FABRICATION OF A COLORIMETRIC PAPER-BASED MICROFLUIDIC SENSOR FOR RESIDUAL POLY-DADMAC DETECTION IN WATER TREATMENT

by

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PREFACE

This work was conducted in the Department of Chemistry, Durban University of Technology, Steve Biko Campus, Durban, South Africa from February 2016 to September 2019. The work was performed under the esteemed supervision of Dr P.S Mdluli with the assistance of Dr M Mlambo.

DECLARATION

I, Sibongile Elizabeth Magubane, hereby declare that this dissertation is wholly my own work and that all the references, to the best of my knowledge, are accurately reported. This work has not been submitted for a degree at any other university, and its only prior publication was in the form of conference papers, journal publications as listed on page iii.

Ms. SE Magubane (Candidate)

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CONFERENCE ATTENDANCE AND JOURNAL PAPERS PUBLISHED

Magubane, S.E., Ntlhoro, S., Mlambo, M., and Mdluli, P.S. 2017. Gold nanoparticles based Lovibond colour filter for quantification of residual poly diallyldimethylammonium chloride in treated potable water. SACI 2017 Conference, Cape Town, South Africa 22-25 June 2017.

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ABSTRACT

Poly– diallyldimethylammonium chloride (poly-DADMAC) is an established coagulant in the treatment of drinking water. Reports have indicated that poly-DADMAC can degrade into a suspected carcinogenic form which is N-nitrosodimethylamine (NDMA). Consequently, water treatment plant operators are required to know the residual concentration of polyelectrolytes at various stages in the treatment process and the eventual quality of the treated water. Historical research has proven that, over the years, a number of methods such as extraction-spectrophotometry, fluorometry and tannic acid have been developed and implemented for the analysis of polymers in drinking water. However, they produced poor linearity, sensitivity and precision, high detection limits or produced false positives due to matrix effects. The laboratory method that has proven to be simple, affordable and accurate is the colloidal titration method. However, this method cannot be used at the plant for quick and accurate monitoring of poly-DADMAC.

In this study, the aim was to fabricate a Lovibond portable colorimetric comparator device based on gold nanoparticles for colorimetric quantification and detection of poly-DADMAC in raw and treated potable water. The colorimetric disk and comparator was fabricated from 14 nm gold nanoparticles with the concentration of poly-DADMAC varying from 1 to 10 mg L^{-1}. The addition of higher concentrations of poly-DADMAC resulted in the aggregation of gold nanoparticles with the colour changing from red to blue. The gold nanoparticles were prepared via the citrate reduction method. Characterisation of the gold nanoparticles was done by ultraviolet-visible (UV/VIS) spectrophotometry and transmission electron microscopy (TEM).

The Lovibond comparator was fabricated with a colour filter disk for the screening of residual poly-DADMAC in raw and potable water. The colorimetric disk was printed on the plastic slide and inserted in the plastic compartment of the comparator. The Lovibond comparator was verified with raw and potable water samples from different sampling points in and around the Mhlathuze river area located in the province of KwaZulu-Natal, South Africa. Preliminary results showed that the developed colorimetric comparator device can visually detect poly-DADMAC concentrations lower than 1 mg L^{-1}.

The colour development was first developed on normal paper and then optimised by UV/VIS spectrophotometry. The method developed has a linear range from zero to 10 mg L^{-1} with the correlation coefficient of R=0.9954.

The effectiveness of the device was investigated by doing a recovery study on a potable water sample. Potable water is water that is suitable for drinking. In this research, potable water refers to tap water. The potable water sample was spiked with 1 mg L^{-1} poly-DADMAC. This exercise was done three times. The acceptance criterion for recovery is 80 to 120%. The 3 recoveries that were obtained are 107.95, 91.26 and 100.3%. The average recovery was 99.84%. This shows that the proposed method can detect poly-DADMAC with the acceptable level of accuracy.

One of the important parameters that a quality method must have is selectivity. This parameter shows that the method can accurately detect the analyte of interest in the midst of different matrices. This was done by analysing the raw water samples together with their treated samples. Physical-chemical parameters were also analysed to show the broader state of the samples. The poly-DADMAC results obtained from the UV/VIS spectrophotometer compared quite well with those obtained from using the Lovibond colorimetric filter.

The limited observation of colours using our eyes is a major contributor of systematic errors during the application of colorimetric devices. Thus, such a limitation can be reduced by using CIELAB system. A gold nanoparticle-based colorimetric CIELAB system for detection of poly-DADMAC in potable and raw water was also demonstrated. The method is based on the application of a paper-based analytical device which is printed on the normal A4 white printing paper. Fully enclosed 6 X 9 hydrophobic wells were fabricated on this paper.

This work provides a clear evidence of the application of CIELAB colour system, and thus, replacing the conventional spectrophotometric technique to quantify polymers. Results of this work showed that the intensity of the fabricated well is proportional to the concentration of the detected polymer. The change in colour (ΔE) was calculated for each fabricated well and clear evidence of the colour change was observed upon the variation of the polymer. Moreover to the application of ΔE, the chromaticity

using CIEYxy was used to verify colour change, it was observed that they followed the expected shift from red to blue, symbolising aggregation due to Van Der Waal inter-particle attractions as a result of the addition of poly-DADMAC.

The results of this experiment were validated using the spectrophotometric technique which further emphasised the appearance of the new surface Plasmon resonance peak formed at 610nm symbolising aggregation. Importantly, the intensity of the new Surface Plasmon Resonance (SPR) peak at 610 nm increased by increasing the concentration of poly-DADMAC.

Comparison of the Lovibond and UV/VIS results showed that there was no significant difference between the two methods. This proved that the fabricated Lovibond colour comparator is capable of the detection of residual poly-DADMAC in water treatment. This therefore implies that plant operators can be able to detect poly-DADMAC at any stage during the water treatment process by using a rapid, user-friendly portable device.

DEDICATION

I dedicate this work to my husband Modise Magubane; my children Pinki, Sibongile, Nondumiso, Joshua and Sindisiwe. Your love and support throughout this journey inspired me to push and never give up.

To my parents, Alfred and Sibongile Zwane, thank you for all your teachings and for always encouraging me to give my best in all that I do.

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ACRONYMS

AuNPs	Gold Nanoparticles
ATP	Adenosine Triphosphate
Conc	Concentration
COD	Chemical Oxygen Demand
CIE	Commission Internationale del ‘Eclairage
EPI-DMA	Epichlorohydrin dimethylamine
DBP	Disinfection by-product
NDMA	N-nitrosodimethylamine
LOD	Limit of Detection
LOQ	Limit of Quantification
LSPR	Localised Surface Plasmon Resonance
$mS\ m^{-1}$	Millisiemens per meter
HPLC	High Performance Liquid Chromatography
NTU	Nephelometric Turbidity Units
mg L-1	Milligrams per litre
TSS	Total Suspended Solids
RGB	Red green blue
TEM	Transmission Electron Microscopy
DADMAC	Diallyl dimethylammonium chloride
SOP	Standard Operating Procedure
Pt-Co	Platinum Cobalt
UV-VIS	Ultra Violet Visible
USEPA	United States Environmental Protection Agency
µPAD	Microfluidic Paper-based Device

Chapter One: Introduction

1.1 Background

Water is the most important and essential component on earth for critical activities of living beings.

By definition, potable or drinking water is water that has acceptable quality in terms of its physical, chemical, and bacteriological parameters so that it can be safely used for drinking and cooking. For this reason, the production of safe drinking water in bulk is vital to sustain livelihoods (Tzoupanos and Zouboulis 2008; John and Trollip 2009). Safe drinking water is an internationally accepted human right (Thompson *et al.* 2007).

Domestic water is normally abstracted from rivers, dams and groundwater, in its raw state, and contains a substantial amount of suspended matter (Mwangi, Ngila and Ndungu 2012). The sad fact is that the water quality of our water resources is deteriorating continuously due to the geometrical growth of population, industrialisation, civilisation, domestic, and agricultural activities including other geological and environmental changes (Ali 2012). As a result, the majority of water supplies require treatment to make the water suitable for domestic and industrial use (Vigneswaran and Visvanathan 1995). It is very rare to find a water source that does not require treatment before being used as a potable-water supply (Binnie *et al.* 2002). Hence, water treatment is quite critical.

Water treatment is a process that basically involves physical, chemical and biological changes that transform raw water into potable water (Cox and Organization 1964). Most water is disinfected for human consumption. Therefore, the treatment process should be able to remove turbidity and organisms harmful to health including the maximum possible removal of organic matter as well (Polasek and Muti 2002).

A very important step in water treatment is the coagulation-flocculation process (John 2008; Tzoupanos and Zouboulis 2008; Ali 2012). This process is widely used due to its simplicity and cost-effectiveness (Tzoupanos and Zouboulis 2008).

Regardless of the nature of the treated sample, coagulation-flocculation is usually included, either as pre- or as post-treatment step (Tzoupanos and Zouboulis 2008). The efficiency of coagulation-flocculation strongly affects the overall treatment performance (John 2008). As a result, the increase of the efficiency of coagulation stage seems to be a key factor for the improvement of the overall treatment efficiency (Tzoupanos and Zouboulis 2008).

Coagulation is a process that combines small particles into larger aggregates (Majam and Thompson 2006; John 2008). During the coagulation and flocculation processes, solids that do not settle easily are converted into larger and heavier settleable solids (Gebbie 2006; Sethumadhavan and Johnson 2006; John 2008). This occurs by the addition and mixing of a coagulant chemical into the raw water (Majam and Thompson 2006; Sillanpää *et al.* 2018). The settleable solids can then be removed by sedimentation and filtration (Gebbie 2006). The particles to be removed from the raw water are usually negatively charged and so the coagulant chemicals used in drinking water treatment are generally positively charged (Majam and Thompson 2006).

1.1.1 Target compound

A wide range of polymers are registered for use in drinking water treatment (John 2008). Two widely used cationic polymers, namely polydiallyldimethyl ammonium chloride (poly-DADMAC) and epichlorohydrin dimethylamine (EPI-DMA), are primary coagulants widely used in the treatment of drinking water in South Africa (Majam, Jonnalagadda and Thompson 2004; Majam and Thompson 2006). They are used singly or in combination with inorganic polymeric coagulants. They are water soluble and have low to moderate molecular weights (Majam and Thompson 2006; John 2008).

This research will focus on poly-DADMAC. Poly-DADMAC is a homopolymer of diallyldimethylammonium chloride with a chemical formula $(C_8H_{16}NCl)n$. The polymer has a molecular weight that is typically in the range of hundreds of thousands of grams per mole, and even up to a million for some products. Poly-DADMAC is the most widely used polymer for water purification due to its high cationic charge content (Hahn and Klute 2012). It is well known to be the most chlorine resistant and operates over a wide pH range (Bolto 1995; John 2008).

1.1.2 Analytical techniques for quantifying poly-DADMAC

The dosing of treatment chemicals in water treatment is of utmost importance. Obviously, a minimum effective amount of treating agent must be maintained in the water for the treatment to have its desired effect. In the same manner, overdosing the treating agent would be at best uneconomical and, at worst, result in damage to the process or the processing equipment (Sethumadhavan and Johnson 2006).

This basically means that the most important factor in water treatment is to ensure that the chemicals that were used during the treatment process are not present in the treated water at quantities that are outside the set limits. For this reason, it is important that the treated water is tested for all these chemicals to ensure that they are indeed within the required limits and that the water is indeed suitable and safe for the intended purpose whether it is for drinking or other uses. Moreover, it is vital to routinely monitor residual levels of poly-DADMAC in the final treated water so as to advise on its removal (Mwangi, Ngila and Ndungu 2012). Potable or drinking water is defined as having acceptable quality in terms of its physical, chemical, and bacteriological parameters so that it can be safely used for drinking and cooking (Gadgil 1998).

Historical research has proven that, over the years, several methods have been developed and implemented for the analysis of polymers in drinking water. These methods included the use of extraction-spectrophotometry, titration, fluorometry, tannic acid, methyl orange and potentiometry, to mention just a few (Becker *et al.* 2004; Jin *et al.* 2006a; Cumming *et al.* 2010; Anik *et al.* 2012; Mwangi *et al.* 2013). The other laboratory-based instrumental technique is size-exclusion chromatography but its limitation is the fact that it has high detection limits and mostly requires dedicated and skilled personnel (Majam and Thompson 2006).

1.1.3 Gold nanoparticles

Drawing from the above mentioned concern, alternative techniques, particularly in the use of nanomaterials, have been suggested in literature for the quantification of poly-DADMAC in water (Gumbi, Ngila and Ndungu 2014). According to Jongjinakool *et al.* (2014), nanotechnology deals with processes that take place on the nanometer scale, that is, from approximately 1 to 100 nm. In nanotechnology, the assembly of metallic nanoparticles has resulted in novel materials with interesting electronic, optical and chemical properties.

Gold nanoparticles (AuNPs) are the most stable metal nanoparticles. They present fascinating aspects such as their assembly of multiple types involving material science, the behavior of the individual particles, size-related electronic, magnetic and optical properties (quantum size effect). Their applications range from catalysis to biology (Daniel and Astruc 2004). These include self-assembly, biolabeling, electron-transfer theories, phase transfer, DNA melting and assays, and crystal growth (Sardar *et al.* 2009)

Of interest, gold nanoparticle colorimetric biosensors have seen significant applications in diagnostics, environmental monitoring,and antibioterrorism supporting unaided, visual readout (Xia *et al.* 2010). Among the conventional methods of synthesis of AuNPs by reduction of gold(III) derivatives, the most popular one for a long time has been that using citrate reduction of $HAuCl_4$ in water, which was introduced in 1951. It leads to AuNPs of ca. 20 nm (Daniel and Astruc 2004).

Equally important, gold nanoparticles exhibit a distinct optical feature commonly referred to as localised surface plasmon resonance (LSPR), which is a broad absorption band in the visible region around 520 nm (Sardar *et al.* 2009). The LSPR is due to the collective oscillations of the electron gas at the surface of nanoparticles (6s electrons of the conduction band for AuNPs) that is correlated with the electromagnetic field of the incoming light, i.e., the excitation of the coherent oscillation of the conduction band (Sardar *et al.* 2009). Thus, the LSPR is absent for AuNPs with core diameter less than 2 nm, as well as for bulk gold. The deep-red color of AuNP sols in water and glasses reflects this localised surface plasmon resonance (Link and El-Sayed 1999; Daniel and Astruc 2004; Sardar *et al.* 2009). This distinct optical feature is of particular interest or importance in this research because the determination is based on colour change.

1.2 Problem statement and justification

The production of safe drinking water is vital to sustain livelihoods. It is, therefore, critical to ensure that water quality is not compromised. During drinking water treatment chemicals are added to raw water to make it potable (Banerjee *et al.* 2008). However, safeguards must be sufficient to ensure that any residual amounts of these chemical by-products and other contaminants in their formulations, do not pose an unacceptable health risk (Wandrey, Hernandez-Barajas and Hunkeler 1999; Jin *et al.* 2006a; Padhye *et al.* 2011; Mwangi *et al.* 2013). More worrisome is the fact that there is evidence that several of these contaminants could have an adverse effect on the health of water consumers if ingested in sufficient quantity over time (Bruzzoniti *et al.* 2004; Cheng 2004).

For instance, reports have indicated that poly-DADMAC can degrade into a suspected carcinogenic form which is *N* -nitrosodimethylamine (NDMA) (Bolto and Gregory 2007; Park *et al.* 2009; Padhye *et al.* 2011). Consequently, the residual concentration of polyelectrolytes at various stages during the treatment process in water should be known (Majam, Jonnalagadda and Thompson 2004; Maldonado, Terán and Guzmán 2012). Moreover, the desired limit of detection for the polymers is 1 mg L^{-1}, which is 10% of the maximum permitted dose for poly-DADMAC (Majam, Jonnalagadda and Thompson 2004; Maldonado, Terán and Guzmán 2012). For this reason, a method that can determine residual poly-DADMAC concentration needs to be established. More importantly, a method that is rapid, easy and can be used onsite will be highly desirable. In this way, plant operators will have enough time to act in case of high poly-DADMAC levels in the system (Maldonado, Terán and Guzmán 2012).

Although, several analytical techniques highlighted in Section 1.1.2 have been used to quantify the amount of poly-DADMAC in water, these methods are, however, reported to be complicated, time-consuming, require several pre-treatment steps and expensive equipment (Gumbi, Ngila and Ndungu 2014). This means that the results will not be available immediately. On the contrary, with a portable meter that can accurately determine poly-DADMAC onsite at any point of the treatment process, the results will be available in time for the operators to make a decision and adjust any dosage where applicable.

1.3 Aim

The aim of this study was to fabricate a colorimetric paper-based microfluidic sensor for the detection of residual poly-DADMAC in water treatment.

1.4 Objectives of the study

- To develop and optimise a method using the UV spectrophotometer for the determination of poly-DADMAC.
- To synthesise and characterise gold nanoparticles.
- To quantitatively determine poly-DADMAC in water by UV spectrophotometer using the synthesized gold nanoparticles. To fabricate and assemble the portable colorimetric device and compare results to those obtained from the UV spectrophotometer.
- To optimise CIEL*a*b*/Yxy colour system for the detection of poly-DADMAC in water.

1.5 Rationale/Significance of the study

The increase in population and improved lifestyles demands for the supply of quality water. Recently, South Africa has been reported to face a serious drought problem. This is not only unique to the Southern region of the continent, it is a world crisis. The demand comes with uncertainty of supplying high quality drinking water at lower cost (cheaper water treatment methods and less laboratory-based testing techniques). In general, the processes involved in drinking water treatment plants consist of coagulation, flocculation, sedimentation, filtration and disinfection. This, therefore, requires very expensive analytical methods to quantify residues of chemicals used during these processes (Bolto 1995).

Current development in nanotechnology involves the application of colorimetric sensors and focuses on gold and silver nanoparticles as they have desirable optical properties (Gumbi, Ngila and Ndungu 2014; Laliwala *et al.* 2014; Li *et al.* 2014; Li *et al.* 2015). The colorimetric detection system for these nanomaterials primarily exploits aggregation which is normally triggered by analytes of interest. The novelty of this work lies in the fabrication of a AuNPs based Lovibond comparator with a colour filter disk for colorimetric screening of *poly-DADMAC* in raw and potable water. The Lovibond comparator is an instrument which was designed way back in the 1800s by Lovibond (Lovibond 1887; Lovibond 1890a).

It is generally used to measure the concentration of the analyte based on the visual comparison of coloured glass or plastic filters that are calibrated for measurement of different concentration levels (Lovibond 1890a). These filters are normally fabricated with organic indicators and ligands which are used to form a detectable colour indicator for an analyte of interest. This is the first report which covers the fabrication of Lovibond comparator colour filters using citrate stabilised AuNPs for colorimetric detection of poly-DADMAC in potable water.

1.6 Dissertation outline

This dissertation is presented in five chapters as described below:

Chapter 1

This chapter covers the introduction, problem statement, aims and objectives of this study. Also covered in this chapter is the Rationale and significance of the work.

Chapter 2

This chapter outlines a literature review of the overall study. This includes detailed literature of poly-DADMAC as a coagulation and flocculation aid in water treatment. Different analysis methods that exist for these determinations are discussed. This chapter also include the background information of the gold nanoparticles synthesis and characterisation techniques used in this study. Optimisation of CIEL*a*b*/Yxy colour system for colorimetric devices fabricated with gold nanoparticles will also be covered in this chapter.

Chapter 3

The detailed experimental procedure for the synthesis and characterisation of gold nanoparticles will be covered in this chapter. This chapter will also include the development of the UV/VIS method, analysis using the portable Lovibond device and optimisation of CIEL*a*b*/Yxy colour system for colorimetric devices fabricated with gold nanoparticles.

Detailed sampling points will also be covered. This includes the different plants that were used and the different stages in the water treatment process where samples were actually taken.

Chapter 4

This chapter outlines the results and discussion obtained from the study of the poly-DADMAC polymer. Included in this chapter are the results for the use and characterisation of gold nanoparticles including their application in the determination of poly-DADMAC in raw and potable water. Results and discussion from the analysis using the portable Lovibond device and optimisation of CIEL*a*b*/Yxy colour system will also be discussed in this chapter.

Chapter 5

This chapter highlights the important achievements, conclusions, and recommendation of this study.

The references that were used to source information for this study are included at the end of chapter 5. An Annexure for the results of the physical-chemical tests are also available after the references.

Chapter Two: Literature review

This chapter reviews the literature related to the use of poly-DADMAC in water treatment. The review presents an overview of the water treatment process. Thereafter the application of poly-DADMAC as a coagulation and flocculation aid in water treatment will follow.

This chapter will be divided into seven sections. Section One explains the importance of water quality. Here, the various treatment steps will be explained. The use of poly-DADMAC is critical in ensuring that the water quality is at an acceptable standard. Section Two discusses the application of poly-DADMAC in water treatment steps. Proper dosing of poly-DADMAC is essential in ensuring that coagulation and flocculation occur as required. Section Three evaluates the different analytical methods for quantifying poly-DADMAC in water. Accurate quantification of poly-DADMAC in the final treated water is of utmost importance in order to ensure that it does not exceed the set limit. Hence, this discussion will highlight the shortcomings of these methods and subsequently the need for a more-effective method.

Section Four introduces gold nanoparticles as the best method of quantifying poly-DADMAC in water. Here the properties of gold nanoparticles will be elaborated. These parameters play a critical role in colour development which is the crux of this research. Section Five describes the method of synthesising gold nanoparticles. The use of citrate reduction in the synthesis of gold nanoparticles and the characterisation techniques will be detailed. Section Six detailed the optimisation of the synthesised gold nanoparticles using colour. Here, the CIEL*a*b*/Yxy colour system for colorimetric devices fabricated with gold nanoparticles will be detailed. Section Seven introduces the Lovibond comparator as it is the underpinning concept in the design of a potable and rapid onsite device for the quantification of poly-DADMAC in water.

2.1 Water treatment

Increasing demand and shortage of clean water sources due to the rapid development of industrialisation, population growth and long-term droughts have become an issue worldwide (Chong *et al.* 2010). Drinking water is water whose quality must ensure the perfect health of the end user. Safe drinking-water is a basic need for human development, health and well-being, and, because of this, it is an internationally accepted human right (Thompson *et al.* 2007). Taken from the natural environment, it must undergo treatments that will render it apt for human consumption (Mwangi, Ngila and Ndungu 2012).

