic acid (TA) produced calibrations of poor linearity, and significant fluctuations in the accuracy of fortified samples. Similar calibration problems were experienced with the rose bengal method. Additionally, the magnitude of the absorbance of the polymer-rose bengal calibration solutions was negligibly different from each other and there was some doubt whether complexation was occurring. The methyl orange (MO) complexation method, although tested extensively, produced poor results. As observed with rose bengal, there was a negligible change in the magnitude of the absorbance values of the different polymer-MO calibration solutions.

Varying degrees of success were achieved with each of the four novel analytical methods developed and tested. The HACH method produced excellent linearity, good precision and accuracy, and the calibrations were valid for over two weeks. The application of the method was demonstrated successfully on samples from the Durban Heights Water Treatment Works (DHWTW). GPC with indirect UV detection using acetone as the chromophore produced erratic results. Substituting acetone with Na_2CrO_4 was a better alternative and produced more stable results but suffered from lower sensitivity than refractive index (RI) detection, hence defeating the objective of the study.

Solid phase extraction (SPE) of polyDADMAC from water was partially successful. Low to nearly zero recoveries were obtained using C_{18} extraction cartridges. Improved recoveries were obtained using tC_{18}, but even so, the results were unacceptably low for quantitative analysis. Colloid titrations were applied successfully for the quantitative determination of polyDADMAC. Although good precision and accuracy was obtained, the method was prone to false positives, it was long and laborious, and required an experienced analyst for the interpretation of results.

Membrane filtration GPC was an excellent technique for sample pre-concentration and analysis of low levels of polymer in water. The enhancement in sensitivity achieved using this operation was 5000 fold, with a further one order of magnitude achieved by evaporative concentration. The limit of detection achieved using this method was between 0.01 and 0.02 mg L^{-1}, which is well within the WHO guideline of 0.05 mg L^{-1} polymer residue in water.

Finally, the risk assessment study revealed that there were minimal risks associated with the production and use of polyDADMAC for drinking water purification. The polymer does however exhibit toxicity to fish and therefore may pose an ecological hazard in the event of its accidental release into the environment.

10.2 Recommendations

It is clear from literature studies that polyDADMAC methods of analysis are the least researched of all the available water treatment polymers. The present study provides a wealth of information, from polyDADMAC synthesis and mechanisms of polymerization, to structure, properties, and analysis. During the course of the research and report preparation, several important aspects were noted that could be used as a basis for the future continuation of this work. As this field of study is of great interest to the water sector, it is hoped to be pursued by the author of this thesis who currently heads the Research and Development unit at Umgeni Water, the second biggest water utility in South Africa. Follow up studies ought to focus on:

- THM formation under plant conditions, as identical conditions can never be simulated accurately in laboratory experiments.

- Extending the scope of the total organic carbon (TOC) and total Kjeldahl nitrogen (TKN) methods for the determination of carbon or nitrogen losses during degradation experiments.

- Conducting stability studies using a more sensitive detector such as an evaporative light scattering detector (ELSD) or a condensation nucleation evaporative light scattering detector (CN ELSD). Reports from manufacturers claim that these detectors are more sensitive than the RI detector by 2 to 3 orders of magnitude and therefore should make a significant difference in the investigations. The special requirements of these detectors are the use of volatile mobile phases.

- Addressing the limitations of indirect UV detection by testing a chromophore that is more sensitive than K_2CrO_4 and that is compatible with the polymer and the mobile phase.

- PolyDADMAC derivatization with fluorescein-5-isothiocyanate (FITC) as there are claims that it has been derivatized even though literature limits the reaction to primary and secondary amines. Alternatively indirect fluorescence detection can be tested that may produce superior polyDADMAC sensitivity.

- PolyDADMAC pre-concentration using C_{18} sulfonic acid as it may improve polymer recoveries by SPE from water.

- Extending the GPC method to include the polyamines, polyacrylamides and any other commonly used water treatment polymer.

APPENDIX 1

RISK ASSESSMENT OF POLYDADMAC

A1.1 Introduction

The level of risk associated with the production and use of polyDADMAC was established using the Drinking Water Treatment Chemicals-Health Effects guidelines [NSF/ANSI 60, 2005]. The risk assessment process involved four basic steps:

- hazard identification
- exposure assessment
- dose-response assessment and
- risk characterization.

PolyDADMAC is synthesized by the polymerization of the DADMAC monomer (mDADMAC). The best available technology can drive the monomer content down to 1% on an active polymer basis. The general level of the monomer is however in the range 1 to 5%.

A1.2 Toxicity Data for PolyDADMAC

A1.2.1 Hazards Identification

Hazards associated with the polymer have been identified as the slippery surfaces caused by accidental spills and the possibility of long term adverse effects in an aquatic environment. It is not considered to be toxic based on studies conducted on laboratory animals but over exposure by ingestion may irritate the gastro intestinal tract. Other potential health effects as a result of over exposure can be summarized as follows:

- Eye contact: may cause irritation or burn eyes
- Skin contact: may cause irritation or burns
- Inhalation: may cause irritation of the respiratory tract.

The toxicological information available from material safety data sheets (MSDS) obtained from overseas manufacturers [SNF, 2002; Tramfloc, 2003] and local companies [Sud Chemie, 1994; Zetachem, 2003] include the following:

- Acute toxicity: *LD_{50}/oral/rat>2000 mg/kg; sensitization, none known.
- Chronic toxicity: **NOAEL/oral/rat/90 day=5000 mg/kg. Two year feeding studies on rats and dogs did not reveal any adverse health effects.
- Irritancy and Corrosiveness: may be slightly irritating to skin and eyes on prolonged exposure, however there is no evidence of corrosiveness.
- Emperical data on effect on humans: considered non-toxic in normal use.

LD_{50} = lethal dose to 50% of test population.
***NOAEL = no observed adverse effects level.*

A1.2.2 Other Information [MSDS SNF, 2003]

- Not mutagenic in Ames test.
- Not mutagenic in micronucleus test on mice.
- Not teratogenic, NOAEL=175 mg/kg.

A1.2.3 Ecological Information [MSDS Tramfloc, 2003]

- Persistence in the environment: no data available.
- The biochemical oxygen demand (BOD)=44000 mg L^{-1}.
- The chemical oxygen demand (COD)=250000 mg L^{-1}.

A1.2.4 Aquatic toxicity

- *Daphnia magna*: LC_{50}/48 h=0.23 mg L^{-1} in 100 mL $CaCO_3$.
- Fathead Minnow (*Pinephales Promelas*): LC_{50}/96 h=6.51 mg L^{-1} in 180 mg L^{-1} $CaCO_3$ containing 10 mg L^{-1} TOC from humic acid and LC_{50}/96 h=0.3 mg L^{-1} in 100 mg L^{-1} $CaCO_3$.
- Fish: LC_{50}/*Danio Rerio*/96 h>10 mg L^{-1} (*OECD 203).
- Daphnids: EC_{50}/*Daphnia Magna*/48 h>10 mg L^{-1} (*OECD 202).
- Algae: not regarded as appropriate test as flocculation characteristics of the product interferes directly with the test medium preventing a homogeneous distribution that invalidates the test.
- Hydrolysis: does not hydrolyze.
- Biodegradation: not readily biodegradable.
- Log P_{ow}: 0.
- Bioaccumulation: does not bioaccumulate.
- Other: effects are rapidly and significantly reduced by the presence of dissolved organic carbon in the aquatic environment. Aquatic toxicity is reduced by factors of 10 to 100 times in the presence of 5 to 10 mg L^{-1} organic carbon as is found in most surface waters.

**OECD=Organization for Economic Cooperation and Development*

A1.3 Toxicity Data for mDADMAC

A1.3.1 Aquatic Toxicity

The LC_{50} at 72 h for Blue Gill Sunfish (*Lempomis Macrochirus*) is 56 mg L^{-1} [Johnson, 1971]. While mDADMAC has not been tested in daphnia, other quaternized monomers have, giving an EC_{50} of greater than 100 mg L^{-1}.

A1.3.2 Acute Toxocity

The acute oral rat LD_{50} for mDADMAC is 3030 mg/kg [Sterner, 1975]. It is non-irritating to the rabbit skin and eyes. Since potential human exposure is very low no further testing was done on the monomer.

A1.3.3 Reproductive/Developmental Toxicity

- No histological, clinical and hematological effects at the *LOAEL in subchronic rat and dog and chronic rat studies were observed.
- No teratogenic or reproductive effects as a result of treating rats with polymers containing monomers were observed.
- mDADMAC is poorly transported across membranes
- mDADMAC is negative in *in vivo* and *in vitro* cytogenic tests and tests for gene mutation.

**LOAEL=lowest observed adverse effects level*

The toxicity data for mDADMAC are summarized in Tables A1.1 to A1.4.

Table A1.1: Acute and chronic toxicity of mDADMAC conducted *in vivo*.

Study	Species	Strain	Result	Reference
Acute Oral Toxicity	Rat	Sprague-Dawley	LD_{50}=3030 mg/kg	Sterner, 1975
Primary Skin Irritation	Rabbit	New Zealand White	None	Vinegar, 1978
Corrosivity	Rabbit	New Zealand White	None	Vinegar, 1978
Acute Eye Irritation	Rabbit	New Zealand White	None	Vinegar, 1978
Segment 1 Multigeneration	Rat	Sprague	NOAEL=1.25 mg/kg/day	Adamik, 1979
Oral (Gavage) Teratology	Rat	Sprague	NOAEL=6 mg/kg/day	Palmer, 1991
Oral Absorption, Distribution and Excretion	Rat	Not Specified	Poorly Absorbed	Easterday, 1965b
13 Weeks Oral Toxicity Feeding Study	Rat	Not Specified	NOAEL=50 mg/kg/day	Sterner, 1976
13 Weeks Oral Toxicity Feeding Study	Dog	Beagle	NOAEL=200 mg/kg/day	Tegris, 1976
Mouse Micronucleus	Mouse	CDI	Negative	Putnam, 1991

Table A1.2: Acute and chronic toxicity of mDADMAC conducted *in vitro*.

Ames Test	Bacteria	*Salmonella Typhimurium*	Not Mutagenic	San, 1991; de Jouffrey, 1996a
Chromosome Aberration Test in Cultured Human Lymphocytes			Negative	de Jouffrey, 1996c
Mammalian Cell Gene Mutation Test Mouse Lymphoma			Negative	de Jouffrey, 1996b

Table A1.3: Environmental fate studies of mDADMAC.

Study	Result	Reference
Effect on Soil Organisms	No Effect	Rieck, 1980e
Anaerobic Aquatic Metabolism	No Effect	Rieck, 1980b
Effects of Microbes on Metabolism	No Effect	Rieck, 1980c
Leachability	No Effect	Rieck, 1980a
Plant Availability	Low Uptake	Rieck, 1980d

Table A1.4: Toxicity tests of mDADMAC conducted on aquatic organisms.

Study	Species	Strain	Result	Reference
Acute Aquatic Toxicity (72 h)	Fish	Blue Gill Sunfish	LC_{50}=56 mg L^{-1}	Johnson, 1971

A1.4 Exposure Assessment

PolyDADMAC is synthesized by the free radical initiated polymerization of mDADMAC, in a two step process, under rigorously controlled conditions. The first step involves the preparation of mDADMAC from the reaction of allyl chloride with dimethyl amine. Some of the controls used are directed at reducing exposure to allyl chloride, a volatile compound that has an OSHA permissible exposure limit of 1 ppm (3 mg m^{-3}). Once the monomer is prepared, it is either:

- polymerized in the same reactor to polyDADMAC
- isolated to remove NaCl and then polymerized, or
- shipped to other manufacturers.

During the polymerization there are essentially no emissions of mDADMAC to air, soil or water. The quality of the product is impeded by the presence of monomer and therefore manufacturers strive for residual monomer levels of 1 to 5%. Employees working in DADMAC production and polymerization facilities wear personal protective equipment (PPE) that includes gloves, safety glasses, and uniforms that are long sleeved and laundered. The potential for worker exposure is very low given that the monomer and polymer are large molecules and essentially has no volatility. The only potential exposure points are material handling for transportation, sampling or cleaning. As for the general population, the only exposure route appears to be through drinking water, given that >95% of the polymer is used in the clarification of drinking water.

To date, residues of polymer have not been detected in treated drinking water and it is assumed that 100% removal occurs during coagulation and flocculation. The

remaining hazard to be considered is therefore the residual amount of monomer present in the polymer that enters the drinking water. The polyDADMAC standard [ANSI/AWWA 451-98] does not deem it compulsory to monitor the product for dimethyl amine or allyl chloride. It is left to the discretion of the product manufacturers.

A1.5 Dose Response Assessment

Assuming a worst case scenario at the maximum permitted use level of 10 mg L^{-1} and 5% of residual monomer [ANSI/AWWA 451-98], the amount of mDADMAC that will enter the final water can be calculated as follows:

$$\begin{aligned} Maximum\ use\ level\ of\ \ polyDADMAC &= 10\ mg\ L^{-1} \\ &= 10 \times 10^{-6}\ kg\ L^{-1} \\ Maximum\ DADMAC\ concentration &= 5\%\ of\ 10 \times 10^{-6}\ kg\ L^{-1} \\ &= \frac{5}{100} \times 10 \times 10^{-6}\ kg\ L^{-1} \\ &= 500\ \mu g\ L^{-1} \\ Daily\ consumption\ of\ \ water &= 2\ L \\ Worst\ case\ daily\ exposure\ to\ DADMAC &= 1000\ \mu g \end{aligned}$$

Since polyDADMAC containing at least 1% mDADMAC residual resulted in a NOAEL of 125 mg/kg bw/day in a 2 generation rat feeding study for reproductive effects, an implied NOAEL for mDADMAC of 1.25 mg/kg bw/day can be calculated [DADMAC HPV Committee, 2005]. For a 70 kg adult, this equates to a daily dose of 87.5 mg/day or 87500 µg/day. Taking into account the worst case estimate of exposure of 1000 µg/day, this converts to 87.5 times less than the given NOAEL. In general, from the experiences at Umgeni Water, the amount of polymer dosed ranges from 1 to 2 mg L^{-1}. This is 5 to 10 times less than the level of the worst case scenario of 10 mg L^{-1}. The level of monomer exposure in this scenario is therefore 5 to 10 times less, bringing the exposure estimate 438 to 875 time less than the given NOAEL. The risk associated with exposure to mDADMAC through drinking water can therefore be deemed insignificant under normal conditions of use. However the risks may become significant in cases of overdosing, accidental spills or dumping.

A1.6 Risk Characterization and Conclusions

Based on the toxicity studies conducted on both the monomer and polymer, there appears to be a negligible amount of risk associated with the production and use of polyDADMAC. This is very reassuring to water authorities, but more importantly to the general public. However, risks may arise as a result of overdosing, spills into drinking water or accidental dumping.

A1.7 Toxicity Testing

Some in-house toxicity tests were conducted on the polymer since these tests were available and carried out routinely in the laboratories of the author.

A1.7.1 Materials and Methods

A1.7.1.1 Test Organism: *Poecilia Reticulata* (1 to 3 weeks old juveniles)

A1.7.1.2 Polymer Test Samples: Polymer samples were prepared at 1; 32; 163 and 4173 mg L^{-1} Z553D in Milli-Q water.

A1.7.1.3 The Control: Milli-Q water was used as the control in the experiment.

A1.7.1.4 Procedure: To 400 mL of the test sample in a 1 L glass beaker was added 5 juvenile guppies. The experiment was monitored over a period of 96 h and the mortality recorded every 24 h.

A1.7.2 Results and Discussion

In the initial experiment three polymer samples at concentrations of 32 mg L^{-1}, 163 mg L^{-1} and 4173 mg L^{-1} Z553D prepared in Milli-Q water were used in the study. Each test was conducted in duplicate. Typical experiments were monitored over a period of 96 h and the mortality recorded after every 24 h. In this experiment, all 30 fish used in the six samples died in the first 4 h. The fish in the control were alive and appeared normal and active. In a second experiment, the polymer concentration was reduced to 1 mg L^{-1}, and the pH was adjusted to 7. It is a well know fact that the juveniles fish species (*Poecilia Reticulata*) are very sensitive to pHs of less than 6 (polymer pH 2 to 4), which may have been responsible for the mortality in the first experiment. The reduction in polymer concentration and the adjustment in pH had no impact on the results and there was 100% mortality within 4 h.

A1.7.3 Conclusions

This study indicated that the polymer was toxic to fish and even though the LC_{50} was not determined it gave a result that was in agreement with that obtained by Johnson [Johnson, 1971].

A1.8 References

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APPENDIX 2

OZONE OUTPUT DETERMINATION

A2.1 Calibration of the Ozone Generator

The unit was turned on for 45 min prior to use for equilibration and production of a constant flow rate of ozone. The ozone output was determined after 40 min by iodometric titration. This involved the preparation and standardization of $Na_2S_2O_3$ for use in determining the ozone dosage by direct and indirect titration methods. The technique for ozone output determination involves determination of the amount of I_2 liberated as a result of the oxidation of a 2% KI solution used in the column.

A2.2 Standardization of *ca* 0.01 N $Na_2S_2O_3$

The number of equivalents of KI oxidized to iodine corresponds to the number of equivalents of $Cr_2O_7^{2-}$ consumed during the oxidation process. The I_2 is then reduced by the $S_2O_3^{2-}$ and the concentration is determined by calculations. The equations for the above sequence of reactions are as follows:

$$Cr_2O_7^{2-} + 14H^+ + 6e^- \rightleftharpoons 2Cr^{3+} + 7H_2O \quad \text{Reduction}$$

$$6I^- \rightleftharpoons 3I_2 + 6e^- \quad \text{Oxidation}$$

$$Cr_2O_7^{2-} + 14H^+ + 6I^- \rightleftharpoons 2Cr^{3+} + 7H_2O + 3I_2$$

The I_2 produced is then titrated with thiosulfate according to the reaction

$$2S_2O_3^{2-} + I_2 \rightleftharpoons S_4O_6^{2-} + 2I^-$$

The titration was done in triplicate and the results (Table A2.1) were used in the calculation of the $Na_2S_2O_3$ concentration.

Table A2.1: Titration of 0.01N $K_2Cr_2O_7$ with *ca* 0.01 N $Na_2S_2O_3$.

	Volume $Na_2S_2O_3$ (mL)		
Solution ID	**Initial**	**Final**	**Titer**
1	0.00	13.04	13.04
2	0.00	13.32	13.32
3	0.00	13.69	13.69
Blank	0.00	0.08	0.08
		Mean	13.27

At equivalence point, the number of equivalents of Cr_2O_7 used corresponds to the number of equivalents of I_2 liberated. This in turn corresponds to the number of equivalents of S_2O_3 required for the reduction of the I_2. The normality was calculated using equations A2.1 and A2.2.

$$equivalents\ Cr_2O_7^{2-} = N_{Cr_2O_7^{2-}} \times V_{Cr_2O_7^{2-}} \qquad A2.1$$
$$= 0.01 \times 10$$
$$= 0.1\ \ equivalent$$

$$equivalents\ Cr_2O_7^{2-} = equivalents\ I_2 \qquad A2.2$$
$$= equivalents\ S_2O_3^{2-}$$
$$= N_{S_2O_3^{2-}} \times V_{S_2O_3^{2-}}$$
$$N_{S_2O_3^{2-}} = \frac{0.1}{13.27}$$
$$= 0.0075N$$

A2.3 Ozone Output Test

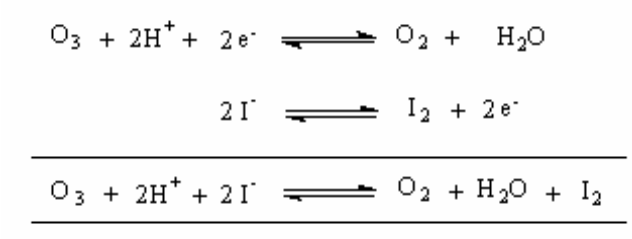
The aim of the first experiment was to determine the effect of ozonation on polyDADMAC. The ozonizer was switched on after directing pure oxygen at 0.5 bars into the unit and adding 2% KI in the three ozone trap impingers for the destruction of excess ozone. The three-way valve V_1 was set to the column by-pass mode and the unit left on for 30 min.

Ozone destruction occurred in impingers 1 and 2. This was followed by the ozone output determination. Four liters of 2% KI prepared in ozone-demand-free water was added to the column via the sample loading port (V_2). This solution was ozonized for 40 min. Ozone oxidizes KI to iodine. Aliquots of 50 mL were removed via V_3 for iodine/ozone determination by titration with standard thiosulfate (Table A2.2).

Table A2.2: Titration of ozonated KI solutions with standard 0.0075 N $S_2O_3^{2-}$.

	Volume $Na_2S_2O_3$ (mL)		
Solution ID	**Initial**	**Final**	**Titer**
1	0.00	93.25	93.25
2	0.00	100.03	100.03
		Mean	96.64

At equivalence point the number of equivalents of S_2O_3 used in the titration corresponds to the number of equivalents of I_2 formed from the oxidation of KI by O_3. The ozone output was then determined by calculations (equations A2.3 to A2.5). The ozone half reactions are as follows:



$$equivalents\ S_2O_3^{2-} = N_{S_2O_3^{2-}} \times V_{S_2O_3^{2-}} \qquad A2.3$$
$$= 0.0075 \times 96.64$$
$$= 0.7248\ equivalents$$

$$equivalents\ S_2O_3^{2-} = equivalents\ I_2 = equivalents\ ozone \qquad A2.4$$
$$= N_{ozone} \times V_{ozone}$$
$$N_{ozone} = \frac{equivalents\ ozone}{V_{ozone}} = \frac{0.7248}{50}$$
$$= 0.0145\ N$$

$$N_{ozone} = \frac{Mass_{ozone}}{equivalent\ Mass_{ozone}} \times \frac{1000}{Volume_{sample}} \qquad A2.5$$
$$Mass_{ozone} = \frac{0.014496 \times 24 \times 50}{1000}$$
$$= 0.0174\ g\ per\ 50\ mL$$
$$= 347\ mg\ L^{-1}\ \left(40\ min\ dose\right)$$

A2.4 Sample Ozonation

The KI solution was removed from the column and replaced with the test sample. A 4 L volume of sample (0.5% Z553D, m/v) was poured into the column while the ozone was switched to the by-pass mode. Impinger 3 placed in series with the column was filled with 2% KI for the destruction of any excess ozone passing through the sample. The sample was ozonized for exactly 40 min. The excess ozone during dosing was consumed in KI impinger trap 3, resulting in the formation of I_2 by oxidation of KI. A 50 mL aliquot of this solution was removed for titration (Table A2.3).

Table A2.3: Titration of ozonated KI solutions with standard 0.0075 N $S_2O_3^{2-}$.

	Volume $Na_2S_2O_3$ (mL)		
Solution ID	**Initial**	**Final**	**Titer**
1	0.00	75.65	75.65
2	0.00	75.65	75.65
		Mean	75.65

At equivalence point the number of equivalents of S_2O_3 used in the titration corresponds to the number of equivalents of I_2 formed from the oxidation of KI by O_3. The mass of ozone was calculated using equations A2.6 to A2.8.

$$equivalents\ S_2O_3^{2-} = N_{S_2O_3^{2-}} \times V_{S_2O_3^{2-}} \qquad A2.6$$
$$= 0.0075 \times 75.65$$
$$= 0.5674\ equivalents$$

$$equivalents\ S_2O_3^{2-} = equivalents\ I_2 = equivalents\ ozone \qquad A2.7$$
$$= N_{ozone} \times V_{ozone}$$
$$N_{ozone} = \frac{equivalents\ ozone}{V_{ozone}} = \frac{0.5674}{50}$$
$$= 0.0113\ N$$

$$N_{ozone} = \frac{Mass_{ozone}}{equivalent\ Mass_{ozone}} \times \frac{1000}{Volume_{sample}} \qquad A2.8$$
$$Mass_{ozone} = \frac{0.0113 \times 24 \times 50}{1000}$$
$$= 0.0136\ g\ per\ 50\ mL$$
$$= 272\ mg\ L^{-1}\ \ (40\ min\ dose)$$

A2.5 Ozone Dosage Calculation

The ozone dosage of the test sample was calculated using two methods, as follows:

Method 1: Indirect Method

$$Ozone\ Dose = Total\ Ozone\ Output - Excess\ in\ Im\ pinger\ 3 \qquad A2.9$$
$$= 347 - 272$$
$$= 76\ mg\ L^{-1}$$

Method 2: Direct Method

This was done by titrating an aliquot of the dosed test sample with a freshly prepared and standardized thiosulfate solution (0.014 N). Standardization was done as described in Section 4.3.2 and the titer volumes are shown in Table A2.4.

Table A2.4: Titration of ozonated test samples with standard 0.014 N $S_2O_3^{2-}$.

	Volume $Na_2S_2O_3$ (mL)		
Solution ID	**Initial**	**Final**	**Titer**
1	0.00	9.60	9.60
2	9.60	20.50	10.90
3	20.50	30.50	10.00
		Mean	10.17

The mass of ozone was calculated using equations A2.10 and A2.11.

$$N_{ozone} = \frac{N_{S_2O_3^{2-}} \times V_{S_2O_3^{2-}}}{V_{sample}} \qquad A2.10$$

$$= \frac{0.01449 \times 10.17}{50}$$

$$= 0.0030\ N$$

$$N_{ozone} = \frac{Mass_{ozone}}{equivalent\ Mass_{ozone}} \times \frac{1000}{V_{sample}} \qquad A2.11$$

$$Mass_{ozone} = \frac{0.0030 \times 24 \times 50}{1000}$$

$$= 3.54\ mg\ \ per\ 50mL$$

$$= 71\ mg\ L^{-1}$$

This value is marginally less than the first value obtained using the indirect method of analysis. The small difference may be attributed to ozone degradation or ozone dissipation as the titration using Method 2 was carried out after standing overnight. The results are however useful and give a good indication that the calculations were correct. The result obtained with the indirect method was obtained immediately after ozonation, and are therefore likely to be more accurate.

APPENDIX 3

THE TANNIC ACID METHOD

A3.1 Results and Discussion

In the first experiment the %T readings for a series of TA-polymer solutions were taken at the wavelengths used by Hanasaki et al. [1985] (Table A3.1). A blank solution of TA containing no added polymer was also included in the experiment.

Table A3.1: %T data obtained for polyDADMAC with the TA method.

Sample ID	Polymer Conc. (mg L^{-1})	%T (554 nm)	%T (830 nm)
Blank	0	99.044	99.703
Std 1	1	92.979	96.455
Std 2	2	92.277	96.092
Std 3	5	95.657	98.868
Std 4	10	95.132	99.298

The corresponding data plots (Figure A3.1) show that at 1 and 2 mg L^{-1} the expected decreasing trend can be observed, although the magnitude of the decrease is very small or can be regarded as negligible. At 5 mg L^{-1} there is an unexpected increase in %T at both wavelengths (*ca* 3%).

