SYNTHESIS OF MAGNETIC SORBENTS MODIFIED WITH *MORINGA OLEIFERA* EXTRACTS FOR THE REMOVAL OF ENVIRONMENTAL ORGANIC POLLUTANTS

MAUREEN MALETSATSI KGATITSOE

A Masters dissertation submitted to the Faculty of Science, University of the Witwatersrand in fulfillment of the requirements of the degree of Master of Science

University of the Witwatersrand, Johannesburg, January 2018

DECLARATION

I declare that this research is my own, unaided work. It is being submitted for the Degree of Master of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

……………………………………..
(Signature of Candidate)

30th day of January 2018

ABSTRACT

Modern developments on nanoparticles (NPs) has led to new innovation in nanoparticles that can be used for the treatment of polluted water. Magnetic NPs are of great interest because of their unique properties and promising applications. Their stability is essential to prevent them from agglomeration, hence, modification with a specific coating layer can assist in the selectivity function and stability of the magnetic NPs.

In this work, a magnetic organic nano-adsorbent was synthesized and investigated for the application in the extraction of nitroaromatic explosive compounds from aqueous solutions. The approach was to find a possible synergy between the adsorptive properties of *Moringa oleifera* and the high surface area of the NPs. The magnetic NPs were utilized for their ferromagnetic properties which assist in the extraction procedure. *M. oleifera* was used as a natural coating agent to functionalize the magnetic NPs and also to provide stability. There are numerous chemical procedures to synthesize magnetite and one of the simplest and convenient procedures, co-precipitation, was used in this study. Magnetite can be easily oxidized under ambient conditions, hence, in order to prevent oxidation, *M. oleifera* extracts were used as a coating agent. The synthesized uncoated magnetite NPs and *M. oleifera* coated magnetite NPs were characterized by powder X-ray diffraction (PXRD), Fourier transform infrared (FTIR), Brunauer, Emmett and Teller (BET) and thermogravimetric analysis (TGA). The *M. oleifera* leaf extract was obtained using pressurized hot water extraction (PHWE) at 80°C and the seed extract was purchased as a pure oil. Both the *M. oleifera* leaf and seed extracts were characterized by FTIR and gas chromatography–mass spectrometry (GC-MS) for possible organic compounds assisting in the stability of the magnetite. From the BET results of the coated magnetic NPs at different times, it was observed that with increasing coating time, the surface area decreased while the pore size increased. To optimize the extraction of nitroaromatic explosive compounds (NAECs), the parameters that were investigated were sample pH, contact time, mass of adsorbent and initial concentration. The optimum value for pH of the sample was found at pH

8 and for the contact time an optimum of 120 min was found. The mass of *M. oleifera* coated magnetic NPs for the maximum removal of explosives were found to be 100 mg. The adsorption capacity of the NAECs was obtained in the range of 0.45 and 0.76 mg g^{-1} at optimum conditions.

During kinetic studies it was observed that the pseudo second order model explained the extent of the adsorption mechanism. It was observed that the Langmuir model was favourable as compared to the Freundlich model. The order of selectivity for the NAECs studied was observed as follows: 2-NT ≈ 3-NT > 4-NT > NB > 2,6-DNT > 1,3-DNB. The limit of detection (LOD) found was 0.37 – 4.47 and 0.45 – 5.66 ng.mL^{-1} for extraction using magnetic NPs coated with *M. oleifera* leaf and seed extract, respectively. The percentage recovery for the extraction of NAECs at optimum conditions was obtained in the range 14 – 46%.

DEDICATION

In loving memory of my mother

Masello Dorah “Mamaza” Kgatitsoe

1967 – 2005

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ABBREVIATIONS AND ACRONYMS

BET	Brunauer, Emmett and Teller
DMBA-TPA	Dimethyl benzanthracene – tetradecanoylphorbol acetate
Fe_3O_4	Magnetite
γ–Fe_2O_3	Maghemite
FTIR	Fourier transform infrared
GC-MS	Gas chromatography mass spectrometry
GC-TOF/MS	Gas chromatography-time-of-flight mass spectrometry
HPLC-UV	High pressure liquid chromatography – ultra violet
LLE	Liquid-liquid extraction
LOD	Limit of detection
LOQ	Limit of quantification
MAE	Microwave-assisted extraction
MDL	Method detection limits
MLE	*Moringa* leaf extract
MO	*Moringa oleifera*
MSE	*Moringa* seeds extract
NAECs	Nitroaromatic explosive compounds
NPs	Nanoparticles
PAGE	Polyacrylamide gel electrophoresis
PHWE	Pressurized hot water extraction

PLE	Pressurized liquid extraction
R^2	Co-efficient of determination
rpm	Rotations per minute
SE	Soxhlet extraction
SDS	Sodium dodecyl sulfate
SPE	Solid-phase extraction
TGA	Thermo-gravimetric analysis
TNT	2,4,6-trinitrotoluene
WHO	World Health Organization

CHAPTER I: INTRODUCTION

This introductory chapter gives the outline on the explosive compounds in the environment. It also highlights the importance of cost-effective natural adsorbents for effective removal of the explosive compounds from wastewater.

1 Introduction

Water pollution is one of the major global problems that we face (Ramakrishna *et al.*, 2006) and it has caused the physiochemical properties of water to be altered (Won *et al.*, 1976). Evidence in literature suggest that pollution generation goes back to anthropogenic sources due to man's livelihood by exploiting natures resources (Rew, 2007). Excessive release of pollutants in the environment has been caused by industrialization and urbanization (Ahmad *et al.*, 2004). The increase in environmental pollution throughout the years has caused many countries, especially developing countries to experience a problem of drinking water supply, sanitation and wastewater treatment (Ramakrishna *et al.*, 2006). The mining industry is one of the main contaminators and is well known for releasing inorganic pollutants in the environment. However, the possibility of unmonitored release of organic pollutants from the mining industry into the environment has been less studied. One such threat is the release of explosive compounds and their combustion products into the environment. The increase of these compounds in the environment is of great concern because of likely health effects (Pennington and Brannon, 2002).

Explosives are mainly used in industries, military, agriculture and mining. Detonation of explosives is usually done in isolated areas and gradually they travel from the site of detonation by leaching into the soil and eventually contaminate groundwater (Kaur *et al.*, 2007). They can be introduced in the environment from unignited explosive in a rock after detonation, discharge from wet blastholes and spillage during transportation (Forsyth *et al.*, 1995). When in water explosive are semi- soluble, thus they travel through subsurface soil and cause contamination to groundwater (Pennington and Brannon, 2002).

New and innovative separation techniques are the key to the removal of these pollutants (Yamaura *et al.*, 2002). According to Tobiszewski *et al.* (2014), some of these waste products occur in trace amounts, making detecting these compounds a challenge. Normal sedimentation usually fails to eliminate soluble impurities from aqueous solutions. The use of adsorbents has been found to be an effective treatment process in the removal of heavy metals (Hua *et al.*, 2012), inorganic (Kuntakapun *et al.*, 2010) and organic compounds (Mohan *et al.*, 2014) from aqueous samples. The application of adsorbents that promote coagulation of pollutants remains one of the most effective methods. The process of coagulation is one of the effective methods for reducing turbidity from water. Aluminium sulfate ($Al_2(SO_4)_3$) and ferric sulfate ($Fe_2(SO_4)_3$); termed alum and ferric respectively, are currently the main adsorbents used to assist in coagulation (Dubey *et al.*, 2017; Menezes *et al.*, 2017)

In developing countries, the use of these commercial sorbents is a challenge because they are expensive and inaccessible. The search of cheaper and readily available but effective sorbents becomes a priority. In this regard, the use of natural coagulants in wastewater treatment has been the focus in recent studies, as a sustainable alternative to costly chemical coagulants (de Paula *et al.*, 2018; Freitas *et al.*, 2018). The application of organic coagulants derived from natural plant extracts such as *Moringa oleifera* (denoted to as *M. oleifera* in this study), *Strychnos potatorum*, Guar gum, *Jatropha curcas* and *Clidemia angustifolia* has gained interest in the treatment of water (Ghebremichael *et al.*, 2005; Pritchard *et al.*, 2010b). The *Moringa* tree is found mainly in parts of Africa and India, with the same species found also in tropical America, Sri Lanka and Malaysia and can grow up to a height of 10 m (Ram, 1994; Pritchard *et al.*, 2010a).

In this study, the application of *Moringa oleifera* as a potential adsorbent for the removal of nitroaromatic explosive compounds (NAECs) from water samples was investigated. The *M. oleifera* extracts were supported on magnetic nanoparticles (NPs) and used for the extraction of six nitroaromatic explosives. The six explosive compounds investigated were nitrobenzene (NB), 2,6-dinitrotoluene (2,6-DNT), 1,3-dinitrobenzene (1,3-DNB), 3-nitrotoluene (3-NT), 2-nitrotoluene (2-NT) and 4-nitrotoluene (4-NT).

CHAPTER II: LITERATURE REVIEW

The background, origin, uses and benefits of *Moringa* for nutritional, health, medicinal and water purification are reviewed in this chapter. Other topics such as, the extraction of organic compounds using modern methods, magnetite synthesis and method validation are also reviewed in detail.

2 Literature review

2.1 Background on *Moringa*

Moringa is the only genus in the monogeneric (flowering plant) family, the *Moringaceae*. There are 13 species contained in this genus, namely: *Moringa arborea*, *Moringa borziana*, *Moringa concanensis*, *Moringa drouhardii*, *Moringa hildebrandtii*, *Moringa longituba*, *Moringa oleifera*, *Moringa ovalifolia*, *Moringa peregrina*, *Moringa pygmaea*, *Moringa rivae*, *Moringa ruspoliana*, *Moringa stenopetala* (Verdcourt, 1985). These species have a variety of sizes, from small herbs to gigantic trees.