During water treatment, incoming raw water is first chlorinated to minimise the growth of fouling organisms on the pipe-work and tanks. The second step is coagulation. Coagulation is an essential mechanism for removing suspended colloidal particles which can cause objectionable turbidity, colour, taste and odour in drinking water **(Figure 2-1)**. It is accomplished by adding special chemical reagents to the raw water (Mwangi, Ngila and Ndungu 2012).

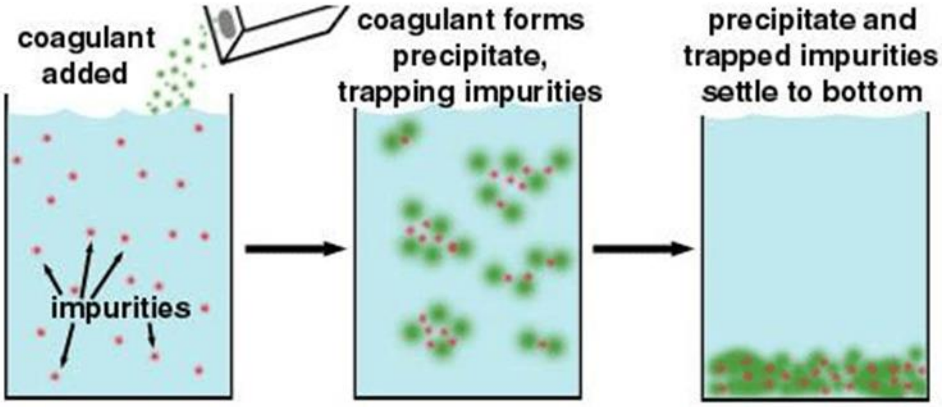


Figure 2- 1: Coagulation process. Source (Olanrewaju *et al.* 2012)

In more detail, coagulation is the process whereby the destabilisation of a given colloidal suspension or solution takes place (Tzoupanos and Zouboulis 2008). The early report of Tzoupanos and Zouboulis (2008) noted that the primary function of coagulation is to overcome the factors that may contribute to the stability of a given water system. The authors revealed that coagulation could be achieve by the use of appropriate coagulants such as aluminium or iron salts. As shown in **Figure 2-2**, coagulation occurs quite early in the process.

Apart from coagulation, flocculation is another important step for the removal of solid particles during water treatment process. Tzoupanos and Zouboulis (2008) refer to flocculation as the induction of destabilised particles in order to come together by forming large agglomerates of particles. Owing to this process, it becomes easier to separate the particles through gravity settling. Moreover, other study (Thompson *et al.* 2007) reported that coagulation together with flocculation are important barriers to microbiological contaminants. Hence, it can be assumed that both techniques are key processes for reducing naturally occurring organic matter and turbidity, which can seriously affect the efficiency of the disinfection process (Thompson *et al.* 2007). Nonetheless, study has shown that coagulation usually completes in a very short period of time (e.g. about 10 s), whereas flocculation occurs usually over a period of 20 to 45 min (Tzoupanos and Zouboulis 2008).

Sedimentation is a process whereby flocs are allowed to settle to the bottom. This occurs in a large tank with low water velocities. Filtration is the final step to remove remaining suspended particles and unsettled flocs (Charles 1998). **Figure 2-2** further depicts the water Treatment Process steps.

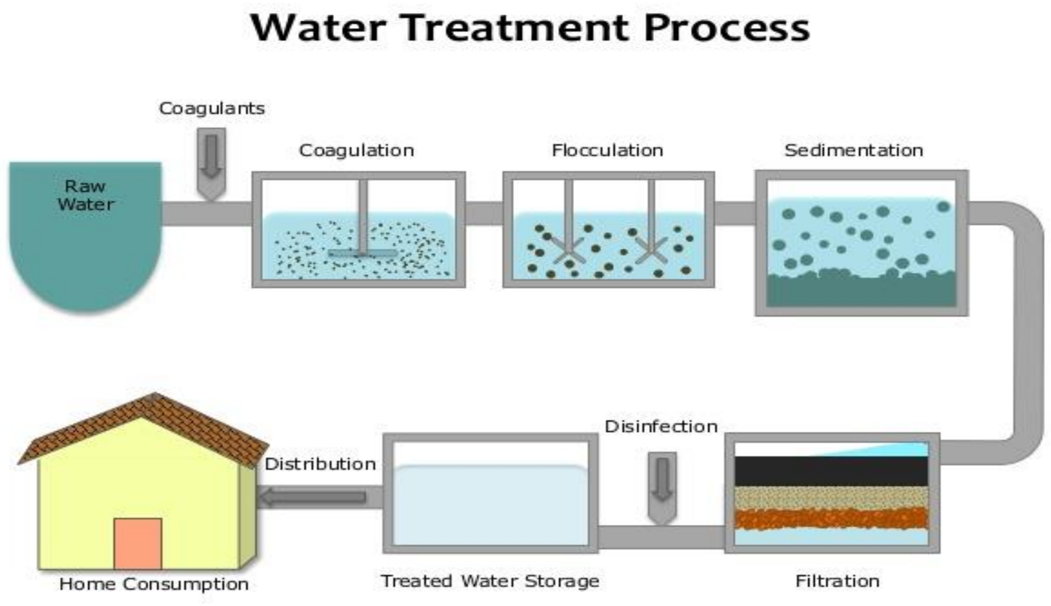


Figure 2- 2: Water treatment process (Source (Mootoor, Oliveira and Patel 2015))

In recent decades, it has been reported in literature that organic polyelectrolytes such as the poly-DADMAC polymer aid greatly in flocculation and coagulation during the treatment process (Bolto and Gregory 2007; Tzoupanos and Zouboulis 2008; Oladoja 2016). This tribute could be due to the numerous sites on the polymer chain which can absorb additional colloidal particles so that a bridging action occurs, and thus precipitation is not impeded (Majam, Jonnalagadda and Thompson 2004). More so, it has been observed that the monomeric units of the polyelectrolytes act as charge neutralising agents by strongly adsorbing onto the surface of certain constituents in raw water (Majam, Jonnalagadda and Thompson 2004). The use of polyelectrolyte in water treatment will lead to the departure for the next section.

2.2 Organic polymers used in water treatment

Historical evidence suggests that synthetic water soluble polymers were developed over 40 decades ago in the 1950s. Importantly, John (2008) considers this development as one of the outstanding breakthroughs in water treatment . Organic polymers are commonly used as primary coagulants or coagulant aids in water treatment. These polymers are commonly referred to as polyelectrolytes due to their ionic nature (Mallevialle, Bruchet and Fiessinger 1984; Gregor, Simpson and Andrew 1993; Maldonado, Terán and Guzmán 2012). Furthermore, the characterisation of polymers is done based on their ionic nature. They can be cationic, anionic or nonionic (Bolto and Gregory 2007). Therefore, a polymer may carry a negative charge (anionic), a positive charge (cationic) or no charge (nonionic) (Gregor, Simpson and Andrew 1993; John 2008).

In a nutshell, cationic polyelectrolytes are polymers that produce positively charged ions when dissolved in water, anionic polymers produce negatively charged ions when dissolved in water and nonionic polyelectrolytes have a balanced or neutral charge (Majam and Thompson 2006; Tzoupanos and Zouboulis 2008). John (John 2008) therefore states that, based on its ionic nature and structure, poly-DADMAC is characterised as a cationic polymer with repeating pyrollidine rings as shown in **Figure 2-3**. The existence of the pyrollidine rings was revealed by the ^{13}C NMR spectroscopic studies conducted on the synthesised product (John *et al.* 2002).

Reports state that poly-DADMAC is the very first quaternary ammonium cationic polymer to be approved by the United States Food and Drug Administration for potable waters (Park *et al.* 2007).

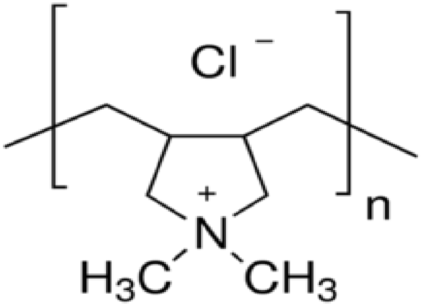


Figure 2- 3: Structure of poly-DADMAC. “n” represent the number of pyrollidine rings

2.2.1 Synthesis of poly-DADMAC

Figure 2-4 depicts the reaction scheme for the synthesis of poly-DADMAC. The first step in the synthesis of poly-DADMAC is the preparation of the Diallyldimethylammonium chloride monomer (mDADMAC) which results from the reaction of allyl chloride with dimethylamine. The non-conjugated diene of DADMAC then undergoes cyclopolymerisation to form a 5-membered ring of poly-DADMAC. The last step is the polymerisation of the monomer by free-radical initiated addition polymerisation (Nozaic, Freese and Thompson 2000; Nozaic *et al.* 2001; Park *et al.* 2007; John 2008).

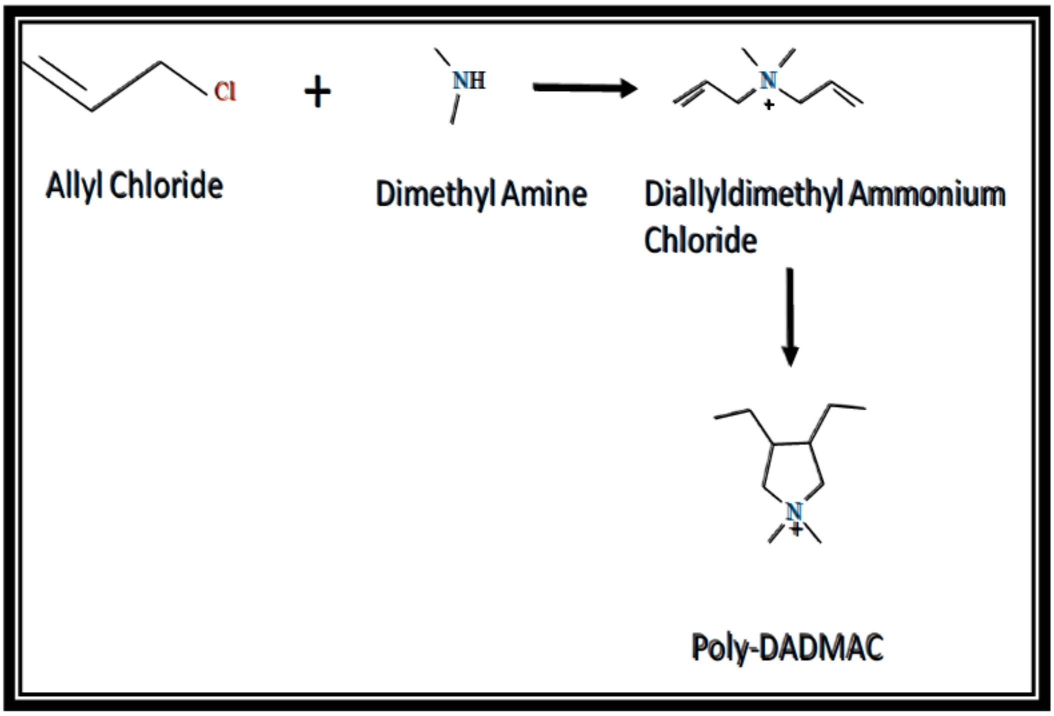


Figure 2- 4: Reaction scheme for the synthesis of poly-DADMAC

2.2.2 Properties of poly-DADMAC

There are two key characteristics that are always cited for organic polyelectrolytes such as poly-DADMAC. These characteristics are molecular weight and amount (or, perhaps, only the type) of ionic charge on the molecule (Hahn and Klute 2012). These polyelectrolyte coagulants used in water treatment are synthetic organic compounds of high molecular-weight and have a strong tendency to be adsorbed on the surfaces of the panicles in an aqueous suspension (Chang *et al.* 1999).

The monomer units of polymers are responsible for one of the useful properties of polymers such as poly-DADMAC. When dissolved in water, these polymers produce highly charged monomers, namely, the DADMAC monomer. (Majam and Thompson 2006; Hahn and Klute 2012). As a result, the DADMAC monomer in poly-DADMAC has a high cationic charge. This is why it is used in the coagulation process during water treatment (Hahn and Klute 2012). The suspended and colloidal solids found in water are generally negatively charged (Murcott and Harleman 1996; Majam and Thompson 2006). As a result, during coagulation, the monomeric units that these polyelectrolytes possess act as charge neutralising agents by strongly adsorbing onto the surface of certain constituents in raw water (Gregor, Simpson and Andrew 1993; Bolto *et al.* 1998; Chang *et al.* 1999; Majam, Jonnalagadda and Thompson 2004; Mwangi, Ngila and Ndungu 2012).

Polyelectrolytes such as poly-DADMAC are water soluble organic polymers that are used as primary coagulants in the treatment of drinking water (Majam, Jonnalagadda and Thompson 2004; Mwangi, Ngila and Ndungu 2012). These polymers are well known to be the most chlorine resistant and operate over a wide pH range (John 2008).

Structurally, the bending poly-DADMAC chain encloses more reaction sites inside the polymer chain. This therefore decreases the chance of the polymer chain to meet other species in the solution. The fact that poly-DADMAC is rigid leads to the conclusion that the polymer will be less active in solution due to limited mobility (Park et al. 2007). Organic polymers have high molecular weight (MW). This is the reason for their high efficiency which implies better flocculation properties (Tzoupanos and Zouboulis 2008). This therefore leads to improved coagulation and flocculation.

2.2.3 Advantages of using poly-DADMAC in water treatment

The use of organic polymers such as poly-DADMAC in water treatment has become increasingly popular in South Africa and world-wide because of the numerous advantages these polymers possess as opposed to the traditional hydrolysing reagents (Polasek and Muti 2002; John 2008).

During their use as either coagulants or coagulant aids, these polymers can improve the quality of the finished water due to the formation of large, more settleable floc, or a stronger and more filterable floc (Sacks *et al.* 1983). This leads to an increase in process stability which is a useful factor especially for cases where the quality of raw water varies (John 2008).

As stated in the report by one of South Africa's big water utilities (Nozaic *et al.* 2001), the cost of coagulation using these polymers was significantly lower than when using inorganic coagulants like aluminium sulphate and ferric chloride. This is due to reduced chemical usage which further reduces chemical handling and storage (Bolto *et al.* 1998; Nozaic, Freese and Thompson 2000). Polymer dosages of 2 to 10% of the quantity of inorganic coagulants are required to achieve equivalent coagulation. This therefore leads to reduced production costs (Nozaic, Freese and Thompson 2000; Polasek and Muti 2002; John 2008; Tzoupanos and Zouboulis 2008).

Furthermore, these polymers do not produce a significant amount of dissolved solids as compared to inorganic coagulants and do not introduce metal ions (Bolto *et al.* 1998; Nozaic, Freese and Thompson 2000; Mwangi, Ngila and Ndungu 2012).

There is a reduction in sludge volume with higher settling rates, which in turn results in an increase in plant capacity (Bolto *et al.* 1998; Edzwald, Pernitsky and Parmenter 2000; John 2008; Mwangi, Ngila and Ndungu 2012). This reduced sludge volume results in a reduction in the frequency of de-sludging the clarifiers (Nozaic *et al.* 2001). In addition to the reduced sludge volume, polymer sludge that is produced tends to be readily dewatered to produce a high solids content sludge (Gregor, Simpson and Andrew 1993; Nozaic, Freese and Thompson 2000; Nozaic *et al.* 2001).

Equally important, polyelectrolytes play a vital role in removing numerous potentially harmful water contaminants. These include suspended sediments, micro-organisms, inorganic and dissolved organics (Lurie, Rebhun and technology 1997).

Sedimentation carryover is reduced, enabling longer filter runs and reduced backwashing (John 2008).

With inorganic coagulants, pH adjustment is often required at 2 points. The first adjustment is done at the head of works for optimum floc formation and after filtration for the reduction of corrosive potential in water. In contrast, organic polymers are not sensitive to pH. This therefore means that pH correction can only be done at the beginning of the process and no post correction is required (Nozaic *et al.* 2001). This basically means that polymers like poly-DADMAC can perform well at any pH and this is an advantage in certain applications as well (Bolto *et al.* 1998; Polasek and Muti 2002; Gebbie 2006; Matilainen *et al.* 2010; Mwangi, Ngila and Ndungu 2012). This, in turn, makes these polymers user-friendly (Polasek and Muti 2002).

In addition, polymers make the use of automatic dosage control possible, with the use of the streaming current detector and feedback control loop (Nozaic, Freese and Thompson 2000; John 2008). These polymers are generally more efficient in lower dosages, wide range of pH, temperature and colloid concentration (Tzoupanos and Zouboulis 2008; Matilainen *et al.* 2010). Moreover, they are chlorine resistant (Nozaic, Freese and Thompson 2000).

2.2.4 Disadvantages of using poly-DADMAC in water treatment

With all the above-mentioned advantages, the use of polymers in water treatment is not entirely without any negative issues. However, the pros greatly outweigh the cons. For this reason, polymers still remain the products of choice for most water and wastewater treatment applications (John 2008).

The removal of turbidity is important in water treatment. Polymers are less efficient in removing turbidity as compared to inorganic coagulants (John 2008). According to the drinking water standard, the turbidity of drinking water must be less than 1 NTU (Standards 2011). The turbidity of water treated with polymers is in the range of 0.2-0.3 NTU. However, for inorganic coagulants such as aluminium sulphate, the obtained turbidity results are below 0.1 NTU (Nozaic *et al.* 2001; John 2008).

The optimum dosage range for polymers is very narrow as compared to inorganic coagulants. This narrow range has a huge effect on turbidity. Under-dosing leads to incomplete particle destabilisation and overdosing leads to re-stabilisation (Majam and Thompson 2006; John 2008). Both cases give rise to high turbidity (John 2008). This therefore means that there is greater sensitivity to incorrect dosages applied (Nozaic *et al.* 2001; John 2008).

Furthermore, at worst, overdosing could result in damage to the processing equipment, particularly in the case of processes involving the use of membranes. This, in turn, stresses the need for the development of improved methods of monitoring and controlling the concentration of water treatment agents in process water (Sethumadhavan and Johnson 2006). More worrisome is the fact that, during water treatment, residual amounts of these polyelectrolytes (unreacted monomers) may persist in solution after the filtration stage. This in turn leads to residual toxicity in the treated water sample (Majam, Jonnalagadda and Thompson 2004; Tzoupanos and Zouboulis 2008). This concern will be addressed in detail in the next section.

2.2.5 Health concerns of residual poly-DADMAC in treated water

Polyelectrolyte formulations contain contaminants from the manufacturing process itself (Mallevialle, Bruchet and Fiessinger 1984; Chang *et al.* 1999; Majam, Jonnalagadda and Thompson 2004). These contaminants are residual monomers, other reactants and reaction by-products. These could potentially affect human health (Mallevialle, Bruchet and Fiessinger 1984; Chang *et al.* 1999; Majam and Thompson 2006). Furthermore, polymers typically contain residual starting materials and oligomers due to polymerisation (Park *et al.* 2009).

Water disinfection is one of the most crucial public health advances of the last century (*Guidelines for drinking-water quality* 2004; Sadiq and Rodriguez 2004; Richardson *et al.* 2007). The destruction of microbial pathogens is essential and involves the use of reactive chemical agents such as chlorine during a process called chlorination (*Guidelines for drinking-water quality* 2004). Chlorination is one of the most widely used disinfection processes in water treatment plants around the world to ensure a safe drinking water (Badawy *et al.* 2012).

However, there has been a concern over disinfection by-products (Mallevialle, Bruchet and Fiessinger 1984; Binnie *et al.* 2002). Numerous cationic polymers such as poly-DADMAC were identified as potential N-Nitrosodimethylamine (NDMA) precursors during water treatment in water that contained chlorine or chloramines or in chlorine-based disinfection processes (Park *et al.* 2007; Park *et al.* 2009). See **Figure 2-5** for the chemical structure of NDMA.

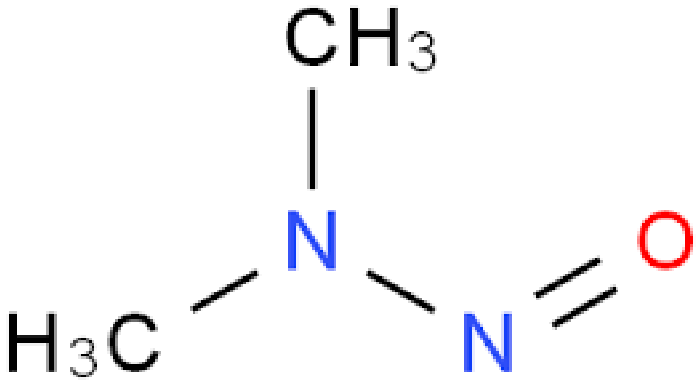


Figure 2- 5: Chemical structure of N-Nitrosodimethylamine (NDMA)

Chlorination of backwash filters fed with poly-DADMAC-coagulated water also formed elevated levels of NDMA (Mitch *et al.* 2003; Krasner *et al.* 2013). It was noted as well that the degradation of these polyelectrolytes in drinking water reservoirs may occur due to interaction with chemicals such as chlorine (Park *et al.* 2007).

NDMA was classified by the United States Environmental Protection Agency (USEPA) as a carcinogen (Choi and Valentine 2002; Park *et al.* 2007). It is actually a member of a family of extremely potent carcinogens, the *N*-nitrosamines (Mitch *et al.* 2003). In fact, their cancer potencies are much more higher than those of the trihalomethanes (Mitch *et al.* 2003).

Several water utilities in Canada and the United States had reported the occurrence of NDMA in the drinking water supplies. As a result, NDMA was identified as a drinking water contaminant (Park *et al.* 2007) and a disinfection by-product (DBP)

(Chang *et al.* 1999; Park *et al.* 2009). This, in turn, has caused drinking water supplies and treatment plants to become a concern (Binnie *et al.* 2002; Park *et al.* 2007). Due to concerns about certain hazardous contaminants in the treated water, the use of polyelectrolytes in the drinking water treatment is not permitted in Switzerland and Japan, Germany and France have established stringent limits on application rates of polyelectrolytes in water treatment plants (Chang *et al.* 1999).

For this reason, the presence of residual polymers in the treated water has encouraged attempts to quantify the amount of polyelectrolyte in various aqueous environments i.e. finished water, sludge, backwash waters etc (Majam and Thompson 2006). However, having established all these facts, the World Health Organisation makes it clear that disinfection should not be compromised in attempting to control disinfection by-products (DBPs) (*Guidelines for drinking-water quality* 2004).

2.3 Analysis methods for poly-DADMAC

To monitor residual concentrations of polyelectrolytes in water treatment, a selective and sensitive analytical method is needed. A number of methods were developed in the past but had some limitations (Gumbi, Ngila and Ndungu 2014).