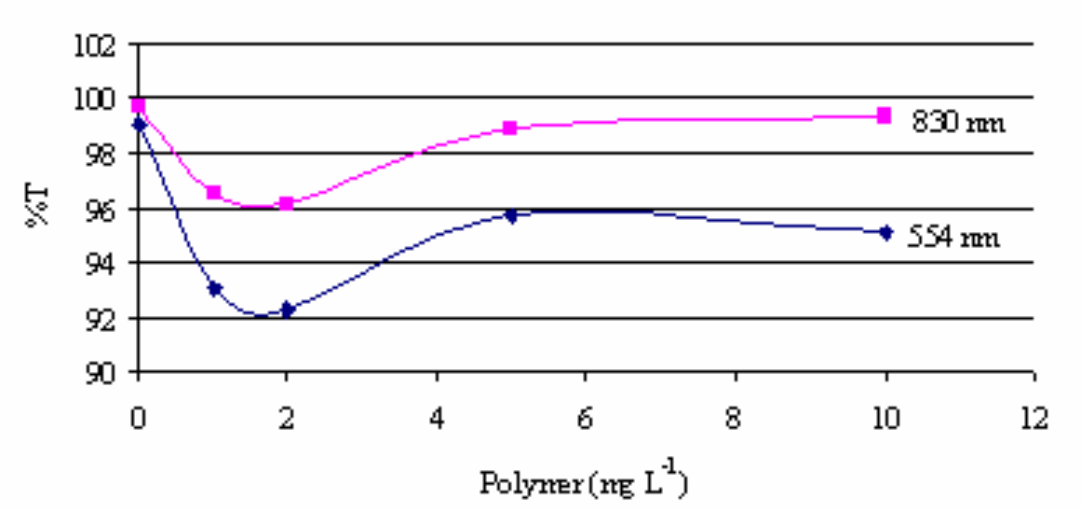


Figure A3.1: Plot of %T as a function of polymer mass at 554 nm and 830 nm.

It was suspected that either the analytical wavelengths were not sufficiently sensitive or the TA added was insufficient, resulting in a charge reversal and the formation of re-stabilized positively charged colloidal particles at high polymer levels. To verify this hypothesis, the standards were treated with a further 5 mL of TA reagent. The results obtained (Table A3.2) showed a better correlation between the amount of polymer and the %T.

Table A3.2: %T data obtained for polyDADMAC with the TA method after the addition of a further 5 mL TA reagent.

Sample ID	Polymer (mg L^{-1})	%T (554 nm)	%T (830 nm)
Blank	0	100	100
Std 1	1	90.350	94.330
Std 2	2	83.649	90.439
Std 3	5	58.029	75.204
Std 4	10	80.731	94.563

The corresponding data plot (Figure A3.2) indicates the expected decreasing trend up to 5 mg L^{-1} of polymer. It can also be noted that the magnitude of the decrease is significant when compared to the previous experiment (Table A3.1). However, the charge reversal phenomenon is observed once more at 10 mg L^{-1} of polymer.

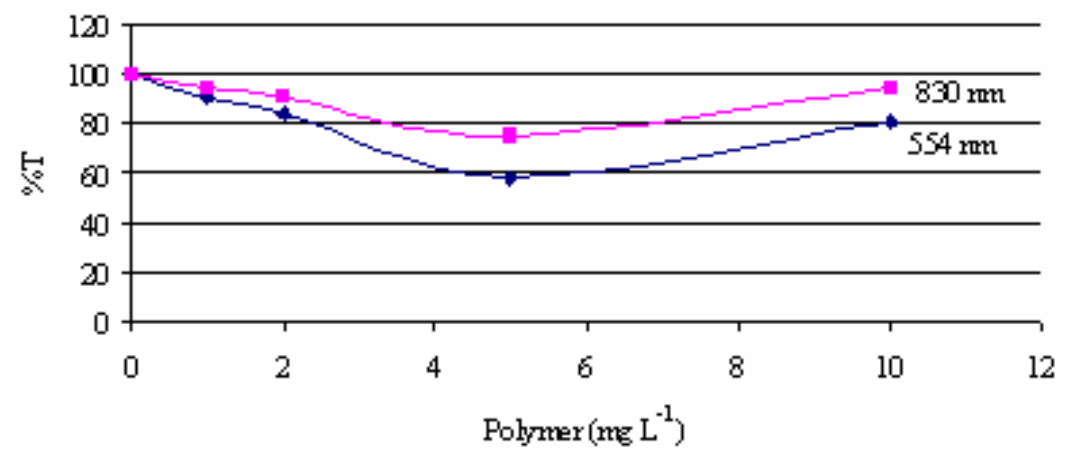


Figure A3.2: Graph of %T as a function of polymer mass at 554 and 830 nm after the addition of a further 5 mL TA.

In view of the above findings it was decided to make use of a 1% TA reagent for all future experiments. To investigate the sensitivity aspect of the analytical wavelength, a control sample (blank) and a 2 mg L^{-1} polymer-TA mixture was scanned in the visible region (Figure A3.3).

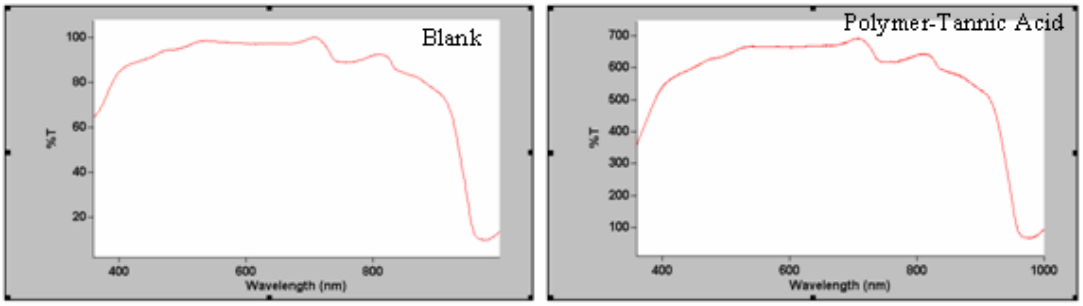


Figure A3.3: UV scans of a TA blank and a 2 mg L^{-1} TA-polymer solution in the wavelength range 800 to 400 nm.

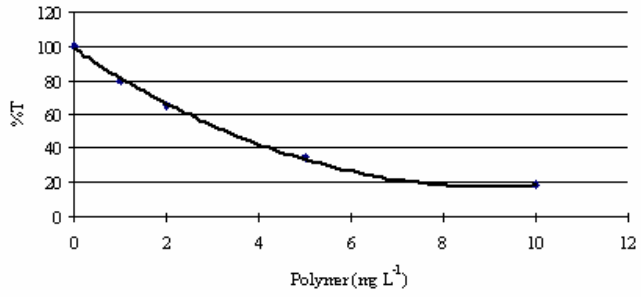
The scans show that both the blank and the test sample exhibit transmittance of light over a broad wavelength range. There are no discreet wavelengths evident in the scans. Transmittance is low in the region less than 400 nm (UV region) and greater than 800 nm (IR region). Only the visible region (800 to 400 nm) was selected as the useful analytical region. Based on %T evidence from the scans, the choice of wavelengths at which to record measurements was not critical. The use of a higher concentration of TA-polymer solutions may be required to reveal further details on the wavelength of optimum transmittance as found by Hanasaki et al. [1985] to be at 553 and 830 nm. Figure A3.2 shows clearly that as more TA is used, a significant change in the %T can be observed. When compared to Figure A3.1 the magnitude of the decrease in %T of the standards is negligible.

In the second experiment the effect of the TA concentration was taken into account. It was increased to 1%. Polymer calibration standards were prepared as previously and a further two test samples were prepared by spiking both tap and Milli-Q water with polymer at a concentration of 2 mg L^{-1}. The %T data measured at 553 nm are shown in Table A3.3.

Table A3.3: %T data obtained for TA-polymer standards and test samples using a 1% TA reagent.

Sample ID	Polymer Conc. (mg L^{-1})	%T (553 nm)
Blank	0	99.882
Std 1	1	79.659
Std 2	2	65.403
Std 3	5	34.546
Std 4	10	18.271
Tap Water	2	67.340
Milli-Q Water	2	60.830

The data were used in regression analysis and the calibration graph (Figure A3.4) generated. Purely by inspection, a non-linear response can be noted, with the regression line having a correlation coefficient $R^2 = 0.894$.



Regression Statistics		
Multiple R	0.95	
R Square	0.894	
Adjusted R Square	0.86	
Standard Error	12.48	
Observations	5.00	STDEV
Intercept ± STDEV	87.49	7.9
Slope ± STDEV	-7.76	1.5
y = -7.76x + 87.49		

Figure A3.4: Linear regression calibration curve for polyDADMAC using the TA method.

Using the linear regression equation, the values for the test samples were found to be 2.64 mg L^{-1} and 3.44 mg L^{-1}. This corresponded to accuracies of 104% and 137% for tap water and Milli-Q water respectively. The method was evaluated further by

repeating the experiment with freshly prepared reagents and calibration standards. However there were no improvements in the linearity of the calibration function and the method was not pursued any further.

A3.2 References

1. Hanasaki T, Ohnishi H, Nikaidoh A, Tanada S, Kawasaki K, Bull. Environ. Contam. Toxicol., 35, 476 (1985).

APPENDIX 4

THE ROSE BENGAL METHOD

A4.1 Results and Discussion

Initial studies were conducted to determine the λ_{max} of the rose bengal dye. A scan of a dilute solution in the visible region of the electromagnetic spectrum (800 to 400 nm) was recorded. The scan (Figure A4.1) shows that λ_{max} is at 549.5 nm.

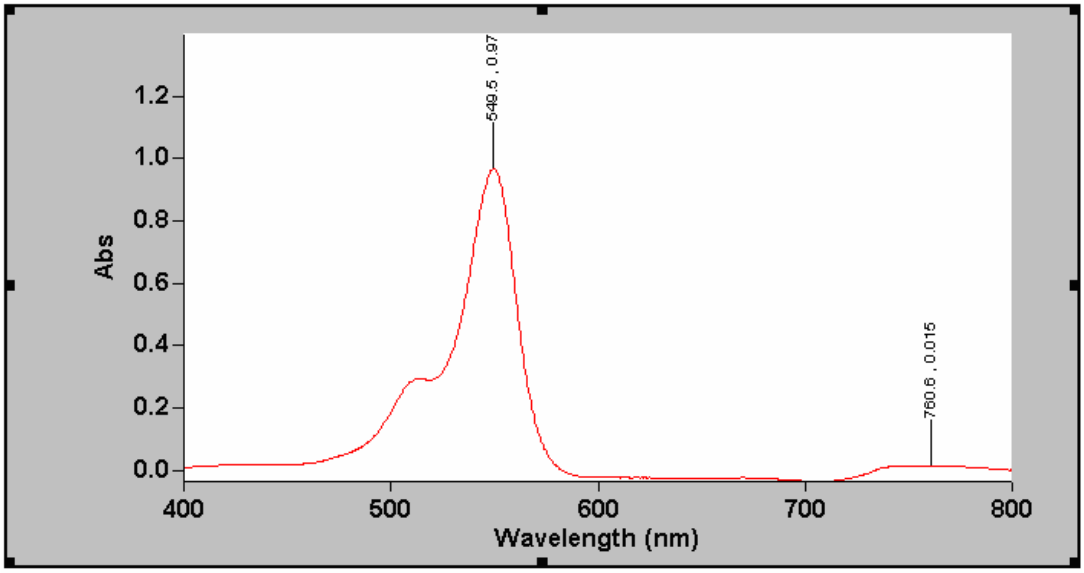


Figure A4.1: UV/VIS absorption spectrum of rose bengal.

When a small amount of polymer was added to the dye there was a color change from pink to light pink, indicating the complexation of rose bengal from solution. Further additions of polymer to the dye solution resulted in additional reductions in the intensity of the color. This was attributed to a continuation of polymer-dye complex formation. Due to the extremely dilute concentrations of both polymer and dye, no physical precipitate was observed. However, the UV/VIS spectrum of the complex (Figure A4.2) shows a change in λ_{max} from 549.5 to 559.1 nm. A decrease in absorbance from 0.970 to 0.738 was also noted.

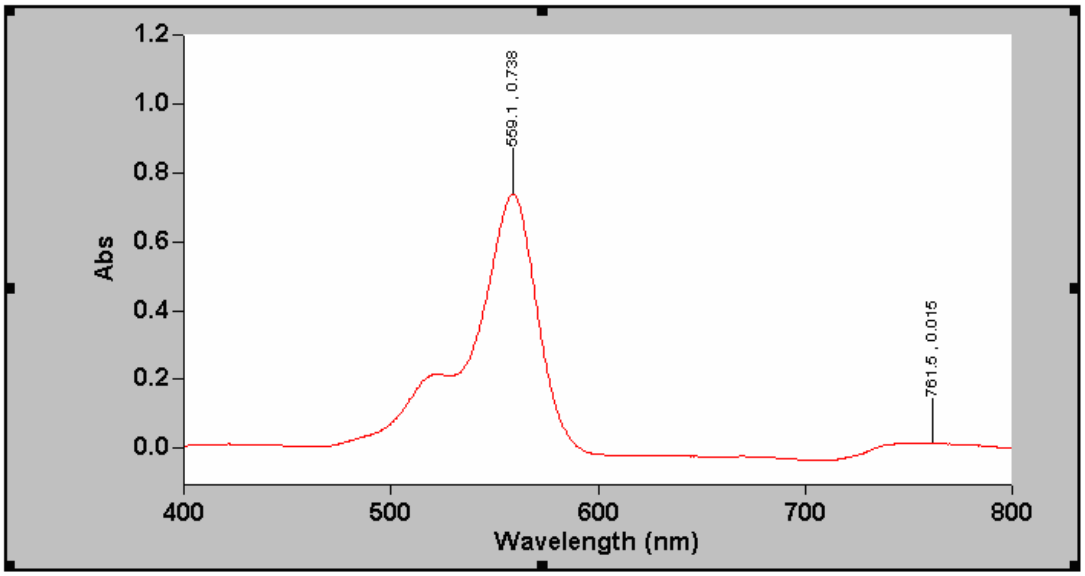


Figure A4.2: UV/VIS spectrum of a polyDADMAC-rose bengal complex.

The fact that the change in optical properties of the solution was accompanied by a change in the absorbance at 549 nm was exploited in an attempt to determine the nature of the relationship between the amount of polymer added to a fixed concentration of a dye solution. The data obtained for a series of polymer-dye solutions (Table A4.1) were used for the preparation of a calibration curve.

Table A4.1: Absorbance data for the polymer-dye calibration solutions.

Sample ID	Polymer Conc.	Absorbance
Blank	0	2.77
Solution 1	1	1.20
Solution 2	2	1.10
Solution 3	3	1.03
Solution 4	4	0.93
Solution 5	5	0.82

A plot of the absorbance-concentration data (Figure A4.3) shows a sharp drop in absorbance between the blank and the first polymer-dye solution. This is followed by a more linear response for all remaining solutions. The plot indicates that the range between 0 to 1 mg L^{-1} is not valid for this method.

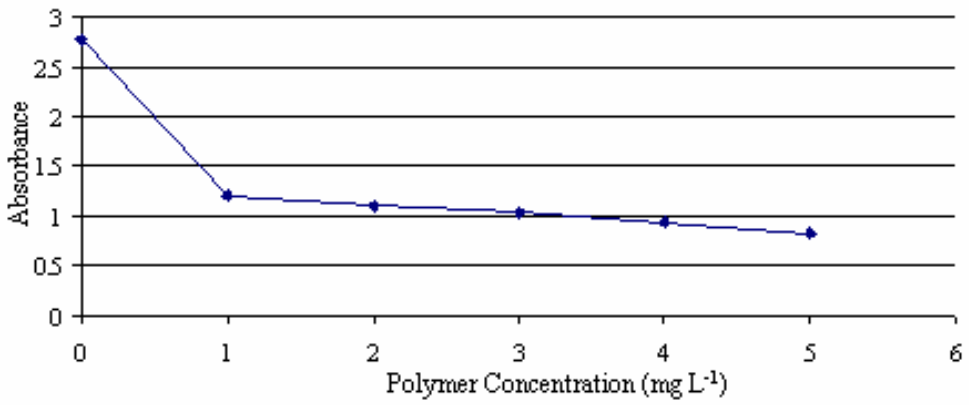


Figure A4.3: Calibration curve obtained with the rose bengal method.

Attempts were made to manipulate the data to obtain a better correlation between the absorbance and the polymer-dye complex. The difference in absorbance between the blank and the test samples (ΔAbs) was used rather than the absolute absorbance (Table A4.2).

Table A4.2: ΔAbs data for the polymer-dye calibration solutions.

Sample ID	Polymer Conc. (mg L^{-1})	ΔAbsorbance (AU)
Blank	0	0
Solution 1	1	1.57
Solution 2	2	1.67
Solution 3	3	1.74
Solution 4	4	1.84
Solution 5	5	1.95

$\Delta Abs = Abs_{blank} - Abs_{sample}$

A plot of the ΔAbs data resulted in a similar trend (Figure A4.4), with no improvement in the linearity of the region 0 to 1 mg L^{-1}.

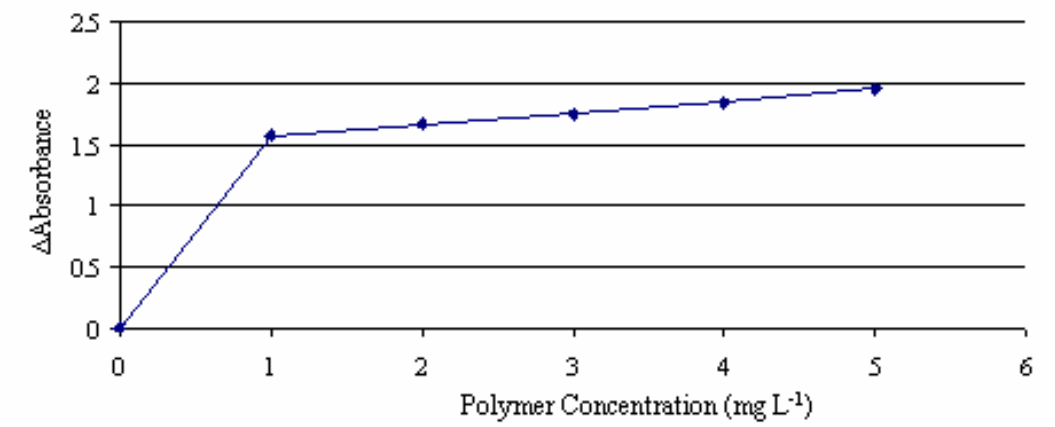


Figure A4.4: Calibration curve obtained using the rose bengal complexation method and ΔAbs data.

Although this is the preferred method as it is independent of and unaffected by the dye concentration, the ΔAbs data had no influence on the method performance. New experiments were designed to investigate whether centrifuging the polymer-dye complex would improve the calibration.

In a second experiment polymer-dye solutions were prepared and absorbance measurements taken both immediately, and after centrifugation (Table A4.3).

Table A4.3: Absorbance data for the polymer-dye solutions before and after centrifugation.

Sample ID	Polymer Conc. (mg L^{-1})	Absorbance		
		Uncentrifuged (a)	Centrifuged (b)	a – b
Blank	0	1.08	0.62	0.46
Solution 1	1	0.44	0.14	0.30
Solution 2	2	0.44	0.09	0.35
Solution 3	3	0.46	0.08	0.38
Solution 4	4	0.48	0.06	0.42
Solution 5	5	0.49	0.06	0.43
Spike	3.5	0.49	0.06	0.43

Use of a plot of the difference in absorbance values (uncentrifuged-centrifuged) as a function of polymer concentration was proposed as a good method to test the calibration function. This procedure was used to remove the polymer-dye complex once formed and prevent it from causing any absorption interference during spectrophotometric measurements. The data plot (Figure A4.5) shows an unusually high value for the blank, resulting in an outlier. This was attributed to the removal of dye from solution during the centrifugation process and hence impacting on the Absorbance. This was unexpected as the blank comprised only rose bengal and no polymer to complex out the dye. The blank solution was expected to give an almost equal response for both uncentrifuged and centrifuged forms, giving rise to a nearly zero absorbance difference.

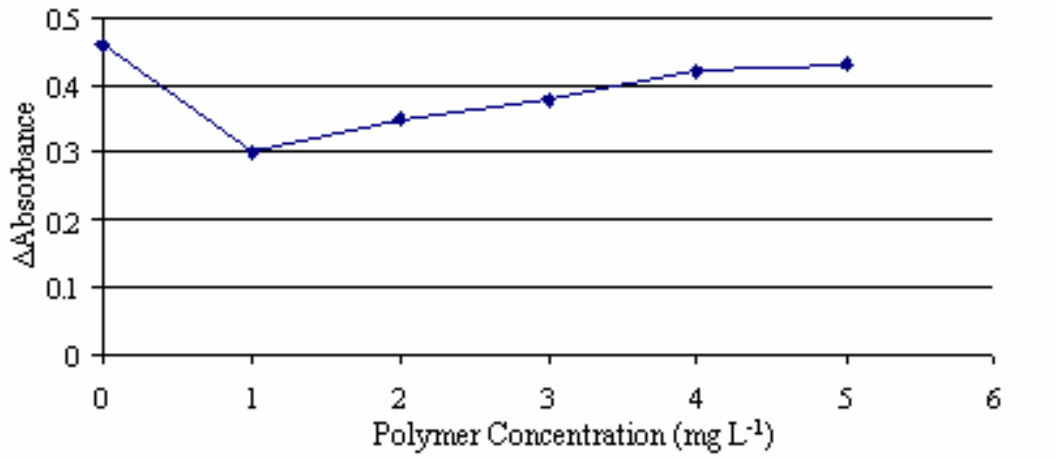


Figure A4.5: Calibration curve obtained using the rose bengal complexation method and ΔAbs data obtained by centrifugation.

Another point to note is that all the absorbance values for the centrifuged samples were extremely small in magnitude, implying that most of the dye was removed during the complexation and centrifugation process. There was concern that either an insufficiently large excess of dye was used in the calibration solutions or that an excessively high concentration of polymer was used as the dosing solution. Apart from the outlier data point for the blank, the remaining points produced a reasonably linear response.

In the third experiment freshly prepared samples of polymer-dye solutions were analyzed (Table A4.4). The blank was found to be affected more adversely than in all previous experiments. The absorbance difference was massive at 1 AU, indicating that the bulk of the dye was removed purely by centrifugation.

Table A4.4: Absorbance data for the polymer-dye solutions before and after centrifugation for Experiment 3.

Sample ID	Polymer Conc. (mg L^{-1})	Absorbance		
		Uncentrifuged (a)	Centrifuged (b)	a - b
Blank	0	1.10	0.10	1.00
Solution 1	1	0.47	0.16	0.31
Solution 2	2	0.49	0.11	0.38
Solution 3	3	0.49	0.08	0.41
Solution 4	4	0.52	0.05	0.47
Solution 5	5	0.55	0.02	0.53
Spike	3.5	0.50	0.06	0.44

This was cause for great concern as it was regarded as an interferent in the actual mechanism upon which the test method was based. A plot of the data (Figure A4.6) shows a similar profile to that previously obtained. Added to the problem experienced with the blank, the absorbance values of the centrifuged test samples were negligibly different from each other, once more reinforcing the fact that not only was the polymer-dye complex removed but also the remaining un-complexed dye that was the indicator of polymer concentration.

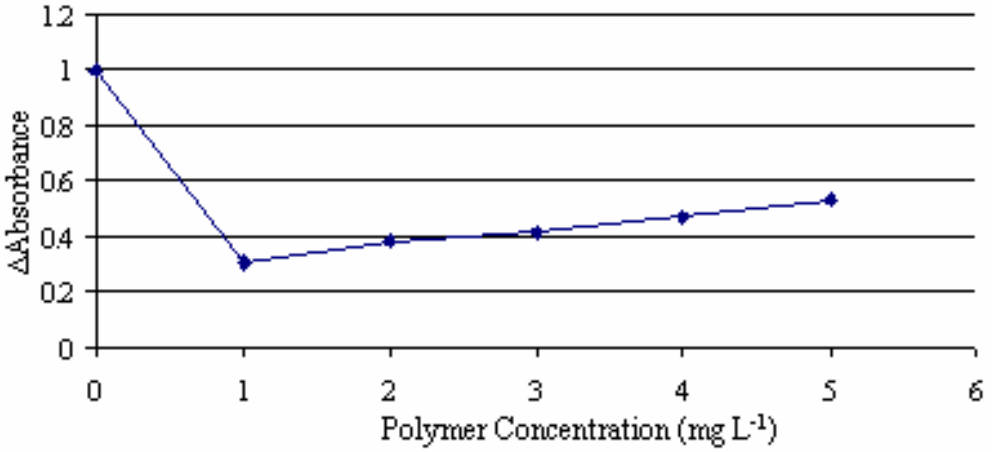


Figure A4.6: Calibration curve obtained in Experiment 3 using the rose bengal method.

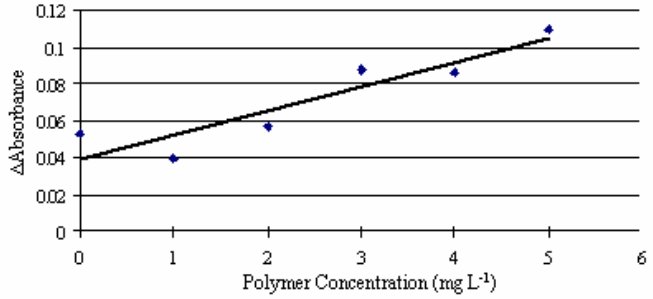
A further problem was that the response to polymer concentration was very weak for both centrifuged and un-centrifuged samples. This is reflected in the very gentle gradient of the curve.

Experiment 4 was conducted in an attempt to resolve the problem experienced with the blank. Finally, the absorbance data (Table A4.5) showed the expected trend.

Table A4.5: Absorbance data for the polymer-dye solutions before and after centrifugation for Experiment 4.

Sample ID	Polymer Conc. ($mg\ L^{-1}$)	Absorbance		
		Uncentrifuged (a)	Centrifuged (b)	a - b
Blank	0	1.03	0.98	0.05
Solution 1	1	0.42	0.38	0.04
Solution 2	2	0.43	0.38	0.06
Solution 3	3	0.43	0.34	0.09
Solution 4	4	0.44	0.35	0.09
Solution 5	5	0.45	0.34	0.11
Spike	3.5	0.43	0.36	0.07

The absorbance for both, uncentrifuged, and centrifuged, is in agreement with the theoretical predictions. They are almost equal in magnitude, and the difference is close to zero, as expected. The absorbance of the test solutions were also greatly improved and are almost an order of magnitude greater than previous runs. However, the magnitude of the response to changing polymer concentration was still very weak. The data were used in regression analysis and a calibration curve (Figure A4.7) with a correlation coefficient R^2= 0.842 was obtained, indicating very poor linearity.