2.1.1 Availability of *Moringa* tree

Moringa plants are found in both tropical and subtropical climates. *Moringa* grows well in loam soils. From all the 13 species, *Moringa oleifera* is the most commonly cultivated (Fahey, 2005). *Moringa oleifera* is a fast-growing tree (also well-known as the drumstick tree, horseradish tree, benzolive tree, ben oil tree, mlonge, marango, mulangay, kelor, nébéday or sajna) that is native to Pakistan, Bangladesh, Afghanistan and to the sub-Himalayan regions of India (Figure 1). It was exploited by the ancient Greeks, Romans and Egyptians and it is now already an essential crop in Ethiopia, India, Sudan, the Philippines and it is being grown in tropical Asia, Latin America, Pacific Island and Africa (Ramachandran *et al.,* 1980). In South Africa, *Moringa* is fast becoming the most consumed herbal plant due to its high nutrient content.

Figure 1: Growing *Moringa oleifera* trees (picture taken from Lebowakgomo, Limpopo province, South Africa)

2.1.2 Uses of *Moringa*

Moringa is an eternal, all edible softwood tree that has long been consumed by humans and is mainly used for herbal medicines and industrial uses. There are many uses for *Moringa* which include home cleaning agent, biogas, blue dye, alley cropping, fertilizer, animal forage, foliar nutrient, green manure, medicine, ornamental paintings, water purification and rope. All these are made from leaves, wood, bark, trunk, seeds, flower nectar or sometimes all the parts of the plant (Fuglie, 1999). According to Fahey (2005), *Moringa* oil is utilized in salads, for fine machine lubrication and in the manufacture of hair care and perfume products. The best known use for *Moringa* is the powdered seeds for drinking water purification and aggregation of contaminants. The seeds are powdered, roasted and steeped for use in curries and tea (Berger *et al.*, 1984; Gassenschmidt *et al.*, 1995). The *Moringa* tree has been promoted as a high source of protein, iron, calcium, vitamin C and carotenoids (Fahey, 2005), making it appropriate for the use in regions where malnutrition is a major concern.

2.1.3 Chemicals in *Moringa*

Phytochemicals are chemical compounds that are produced by plants (Krishnaiah *et al.*, 2007). Several of these chemical compounds are responsible for the plant's health, colour, smell, texture or flavour. However, phytochemicals do not have important nutrients for humans but medicinal properties. Fahey *et al.* (2001) and Bennett *et al.* (2003) examined the phytochemicals of *Moringa* species and from the studies it was found that the *Moringa* family is rich in compounds comprising of common sugars such as rhamnose and it is also fairly rich in a distinctive group of compounds called glucosinolates and isothiocyanates. Other main substituents found include, proteins, amino acids, chlorophylls (Ghebremichael *et al.*, 2005; Krishnaiah *et al.*, 2007), etc. Compounds shown in Figure 2 have been reported to having antibacterial, anticancer and hypotensive activity. The *Moringa* family furthermore is rich in a number of minerals and vitamin and other phytochemicals such as carotenoids and flavanols (Fahey, 2005).

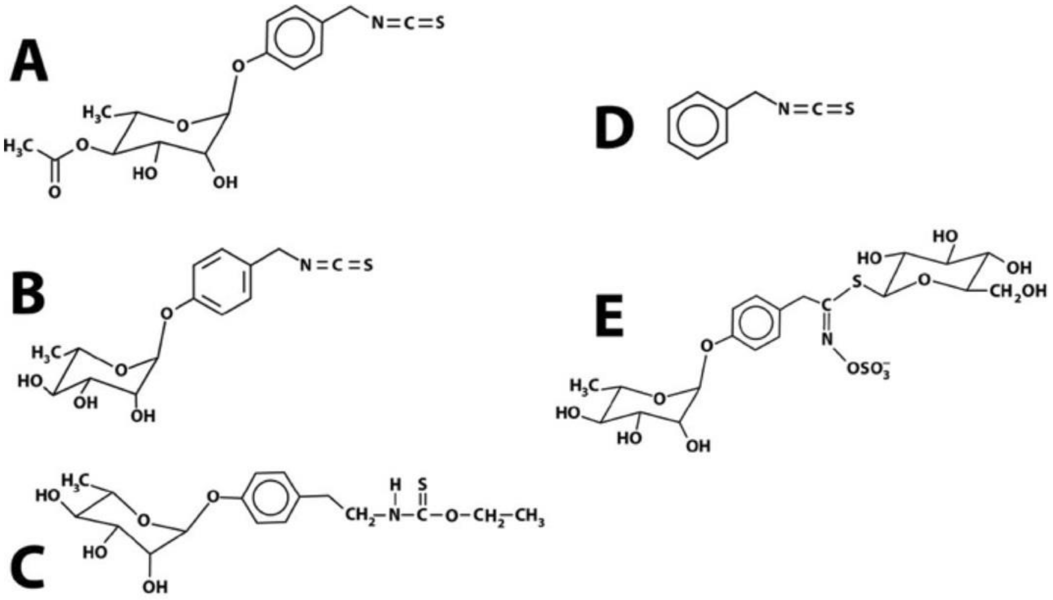


Figure 2: Chemical structures of phytochemicals found in *Moringa* species. **A.** 4-(4'-O-acetyl-a-L-rhamnopyranosyloxy) benzyl isothiocyanate, **B.** 4-(-L-rhamnopyranosyloxy) benzyl isothiocyanate, **C.** Niazimicin, **D.** Benzyl isothiocyanate, **E.** 4-(a-L-rhamnopyranosyloxy)benzyl glucosinolate (http://moringaceae.org, accessed on 02/08/2015)

A study by Gassenschmidt *et al.* (1995) conducted experiments with the pure protein of *M. oleifera*, in order to gain a better understanding into the flocculant mechanism and activity. The flocculant protein has been found to be homogenous by polyacrylamide gel electrophoresis (PAGE) under non-denaturing conditions and using sodium dodecyl sulfate (SDS) – PAGE. The molecular masses of the protein ranged from 6.5 – 7.0 kDa and isoelectric point that exceed pH 10 was found. Gassenschmidt *et al.* (1995) proposed that the protein's flocculant activity is from the cationic peptides. The flocculation protein was not characterized in this study, only organic compounds found in *M. oleifera* extracts were investigated using an analytical techniques.

2.1.4 Nutritional and health benefits of *Moringa*

Moringa oleifera has been endorsed as a natural nutrient to combat malnutrition (Fahey, 2005). *M. oleifera* has also shown great interest as a dietary supplement in regions where there is nominal access to healthcare. It can be used as a dietary supplement due to its increased vitamin, mineral content, antibacterial, antiulcer and anti-carcinogenic properties. The whole *Moringa* tree is edible and all parts of the tree (Figure 3) have been consumed for years by humans (Fahey, 2005). The leaves can retain their nutritional value for months without being refrigerated. The leaves can be freshly eaten, cooked or eaten in powder form by putting it into various foods such as yoghurt, porridge, beverages, etc. In some parts of Africa, the leaves are used as a vegetable, for example spinach (Guevara *et al.*, 1999; Siddhuraju and Becker, 2003; Farooq *et al.*, 2012). According to the Trees for Life organization, *Moringa* leaves have more calcium than milk, more iron than spinach, more Vitamin A than carrots, more potassium than bananas, more Vitamin C than orange and the protein value competes with that of eggs and milk (Faizi *et al.*, 1998).

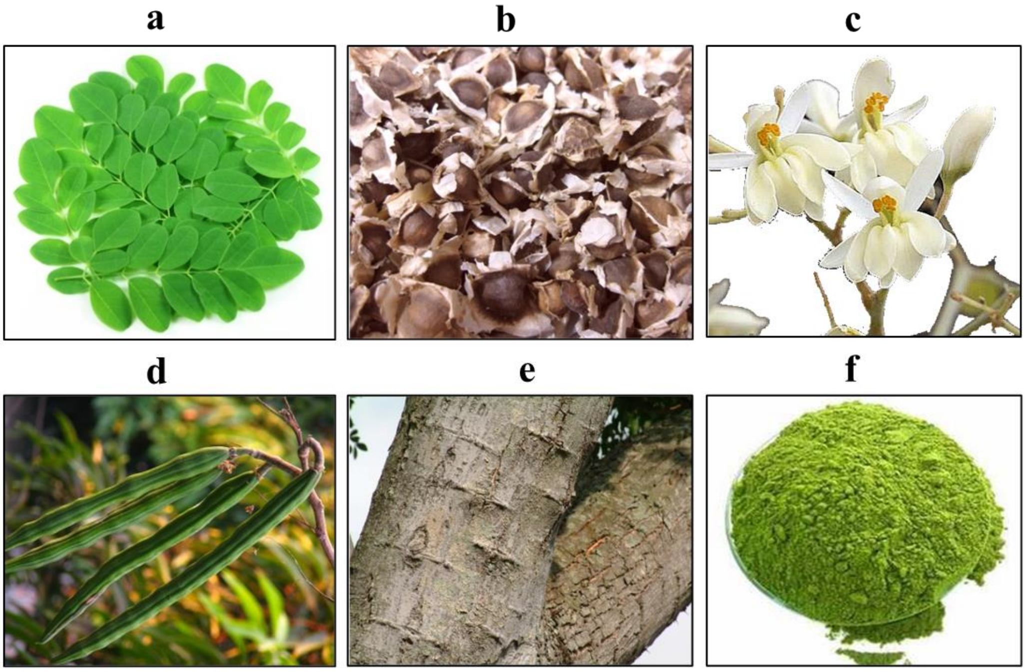


Figure 3: *Moringa oleifera* (a) leaves, (b) seeds, (c) flower, (d) fruit, (e) bark and (f) powder (Price, 1985).

