SURFACE AND ADSORPTIVE PROPERTIES OF *MORINGA OLEIFERA* BARK FOR REMOVAL OF METALS FROM AQUEOUS SOLUTIONS

by

ROBERT LONDI MNISI

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Supervisor: Dr. P. P. Ndibewu
Co-supervisor: Prof. N. S. Mokgalaka

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DECLARATION

“I hereby declare that the thesis submitted for the degree Doctor Technologiae: Chemistry, at the Tshwane University of Technology, is my own original work and has not been previously submitted to any other institution of higher education. I further declare that all sources cited or quoted are indicated and acknowledged by means of a comprehensive list of references.”

Robert Londi Mnisi
DEDICATIONS

This work is dedicated to my family: My wife Philile, and my two boys; Snenkosi and Onalenna for ‘allowing’ me to pursue a dream that seemed elusive, and that left them without a father figure.

“In the end,
We will remember not the words of our enemies,
But the silence of our friends”

Martin Luther King Jr.
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To Him who declares the end from the beginning be the Glory, Honour, Praise, Worship, Adoration, Majesty and Lordship for ever and ever. “I hold true your promise in Isaiah 43:2”.
ABSTRACT

Technological innovations into metal recoveries from waste streams have been limited by factors such as low metal recoveries; costs and technical expertise associated with the application of the technologies, especially in disadvantaged communities; extensive use of chemicals and, that most of the techniques are inappropriate for low metal concentrations. This is where the hypothesis of the study is premised: that a plant-based natural polymer at different stages of chemical treatments can be used to remove metallic contaminants from aqueous solutions. The bark of *Moringa oleifera*, a naturally growing plant native in India, Mexico and Middle East, was used to test the validity of this hypothesis.

To achieve this, bark samples were characterised to investigate its surface properties using N₂-BET; Fourier Transform infrared (FTIR) spectroscopy; scanning electron microscopy (SEM) coupled with an energy dispersive x-ray spectroscopy (EDS); a CHNS elemental analyser to determine the proximate elemental composition of C, H, N, O and S; an x-ray diffractometer (XRD) to investigate sample consistency and crystallographic properties. Preliminary adsorption experiments were performed in batch using a 300 µm diameter pulverized bark sample. These were optimized for pH, contact time, initial metal concentration, temperature and adsorbent dosage using VO₂⁺ as an adsorbate.

The results show a relatively high adsorption capacity (48.8 mg/g), within 1 h of reaction time with maximum adsorption in acidic medium, at pH 4.6. pH was found to greatly influence the adsorption process. Adsorption data were fitted using Langmuir and
Freundlich isotherms and correlation coefficient values were higher ($r^2 = 0.999$) with the latter, suggesting a better fit therein.

Aliphatic polyamines of increasing chain length (ethylenediamine, $\text{C}_2\text{H}_8\text{N}_2$, (EDA), diethylenetriamine, $\text{C}_4\text{H}_{13}\text{N}_3$, (DETA), triethylenetetramine, $\text{C}_6\text{H}_{18}\text{N}_4$, (TETA) and tetraethylenepentamine, $\text{C}_8\text{H}_{18}\text{N}_5$, (PETA) were immobilized on the surface of the bark sample and their effects on adsorption investigated. Characterization results show a significant increase in the amount of nitrogen-based groups on the surface of the adsorbent (17, 19, 29 and 27%) with an increase in polyamine chain length, polyamine density and surface area. Batch experiments on the amine-modified adsorbent show a significant increase in the amount of $\text{VO}_2^+$ adsorbed in the order: DETA$>$EDA$>$TETA$>$TEPA, representing percentage increases of 155, 146, 67 and 42%, respectively.

Polar water soluble biomolecules in the broth of the bark were extracted and used to synthesize gold nanoparticles, with $\text{AuCl}_4$ as a precursor salt. SPR bands confirmed the formation of gold nanoparticles at 520 nm, in agreement with conventional synthetic protocols. HRTEM micrographs show the presence of a variety of shapes, (spherical, triangular, elongated) with the predominance of spherical nanoparticles. Variable sizes were obtained with average diameters around 8 nm, with a predominance of very small nanoparticles less that 2 nm. Furthermore, the nanoparticle colloidal suspension was used to determine the concentration of lead ions in aqueous solutions using batch experiments. Lead was successfully determined and analytical figures of merit applied thereto.
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CHAPTER 1

1.0 INTRODUCTION

1.1 BACKGROUND AND MOTIVATION

Pollution of the environment by man through anthropogenic sources is an age-old practice of mankind. In this way, man has been his own enemy since time immemorial by polluting and contaminating the very resources that he needs for his survival. This happens through man’s quest to obtain as much of natural resources as possible. Unfortunately, the unsustainable means of extraction and processing affect the very principles of sustainable development as enshrined in international environmental protection instruments such as the Rio Declaration of 2002. Some of the resulting environmental challenges are evident in the mining, agricultural, processing and manufacturing sectors; where millions of dollars are accumulated in profit at the expense of environmental quality and sustenance.

These industrial operations produce large amounts of wastes (synthetic or natural) which are disposed of into the environment, whether treated or not. The composition of these wastes is variable depending on the type of industry, but may be broadly classified to contain organic and inorganic contaminants, such as metals. These contaminants have a cumulative effect on the quality of receiving waters, especially if untreated or poorly treated. Such effects result in eutrophication, high biochemical
oxygen demand (BOD) and chemical oxygen demand (COD) and acid mine drainage (AMD). These negatively affect aquatic life and may decimate or even render some ecologically significant aquatic species extinct.

Among these substances, metals have been identified as priority environmental pollutants, since they are easily transported, bio accumulate, are refractory and non-biodegradable in the environment, as well as toxic to plants and animals. Their toxicity in animals is through binding to thiol groups or other binding sites in enzymes, thereby causing conformational changes in the enzymes (Sharma et al., 2008; Goering, 1993), which also explains why some of them, such as Hg, are carcinogenic. Metal toxicity in animals is dependent upon its chemical form (Craig, 1986), its dosage (Xu et al., 2011) and the diet of the animal (Chan et al., 2004).

Natural inputs of metals into the environment are minimal and can rarely put a strain on biotic systems. Most of the metal pollution is from anthropogenic sources. Metals enter aquatic ecosystems as dissolved and solid wastes from domestic, industrial, and agricultural runoffs. Many industries, such as the automotive, metal-producing, electroplating, battery and electric cable manufacturing, mining, tannery, steel and textile, release metals such as Cd, Cu, Cr, Ni, and Pb into wastewaters (Dermirezen et al., 2007). Considerable trace metal concentrations have also been found in surface and sea waters which originate or pass through industrial activities, such as tanneries and paper mills. Such contaminations result in high concentrations of metals in plants,
sediment, and water, which demonstrate that the spatial distribution of metals in the environment, and the sources thereof are broad.

Generally, most metals have high soil-to-plant transfer ratios (Satarug et al., 2003) and high bioaccumulation factors (Mishra & Malik, 2012), suggesting that they prefer to accumulate in plant and animal tissues than to be dissolved in water or be adsorbed onto sediment particles. This means that higher than average concentrations of metals can be found in plant and animal tissues exposed to metal pollution.

The identification and removal of metals from waste water, as well as in receiving water is critical in keeping with principles of sustainable development. The development and application of effective, affordable and cleaner technologies of metal removal in keeping with emergent metal pollutant forms and concentrations is also vital. Conventional methods of metal removal such as coagulation-flocculation, flotation, electrochemical treatment (Guo et al., 2008; Bailey et al., 1999), chemical precipitation, ion exchange, electrolysis (Kurniawan et al., 2006; Wang et al., 2003), solvent extraction, ion-exchange and reverse osmosis (Sharma et al., 2006) have been applied with some degree of success. They, however, have some limitations such as the high capital and maintenance costs associated with their operation; the generation of large amounts of metal containing sludge which further creates problems of disposal of the sludge (Rubio et al., 2002). Some of these techniques have low recoveries and are inappropriate for low pollutant concentrations and make use of large solvent volumes during operation (Ngah & Hanafiah, 2008; O'Connell et al., 2008). These limitations necessitate
continued research and development of technologies and techniques that will remove metal contaminants from water at reasonable costs, with high efficiency and with minimum waste output.

Adsorption is recognized as an efficient and economic technique for the sequestration of metallic pollutants from aqueous solutions (Ndibewu et al., 2014; O’Conell et al., 2008). This method is based on the premise that the adsorbents have a high adsorption capacity - a factor that is dependent upon the surface properties of the adsorbent, pollutant (adsorbate) concentrations, as well as chemical characteristics of the adsorbent (O’Conell et al., 2008; Dermibus, 2008; Babel & Kurniawan, 2003). Activated carbon has been the most widely used adsorbent since the advent of research into the removal of metals from aqueous systems. Due to the high costs associated with the production and application of adsorption technologies such as activated carbon, synthetic resins and cation-exchangers, scientists are now actively exploring the use of natural and plant based adsorbents. A wide range of such adsorbents have been explored based on properties such as cost, effectiveness, local availability, technical feasibility and engineering applicability (Xiaomin et al., 2008).

Most industrial processes produce considerable amounts of solid waste as sludge or waste dumps. Recent research focus has shifted into using much of the waste material as possible, since they often present an aesthetic nuisance to the environment, apart from the associated health and environmental effects. It is therefore not uncommon to find that most adsorption studies are performed on simple wastes and or substances
such as grape stalk wastes (Villaescuca et al., 2004), rice husk ash and neem bark (Bhattacharya et al., 2006), sago waste (Quek et al., 1998), maize leaves (Babarinde et al., 2006), orange peels (Xiamomin et al., 2008), bagasse (Dinesh & Singh, 2002), Sawdust (Argun, 2007) and fly ash (Gupta & Ali, 2004).

However, plant-based adsorbents have been found to have major limitations during application, in that they tend to have problems such as low adsorption capacity, high chemical oxygen demand (COD) and biological oxygen demand (BOD) as well as high total organic carbon (TOC). This is attributed to the release of soluble organic compounds in the plant-based adsorbents (Gaballah et al., 1997; Nakajima & Sakaguchi, 1990) into the aqueous environment. This then necessitates that potential adsorbents are chemically modified prior to application to extract the soluble organic compounds so as to remove or minimize colouration of the aqueous solution. This also enhances the chelating efficiency and, depending on the degree of chemical modification, the chemically modified adsorbents are usually stable, have a high surface area, are highly reactive and result in shorter equilibration times (Ndibewu et al., 2011). Different pre-treatment methods utilizing different types of modifying agents have been explored such as base solutions (calcium hydroxide, Ca(OH)$_2$ or water softener; sodium carbonate, Na$_2$CO$_3$ or washing soda), mineral and organic acids (hydrochloric acid, HCl; nitric acid, HNO$_3$ and sulphuric acid, H$_2$SO$_4$), oxidizing agents (hydrogen peroxide, H$_2$O$_2$) and organic compounds (ethylenediamine, HN(CH$_2$CH$_2$NH$_2$)$_2$; formaldehyde, CHOH and methanol, CH$_3$OH) (Ngah & Hanafiah, 2008).
In spite of its wonderful properties and high adsorbent capabilities, activated carbon has cost-related limitations which restrict its application especially in deprived communities where income margins are low. Consequently, the use of naturally occurring substances is not uncommon, and they are also widely used as adsorbents in removing metals from the environment. These include, but not limited to, natural zeolites (Erdem et al., 2004), which may be expressed as two different formulas, an oxide formula \((\text{M}_2/\text{nO.} \text{Al}_2\text{O}_3.\text{xSiO}_2.\text{yH}_2\text{O})\) and an idealized formula \((\text{M}_x/\text{n[(AlO}_2)_x(\text{SiO}_2)_y].\text{wH}_2\text{O})\), clays, with chemical formula \((\text{AL}_{2}\text{Si}_{3}\text{O}_{5}(\text{OH})_{4})\) (Bhattacharyya & Gupta, 2007), chitosan (Wan Ngah et al., 2002) and coal, for example anthracite, peat, lignite, subbituminous and bituminous coal (Babel & Kurniawan, 2003). The interest in this work is to utilize a plant-based material (Moringa oleifera bark, MOB) as a potential adsorbent for the removal of metals from aqueous solutions.

1.2 RESEARCH PROBLEM

Metal concentrations in the natural environment are only at the trace level and can rarely affect biological systems, except for a few of the most toxic elements. This means that most of the reported metal concentrations and the negative effects, thereof, are due to anthropogenic activities. In order to minimise environmental damage, there is a need for cleaner and more environmentally friendly technologies for water treatment, which will result in water bodies and waste streams that meet specific legal requirements. Conventional metal removal technologies have economic and operational limitations, which restrict their use by the broader communities, especially the poor. Therefore, the
search for alternative metal removal technologies that appeal to the needs of the broader society is still not fulfilled.

The bark of *Moringa oleifera*, a naturally growing drought resistant plant that grows throughout the season is proposed in this study as a potential biosorbent for the removal of metal contaminants. This plant has been a subject of research due to its unique nutritional (Makkar & Becker, 1996), therapeutic (Chumark *et al.*, 2008; Verma *et al.*, 2009; Singh *et al.*, 2009) and water coagulation properties (Ndabigengesere & Narasiah, 1998). These properties are believed to be attributed to the presence of bioactive chemical constituents such as amino acids, fatty acids, vitamins, nutrients, glucosinolates and phenolics, some of which possess metal chelating properties (Ndabigengesere *et al.*, 1995). *M. oleifera* contains considerable amounts of cellulose, interlinked with lignin in its structure (Ndibewu *et al.*, 2011), which are believed to favour the adsorptive capacity of this plant.

Lignin is a complex biopolymeric heterogeneous molecule which is endowed with many different functional groups, including methoxy (-OCH₃), hydroxy-aliphatic (-OH-C-C), carboxyl (-COOH) and phenolics (Dermibas, 2004), which may actively participate in bond formation with metallic species in aqueous solutions. Lignin also has an aromatic, three-dimensional polymer structure (Lalvani *et al.*, 2000) and is insoluble in water, making it ideal for water purification applications. These properties have led to research and application into the use of *M. oleifera* seeds and leaves, mostly for nutritional and water coagulation purposes. However, information on the use of the bark for metal
sequestration from aqueous systems is missing or scanty and the associated mechanisms have not been explored, hence, the motivation of this work.

Since the leaves and seeds of the plant are edible and consumed all throughout the season, the bark has no known or reported economic use. This renders the bark as a non-competitive, cheap and readily available agricultural resource. Also, in spite of the overwhelming reported usage on *M. oleifera*, there are glaring information gaps that exist in understanding the mechanisms responsible for the water purification capabilities of *M. oleifera*, including biosorption (Ndibewu, *et al.*, 2011). These gaps have motivated the current research into investigating the surface and adsorptive properties of the bark for possible application in removing metal contaminants from aqueous solutions. Technologies using these types of materials will add value to a wasted agricultural by-product, thereby promoting environmental sustainability in developing countries, including South Africa.

1.3 HYPOTHESIS

The bark of *Moringa oleifera* can be used as an adsorbent to remove metals from aqueous solutions.
1.4 RESEARCH OBJECTIVES

The main aim of this work was to investigate the potential of using the bark of *M. oleifera* to remove metal contaminants from aqueous solutions. The specific objectives of the study were to:

1.4.1 characterize the bark of *M. oleifera* to understand its surface properties;
1.4.2 chemically modify the bark of *M. oleifera*;
1.4.3 investigate optimal adsorption conditions for selected metals;
1.4.4 determine adsorption capacity of *M. oleifera* bark for selected metals;
1.4.5 elucidate the adsorption mechanism of selected metals on *M. oleifera* bark;
1.4.6 synthesize novel products from *M. oleifera* bark extractibles; and
1.4.7 apply *M. oleifera* bark products at optimized conditions to remove metals from synthetic samples.
CHAPTER 2

2. LITERATURE REVIEW

2.1 PHYSICOCHEMICAL CHARACTERIZATION OF ADSORBENTS

Understanding surface properties of adsorbents for pollutant removal from waste water is critical in their design, optimization and application. Adsorbents vary greatly in pore characteristics, surface functionality, thermal and chemical stability in different aqueous media and in their chemical composition. These factors influence the suitability of the proposed adsorbent in water purification.

Even though there is considerable literature evidence to support the use of *M. oleifera* plant parts as effective water coagulants (Sànchez-Martin *et al.*, 2010; Ghebremichael *et al.*, 2005; Ndabigengesere & Narasiah, 1998; Ndabigengesere *et al.*, 1995), as well as their ability to significantly reduce coliforms (Poumaye *et al.*, 2012; Sengupta *et al.*, 2012), literature that explores surface and pore characteristics of the plant bark is missing or scanty (Ndibewu *et al.*, 2011). Therefore, the underlying mechanism(s) responsible for the reported capabilities of *M. oleifera* in water purification have not been fully explored, hence, are not clearly understood. The reported characterization protocols for the bark of *M. oleifera* and the results presented herein aim to fill that literature void to better understand the physicochemical properties of *M. oleifera* bark, as a proposed adsorbent.
The use of natural plant-based adsorbents has received much attention lately, in promotion of green chemistry principles. Most are non-competitive agricultural by-products; thereby rendering them readily available, at low costs and, depending on the surface properties, some require minimum chemical modification. In this regard, different adsorbents have been applied widely to remove different types of pollutants (organic and inorganic), with varying degrees of success. Such adsorbents include, but not limited to, rice husks and maize cobs (Abdel-Ghani et al., 2007), tea and coffee wastes (Orhan & Buyukgungor, 1993), hazelnut shells (Cimino et al., 2000), peanut hull (Hashem et al., 2007), sawdust (Bozić et al., 2013), different bark samples (Khokhotva & Waara, 2010; Naiya et al., 2009), apple wastes (Maranon & Sastre, 1991), tea leaves (Yang & Cui, 2013), wool fibres (Monier et al., 2010), banana peels (Albarelli et al., 2011), modified cellulosic materials (Zaki et al., 2012), modified lignin (Demirbas, 2004), modified sugar beet pulp (Altundogan, 2005), and different agricultural by-products. The major challenges limiting a large scale application of these adsorbents are related to their adsorption capacity, their re-usability, the presence of toxic water soluble organics in the plant based adsorbent, generally low surface area, chemical and thermal instability, and the amount of sludge generated during operation (Veglio & Beolchini, 1997). This has led to some fundamental research questions which can be better addressed by exposing the adsorbent to characterization techniques, wherein avenues for chemical modification can be identified and explored. This study will address some of these research questions through the exploration of various characterization techniques.
The type of characterization technique to be used depends on the information that is sought from the adsorbent. Some of these techniques are generally used to elucidate surface properties with respect to chemical reactivity of the materials being characterized. For purposes of this study, characterization techniques were particularly used as a tool to investigate physicochemical properties of the adsorbent. Techniques employed were also to study the interaction of the adsorbent with metallic pollutants at different reaction conditions with respect to varying degrees of chemical modification. Other authors have used physicochemical characterizations in similar studies, such as Brunauer Emmett and Teller gas adsorption (N$_2$-BET) (Fu et al., 2010; Passe-Coutrin et al., 2008), Fourier transform infra-red spectroscopy (FT-IR) (Fu et al., 2010), high resolution electron scanning microscopy (HRSEM) (Boufi et al., 2011), thermogravimetric analysis (TGA) (Lin et al., 2010), high resolution transmission electron microscopy (HRTEM) (Sun et al., 2013; Smitha et al., 2009), energy dispersive x-ray spectroscopy (EDS) (Taurozzi et al., 2008), zeta potential measurement (Prathna et al., 2011), UV-vis spectrometry (Gupta et al., 2011); x-ray diffractometry (XRD) (Alila et al., 2009) and particle size analysis (PSD).