Regression Statistics		
Multiple R	0.92	
R Square	0.842	
Adjusted R Square	0.80	
Standard Error	0.01	
Observations	6.00	STDEV
Intercept ± STDEV	0.04	0.01
Slope ± STDEV	0.01	0.00
y = 0.01x + 0.04		

Figure A4.7: Linear regression line obtained with the rose bengal method.

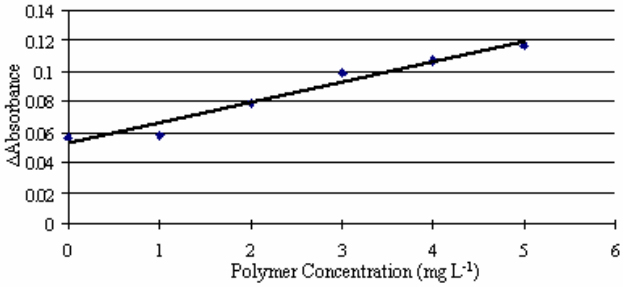
The slope also indicates very poor sensitivity of the method. The analysis of the fortified test sample gave a result of 3.41 $mg\ L^{-1}$, which was calculated as 97% of the true value.

The final experiment with this method was repeatable and produced similar results to Experiment 4 (Table A4.6). The blank produced good results for both centrifuged and uncentrifuged samples.

Table A4.6: Absorbance data for the polymer-dye solutions before and after centrifugation, final experiment.

Sample ID	Polymer Conc. (mg L^{-1})	Absorbance		
		Uncentrifuged (a)	Centrifuged (b)	a - b
Blank	0	1.04	0.98	0.06
Solution 1	1	0.43	0.37	0.06
Solution 2	2	0.44	0.36	0.08
Solution 3	3	0.44	0.34	0.10
Solution 4	4	0.44	0.34	0.11
Solution 5	5	0.45	0.33	0.12
Spike	3.5	0.43	0.34	0.09

Once more it was found that although the magnitude of the absorbance was sufficiently high, the response with increasing polymer concentrations was very small to almost negligible. Using the data for regression analysis, the calibration curve obtained (Figure A4.8) shows a slight improvement in the degree of linearity with a correlation coefficient $R^2 = 0.961$.



Regression Statistics		
Multiple R	0.98	
R Square	0.961	
Adjusted R Square	0.95	
Standard Error	0.01	
Observations	6.00	STDEV
Intercept ± STDEV	0.05	0.00
Slope ± STDEV	0.01	0.00
y = 0.013x + 0.05		

Figure A4.8: Linear regression line obtained with the rose bengal complexation method and ΔAbs data.

However, sensitivity was very poor, as can be noted from the slope of the calibration function. The method was plagued with problems and produced a very weak response for increasing polymer concentrations. The method exhibited very little potential for application to real samples even although the spiked sample gave an accuracy of 97%. There was some doubt that there was any complexation taking place as the absorbance variation from the lowest to the highest standard was 0.06 AU. Instrument drift is known to produce similar or even higher values. The results were therefore viewed with skepticism and no further work was conducted with this method of polymer complexation.

APPENDIX 5

THE METHYL ORANGE METHOD

A5.1 Results and Discussion

Standard solutions of the MO-polyDADMAC complex were scanned to determine the relationship between absorbance and polyDADMAC concentration. The UV/VIS scans of each solution (Figure A5.1) showed no trend or relationship.

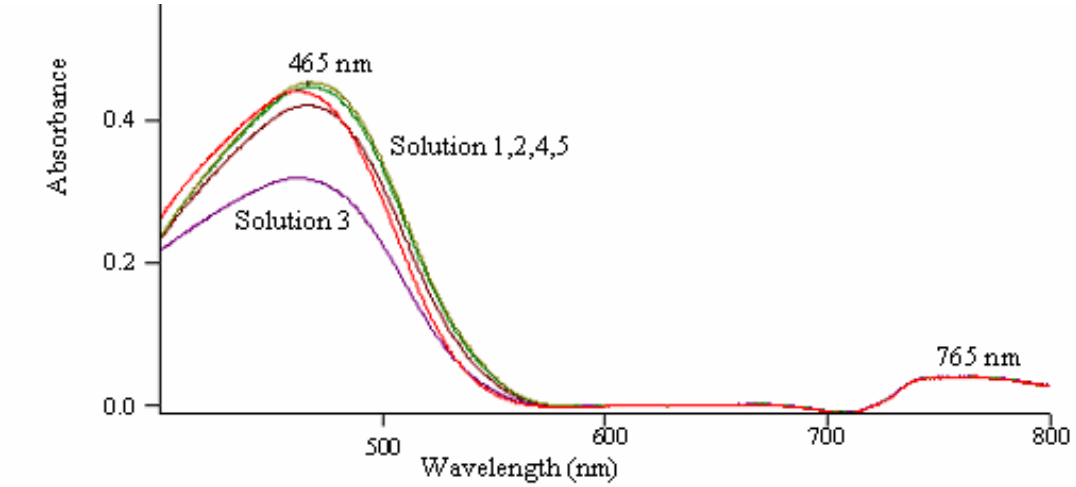


Figure A5.1: Overlay of UV/VIS scans for the MO-polyDADMAC solutions.

Only the wavelength of maximum absorbance (λ_{max}) occurred repeatedly at 465 and 765 nm. The corresponding absorbance values (Table A5.1) show no correlation between absorbance and concentration of the standards.

Table A5.1: Absorbance readings for the 0 to 4 mg L^{-1} MO-polymer standard solutions.

Polymer (mg L^{-1})	λ_{max1}	Absorbance 1	λ_{max2}	Absorbance 2
0	465	0.441	765	0.04
1	465	0.319	765	0.04
2	465	0.421	765	0.04
3	465	0.440	765	0.04
4	465	0.454	765	0.04

It was suspected that the complex did not form as expected for unknown reasons or, alternatively, it was speculated that the polymer amounts added were too small and resulted in a negligible change in the absorbance intensity. A follow-up experiment was conducted in which the concentration of the polymer stock solution was increased by an order of magnitude to 10 000 mg L^{-1}. Once more the results (Table A5.2) showed no trend or relationship between the polymer added and the absorbance intensity at either of the two wavelengths.

Table A5.2: Absorbance readings for MO-polymer standard solutions.

Polymer (mg L^{-1})	λ_{max1}	Absorbance 1 465 nm	λ_{max2}	Absorbance 2 765 nm
0	465	0.726	765	0.128
10	465	0.837	765	0.129
20	465	0.851	765	0.128
30	465	0.856	765	0.129
40	465	0.867	765	0.130

During the experiment, a color change from orange to red was observed upon addition of the polymer. The UV/VIS scan (Figure A5.2) however, showed no change in the absorption spectrum when a control sample of MO solution containing zero polymer was compared to a test sample containing 20 mg L^{-1} of polymer. Only a difference in the intensity can be observed with the test sample having a higher absorbance.

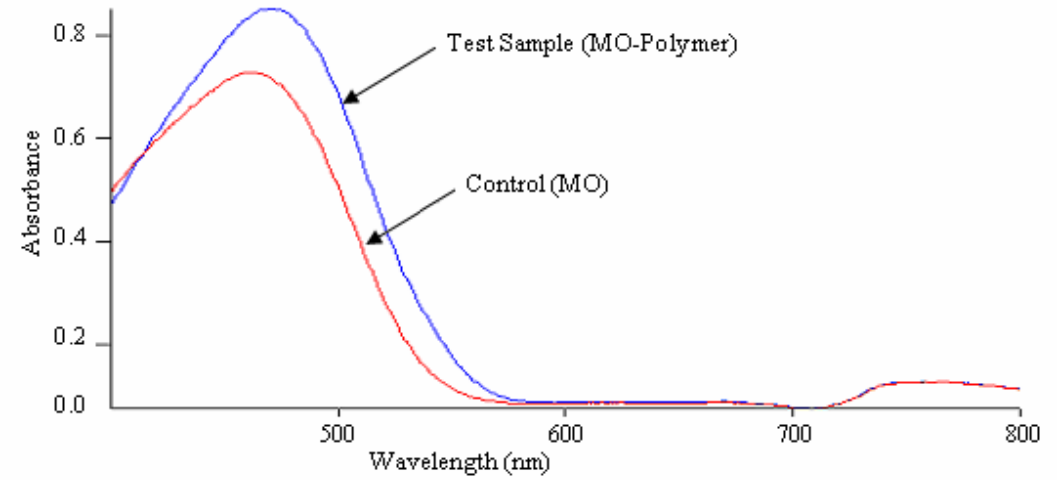
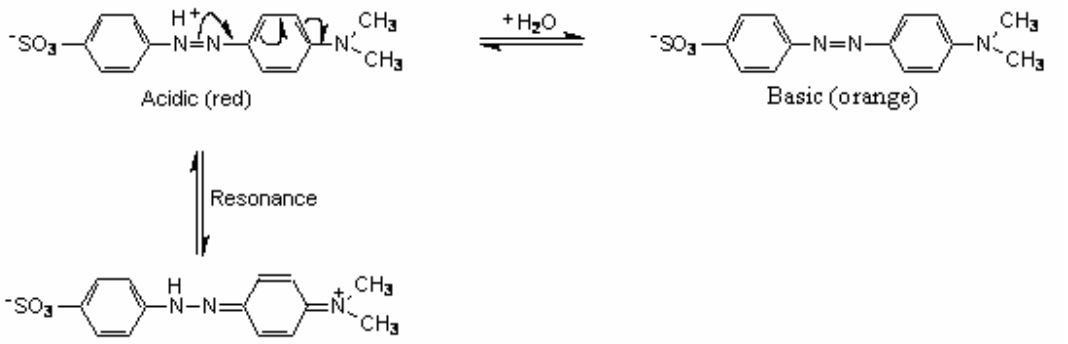


Figure A5.2: Overlay of UV/VIS spectra of the control and test sample of a MO-polyDADMAC complex.

The color change from orange to red can be explained by the fact that MO is a pH sensitive dye known to undergo this change. In the above case, the polymer added to MO was at an acidic pH and the acidic and basic forms are explained by Scheme A5.1.



Scheme A5.1: Structural changes of MO resulting in the transformation from orange (basic form) to red (acidic form).

During the MO experiments the conductivities of a control and test sample were measured. The aim was to determine the effect, if any, that complexation had on the solution conductivity and whether a relationship could be established between conductivity and the amount of polymer added to the standards. Using both polymer stock solutions previously prepared (1000 mg L^{-1} and 10 000 mg L^{-1}), four MO-polymer samples were prepared and the conductivities recorded (Table A5.3).

Table A5.3: Control and test samples used for conductivity experiments with 1000 mg L^{-1} and 10000 mg L^{-1} polymer stock solution.

Sample ID	Polymer Conc. (mg L^{-1})	Volume MO (mL)	Conductivity (mS m^{-1})
Control	1000	0	23
Test	1000	5	23
Control	10000	0	169
Test	10000	5	175

The conductivities of the dilute polymer solutions remained identical, and were only marginally different to the more concentrated solutions. The technique of complexation-spectroscopy showed little potential for further development in polymer analysis.

A5.2 Optimization of MO Absorbance

A series of new experiments was designed as a continuation of the MO ion association investigation. The new approach used was to first establish the detector linear operating range for MO and follow up with the complexation experiments using MO-polymer standards having absorbance values in the optimum absorbance range of the detector. A new MO stock solution (500 mg L^{-1}) was prepared together with a series of dilute MO standard solutions. The first standard was scanned once more to confirm the MO absorption spectrum (Figure A5.3). The scan was similar to that obtained previously (Figure A5.1).

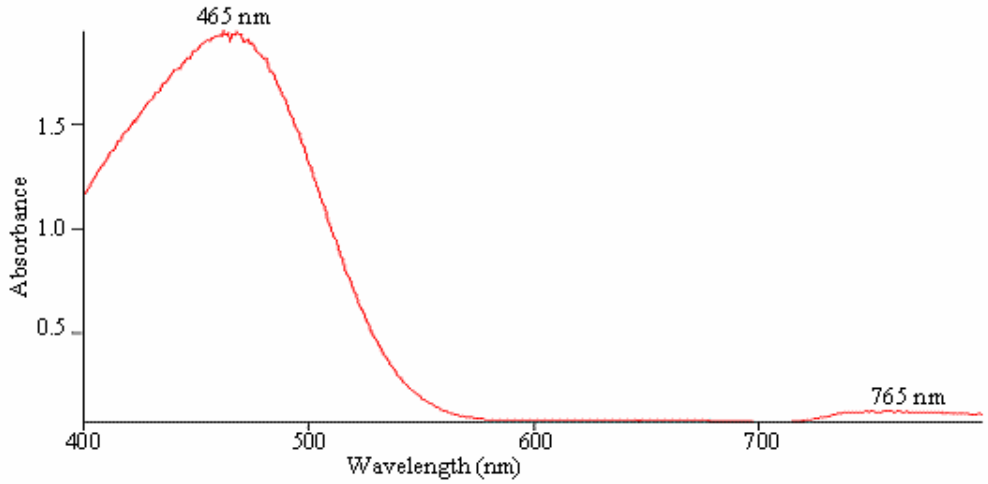


Figure A5.3: UV/VIS scan of MO at 5 mg L^{-1} and a wavelength range of 800 to 400 nm.

The absorbance values of the remaining standards were read at 465 nm (Table A5.4). Each solution was measured in triplicate.

Table A5.4: Absorbance-concentration data for the high range MO standards at 465 nm.

MO Conc. ($mg\ L^{-1}$)	Absorbance (AU)
5	1.89
5	1.88
5	1.87
10	344
10	3.48
10	3.47
20	3.79
20	3.85
20	3.80
40	3.87
40	3.72
40	3.70
50	3.70
50	3.78
50	3.74

A plot of the above data (Figure A5.4) shows excessive curvature, indicating that the MO standards were outside the linear range of the detector.

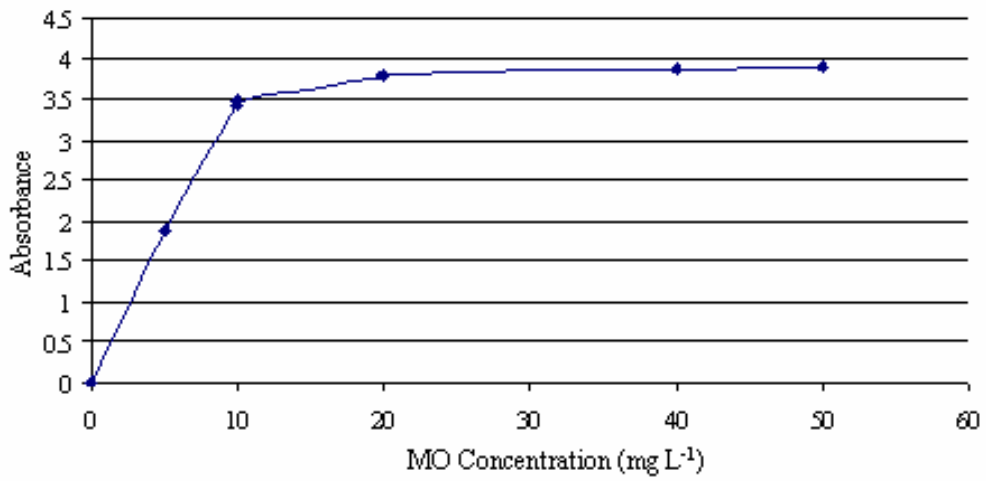


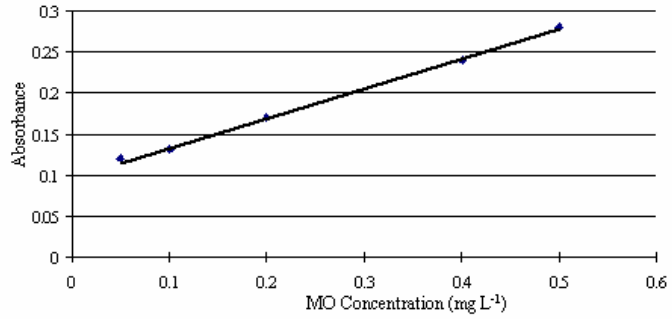
Figure A5.4: A plot of the MO absorbance as a function of concentration.

The above plot shows that all MO concentrations greater than 10 $mg\ L^{-1}$ were too concentrated to be measured accurately. This exercise was extended by preparing a set of new MO solutions that were two orders of magnitude lower. The absorbance data (Table A5.5) indicate that the values are rather low.

Table A5.5: Absorbance-concentration data for the intermediate range MO standards at 465 nm.

MO Conc. ($mg\ L^{-1}$)	Absorbance (AU)
0.05	0.12
0.1	0.13
0.1	0.13
0.1	0.13
0.2	0.17
0.2	0.17
0.2	0.17
0.4	0.24
0.4	0.24
0.4	0.24
0.5	0.28
0.5	0.28
0.5	0.28

Although regression analysis of the data (Figure A5.5) indicated good linearity (R^2 = 0.998), the MO solutions were not in the optimum concentration range for UV/VIS absorption spectroscopy. For most absorbance detectors the maximum absorbance is usually in the region 1.2 absorbance units full scale (AUFS).



Regression Statistics		
Multiple R	1.00	
R Square	0.998	
Adjusted R Square	1.00	
Standard Error	0.00	
Observations	13.00	STDEV
Intercept ± STDEV	0.10	0.001
Slope ± STDEV	0.37	0.004
y = 0.37x + 0.10		

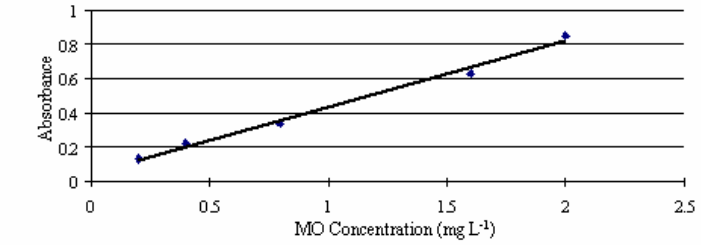
Figure A5.5: Linear regression line for MO standards in the range 0.05 to 0.5 $mg\ L^{-1}$.

A third range of MO solutions (0.2 to 2 $mg\ L^{-1}$) was prepared and analyzed. The absorbance readings were not consistent with the expected trend. The readings were similar to those obtained in Table A5.5. The spectrophotometer was recalibrated and the standards reanalyzed (Table A5.6).

Table A5.6 : Absorbance-concentration data for the low range MO standards at 465 nm.

MO Conc. ($mg\ L^{-1}$)	Absorbance (AU)
0.2	0.13
0.2	0.13
0.2	0.13
0.4	0.22
0.4	0.22
0.4	0.22
0.8	0.34
0.8	0.34
0.8	0.34
1.6	0.63
1.6	0.63
1.6	0.63
2	0.85
2	0.85
2	0.85

The data were used in regression analysis and the regression line (Figure A5.6) showed slightly poorer linearity, with a correlation coefficient $R^2 = 0.992$. This was chosen as the optimum MO concentration operating range. This range was used as the basis for all future ion association reaction experiments with polymers.



Regression Statistics		
Multiple R	1.00	
R Square	0.992	
Adjusted R Square	0.99	
Standard Error	0.03	
Observations	15.00	STDEV
Intercept ± STDEV	0.05	0.01
Slope ± STDEV	0.39	0.01
y = 0.38x + 0.05		

Figure A5.6: Linear regression line for MO standards in the range 0.2 to 2 $mg\ L^{-1}$.

Using this information of the best operating range for MO, a series of new experiments were conducted using ion association. Since the ideal absorbance (0.85) was achieved with a 2 $mg\ L^{-1}$ solution of MO, this was selected as the solution for the preparation of the polymer standards. Absorbance measurements (Table A5.7) indicated that all absorbance values were similar.

Table A5.7: Absorbance-concentration data for the MO-polymer standards.

Sample ID	Polymer Conc. (mg L^{-1})	Absorbance (AU)
Std 1	250	0.26
Std 1	250	0.26
Std 1	250	0.26
Std 2	500	0.29
Std 2	500	0.29
Std 2	500	0.29
Std 3	750	0.26
Std 3	750	0.26
Std 3	750	0.26
Std 4	1000	0.26
Std 4	1000	0.26
Std 4	1000	0.26
Std 5	1250	0.26
Std 5	1250	0.26
Std 5	1250	0.26

Two factors were considered to be possible contributors to this phenomenon. The first was a loss of the MO during filtration and the second was the use of an excessively high concentration of the stock polyDADMAC causing the total removal of MO by ion association. It is important to remember that the quantity being measured is the residual MO after complexation. To assess the extent of MO removal by filtration a 2 mg L^{-1} solution was analyzed by spectrophotometry before and after filtration through 0.45 µm syringe type filters. The result (Table A5.8) shows a negligible difference in absorbance and that are similar to that obtained for the 2 mg L^{-1} solution previously (Table A5.6).

Table A5.8: Absorbance results for unfiltered and filtered MO solutions.

MO Unfiltered		MO Filtered	
ID	**Absorbance**	**ID**	**Absorbance**
Replicate 1	0.85	Replicate 1	0.83
Replicate 2	0.85	Replicate 2	0.83
Replicate 3	0.85	Replicate 3	0.83

To test whether the second factor (excessively high polyDADMAC concentration) was a potential cause of the low absorbance obtained, the amount of polymer added to prepare the standards was reduced by an order of magnitude. The absorbance measurements are shown in Table A5.9.

Table A5.9: Absorbance readings of MO-polymer standards in the range 25 to 125 mg L^{-1}.

Sample ID	Polymer Conc. (mg L^{-1})	Absorbance (AU)
1	25	0.56
1	25	0.56
1	25	0.56
2	50	0.56
2	50	0.56
2	50	0.56
3	75	0.56
3	75	0.56
3	75	0.56
4	100	0.56
4	100	0.56
4	100	0.56
5	125	0.55
5	125	0.55
5	125	0.55

The absorbance values were higher than those of the previous run (Table A5.7) but still no trend existed. Although the absorbance intensities were sufficiently high to work with, there was essentially no change in the absorbance of the MO solution when increasing amounts of polymer was added. There was some doubt as to whether the MO-polymer was being formed or whether it absorbed at the wavelength for MO. The results obtained thus far were unsuitable for the purpose of a calibration curve.

Another attempt was made at the calibration, and fresh standards were prepared using the 5000 mg L^{-1} polyDADMAC stock and 2 mg L^{-1} MO solution. In this experiment, the results showed a poor correlation of concentration and absorbance (Table A5.10) that could not be explained. Visual inspection of the MO-polymer solutions however did show an expected decrease in the intensity of the color with an increase in polymer addition. After standing overnight, the color changed from red to yellow, indicating the basic form of MO. On treating the above solution with 100 µL of HCl (0.1 M), there was no change in color to the acidic form, as anticipated.

Table A5.10: Absorbance-concentration data for MO-polymer standards.

Sample ID	Polymer Conc. (mg L^{-1})	Absorbance (AU)
Std 1	250	0.47
Std 1	250	0.47
Std 1	250	0.47
Std 2	500	0.41
Std 2	500	0.41
Std 2	500	0.41
Std 3	750	0.61
Std 3	750	0.61
Std 3	750	0.60
Std 4	1000	0.61
Std 4	1000	0.61
Std 4	1000	0.61
Std 5	1250	0.61
Std 5	1250	0.61
Std 5	1250	0.61

Either an irreversible reaction had taken place or the solution was still in the basic pH region. The absorbance values (Table A5.11) were significantly different to the original values in Table A5.10.

Table A5.11: Absorbance-concentration data for MO-polymer standards after reacting overnight.

Sample ID	Polymer (mg L^{-1})	Absorbance (AU)
Std 1	250	0.5
Std 1	250	0.5
Std 1	250	0.5
Std 2	500	0.25
Std 2	500	0.25
Std 2	500	0.25
Std 3	750	0.22
Std 3	750	0.22
Std 3	750	0.22
Std 4	1000	0.23
Std 4	1000	0.23
Std 4	1000	0.22
Std 5	1250	0.23
Std 5	1250	0.23
Std 5	1250	0.23

The poor reproducibility of the absorbance measurements after re-analysis suggested that the reaction conditions were not well controlled and that the reaction could have gone to completion or equilibrium after standing overnight. Evidence of this is found later in Figure A5.7. All solutions except for solution 1 have higher absorbance when

analyzed immediately after preparation and significantly lower absorbance when measured after reacting overnight.

A new experiment was designed taking into account all factors that affected the results in previous runs. The volume of acid was increased by an order of magnitude to 1 mL. The absorbance values were read immediately and after standing overnight (Table A5.12).

Table A5.12: Absorbance readings for MO-polymer standards measured immediately and after reacting overnight, repeat experiment.

Sample ID	Polymer Conc. (mg L^{-1})	Absorbance 1 (immediate)	Absorbance 2 (overnight)
Std 1	250	0.82	0.81
Std 2	500	0.55	0.28
Std 3	750	0.48	0.23
Std 4	1000	0.44	0.21
Std 5	1250	0.47	0.23

A plot of the data (Figure A5.7) shows significant curvature and lower absorbance values after reacting. This indicates that the ion association reaction was not spontaneous and proceeded at a slow rate overnight. In addition to this there was a poor correlation between the amount of polymer and the absorbance. Furthermore, absorbance values of each solution are similar, except for the first solution.

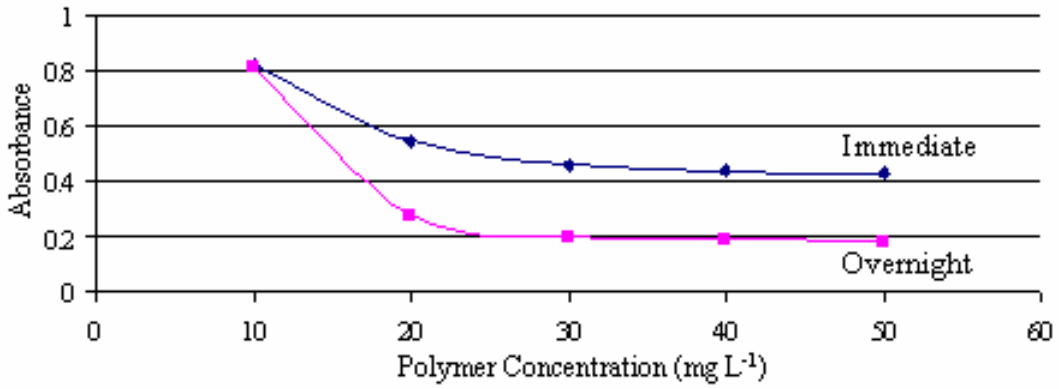


Figure A5.7: Absorbance curves for MO-polyDADMAC standards taken at two different times.