There is significant health benefits of the consumption of *Moringa oleifera*, thus making the nutritional properties of *Moringa* well known especially consumption in conditions where starvation is prominent. Lockett (2000) stated that in many cultures throughout the tropic, it is very difficult to differentiate between food and medicinal uses of plants (such as bark, flowers, fruit, leaves, nuts, roots, tubers and seeds) since the plants are widely used in the communities for both nutritional and health benefits. The leaves have an abundance of iron and due to this, it is used to fight malnourishment, especially amongst nursing mothers, infants and also expecting mothers (Fahey, 2005; Farooq *et al.*, 2012). In south India, the leaves are included in children's and infants' diet due to high β-carotenes which inhibits blindness (vitamin A deficiency) (Ghasi *et al.*, 2000).

The *M. oleifera* tree has more nutrients than most food sources as shown in Table 1. All parts of the plant can be used for nutritional, health and other uses. The use of different parts of the plant are summarised in Table 2.

Table 1: The nutritional properties of *M. oleifera* when compared to that of everyday foods (Palada, 1996)

Nutrients	Content
Calcium	4 times more than Milk
Iron	25 times more than Spinach
Potassium	3 times more than Bananas
Protein	2 times more than Yoghurt
Vitamin A	4 times more than Carrots
Vitamin C	7 times more than Oranges

Table 2: Possible uses of all parts of *M. oleifera* (Palada, 1996)

Part of the *Moringa* tree	Uses
Bark	Health
Flower	Health
Gum	Health
Leaves	Nutritional supplement, plant growth spray, animal feed, biomass, food and medicinal
Pods	Nutritional supplement, food and medicinal
Roots	Health
Seeds	Water purification, skin care, medicinal, oil and animal feed
Trees	Alley cropping and erosion control

The young green pods are also very nutritious and highly consumed by humans especially by the Indian communities. They are prepared by boiling and eaten like green beans. The seed pods are known to be rich in free leucine. Seeds are eaten green before they change to a yellow colour (Foidl *et al.*, 2001). *Moringa* is imported as a food supplement to prevent and treat malnourishment in some parts of the world where poverty and starvation are primary issues. The distribution of *Moringa* in the world and countries experiencing malnutrition is shown in Figure 4.

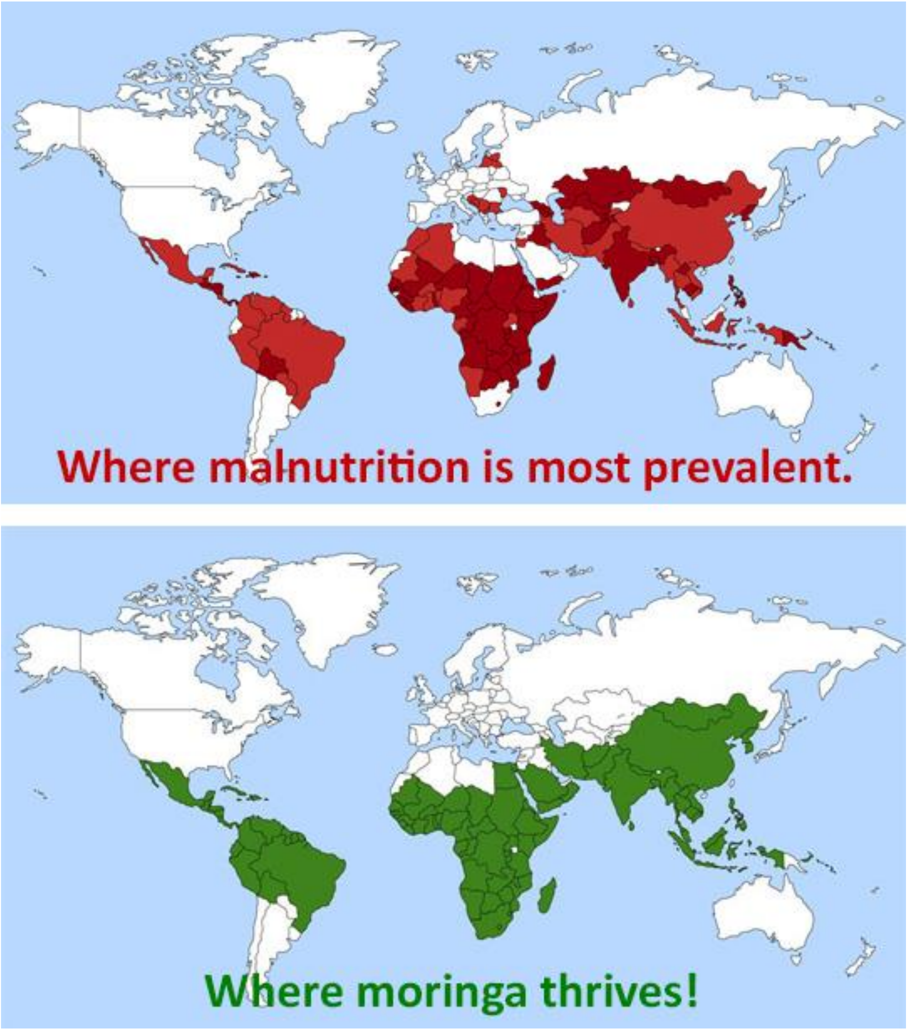


Figure 4: Countries where malnutrition is predominant (red) and also where *Moringa* is grown (green) (www.treesforlife.org, accessed on 07/05/2016)

2.1.5 Medicinal benefits of *Moringa*

Herbal medicines are mostly made up of medicinal plants, and these have long been used for treating illnesses and diseases all over the world (Kosalec *et al.*, 2009). The demand of medicinal plants is more with increasing population. The World Health Organisation (WHO) has recognised herbal medicine as important as synthetic medicine and as a source of primary health care (Rukangira, 2001). Numerous species are used medicinally, essentially for herbal remedies and are also used as sources of very potent and powerful drugs (Ahmad *et al.*, 1998). For the past two decades there has been a substantial increase in the utilisation of plant-based medicine. This is due to their availability, minimal side effects and suitability to the majority of the people of the third world countries (Rout *et al.*, 2013). *M. oleifera* is amongst these plants because it has exceptional nutritional and medicinal properties (Farooq *et al.*, 2012). *Moringa* is legendarily known as the “Miracle Tree” on various literature and research works. This name is given since it contains high nutritional content in all parts of the tree (Fahey, 2005). In Thailand, *Moringa* is commonly known as the “Wonder Tree” due to its therapeutic properties against diabetes, cancer, rheumatoid arthritis and other life threatening diseases (Chumark *et al.*, 2008).

The use of *Moringa* and its extracts in creams, oils, powders, salves, porridges, decoctions, poultices and emollients for the prevention or treatment of disease or infection is known but not much clinical studies have been done (Palada, 1996). The overabundance of traditional medicine references verifying *Moringa oleifera*’s health-giving power, and scientific proof of the well-known uses of *Moringa oleifera* is evolving to support some of the claims made (Fahey *et al.*, 2001; Fahey, 2005). In many of the scientific literature, *M. oleifera* has been cited as having hypotensive, antiulcer, antibiotic, anti-inflammatory, anti-asthmatic, hypoglycaemic, analgesic, antitrypanosomal, hypocholesterolemic and antioxidant activity, along with having significant efficiency in water purification by antibiosis, flocculation and sedimentation (Fahey, 2005).

Fewer studies have been done on the effectiveness of *Moringa* on human beings and the studies were not supported by controlled medical trials. For instance, in a report by Shaw and Jana (1982), it was reported that *Moringa* is a powerful healing remedy for urinary tract infection; however it offers no source of evaluation or control subjects. Therefore, to this degree *Moringa* is and will not be acknowledged for its remedial or nutritional properties by Western-trained medical practitioners (Fahey, 2005). Several published studies involving *in vitro* and *in vivo* trials provide some logical support for some claims that have been provided by traditional folklore (Verma *et al.*, 2009).

For long *Moringa* species have been recognized by traditional medicine specialists as having a benefit in cancer therapy (Hartwell, 1967). Compounds **A** and **B** in Figure 2 have been studied for their cancer preventive potential (Fahey *et al.*, 2004). Studies by Murakami *et al.* (1998) and Guevara *et al.* (1999) show that compounds **A** and **C** are potent inhibitors of phorbol ester (TPA)-induced Epstein-Barr virus and they are responsible for early antigen activation in lymphoblastic cells. Compound **C** was shown to inhibit tumour promotion in mice two-stage dimethyl benzanthracene – tetradecanoylphorbol acetate (DMBA-TPA) tumour models. Further studies done by Bharali *et al.* (2003) have shown skin tumour prevention when *Moringa* seed pod extracts are eaten. According to Farooq *et al.* (2012), *M. oleifera*'s seed kernels shows a promising effect on the treatment of bronchial asthma. The leaves of *Moringa oleifera* contain high phenolic content which can help decrease atherosclerosis vascular disease by removing hydrogen oxide radicals (Chumark *et al.*, 2008). The leaves also possess antibacterial activity which can be used in constraining gram negative and positive bacteria (Peixoto *et al.*, 2011).

2.1.6 Water purification

Ground water from boreholes serves as a primary source of drinking water. It is not only important for living systems; it is an essential part of the hydrological cycle of the earth. Ground water contamination is one of the major challenges we face in South Africa. This is due to human activities, such as, unintended spills and leakages from chemicals, acid mine drainage, fuels (Montgomery and Elimelech, 2007), etc. Agricultural activities contribute as non-point sources of ground water contamination, these activities include, the use of fertilisers and pesticides (Pye and Patrick, 1983). Other sources of contamination involve, improper management and handling of explosives in mining sites (Forsyth *et al.*, 1995).