Historical research proved that, over the years, a number of methods have been developed and implemented for the analysis of polymers. These methods included the use of extraction-spectrophotometry, polarography, two-phased titration, flow injection analysis and potentiometry to mention just a few (John 2008; Maldonado, Terán and Guzmán 2012). However, they produced poor linearity, sensitivity and precision, or produced false positives due to matrix effects (John 2008).

Another laboratory-based instrumental technique that was used for determining poly-DADMAC in water is HPLC (High Performance Liquid Chromatography) using a size exclusion column (Majam and Thompson 2006; Maldonado, Terán and Guzmán 2012). However, it produced a high detection limit of 4300 mg L^{-1} (Majam and Thompson 2006). For quantificaion of residual polymers in water to be successful, the desired detection limit is 1 mg L^{-1} which is 10 % of the maximum permitted dose for polydadmac or lower (Majam and Thompson 2006). Equally concerning, most of these instruments are operated in the laboratory setting and require skilled personnel (Majam, Jonnalagadda and Thompson 2004; Majam and Thompson 2006; John 2008; Gumbi, Ngila and Ndungu 2014).

The Tannic acid method yielded bad linearity and there were significant fluctuations in the results of tap water (Majam and Thompson 2006). The Rose Bengal method produced poor linearity and there was evidence of an unwanted complex forming (John 2008). The methyl orange method involves the complexation of polydadmac with methyl orange (John 2008). Further to this, the above mentioned method yielded poor linearity and low absorbances (John 2008).

Fluorescent tagging is another method that was used for determination of residual polydadmac in treated water (Mwangi, Ngila and Ndungu 2012). However, it yielded a detection limit of 10–40 mg L^{-1} therefore not suitable for trace-level analysis. Quantitative determination was also done by 1H NMR spectroscopy. However, this was still not successful due to poor sensitivity (Mwangi, Ngila and Ndungu 2012).

Another research group looked at spectrophotometric detection of poly-DADMAC by exploiting the flocculation properties of poly-DADMAC with the formation of simple and convenient ion association flocculants. The polyelectrolyte was reacted with (4-hydroxy-1-napthylazo) benzene-sulfonic acid which forms a coloured colloid ion pair, followed by the determination by UV spectrophotometry (Gumbi, Ngila and Ndungu 2014).The method had a linear range of 0.1 – 1.8 mg L^{-1} and a detection limit of 0.07 mg L^{-1} (Mwangi, Ngila and Ndungu 2012).

Another group of authors reported the use of fluorescent tagging for the quantification of poly-DADMAC in water (Becker *et al.* 2004). They used pre- and post fluorescent tagging of poly-DADMAC to determine low levels of the polymer in various water samples (Becker *et al.* 2004). This method yielded detection limits of 10 – 40 mg L^{-1}.

These methods are either complicated, require several pre-treatment steps, expensive or very time consuming., They may, therefore, not be suitable for routine analysis of poly-DADMAC in water (Gumbi, Ngila and Ndungu 2014).

On the contrary, colloidal titration is another method that is reported to be simple and seemed to be satisfactory (John 2008). John (2008) noted that colloidal titration is one of the cheapest and relatively cheapest methods for this analysis. According to his report, colloidal titration is based on the reaction between cationic and anionic polyelectrolytes. When mixed in solution together, a charge neutralisation reaction will occur and the reaction is stoichiometric in the sense of 1:1 charge compensation.

The determination of the concentration relies on the detection of the end-point and the validity of the assumption of the 1:1 stoichiometry. End point can be detected visually or by using a spectrophotometer. Visual detection has led to under- or over-estimation of the concentration. This method of using metachromatic dyes improved the end-point detection and detection limits when using UV-VIS spectrophotometer. This is known as metachromatic titration (John 2008).

However, with all this improvement the following problems still exist. It is documented in the literature that during metachromatic titration, a small volume of indicator is used for the analysis (Bond, Templeton and Graham 2012; Gumbi 2013). During such titrations positive ionic species are known to interfere with the analysis (Shah and Mitch 2011). This results in precipitation reactions between the titre, titrant and the indicator during titration due to the small volumes that are used. This leads to inaccurate results (Gumbi 2013). The only way to overcome this is to increase the volume of the indicator (John 2008). The aforementioned precipitation and small volume issues justify the need for this problem to be addressed. This method will therefore be further investigated and improved in this study.

2.4 Gold nanoparticles

Section 3 has basically confirmed the fact the only method that proved to be satisfactory is the colloidal titration. However, weakness of this method is the use of indicators and the problems that come with it. The solution to this weakness is the use of gold nanoparticles.

Gold nanoparticle chemistry and physics have emerged as a broad new sub-discipline in the domain of colloids and surfaces. The unusual optical properties of small gold particles, their size dependent electrochemistry, and high chemical stability have made them the model system of choice for exploring a wide range of phenomena including self-assembly, biolabeling, catalysis, electron-transfer theories, phase transfer, DNA melting and assays, and crystal growth (Sardar *et al.* 2009). The electronic and optical properties of nanoparticles are determined by both their size and shape. Metal nanoparticles have mainly been studied because of their unique optical properties (Link and El-Sayed 1999).

As briefly stated Chapter 1, Section 1.3, gold nanoparticles (AuNPs) have unique and very interesting physical–chemical properties. In addition to this, their optical properties are of interest to this present study. Gumbi, Ngila and Ndungu (2014) point out that, like other metal nanoparticles, AuNPs undergo plasmon resonance. As depicted in **Figure 2-6**, plasmon resonance occurs when the frequency of the incident electromagnetic radiation resonates with the oscillation of the delocalised electron cloud present on the nanoparticle surface. This localised surface plasmon resonance frequency lies in the visible range for AuNPs. This LSPR is very sensitive to: (i) the diameter of the AuNPs, (ii) the surrounding surface chemistry, and (iii) the aggregation of AuNPs. For this reason, it has found use as a probe for the detection of large and small bio-molecules, various organic molecules and some inorganic ions (Liu *et al.* 2012; Gumbi, Ngila and Ndungu 2014).

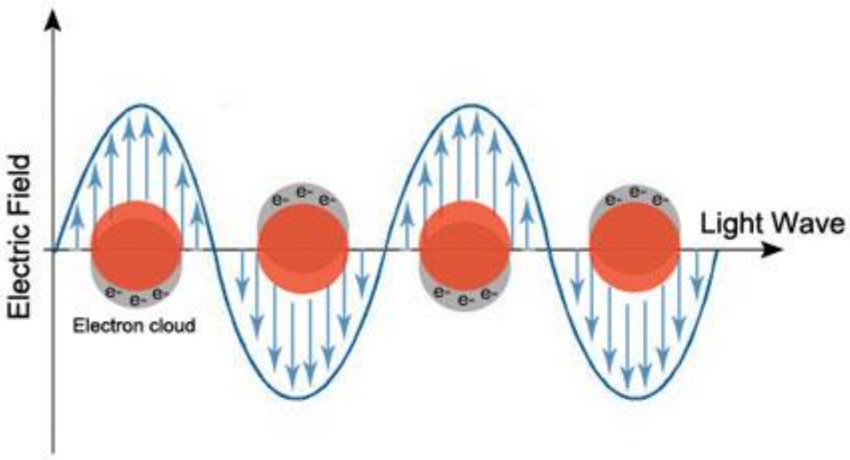


Figure 2- 6: Basics of localized surface plasmon resonance (LSPR) of gold nanoparticles due to collective oscillation of surface electrons with incident light at a specific wavelength (DATTILA 2017)

Of significance, Khosroshahi (2017) claims that optical properties of AuNPs are tuneable. The author revealed this could be achieved by modifying its size, shape, surface chemistry, or aggregation state. For example, Polte *et al* demonstrated that for small monodisperse AuNPs (~30 nm), the LSPR causes an absorption of light in the blue-green portion of the spectrum (~450 nm) while red light (~700 nm) is reflected, yielding a rich red colour (Polte *et al.* 2010) .

Equally essential, Verma, Singh and Chavan (2014) observed that as particle size increases, the wavelength of LSPR related absorption shifts to longer, redder wavelengths (**Figure 2-7**). Consequently, and as shown in **Figure 2-8**, the red light is absorbed, and blue light is reflected, yielding solutions with a pale blue or purple colour (Verma, Singh and Chavan 2014). As particle size continues to increase toward the bulk limit, LSPR wavelengths move into the IR portion of the spectrum and most visible wavelengths are reflected, giving the nanoparticles clear or translucent colour (Merza *et al.* 2012).

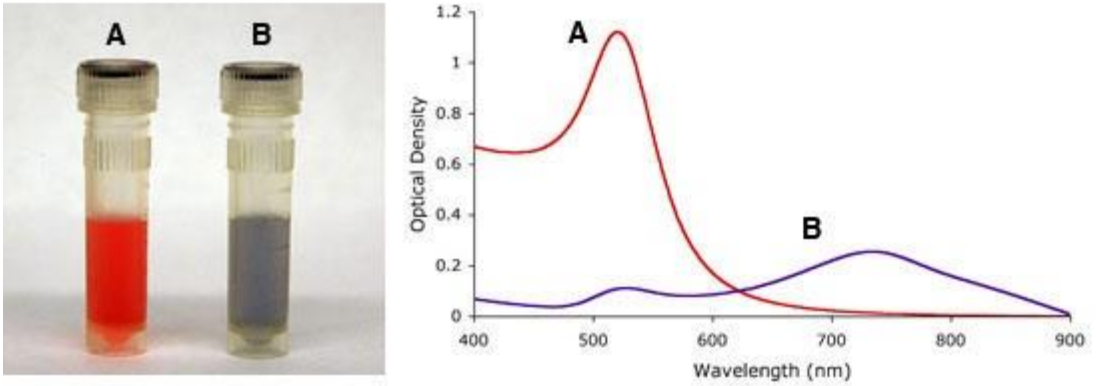


Figure 2- 7: Visual appearance and UV-VIS spectra of monodisperse (A) and sodium chloride (NaCl) induced agglomeration (B) of 15 nm gold nanoparticles (Source (Cytodiagnostics 2019))

Figure 2- 8: Colours of various sized monodispersed gold nanoparticles (Source (Sigma-Aldrich 2019))

Moreover, it is generally acknowledged in the literature that the properties of materials change at the nanoscale (Eustis and El-Sayed 2006; Yang *et al.* 2015; Demkowicz 2019). For instance, it is widely reported that the element of gold is gold in colour when in their bulk or macroscale (Eustis and El-Sayed 2006). In contrast, the colour characteristic of gold is red or purple depending on the aggregation when in nanoscale (Eustis and El-Sayed 2006). Owing to this unique attribute, the formation of gold nanoparticles can, therefore, be observed by a change in colour since small nanoparticles of gold are red (Eustis and El-Sayed 2006).

2.5 Methods for the synthesis of gold nanoparticles

Nanotechnology is the science of the small, the very small. It basically deals with processes that take place on the nanometer scale, that is, from approximately 1 to 100 nm (Wang 2018). It is the use and manipulation of matter at a tiny scale. At this size, atoms and molecules work differently, and provide a variety of surprising and interesting uses (Jongjinakool *et al.* 2014). Although established over two decades ago, nanotechnology still remains a fascinating area of research for the development and application of novel and useful materials (Wang 2018).

While nanotechnology has found usefulness in various areas of research and academic study, current development in nanotechnology involves the application of colorimetric sensors focus on gold and silver nanoparticles as they have excellent optical properties (Magubane *et al.* 2019b). Importantly, nanoparticles such as AuNPs with diameter from 15 – 20 nm can be generated by the reduction of auric-chloride with tri-sodium citrate (Verma, Singh and Chavan 2014). Particularly, AuNPs of 15 – 20 nm size range have attracted attention for fabrication of smart sensing devices in biomedical sciences as diagnostic tools (Wang 2018).

According to Verma, Singh and Chavan (2014), citrate capped AuNPs are negatively charged, and thus could be exploited for electrostatic interactions with some positively charged biomolecules like antibodies. When it comes to the synthesis of AuNPs and to match the enlarging demand of AuNPs, Verma, Singh and Chavan (2014) had advised that more attention should be paid on the effective synthesis method to match the enlarging demand of AuNPs. Cognisance of the aforementioned authors' advice will be considered when synthesising AuNPs.

While many methods have been developed for this synthesis in the past studies, (Haiss *et al.* 2007; Verma, Singh and Chavan 2014) however argued that the citrate reduction method has remained the best option to fit the enlarging demand of AuNPs. Moreover, citrate reduction has an advantage over other techniques in that it is relatively inexpensive, reductant and there is low pollution in the reaction (Haiss *et al.* 2007; Verma, Singh and Chavan 2014). Of interest, and highly relevant to this study, Verma, Singh and Chavan (2014) hinted that rapidly pouring a certain amount of tri-sodium citrate solution into a boiling solution of 10 - 25 mM chloroauric acid produces narrowly distributed AuNPs. Ultimately, the AuNPs synthesised this way are noted to be biocompatible and easily handled in method applications.

Furthermore, in the citrate reduction method, before the addition of the reducing agent, the gold is in solution in the Au^{+3} form. When the reducing agent is added, gold atoms are formed in the solution, and their concentration rises rapidly until the solution exceeds saturation. That is when particles then form in a process called nucleation (**Figure 2-9**). The remaining dissolved gold atoms bind to the nucleation sites and growth occurs (Polte *et al.* 2010; Merza *et al.* 2012).

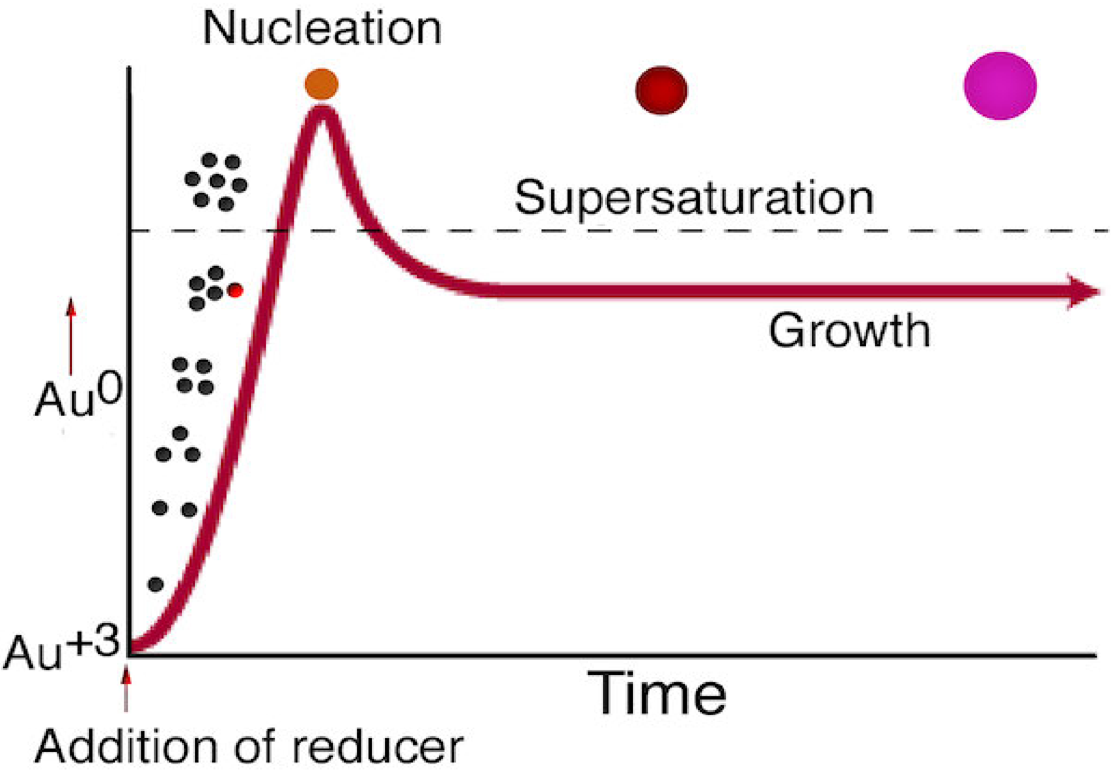


Figure 2- 9: Nucleation (Source (MRSEC EDUCATION GROUP 2019))

2.6 Colour optimisation

There is currently a steady increase in research to design microfluidic paper-based (µPAD) analytical and diagnostic devices since the initial report in 2007 by Whiteside's group (Martinez *et al.* 2007; Carrilho, Martinez and Whitesides 2009a). As a result of this report, there is growing interest to many researchers to try and improve the fabrication of these devices. Thus, the µPADs have gained a great prospective in many applications, especially in resource-limited areas. Such interest is due to the fact that they can easily be fabricated and require no sophisticated laboratory and equipment setting to operate (Dungchai, Chailapakul and Henry 2011).

Of interest, there are many fabrication techniques which have been reported, such as photolithography (Martinez *et al.* 2009), screen printing (Dungchai, Chailapakul and Henry 2009), wax printing (Karita and Kaneta 2014), and cutting (Carrilho, Martinez and Whitesides 2009b). These µPADs have been designed to use different detection systems, among those include, colorimetric (Martinez *et al.* 2009), fluorescence (Thom *et al.* 2014), electrochemical (Dungchai, Chailapakul and Henry 2010). Some of these detection systems, such as the colorimetric methods, can easily be imaged using portable smartphone or digital camera because they can easily be designed to suit integrated smartphones' applications (Lopez-Ruiz *et al.* 2014).

It has been demonstrated that the digital image analysis using digital camera or a smartphone could allow the quantitative analysis of poly-DADMAC by reading out the colour information, especially the RGB value of the detection zone of µPAD. The RGB values provided the information of the analyte concentration based on the RGB channel or grey scale value. This gives the concentration of the analyte based on the corresponding grey scale values (Martinez *et al.* 2009; Vella *et al.* 2012; Sicard *et al.* 2015; Kim, Koo and Yun 2017). Moreover, there has been an increase in the development of the different smartphone applications which are used for image analysis (Lopez-Ruiz *et al.* 2014). These systems have been demonstrated as good devices for application at point-of-care and for limited resource environment (Kanchi *et al.* 2018). However, the grey scale measurement has some limitation in quantitative analysis because of the variation of colour with time. Thus, it is always advisable that image analysis time is optimised before these detection systems are sent for any external validation (Magubane *et al.* 2019a).

In order to improve grey scale value of µPAD system, herein, this study reports on the application of gold nanoparticles for developing simple CIE L*a*b* devices for detection poly-DADMAC in potable waters using the µPAD system (Magubane *et al.* 2019a). Gold nanoparticles and other noble metals such as silver nanoparticles have shown an increased use as chemical sensors in colorimetric devices especially for the detection of various analytes in the environmental, biological and pharmaceutical samples (Zhou, Yang and Xu 2012; Jongjinakool *et al.* 2014).

Their applications as colorimetric sensors are owed upon their optical and electronic properties which is called localised surface plasmon resonance (LSPR). This LSPR is one of the unique properties of these metals making them to be easily applied as colorimetric sensors (Magubane *et al.* 2019a). The LSPR is generated by the oscillations of electrons in their conduction band which is normally induced on the surface of the metal when they interact with electromagnetic waves. Thus, this interaction provides the change in colour of noble metal which is dependent on their morphologies and size (Ray 2010).

It is without any doubt that this property is always taken into account when designing colorimetric devices. These noble metals especially their nanoparticulate system have been used as colorimetric sensor for analytes such as metal ions (Kim *et al.* 2009), proteins (Dang *et al.* 2009; Chai *et al.* 2010), amino acids (Zhou, Yang and Xu 2012), glucose, Adenosine Triphosphate (ATP), anticancer drugs, DNA and Vitamin C. All these colorimetric assays had offered robust devices which are easy to operate and require no sophisticated training and equipment. It is therefore without any doubt that the combination of LSPR induced by metal nanoparticles and application International Commission on Illumination, CIE (Commision Internationale del'Eclairage) colour system to develop colorimetric could offer improved systems and protocols that would generate more understanding of the interaction of molecules with the surface of nanomaterials (Magubane *et al.* 2019a).

The CIE L*a*b* colour system contains three coordinates, L*, a*, and b*. The L* variable represents the lightness, and (a* + b*)^{½} and Tan^{-1} (b*/a*) are both indicating chroma (C_{ab}) and hue angle (h_{ab}), respectively. In order to calculate the colour difference (ΔE) between an objective colour and the reference, one is normally given by the following equation (Hirayama *et al.* 2000; Maskan 2001).

$$\Delta E = \sqrt{(a_x - a_0)^2 + (b_x - b_0)^2 + (L_x - L_0)^2} \qquad (2.1)$$

In this equation x and 0 represent objective and reference, respectively. The application of this equation has been previously demonstrated using the change in colour due to the application of universal indicator for different pH ranges. However, it was noted that their approach did not cover a wide pH ranges. It is normally known that a* coordinate represents the redness and greenness for the positive (+a*) and negative (-a*) coordinates, respectively. Generally, the value of a* is given by a*= C_{ab} × Cos (h_{ab}). Komatsu and co-workers demonstrated that the red and yellow colours which appeared at low pH were corresponding to small hue angles (Komatsu *et al.* 2016). That observation was attributed to the change in the a* coordinate.

The above mentioned authors concluded that it was highly impossible to use a* value only for measuring pH which is obviously as a result of the combination of different colours. Reports have shown that multiple colours systems have received much attention in various colorimetric devices, such as for detection of heavy metals, and in areas of enzymatic assay development. All these assays were easily fabricated to µPAD system. This study, therefore, will apply the optical behaviour of gold nanoparticles to design a quantitative measurement of poly-DADMAC in potable water. Furthermore, the optimisation of an improved simple colorimetric method to detect poly-DADMAC in water using CIEL*a*b*/Yxy colour system from gold nanoparticles will be demonstrated.

2.7 LOVIBOND Comparator

The Lovibond comparator is an instrument which was designed way back in the 1800 by Lovibond (Lovibond and Colourists 1887; Lovibond 1890b). The Lovibond comparator is used to measure the concentration of the analyte based on the visual comparison of coloured glass or plastic filter. These filters are normally calibrated to allow the measurement of different fabricated concentration levels. The filters are normally fabricated with organic indicators and ligands which are used to form a detectable colour indicator for analytes of interest.

The Lovibond comparator is mainly used in water analysis. Up to date, there are no available reports which cover the fabrication of Lovibond comparator colour filters using gold nanoparticles for detection of poly-DADMAC in potable water. In this study, citrate capped gold nanoparticles were fabricated on the plastic Lovibond filters and tested as colorimetric device to test for poly-DADMAC in potable and raw water. In this device, the quantification of poly-DADMAC was achieved by rotating the Lovibond filters using standardised lighting, until the closest colour matched with the sample.