2.1.1. Surface properties of adsorbents using the N$_2$-BET gas adsorption method

The N$_2$-BET method is based on the adsorption of low pressure gas molecules onto the surface of a solid material, wherein the gas molecules form a thin monomolecular layer of adsorbed molecules (Ndibewu et al., 2014; Sing, 2001). The amount of gas
molecules adsorbed can then be used to determine pore characteristics of the material/adsorbent with respect to the surface area, pore volume, pore size, particle size distribution (PSD) and, to determine the likely mechanism of gas adsorption. The information obtained therein is essential in such fields as catalysis, ceramics, pharmaceuticals, gas sensors and filter characterization (Jaroniec & Kruk, 2003). Environmental chemists use such information in determining the type and size of pollutants that can be adsorbed; the strength and type of interactions between the adsorbate and adsorbent - which has implications on the ease of desorption and, hence the re-usability of the adsorbent.

Various methods have been developed to investigate surface properties of materials, but intrusive and adsorption methods are the most widely used. According to Sing (1998), gas adsorption offers the best method for the characterization of porous materials, wherein the amount of gas adsorbed on a solid material is a direct measure of the porous properties and structure of the material (Gregg & Sing, 1982). Of the many gases and vapours readily available, such as N₂, Ar, Kr and CO₂, which could be used as adsorptive gases, N₂ has remained universally pre-eminent (Groen et al., 2003). It has been extensively used for routine quality control, as well as for investigating new materials (Sing, 2001). This then informs the widespread usage of nitrogen gas, as it also gives more accurate surface area results than other adsorptives. The other gases have limitations which restrict their usage as general purpose adsorptives. For instance, Ar fills micropores at much higher pressures than N₂; Kr is
ideal for the determination of low surface areas and CO\textsubscript{2} is ideal for pore sizes with diameters less than 1.5 Å (Odler, 2003).

When the quantity of gas on a surface is measured systematically over a wide range of relative pressures at constant temperature, the result is an adsorption isotherm of that material. The adsorption isotherm can be extended to include a desorption branch which shows the systematic desorption of the adsorptive from the surface of the adsorbent. Nitrogen adsorption-desorption isotherms, over a wide range of relative pressures ($P/P_0$), can provide information on pore size distribution in the micro-(< 2 nm), meso- (between 2 and 50 nm) and macro- (larger than 50 nm) diameter ranges (Wu et al., 1999). Furthermore, within each of these ranges the pore size model is predicted based on mathematical models, which accommodate mono- and multi-layer adsorption so that it extends beyond the microporous range into the meso- and macroporous ranges.

This has led to the classification of the geometry of the pore surface openings as either slit-shaped or cylindrically shaped (Serwicka, 2000). It has been thought that pore geometry is largely influenced by hysteresis phenomenon, wherein the presence of slit-shaped pores seems to result from a delayed condensation process beyond mesopore filling on the adsorption branch. However, it has since been found that the desorption branch may be dependent on network percolation effects (Seaton, 1991). However, the desorption loop does not necessarily follow the adsorption curve, even though hysteresis loops are generally associated with the filling and emptying of mesopores.
This then explains why desorption branches of the isotherms differ. Consequently, the International Union of Pure and Applied Chemistry (IUPAC) classified the gas adsorption-desorption curves according to the resulting shapes of the isotherms into six basic categories depicted in Figure 2.1.

![Figure 2.1: IUPAC classification of nitrogen adsorption-desorption curves through physisorption (Ssing et al., 1985)](image)

This classification provides a basis for understanding the type of interactions between the adsorbate and the adsorbent, accounting for both branches of the isotherm (adsorption and desorption). However, this classification represents a simplification of the experimental physisorption conditions as some adsorbents are composite and the surface properties thereof may be complex. Also, this classification is suitable for a
single-component adsorptive within its condensation ranges, beyond which deviations are evident.

Type I isotherms are formed based on the assumptions that the adsorbent surfaces are homogeneous; that there are no lateral interactions between molecules, and that only monolayer adsorption is permitted. In its characteristic form, the Type I isotherm is concave at low relative pressures ($P/Po$), suggesting rapid adsorbate adsorption at low pressures. The narrow range of relative pressures needed to attain the plateau is indicative of a limited range of pore sizes, suggesting predominantly monolayer micropore filling of pores. The amount adsorbed by a unit mass of solid approaches a unit value as $P/Po$ approaches 1, hence, the plateau (Figure 2.1). The appearance of this plateau is indicative of a nearly small external surface area of the adsorbent (Rouquerol et al., 1998).

The type II isotherm is characterized by a concave curve to the $P/Po$ axis at low relative pressures, then an almost horizontal plateau which then assumes a convex curve to the $P/Po$ axis. It indicates the formation of an adsorbed layer whose thickness increases progressively with an increase in relative pressures until $P/Po$ approaches 1 (Rouquerol et al., 1998). The inflection point on the curve, often called the ‘knee of the curve’ or point B, signifies a transition from monolayer to multilayer coverage. At this point, the micropores, characteristic of monolayer coverage, have been filled and the adsorption progresses to meso- and macropores. The sharpness of this point gives an indication of the strength of the interaction between the adsorbent and the adsorbate, and gives
value to the monolayer capacity (Gregg & Sing 1982). Type II isotherms are most frequent with materials that are non-porous or macroporous or with powders whose diameters exceed micropores (Sing, 1998).

Type III isotherms are characterized by a concave curve through the length of the $P/Po$ axis. There is no inflection point, suggesting poor interaction between the adsorbent and the adsorbate. These types of isotherms are generally not common.

Type IV isotherms are characterized by an adsorption branch that is similar to the Type II isotherm, except for the occurrence of a hysteresis loop at higher $P/Po$ values. The hysteresis loop is associated with the filling and emptying of mesopores by capillary condensation. The lower branch of the loop represents the progressive addition of gas molecules to the system and the upper part represents the progressive withdrawal of the same (Rouquerol *et al.*, 1998). Type IV isotherms are common, but the shape of the hysteresis loop differs between systems.

In Type V isotherms, the adsorption branch of the curve is similar to a Type III isotherm, except for the occurrence of a hysteresis loop. The convex shape to the $P/Po$ axis is indicative of a weak interaction between the adsorbate and the adsorbent. The shape of the hysteresis loop is also variable, depending on the type of material, but is generally associated with the mechanism of pore filling and emptying.
Type VI isotherms are rare, but generally result from layer by layer adsorption on a highly uniform surface.

The strength of the interaction between the adsorptive and the adsorbent plays a phenomenal role in explaining the supposed mechanism(s) of adsorption. Apart from the use of the isotherms to determine the strength of the interaction, other mathematical models can be utilized. Based on the adsorption of gas molecules on porous materials, two types of mechanisms are predominant; physisorption (physical adsorption) and chemisorption (chemical adsorption). Physisorption results from weak intermolecular attractions ($\Delta H^o = 10$ to $40 \text{ kJ/mol}$), mainly by van der Waals forces and hydrogen bonding, whereas chemisorbed interactions are stronger ($\Delta H^o = 100$ to $400 \text{ kJ/mol}$) and result from covalent intermolecular bonding (Okamoto & Miyamoto, 2001). Consequently, gas adsorptions in physisorption results in either monolayers or multilayers which are reversible and fast, whereas in chemisorption the adsorption results mainly in multilayers, whose desorption is not reversible and kinetically slow (Singh, 1998).

The relationship between the amounts of adsorbate adsorbed per unit weight of adsorbent and the concentration of adsorbate in bulk solution at a given temperature under equilibrium conditions is given by an adsorption isotherm. Adsorption isotherm data are often fitted with adsorption models which are often semi-empirical and derived from observations of large sets of adsorption data (Noeline et al., 2005), wherein they offer information on thermodynamic parameters, affinity constants, and maximum
adsorption amount for the adsorption process. These properties help in further expounding the adsorption potential of the adsorbent.

2.1.2. Zeta potential measurements

Zeta potential is the potential difference that exists between the dispersion medium and the stationary layer of fluid attached to the dispersed particle in a colloidal system. At this interface the bulk of the aqueous medium is separated from the colloidal particle by a sheath. The colloidal particle, therefore moves about in the medium with a certain quantity of the surrounding liquid and its contained charge. Consequently, the Zeta potential value depends on the surface charge of the particle as well as on the thickness of the electrical double layer surrounding the particle (Hunter, 1981). This dependence extends the description of zeta potential measurements to include solution conditions such as pH and ion concentration. For instance, the presence of high levels of ions in solution compress the electric double layer, thereby zeta potential values are affected (Lin & Juang, 2002).

Zeta potential measurements have found much application in colloidal sciences to understand colloid interactions and have been used to describe, predict and control the stability of a colloidal system (Hunter, 1981), since they give value to the surface charge and by extension, its ability to interact with other molecules.
2.2 CHEMICAL MODIFICATION OF NATURAL BIOSORBENTS

Pollutant removal from contaminated drinking water remains a challenge - a concern that has also been prioritized by the United Nations through the Millennium Development Goals (MDGs). Different techniques have been applied to reduce the pollution load in riverine systems with varying degrees of success. Conventional techniques for pollutant removal from aqueous solutions include precipitation, ion exchange, reverse osmosis, electrolysis, membrane filtration (Barnejee et al., 2012) and, more recently, adsorption (O’Connell et al., 2008). However, most of these methods have been found unsuitable in that they are relatively expensive, have low pollutant recoveries, are inappropriate for low pollutant concentrations, have high energy and chemical requirements, generate large amounts of sludge and incur high costs associated with large scale application (Mishra & Malik, 2012; Dermibus, 2008). These limitations restrict their use in large scale applications, especially in developing countries and poor communities.

Biosorption is now recognized as a potentially efficient and economic method for the sequestration of pollutants from aqueous solutions, especially inorganic pollutants (Krishnani et al., 2008). Biosorption involves the use of biological materials (such as plant parts and microorganisms) that form complexes with metal ions using their ligands or functional groups. The complexes formed can help explain the adsorption potential of the adsorbent, vis-à-vis the adsorption capacity of the adsorbent (Wu et al., 2012); adsorption kinetics and thermodynamics of the adsorption process (Hu et al., 2010); re-
usability of adsorbent (Reddy et al., 2010), and possible mechanisms of adsorption (Sheng et al., 2004), using appropriate mathematical models. Different biosorbents have been applied with success, and include grape stalk wastes (Pujol et al., 2013), rice husks ash (Chakraborty et al., 2011), neem bark (Naiya et al., 2009), *Moringa oleifera* bark (Mnisi et al., 2012), barley straws (Pehlivan et al., 2009), orange peels (Schiewer & Iqbal, 2010), bagasse (Jiang et al., 2009) and sawdust (Naiya et al., 2009).

Biosorption is based on the fact that the adsorbents have a high adsorption capacity for the target pollutant(s), a property that is largely dependent upon the surface properties of the adsorbent, as well as the concentration and chemical characteristics of the pollutant (Dermibus, 2008; Sheng et al., 2004). Adsorbent surface properties define the type and size of pollutants that can be sequestered, the reaction potential of the adsorbent, as well as the type and strength of chemical bonding that results from the interaction. As such, recent research has focused on modifying surface properties of biosorbents to improve their adsorption properties to suit the adsorption of targeted organic and inorganic contaminants (Saikia et al., 2013; Chang et al., 2012).

2.2.1 Surface modification of adsorbents

Surface modification entails applying chemical and physical techniques to change the surface chemical properties of a material to improve its adsorptive potential. Chemically modified adsorbents at different degrees of functionality have been applied to remove various target analytes/pollutants with success, wherein the adsorption capacity,
stability of biosorbents, surface area, and chemical reactivity of materials were improved 
(Mnisi et al., 2012; Dermibus, 2008). The advantages of surface modification were 
clearly demonstrated, thereof.

Understanding the mechanism(s) responsible for the observed adsorption is critical in 
deciding the type of chemical or physical modification that is suitable for a specific 
adsorbent. Microbially mediated adsorption is facilitated by viable microbial cells at the 
cell wall, and is dependent upon the cell’s metabolism (Veglio & Beolchini, 1997). It is 
often associated with the microbial cell’s defence system, which reacts in the presence 
of a metal or a stress-induced situation – a similar mechanistic pathway to 
phytoremediation. Therefore, biosorption is not a spontaneous process, since time is 
required to allow for metabolic processes to take effect.

However, when using lignocellulosic biomass and related adsorbents, the mechanism is 
not dependent on metabolism, but is a spontaneous and reversible process. This 
process is dependent upon the interaction between the pollutant (organic or inorganic) 
with the functional groups on the surface of the biomaterial. The cell walls of these 
biomaterials contain considerable proportions of cellulososes interlinked with lignin in its 
structure. Lignin is a complex biopolymeric heterogeneous molecule mainly composed 
of polysaccharides, proteins and lipids (Veglio & Beolchini, 1997), which are endowed 
with many different functional groups, including methoxy (-OCH₃), hydroxyl (-OH), 
carboxylate (-COOH), sulphates (-SO₄²⁻), phosphates (-PO₄³⁻) phenolic and amino (-
NH₂) groups (Sharma et al., 2004; Veglio & Beolichini, 1997).
With all these properties and functional groups, the biosorbent exhibits chemical characteristics of an ion-exchange resin or activated carbon, wherein the ion-exchange potential has been greatly enhanced. This is provided for by the electrostatic attraction that exists between the adsorbent surface and the target pollutant, especially with metals. Therefore, physical adsorption associated with the formation of weak van der Waals forces is the predominant and most common mechanism of adsorption; hence the adsorptions are reversible and require minimum energy expenditure. Furthermore, the presence of weakly bound exchangeable divalent cations on the adsorbent (such as Ca$^{2+}$ and Mg$^{2+}$) can effectively exchange with pollutant ions such as Co$^{2+}$, Cd$^{2+}$ and Cu$^{2+}$ (Veglio & Beolichini, 1997), resulting in the biosorptive uptake of these metals. Alternatively, the interaction of the metal pollutant with the reactive functional groups can result in the formation of a water insoluble adsorbent-metal complex. This complex can form from mono- or poly-dentate ligands on the adsorbent; thereby facilitating the removal of the metal from aqueous solutions through precipitation.

Therefore, the type and chemical properties of biomolecules (functional groups) on the surface of an adsorbent define its chemical reactivity to specific pollutants. It is therefore not surprising that recent studies have focused on chemical surface modification to obtain novel adsorbent properties which can adsorb and remove specific target analyte groups from aqueous solutions such as dyes (Zhou et al., 2014; Saikia et al., 2013), antibiotics (Rivera-Utrilla et al., 2013; Gao et al., 2012), phenolic compounds (Yousef et al., 2011; Ming et al., 2006), polycyclic aromatic hydrocarbons (PAHs) (Vidal et al.,
Surface functionalization of biosorbents also improves its physical and chemical attributes to enhance their affinities toward metal ion uptake from aqueous solutions. Additionally, the adsorption capacity increases with an increase in specific surface area due to an increase in the availability of a number of adsorption sites, while pore size and micropore distribution are closely related to the composition of the adsorbents and the type of biomass raw material used (Srivastava et al., 2008). The type of pores and pore volumes thereof, define the type and size of adsorbate (metals) that can be adsorbed, respectively (Singh, 1998), and these properties are further enhanced through surface modification (Chang et al., 2013). Improved adsorption capacities have been reported for chemically modified adsorbents such as M. oleifera leaves (209.54 mg/g) (Reddy et al., 2010), bagasse (101.01 mg/g) (Jiang et al., 2009) and barley straw (31.71 mg/g) (Pehlivan et al., 2012). Chemical modification can also improve the pore characteristics of the adsorbent, vis-à-vis; the pore volume and surface area (Mnisi et al., 2012).

2.2.2 Surface modification of adsorbents using amines

Adsorbent modification for metal removal can be achieved using different chemical techniques. Amine-based adsorbent functionalization has been studied widely for their application in removing metal ions from aqueous solutions (Xu et al., 2011; Liu & Bai, 2010). This is attributed to their strong chelation properties to transitional metal ions.
(Lam et al., 2006), and that the modified biosorbents are usually chemically stable yet highly reactive (Xu et al., 2011). Polyamines have been used to functionalize silica, polystyrene (Yoshitake et al., 2005), cellulose (Qu et al., 2010), polyacrylonitrile fibres (Torres et al., 2006), and poly(glycidyl methacrylate polymers (Atia et al., 2007).

2.3 SYNTHESIS OF GOLD NANOPARTICLES

The synthesis and application of different products of nanomaterials have been receiving much attention due to their novel properties, applications and the wide range of noble metals that can be utilized. Metal nanoparticles have found applications in biomedicine (Daniel & Astruc, 2004), catalysis (Campbell et al., 2002), electronics (Maier et al., 2001) and in environmental applications (Tratnyek & Johnson, 2006). These applications are premised on an understanding of the chemistry of the synthetic route so that different novel products can be produced from modelling the initial reaction conditions. Noble-metal nanoparticles have, especially, attracted extensive research interest due to their unique features such as optical, electronic, magnetic and catalytic properties (Zargar & Hatamie, 2013).

For instance, gold nanoparticles are used extensively since they possess interesting physico-chemical properties such as biocompatibility (Daniel & Astruc, 2004), thermal stability (Sau & Rogach, 2010), chemical stability, size related electronic, magnetic and optical properties (Gupta et al., 2011), which then influence their application as sensors in environmental (Zargar & Hatamie, 2013; Sun & Xia, 2002) and medical applications.
(Zhang et al., 2009; Daniel & Astruc, 2004). The wide range of different applications imply that different properties are desired, therefore, the synthetic protocols employed therein vary greatly to produce gold nanoparticles of differing chemical properties.