Currently, chemical coagulants used are expensive and toxic, thus a practical alternative is needed to manage contamination (Guida *et al.*, 2014; Freitas *et al.*, 2018). Natural coagulants have been of great interest in water treatment industry especially in developing countries with limited financial resources (Shukla *et al.*, 1988; Ndabigengesere and Narasiah, 1998). *Moringa oleifera* seed extracts is one of the promising natural coagulants (Shan *et al.*, 2017; de Paula *et al.*, 2018). Laboratory studies on the coagulation properties of *M. oleifera* seeds have identified the active agent for coagulation as a dimeric cationic protein with a molecular weight estimate at 13 kDa (Jahn, 1988; Ndabigengesere *et al.*, 1995). The seeds have also been found to be very effective in reducing micro-organisms in water and the turbidity of water (Berger *et al.*, 1984; Jahn, 1988; Ndabigengesere and Narasiah, 1998). The latter is shown in Figure 5.

Figure 5: *Moringa oleifera* seeds used for water purification (Shah, 2012)

2.1.7 Industrial, Agricultural and other benefits of *Moringa*

Moringa oleifera seed oil extract has been of great value in industries. The oil has been used for lubricating machinery because it does not deteriorate easily or become thick and sticky (Ferrao and Ferrao, 1970). It is also utilized in cooking as a vegetable cooking oil because of its high smoking point (Ramachandran *et al.*, 1980). The perfume industries use *Moringa* oil for the stability of the scents due to its ability to retain and absorb volatile substances (Foidl *et al.*, 2001).

There are other benefits of *M. oleifera* mentioned in literature. These include, improvement of male sexual desire and performance using *M. oleifera* leaf extract (Prabsattroo *et al.*, 2012). The roots' aqueous extracts are used to treat the infertility effect (Shukla *et al.*, 1988). However, the compounds responsible for these benefits are not fully known and need to be accurately researched.

2.2 Extraction of organic compounds using modern methods

According to Munshi and Bhaduri (2009) when analysing solid samples, extraction is known as the most essential stage in sample preparation. Typical sample preparation techniques that depend on extraction with solvents have been used for many years. These include sonication extraction (Fu *et al.*, 2009; Wenrui *et al.*, 2009; Hoai *et al.*, 2010), liquid-liquid extraction (LLE) (Jiawei *et al.*, 2008; Kuranchie-Mensah *et al.*, 2012) and Soxhlet extraction (SE) (Camel, 2001). These classical extraction techniques, nevertheless, require large volumes of organic solvents that are harmful to the environment (Teo *et al.*, 2010). Modern technology has given rise to the development of simpler and faster extraction techniques that use less solvents such as, pressurized liquid extraction (PLE) (Matshediso, 2015), microwave-assisted extraction (MAE) (Saleh *et al.*, 2017), solid-phase extraction (SPE) (Saadati *et al.*, 2012) and pressurized hot water extraction (PHWE) (Matshediso, 2015). Comparison of the different extraction techniques is given in Table 3:

The modern method used in the extraction of organics is PHWE (Teo *et al.*, 2010; Makita, 2015; Matshediso, 2015; Gbashi *et al.*, 2016; Tavengwa *et al.*, 2016). PHWE generally comprises of a water supply, an oven for maintaining the temperature of the solvent, a pump for the transport of the solvent, an extraction cell kept at a certain pressure and a collection flask for the extract. There are four progressive steps for the extraction mechanism of PHWE and these steps take place in the extraction cell packed with sample material: (1) desorption of solutes from several active sites in the sample matrix under pressurized and high temperature conditions, (2) the extraction fluid diffuse into the matrix, (3) the solutes separate themselves from the sample matrix into the extraction liquid depending on the matrix and (4) the solutes are eluted out of the extraction cell into the collection flask (Ong *et al.*, 2006).

Table 3: Comparison of the extraction methods

	Extraction		
	Solvents	Solvent Volume (mL)	Time
PHWE	Water	40 – 50	5 – 30 min
LLE	Ethylacetate, hexane, methyl tert-butyl ether, methylene chlorine	300 – 500	18 – 24 h
SPE	Acetone, acetyl acetate, chlorinated solvents, hexane, methanol	Less than 100	40 – 70 min
SE	Acetone, dichloromethane, hexane, methanol, toluene	300	4 – 48 h
PLE	Acetone, dichloromethane, hexane	15 – 40	2 – 20 min
MAE	Acetone, methanol	25 – 50	30 – 60 min

Pressurised hot water extraction (PHWE) is an environmentally friendly solvent extraction method because it uses water that is pressurized at controlled pressure and high temperature conditions. The polarity of water can be changed to almost that of alcohols by controlling the pressure (22 MPa) and temperature (374°C); hence, it can be used to dissolve medium and low polarity analytes (Wallace *et al.*, 2007; Nieto *et al.*, 2010). The fact that PHWE uses water (without any consumption of organic solvents) has proved to be advantageous since water is non-toxic, easily accessible, and recyclable or can be disposed with little environmental effects (Wallace *et al.*, 2007; Zaibunnisa *et al.*, 2009). Therefore, PHWE is slowly becoming an effective and cheap method of extraction for less polar organic components from environmental sediments, soil and plant material. Also, a PHWE system can be easily assembled using cheap components in a laboratory.

2.3 Magnetic nanoparticles

Nanoparticles (NPs) are nano-sized particles ranging from 1 to 100 nm (Lu *et al.*, 2007). They are of great interest with promising applications because they have a distinctively large particle surface area (Giraldo *et al.*, 2013). Studies on metal oxide nano-adsorbents have revealed impressive properties in comparison to their bulk form. These include greater catalytic activity, elevated adsorption capacity, elevated dispersion degree and tremendous paramagnetism behaviour (Roco, 2003; Perez, 2007). When metal oxides are applied as nano-adsorbents, these properties assist in fields such as air pollution control, medicine, biotechnology and water treatment (Hua *et al.*, 2009). NPs that exhibit magnetism are referred to as magnetic NPs. Iron oxide NPs are known to be ferromagnetic and one of the iron oxide that is of great interest is magnetite (Fe_3O_4). It has the ability to be permanently magnetized, hence it is also called magnetic iron oxide. Another important ferromagnetic iron oxide is ferrite. Ferrites are ferric oxides (α-Fe_2O_3) that make up ceramic-like ferromagnetic materials.

Both magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) nanoparticles can be used for the adsorption of contaminants. The process of extraction of these contaminants occurs either by adsorption or reduction. Magnetite NPs are preferred because they have better ferromagnetic properties which makes isolation of analytes from solution an easy procedure (Sugimoto, 1999). According to a study done by Hu *et al.* (2004), the removal of heavy metals using Fe_3O_4 nano-adsorbents occurs by both chemical and physical adsorptions. At different pH conditions polarization of the oxygen atoms on the surface of the iron oxides (Fe_3O_4 and γ-Fe_2O_3) NPs can occur. For example, at pH values that are under the zero point of charge (pH_{ZPC}), the surface has a positive charge that tends to attract negatively charged pollutants (Hu *et al.*, 2005, 2006). This suggests that the adsorption of contaminants by Fe_3O_4 and γ-Fe_2O_3 nanoparticles is highly dependent on pH.

2.3.1 Applications

Interest in magnetic NPs is growing due to its vast application in different fields such as in biological, chemical and medicinal studies. The uses include data storage (Hyeon, 2003), dissolution of enzymes and proteins (Sutor, 1997; Koneracká *et al.*, 1999; Elaissari and Bourrel, 2001), DNA and RNA purification (Elaissari and Bourrel, 2001), environmental remediation (Elliott and Zhang, 2001; Takafuji *et al.*, 2004), magnetic fluid (Chikazumi *et al.*, 1987), magnetic resonance imaging (MRI) procedures in cancer diagnosis and therapy (Sousa *et al.*, 2001; Lacava *et al.*, 2002; Li *et al.*, 2005; Mornet *et al.*, 2006), etc.

In analytical chemistry, magnetic NPs are used for extraction and pre-concentration, and the procedure is more simple, faster and precise than those used customarily (Sheykhaghaei *et al.*, 2016). The advantage of using magnetic NPs is that the target materials are isolated from the solution by an easy procedure. Synthesis of Fe_3O_4 nanoparticles that are non-toxic, inexpensive, effective and scalable are greatly preferred for applied research, like in the study by Giraldo *et al*. (2013).

2.3.2 Synthesis

The dimension of the nanoparticle gives it its properties and this entirely depends on the controlled monodisperse size of the magnetic nanoparticle (Laurent *et al.*, 2008). According to Bradley *et al.* (2000) and Zhang *et al.* (2006), the two essential features that gives the NPs its widely used properties (electrical, optical or magnetic) are morphology and size. Hence, the route of synthesis of NPs is of major importance in obtaining consistency in the morphology and size distribution (Rao *et al.*, 2000; Liu *et al*., 2005).

Several chemical procedures have been implemented on the synthesis of magnetite. The colloidal nature of magnetic NPs makes the synthesis to be a rather complex procedure

(Laurent *et al.*, 2008). The different procedures for synthesis include, flow injection synthesis (Salazar-Alvarez *et al*., 2006), sol-gel, micro-emulsion (Albornoz and Jacobo, 2006), co -precipitation (Lu *et al*., 2007; Laurent *et al*., 2008), high temperature reactions (Wan *et al.*, 2005), etc. Experimental conditions are significant in order to produce magnetic particles that are stable, remain uniformly distributed in solutions and have a particular particle size (Laurent *et al.*, 2008). The smaller particle size of magnetic NPs steer researchers to unique and innovative properties, especially superparamagnetism (Raj and Moskowitz, 1990; Leslie-Pelecky and Rieke, 1996). It is also essential to have a process that is reproducible and can be up scaled without any difficult additional procedures involved (e.g. purification step).