A color change was also noted from red to yellow. Since the solutions were not treated with base (only spiked with polyDADMAC, at acidic pH), there was little chance of a pH change causing the color change. The results suggested that there was either a total removal of MO to form a yellow complex or there may have been a swap of quaternary sites with polyDADMAC. At this stage it was realized that the MO ion association method was plagued with problems and all further experiments in this area were suspended. Poor control of experimental variables affected the reaction, resulting in a bad correlation of the absorbance data with the quantity of polymer.

However some HPLC investigative work continued on MO solutions in the hope of identifying and confirming the MO-polymer complex formation.

New experiments were then conducted using the polyDADMAC synthesized in Section 3.3.3, to determine the nature and properties of the resulting MO-polymer complex. It was hoped that the resulting complex would be soluble in the phosphate buffer mobile phase for the GPC of polyDADMAC (Section 2.5.1) and would form a strongly UV active species.

A5.3 GPC of a MO-Polymer Standard

The objective here was to gain more insight into the nature and properties of the MO-polymer complex and to make an assessment of whether the resulting complex could be exploited to improve the detection sensitivity of polyDADMAC by GPC with PDA UV detection. It was hoped that the complex could be used as a "tagged" polymer with MO, providing the chromophores required for detection. The experiments commenced with the preparation of two solutions, a control and a test sample (MO-polymer) for investigating of the UV/VIS absorption spectrum of the complex. A control containing only MO (and no polymer) was used to establish any differences in the absorption spectra before, and after polymer addition. Visual inspection of the solutions showed that there was a definite change in the intensity of the color (dark orange to light orange) upon the addition of polymer. This indicated that MO was removed during the formation of the MO-polymer complex. Spectrophotometric analysis of the two solutions resulted in a broad absorption band ranging from 600 to 400 nm at an intensity that was well over the detector range (Figure A5.8).

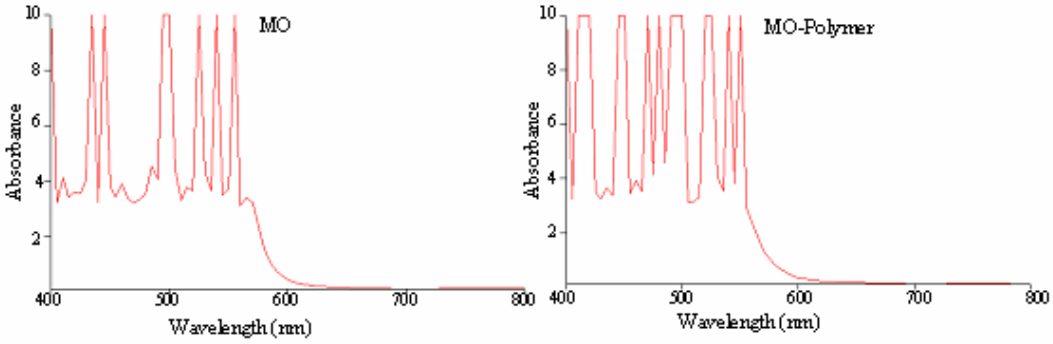


Figure A5.8: UV/VIS spectra of the control and test samples.

Since the solutions were not diluted further at the time of the study, spectral differences were not established. The control and test samples were then analyzed by GPC using only a guard column and RI detection to establish if the MO and the MO-polymer complex could be separated from each other. The removal of the analytical column was a precautionary measure for column protection in the event of plugging due to precipitation of the MO-polymer complex. Visual inspection revealed no precipitate when a few drops of the MO-polymer solutions were added to the GPC mobile phase. Nevertheless, the added precautions were necessary. The overlay GPC chromatogram of the control and test sample with RI detection

(Figure A5.9) shows a distinct polymer peak in the test sample. The control shows no discreet peaks but careful inspection of the chromatogram shows a low intensity broad peak between 6 and 7 min. Considering the fact that MO was in an ionized state, it was likely to interact strongly with the column by non-size exclusion mechanisms and account for the peak.

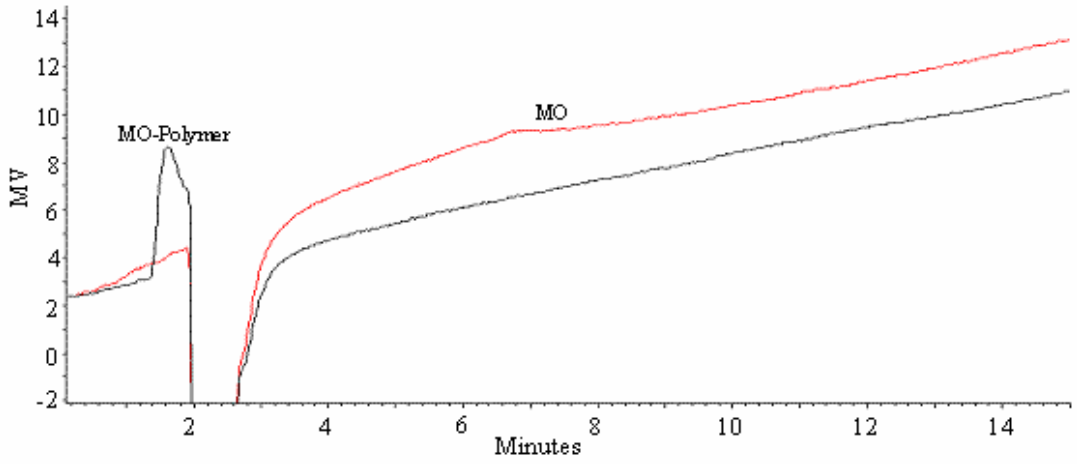


Figure A5.9: Overlay of GPC chromatograms of MO and MO-polyDADMAC using the Ultrahydrogel guard column for separation and RI detection.

Focusing on the early eluting peak in the test sample, it could not be established whether the peak was that of the polymer alone or the MO-polymer complex or both. The polymer in a complexed form could be negligibly different in size to the uncomplexed polymer and hence elute together as one peak in GPC. In general, GPC requires that the difference in molecular weight be at least an order of magnitude from each other for effective separation to occur.

When the same samples were analyzed by GPC with PDA UV detection, the GPC chromatograms showed a distinct difference from each other (Figure A5.10) as well as from the GPC RI chromatogram (Figure A5.9).

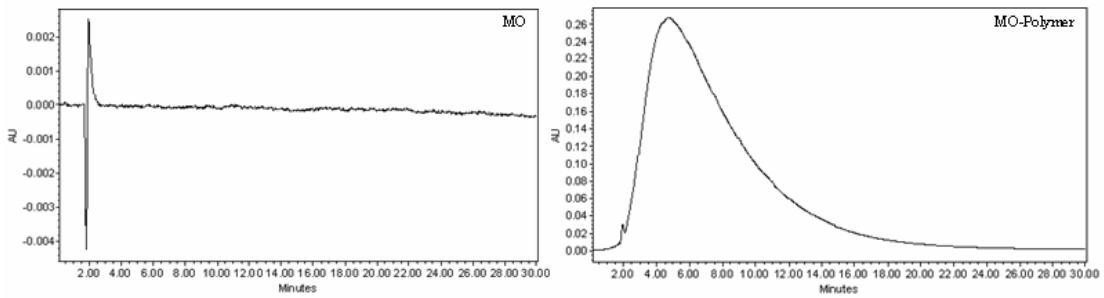


Figure A5.10: GPC chromatograms of MO and MO-polyDADMAC using PDA UV detection.

The MO shows a single peak of low intensity at 2 min. This was not expected since MO is a highly UV active compound with a strong absorption at 465 nm. The reason

for the absence of the MO peak was of concern and warranted further investigation. The MO-polymer on the other hand produced a peak that was possibly the MO peak, or its corresponding complex, or both. The low intensity peak preceding the main peak appears to be the same peak as that found in the control but appears suppressed due to the scaling of the chromatogram. Identical profiles were obtained when repeat injections were done. A second concern at this stage was the broad peak observed for the test sample when only the GPC guard column was used. The peak width was approximately 20 min. This implied that the elution time would be in the order of several hours with the analytical column connected in series. It was therefore a wise choice to use the guard column alone for this investigation. It was still unclear whether the broad peak was the result of adsorption or just due to an excessively high concentration of analyte (MO-polymer or MO). Further MO solutions containing 0 to 4 mg L^{-1} polymer were analyzed by GPC RI (Figure A5.11).

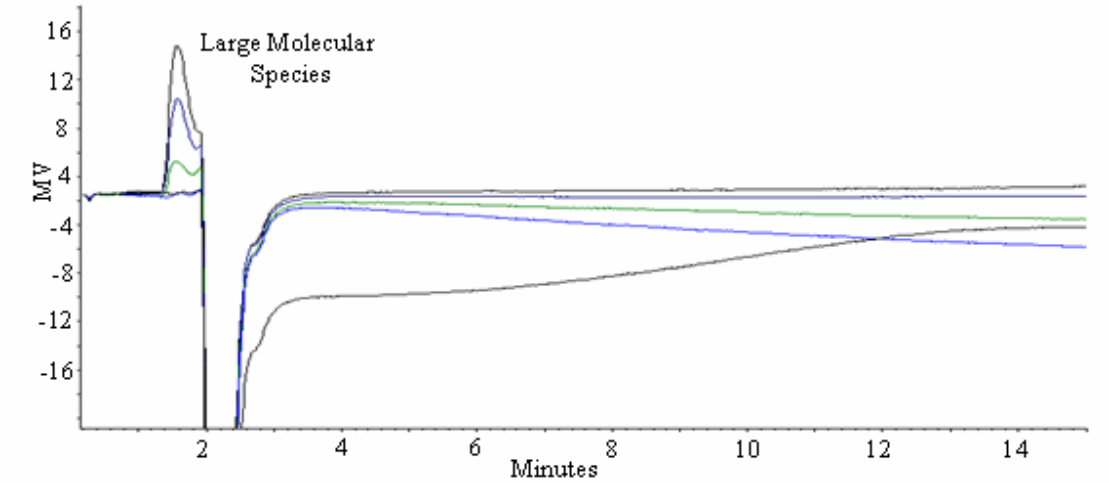


Figure A5.11: Overlay of GPC chromatograms of MO solutions containing 0 to 4 mg L^{-1} of Z464N with RI detection and the Ultrahydrogel guard column.

The linear response to polymer concentration confirms the identity of the polymer peak. However, it remained unknown whether this was the polymer alone of the MO-polymer complex or a combination of both. Therefore GPC with RI detection did not reveal any significant information regarding complexation or ion association with MO. Similarly GPC with PDA UV detection provided no important details (Figure A5.12).

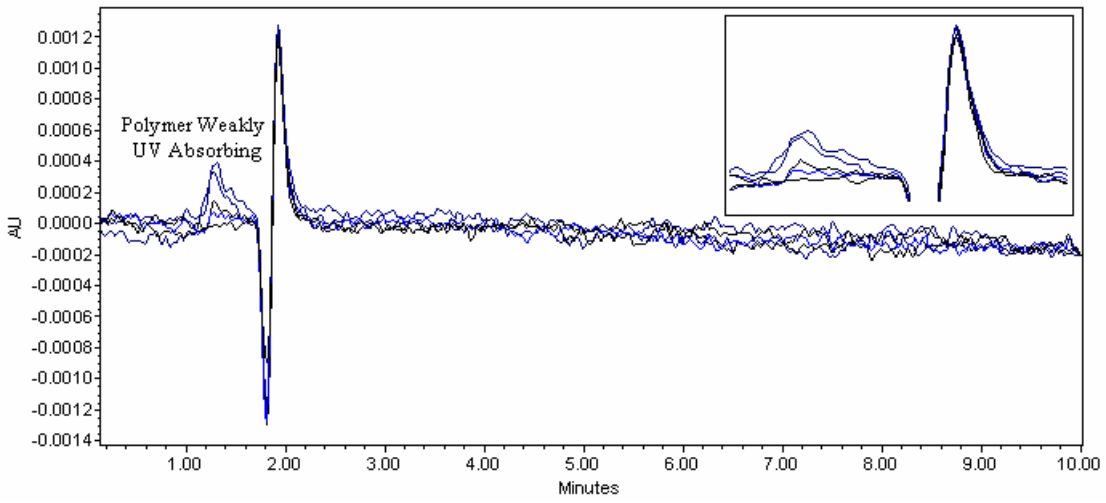


Figure A5.12: Overlay of GPC chromatograms of MO-polyDADMAC solutions containing 0 to 4 mg L^{-1} of Z464N with PDA UV detection.

A small poorly defined peak can be observed at 1.5 min but was of little value since the sensitivity was worse than that achieved with RI detection. This peak was assigned to the polymer alone as in the complexed form it would give rise to a strongly absorbing large peak. The peak at 1.9 min was also present in blank as shown in Figure A5.13 and was therefore assigned to Milli-Q water.

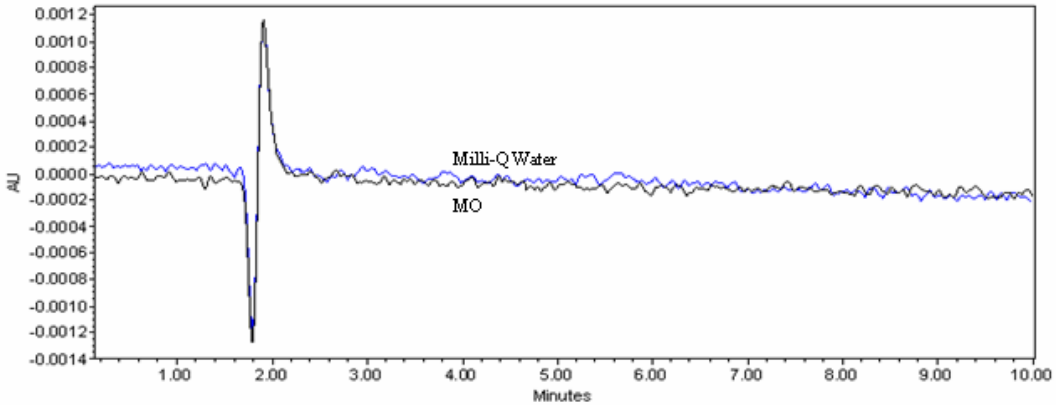


Figure A5.13: Overlay of GPC chromatograms of MO and Milli-Q water obtained with PDA UV detection.

The results were viewed with great caution as there were no signs of the highly absorbing MO peak anywhere in the chromatogram. MO should have produced a massive peak at 465 nm, as determined by the scans of MO solutions previously (Figure A5.1 to A5.3). In addition, the color of the test and control samples was sufficiently intense to be visible by the naked eye and so there were no reasons for the absence of a detector response.

The investigations were continued to establish the cause of the "missing" MO peak. Fresh solutions of MO, MO-polymer and polymer were prepared for this exercise. All solutions were acidified with an excess of HCl to suppress color changes due to

pH fluctuations. All three solutions were chromatographed with PDA UV detection (Figure A5.14).

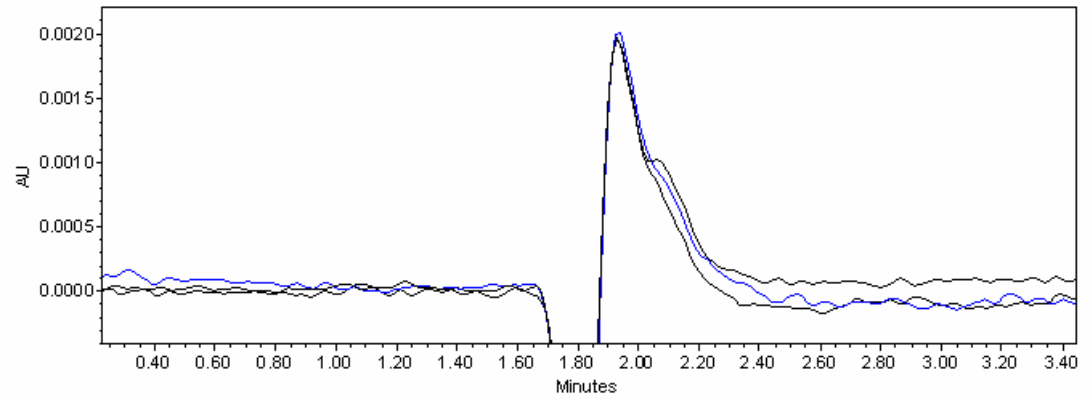


Figure A5.14: Overlay of GPC chromatograms of MO, MO-polyDADMAC and polyDADMAC with PDA UV detection at 465 nm.

A short experiment was conducted by passing the MO, MO-polymer, and polymer solution through the detector after removing the separating column. The chromatogram (Figure A5.15) shows a massive absorbance for the dye and dye polymer complex and no absorbance for the polymer.

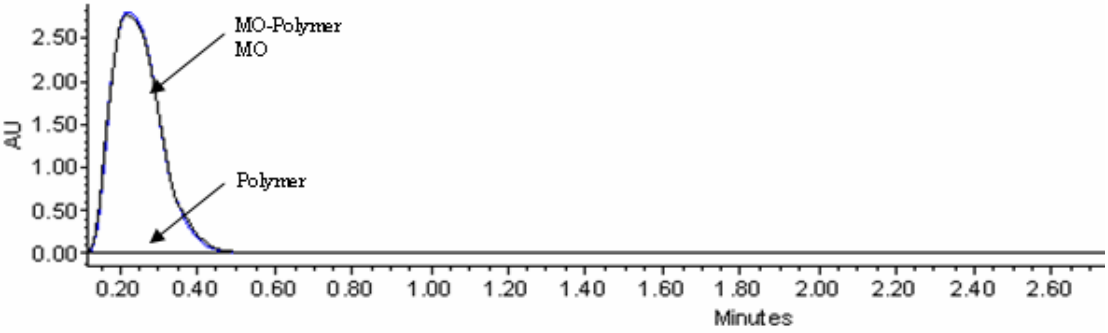


Figure A5.15: Overlay of the PDA detector response of MO-polyDADMAC, MO and polyDADMAC measured at 465 nm without the GPC separation column.

After a lengthy investigation results were obtained that were in accordance with the expected trend. The MO and MO-polymer were highly UV absorbing and the polymer on its own was non-absorbing.

Results of this experiment were very encouraging, and further experiments followed immediately, using a similar test with the guard and analytical columns connected. The GPC PDA overlay chromatograms (Figure A5.16) show the small peaks at 1.9 min previously established as originating from Milli-Q water.

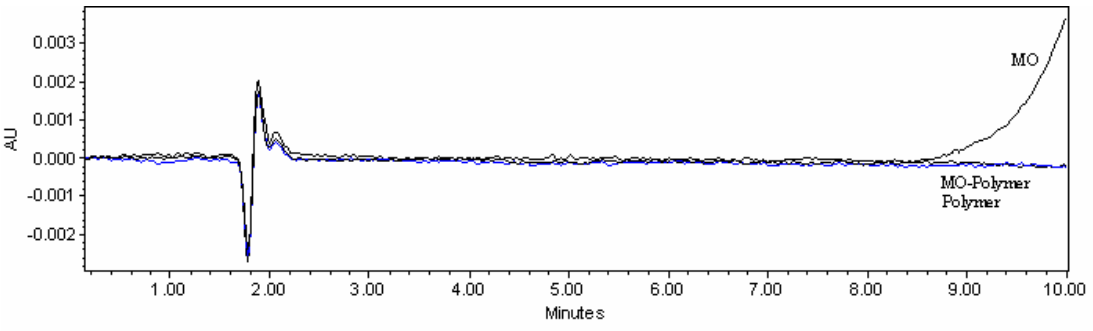


Figure A5.16: Overlay of GPC chromatograms of MO, MO-polyDADMAC and polyDADMAC using the guard column and a wavelength of 465 nm.

In addition to this, a huge peak can be observed emerging at *ca* 9 min for MO. Immediately it was realized that the dye or dye-polymer complex was eluting late in the chromatogram and that the run time had to be increased. An increase in the run time from 10 to 35 min immediately showed the expected profile for the solutions (Figure A5.17). The profile shows a high absorbance for MO and MO-polymer and non-absorbing for the polymer solution.

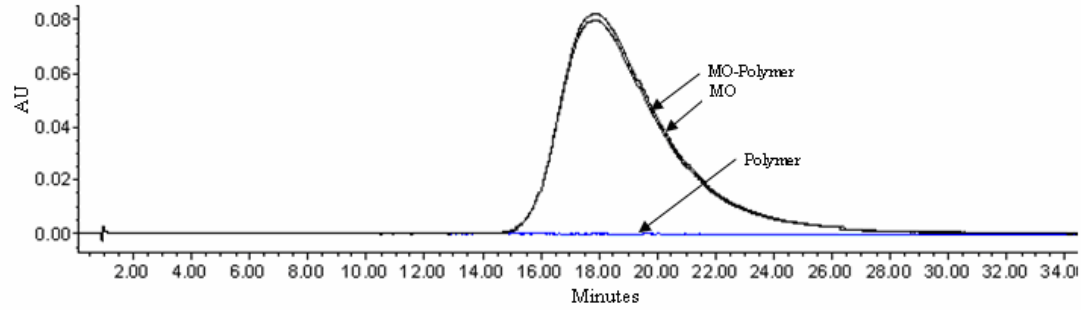


Figure A5.17: Overlay of GPC PDA chromatograms for MO, MO-polyDADMAC and the polyDADMAC using the guard column, a wavelength of 465 nm and a run time of 35 min.

Another prominent feature of the GPC profile is the broad peak observed for the two solutions. This is indicative of strong adsorption in the column. Therefore there was merit in using the guard column alone as the elution of the dye and dye-polymer complex would have required a run time of several hours or in the worst case scenario it could remain permanently adsorbed on the column and eventually poison the column. The profiles for the MO and MO-polymer are identical and indicate that only MO is being detected whilst the absence of a peak early in the chromatogram suggests that the polymer complex was not in a form that could be detected by GPC. The polymer solution shows no absorption of radiation as expected. Using the knowledge of the late elution characteristic of MO, the samples were chromatographed with RI detection (Figure A5.18).

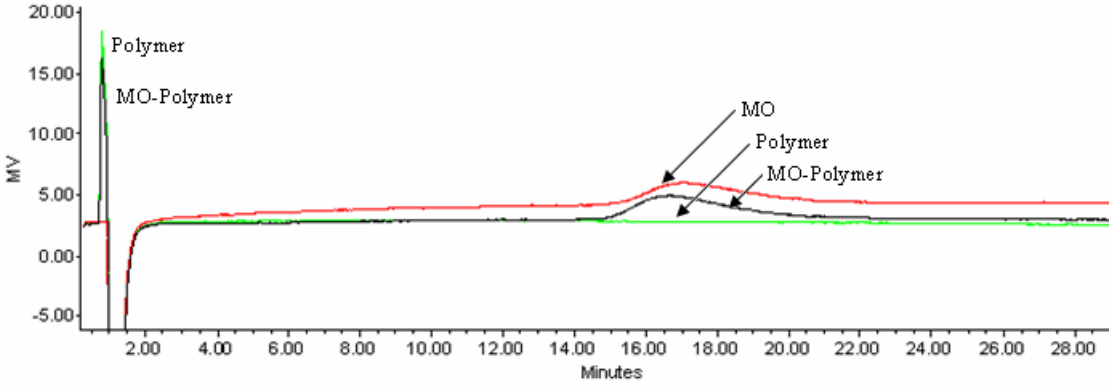


Figure A5.18: Overlay of GPC RI chromatograms of MO, MO-polyDADMAC and the polyDADMAC solution and the polymer using the guard column.

The MO solution gives rise to a peak at 18 min. The MO-polymer solution is interesting and gives rise to two peaks, one at *ca* 1 min and the second at 18 min. The latter originates from MO and the former from the polymer. Similarly, the polymer solution gives rise to a peak at *ca* 1 min and no absorption for MO.

Using the GPC results from Figures A5.17 and A5.18, it can be concluded that the MO reagent used in the ion association did not form a complex that could be detected by GPC with PDA UV detection. Complexation did however take place as noted by visual inspection of the color of the MO-polymer standards previously prepared (Section A4.2). The complex did not appear to remain stable enough for GPC analysis. It could have decomposed to form two components, the polymer and MO, as shown clearly by Figure A5.18, but this is unlikely.

It can be concluded that when using GPC with PDA UV detection, only MO could be detected. The GPC chromatogram showed no evidence of the formation of a UV/VIS absorbing polymeric species, as indicated by the absence of a peak in the high molecular weight region of the chromatogram (Figure A5.17). It could however be speculated that the complex formed but was not identifiable by GPC.

An attempt was made to analyze the MO, MO-polymer and the polymer solutions by reversed phase HPLC to ensure that no components were missed out due to poor resolution of the GPC technique. A µBondapak C_{18} column (Waters) of dimensions 3.6 mm x 30 cm and 10 µm particle sizes was used together with a mobile phase of 10 mM ammonium acetate prepared in methanol. The overlay chromatogram (Figure A5.19) shows only the presence of the MO.

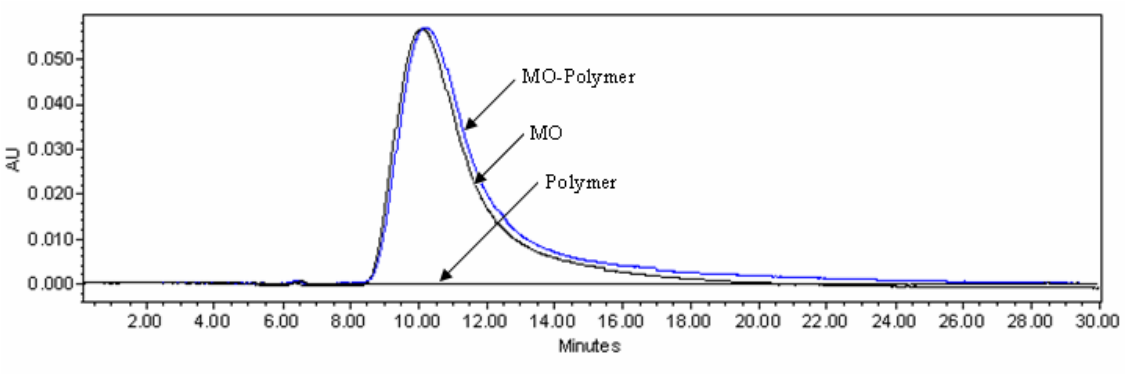


Figure A5.19: The reversed phase overlay chromatograms of MO, MO-polyDADMAC and the polyDADMAC solution.