2.3.1 Conventional gold nanoparticle synthetic routes

Nanoparticles can be referred to as particulate materials with a dimension that is at least less than 100 nm (Dubey et al., 2010). The formation of gold nanoparticles with such diameter ranges results from at least four processes: the reduction of the initial gold in the precursor salt, the heterogeneous nucleation of particular materials, growth and then ripening of the resulting particles (Gan et al., 2012).

Most methods of gold nanoparticle synthesis are based on the initial work by Turkevich and co-workers, wherein gold (III) is reduced to elemental gold at controlled reaction conditions (Tukervich et al., 1951). Generally, auric acid, often called gold tetrachloride (HAuCl₄·3H₂O) is the most commonly used precursor salt to provide the gold seed as Au³⁺. The reduction of the Au³⁺ to Au⁰ is normally facilitated by the addition of a reductant, which may also act to stabilize the colloidal nanoparticle suspension.

The type, strength and chemical properties of the reductant determine the physico-chemical properties of the resulting nanoparticles. Consequently, research focus has been on applying different chemical reductants to obtain novel nanoparticles, which can
be used in the different fields of nanotechnology. This has seen a shift from the original synthesis wherein tri-sodium citrate (Na$_3$C$_6$H$_5$O$_7$$\cdot$2H$_2$O) is used to synthesize small and spherical gold nanoparticles. Depending on the intended end-use of the materials, most nanotechnology fields are interested in size and shape tunable synthetic protocols.

Tri-sodium citrate and sodium borohydride (NaBH$_4$) have been conventionally used as chemicals of choice for most of the research since they produce similar nanomaterial products (Gupta et al., 2011; Kimling et al., 2006). Advances in nanotechnological applications have seen a need for additional size and shape options from the original arbitrary 'small and spherical' classification (Bates & Frenkel, 1998). This need can be addressed by (i) altering the identity of the chemical species responsible for the reduction (Safavi & Zeinali, 2010), so as to model specific size and shape outcomes, (ii) introducing an additional size separation step during the synthesis (Xu et al., 2007) to selectively separate the targeted shape(s) and size(s) of gold nanoparticles and, (iii) exploration of other non-chemical reduction methods, such as the use of UV irradiation (Kimling et al., 2006). As such, different novel nanoparticle properties can be modelled and applied in diverse fields of nanotechnology.

Self-assembly of nanoparticles in aqueous solutions is a common problem during and after synthesis. Therefore, capping agents (protective agents or stabilizers) are added to prevent self-aggregation of the nanoparticles and, to some extent, control size and shape (Quantanilla et al., 2010). Nanoparticle stabilization can be achieved through electrostatic stabilization, wherein adjacent particles repel against each other based on
the net charge of the electric double layer of the nanoparticles. Alternatively, steric stabilization is common, achieved by the coordination of sterically bulky molecules such as polyamines, which act as protective shields on the metal surface (Zhou et al., 2009).

Depending on the intended use of the gold nanoparticles, a range of capping agents can be used at optimum experimental conditions. Due to their chemical nature, capping agents can have a chemical effect on the quality and reactivity of the resulting nanomaterial in production. For instance, catalytic activity of gold is detrimentally affected when strong covalently bonding capping agents (such as phosphine molecules) are present, even in minute amounts (Martra et al., 2003). Conversely, in most applications the addition of capping agents has a beneficial value as they improve the stability and reactivity of the nanoparticles (Prathna et al., 2011; Aryal et al., 2006), hence improving their usage. Therefore, central to the synthesis of ideal nano-products are the choice of capping agent, the amounts involved during the synthesis and the optimum reaction conditions.

A wide range of capping agents have been used with some degree of success, but can be generally classified into: polymers and block copolymers; P, N, and S donors (e.g. amines, thiols, phosphanes); solvents such as tetrahydrofuran (THF), THF/MeOH; long chain alcohols; surfactants and organometallics (Zhou et al., 2009). Within the scope of environmental applications, most of the surface functionalizing ligands contain considerable amounts of nitrogen and sulphur - important elements with interesting chelating properties. These normally bind onto the surface of the nanoparticle through
the thiol group; simultaneously exposing the nitrogen-based terminal functional groups in the aqueous medium. These negatively charged functional groups, together with the amine groups, provide the electrostatic contribution necessary for the nanoparticle to be reactive to the target analyte (Gupta et al., 2011). Some capping agents can play a dual role during the synthesis in that they can reduce the gold and subsequently stabilize the resulting nanoparticles (Boufi et al., 2011).

2.3.2 Green synthesis of gold nanoparticles

The principles of green chemistry are based on chemical research and engineering that encourages the design of products and processes that minimize the use and generation of hazardous substances (Smitha et al., 2009). Common synthetic protocols for gold nanoparticles make use of commercial chemicals, most of which have adverse health and environmental effects. Even though gold nanoparticles are considered biocompatible, current chemical synthetic routes may lead to the presence of undesirable chemical constituents in the final product. Environmentally benign methods of synthesis are therefore considered, wherein natural biomolecules are utilized to simultaneously function as a reductant and as a capping agent. This also has an added advantage of making the gold nanoparticles more biocompatible (Smitha et al., 2009).

Biological synthesis of gold nanoparticles using microorganisms such as bacteria (Rajasree & Suman, 2012), fungi (Castro et al., 2014), algae (Sharma et al., 2014) and yeast (Yamal et al., 2013) have been reported, with limited success - due to the
sensitivity of the microorganisms to changes to the external environment. Therefore, the use of natural, readily available, low-cost agro-wastes such as palm oil mill effluent (POME) (Gan et al., 2012), and plant mediated biosynthesis (Basavegowda et al., 2014; Smitha et al., 2009) have received keen attention. The nanoparticles produced from these simple and eco-friendly synthetic protocols have morphologies that are comparatively similar to those synthesized from the conventional methods (Parsons et al., 2007).

Plant mediated biosynthesis is premised on an understanding that the bioactive biomolecules can be extracted from the plant part for use as a reductant and or capping agent. The chemical composition of the extract may be variable, but generally contain proteins, flavonoids, reducing sugars and some alkaloids (Gan et al., 2012). These contain important functional groups such as C=O, COOH and N-H which are capable of gold reduction and subsequently, stabilization/capping of the resulting nanoparticles.

The emergence of these environmentally benign synthetic protocols has culminated into an increase in research into plant mediated biosynthesis of transition metal nanomaterials. Various authors have reported on the successful plant-mediated reduction of the initial gold precursor salt using extracts from flowers (Vidhu & Philip, 2014), fruits (Basavegowda et al., 2014), leaves (Dubey et al., 2013), seeds (Jayaseelan et al., 2013) and barks (Yallappa et al., 2013). However, to the best of our knowledge in literature, the use of *M. oleifera* plant parts for the synthesis of metal nanoparticles has not been reported. This is in spite of the widespread use of the plant
for a wide variety of purposes, which are based on the amazing physicochemical properties reported in literature (Ndabigengesere et al., 1995).

### 2.4 THEORETICAL ADSORPTION MODELLING

Among the available techniques for waste water treatment, adsorption has rendered itself as a viable alternative; mainly due its simplicity, low costs associated with its application and high reported efficiencies (Bhattacharya et al., 2006). This is further supported by the large feed stocks that are available as potential adsorbents, most of which is biomass from agricultural waste products (Ndibewu et al., 2006). These potential adsorbents have different physicochemical properties, which culminate into different adsorption protocols; hence it is imperative to understand the theoretical basis for adsorption.

#### 2.4.1 Types of adsorption isotherms

An adsorption isotherm is an expression that depicts relationships between the amount of adsorbate adsorbed per unit weight of adsorbent and the concentration of adsorbate in bulk solution at a given temperature under equilibrium conditions. An adsorption equilibrium is established when the amount of adsorbate adsorbed onto the adsorbent is equal to the amount being desorbed. At this stage, the equilibrium concentrations in both phases are constant (Hanafiah et al., 2012).
Adsorption equilibrium is expressed in terms of an adsorption equation, where the parameters defined therein express the interaction of the adsorbent with the metal, adsorption mechanisms, surface properties and, consequently, culminate into adsorption isotherms. Adsorption isotherm data are often fitted based on mathematical models which are usually semi-empirical and are derived from observations of large sets of adsorption data (Liu et al., 2012). By combining these data series, it is then possible to look for a theoretical model that fits rather well to the experimental data; thereby explaining one adsorption phenomenon or another. The model fit can offer information on thermodynamic parameters, affinity constants, and maximum adsorption amount for the adsorption process.

Equilibrium isotherm models are usually classified into empirical equations and mechanistic models, based on the mechanism of metal ion biosorption (Wang & Chen, 2006). Mechanistic models can be used not only to represent, but also to explain and predict the experimental behaviour (Pagnanelli et al., 2002). Several empirical models have been proposed to describe batch biosorption equilibrium, but the most common and widely used for single solute systems are the Langmuir, Freundlich and Brunauer-Emmett-Teller (BET) adsorption isotherm models.

2.4.1.1 Langmuir Adsorption Isotherms

Langmuir (1918) proposed an adsorption model for a single component gas-solid system. This model assumes that; (i) the uptake of metal ions occurs on a homogenous
surface by monolayer adsorption on adsorption sites, (ii) wherein all the adsorption sites have the same affinity and, that (iii) adsorption at one site is independent of adsorption at adjacent sites (Wong et al., 2003). The mathematical representation of this isotherm is:

\[ q = q_{\text{max}} \left( \frac{bC_{\text{eq}}}{1 + bC_{\text{eq}}} \right) \]  

(Eqn. 2.1)

where, \( q \) is the quantity of adsorbent required to form a single monolayer on unit mass of adsorbent (mg/g) at equilibrium, \( q_{\text{max}} \) is the maximum amount adsorbed on unit mass of the adsorbent (mg/g) when the equilibrium concentration is \( C_{\text{eq}} \) (mg/L) and \( b \) is the Langmuir constant for the ion exchange capacity and is indicative of the adsorption affinity or efficiency of the biomass.

The Langmuir constant, \( q_{\text{max}} \), is influenced by experimental reaction conditions such as pH, temperature, dosage amounts of adsorbents and concentration of analyte. Therefore, primary data to test the validity of this model is obtained using batch adsorption experiments at optimized conditions, from which the Langmuir adsorption parameters are empirically determined. However, the assumptions of this isotherm suggest that metal adsorption is dependent on adsorption sites, so that, generally;
Therefore, the Langmuir isotherm equation can also be presented to clearly illustrate the contribution of the adsorption sites:

\[
Q = Q_{\text{max}} \left( \frac{LC}{1 + LC} \right)
\]  
(Eqn. 2.2)

where \( Q \) is the solid concentration of retained compound on the adsorbent; \( Q_{\text{max}} \) is the adsorption capacity of the adsorbent; \( L \) is an affinity constant for the adsorbent, given by:

\[
L = Q_{\text{max}} \left( \frac{Q}{C(Q_{\text{max}} - Q)} \right)
\]  
(Eqn. 2.3)

where \( (Q_{\text{max}} - Q) \) is the solid concentration of free adsorption sites and, \( C \) is the equilibrium concentration. Plotting a graph of the linearized form of this equation gives a
linear response with slope \(-L\), and the intercept at \((Q_{\text{max}} L)\), from which the distribution coefficient \((K_d)\) of the system is determined (Limousin et al., 2007).

The features of the Langmuir isotherm model can also be expressed in terms of the dimensionless separation factor, \(R_L\), which defines whether the adsorption is favourable or not (Zhang et al., 2010). The separation factor is expressed as:

\[
R_L = \frac{1}{1 + bC_o}
\]  
  (Eqn. 2.4)

where \(b\) and \(C_o\) are derived from the Langmuir isotherm. The value of \(R_L\) lies between 0 and 1 for a favourable adsorption, while \(R_L > 1\) represents an unfavourable adsorption, and \(R_L = 1\) represents a linear adsorption, while an irreversible adsorption is represented by \(R_L = 0\) (Naiya et al., 2009). To confirm a good model fit, experimental data should depict correlation coefficient values \((R^2)\) above 0.99.

Although the Langmuir adsorption model is widely used, its linearization using experimental data often gives poor results in the form of a convex curve (Limousin et al., 2007), a deviation from the theoretical ‘H’ shape (Fig. 2.1), originally reported by Giles and co-workers (1974). This deviation is attributed to (i) the existence of several types of adsorption sites, (ii) the decrease of adsorption energy as the fraction of occupied sites increases and, (iii) competition between two adsorbing species (Limousin et al., 2007). These findings are in contrast to the assumptions initially proposed by
Langmuir. Consequently, the general Langmuir adsorption model has been modified to accommodate these limitations (Murali & Aylmore, 1983a; Sposito, 1980).

2.4.1.2 The Extended Langmuir adsorption model

Commercial applications of adsorption data involve mixtures containing two or more components rather than pure gases. As such, the adsorption of one component can affect the adsorption of the other; thereby limiting possible application of the single component system of the Langmuir adsorption model. Depending on the type and strength of interactions between the adsorbed molecules, it is possible that during the adsorption of one or more components, one component may decrease, increase or not have an effect at all on the adsorption of the other (Foo & Hameed, 2010). By ignoring these interactions and assuming that the only change is in the reduction of the available active sites with each adsorption, the Langmuir adsorption model can be modified to show a binary metal adsorption system:

$$q_i = \frac{q_i^0 b_i c_i}{1 + \sum_{k=1}^{n} b_k c_k}$$  \hspace{1cm} \text{(Eqn. 2.5)}$$

where $q_i$ is the uptake of component $i$ in the multicomponent system; $C_k$ ($k = 1, 2, \ldots n$; where $n$ is the number of components) is the equilibrium concentration of each component in the system; $q_i^0$ and $b_i$ are Langmuir constants, which can be obtained
from a single isotherm for each metal and substituted into the equation (Al-Ashesh et al., 2000).

2.4.1.3 Freundlich adsorption isotherm model

The Freundlich isotherm model is an empirical model which assumes heterogeneous surfaces on the adsorbent with non-uniform distribution of heats of adsorption, and is applicable for multilayer adsorption of adsorbates (Foo & Hameed, 2010). The theory of this model informs that the ratio of the amount of solute adsorbed onto a given mass of sorbent to the concentration of the solute in the solution is not constant at different concentrations (Ho & Ofohaja, 2005). Therefore, the amount adsorbed is a summation of adsorption all adsorption sites, with the stronger binding sites occupied first. The Freundlich isotherm is commonly expressed as:

\[ q_e = k_f C_{eq}^{1/n} \]  
(Eqn. 2.6)

where \( q_e \) is the equilibrium metal uptake capacity (mg/g); \( C_{eq} \) is the residual metal ion in solution (mg/L); \( K_f \) is a Freundlich constant (L/g) characteristic of the system indicating the relative adsorption capacity of the adsorbent and \( n \) is a dimensionless parameter indicating adsorption intensity of the system. If the system obeys Freundlich adsorption isotherms, then a plot of ln \( q_e \) versus ln \( C_e \) yields a straight line, from the linearized form of the equation:
\[ \log q_e = \log k_f + \frac{1}{n} \log C_e \]  \hspace{1cm} \text{(Eqn. 2.7)}

Based on the above equation (eqn. 2.7), and from the intercept and slope, the value of the constants \( K_f \) and \( 1/n \), respectively, are obtained (Limousin et al., 2007). The value of the slope \( (n) \) ranges between 0 and 1 and is a measure of adsorption intensity or surface heterogeneity of the adsorbent - becoming more heterogeneous as its value gets closer to zero (Foo & Hameed, 2010). A value below unity implies chemisorptions process where \( 1/n \) above one is an indicative of cooperative adsorption (Haghseresht & Lu, 1998).

2.4.1.4 Langmuir-Freundlich (Sips) Adsorption isotherm model

The Langmuir-Freundlich (LF) adsorption isotherm model, also known as the Sips isotherm, was proposed by Sips (1948). It addresses some of the limitations of the Langmuir and Freundlich isotherms; hence it resembles the format of the Langmuir isotherm model, but is modified according to the Freundlich isotherm to satisfy Henry’s law behaviour at the equilibrium adsorption of the adsorbates (Bautista et al., 2002). At low adsorbate concentrations, it reduces to Freundlich isotherm; while at high concentrations, it predicts monolayer adsorption capacity characteristic of the Langmuir isotherm (Foo & Hameed, 2010). As such, the mathematical expression representing the LF isotherm is a function of the concentration, pH and temperature of the system.
The application of the LF isotherm model allows for the modelling of both homogeneous and heterogeneous distributions at both high and low concentrations (Umpleby et al., 2001) - a major limitation of the Langmuir and Freundlich adsorption isotherms. Rau and co-workers (2003) studied adsorption of arsenate on metal-oxides using different adsorption models and they concluded that the LF model better describes their data. The mathematical expression of the LF isotherm allows for the prediction of a heterogeneity constant, as well as the adsorption capacity (Onyango et al., 2004) for the adsorption system. The isotherm can be expressed as:

\[ q_e = \frac{k_{LF} c_e^\alpha}{1 + b_{LF} c_e^\alpha} \quad \text{(Eqn. 2.8)} \]

where \( K_{LF} \) (mmol\(^{1-\alpha} \) L\(^{\alpha} / \)g) and \( b_{LF} \) (L/mmoll)\(^\alpha\) are the LF constants, \( \alpha \) is the heterogeneity coefficient. The adsorption capacity, \( q_m \), is obtained from the ratio of \( K_{LF} / b_{LF} \). The LF isotherm model reduces to the Langmuir isotherm when \( \alpha = 1 \) and \( b_{LF} = K_L \), and \( K_{LF} = q_m K_L \) (Vilar et al., 2005).

2.4.2 Adsorption Kinetics

The rates at which metal ions are transferred from the bulk solution to the adsorbent surface and are accumulated there determine the kinetics of the adsorption (Gupta & Bhattacharyya, 2011). By extension, the reaction pathways and possible mechanisms thereof are explained (Plazinski et al., 2009). Prediction of the rate at which adsorption
takes place is an essential factor in the successful design of an adsorption based water purification system and is required for selecting the optimum operating conditions for a full-scale batch metal removal process (Liu et al., 2012).

Adsorption kinetics are affected by the chemical properties of the adsorbent material under investigation, vis-à-vis; its degree of ionization at the surface, the types of functional groups at the surface, and the degree to which these properties change when the adsorbent interact with the bulk solution (Gupta & Bhattacharyya, 2011). As such, most of the kinetic interactions occur at the adsorbent-solution interface. The adsorption of metal ions on the solid surface include; (i) the transfer of the metal ion from the bulk solution to the liquid-solid interface which takes place through convection, (ii) the diffusion of the metal ion through the boundary layer that surrounds the solid particles (film diffusion), (iii) the diffusion of the metal ions on the surface (surface diffusion) and interior (pore diffusion) of the solid particle, and (iv) the adsorption of the metal ions to active adsorption sites on the adsorbent’s surface (Malamis & Katsou, 2013; Plazinski et al., 2009).