Co-precipitation

Co-precipitation process is one of the classic methods for synthesizing magnetic particles. It is one of the simplest and convenient chemical processes to produce magnetic nanoparticles. Iron magnetite (Fe_3O_4) NPs are formed by the maturing of ferric and ferrous ions in a basic aqueous medium. The chemical reaction for magnetite is shown in Equation 1:

$$Fe^{2+} + 2Fe^{3+} + 8OH^{-} \rightarrow Fe_3O_4 + 4H_2O \qquad (1)$$

The arrangement, shape and size of Fe_3O_4 greatly depends on ratio of Fe^{2+}/Fe^{3+}, temperature of the reaction, the type of salts used (for example, chlorides, nitrates or sulfates), ionic strength and pH of the media (Lu *et al* ., 2007). The stability of Fe_3O_4 is of concern because when under ambient conditions, they oxidize easily to γ-Fe_2O_3 (Lu *et al*., 2007; Laurent *et al.*, 2008). The reaction must therefore take place under non-oxidizing conditions (Jolivet *et al*., 2004; Lu *et al*., 2007; Laurent *et al.*, 2008).

There are two phases involved in the co-precipitation method, namely, nucleation and growth. The nucleation process is short-lived and occurs when critical supersaturation of the ferric and ferrous species is reached. The growth phase is when the nuclei slowly grow due to the dispersion of the solutes to the exterior of the crystal (Tartaj *et al.*, 2006; Lu *et al*., 2007; Laurent *et al.*, 2008). A successful synthesis will lead to monodispersion of magnetic NPs. The key advantage of the co-precipitation process is that the amount of product produced is large. However, size distribution cannot be controlled for the reason that kinetic factors usually control crystal growth (Boistelle and Astier, 1988; Sugimoto, 2003).

Thermal decomposition

This process is used to synthesize high quality superparamagnetic magnetite NPs (Sun and Zeng, 2002; Maity and Agrawal, 2007). It involves thermal breakdown of organometallic compounds in highly steaming organic solutions (temp. 100 – 320°C) containing stabilizing surfactants (Park *et al.*, 2004; Redl *et al.*, 2004; Sun *et al.*, 2004). The organometallic precursors comprise of:

- Metal acetylacetonates, ($[M(acac)_n]$): M = Co, Cr, Fe, Mn, Ni; n = 2 or 3; acac = acetylacetonate
- Metal cupferronates ($[M^x Cup_x]$): M = metal ion; Cup = $C_6H_5N(NO)O-$
- Carbonyls ($R_2C = O$)

Fatty acids, oleylamine and oleic acids are generally used as stabilizing surfactants (Jana *et al*., 2004; Samia *et al.*, 2005; Li *et al.*, 2006). Specific size and morphology of magnetic NPs are controlled by the amounts of the initial reagents including the surfactant, organometallic compounds and the solvent. The other parameters that are essential to the size and morphology are reaction time and temperature (Lu *et al.*, 2007). The advantage of the thermal decomposition method is that the NPs produced have a controlled shape and a scalable yield (Lu *et al.*, 2007).

Hydrothermal process

Hydrothermal synthesis is done by adding oxidizing or reducing agents to an aqueous mixture of ferric and ferrous salt aqueous solution under hydrothermal conditions (Fan *et al.*, 2001; Deng *et al.*, 2003; Wang *et al.*, 2004). It is done under controlled pressure (< 2000 psi) and temperature (< 200°C) such as in autoclaves or reactors. This synthesis approach produces a broad size of nanoparticles (Butter *et al.*, 2005; Giri *et al.*, 2005; Mao *et al.*, 2006; Zhu *et al.*, 2007). The synthesis is driven by an overall phase transfer and division mechanism that transpire as the liquid, solid and solution integrate (Euliss *et* *al.*, 2003; Wang *et al.*, 2005). The parameters that are essential for the outcome of the reaction are time, temperature and solvent (Chen and Xu, 1998).

Microemulsion

Microemulsion is when two liquid solutions that are incapable of mixing disperse and due to being physically and chemically stable, one or both the liquid solutions form a stable interfacial film of surfactant particles (Langevin, 1992). For instance, water and oil are immiscible and during microemulsions, the liquid phase diffuses into microprecipitations enclosed in a single layer of surfactant molecules (micelle of constant hydrocarbons). The size of the inverse micelles can range from generally 1 – 50 nm and it is dependent of the ratio of the number of moles of the reactants – surfactant and water (Paul and Moulik, 2001).

If the microemulsion of equivalent liquid solutions with ideal reactants and conditions, the microprecipitations will constantly collide, form masses and breakdown again and lastly aggregate in micelles (Gupta and Gupta, 2005). The precipitate can be extracted out by centrifuging or filtering. This method thus can be used for the synthesis of nanoparticles by serving as a nanoreaction vessel (Lu *et al.*, 2007).

The size distribution of NPs produced with microemulsion is relatively narrow, ranging from 2 – 5 nm (Tan *et al.*, 2003). The disadvantage of the NPs produced by this method is that the operational window is generally small and the yield is low when compared to that produced using co-precipitation and thermal decomposition. Microemulsion cannot be upscaled as it uses a high volume of solvent to produce a low yield product (Lui *et al.*, 2007).

Other methods of synthesis

There are many other methods that can be used to synthesize magnetic NPs. These methods include:

- Sol-gel reaction which involves condensation and hydroxylation of precursor particles in solution, leading to a “sol” of nanometric molecules (Kojima *et al.*, 1997; Liu *et al.*, 1997; Laurent *et al.*, 2008).

- Flow Injection reaction which involves synthesis of limited size distributed magnetic nanoparticles (2 – 7 nm) by creating a reaction zone constraint in different media, e.g. emulsions, etc. This method has a high reproducibility because the process can be controlled externally, in continuous flow and laminar conditions and also seamless homogeneity (Salazar-Alvarez *et al.*, 2006).
- Polyol technique (Fievet *et al.*, 1989), aerosol technique (Gonzalez-Carreno *et al.*, 1993; Pecharromán *et al.*, 1995) and sonolysis (Puntes *et al.*, 2001, 2002; Rotstein and Tannenbaum, 2002).

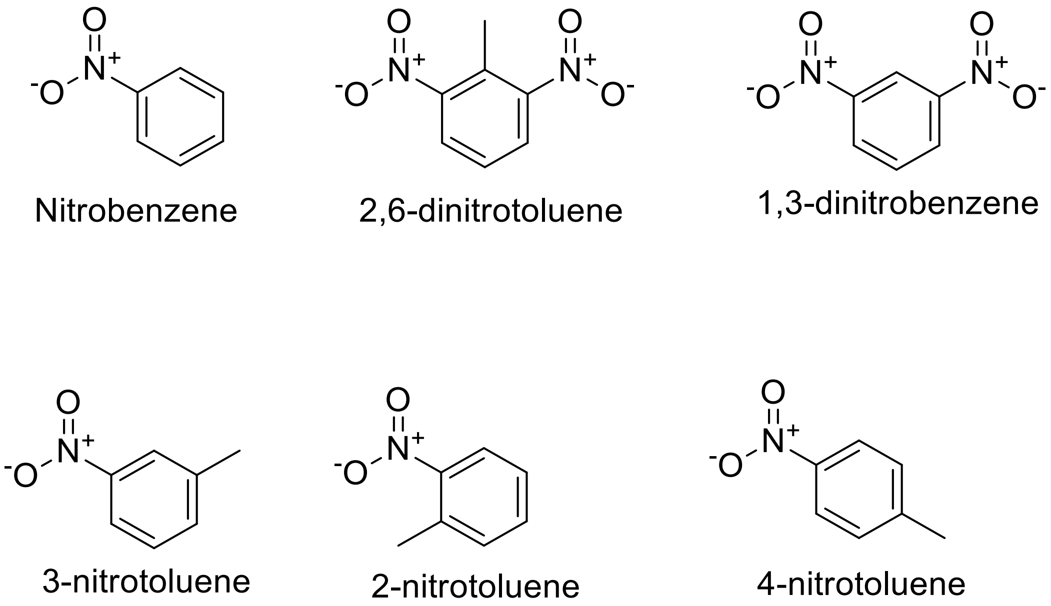
2.3.3 Stability and functionalization

Despite the fact there have been substantial developments in the synthesis of magnetic NPs, there is still a problem of aggregation or precipitation affecting the stability of magnetic NPs. Ferromagnetic magnetic NPs are also sensitive in an oxidizing atmosphere making their stability an important condition for their use (Lu *et al.*, 2007). Several strategies have therefore been used in order to prevent aggregation and oxidation of the surface of nanoparticles.

Many studies have successfully coated magnetic NPs by using amino silane (Yamaura *et al.*, 2002; Ma *et al.*, 2003), γ-methacryloxypropyltrimethoxysilane (Tavengwa *et al.*, 2017), oleic acid (Zhang *et al.*, 2006) and alkyl phosphonate/phosphate (Sahoo *et al.*, 2001). The coating has also been done in order to give NPs functionality, where the coatings functional group can assist in enhancing the selectivity of NPs towards target compounds. Both organic coating (such as surfactants and polymers) (Euliss *et al.*, 2003; Liu *et al.*, 2004; Kim *et al.*, 2005) and inorganic coatings that include silica, silver and gold (Liu *et al.*, 1998; Sobal *et al.*, 2002; Kobayashi *et al.*, 2003) have been used. Functionalizing the surface of NPs helps by extraction compounds according to their chemical nature. This is important especially in the analysis of contaminants that exist in trace quantities.