Although the MO method was extensively evaluated, all attempts at producing a tagged polymer for GPC PDA analysis and reversed HPLC analysis were unsuccessful. Visual inspection of the test solutions however did suggest some degree of complex formation or reorganization of complexes. Spectrophotometric analysis of the resulting complex produced a poor correlation of absorbance with polymer concentration.

APPENDIX 6

THE HACH METHOD

A6.1 Results and Discussion

The experimental procedure involved the preparation of a series of polyDADMAC standards for the purpose of a calibration curve. In the first experiment three solutions (0 to 6 mg L^{-1}) were tested. The highest concentration standard was scanned on a spectrophotometer from 800 to 400 nm. The absorption spectrum (Figure A6.1) of the solution has two absorption maxima (λ_{max}), the first at 545 nm and the second at 575 nm.

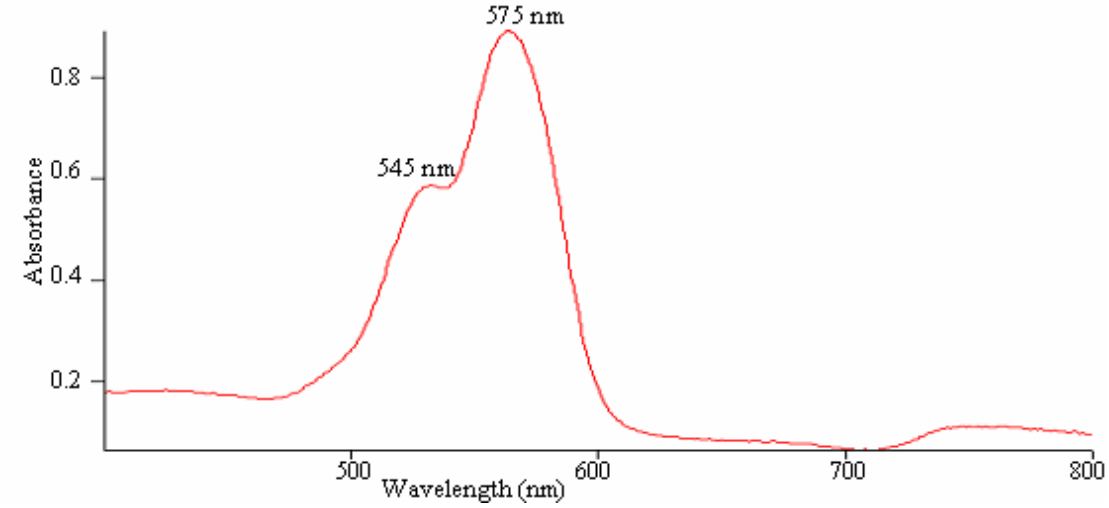


Figure A6.1: Absorption spectrum of a polyDADMAC standard after treatment with HACH reagents.

Absorbance readings were taken at the more sensitive wavelength of 575 nm (Table A6.1).

Table A6.1: Absorbance readings of polyDADMAC calibration standards using the HACH method.

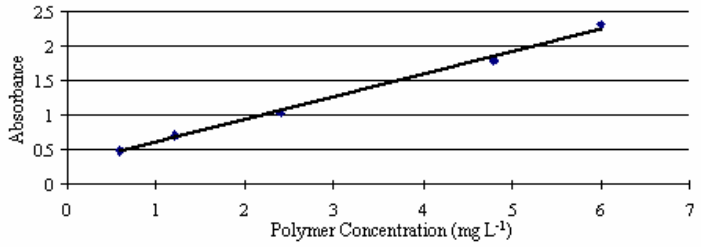
Standard ID	Polymer Conc. (mg L^{-1})	Absorbance (AU)
Std 1	0	0.15
Std 1	0	0.16
Std 1	0	0.16
Std 2	1.2	0.34
Std 2	1.2	0.34
Std 2	1.2	0.34
Std 3	6.0	0.74
Std 3	6.0	0.74
Std 3	6.0	0.74

The data exhibited the expected trend, although not linearly. The second experiment was planned with a full range of calibration standards together with a calibration check sample and a fortified tap water sample to test for calibration accuracy and method accuracy respectively. A blank prepared from 25 mL Milli-Q water was also included in the run. The absorbance values of the solutions (Table A6.2) show the expected trend.

Table A6.2: Absorbance readings of polyDADMAC calibration standards using the HACH method.

Sample ID	Polymer Conc. (mg L^{-1})	Absorbance (AU)
1	0.6	0.48
1	0.6	0.48
1	0.6	0.49
2	1.2	0.70
2	1.2	0.70
2	1.2	0.69
3	2.4	1.03
3	2.4	1.03
3	2.4	1.03
4	4.8	1.78
4	4.8	1.78
4	4.8	1.79
5	6.0	2.30
5	6.0	2.31
5	6.0	2.31
Blank	0	0.29
Blank	0	0.29
Blank	0	0.28
QC	3	1.23
QC	3	1.22
QC	3	1.22
Tap Water	3	1.15
Tap Water	3	1.15
Tap Water	3	1.15

The data were used in linear regression analysis and the linear regression line (Figure A6.2) shows excellent linearity with a correlation coefficient $R^2 = 0.996$. Other regression statistics (standard error, STDEV of slope and intercept) provide supporting evidence of good linearity.



Regression Statistics		
Multiple R	1.00	
R Square	0.996	
Adjusted R Square	1.00	
Standard Error	0.05	
Observations	15.00	STDEV
Intercept ± STDEV	0.27	0.02
Slope ± STDEV	0.33	0.01
y = 0.33x + 0.27		

Figure A6.2: Linear regression line obtained for the polymer calibration standards.

Using the linear regression equation the accuracy of the calibration was tested by calculating the concentration of polymer observed in the QC sample as well as the fortified tap water sample. The QC sample gave a value of 96 % accuracy and tap water 89%. The method exhibited excellent potential for residual polymer analysis and warranted further investigation. The accuracy of the calibration was tested by recording the absorbance values of the standards, QC and tap water samples on three different days (Table A6.3).

Table A6.3: Accuracy data of polymer solutions in the concentration range 0.6 to 6.0 mg L^{-1} obtained using the HACH method.

Sample ID	Polymer Conc. (mg L^{-1})	% Accuracy		
		Day 1	Day 2	Day 6
Std 1	0.60	102	100	93
Std 1	0.60	104	101	93
Std 1	0.60	105	102	93
Std 2	1.20	105	105	102
Std 2	1.20	106	104	103
Std 2	1.20	105	104	102
Std 3	2.40	96	95	93
Std 3	2.40	96	95	93
Std 3	2.40	96	95	93
Std 4	4.80	96	94	93
Std 4	4.80	96	94	93
Std 4	4.80	96	93	93
Std 5	6.00	103	101	98
Std 5	6.00	103	101	98
Std 5	6.00	103	100	98
Cal Chk	3.00	96	95	94
Cal Chk	3.00	96	95	94
Cal Chk	3.00	96	95	93
Tap Spike	3.00	88	86	84
Tap Spike	3.00	88	86	84
Tap Spike	3.00	88	86	85

The first readings were taken on the same day as the calibration (Day 1), one day after preparation (Day 2) and six days after preparation (Day 6). The original calibration curve and regression equation from Day 1 was used in the calculation of the observed polymer concentration and the corresponding accuracy determined. A plot of the data in Figure A6.3 indicates that good accuracy is achieved with this method. Using all data (n=21), over the three different days the accuracy was within 100±10% except for the last three data points. A second accuracy experiment was conducted in which tap water was used instead of Milli-Q water. The samples were fortified with polymer (15 mg L^{-1}) in the range 0 to 6.0 mg L^{-1}.

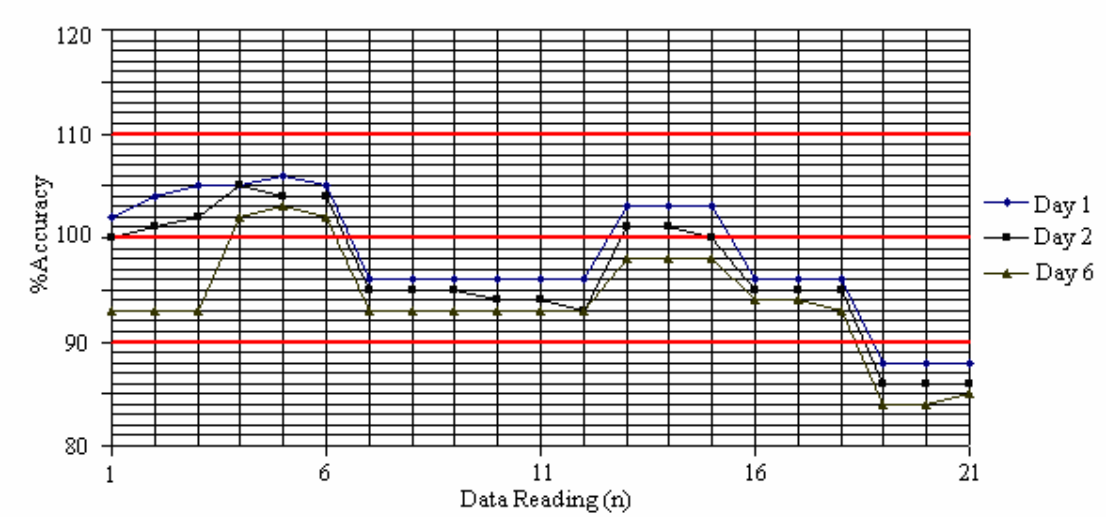


Figure A6.3: Plot of accuracy data for polymer solutions in the concentration range 0.6 to 6.0 mg L^{-1}.

The accuracy was in the range 58-115% (Table A6.4). The low accuracy result of 58% at 4.8 mg L^{-1} was regarded as an outlier. The method precision was assessed by reading the absorbance of all the standards (prepared in Milli-Q water) and fortified tap water samples ten times.

Table A6.4: Accuracy data for polyDADMAC solutions in tap water.

Sample ID	Polymer Fortified Conc. (mg L^{-1})	Polymer Observed Conc. (mg L^{-1})	Absorbance (AU)	Accuracy (%)
Tap 1	0	0.24	0.359	
Tap 2	0	0.24	0.3575	
Tap 3	0	0.24	0.3572	
Tap 4	0.60	0.6	0.4801	102
Tap 5	0.60	0.6	0.479	101
Tap 6	0.60	0.6	0.477	100
Tap 7	1.20	1.40	0.7334	115
Tap 8	1.20	1.36	0.7199	112
Tap 9	1.20	1.32	0.7134	110
Tap 10	2.40	1.76	0.855	73
Tap 11	2.40	1.76	0.8517	73
Tap 12	2.40	1.72	0.8495	72
Tap 13	4.80	2.80	1.1925	58
Tap 14	4.80	2.80	1.1913	58
Tap 15	4.80	2.76	1.1854	58
Tap 16	6.00	4.40	1.744	74
Tap 17	6.00	4.40	1.7363	74
Tap 18	6.00	4.40	1.7342	74

The absorbance values were substituted in the linear regression equation previously determined (Figure A6.2) and the results calculated. These results were used to determine the method precision (Table A6.5).

Table A6.5: Precision data for polymers prepared in Milli-Q and tap water.

Polymer (mg L^{-1})	%RSD in Milli-Q Water (n=10)	%RSD in Tap Water (n=10)
0.60	0.7	1.0
1.20	0.6	1.1
2.40	0.2	0.5
4.80	0.1	0.4
6.00	0.2	0.2

In Milli-Q water, the precision ranged from 0.1 to 0.7% RSD. A similar trend was observed with tap water fortified with polyDADMAC. The precision was marginally poorer; it was in the range 0.2 to 1.1% RSD. The overall precision was excellent at less than 1.2%. Lastly, the limits of detection were determined for the HACH method. This was achieved using absorbance readings from ten blank tap water samples (Table A6.6).

Table A6.6: Detection limit of absorbance data of ten blank samples of tap water.

Sample ID	Absorbance (AU)	Polymer Conc. (mg L^{-1})
1	0.3439	0.19
2	0.3334	0.16
3	0.3335	0.16
4	0.3318	0.16
5	0.3313	0.16
6	0.3339	0.16
7	0.3322	0.16
8	0.3287	0.15
9	0.3278	0.14
10	0.3293	0.15
AVE		0.16
STDEV		0.012

The absorbance data were substituted in the regression equation previously established (Figure A6.2) to obtain the corresponding concentration of polymer. The detection limit was calculated using equation A6.1.

$$L_D = x + 4.7\sigma \qquad A6.1$$

$L_D = limit\ of\ detection$

$x = mean$

$\sigma = standard\ deviation$

$4.7 = factor\ for\ 95\%\ confidence$

The detection limit, L_D was found to be 0.22 mg L^{-1}.

A6.2 Application of the HACH Method

Samples of the raw water after pre-chlorination and polymer dosing were collected from the DHWTW. A sample of sludge scraped off the walls of the canal leading to the pulsators was also collected for analysis. The samples were analyzed together with spiked aliquots of a 10 µg mL^{-1} polymer spike solution. The absorbance values were measured at 575 nm (Table A6.7) and the corresponding polymer masses were determined using the linear regression equation previously determined. The mass observed ($Mass_{observed}$) is the experimental result obtained for the amount of polymer present in the samples as determined by applying the HACH method. It involved the preparation of a calibration curve using a series of polyDADMAC standards followed by linear regression analysis. The regression line given by equation A6.2 was used to determine the mass of polymer present in the test and fortified samples.

$$y = 0.013x + 0.28 \qquad A6.2$$

The theoretical mass ($Mass_{theoretical}$) is the amount of polymer expected to be present in the sample after fortification and is determined by calculations. The mass observed and theoretical are determined using equations A6.3 and A6.4.

$$Mass_{observed} = \frac{(Absorbance - 0.28)}{0.013} \qquad A6.3$$

$$Mass_{theoretical} = Mass_{observed} + Mass_{spike} \qquad A6.4$$

$$\%Recovery = \frac{Mass_{observed}}{Mass_{theoretical}} \times 100$$

The results exhibited good recoveries and confirmed the presence of polymers. The amount of polymer in the raw water was 4.4 µg per 25 mL of sample, which converts to 176 µg L^{-1}.

Table A6.7: Absorbance and percentage recovery data determined for polyDADMAC using the HACH method of analysis.

Sample ID	Absorbance (AU)	Mass Theoretical (µg)	Mass Observed (µg)	% Recovery
Dbn Raw	0.3378	4.4	4.4	
Dbn Raw	0.3368	4.3	4.3	
Dbn Raw	0.3394	4.5	4.5	
Mean		**4.4**	**4.4**	
Dbn Raw + 4 mL polymer	0.7688	44.4	37.3	84
Dbn Raw + 4 mL polymer	0.7682	44.4	37.2	84
Dbn Raw + 4 mL polymer	0.7678	44.4	37.2	84
Dbn Raw + 8 mL polymer	1.3218	84.4	79.5	94
Dbn Raw + 8 mL polymer	1.3195	84.4	79.3	94
Dbn Raw + 8 mL polymer	1.3203	84.4	79.4	94
Dbn Raw + 10 mL polymer	1.4123	104.4	86.4	83
Dbn Raw + 10 mL polymer	1.4161	104.4	86.7	83
Dbn Raw + 10 mL polymer	1.4044	104.4	85.8	82
Dbn Canal Sludge	0.6371	27.2	27.2	
Dbn Canal Sludge	0.6245	26.3	26.3	
Dbn Canal Sludge	0.6193	25.9	25.9	
Mean		**26.5**	**26.5**	
Dbn Canal Sludge + 4 mL polymer	1.3197	66.5	79.3	119
Dbn Canal Sludge + 4 mL polymer	1.3101	66.5	78.6	118
Dbn Canal Sludge + 4 mL polymer	1.3089	66.5	78.5	118

The amount of polymer found in the sludge was 26.5 µg in 12.5 g, which converts to 2.12 µg g^{-1}. There was an over recovery on the sludge sample but, even so, recovery values ranging from 80 to 120% are often analytically acceptable for many standard and EPA methods of analysis.

APPENDIX 7

INDIRECT UV DETECTION

A7.1 Results and Discussion

As a first choice of the chromophore, acetone is readily available, soluble in the mobile phase KH_2PO_4, and is known to absorb at 280 nm. First, the absorbance profile of both acetone and the mobile phase was required in order to select the optimum wavelength for monitoring. A series of solutions (Table A7.1) was prepared for this exercise and scanned by UV/VIS spectrophotometry in the wavelength range 800 to 200 nm.

Table A7.1: Solutions used for the determination of UV/VIS absorption profiles of acetone-KH_2PO_4 mixtures.

Solution ID	Description
Acetone 1	Milli-Q water in glass cuvette
Acetone 2	Milli-Q water in quartz cuvette
Acetone 3	5 mL KH_2PO_4 + 500 µL acetone
Acetone 4	Contaminated solution
Acetone 5	5 mL KH_2PO_4 + 1000 µL acetone
Acetone 6	5 mL KH_2PO_4 + 2000 µL acetone
Acetone 7	Acetone neat
Acetone 8	KH_2PO_4 neat

The solutions were prepared in such a manner so as to establish impact of each reagent on the UV spectra of the test solutions (Acetone 1 to 8). In addition, the use of the quartz cuvette was to ensure that the glass cuvette was not contributing to the absorbance profiles. An overlay of the scans (Figure A7.1) indicates that there are no apparent differences in the spectra of each solution.

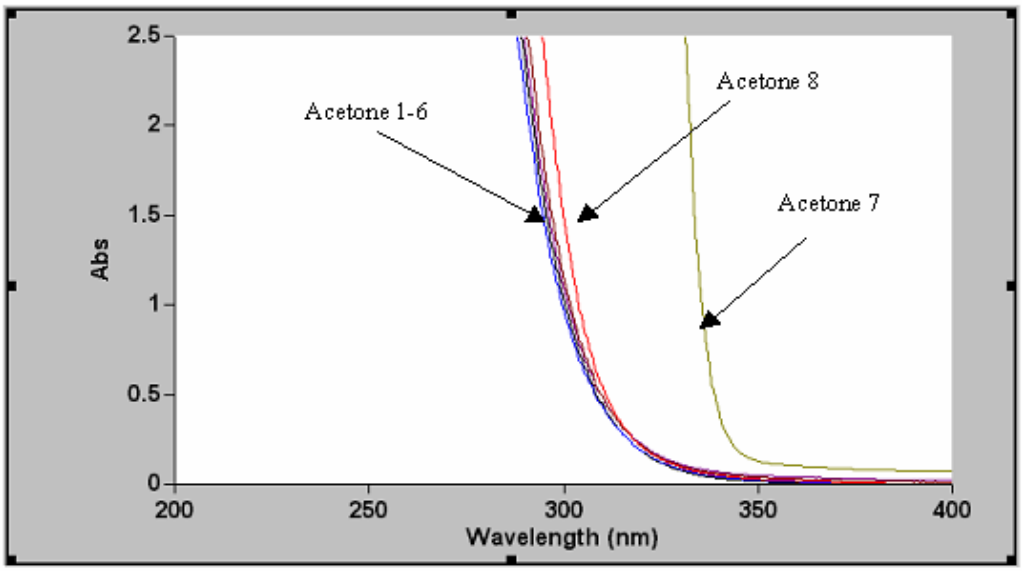


Figure A7.1: Overlay of UV spectra of the solutions labeled Acetone 1 to 8 as shown in Table A7.1.

More importantly, there appears to be no significant difference between the spectra of acetone and the KH_2PO_4 mobile phase (Figure A7.2). Both solutions show absorption of radiation from 350 nm.

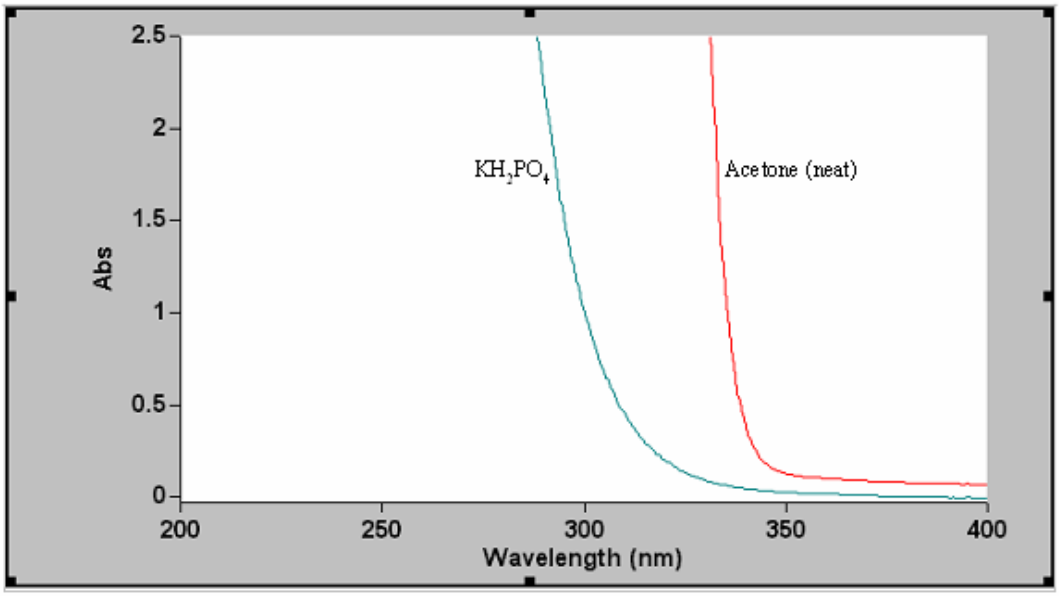


Figure A7.2: Overlay of UV spectra of acetone and KH_2PO_4 MP.

All solutions were transparent in the visible region, they lacked of absorbance in the region 800 to 400 nm (not shown in Figure A7.2), and exhibited strong absorbance in the UV region less than 350 nm. The UV scans suggested that acetone was of no use as a chromophore for use in combination with KH_2PO_4. The requirement for effective indirect UV detection is to have a UV active reagent which when added to the mobile phase will produce a high background absorption at a discreet wavelength (λ_{max} of the chromophore) while simultaneously the mobile remains transparent at that particular wavelength. When the analyte passes the detector there would be a reduction in the absorbance, producing a peak, the intensity of which can be measured and correlated to the concentration of polymer. The results obtained by spectrophotometry were not as anticipated, and hence it was decided to verify the results.

In the next experiment the UV spectra of two samples comprising the MP and MP-acetone (Acetone 6 and 8) were measured using the PDA UV detector. This was achieved by disconnecting the column and injecting the samples into the HPLC system that was equilibrated with Milli-Q water. The 3-D chromatogram (Figure A7.3) shows a significant difference in the UV absorption spectrum to that obtained by spectrophotometry (Figure A7.2).

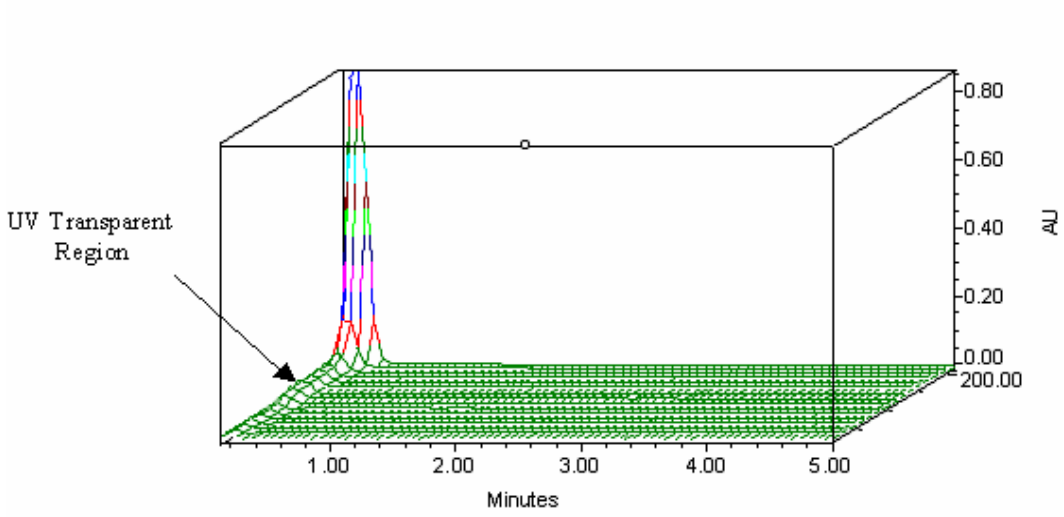


Figure A7.3: 3-D UV absorption profile of KH_2PO_4 by PDA UV detection.

It is noted that the mobile phase shows a greater transparency in the UV region (transparent from 400 to 250 nm) than observed by spectrophotometry (Figure A7.2) (transparent from 400 to 350 nm). The PDA results are more reliable than spectrophotometry as the former results were in agreement with general absorbance data obtained for acetone in literature [Kemp, 1991]. Acetone absorbs at 280 nm and it is also a well known fact that water absorbs in the region less than 205 nm and air less than 200 nm (vacuum UV region) that accounts for the absorbance from 250 to 200 nm. Analysis of MP-acetone also gave greater confidence in the results obtained on the PDA detector (Figure A7.4).

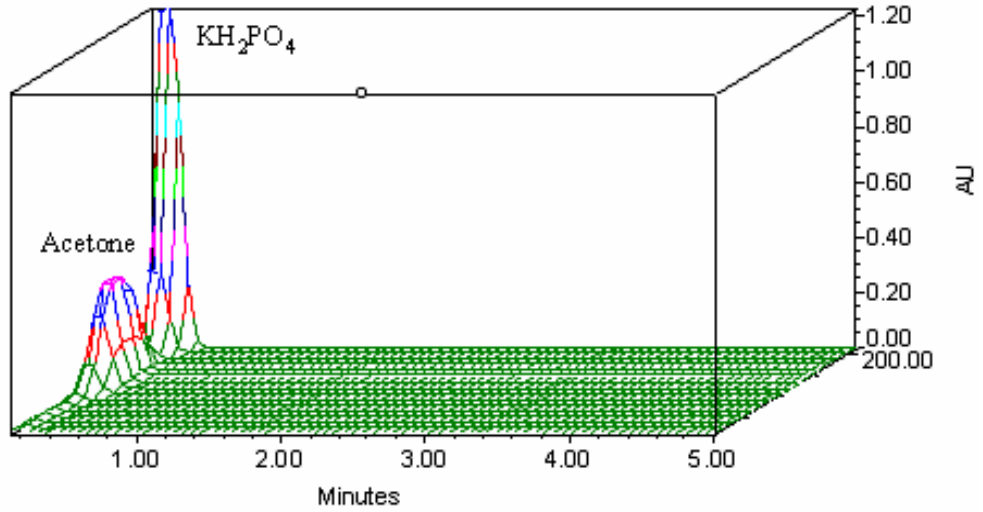


Figure A7.4: 3-D chromatogram of MP-acetone by PDA UV detection.