2.8 Conclusion

In summary, this chapter has explicitly highlighted the importance of quantification of poly-DADMAC in potable water. More importantly, the use of gold nanoparticles as underpinning materials for the design and development of portable µPAD colorimetric sensor was further discussed. In addition, the chapter presents CIEL*a*b*/Yxy colour system as an innovative method for the optimising of the colorimetric device. The next chapter will, therefore, present the research methodology and design. This will include a detailed explanation of the fabrication of the device as well as the Standard Operation Procedure (SOP) for its use in the detection of poly-DADMAC in water.

Chapter Three: Methodology

The research methodology and design adopted in this study is detailed in this chapter. The synthesis and characterisation of gold nanoparticles will first be described. Subsequently, the methodology used in the design, fabrication and optimisation of the Lovibond portable colorimetric sensor including the various experimental work and analyses that were conducted will be described in-depth.

3.1 Chemicals and reagents

All reagents were of analytical grade and were used as received. High purity water was prepared by means of an Elix water purification system. The chemicals that were used for the preparation of the AuNPs solution were Gold (III) chloride tri-hydrate ($HAuCl_4.3H_2O$) and tri-sodium citrate dehydrate ($Na_3C_6H_5O_7.2H_2O$). The poly-DADMAC calibration standards were prepared from a stock solution prepared from poly-DADMAC at 35 % wt (average molecular weight 100 000) ($C_8H_{16}ClN$). These chemicals were purchased from Sigma Aldrich (South Africa) and needed no further purification. All chemicals were of Analytical Reagent Grade. All solutions were stored in plastic containers after preparation.

3.2 Synthesis of Gold nanoparticles (AuNPs)

Following the previously reported method (Turkevich, Stevenson and Hillier 1951), AuNPs were prepared by the citrate reduction method as depicted in **Figure 3-1**. A solution of 0.001 M tetrachloroaurate was filtered through a rinsed 0.2 µm filter into a clean designated beaker. The total volume was 250 mL. On a hotplate; the solution was brought to a reflux at 250 °C stirring at 900 rpm. A solution of 0.04 M tri-Sodium Citrate was filtered through the rinsed 0.2 µm filter into a clean designated beaker. Once reflux had begun, a calculated amount of the 0.04 M tri-sodium citrate solution was quickly added to the centre of the flask as one bolus. The solution turned grey in 1 - 2 minutes. The heat was then reduced to a gentle reflux. The solution continued to change colours from grey → dark purple → burgundy → red → red-orange. The reflux was continued until the red-orange colour was stable for 5 minutes. The heat was switched off; stirring was allowed to continue for 3 hours and cooled to room temperature.

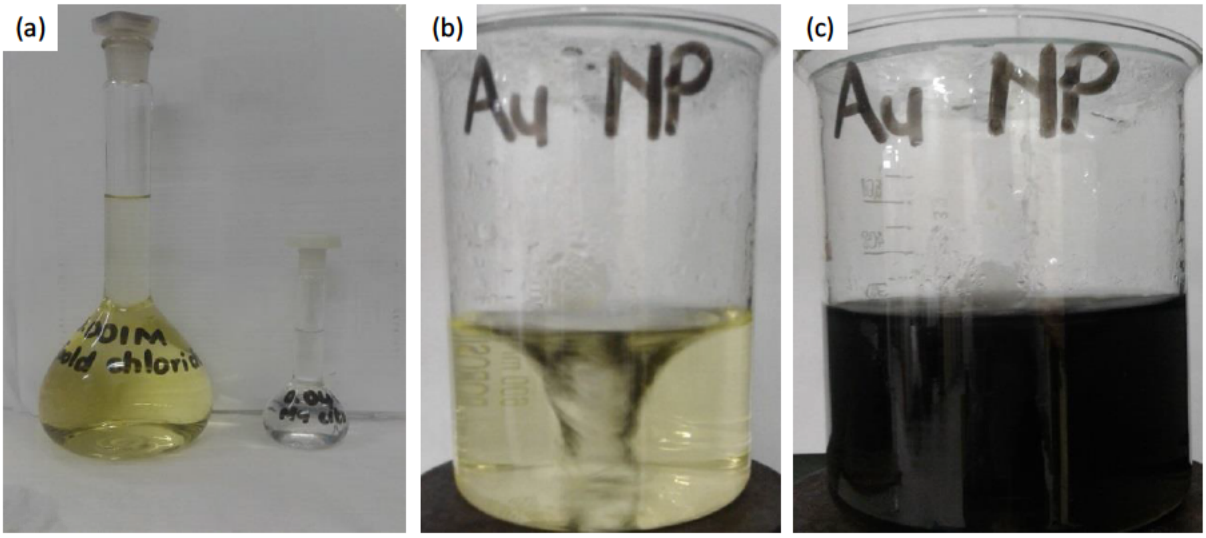


Figure 3- 1: Preparation of gold nanoparticles. (a) – 0.001 M solution of gold chloride and 0.04 M solution of tri-sodium citrate; (b) – gold solution during stirring just after adding the solution of tri-sodium citrate; (c) – gold nanoparticles solution after 3 hours of stirring.

3.2.1 Characterisation of the synthesised AuNPs

The prepared gold nanoparticles were characterised using the UV spectrophotometer. The Varian Cary 60 UV-VIS spectrophotometer was also used for the optimisation and verification of the process. The UV spectrophotometer was purchased from Chemetrix. The scanning range was set at 800 to 400 nm. Transmission electron microscope (TEM) images were obtained using a JEM-2100F at 200 kV. The TEM grids were prepared by depositing approximately 10 µL of the solution obtained after centrifugation and allowed to dry in air.

The concentrated gold nanoparticles solution **(Figure 3-2A)** was diluted three times (3X) before use. The dilute solution (**Figure 3-2B**) was used for characterisation and colour development throughout the research**.**

Figure 3- 2: Solution A is the concentrated solution AuNPs and solution B is the dilute solution of AuNPs

3.3 Preparation of poly-DADMAC standards

For this research, it was crucial that all glassware and magnetic stirrer bars used in these syntheses be thoroughly cleaned in aqua regia (HCl/HNO_3 3:1, v/v) and rinsed with de-ionised water prior use. This was done in order to avoid contamination as much as possible. A mass of 1.09 g of poly-DADMAC (35 % weight) was weighed into a plastic weighing boat and transferred into a 1000 mL volumetric flask. De-ionised water was used to make up to the volume of 1000 mL resulting to a 400 mg L^{-1} poly-DADMAC solution. This solution was mixed thoroughly by inverting the flask 20 times. It was then transferred to a plastic container. A 50 mg L^{-1} working stock solution was prepared by pipetting 12.5 ml of the 400 mg L^{-1} stock solution into a 100 ml volumetric flask and making up to the mark with de-ionised water. This solution was mixed thoroughly by inverting the flask 20 times and labelled correctly. The calibration standards for poly-DADMAC were then prepared from the 50 mg L^{-1} working stock solution.

3.4 Design and fabrication of the 6 X 9 wells paper device (μPAD)

The wax patterns for the 6 X 9 μPAD were designed using CorelDraw Home and Student X7. The designed μPADs were printed on a sheet of normal A4 paper by a wax printer (ColorQube 8570N, Xerox, SA), followed by heating at 120 °C for 2 min on heating hot plate. The colorimetric device depicted in **Figure 3-3** employed the flocculation of gold nanoparticles, mainly focusing on the addition of different higher concentrations of poly-DADMAC solutions. A 6 X 9 wells paper device was printed as shown in **Figure 3-3 (d)**.

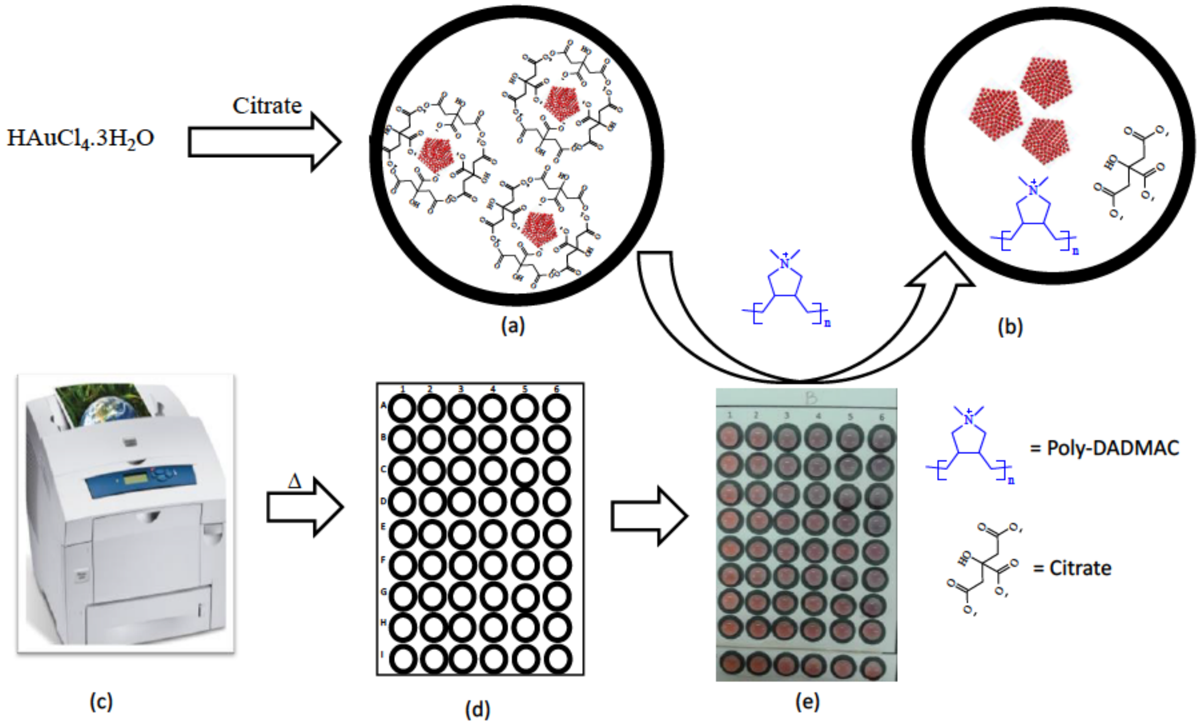


Figure 3- 3: Diagram of the fabrication of gold nanoparticles enabled μPAD. (a) depicts the formation of citrate stabilised gold nanoparticles after reacting gold chloride with tri-sodium citrate, (b) after addition of poly-DADMAC, the poly-DADMAC replaces some of the citrate ions from AuNPs surface, (c) the wax printer used to print the designed μPAD, (d) the designed μPAD after heating on the hotplate to produce hydrophobic wax barriers, (e) different colours after the addition of different concentrations and volumes of poly-DADMAC to the gold nanoparticles.

3.4.1 Fabrication of Cuvette and Lovibond colorimetric filter

CorelDraw home and student X7 were used for the design of Lovibond colour filter. The colour filter was printed on a plastic slide using a wax printer (ColorQube 8570DN, Xerox). Firstly, the AuNPs based colorimetric screening of poly-DADMAC in the potable water was proven using previously developed methods (Mwangi, Ngila and Ndungu 2012; Gumbi, Ngila and Ndungu 2014) where cuvettes were used for analysis **(Figure 3-4)**.

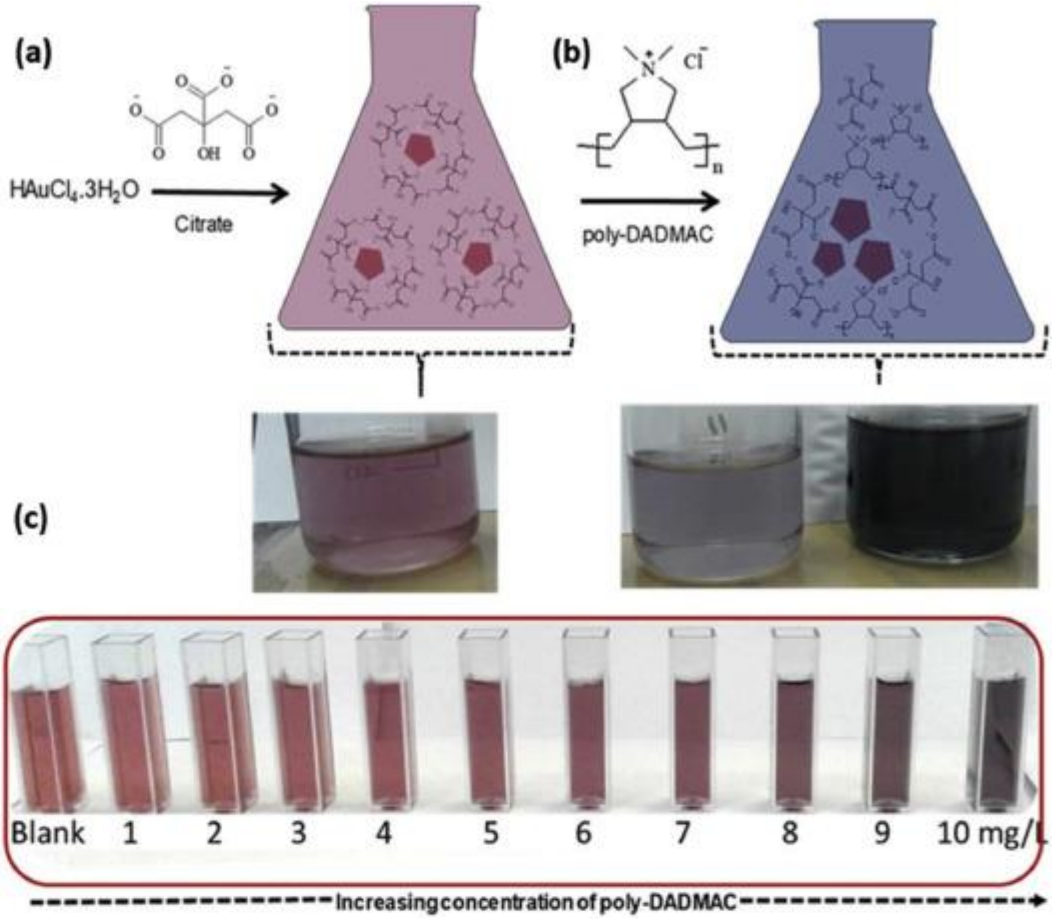


Figure 3- 4: General scheme for interaction of poly-DADMAC with citrate stabilised gold nanoparticles, where (a) is citrate stabilized AuNPs, (b) after the addition of stoichiometric amount of poly-DADMAC and (c) variation of colour as concentration of poly-DADMAC *increase from* 1.0 to 10 mg L^{-1}.

The colours in **Figure 3-5 (a) and (c)** were obtained by preparing poly- DADMAC standards from 1 (far left) to 10 mg L^{-1} (far right) in units of 1. Into each cuvette, 3 mL of AuNPs (at 3 X dilution) was transferred followed by the addition of 100 µL of each standard to the appropriate cuvette. This equates to 30X dilution of the standard or sample. The complimentary reading for each colour was printed at angle of 90º of the complimentary colour in **Figure 3-5 (c**). The colorimetric test was conducted using cuvettes which are shown in **Figure 3-5 (b)**. The blue colour became more intense as the poly-DADMAC concentration increased.

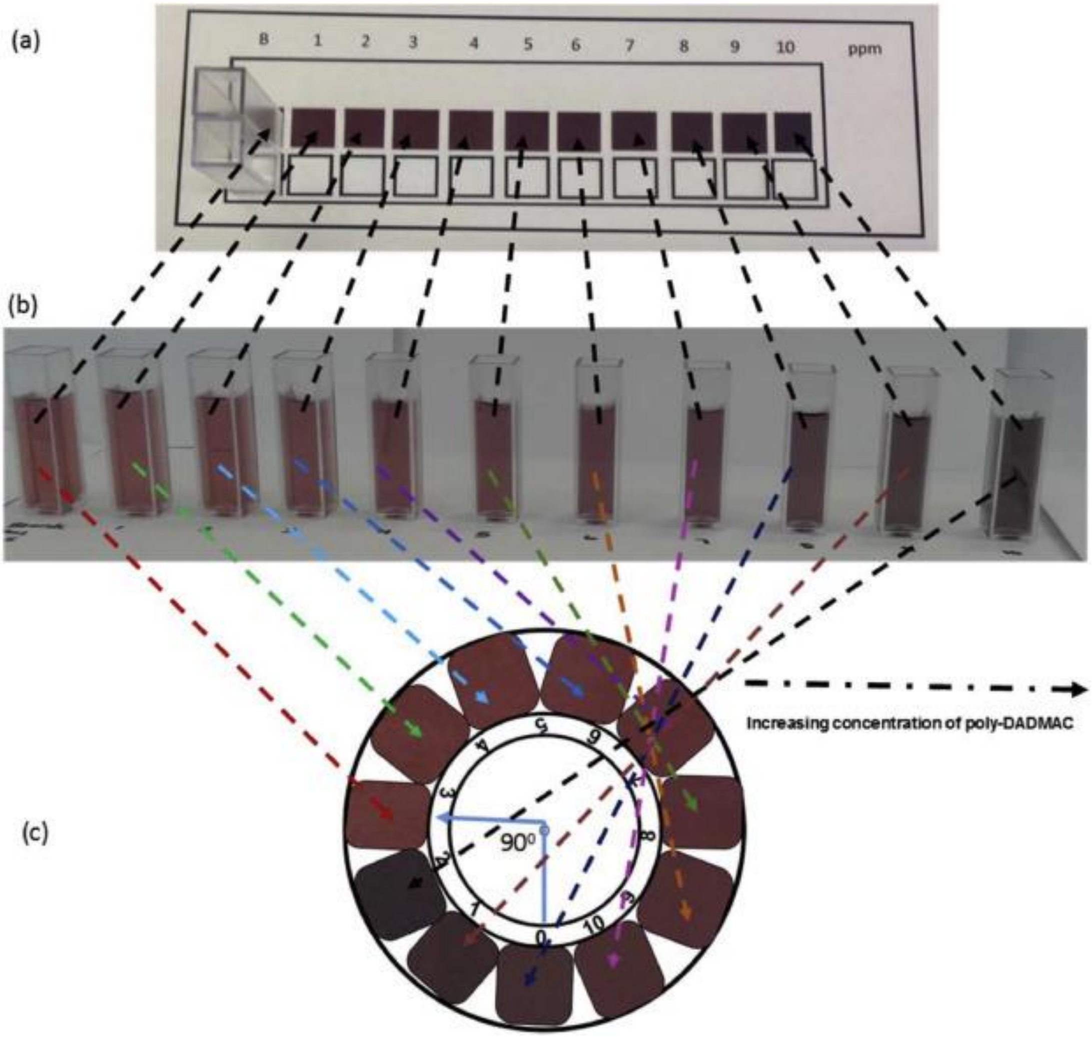


Figure 3- 5: Showing the designed colorimetric filters from AuNPs where (a) cuvette colorimetric devices, (b) AuNPs based Lovibond color filter and (c) Lovibond disk with AuNPs at different concentration of *poly-DADMAC*.

3.4.2 Optimisation of Colorimetric filter

The areas and RBG values of the reservoirs were measured using the public domain software Image J (National Institutes of Health). RBG values were converted to CIE L*a*b*/Yxy colour system using an online generator. Each µPAD was cut to a 10 ×150 mm piece, and then was prepared by applying appropriate volumes of primary gold nanoparticles and poly-DADMAC solutions to the reaction reservoirs via a micropipette as shown in **Table 3-1**. The resultant colours are shown in **Figure 3-6**.

Table 3-1: Shows the quantity of poly-DADMAC and gold nanoparticles added into each well. Where [P], vP and Au represent the concentration of poly-DADMAC, volume of poly-DADMAC and volume of gold nanoparticles, respectively.

Wells	1			2			3			4			5			6		
	[P]	vP	Au	[P]	vP	Au	[P]	vP	Au	[P]	vP	Au	[P]	vP	Au	[P]	vP	Au
A	0	0	250	2	10	250	2	20	250	2	30	250	2	40	250	2	50	250
B	0	0	250	1.8	10	250	1.8	20	250	1.8	30	250	1.8	40	250	1.8	50	250
C	0	0	250	1.6	10	250	1.6	20	250	1.6	30	250	1.6	40	250	1.6	50	250
D	0	0	250	1.4	10	250	1.4	20	250	1.4	30	250	1.4	40	250	1.4	50	250
E	0	0	250	1.2	10	250	1.2	20	250	1.2	30	250	1.2	40	250	1.2	50	250
F	0	0	250	1	10	250	1	20	250	1	30	250	1	40	250	1	50	250
G	0	0	250	0.8	10	250	0.8	20	250	0.8	30	250	0.8	40	250	0.8	50	250
H	0	0	250	0.6	10	250	0.6	20	250	0.6	30	250	0.6	40	250	0.6	50	250
I	0	0	250	0.4	10	250	0.4	20	250	0.4	30	250	0.4	40	250	0.4	50	250

Figure 3- 6: Colour development tested on paper.

3.5 Sampling

Samples were collected from rivers, lakes and water treatment plants. The samples were collected in clean 500 mL polyethylene sample containers. During sampling, the sample containers were first rinsed with the sample twice before collecting the sample. The containers were then tightly closed and labelled appropriately. During transportation, the samples were kept in cooler boxes while avoiding direct sunlight. Upon arrival to the lab they were given unique identification numbers.

The physical-chemical quality results of these water samples are in **Annexure 1** and they were determined by using the instrumentation that is stated in section 3.6.1. The raw samples were collected from lake Nsezi or Nsezi raw (GPS: 28.7475°S, 31.9715°E), lake Mzingazi or Mzingazi raw (GPS: 28.7620°S, 32.0894°E), Mhlathuze river or Mhlathuze raw (-28° 45' 43.09" S, 31° 53' 35.84"E) and lake Cubhu or ESikhaleni raw (GPS: 28.7475° S, 31.9715° E).

Treated water samples were taken from the treatment plants that are situated around the lakes mentioned above. These plants are Nsezi Water Treatment Plant (31.982450E -28.748700 S), Ngwelezana Water Purification Works (Mhlathuze final) (31.824734 E -28.772215 S), Mzingazi Water Works (32.077021E -28.760124 S) and ESikhaleni Water Treatment Works (31.955244 E -28.845570 S). All these sampling sites are located in South Africa, Richards bay. See **Figure 3-7** for these sample locations.

