

**COMPARISON OF METACHROMATIC
TITRATION VERSUS A NOVEL NANO-GOLD
TAGGING TECHNIQUE FOR THE DETECTION
AND QUANTIFICATION OF
POLYDIALLYLDIMETHYLAMMONIUM
CHLORIDE IN WATER SAMPLES**

By

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As the candidate's supervisor I have approved this thesis/dissertation for submission

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Date

Preface

The experimental work described in this dissertation for Masters was carried out in the School of Chemistry and Physics, University of KwaZulu-Natal, Westville campus from February 2011 to February 2013, under the close supervision of Doctor Patrick Ndungu and co-supervised by Prof J. Catherine Ngila (University of Johannesburg).

These studies represent original work by the author and have not otherwise been submitted in any form for degree or diploma to any tertiary institution. Where use has been made of the work of others it is duly acknowledge in the text.

This dissertation has been prepared according to the format outlined in the guidelines from the Faculty of Science and Agriculture of UKZN, (FHDR Approved 13 March 2007).

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Declaration 2 – Scientific Communications

Details of contribution to publications that form part and include research presented in this thesis.

Presentation

Parts of this work have been presented in two international conferences and one local conference.

List of conferences

1. 4th SEANAC 2012 international conference Maputo, Mozambique between 7 to 12 July 2012.
Title of paper presented: **Synthesis of gold nanoparticles and application in environmental analysis for the quantification of polydiallyldimethylammonium chloride in water**
Authors: Bhekumuzi Prince Gumbi, Patrick Ndungu (Supervisor), Catherine Ngila (Co-supervisor)
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Title of paper presented: **Application of metachromatic polyelectrolyte titration in environmental analysis for the quantification of polydiallylmethylammonium chloride in water**
Authors: Bhekumuzi Prince Gumbi, Patrick Ndungu (Supervisor), Catherine Ngila (Co-supervisor)
3. College of Agriculture and engineering symposium 2012, Pietermaritzburg, South Africa, 29 October 2012.
Title of paper presented: **Application of metachromatic polyelectrolyte titration in environmental analysis for the quantification of polydiallylmethylammonium chloride in water**
Authors: Bhekumuzi Prince Gumbi, Patrick Ndungu (Supervisor), Catherine Ngila (Co-supervisor)

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Abstract

This study has developed a sensitive, accurate, and relatively cheap methodology for the detection and quantification of polydiallyldimethylammonium chloride (poly-DADMAC) in water samples. Poly-DADMAC is a cationic polyelectrolytes used in drinking water treatment facilities for the removal of particles and concentration of sludge. Poly-DADMAC breakdown during the disinfection stage to carcinogenic compounds and its occurrence in home taps, may pose a health risk to end-users of the treated water.

Upon review of the literature and evaluation of common analytical methods such as NMR, LC-MS, Raman and metachromatic titration for possible routine analysis of poly-DADMAC in water samples, it was established that NMR, LC-MS and Raman techniques were found not suitable for routine analysis of poly-DADMAC. One of the method that has been extensively used in the past is the metachromatic titration technique. It is one of the oldest analytical methods for detection of poly-DADMAC in water samples. However, this method suffers from poor detection limits and is not very accurate in identifying the endpoint.

In this study we solved this problem by employing a sensitive Ocean Optics Spectrometer equipped with a dip probe reading absorbance in-situ. There was good improvement in terms of precision and accuracy with a lower limit of detection of 0.1 mg L^{-1} . It was easier to determine the endpoint compared to previous reported techniques in the literature. Validation studies revealed that the method is suitable for routine analysis of poly-DADMAC.

Secondly we developed a novel method for detection of poly-DADMAC in water samples based on a colourimetric gold nanoparticle (Au-NP) probe. We synthesised and fully characterized different Au-NPs with TEM and UV-Vis spectroscopy. The novel method was validated and the results showed that this method could be used for the routine analysis of poly-DADMAC. This method was found to have a lower limit of detection down to $5 \text{ } \mu\text{g L}^{-1}$ and a relatively better selectivity compared to its monomer, diallyldimethylammonium chloride (DADMAC). The

performance of the various methods employed in this study was compared on the basis of limits of detection (LOD), limit of quantification (LOQ) and selectivity.

Keywords: polyelectrolytes, polydiallyldimethylammonium chloride (poly-DADMAC), diallyldimethylammonium chloride (DADMAC), metachromatic titration, gold nanoparticles and tri-sodium citrate.

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Table of contents

Preface.....	ii
Declaration 1 – Plagiarism	iii
Declaration 2 – Scientific Communications.....	iv
Abstract.....	v
Acknowledgements	vii
Table of contents	ix
List of Figures.....	xiv
List of Tables	xvii
Abbreviations	xix
Chapter 1: Introduction	1
1. General introduction	1
1.1 Research motivation.....	5
1.2 Problem identification.....	5
1.3 Hypothesis.....	6
1.4 Aims and objective	6
1.5 References	7
Chapter 2: Polyelectrolytes in water treatment.....	9
2. Introduction to polyelectrolytes	9
2.1 Classification of polyelectrolytes.....	9
2.2 Non-water treatment uses of polyelectrolytes.....	10
2.2.1 Materials science and nanotechnology.....	10
2.2.2 Membrane science.....	11
2.2.3 Pulp and paper industry.....	12
2.2.4 Selected industrial uses of polyelectrolytes.....	13
2.2.5 Polyelectrolytes in cosmetics, foods, and pharmaceutical products.....	13

2.2.6	Medical applications	14
2.3	Water treatment uses of polyelectrolytes	14
2.3.1	Polyelectrolytes used in drinking water treatment	15
2.3.2	Flocculation and coagulation	18
2.4	Polyelectrolytes as contaminants in drinking water	20
2.4.1	Use of Poly-DADMAC in water treatment	22
2.4.1.1	Synthesis of poly-DADMAC.....	22
2.4.1.2	Application of poly-DADMAC in water treatment.....	23
2.4.1.3	Regulatory aspects of Poly-DADMAC in water treatment	23
2.5	Analytical methods for quantification of poly-DADMAC and other polyelectrolytes	24
2.5.1	Colloid titration.....	24
2.5.2	Tagging.....	25
2.5.3	NMR.....	26
2.5.4	Chromatographic methods	27
2.5.5	Summary of analytical methods	27
2.6	Conclusions	28
2.7	References	30
 Chapter 3: Investigation of selected analytical methods.....		38
3.	Silanization of glassware	38
3.1	Preparations of poly-DADMAC stock and working solution	39
3.2	LC-MS methods.....	39
3.2.1	Chemicals.....	40
3.2.2	Instrumentation.....	40
3.2.3	Procedure.....	40
3.2.4	Results and discussion	41
3.2.5	Final Comments and Observations	43
3.3	NMR spectroscopy	44
3.3.1	Reagents and chemicals.....	44
3.3.2	Instrumentation.....	45

3.3.3	Procedure.....	45
3.3.4	Results and discussion	45
3.3.5	Final Comments and Observations	48
3.4	Raman spectroscopy	49
3.4.1	Reagents and Chemicals.....	50
3.4.2	Instrumentation.....	50
3.4.3	Procedure.....	50
3.4.4	Results and discussion	50
3.4.5	Final comments and observations	51
3.5	Overall Conclusions	51
3.6	References	53

Chapter 4: Application of metachromatic polyelectrolyte titration in environmental analysis for the quantification of polydiallyl methyl ammonium chloride in water..... 56

4.	Synopsis	57
4.1	Introduction	57
4.2	Experimental Methods.....	59
4.2.1	Chemicals and reagents.....	59
4.2.2	Silanization of glassware	59
4.2.3	Instrumentation.....	60
4.2.4	Standardisation of potassium salt poly (vinyl sulfate).....	60
4.2.5	Analysis of poly-DADMAC	61
4.3	Results and discussion.....	62
4.3.1	Standardization of PVSK.....	62
4.3.2	Analysis of poly-DADMAC	63
4.3.3	Direct plot of the volume of PVSK against poly-DADMAC concentration	64
4.3.4	Charge density approach	66
4.3.5	Spectrophotometric endpoint determination	68
4.4	Method development.....	72
4.4.1	Precision, accuracy and robustness statics studies	73

4.4.2	Matrix effect, limit of detection and application.....	75
4.5	Comparison of the proposed dip probe method of detecting the endpoint with methods in the literature.....	77
4.6	Conclusion.....	78
4.7	References	80

Chapter 5: Synthesis of gold nanoparticles and application in environmental analysis for the quantification of polydiallyldimethylammonium chloride in water

5.	Synopsis	83
5.1	Introduction	83
5.2	Experimental.....	85
5.2.1	Reagents and chemicals.....	85
5.2.2	Instrumentation.....	85
5.2.3	Preparation of solutions of gold nanoparticles.....	86
5.2.4	Preparation of stock solution of poly-DADMAC.....	88
5.2.5	Detection and quantification of poly-DADMAC.....	88
5.3	Results and discussion.....	89
5.3.1	Characterization of gold salt in aqueous solution.....	89
5.3.2	Characterization of gold nanoparticles.....	90
5.3.2.1	Characterization of gold nanoparticles A1 solution	90
5.3.2.2	Characterization of gold nanoparticles A2 solution	91
5.3.2.3	Characterization of gold nanoparticles A3 solution	93
5.3.3	Characterization of gold nanoparticle poly-DADMAC aggregates	95
5.3.3.1	Characterization of A1-poly-DADMAC aggregates	95
5.3.3.2	Characterization of A2-poly-DADMAC complex	98
5.3.3.3	Characterization of A3-poly-DADMAC aggregate.....	100
5.3.4	Detection of poly-DADMAC with the A1 gold nanoparticle solution.....	103
5.3.5	Development and validation of analytical protocols using the A1 gold nanoparticle solution	103
5.3.5.1	Limit of detection and limit of quantification.....	106

5.3.5.2	Precision	107
5.3.5.3	Recovery	109
5.3.5.4	Ruggedness (robustness).....	110
5.3.5.5	Effect of pH.....	110
5.3.5.6	Effect of Au-NP solution volume	112
5.3.5.7	Effect of time on stability of poly-DADMAC reaction with Au-NPs.....	113
5.3.5.8	Effect of temperature on stability of poly-DADMAC reaction with Au-NPs 114	
5.3.5.9	Selectivity for poly-DADMAC in the presence of specific ionic compounds 115	
5.3.5.10	Matrix effects: selectivity and interference studies	116
5.3.5.11	Selectivity for poly-DADMAC in the presence of other nitrogen- containing compounds.....	117
5.3.5.12	Selectivity for poly-DADMAC in the presence of surfactants.....	119
5.3.5.13	Stability of poly-DADMAC under storage conditions	120
5.3.6	Poly-DADMAC standards prepared from A2 and A3 solution	121
5.4	Conclusions	122
5.5	References	124

Chapter 6: General Conclusion 126

Appendices	I
A 1: LC-MS data.....	I
A 2: NMR data	III
A 3: Raman data	VI
A 4: metachromatic titration data.....	VII
A 5: colloidal gold method data	VIII

List of Figures

Figure 1. 1: Molecular structures of N-Nitrosamines	4
Figure 2.1: Advanced drinking water treatment system flow chart showing the chlorination, flocculation, coagulations and sand filtration steps.....	15
Figure 2.2: Diagram shows the destabilization of colloidal matter to form micro-flocs after addition of polyelectrolytes, in a coagulation process.....	19
Figure 2.3: Diagram shows agglomeration of micro-flocs to form macro-flocs after further addition of polyelectrolytes, in a flocculation process.	19
Figure 2.4: The synthesis of poly-DADMAC by free-radical initiated addition of DADMAC...	22
Figure 4.1: Photograph of the colour change of the colloidal titration from blue to purple.	61
Figure 4.2: Volume of the titrant (PVSK) corrected for blank versus known concentrations of poly-DADMAC (for data see Appendix A4.1)	65
Figure 4.3: Plot used to determine the charge density of poly-DADMAC (0.0048 eq g^{-1}).....	67
Figure 4.4: Absorbance spectra of a 50 mL sample containing 5 mg L^{-1} poly-DADMAC and 0.2 mL TBO indicator, after addition of increasing quantities of 0.2 mL of PVSK. The arrows show a decrease at 634 nm and an increase at 515 nm.....	69
Figure 4.5: Graph spectrophotometric titration of a solution containing 5 mg L^{-1} poly-DADMAC concentration at 634 (recording disappearance of blue) and 515 nm (recording the emergence of the purple-pink colour).	70
Figure 4.6: Titration curves of poly-DADMAC standards obtained by recording the absorbance at 634 nm versus PVSK volume added.	71
Figure 4.7: Photograph of the arrangement used for poly-DADMAC titration using a dip probe and spectrometer.....	73

Figure 5.1: Schematic diagram for the reduction of a gold salt with reducing agents (ascorbic acid, sodium borohydride or citrate salt) in presence of the capping agent tri-sodium citrate into Au-NPs. 86

Figure 5.2: Panel (A) is a UV-Vis spectrum of the gold salt (Au^{3+} ions) in aqueous solution and Panel (B) is a photograph of the gold salt in aqueous solution. 89

Figure 5.3: Panel (a) is a UV-Vis spectrum of the gold nanoparticles synthesized by the citrate salt method and panel (B) is a photograph of gold nanoparticles stabilized with citrate ions. 90

Figure 5.4: Panel (B) is a TEM image of the gold nanoparticles and panel (A) is the particle size distribution as measured from over 200 nanoparticles from several TEM images. ... 91

Figure 5.5: Panel (A) shows the UV-Vis spectrum of the gold nanoparticle seed solution (reduction with sodium borohydride) and panel (B) is a photograph of the gold nanoparticles synthesized by the seed growth method. 92

Figure 5.6: Panel (A) is a TEM image of the gold nanoparticle seed solution, panel (B) is the particle size distribution of the seed solution and panel (C) is the particle size distribution of the growth solution. 93

Figure 5.7: Panel (A) is UV-Vis spectrum of the gold nanoparticles prepared by stock solution A3 method (reduction of gold salt with ascorbic acid), (B) is photo image of the gold nanoparticle solution. 94

Figure 5.8: Panel (A) is TEM image of the gold nanoparticle solution A3 and panel (B) is the particle size distribution of stock solution A3. 95

Figure 5.9: Panel (A) is a UV-Vis spectrum of the gold nanoparticle solution A1 aggregated by poly-DADMAC and panel (B) is a photograph of the poly-DADMAC-gold nanoparticle aggregate solution. 97

Figure 5.10: Panel (A), (B) and (C) are TEM images of the poly-DADMAC-gold nanoparticle aggregates prepared from solution A1, and panel (c) is the particle size distribution as measured from over 200 nanoparticles from several TEM images. 98

Figure 5.11: Panel (A) is a UV-Vis spectrum of the gold nanoparticle solution A2 aggregated by poly-DADMAC and panel (B) is a photograph of the poly-DADMAC-gold nanoparticle aggregates. 99

Figure 5.12: Panels (A), (B) and (C) are TEM images of the poly-DADMAC-gold nanoparticle aggregates formed from solution A2 and panel (D) is the particle size distribution as measured from over 100 nanoparticles from several TEM images.....	100
Figure 5.13: Panel (A) is a UV-Vis spectrum of the gold nanoparticle solution A3 aggregated by poly-DADMAC and panel (B) is a photograph of the poly-DADMAC-gold nanoparticle aggregates.	101
Figure 6.14: Panel (A, B and C) is TEM of the gold nanoparticles solution A3 aggregated by poly-DADMAC and panel (D) is a histogram of the poly-DADMAC-gold nanoparticles aggregates.....	102
Figure 5.15: UV-Vis spectra of the A1 gold nanoparticle solutions mixed with various concentrations of poly-DADMAC.	104
Figure 5. 16: Panel (A) is a integrated area under the curve between 600 – 800 nm calculated by using Origin software and panel (B) is a calibration curve plotted from the integrated area under the curve between 600 – 800 nm versus various concentration of poly-DADMAC.	105
Figure 5.17: Calibration curves plotted from the peak absorbance at 690 nm (Panel (A) range 15 – 50 $\mu\text{g L}^{-1}$ and panel (B) range 55 – 100 $\mu\text{g L}^{-1}$), at 526 nm (panel (C)) and the ratio of the peak absorbance's at 690 and 526 nm (panel (D))......	106
Figure 5.18: UV-Vis spectra of results for pH studies at pH values of 4, 7.0 and 8 corresponding to acidic, neutral and basic.	111
Figure 5. 19: Effect of temperature on the plasmon band of the Au-NP poly-DADMAC aggregates.....	115
Figure 5.20: Effect of dissolved solids in tap water.....	117
Figure 5.21: UV-Vis spectra of 8.0 mg L^{-1} hexadecylamine and cetylpyridinium chloride spiked with 50 $\mu\text{g L}^{-1}$ poly-DADMAC and analysed with the Au-NPs.....	118
Figure 6. 22: UV-Vis spectrum of surfactant, poly-DADMAC and Au-NP aggregates.....	119
Figure 5.23: Plot of absorbance at 526 nm versus concentration of poly-DADMAC. Standards were prepared from A2 solution of Au-NPs.....	121
Figure 6. 24: A plot of absorbance at 526 nm versus concentration of poly-DADMAC. Standards were prepared from A3 solution with a correlation coefficient 0.93.	122

List of Tables

Table 1.1: Comparison between polyelectrolytes flocculants and inorganic flocculants	2
Table 2.1: Polymers accepted for use in drinking water treatment [94]	16
Table 2.2: Polyelectrolytes accepted for use in drinking water treatment and their contaminants [94]	21
Table 2.3: Physico-chemical properties of poly-DADMAC.	22
Table 3. 1: Gradient elution of poly-DADMAC at flow rate of 0.2 ml/min, 5 μ L injection volume	41
Table 4.1: Equivalence of PVS-K as determined by using the indicated solutions of CPC.	62
Table 4.2: Evaluation of three curves for precision used for poly-DADMAC analysis	74
Table 4.3: Evaluation of accuracy of the calibration curves used for poly-DADMAC	74
Table 4.4: : Evaluation of robustness of calibration curves used in the poly-DADMAC analysis.	75
Table 4.5: Limit of detection and limit of quantification studies.	75
Table 4.6: Summary of results of calibration curves	76
Table 5.1: Synthesis of gold nanoparticle stock solutions.	87
Table 5.2: Preparation of poly-DADMAC standards.	88
Table 5.3: Summary of the solutions of Au-NPs A1, A2, and A3; UV-Vis peak absorbance colour, size distribution and shape; before and after addition of poly-DADMAC... ..	102
Table 5.4: Limit of detection and quantification for poly-DADMAC by forming aggregate with Au-NPs from solution A1.	107
Table 5. 5: Inter-day and intra-day results for poly-DADMAC analysis.	108

Table 5.6: Recovery results for 30 $\mu\text{g L}^{-1}$ poly-DADMAC solution with 10 and 30 $\mu\text{g L}^{-1}$ spikes.	109
Table 5.7: Recovery on selected concentrations of poly-DADMAC at slightly basic pH 7.5. ..	111
Table 5.8: Effect of changes in the initial volume of Au-NPs on the recovery of poly-DADMAC at a set concentration.....	112
Table 5.9: Results for time studies.....	114
Table 5.10: The % recovery from different salt solutions spiked with 50 $\mu\text{g L}^{-1}$ poly-DADMAC.	116
Table 5.11: Selectivity results for structurally similar species.	118
Table 5.12: Results for surfactants study.....	120
Table 6.1: Comparison of metachromatic method against a novel Au-NP probe method for detection and quantification of poly-DADMAC based on precision and selectivity.	128

Abbreviations

Au-NPs	Gold nanoparticles
AWWA	American Water Works Association
ASTM	American Society for Testing Materials
CETIC	Council of Chemical Manufactures Factories
CPC	Cetylpyridinium Chloride
DMFs	Direct methanol fuel cells
DNA	Deoxyribonucleic acid
DADMAC	Diallyldimethylammonium chloride
HPLC	High Performance Liquid Chromatography
LOD	Limit of Detection
LOQ	Limit of Quantification
LPR	Liquid-Phase polymer-Base
LBL	Layer-by-Layer
NDMA	N-nitrosodimethylamine
NMR	Nuclear Magnetic Resonance
PEC	Polyelectrolyte complexes
PAE	Polyallylamine hydrochloride

PAA	Polyacrylic acid
Poly-DADMAC	Polydiallyldimethylammonium chloride
PVSK	Potassium salt of polyvinyl sulfate
R & D	Research and development
TBO	Toluidine Blue O
TEM	Transmission Electron Microscopy
UV-Vis	Ultraviolet-Visible Spectroscopy
WHO	World Health Organisation

Chapter 1: Introduction

1. General introduction

Polyelectrolytes are organic polymers that represent a class of compounds, which derive their unique properties mainly from the density and distribution of positive or negative charges along the macromolecular chain [1]. There are two major classes of polyelectrolyte: natural and synthetic polyelectrolytes. Like other polymers, polyelectrolytes are widely used in industrial processes, such as, pulp and paper manufacturing [2], paint industry and water treatment sectors [2, 3]. They have also found use in the pharmaceutical and cosmetics industries for the preparation of hair conditioners, shower shampoos, drugs and for most skin care products [4-6]. Research has shown that many such polyelectrolytes can enter the environment through drinking and waste water treatment plants; disperse, and persist to greater extent than first anticipated. Little is known about the fate of polyelectrolytes in the environment and they are now considered by many regulatory organisations as emerging pollutants [7]. Therefore, they have been the subject of extensive investigation for several decades and still continue to be an active area of research.

For decades, synthetic polyelectrolytes based on various monomers like acrylamide, acrylic acid, diallyldimethylammonium chloride (DADMAC), and styrene sulfonic acid, have been widely used in water treatment facilities as primary coagulants, flocculants and filtration aids. Since suspended particles and solids found in natural waters are assumed to be negatively charged [8], it is for this reason water treatment processes rely on the use of positively charged polyelectrolytes called cationic polyelectrolytes (negative charged polyelectrolytes are called anionic polyelectrolyte). Cationic polyelectrolytes are either jointly used with inorganic coagulants (alum, ferric chloride) to enhance coagulation/flocculation and strengthen flocs, or alone as a primary coagulant [9]. Anionic polyelectrolytes sometimes are added in very low doses to strengthen positively charged hydroxide flocs, generated by inorganic coagulants. High

charge density cationic polyelectrolytes such as polydiallyldimethylammonium chloride (poly-DADMAC) are used alone as primary coagulants/flocculant in dosages of a few mg L⁻¹ [10].

Flocculation is the agglomeration of tiny particles by polyelectrolytes (for example) to form flocs which settle and cause clarification of the system [11]. In an ideal situation, the majority or all of the added polyelectrolyte will settle down with destabilized particles and be removed from the process with the sludge. But since this is not always the case, significant amounts of polyelectrolytes remain in the treated water. For this reason, dosing with these polyelectrolytes is strictly regulated in many countries, but there is little or no legalisation for residual amounts that remain after treatment of water [10]. There are health concerns over residual polyelectrolytes and associated contaminants in treated water [7]. Benefits arising from polyelectrolyte usage compared to inorganic coagulants and poor toxicological data on polyelectrolytes have overshadowed health concerns for decades [7]. Some of these advantages are tabulated in Table 1.1.

Table 1.1: Comparison between polyelectrolytes flocculants and inorganic flocculants

Conditions	Polyelectrolytes flocculants	Inorganic flocculants
Concentration	Small dosage needed in mg L ⁻¹	Large amounts are required to cause solid-liquid separation
pH	Can be used in wide pH ranges	Highly sensitive to pH
Applicability	Work for any system	Applicable only to few disperse systems and do not work for others.
Effectiveness	Coagulate very fine particles	They do not coagulate very fine particles.
Cost	Cheap to manage	Expensive
Disposal	Concentrated sludge is produced.	Produces a lot of sludge whose disposal is a major problem.

Due to these disadvantages, inorganic flocculants have almost been abandoned. The United States Environmental Protection Agency (USEPA) has accepted more than 1000 polyelectrolytes for use in the production of drinking water [7]. More than 50% of the water treatment plants in

the United States use polyelectrolytes to improve treatment efficiency and over 70% of water treatment plants in South Africa have adopted various polyelectrolytes in their water treatment process [12]. Organisations such as the American Water Works Association (AWWA), American Society for Testing Materials (ASTM), Council of Chemical Manufacturers Federations (CEFIC), the European Committee for Standardization, the National Sanitation Foundation International, and the American National Standards Institute provide standards for the maximum dosage of polyelectrolytes ($10 - 100 \text{ mg L}^{-1}$) that can be used in water treatment.

Although dosing with polyelectrolytes is strictly regulated, research has shown that there is need for legislation of monomers and polyelectrolyte residuals that persist after water treatment as well. One of the disadvantages of polyelectrolytes is that they often contain contaminants from the manufacturing process such as toxic residual monomers, other reactants, and reaction by-products that could potentially affect human health [7]. The lack of legislation can be attributed to the low sensitivity, relatively high cost, large amount of time and effort, and lack of validation protocols with the existing methods to determine residual polyelectrolyte in water. As low levels of these polyelectrolytes are very difficult to quantify, polyelectrolyte residual levels remaining in finished water are not measured and cannot be regulated easily according to the World Health Organisation (WHO) [11]. The Cooperative Research Centre for water quality and treatment, Australia identified residual polyelectrolyte detection as an area in need of further research [8].

In recent years, there has been a growing concern over the fate of polyelectrolytes within the water treatment process and its presence in the environment at large [9, 13]. Reviews on the fate of polyelectrolyte in water treatment processes, have suggested that they breakdown at the disinfection stage, where disinfectants like chlorine are added and result in dangerous by-products. N-Nitrosamines (examples in Figure 1.1) are an emerging group of contaminants that are an example of disinfection by-products known to be carcinogenic at ng L^{-1} levels. These compounds are frequently detected in water and wastewater systems in many countries. To investigate the mechanisms of nitrosamine formation from commonly employed water treatment polymers, many studies have focused on polydiallyldimethylammonium chloride (poly-DADMAC), since it is one of the most common used cationic polyelectrolytes in drinking water treatment plants.

Results show, that upon ozonation, poly-DADMAC may yield N-nitrosodimethylamine (NDMA) at levels up to two orders of magnitude higher than the standard guidelines. This development, in part, can be traced back to the initial work by Choi and Valentine [14] where they demonstrated that NDMA is a disinfection by-product formed during chlorination steps within the water treatment process. NDMA is a suspected carcinogen and classified as a B2 carcinogen. Recently, various authors have reviewed the links between the presence of NDMA in treated water and the use of poly-DADMAC and other polyelectrolytes and on the mechanisms, kinetics and precursors that can lead to NDMA formation [1, 15-17]. In addition, Huang et al. [18] reported on the kinetics and mechanisms for the formation of NDMA from poly-DADMAC solutions exposed to monochloroamine. A follow-up study detailed the physical chemical processes for the formation of NDMA from poly-DADMAC when exposed to ozone [19]. Thus, from an environmental and human health risk perspective, there is a strong need to rapidly determine the amount of residual poly-DADMAC in a water treatment process before any type of chlorination or ozonation steps.

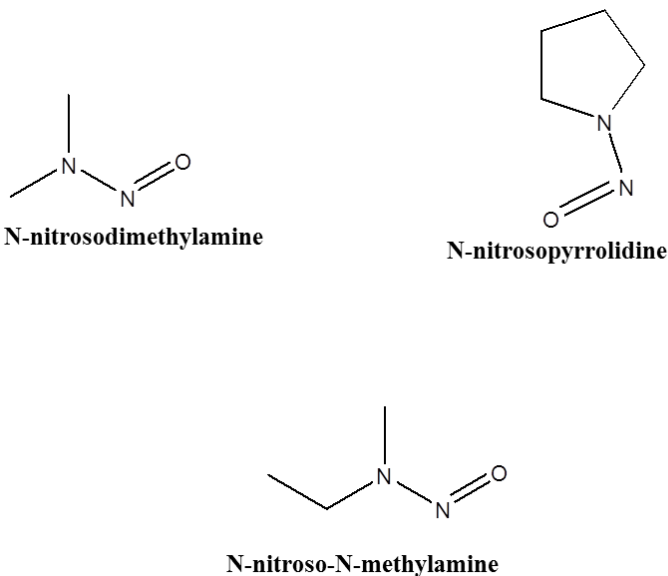


Figure 1.1: Molecular structures of various N-nitrosamines

1.1 Research motivation

The chemical structure and physical properties of polyelectrolytes determine their toxicity towards aquatic organisms [20]. Quaternary amine polyelectrolytes, such as poly-DADMAC, (experimentally) have been found to be toxic at very low concentrations (below 1 mg L^{-1}) [21-23]. Also, there is evidence that poly-DADMAC monomer (DADMAC) is absorbed by the gastrointestinal tract and distributed all over the body [9]. Furthermore, disinfection by-products of poly-DADMAC are known to be carcinogenic at ng L^{-1} levels (structures shown in Figure 1.1). Our literature review on polyelectrolytes suggests that poly-DADMAC is the most used polyelectrolyte in South Africa [12, 24, 25]. Recently, poly-DADMAC and its monomer have been detected in drinking water (taps) and environmental waterways [9, 13, 26].

Detection of polyelectrolytes in tap water is evidence which presents another scenario that polyelectrolytes are not completely removed with flocs (coagulation stage), and that they are present in the disinfection stage and their disinfection by-products might be present in drinking water as well. These can pose a threat to the South African people, who depend entirely on the drinking water from water treatment facilities. In South Africa there is no legislation on polyelectrolytes used in water treatment and their residues [24]. Our review on this work, suggests that incorrect dosing, absence of legislation and unavailability of reliable analytical methods to monitor polyelectrolytes after dosing and to ensure they are absent at the disinfection stage, is the reason these molecules are present in tap water in homes.

1.2 Problem identification

The non-toxicity of poly-DADMAC depends on its absence in the disinfection stage. A selective, sensitive and relatively cheap method is needed to monitor residual concentrations of polyelectrolytes in water treatment facilities and to ensure their absence in the disinfection stage. Current methods are complicated, time-consuming, and expensive and are not suitable for monitoring in developing countries like South Africa, or a technical laboratory.

1.3 Hypothesis

Gold nanoparticle tagging as a probe-based colourimetric method is a suitable technique for detection of poly-DADMAC and is superior to the existing standard techniques of liquid chromatography mass spectroscopy (LC-MS), nuclear magnetic resonance (NMR) and Raman spectroscopy.

1.4 Aims and objective

The main aim of this research was to develop a novel, sensitive, accurate, and relatively cheap methodology that uses gold nanoparticles as a probe to detect and quantify poly-DADMAC at $50 \mu\text{g L}^{-1}$. In addition, the limitations and advantages of existing techniques were to be compared with the novel gold-tagging method. The aim was to be achieved via the following objectives:

- i. Repeat several selected protocols from the literature that can be easily implemented in-house; these included, NMR, LC-MS, and colloidal/metachromatic titration.
- ii. Determine the limitations of the metachromatic titration technique.
- iii. Determine a suitable synthesis method for gold nanoparticles and fully characterize the gold nanoparticles.
- iv. Determine whether gold nanoparticles can be used to detect poly-DADMAC and quantify the polyelectrolyte at $50 \mu\text{g L}^{-1}$ levels.
- v. Explore validation procedures for the novel methodology.

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Chapter 2: Polyelectrolytes in water treatment

2. Introduction to polyelectrolytes

Polyelectrolytes are polymers with ionisable groups with either a net positive or negative charge at near neutral pH [1-5]. Polyelectrolytes have their charge distributed along a macromolecular chain, which define their unique physico-chemical properties. Examples of polyelectrolytes are polystyrene sulfonate, polyacrylic, DNA, poly-DADMAC, proteins and polymethacrylic acid. This simple list shows that polyelectrolytes can include synthetic and natural polymer molecules [6-8].

In water they are generally soluble, and can dissociate, leaving charges on polymer chains and releasing counter ions in solution. Their solubility is driven by the electrostatic interactions between water and the charged monomer that makes up the polymer backbone [5]. The electrostatic interactions, the length of the polymer chain, hydrophobic interactions, H-bonding, and rigidity of the molecule all play a role in the physico-chemical properties of polyelectrolytes. The unique physico-chemical properties of the various polyelectrolytes known are subject to intense research and development (R&D) and the synthesis of new ones remains an active field of research [3, 9, 10]. Although the reasons behind the physical and chemical properties of polyelectrolytes are still not fully understood, they are used in numerous industrial, commercial, and basic R&D applications [11-15].

2.1 Classification of polyelectrolytes

Polyelectrolytes are classified into various types, and this can be based on origin, composition, molecular architecture, charge and/or electrochemistry. Based on origin they can be classified as natural polyelectrolytes, synthetic polyelectrolytes or chemically modified biopolymers. When

using composition as the classification system, they can be classified as homopolymers or copolymers. Some authors use molecular architecture to categorize polyelectrolytes, and refer to the molecules as linear, branched or cross-linked. Based on electrochemistry they are classified as polyacids/polyanions, polybases/polycations and polyampholytes [2]. Also based on charge, polyelectrolytes can be classified into anionic, cationic and amphoteric [9]. Interactions between polyelectrolytes and oppositely charged amphiphiles have been a focus area of research for most scientists for the last twenty years, due to their importance both in fundamental polymer physics/biophysics, and biological and industrial applications [9, 16-20].

2.2 Non-water treatment uses of polyelectrolytes

Polyelectrolytes used in water treatment are of high molecular weight and high charge density [21]. Most are synthetic cationic polyelectrolytes, which are soluble in water and with a tendency to adsorb [22]. Cationic polyelectrolytes are known to be toxic compared to anionic polyelectrolytes and non-biodegradable. In aqueous solution they are independent of pH, adopt a coil configuration and become less permeable [23]. Therefore properties of polyelectrolytes for non-water treatment differ from those used in water treatment purposes. Polyelectrolytes for non-water treatment are sensitive to pH (drug delivery polyelectrolytes) [24], define surfaces (used in synthesis and coating) [25], mostly natural and biodegradable (used in medical applications) [26-28], low molecular weight and permeable (used in industries) [29-31]. This section highlights a few selected examples of non-water treatment uses of polyelectrolytes.

2.2.1 Materials science and nanotechnology

Polyelectrolytes have been employed in synthesis for many years. Miguel et al [32], reported on the use of carbon nanotubes as templates for one-dimensional nanoparticle assemblies. This method makes use of polyelectrolytes for wrapping carbon nanotubes and providing them with adsorption sites for electrostatically driven nanoparticle deposition. Hillel et al. [33] reported techniques for synthesizing inorganic oxide thin films from low-temperature liquid of various sulfonated polyelectrolyte multilayers.

Chen et al. [34] reported on the synthesis of colloidal gold nanoparticles in a quaternary ammonium-based room-temperature ionic liquid by gamma-radiation for the first time. Huang et al. [35] reported on the controlled synthesis of stable gold nanoparticles in quaternary ammonium ionic liquids by simply heating in air at 135 – 145 °C, and gold nanoparticles (Au-NP) synthesized by this simple and environmentally friendly reaction can be realized in several minutes and also exhibit high stability and good reproducibility, providing a new facile method for metal nanoparticle preparation. Choi et al. [36] reported the spontaneous deposition of gold nanoparticle nanocomposite on polymer surfaces through sol-gel chemistry.

Xia et al. [37] reported on a novel biosensor using probe based deoxyribonucleic acid (DNA), unmodified gold nanoparticles, and cationic polyelectrolytes to detect a broad range of target analytes. Chen et al. [38] studied the protection of Au-NP with cationic polyelectrolytes. Weaver et al. [39] reported the synthesis of small gold crystallites by photoresponsive polyelectrolytes. Muller et al. [40] also reported the synthesis of anionic and cationic nanoparticles of polyelectrolyte complexes.