The overall sorption rate of any adsorption may be controlled by any of these steps, depending on which one is the slowest; thereby defining the rate determining step. The stages that define the overall rate may occur in series or parallel. If they take place in parallel and are independent of each other, then the total rate is given by the sum of the rates at each stage. However, if they occur in series, then at steady state they have the same rate, which is equal to the overall rate (Malamis & Katsou, 2013). Theoretically, if
step (i) is the slowest, then the adsorption process is transport-limited and the actual interactions between the solid and the metal have no significant contribution to the overall rate. When step (ii) is the rate determining step, then the physical diffusion of the metal through the thin film influences the overall outcome of the adsorption. Only when steps (iii) and (iv) are the slowest does the rate depend on a chemical process (Gupta & Bhattacharyya, 2011). However, rapid mechanical shaking of the solution renders the metal ions readily available at the surface boundary layer, hence eliminating step (i) as the rate determining step (Malamis & Katsou, 2013; Plazinski et al., 2009). Therefore, the total rate of adsorption is defined either by step (ii) or (iii) or by the rates of both (Malamis & Katsou, 2013). However, Plazinski and co-workers (2009) concluded that step (iv) is the rate determining step, and referred to the process taking place as the surface reaction step. Their underlying assumption is that the rate of the transfer of solute molecules from the solution to the adsorbed phase either governs the overall rate of the sorption process or at least is partially involved in it.

The rate determining steps are dependent on the experimental conditions, which may influence the speed of a chemical reaction, hence help attain equilibrium at a reasonable time. To help explain the underlying mechanisms several reaction kinetic and diffusion models have been proposed and successfully applied to study adsorption processes (Febrianto et al., 2009), and the most commonly used in liquid-solid phase studies are the pseudo-first order, the pseudo second order and Elovich’s model.

Summarily, this literature background lays the foundation for better understanding of the processes and mechanisms to be encountered in the text of this document.
CHAPTER 3

3.0 EXPERIMENTAL

3.1 INTRODUCTION

This study aims to investigate a plant-based material (*Moringa oleifera* bark, MOB) as a potential adsorbent for the removal of metals from aqueous solutions. The *Moringa oleifera* bark samples were collected from a unique site (Botswana, Southern Africa) with conditions favourable to growth of this particular type of plant. Some of these conditions include a semi-arid climate and quite often prolonged drought periods during the year (for more than six months). Plant samples were collected and bagged in sterile plastic containers and transported to the laboratory in South Africa where appropriate sample pre-treatment procedures were undertaken prior to batch experiments.

Access to water and its quality, thereof, are important development benchmarks in South Africa, particularly now that water scarcity is increasingly becoming a major concern. In general, investigations into both cheap water purification methods and sustainable supply of quality water to poorer communities are a challenging task. This task is complicated due to the huge volumes of contaminated or poorly treated waste water from industrial effluents which are discharged into rivers, leading to deterioration of the quality of water. Thus, sustainable supply is hampered, as the only source of
water supply for human consumption, agricultural production and animal husbandry is compromised.

Therefore, water quality defining parameters were also determined in this study when preparing simulated environmental conditions. Characterization of materials was achieved via the use of the state-of-the art analytical instrumentations: PerkinElmer ATR-FT-IR spectrometer, Shimadzu UV-1601 spectrophotometer (Japan), Micromeritics ASAP N₂-BET analyzer and a Nanoseries (UK) Malvern zetasizer. The percentage C, H, N and S contents were determined for the various samples using a NA Carlo Erba instrument and elemental percentage of O determined by mass difference, while inductively coupled plasma–optical emission spectrometry (ICP-OES) and inductively coupled plasma–mass spectrometry (ICP-MS) were employed for analysis of samples due to their sensitivity, accuracy and precision, and versatility. All the chemicals used were of analytical reagent grade while deionised doubly distilled (DDD) water was used throughout the experimental studies.

Crystallographic properties and consistency of the *Moringa oleifera* bark samples were investigated using a Bruker D8 Advance X-ray diffractometer. A high resolution scanning electron microscopy (HRSEM) imaging and energy dispersive X-ray analysis (EDX) were employed to analyze unmodified and chemically modified different bark sample treatments.
3.2 SAMPLING AND ADSORBENT PREPARATION

*Moringa oleifera* bark samples were collected from a farm at the University of Botswana, Gaborone, southern Africa (Latitude: 24 39’ 40”, Longitude: 25 55’ 54”) (Figure 3.1). Gaborone has a hot semi-arid climate with more sunny days per year, wherein precipitation is scant and erratic with more rainfall within the summer months, between October and April. These conditions are good for *M. oleifera* propagation as it can survive harsh conditions, including drought.

![Figure 3.1: A map of the Republic of Botswana, showing Gaborone where sample collection was done](image)

Specific sampling site, University of Botswana in Gaborone with latitude 24 39’ 40” and longitude 25 55’ 54”

Bark samples were collected from the same tree, to minimize variations in the chemical constitution of the samples collected. Samples were collected four (4) times over a three (3) year period between 2011 and 2014. Samples were collected by cutting off a branch from the main trunk and then de-barking it using a stainless steel pre-cleaned and sterilized blade. They were then washed with copious amounts of water to remove adhering dirt, after which they were air-dried for seven days. An example of an air-dried sample is shown in Figure 3.2.

![Figure 3.2: Air dried bark samples of M. oleifera](image)

The dry bark samples were then pulverized using a high speed mechanical crusher with stainless steel blades (Ika® A11 Basic, Germany) (Figure 3.3A) and the resulting powder boiled in de-ionized water at 90 °C to dissolve chlorophyll and other soluble organics. It was then dried in an oven at 110 °C until a constant weight was obtained, after which it was passed through a 300 µm sieve (Figure 3.3B) and stored in small sterilized glass vials. The 300 µm particle sizes were used for adsorbent
characterization, batch adsorption experiments and for extracting water soluble biomolecules to synthesize gold nanoparticles.

FIGURE 3.3: (A) A high speed mechanical sample crusher, and, (B) Fractional separation of pulverized sample based on particle size of mechanical sieves

3.3 GLASSWARE, REAGENTS AND APPARATUS

All glassware used in this work were washed using liquid soap, rinsed in distilled water and then soaked in 10% HNO₃ (55%) overnight, after which they were rinsed in deionized water. All chemicals used were of analytical grade. Commercial metal standard solutions (1000 mg/L) used were procured from Merck Chemicals, South Africa. Polyamine solutions (>98% purity) and the gold precursor salt, HAuCl₄·3H₂O, were procured from Sigma Aldrich, Germany, and were used without any further alterations.
3.4 PHYSICOCHEMICAL CHARACTERIZATION OF ADSORBENT

3.4.1 Fourier Transform infrared (FTIR) analysis

Solid samples and liquid bark extracts (broth) of *M. oleifera* (unmodified and chemically modified) were analysed for their IR active biomolecules using an FTIR spectrometer. The FTIR spectra (4000 – 400 cm\(^{-1}\)) were recorded using a PerkinElmer Spectro65 FT-IR spectrometer (USA), with 8 scans and 4 resolutions (cm\(^{-1}\)). The instrument was coupled to an OptKBr (8000 – 30 cm\(^{-1}\)) beamsplitter and a LiTaO\(_3\) (15700 – 370 cm\(^{-1}\)) detector. The data was acquired in a strong apodization mode and further normalized after filtration with Savitsky-Golay polynomial-convolution filters to reduce noise.

3.4.2 Proximate elemental analysis of *M. oleifera* bark

To determine the elemental composition, a CHNS analyzer (NA 1500 series, Carlo Erba instruments) was used to determine the percentage weight composition of C, H, N and S. Elemental percentage of O was determined by mass difference. Completely dried samples were milled to form particle diameters > 2mm. Sample masses between 4 and 6 mg were weighed using the instrument balance. Instrument calibration was performed using standards and a blind standard before samples were loaded onto the autosampler.
3.4.3 X-ray diffraction (XRD) analysis

*Moringa oleifera* bark sample consistency and crystallographic properties were investigated using an X-ray diffractometer. Standard X-ray diffraction patterns were obtained on a Bruker D8 Advance X-ray diffractometer (USA) fitted with a copper tube (CuK\(_\alpha\) radiation source, \(\lambda = 1.5408\) Å), monochromator and scintillation detector. Diffraction patterns were run using a 1° fixed divergence slit, a step size of 0.015°, 2.5 s per step and a total run time of 140 min at 40 kV and 35 mA. The instrument was calibrated using a silica calibration standard. There was no pre-treatment of the sample, save to pulverize it using a pestle and mortar (50 µm diameter) and then homogenizing it. Samples were pressed into sample holders using customised sample press and gentle roughened.

3.4.4 Surface properties of *Moringa oleifera* bark

3.4.4.1 High resolution scanning electron microscopy (HRSEM) imaging and energy dispersive X-ray analysis (EDX)

Different bark sample treatments (unmodified and chemically modified) were analyzed for their surface morphologies using a JEOL JSM 639OLV focused ion beam - scanning electron microscopy (FIB-SEM) coupled with a Noran Six 200 Energy Dispersive X-ray (EDX) analyzer at an accelerating voltage of 5 kV. Samples were adhered onto the conductive tape on the stub and sputter-coated with gold-palladium nanoparticles since
preliminary imaging revealed that the samples had irregular surfaces and were charging. The instrument had an energy dispersive mode which was used to detect the surface elemental composition of the bark material.

3.4.4.2 Nitrogen adsorption-desorption BET surface area determination

BET specific surface area and porosity determinations were performed using nitrogen adsorption-desorption isotherms obtained using a Micromeritics ASAP, 2010, N₂-BET analyzer (USA). The pulverized sample was degassed for 12 h at 383 K under vacuum - a temperature determined from preliminary investigations using a thermogravimetric analyzer (TGA). This was done to remove any moisture, adsorptives or adsorbed contaminants that may have been on the surface. Then 20.00 mg of the dry sample was introduced into the instrument and the nitrogen adsorption-desorption carried out at 77 K. The adsorption – desorption data generated therein was mathematically computed using the modified BET equation:

\[
\frac{P}{w\left(\frac{P_0}{P}\right) - 1} = c - 1 \left(\frac{P}{P_0}\right)^{1/c - 1}
\]

(Eqn. 3.1)

where \(w\) is the weight of gas adsorbed at relative pressure, \(P/P_0\); \(W_m\) is the weight of the adsorbate consisting of monolayer surface coverage and \(c\) is the BET constant. A
plot of \( \frac{1}{W(\frac{Po}{P} - 1)} \) against \( \frac{P}{Po} \) gives a linear curve from which the slope and intercept were used to determine \( W_m \), using the relation:

\[
W_m = \frac{1}{s + i}
\]

(Eqn. 3.2)

where \( s \) is the slope and \( i \) is the y-intercept. The BET surface area \( (S_{BET}) \) of the adsorbent was then calculated using Equation 3.3, at low pressure regions within which linearity is observed (Sing, 2001):

\[
S_{BET} = \frac{w_m N_o \sigma_o}{M}
\]

(Eqn. 3.3)

where \( S_{BET} \) is the specific surface area \( (m^2/g) \); \( N_o \) is Avogadro’s number, \( M \) is the molecular weight \( (g/moL) \) of the adsorbate, and \( \sigma_o \) represents the settlement area of a molecule of nitrogen \( (1.62 \times 10^{-19} \text{ m}^2/\text{moL}) \).

The macroporous surface area \( (S_{macro}) \) and external surface area \( (S_{ext}) \), as well as the micropore volume \( (V_{mi}) \) were evaluated by the \( t \)-plot method. The Barret-Joyner-Halenda (BJH) method was used to determine the particles size distribution (PSD) and the mesopore volume \( (V_{me}) \). The mean pore diameter, \( D_p \), was calculated from:
\[ D_p = \frac{4V_T}{S} \]  
(Eqn. 3.4)

where \( V_T \) is the total volume of pores, and \( S \) being the BET surface area. The pore volume of the adsorbent was obtained by applying the \( t \)-plot method using De Boer's equation:

\[
t(\text{Å}) = \sqrt{\frac{13.99}{\log \left( \frac{P}{P_o} \right) + 0.034}}
\]  
(Eqn. 3.5)

The resulting plot of volume adsorbed (g/cm\(^3\)) versus \( t(\text{Å}) \) was then used to determine the total surface area of the pores, \( S_t \), from the slope, \( s \), and Equation 3.6:

\[
s_t = \frac{V_{Adsl}^{STP}(15.47)}{t(\text{Å})} = s \times 15.47
\]  
(Eqn. 3.6)

where the value 15.47 is the conversion factor of the gas volume to liquid volume, and \( V_{Adsl}^{STP} \) is the volume of adsorbed gas converted to standard conditions of temperature and pressure.
3.4.4.3 Determination of the particle size distribution (PSD)

The evaluation of PSD of the adsorbent is critical since size and pore distributions have a great influence on the characteristics and performance of the adsorbing material. It determines the contribution of each pore volume contained in each pore size to the total volume of the sample. The molecules adsorbed thereon behave as being in the liquid state, hence a vapor – liquid interface exists between the gas and the already adsorbed molecules (Pràdanos et al., 1996). This then forms the basis for the use of Kelvin’s equation:

\[ r_k = \frac{-2\gamma_m}{RT \ln \left( \frac{P}{P_o} \right)} \cos \theta \]  

(Eqn. 3.7)

where \( \gamma \) is the surface tension of nitrogen at its boiling point, \( \gamma_m \) is the molar volume of liquid nitrogen (34.7 cm\(^3\)/mol), \( R \) is the gas constant, \( T \) is the boiling point of nitrogen (77K), \( P/P_o \) is the relative pressure of nitrogen and \( r_k \) is the Kelvin radius of the pore. The pore volume, \( r_p \), was corrected, since already there is a layer of adsorbed gases on the wall of the adsorbent. It, therefore, becomes:

\[ r_p = r_k + t \]  

(Eqn. 3.8)
where $t$ represents the thickness of the adsorbed layer on the surface and can be expressed using De Boer's equation (Eqn. 3.5). The determination of $r_p$ allows for the evaluation of PSD of the adsorbent using a method proposed by Barret and co-workers (1951). They proposed a calculation method for the distribution curves of the pore volume as a function of pore diameter from nitrogen adsorption-desorption data. This is achieved by correlating $r_p$ with the volume adsorbed, $V_a$, so as to obtain the necessary gas volume to fill by condensation of all pores with a radius $< r_p$. The derivative of this function ($dV/dr_p$) gives the differential distribution of specific volume of pores with respect to $r_p$.

### 3.5 ADSORPTION STUDIES OF SELECTED METALS ON *M. OLEIFERA* BARK

The adsorption potential of *M. oleifera* bark was investigated for the removal of selected metals (Cd, Cr, Co and V) from simulated aqueous samples. Due to the nature of plant-based adsorbents, metal adsorption is largely dependent on pH, temperature, initial metal concentration, adsorbent dosage, and contact time. Therefore, optimum metal adsorption conditions of *M. oleifera* were investigated by monitoring these parameters using the batch adsorption technique for a one component metal system. These parameters also enabled the determination of thermodynamic and kinetic parameters of the adsorption to better understand the underlying mechanistic pathways responsible for the observed adsorptions, or lack of. Unless otherwise stated, the batch adsorption experiments were carried out in triplicates at room temperature ($25 \pm 2 ^\circ C$), using a 1.0 $\pm$ 0.05 g adsorbent mass, initial metal concentration of 5 mg/L, for 1 h and at pH 4.6.
Sample mixtures were agitated in a thermostatically controlled water bath at 100 rpm for the specified time period. At the end of each experiment, the mixture was filtered using a 0.45 μm nylon membrane filter and metal determinations performed using an optimized inductively coupled plasma optical emission spectrometer (ICP-OES). The adsorption capacity and percentage adsorbed for each element were subsequently determined by applying equations 3.9 and 3.10, respectively:

\[
Q_t = \frac{(C_o - C_e)\nu}{w}\frac{1000}{1000}
\]

(Eqn. 3.9)

\[
\% \text{ Removal} = \frac{C_i - C_o}{C_i} \times 100\%
\]

(Eqn. 3.10)

where, \(Q_t\) is the adsorption capacity (mg/g) at time \(t\), \(C_i\), \(C_o\) and \(C_e\) are the initial, outlet and equilibrium concentrations of the metal in mg/L, respectively. \(V\) is the volume of metal in solution (L) and \(w\) is the total amount of adsorbent in g.

3.5.1 Effect of pH on the adsorption of metal ions onto \(M. \text{ oleifera}\) bark

The optimum adsorption of Cr(VI), Co(II), Cd(II) and V(V) onto \(M. \text{ oleifera}\) bark was investigated by performing batch adsorption experiments at different pH solutions from pH 2, 4, 6, 7, 8, 10 and 12. This was achieved by a drop-wise addition of 1.0 M HNO_3 or
1.0 M NaOH to the reaction mixture, depending on the desired solution pH. The pH was determined using a portable pH meter (Thermo Scientific Orion 4 Star pH/conductivity meter, USA) calibrated using commercial calibration buffer solutions (pH 4, 7 and 10).

3.5.2 Effect of adsorbate concentration on the adsorption of metal ions onto *M. oleifera* bark

The effect of metal concentration on the adsorption of metals onto *M. oleifera* bark was investigated by performing batch adsorption experiments for the adsorption of Cd(II), Cr(VI), Co(II) and V(V). The initial concentrations of these metals were varied as follows: 1, 5, 10, 20, 50, 80, 100, 200 and 500 mg/L in 100 mL polyethylene containers in a thermostatically controlled water bath. The same analytical procedure was then followed until metal determination using an ICP-OES.

3.5.3 Effect of adsorbent dosage on the adsorption of metal ions onto *M. oleifera* bark

The optimum mass of adsorbent for the adsorption of Cd(II), Cr(VI) and Co(II) was determined by varying the initial masses (0.05, 0.5, 1, 2 and 5 g) of *M. oleifera* bark at pre-set experimental conditions. The reaction mixture was agitated at the set time period in a thermostatically controlled water bath.
3.5.4 Effect on contact time on the adsorption of metal ions onto \textit{M. oleifera} bark

To investigate the effect of the amount of contact time between the adsorbate and adsorbent, batch experiments were designed with different contact times (5, 10, 20, 30, 60, 80, 100, 120, 150 and 180 min) for the adsorption of Cd(II), Cr(VI) Co(II) and V(V) on \textit{M. oleifera} bark. Samples were immediately filtered at the end of the pre-set time period to minimize prolonged contact between the adsorbate and adsorbent.