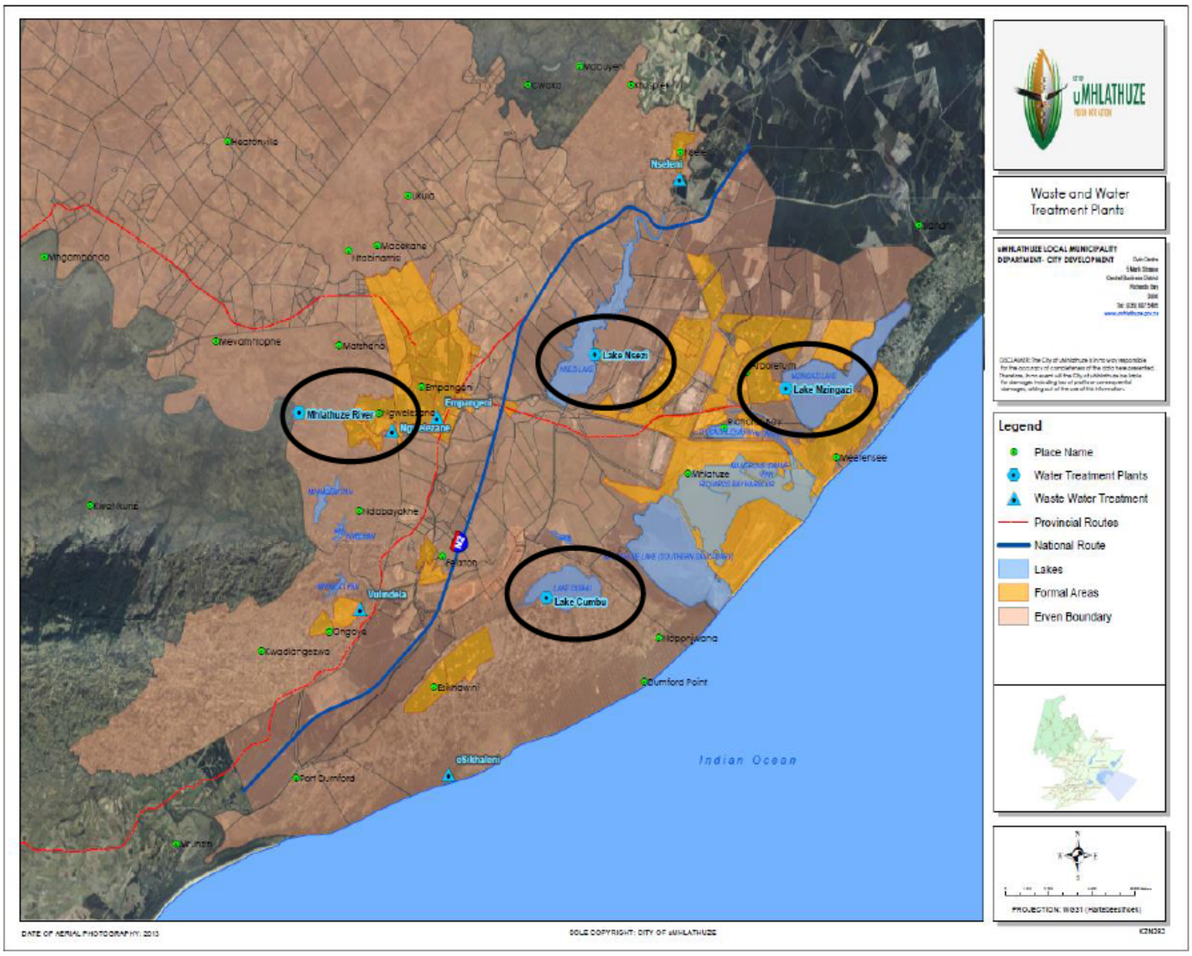


Figure 3- 7: MAP showing the sampling points in Richards bay, KwaZulu-Natal, South Africa

3.5.1 Dilution and dosage of poly-DADMAC

Before use, poly-DADMAC is diluted with water inside a mixing tower. **Figure 3-8** shows the tank in which the poly-DADMAC polymer is diluted with water and mixed thoroughly before dosing. Samples were also taken from the mixing tower, the treated water reservoir and the taps where the end-user gets the treated water. The samples were collected in 500 mL polyethylene containers and labelled accordingly. 3 mL of AuNPs solution was added to plastic cuvettes and 100 µL of each poly-DADMAC standard was subsequently added into this vial, and the solutions were scanned using UV–visible spectrophotometer for validation of the test.

Figure 3- 8: Mixing tower where poly-DADMAC is diluted

3.6 Analytical Methods

The Ultraviolet (UV)-Visible (Vis) spectra were measured using a Cary 60 spectrophotometer. This spectrophotometer was equipped with a double beam, Czerny-Turner monochromator, 190-1100 nm wavelength range, full spectrum Xenon pulse lamp single source and dual silicon diode detector. The instrument was supplied by Chemetrix, South Africa, agents for Agilent. Raw data was captured and analyzed using the spectrometer Cary win software. Samples were transferred to 1.0 mL disposable cuvettes and placed in the cuvette holder with a 1 cm path length.

3.6.1 Physical tests analysis

Different instruments were used for the physical tests. The pH and conductivity tests were analysed by using the autotitrator from Metrohm. The autotitrator was equipped with a pH electrode (6.0253.100) and a conductivity probe (6.0912.110) both purchased from Metrohm. Both probes were conditioned with standards (as per the manufacturer’s recommendations) before use every day.

Turbidity, in Nephelometric Turbidity Units (NTU), was measured with a HACH 2100Q turbidimeter. The total suspended solids (TSS) were determined by gravimetric technique. For the analysis of Chemical Oxygen Demand (COD), the samples were first digested at 148°C for 2 hours using a Nanocolor digester. Thereafter the digested samples were analysed using the Nanocolor photometer at a wavelength of 620nm.

3.6.2 Quality assurance

The poly-DADMAC calibration standards that were prepared were used to construct a calibration curve. For the correlation coefficient to be accepted, it had to meet the acceptance criterion of 0.995. From the calibration curve, the limit of detection (LOD) and limit of quantification (LOQ) were calculated. Accuracy and recovery studies were also done in order to prove the selectivity and specificity of the method.

3.7 Lovibond comparator

The Lovibond comparator device was setup as shown in **Figure 3-9**. All colours were placed at 90° to the corresponding concentration for easy reading in the Lovibond comparator. The Lovibond comparator is operated by reading the corresponding concentration of the sample from displayed concentrations (1 – 10 mg L^{-1} fabricated on the plastic slide). This is done by moving the colour filter clockwise until the colour of test tube A matches with one color in the two test tubes, test tube A with 3 mL of AuNPs and the other (test tube B) with reference water samples, then 100 μL of potable water samples is added into tube A.

These two test tubes are then inserted into their compartments as shown in **Figure 3-9 (c)**. The system is calibrated to be read within 1min. In cases where readings are done beyond the 1 min calibrated time, AuNPs tend to aggregate and then flocculate out of the solution, thereby leaving water to become clear.

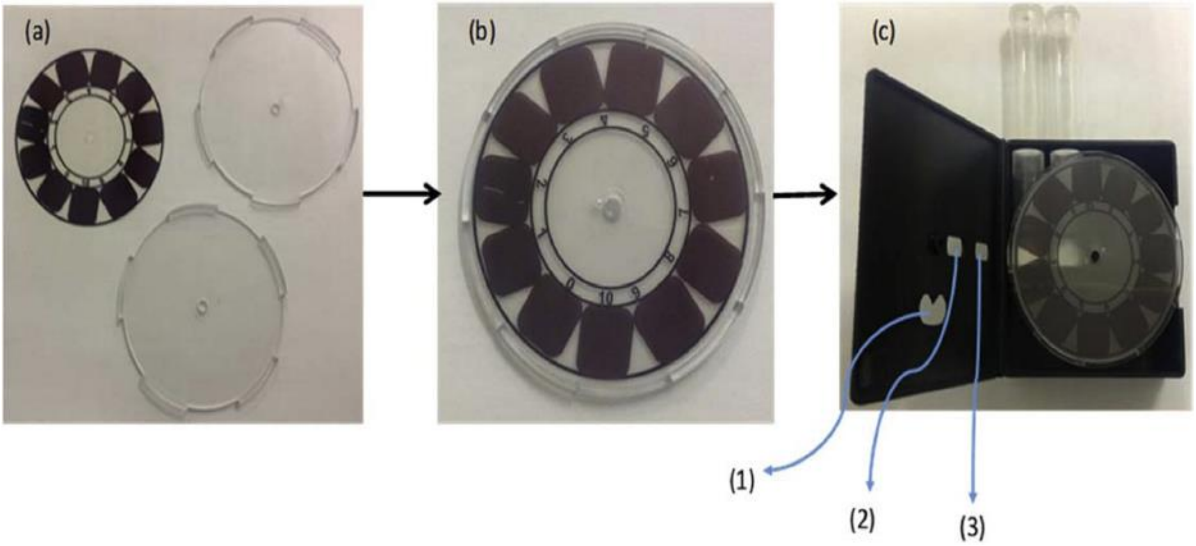


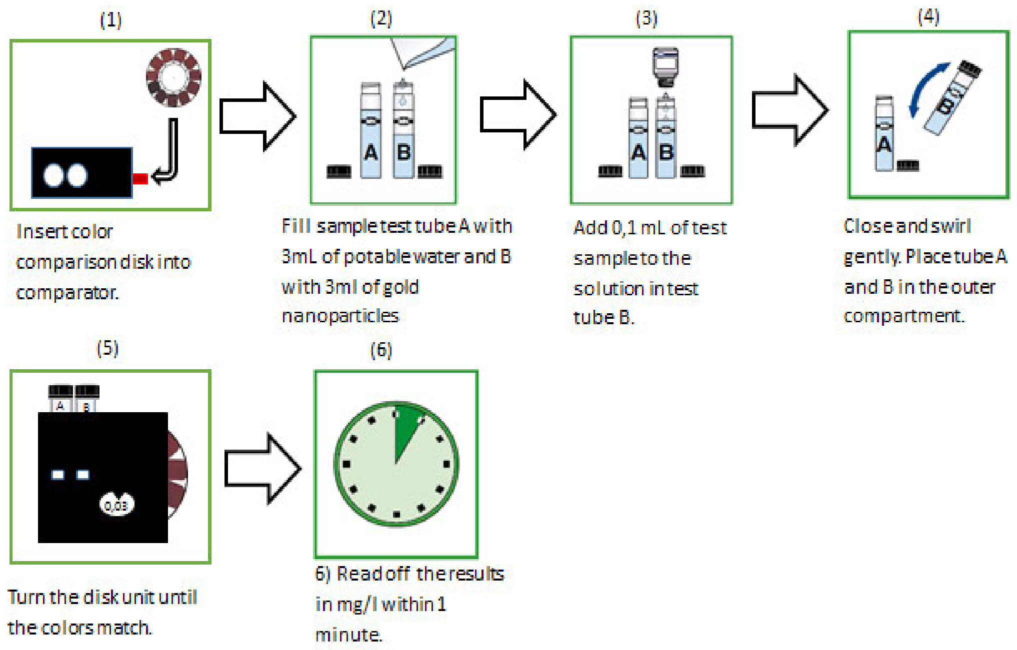
Figure 3- 9: **Lovibond comparator with color filters (a) before assembly, (b) after assembly and (c) inserting cuvettes into the device**.

The final Lovibond comparator device which can be packaged as shown in **Figure 3-10** has the following components to enable proper testing: solution of AuNPs, four sample test tubes and a mirror to reflect light into the system.

Figure 3- 10: The final assembled Lovibond kit with the colorimetric disk inserted into the Lovibond comparator (a) package, (b) sample test-tube, (c) AuNPs container and (d) Lovibond comparator inserted with color disk.

3.8 Standard Operating Procedure (SOP)

The SOP has six steps making it very user friendly, simple and robust. It can be noted that the first step is to fill up the two test tubes, test tube B with 3 mL of gold nanoparticles and the other (test tube A) with reference water sample. Then 100 µL of potable water samples is added to tube B. Invert to mix gold nanoparticles with potable water. These two test tubes are then inserted into their compartment as shown in **Figure 3-11**. This is followed by reading the corresponding concentration of the sample from the displayed concentrations (1 – 10 mg L^{-1}) fabricated on the plastic slide. This is done by moving the colour filter until the colour of test tube B matches with one colour in the gold nanoparticle colour filter. As soon as the colours match, the exact concentration will appear on the colorimetric board. The results will be read off in mg L^{-1}. The system is calibrated to be read after 1 minute. In cases where readings are done over the 1 minute calibrated time, gold nanoparticles turn to form aggregated particles which flocculate out of the solution thereby leading water becoming clear.



(1) Insert color comparison disk into comparator.

(2) Fill sample test tube A with 3mL of potable water and B with 3ml of gold nanoparticles

(3) Add 0,1 mL of test sample to the solution in test tube B.

(4) Close and swirl gently. Place tube A and B in the outer compartment.

(5) Turn the disk unit until the colors match.

(6) 6) Read off the results in mg/l within 1 minute.

Figure 3- 11: Schematic diagram showing the standard operation procedure for testing poly-DADMAC using Lovibond colorimetric disk which is inserted into Lovibond comparator

In summary, this chapter has exhaustively explained the research approach and the experimental design strategy carried out in the study. The results and discussion of the study will be presented in the next chapter.

Chapter Four: Results and discussion

This Chapter presents the results and discussion of the study. The data obtained from the study was analysed in relation to the research objectives as outlined in Chapter One, Section 1.5, that is:

- To synthesise and characterise gold nanoparticles
- To develop and optimise a method using the UV spectrophotometer for the determination of poly-DADMAC
- To optimise CIEL*a*b*/Yxy colour system for this determination
- To quantitatively determine poly-DADMAC in treated water using the UV spectrophotometer.
- Assemble the portable colorimetric device and compare results to those obtained from the UV spectrophotometer

4.1 Characterisation of gold nanoparticles (AuNPs)

The optical and structural properties of the synthesized AuNPs were confirmed by using the UV–VIS spectrophotometer and TEM, as shown in **Figure 4-1**. Gold nanoparticles had a surface Plasmon resonance (SPR) peak at 502 nm. This has been shown before for UV–VIS spectrophotometer being used as an evidence of the size and the morphology of metal nanoparticles (Verma, Singh and Chavan 2014).

The size of AuNPs is very critical for this study because it determines the optical properties for nanoparticles used for the colorimetric test. According to Mock *et al.* 2002; Kelly *et al.* 2003, the optical properties of metal nanoparticles vary with size and morphology. As a result, a method for synthesis of AuNPs with an average size of 14 nm was standardised. The TEM characterisation technique is critical for size determination and morphology visualising. This is due to the fact that larger variation in size would have a huge effect on the colorimetric test (Mock *et al.* 2002; Kelly *et al.* 2003) . See TEM image in **Figure 4-1(b)**. The colour of AuNPs solution was deep red-orange and it absorbed at a SPR wavelength peak of 502 nm as shown in **Figure 4-1(a)** and **Figure 3-2 B**. When colloidal AuNPs were introduced into a *poly-*

DADMAC solution, the deep red-orange color slowly disappeared, and the blue color appeared as shown in **Figure 3-4** (a) to (c). This colour change is due to the formation of the AuNPs aggregates as a result of poly-DADMAC.

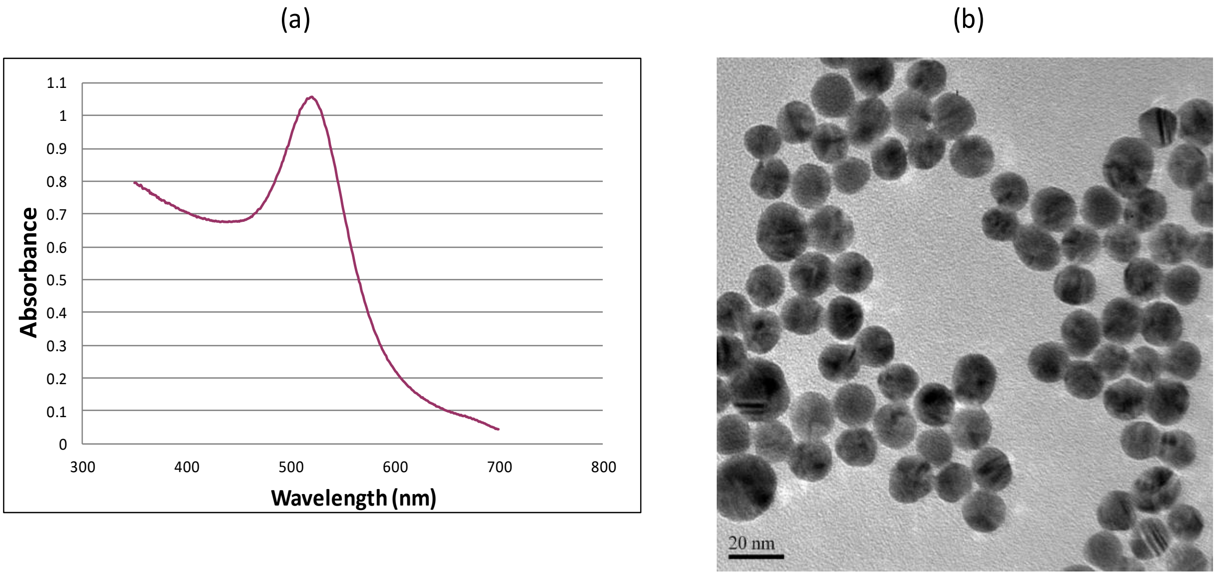


Figure 4- 1: (a) UV visible spectrum and (b) TEM image of gold nanoparticles that were used to fabricate the colorimetric device.

4.2 Aggregation of gold nanoparticles with poly-DADMAC

During the aggregation of AuNPs, the poly-DADMAC replaces some of the citrate ions from AuNPs surface, and therefore destabilises the colloid with aggregate formation. This aggregation causes a blue-shift of the plasmon band as shown in **Figure 4-2**. The figure visibly shows the shift in the plasmon band at a concentration of 100 mg L^{-1} poly-DADMAC. In **Figure 4-2**, the peak at 526 nm is due to AuNPs aggregates and the peak at 602 nm is due to the formation of the AuNPs- poly-DADMAC aggregates. A critical point worth mentioning is that disposable plastic cuvettes were used in order to avoid cuvettes being stained with the deep red or blue colour of the gold nanoparticles and poly-DADMAC. This was done to ensure the accuracy of the colour by minimising any other interference.

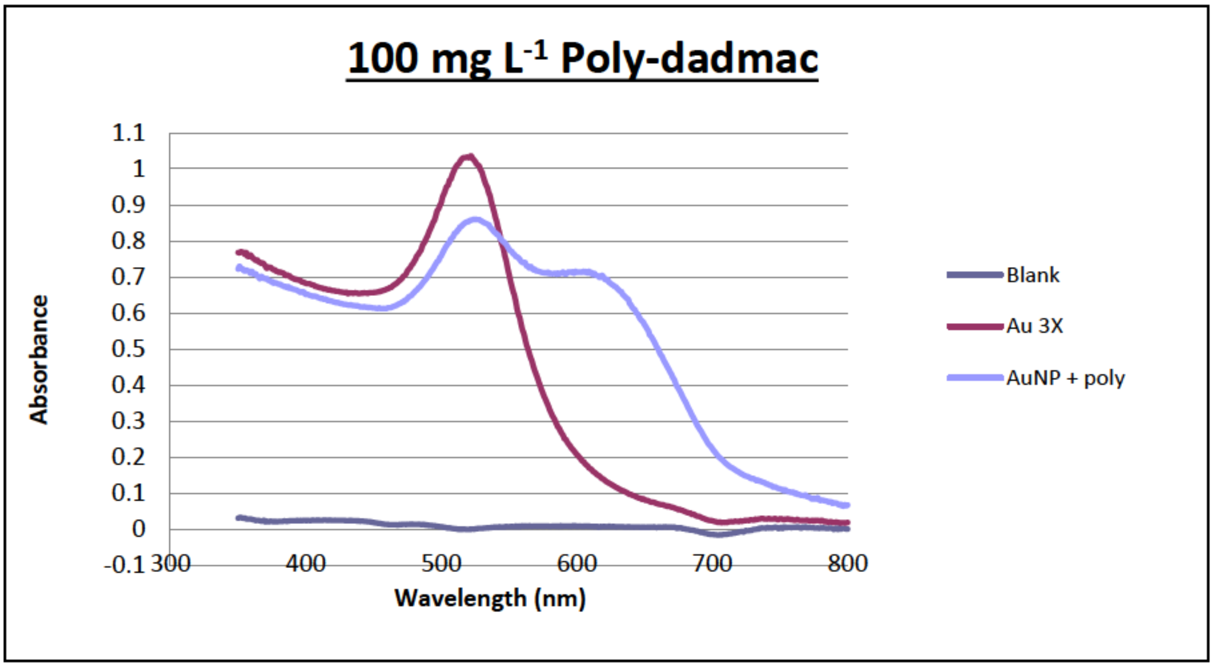


Figure 4- 2: Shift of the plasmon band to longer wavelengths caused by the formation of the AuNP-poly-DADMAC aggregate.

4.3 Optimisation of the Colorimetric filter

The shift in the plasmon band can further be confirmed in **Figure 4-3**. Poly-DADMAC was added to AuNPs at different aliquots and concentrations. It was established that the blue colour became more intense as the *poly-DADMAC* concentration and volume increased.

The AuNPs solution was used at 3 times dilution. Hence the 3X. For instance, in **Figure 4-3 (e),** 3ml of the AuNPs was transferred to a plastic cuvette and analysed as Au 3X. This solution was scanned and produced a peak at 520 nm. Thereafter, to the same Au 3X solution, a 20 µL of 10 mg L^{-1} poly-DADMAC was added. Upon scanning it was discovered that there was a peak showing at 650 nm. This was due to the SPR signalling the presence of poly-DADMAC. Subsequent additions of poly-DADMAC were done until the total volume of poly-DADMAC added was 100 µL. it should be noted that, with every addition of poly-DADMAC, the absorbance of the peak at 650 nm increased.