2.4 Explosive compounds

An explosive material is an element in its pure form or mixture, capable of generating an explosion by a chemical reaction. The explosion releases heat and produces a gas (Akhavan, 2011; Mahadevan, 2013). There are two main categories for explosives, primary and secondary explosives. The secondary explosives are different to the primary explosives in that they are less susceptible to detonating when exposed to heat (Pichtel, 2012). These two categories comprises of both inorganic and organic explosive compounds. The secondary organic explosives consist of emulsions, RDX, Pentaerythritol tetranitrate (PETN) and nitro-based explosives. Current military explosives are generally nitro-based explosive compounds (Yew *et al.*, 2016). These compounds tend to self-oxidize and their intermediate compounds produced can be very soluble and volatile. These intermediate compounds can impact the health of humans and other species in the environment (Luning Prak and O'Sullivan, 2006; Olson *et al.*, 2015). The structure of the nitro-based explosive compounds have nitro ($–NO_2$) group(s) directly connected to a carbon atom (Yew *et al.*, 2016). Various known nitroaromatic explosive compounds consisting of a nitro group bonded to the aromatic ring are shown in Figure 6, and they were extracted in this study using the synthesized nano-adsorbent.



Nitrobenzene

2,6-dinitrotoluene

1,3-dinitrobenzene

3-nitrotoluene

2-nitrotoluene

4-nitrotoluene

Figure 6**:** The nitroaromatic explosive compounds (NAECs) that were extracted in this study

2.4.1 Source, applications and fate of explosive compounds

The introduction of explosives in the water system is a major problem which impacts the water quality. Explosives are predominantly used for military purposes, mining procedures, industries and agricultural activities. Explosives are dumped in the ocean, charred or set off in isolated areas (Yinon, 1990; Narayanan, 1996). Due to these dumping practices, the explosives ultimately leach into the soil and travel from the contamination site into water streams. Explosives are also introduced in the soil and water systems during manufacturing procedures, incomplete explosive bursts and leaching from undetonated landmines (Mayfield *et al.*, 2006). A 2,4,6-trinitrotoluene (TNT) industrial plant is estimated to produce about 2 mega litres of water per day (Yinon, 1999). The solubility of TNT is 150 mg L^{-1} meaning that there is a possibility of the migration into groundwater and surface aquifers (Talmage *et al.*, 1999). Manufacturing of TNT produces co-pollutants which include, 1,3-DNB, 2-nitrotoluene, 3-nitrotoluene, 4-nitrotoluene, 2,4-dinitrotoluene, 2,6-dinitrotoluene and 1,3,5-trinitrobenzene (TNB) (Rieger and Knackmuss, 1995).

An increased accumulation of the explosive compounds can prove fatal to animals, aquatic animals and humans, hence, detecting and monitoring of these compounds in the environment is essential (Pichtel, 2012; Thurman and Ferrer, 2012). A study by Paden *et al.* (2008) showed that TNT can be highly toxic when exposed to adult bullfrogs. Its degradation products (especially dinitrotoluenes) are known to rapidly penetrate into the skin and this can lead the development of methemoglobin when severely exposed (Yinon, 1990; Narayanan, 1996). Depending on the degree of exposure, the toxic effects may vary from a minor headache to serious harm to internal organs.

2.4.2 Regulation on explosive waste and pollution

Regulatory policies need to be put in place to control the discharge of explosives into the environment (Adler *et al.*, 2007). In South Africa, dinitrotoluene (DNT) and 2,4,6-trinitrotoluene (TNT) are classified as suspended human carcinogens under the Hazardous Chemical Substances Regulations (1995). The set exposure limits in drinking water are 0.15 mg L^{-1} and 1.5 mg m^{-3} for DNT and TNT, respectively. The release of 2,4-DNT is limited to 0.065 mg L^{-1} (Strydom and King, 2009). Table 4 gives the maximum concentration of explosive compounds that is estimated to not produce any carcinogenic outcome after exposure (USEPA).

Table 4: Maximum exposure limits (mg L^{-1}) in drinking water (Griffiths *et al.*, 2012)

Compounds	1 – 10 days of exposure*	Lifetime of exposure#
1,3-dinitrobenzene	0.04	0.001
2,4-dinitrotoluene	1	0.005
2,6-dinitrotoluene	0.4	0.005
2,4,6-trinitrotoluene	0.02	0.002

*assuming that a 10 kg child drinks 1L of water/day # assuming that a 70 kg adult drinks 2L of water/day

2.4.3 Various methods for the preconcentration of explosives

It is essential to determine explosive compounds and their by-products at reasonably low concentrations in order to monitor them in the environment. For good reliable and accurate results, it is important to choose wisely and appropriately the preconcentration methods. Methods such as, column concentration (Talmage *et al.*, 1999), LLE (Furton *et al.*, 2000; Calderara *et al.*, 2003), SFE (Marple and LaCourse, 2005) and many others have been used together with high pressure liquid chromatography (HPLC) and gas chromatography (GC) to preconcentrate and detect nitroaromatic explosive compounds. Numerous studies have used SPME method together with GC (Barshick and Griest, 1998; Psillakis and Kalogerakis, 2001; Calderara *et al.*, 2003) and HPLC (Furton *et al.*, 2000; Halasz *et al.*, 2002; Monteil-Rivera *et al.*, 2004) to determine organic explosives in the ecosystems. In this study, *M. oleifera* coated magnetic NPs were applied as nano-adsorbents for the extraction of six NAECs and analysis was done using HPLC coupled to an ultra violet (UV) detector.

2.5 Kinetic Modelling

Kinetic modelling was used to understand the mechanism and rate controlling stages affecting the kinetics of adsorption. The Lagergren pseudo-first order and pseudo-second order models were used. The model with the highest R^2 value was chosen to as the model that explains the extent of adsorption of explosive compounds onto the nano-adsorbent. The pseudo first order indicates a physisorption interaction while the pseudo-second order indicate a chemisorption interaction.

The pseudo-first order equation is given in Equation 2 (Li *et al.*, 2003). After Equation 2 has been linearized, the model equation becomes Equation 3. The plot of $\ln(q_e - q_t)$ against t when plotted gives a straight line in which k_1 is the gradient and $ln\, q_e$ is the intercept.

$$\frac{dq_t}{dt} = k_1(q_e - q_t) \tag{2}$$

$$ln(q_e - q_t) = ln\, q_e - k_1 t \tag{3}$$

where q_t and q_e are measured in mg g^{-1} and represent the of adsorbed nitroaromatic explosive on the adsorbent at time t(min) and at equilibrium, respectively. k_1 is the pseudo-first-order rate constant given in per min.

The pseudo-second order equation is given as Equation 4 (Wang *et al.,* 2007). The linearized form of the pseudo second order model is given as Equation 5. A plot of $\frac{t}{q_t}$ against t gives a straight line if the model is applicable. q_e and k_2 can be determined from the calculated gradient and intercept, respectively.

$$\frac{dq_t}{dt} = k_2(q_e - q_t)^2 \quad (4)$$

$$\frac{t}{q_t} = \frac{1}{k_2{q_e}^2} + \frac{1}{q_e}t \quad (5)$$

where, k_2 measured in g mg^{-1} min^{-1} represents the rate constant sorption.

2.6 Adsorption isotherm modelling

Adsorption isotherms were used to explain the mechanism of adsorption of analytes onto the adsorbent. The Freudlich and Langmuir isotherms were used to define the interactions of explosive compounds onto the nano-adsorbent.

The Freundlich isotherm is based on the assumption that the adsorbent has a heterogeneous surface which can allow for multilayer adsorption. The model equation is given by Equation 6 (Ghiaci *et al.*, 2004). The linearized form of Equation 6 is given by Equation 7. A plot of lnq_e against lnC_e gives a straight line in which $\frac{1}{n}$ and K_f can be determined from the calculated gradient and intercept, respectively.

$$q_e = K_f C_e^{\frac{1}{n}} \quad (6)$$

$$lnq_e = lnK_f + \frac{1}{n} lnC_e \quad (7)$$

where q_e and C_e, both measured in mg L^{-1} represents the amount adsorbed at equilibrium and the concentration at equilibrium, respectively. K_f (L g^{-1}) is the Freudlich isotherm constant that is related to the adsorption capacity and n represents the intensity of the adsorption. If n is in the range $0 < n < 1$, then there is a favourable adsorption reaction.

The Langmuir isotherm is based on the assumption that the adsorbent has a homogenous surface which only allows for monolayer adsorption. It also assumes that all sites the on the surface of the adsorbent are equal (Atkins and de Paula, 2010). The model equation is given as Equation 8. The linearized form of Equation 8 is given as Equation 9. A plot of $\frac{1}{q_e}$ against $\frac{1}{lnC_e}$ and from the slope and the intercept b and q_m are calculated, respectively.

$$q_e = \frac{q_m b C_e}{1+bC_e} \tag{8}$$

$$\frac{1}{q_e} = \frac{1}{q_m b C_e} + \frac{1}{q_m} \tag{9}$$

where q_e and q_m (mg g^{-1}) represent the amount of adsorbate adsorbed and the maximum adsorption adsorbate adsorbed, respectively. b (L g^{-1}) is the Langmuir isotherm constant and C_e (mg L^{-1}) is the equilibrium concentration.