3.5.5 Effect of solution temperature on the adsorption of metal ions onto \textit{M. oleifera} bark

Batch adsorption experiments were designed using the same experimental procedure as before, but varying the temperature at which the reaction is allowed to take place. A temperature controlled water bath was used to facilitate adsorption with agitation at 25, 35 and 45 \textdegree C. Preliminary investigations showed that the reaction temperature could not be set beyond 45 \textdegree C, as the metal anchoring biomolecules in the bark seem to become inactive, hence no adsorption was observed.
3.6 EQUILIBRIUM ISOTHERMS AND KINETIC STUDIES ON THE ADSORPTION OF METAL IONS ONTO M. OLEIFERA BARK

3.6.1 Equilibrium isotherms for the adsorption of vanadium onto M. oleifera bark

The batch adsorption technique was used to generate data for determining sorption isotherms for the adsorption of V(V) onto the bark of M. oleifera. This was achieved by equilibrating 1.0 ± 0.01 g pulverized sample for 1 h at 25 ± 2 °C in a thermostatically controlled water bath with constant stirring at 100 rpm. The pH was adjusted to pH 4.6 with a stepwise addition of 1.0 M HNO₃ or 1.0 M NaOH, depending on the initial pH of the solution. Different experiments were designed with varying concentrations of V(V): 1, 10, 20, 30, 40, 80, 100, 150, 200 and 300 mg/L obtained from dilutions of commercial 1000 mg/L V(V) standards. The samples were then immediately filtered using a 0.45 µm nylon membrane filter and the residual metal concentrations determined using an ICP-OES. The data obtained was used to determine equilibrium constants, which were then used to model the adsorption using common adsorption isotherms.

3.6.2 Kinetic studies on the adsorption of vanadium onto M. oleifera bark

To determine the degree to which metal adsorption onto M. oleifera bark conforms to common kinetic model equations, adsorption data were generated from the adsorption of 1.0 ± 0.01 g pulverized sample with 20 mg/L V(V) in a thermostatically controlled water bath at 25 ± 2 °C at 100 rpm, maintaining a total volume of 250 mL of solution.
The pH of the solution was adjusted to pH 4.6 by the stepwise addition of 1.0 M HNO$_3$ or 1.0 M NaOH. Aliquots from the reaction medium were collected using a 5 mL calibrated sterilized syringe at time intervals of 5, 10, 20, 30, 40, 60, 90, 120, 150, 180, 210 and 240 min into 50 mL volumetric flasks and diluted to volume with de-ionized water. An ICP-OES spectrometer was used to determine the residual metal concentrations, from which mathematical kinetic equations (Pseudo-first and second order equations and intra-particle diffusion models) were used to model the adsorption.

3.7 DATA QUALITY PROTOCOLS

To ensure the data generated from the batch adsorption experiments was reproducible and reliable, a number of protocols were employed. Firstly, all experiments were replicated three times and the relative standard deviation (RSD) monitored for each result to be within the 5% upper range, failing which, the result was rejected and the experiment repeated until an acceptable range was obtained. Reagent blank samples were prepared with each batch and treated as samples. Instrumental outputs for each experiment were corrected using the relation:

$$C_c = C_m - C_b$$

(Eqn. 3.11)
Where, $C_{c}$ is the corrected metal concentration in solution after adsorption, $C_{m}$ is the metal concentration as determined from the instrumental output, and $C_{b}$ is the metal concentration in the blank sample.

### 3.8 SURFACE MODIFICATION OF ADSORBENT USING POLYAMINES

*M. oleifera* bark samples were chemically functionalized by immobilizing different aliphatic polyamines of increasing chain length on the surface of the adsorbent. The polyamines used, in increasing chain length, are ethylenediamine (EDA, C$_2$H$_8$N$_2$), diethylenetriamine (DETA, C$_4$H$_{13}$N$_3$), triethylenetetramine (TETA, C$_6$H$_{18}$N$_4$) and tetraethylenepentamine (PETA, C$_8$H$_{23}$N$_5$) procured from commercial suppliers. For each experiment, 1,4-dioxane was added to 5.00 g pulverized adsorbent in separate flasks to activate the adsorbent pores. The mixture was degassed with argon gas for 20 min, after which 50 mL of each polyamine was introduced into each flask and then sealed with a silicon rubber. The mixture was then allowed to react at room temperature (23 ± 2 °C) with constant stirring for 12 h. The adsorbents were then separated by a Buchner funnel using a 0.45 µm nylon membrane filter and then repeatedly washed with deionized water to remove unadsorbed amine molecules. The amine functionalized adsorbents were then put in a desiccator to dry until characterization using FTIR spectroscopy, FIB-SEM photography, CHNS elemental analyzer and N$_2$-BET analysis.
3.9 SYNTHESIS OF GOLD NANOPARTICLES USING *M. OLEIFERA* BROTH

3.9.1 *M. oleifera* broth extraction

*M. oleifera* bark extract was obtained by putting 40 g of the pulverized bark sample into a 500 mL container with 300 mL of deionized water and then heating the mixture for 30 min at 75 °C with constant agitation. The extract obtained was filtered under vacuum using Whatman filter paper (No. 41) and stored in glass vials. This solution, always used fresh, was considered to be the stock *M. oleifera* broth solution and was hypothesized to act both as a reducing and as a stabilizing agent during the synthesis.

3.9.2 Green synthesis of gold nanoparticles using *M. oleifera* broth

Complete reduction of Au$^{3+}$ to Au$^0$ during the synthesis of gold nanoparticles is evidenced by a visual and gradual colour change from yellow, characteristic of the gold precursor salt, to a wine red colour, characteristic of the formation of colloidal gold nanoparticles. Tetrachloroauric acid (HAuCl$_4$.3H$_2$O) was used as the precursor salt, wherein a concentration of 2.0 mM was prepared, from which dilutions were performed to obtain working concentrations for the different experiments. The synthesis was conducted by monitoring the effects of varying pH, volume and percentage of *M. oleifera* broth, concentration of the precursor salt and contact time to the quality of the nanoparticles formed. Typically, the synthesis conditions were set at a volume ratio of 5:2 (HAuCl$_4$.3H$_2$O to *M. oleifera* broth) at optimized experimental conditions of pH 3.4;
synthesis time and temperature of 60 min and 90 °C, respectively; 0.2 mM concentration of HAuCl₄·3H₂O and 300 rpm stirring rate, unless otherwise stated.

The effect of pH on the quality of the synthesized nanoparticles was investigated by designing experiments with varied solution pHs, attained by adjusting the pH of the reaction media to pH 2, 4, 6, 8 and 10, by a drop wise addition of 1.0 M HNO₃ or NaOH, depending on the desired pH value. The effect of the initial concentration of the gold precursor (HAuCl₄·3H₂O) salt was investigated by varying its initial concentration between 0.125, 0.25, 0.5, 1.0 and 2.0 mM in separate experiments. To study the effect of percentage (%) broth used, the broth was diluted with deionized water to provide percentage broth volumes of 100, 75, 50, 25 and 10 mL, maintaining the HAuCl₄ volume at 50 mL, and the volume ratios used are presented in Table 3.1. The effect of the volume of broth was investigated by assuming that the extracted broth solution is 100 % of the M. oleifera extract, from which varying volumes (4, 6, 8, 10, 15 and 25 mL) were added into the reaction vessels. Effects of varying reaction time on the quality of the gold nanoparticles formed were investigated by taking 5 mL aliquots of the reaction mixture at predetermined time intervals of 0, 5, 10, 15, 30 and 40 min using a pre-cleaned syringe. After each experiment, a portion of the colloidal suspension was cooled to room temperature, centrifuged at 14 000 rpm for 10 min and then frozen at -80 °C before freeze-drying them to obtain crystalline gold nanoparticles. The other aqueous portion was reserved for further experiments and characterizations that required aqueous samples.
TABLE 3.1: Volume ratios of *M. oleifera* broth and gold precursor salt used to investigate percentage broth effects on gold nanoparticles synthesis.

<table>
<thead>
<tr>
<th>Percentage Extract (Extract)</th>
<th>Volume of Extract (mL)</th>
<th>Volume of HAuCl₄ (mL)</th>
<th>Deionized water (mL)</th>
<th>Total volume (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>20</td>
<td>50</td>
<td>0</td>
<td>70</td>
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<tr>
<td>75</td>
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<td>10</td>
<td>2</td>
<td>50</td>
<td>18</td>
<td>70</td>
</tr>
</tbody>
</table>

3.9.3 Characterization of synthesized gold nanoparticles

The obtained gold particles at the different treatments of pH, time, precursor concentrations and broth amounts were characterized for surface and spectral properties as well as size and shape determinations. Infrared spectra were obtained using a PerkinElmer Spectro65 FT-IR spectrometer (USA), with 8 scans and 4 resolutions (cm⁻¹). A Micromeritics ASAP 2010 surface area and porosity N₂-BET analyzer was used to determine the surface area and pore characteristics. Due to sample size limitations; BET analysis was done for the particles resulting from the 2.0 mM concentration of the precursor salt. Surface plasmon resonance measurements were obtained using a Shimadzu UV-1601 spectrophotometer (Japan) operated on a 1 nm resolution. Size and morphological analyses were done using a JEOL-2100 high resolution transmission electron microscopy (HRTEM), Japan, operated at 200 kV. Samples were prepared by drop casting 5 μL on a 300 mesh carbon coated copper grid (Agar scientific) and allowed to dry at room temperature. Surface properties of the
materials were determined using a JEOL JSM-7600F Field emission high resolution scanning electron microscope (HRSEM) coupled to an energy dispersive x-ray (EDX), Japan. Samples were adhered onto a conductive tape on the stub and mounted.

Zeta potential measurements were performed using a Malvern Zetasizer Nanoseries (UK). Aqueous gold nanoparticle samples were diluted 1:20 parts in de-ionized water in a 12 mm glass cuvette. The cuvette was covered using a universal dip cell kit and put into the sample compartment and measurements performed. Particle size distribution (PSD) for the nanoparticles was also simultaneously determined.

3.9.4 Colorimetric assay of lead ions based on gold nanoparticles aggregation

Metal ions in solution can be quantified by monitoring the aggregation behaviour of the gold nanoparticles in the presence of these ions. This monitoring can be done visually; since gold nanoparticles tend to change colour between their dispersed and aggregated forms, or spectrophotometrically through observations of changes in the SPR bands between their dispersed and aggregated forms. In this section, aqueous gold nanoparticles synthesized under different experimental conditions of pH, precursor concentration, broth amounts and different synthetic times were used to determine aggregation behaviour of these particles in the presence of Lead (Pb). This was done with a view to determine the concentration of Pb in a synthetic solution spectrophotometrically or colorimetrically.
To investigate the potential of manipulating the aggregation behaviour of gold nanoparticles to quantify the concentration of lead ions in water, triplicates of 800 μL of different concentrations of lead (2.5, 5, 10, 25, 50 and 100 mM) were individually added to 500 μL of a gold nanoparticle colloidal solution at room temperature (23 ± 2 °C), pH 4.62 and allowed to react for 24 h. The effect of pH was monitored by using gold nanoparticles synthesized under different pH conditions (pH 2, 4, 6, 8, 10), maintaining all the other parameters constant. Gold nanoparticles synthesized from different broth percentages were used to investigate their effect on the determination of lead in aqueous solutions. This was done by taking triplicates of 500 μL of the different broth formulations (100, 75, 50, 25 and 10%) and adding them to 800 μL of a 100 mM Pb and allowed to react for 24 h at room temperature (23 ± 2 °C) and at pH 4.62. All analyses were done in triplicate and statistical estimations performed.

The resulting gold nanoparticle-metal complex was characterized using the same protocols as above. Spectral properties of this complex were investigated using a Shimadzu UV-1601 spectrophotometer (Japan) to observe the SPR band behaviour at different analytical conditions on the extent to which the wavelength shifts, corresponding to the degree of nanoparticle aggregation. A calibration curve was constructed from which analytical figures of merit, such as limits of detection (LOD), limits of quantification (LOQ), sensitivity, accuracy and linear range, were determined. A student’s t-test was performed on the data at the 95% confidence interval to deal with suspected outlier data points.
4.1 PHYSICOCHEMICAL CHARACTERIZATION OF *M. OLEIFERA* BARK

An in-depth investigation into the physicochemical properties of a potential adsorbent helps improve the understanding of the potential applications of the material proposed, as well as the degree to which it can be chemically modified to improve its applicability. Such investigations were undertaken on *M. oleifera* bark, to determine the adsorptive potential of the material with respect to surface area, pore volume, porosity, surface functional group morphology, proximate elemental distributions, thermal stability and crystallinity. These factors are critical in the design, optimization and operation of the material.

4.1.1 Pore and surface characteristics of *M. oleifera*

The N$_2$ adsorption-desorption isotherm for *M. oleifera* (Figure 4.1) shows a classical type II adsorption curve with a H3 hysteresis loop, according to the IUPAC classification of 1994 (Sing *et al.*, 1985).
FIGURE 4.1: N₂-BET adsorption-desorption isotherm for unmodified *M. oleifera* bark

There is a sharp initial adsorption at low relative pressure \((P/P_0)\) below 0.05, which suggests the existence of a micropore structure in agreement with Serwicka (2000) even though very slightly. This is also typical of a characteristic sub and monolayer formation on the surface of the adsorbent. Beyond this point, mono and multi-layer adsorption are operative, since the micropore sites are filled up and larger pores, at high \(P/P_0\) values, are occupied. IUPAC (1994) classified adsorbent pore sizes as microporous (<2 nm), mesoporous (2 to 50 nm) or macroporous (more than 50 nm); wherein the pore sizes in *M. oleifera* bark were found to be 1.39 nm, qualifying the bulk of the material as macroporous. The sharp curve in Figure 4.1 (commonly referred to as
B or the knee of the isotherm) is indicative of a strong adsorbate-adsorbent interaction; an ideal property in adsorption technology development which is further confirmed by the negative C value obtained from equation 4.1:

\[
\frac{P}{n^a (P_o - P)} = \frac{1}{n^{a_m} c} + \left(\frac{c - 1}{n^{a_m} c} \right) \frac{p}{P_o}
\]

(Eqn. 4.1)

where, \(n^a\) is the amount of the gas adsorbed at the relative pressure \(P/P_o\), \(n^{a_m}\), the monolayer capacity and C, the dimensionless BET constant which is related to the enthalpy of adsorption in the first adsorbed layer.

Even though the material is mostly microporous, bulk/capillary condensation of the adsorbing gas is evident resulting in the steep upward curve at high \(P/P_o\), even though the curve is observed to increase steadily. This, therefore, suggests that there is no meaningful mesoporous adsorption as most of the adsorption occurs predominately between the micro and macroporous ranges. These findings form critical building blocks in describing the possible mechanistic behaviour of the bark in binding particles together to form flocs, thus, its biosorption behaviour could be elucidated.

The shape of the adsorption curve (Figure 4.1) and the associated hysteresis loop thereof, suggest that adsorption is dependent on the formation of a polymolecular layer of an adsorbate on the surface of the adsorbent and, also in the pores (Collet et al., 2008), thereby multilayer adsorption is facilitated. The shape of the hysteresis loop
suggests the existence of ink-bottle pores typically with narrow necks/entrances and wide bodies. These observations are similar to those observed by other authors (Wlodarczyk-Stasiak & Jamroz, 2009). This pore geometry is particularly advantageous in adsorption technology as the resultant total pore volume is relatively large, availing more adsorption space; thereby improving the adsorption capacity of the material. In this study, the pore volume characteristics, presented in Table 4.1, were obtained by applying the \( t \)-plot method from De Boer’s equation (Equation 3.5), the patterns of which are presented in Figure 4.2.

**TABLE 4.1:** Pore characteristics of the unmodified *M. oleifera* bark obtained from \( \text{N}_2 \) adsorption data at 77 K.

<table>
<thead>
<tr>
<th>Adsorbent</th>
<th>( S_{\text{BET}} ) (m(^2)/g)</th>
<th>( S_L ) (m(^2)/g)</th>
<th>( S_{\text{ext}} ) (m(^2)/g)</th>
<th>Pore diameter (Å)</th>
<th>( S_t ) (m(^2)/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. oleifera</em> bark</td>
<td>1.79</td>
<td>295.05</td>
<td>41.06</td>
<td>52.04</td>
<td>2.40</td>
</tr>
</tbody>
</table>
FIGURE 4.2: A $t$-plot curve of the chemically unmodified *M. oleifera* bark adsorbent

The presence and operation of micropores in *M. oleifera* is further confirmed by the straight line in Figure 4.2, which when extrapolated does not pass through the origin. This, according to Dollimore and Heal (1964), is indicative of the filling of micropores, and in this case, occurs at low relative pressures. The calculated total surface area of the pores, $S_t$, is 2.40 cm$^3$/g (Table 4.1) and is different from the $S_{BET}$ (1.79 m$^2$/g), which implies operative microporosity at these pressure ranges (Dollimore & Heal, 1964).

Application of De Boer's equation also permits for the identification of mesopores in the adsorbent. This manifests itself in the $t$-plot as two different curves with different slopes, which would then give two $S_t$ values (Dollimore & Heal, 1964). This phenomenon is not observed with *M. oleifera*, further confirming earlier conclusions (from Figure 4.1) that mesopore filling is negligible.
Surface properties of an adsorbent can also be partially explained with respect to the surface area of the material. The surface area of a solid material can be calculated from the gas adsorption isotherm which is based on the application of the modified BET equation. A plot of $1/w[P/P_0 - 1]$ against $P/P_0$ gives a linear curve (Figure 4.3), from which the BET surface area ($S_{BET}$) was determined to be 1.79 m$^2$/g.

Pore properties of an adsorbent do not exclusively define adsorption properties of a particular adsorbent. For instance, Siswoyo and co-workers (2014) recorded a surface area of 413 m$^2$/g with a corresponding adsorption capacity of 9.2 mg/g, which is significantly lower than that obtained in this study (48.8 mg/g), even though the reported surface area is significantly lower (1.79 m$^2$/g). This suggests that the adsorption capacity of a material is also influenced by other factors apart from its pore properties. The type and amounts of molecules that characterize the surface of the adsorbent also play a significant role in adsorption through a different adsorption mechanism. Surface properties are, therefore, critical components in elucidating or improving the adsorption potential of an adsorbent. In light of this, surface modification of adsorbent is therefore not an uncommon research route.
As with other adsorbents where N₂-BET is used to determine porosity, the range of linearity or applicability of the BET equation is restricted to low pressure regions ($p/p_o \sim 0.3$), and is dependent on the adsorptive, adsorbent and the operational temperature (Sing, 2001). In the results presented herein, linearity was lost beyond this range (Figure 4.3), in keeping with the pressure range within which micropore filling occurs. The formation of the monolayer is restricted to micropore filling, beyond which, progression to multilayer is evident. This is based on the assumption that the smaller pores become completely filled with nitrogen since the saturation vapour pressure in a small pore is reduced by the effect of surface tension to agree with Wlodarczyk-Stasiak & Jamroz (2009).
Pore characteristics can further be determined based on the particle size distribution (PSD) of the material and it determines the contribution of each pore volume contained in each pore size to the total volume of the sample. The relationship obtained therein between the statistical thickness from De Boer and Kelvin’s equations and total pore radius, \( r_p \), is presented in Figure 4.4, which shows that the pore radius increases with an increase in the relative pressure and an exponential increase is observed at \( P/P_o \sim 0.6 \), a point at which multilayer adsorption is preferred.