In manufacturing processes Susan et al. [41] reported the use of polyelectrolytes to alter the surface of microchannels imprinted in two plastics. In the pharmaceutical drug industry the biggest challenge is to deliver drugs at the right moment, in the right place and at an optimum concentration in human body. Semi-permeable polyelectrolytes are used extensively in this area of research. Recently more advanced polyelectrolyte microcapsules with small dimensions have been developed by the layer-by-layer (LBL) technique and found to deliver drugs better than other techniques of delivery [42-44]. The LBL technique is based on sequential adsorption of oppositely charged substances (polyelectrolytes, nanoparticles, nanotubes, proteins and lipids) onto charge templates, to prepare nanostructured materials with defined composition and shape called polyelectrolytes multilayers. These polyelectrolyte multilayers are promising as nanoreactors and have been used in nanomaterial synthesis [45-47].

2.2.2 Membrane science

Recently, polyelectrolytes are used in both the manufacture and modification of nanomembranes. In modification, polyelectrolytes are used to improve the thermal and chemical stability of other

membranes and, to fill pores for better permeability. This type of membrane is used most in medicine [48-52]. In membranes manufacture, polyelectrolytes are used as major component of the membrane; these types of membrane are used mostly in fuel cells [53-58].

Fuel cells have recently received much attention as an alternative energy source. Among the different types of fuel cells, direct methanol cells (DMFCs) are promising clean-energy source because they efficiently convert fuel to electricity without the emission of pollutants into cars [59]. However, the poor performance of DMFCs is attributed to methanol crossover through the polyelectrolyte membrane from the anode to the cathode. Sung et al. [59] demonstrated the use of solvent casting method after mixing nafion solutions with nanostructured sulfonated polyhedral oligomeric silsesquoxane fillers to synthesize organic polyelectrolyte membranes for fuel cell application. Polyelectrolyte membranes exhibited a lower degree of methanol penetration and higher proton conductivity, which was a good result. Chen et al. [60] also synthesised hybrid cross-linked membranes for DMFCs from sulfonated poly-ether sulfones.

2.2.3 Pulp and paper industry

Paper exists because of the tendency of fibers to stick together [61]. In the process of papermaking, the strength of the wet web is of critical importance, when the web is transferred without a support from the presses into the dryer section, insufficient adhesion among fibers may result in costly breaks. Gärdlund et al. [62] reported the use of polyelectrolyte complexes (PEC) as strength additives for different pulps used for production of fine paper. The polyelectrolytes that were employed in this study were a polyamideamine epichlorohydrin (PAE), traditionally used as a wet-strength additive, and carboxymethylcellulose. Nikolaeva [63] also studied the effect of polyelectrolytes on the wet-web strength of paper. Gärdlund et al. [62] investigated the polyelectrolytes of polyallylamine hydrochloride (PAH) and PEC of PAH and polyacrylic acid (PAA) as strength additives. Reye et al. [64] reported on the enhancement of cellulose catalysis of wood pulp fiber by cationic polyelectrolytes. Many other researchers have investigated the use of polyelectrolytes in paper making to date [61, 65-69]. The pulp and paper industry is a very water-intensive industry and can consume 60 m³ of freshwater per ton of paper produced, while there are 300 million metric tons of papers and paperboard produced per year [67]. A large

amount of waste containing polyelectrolytes is generated in the pulp and paper industry. Furthermore, polyelectrolytes are added during the waste treatment stage and some of the polyelectrolytes are by-products of wood extracts such as lignosulfonates [70]. Significant amounts of polyelectrolytes enter rivers through this industry.

2.2.4 Selected industrial uses of polyelectrolytes

In industry, cationic polyelectrolytes are the most used polyelectrolytes for processes including oil water separation [71-74], corrosion control [75-78], flocculation of metal ores [70], improvement of lime precipitation processes [79, 80] clarification of titanium sulfate liquor [81, 82], removal of heavy metals and killing viruses [83-85]. This extensive use of polyelectrolytes in heavy industry signifies the threat they can pose to the environment.

2.2.5 Polyelectrolytes in cosmetics, foods, and pharmaceutical products

When used in cosmetic formulations, their primary function is generally described as film formers and antistatic agents. Polyelectrolytes are used in various cosmetic products, for example, cleansing agents such as shampoos, liquid hand soaps, body scrubs and facial cleansers; and also in conditioning agents including hair conditioners and moisturizers [86, 87]. The combination of cationic polyelectrolytes and anionic surfactants in formulations is one of the two technologies that led to the development of the 2-in-1 shampoo. Deo et al. [87] studied the interactions of hydrophobically modified polyelectrolytes with nonionic surfactants. Lohead developed hydrophobically-modified cross-linked polyelectrolytes as polymeric emulsifiers for the triggered release of the oil phase when applied to the substrate- this has found use in sports sunscreens [88]. Pharmaceutical products are also suspected of containing polyelectrolytes that break down to compounds which pose a threat to the environment.

2.2.6 Medical applications

In medicine, polyelectrolytes are emerging as a solution in controlling drug delivery. Most recent advances showed that specific biological activities can be induced by incorporation of bioactive species in polyelectrolytes and layer-by-layer (LBL) deposition method for polyelectrolyte multilayer films, a versatile route toward thin film was discovered [89]. Biomedical applications of natural rubber (NR) latex, mostly in dry membranes, have motivated research into novel Zhang et al. [90] reported the fabrication of LBL films of NR alternated with polyelectrolytes. Viera et al. [91] reported the strategy to deliver cis-platin to melanoma cells by sandwiching the drug between two oppositely charged polyelectrolytes. Apart from drug delivery, Daniels et al. [92] reported the use of polyelectrolytes as osmotic agents for peritoneal dialysis.

2.3 Water treatment uses of polyelectrolytes

The United States Environmental Protection Agency (USEPA) have accepted more than 1000 different types of polyelectrolytes for use in the treatment of waste water and processing of drinking water [23,93,94]. More than 50% of the water treatment plants within the United States use polyelectrolytes to improve treatment efficiency [94], while 75% of the water treatment plants in South Africa have adopted various polyelectrolytes in their water treatment process [95]. Polyelectrolyte applications in potable water production and industrial waste water treatment are in the coagulation and flocculation steps, and dewatering of treatment plant sludge. Figure 2.1 illustrates a potential process train for potable water, highlighting the stages where one may use a suitable polyelectrolyte.

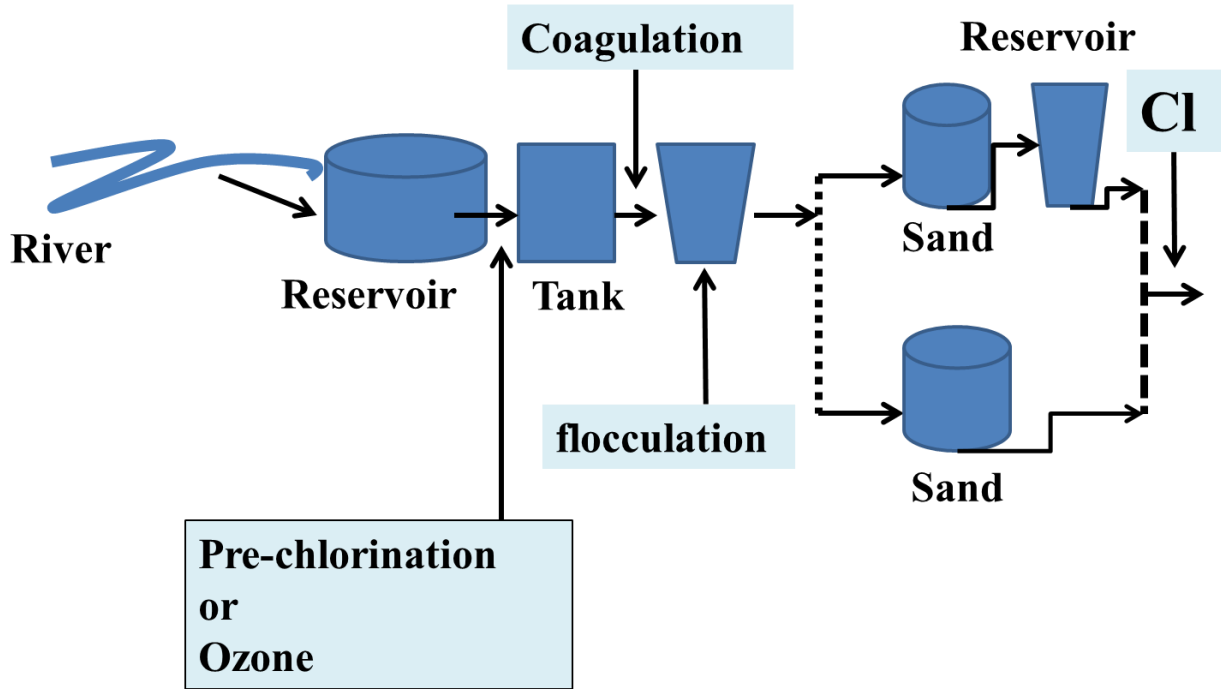


Figure 2.1: Advanced drinking water treatment system flow chart showing the chlorination, flocculation, coagulations and sand filtration steps.

Polyelectrolytes that are added during water treatment are thought to be removed with the floc; but there is a possibility that low concentrations may remain in the water when chlorine or ozone is added at a later treatment stage. As shown in Figure 2.1 in the pre-chlorination stage, in some plants the contact time of chlorine and polyelectrolytes is increased due to addition of oxidants before the coagulation tank [96]. This gives enough time for the reaction between polyelectrolytes and oxidants to yield more by-products (nitrosamines). Little is known about the by-products which are released by most polyelectrolytes in water treatment processes [97].

2.3.1 Polyelectrolytes used in drinking water treatment

Table 2.1 lists the polyelectrolytes that, according to Letterman’s article, are accepted for use in drinking water treatment [94]. Polyelectrolytes are listed in alphabetical order. In table 2.1,

acrylamide polyelectrolytes, that are sometimes called coagulant-aid polymers, have acrylamide as a monomer or co-monomer, a high molecular weight, and a significant number of negatively charged sites (anionic).

Table 2.1: Polymers accepted for use in drinking water treatment [94]

Common Name and Abbreviation	CAS Registry	Chemical Name
Acrylamide polymers Polyacrylamide (nonionic polyacrylamide, PAM)	9003-05-8	2-Propenamide, homopolymer $(C_3H_5NO)_n$
Acrylamide-acrylic acid copolymer (PAM-PAA)	9003-06-9	2-Propenoic acid, polymer with 2-propenamide $(C_3H_5NO.C_3H_4O_2)_n$
PAM-PAA (Na salt)	25085-02-3	2-Propenoic acid, sodium salt, polymer with 2-propenamide $(C_6H_4O_2.C_3H_5NO .Na)_n$
Poly-DADMAC (Diallyldimethylammonium chloride, homopolymer)	26062-79-3	N,N-Dimethyl-N-2-propenyl-2-propen-1-aminium chloride, homopolymer $(C_8H_{16}N.Cl)_n$
Epi-DMA polymer Epichlorohydrin-dimethylamine, polymer	25988-97-0	N-Methyl methanamine, polymer with (chloromethyl) oxirane $(C_2H_7N.C_3H_5ClO)_n$
Epi-DMA*	39660-17-8	Poly [(dimethylimino) (2-hydroxy-1,3-propanediyl) chloride] $(C_5H_{12}NO.Cl)_n$
Epi-DMA with ethylenediamine†	42751-79-1	1,2-Ethanediamine polymer with (chloromethyl) oxirane and N-methyl methanamine $(C_2H_5N.C_3H_5ClO.C_2H_7N)_n$
Additional epichlorohydrin-based polyamine polymers Epichlorohydrin with monomethylamine	31568-35-1	Methanamine, polymer with (chloromethyl) oxirane $(CH_5N.C_3H_5ClO)_n$

Common Name and Abbreviation	CAS Registry	Chemical Name
Epichlorohydrin with a polyamine	27029-41-0	N,N-dimethyl-1,3-propanediamine polymer with (chloromethyl) oxirane (C ₅ H ₁₄ N ₂ .C ₃ H ₅ ClO) _n
Polyethylenimine Polyethyleneimine	9002-98-6	Aziridine, homopolymer (C ₂ H ₅ N) _n
PEI*	25988-99-2	Ethanimine, homopolymer (C ₂ H ₅ N) _n
PEI*	26913-06-4	Poly(imino-1,2-ethanediyl) (C ₂ H ₅ N) _n
Polyethylene polyamine polymers Polyethylene polyamine (polyalkyleneamine)‡	8660-40-1	1,2-Dichloroethane polymer with ammonia (C ₂ H ₄ Cl ₂ .H ₃ N) _n
Polyethylene polyamine‡	29320-38-5	1,2-Dichloroethane polymer with ammonia (C ₂ H ₄ Cl ₂ .H ₃ N) _n
Polyethylene polyamine§	49553-92-6	1,2-Ethanediamine polymer with 1,2-dichloroethane (C ₂ H ₈ N ₂ .C ₂ H ₄ Cl) _n
Melamine-formaldehyde polymers	9003-08-1	1,3,5-Triazine-2,4,6-triamino polymer with formaldehyde (C ₃ H ₆ N ₆ .CH ₂ O) _n
Mannich reaction-modified polyacrylamide (AMPAM)	25765-48-4	N,N-[dimethyl(amino)methyl] 2-propenamide, homopolymer (C ₆ H ₁₂ N ₂ O) _n
Poly(DMAEMA) (N,N-dimethylaminoethyl methacrylate, homopolymer)	25154-86-3	2-Methyl-2-propenoic acid, 2-(dimethylamino) ethyl ester, homopolymer (C ₈ H ₁₅ NO ₂) _n
*The compound is identified by its repeating structural units or by the use of a different monomer nomenclature.		
†This compound contains the monomer ethylenediamine in addition to the monomer formed from epichlorohydrin and dimethylamine.		
‡The reason for a multiple listing by Chemical Abstract Service is not known.		
§This compound is structurally similar to the preceding compound but is produced using a different starting material		

Most acrylamide polymers are made by free-radical polymerization. According to Letterman's article [94] poly-DADMAC, epi-DMA and amine-formaldehyde are the most widely used polyelectrolytes in drinking water treatment [21,95]. Poly-DADMAC and epi-DMA were found to be widely used in South Africa as reported by Majam et al. [121]. Polyelectrolytes have a strong tendency to adsorb onto surfaces of particles in aqueous suspension, and that is the main reason they are widely used in water treatment processes.

The water industries are responsible for producing safe drinking water for people and all organisms in rivers, lakes and oceans. To keep water safe, polyelectrolytes are required to mix with turbid natural water for removing solid waste material before filtration. However, some authors have used polyelectrolytes in unique ways for treating waste or drinking water. For example, Ge et al. [98] reported polyelectrolyte-promoted forward osmosis-membrane distillation (FO-MD) hybrid processes for dye wastewater treatment. Patel et al. [99] studied the removal of oil from oily water produced from petroleum industry by adsorption in polymer nanocomposites. These composites were prepared from cationic polyelectrolytes and sodium bentonite. Jadhav et al. [100] studied the removal of heavy metal ions from industrial effluent by polyelectrolytes. Metal species can be removed from the aqueous solutions with liquid-phase polymer-base (LPR). The LPR technique removes ionic species by functional groups of water-soluble polyelectrolytes. Grachek et al. [101] reported the use of water-soluble polyelectrolytes based on polyacrylonitrile in wastewater treatment and also studied polyelectrolyte flocculants based on nitron fiber scrap for use in treatment of wastewater of finishing-and-dyeing production.

Flocculants based on polyelectrolytes are widely used for treating water and intensifying separation of various natural dispersions. It is known that flocculant performance is determined not only by polymer characteristics, but also by the wastewater composition.

2.3.2 Flocculation and coagulation

The main aim of introducing polyelectrolytes in water treatment is to induce flocculation and coagulation processes for the removal of suspended solid particles (colloidal matter) [102]. All waters, especially surface water, contain both dissolved and suspended particles, which are often

assumed to be negatively charged. In suspension particles repel each other and they cannot stay together (stay stable in solution), and as a result they will remain in suspension. Coagulation involves processes where polyelectrolytes are added to a destabilized suspension or affect the surface of water [103]. In coagulation, polyelectrolytes overcome the factors that keep particles apart such as repulsion forces, and enable the particles to come together to form micro-flocs (flocs are clusters of small particles) as shown in Figure 2.2 [104].

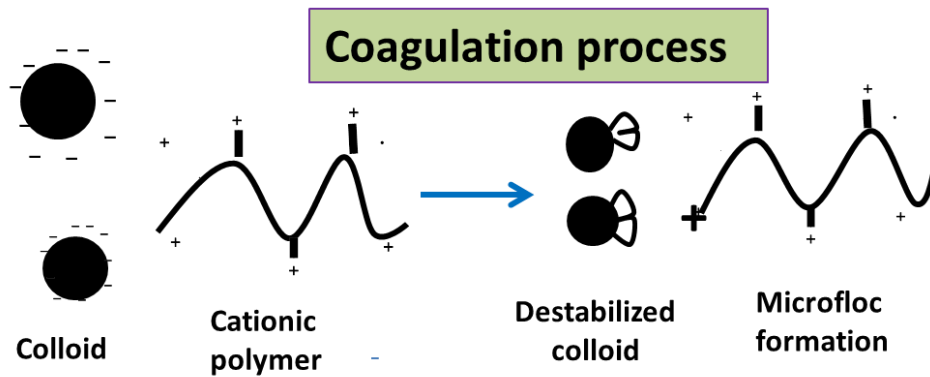


Figure 2.2: Diagram shows the destabilization of colloidal matter to form micro-flocs after addition of polyelectrolytes, in a coagulation process.

In the flocculation process polyelectrolytes are further added to induce the agglomeration of micro-flocs to form macroflocs (bigger particles see Figure 2.3) and these particles are dense enough to settle down [103]. Macro-flocs settle or precipitate out of water and are removed as sludge.

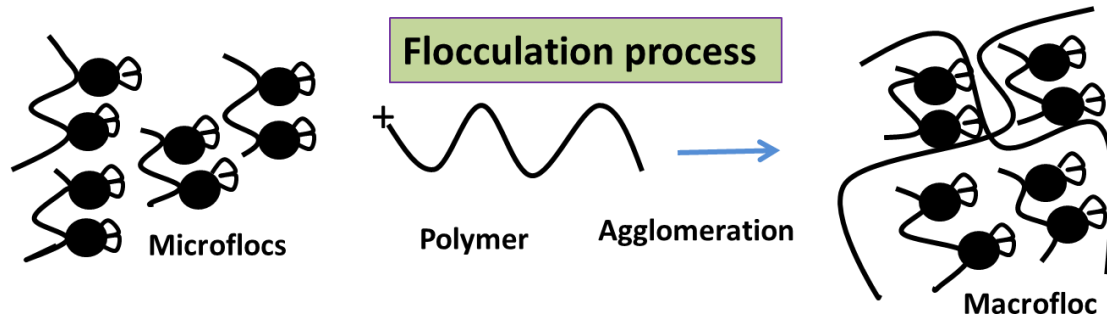


Figure 2.3: Diagram shows agglomeration of micro-flocs to form macro-flocs after further addition of polyelectrolytes, in a flocculation process.

2.4 Polyelectrolytes as contaminants in drinking water

The application of polyelectrolytes in coagulation/flocculation processes for water purification has been normal practice for over 30 years and dates back to the 1970's. This is because of the advantages associated with the use of polyelectrolytes in water treatment compared to alum. These include reduced levels of aluminium in treated water, a lower coagulant dose requirement, a smaller volume of sludge, a smaller increase in the ionic load of the treated water, and cost savings of up to 25 – 30% [93]. But there are some disadvantages, such as, a greater sensitivity to incorrect dosage, and with larger turbidity and natural organics in the water, the removal can be less efficient.

The main concerns with polyelectrolytes are the environmental factors [105]. Polyelectrolytes used in a water treatment plants may contain a number of contaminants. These could include residual monomers, chemicals used in the production of the polyelectrolyte, and reaction by-products formed during the manufacturing processes.

The major problems are monomers used in the production of polyelectrolytes. These monomers have been shown to be carcinogenic, and hence they are a potential health hazard when present in the polyelectrolyte [97]. The information regarding the structure of polyelectrolytes can also be used to predict their toxicity effect [105].

Table 2.2 is a list of the contaminants that the assessment by Letterman and Pero [94] determined might be found in polyelectrolytes that were included on the USEPA list of accepted polyelectrolytes. In Table 2.2 “direct evidence” means that the pollutants were detected and their levels have been reported in the literature. For the contaminants listed under ‘indirect or inconclusive evidence’, measurements indicating the presence or absence have not been reported.

Table 2.2: Polyelectrolytes accepted for use in drinking water treatment and their contaminants [94]

Polyelectrolytes	Pollutants
Direct evidence	
Acrylamide	Acrylamide Hydroxypropionitrile Isobutyronitrile
Epi-DMA	Cycidol 1,3-Dichloro-2-propanol 3-Chloro-1,2-propanediol 2,3-Dichloro-1-propanol
Poly-DADMAC	DADMAC
Indirect or inclusive evidence	
Epi-DMA	Dimethylamine
Epi-MMA	Methylamine
Melamine-formaldehyde polymer	Melamine Methylolmelamines Formaldehyde
PEI	Ethylamine
Poly-DADMAC	Dimethylamine
Speculative	
Poly-DADMAC	Allyl chloride Diallyl ether 5-Hexanal

In some cases, these are compounds used in making the product and the chemistry of the process suggests that residual amounts might exist in the finished product. In this project focus is more on poly-DADMAC since it is used the most in South Africa for water treatment.

2.4.1 Use of Poly-DADMAC in water treatment

2.4.1.1 Synthesis of poly-DADMAC

The manufacture of poly-DADMAC involves two sequential steps: formation of the monomer and its polymerization. The monomer is usually formed by the reaction of a stoichiometric excess of allyl chloride with dimethylamine in aqueous solution as shown in Figure 2.4 and its physico-chemical properties are listed in Table 2.3.

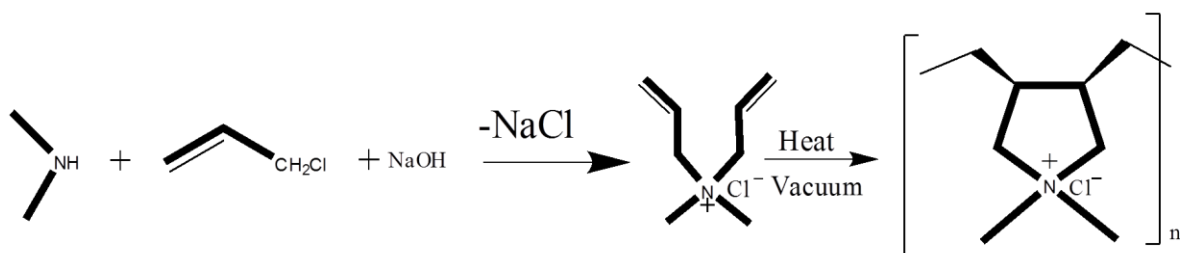


Figure 2.4: The synthesis of poly-DADMAC monomer, and the polymer by free-radical initiated addition of DADMAC.

Table 2.3: Physico-chemical properties of poly-DADMAC.

Physical properties	Description
Molecular weight	100000 – 500000 Da
Form (phase)	Clear, liquid
Colour	Light yellow
pH	5 – 8 at 25 °C
Initial boiling point	100 °C at 1.013 hPa
Flash point	> 100 °C – closed up
Vapour pressure	27 – 40 hPa 25 °C
Water solubility	Completely soluble
Partition coefficient octanol/water	$\log P_{ow} < 10$

2.4.1.2 Application of poly-DADMAC in water treatment

Poly-DADMAC has been widely used in drinking water treatment for the last 36 years as a primary coagulant. It is particularly useful in water treatment applications because colloidal particles in natural waters and wastewater are generally negatively charged. Poly-DADMAC is also used extensively in the paper industry as an anti-static agent, and in the textile industry as a dyestuff additive. In sewage, poly-DADMAC is used for clarification and dewatering of effluent. Poly-DADMAC is designed to sorb to colloidal matter to produce a neutral precipitate and sorption is thought to be irreversible. The industrial application of poly-DADMAC is expected to release zero amounts of this polyelectrolyte in waterways, because poly-DADMAC is thought to precipitate together with colloidal matter and thus is removed from the water column in the process [106]. However, to prevent the contamination of potable water, dosing with this polyelectrolyte is strictly regulated in many countries [107].

2.4.1.3 Regulatory aspects of Poly-DADMAC in water treatment

The maximum dose of poly-DADMAC that may be used in potable water production is regulated in many countries. For example, in the United States of America (USA) and Spain, it is regulated to be 25 mg L⁻¹ and 10 mg L⁻¹ respectively [107, 108]. Also the residual amounts of poly-DADMAC permitted in finished drinking water after treatment is 50 µg L⁻¹ [108, 107]. Several authors have shown that poly-DADMAC can be a hazardous substance if it is present at the disinfection stage (addition of oxidants Figure 2.1, p. 15) in a water treatment plant [109-115] and remains in drinking water [116-118]. Most water treatment plant in South Africa employ poly-DADMAC in their drinking water production processes compared to other countries [95], and ironically there is no legislation or regulation in place for poly-DADMAC in South Africa [105] owing to the unavailability of a reliable method to quantify poly-DADMAC in water. Thus, analytical methods that determine low levels of poly-DADMAC in water are necessary, especially considering that legislation to protect what is increasingly becoming a precious resource globally, will become more stringent in the near and long term.

2.5 Analytical methods for quantification of poly-DADMAC and other polyelectrolytes

A number of analytical methods have been developed for analysis of poly-DADMAC in relatively clean water. The following methods have been suggested for the detection of poly-DADMAC:

- Turbidimetry/nephelometry
- Spectrofluorimetry
- Spectrophotometry
- Viscometry
- Colloidal titration
- Luminescence titration
- Chromatography and NMR
- Tagging

Selected methods will be reviewed below; most of these methods are limited due to lack of specificity toward poly-DADMAC. Colloidal titration is the most widely used method for the detection of poly-DADMAC.

2.5.1 Colloid titration

Colloid titration was the name given to the quantitative volumetric analysis of polyelectrolytes in solution by Terayama in 1952 [106]. Colloidal titration has been used successfully for the quantification of poly-DADMAC in water solution [106, 119]. Colloidal titration is based on the reaction between cationic and anionic polyelectrolytes [21]. When these polyelectrolytes are mixed together in the solution a neutralisation reaction will occur. This reaction is stoichiometric in the sense of 1:1 charge compensation. If the charge density (or equivalent weight) of polyelectrolyte is known, that polyelectrolyte can be used as the titrant to determine the charge or equivalent of the oppositely charged polyelectrolytes with unknown charge to find the concentration, provided that the method of detecting the endpoint is available and that the

assumption of 1:1 stoichiometry is valid (a recently modified colloidal titration by Cumming [106]). Currently there are two widely used ways of determining the endpoint, namely visual titration and spectroscopy. Visual detection of the endpoint for direct titration methods is difficult, consequently several automatic titrimetric methods have been reported for colloidal titration, double wavelength photometry [120], potentiometry [121], changes in turbidity [122], streaming current detection [21], conductometry [106, 123] and colour change of metachromatic dye visually or spectrometrically [106,119,124,125]. The endpoint of colloid titration has been determined by many other methods ever since colloid titration was introduced in 1952.

Colloid titration methods have also been applied as routine analysis for quaternary compounds. During the production of long chain quaternary ammonium compounds, fast yet accurate analytical methods are needed to control the process. Wang et al. [126] reported the potentiometric titration of long-chain quaternary ammonium compound using sodium tetraphenyl borate and a platinum electrode system to detect the endpoint, and found the method to be superior in precision and accuracy to visual methods [126]. Jonnalagadda et al. [121] developed a photometric titration method for the determination of organic polymers used in water treatment which eliminates interferences from other absorbing species in solution, since the wavelength was fixed at 631 nm. Mocchiutti et al. [125] reported on the key things that must be considered in the determination of polyelectrolyte concentration by colloidal titration, these included the use of buffers, pumps, the increments used to add the titrant, the staining of glassware by the indicator, and the time taken to form the color-absorbing complex. Analysis of poly-DADMAC in water with colloidal titration was studied by both Cumming et al. [106] and John et al. [119], and the difficulties they reported on were the reversal of the titration after endpoint, colour of the endpoint was not clear, no standard method to determine endpoint and use of sophisticated calculations to determine the end-point. With colloidal titration the endpoint determination has been the focus of research since 1952.

2.5.2 Tagging

Copolymerization of acrylamide or DADMAC with small amounts of an amine-functionalised monomer yielded a flocculent that can be tagged with a reactive fluorophore before or after use

and detected at low concentration [127]. Tagging with fluorescein isothiocyanate yields polymers having high fluorescence intensity. The fluorescein chromophore is light-sensitive and suffers interferences from organic matter in natural waters, but ways of overcoming these problems were developed [127]. In natural water samples size-exclusion chromatography separates residual polyelectrolyte from interfering impurities and allows it to be quantified by fluorescence [127]. Eldrige et al. [127] reported detection of polyelectrolytes at trace levels in water by this fluorescent tagging method, and estimated the detection limits of tagged poly-DADMAC to be ~ 10 to $40 \mu\text{g L}^{-1}$ in clean water and higher in treated potable water. Many tagging methods have been developed for other polyelectrolytes as well [128, 129].

Another new exciting method is the absorption of polyelectrolytes onto charged nanoparticles [130-133]. In this method silver-coated silica nanoparticles are synthesized and coated with pyrenyl acrylic and methyl ester polyelectrolytes, and later detected with a Raman spectroscopy.

In this work gold nanoparticles (Au-NPs) were added to poly-DADMAC solution to form aggregates, that were later detected and quantified with ultra-violet visible spectroscopy (UV-vis). To determine whether Au-NPs are tagged on polyelectrolytes or adsorbed is area that needs more research.

2.5.3 NMR

Organic polyelectrolytes are utilized in wastewater treatment, but their fate after use is poorly understood. Analytical methods used for polymer determination in less complex systems appear to fail when applied to wastewater systems, contributing to the lack of knowledge [134]. Dentel et al. [134] reported the determination of cationic polymer concentrations by NMR spectroscopy using a strong linear relationship between polymer concentration and either height or area of the specific peak. The detection limit of this method was determined to be $< 0.5 \text{ mg L}^{-1}$. Use of the method was exemplified by analysis of anaerobically digested sludges for residual polymer following a range of dosages. Recently, John et al. [119] showed that it is difficult to detect poly-DADMAC with NMR.

2.5.4 Chromatographic methods

Liquid chromatography-mass spectrometry (LC-MS) has evolved as an important technique for the quantification of polyelectrolytes in environmental samples. Despite being a sensitive method and able to eliminate possible interferences almost completely, there is some hesitation in accepting it as a reliable method for polyelectrolyte analysis owing to it being expensive and complicated. Esparza et al. [108] and Jin et al. [107] separately reported the use of this technique for the detection of poly-DADMAC monomer, DADMAC, in water samples with a limit of quantification as low as $25 \mu\text{g L}^{-1}$ and $0.1 \mu\text{g L}^{-1}$ respectively [107, 108]. Guo et al. [135] and Li et al. [136] also reported the application of LC-MS in the analysis of quaternary ammonium compounds at trace levels in water [135, 136]. An extensive literature search undertaken indicates that there has been little or no published report on the application of LC-MS to poly-DADMAC or polyelectrolytes, except for the application of gel permeation chromatography [119].

2.5.5 Summary of analytical methods

Many of methods for the analysis of polyelectrolytes and quaternary compounds were mentioned in this Section 2.5 of the literature review. But some of these methods suffer from interferences, detection limits, expensive equipment, repeatability and they are hardly reproducible. In general, the sensitivity of these methods is insufficient for regulatory purposes and, in some situations, the reliable identification of the polyelectrolyte is not ensured. In this work recent published methods were investigated for the analysis of poly-DADMAC in water solutions.

Although colloidal titration is the oldest method for the analysis of polyelectrolytes (1952) in water solutions, it was also included for investigation. It is the only method that is extensively used for analysis of polyelectrolytes, because it is easy to implement, cheap, and is not time-consuming. But reproducibility and interferences are problems arising from difficulties in endpoint detection and calculation of concentration of the analyte from calibration curves. As a result a reliable way of determining the end point and calculation of the concentration from calibration curves has been a point of research since 1952 until today. The following methods

were investigated based on repeatability, interferences, throughput, availability, time and cost of the methods:

- LC-MS techniques
- NMR spectroscopy methods
- Raman spectroscopy method
- Colloidal titration
- Tagging: Indirect UV-Vis detection of poly-DADMAC, a novel method utilising gold nanoparticles

The above techniques were selected for investigation because they are known to be reliable, specific and have higher sensitivity accompanied by lower limits of detection. These methods have been applied in different matrixes and eliminated interferences which have been encountered with other methods. LC-MS and NMR methods are accurate and precise, but they are expensive and only selected laboratory in developed countries have this equipment. But the health threat posed by polyelectrolytes in the environment makes it harder to ignore these methods. The remaining methods rely on UV-Vis spectroscopy, which is cheap, but they are not reliable owing to selectivity and reproducibility. Only two methods (colloidal titration and indirect UV-Vis detection of poly-DADMAC by using gold nanoparticles) investigated here were found to be useful in this study and, were considered for publication, and are presented in chapter 4 and 5 in the form of extended papers suitable for dissertation chapters.

2.6 Conclusions

Polyelectrolytes are polymers with ionisable groups with either a net positive or negative charge at near neutral pH and can be classified by origin, composition, molecular architecture, charge and/ or electrochemistry. Like other polymers, polyelectrolytes are widely used in industrial processes; such as, pulp and paper manufacturing, paint industry and water treatment sectors. They have also found use in the pharmaceutical and cosmetics industries for the preparation of hair conditioners, shower shampoos, drugs and for most skin care products. One of the biggest

uses of polyelectrolytes is in water treatment processes. There have been recent reports in the literature that widely caution us on the use of polyelectrolytes since there are different paths polyelectrolytes can enter the environment. The problem with polyelectrolytes in water treatment processes is mainly concern over health effect with their monomers, contaminants and degradation to more dangerous by-products (classified as emerging contaminants) considered to be carcinogenic, especially at the disinfection stage. Hence the need for reliable analytical methods to detect low levels of polyelectrolytes at various stages before disinfection and finished water. Current methods are expensive, complicated and time-consuming, while older methods are outdated and not capable of quantifying low levels of poly-DADMAC with certainty.

2.7 References

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Chapter 3: Investigation of selected analytical methods

The following chapter fully describes the investigation of three used analytical techniques, namely LC-MS, NMR and Raman spectroscopy for detection and quantification of poly-DADMAC in water [1,2,3,4,5]. Based on difficulties encountered during the construction of the calibration curves and unsuccessful validation, we found these techniques unsuitable for routine analysis of poly-DADMAC in water samples.

3. Silanization of glassware

Since the charged species, namely, the anionic and cationic polyelectrolytes can easily adsorb onto glass surfaces, the glassware was subjected to a silanization process [1]. Poly-DADMAC adsorbed on the walls of the glassware containers and caused the concentration prepared to differ from the initial calculated concentration [1].

All water used in the preparation of solutions, in the titrations, and for final rinsing of glassware was Mill-Q ultrapure water (Mill-Q). Mill-Q water was obtained from a Millipore Elix system bought from Microsep South Africa and had a resistivity of 18 M Ω cm. but for cleaning and soaking of glassware double distilled water was used. All chemicals were of analytical grade, purchased from Sigma Aldrich, Germany through Capital Lab Supplies, South Africa except were stated otherwise.

For the silanization procedure, the glassware was washed with 10% nitric acid solution (overnight soak), rinsed thoroughly with double distilled water and then five times with mill-Q water and then air dried. The glassware (volumetric flasks, conical flasks, burettes, and pipettes)

were then coated with the silanization solution. The silanization solution was prepared in small volumes, as needed, by mixing 2.0 mL of dichloro-1-fluoroethane (98% w/w) and 2.0 mL of a 2% dimethyldichlorosilane. Once coated, all glassware was allowed to dry for 24 hours. The glassware was then rinsed with double distilled water until foaming ceased, rinsed again with Milli-Q water, air dried and acid washed again. Quick visual test to ensure the glassware was silanized was to run a drop of water over the surface, beading was taken as a good indication the procedure had been successful. Also, for the proceeding Chapters 4 and 5, the same procedure described in this section was followed for the preparation of water, glassware and solutions.