To determine the whether the sorption method is favoured, a dimensionless separation parameter (R_L) can be estimated by Equation 10 below (Hall *et al.*, 1966):

$$R_L = \frac{1}{1+bC_i} \tag{10}$$

where C_i measured in mg L^{-1} represents the initial explosive compounds concentration and the Langmuir constant is given as b in L g^{-1}. The type of adsorption when the R_L value is:

- equal to 0 – irreversible
- between 0 and 1 – suitable
- equal to 1 – linear
- greater than 1 – unsuitable

2.7 Method validation

Method validation is the procedure done to prove that an analytical method/technique is up to standard for its proposed purpose (Green, 1996). The use of analytical methods for quantitative determination of organics, drugs, ions and many others is important in generating reliable and reproducible data. Method validation forms part of quality assurance and quality control procedures to ensure that the results are reliable (Saadati *et al.*, 2013). Thus, it is vital to use well-characterised and fully validated analytical methods to produce reliable results that can be interpreted appropriately. It is known that extraction techniques used in isolating analytes from complex samples contributes to over 30% of error in the reported value (Poole *et al.*, 2000). It is key to acknowledge that each technique and method has its own limitations, which will differ from analyte to analyte (Findlay *et al.*, 2000; Shah *et al.*, 2000). It is therefore, essential to define the limitations and quantity of the amount of error when a particular method is used. There are various parameters used to assess quality assurance of the method. These include, accuracy, precision, linearity, limit of detection (LOD), limit of quantification (LOQ), reproducibility, repeatability and extraction efficiency.

2.7.1 Linearity

Linear regression model is used to test linearity by looking at the response of the instrument toward the extracted analyte and the spiking concentration. The R^2 value known as the coefficient of determination is defined as the proportion of variance described by the regression model and it useful as a measure of success of predicting the dependent variable from the independent variables (Nagelkerke, 1991). The R^2 coefficient is used to calculate the fit of the data along a regression line. Its value ranges from 0 to 1 and the values are an indication of the degree of confidence in predicting the dependent variable using a linear regression equation. When R^2 value is 0, this is an indication that dependent variable cannot be predicted from the independent variables and 1 indicates that the regression line passes through all the data points and the predicted value is totally reliable (Magee, 1990; Nagelkerke, 1991).

2.7.2 Limit of detection and quantification

Determination of the limit of detection (LOD) and limit of quantification (LOQ) are one of the most vital steps in method development. LOD and LOQ are used to determine the lowest concentration of an analyte that can be reliably detected or quantified by an analytical procedure (Armbruster and Pry, 2008; Shrivastava and Gupta, 2011). Detection and quantification limits are related to matrix effects, chemical purity, sensitivity of the instrument and sample preparation (Knoll, 1985; Saadati *et al.*, 2013). Various methods can be used to determine the LOD and LOQ. These include, blank determination, calibration curve slope and signal-to-noise (S/N) ratio.

The blank determination method uses the mean and standard deviation of the blank to estimate the LOD and LOQ. The LOD and LOQ are calculated using Equations 11 and 12, respectively.

$$LOD = \bar{x}_{blank} + 3SD_{blank}, \tag{11}$$

$$LOQ = \bar{x}_{blank} + 10SD_{blank}, \tag{12}$$

$\bar{x}$ represents the mean and SD the standard deviation of the blank.

In this method both interference and baseline noise are taken into account. Interference may arise due to matrix effects in the method making this method unsuitable for this analytical process.

LOD can also be determined using the calibration curve slope. This method uses calibration curve with a linear regression equation (Equation 13). This method can only be used when no background noise is observed and this is next to impossible in a typical analytical process.

$$LOD = a + \frac{3 \times SD_y}{b}, \tag{13}$$

where a is the y intercept of the calibration linear equation, SD_y denotes the standard deviation of y and b is the slope of the calibration curve.

The method of S/N ratio is used when the response in a chromatographic instrument display noise (baseline noise) in the absence of an analyte. Around the retention time of the analyte of interest, the highest peak noise from the blank is recorded and using a calibration curve regression equation, the concentration of the analyte that would give 3 times the peak height of the noise signal is predicted. The S/N ratio is calculated using Equation 14.

$$LOD = \frac{2H}{h}, \tag{14}$$

where H is peak height of the analyte of interest and h is the peak height of the background noise.

This method is used when the instrument's background noise is of concern and it is used to determine the performance of the instrument in the analytes of interest (Guideline, 2005; Saadati *et al.*, 2013). Hence, this method was applied in this research.

CHAPTER III: RESEARCH OBJECTIVES

This chapter focuses on the main and general objectives of the research work. The novelty is also discussed.

3 Research objectives

3.1 Main objective

To prepare a nano-adsorbent coated with *Moringa oleifera* extracts for the removal of nitroaromatic explosive compounds (NAECs) in aqueous samples.

3.2 Specific objectives

a. To prepare magnetite nanoparticles
b. To extract organic compounds from powdered *M. oleifera* leaves using pressurized hot water extraction (PHWE) method
c. To modify the magnetic NPs with *M. oleifera* leaf and seed extracts
d. To characterize the synthesized magnetic nano-adsorbent for functional groups on the surface
e. To determine the surface area, pore volume and pore size of the coated and uncoated magnetic NPs using Brunauer, Emmett and Teller (BET)
f. To determine the thermal behavior of the coated and uncoated magnetic NPs using Thermogravimetric analysis (TGA)
g. To optimize the extraction of NAECs using the modified magnetite
h. To investigate the extent and mechanism of adsorption of nitroaromatic explosive compounds on the magnetic nano-adsorbent
i. To validate the applicability of *M. oleifera*–coated magnetite as a viable nano-adsorbent in the extraction of explosive compounds from aqueous samples.

3.3 Research questions

a. Will the synergy of the magnetite NPs and *M. oleifera* extracts provide better selectivity towards the nitroaromatic explosive compounds?
b. Will the *M. oleifera*–coated magnetite provide a viable alternative in the extraction of NAECs from aqueous samples?

3.4 Novelty

Modification of magnetite using synthetic agents for the extraction of organic pollutant has been done previously. In this study, the modification was done using natural organic extracts and this is the first of its kind in the extraction of nitroaromatic explosives in aqueous samples. The goal was to combine the increased surface area of the nanoparticles and the selectivity of the extracts to produce a highly selective nano-adsorbent with a high adsorption capacity and extraction efficiency.

3.5 Motivation

In South Africa, the fresh water quality is decreasing due to an incline in pollution, industrial activity, agriculture, destruction of wetlands, mining, etc. Mine effluent allow for an increase in various pollutants including explosive compounds thus causing a decrease in the quality of water (Ekwanzala *et al.*, 2017; Winde *et al.*, 2017). Hence, the search for green methods that are also cheap is essential since synthetic agents are expensive. Organic agents are readily accessible and easy to extract, thus the coating of NPs with organic extracts can be a solution. *Moringa* is one of the plants that can be utilized because it is abundant in many parts of the world and it is a widely cultivated. However, limited studies have been done on this plant especially on its ability to purify water especially using coated *M. oleifera* extracts.

CHAPTER IV: MATERIALS AND EXPERIMENTAL METHODS

This chapter provides more detail on the chemicals, samples and experimental methods developed to generate data that assisted in achieving the research objectives mentioned in Chapter III. The main experimental methods included:

- Synthesis and coating of magnetic nanoparticles
- Characterization of the magnetic NPs and analysis of NAECs
- Optimization of the extraction parameters such as, sample pH, contact time, amount of adsorbent and initial concentration

4 Materials and experimental methods

4.1 Chemicals and reagents

Ferrous sulphate (90%) was purchased from Saarchem (Pty) Ltd (Krugersdoorp, South Africa), iron(III) nitrate nonahydrate (≥98%) from Sigma-Aldrich (Johannesburg, South Africa) and the sodium hydroxide pellets were purchased from Associated Chemical Enterprises (Johannesburg, South Africa).

Six explosive compounds of analytical grade namely: nitrobenzene (NB), 1,3-dinitrobenzene (1,3-DNB), 2,6-dinitrotoluene (2,6-DNT), 3-nitrobenzene (3-NT), 2-nitrotoluene (2-NT) and 4-nitrotoluene (4-NT) were purchased from Sigma Aldrich (Steinheim, Germany). From the six explosives, 2,6-DNT, 1,3-DNB and 4-NT were in solid state while the rest were in liquid state.

Deionized water purified using the Milli-Q-R04 system ultrapure (Millipore, Billerica, Massachusetts, USA) was used in preparing all the aqueous solvents. HPLC grade acetonitrile and hexane were purchased from Associated Chemical Enterprises (Johannesburg, South Africa) while ethanol and methanol were purchased from VWR International Ltd (Atlanta, USA) and Merck (Johannesburg, South Africa), respectively.

The *M. oleifera* leaves powder used for producing the extract in this study was purchased from the *Moringa* farms in the Limpopo province, South Africa. The powder was kept in darkness at ambient conditions. A pure *M. oleifera* seed extract oil was purchased from a herbal shop in Johannesburg, South Africa and it was stored in a refrigerator at 4°C.

4.2 Preparing stock solutions

Explosives stock solutions were made by dissolving the compounds in an acetonitrile:water (50:50 v/v) solution. From the stock solutions, the working solutions were prepared regularly through a series of dilutions and used as calibration standard solutions. When not in use, the stock solutions were stored at 4ºC in a refrigerator.

4.3 Instrumentation

Every pH measurements were done using a calibrated Mettler Toledo pH meter (Schwerzenbach, Switzerland). The Elma® Transsonic T460 (Singen, Germany) ultrasonic bath was used for degassing solvents. The Labcon shaker (Krugersdoorp, South Africa) was used for shaking the solutions during adsorption studies. Hettich Rotofix 32A Centrifuge (Tuttlingen, Germany) was utilized for centrifuging. Weighing was done on Kern ABT 220-4NN analytical balance (Stuttgart, Germany).

Brunauer, Emmett and Teller (BET) analysis was used in this study to determine the surface area, pore volume and pore size of the coated and uncoated magnetic NPs. The instrument used was a Micromeritics Flow Prep 060 unit (Aachen, Germany). A mass of 0.2 g of each sample were degassed in N_2 for 4 h at a temperature of 150°C before being analyzed. A Perkin Elmer TGA 6000 (Massachusetts, USA) was used to assess the thermal behaviour of the synthesized magnetic NPs. For the analysis, approximately 10 mg of each samples were heated at a constant rate of 10°C min^{-1} under nitrogen gas, at a flow rate of 50 mL min^{-1}, from 30 – 900°C. Pyris^{TM} software from Perkin Elmer was used for the analysis of data.