Contrary to the spectrophotomtric result (Figure A7.2, Acetone 3, 5, 6) which produced a single broad absorption band at less than 350 nm, the PDA detector produced two discreet absorbance bands, indicating that acetone could be used effectively as a chromophore by having no overlapping absorptions with the MP. The desired absorbance of the acetone peak was 1 AUFS. A new mobile phase containing 50 mL of KH_2PO_4 and 2 mL of acetone was then prepared. The solution was injected

into the HPLC system as before but the UV spectrum showed a single broad absorption band (Figure A7.5) indicating an excessively high acetone content.

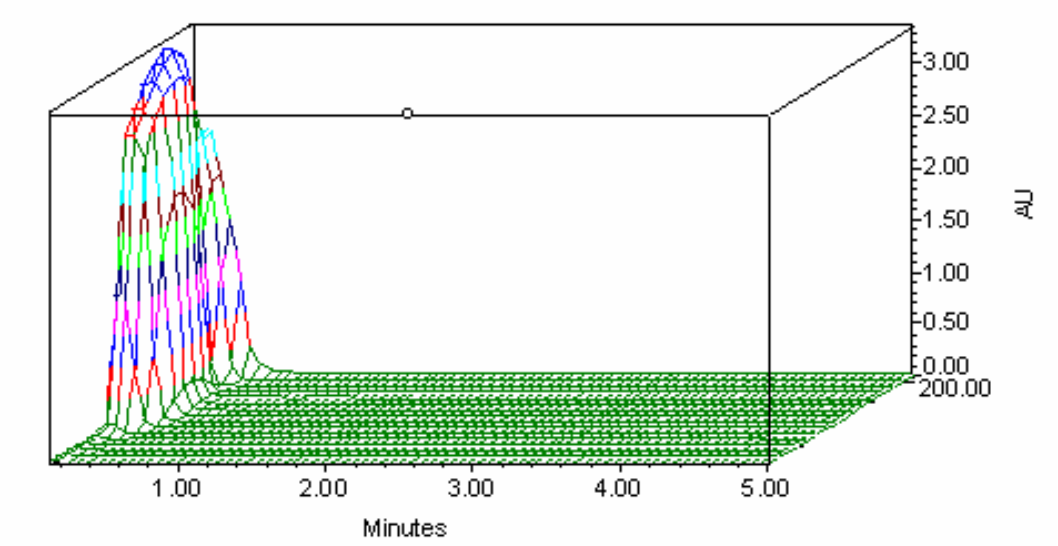


Figure A7.5: 3-D UV absorption profile of the MP-acetone by PDA UV detection.

The reason for the two discreet bands for the MP-acetone solution (Figure A7.4) may be explained by the fact that the solution was analyzed three days after spectrophotometry. Evaporation losses may have caused a reduction in the acetone level of the solution resulting in good separation of the component absorption bands. Acetone at a level of 500 µL was also excessive and produced a broad absorption band. A volume of 50 µL acetone per 50 mL MP produced optimum absorbance values (Figure A7.6).

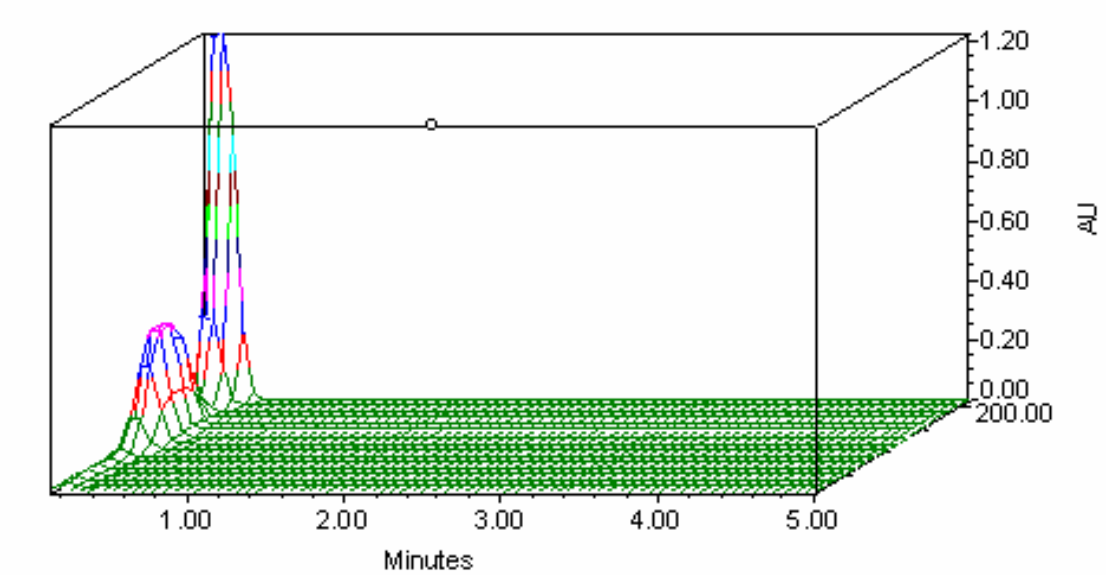


Figure A7.6: 3-D UV absorbance profile of 50 µL acetone per 50 mL MP.

All further attempts at optimization were fruitless and resulted in a heavily overlapping band for all acetone volumes greater than 50 µL. The absorption profile of Figure A7.6 was considered to be the best compromise and it can be noted that it is

not significantly different to that of Figure A7.4. Unfortunately, the desired absorbance of 1 AU for acetone was not achieved.

The above acetone content was then incorporated into the mobile phase for GPC analysis with PDA indirect UV detection. The λ_{max} of acetone was 280 nm and monitoring was done from 400 to 200 nm. A test solution of Z553D (0.02%) was used in the analysis. The sample was injected four times (Figure A7.7).

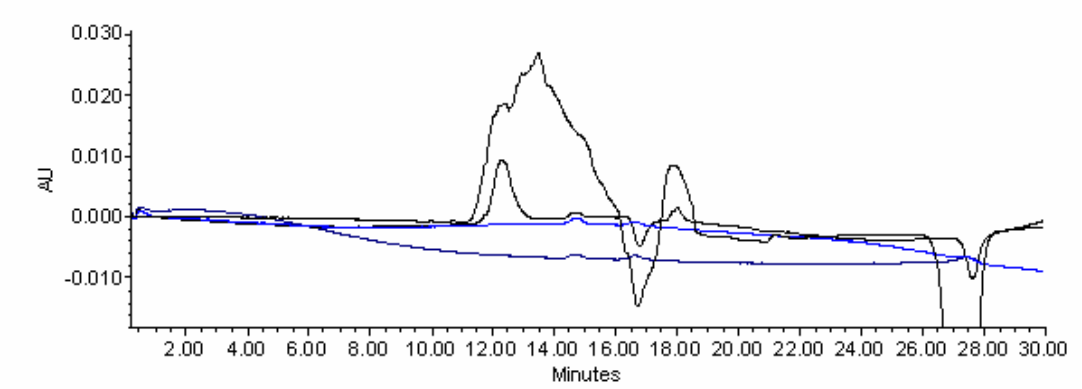


Figure A7.7: Overlay chromatograms of Z553D obtained by indirect UV detection.

Each injection showed a different peak profile, with very low sensitivity. Only the first injection showed a profile resembling that of the test polymer. The investigation into the poor peak reproducibility continued and the sample was re-injected twice more. The peak profiles (Figure A7.8) were totally different from each other.

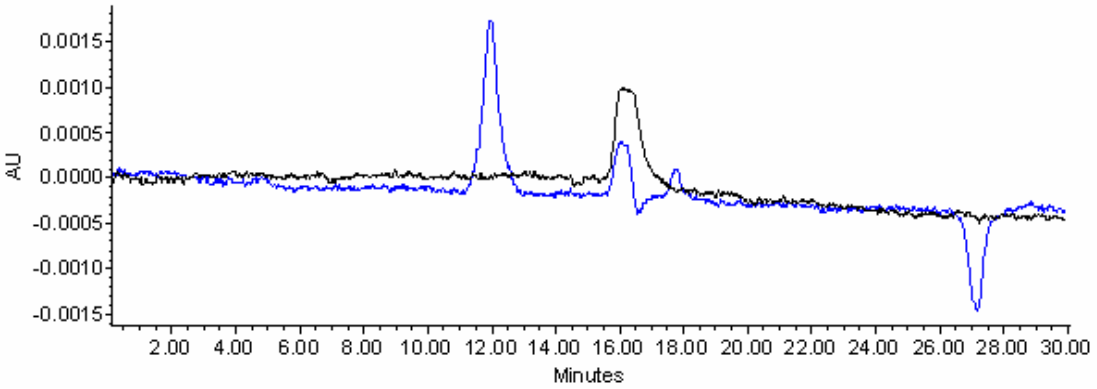


Figure A7.8: Overlay of GPC chromatograms of Z553D obtained by indirect UV detection.

It was first thought that the polymer was being retained in the GPC column. The run time was therefore increased from 30 to 60 min, but even so there was no evidence of a polymer peak (Figure A7.9).

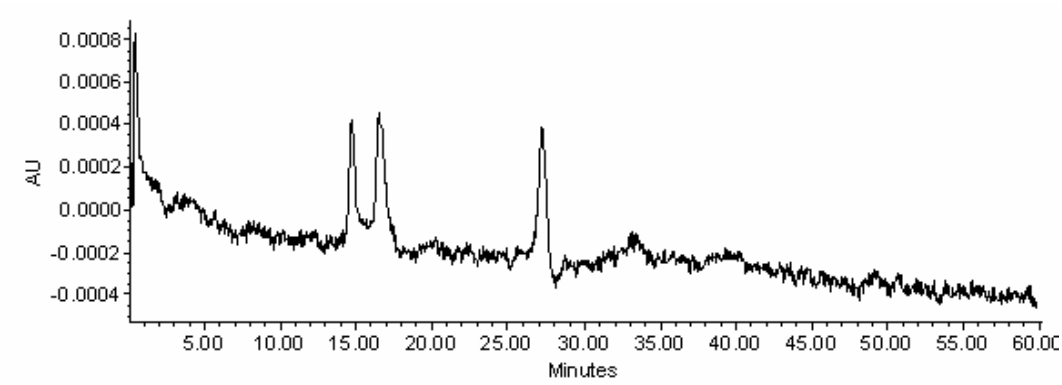


Figure A7.9: GPC chromatogram of Z553D with an increased run time of 60 min.

From the characteristics of the baseline it was suspected that no sample was being injected and that there was a blockage in the injection system of the instrument. A blank sample of Milli-Q water was injected (Figure A7.10) as a system test.

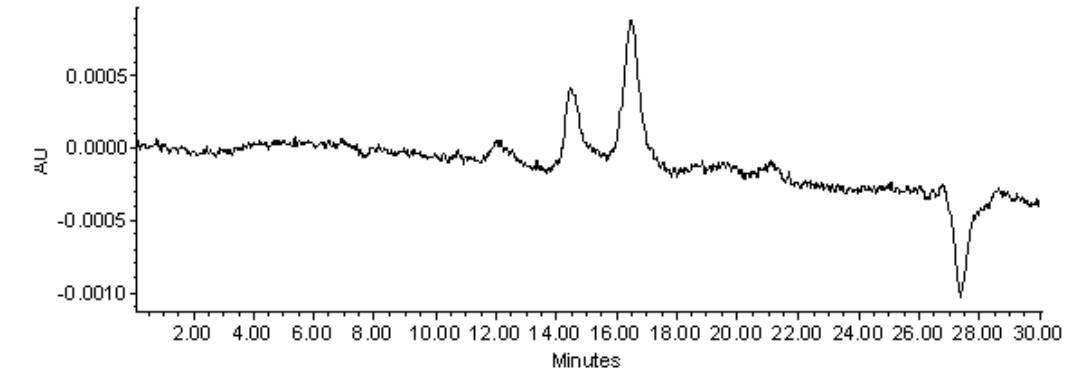


Figure A7.10: GPC chromatogram of Milli-Q water obtained by indirect UV detection.

The characteristic water dip was evident at 27 min and two low-intensity peaks of unknown origin were observed at 14 to 16 min. The results of the investigation were inconclusive. Although an injector blockage was suspected, a freshly prepared mobile phase was used and the test sample re-analyzed. No polymer peak was observed at the expected retention time of 12 min (Figure A7.11).

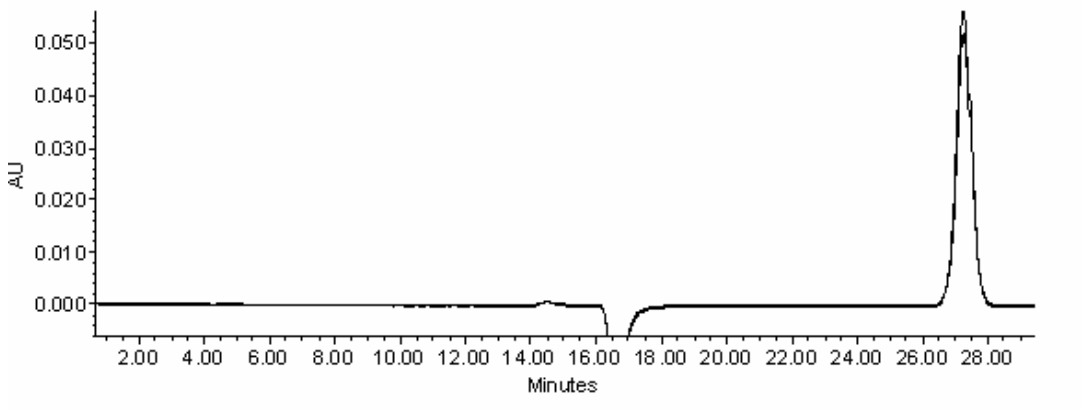


Figure A7.11: GPC chromatogram of Z553D run with a freshly prepared MP.

Since initial studies with acetone did not give the desired result, namely a sensitive response for polyDADMAC, a new chromophore, the chromate ion $CrO4^{2-}$, was selected and tested for polymer analysis. The MP was prepared for GPC to which was added 10 mM Na_2CrO_4. The UV/VIS spectrum was acquired on the spectrophotometer. The absorbance was found to be excessively high and the amount of Na_2CrO_4 was reduced to 0.2 mM. The UV/VIS spectrum of the MP and MP-Na_2CrO_4 combination show a distinct difference in the absorption characteristics of the two solutions (Figure A7.12).

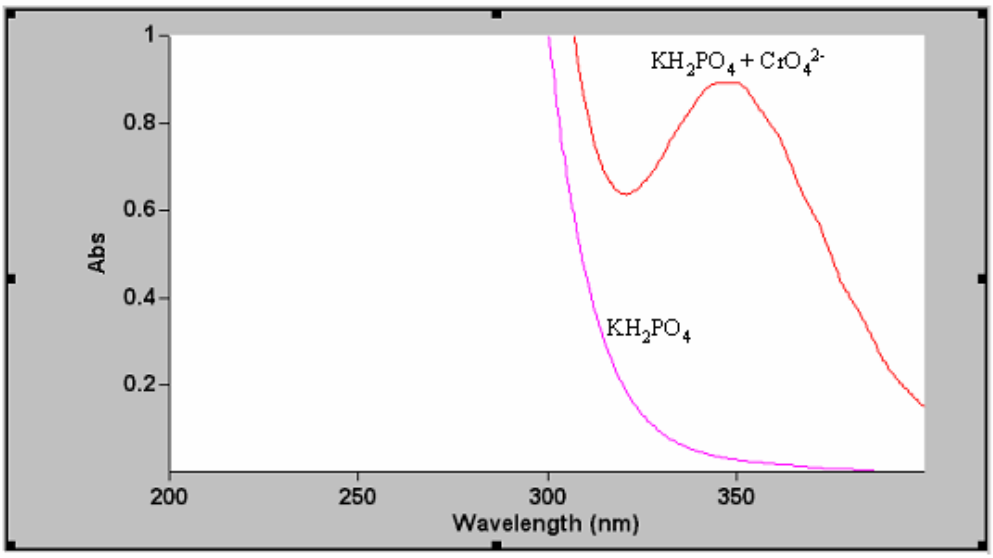


Figure A7.12: Overlay of UV/VIS absorption spectra of the MP and MP-Na_2CrO_4 combination.

The λ_{max} occurred at 350 nm and was well separated from the absorption of the MP. The MP containing the chromophore was then prepared for GPC analysis at the optimized concentrations as determined by spectrophotometry. The polymer sample of Z553D prepared from previously purified crystals of polymer was chromatographed (Figure A7.13) using RI detection.

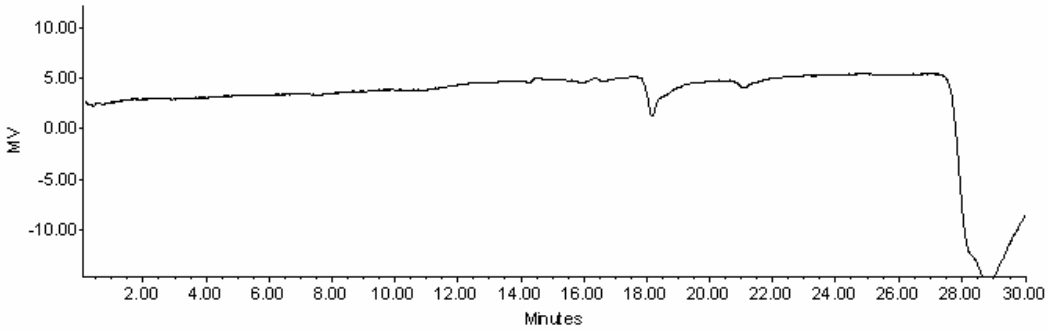


Figure A7.13: GPC chromatogram of Z553D with RI detection.

No polymer peak was observed. Fresh reagents were prepared and the test sample chromatographed under different MP conditions. The first two injections were done under standard MP conditions (KH_2PO_4, pH 2.3). This was regarded as a system test as it produces a known polymer peak profile. This was done in response to the poor

results obtained in the previous experiments with acetone. The first injection produced a flat baseline and was attributed to an electronic detector fault that was experienced randomly after five years of operation. The second injection produced the expected profile (Figure A7.14).

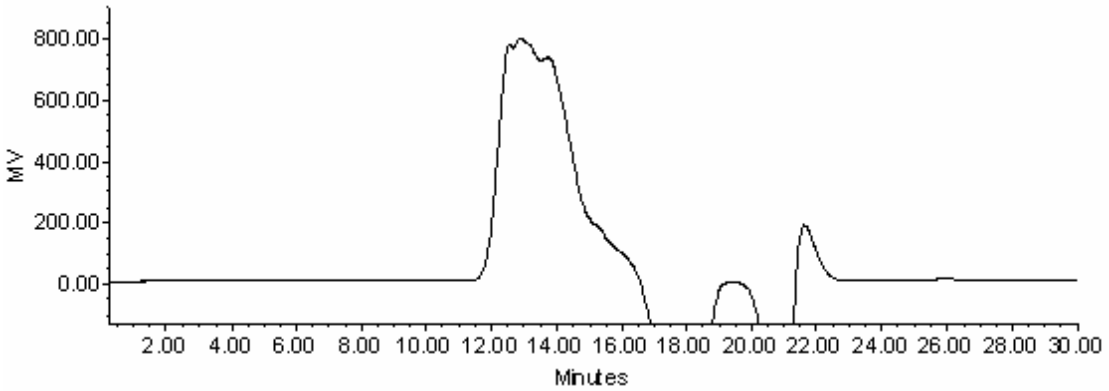


Figure A7.14: GPC chromatogram of Z553D with RI detection and standard operating conditions used as the system test.

The third injection was done with the chromophore $CrO4^{2-}$ added to the MP (Figure A7.15).

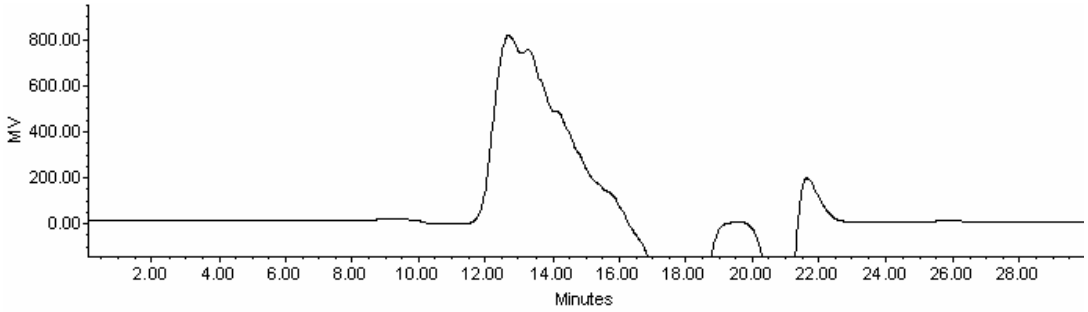


Figure A7.15: GPC chromatogram of Z553D with the MP containing the CrO_4^{2-} chromophore.

The overlay chromatogram (Figure A7.16) showed that the added chromophore did not affect or interfere with the normal GPC process as the profiles are almost identical.

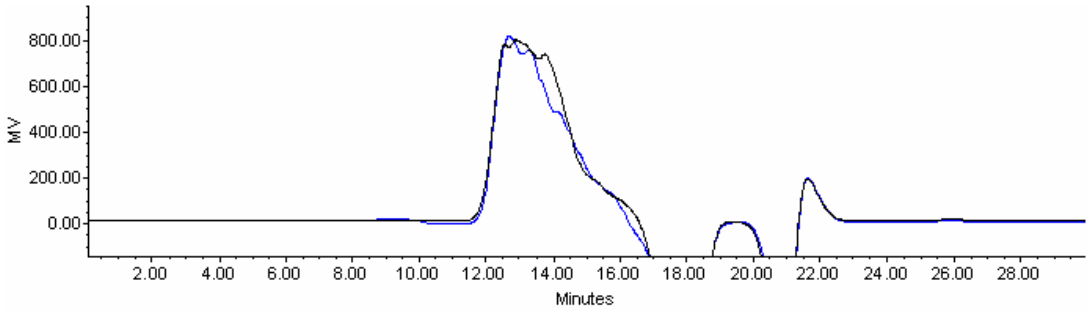


Figure A7.16: Overlay of GPC chromatograms of Z553D under standard MP conditions and with added CrO_4^{2-}.

Since the chromate ions were shown to be a good candidate chromophore for indirect UV detection, a full assessment of the technique was made. The procedure involved analysis of six test solutions of Z553D with decreasing injection volumes (100 to 1 µL). To ensure that the system was fully operational, the test solutions were first analyzed by GPC with RI detection. This was found to be extremely useful and problems could be addressed more easily. In this exercise it was discovered that all data acquired with RI detection did not produce the expected polymer peak profile. On careful inspection of the solutions it was noted that there was small quantities of precipitate visible in the sample vials. There was a possibility that this problem may have resulted in partial or full blockage of the injector. For this reason, no UV data was collected for the samples.

A fresh polymer stock solution (0.5%, m/v) was prepared using Z553D and analyzed by GPC RI (Figure A7.17).

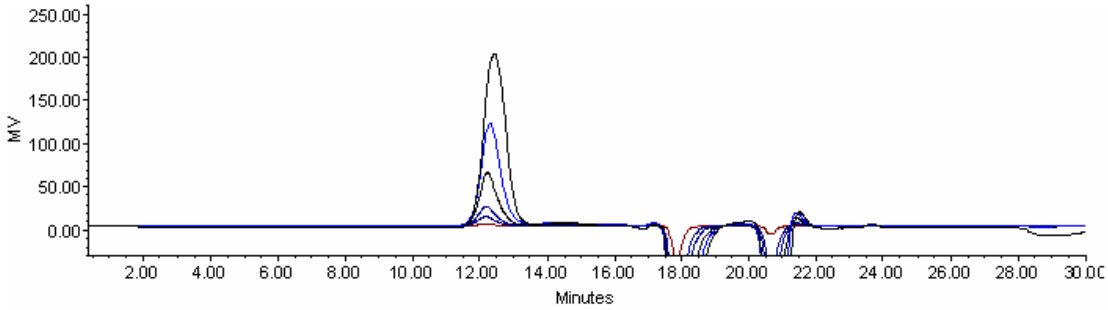


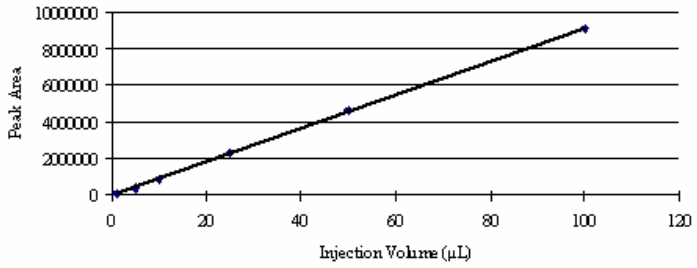
Figure A7.17: Overlay of GPC chromatograms of 0.5% Z553D with RI detection and injection volumes ranging from 100 to 1 µL.

The chromatogram indicates that the polymer peaks are symmetrical, with a good correlation between the detector response and the amount of polymer injected. The peak areas and corresponding injection volumes are shown in Table A7.2.

Table A7.2: Injection volume and peak area data for 0.5% Z553D solutions with RI detection.

Injection Volume (µL)	Peak Area
100	9120521
50	4641087
25	2316062
10	865466
5	383382
1	63227

The data were used in regression analysis and the regression line obtained (Figure A7.18) showed excellent linearity with a correlation coefficient $R^2 = 0.9997$.



Regression Statistics		
Multiple R	1.00	
R Square	1.00	
Adjusted R Square	1.00	
Standard Error	55544	
Observations	6	STDEV
Intercept ± STDEV	-27501	30824
Slope ± STDEV	91909	655
y = 91910x - 27502		

Figure A7.18: Relationship between peak area and injection volume with RI detection.

The remaining regression statistics shows a goodness-of-fit of the calibration data to be satisfactory. All conditions were maintained except for the use of the PDA UV detector instead of the RI detector. The PDA overlay chromatogram (Figure A7.19), extracted at 350 nm, did give a response but there was significant detector instability and baseline drift.