3.1 Preparations of poly-DADMAC stock and working solution

A volume of 1.0 mL of the poly-DADMAC (35 w/w% bought from sigma Aldrich) solution (1.0889 g) was transferred into a 1000 mL volumetric flask and ultrapure water was added up to the mark. After mixing thoroughly, the solution (381.1 mg L^{-1}) was transferred into a plastic container. This solution was then used to make a 50 mg L^{-1} solution of poly-DADMAC.

3.2 LC-MS methods

In this study the Gemini-NX column was selected for the analysis of poly-DADMAC because it has a wide pH range between 1 and 12, high sample loading and can withstand high pressures which meet the conditions required to elute poly-DADMAC [2] and is similar to a column used to elute quaternary ammonium compounds by means HPLC [2-6]. Booth gradient and isocratic elution were previously proposed for the elution of poly-DADMAC monomer and quaternary amines [2, 5]. A mobile phase of 45% acetonitrile and 55% aqueous buffer (formic acid/ammonium formate, pH 4) was reported by Esparza et al to elute poly-DADMAC monomer within 1.5 minutes. All parameters used here were previously proposed to elute quaternary amines from the column. In addition Jin et al. [5] showed that a dilute solution of poly-

DADMAC could be used on an HPLC-MS system. But the authors focused on the detection of the monomer and did not report on the fate of the polymer in their experiments.

3.2.1 Chemicals

Polydiallyldimethylammonium chloride 35%, acetonitrile and methanol (chromosov grade, 99%), formic acid and ammonium formate (analytical grade, 99%) were used without further purification.

3.2.2 Instrumentation

Liquid chromatography-electro-spray ionization mass spectrometry (LC-ESI-MS) (Agilent series, G 244-90091, USA) in positive mode was used to analyse the poly-DADMAC in the water samples. The analyte was separated by using an Agilent series HPLC (G1316A, USA) equipped with a quaternary gradient pump, column oven, detector (G1315D) and an auto-sampler. The analytical column was a Gemini C₁₈ 110A (150 mm x 2 mm, 3 μm; Phenomenex USA, purchased through Industrial Analytical, South Africa).

3.2.3 Procedure

The mobile phase used for eluting the poly-DADMAC from HPLC column consisted of acetonitrile and formic acid/ammonium formate (preparation see Appendix 1) buffer (pH 4) at a flow rate of 0.2 ml/min. Gradient elution was performed as a linear gradient from 45% A for 10 minute and held at 60% B for 2 minutes, followed by stepwise elution 60% B and 40% A to 0% B and 100% A as shown in Table 3.1. The injection volume was 5 μL. The HPLC was connected to a single quadropole mass spectrometer equipped with an ion-trap operated in positive ionization mode. The entire column effluent from the HPLC was directed into mass spectrometer without flow splitting. In the positive mode, typical ion source parameters were used as follows: ESI capillary voltage 4 kV, desolvation temperature 350 °C. Nitrogen was used as the

desolvation gas with a flow rate of about 400 L h⁻¹ and as the cone gas with a rate of 60 L h⁻¹ from a pressurized system cleaner. The LC-ESI-MS maximum pressure is 200 bar [2, 5, 7].

Table 3. 1: Gradient elution of poly-DADMAC at flow rate of 0.2 ml/min, 5 µL injection volume

Mobile Phase	Acetonitrile (A)	Formic acid/ammonium formate (B)	Time/min
Solvent ratio 1 st (%)	45	55	10
Solvent ratio 2 nd (%)	40	60	2
Solvent ratio 3 rd (%)	60	40	stepwise
Solvent ratio 4 th (%)	100	0	stepwise

3.2.4 Results and discussion

Figure 3.1 shows chromatogram of a water solution spiked with poly-DADMAC, mobile phase was 45% acetonitrile, 55% aqueous buffer and eluted through Gemini-NX column, as expected there was no peak of poly-DADMAC observed because of its UV-Vis inactiveness.

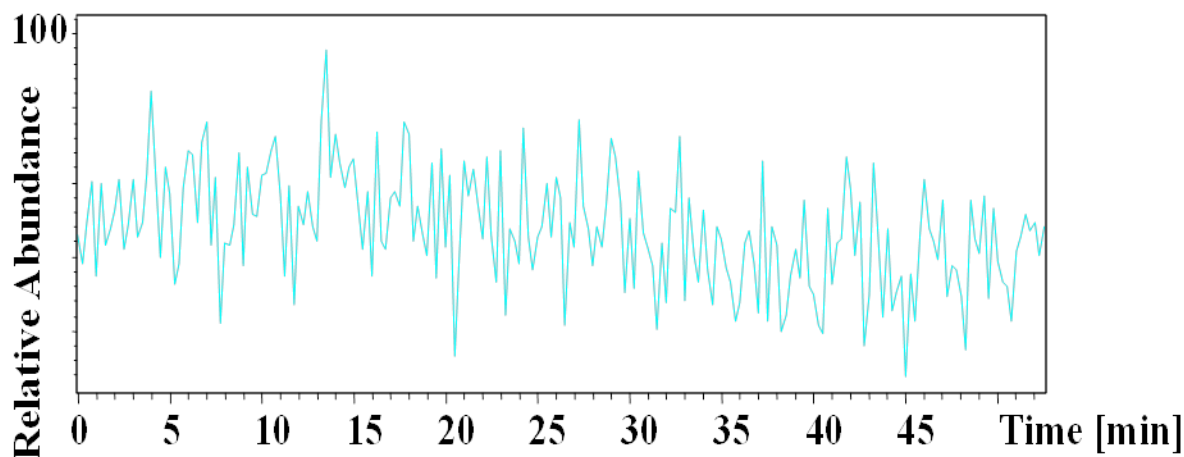


Figure 3.1: Chromatogram of a water solution spiked with poly-DADMAC spiked water solution, eluted through Gemini – NX column with 55% acetonitrile and 55% aqueous buffer solution.

There was no peak of poly-DADMAC observed in the MS spectrum after 45 minutes either. Different parameters were optimized and gradient elution was also performed (conditions shown

in Table 3.1, p. 44), and still there was no sign of poly-DADMAC in the MS spectrum. It was previously reported by Jin et al. [5] that 95% of an aqueous buffer (trifluoroacetic acid/heptafluorobutyric acid) is required to elute the monomer (high pressures were required to flow aqueous solution in the column).

Although our column can withstand high pressure conditions, our LC-MS system was designed to operate at a lower pressure (below 200 bar). An attempt to change the mobile phase buffer to 95% would result in an automatic shutdown of the LC-MS system which means an expensive high pressurized LC-MS system was needed with no guarantee that would work. Considering the cost of the method and column, the experiments were stopped. Poly-DADMAC was thought to have adsorbed onto the stationary phase and accumulated inside the column. The column was placed in an HPLC which was not connected to the MS detector and which could operate under high pressure for cleaning off the poly-DADMAC and removal of buffers for eventual storage of the column. In a separate experiment, poly-DADMAC was injected directly into the MS detector and the results obtained are shown in Figure 3.2.

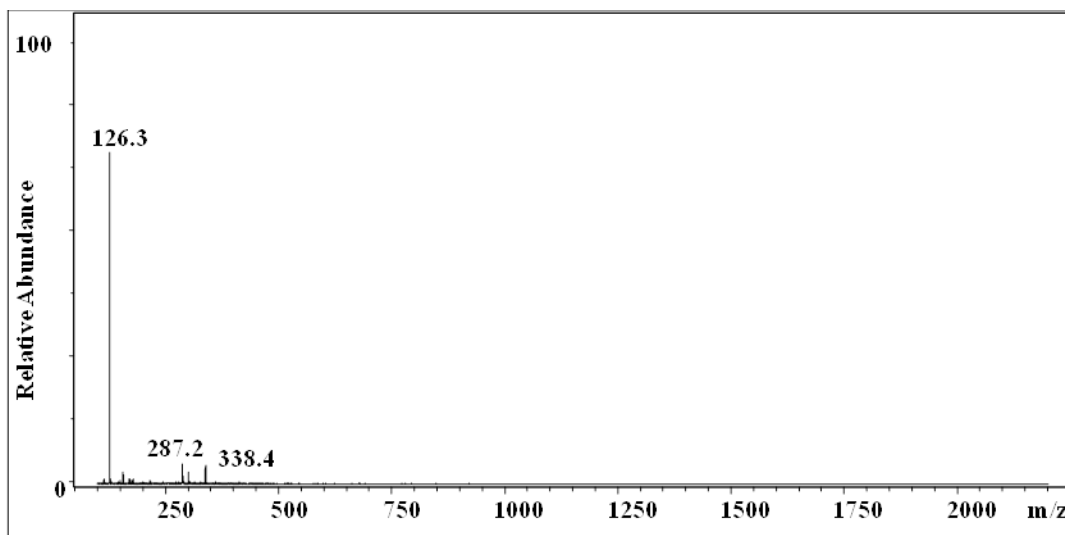


Figure 3.2: Mass spectrum of DADMAC obtained by injecting 1 mg L⁻¹ of poly-DADMAC straight into the mass spectrometer at 70 kV voltage.

Figure 3.2 shows the mass spectrum of poly-DADMAC with 126 m/z as the dominant ion which corresponds to the mass of DADMAC previously reported by Esparza et al. [2]. It was difficult to identify whether DADMAC was the result of poly-DADMAC breakdown inside the MS or chemical impurities from the manufacturing company. Peaks at 287.2 and 338.4 are solvent contaminants. An attempt to construct a calibration curve failed due to the ion detector being flooded with ions as result of poly-DADMAC or DADMAC breakdown and in some cases the DADMAC peak was not present. This led to the assumption that maybe the poly-DADMAC may have decomposed completely ('burned') inside the ionization chamber instead of fragmenting in a reproducible manner.

3.2.5 Final Comments and Observations

Existing LC-MS methods are not suited for the analysis of poly-DADMAC in water. Apart from being expensive, it was shown in this preliminary study that these methods are useful for the determination of quaternary monomers and not polyelectrolytes since it was not clear whether the poly-DADMAC was eluted from the column to the mass spectrometer. Although the method did not work, an improved method that uses volatile and high ionic strength buffers which may prevent the adsorption of poly-DADMAC are recommended. Poly-DADMAC was effectively used as displacer for cation-exchange in displacement chromatography [8]. The ability of poly-DADMAC to displace protein in the chromatography depends on the molecular weight (poly-DADMAC) and ionic strength of the buffer (mobile phase). We assume if these conditions are reversed it might be possible to elute poly-DADMAC from an LC-column through into the MS system. Injecting poly-DADMAC directly into the mass spectrometer might work in the MSⁿ mode which will prevent the ion detector from being flooded. Interfacing mass spectrometry with gel permeation chromatography might be another option [7] or the use of liquid chromatography that operates at so-called ultra-high pressures (above 200 bar).

3.3 NMR spectroscopy

Recent advances in nuclear magnetic resonance (NMR) spectroscopy allow for the analysis of analytes at lower concentrations in complex matrixes due to the wide linear dynamic signal digitization, and the fact that this is a non-destructive technique that can selectively detect a large number of compounds simultaneously [9]. All these advantages make proton NMR well suited for rapid identification and quantification of analytes using well-resolved peaks [10]. NMR has been also utilised to quantify analytes of low molecular weight [11]. NMR have been used in quality control of food and drug processing for many years [10, 12, 13]. Chang et al. [14] reported detection of polyelectrolytes below 0.5 mg L^{-1} in water. Application of NMR to quaternary compounds was reported by Zuppi et al. [11]

Despite the advantages (qualitative and quantitative information at the same time within a few minutes) provided by NMR spectroscopy, compared to other techniques and its wide application, it has not been used as a quality control technique for polyelectrolytes [10]. NMR spectroscopy suffers from poor sensitivity compared to other techniques such as mass spectrometry, HPLC and fluorimetry, because of the dependence of NMR peaks to solution matrix effects such as pH, metal and molecular interactions [10]. Furthermore, comparing the spectrum obtained from different instruments is difficult without the knowledge of how the sample was prepared and parameters used for data acquisition. Poly-DADMAC can be detected at lower concentrations compared to other polyelectrolytes due to its higher charge density [1]. This study was undertaken to determine whether the higher charge of poly-DADMAC does enhance sensitivity and whether NMR spectroscopy can be used as a quality control technique as is done in food processing.

3.3.1 Reagents and chemicals

Poly-DADMAC 35% solution was purchased from Sigma Aldrich through Capital Lab Supplies from Germany), chloroform- d_1 and methanol- d_1 both were purchased from Merck, Germany. After use NMR tubes were cleaned with aqua regia to remove adsorbed poly-DADMAC.

3.3.2 Instrumentation

NMR spectra were recorded with a Bruker Ultra operating at 400 MHz and utilising Topspin software.

3.3.3 Procedure

Solutions of poly-DADMAC were prepared in NMR-grade methanol- d_1 to prevent the interference of water molecules (methanol and poly-DADMAC are miscible). To construct calibration curves poly-DADMAC standards with concentrations of 10, 30, 50, 70 and 100 mg L⁻¹ were prepared [15] and analysed by means NMR [7, 15].

3.3.4 Results and discussion

To construct a calibration curve, poly-DADMAC standards with concentrations of 10, 20, 30, 50, 70 and 100 mg L⁻¹ were prepared and analysed with NMR. These high concentrations were chosen since Xu et al. [10] reported poor recovery of polyelectrolytes at lower concentrations. In this study poly-DADMAC was dissolved in methanol- d_1 rather than in D₂O; compared to a method that involved the heating of the polyelectrolyte for several hours to remove water reported by Chang et al. [14]. In this case, this method was not applied, since the boiling point of poly-DADMAC is close to that of water. Water molecule interferes with the analysis of most polyelectrolytes [13]. John et al. [7] used this method of heating in qualitative analysis of poly-DADMAC while in quantitative analysis poly-DADMAC is very dilute and might evaporate together with water due to the closeness of the boiling points and thus lead to faulty concentrations [7, 16].

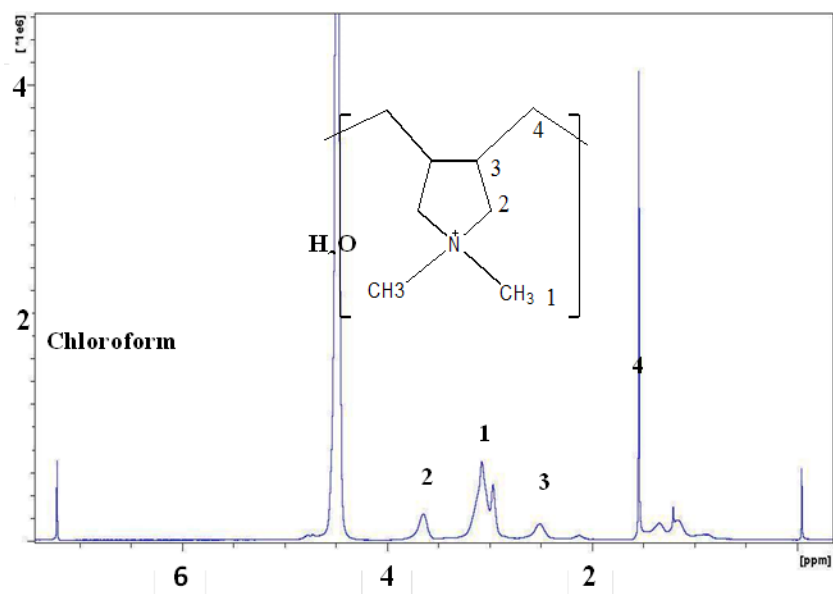


Figure 3.3: NMR spectrum and structure of poly-DADMAC at high concentration (50 mg L^{-1} prepared in water and later substituted with methanol).

The NMR spectrum of poly-DADMAC is shown in Figure 4.3, and was similar to that observed in the literature [16]. The chemical shifts observed were assigned as follows: methyl – 3.1 ppm (1), methylene – 3.65 ppm (2), methine – 2.5 ppm (3), methylene – 1.5 ppm (4), water – 4.5 ppm and chloroform-D – 7.23 ppm. Peak at 3.1 ppm (integrated peak area using TopspinTM software) was used for the analytical quantification of poly-DADMAC in water solution. Although water has been regarded as an interfering species in NMR analysis, here it can be seen from the spectrum that poly-DADMAC peaks are well-resolved from water and chloroform- d_1 [14]. Water is usually removed by heating polyelectrolytes for several hours above 100°C to dryness and later D_2O is introduced to substitute the water molecules [14]. This heating method is useful in qualitative analysis while in this case it is not applicable due to the closeness of the boiling points of poly-DADMAC and water. This method requires that poly-DADMAC be heated at 132°C for 6 hours. In dilute solution poly-DADMAC might evaporate with water and lead to faulty concentrations being reported [7].

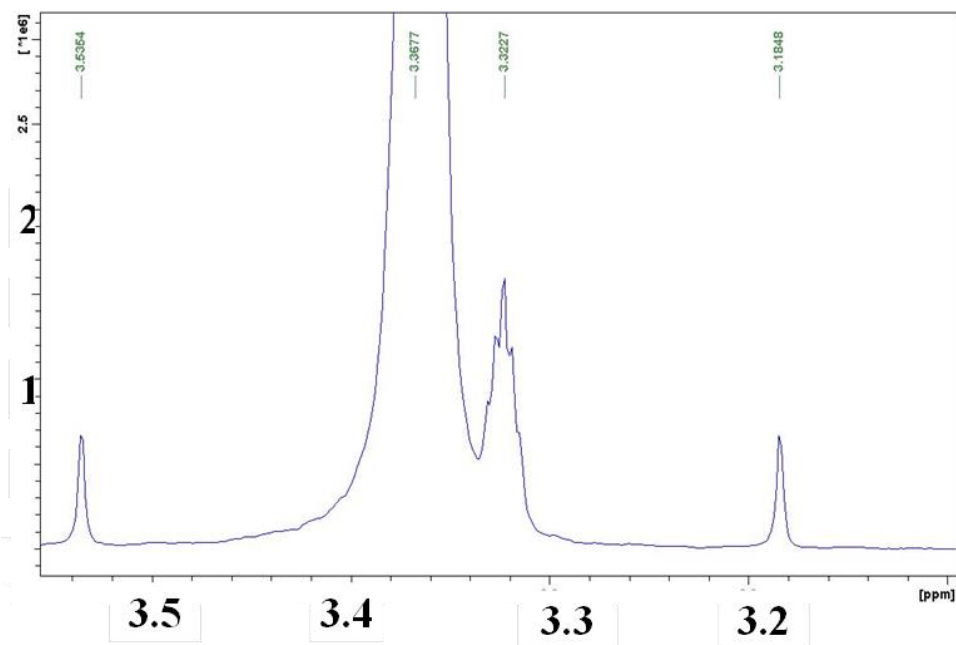


Figure 3.4: NMR spectrum of 10 mg L⁻¹ poly-DADMAC solution prepared in methanol.

To prevent the loss of poly-DADMAC through evaporation, it was dissolved in methanol because in this solvent it is completely miscible even at room temperature unlike chloroform-*d*₁. Sometimes thioyl chloride is used to dissolve polyelectrolytes for NMR analysis [17]. The NMR spectrum of 10 mg L⁻¹ of poly-DADMAC standard is shown in Figure 5.4. The spectrum shows that the poly-DADMAC peaks were well separated from that of methanol-*d*₁ and the HPLC grade methanol used to prepare the stock solution of poly-DADMAC [18]. A calibration curve was constructed from the peak height of the CH₃ (This peak is well resolved and distinct) of poly-DADMAC versus the concentration of poly-DADMAC as shown in Figure 4.5. The Calibration curve is linear over the concentration range of 10 – 100 mg L⁻¹ with R² of 0.9928 and slope of 0005000.

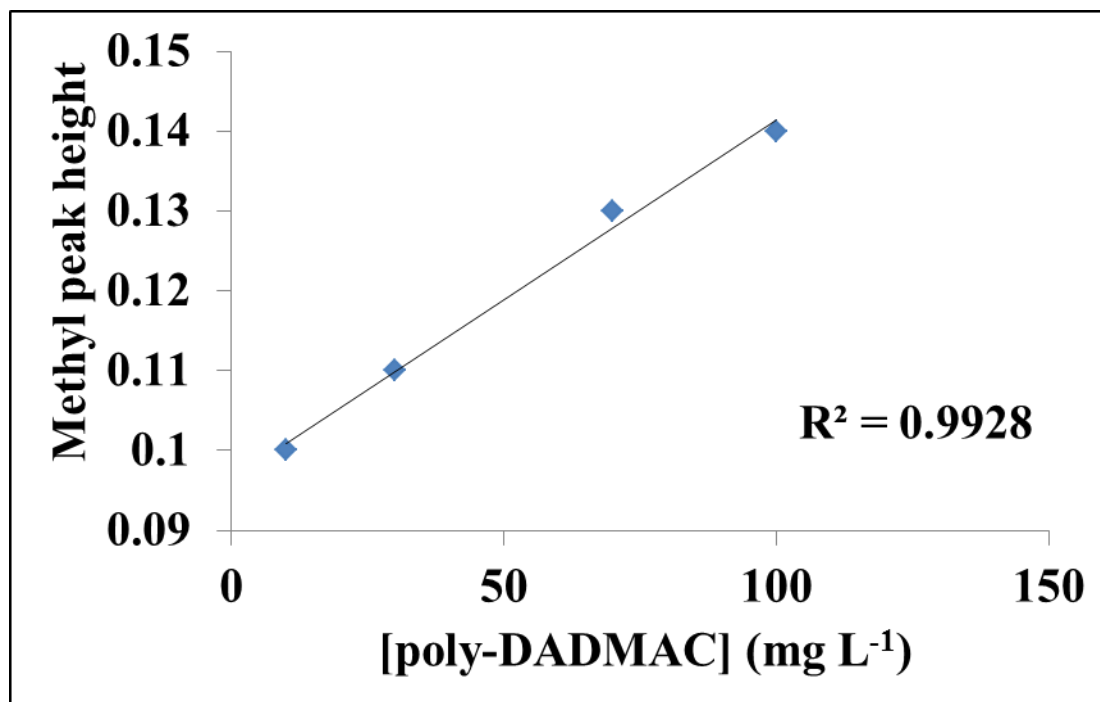


Figure 3.5: Calibration curve constructed from peak height of CH₃ versus poly-DADMAC concentration with an equation $y = 0.0005x + 0.0963$ ($R^2 = 0.9928$) in concentration range between 10 – 100 mg L⁻¹.

Regression analysis showed that a good linear relationship between poly-DADMAC concentration and peak height exists, but the sensitivity was very poor and attempts to detect poly-DADMAC below 1 mg L⁻¹ were unsuccessful with this method. Chang et al. [14] reported good recovery of polyelectrolytes with a NMR method at concentrations above 250 mg L⁻¹. Considering the limit of detection required for poly-DADMAC detection in the environment (50 µg L⁻¹), the effect of incorrect dosage in water treatment, and the time and cost of this method, further improvement of this method was stopped.

3.3.5 Final Comments and Observations

Poly-DADMAC needs to be detected at trace levels and small changes in the dosage of poly-DADMAC in a water treatment plant will result in a large effect [19]. A sensitive method with a low limit of detection is required for routine analysis of polyelectrolytes both in the environment and water treatment plants. The NMR method suffers from poor sensitivity with this

macromolecule, and similar results have been reported in the literature [11]. A pre-concentration step was recommended if this method is to be used [11]. Removing water from poly-DADMAC must be done carefully to prevent the loss of poly-DADMAC itself due to evaporation. In this study poly-DADMAC was analysed without water being removed from the solution. Our calibration curve had a wider concentration range 10 – 100 mg L⁻¹ with a slope of 0.0005000 [20] which means the method is not sensitive to polyelectrolyte with high molecular weight as opposed to lower molecular weight polyelectrolytes [20].

3.4 Raman spectroscopy

Surface enhanced Raman spectroscopy (SERS) and resonance Raman spectroscopy have been recognised as sensitive detection techniques with high levels of molecular specificity (detection limit below 1 µg L⁻¹) [21]. Recently, Raman spectroscopy has been accepted as a low-cost and reliable technique across a broad range of applications, matrices and sample types [21]. Raman spectroscopy is no longer used as a last option and has been employed as a routine method in many fields due to several qualities (fast measurements, easy handling, accuracy, reliability and possibility of on-line monitoring with fibre-optic cables) when compared to existing analytical approaches [21, 22].

Raman spectroscopy has been widely used in the pharmaceutical, environmental and polymer industries as a quantitative technique [21, 23-29]. Despite the wide application of Raman spectroscopy in many fields, there is very little (or nil) reported on the use of this technique for the analysis of polyelectrolytes. Poly-DADMAC has been detected in qualitative analysis with Raman spectroscopy and shows well-resolved peaks, and it has been incorporated in other analytical methods and reported to have resulted in enhanced Raman signal of other chemicals [30-34]. Based on our knowledge, no analytical method based on Raman spectroscopy for the quantification of poly-DADMAC has been reported in the open literature. The following study was undertaken to determine whether Raman spectroscopy could be used to direct or indirectly selectively quantify poly-DADMAC in water solution at dilute concentration.

3.4.1 Reagents and Chemicals

Poly-DADMAC 35% (was purchased from Sigma Aldrich through Capital Lab Supplies from Germany) and To prepare all solutions, Mill-Q water with a resistivity of 18 M Ω cm was used and was obtained from a Millpore Elix system bought from Microsep South Africa.

3.4.2 Instrumentation

Advantage 532 utilising laser (solid state 532 nm, ND Yag crystal), detector (charged couple device (CCD)), and gratings (1800 lines per mm) and was bought from Delta Nu, USA.

3.4.3 Procedure

A poly-DADMAC stock solution was prepared followed by series dilution to obtain working standards. Poly-DADMAC standards ranging from 30 – 90 $\mu\text{g L}^{-1}$ were analysed by Raman spectroscopy.

3.4.4 Results and discussion

Raman spectroscopy analyses of dilute concentrations of poly-DADMAC were performed and the results are shown in Figure 4.6. The spectrum of poly-DADMAC obtained was similar to that reported by Park et al. [31] at high concentration. The intensity of the peak at around 3000 cm^{-1} was reported to be directly proportional to the concentration of poly-DADMAC in the solution [30], since it decreased when poly-DADMAC was exposed to chlorine. This peak is due to the CH_2 cyclic stretching vibration in poly-DADMAC [31]. In this study different concentrations of poly-DADMAC were prepared and analysed with Raman spectroscopy. The analysis of this peak gave no relationship between poly-DADMAC concentration and peak intensity (around 3000 cm^{-1}) in dilute solution as opposed to the high concentrations observed by Park et al. [31].

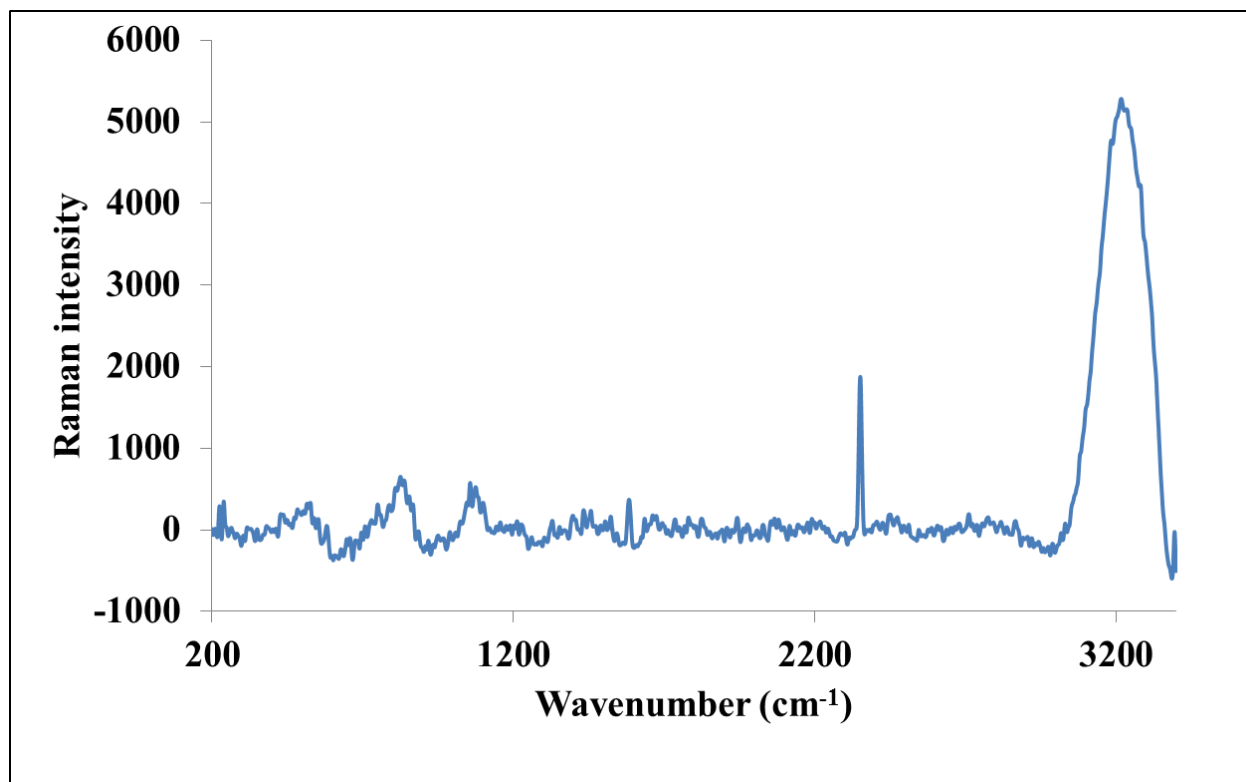


Figure 3.6: Raman spectrum of 90 µg L⁻¹ of poly-DADMAC in water solution.

3.4.5 Final comments and observations

Raman spectroscopy was able to detect poly-DADMAC at dilute concentrations but was unable to produce a signal that varied with concentration that could be used to quantify poly-DADMAC. Gold nanoparticles are known to enhance the Raman signal of many analytes at dilute concentration and assist in quantification those analytes [24, 35-38]. Enhanced Raman detection of the analyte in the presence of gold nanoparticles would be a viable future study for poly-DADMAC.

3.5 Overall Conclusions

Five analytical methods were investigated; only two were determined to be useful in quantification of polyelectrolytes. These two methods were colloidal titration and gold nanoparticles synthesised from a citrate salt and these two successful and in-depth investigations

are presented in Chapters 4 and 5 respectively. The other methods were not ruled out completely. With careful observations, and experience gained, some recommendations were made; specifically, more time is needed to develop the other techniques, significant investment in more modern instrumentation may yield better results (not guaranteed), increased complexity in terms of protocols, and in some cases use of more chemicals (see HPLC-MS). In the future these methods could be useful.

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Chapter 4: Application of metachromatic polyelectrolyte titration in environmental analysis for the quantification of polydiallylmethylammonium chloride in water

Metachromatic titration is an analytical method used since 1952 for the analysis of polyelectrolytes in water samples. A detailed literature review on this work suggested that endpoint detection is a limitation for this method. We have undertaken this work to investigate an easy way of determining the endpoint with fewer errors compared to literature methods. We present a novel way of detecting the endpoint based on an Ocean Optics dip probe technique. A significant amount of work was done in terms of developing calibration curves and validating the new technique, for this reason we present this metachromatic titration technique as a separate chapter.

4. Synopsis

Polydiallyldimethylammonium chloride (poly-DADMAC) is a water soluble polymer that easily ionizes when dissolved in water. This cationic polyelectrolyte is mainly used as a flocculant within the water treatment industry, but little is known of its toxicological properties or its fate in the environment. It is often assumed that the polyelectrolyte sorbs onto solid surfaces in the water treatment stream and may be removed with the sludge or by a sand bed filter; which may not always be the case. In any event, reliable analytical techniques are needed for the determination of poly-DADMAC in matrices of environmental relevance. Metachromatic polyelectrolyte titration was used to quantify poly-DADMAC in various model and tap water samples. The technique provides an alternative to expensive and complicated techniques (e.g. liquid chromatography-mass spectrometry [1]). The method involves the titration of poly-DADMAC with the potassium salt of polyvinyl sulfate using *o*-toluidine blue as an indicator, and observing the colour change from blue to purple-pink, with significant decrease in the intensity of the peak absorbance at 634 nm or absorbance of the emerging peak at 515 nm. Our initial work in this area is presented, and we demonstrate that the detection limit for this method is below 0.1 mg L^{-1} in model water and between $0.5 - 1 \text{ mg L}^{-1}$ for selected water samples, including tap water where poly-DADMAC is normally detected [1, 2].

4.1 Introduction

Recently several methods have been developed to monitor poly-DADMAC in water treatment plants, rivers and dams; but, these methods can be expensive, sophisticated or time-consuming. Examples were given in the literature review in chapter 2. Colloidal titration is one of the oldest, cheapest, relatively simple and widely accepted analytical methods for the analysis of polyelectrolytes [3]. Colloidal titration has been used for the successful quantification of poly-DADMAC in water solutions [4-7]. Colloidal titration is based on the reaction between cationic and anionic polyelectrolytes [8]. When these polyelectrolytes are mixed in the solution together, a charge neutralisation reaction will occur, and the reaction is stoichiometric in the sense of 1:1 charge compensation. If the charge density (or equivalent weight) of the polyelectrolyte is

known, then the known polyelectrolyte can be used as a titrant to determine the charge or equivalence of the oppositely charged unknown polyelectrolyte. The concentration can then be determined, provided that the method of detecting the endpoint is reliable and that the assumption of 1:1 stoichiometry is valid. Typically, the endpoint is determined visually or by a spectroscopic technique. Visual detection of the endpoint for the direct titration method can be difficult and lead to under, or over-estimation of the concentration. Several automatic titrimetric methods have been reported for colloidal titration, including double wavelength photometry, potentiometry [9], turbidimetry and conductometry [4].

Recently, Cumming et al [4], Mocchiutti et al. [6] and John [5], separately introduced a unique spectroscopic method of detecting the endpoint or equivalence point in colloidal titration. In each case, the authors used a dye that underwent a colour change to shorter wavelengths and decreased in colour intensity; such dyes are referred to as metachromatic dyes; and hence such titrations can also be called metachromatic titration. Both John [5] and Cumming et al. [19], confirmed that the endpoint detection was improved (with a lower limit of detection) when using UV-Vis spectroscopy to determine the endpoint. However, their methods required small volumes, and this led to smaller volumes of indicator being added [5, 6, 9]. In metachromatic titration positively charged ionic species are known to interfere with the analysis [10]; and the only way to overcome this is to increase the amount of indicator. Many automatic titration methods have been developed for detecting the endpoint in colloidal titration since 1952 [3, 8, 11-13], but besides the work of Cumming et al. [4] and John [5] there is very little in the literature regarding UV-Vis spectroscopy endpoint detection techniques, especially for poly-DADMAC. This can be attributed to the time-consuming nature of the procedure, and precipitation reactions occurring between titre, titrant and indicator during titration due to the use of the small volumes needed.

In this work we have adapted the colloidal titration method for the analysis of poly-DADMAC and applied various techniques to improve the endpoint determination and ultimately the sensitivity of the method. One of the significant differences in our approach is the use of a fibre optic dip probe. One of the main advantages with the dip probe includes no need to transfer samples to expensive and fragile quartz cuvettes. It has been shown that poly-DADMAC can sorb to glass surfaces, thus leading to inaccuracies in the measurement, and the indicator can

stain quartz surfaces, which then requires specialized cleaning after every run [6]. Other advantages with the dip probe include faster measurements with a relatively quick response (less than a second upon addition of titrant); larger volumes can be used to prevent unwanted precipitation via increasing the indicator concentration to overcome interferences from positively charged species; and the dip probe is also much quicker in operation, typically taking less than 1 second per reading compared with 10 seconds for most peristaltic pump based flow cell sipper systems and is not affected by cross-contamination between titration runs [6].