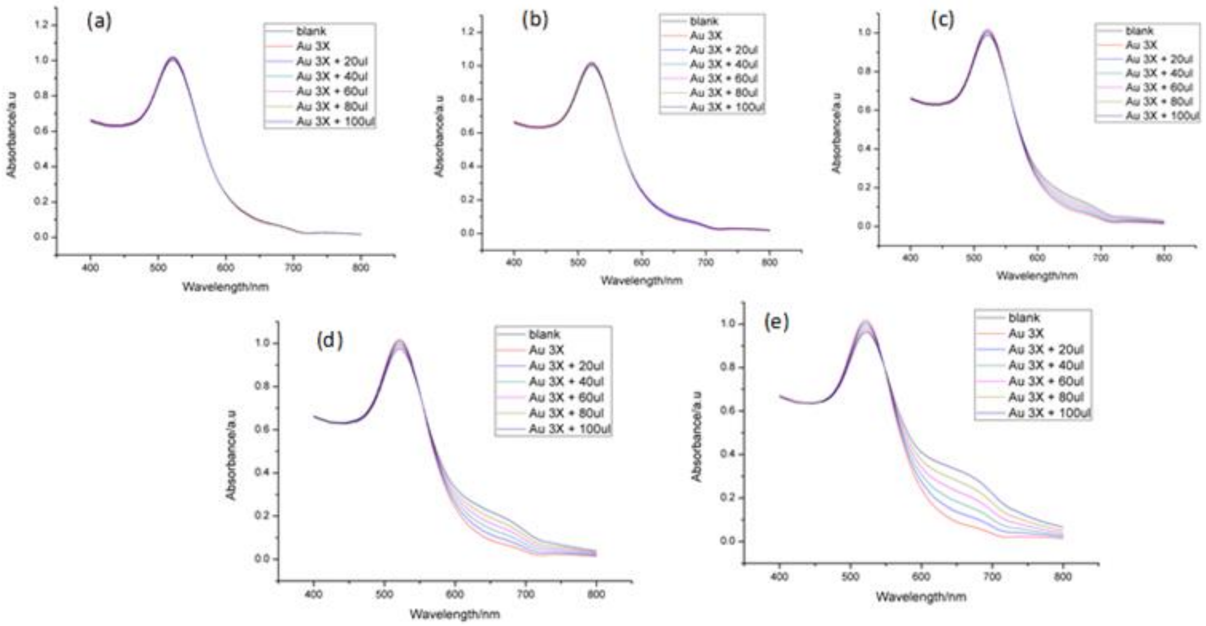


Figure 4- 3: Change in UV visible spectra of AuNPs after the addition of different poly-DADMAC concentrations where (a) 0.5 mg L^{-1}, (b) 1 mg L^{-1}, (c) 3 mg L^{-1} (d) 5 mg L^{-1} and (e) 10 mg L^{-1}. Note that 3 ml of AuNPs was used for each test.

Colour images of **Figure 4-3** are also shown in **Figure 3-4** and **3-5** (Chapter 3; Section 3.4.1). The actual results of these standards are in **Table 4-1**. The table also depicts how the UV scans compared to the results obtained with the Lovibond results. These results were in agreement with reported spectrophotometric studies on this application (Gumbi, Ngila and Ndungu 2014; Manickum *et al.* 2015).

It can be noted that these results prompted us to establish that the variation in colour as the concentration of poly-DADMAC increases can be incorporated to fabricate our portable colour filters (both paper and Lovibond filters). These colour filters can be used to screen the concentration of poly-DADMAC on-site without having to use sophisticated equipment which are currently employed in the laboratory. The fabricated colour filters are shown in **Figure 3-5 (a)** and (c). The filters were calibrated for poly-DADMAC concentration ranging from 1 to 10 mg L^{-1}. The Lovibond colour filter device and inserting of cuvettes are shown in **Figure 3-9**.

Table 4- 1: Comparison of the UV and Lovibond results.

Sample Name	Abs @ 521.015 nm	Abs @ 637.008 nm	Ratio @ (A634.008 nm /A521.015 nm)	Poly-DADMAC conc (mg L^{-1})		
				UV Raw conc	UV Calculated	Lovibond
0,5 mg L^{-1} poly	0.9779	0.1031	0.1054	0.0139	0,417	0-1
1 mg L-1 poly	0.9755	0.1137	0.1165	0.028	0,84	1
3 mg L-1 poly	0.9664	0.1675	0.1734	0.1002	3,006	3
5 mg L-1 poly	0.9531	0.2263	0.2374	0.1816	5,448	5
10 mg L-1 poly	0.9406	0.3307	0.3516	0.3266	9,798	10
Poly mixer @ 5000X	0.9186	0.4384	0.4773	2431.222	72936,66	>>10*
Poly mixer @ 2000X	0.8243	0.7986	0.9689	2221.4693	66644,079	>>10*
Poly mixer @ 20 000X	0.9647	0.1916	0.1986	2645.4548	79363,644	>>10*
Mhlathuze Raw	0.9853	0.0965	0.0979	0.0044	0,132	0-1
Mhlathuze Final	0.9909	0.1019	0.1029	0.0106	0,318	0-1
Mzingazi Final	0.9799	0.0978	0.0998	0.0067	0,302	0-1
Mzingazi Raw	0.9901	0.1021	0.1031	0.0109	0,327	0-1
Nsezi Final	0.9943	0.1116	0.1122	0.0225	0,675	0-1
ESikhaleni raw	0.9886	0.1062	0.1074	0.0164	0,492	0-1
ESikhaleni Final	0.9866	0.0999	0.1013	0.0086	0,258	0-1
Nsezi Raw	0.9761	0.0924	0.0946	0.0002	0,006	0-1

4.4 Calibration curve

From the calibration standards peaks obtained, the calibration curves and sample results were read using 3 methods. The first method is using the absorbance of the Gold nanoparticles at 521.02 nm, the second one is using the peak at 637.01 nm and the third method is using the ratio at the peak absorbance at 637.01nm and 521.02nm.

4.4.1 Comparison of the calibration curves

For the first method of using the peak absorbance at 521.02 nm, the calibration curve in **Figure 4-4** was obtained. For the second and third methods, the calibration curves are shown in **Figures 4-5** and **4-6** respectively. From these calibration curves, the exact standard concentrations were calculated using the linear equation which is **y = mx + c** from Beer's law equation A=ϵbc. The results are shown in **Tables 4-2, 4-3 and 4-4**. Of the 3 methods that were used, the ratio method is the preferred method according to literature (Gumbi, Ngila and Ndungu 2014).

Table 4- 2: Calibration data at 521.02 nm

Polydadmac Std conc (mg/l)	Std conc at 30X dil (mg/l)	Absorbance	y-int	slope	Raw Poly conc (mg/l)	Calc Poly conc (mg/l)
0,5	0,017	0,977901647	0,9783	-0,1203	0,003	0,099
1	0,033	0,975544781	0,9783	-0,1203	0,023	0,687
3	0,100	0,966352064	0,9783	-0,1203	0,099	2,980
5	0,167	0,953077832	0,9783	-0,1203	0,210	6,290
10	0,333	0,940623296	0,9783	-0,1203	0,313	9,396

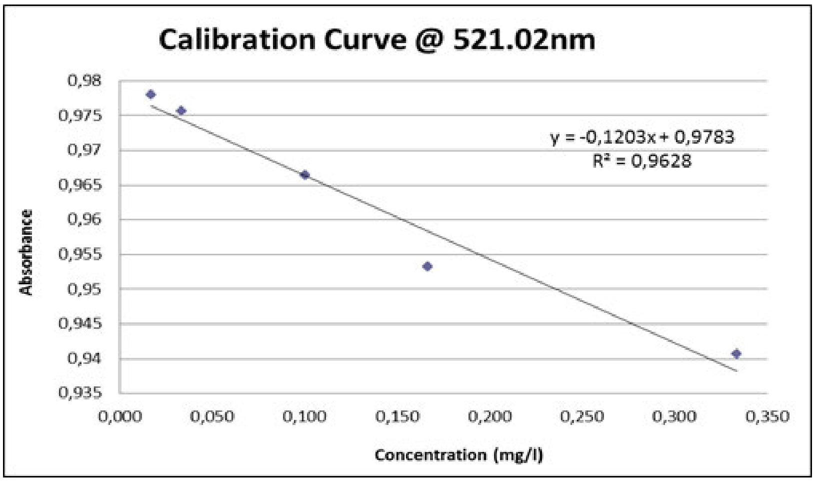


Figure 4- 4: Calibration curve @521.02 nm

Table 4- 3: Calibration data from the absorbance peak at 637.01 nm

Polydadmac Std conc (mg/l)	Std conc at 30X dil (mg/l)	Absorbance	y-int	Slope	Raw Poly conc (mg/l)	Calc Poly conc (mg/l)
0,5	0,017	0,103105124	0,0937	0,7273	0,013	0,388
1	0,033	0,113688488	0,0937	0,7273	0,027	0,824
3	0,100	0,167519998	0,0937	0,7273	0,101	3,045
5	0,167	0,226288568	0,0937	0,7273	0,182	5,469
10	0,333	0,330699187	0,0937	0,7273	0,326	9,776

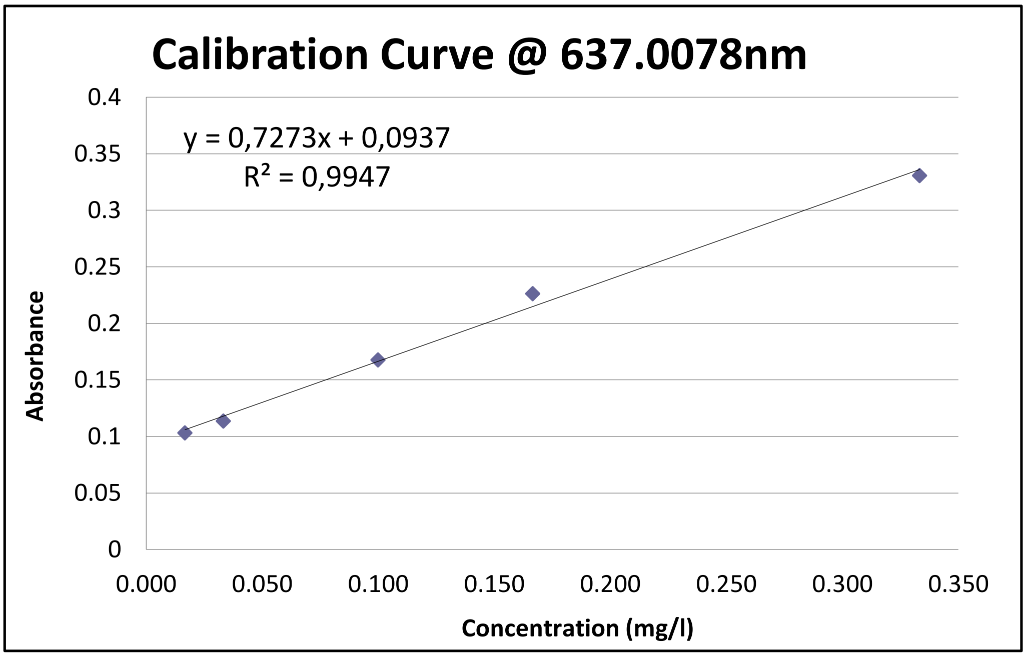


Figure 4- 5: Calibration curve @ 637.01 nm

Table 4- 4 Calibration data @ Ratio ($A_{637.01\ nm}/A_{521.02\ nm}$)

Polydadmac Std conc (mg/l)	Std conc at 30X dil (mg/l)	Absorbance	y-int	slope	Raw Poly conc (mg/l)	Calc Poly conc (mg/l)
0,5	0,017	0,1054	0,0945	0,7872	0,0139	0,417
1	0,033	0,1165	0,0945	0,7872	0,0280	0,840
3	0,100	0,1734	0,0945	0,7872	0,1002	3,005
5	0,167	0,2374	0,0945	0,7872	0,1816	5,447
10	0,333	0,3516	0,0945	0,7872	0,3266	9,797

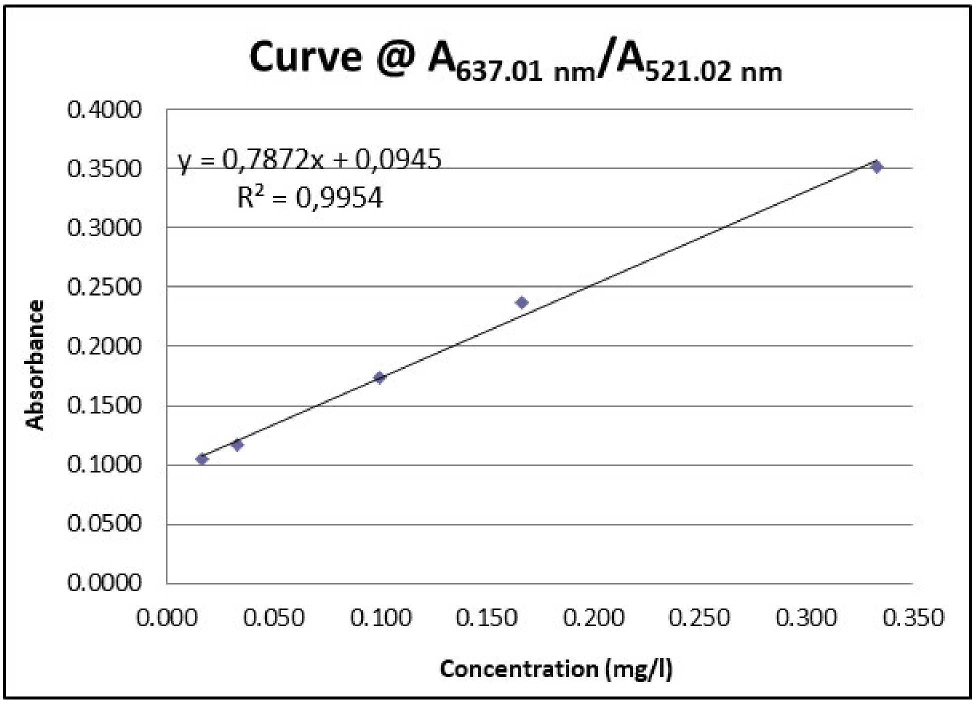


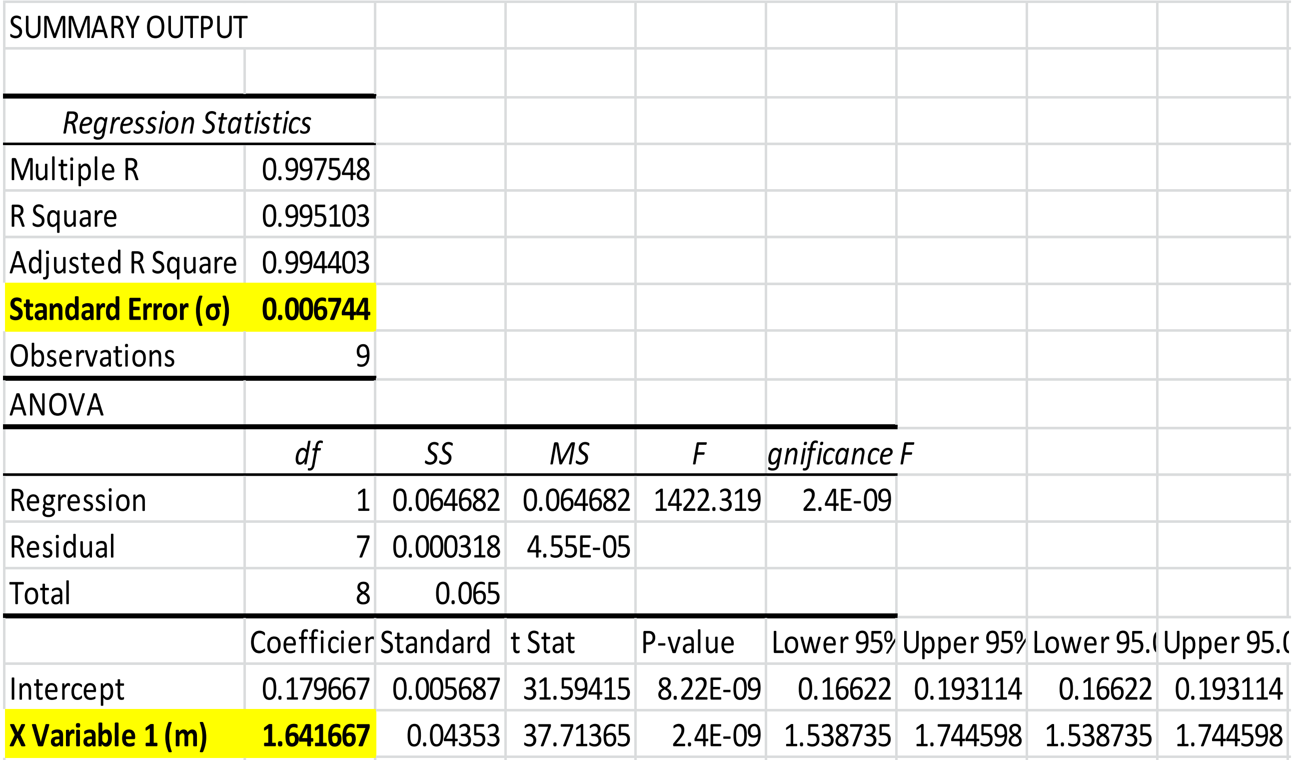
Figure 4- 6: Calibration curve @ ratio ($A_{637.01\ nm}/A_{521.02\ nm}$)

4.4.2 Calculation of the LOD and LOQ

The LOD and LOQ were calculated from the regression data using the Standard Deviation of the response (σ) and the slope of the curve (S) as highlighted in **Figure 4-7**. A calibration curve was constructed using the absorbance ratio method described in section 4.4.1. **Table 4-5** shows the calibration standards concentrations and absorbances thereof. The LOD was calculated as 3.3σ/S and the LOQ as 10σ/S. The results obtained were LOD = 0.0136 and 0.0411 mg L^{-1} for the LOQ.

Table 4- 5: Calibration standards

Std Number	Poly-dadmac (mg/l)	Absorbance ratio
1	0.04	0.25
2	0.06	0.28
3	0.08	0.30
4	0.1	0.34
5	0.12	0.38
6	0.14	0.42
7	0.16	0.44
8	0.18	0.47
9	0.2	0.51



SUMMARY OUTPUT								
Regression Statistics								
Multiple R	0.997548							
R Square	0.995103							
Adjusted R Square	0.994403							
Standard Error (σ)	**0.006744**							
Observations	9							
ANOVA								
	df	*SS*	*MS*	*F*	*gnificance F*			
Regression	1	0.064682	0.064682	1422.319	2.4E-09			
Residual	7	0.000318	4.55E-05					
Total	8	0.065						
	Coefficier	Standard	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.(	Upper 95.(
Intercept	0.179667	0.005687	31.59415	8.22E-09	0.16622	0.193114	0.16622	0.193114
X Variable 1 (m)	**1.641667**	0.04353	37.71365	2.4E-09	1.538735	1.744598	1.538735	1.744598

Figure 4- 7: Regression data

4.5 Flocculation of gold nanoparticles

The design scheme of the colorimetric device depicted in **Figure 3.3** employed the flocculation process of gold nanoparticle, with the main focus on the addition of poly-DADMAC solutions. Interestingly, it was observed that when 100 µl of gold nanoparticles was dropped on the printed well, the droplet did not flow through and it stayed intact within the printed well. This phenomenon is well documented for wax patterning because the wax printing process is known to create a hydrophobic pattern after heating wax ink leading to its diffusion through the paper. In addition, the fact that a normal A4 printing paper was used for this fabrication, it was again observed that these droplets could not penetrate the paper because of the hydrophobic nature of paper. The combination of hydrophobicity of A4 paper and wax pattern created stable drops which were used for colorimetric assay.

There are few articles which reported the colorimetric devices using gold nanoparticle. Notable in all these reported procedures, cuvettes were mainly used in conjunction with the spectrophotometric analysis (Magubane *et al.* 2019a). Herein the researcher has managed to demonstrate the application of the intensity variation during the flocculation process of gold nanoparticle as a result of different poly-DADMAC concentration.

The flocculation process is depicted in **Figure 3-3**. Clearly, the citrate stabilised gold nanoparticles have the carboxyl end of the citrate facing towards the surface of isotropic nanoparticles as shown in **Figure 4-7**. This arrangement is very critical for stabilising gold nanoparticles in solution.

The stability of gold nanoparticles is caused by the surface charge that attracts a thin layer of citrate ions of opposite charge to the nanoparticle surface. This double layer of citrate ions travels with the nanoparticle as it diffuses throughout the solution **(Figure 4-7)**. The electric potential at the boundary of the double layer is known as the Zeta potential of the particles and has values that normally range from -100 mV to +100 mV.

The Zeta potential is normally used as a predictive system of gold nanoparticles stability in solution. It has been shown that all nanoparticulate systems with Zeta potential values which are greater than +25 mV or less than -25 mV are typically indicative high degrees of stability. However, an immediate disturbance of double layer could result to low zeta potential value which will eventually aggregate due to Van Der Waal inter-particle attractions as a result of the addition of poly-DADMAC.

The schematic demonstration of the double layer disturbance is shown in **Figure 4.7**. A clear pictorial demonstration of the evolution of bluish colour due to variation of poly-DADMAC concentration is shown in **Figure 4.7(b)**.