**FIGURE 4.4**: Variation of statistical thickness of adsorbed layer from de Boer’s equation, pore radius from Kelvin equation and the total pore radius

The determination of \( r_p \) allows for the evaluation of the particle size distribution (PSD) of the adsorbent using a method proposed by Barret and co-workers (1951), commonly known as the BJH method. The results of this analysis are presented in Figure 4.5 and
reflect the distribution for both branches of the hysteresis loop (adsorption and desorption curves).

The PSD curve for both the adsorption and desorption branches show pore diameter ranges between 2.5 and 50 nm with a mean distribution centred around 8 nm. The pore sizes for the adsorption branch are centred at 8 nm, and at 7.5 nm for the desorption curve, indicating that *M. oleifera* pore sizes are distributed along the micro and sub-mesoporous ranges. There is no significant difference between the maxima of both curves, suggesting minimum pore network effects and the apparent absence of tensile strength effects on the adsorption branch. This effect had earlier been observed by Groen *et al.* (2003). This implies that both curves can be used for pores size calculations and that pore filling and emptying follow the same mechanisms with respect to the kinetics involved.
4.1.2 Scanning Electron Microscopy with energy dispersive capabilities (SEM-EDS)

Surface morphological features of the adsorbent were determined using SEM and the images are presented in Fig. 4.6 (A) and (B). The micrographs show that the surface of *M. oleifera* bark has slit-like pores and an open pore structure, which is line with the pore geometry described earlier. This geometry provides a high internal surface area, which is critical in metal adsorption.

![SEM micrographs of the unmodified *M. oleifera* bark at low magnification (A) and at high magnification (B)]

**FIGURE 4.6**: SEM micrographs of the unmodified *M. oleifera* bark at low magnification (A) and at high magnification (B)

4.1.3 Elemental Composition of *M. oleifera* bark

Total amounts of CHNS in the bark were determined using an elemental analyzer. The same technique was used to determine the amounts of N before and after amine functionalization. Blank run and K-factor calibrations were performed to improve on
accuracy of the technique. Standard samples with known C, H and N contents were also used to ascertain the level of accuracy in the determinations. Replicated results from the CHNS analyser showed an error of less than 5% for all the elements investigated. Table 4.2 shows the results obtained therein.

TABLE 4.2: CHNS results of the chemically unmodified *M. oleifera* bark.

<table>
<thead>
<tr>
<th>Adsorbent</th>
<th>C</th>
<th>H</th>
<th>N</th>
<th>S</th>
<th>O</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. oleifera</em> bark</td>
<td>39.5±0.56</td>
<td>5.6±1.36</td>
<td>1.07±0.18</td>
<td>0.234±0.07</td>
<td>53.7±1.8</td>
</tr>
<tr>
<td><em>M. oleifera</em> seed</td>
<td>44.8±0.00</td>
<td>5.9±0.00</td>
<td>0.8±0.00</td>
<td>0.9±0.00</td>
<td>47.6±0.0</td>
</tr>
</tbody>
</table>

*a*Estimated by difference  
*b*Sharma *et al.*, 2006

Comparatively, the results obtained herein are comparable with results obtained from the elemental analysis of the seeds of *M. oleifera* reported in literature (Sharma *et al.*, 2006). The results confirm the lignocellulosic nature of the material, since the percentage compositions of C and H are relatively high. The protein nature of the bark is revealed by the elevated percentages of N and S, which are however, lower than those obtained from the seeds (Sharma *et al.*, 2006).

Percentage nitrogen, indicative of the degree of amination, was similarly determined after surface functionalization of the bark with aliphatic polyamines of different chain lengths, and the results presented thereof are averages of replicate analyses.
4.1.4 Spectral properties of the *M. oleifera* bark

4.1.4.1 FTIR spectra of the unmodified *M. oleifera* bark

A FT-IR spectrum of the unmodified *M. oleifera* bark is shown in Figure 4.7. It shows a broad and distinctive curve which peaks at 3352 cm\(^{-1}\) region, indicating the presence of hydroxyl groups. A weak band at 2945 cm\(^{-1}\) is indicative of C-H stretching frequencies, confirming its lignocellulosic nature. Stretching frequencies showing the presence of primary and secondary amides (R-NH\(_2\) and R-NH, respectively) are shown at 1630 cm\(^{-1}\). The presence of carboxylic acids (R-COOH) is indicated by the band located at 1321 cm\(^{-1}\). At 1040 cm\(^{-1}\) there is an elaborate peak, suggesting the presence of thio-carbonyl (C=S) group.

![FTIR spectrum of the unmodified M. oleifera bark powder](image)

FIGURE 4.7: FT-IR spectrum of the unmodified *M. oleifera* bark powder
Other authors have characterized the seeds and leaves of *M. oleifera* and it is generally agreed that the plant is endowed with many functional groups such as hydroxyl (R-OH), carboxyl (R-COOH), carbonyl (R-C=O), amides (R-NH₂), ketones (R-C=0-R) and aldehydes (R-C=OH) (Alvesa *et al.*, 2010; Reddy *et al.*, 2010), some of which have been identified herein.

4.2 OPTIMIZATION OF EQUILIBRIUM ADSORPTION PARAMETERS OF *M. oleifera* USING V(V)

Vanadium (V) was used as an adsorbate to investigate and optimize adsorption conditions that define the adsorption process. The chemistry and speciation behaviour of vanadium, a priority pollutant with adverse health and environmental effects, is well documented and, therefore, is an ideal element for optimization experiments. The parameters investigated herein include pH, contact time, dosage mass, concentration of adsorbate and the temperature at which the reaction proceeds.

4.2.1 Effect of pH on adsorption of V(V) onto *M. oleifera* bark

The pH of an aqueous solution has an important role in biosorption of metals as it affects the network of negative charges on the surface of the adsorbent, as well as the physicochemistry and hydrolysis of the metal. Percentage removal of V(V) ion as a function of pH is shown in Figure 4.8.
FIGURE 4.8: Influence of pH on the adsorption of 5 mg/L V(V) onto the bark of 1.00 g *M. oleifera* at 25 °C for 1 h contact time

Figure 4.8 shows that the amount of V(V) adsorbed increases with an increase in pH until pH 6. Beyond this point, the amount adsorbed decreases as the pH is increased to basic conditions until pH 12 – the highest pH value investigated. These results emphasize the significance of solution pH on the adsorption capabilities of vanadium onto *M. oleifera* adsorbent, which can also be attributed to the chemical nature of the adsorbent, as well as the speciation chemistry of vanadium in aqueous solutions.

pH influences both the metal chemistry in solution and the protonation/deprotonation of metal surfaces, a mechanism reported elsewhere (Peacock & Sherman, 2004). In aqueous solutions vanadium exists in different hydrolyzed forms depending on its
concentration and pH. Speciation diagrams for vanadium suggest in aqueous solutions that the element exists in its cationic forms at low pH values, and an increase in hydroxide ions (increasing pH) increases the predominance of the anionic species (Figure 4.9). The importance of this is possibly owed to the fact that *M. oleifera* is largely composed of a cationic polymeric protein of high molecular weight (Vieira *et al.*, 2010). Therefore, a change in the pH of the solution causes a net change in the overall charge of the adsorbing protein at the active sites of the adsorbent (Kwaambwa & Maikokera, 2007). In acidic solutions, about 90% of the amino acid molecules contain cationic sites (Wlodarczyk-Stasiak & Jamroz, 2009). Consequently, the protein is protonated, and so few active sites are available for the adsorption of the cationic V species using common ion exchange mechanisms. Subsequently, the recorded percentage adsorption is low in acidic conditions. Furthermore, low pH concentrations present a high concentration of H$_3$O$^+$ ions in solution, which also have the propensity to compete for the available active sites with the metal ions; thereby facilitating competitive adsorption. However, due to their high concentrations at these low pH values, they out-compete the metal ions and remain adsorbed, leaving most of the metal ions unbound.
As the solution pH is increased beyond pH 4, weakly acidic surface functional groups, such as the carboxylic group (-COOH) of the amino acid group (-NH₂) are progressively deprotonated as carboxylate (-COO⁻) ligands, simultaneously protonating the amino group (NH₃⁺). Such positively charged NH₃⁺ would facilitate binding between the bark and the vanadium metal. At these pH values (and beyond), the protein becomes deprotonated and the vanadium exists as the vanadate ion, HVO₄²⁻. The same observations had been reported by Peacock & Sherman (2004). This anion adsorbs
onto the adsorbent, possibly by a ligand exchange process, where the OH\(^-\) released are consumed in neutralizing the H\(^+\) for the deprotonation reactions. This explains why the adsorption decreases linearly at high pH values (Figure 4.8).

The implication of Figure 4.8 is that *M. oleifera* bark is sensitive to changes in solution pH during metal adsorption. The shape of the curve suggests an ease with which the adsorbed metals can be desorbed from the adsorbent using weakly basic desorbing solutions.

4.2.2 Effect of contact time on adsorption of V(V) onto *M. oleifera* bark

In order to determine equilibration time of the adsorption of V(V) onto *M. oleifera* bark and the associated kinetics thereof, the adsorption of V(V) was investigated as a function of contact time, and the results are presented in Figure 4.10.
A rapid initial rate of adsorption within the first 4 h is evident, after which it plateaus off, denoting the attainment of equilibrium. The initial rapid rate is owed to the presence of empty adsorption sites on the adsorbent, creating a necessary gradient for a rapid adsorption of the adsorbate. This rate decreases with time, as the amount of available sites decrease on the cell walls of the adsorbate after occupation by the metal ions.

4.2.3 Effect of initial concentration on the adsorption of V(V) onto *M. oleifera* bark

High metal ion concentrations in adsorption experiments imply increased incidences of their adsorption onto the adsorbent, provided the system has not attained equilibrium.
This phenomenon was investigated by increasing the concentration of V(V) ions in solution between 5 and 20 mg/L, and the results are presented in Figure 4.11.

FIGURE 4.11: Effect of increasing concentration of vanadium on its adsorption onto *M. oleifera* bark at 25 °C, pH 4 using 1.00 g adsorbent mass for 4 h

According to the trend observed in Figure 4.11, increasing the concentration of the adsorbate does not necessarily result in a significant increase in metal adsorption. Concentration equilibrium is achieved even at low concentration values below 4 mg/L, wherein the initial adsorption rate was higher. The graph, however, shows maximum percentage uptake of vanadium at less than 50%, indicating that adsorbate concentration does not play a significant role in the adsorption of vanadium as the other parameters such as dosage mass, temperature, contact time and pH. Furthermore, the
graph shows a steady decrease in adsorption as the concentration is increased beyond 5 mg/L, implying a predominance of desorption over adsorption of the metal ions.

4.2.4 Effect of adsorbent dosage on the adsorption of V(V) onto *M. oleifera* bark

The effect of increasing the initial dosage (biomass) of the adsorbent was investigated by varying the mass between 0 and 250 mg while keeping all the other parameters constant. The results are presented in Figure 4.12.

![Graph](image)

**FIGURE 4.12:** Effect of increasing amounts of *M. oleifera* mass on the degree of adsorption of 5 mg/L vanadium, at pH 4, 25 °C and for 24 h contact time

Figure 4.12 shows a rapid increase in the percentage of vanadium adsorbed with an increase in adsorbent dosage, until a steady adsorption at around 150 mg of adsorbent. The graph plateaus off at this point until 250 mg of adsorbent (corresponding to 82% V
adsorbed). This indicates that an optimum adsorbent mass for maximum occupancy of the active sites of the adsorbent is achieved at around 150 mg at these adsorption conditions.

4.2.5 Effect of temperature on the adsorption of V(V) onto *M. oleifera* bark

Temperature fluctuations affect solution chemistry, including adsorption processes. The effect of solution temperature on the degree of adsorption of V(V) onto *M. oleifera* bark was investigated by altering the temperature between 20 °C and 60 °C. Results obtained in this study are presented in Figure 4.13.

![Figure 4.13: Effect of varying temperature on the degree of adsorption of 5 mg/L V(V) onto 1.00 g *M. oleifera* bark at pH 4 for 24 h](image)

FIGURE 4.13: Effect of varying temperature on the degree of adsorption of 5 mg/L V(V) onto 1.00 g *M. oleifera* bark at pH 4 for 24 h
Figure 4.13 shows the temperature dependence of adsorption, as the amount adsorbed fluctuates with a change in temperature. Highest amounts of metal are adsorbed when the solution temperatures are low (20 to 25 °C). However, temperature increases beyond this point exhibits an inverse amount of metal adsorption until it plateaus off at 45 to 60 °C. This can be explained by the fact increasing the temperature beyond 30 °C favours thermodynamic principles, wherein the adsorbate gains enough kinetic energy and is then dislodged from the active sites, hence the observed decrease in adsorption with an increase in temperature.

Increasing the temperature beyond 60 °C (to 80 and 100 °C) did not show any evidence of adsorption, largely attributed to the proteinaceous nature of *M. oleifera* which starts to lose its quaternary arrangement and the bioactive surface functional groups collapsing; thereby hindering active adsorption. This is especially true considering that the adsorption is independent of metabolism, wherein the plant cells would acclimatize and still facilitate adsorption.

### 4.3 EQUILIBRIUM ADSORPTION STUDIES OF V(V), CR(VI), CO(II) AND CD(II) IONS ONTO *M. OLEIFERA* BARK

Common metallic ions of environmental and health significance (V(V), Cr(VI), Co(II) and Cd(II)) were used to investigate the adsorption performance of *M. oleifera* in single-
component systems. Preliminary optimization results revealed that maximum metal adsorption is favoured in acidic medium (pH 4.62), using 150 mg biomass, at 25 °C, using 2 mg/L adsorbate concentration for a 4 h contact time. Batch adsorption experiments were set-up and these parameters applied to determine adsorption capacity and the percentage metal ions adsorbed.

4.3.1 Effect of metal ion concentration on their adsorption onto *M. oleifera* bark

Increasing metal ion concentrations in aqueous solutions also improves chances of their occupation into available active sites to facilitate adsorption. This phenomenon was investigated for Cd(II), Co(II) and Cr(VI) by increasing the initial concentration between 5 and 100 mg/L at experimental conditions, and the results are presented in Figure 4.14.
FIGURE 4.14: Effect of increasing metal concentrations on their adsorption onto *M. oleifera* bark at experimental conditions of pH 4.62, 25 °C, for 1 h contact time and using 150 mg adsorbent mass.

Figure 4.14 shows that all the metal ions follow a similar trend of adsorption as the initial metal ions concentration is increased from 1, 5, 10, 20, 50 and 100 mg/L. Generally, percentage metal adsorbed increases between 1 and 10 mg/L, with Cr(VI) recording the highest percentage adsorption and Co(II) the lowest. However, beyond the 10 mg/L metal concentration, the amount adsorbed decreases for all the ions investigated, instead of the normal plateau after equilibrium had been attained. This observation, though uncommon, maybe be due to the relatively low surface area (1.79 m²/g) and pore volume of the material for adsorption, wherein adsorption equilibrium is attained at low metal ion concentrations. However, as the metal ion concentration is increased
beyond this point the metal ions are desorbed from the active sites until at high concentrations.

4.3.2 Effect of solution pH on the adsorption of metal ions concentration onto \textit{M. oleifera} bark

The effect of solution pH on the adsorption of Cd(II), Cr(VI) and Co(II) was investigated and the results are shown in Figure 4.15. Rapid adsorption is observed for all ions at pH values less than 4, and from pH 5 an equilibrium state is attained.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.15.png}
\caption{Adsorption of metal ions onto \textit{M. oleifera} bark in solutions of varying pH conditions}
\end{figure}
At highly acidic conditions (pH ≤ 4), the *M. oleifera* adsorption sites are probably occupied mostly by the hydronium ions; thereby having a competitive advantage over the metal ions. Increasing the pH of the solution avails hydroxide ions which deprotonate the bioactive functional groups so that the metal ions are favoured to occupy the adsorption sites of the adsorbent possibly involving electrostatic attractions. This then explains the improved adsorption with an increase in the pH of the solution until at pH 5, wherein maximum adsorptions are recorded for each of the ions (Cd(II), 93.6 %, Cr(VI), 99.6 % and Co(II), 87.2 %).

In aqueous solutions of varying pH values, Cr exists in different ionic species (Figure 4.16) which influence its redox potential. Since the bioactive molecules at the adsorbing sites are proteinaceous in nature, they tend to be highly protonated in acidic conditions in agreement with an interpretation provided in literature by other workers (Wlodarczyk-Stasiak & Jamroz, 2009), suggesting that electrostatic attractions are limited. Furthermore, as Figure 4.16 suggests, Cr(VI) exists in its protonated forms in these acidic conditions and the degree of protonation decreases with an increase in pH. These observations best explain the low percentage Cr(VI) adsorbed at pH ≤ 4, which increases with an increase in solution pH.

As the pH is increased there is, however, a progressive protonation of Cr(VI) so that it exists in its protonated state. Concurrently, the surface functional groups of the *M. oleifera* bark are progressively deprotonated, which then provide an optimum
environment for adsorption of the metal into the active sites of the bark. The amount adsorbed is therefore significantly increased until equilibrium is reached at pH 5.

![Speciation patterns of chromium in aqueous solutions with changing pH conditions](image)

**FIGURE 4.16:** Speciation patterns of chromium in aqueous solutions with changing pH conditions (Adapted from Manahan, 2000)

The chemical behaviour of cobalt in aqueous solutions is predominately controlled by the Co(II) oxidation state (Collins & Kinsela, 2010). The adsorption trend of Co(II) onto
M. oleifera bark in solution at varied pHs is shown in Figure 4.15. It can be seen from this graph (Figure 4.15) that there is a rapid initial adsorption of Co(II) at acidic conditions and equilibrium attained at pH 5.