4.2 Experimental Methods

4.2.1 Chemicals and reagents

Polyvinyl sulfate potassium salt (99%, PVSK), toluidine blue-O (80%, TBO) indicator, poly-DADMAC 35% (M.W. ~ 100000), and hexadecylpyridinium chloride monohydrate, also known as cetylpyridinium chloride (99 – 102%, CPC), were all purchased from Sigma Aldrich, and used without any further purification or treatment steps.

To prepare all solutions, Mill-Q water with a resistivity of 18 M Ω cm was used and was obtained from a Millpore Elix system bought from Microsep South Africa.

4.2.2 Silanization of glassware

Since the charged species, namely, the anionic and cationic polyelectrolytes can easily adsorb onto glass surfaces, the glassware was subjected to a silanization process [1]. This was done by cleaning the glassware with 10% nitric acid solution (overnight soak), rinsing thoroughly with demineralised water and then five times with mill-Q water and then air drying the glass. The glassware (volumetric flasks, conical flasks, burettes, and pipettes) were then coated with the silanization solution. The silanization solution was prepared in small volumes, as needed, by mixing 2.0 mL of dichloro-1-fluoroethane (98% w/w) and 2.0 mL of a 2% dimethyldichlorosilane. Once coated, all glassware was allowed to dry for 24 hours. The

glassware was then rinsed with demineralized water until foaming ceased, rinsed again with Milli-Q water, air dried and acid washed again. Quick visual test to ensure the glassware was silanized was to run a drop of water over the surface, beading was taken as a good indication the procedure had been successful.

4.2.3 Instrumentation

An Ocean Optics spectrometer (HR 2000+ high resolution), a Tungsten halogen light source, CUV 1 cm cuvette holder, quartz cuvette (4 mL) and dip probe (T300 – RT-UV-VIS, EOS – 1212277) were bought from Ocean Optics company, The Netherlands and were used for titration experiments. All spectra were collected and analysed by using the Ocean Optics Spectrasuite software. A pH meter (827 pH lab) and electrode (model 60220 100, pH range 0 to 14, temperature tolerance 80 °C and 3 M KCl) were bought from Metrohm, Switzerland.

4.2.4 Standardisation of potassium salt poly (vinyl sulfate)

It is critical that the charge density of the anionic polyelectrolyte, potassium salt poly vinyl sulfate (PVSK), is known so that the charge density of the unknown (the cationic polyelectrolyte poly-DADMAC) can be determined, and the concentration calculated.

A mass of 0.5000 g of PVSK was weighed directly into a 1.0 L volumetric flask and the solution made up to the mark with Milli-Q water (note that PVSK dissolved completely after 2 minutes). A stock solution of cetylpyridinium chloride was prepared by dissolving 0.0700 g of solid in a 1.0 L volumetric flask and making up to the mark with Milli-Q water. The normal charge of the PVSK solutions was determined by titration with cetylpyridinium chloride. For the titration, 10.0 or 5.0 mL of the cetylpyridinium chloride stock solution and 0.2 mL TBO indicator (0.1002 g weighed into the 100 mL volumetric flask) were placed in a silanized conical flask, and the required volume of Milli-Q water was added to make a 20.0 mL solution. After stirring for ~15 minutes, PVSK solution was added (very slowly) drop-wise from a graduated 10.0 mL (silanized) burette until the colour changed from blue to purple as shown in Figure 4.1.



Figure 4.1: Photograph of the colour change of the colloidal titration from blue to purple.

To ensure the reaction was complete, and the titration had not been done too quickly, the solution was allowed to stand for a couple of hours to make sure a return to blue did not occur. Three runs were performed and were within tolerance volume of 0.20 mL. Blank titration of the indicator was also performed, and the blank titration titre deducted from cetylpyridinium chloride titre.

A 0.5 M of acetate buffer was prepared. 14.31 mL of 17.465 M acetic acid was added into a 0.5 L volumetric flask and 19.2665 g of ammonium acetate weighed into a separate 0.5 L volumetric flask, and in both cases Milli-Q water was used to fill the flasks up to the mark. Then buffers of pH 7 and 4 were prepared by mixing the two solutions and measuring the pH of the solution, with a pH meter. The buffers were used to control the pH during titration.

4.2.5 Analysis of poly-DADMAC

A mass of 0.5046 g of PVSK was weighed and transferred to a 1.0 L volumetric flask, dissolved and the volume made up to the mark with Milli-Q water. Stock solution of 400 mg L⁻¹ of poly-DADMAC was prepared, and a series of dilutions made (1, 2, 3, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35 and 40 mg L⁻¹). For the titration, 0.2 mL of TBO indicator was added to the solutions of poly-

DADMAC that were in a pre-treated (silanized) conical flask, and the endpoint change from blue to purple-pink was observed when PVSX was added drop-wise from a 10.0 mL silanized burette. All experiments were done in triplicate and results were validated based on accuracy, precision and matrix.

4.3 Results and discussion

4.3.1 Standardization of PVSX

PVSX has a nominal charge density of 6.17 meq g^{-1} , and using this value, the concentration of unknown polyelectrolytes can be determined [4]. Alternatively, the actual equivalence of PVSX can be determined by using a suitable cationic surfactant, such as, zephiramine (benzalkonium chloride) or cetylpyridinium chloride. By using 5 different concentrations of CPC, the normality of the PVSX was determined, and the results are presented in Table 4.1.

Table 4.1: Equivalence of PVSX as determined by using the indicated solutions of CPC.

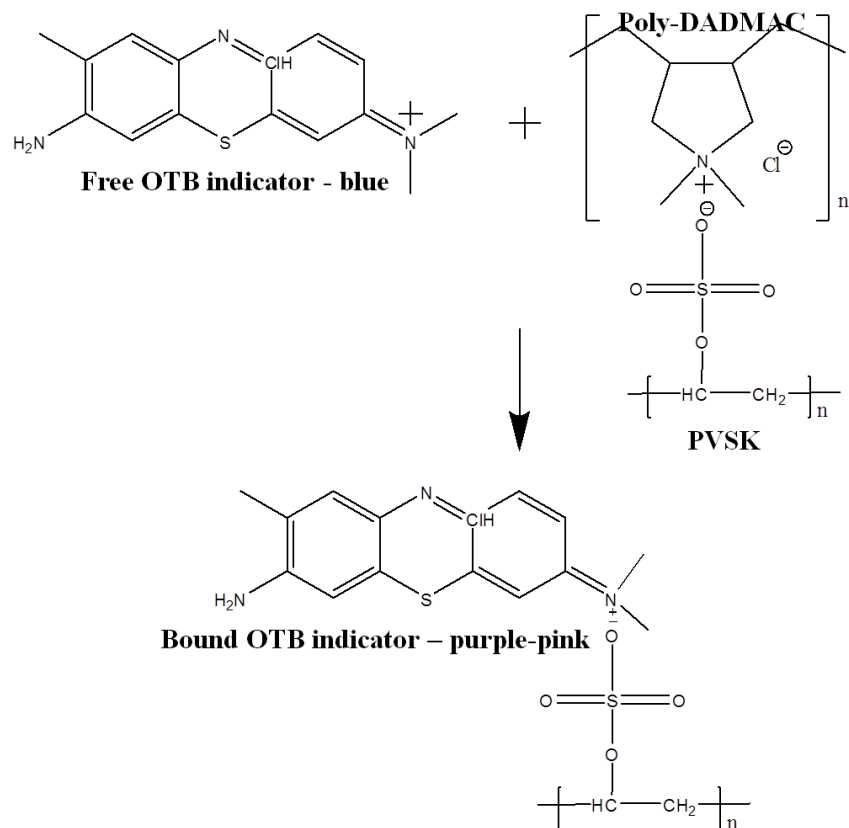
Concentration of CPC (M) $\times 10^{-5}$	Moles of CPC $\times 10^{-7}$	Average endpoint (L) $\times 10^{-3}$	Normality of PVSX (eq L ⁻¹) $\times 10^{-4}$	Concentration of PVSX (g L ⁻¹)
0.8441	1.6882	0.273	6.183	0.1002
1.152	2.304	0.346	6.659	0.1078
2.532	5.064	0.00079	6.410	0.1038
3.448	6.895	0.00112	6.151	0.0996

To find the normality of PVSX, we assumed a 1:1 stoichiometry between CPC and PVSX, and converted from normality of CPC to normality of PVSX, and then used the corrected endpoint to find the equivalence of PVSX. The corrected endpoint was determined by subtracting the values of the blank titrations from the actual titration between CPC and PVSX. The average equivalence for PVSX was $0.6376 \pm 0.014 \text{ meq L}^{-1}$, which was similar to what has been reported in the literature [4]. One of the main reasons the polymer is standardized before use, is due to the fact that some commercial samples will vary in the degree of esterification, and as such it may not be always 100%, which will introduce some deviation from expected normality values [13]. The

solution of PVSK was found to be stable and was only re-calibrated if the blank titration varied more than 15% from the initial blank titration. All solutions were used within 1 month of preparation.

4.3.2 Analysis of poly-DADMAC

During the colloidal titration of poly-DADMAC with PVSK, the endpoint is determined when the indicator, TBO, changes from blue to purple/pink. This colour change (as shown in Figure 4.1), occurs when there is a slight excess of the anionic PVSK titrant. Poly-DADMAC (cationic polymer) and PVSK form a preferential complex or colloid as shown in Scheme 4.1. When all of the analyte has reacted with PVSK, the excess PVSK begins reacting with the TBO indicator resulting in a colour change. However, the literature has shown that the visual detection method can vary from study to study, due to the operators experience with the observation of the colour change [14]. For example, the colour change of the metachromatic dye binding on to the anion has been described as a change from blue to pink [13], purple [15], reddish-purple [3], bluish purple [16], or purple-pink [4-6]. Furthermore, the solution can sometimes become colourless prior to the colour change [13] or a precipitate can form. This transition is usually attributed to flocculation of the neutral colloid [4]. Recently, researchers have been focused on finding a suitable endpoint detection technique and an easy way of processing data [4, 5].



Scheme 4.1: Schematic diagram of the reaction occurring in the colloidal titration of poly-DADMAC with PVSK and with TBO as an indicator.

In the past, work on the colloidal titration to quantify poly-DADMAC was accomplished by means of a calibration curve constructed from the direct plot of titrant volume against poly-DADMAC concentration [5, 6, 9]. These processing techniques were later criticised by Cumming et al. [4] where they suggested that at the point the colour change is observed, the amount of PVSK added is not related to the amount of poly-DADMAC in solution, but it is related to charge density on poly-DADMAC.

4.3.3 Direct plot of the volume of PVSK against poly-DADMAC concentration

In this work, we have evaluated two data processing approaches based on the direct plot of PVSK volume versus concentration of poly-DADMAC and the charge density approach. In

addition we have compared two techniques, visual titration and spectrophotometric titration with a fibre optic probe. Results for the direct plot of PVSK volume versus concentration of poly-DADMAC are shown in Figure 4.2. The linear regression equation was $y = 0.1495 + 0.0248x$ with a correlation coefficient of 0.999.

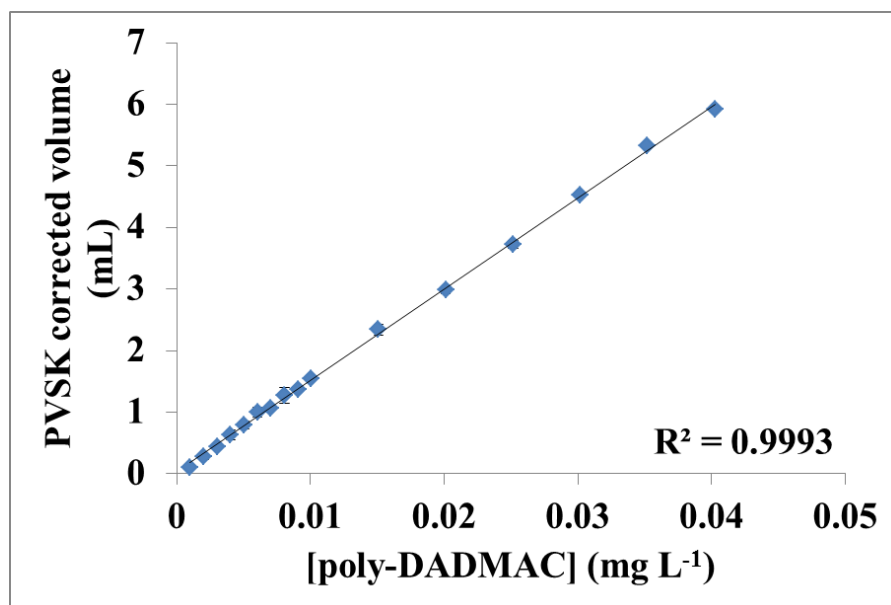


Figure 4.2: Volume of the titrant (PVSK) corrected for blank versus known concentrations of poly-DADMAC (for data see Appendix A4.1)

To determine the accuracy of this data processing technique recovery studies were performed. Poly-DADMAC solutions with known concentration (6, 17 and 23 mg L⁻¹) were prepared in triplicate from independent stock solutions. Recovery results for solutions tested were always close or equal to 100% and the percent error for all the solutions was below 1% which is in line with published method development guidelines [17]. These results suggest that the method used is accurate and precise (endpoint within 0.2 mL). However, we found the repeatability of the method was poor when using PVSK that was from a container that had been opened 6 months before. With the old PVSK, there was a constant reversal of the endpoint which led to continual addition of PVSK and thus inconsistent results. Replacement of the PVSK with a new commercial sample improved repeatability.

4.3.4 Charge density approach

In the charge density approach the normality of PVSK is required and this was determined by standardising the PVSK with CPC. The normality of the poly-DADMAC solution is then determined using equation (1).

$$N_{poly-DADMAC} = \frac{(V(PVSK)-Vblank) \times N(PVSK)}{V_{poly-DADMAC}} \quad [1]$$

In this expression, $N_{poly-DADMAC}$ is the normality of the poly-DADMAC solution (eq L^{-1}), $V_{poly-DADMAC}$ is the volume of the poly-DADMAC solution in mL, N_{PVSK} is the normality of PVSK solution (eq L^{-1}) and V_{PVSK} is the volume of the PVSK solution added in mL. The normality of poly-DADMAC in eq L^{-1} was plotted against the concentration of poly-DADMAC in g L^{-1} and the results are shown in Figure 4.3. The slope of the line is the equivalent weight of poly-DADMAC, and was determined to be 0.0048 eq g^{-1} , which compares well with the literature [4].

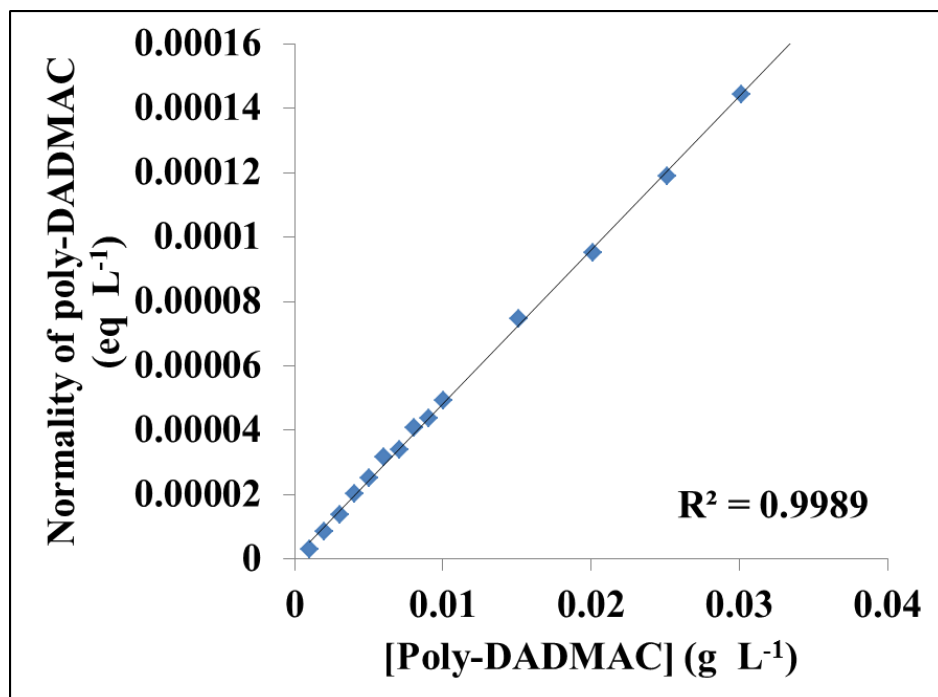


Figure 4.3: Plot used to determine the charge density of poly-DADMAC (0.0048 eq g⁻¹).

Although it was not necessary, care was taken to make sure the volume of poly-DADMAC used was constant so as to allow for a meaningful comparison between the direct plot of PVSK volume versus concentration of poly-DADMAC and the charge density technique. Both methods have a similar and high R² value (0.999), indicating either processing method is suitable.

The concentration of an unknown poly-DADMAC solution can be determined from the charge density (equivalent weight) of poly-DADMAC by using equation 2.

$$C_{polyDADMAC} = N \times EqW \quad [2]$$

$C_{poly-DADMAC}$ is the concentration of the unknown solution of poly-DADMAC, N is the normality of the solution in eq L⁻¹ and EqW is the equivalent weight of the poly-DADMAC in g eq⁻¹.

To test the accuracy of this approach recovery studies were performed. Three solutions of poly-DADMAC (0.006, 0.017 and 0.023 g L⁻¹) were prepared from an independent stock solution of poly-DADMAC and their normality was determined by using PVSK as described above. Using equation 2, the normality of the tested solutions were 2.888×10^{-5} , 8406×10^{-5} and 1.151×10^{-4}

eq L⁻¹. The corresponding concentrations were 0.006, 0.017 and 0.024 g L⁻¹ respectively. For all solutions tested, the recovery studies were close to 100% and the percent error less than 1%. These results show that the charge density approach can be an accurate method for the analysis of poly-DADMAC.

The normality of poly-DADMAC calculated by using equation 1 (0.0019 eq L⁻¹) was close to the theoretical value estimated from the molar mass of the monomer unit of poly-DADMAC and the mass weighed (0.0024 eq L⁻¹) [13].

4.3.5 Spectrophotometric endpoint determination

Fresh working stock solutions were prepared, as described above, and used for the spectrophotometric method. Standardisation of PVSK and titration of poly-DADMAC were done as described previously. The normality of PVSK was calculated from the equivalence point of the titration with cetylpyridinium chloride and found to be 9.33×10^{-4} eq L⁻¹. Normality of poly-DADMAC corresponding to the concentration in g L⁻¹ was calculated from the normality of PVSK as described in equation 1. The normality of poly-DADMAC was plotted against the corresponding concentration of poly-DADMAC and the relation was found to be linear with a straight line equation ($y = 0.00700x - 3 \times 10^{-9}$) and coefficient of correlation (0.996).

With this technique, the endpoint was determined by measuring the absorbance at 634 nm (blue colour of the free TBO indicator) or at 515 nm (purple-pink colour of bound TBO indicator). Figure 5.4 shows the absorbance spectra obtained during the titration of a 50 mL solution containing 5 mg L⁻¹ of poly-DADMAC and 0.2 mL TBO indicator. The titration was done by slowly adding 0.2 mL of PVSK (10^{-4} eq L⁻¹). It should be noted in this case that initial absorbance of solution with poly-DADMAC was similar to that of the indicator without poly-DADMAC. Figure 4.4 also shows that at the start of the titration the maximum absorbance was at a wavelength of 634 nm.

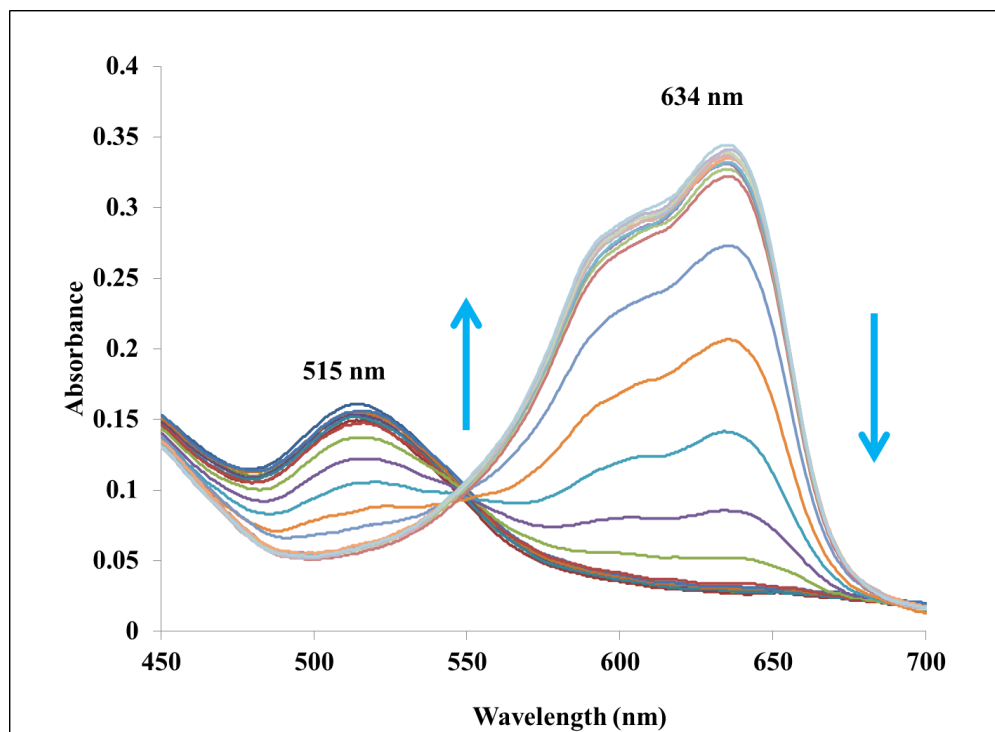


Figure 4.4: Absorbance spectra of a 50 mL sample containing 5 mg L⁻¹ poly-DADMAC and 0.2 mL TBO indicator, after addition of increasing quantities of 0.2 mL of PVSK. The arrows show a decrease at 634 nm and an increase at 515 nm.

The spectra shows that once all poly-DADMAC has been neutralized by PVSK reacts with the indicator and the bound indicator changes colour and gives rise to the growth of the peak observed at 515 nm and consequently the loss of absorbance of the peak at 634 nm due to the free from the indicator. The decrease in absorbance at 634 nm was greater than the increase of the absorbance at 515 nm. For this reason, the wavelength value of 634 nm is often used to follow the titration [5, 6, 9]. In this work both wavelengths were compared for endpoint determination.

Figure 4.5 shows the absorbance at 634 nm and 515 nm versus the volume of PVSK added to the solution of poly-DADMAC. During the initial stage of the titration, when PVSK binds onto poly-DADMAC, there is little change in the absorbance of the solution and hence there is a plateau region from 0 – 1.8 mL. At the break point there is a rapid change with the absorbance values, where excess PVSK starts to react with the TBO indicator. Once the indicator has completely reacted with the PVSK, a second plateau region is observed. The increment points

observed by researchers before were not absent here [4], indicating some improvement in the sharpness of this endpoint when the probe is used.

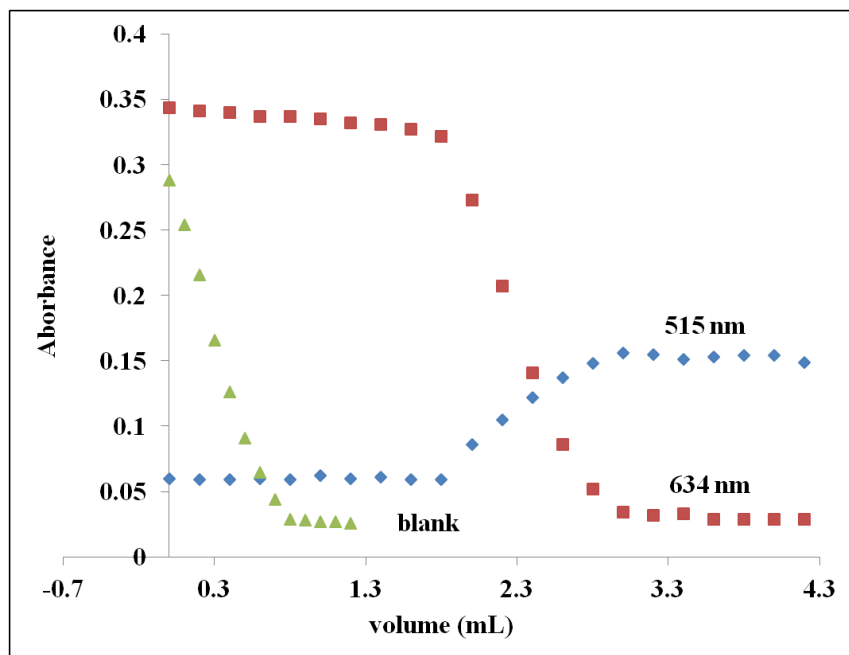


Figure 4.5: Graph spectrophotometric titration of a solution containing 5 mg L^{-1} poly-DADMAC concentration at 634 (recording disappearance of blue) and 515 nm (recording the emergence of the purple-pink colour).

With respect to the curve at 634 nm, there are three potential endpoints on these titration curves, the upper and lower break point and the inflection point. The upper break corresponds to the value obtained after deducting the relevant values for the blank, and the lower break corresponds to the colour change observed when doing a visual titration. Similar description applies to the curve at 515 nm. In this proposed method both wavelengths gave a sharp endpoint unlike before where even double beams spectrophotometer did not give a distinct endpoint for the emerging colour curve [4, 6, 9]. The endpoint obtained from upper break point of the 634 nm curve agrees with lower break point of the 515 nm curve. This indicates that our proposed method is sensitive when compared to other methods in the literature [4, 8, 11, 18].

The data from the titrations of selected poly-DADMAC standards ($0.5 - 5 \text{ mg L}^{-1}$) are presented in Figure 4.6. The upper break point at 634 nm was used as the endpoint for the titration. In the current method, this point was clear and showed excellent repeatability when compared to other methods in the literature, where the actual endpoint was calculated by taking the average value between the last point on the plateau and first point on the sigmoidal transitional curve, with the assumption being that the endpoint lies somewhere between the two points [4, 5, 9, 19].

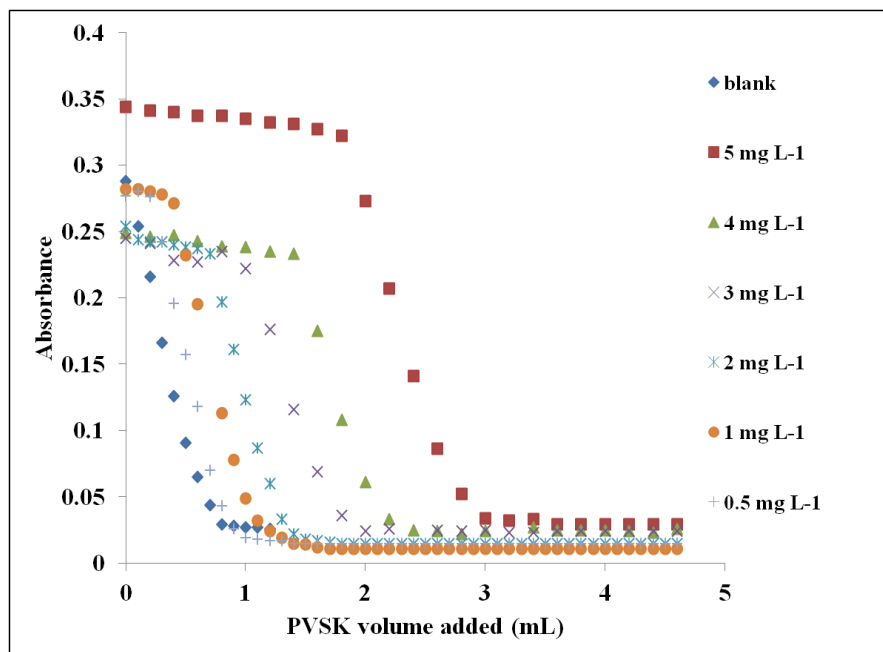


Figure 4.6: Titration curves of poly-DADMAC standards obtained by recording the absorbance at 634 nm versus PVSX volume added.

Using this method simplifies the mathematical modelling needed to establish the end point if the titration curve is not clearly defined. For example, Cumming et al. [4] used a comparatively sophisticated Matlab program to establish the endpoints. Whereas the practise of taking the tangents along the sigmoidal part of the curve, are widely used method in literature [5, 6, 9]. Subtraction of the blank and timing these titration reactions were unnecessary, when the average of the last two break points of the upper plateau were used in spectrophotometric titration, a method that has been shown to be useful in the literature [20].

4.4 Method development

Approximately 0.5000 g of dry PVSK was weighed into a 500 mL plastic beaker; 200 mL of mill-Q water was added into the beaker. After stirring overnight, some small amount of gelatinous material remained in the beaker; however, after transferring to a 1.0 L volumetric flask (silanized), and upon diluting to the mark with Mill-Q water the gelatinous materials disappeared. After thoroughly mixing, the solution was immediately transferred into plastic container, and stored until used for further experiments. PVSK solutions used were stored for up to one week. The solution of PVSK was standardized as described previously. Poly-DADMAC stock solution was prepared in 1.0 L volumetric flask (Salinized) by dissolving 1.1400 g of poly-DADMAC (35% w/w) with mill-Q water. After stirring, the solution was diluted up to the mark with Mill-Q water, and the solution was transferred into the plastic container. Solutions of poly-DADMAC were stored in cool place and were kept for no more than 1 month. Poly-DADMAC standards were titrated the same way as in the standardisation with a freshly prepared standardised PVSK.

The spectrophotometric determination of the endpoint was carried out using arrangement shown in Figure 4.7. The absorbance was measured *in-situ* with a dip probe.

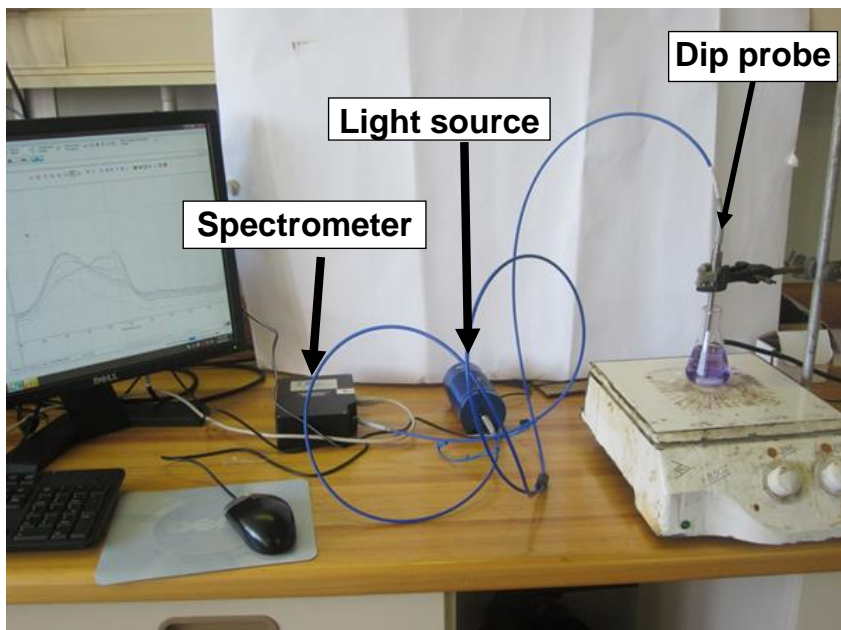


Figure 4.7: Photograph of the arrangement used for poly-DADMAC titration using a dip probe and spectrometer.

The method validation was carried out by using the same set-up.

4.4.1 Precision, accuracy and robustness statics studies

To determine the precision of the proposed method, a blank and 5 mg L^{-1} (7 replicate for each prepared at different times of the day) solution of poly-DADMAC from independent stock solutions were prepared using Mill-Q water. Both the solution blank and the 5 mg L^{-1} poly-DADMAC solutions were titrated against the standardised PVSK solution and the results obtained shown in Table 4.2. The volume of the endpoint was found to range from 0.3 to 0.5 mL and from 1.8 to 2 mL respectively. For all titrations, the deviation in the volume was 0.2 mL. This result reveals a good repeatability for the proposed methods.

Table 4.2: Evaluation of three calibration curves for precision used for poly-DADMAC analysis

Parameters	Calibration curve 1	Calibration curve 2	Calibration curve 3
Slope (m)	0.1495	0.004800	0.00700
Intercept	0.00248	6×10^{-7}	3×10^{-9}
Precision (mL)	0.2	0.2	0.1
Repeatability (mL)	0.3 – 0.5	0.3 – 0.5	0.6 – 0.8

The accuracy of the calibration curves were determined by preparing a 2 mg L⁻¹ solution of poly-DADMAC from independent stock solutions. Samples were prepared in triplicate and analysed as described in Section 4.5. Recovery studies were performed as shown in Table 4.3. The normality of this solution was 0.000013995 eq L⁻¹ and corresponding concentration was 1.989 mg L⁻¹ for calibration curve 3. Recovery studies results reveal that the proposed method was accurate with a recovery close to 100% (99.45%) and the percentage error was below 1%.

Table 4.3: Evaluation of accuracy of the calibration curves used for poly-DADMAC

Parameters	Calibration curve 1	Calibration curve 2	Calibration curve 3
Slope (m)	0.1495	0.004800	0.007000
Intercept	0.00248	6×10^{-7}	3×10^{-9}
Accuracy model water	95 – 105%	95 – 105%	98 – 100%
Accuracy in tap water	Not possible	Not possible	80 – 100%

Robustness was investigated by varying the volume of the TBO indicator added during titration from 0.1 to 0.3 mL and changing the pH of the solution by 1 pH unit (by adding acetic acid or sodium hydroxide (NaOH) dropwise), results shown in Table 4.4. The initial absorbance in the titration curve as a result of varying the indicator volume did not affect the endpoint of the proposed method. This proposed method of determining endpoint was found to be independent of indicator volume added as opposed to visual titration and other methods in the literature [4-6].

To change the pH of the solution and ionic strength, acetate buffer, acetic acid or sodium chloride were used respectively. With the conditions used in this investigation, no significant effect was observed with both changes in pH and ionic strength, similar to what has been reported in the literature [6]. These observations are in line with current theories whereby quaternary compounds are not affected by changes in pH and ionic strength of the solution [6,

14]. However, at high pH precipitates were observed. Thus experiments were done at the natural pH of the poly-DADMAC solutions (pH 7). These results show that the method is suitable for routine monitoring.

Table 4.4: Evaluation of robustness of calibration curves used in the poly-DADMAC analysis.

Parameters	Calibration curve 1	Calibration curve 2	Calibration curve 3
Slope (m)	0.1495	0.004800	0.007000
Intercept	0.00248	6×10^{-7}	3×10^{-9}
Robustness (recovery)	70 – 130	70 – 130	80 – 100
Indicator	Affected	Affected	Not affected
pH	Not affected	Not affected	Not affected
ionic strength	Not affected	Not affected	Not affected

4.4.2 Matrix effect, limit of detection and application

The proposed method was investigated for the quantification of poly-DADMAC in tap water. Four solutions of poly-DADMAC (0.1, 0.5, 1 and 5 mg L⁻¹) were prepared from independent stock solutions, and were prepared in triplicate with tap water. The solutions were analysed as described in Section 4.5 and results are shown in Table 5.5. Titration curves for the blank tap water were similar to the Mill-Q water blanks with similar profiles at 515 and 634 nm; specifically no discernible plateau levels. Recoveries of the spiked tap water samples 0.5, 1.0 and 5.0 mg L⁻¹ of poly-DADMAC were found to be 80%, 100% and 100% respectively. The proposed method was able to quantify 0.5 mg L⁻¹ in tap water (80% recoveries) and was unable to either detect or quantify 0.1 mg L⁻¹ of the spiked poly-DADMAC in the tap water sample.