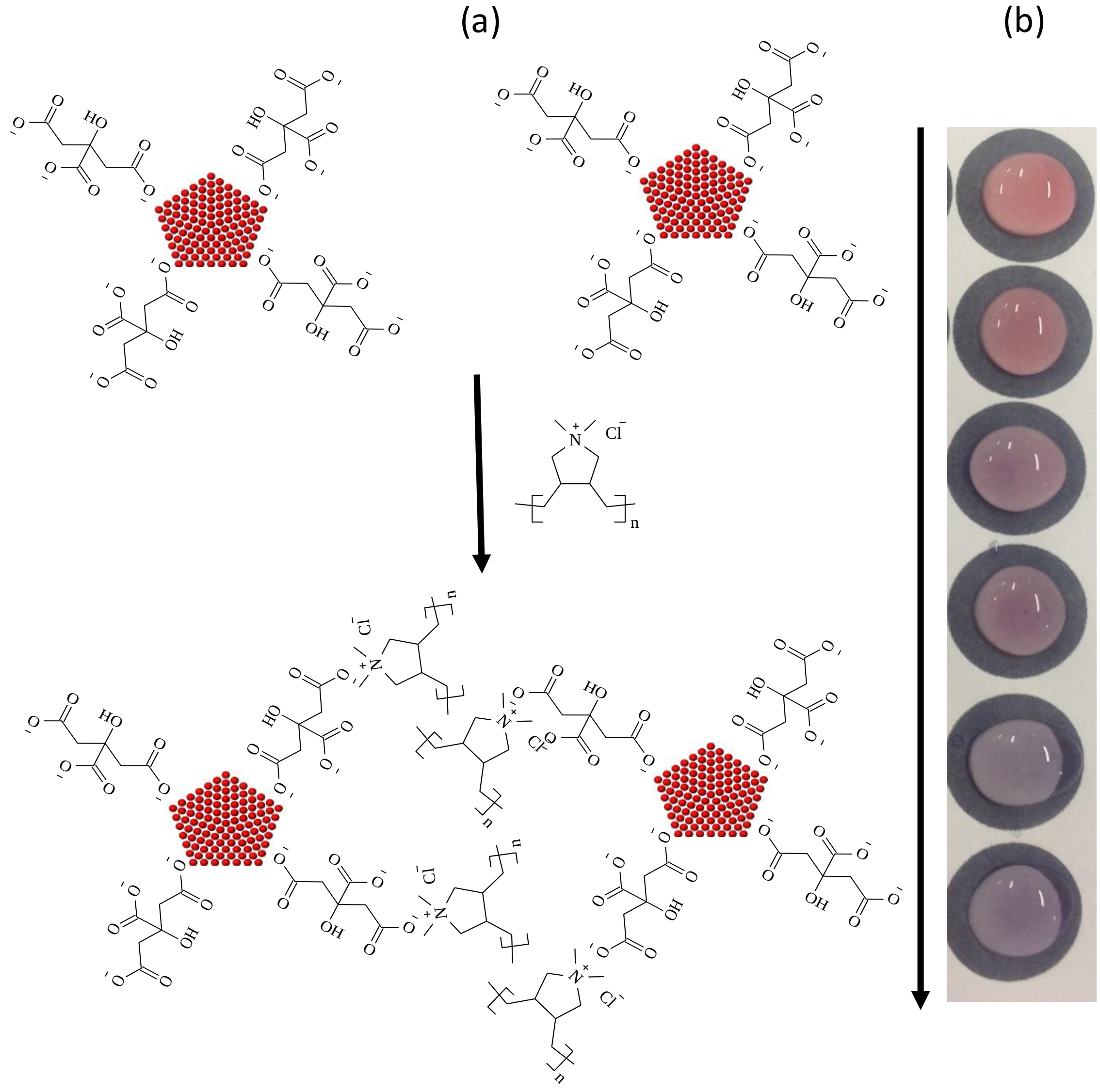


Figure 4- 8: The schematic demonstration of the double layer disturbance

4.5.1 Validation of the flocculation process using UV/VIS spectrophotometer

The aggregation of gold nanoparticles was further validated using the UV/Vis spectrophotometer; results of this are shown in the spectrum **(Figure 4-8)**. It was clearly observed that there was a clear appearance of the new red-shifted surface Plasmon resonance peak at 610 nm symbolising aggregation. Importantly, the intensity of the new SPR peak at 610 nm increased with an increase in the concentration of poly-DADMAC. This was indicative of the well-known phenomenon of gold nanoparticle where the interaction of light with isotropic gold nanoparticles would produce a strong SPR peak at around 520 nm. However, the immediate change to form anisotropic nanoparticles alters the incident rays interaction with the surface of gold nanoparticles and thus producing a second red-shifted SPR peak. This optical property of gold nanoparticles was very significant in the optimisation of the proposed colorimetric device.

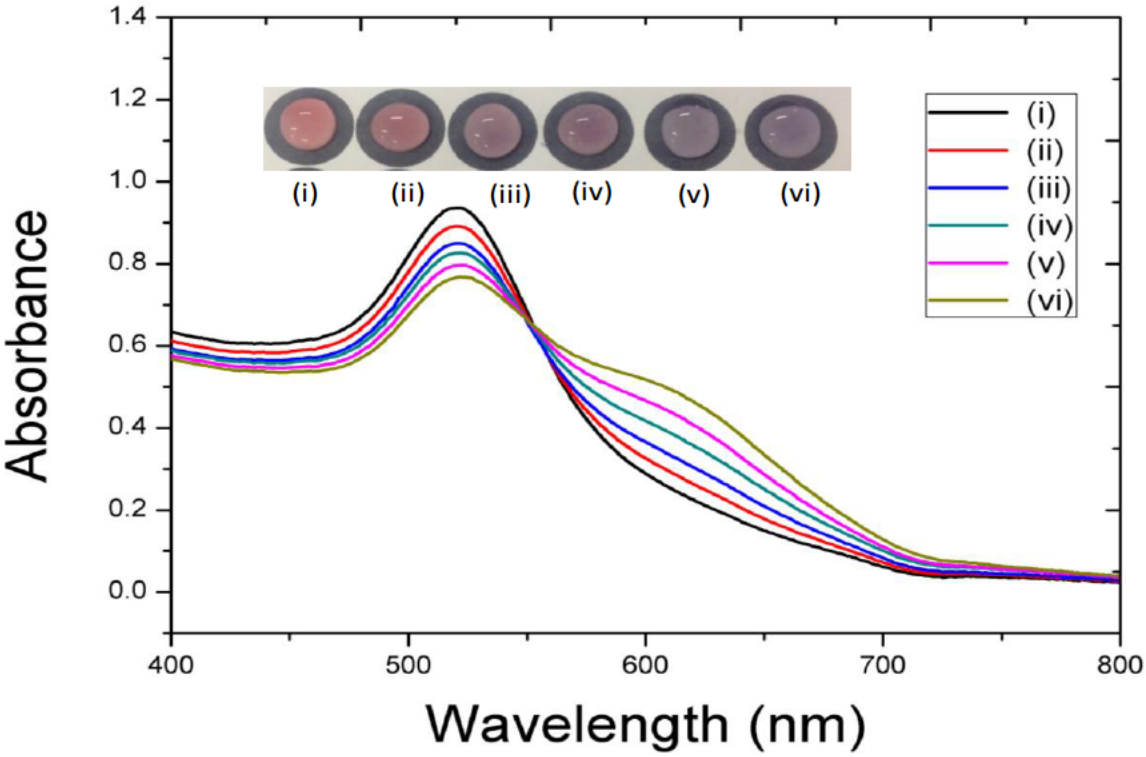


Figure 4- 9: The aggregation of gold nanoparticles

4.5.2 Optimisation of CIEL*a*b*/Yxy colour system for colorimetric devices fabricated with gold nanoparticles

The limited observation of colours using our eyes is a major contributor of systematic errors during the application of colorimetric devices. Thus, such a limitation can be reduced by using CIE L*a*b*/Yxy. The CIE L*a*b* data of the calculated ΔE are given in **Figure 4.9**. In **Figure 4.9(a)**, the calculated ΔE values for each column of the given colorimetric devices given in **Figure 4.9(b)** indicated that there was colour change in each column.

Figure 4.9(a) was sectioned into two regions, red (ΔE 40 to 46) and blue (ΔE 26 to 40). It is noted in support of **Figure 4.9(b)** that ΔE for column 1 and 2 is higher than all others. However, it is apparent that ΔE for rows H and I for column 1 and rows F, G and H for column 2, they had fallen in the blue region, even though the visual observation of device in **Figure 4.9(b)** seemed to suggest that these rows were supposed to be in the red region.

This apparent deviation may be due to systematic errors during the application of image J where certain part of the hydrophobic barrier may have been included into the analysis thus causing a deviation in the data as shown in **Figure 4.9(a).** The other may have been the fact that when this multiplex system was analysed all rows were done then later analysed. This seemed to suggest that, for all future gold nanoparticle colorimetric devices, multiplex system is discouraged. This may be due to the fact that the working principle of this device is based on the flocculation of gold nanoparticles, thus, if this was allowed to continue longer than the expected, gold nanoparticles would coagulate leading to diminishing of the expected blue colour.

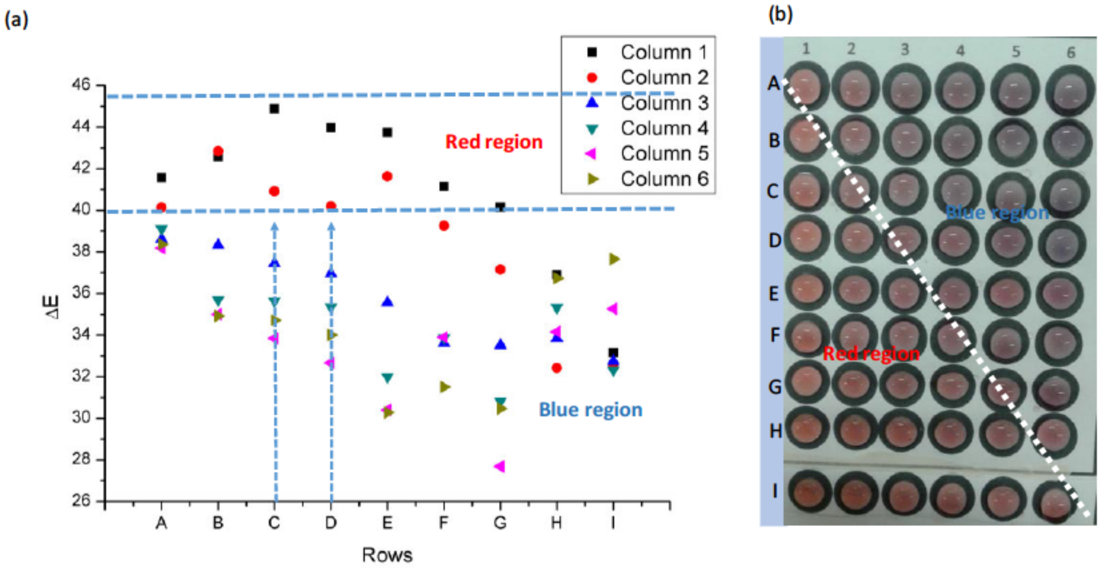


Figure 4- 10: The CIE L*a*b* data of the calculated ΔE

Further analysis of CIE chromaticity was investigated as shown in **Figure 4-11**. This analysis was based on defining the three standard primary colours which were combined to produce possible perceived colours. The three standard primaries were based on the 1931 CIE, called X,Y and Z which were referred to as imaginary colours. These primaries were used because they provide an international standard definition for all colours and the negative value colour matching is normally eliminated. The Y primary in the CIE was added to match the response of the human eyes. The quantities of X, Y and Z primaries are required to match any colour with the spectral energy distribution P (λ).

The horseshoe-shaped CIEL Yxy shown in **Figure 4-11** confirmed the aggregation of gold nanoparticle by shifting from normal pink colour of gold nanoparticles to blue. The apparent observation is that of A1 to I1 which are located in the red region, indicate that in the absence of poly-DADMAC no aggregation is induced.

Apart from cell G2, which was observed to have shifted towards the yellow region, this is normal for gold nanoparticles aggregate. The flocculation test, if not read quickly, could lead to clearing of gold solution, thus, resulting to this abnormality.

The chromaticity diagram had assisted us to optimise and to determine the optimum amount of poly-DADMAC that is required for each test. It could be observed that the cells H1 to H6 all remain concentrated within the red region with a slight shift towards the blue region. All other cells confirmed that increasing the concentration of poly-DADMAC will lead to a more intense blue colour development which can be optimized to develop a colorimetric assay.

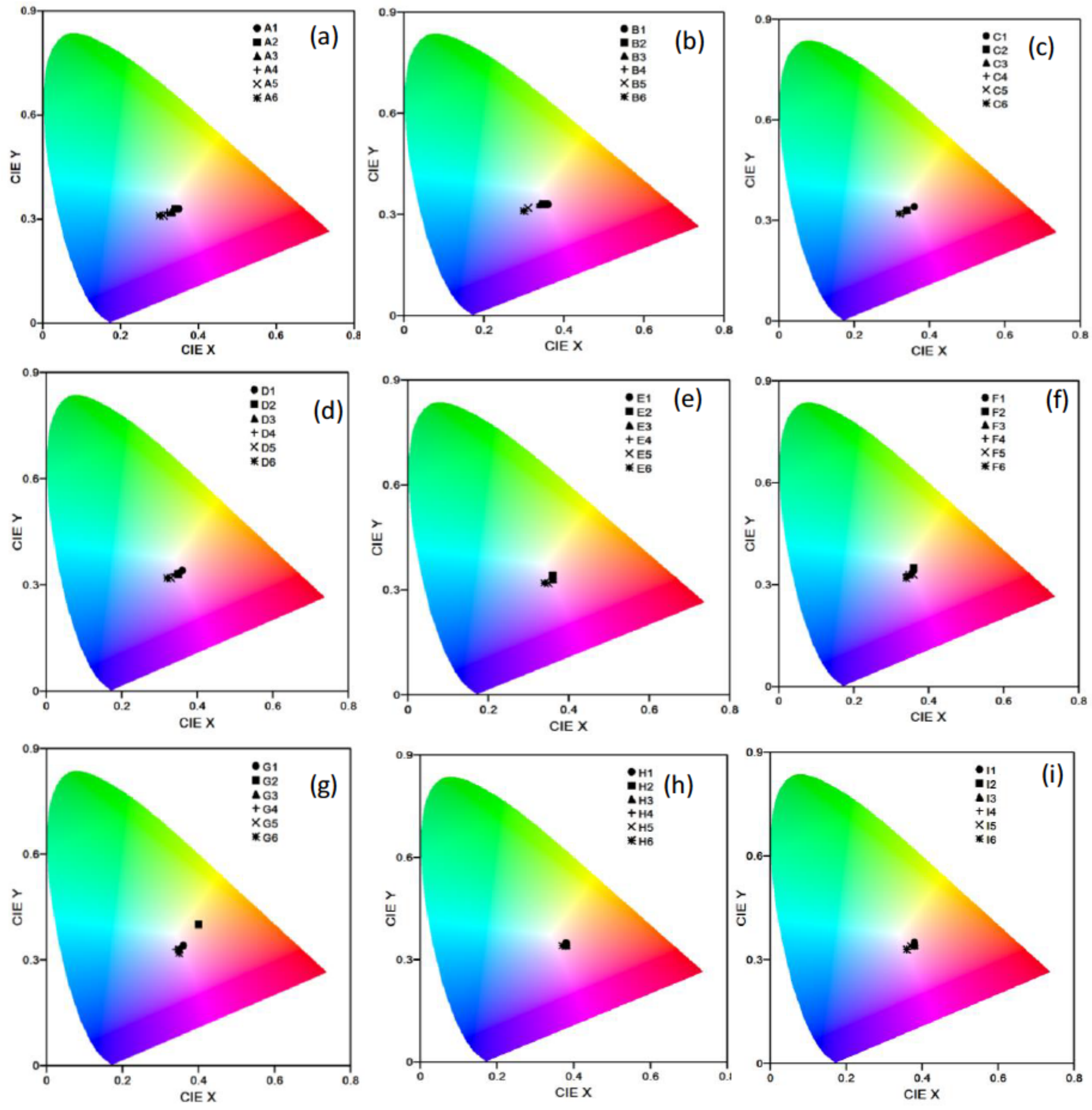


Figure 4- 11: Horse CIE lab image of the evolution of gold colours aggregation

4.6 Analysis of samples by UV-VIS spectrophotometer

The samples were analysed in the same way as the calibration standards. To each plastic cuvette, 3 mL of Au-NP (at 3 X dilution) was transferred followed by the addition of 100 µL of sample in aliquots of 20 µL. The UV spectrophotometer scans obtained are discussed below.

4.6.1 Analysis of poly-DADMAC in mixing tank

Figure 4-12 depicts the results of poly-DADMAC in the mixing tank where it is diluted with water before use. The dilutions that were used are 2000, 5000, and 20 000 X dilution for a, b and c respectively. From the scans in **Figure 4-12**, the additions of the 20 µL aliquots of sample can be clearly seen for the poly mixer samples because the poly-DADMAC concentration in them is quite high.

Moreover, the colorimetric result obtained in **Figure 4-12**(a) was a deep blue. This is an indication of the high concentration of poly-DADMAC in the sample. Similar trend could also be observed in **Figure 4-12** (b) and (c). The high result in **Figure 4-12**(a) was expected because the dilution factor was the lowest of the 3 dilutions made. This high result is also confirmed by the high absorbance of the peak at 637 nm.

The result of **Figure 4-12**(a) was also supported by the Lovibond image in **Figure 4-13**. The said image visibly confirmed the deep blue colour which is an indication of the high presence of poly-DADMAC.

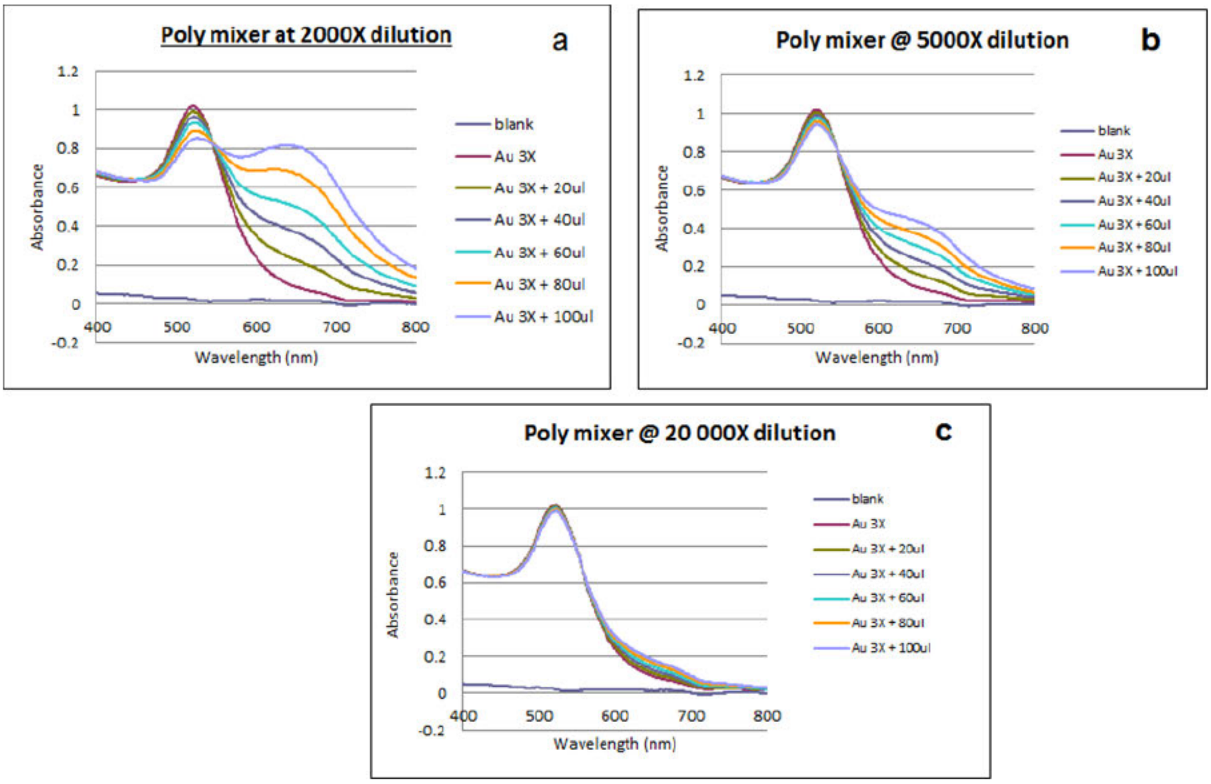


Figure 4- 12: UV scan for poly-DADMAC mixing tank where the poly-DADMAC is diluted in (a) 2000; (b) 5000; and (c) 20 000 times

Figure 4- 13: Lovibond colorimetric indication of poly-DADMAC in mixing tank (arrow pointing to the blue colour)

4.6.2 Analysis of poly-DADMAC in water samples

The results of the screening of poly-DADMAC in the four selected water samples are presented in this section.

Figure 4-14 below shows the results obtained from the Mhlathuze raw and final water samples. The colorimetric results obtained suggest that there is very little or no poly-DADMAC in both samples. It can be visually observed as well that there is no significant difference in colour between the water sample and the AuNPs in both the raw and final samples (A1 and B1).

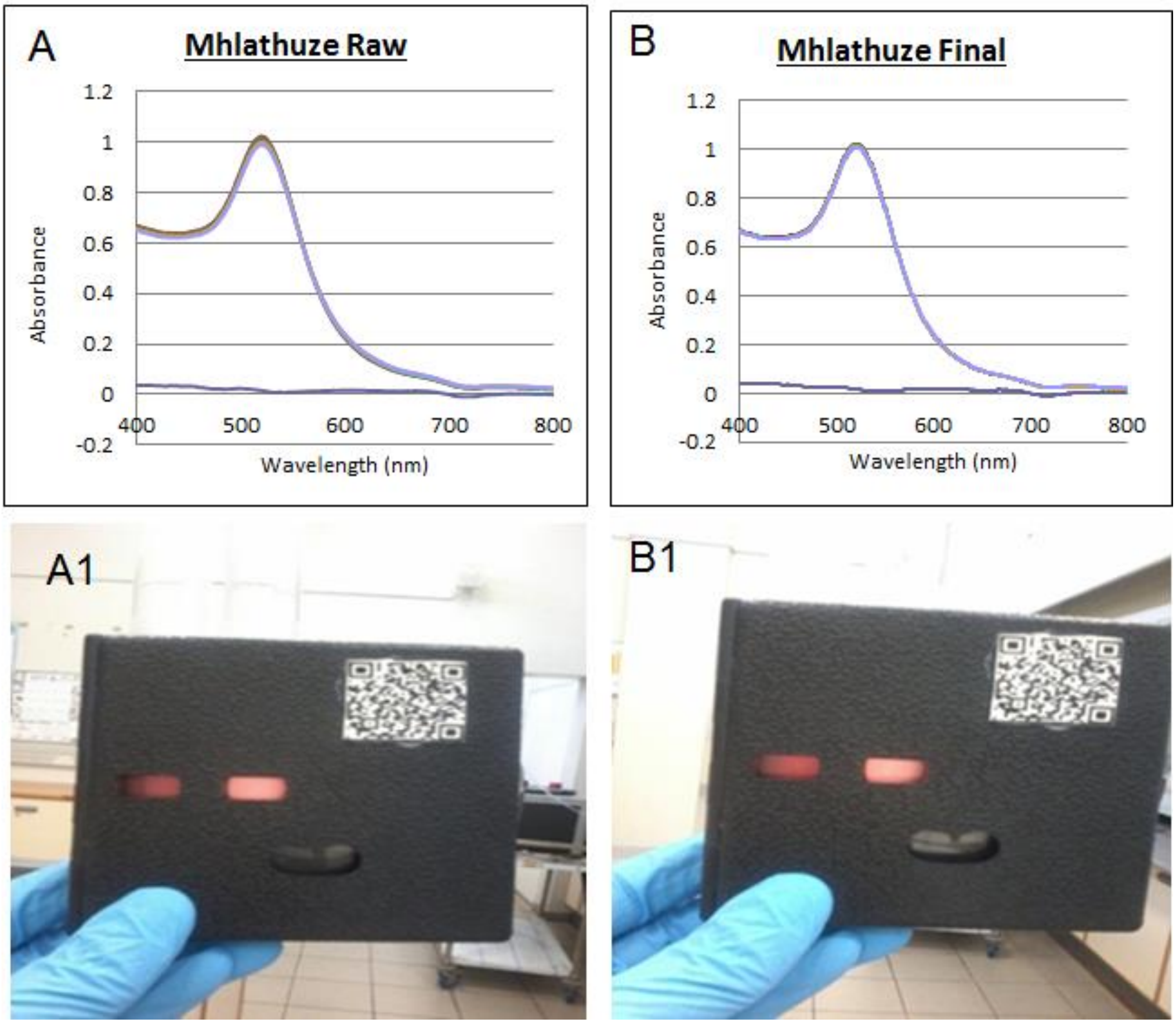


Figure 4- 14: A and B are the UV scans of Mhlathuze water before and after treatment respectively, A1 and B1 are Lovibond results before and after treatment respectively.