4.3.3 Effect of contact time on the adsorption of metal ions onto M. oleifera bark

Determination of the amount of contact time between an adsorbent and the adsorbate during adsorption can be phenomenal in explaining the kinetics of the adsorption process. Figure 4.17 shows that there is rapid adsorption of the metal ions investigated as equilibrium is attained within 20 min from the onset of the reaction. The amount adsorbed increases in the order: Cd(II) < Co(II) < Cr(VI) corresponding to percentage removals of 39, 62 and 98, respectively. The low Cd adsorption is attributed to its very low electron affinity, implying that the predominance of electrostatic attractions is greatly inhibited, so that the observed adsorption could be through some other pathway such as ligand exchange.
4.3.4 Effect of dosage mass on the adsorption of metal ions onto *M. oleifera* bark

Different dosage masses were used to investigate the extent to which metal adsorption is affected by varying adsorbent mass. The results are presented in Figure 4.18 for Co(II), Cd(II) and Cr(VI) for adsorbent mass ranges of 0.5, 1, 2 and 5 g.
FIGURE 4.18: Effect of varying adsorbent mass on the adsorption of metal ions from aqueous solutions at pH 4, for 4 h, at 5 mg/L adsorbate concentration and at 25 °C

The graph (Figure 4.18) shows that increasing the amount of adsorbent mass results in an increase in the amount of metal ions adsorbed onto the adsorbent. This increase eventually culminates into a plateau at 1.00 g adsorbent mass, which corresponds to the attainment of equilibrium. This observation holds true for all the elements under investigation, suggesting that increasing adsorbent mass increases available adsorption sites until equilibrium, beyond which the adsorption performance is not affected by any adsorbent additions - a phenomenon that has been previously observed in similar studies (Argun et al., 2007).
4.3.5 Effect of solution temperature on the adsorption of metal ions concentration onto *M. oleifera* bark

Increasing solution temperature improves the kinetic and thermodynamic capabilities of the reacting species in solution. Metal ion adsorption onto *M. oleifera* bark was investigated by varying the solution temperature from 20, 25, 35, 45 to 60 °C, and the results are presented in Figure 4.19.

![FIGURE 4.19: Effect of adsorption of Cd(II), Cr(VI), Co(II) and V(V) onto M. oleifera bark at different solution temperatures](image)

In Figure 4.19, it is observed that each element achieves maximum percentage adsorption at the temperature range between 20 and 60 °C, suggesting the ease with which adsorption is facilitated within this range. This is to be expected, as a temperature
increase significantly increases the rate of bombardment of the reacting species in solution (Aksu, 2001) as it impacts on the kinetic and thermodynamic parameters.

However, as the solution temperature is increased beyond 25 °C for V(V), there is a significant decrease in the amount adsorbed. This is largely attributed to the solution chemistry of vanadium and the effect of pH upon heating. This experiment was carried out at pH 4.6, and heating the solution increased the ionization potential of the aqueous solution so that more $H^+$ ions were generated from the dissociation of water molecules. These $H^+$ ions have a competitive advantage over the V(V), especially since at acidic conditions the vanadium predominantly exists in its cationic forms (Figure 4.9). This explains why the adsorption decreases as more $H^+$ ions are generated and outcompete the vanadium ions for the active sites.
4.4 SURFACE MODIFICATION OF ADSORBENT USING POLYAMINES

4.4.1 Immobilized amine content and polyamine density

The amount of nitrogen-based groups immobilized onto the surface of the adsorbent was investigated and the results are presented herein, reported as percentage nitrogen (Figure 4.20). The results show that the amine content (presented as percentage nitrogen) was improved after the addition of different polyamines of varying chain lengths at diverse degrees of functionality but under the same experimental conditions. The degree of amination of the adsorbent is in the order: TETA>TEPA>EDA>DETA. The results indicate that the use of polyamines with a high amine content can increase the overall amine (as nitrogen) content immobilized on the adsorbent. Table 4.3 reports the amine content (reported as percentage nitrogen) for each group and the ratios thereof.
Polyamine density is defined as the number of polyamine molecules immobilized on the adsorbent per unit area (Liu & Bai, 2010). Results of polyamine density immobilized onto the adsorbent are presented in Table 4.3. It was observed that increasing the molecular chain of the polyamines results in corresponding increases in the polyamine density on the adsorbent in the order; TEPA>TETA>DETA>EDA (Table 4.3). Theoretically, this is to be expected as the chemical compositions of the polyamines depict the number of amine groups as 2, 3, 4 and 5 for EDA, DETA, TETA and TEPA, respectively. The question, therefore, is the ease of reactivity of the polyamines with the adsorbent and the accessibility of the active sites of the adsorbent for bond formation with the polyamines.

FIGURE 4.20: Degree of amination of the *M. oleifera* bark with different aliphatic polyamines, compared with the unmodified *M. oleifera* bark (UMOB)
Table 4.3: N$_2$-BET and elemental analysis of polyamine functionalized *M. oleifera* adsorbent.

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>EDA</th>
<th>DETA</th>
<th>TETA</th>
<th>TEPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amine content (% wt)$^a$</td>
<td>1.23</td>
<td>1.25</td>
<td>1.35</td>
<td>1.32</td>
</tr>
<tr>
<td>Ratio of amine content $^b$</td>
<td>1</td>
<td>1</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Surface area (m$^2$/g)$^c$</td>
<td>7.23</td>
<td>7.07</td>
<td>5.56</td>
<td>1.11</td>
</tr>
<tr>
<td>Surface area increase (%)$^d$</td>
<td>304</td>
<td>295</td>
<td>211</td>
<td>-38</td>
</tr>
<tr>
<td>Polyamine density (mmol/g)$^e$</td>
<td>0.17</td>
<td>0.18</td>
<td>0.24</td>
<td>1.19</td>
</tr>
<tr>
<td>Correlation coefficients$^f$</td>
<td>0.80</td>
<td>0.91</td>
<td>0.88</td>
<td>0.78</td>
</tr>
</tbody>
</table>

$^a$ determined from CHN analysis, $^b$ EDA/polyamine, $^c$ BET surface area, $^d$ determined from surface area for unmodified bark, $^e$ amine content/surface area, $^f$ calculated from FT-IR data.

4.4.2 Surface characterization analysis

FIB-SEM images showing the surface morphology of the *M. oleifera* bark at different degrees of amination are shown in Figure 4.21. The images show the distribution of the polyamines on the surface of the adsorbent. It is noticed that the polyamine density seems to be highest for the DETA and TEPA-functionalized adsorbents (Figure 4.21, A and C) than for EDA and TETA-functionalized adsorbents (Figure 4.21, B and D).
Figure 4.21: FIB-SEM images showing the surface morphology of the *M. oleifera* bark at different stages of functionalization; (A) unmodified, (B) EDA-functionalized, (C) DETA-functionalized, (D) TETA-functionalized, (E) TEPA-functionalized and (F) a closer morphology of the active sites and how the adsorbate adsorbs itself on the surface of the adsorbent.
Surface functionalization of the *M. oleifera* bark with low chain polyamines results in a corresponding increase in N₂-BET surface area. This trend, however, does not hold for the long chain polyamines as the surface decreases significantly. This increase in surface area also improves the reactivity of the bark to the metal ions.

4.4.3 Spectral analysis

Spectral comparisons between the unmodified and amine-modified *M. oleifera* bark samples obtained using a FTIR are presented in Figure 4.22. The figure shows that as the degree of amination is improved in the order UMOB < DETA < EDA < TETA, an absorption peak emerges at 3470 cm⁻¹ and increases in intensity in that specific order. This peak emergence shows the introduction of acidic phenolic and alcoholic functional groups, which are probably due to the effect of the 1,4-dioxane (Peacock & Sherman, 2004), which was added to activate the adsorption sites before the modification process. This means that the washing step was not effective in removing the activation agent; hence it became part of the intermediate step prior to the desired MOB-amine complex reaction.
FIGURE 4.22: FTIR spectra of *M. oleifera* bark (UMOB) before and after chemical modification with polyamines (EDA, DETA, TETA and TEPA)

Of note is that this region (~3500 cm\(^{-1}\)) represents the stretching frequencies for N-H amides, representing primary and secondary amines. Therefore, addition of amines of increasing chain length and, hence amine density, also improves the surface functionality of the adsorbent. In contrast with the UMOB, there is a steady increase and magnification of a peak at 1635 cm\(^{-1}\), which is also observed to increase in the order; DETA < EDA and TETA. This increase is possibly due to the introduction of N-H primary amines to the surface of the adsorbent, which then reacts with the cellulose to form
cellulosic amine-cross-linked biopolymers, also observed by other researchers (Li et al., 2008).

There are no observed differences in peak formation or magnification in TEPA functionalized adsorbent, both at 3500 cm\(^{-1}\) and 1635 cm\(^{-1}\), even though TEPA contains the longest amine chain (high amine density). Correlation coefficients calculated for the adsorbent-amine reaction were low for the TEPA functionalized adsorbent (Table 4.3), implying lower reactivity between TEPA and the adsorbent.

4.4.4 Adsorption performance of amine-modified bark with vanadium

The adsorption performance of the amine-modified *M. oleifera* bark was investigated using V(V) as an adsorbate. After VO\(_2^+\) adsorption, the amine content was determined to be in the same order (TETA > EDA > DETA) as with the amination step observed earlier (Figure 4.20) for the low chain polyamine functionalized adsorbent. A direct proportionality was observed in the amine content before and after VO\(_2^+\) adsorption with the adsorption performance, and following the same order (Figure 4.23). However, there is no significant change in amine content for the TEPA-functionalized adsorbent after VO\(_2^+\) adsorption. This suggests that the N-based functional groups did not participate in the formation of the V-adsorbent complex. It would seem that there was also poor adsorption/reaction between the adsorbent and TEPA (Figure 4.20 and Table 4.3).
Additionally, the calculated amination correlation coefficients (Table 4.3) were found to be low for the *M. oleifera*-TEPA mixture than with the other combinations.

![Percentage nitrogen levels in the modified adsorbent before and after vanadium adsorption](image)

**FIGURE 4.23**: Percentage nitrogen levels in the modified adsorbent before and after vanadium adsorption

The low amine content for the TEPA-functionalized adsorbent can be further explained by considering the size of the longer chain polyamines relative to the others, which lead to steric effects, wherein the size of the molecule prevents more long chain polyamines from accessing and reacting with the functional groups on the surface of the adsorbent (Minelli *et al.*, 2000). The long chain polyamines also have a tendency of reacting with more terminal functional groups on the adsorbent surface, due to its many functional groups contained, thus consuming more of the active available sites. This is evidenced
by low surface areas where chemical modification was performed using the longer chain polyamines, especially TEPA (Table 4.3).

Figure 4.24 shows that the adsorption of vanadium by the modified adsorbents was enhanced in the order: DETA > EDA > TETA > TEPA, representing percentage increases of 155, 146, 67 and 42, respectively. Evidently, TEPA functionalized adsorbent recorded the lowest adsorption potential probably due to the poor amination as reported earlier (Figures 4.21, 4.22 and 4.23).

FIGURE 4.24: Effect of amination on the adsorption performance of M. oleifera bark adsorbent at different degrees of modification
4.5 SYNTHESIS, CHARACTERIZATION AND APPLICATION OF GREEN SYNTHESIZED GOLD NANOPARTICLES

The synthesis and formation of gold nanoparticles in the presence of a reducing agent can be visually monitored by distinct colour changes from pale yellow, characteristic of the gold chloride salt, to an intense wine red colloidal suspension, distinctive of the formation of gold nanoparticles. The colour changes are characteristic of the surface plasmon resonance (SPR) of different sizes of gold nanoparticles in solution. The formation of these particles occurs at specific wavelengths in the UV-vis region, and would occasionally manifest some shifts which are dependent on the initial synthesis conditions; thereby resulting in nanoparticles of different sizes and shapes. Gold nanoparticles are the aggregates of Au atoms at nanoscale, which are red in colour because of the absorption by their surface plasmon oscillation that peaks at 520 nm (Sujitha & Kannan, 2013).

4.5.1 Reduction and stabilizing potential of *M. oleifera* biomolecules

Conventional synthetic protocols for gold nanoparticles make use of chemical reagents, which facilitate the reduction of Au$^{3+}$ to Au$^{0}$ and to stabilize the resulting double layer of gold nanoparticles (Gan *et. al.*, 2012). In the results presented herewith, the broth of *M. oleifera* bark was investigated as a potential alternative to these chemicals with respect to their specific roles during the synthesis.
Colorimetric and spectral observations reveal that the broth of *M. oleifera* bark was able to reduce Au$^{3+}$ to Au$^{0}$, resulting in the production of gold nanoparticles characterized by the wine red colouration at the theoretical wavelength of gold nanoparticles synthesis in the 530 - 550 nm range reported by Muangnapoh *et al.* (2010). Intermediate colour changes (from yellow to wine-red) were observed, which appeared/disappeared at different rates as a function of the initial synthesis conditions. This, together with other characterization techniques, proved the reduction potential of the broth in synthesizing gold nanoparticles. Surface plasmon resonance (SPR) bands for gold nanoparticles synthesized under different percentage amounts of the broth are presented in Figure 4.25.

![SPR bands for different gold nanoparticles synthesized under varying amounts of *M. oleifera* broth](image)

**FIGURE 4.25**: SPR bands for different gold nanoparticles synthesized under varying amounts of *M. oleifera* broth
Figure 4.25 shows that an increase in the amount of added broth results in an increase in the intensity of the absorbing species. Even very small amounts of the broth (2 mL broth in a total solution volume of 70 mL) produce significant SPR bands in the UV-vis spectra with perfect alignment between the experimental and theoretical wavelength of gold nanoparticle formation. This suggests that the threshold ratio of precursor salt to amount of broth is largely dependent on the amount of precursor salt than they are on the amount of broth. On the contrary, the lowest concentration of precursor (0.125 mM) salt did not show evidence of gold nanoparticle formation, since the Au\(^0\) was below the stoichiometric equivalence for the reduction to be effected.

Spectral intensity of the colloidal suspension increases as the amount of broth is increased during the synthesis. The intensity increase is directly proportional to the amount of gold nanoparticles produced at each experimental condition. These observations are attributed to the notion that as the amount of broth is increased, the amount of gold-reducing bioorganic molecules are also increased, availed and they facilitate the bioreduction.

The chemical composition of the broth is variable, but generally contains polar water soluble extractable biomolecules, such as proteins and carbohydrates. These molecules are believed to play a significant role in the bioreduction and subsequent stabilization/capping of the nanoparticles. Preliminary characterization results on the elemental composition of *M. oleifera* bark show that it contains considerable quantities of nitrogen and sulphur (Table 4.2), a basis to suggest the presence of nitrogen and
sulphur based water soluble molecules that are likely to participate in the bioreduction and functionalization of the surface of the gold nanoparticle surface to generate a negatively charged electric double layer. It has been suggested (Boufi et al., 2011) that the amino group in the protein plays a dual role: as a reductant and as a capping agent implying surface functionalization was effected through the terminal thiol moiety to form the Au-S bond in agreement with results obtained by co-authors (Ding et al., 2012).

Chemical reactivity of gold nanoparticles is dependent on the type and degree of chemical functionalization. Different capping agents have been used (Ding et al., 2012; Boufi et al., 2011) with different degrees of success, depending on the intended use of the particles. In this study, no additional surface functional ligands were added and the strength of the water soluble biomolecules was investigated as potential stabilizing agents during the synthesis. It is believed that the amino group (\(-\text{NH}_2\)) in the broth solution formed weak covalent bonds at the surface of the nanoparticle through the sulphur atom; thereby functionalizing its surface and improving its chemical reactivity and stability. Cysteine \((\text{C}_3\text{H}_7\text{NO}_2\text{S})\) and methionine \((\text{C}_5\text{H}_{11}\text{NO}_2\text{S})\) (common compounds in the bark) stabilize by exposing their terminal functional groups such as carboxylate \((-\text{COO}^-\)) and amines \((\text{R-NH}_2\)) thereby activating them. These populate the surface of the nanoparticle thereby providing a protective layer of alternate functional groups. This serves an important phenomenon, in that this alternate arrangement masks the amines and prevents complete collapse of the nanoparticles through aggregation. This eliminates the need to apply capping agents to prevent spontaneous aggregation of the nanoparticles, as is common practice when using other synthesis techniques. However,
some uncontrolled aggregation is observed due to the complex nature of the biomolecules found in the broth, wherein they cause undesirable side reactions due to their reactivity with other chemical species in the surrounding medium.

Zeta potential measurements give value to the surface charge of the nanoparticle, thereby indicating colloidal stability, and the ability of the nanoparticle to react with other molecules. In the conventional gold nanoparticle synthesis using the citrate reduction method, this surface charge is provided for by the citrate ions \((\text{HO[CH}_2\text{COO}^\text{-}]_2\text{COO}^-)\) which populate the particle surface after reducing the gold. However, in this synthetic route, the negative charge is provided for by the water soluble functional groups which characterize the identity of the biomolecules involved in the bioreduction and the subsequent stabilization. FTIR data has identified these functional groups to include carboxylates (-COO\text{-}), alcohols (-OH), hydroxides (-OH), sulphides (R-S-R) and amines (R-NH\text{2}). Therefore, the stability results presented herein are influenced by the degree to which these functional groups participate during the synthesis. Figure 4.26 shows a summary of the relative Zeta potential values for the nanoparticles synthesized at different conditions of pH, synthesis time, precursor concentration and amount of broth. The results show average values around -20 mV, a threshold value for colloidal stability reported elsewhere (Sun et al., 2013), implying relative stability of the nanoparticles.
FIGURE 4.26: Zeta potential values for gold nanoparticles synthesized under varying conditions of pH, synthesis time, precursor concentration and broth amounts

4.5.2 Effect of initial HAuCl₄ concentration on the formation of gold nanoparticles

The formation of nanoparticles can only happen when the precursor concentration is within a suitable range for nucleation to occur (Gan et. al., 2012), and this range differs between different systems, hence the need to investigate specific cases. The formation of these nanoparticles was monitored using a UV-vis spectrometer, due to the intense colour changes shown by these particles owing to the collective oscillation of free conduction electrons induced by an interacting electromagnetic field (Smitha et al., 2009). UV-vis spectra of gold nanoparticles synthesized under different precursor concentrations are presented in Figure 4.27.
FIGURE 4.27: UV-vis-NIR absorption spectra of gold nanoparticles synthesized at different initial concentrations of HAuCl₄ (2.0, 1.0, 0.5, 0.25 and 0.125 mM)

The spectra in Figure 4.27 show that as the concentration of the precursor salt is increased, there is a direct increase in the intensity of the absorption. For the concentrations: 1, 0.5 and 0.25, a single surface plasmon band is shown, characteristic of the presence of small spherical nanoparticles in agreement with work done by Smitha et al. (2009). However, with the lowest experimental concentration (0.125 mM) the absorption spectra show no absorption, suggesting no formation of gold nanoparticles,
a phenomenon earlier reported by Gant and co-workers (2012). At 0.25 mM precursor concentration, there is evidence of the formation of gold nanoparticles, contrary to earlier findings by the same authors (Gant et al., 2012), suggesting better reducing potential of the broth at these experimental conditions. At 0.5 mM precursor concentration, there is a $\lambda_{\text{max}}$ shift to a higher wavelength, away from the theoretical wavelength characteristic of the formation of gold nanoparticles. This observation, though unique in this study, has been observed previously (Phillip, D., 2010; Smitha et al., 2009) and is associated with a move towards anisotropy and possible self-aggregation of the nanoparticles.