Table 4.5: Limit of detection and limit of quantification studies.

Parameters	Calibration curve 1	Calibration curve 2	Calibration curve 3
Slope (m)	0.1495	0.004800	0.007000
Intercept	0.00248	6×10^{-7}	3×10^{-9}
Linearity	(1 – 40) mg L ⁻¹	(1– 40) mg L ⁻¹	(0.5 – 5) mg L ⁻¹
Correlation coefficient	0.999	0.999	0.996
LOD in model water	1 mg L ⁻¹	1 mg L ⁻¹	0.1 mg L ⁻¹
LOD in tap water	No detection	No detection	0.5 mg L ⁻¹
LOQ in model water	1 mg L ⁻¹	2 mg L ⁻¹	0.5 mg L ⁻¹
LOQ in tap water	not possible	not possible	0.5 mg L ⁻¹

The upper plateau was used to determine the limit of detection of the proposed method. Limit of quantification and limit of detection were the same and found to be 0.1 mg L⁻¹ in model water and 0.5 mg L⁻¹ in tap water for the proposed method. It should be noted that, visually it was very difficult to observe the colour change with the TBO indicator and in some cases there was no visual detection of the end point.

The proposed method was successfully applied for the detection of poly-DADMAC in tap water. The significant drawbacks in colloidal titration are interferences from positive ions and the inability to distinguish between polyelectrolytes (selectivity) [4]. Poly-DADMAC has been detected in tap water, rivers and dams [1, 2]. A cheaper method that is capable of detecting poly-DADMAC in such samples is needed. The colloidal titration method is not capable of detecting poly-DADMAC in tap water because of the dissolved solids, ions and inability of the TBO indicator to change colour especially in the presence of such interferences [11, 20]. Results are summarized in Table 4.6. Recently Cumming et al. [4] improved on the colloidal titration method, but they failed to detect poly-DADMAC in tap water that was exposed to clay. John et al. [5] was able to detect poly-DADMAC in various aqueous matrices, and reported the lower limit of detection to be approximately 0.3 mg L⁻¹ [5].

Table 4.6: Summary of results of calibration curves for comparison with literature methods.

Parameters	Calibration curve 1	Calibration curve 2	Calibration curve 3
Axis	PVSK volume versus [poly-DADMAC] (visual)	Normality versus [poly-DADMAC] (visual)	Normality versus [poly-DADMAC] (dip probe)
Endpoint determination	Blue to purple-pink	Blue to purple-pink	Average of break point and last plateau point
Equation	$y = 0.1495x + 0.02$	$0.0048x + 6 \text{ E-}07$	$Y = 0.007x + 3 \text{ E-}09$
Slope (m)	0.1495	0.0048	0.007
Intercept	0.00248	6×10^{-7}	3×10^{-9}
Blank (mL)	0.4	0.4	0.8
Normality of PVSK eq L⁻¹	6×10^{-4}	6×10^{-4}	9×10^{-4}
Equivalence weight of poly-DADMAC eq g⁻¹	N/A	0.0048	0.007

4.5 Comparison of the proposed dip probe method of detecting the endpoint with methods in the literature

The proposed method displayed good repeatability and higher sensitivities than earlier published methods in the literature. These changes were attributed to the use of the spectrophotometer, dip probe, and the computer software. All chemicals used were new, and PVSK has been shown to have an unpredictable shelf-life and is regarded as a source of poor repeatability in colloidal titration [5]. Other factors that contributed to an improvement in the colloidal titration method included:

- Burette [4, 5] and titrant reservoir [9] were replaced by a micro pipette equipped with a plastic tip to ensure a constant addition of the titrant. This eliminated the adsorption of polyelectrolyte on the walls of the burette. The changes in flow rate due to height differences caused by the solution in the burette and the homogenous solution was always ensured compared to still solution in the reservoir and burette.
- Adjusting the pH with buffers [5, 9] was eliminated and a pH of 7 was established as an optimum pH, since at basic pH TBO indicator precipitates out of solution. This eliminated errors that might be introduced by buffers by affecting ionic strength.
- The amount of indicator used was 200 μL which is larger than the 40 μL used in literature [4]. The calibration curve was found to be independent of the amount of indicator added.
- Spectrasuite software was found to be user-friendly and allowed for the acquisition of a full spectrum in a relatively short period of time (1000 full scan spectrums per second); thus allowing for the measurement of both the absorbance at 634 nm and 515 nm at the same time and thus eliminating the use of a double beam spectrophotometer [6].
- The titration curves with all the standards had clear plateaus, and sigmoidal segments. No increment observed usually generated by adsorption of material in a quartz cell [6].
- The endpoint was taken as an average between the last point of plateau and first break point. This endpoint point was sharp, accurate, precise and independent of initial absorbance. This method eliminates the use of expensive and complicated software, and

simplifies the data processing thus making it easier to implement in day-day analysis in a technical laboratory.

- The use of the dip probe in colloidal titration completely eliminated the need for peristaltic pumps. This eliminated errors that might arise during transportation of the sample, and problems reported in the literature whereby precipitation of poly-DADMAC in peristaltic pump tubes was observed [6].
- The time taken to reach the detector was reduced to less than 1 second compared to 15 seconds with most peristaltic pump [5, 6, 9]. This ensured the consistency in the calibration curves.
- Elimination of UV-Vis cells [4, 5, 9] which limit the volume used in the titration to 4 mL or less, and since this method uses a dip probe constructed from stainless steel, it avoids the indicator staining the cell and leading to the wrong absorbance being recorded.
- There was good agreement between the endpoint obtained by following the disappearance of blue and emergence of purple-pink. The titration curve constructed from measuring the absorbance of the emerging purple-pink colour was sharp compared to the literature [6].
- The need to measure the flow rate of the solution was not necessary; absorbance was measured *in-situ* with a dip probe. Variation in flow rate might cause the absorbance to fluctuate and lead to a poor titration curve [9].

4.6 Conclusion

Modification of the colloidal titration was successful as was confirmed by the easy way of determining the endpoint compared to previous complicated computer programs used in endpoint calculation. Furthermore, a number of potential errors associated with the use of spectrophotometers in colloidal titration were eliminated by the use of the dip probe. Variations in pH might cause precipitation during titration, but the use of buffers was found unnecessary in this work. Titration curves constructed from both wavelengths (515 nm and 634 nm) were found to be useful, except in tap water where it was not possible to construct a reputable titration curve from 515 nm. The limit of detection was determined to 0.1 mg L⁻¹ in Mill-Q water and 0.5 mg L⁻¹

¹ in tap water (the presence of the plateau was use as a measure). While the limit of quantification was found to be 0.5 mg L⁻¹ both in tap and model water. The equivalent weight (charge density) of poly-DADMAC was determined to be 0.007000 eq g⁻¹ accord once with, the literature value [Cumming et al. [4]]. The proposed method was validated and found to be precise, repeatable and accurate. The proposed method can be use water treatment plant for the routine determination of water quality.

4.7

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Chapter 5: Synthesis of gold nanoparticles and application in environmental analysis for the quantification of polydiallyldimethylammonium chloride in water

Poly-DADMAC is UV-inactive and in low concentration may be detected by sophisticated detectors such as mass spectrometry. From an extensive literature review undertaken in this study, no information was found to be available on a colourimetric method based on gold nanoparticles aggregation for the detection of poly-DADMAC. This chapter describes a novel method for the detection and quantification of poly-DADMAC. This work commenced by synthesizing gold nanoparticles, and then fully developing and validating the method for the detection and quantification of poly-DADMAC.

5. Synopsis

Gold nanoparticles are increasingly being used as a colourimetric probe for the detection and quantification of several target species of interest. Often the methods are seen to be quick, cost effective, and highly sensitive. The polyelectrolyte, polydiallyldimethylammonium chloride (poly-DADMAC), is extensively used in the water treatment, cosmetics, and paper making industries. In recent years this polymer is slowly being recognized as a widespread environmental contaminant found in drinking and surface water and hence there is an increasing need for methods that can rapidly detect and quantify this contaminant. Gold nanoparticles were synthesized by using different methods (citrate reduction, seed growth and ascorbic acid reduction method), characterized with UV-Vis spectroscopy and TEM, and investigated for detection of poly-DADMAC in aqueous solution.

We present a colorimetric method based on gold nanoparticle aggregation. The protocol developed has linear ranges between $10 - 100 \mu\text{g L}^{-1}$ ($R = 0.99$) and $1 - 10 \text{mg L}^{-1}$ ($R = 0.93$) with a lower limit of detection of $0.54 \mu\text{g L}^{-1}$ and 1mg L^{-1} respectively. We show the citrate based method has excellent intermediate precision, relatively quick analysis times, requires no extraction or derivatization methods, and is robust and rugged. The simplicity and ease of this method makes it potentially suitable for rapid screening and routine analysis of poly-DADMAC in environmental samples and water treatment plants when compared to other methods in the literature [1].

5.1 Introduction

Polydiallyldimethylammonium chloride (poly-DADMAC) is one of the most commonly used polyelectrolytes in wastewater and potable water treatment plants both for coagulation and as coagulant [2] or flocculent aid to strengthen flocs and improve settle-ability [2-6]. In recent years, there has been a growing concern over the fate of poly-DADMAC within the water treatment process and its presence in the environment at large. Choi and Valentine [7] demonstrated that degradation of poly-DADMAC to N-nitrosodimethylamine (NDMA) might be

the fate of poly-DADMAC in water treatment plant. NDMA is a suspected carcinogen [3, 8-12] and classified as a B2 carcinogen [13]. Thus from an environmental and human health risk perspective there is a strong need to rapidly determine the amount of residual poly-DADMAC in a water treatment process before any type of chlorination or ozonation steps.

There is great interest in the exploration of metal or semiconductor nanoparticles for the detection of emerging contaminants in the environment. Au-NPs, with the size range between 1 – 100 nm have been a focus point of research because of their unique physical and optical properties. The colour change in Au-NP – based colourimetric analytical method is important because indicates the interaction, which can be followed by the naked eye alone and minimizes the use of sophisticated instruments. The colour change of Au-NPs is due to changes in size, shape, capping agents, of the medium refractive index, as well as their aggregation. Since the early work of Mirkin [14] and Storhoff [15], many analytical methods based on Au-NP probes have been developed for detection of different analytes in complex matrices.

The detection method of thiol-containing amino acids based on Au-NPs was reported by Zhang et al. [16]. Selevraj et al. [17] reported Au-NPs as probes for the detection of the anti-cancer drug, 5-fluorouracil. Also, Menon et al. [18] reported an analytical method for detection of anti-cancer drug gemcitabine based on citrate stabilized Au-NPs. Yang et al. [19] reported a fast method for the detection of metal ions using L-cysteine functionalized Au-NPs. Liang et al. [20] reported the detection of melamine in complex matrices based on cysteamine-modified Au-NPs.

In this chapter, we synthesise different citrate stabilized Au-NPs and explore them for the detection of poly-DADMAC, and also provide a fully validated and sensitive spectroscopic technique to detect and quantify poly-DADMAC at low concentrations. At the time of writing this dissertation, and to the best of our knowledge, it is the first time citrate capped gold nanoparticles (Au-NPs) are used in a UV-Vis based method for the detection and quantification of low levels of poly-DADMAC in water. With this method, we are able to detect poly-DADMAC at concentrations below $5 \mu\text{g L}^{-1}$, and selectively quantify it in the presence of its monomer DADMAC, and various cations.

5.2 Experimental

5.2.1 Reagents and chemicals

Gold(III) chloride trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$) 99.9% tri-sodium citrate, ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$) 99%, and polydiallyldimethylammonium chloride (poly-DADMAC) 35% wt. (average molecular weight 100,000) ($\text{C}_8\text{H}_{16}\text{ClN}$) were purchased from Sigma Aldrich and were of analytical grade. All chemicals were used without further purification. All glassware used were silanized (see section 3.1 for procedure) to prevent the adsorption of poly-DADMAC and other charged species. Plastic containers were used to store solutions.

5.2.2 Instrumentation

UV-Vis spectra were measured on an Ocean Optics spectrometer (model HR2000+ manufactured by Ocean Optics at EW Duiven in The Netherlands) and the data was captured and analyzed using the Spectrasuite software. Samples were transferred to a 1.0 mL quartz cuvette and placed in the cuvette holder (Ocean Optics CUV-UV with a 1.0 cm path-length bought from Ocean Optics at EW Duiven in The Netherlands). The light source used was a tungsten halogen (Ocean Optics) based module. The light was passed through a fiber optic cable, then the cuvette holder, and finally via a second fiber optic cable (both cables were from Ocean Optics - QP 600-2-vis-BX model 727-733-2447 suitable for 400 – 2100 nm) to the spectrometer. For transmission electron microscopy (TEM) analysis, samples were prepared by dipping a 200 mesh copper grid (Formvar support film) in the solution of interest, air drying on filter paper, and then analyzed with a JOEL 1010 transmission electron microscope (Jeol, Tokyo and Japan). FTIR spectra were obtained on a Perkin Elmer Spectrum 100 series spectrometer (bought from PerkinElmer at Waltham, United States of American) with a universal ATR sampling accessory.

5.2.3 Preparation of solutions of gold nanoparticles

The preparation of the Au-NP solution A1 was done by using well-known reduction methods [21]. A mass of 0.4768g of the gold salt was added to 400 mL of ultrapure water. The gold solution was then heated on a hotplate, and 10 mL of 0.2746 M tri-sodium citrate was added to the boiling gold solution. The solution was stirred and carefully observed. The solution colour changed from yellow to colourless and finally to deep red. After the appearance of the red colour, the solution was immediately taken off the hotplate and left to cool to room temperature. After cooling to room temperature the Au-NP solution was transferred to a 2000 mL volumetric flask, which was then filled to the mark with ultrapure water. The solution was mixed thoroughly and no precipitate was observed. Au-NPs were characterized with UV-Vis and TEM in solution. Other solutions of Au-NPs, namely A2 and A3 were prepared by the same procedure as shown in chapter 3, section 3.7.3 p. 49, but without heating (prepared in room temperature), using sodium borohydride (seed growth method) and ascorbic acid respectively. Synthesis of Au-NPs was summarized in Figure 5.1 and Table 5.1.

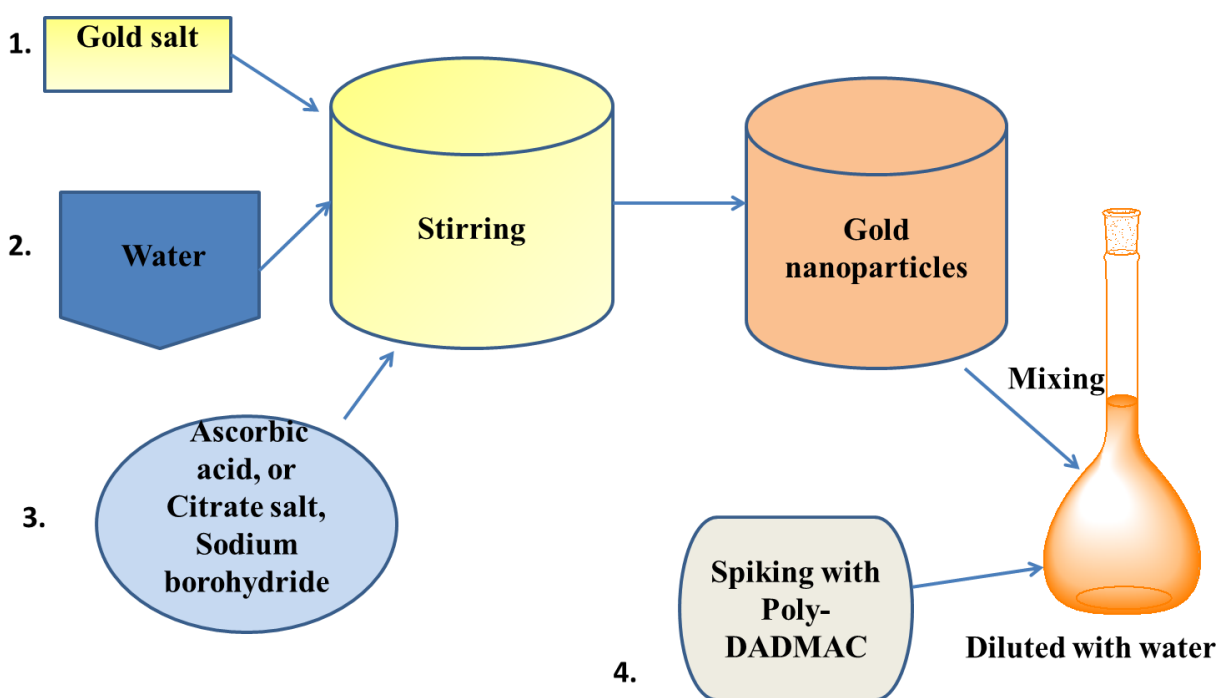


Figure 5.1: Schematic diagram for the reduction of a gold salt with reducing agents (ascorbic acid, sodium borohydride or citrate salt) in presence of the capping agent tri-sodium citrate into Au-NPs.

Table 5.1: Synthesis of gold nanoparticle stock solutions.

Au-NPs nanoparticles solution								
Stock solution	Method	Reducing Agent	Sodium Citrate Concentration (M)	Gold salt (M)	Capping agent (Na₃C₆H₅O₇·2H₂O)	Reducing Agent added (mL)	Volume of stock solution (mL)	T(°C)
A1	Stock solution	Na ₃ C ₆ H ₅ O ₇ ·2H ₂ O	0.2746	3.026 x 10 ⁻³	-	10	2000	100
A2	Seed	NaBH ₄	1.864	6.696 x 10 ⁻⁴	1.274 x 10 ⁻³	0.06	10	25
	growth	C ₆ H ₈ O ₆	0.05453	6.697 x 10 ⁻⁴	Poly-DADMAC (1-100) mg L ⁻¹	0.060	10	25
	Seed growth method	0.05 ml of seed solution was added into growth solution. Then solution was stirred for 15 minutes before analysis.						
A3	Stock solution	C ₆ H ₈ O ₆	0.05453	6.696 x 10 ⁻⁴	1.274 x 10 ⁻³	0.06	100	25

5.2.4 Preparation of stock solution of poly-DADMAC

A volume of 1.0 mL of the original poly-DADMAC solution (1.0889 g dissolved into 1 L volumetric flask) was transferred into a 1000 mL volumetric flask and ultrapure water was added up to the mark. After mixing thoroughly, the solution was transferred into a plastic container. This solution was then used to make a 50 mg L⁻¹ solution of poly-DADMAC.

5.2.5 Detection and quantification of poly-DADMAC

A volume of 25 mL of ultrapure water was added into a 50 mL volumetric flask, and then the required amount (e.g. 10 µL for a 50 µg L⁻¹ solution) of a 50 mg L⁻¹ poly-DADMAC solution was added to the contents within the flask. A volume of 20 mL of the Au-NP solution A1 was then added to the flask. It was noted during this addition step that the Au-NP solution changed colour from red to blue. The solution was made up to the mark, mixed and analyzed within 20 minutes. This was done to avoid the coagulant effect of poly-DADMAC. Several standards of poly-DADMAC (10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, and 130 µg L⁻¹) were prepared using this method. A2 and A3 gold nanoparticle solutions were used in a similar manner to prepare more calibration curves for analysis of poly-DADMAC. The preparation of standards is summarized in Table 5.2.

Table 5.2: Preparation of poly-DADMAC standards.

Au-NP solution	Volume of Au-NPs (mL)	Working stock solution of poly-DADMAC (mg L⁻¹)	[poly-DADMAC] volume from stock solution (µL)	Final volume of solution (mL)	Concentration of Poly-DADMAC standards (mg L⁻¹)	Calibration curve range (mg L⁻¹)
A1	20	50	10	50	0.01	(0.01 – 0.13)
A2	10	763	130	100	1	(1 – 100)
A3	10	763	13	5	2	(2 – 10)

5.3 Results and discussion

5.3.1 Characterization of gold salt in aqueous solution

The gold salt was dissolved in water and produced a characteristic yellow colour of Au^{3+} ions in aqueous solution shown in Figure 5.2 (B). The gold solution was further characterized with UV-Vis spectroscopy and the results are shown in Figure 5.2 (A). The UV-Vis spectrum in Figure 5.2 (A) shows the characteristic peak of Au^{3+} ions at around 290 nm. Since the gold salt exhibited only one peak, which was expected, it was assumed gold salt was pure and was used without further purification.

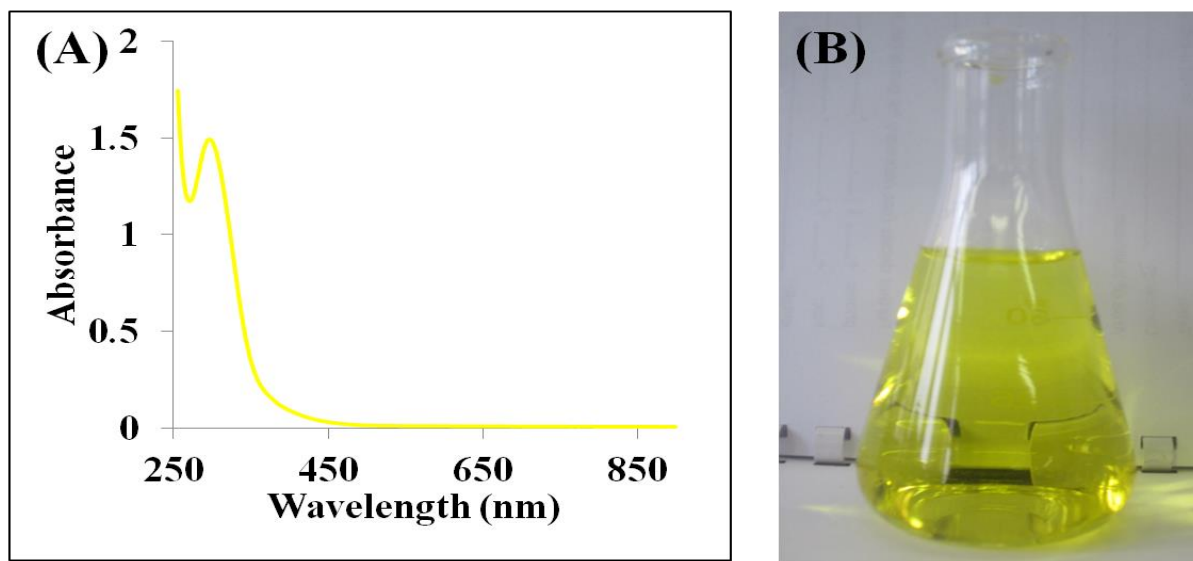


Figure 5.2: Panel (A) is a UV-Vis spectrum of the gold salt (Au^{3+} ions) in aqueous solution and Panel (B) is a photograph of the gold salt in aqueous solution.

5.3.2 Characterization of gold nanoparticles

5.3.2.1 Characterization of gold nanoparticles A1 solution

Visually, the change of the yellow coloured gold solution to a type of deep red is a confirmation of the reduction of Au^{3+} to Au^0 in the solution. This colourful and clear change is a quick and easy way to confirm the formation of Au-NPs. Further and more detailed characterization with UV-Vis spectroscopy is presented in Figure 5.3. The UV-Vis spectrum (Figure 5.3) of the Au-NPs shows a characteristic peak at 526 nm [18]. In ultrapure water, the absorbance measured at 526 nm retained 98% of its original value after 6 months. These results prove that the Au-NPs in the presence of tri-sodium citrate are stable for relatively long periods of time.

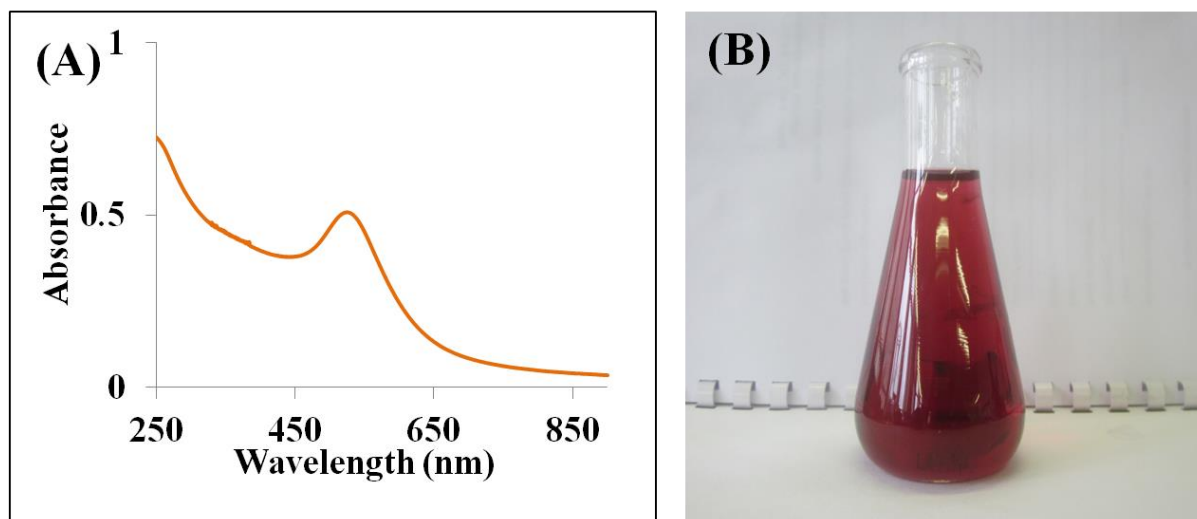


Figure 5.3: Panel (a) is a UV-Vis spectrum of the gold nanoparticles synthesized by the citrate salt method and panel (B) is a photograph of gold nanoparticles stabilized with citrate ions.

A typical TEM image of the Au-NPs with a size distribution histogram is shown in Figure 5.4. From the TEM analysis, it was observed that most of the Au-NPs were slightly ellipsoidal with an average diameter (long axis) of 27 ± 3 nm (analysis of several TEM images and 228 nanoparticles). The particle size distribution is a key factor for most applications that use Au-NPs and despite the wide range of sizes observed, 16 – 40 nm, the size distribution range of 24 – 30 nm was fairly narrow.

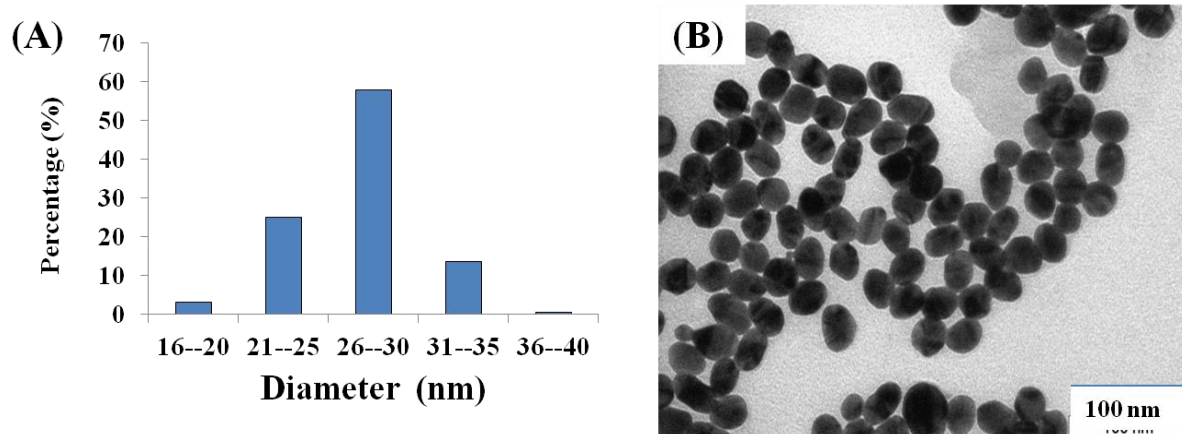


Figure 5.4: Panel (B) is a TEM image of the gold nanoparticles and panel (A) is the particle size distribution as measured from over 200 nanoparticles from several TEM images.

5.3.2.2 Characterization of gold nanoparticles A2 solution

The UV-Vis spectrum in Figure 5.5 (A) shows that the characteristic peak of Au-NPs at 526 nm was still in the same position for the A2 solution, when compared to the A1 solution. Also the colour of Au-NPs after synthesis was purple as shown in Figure 5.5 (B).

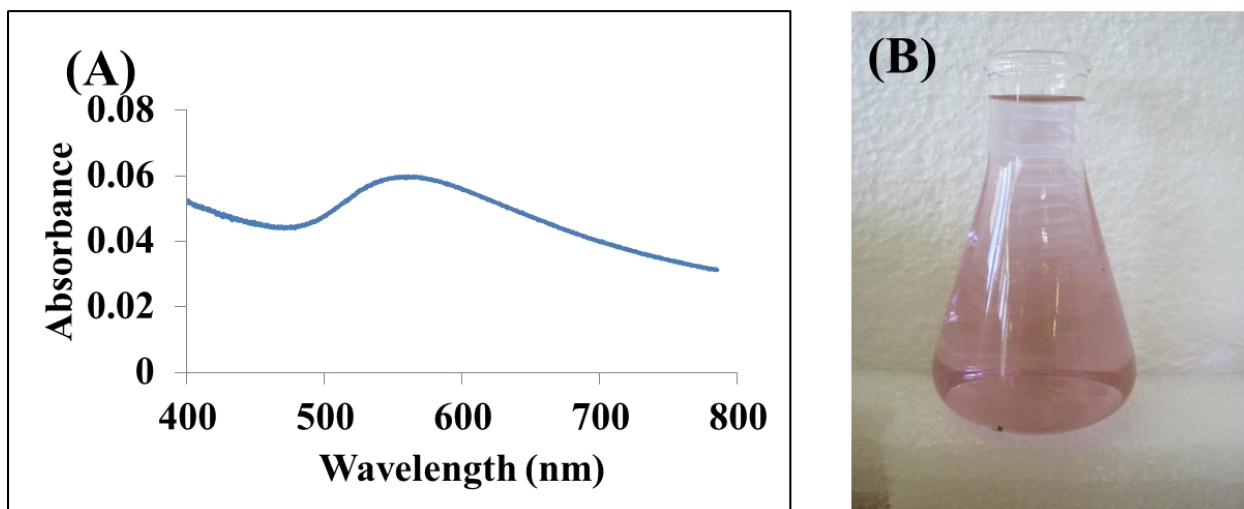


Figure 5.5: Panel (A) shows the UV-Vis spectrum of the gold nanoparticle seed solution (reduction with sodium borohydride) and panel (B) is a photograph of the gold nanoparticles synthesized by the seed growth method.

The A2 gold nanoparticle solution was further characterized with TEM, and the results are shown in Figure 5.6. The TEM image of the Au-NP A2 solution shows that the particles were spherical and dispersed in solution. The particle size distribution of Au-NPs obtained from reduction of the gold salt with sodium borohydride in the presence of citrate ions (called seed solution) was between 2 – 7 nm (70%) and the size distribution of Au-NPs obtained from reduction of the gold salt with ascorbic acid in the presence of poly-DADMAC called growth solution was between 5 – 10 nm (70%). Both seed and growth solution showed poor stability, and precipitated after 2 days.

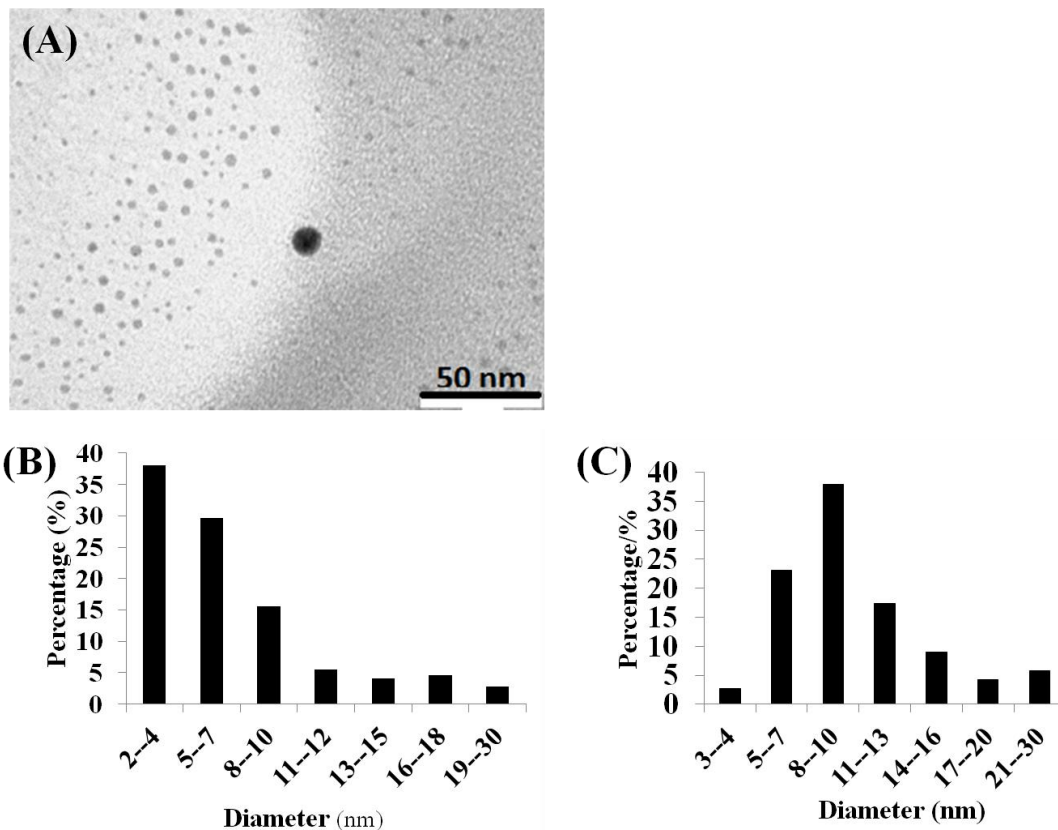


Figure 5.6: Panel (A) is a TEM image of the gold nanoparticle seed solution, panel (B) is the particle size distribution of the seed solution and panel (C) is the particle size distribution of the growth solution.

5.3.2.3 Characterization of gold nanoparticles A3 solution

Au-NP solution A3 was synthesised by reduction of the gold salt with ascorbic acid in the presence of citrate ions as capping agents. The A3 solution was characterized with both the naked eye and UV-Vis spectroscopy, and the results are shown in Figure 5.7. Addition of ascorbic acid into solution of gold salt caused the colour to change from yellow to reddish. The colour change is always attributed to the reduction of Au^{+3} to Au^0 which indicates the formation Au-NPs in solution.

The results observed were further confirmed with UV-Vis spectroscopy. Figure 5.7 (A) shows the UV-Vis spectrum of the Au-NP solution obtained from reduction of the gold salt with ascorbic acid, in the presence of citrate ions, with the characteristic peak of Au-NPs at 526 nm. Here citrate ions acting as capping agents to stabilize the Au-NPs in the solution.