Figure 4-15 below shows the results obtained from the ESikhaleni raw and final water samples. The colorimetric results obtained suggest that there is very little or no poly-DADMAC in the samples. It can be visually observed as well that there is no significant difference in colour between the water sample and the AuNPs in both the raw and final samples (**C1 and D1**).

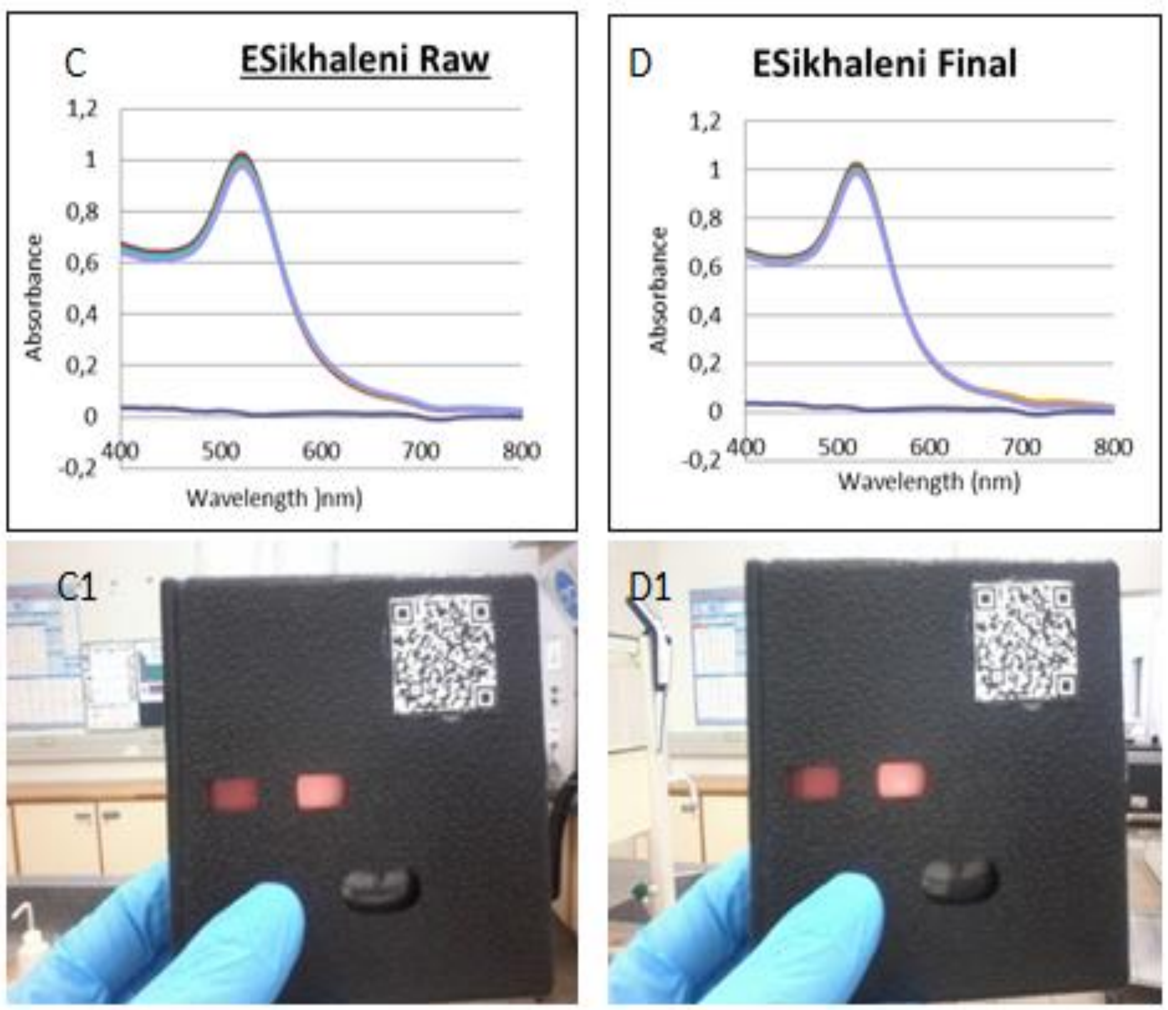


Figure 4- 15: C and D are the UV scans of ESikhaleni water before and after treatment respectively, C1 and D1 are Lovibond results before and after treatment respectively

Figure 4-16 below shows the results obtained from the Mzingazi raw and final water samples. The colorimetric results obtained suggest that there is very little or no poly-DADMAC in the samples. It can be visually observed as well that the there is no significant difference in colour between the water sample and the AuNPs in both the raw and final samples (**E1 and F1**).

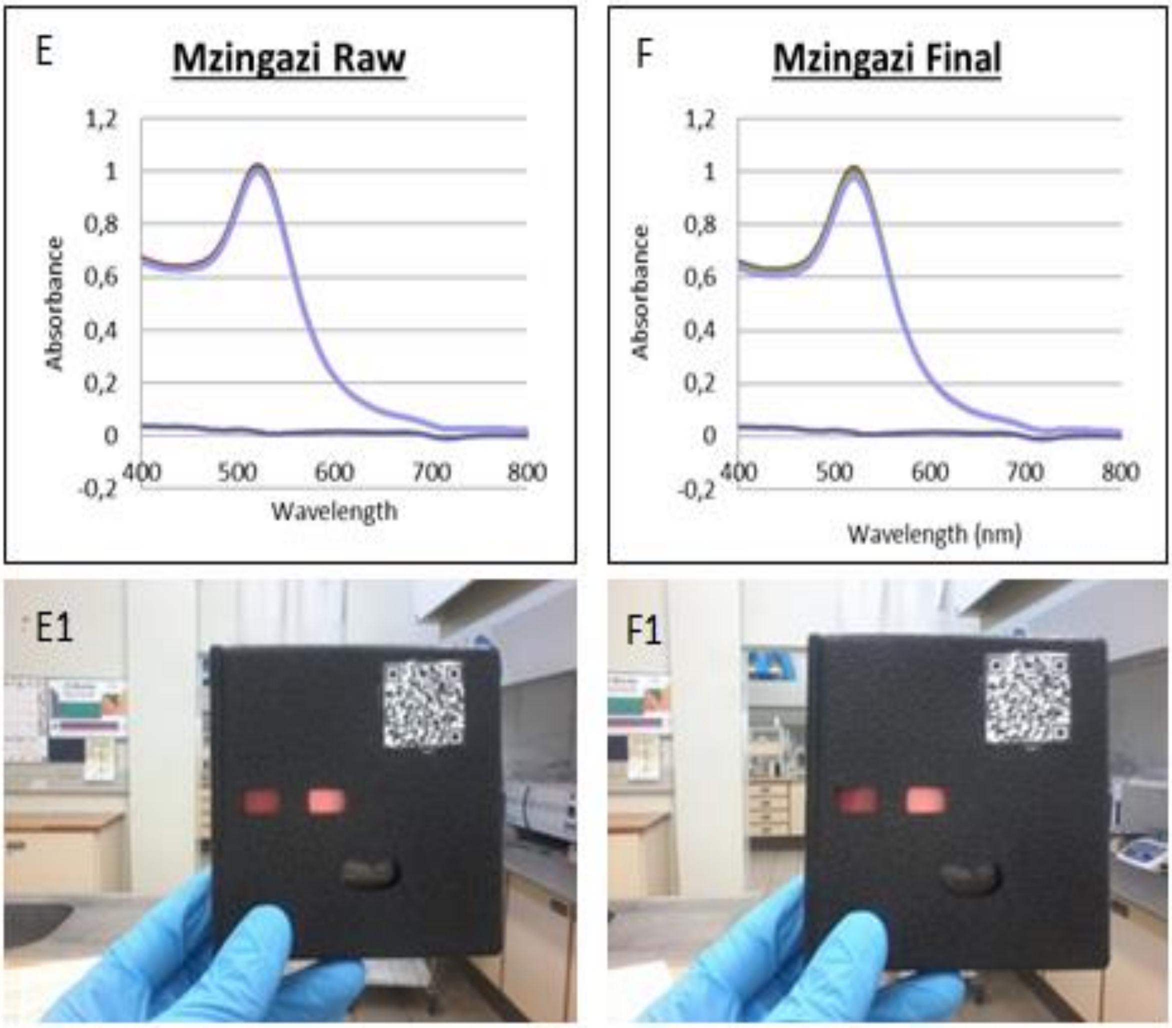


Figure 4- 16: E and F are the UV scans of Mzingazi water before and after treatment respectively, E1 and F1 are Lovibond results before and after treatment respectively

Figure 4-17 below shows the results obtained from the Nsezi raw and final water samples. The colorimetric results obtained suggest that there is very little or no poly-DADMAC in the samples. It can be visually observed as well that the there is no significant difference in colour between the water sample and the AuNPs in both the raw and final samples (**G1 and H1**).

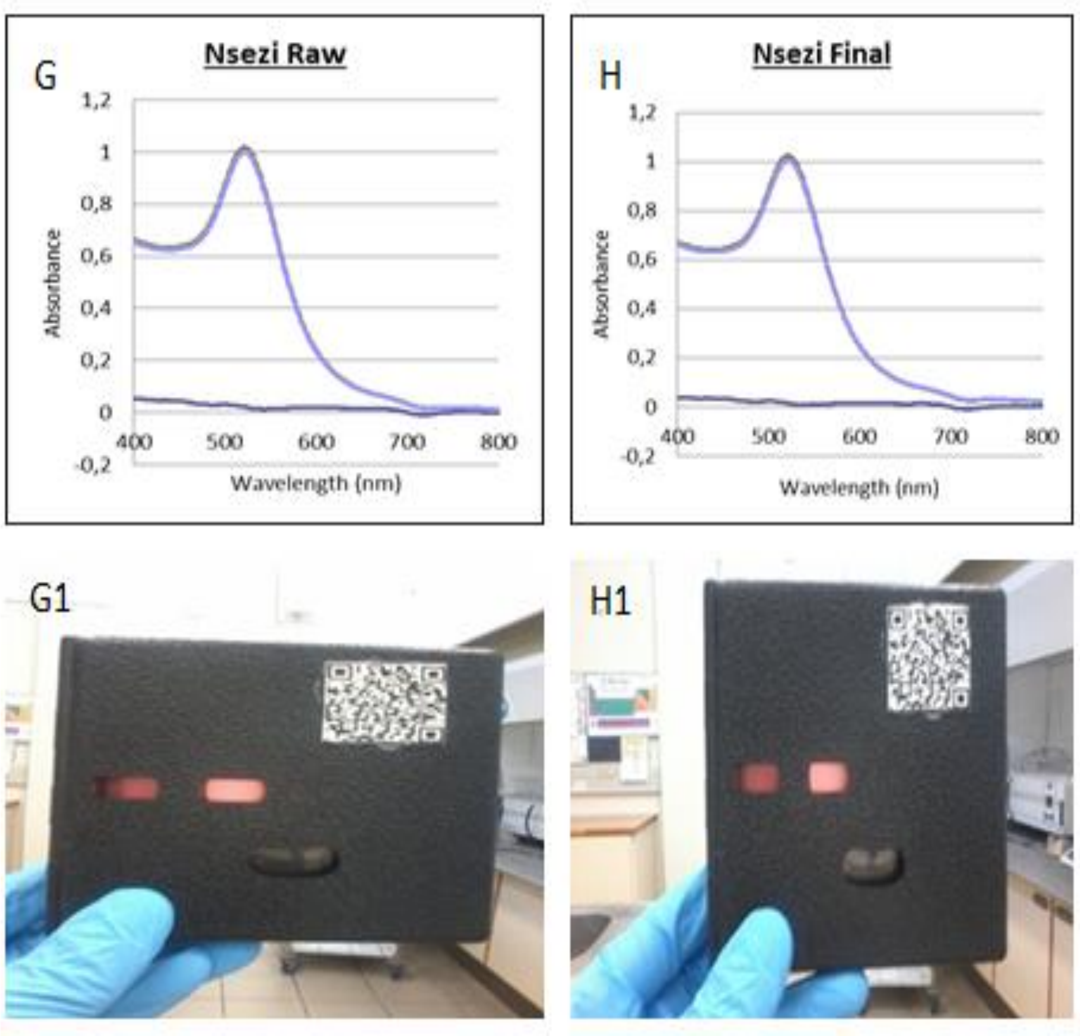


Figure 4- 17: G and H are the UV scans of Nsezi water before and after treatment respectively, G1 and H1 are Lovibond results before and after treatment respectively

From the results above, there was no poly-DADMAC in the raw and treated water samples. This indicates that the raw water is not contaminated with poly-DADMAC either from industries or activities that occur around the rivers in question. Since poly-DADMAC is added during water treatment, it is critical to ensure that it meets the requirement of not exceeding 1mg L^{-1} in the final product.

4.7 Recovery study

This section aimed to demonstrate that the colorimetric method is accurate and selective at the same time. Accuracy is the agreement between the obtained value and the true value of the analyte of interest. Selectivity is the ability of the method to specifically detect the analyte of interest in the sample.

To prove these parameters, a treated water sample was spiked with a known concentration of the poly-DADMAC standard. To calculate recovery, the following formula was used: Recovery = (($X_{sp\ ked}$ – $X_{original}$)/spike conc) * 100. Where:

X_{spiked} = concentration of the spiked sample

$X_{original}$ = concentration of the unspiked sample (original)

Spike conc = concentration of the spike standard in the sample

Three poly-DADMAC standards were prepared (1, 5 and 10 mg L^{-1}) and used for calibration. The preparation was done as shown in **Table 4-5** and the resultant calibration curve is in **Figure 4-6**

Table 4- 6: Standard and sample preparation for recovery

Sample Name	Vol poly (ml)	Vol AuNP (ml)	Dilution factor	Poly Calc Conc (ppm)
AuNP Blank	0	3	0	0,00
1ppm poly	0,1	3	30	0,03
5ppm poly	0,1	3	30	0,17
10ppm poly	0,1	3	30	0,33
X original	0,1	3	30	-
X spiked A	0,1	3	30	-
X spiked B	0,1	3	30	-

A calibration curve was constructed from the calibration standards above. The regression line obtained was y = 1.102x + 0.1657. This regression line was used to calculate the raw concentrations of poly-DADMAC. The calculated concentrations were obtained by multiplying the raw concentrations by the dilution factor of 30. From there, the recoveries were calculated using the given formula. The results are shown in **Table 4.6**.

The recoveries obtained in **Table 4-6** all meet the acceptance criterion of 80 to 120 %. This therefore means that this method is indeed selective. It can accurately detect poly-DADMAC in the sample.

Table 4- 7: Recovery data

Sample Name	Wavelengths (nm)	Absorbances	Ratio $(A_{621,00}/A_{521.01})$	Poly Raw Conc (mg L^{-1})	Poly Calculated conc (mg L^{-1})	Recovery (%)
AuNP Blank	621.00	0.1507	0.1467	-0.0173	-0.5184	-
	521.01	1.0278				
1 mg L^{-1} poly	621.00	0.2181	0.2210	0.0501	1.5042	
	521.01	0.9871				
5 mg L^{-1} poly	621.00	0.3374	0.3541	0.1710	5.1290	
	521.01	0.9529				
10 mg L^{-1} poly	621.00	0.4887	0.5288	0.3295	9.8851	
	521.01	0.9242				
X original	621.00	0.1612	0.1624	-0.0030	-0.0909	
	521.01	0.9926				
X spiked A	621.00	0.2021	0.2054	0.0360	1.0795	**107.95**
	521.01	0.9842				
X spiked B	621.00	0.1978	0.2025	0.0334	1.0030	**100.30**
	521.01	0.9767				
X spiked C	621.00	0.1898	0.1992	0.0304	0.9126	**91.26**
	521.01	0.9527				

4.8 Discussion of results

Reports have indicated that the required standard dosage of polyelectrolytes that can be added in water treatment should be set between 10 and 100 mg L^{-1}, these studies have also reported that the allowable residual amount of poly-DADMAC must be at 50 µg L^{-1} for potable water (Letterman and Pero 1990; Jin et al. 2006b; Manickum et al. 2015) . It is therefore without any doubt that the fabricated Lovibond disk which is reported here can contribute very immensely in screening on-site samples for excess levels of poly-DADMAC. These systems can be used for on-site screening, in cases where there is an imminent variation of the concentration level of poly-DADMAC samples can be taken into the well-established laboratory for further quantitative analysis of the suspected water. Overall, results of these tests obtained from the colorimetric device were in range of 1-0.10 mg L^{-1} for all potable and raw water samples from Mhlathuze, Mzingazi, Nsezi and eSikhaleni. This was obtained by comparing the colour of water after the addition of gold nanoparticles and the fabricated disk. The validated spectrophotometric results from Mhlathuze, Mzingazi, Nsezi and eSikhaleni are given in Table 4.1 as well.

The poly-DADMAC concentration was found to be from 0.0002 to 0.0225 mg L^{-1}. In order to validate high concentrated samples, three poly-DADMAC mixing tanks samples (Poly mixing tank @ 5000X, Poly mixing tank @ 2000X and Poly mixing tank @ 20 000X) were obtained from Mhlathuze water treatment plant. These samples were diluted at 50000, 2000 and 20000 respectively and were tested using the spectrophotometric technique. The concentration of poly-DADMAC was found to be 2431, 2221 and 2645 mg L^{-1} respectively. The blue colour due to coagulation of gold nanoparticles was obtained.

Upon the application of Lovibond colorimetric disk, it was observed that these samples were above the calibrated colour associated with 10 mg L^{-1} which is the highest concentration of the fabricated colour filter disk. The physical-chemical water quality test results for turbidity, conductivity, TSS, COD and Colour are also reported in **Table 4.1**. Results of the turbidity level was found to range from 0.35 to 89.5 NTU for both raw and final portable water from the sampled site. The turbidity level did not have any major effect into the measured level of poly-DADMAC. Thus Lovibond colorimetric disk which is reported here was not affected by potential matrices which may have been in the potable water.

In summary, a simple and rapid colorimetric disk was developed using AuNPs for detecting *poly-DADMAC*. The newly designed colorimetric disks were successfully applied to the screening of *poly-DADMAC* in potable water from selected areas. These tests can be completed with 10 min and they have advantages in portability and disposability since it is small, light, and inexpensive. It should be emphasised that the two colorimetric methods need no calibration curve during their usage since the concentration of the sample is determined directly by the reference known amounts based on the colour of the disk. However, more substantial works was conducted to validate these results using UV–visible spectrophotometric method. As a result, the proposed colorimetric disks are available alternatives to conventional spectrophotometric methods in the analysis of several polyelectrolytes in the field.

Chapter Five: Conclusion and Recommendations

5.1 Conclusion

The aim of this study was to fabricate a gold nanoparticle based Lovibond colour filter for quantification of residual poly-DADMAC in treated potable water.

Gold nanoparticles were successfully synthesized using the citrate reduction method. The solution was a deep red colour, which is what was expected. Upon optimisation by the UV-VS spectrophotometer, it was observed that the synthesised gold nanoparticles had a surface Plasmon resonance peak at 520 nm. The average size for these gold nanoparticles was 14 nm. TEM images also proved the size and morphology of these gold nanoparticles.

A method was developed and optimised on the UV-VIS spectrophotometer. Calibration standards from 1 to 10 mg L^{-1} were read at 30X dilution and produced a curve with a correlation coefficient of 0.9954 which points to good linearity. The recovery study that was done has successfully proven and verified the accuracy of the method. Selectivity was also validated by analysing samples of different matrices.

The Lovibond comparator was successfully assembled and an SOP which is user-friendly was written up. Quantification of poly-DADMAC was carried out using the UV-VS spectrophotometer and the results were compared to those obtained from the Lovibond comparator. There was no significant difference between the results. This proved that the Lovibond comparator’s results can be trusted.

In this research, we have also successfully illustrated the application of CIEL*a*b*/Yxy to demonstrate the development of gold nanoparticles enabled paper colorimetric for screening of poly-DADMAC. It was shown that the chromaticity diagram could be very significant to study the intensity of the colours produced by gold nanoparticles during flocculation.

This research could pave ways to revolutionized colorimetric assays development. We have also developed a simple and cheap multiplex gold nanoparticles paper based 6x9 wells system. The 6x9 wells system could enable many more application for quickly screening of various water samples using cheap paper based material.

5.2 Recommendations

Further studies are required that will upgrade the comparator from reading results manually to making it more digital. Visually reading the colour results is dependent on what the individual sees. However, getting digital results will eliminate the risk of misinterpretation of colour. This will greatly improve the accuracy in using the device correctly.

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Table Physicochemical properties of water in the studied sources.

		pH	COD[a] (mg L-1)	Conductivity (mS/m)	TSS[b] (mg L-1)	Turbidity (NTU)	Colour Pt-Co
Nsezi	Raw	6.70	22	35.9	19.8	54.7	361
	Treated	7.06	15	30.7	<1.0	0.52	<2.0
Mzingazi	Raw	7.10	18	99.5	20.4	7.52	38.1
	Treated	7.01	13	35.3	<1.0	0.74	5.2
ESikhaleni	Raw (Cubhu)	6.37	16	68.8	32.2	3.14	36.6
	Treated	6.96	12	68.4	<1.0	0.52	6.7
Mhlathuze	Raw	6.95	24	30.7	25.2	89.5	457
	Treated	6.76	44	31.1	<1.0	0.62	15

(a) Chemical Oxygen Demand, (b) Total Suspended Solids