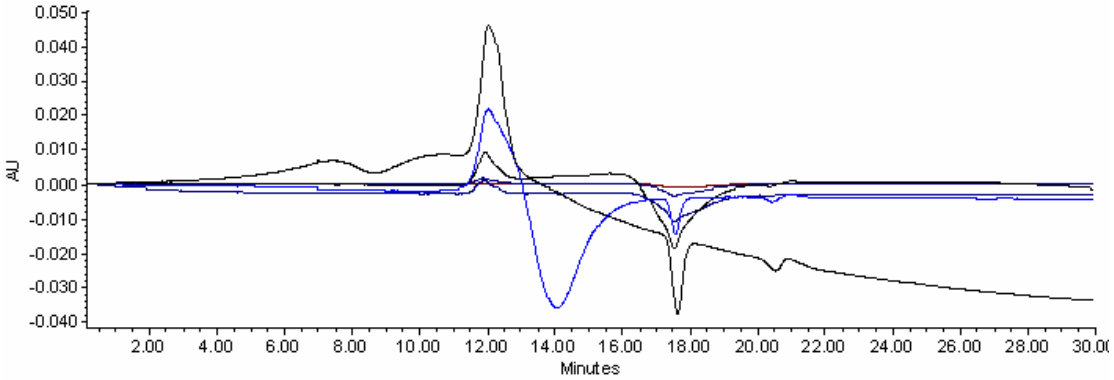


Figure A7.19: Overlay of chromatograms of 0.5% Z553D solutions with indirect UV detection.

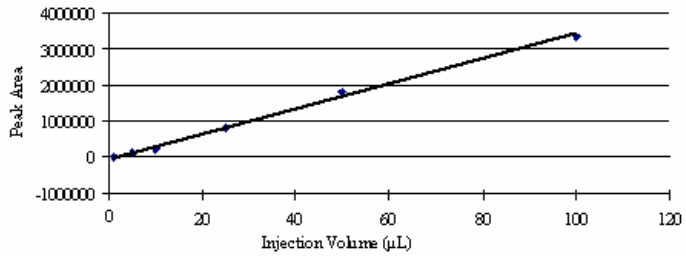
The detector response was assessed by acquiring the peak areas and injection volumes (Table A7.3).

Table A7.3: Injection volume and peak area data for 0.5% Z553D solutions with indirect UV detection.

Injection Volume (µL)	Peak Area
100	3360512
50	1801514
25	803272
10	239064
5	116874
1	nd

nd=not detected

The data were used in linear regression analysis. The regression line (Figure A7.20) indicated good linearity for a newly developed technique, with a correlation coefficient $R^2 = 0.998$.



Regression Statistics		
Multiple R	1.00	
R Square	0.998	
Adjusted R Square	1.00	
Standard Error	71723	
Observations	6	STDEV
Intercept ± STDEV	-47989	39804
Slope ± STDEV	34578	847
y = 34578x - 47989		

Figure A7.20: Relationship between peak area and injection volume of Z553D with indirect UV detection.

To compare the two detection methods in terms of sensitivity, the RI and UV data were plotted together (Figure A7.21). The plots indicate clearly that the RI response has a steeper gradient and is therefore superior in sensitivity to the PDA UV detector.

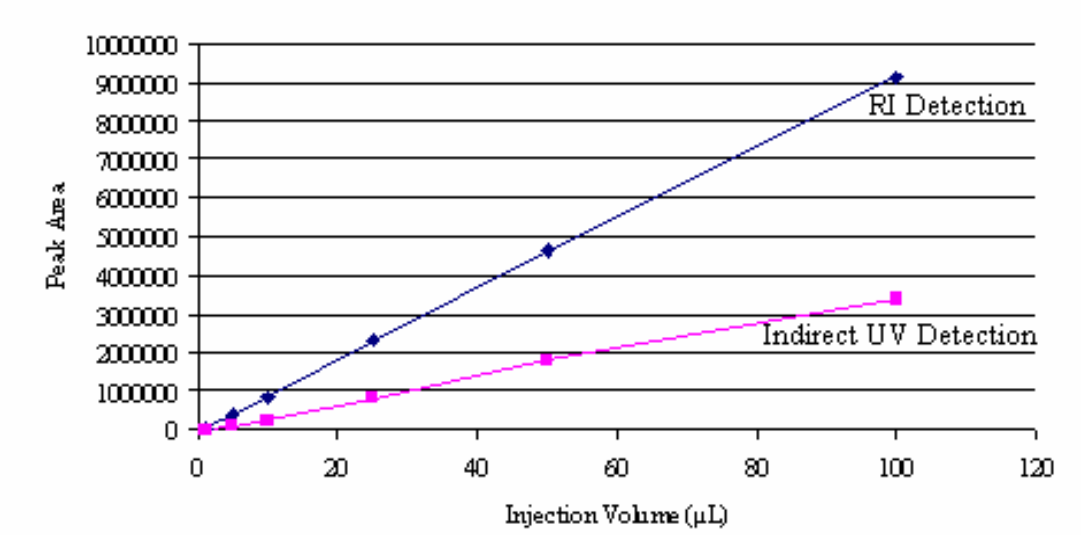


Figure A7.21: Plot of RI and UV detector response as a function of polymer injection volume.

In conclusion, the indirect UV detection method with $CrO4^{2-}$ at a concentration of 0.2 mM was developed successfully. However it proved to be of limited value for residual polymer analysis as the chromophore did not appear to be sensitive enough.

A7.2 References

1. Kemp W, Organic Spectroscopy, 3rd Edition, The Macmillan Press LTD, London, 247 (1991).

APPENDIX 8

SOLID PHASE EXTRACTION

A8.1 Results and Discussion

Initially, a series of experiments was conducted to test if the predicted mechanism occurred. The first test was to observe if there was any interaction between the polymer and the solid phase without any chemical modification. A small volume of polymer (2 mL of 0.1% Z553D) was pumped through the C_{18} extraction cartridge using a syringe and the effluent collected in an HPLC vial. Subsequent GPC analysis (Figure A8.1) indicated that, as predicted, the charged polymer did not interact with the solid phase and was present in the effluent.

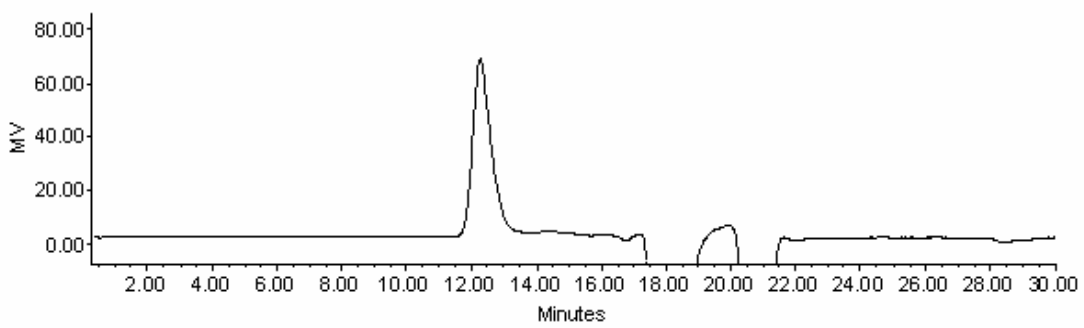


Figure A8.1: GPC chromatogram of Z553D after passing through the C_{18} extraction cartridge without chemical modification.

This was followed by another experiment in which the C_{18} material was first chemically modified with the ion pair reagent hexane sulfonic acid prior to sample extraction. The effluent was filtered into a vial for analysis. The C_{18} extraction cartridge was then treated with 2 mL of the elution reagent and collected in a vial. The samples together with the 0.1% Z553D reference polymer were analyzed by GPC (Figure A8.2).

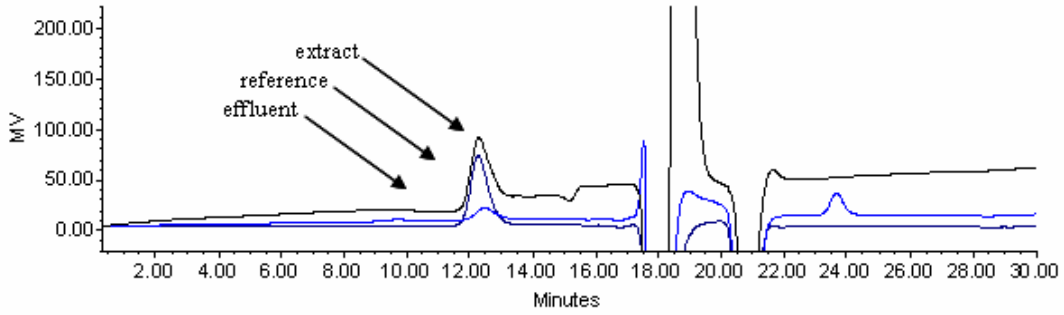


Figure A8.2: Overlay of GPC chromatograms of Z553D effluent, extract and the reference polymer after passing through the C_{18} extraction cartridge.

The overlay chromatograms indicate that both the effluent and extract show the presence of polymer. The peak area data shown on the chromatogram (effluent 614598; extract 2781670; reference 2835467) show that *ca* 22% of the

polymer is lost in the effluent. This could be a result of polymer breakthrough by overloading of the solid. The extract on the other hand indicates an extraction efficiency of 98 % when compared to the area of the reference polymer solution. The total of 120% recovery may be due to measurement errors associated with the method.

The results were encouraging and sample extraction and analysis commenced, beginning with one liter volumes containing 2 mg L^{-1} Z553D in Milli-Q water. The sample was extracted followed by elution in 10 mL of the elution reagent. This resulted in a concentration factor of 100, to give a theoretical polymer concentration of the extract of 200 mg L^{-1}, which was well within the method detection limit of 5 mg L^{-1}. The GPC chromatogram of the extract (Figure A8.3) shows an absence of the polymer peak at the expected retention time of 12 min.

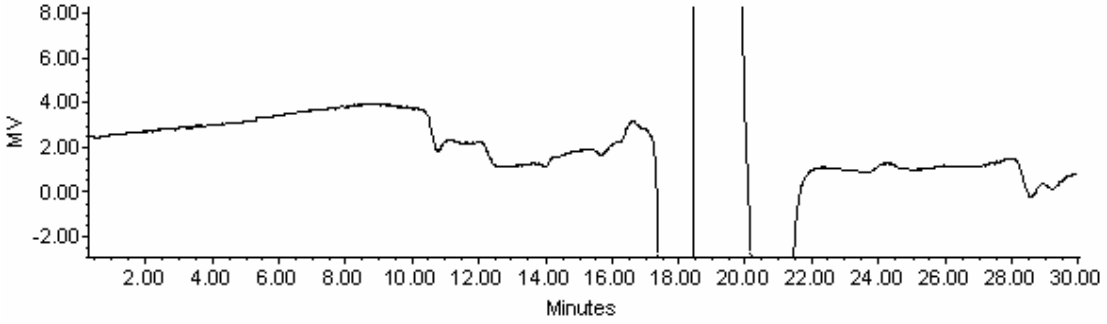


Figure A8.3: GPC chromatogram of a Z553D extract using C_{18} extraction cartridges.

It was speculated that two factors may have contributed to this problem: the ion pair reagent may have been "washed off" the sorbent by the 1 L of liquid sample, leaving the sorbent surface essentially unmodified, and the possibility existed that the polymer was strongly adsorbed by the modified sorbent and not eluted by the elution reagent. To test the first theory, a new sample was prepared (0.005 g L^{-1} Z553D), to which was added 3 g of hexane sulfonic acid. The sample was extracted and analyzed by GPC (Figure A8.4).

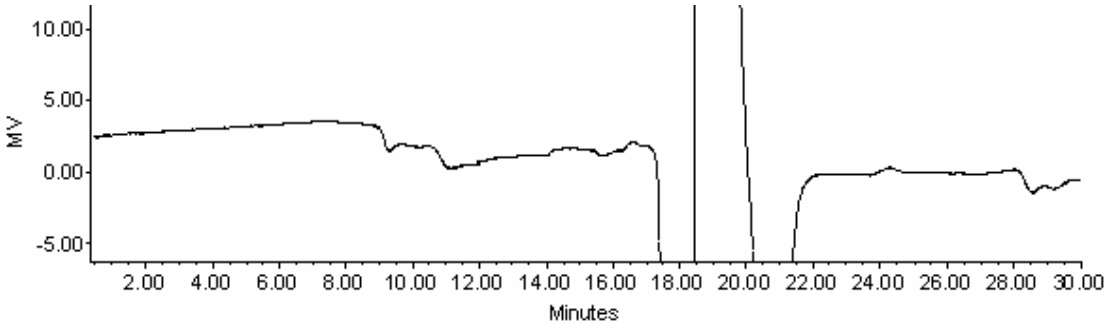


Figure A8.4: GPC chromatogram of a Z553D C_{18} extract after the addition of ion pair reagent.

The addition of the hexane sulfonic acid ion pair reagent to the sample did not have any observed effect on the extraction efficiency. The polymer content of the sample was increased by an order of magnitude to 0.05 g L^{-1} with 3 g ion pair reagent. The sample was extracted and eluted in 10 mL elution reagent to give a theoretical

concentration of 5 g L^{-1}. The GPC chromatogram (Figure A8.5) shows a broad peak eluting from 9 to 17 min and which did not approximate the expected polymer peak profile. The same sample was re-injected but the broad peak was greatly reduced and contamination of the HPLC injector was suspected.

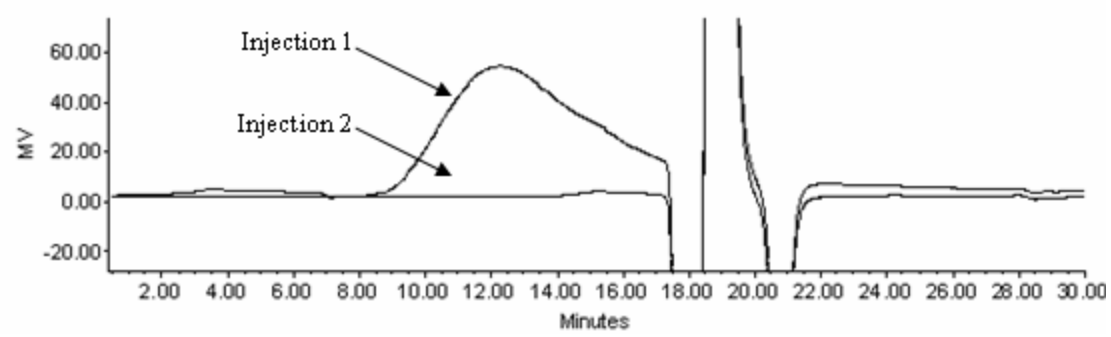


Figure A8.5: Overlay of GPC chromatograms of replicate injections of a 0.05% Z553D extract.

Analysis of a reference polymer solution (0.1% Z553D) as a system test indicated that the GPC system was fully functional (Figure A8.6).

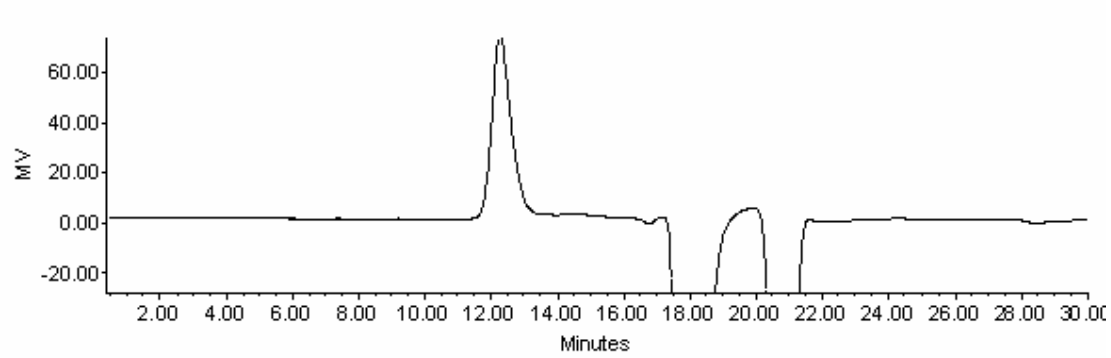


Figure A8.6: GPC chromatogram of a 0.1% Z553D reference polymer.

The lack of polymer detection of the C_{18} extracts necessitated the re-assessment and evaluation of the instrument's detection limit. New polymer solutions were used. Analysis of the standards from 0.001% to 0.02% (Figure A8.7) shows that no peak was detected at a level of 0.001% concentration.

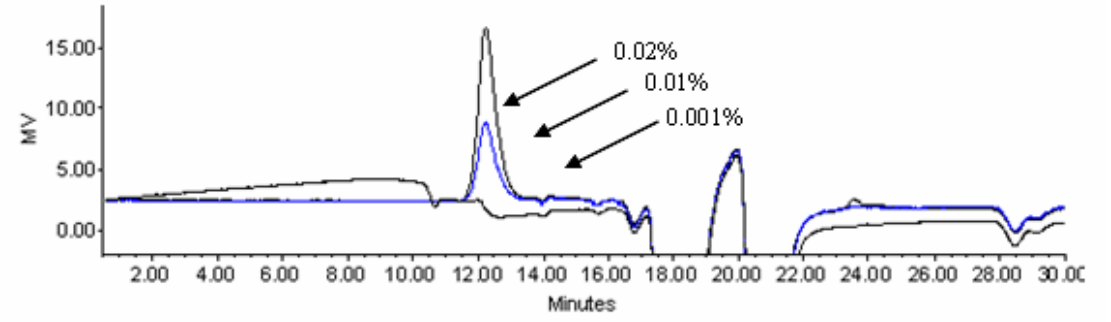


Figure A8.7: Overlay of GPC chromatograms of 0.001 to 0.02% polymer solutions.

The estimated detection limit of 0.005% previously established was therefore confirmed once more. The investigation into poor polymer recoveries was continued with an increase in the spike concentration of the test samples to a value greater than the instrument detection limit of 0.005%. The aim of using this concentration was to test for the presence of polymer in the effluent during extraction in order to determine whether the polymer was strongly adsorbed by the sorbent or was breaking through.

A new sample of Z553D was prepared at a spike concentration of 0.01%. Since this value is greater than the detection limit, the effluent could be monitored for polymer presence. During sample extraction, a small volume of the effluent was filtered into a vial for GPC analysis. After extraction, the C_{18} was wetted with a small volume of elution reagent for 45 min, after which it was extracted in 10 mL of the reagent (theoretical concentration 1%). Both solutions were chromatographed (Figure A8.8) and polymer presence was found in both vials, suggesting that a significant amount of polymer was lost during sample extraction.

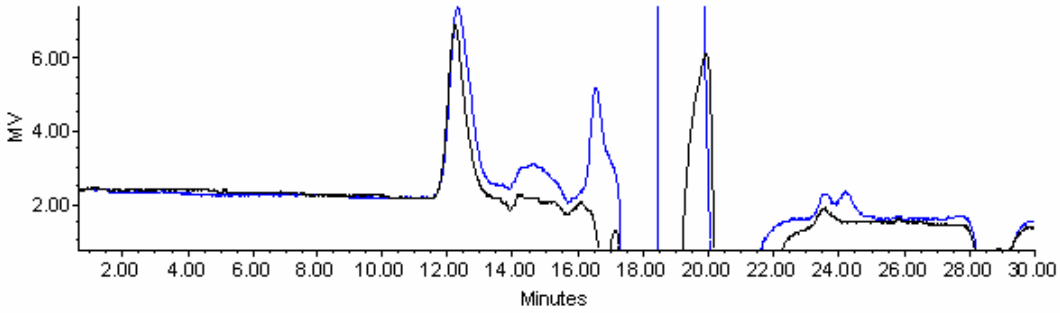


Figure A8.8: Overlay of GPC chromatograms of the effluent and extract of Z553D at a spike concentration of 0.01%.

The polymer concentration and percentage recovery of the effluent was estimated based on the 0.1% reference polymer from previous experiments (Figure A8.6), and calculated using equations A8.1 and A8.2.

$$Concentration_{effluent} = \frac{area_{effluent}}{area_{std}} \times concentration_{std} \qquad A8.1$$

$$= \frac{188589}{2835467} \times 0.1$$

$$= 0.0066\,\% \quad (theoretical\ value = 0.01\%)$$

$$\%Recovery = \frac{0.0066}{0.01} \times 100 \qquad A8.2$$

$$= 66\,\%$$

This result must however be used with caution as it does not necessarily imply that such losses occur with all samples. The large losses in this case may be due to overloading of the C_{18} with the intentionally high spike concentration of the sample. The concentration and percentage recovery of the extract was calculated using equations A8.3 and A8.4.

$$Concentration_{extract} = \frac{area_{effluent}}{area_{std}} \times concentration_{std} \qquad A8.3$$
$$= \frac{227403}{2835467} \times 0.1$$
$$= 0.008\% \quad (theoretical\ value = 0.01\%)$$

$$\%Recovery = \frac{0.008}{1} \times 100 \qquad A8.4$$
$$= 0.8\%$$

This result demonstrates that extraction efficiency is negligible and most of the polymer is lost in the effluent. The effluent and extract add up to a total polymer content of 67% and the remaining 33% has not been accounted for. This was attributed to adsorption of polymer onto glass surfaces such as that of volumetric flasks and apparatus (funnels, filters, syringes, tubing, etc) used for sample preparation and processing. A repeat of the experiment with two fresh samples showed no polymer recovery in the extract (Figure A8.9).

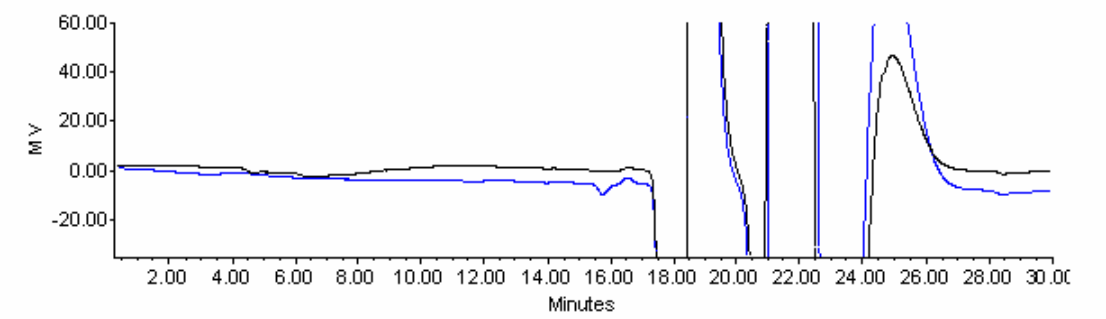


Figure A8.9: Overlay of GPC chromatograms of the polymer extracts at spike concentrations of 0.01%.

The sample volume was reduced from 1000 mL to 100 mL (0.0001%). Sample extraction was carried out and eluted in 2 mL of reagent to give a theoretical concentration of 0.005% (detection limit). The motivation for this low value was to test for whether the C_{18} extraction media was being overloaded with polymer. Even at this low polymer content, the GPC chromatogram showed the absence of a polymer peak.

A new experiment was designed to observe the effect of loading onto the C_{18}, and simultaneously confirm the initial experiment conducted to test the potential of polymer extraction by C_{18}. The test sample (2 mL of 0.1% Z553D) was loaded onto a pre-treated C_{18} cartridge and both effluent and extract were chromatographed (Figure A8.10a). This was followed by the extraction and analysis of a 5 mL aliquot of the test sample (Figure A8.10b). The effluent of the first sample shows that no polymer is present in the effluent, indicating a total polymer removal. The extract on the other hand shows an unfamiliar broad peak eluting in the region 11 to 17 min. This was encountered before and was thought to be contamination. The unknown peak co-eluted and totally masked the test polymer peak. The polymer peak presence could not be confirmed. The second sample shows the presence of polymer in the

effluent and suggested that there was polymer breakthrough occurring. The extract shows a polymer peak as well as the unknown peak on the shoulder of the polymer peak.

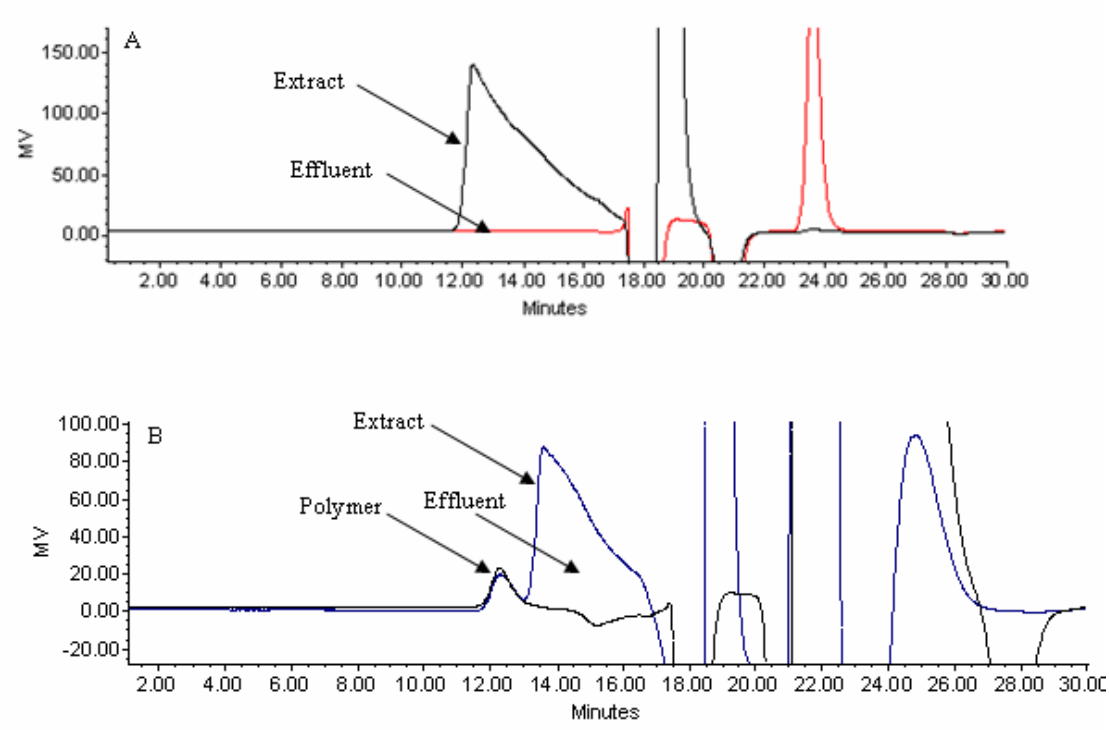


Figure A8.10: Overlay of GPC chromatograms of the effluent and extract of (a) the 2 mL 0.1% polymer sample and (b) the 5 mL 0.1% polymer sample.

There was some uncertainty whether the reagents were causing the unknown peak or whether it originated from another source (contamination). Three aliquots of the polymer were treated individually with each of the three reagents (elution reagent, conditioning A and conditioning B) in a 1:1 ratio of sample:reagent. The GPC profiles (Figure A8.11) indicate no adverse effects of the reagents on the polymer peak except for the slightly suppressed polymer peak with conditioning solution B. There were no traces of the broad peak observed during solid phase extraction.