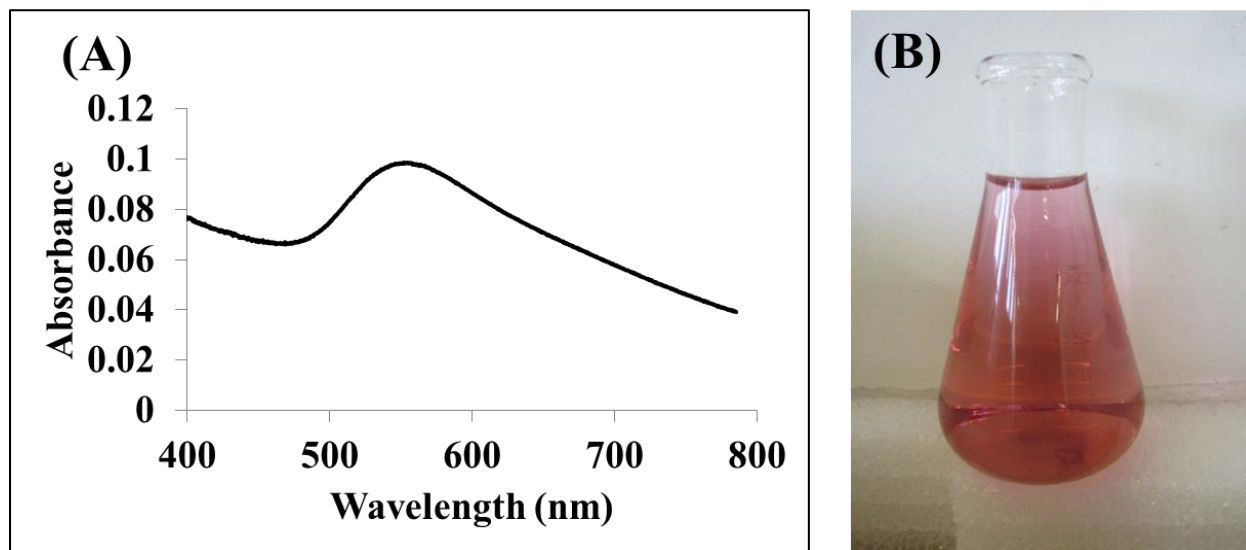


Figure 5.7: Panel (A) is UV-Vis spectrum of the gold nanoparticles prepared by stock solution A3 method (reduction of gold salt with ascorbic acid), (B) is photo image of the gold nanoparticle solution.

Figure 5.8 (A) shows that ascorbic acid made Au-NPs that were spherical and partially dispersed in the solution. The size distribution result shown in Figure 5.8 (B) shows that 75% of the Au-NPs have diameters between 8 – 13 nm. The shapes of these Au-NPs were not significantly different from the other Au-NP solutions A1 and A2, but the size distribution was different for each solution. The average diameter was 11 ± 3 nm based on spherical particles. Some elongated, and triangle shapes Au-NPs were also observed.

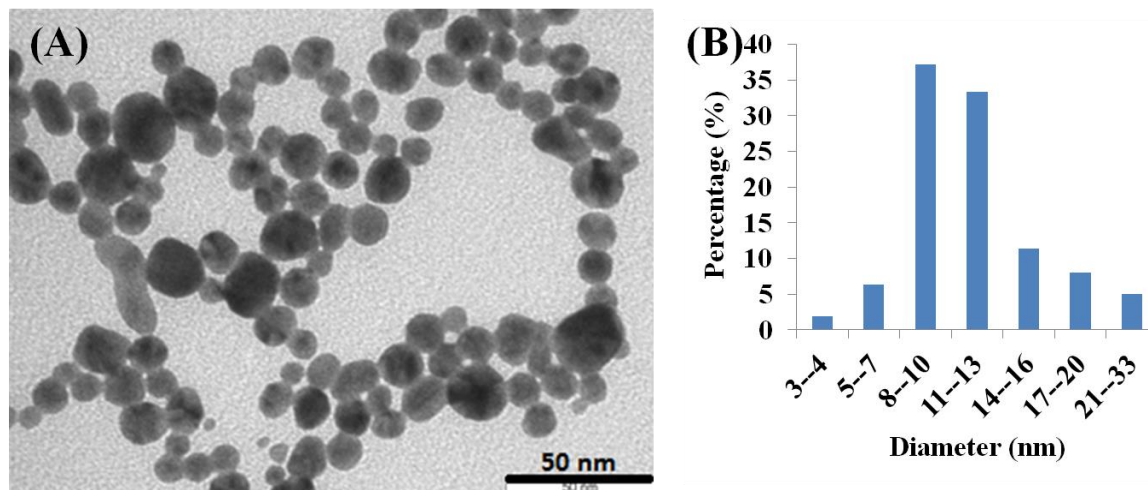
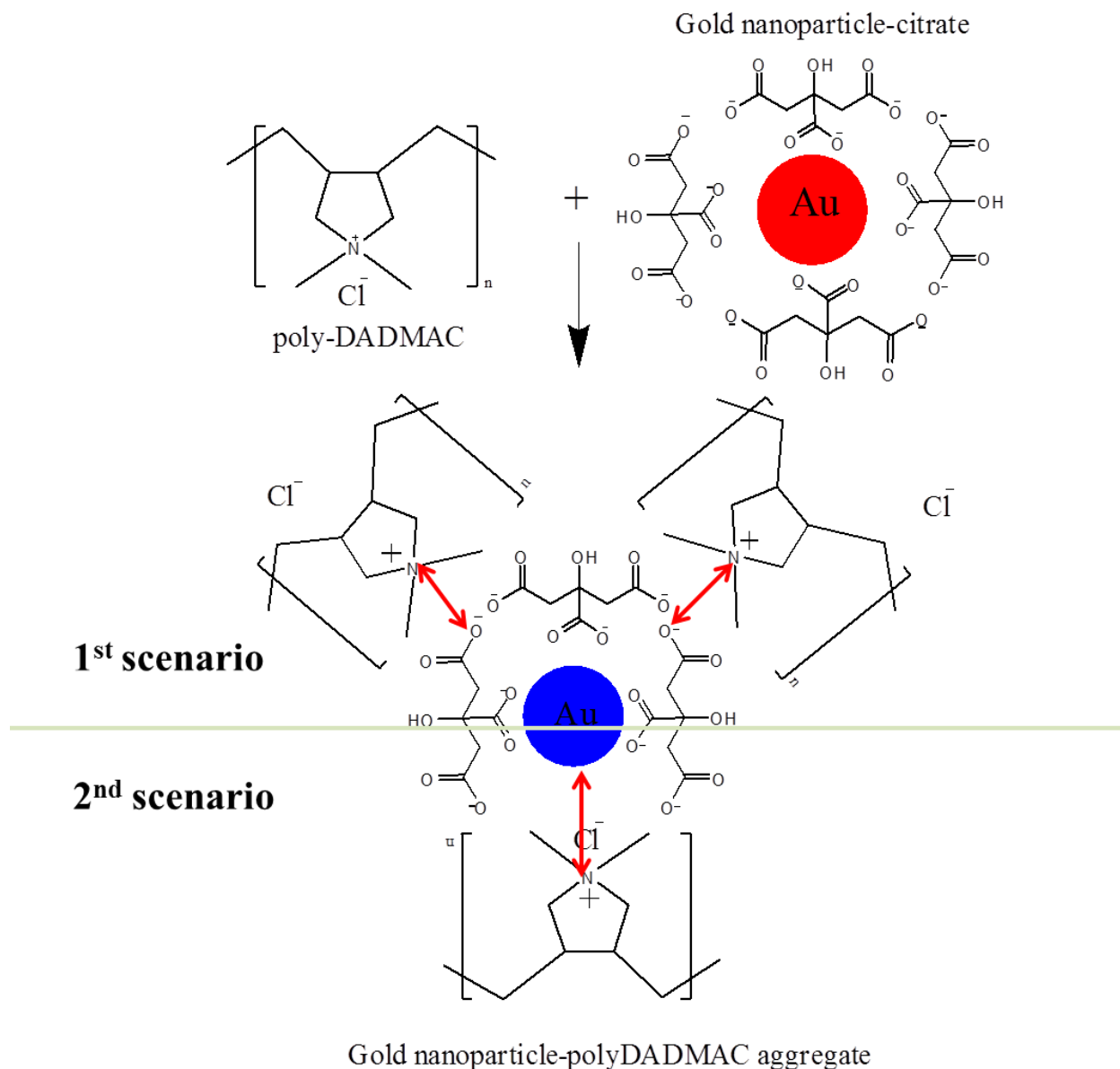


Figure 5.8: Panel (A) is TEM image of the gold nanoparticle solution A3 and panel (B) is the particle size distribution of stock solution A3.

5.3.3 Characterization of gold nanoparticle poly-DADMAC aggregates

5.3.3.1 Characterization of A1-poly-DADMAC aggregates

When gold nanoparticles were introduced into the poly-DADMAC solution, the intense red colour of the Au-NPs decreased and there was a slow appearance of a blue colour. The colour change is due to the shift of the plasmon band to longer wavelengths as the Au-NPs aggregate and this result is similar to what has been reported in the literature [18, 22-26]. The blue colour is attributed to the formation of aggregates between the Au-NPs and poly-DADMAC. In Scheme 5.1 we show two possible scenarios, whereby the poly-DADMAC has substituted the citrate ions on the surface of the Au-NPs, or the poly-DADMAC has simply adsorbed onto the citrate ions surrounding the Au-NPs. The first scenario is the most likely due to the high affinity of Au-NPs for nitrogen and cationic molecules versus citrate ions [18, 22, 24, 26].



Scheme 5.1: Schematic diagram showing aggregation of gold nanoparticles prepared from reduction of a gold salt with tri-sodium citrate upon addition of poly-DADM MAC to form gold nanoparticle-poly-DADM MAC aggregates (GNPs-PDC).

The aggregates of Au-NPs and poly-DADM MAC were characterized with UV-Vis spectroscopy and TEM, and typical results for the various samples are presented in Figure 5.9. The first panel, Figure 5.9 (A), shows two peaks, the original peak observed at 526 nm (due to the excess Au-NPs), and a second peak at 690 nm. The 164 nm shift to longer wavelengths is due to the formation of aggregates, and this was confirmed by TEM analysis.

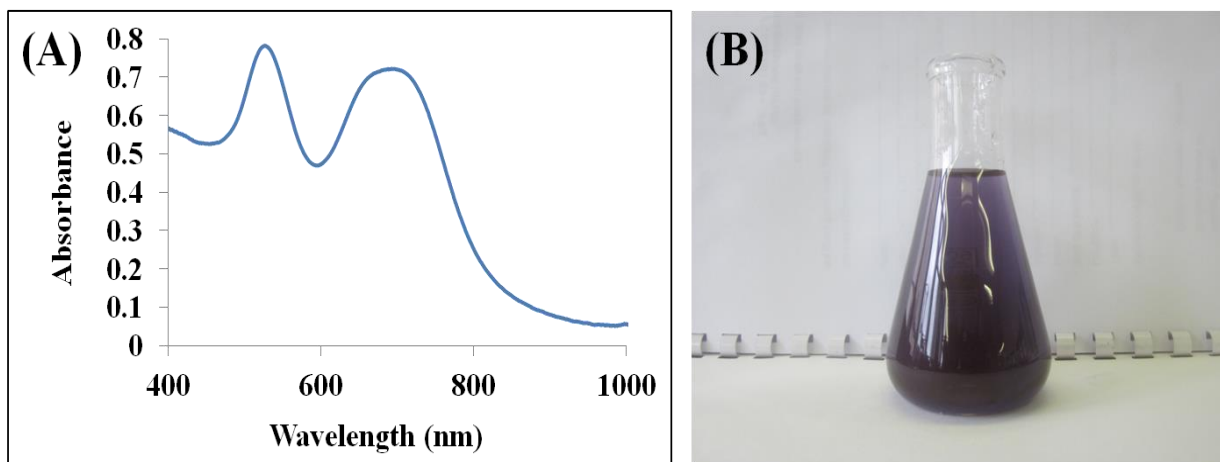


Figure 5.9: Panel (A) is a UV-Vis spectrum of the gold nanoparticle solution A1 aggregated by poly-DADMAC and panel (B) is a photograph of the poly-DADMAC-gold nanoparticle aggregate solution.

The before (Figure 5.4 (B)) and after (Figure 5.10 (A, B & C)) analysis with TEM did not clearly show a difference in terms of size and shape of the particles, as reported by some authors [18, 22, 26]. The factors that can cause the shift in the plasmon peak are the size or the shape of the Au-NPs [27]. The particle size analysis for the aggregates (Figures 5.10 (A), (B) & (C) and other images included in the Appendix A5.3), revealed that the Au-NPs have a similar morphology to the starting solution, and thus the shift in the plasmon resonance peak cannot be due to change in size (average was between 27 ± 3 nm before and 28 ± 3 nm after addition of poly-DADMAC) or shape (remains ellipsoidal before and after addition of poly-DADMAC) of the Au-NPs. This shift in the plasmon resonance peak was attributed to the aggregation of Au-NPs in the solution, as shown in Figure 5.10 (A) which was absent in Figure 5.4 (B) the starting solution of Au-NPs before addition of poly-DADMAC.

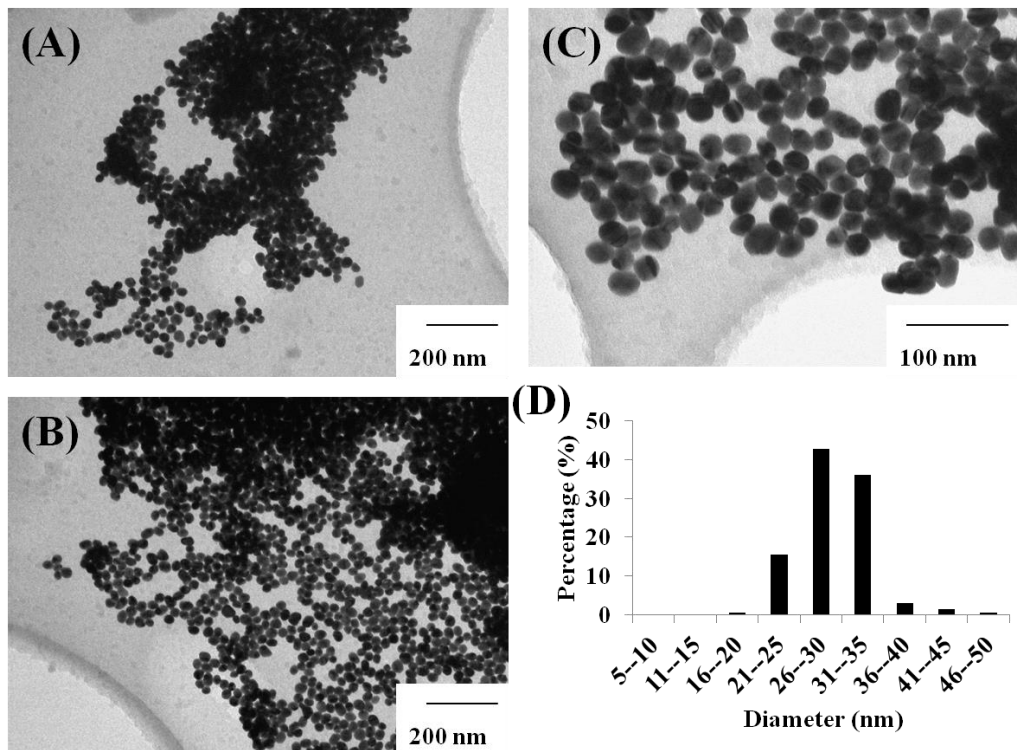


Figure 5.10: Panel (A), (B) and (C) are TEM images of the poly-DADMAC-gold nanoparticle aggregates prepared from solution A1, and panel (c) is the particle size distribution as measured from over 200 nanoparticles from several TEM images.

5.3.3.2 Characterization of A2-poly-DADMAC complex

The Au-NP A2 solution was prepared in the presence of poly-DADMAC to form Au-NP aggregates (A2-poly-DADMAC to distinguish between the different Au-NP solutions used). A2-poly-DADMAC aggregates were characterized with UV-Vis spectroscopy and TEM, and the results are shown in Figure 5.11. The UV-Vis spectrum shown in Figure 5.11 (A), shows that the characteristic peak of the gold nanoparticles changed from 526 nm to 540 nm, and is most likely due to a slight increase in the average diameter of these Au-NPs. There was no extended peak at longer wavelengths as was observed in Figure 5.9 (A) (A1-poly-DADMAC aggregates), and the absence of such a peak is a good indication that the Au-NPs grew into larger particles instead of forming aggregates. To was confirm this we did some TEM investigations.

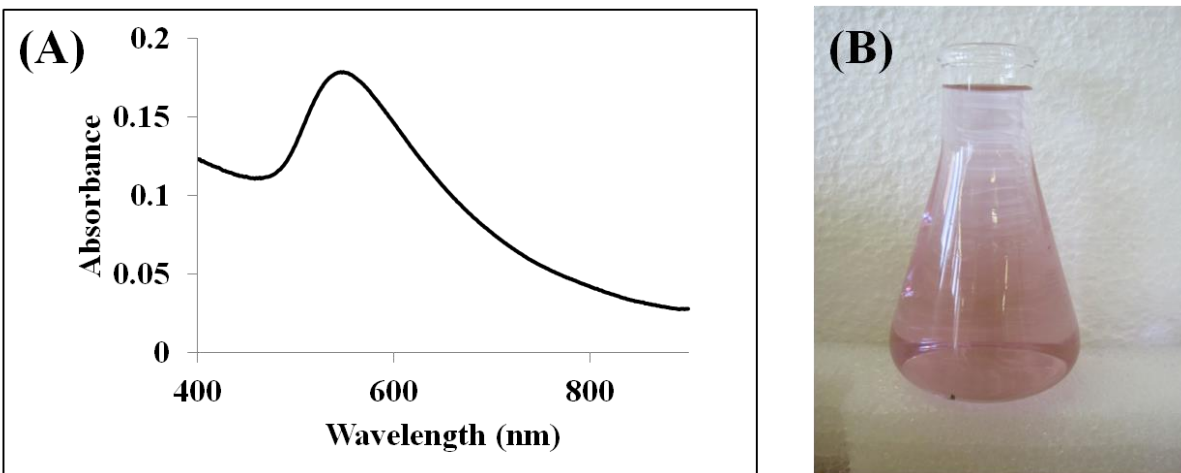


Figure 5.11: Panel (A) is a UV-Vis spectrum of the gold nanoparticle solution A2 aggregated by poly-DADMAC and panel (B) is a photograph of the poly-DADMAC-gold nanoparticle aggregates.

Figure 5.11 (B) is a photograph of the A2-poly-DADMAC aggregates which shows that the colour of the solution remains the same after the addition of poly-DADMAC.

Figure 5.12 (A, B, and C) shows the TEM image of the A2-poly-DADMAC complex with spherical Au-NPs as in the A2 solution and with particles attached to poly-DADMAC (grey material). The size distribution of the Au-NP complex was different from the A2 Au-NP solution. The A2 solution had particle diameters between 5 – 10 nm (13 ± 2 nm), whereas the A2-poly-DADMAC complex has particle diameters between 150 – 300 nm (300 ± 50 nm). The increase of the average size of gold nanoparticles is confirmation of interaction between poly-DADMAC and Au-NPs in the solution. This solution was not stable and precipitated after 3 hours of preparation.

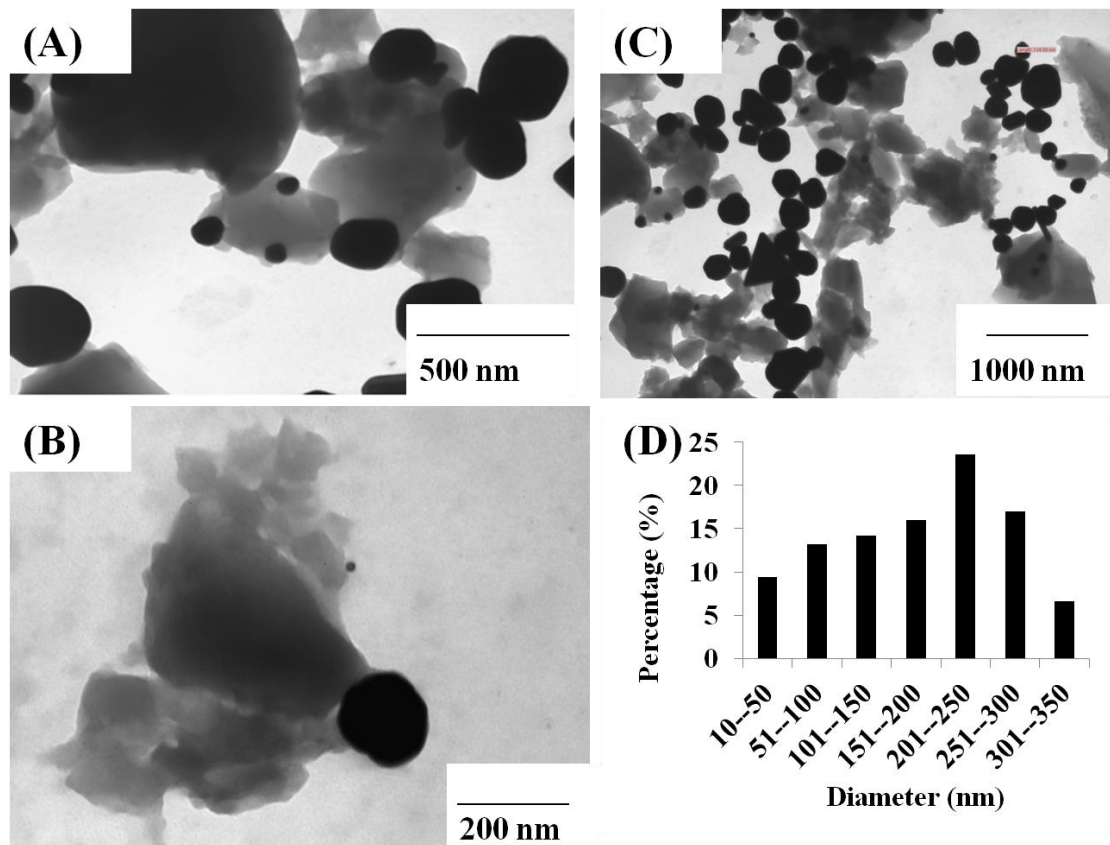


Figure 5.12: Panels (A), (B) and (C) are TEM images of the poly-DADMAC-gold nanoparticle aggregates formed from solution A2 and panel (D) is the particle size distribution as measured from over 100 nanoparticles from several TEM images.

5.3.3.3 Characterization of A3-poly-DADMAC aggregate

The Au-NP A3 solution was prepared by the reduction of the gold salt with ascorbic acid in the presence of citrate ions. Then the stock solution was made, and poly-DADMAC was added to separate aliquots of the stock solution of A3 to synthesise A3-poly-DADMAC aggregates. The A3-poly-DADMAC aggregates were analyzed both with UV-Vis spectroscopy and TEM; and the results are shown in Figures 5.13 and Figure 5.14 respectively. The UV-Vis spectrum in Figure 5.13 (A) shows change from the Au-NP peak at 526 nm to 538 nm and slight shift in the absorbance peak with addition of poly-DADMAC, for A3-poly-DADMAC complex. Also Figure 5.13 (B) shows the unchanged colour of A3 Au-NPs after addition of poly-DADMAC.

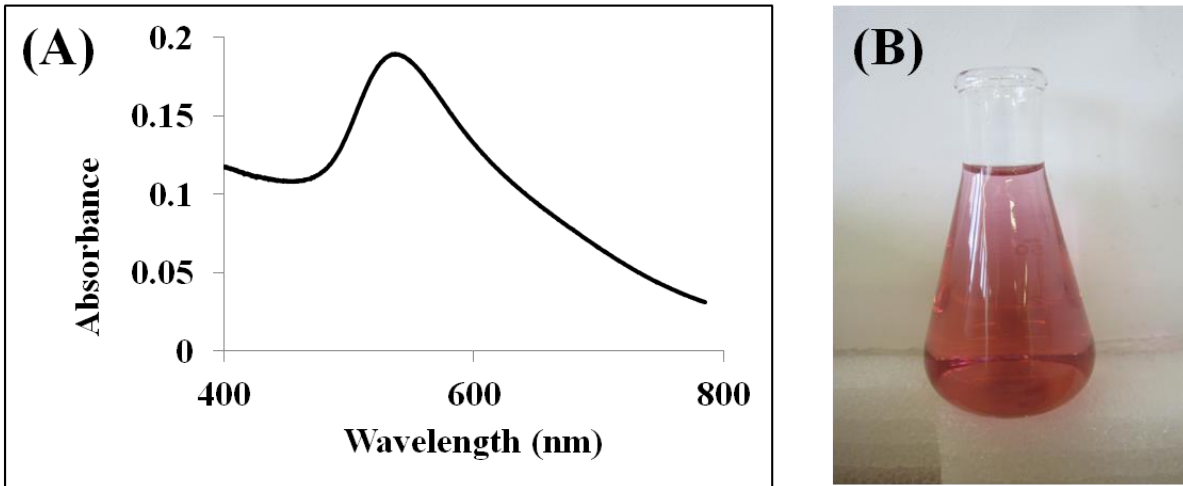


Figure 5.13: Panel (A) is a UV-Vis spectrum of the gold nanoparticle solution A3 aggregated by poly-DADMAC and panel (B) is a photograph of the poly-DADMAC-gold nanoparticle aggregates.

Figure 5.14 (A, B and C) reveals changes in A3 Au-NPs aggregated with poly-DADMAC with respect to shape (changes from uniform spherical shape to mixed spherical with polyhedral, triangular/prism and nucleated) compared with A3 Au-NPs. The histogram shown in Figure 5.14 (D) reveals that 80% (70 ± 10 nm) of A3 Au-NP diameters after the addition of poly-DADMAC are between 31 – 100 nm compared with the original A3 Au-NPs with diameters between (8 – 14) nm. These results (changes in size) show that poly-DADMAC interacted with the A3 Au-NPs. The size distribution revealed that the three synthesized Au-NPs (i.e. A1, A2, and A3) interacted differently with poly-DADMAC. Au-NP solutions, using A1 solutions, with varying concentrations of poly-DADMAC were subsequently prepared.

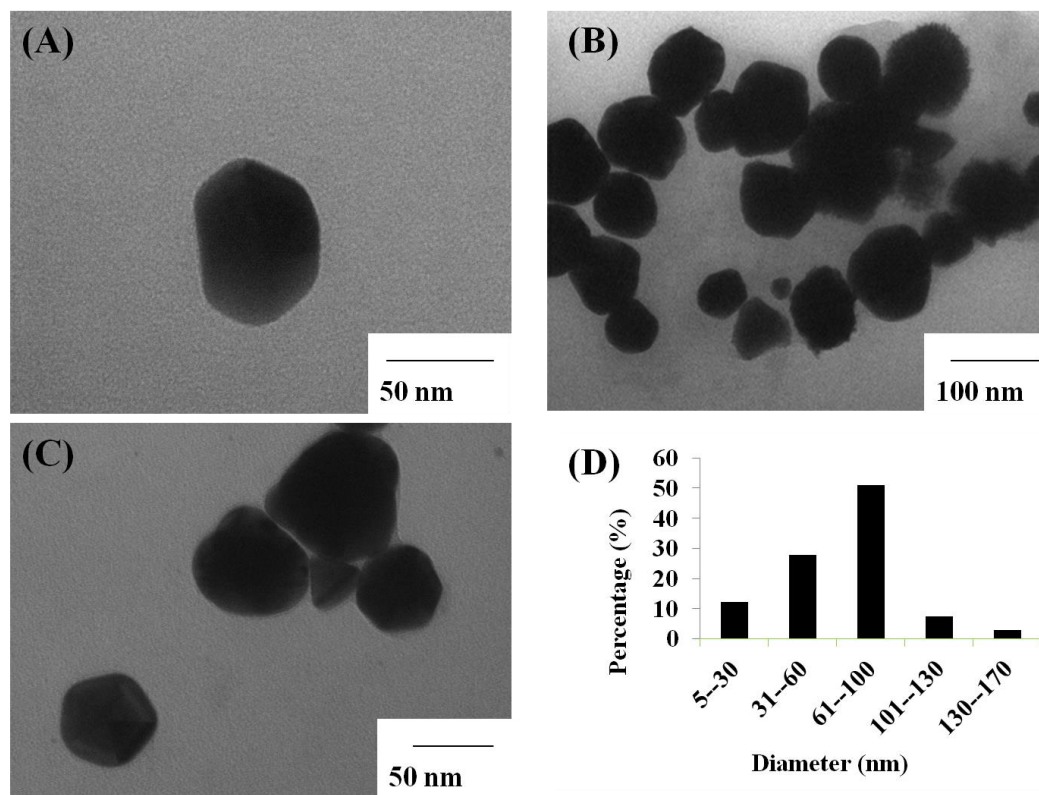


Figure 6.14: Panels A, B and C are TEM images of the gold nanoparticles that were in solution A3 after mixing with poly-DADMAC and panel (D) is a histogram of the gold nanoparticles.

The characterization of the Au-NP solutions A1, A2 and A3 is summarized in Table 5.3.

Table 5.3: Summary of the solutions of Au-NPs A1, A2, and A3; UV-Vis peak absorbance colour, size distribution and shape; before and after addition of poly-DADMAC.

	Before addition of poly-DADMAC			After addition of poly-DADMAC		
	A1	A2	A3	A1	A2	A3
Solutions	A1	A2	A3	A1	A2	A3
Colour	Deep red	Purple	Reddish	Blue	Purple	Reddish
UV-Vis peak (nm)	526	526	526	690	540	538
Shape	Slightly ellipsoid	Spherical	Spherical	Spherical	Spherical, triangle, hexagonal and rods	Spherical, triangle, prism and hexagonal
Size distribution (nm)	27 ± 3	11 ± 3	12 ± 5	28 ± 3	300 ± 50	70 ± 10

5.3.4 Detection of poly-DADMAC with the A1 gold nanoparticle solution

Au-NP solutions with different concentrations of poly-DADMAC were prepared from three Au-NP solutions A1, A2 and A3. These solutions were later analyzed with UV-Vis spectroscopy to determine whether a linear relationship between the concentration of poly-DADMAC in the solution and the absorbance of the Au-NPs existed.

For the A1 solution the absorbance of Au-NPs at 526 nm in Figure 5.9 (A) was found to be inversely proportional to the concentration of poly-DADMAC in the solution. The newly observed extended plasmon band in Figure 5.9 (A) was found to be directly proportional to the concentration of poly-DADMAC in the solution. Both absorbance peaks gave a linear relationship with a good correlation coefficient. Calibration curves from these relationships were developed and validated, and described in section 5.4.5.

5.3.5 Development and validation of analytical protocols using the A1 gold nanoparticle solution

For residual analysis of poly-DADMAC in water treatment processes, a value of $50 \mu\text{g L}^{-1}$, as the lower limit, has been recommended [5, 28, 29]. Thus, to ascertain whether Au-NPs could be used for poly-DADMAC analysis we prepared a series of standards from $10 - 130 \mu\text{g L}^{-1}$. The UV-Vis spectra of these solutions are presented in Figure 5.15.

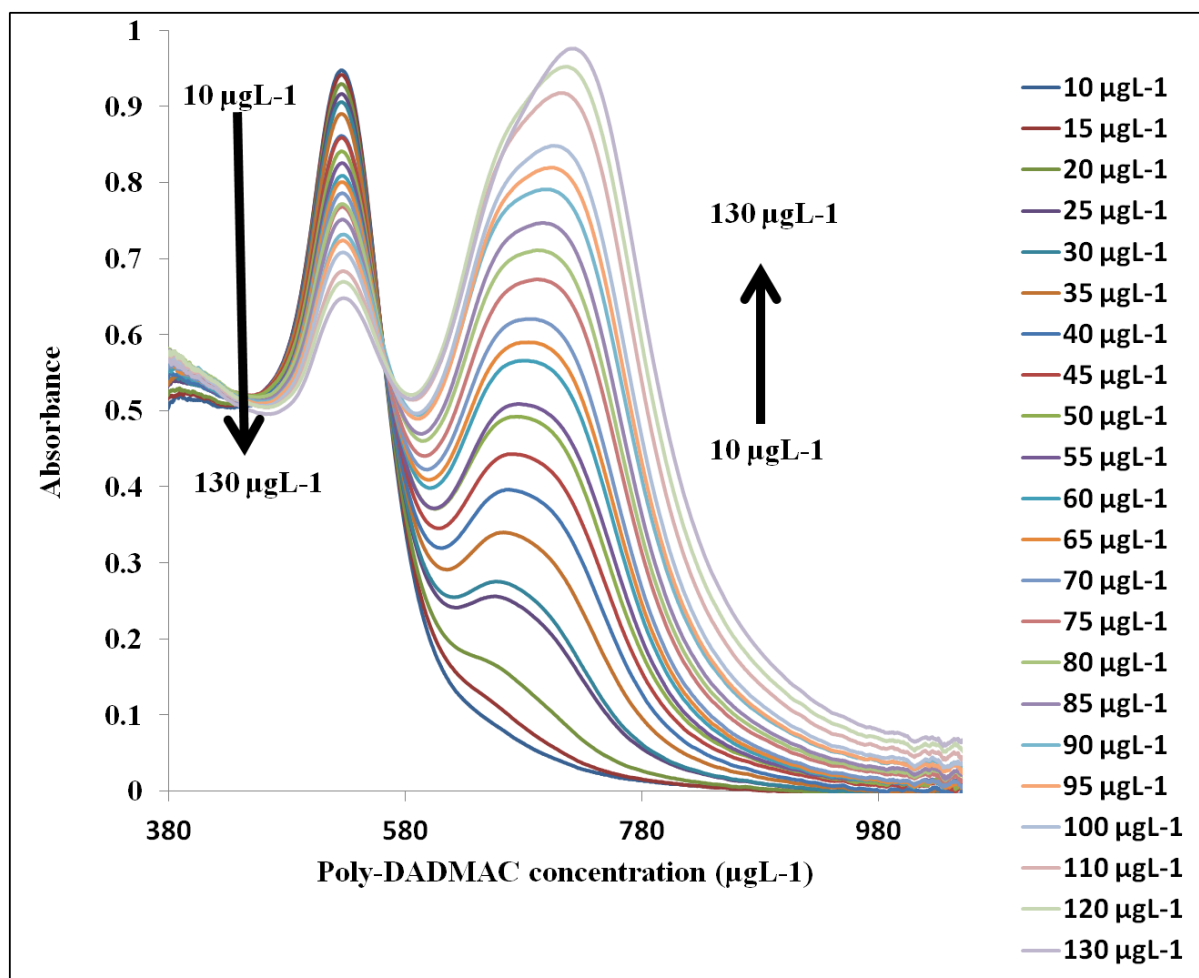


Figure 5.15: UV-Vis spectra of the A1 gold nanoparticle solutions mixed with various concentrations of poly-DADMAC.

From Figure 5.15, the peak at 690 nm (due to the aggregates) increased in intensity with an increase in the concentration of poly-DADMAC, and the peak at 526 nm, due to the free citrate stabilized Au-NPs, decreased in intensity with an increase in concentration of poly-DADMAC.

By means of least squares regression analysis, we then compared the linearity of the absorbance values derived from the two peaks, area of the peak at 690 nm, and the absorbance ratio $A(690)/A(526)$, and the results are presented in Figures 5.16 and 5.17.

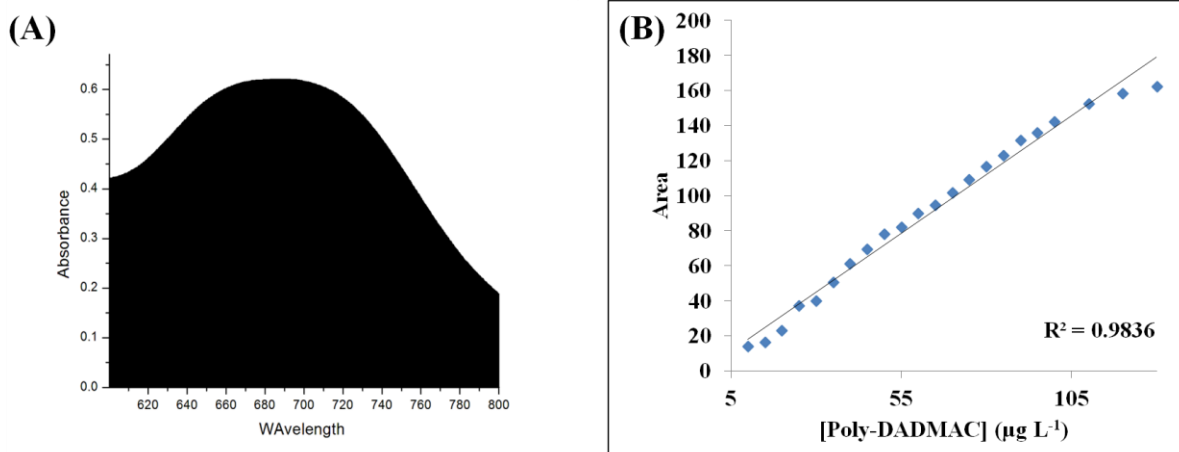


Figure 5. 16: Panel (A) is a integrated area under the curve between 600 – 800 nm calculated by using Origin software and panel (B) is a calibration curve plotted from the integrated area under the curve between 600 – 800 nm versus various concentration of poly-DADMAC.