The sizes of the nanoparticles varied greatly with the amount of precursor added. Transmission electron microscopy (TEM) micrographs (Figure 4.28) show that as the precursor concentration is increased, there is a corresponding increase in the number of particles of different sizes, maintaining their dispersed forms in solution.

FIGURE 4.28: TEM micrographs showing gold nanoparticles synthesized from 0.5 mM (A), 1 mM (B) and 2 mM (C), of HAuCl$_4$
Zeta potential measurements reveal that as the concentration of the precursor salt is increased, the surface of the nanoparticles becomes more negatively charged (Figure 4.29). This translates to a more reactive material as a negative surface charge favours adsorption mechanisms such as electrostatic attractions and ion-exchange.

![Graph showing Zeta potential values](image)

**FIGURE 4.29:** Gold nanoparticle Zeta potential values obtained with an increase in precursor concentration

However, this chemical reactivity may have undesirable effects on the chemical stability of the gold nanoparticles in solution. They tend to react easily with surrounding chemical species, especially since the *M. oleifera* broth is a complex heterogeneous chemical mixture, and also with adjacent nanoparticles. This chemical reactivity results in a collapse of the double layer and the nanoparticles aggregate, especially at high concentrations of the initial precursor salt. These aggregates eventually precipitate out.
of solution, as observed by Safavi & Zeinali (2010), so that the results presented in Figure 4.30, are representative of the non-aggregated gold nanoparticles. Figure 4.30 shows a comparative analysis of the Zeta potential values of the same sample, separated by a 12 month period from date of synthesis.

**FIGURE 4.30:** Zeta potential values on the effect of time on the chemical stability of the gold nanoparticles over a 12 month period

The figure shows that the surface charge is diminished with time over the 12 month period in all precursor concentration ranges, except when the concentration is lowest (0.125 mM). This observation impacts negatively on the possible applications of these nanoparticles as their stability cannot be sustained.
4.5.3 Effect of solution pH on the formation of gold nanoparticles

A change in solution pH during the synthesis of gold nanoparticles affects the reducing potential of the system in facilitating the reduction of $\text{Au}^{3+}$ to $\text{Au}^{0}$. This phenomenon was investigated by altering the pH of the synthesis environment between pH 2 and 10, results of which are presented in Figure 4.31.

**FIGURE 4.31**: UV-vis-NIR SPR absorption spectra of gold nanoparticles synthesized at different pH conditions

In Figure 4.31, the SPR bands are generally centred at 520 nm, which is the theoretical wavelength at which gold nanoparticles are formed (Gupta et al., 2011), and is largely influenced by the solution pH (Hamaguchi et al., 2010). As the pH is decreased from pH 10 to pH 6, there is a corresponding increase in the intensity of the SPR bands,
suggesting more particles are synthesized as the reaction media is acidified. This relationship is inverse to the nanoparticle sizes formed at these pH ranges, as smaller sizes are particularly favoured at basic conditions (Hamaguchi et al., 2010). A visual inspection during the synthesis showed that as the pH became more basic (from pH 6) it took more time for the appearance of the wine-red colour. This implies that the synthesis is more spontaneously favoured in acidic media, as the absorption intensities are also higher therein. However, as the solution medium becomes strongly acidic (pH 2), the SPR band shows the emergence of a shoulder at higher wavelength. At this point, the synthesized nanoparticles are beginning to aggregate, a phenomenon that has been observed previously (Salcedo & Sevilla, 2013; Basu et al., 2007). This could be attributed to the neutralization of the surface charge of the nanoparticle by the constant acidification of the reaction medium, which then increases the electrostatic attractions between adjacent particles, thereby reducing the hydrodynamic distance between them. This also explains why $\lambda_{\text{max}}$ shifts to higher wavelength at pH 4 away from the theoretical 530 nm. Interestingly, though, the colour does not change except to appear intense as the solution pH is decreased.

The rapid random protonation of the surface of the nanoparticles is translated into a reduction in the Zeta potential values of the material as the pH value is decreased (Figure 4.32). This reduction then explains the emergence of the SPR shoulder at pH 2, due to a progressive selective reduction in the chemical stability of the materials causing adjacent particles to be attracted to each other, hence aggregation.
4.5.4 Synthesis of gold nanoparticles at different synthesis times

The effect of synthesis time on the production of gold nanoparticles was investigated by varying synthesis time between 0 and 30 minutes and the outcomes monitored using a UV-visible spectrophotometer, as well as colorimetrically. Progressive colour changes were observed from the onset of each of the experiments from a cloudy yellow suspension (from the combination of the broth and the crystal yellow HAuCl$_4$.3H$_2$O) to a thick wine red colouration (Figure 4.33, insert), indicative of the presence of gold nanoparticles. UV-vis spectra of the nanoparticles are presented in Figure 4.33 and reveal that gold nanoparticle production is a spontaneous and time dependent process.
Figure 4.33 shows that within 5 minutes of the onset of the reaction, there is evidence of the presence of gold nanoparticle in solution. In all the experiments conducted, the absorption intensity shows a direct proportionality with synthesis time. A plot of average absorbance at the $\lambda_{\text{max}}$ versus time (Figure 4.34) shows a steady increase in intensity with time. This increase plateaus off after 15 minutes of synthesis suggesting that at this point, there is no net change in the SPR properties of the UV-spectra, whereby the spectral properties of the nanoparticles are similar.
FIGURE 4.34: Temporal spectral patterns of gold nanoparticles from average $\lambda_{\text{max}}$ values

4.5.5 N$_2$-BET surface area and pore characteristics of gold nanoparticles

N$_2$-gas adsorption-desorption isotherms are widely used to determine the surface area and pore size distribution for various solids. The Brunauer-Emmett-Teller (BET) method was applied in this study, whose theory is an extension of the Langmuir theory with its major assumptions. Nitrogen gas was used in this investigation to determine the surface area and porosity of the green synthesized gold nanoparticles from *M. oleifera* bark. The resulting adsorption-desorption isotherm for the gold nanoparticles is shown in Figure 4.35.
FIGURE 4.35: N₂ adsorption-desorption curve at 77 K for green synthesized gold nanoparticles using *M. oleifera* broth extract and 2.0 mM HAuCl₄

Figure 4.35 shows that the adsorbent is largely mesoporous which is indicative of multilayer adsorption. The desorption curve is characteristic of an H3 hysteresis loop, indicating weak adsorbate-adsorbent interaction. This is a good property since the adsorbate can be removed without the use of strong desorption chemicals. As such, the regenerative capabilities can be explored, wherein the adsorbent can be used repeatedly without losing its adsorptive properties. The adsorption branch follows type VI, since there is no evidence of micropores; hence no monolayer adsorption is favoured. Capillary condensation of the adsorbing species is evident at 0.9 < P/P₀ > 1, indicative of macroporous adsorption, even though it is only slightly.

The surface area was found to be 6.23 m²/g, the total pore volume being 0.00817 cm³/g and with the surface area corresponding to the synthesized nanoparticles to be 5.25
m²/g and is much smaller than that of commercial activated carbon (790 m²/g) reported in literature (Lee et al., 2001), hence, this research was focused on chemical modification to improve chemical functionality.

4.5.6 Colorimetric assay of lead ions based on the aggregation behaviour of gold nanoparticles

The aggregation behaviour of the synthesized gold nanoparticles was investigated with a view to explore possibilities for the determination of lead ions in solution, colorimetrically as well as spectrophotometrically through changes in their absorption spectra. This, according to Smitha and co-workers (2009), is premised on the understanding that when the nanoparticle solution environment is altered (lowering the pH, introduction of a chelating ligand or altering the salt concentration), gold nanoparticles tend to aggregate by reducing the average hydrodynamic radius between adjacent particles. This change is facilitated, in part, through interaction between the surface functional groups, the capping agents and any added external substrate, such as a metal in this case. In this study, no commercial reducing agents, capping or stabilizing agents were added. Instead, water-extractable biomolecules were employed, and the quality of the resulting nanoparticles is investigated herein in determining the presence and concentration of lead ions in aqueous solutions.
Spectral properties of the colloidal gold nanoparticles at different precursor concentration, amount of broth and pH were investigated after the addition of different concentrations of lead ions. SPR bands for the effect of precursor concentration on the aggregation of gold nanoparticles are presented in Figure 4.36.

FIGURE 4.36: UV-vis spectra of gold nanoparticles after addition of varying concentrations of Pb ions compared with the spectrum of the dispersed (insert), and the corresponding stepwise colour changes (insert)

The addition of lead ions results in a significant decrease in the absorption intensity of the incident electromagnetic radiation (EMR) passing through the solution, as compared
to when the nanoparticles are dispersed in solution (Figure 4.36). This means that the amount of EMR passing through the colloidal suspension was expressively increased, signifying a dispersion medium that is less concentrated in EMR absorbing colloidal matter. This culminates from the colloidal particles settling out of solution after aggregation, which is facilitated by a collapse of the electrostatic component of the double layer through the addition of lead ions, which, themselves act to reduce the repulsive behaviour of the nanoparticle surfaces.

The SPR band characteristic of gold nanoparticles was centred at 530 nm, and is the responsible band for the observed wine-red colour at this wavelength ranges in agreement with results obtained in a similar experiment (Patungwasa & Hodak, 2008). However, as metal ions are introduced into the system, the SPR peak had an insignificant shift to higher wavelengths (from 530 nm to 540 nm). Correspondingly, the resultant colour change from wine-red was also very slight, giving a faint blue colour for all the concentration ranges investigated. A closer investigation into Figure 4.36 shows a very weak shoulder at 750 nm, characteristic of a weakly aggregated colloidal suspension. This may also explain the slight colour shift; characteristic of neither the dispersed nor aggregated colloidal gold nanoparticles.

The position and magnitude of an absorption band in Figure 4.36 is dependent on the size of the particles which influences the degree of aggregation of the nanoparticles as equally observed by Mulvaney (1996) earlier. TEM micrographs (Figure 4.37) show that the gold nanoparticles, in the absence of the metal, were dispersed in solution (non-
aggregated) and the average hydrodynamic radius was small; hence the absorption band was elaborate and broad, in agreement with previous work (Link & El-Sayed, 1999). The reduced hydrodynamic radius results from the presence of a large number of very small nanoparticles (Figure 4.39), which aggregate easily when a charge altering external stimulus is introduced. As different concentrations of lead were added (Figure 4.37, a-d), there was a corresponding reduction in the inter-particle distance forming clusters or aggregates of metal-nanoparticle complexes.

FIGURE 4.37: TEM micrographs of gold nanoparticles after the addition of different concentrations of lead ions: a) 10 mM, b) 25 mM, c) 50 mM, d) 100 mM

The extent of the aggregation, and by extension the colorimetric determination, is affected by the sizes and shapes of the nanoparticles in solution. Size and shape of particles determine the optical properties of the bulk solution. In this study, variable shapes and sizes of particles were obtained; (Figure 4.38 and 4.39, respectively) which are believed to have played a significant role in determining the extent of aggregation.
FIGURE 4.38: TEM micrographs of colloidal gold nanoparticles showing different shapes: (a) spherical, (b) triangular and, (c) nanorods

FIGURE 4.39: TEM micrographs of colloidal gold nanoparticles showing different size ranges (a & b)

4.5.7 Spectrophotometric determination of lead ions in aqueous solutions

The amount of lead in aqueous solutions can be determined using spectrophotometric techniques by making use of calibration curves. Therefore, the nanoparticles synthesized herein were used to develop a spectrophotometric method to determine
lead concentrations in synthetic aqueous solutions. This investigation makes use of the water soluble bioorganic molecules extracted from the broth as chelating agents to induce nanoparticle aggregation, which culminates in spectral changes resulting in a visual colorimetric determination.

To evaluate the performance of the determination, analytical figures of merit were employed. A UV-vis spectrometer calibration curve is presented in Figure 4.40, and shows a good linearity ($r^2 = 0.9878$) over the calibration range (5.0 – 100 mM) for n= 5. Generally, percentage recoveries for the method were high and are presented in Table 4.4, except for the lowest calibration concentration.

FIGURE 4.40: Linear relationship between metal concentration and the instrument response
TABLE 4.4: Percentage recoveries obtained for the spectrometric determination of Pb using gold nanoparticles.

<table>
<thead>
<tr>
<th>Concentration (mM)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
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</tr>
<tr>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>100</td>
<td>132</td>
</tr>
</tbody>
</table>

TABLE 4.5: Assay validation sheet for the spectrophotometric determination of Pb using green synthesized gold nanoparticles.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
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<td>Accuracy</td>
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<td>Linear range</td>
<td>5 – 100 mM</td>
</tr>
<tr>
<td>LOD</td>
<td>9.9 mM</td>
</tr>
<tr>
<td>LOQ</td>
<td>30</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>0.418 au/mM</td>
</tr>
</tbody>
</table>
CHAPTER 5

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Evaluation of research objectives

The potential use of the bark of *M. oleifera* for the removal of metal ions from aqueous solutions was demonstrated in this work using simulated environmental samples for different metals at different environmental conditions.

Surface characterization techniques revealed the surface of the bark with a comparatively small surface area \((S_{\text{BET}} = 1.79 \ \text{g/m}^2)\), which is, however, compensated for by the high degree of polyfunctional groups contained therein, as depicted by FTIR spectra. The adsorption surfaces were found to be neither strictly homogenous nor heterogeneous, but the adsorption was better explained using the Freundlich isotherm model \((r^2 = 0.999)\) than the Langmuir model \((r^2 = 0.997)\). Adsorption at these surfaces was found to be in the micro- and macroporous ranges, with the presence of slit-like pore geometry. The adsorption capacity was found to be 48.8 mg/g with metal removal percentages of over 95% obtained using V(V) as an adsorbate at optimized adsorption conditions (pH 4.6, 25 °C for 4 h contact time using 150 mg adsorbent mass). Chemical modification of the bark was successfully done using aliphatic polyamines. Characterization of the amine-modified bark showed improved percentages of amination of up to 25%; confirmed using FTIR
spectra and CHNS analysis. A significant (p>0.05) improvement in the adsorption potential of the bark was observed after amination. Gold nanoparticles were successfully synthesized, characterized and applied to remove up to 100 mM lead (Pb) from synthetic aqueous solutions. Polar water soluble biomolecules were extracted from the bark and successfully used to reduce Au$^{3+}$ to Au$^{0}$ leading to the formation of gold nanoparticles.

5.2 Characterization of *M. oleifera* bark

Surface and spectral properties of the bark sample at different degrees of chemical treatments were successfully determined using different techniques (FTIR, HRSEM, N$_2$-BET physisorption). Conclusively, the techniques revealed that the bark of *M. oleifera* has a low surface area, a major limitation in the development of adsorption technologies. However, the pore surfaces are endowed with a lot of functional groups, some of which have metal anchoring properties. The adsorption capacity is therefore improved, in spite of the low surface area.

5.3 Adsorption potential of *M. oleifera* bark

*M. oleifera* bark can adsorb metals (Co(II), Cd(II), V(V) and Cr (VI)) from aqueous solutions at optimized conditions in a one-component system. An experimental adsorption capacity of 48.8 mg/g was obtained and is superior to other plant-based adsorbents. The pH of the adsorbing medium greatly affects metal adsorption and
acidic conditions are preferred. *M. oleifera* bark is sensitive to temperatures above 60 °C, wherein adsorption decreases significantly.

### 5.4 Surface modification of *M. oleifera* bark

The surface properties of *M. oleifera* bark can be improved through the immobilization of aliphatic polyamines on its surface. This functionalization also improves the chemical reactivity of the bark as metal recoveries are improved. The adsorption of vanadium, V(V), using the amine-modified bark was increased by 155, 146, 67 and 42%, respectively for diethylenetriamine, HN(CH₂CH₂NH₂)₂, ethylenediamine, HN(CH₂CH₂NH₂)₂, triethylenetetramine, (CH₂NHCH₂CH₂NH₂)₂, and tetraethylenepentamine HN(CH₂CH₂NHCH₂CH₂NH₂). The larger polyamine molecules are hindered from effective interaction with the adsorbent due to steric effects.

### 5.5 Synthesis of novel products from *M. oleifera* bark

Polar water soluble biomolecules extracted from the broth of *M. oleifera* bark can facilitate the reduction of Au³⁺ to Au⁰ resulting in the formation of gold nanoparticles. These nanoparticles have limited stability in the colloidal solution due to uncontrollable size and shape properties during synthesis. This further limits their application in removing metal ions in that they self-aggregate without even the
introduction of an external stimulus, such as a metal. The synthesized nanoparticles can successfully remove Pb in a single-component metal system.

5.6 Contribution of the study

As reported in the literature section, M. oleifera is widely used for a wide range of applications, even though the scientific basis for the usage is not well documented. This study has been able to illustrate:

- The surface and adsorptive properties of M. oleifera bark,
- The possibility for chemical functionalization of the bark to improve on chemical functionality,
- That the broth of the bark has strong reducing potential, as well as a moderate potential for chemical stabilization of gold nanoparticles.

5.7 Limitations of the current study

Adsorption of an adsorbate by an adsorbent is largely governed by various mechanistic pathways at the adsorbate-adsorbent interface or in the pore spaces of the adsorbent, and by the thermodynamics of the adsorbed molecules. This study was able to characterize pore properties of M. oleifera bark, but the following short-comings were observed as major limitations:
5.7.1 Lack of sufficient literature on the chemical profile of the bark, as well as information on the surface chemistry of the bark,

5.7.2 Lack of geometrical investigations into the coordination chemistry of the metal in the adsorbent pores, as well as the mechanism of adsorption,

5.7.3 Lack of comprehensive experimental data on the thermodynamics of the surface coverage of the *M. oleifera* bark.

5.8 Recommendations

It is recommended that:

5.8.1 An investigation into the chemical profile of the bark be considered,

5.8.2 An investigation into the thermodynamics of the adsorption be considered,

5.8.3 The study be extended to investigate spatial variations of *M. oleifera*,

5.8.4 The material be investigated in a simulated multi-element system

5.9 Future investigations

5.9.1 Further characterization of the bark to obtain more information on the preferred adsorption mechanism,

5.9.2 Further development of the green synthetic route developed in this study to control for sizes and shapes of nanoparticles,

5.9.3 Applications of *M. oleifera* bark samples at different treatments for various selected environmental samples, e.g. wastewaters.
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