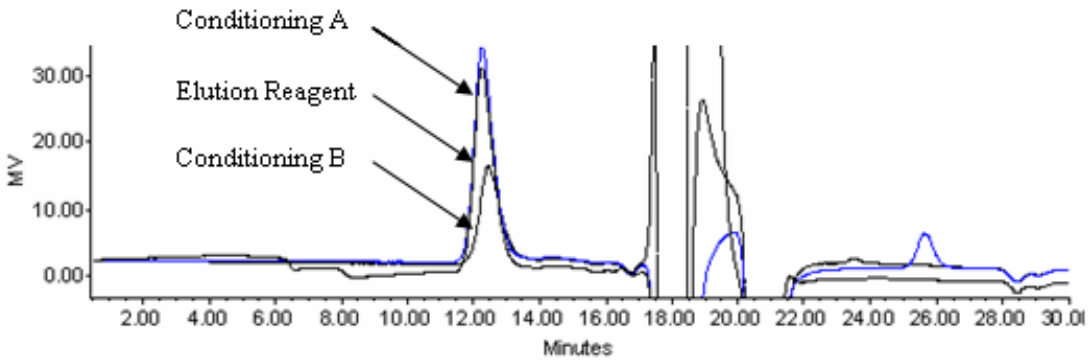


Figure A8.11: Overlay of GPC chromatograms of the reference polymer with the three solid phase extraction reagents.

Further work investigating the unknown peak was carried out. It was thought that wetting the C_{18} extraction cartridge with elution reagent for the extended period was causing degradation of the extraction media. To verify this two further polymer samples (0.1% Z553D) were processed. The effluents from both extracts were collected and analyzed. Thereafter, the one cartridge was eluted and analyzed. The second cartridge was wetted with the eluting reagent for 30 min prior to elution and analysis. The GPC chromatogram of the normal (Figure A8.12a) and wetted extracts (Figure A8.12b) show an unexpected trend.

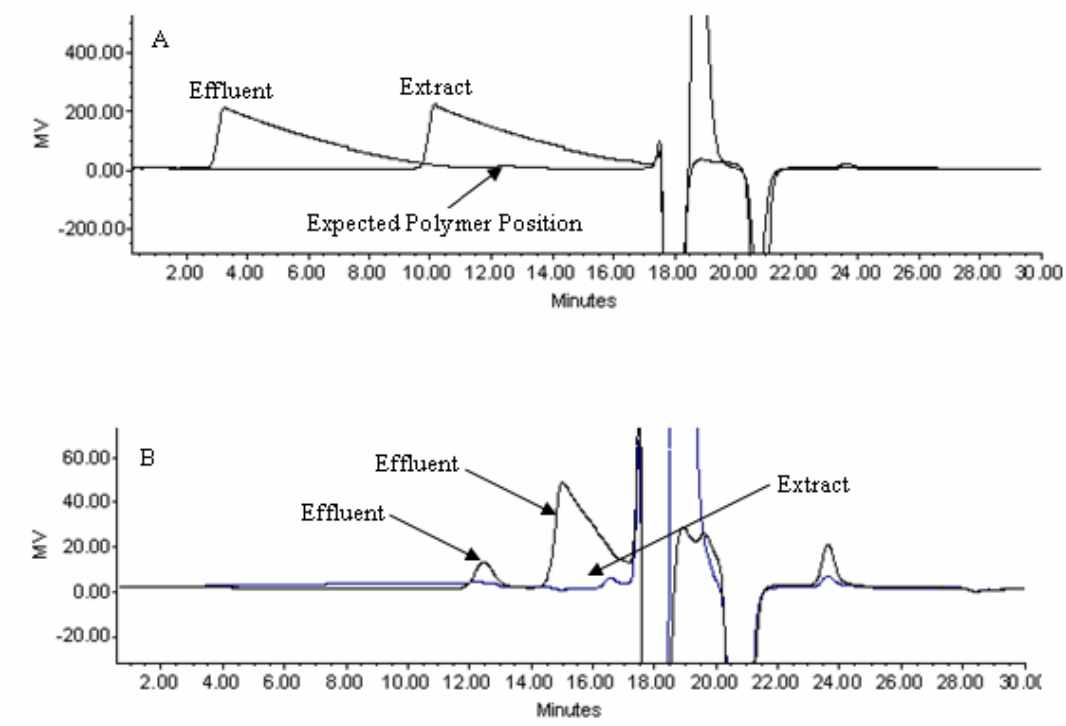


Figure A8.12: Overlay of GPC chromatograms of the effluent and extract of the (a) "normal" and (b) the wetted sample extracts.

The normal sample shows prominent peaks occurring at different retention times in both the effluent and extract, which added to the confusion. The wetted sample shows a polymer peak in the effluent, followed by a reduced interferent peak. The polymer peak is absent in the extract. The origin of the peak was still unknown but it was strongly suspected that it was originating from the sorbent material. Further work was conducted on more samples. The detection of polymer in the extract and effluent was random and poor reproducibility of the results was experienced. It was suspected that this random occurrence was the result of weak polymer-sorbent interactions. The polymer and ion pair reagent was breaking through by the action of the solvent.

To test this theory, 2 mL of polymer (0.1%) was loaded on to a pre-treated cartridge and followed up by a washing process with 500 mL of Milli-Q water. This was followed by extraction in 2 mL of elution reagent, and analysis. The GPC chromatogram (Figure A8.13a) shows the presence of a polymer peak. The experiment was repeated (Figure A8.13b) and showed good reproducibility.

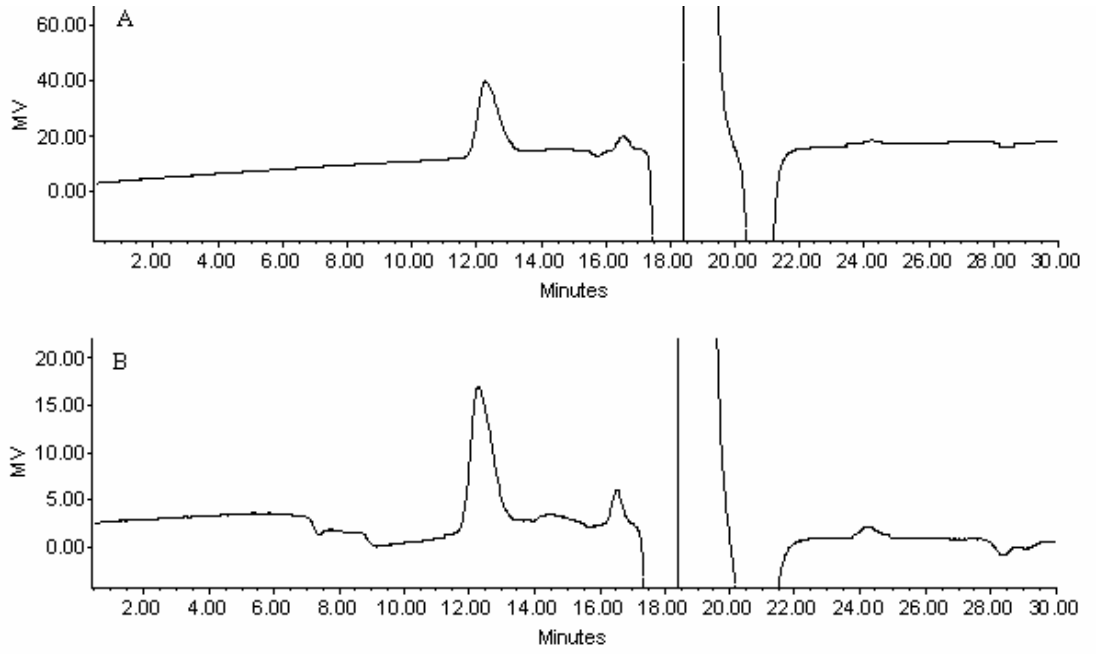


Figure A8.13: GPC chromatogram of (a) a polymer extract by solid phase extraction and (b) a repeat experiment.

A similar experiment was conducted but the rinsing was carried out with 250 mL of Milli-Q water. The extract showed a significant improvement in recovery based on observation of the polymer peak size. The final extraction was carried out using a 50 mL rinse with Milli-Q water. Again there was a visible increase in the polymer recovery. The relative change in polymer recoveries as a function of rinsing volume is shown in Figure A8.14.

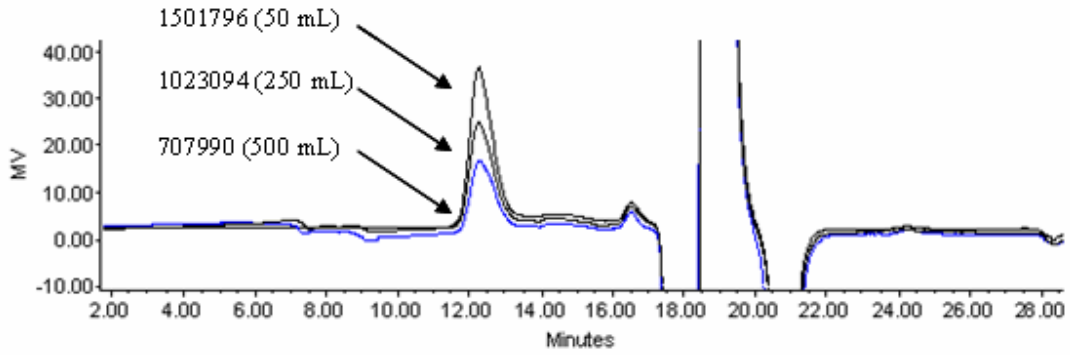


Figure A8.14: Overlay of GPC chromatograms of polymer extracts with decreasing rinse volumes.

The observed trend confirmed the following:

- There is some degree of interaction of the polymer with the sorbent extraction medium
- An increase in the sample volume tends to weaken the forces of interaction between the polymer and the solid phase medium
- When large sample volumes are loaded the interaction of the polymer and the sorbent is negligible, resulting in nearly zero recoveries

- Solid phase extraction appears to be effective with only small sample volumes.

The practical importance of the above renders the technique of little use in residual polymer analysis that requires extraction from large sample volumes.

Since the experiments with the C_{18} phase produced very erratic results, a new solid phase, tri-functionally bonded C_{18} (tC_{18}) was tested. The new phase was a tri-functionally bonded phase with higher capacity and improved chemical stability. The activation, extraction and elution procedures were identical to those used for the C_{18} experiments. The recovery results were reproducible (Figure A8.15), giving percentage recoveries of 33%, 40% and 40% for each of the three samples (50 mL 0.004% polymer).

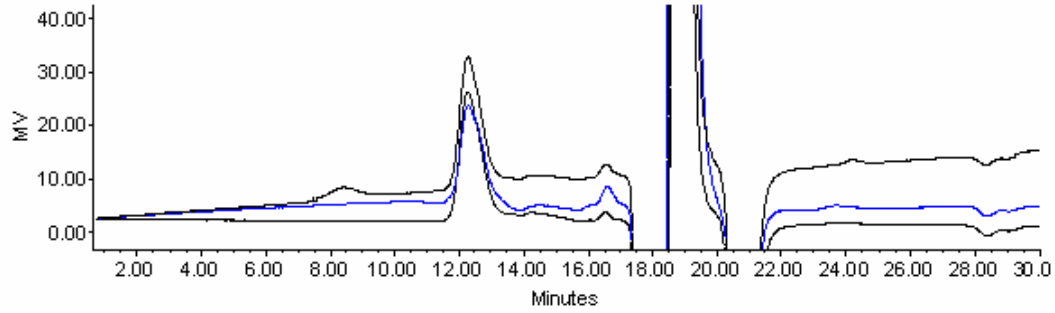


Figure A8.15: Overlay of GPC chromatograms of 50 mL sample volumes extracted using tC_{18} solid phase cartridges, at neutral pH.

The technique was significantly improved with the tC_{18} solid phase. A further two samples with increased sample volumes of 250 and 500 mL were extracted. Polymer peaks were detected in the extract (Figure A8.16) at a level far in excess to that achieved with normal C_{18}.

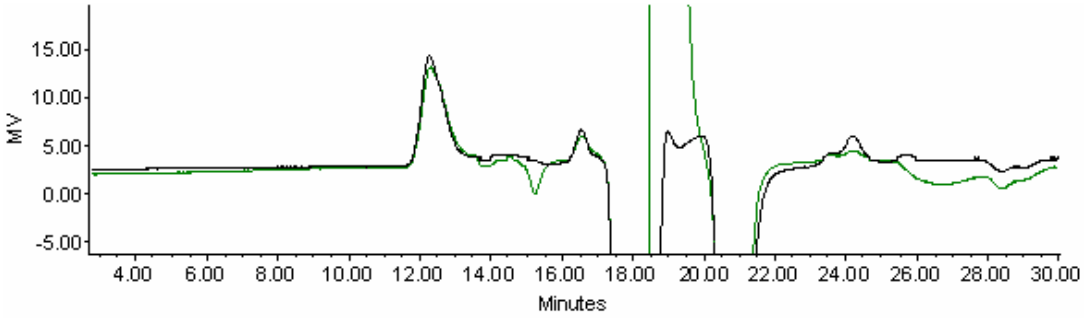


Figure A8.16: Overlay of GPC chromatograms of 250 and 500 mL sample volumes extracted using tC_{18} solid phase cartridges, at neutral pH.

The recoveries were calculated to be 18% and 19% for the two samples. The main reason for the low extraction efficiency of the solid appeared to be weak polymer-solid phase interactions that resulted in a breakthrough of polymer. This was aggravated by large solvent volumes.

As alternative reagents were not available during this study, the final experiment with solid phase extraction was conducted using Amberlite XAD resin. The mode of

action was cation exchange. The resin was activated with 10 mL of 2 M HCl prior to sample extraction (500 mL, 2 mg L^{-1} Z553D). The elution reagent was used for the removal of polymer from the resin. GPC analysis (Figure A8.17) showed no recovery of polymer with this method of extraction, suggesting that the polymer interacts irreversibly with Amberlite XAD resin.

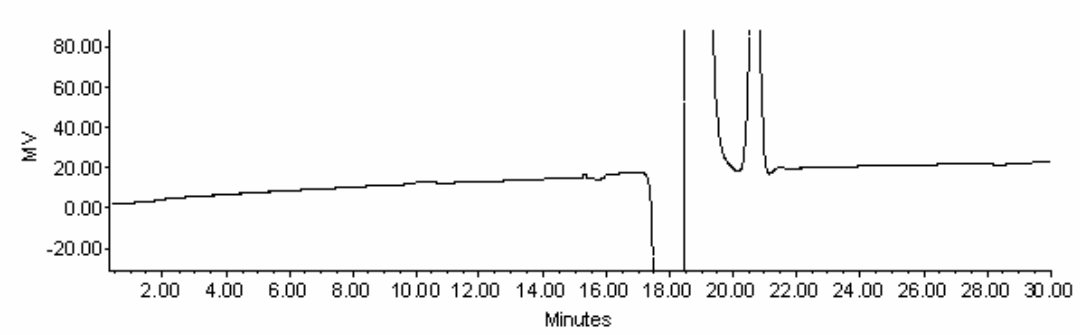


Figure A8.17: GPC chromatogram of Amberlite XAD resin extract of a polymer sample.

The analysis of polymers by the colloid titration method and ultrafiltration GPC was described elsewhere (Chapters 8 and 9 respectively).

APPENDIX 9

ELECTRON MICROSCOPY

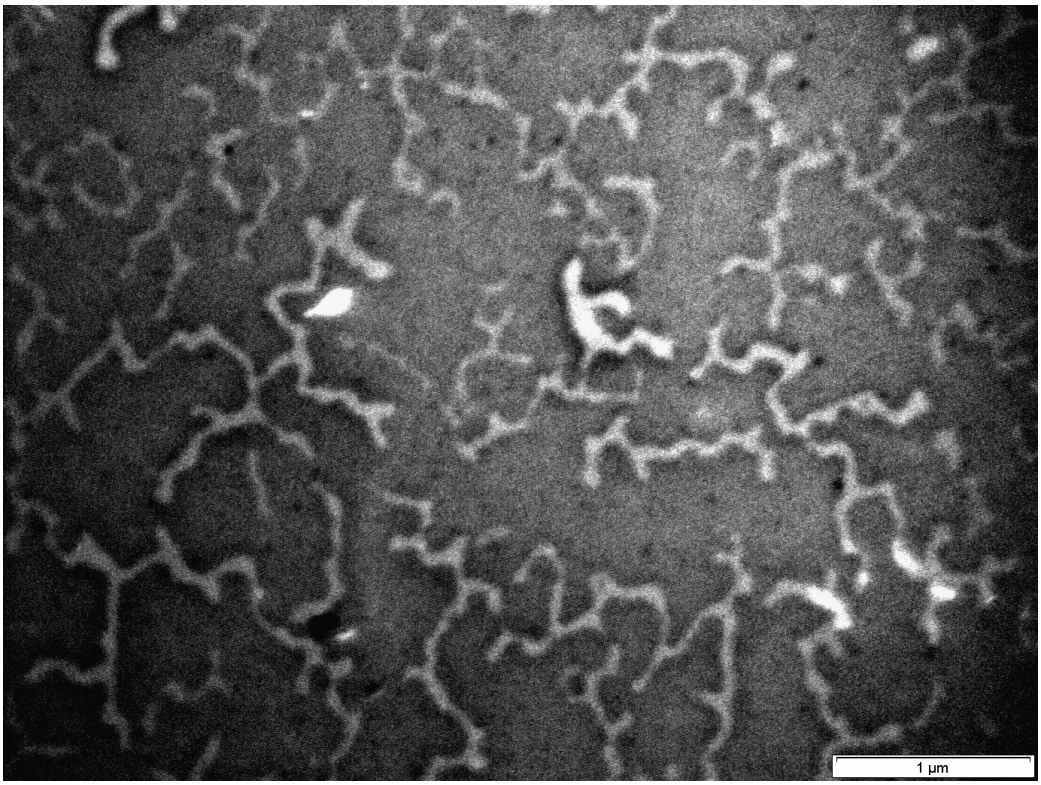


Figure A9.1: TEM magnified view of PolyDADMAC.

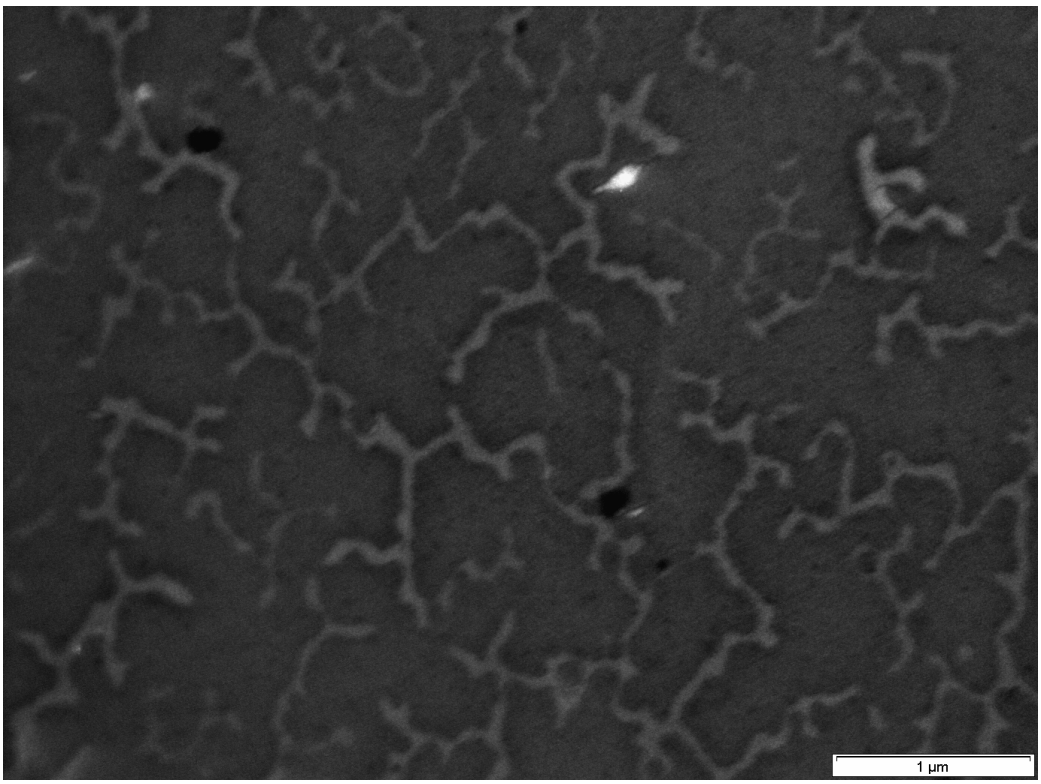


Figure A9.2: TEM magnified view of PolyDADMAC.

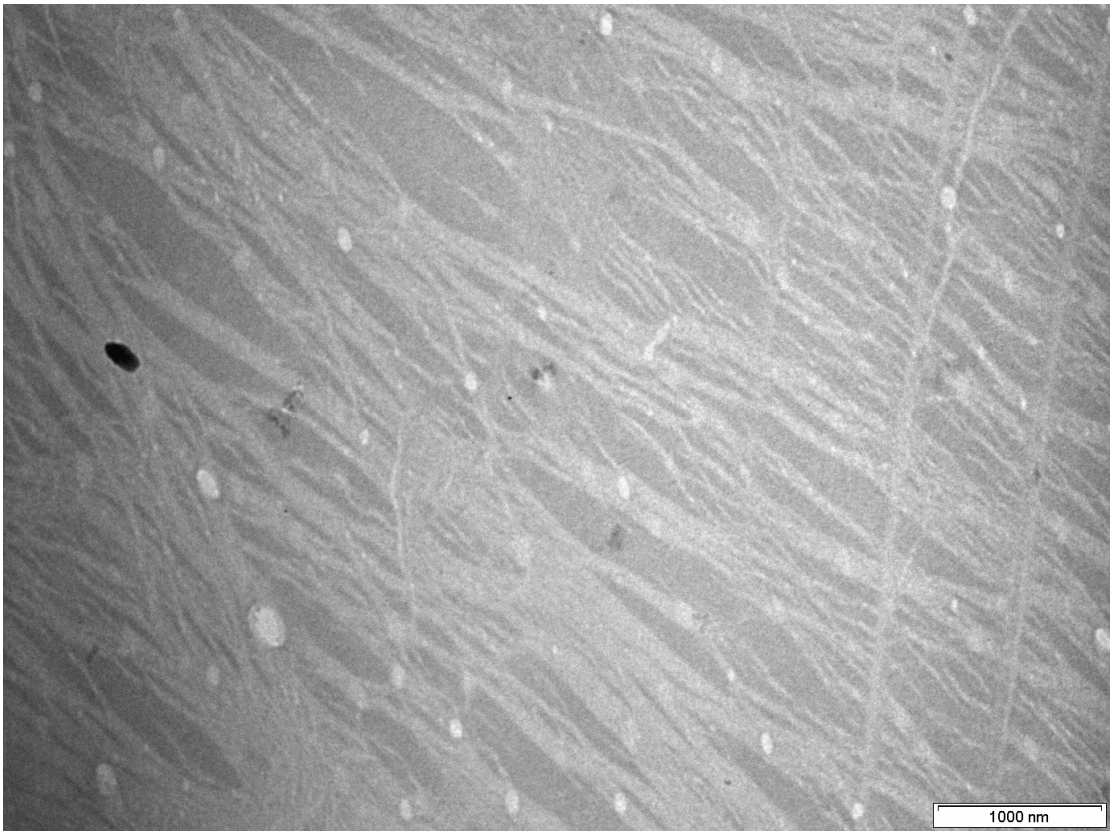


Figure A9.3: TEM magnified view of PolyDADMAC.

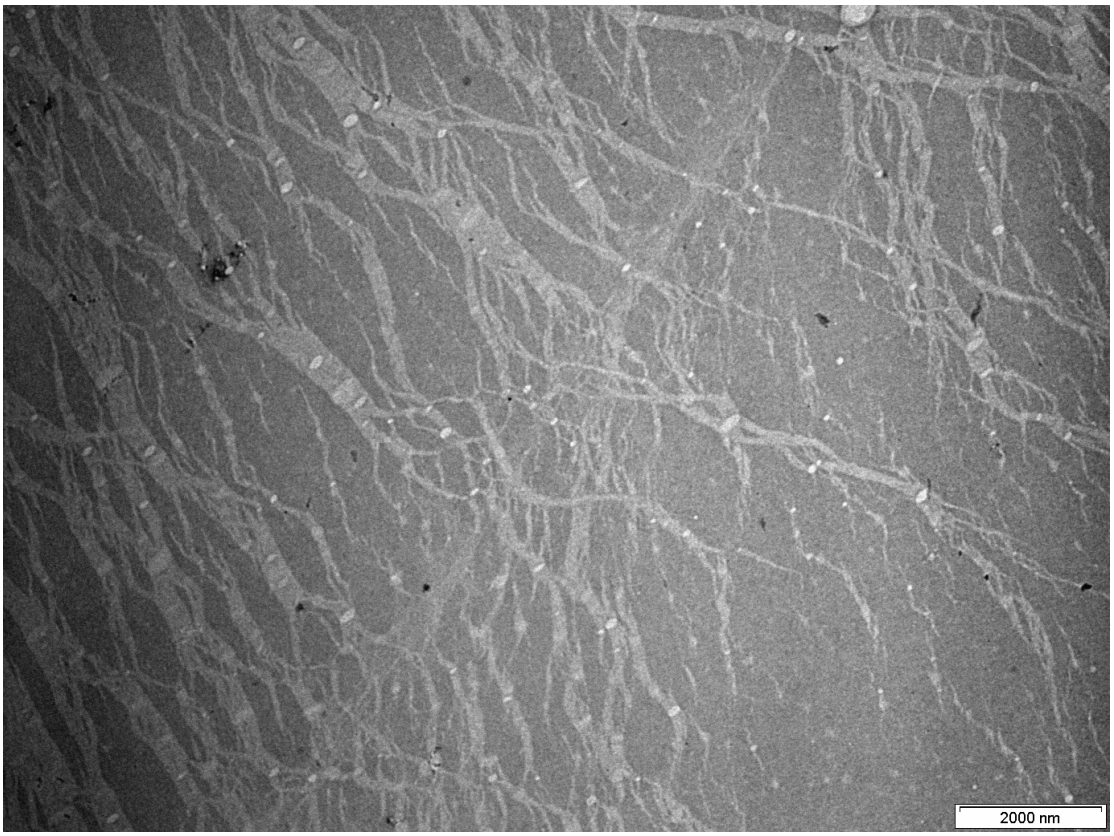


Figure A9.4: TEM magnified view of PolyDADMAC.