The area of the extended plasmon band was determined by using Origin software. The area of the extended plasmon band between 600 and 800 nm of various poly-DADMAC standards as plotted against the corresponding concentration of poly-DADMAC and The result is shown in Figure 5.16 (B). The area of this region increased with an increase in the concentration of poly-DADMAC. Figure 5.16 (B) shows poly-DADMAC standards with concentration ranges from 10 to 130 $\mu\text{g L}^{-1}$, with a correlation coefficient of 0.9836 and a gradient of 1.3 ($y = 1.3436x + 4.4482$).

Irrespective of the response parameter used to construct the calibration curve, excellent R^2 values were obtained; however, the mixture of Au-NPs and poly-DADMAC consists of aggregates and nanoparticles with various sizes and shapes. In such cases the maximum wavelength shifts. We attempted to account for this problem by employing three different scenarios: scenario 1 is an ‘area under the curve’ method which was explained above; scenario 2 we plotted the calibration curve using two concentration ranges, as shown in Figure 5.17 (A and B), which improved the correlation coefficient from 0.94 (range 10 – 130 $\mu\text{g L}^{-1}$) to 0.99 (15 – 50 $\mu\text{g L}^{-1}$ or 55 – 100 $\mu\text{g L}^{-1}$). Scenario 3 was based on experimental and theoretical studies which have shown a more accurate determination of Au-NP aggregate concentration can be determined by using absorbance ratios (as in Figure 6.17 (D)) and the slight improvement in R^2 with our results supports these earlier results [30].

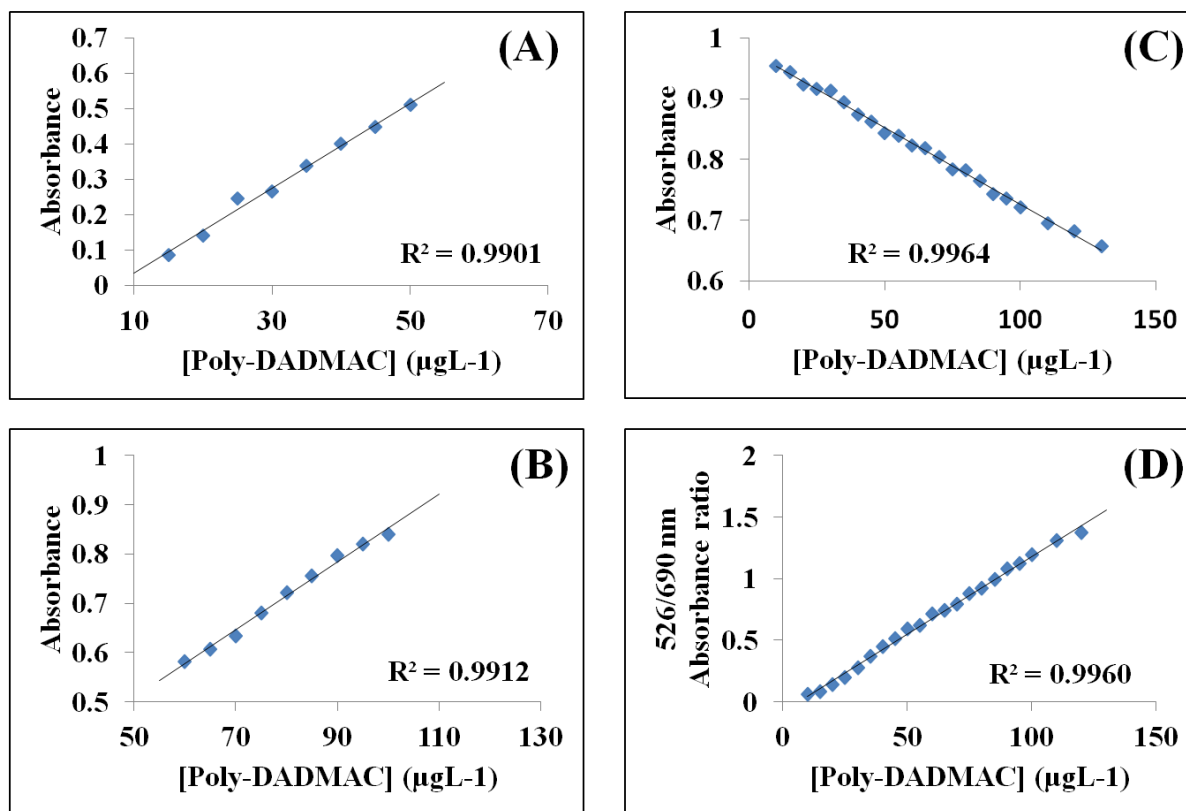


Figure 5.17: Calibration curves plotted from the peak absorbance at 690 nm (Panel (A) range 15 – 50 $\mu\text{g/L}$ -1 and panel (B) range 55 – 100 $\mu\text{g/L}$ -1), at 526 nm (panel (C)) and the ratio of the peak absorbance's at 690 and 526 nm (panel (D)).

5.3.5.1 Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were determined from the calibration curves and the results are presented in Table 5.4. The LOD and LOQ were calculated as $3.3\sigma/S$ and $10\sigma/S$ respectively, where S is the slope of the calibration curve and σ (calculation see Appendix A5) is the standard deviation of the response factor.

From Table 5.4, it is clear the use of a single peak results in a slightly decreased detection limit with the aggregate peak (690 nm), and a significantly poor detection limit with the Au-NPs peak (526 nm). It is interesting to note the use of the area of the peak curve gives a slightly better result than the ratio of the peak absorbance.

Table 5.4: Limit of detection and quantification for poly-DADMAC by forming aggregate with Au-NPs from solution A1.

Calibration curve	Linearity range ($\mu\text{g L}^{-1}$)	R²	STDEV R factor	LOD ($\mu\text{g L}^{-1}$)	LOQ ($\mu\text{g L}^{-1}$)
Area	25 – 100	0.9969	0.1359	0.32	0.944
A at 690 nm	10 – 50	0.9901	0.001367	0.49	1.47
A at 690 nm	55 - 100	0.9912	0.001377	0.80	1.52
A at 526 nm	10 – 130	0.9958	0.02270	29.1	88.1
A₍₆₉₀₎/A₍₅₂₆₎	10 – 130	0.9968	0.002127	0.54	1.64

5.3.5.2 Precision

Precision is the closeness of agreement (degree of scatter) between a series of measurements obtained by analysing solutions with a known concentration that covers the calibration curve range (lower, middle and high) under the prescribed conditions and is always associated with repeatability, and reproducibility [31]. Since this study was done in one laboratory, we cannot report on the repeatability of the procedure, but we can present result on the intermediate precision of the protocol. Intermediate precision is the total precision under varied conditions within-laboratory [31]. Repeatability was determined by preparing three sets of different standards (three runs each) of poly-DADMAC with concentrations of 30, 60 and 90 $\mu\text{g L}^{-1}$ from an independent stock solution, and samples were analysed in triplicate. Standards were chosen to cover the extremes and middle of the calibration curves. Inter-day and intra-day analyses were done to determine the intermediate precision of the proposed calibration method. These standards were prepared at different times and on different days, and the results obtained were used to study the intra-day variation. The same procedure was used to prepare and study inter-day variation. The relative standard deviation (%) of the results was used as measure of the precision [32]. Results of inter-day and intra-day precision are shown in Table 5.5. The relative standard deviation (RSD) for both inter-day and intra-day data, for the calibration curves was between 0.1 and 0.7% (Table 5.5). The calibration curves were constructed as shown in figure 5.17, and the means in Table 5.5 refer to the mean of the response parameter used to construct the calibration curve.

Table 5.5: Inter-day and intra-day results for poly-DADMAC analysis.

Condition	Calibration Curve	standard ($\mu\text{g L}^{-1}$)	Mean	STDEV	% RSD
Inter-day variation	Area	30	44.871	0.241	0.534
		60	88.636	0.110	0.124
		90	131.707	0.665	0.505
Intra-day variation		30	42.378	0.222	0.530
		60	90.533	0.492	0.543
		90	123.949	0.190	0.153
Different Analyst		30	12.331	6.858	55.618
		60	118.036	2.851	2.414
		90	167.588	0.124	0.074
Inter-day variation	Absorbance at 526 nm	30	0.917	0.002	0.189
		60	0.825	0.002	0.242
		90	0.726	0.008	1.076
Intra-day variation		30	0.860	0.006	0.747
		60	0.703	0.003	0.457
		90	0.721	0.003	0.367
Different analyst		60	0.721	0.022	3.073
		90	0.623	0.006	1.032
Inter-day variation		Absorbance at 690 nm	30	0.287	0.002
	60		0.558	0.001	0.104
	90		0.801	0.006	0.695
Intra-day variation	30		0.271	0.001	0.213
	60		0.569	0.004	0.703
	90		0.764	0.005	0.604
Different analyst	60		0.648	0.026	3.962
	90		0.820	0.003	0.352
Inter-day variation	$A_{(690)} / A_{(526)}$		30	0.313	0.001
		60	0.676	0.001	0.161
		90	1.10	0.018	1.65
Intra-day variation		30	0.315	0.003	0.962
		60	0.809	0.004	0.452
		90	1.06	0.015	1.37

In all cases RSD was found to be below 20% precision benchmark except for the 30 $\mu\text{g L}^{-1}$ standard used by the different analyst. The RSD range (0.1 – 0.7%) demonstrate excellent repeatability and good precision for the proposed method. This work was also performed by a different analyst (Ekemena Oseghe). The %RSD values shows excellent repeatability for developed method.

5.3.5.3 Recovery

For the validation of a method, acceptable criteria include LOQ, LOD, precision, and accuracy. Accuracy can only be determined by using acceptable reference materials, which are lacking for poly-DADMAC due to the nature of the polymer. Thus, instead of an adequate accuracy determination, we performed recovery experiments.

Solutions of poly-DADMAC with known concentrations were prepared from independent stock solutions. These solutions were then spiked with different concentrations of poly-DADMAC from different stock solutions. The final solutions were mixed gently and then analyzed. All samples were done in triplicate, and the percent recovery was calculated from the mean of the amount determined over the theoretical/expected amount. The % error was determined from mean compared with the added concentration. Results are presented in Table 5.6.

Table 5.6: Recovery results for 30 $\mu\text{g L}^{-1}$ poly-DADMAC solution with 10 and 20 $\mu\text{g L}^{-1}$ spikes.

Calibration Curve Used	Initial Concentration ($\mu\text{g L}^{-1}$)	Amount Added ($\mu\text{g L}^{-1}$)	Amount Recovered ($\mu\text{g L}^{-1}$)	Surrogate Recovery	% Recovery	% Error
Area	30	10	39.01 ± 0.57	9.01	97.5	-2.45
	30	20	46.02 ± 0.10	16.02	92.0	-7.96
Absorbance at 526 nm	30	10	44.40 ± 0.08	14.4	111	11.0
	30	20	53.60 ± 0.69	23.6	107	7.20
Absorbance at 690 nm	30	10	38.65 ± 0.68	8.65	96.6	-3.37
	30	20	44.28 ± 0.05	14.3	88.6	-11.5
$A_{(690)}/A_{(526)}$	30	10	36.95 ± 0.83	6.95	92.4	-7.61
	30	20	44.37 ± 0.13	-14.37	88.7	-11.3

In all cases the percent recovery as above 50% and the percentage was below 20% in line with validation protocols recommended by Peters et al. [31]. Based on recovery data, developed method was found to be accurate.

5.3.5.4 Ruggedness (robustness)

The ruggedness of an analytical method is a measure of how well the protocol can withstand small changes that might occur during routine analysis due to various factors that may deviate from the experimental conditions described in the original procedure [33]. The ruggedness of the proposed method was tested by changing the volume of the Au-NP solution used, adjusting the pH, changing the time of incubation/analysis, and the temperature. Here only one experimental parameter was changed at a time while others were kept constant.

5.3.5.5 Effect of pH

The pH of the solution was studied because the proposed method was assumed to be based on electrostatic interaction between the quaternary nitrogen and citrate ions. In acidic conditions, it is likely that everything in the solution will be protonated and this might affect the interactions between poly-DADMAC, citrate ions on the Au-NPs, and the Au-NPs. To test the effect of pH, two samples were prepared. In one solution the pH was adjusted by using NaOH to alter the pH from the natural pH 7.0 to 7.5 and the sample was successfully analyzed following the procedure explained above. A similar experiment was repeated at an acidic pH of 6.5 (adjusted by using hydrochloric acid) and it was impossible to quantify poly-DADMAC in solution. An acidic pH is well known to destabilize citrate capped gold nanoparticles, and as expected the UV-Vis spectra of such samples showed broad peaks with peaks at longer wavelengths and the Au-NP peaks were slightly shifted with relatively low intensities as shown in Figure 5.18.

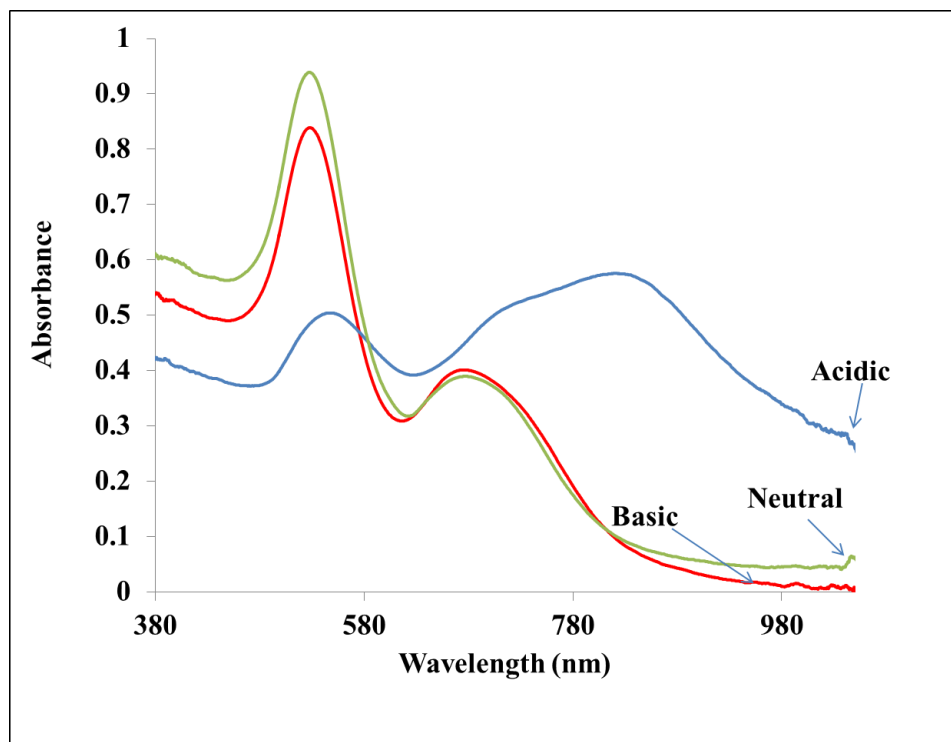


Figure 5.18: UV-Vis spectra of results for pH studies at pH values of 4, 7.0 and 8 corresponding to acidic, neutral and basic.

The recoveries at basic pH 7.5 are presented in Table 5.7.

Table 5.7: Recovery on selected concentrations of poly-DADMAC at slightly basic pH 7.5.

Calibration Curve Used	Initial Concentration ($\mu\text{g L}^{-1}$)	Amount Recovered ($\mu\text{g L}^{-1}$)	% RSD	% Recovery	% Error
Area	30	31.7 ± 1.04	3.28	105	5.61
	90	94.3 ± 1.52	1.61	105	4.75
Absorbance at 526 nm	30	21.3 ± 10.3	48.2	71.1	-28.9
	90	99.2 ± 5.77	5.82	110	10.2
Absorbance at 690 nm	30	32.0 ± 0.81	2.90	107	6.72
	90	92.6 ± 0.64	0.689	103	2.90
$A_{(690)}/A_{(526)}$	30	29.8 ± 0.20	0.671	99.2	-0.811
	90	94.3 ± 2.21	2.35	105	4.82

Varying the pH of the solution by 0.5 units to basic, did not have any significant effect both on absorbance and area. In all cases percent recovery were above 50% and percent error for recovery was below 20% (except for absorbance at 526 nm) in line with validation protocols recommended by Peters et al. [31].

5.3.5.6 Effect of Au-NP solution volume

The volume of the Au-NP solution used to detect poly-DADMAC was varied because the method relies on the need for excess Au-NPs in the solution. During the preliminary method development experiments whereby the volume of Au-NPs solution was varied (5 – 30 mL), it was observed that the absorbance values tend to shift and broaden quite extensively, and the appearance of the blue colour was found to be very sensitive to the Au-NP concentration. Thus fixing the volume of Au-NPs to 20 mL was necessary to ensure good linearity of the calibration curves. In this instance, the slight variation of volume was used to test the ruggedness of the protocol. Results on volume perturbations are presented in Table 5.8.

Table 5.8: Effect of changes in the initial volume of Au-NPs on the recovery of poly-DADMAC at a set concentration.

Calibration Curve Used	Volume of Au-NPs Used (mL)	Concentration of poly-DADMAC ($\mu\text{g L}^{-1}$)	Amount Recovered ($\mu\text{g L}^{-1}$)	% RSD	% Recovery	% Error
Area	18	60	65.19 ± 0.19	0.2880	108.6	8.65
	22	60	72.29 ± 0.29	0.4060	120.5	20.5
Absorbance at 526 nm	18	60	109.3 ± 2.27	2.080	182.2	82.20
	22	60	136.3 ± 0.83	0.6110	227.1	127.1
Absorbance at 690 nm	18	60	61.08 ± 0.68	1.110	101.8	1.80
	22	60	63.73 ± 0.18	0.2840	106.2	6.21
$A_{(690)}/A_{(526)}$	18	60	71.19 ± 0.44	0.6140	118.64	18.60
	22	60	64.49 ± 0.16	0.2470	107.2	7.22

Compared with Table 5.6 and Table 5.7, Table 5.8 has relatively high % recovery values for calibration curves based on absorbance at 526 nm (182.7% and 227.1%) due to the dependence of the calibration curve on excess Au-NPs in solution. Although the other calibration curves were within the prescribed acceptable limits [31], this highlights the importance that the volume can play, and if these calibration curves are to be adapted in a routine analysis scenario, then appropriate adjustments may need to be made.

5.3.5.7 Effect of time on stability of poly-DADMAC reaction with Au-NPs

Poly-DADMAC is a well-known powerful coagulant used in water treatment processes. Thus, in some initial experiments, whereby the poly-DADMAC concentration was relatively high, a precipitate would form that could be seen with the naked eye, and the absorbance could not be measured reliably due to light scattering. After a few days the precipitate would settle to the bottom of the glassware and leave a clear solution on top. Because of these observations an optimum time (analyzing solutions within 5 minutes after preparation) where the method can be performed with a degree of accuracy and precision was needed.

To determine the resistance of the methods with respect to time, a $30 \mu\text{g L}^{-1}$ poly-DADMAC solution was prepared triplicate, mixed with Au-NPs, and the spectrum was taken every 5 minutes for 50 minutes. For area, peak absorbance at 526 nm and 690 nm, and absorbance ratio ($A_{(690)} / A_{(526)}$), the RSD values were 0.435, 0.006, 0.003, and 0.005 respectively. The % RSDs were 0.94, 0.69, 1.1, and 1.6 respectively and the results are presented in Table 5.9. These results demonstrate that this method can be performed within 50 minutes with no significant loss in the degree of accuracy and precision and again highlights the possibility of adapting this method in routine analysis laboratory.

Table 5.9: Results for time studies

Time	Area	Absorbance at 526	Absorbance at 526
5	45.873	0.901	0.291
10	45.551	0.898	0.291
15	46.165	0.902	0.296
20	46.649	0.879	0.296
25	46.689	0.897	0.298
30	46.569	0.892	0.297
35	46.801	0.892	0.296
40	46.584	0.891	0.301
45	47.036	0.895	0.301
50	46.719	0.894	0.299
Mean	46.463	0.891	0.296
RSD	0.435	0.006	0.003
%RSD	0.9353	0.690	1.120

5.3.5.8 Effect of temperature on stability of poly-DADMAC reaction with Au-NPs

The aggregation mechanism and kinetics may be affected by temperature. Poly-DADMAC is a polymer and as such it is susceptible to changes in viscosity with temperature and hence its properties might change significantly.

To study the resistance of the method towards temperature a solution of 30 $\mu\text{g L}^{-1}$ and 90 $\mu\text{g L}^{-1}$ of poly-DADMAC were prepared in triplicate. The solutions were heated to 80 °C in a water bath, and at 80 °C, 70 °C, 60 °C, 50 °C, 40 °C, and 30 °C. The different solutions were mixed with Au-NPs as described above. The UV-Vis spectra are presented in Figure 5.19.

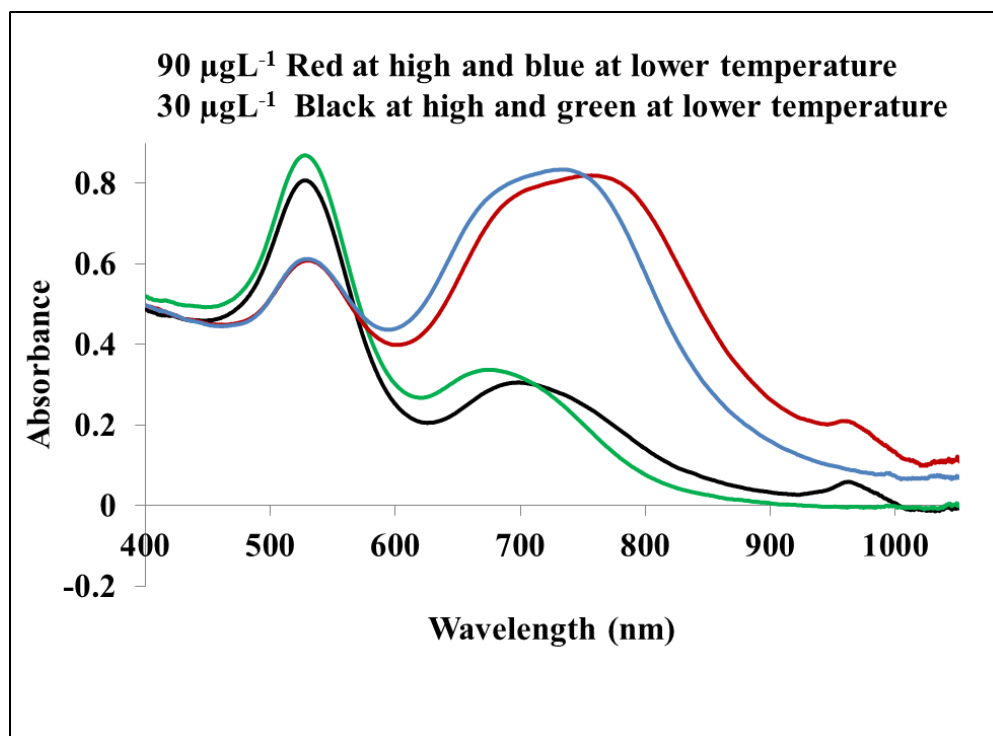


Figure 5.19: Effect of temperature on the plasmon band of the Au-NP poly-DADMAC aggregates.

At higher temperatures the peak of the poly-DADMAC-Au-NP aggregates shifted to longer wavelengths, and a new absorption peak at 950 nm emerged. The original plasmon band was observed at temperatures below 40 °C and the peak at 950 nm disappeared.

5.3.5.9 Selectivity for poly-DADMAC in the presence of specific ionic compounds

Separate solutions of aluminium nitrate, calcium nitrate, sodium chloride, and zinc chloride were prepared and spiked with 50 µg L⁻¹ of poly-DADMAC. The concentration of the salts was 0.04, 0.1 and 8 mg L⁻¹. A separate blank with no poly-DADMAC, was also prepared. The results for the percentage recoveries are presented in Table 5.10.

Table 5.10: The % recovery from different salt solutions spiked with 50 µg L⁻¹ poly-DADMAC.

Salt Used	% Recovery			Blank
	8	0.1	0.04	(% recovery)
Concentration of the salt (mg L ⁻¹)				0.04
Aluminium salt	109.8 ± 9.82	105.2 ± 5.25	99.33 ± 0.667	12.68 ± 0.050
Calcium nitrate	109.2 ± 8.20	106.2 ± 6.17	103.9 ± 3.91	12.62 ± 0.050
Sodium chloride	102.6 ± 2.64	102.6 ± 2.64	102.6 ± 2.64	11.30 ± 0.100
Zinc chloride	101.4 ± 1.36	101.4 ± 1.36	101.4 ± 1.36	10.05 ± 0.100

For all samples tested the recoveries were between 99 and 110%, and the blanks were between 10 – 13%. The blanks demonstrate these ions can interfere with the method, albeit at very low level. The recoveries of the spiked samples do show that the method can be selective toward poly-DADMAC in the presence of interfering species.

5.3.5.10 Matrix effects: selectivity and interference studies

Selectivity can be defined as the degree to which a method can quantify the analyte accurately in the presence of interfering species that are likely to be present in the sample matrix [31]. Selectivity was assessed qualitatively based on the significance of the interference and recovery studies with the chemical species as described Section 5.4.5.7.

Initial tests were done by preparing a 50 µg L⁻¹ poly-DADMAC solution with tap water. Poly-DADMAC was detected and the recovery was 70%; however the % RSD and percentage error were above the stipulated limits of 2% and 20% respectively shown in Figure 5.20. The tap water had a total dissolved solid (TDS) value of 10 mg L⁻¹, and it is due to these interferences that low recoveries resulted. This result implies that the poly-DADMAC sorbs onto the dissolved solids, and some remained within the solution. This could have some implications for water treatment processes whereby the assumption that all of the poly-DADMAC is removed may not be valid.

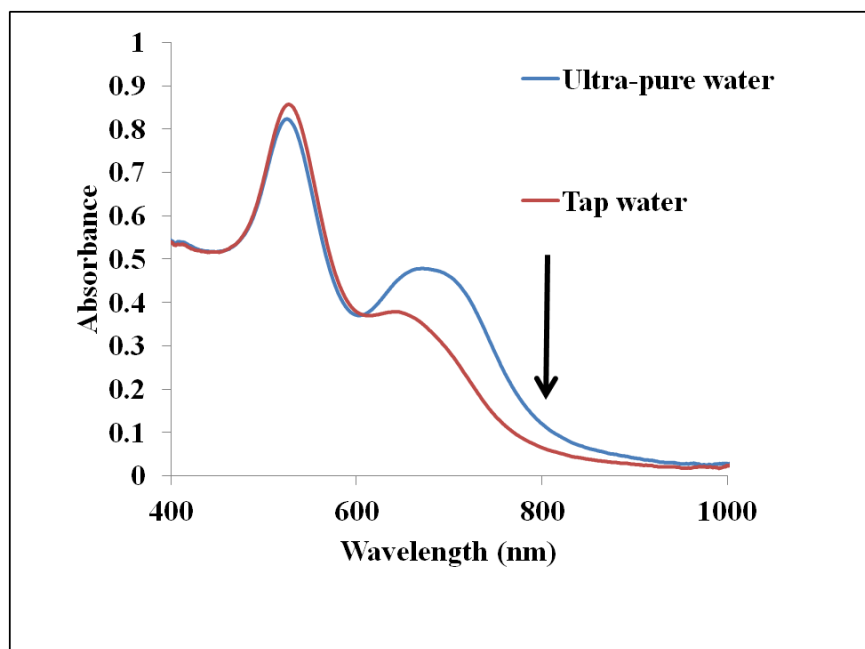


Figure 5.20: Effect of dissolved solids in tap water.

5.3.5.11 Selectivity for poly-DADMAC in the presence of other nitrogen-containing compounds

The interaction of poly-DADMAC with Au-NPs is possible because of the affinity of Au-NPs to the nitrogen-containing moiety on the polyelectrolyte. Thus to determine the selectivity, solutions of choline chloride, hexadecylamine, cetylpyridinium chloride, and DADMAC were prepared and spiked with $50 \mu\text{g L}^{-1}$ of poly-DADMAC. A blank without nitrogen-containing compounds was prepared by spiking with $50 \mu\text{g L}^{-1}$ of poly-DADMAC solution.

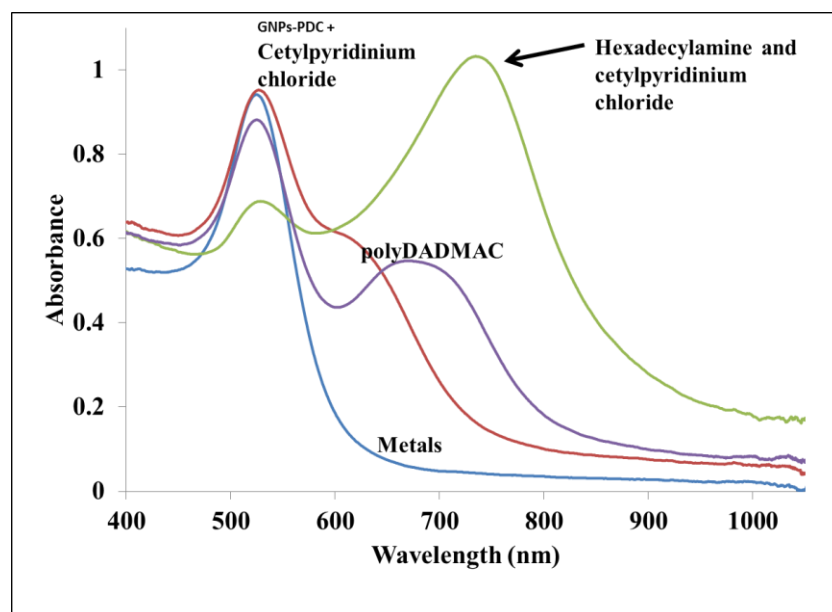
Table 5.11 presents the % recovery for the various solutions. For DADMAC and choline chloride spiked solutions the recoveries were between 99 and 104%, and the % errors were ± 5 . DADMAC monomer is usually present in small amounts in the poly-DADMAC used for various applications.

Table 5.11: Selectivity results for structurally similar species.

Compound used	%Recovery			Blank
Concentration of Compound used($\mu\text{g L}^{-1}$)	8	0.1	0.04	0.04
DADMAC	104.5 ± 4.49	98.12 ± -1.88	98.56 ± -1.42	11.04 ± 0.279
Choline chloride	94.754 ± -5.25	102.1 ± 2.06	99.10 ± -0.899	10.77 ± 0.050
Hexadecylamine	Not Possible	Not Possible	Not Possible	Not Possible
Cetylpyridinium chloride	Not Possible	Not Possible	Not Possible	Not Possible

The large levels of DADMAC used in these experiments highlight how this method is very sensitive and selective to poly-DADMAC versus its monomer, which further highlights the potential to adopt this method in routine analysis for low levels of poly-DADMAC.

With solutions of hexadecylamine and cetylpyridinium chloride, the detection of poly-DADMAC was not possible because these surfactants formed aggregates with the Au-NPs with an intense peak at 700 nm (Figure 5.21). Cetylpyridinium chloride is known to have properties similar to poly-DADMAC and is often used as a primary standard in metachromatic titration methods for the quantification of poly-DADMAC [34].

**Figure 5.21: UV-Vis spectra of 8.0 mg L^{-1} hexadecylamine and cetylpyridinium chloride spiked with $50 \mu\text{g L}^{-1}$ poly-DADMAC and analysed with the Au-NPs.**

Due to the large interference from hexadecylamine and cetylpyridinium chloride, a comparison with non-nitrogen containing surfactants was carried out.

5.3.5.12 Selectivity for poly-DADMAC in the presence of surfactants

To determine whether poly-DADMAC can be detected in the presence of surfactants, separate surfactant solutions were prepared as described for other interfering species, and then spiked with $50 \mu\text{g L}^{-1}$ of poly-DADMAC. Figure 5.22 shows the UV-Vis spectra of the various surfactants with the $50 \mu\text{g L}^{-1}$ spiked poly-DADMAC and Au-NP aggregates. Although the figure shows that the poly-DADMAC can be detected in the presence surfactants, it also shows that the aggregate peak due to Au-NPs and poly-DADMAC is slightly suppressed and is inversely proportional to the concentration of surfactant in the solution.

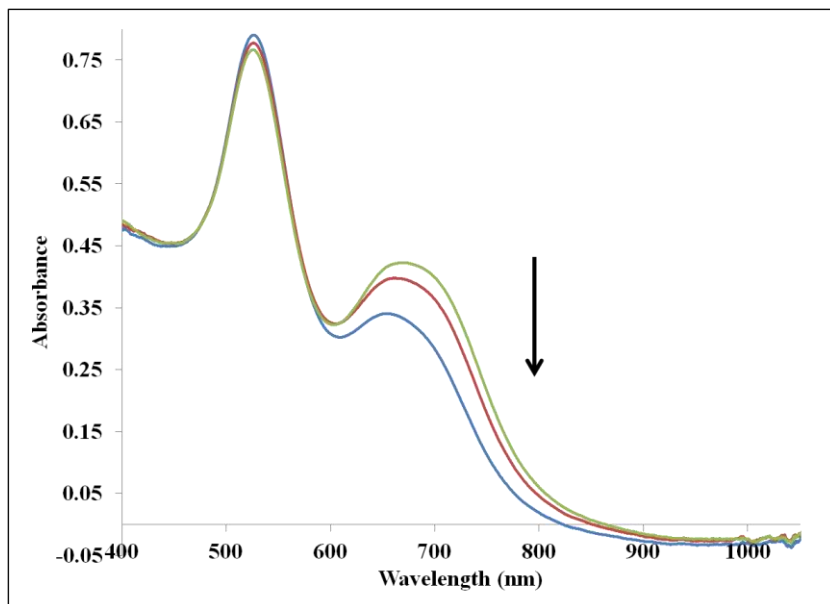


Figure 6. 22: UV-Vis spectrum of surfactant, poly-DADMAC and Au-NP aggregates.

The recoveries for the surfactants were above 90% for 0.1 and 0.04 mg L⁻¹ solutions, and are presented in Table 5.12. At relatively high concentration (8.0 mg L⁻¹), poly-DADMAC could be detected and quantified in the presence of pluronic and polyethylene glycol, but not polyvinylpyrrolidone.

Table 5.12: Results for surfactants study.

Surfactant used	% Recovery			Blank
	8	0.1	0.04	0.04
Concentration of surfactant ($\mu\text{g L}^{-1}$)				
Pluronic	76.78 \pm -23.22	90.63 \pm -9.36	95.80 \pm -4.20	Not detected
Polyvinylpyrrolidone	Not possible	95.33 \pm -4.66	95.33 \pm -4.66	Not detected
Polyethylene glycol	95.39 \pm -4.99	95.62 \pm -4.37	99.79 \pm -0.20	10.82 \pm 0.08

Surfactants are commonly used in pharmaceutical and cosmetic products. In addition, they are increasingly being found in various water samples in the environment and at water treatment facilities [35]. These results are encouraging in terms of the ability of the method to selectively detect and quantify poly-DADMAC in the presence of such species.

5.3.5.13 Stability of poly-DADMAC under storage conditions

Stability is defined as the chemical stability of an analyte in a given matrix under specific conditions for given time intervals [31]. For the method to be considered reliable, the analyte must be stable for the entire analytical procedure. To test the stability of the protocol, solutions were prepared and used up to 6 months after initial preparation. In addition, Au-NPs and poly-DADMAC aggregates were prepared at different times of the day. There were no significant differences observed between these samples and those analysed initially. Although the solutions were considered stable, it is recommended that a fresh solution of poly-DADMAC be used as often as possible. This is due to the strong tendency of poly-DADMAC to adsorb onto surfaces.

5.3.6 Poly-DADMAC standards prepared from A2 and A3 solution

For A2 and A3 there was no extended peak observed when poly-DADMAC was introduced in the solution. But a series of poly-DADMAC standards were prepared and analyzed with UV-Vis. Poly-DADMAC standards prepared from the A2 solution gave no relationship between absorbance and concentration of poly-DADMAC in the solution as shown in Figure 5.23.

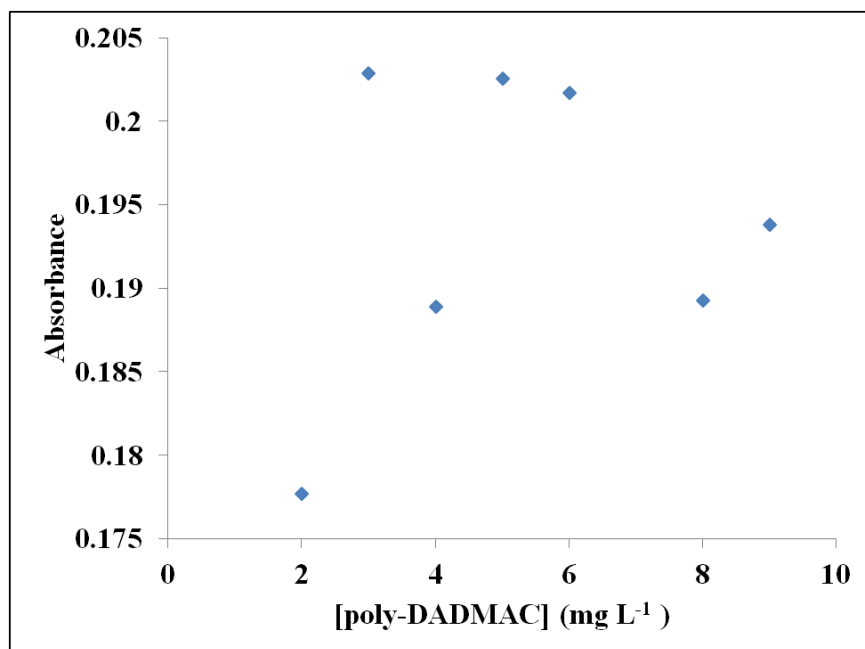


Figure 5.23: Plot of absorbance at 526 nm versus concentration of poly-DADMAC. Standards were prepared from A2 solution of Au-NPs.

On the other hand, the poly-DADMAC standards prepared from A3 solution show a linear relationship between absorbance at 526 nm and concentration poly-DADMAC in the solution. Figure 6.24 shows the graph of absorbance at 526 nm plotted against the concentration of poly-DADMAC. Although the correlation coefficient was good, it was difficult to repeat the experiment and led to poor precision and repeatability, because of varying Au-NP diameters and large uncontrollable aggregates as observed by TEM. Due to these factors and time this method was not validated, because it needed further development of the synthesis of Au-NPs. Only

calibration curves constructed from the A1 solution were validated and developed as a method of detection for poly-DADMAC in different matrices.

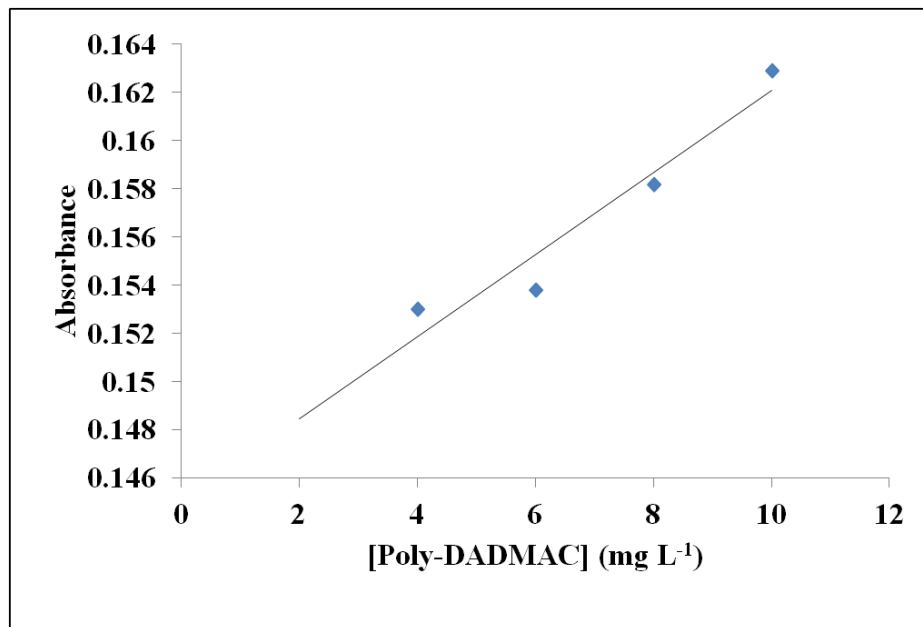


Figure 6. 24: A plot of absorbance at 526 nm versus concentration of poly-DADMAC. Standards were prepared from A3 solution with a correlation coefficient 0.93.

5.4 Conclusions

Three Au-NPs solutions namely A1, A2, and A3, were successfully synthesised and characterised with UV-Vis spectroscopy and TEM. These Au-NPs were explored for the quantification of poly-DADMAC in solution. Results showed that all synthesised Au-NPs interacted with poly-DADMAC in solution. Au-NPs A2 and A3 were prepared by seed growth and stock solution methods respectively. These Au-NPs yielded no useful relationship between absorbance of Au-NPs and the concentration of poly-DADMAC in solution. Au-NPs A1 prepared from citrate reduction provided a linear relation between absorbance of Au-NPs and concentration of poly-DADMAC in the solution. A method of detecting poly-DADMAC in solution was developed based on Au-NPs A1.

The proposed analytical method is simple, sensitive, and cost-effective. The method does not require any pre-treatment of the samples or any sophisticated extraction techniques. Validation data presented here demonstrate that the method is accurate and precise. We used different techniques (area method, absorbance at 690 nm, absorbance at 526 nm and absorbance ratios at 690/526) to construct calibration curves.

For the area method, the lower limit of detection and lower limit of quantification were 0.5 and 1.5 $\mu\text{g L}^{-1}$ respectively. Linearity ranges were between 25 – 100 $\mu\text{g L}^{-1}$ with $R^2 = 0.9969$. The calibration curve constructed from the absorbance at 690 nm gave linearity between 10 – 100 $\mu\text{g L}^{-1}$. The LOD and LOQ were ranging from 0.4 to 1.60 $\mu\text{g L}^{-1}$. The calibration curve at 526 nm had linearity between 10 – 130 $\mu\text{g L}^{-1}$ and a LOD and LOQ of 29.1 and 88.1 $\mu\text{g L}^{-1}$ respectively. The last calibration curve was constructed from the absorbance ratio at 526/690 and was linear between 10 and 130 $\mu\text{g L}^{-1}$ and the LOD and LOQ were 0.54 and 1.64 $\mu\text{g L}^{-1}$ respectively. All calibration curves had similar LOD and LOQ values except the calibration curve at 526 nm. Our proposed method was found to be precise and, was also performed by different analyst.

The selectivity and ruggedness (pH, temperature and volume) of the protocol was tested by analysing various solutions with possible interfering species. Inorganic ions had no significant effect on the detection and quantification of poly-DADMAC. The monomer did not interfere with detection and quantification of the polymer; however, nitrogen containing surfactants (hexadecylamine and cetylpyridinium chloride completely suppressed the detection of poly-DADMAC. Surfactants without any nitrogen groups did not interfere significantly at low concentrations. It was found possible to detect poly-DADMAC in tap water provided that water is filtered for dissolved solids.

Currently, we are investigating the possibility of using this technique on real environmental samples, and water samples from water treatment plants. Furthermore, its limitations in complex matrices that may contain other nitrogen containing compounds needs further study.

5.5 References

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Chapter 6: General Conclusion

Polyelectrolytes are water soluble polymers with ionisable groups in their chains and are used in drinking and wastewater for the removal of particles and varying concentrations of sludge. They are classified by their origin, composition, molecular architecture, charge and electrochemistry. Polyelectrolytes are widely used in industrial processes, such as, pulp and paper manufacturing, paint industry and water treatment sectors. They have also found use in the pharmaceutical and cosmetics industries for the preparation of detergents, shower shampoos, drugs and for most skin care products. In this study we focused on the main use of polyelectrolytes in drinking water treatment processes. Polyelectrolytes enter the environment through water treatment pathway (main path) and persist to a greater extent than first anticipated.

To determine a suitable analytical method for detection and quantification of poly-DADMAC in water samples, five methods were investigated: LC-MS, NMR spectroscopy, Raman spectroscopy, metachromatic titration and gold tagging method. For the LC-MS method, poly-DADMAC was injected in the system but was not detected by the MS detector. It was not clear whether the poly-DADMAC eluted from the column to the MS, or not. Poly-DADMAC is used as a displacer in displacement chromatography, and it is therefore likely to remain inside column. Due to the difficulties faced in trying to elute poly-DADMAC, LC-MS was found not to be suitable for routine analysis of poly-DADMAC. However, based on the literature (displacement chromatography) we recommend buffers with a high ionic strength to be used as the mobile phase for elution of poly-DADMAC from columns.

With NMR it was possible to detect poly-DADMAC at trace levels, however, the construction of calibration curves was difficult due to poor repeatability of the method. Our calibration curve on this method had a linearity range between 10 – 100 mg L⁻¹ and a slope of 0.0005. Detection of poly-DADMAC below 1 mg L⁻¹ was impossible, and this limits its application due to the fact that the WHO permitted amount of poly-DADMAC in drinking water is 50 µg L⁻¹. On the basis of the poor detection limit which was above the maximum allowed level of the analyte, the NMR

technique was considered unsuitable for application in environmental water systems. In addition, even though the permissible dose of poly-DADMAC can be 20 mg L^{-1} , which is within the linear range of the NMR technique, the latter suffers poor precision and accuracy and therefore is not recommended for routine analysis of poly-DADMAC in water treatment plants.

Raman spectroscopy was found to detect poly-DADMAC below $50 \text{ } \mu\text{g L}^{-1}$ but failed to produce a quantifiable signal that can be used to construct a calibration curve. In an effort to achieve a reproducible signal, we concluded that Raman spectroscopy could not be recommended for routine analysis of poly-DADMAC but we have identified it as an area that needs further investigation. Our investigations have shown that Raman enhancement detection of the analyte in the presence of gold nanoparticles would be a viable technique for future studies.

For the metachromatic titration method, we were able to improve the endpoint detection. This improved the calculation of precision and accuracy. Errors associated with the use of spectrophotometers were eliminated by the use of a stainless steel dip-probe. Titration curves constructed from both wavelengths (515 nm and 634 nm) were found to be useful, except in tap water where it was not possible to construct a repeatable titration curve using the wavelength at 515 nm. The limit of detection was determined as 0.1 mg L^{-1} in model water and 0.5 mg L^{-1} in tap water (the presence of the plateau was used as a measure), and the limit of quantification was 0.5 mg L^{-1} both in tap and model water, results are shown in Table 6.1. The equivalent weight (charge density) of poly-DADMAC was determined to be 0.007 eq g^{-1} . Validation results suggest that the proposed method can be used in water treatment plants for routine analysis of poly-DADMAC.

A novel method based on a Au-NP probe was developed for analysis of poly-DADMAC in water samples. Au-NPs were synthesised and characterised with UV-Vis spectroscopy and TEM. Using the gold tagging method, it was possible to detect and quantify poly-DADMAC at concentrations below $50 \text{ } \mu\text{g L}^{-1}$. The proposed analytical method is simple, sensitive, and cost-effective. The developed method does not require any pre-treatment of the samples or any sophisticated extraction techniques. Validation data presented here demonstrate that the method is accurate and precise compare to metachromatic titration (Table 6.1). The lower limit of detection and lower limit of quantification were 0.5 and $1.5 \text{ } \mu\text{g L}^{-1}$ respectively and results are shown in Table 6.1. Linearity ranges were between $10 - 100 \text{ } \mu\text{g L}^{-1}$ with correlation coefficient,

R^2 , of 0.99 in all cases. The selectivity and ruggedness of the protocol was tested by analysing various solutions with possible interfering species. Inorganic ions had no significant effect on the detection and quantification of poly-DADMAC. The monomer did not interfere with detection and quantification of the polymer; however, nitrogen containing surfactants (hexadecylamine and cetylpyridinium chloride) completely suppressed the detection of poly-DADMAC. Surfactants without any nitrogen groups did not interfere significantly at low concentrations.

Validation results for the developed novel method suggest the method is suitable for routine analysis of poly-DADMAC in various water samples. Currently, we are investigating the possibility of using this technique on real environmental samples, and samples from water treatment plants. Furthermore, its limitations in complex matrices that may contain other nitrogen containing compounds, needs further study. We recommend a further investigation in following techniques: NMR, LC-MS and Raman spectroscopy for possible application in routine analysis and quality control of poly-DADMAC.

Table 6.1: Comparison of metachromatic method against a novel Au-NP probe method for detection and quantification of poly-DADMAC based on precision and selectivity.

Criteria	Metachromatic method	Au-NP probe method
LOD	0.1 – 0.5 mg L ⁻¹	0.5 – 0.8 µg L ⁻¹
LOQ	0.5 mg L ⁻¹	1.5 µg L ⁻¹
Linearity	0.5 – 40 mg L ⁻¹	10 – 100 µg L ⁻¹
Selectivity	Not metals, polyelectrolytes containing and monomer	Selectivity to metals, monomer and not to polyelectrolytes containing nitrogen
Surfactants	Possible to detect poly-DADMAC	Possible to detect poly-DADMAC

Appendices

A 1: LC-MS data

A 1.1 LC-MS Experiment

All LC-MS experiments conducted are described in details in Chapter 3, Section 3.3. LC-ESI-MS was kept in positive mode and the Gemini C₁₈ 110A column was used for separation of poly-DADMAC. The mobile phase used for eluting the poly-DADMAC from the HPLC column consisted of acetonitrile and formic/ammonium formate buffer (see section 3.3) at a flow rate of 0.2 mL min⁻¹. Gradient elution was performed as linear gradient for 2 minutes.

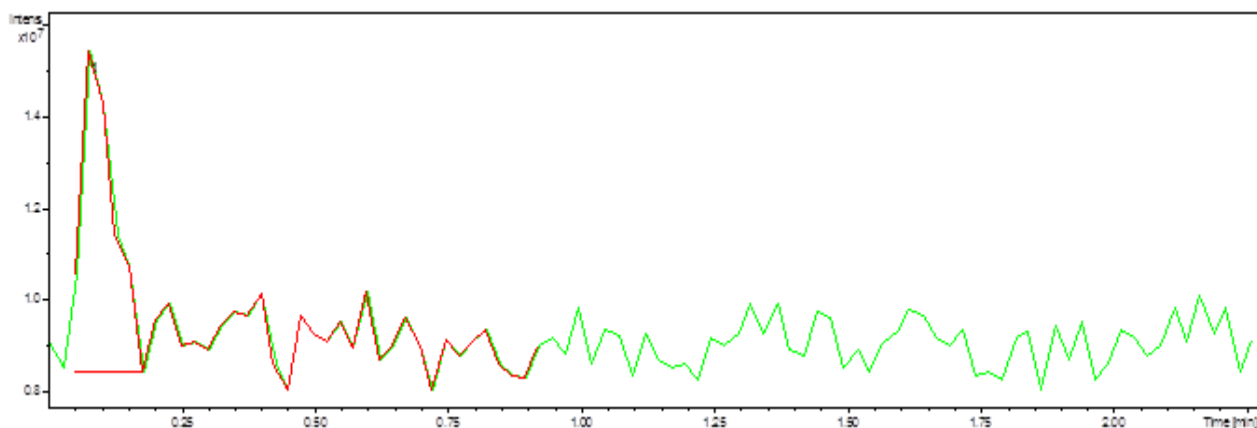


Figure A1.1: Chromatogram of 1 mg L⁻¹ poly-DADMAC standard solution after 2 minutes of elution time.

The expected peak of poly-DADMAC was not present in the MS spectrum and poly-DADMAC standards were run for different times 2 minute, 18 minutes and 45 minutes shown in Figure A1.1, 2 and 3 respectively. Poly-DADMAC was injected direct into the MS a peak corresponding to the monomer of poly-DADMAC at 126 m/z was observed as can be seen in Figure A1.4.

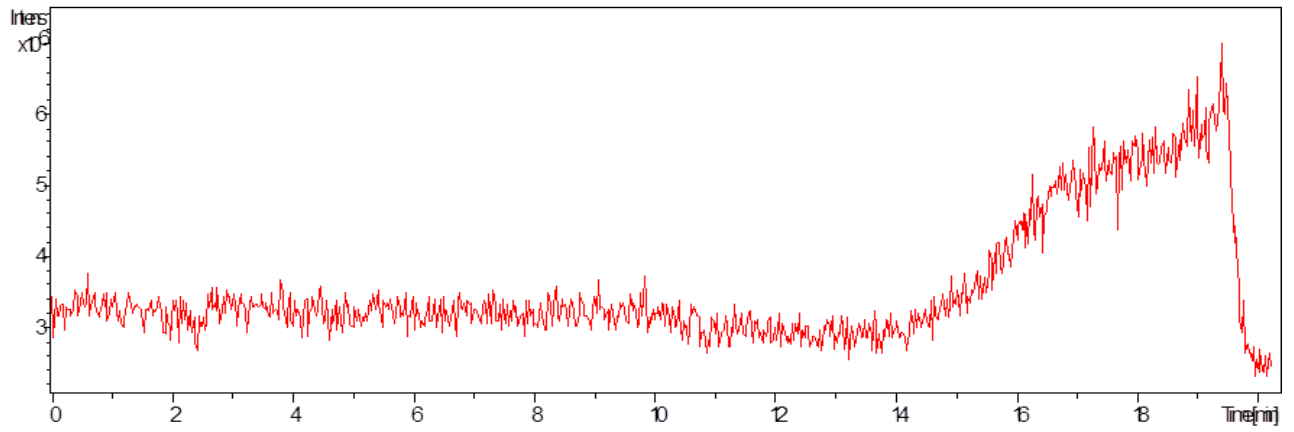


Figure A1.2: Chromatogram of 1 mg L⁻¹ poly-DADMAC standard solution after 18 minutes of elution time.

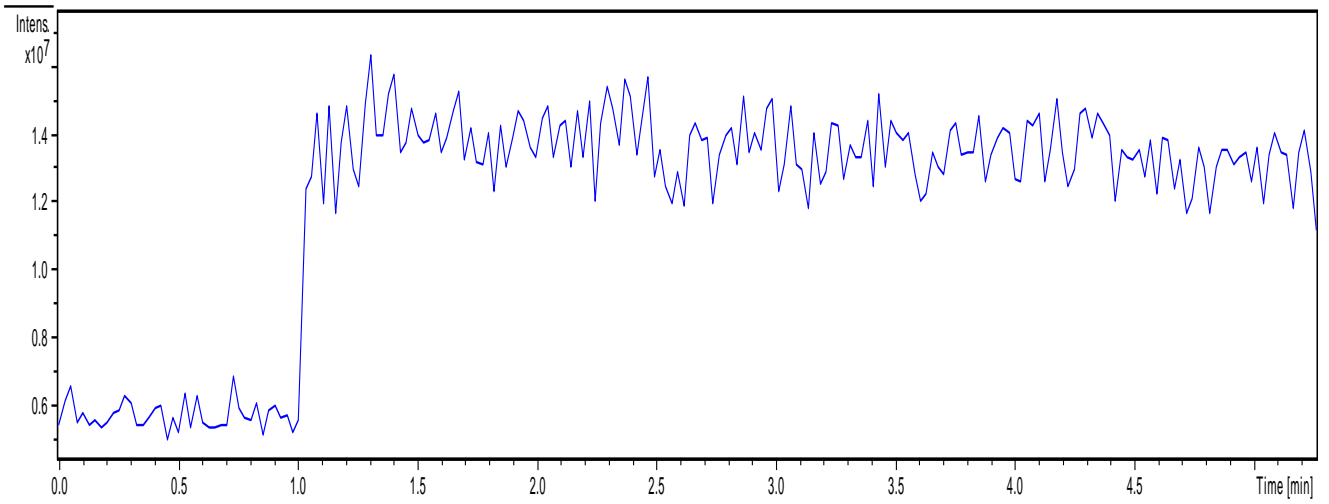


Figure A1.3: Chromatogram of 1 mg L⁻¹ poly-DADMAC standard solution after 45 minutes of elution time.

A 2: NMR data

A2.1 NMR data

All NMR experiments were done as described in Chapter 3 Section 3.4) and the results were presented in chapter 3 section 3.4 (pp. 56) as well. Peak at 3.1 ppm was used for the analytical quantification of poly-DADMAC in water solution.

Solutions of poly-DADMAC were prepared in methanol- d_1 to prevent the interference of water molecules (methanol and poly-DADMAC are miscible). To construct a calibration curve poly-DADMAC standards with concentration values of 10, 20, 30, 50, 70 and 100 mg L⁻¹ were prepared and analysed with NMR. NMR spectra were recorded by using a Bruker Ultra operating at 400 MHz with Topspin software to analyze and integrate the relevant peaks.

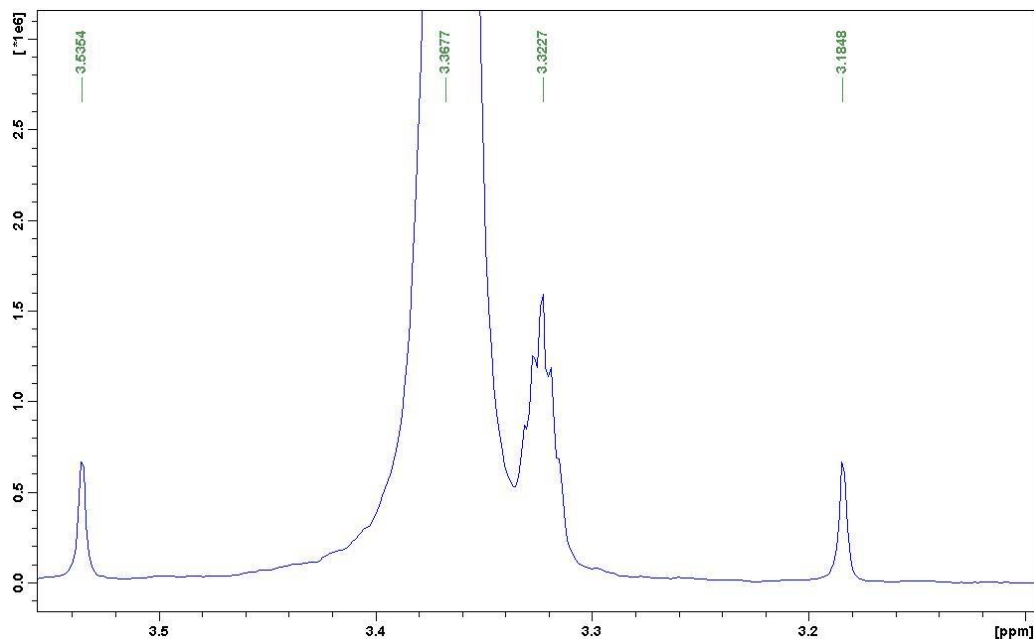


Figure A2.1: NMR spectrum of 10 mg L⁻¹ poly-DADMAC solution prepared in methanol- d_1 .

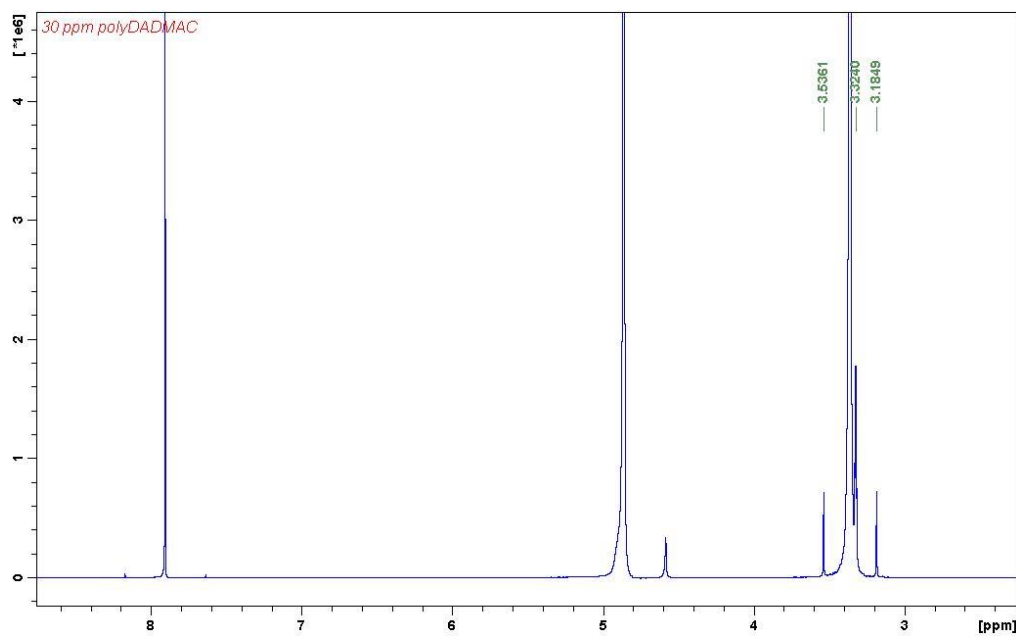


Figure A2. 2: NMR spectrum of 30 mg L⁻¹ poly-DADMAC solution prepared in methanol-*d*₁.

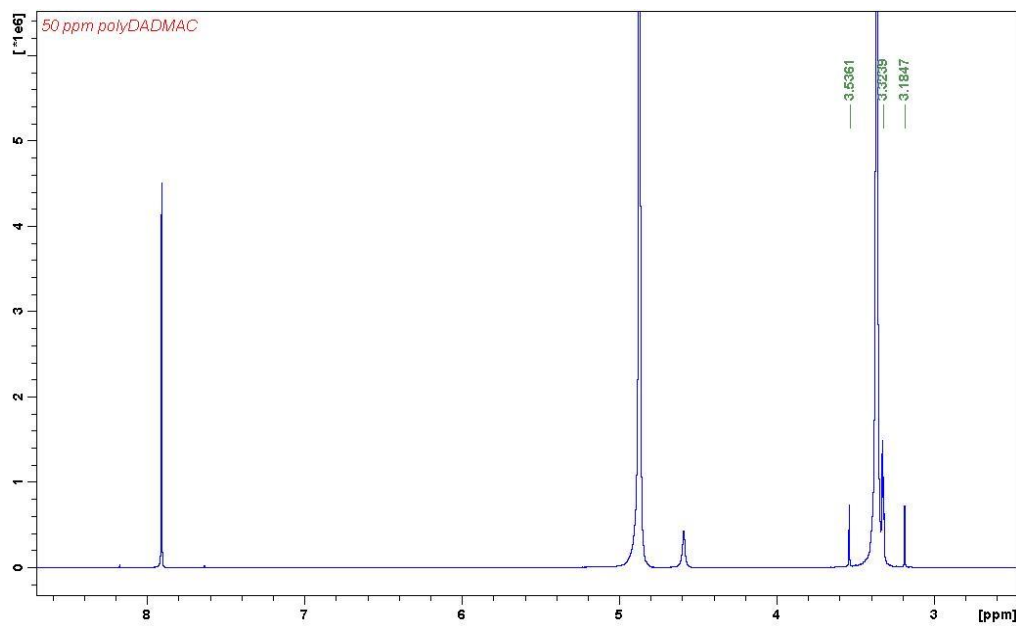


Figure A2. 3: NMR spectrum of 50 mg L⁻¹ poly-DADMAC solution prepared in methanol-*d*₁.

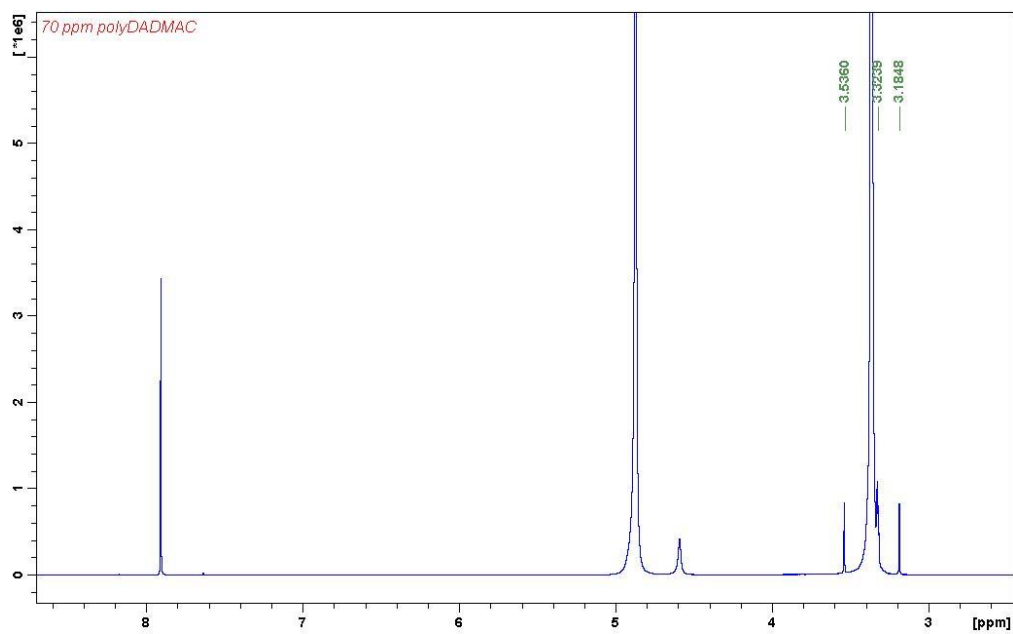


Figure A2. 4: NMR spectrum of 70 mg L⁻¹ poly-DADMAC solution prepared in methanol-*d*₁.

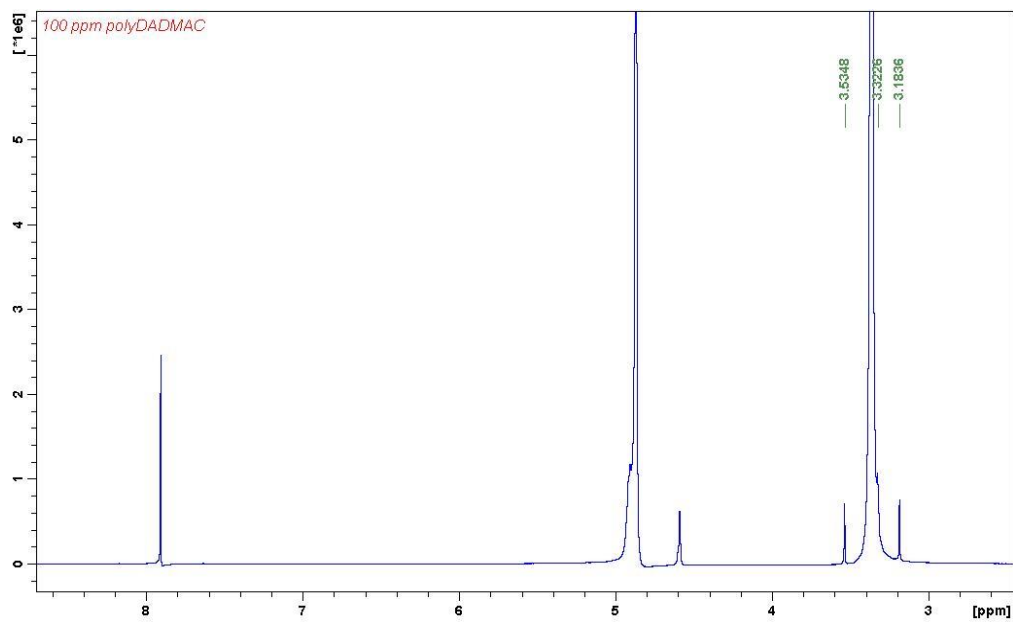


Figure A2. 5: NMR spectrum of 100 mg L⁻¹ poly-DADMAC solution prepared in methanol-*d*₁.

A 3: Raman data

A 3.1 Raman Data

All Raman experiments were done as explained in Chapter 3, Section 3.5.

A poly-DADMAC stock solution was prepared followed by series dilution in water to obtain working standards. Poly-DADMAC standards ranging from 30 – 90 $\mu\text{g L}^{-1}$ were analysed with Raman spectroscopy.

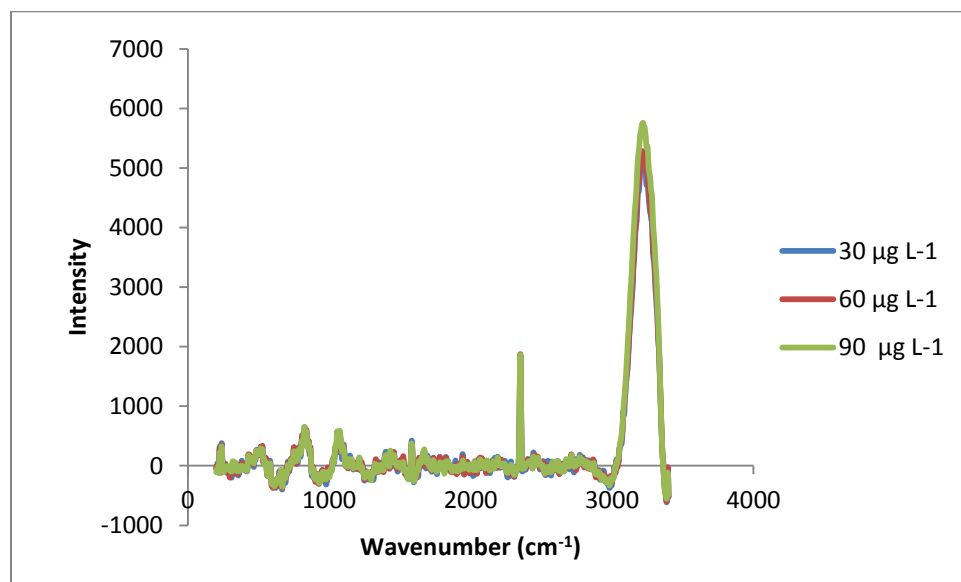


Figure A3. 1: Raman spectra a plot of poly-DADMAC standards (30, 60 and 90 $\mu\text{g L}^{-1}$) in the same axis.

Figure A3.1 shows there was no change in the Raman signal when the concentration of poly-DADMAC was increased from 30 to 90 $\mu\text{g L}^{-1}$. This were described in more depth in Chapter 4.

Raw Raman Excel data is included on the disc provided in **Folder A3.1 Raman data**.

A 4: metachromatic titration data

All experiments were carried out as described in Chapter 5 section 5.3. PVSK was standardized with cetylpyridinium chloride prior to titration. Poly-DADMAC was titrated with PVSK using TBO as an indicator. The endpoint was followed both visually and by means of a spectrophotometer. Visual endpoint was followed by taking the colour change from blue to purple. Spectrophotometric titration was done by measuring the absorbance of the solution at 634 nm and plotting it against volume. The end point was determined from the plot of Absorbance against volume of PVSK added.

- Folder A4.1 Visual titration contains data used for construction of Table 5.1, Figure 5.2 and Figure 5.3.
- Folder A4.2 Spectrophotometric titration contains data used in the construction of Figure 5.4, Figure 5.5 and Figure 5.6

A 5: colloidal gold method data

All experiments were done as described in Chapter 5 section 5.3.

Appendix 5 present data for the synthesis of different citrate stabilized Au-NPs, detection of poly-DADMAC and validation. The following folders are in the Disc.

- A5.1 Characterization of gold salt solution
- A5.2 Characterization of poly-DADMAC Au-NPs aggregate
- A5.3 Characterization of poly-DADMAC Au-NPs aggregate
- A5.4 Detection of poly-DADMAC