A Tensor 27 Bruker FTIR spectrometer (Ettlingen, Germany) was used to identify functional groups on the magnetic NPs and the *M. oleifera* leaf (dehydrated) and seed extracts. The spectra were done at a frequency between 400 and 4000 cm^{-1}. The OPUS® spectroscopy software was used to obtain the IR spectra. Powder X-ray Diffraction (PXRD) was utilized to characterize the crystalline structure of the coated and uncoated magnetic NPs. For this study, the D2 Bruker Powdered X-ray Diffractometer (Karlsruhe, German), using Cu K_α radiation (1.5418 Å) was used to identify the structure of the synthesized magnetic NPs and obtain the size of their crystalline domain structure. The Debye-Scherrer equation shown in Equation 15, was used to estimate the size of the magnetic NPs.

$$\bar{d} = \frac{57.3k\lambda}{\beta cos\theta} \tag{15}$$

where $\bar{d}$ is the average size of the crystalline domain structure. k and λ represents the shape factor and wavelength of radiation equivalent to the CuK_α peak, respectively. β is the line broadening in radian obtained from the full-width at half maximum and θ

represents the Bragg’s angle. The 57.3 value is the conversion of β to degrees from radians given as $\frac{180}{\pi}$.

The GC Agilent© 7890B and MS Pegasus 4D GC X GC from LECO® (Michigan, USA) was used to characterize the organic composition of *M. oleifera* extracts. The column used was a 30 m long BPX-5 fused silica column with 250 µm film thickness. The carrier gas used was helium flowing at 1 mL min^{-1}. A volume of 1 µL of sample was injected in splitless mode at 300°C. The temperature program was set at initial 50°C and ramped to 280°C over a total run period of 10 min. Acquisition was done at a rate of 10 spectra per second for the mass to charge ratio (m/z) of 30 – 800. Ionization of ions was done at -70 eV. A Bischoff HPLC system (Leonberg, Germany) which consists of a HPLC compact pump, Lambda 1010 UV-vis detector set at a maximum wavelength of 254 nm, LC-CaDI interface and a variometer was used. The Ascentis® RP-amide column purchased from Sigma-Aldrich (Johannesburg, South Africa) was used for the separation of the nitroaromatic explosive compounds. Acetonitrile/water (50:50, v/v) was used as a mobile phase in isocratic mode and the flow rate was marinated at 1 mL min^{-1}.

4.4 Calibration of HPLC

A four point calibration was done for the six NAECs in the range of 200 – 1500 µg L^{-1}.The linearity was reported as the coefficient of determination (R^2). The calibration equations were used to quantify the explosive compounds from aqueous samples. Each standard was analysed in triplicate and the standard deviation (SD) denoted the error in the reported values.

4.5 Synthesis of magnetite

Synthesis was done via co-precipitation of ferrous and ferric salts in alkaline medium. This was done by heating 180 mL of an aqueous solution containing 5.6 mmol of Fe^{2+} and 11.2 mmol of Fe^{3+} to 50ºC and then adding 1.5 M NaOH while stirring vigorously for 30 min. The reaction temperature was then increased and maintained at 90ºC for an additional 30 min. This reaction was carried out in inert atmosphere of N_2 to avoid oxidation of the magnetite. This was done by purging the reaction with N_2 gas for 30 min while adding NaOH, after this time the reaction was closed. The final product was a black precipitate that was collected by an external magnetic field. The product was washed with a mixture of water and ethanol (1:1, v/v) and finally dried in a vacuum.

4.6 Preparation of the *Moringa oleifera* leaf extract using PHWE

The setup of the PHWE system is illustrated in Figure 7. Deionized water was used for the extraction of polar and semi-polar metabolites from the *M. oleifera* powder. A total mass of 2.5 g of powder and diatomaceous earth (1:1 w/w) was packed into the extraction cell. This was equivalent to the maximum mass of *M. oleifera* leaves powder that the extraction cell could hold. The extraction was done at an optimized temperature (100ºC) and high pressure (<10kPa). At this temperature and pressure, the polarity of water changes slightly to be less polar at the preheating coil. The water was pumped at 2 mL min^{-1} for the first 10 min to allow the deionized water to fill the cell more rapidly. The flow rate was then decreased to 1 mL min^{-1} and the extract was collected in a collection vial for a further 50 min. The extract was stored in a refrigerator at 4ºC. Before characterization the extracts were kept at ambient temperature for 3 h. The PHWE setup was assembled by installing a PHWE cell in an old Hewlett Packard 5890 GC oven combined with a GenTech 515 HPLC pump (Milford, USA).

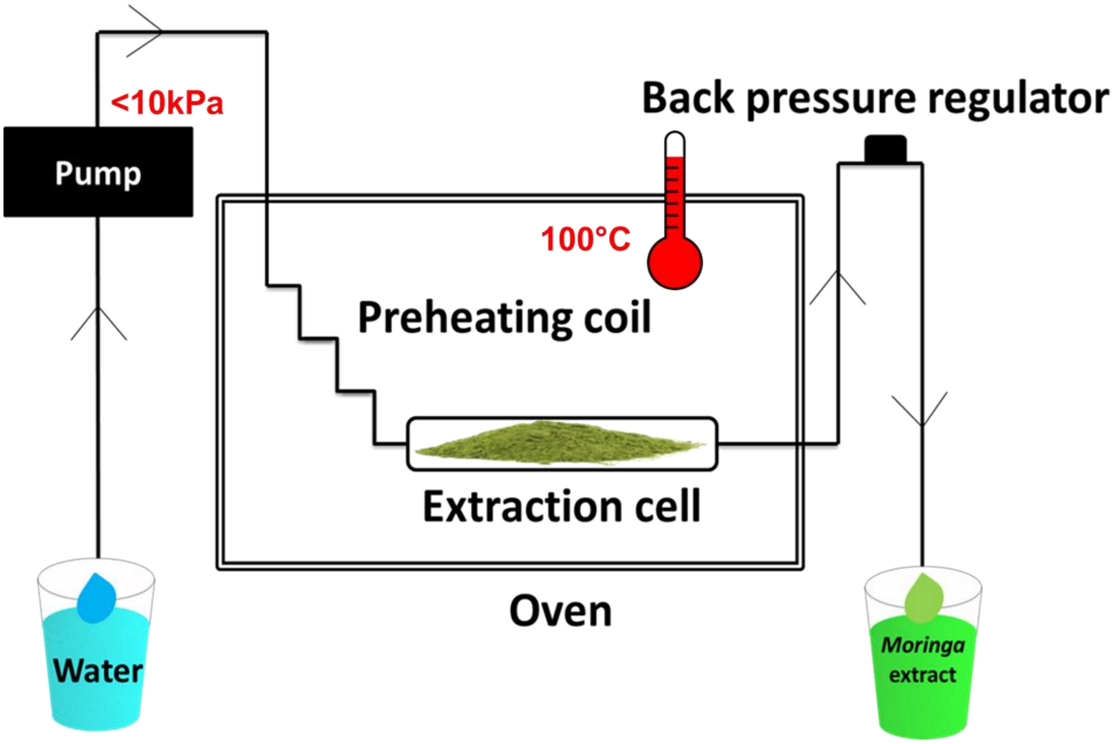


Figure 7: A schematic of a general instrumentation set-up of PHWE system with an extraction cell packed with *M. oleifera* leaves

4.7 Coating of magnetite

A coating procedure previously reported by Kan *et al.* (2010) was used with a few modifications. The magnetite NPs were coated with both *M. oleifera* leaf and seed extracts separately. A volume of 10 mL of leaf extract was added dropwisely in a 6 ml mixture of ethanol and water (1:1 v/v) containing 1 g of Fe_3O_4 nanoparticles. The reaction was stirred vigorously at 70°C for a specific period of time under N_2 gas to avoid oxidation. An external magnetic field was used to collect the product. Ethanol and water (1:1 v/v) were used to wash the coated magnetite and dried under vacuum. A black-dark greenish powder was obtained. For the oily *M. oleifera* seed extract, the same procedure was followed except that the Fe_3O_4 nanoparticles was suspended in 6 mL hexane. Washing of the *M. oleifera* seed extract–coated NPs was also done with hexane. The end product was a black oily paste that was magnetic when an electric field was applied.

The degree of coating over a period of time was optimized. The coating times investigated were 0, 4, 6 and 8 h. The extent of coating was evaluated by comparing the surface area of the coated NPs over time and the overall % weight loss when the coated NPs was heated up to 1000°C. The coating time recorded as 0 h refers to the uncoated Fe_3O_4.

4.8 Optimization experiments

4.8.1 The effect of sample pH

To investigate the effect of pH, the explosive solutions were controlled using 1.5 M NaOH and 0.5 M HCl to attain a pH range of 3 – 11. In 50 mL containers containing 50 mg of adsorbent (magnetic NPs modified with *M. oleifera* leaf extract), 20 mL of pH adjusted solutions of NAECs concentration of 1 mg L^{-1} were then transferred. The mixture was agitated for 40 min at 150 rpm and allowed to stabilize (nano-adsorbent allowed to separate from the solution). An external magnetic field was used to isolate the coated magnetic NPs from the mixture and the filtrate was analysed for NAECs content using high pressure liquid chromatography – ultra violet (HPLC-UV).

4.8.2 The effect of contact time

To investigate the effect of contact time, 20 mL of 1 mg L^{-1} explosive solutions were prepared at the optimized pH. The solutions were transferred in 50 mL containers containing 50 mg of magnetic NPs modified with *M. oleifera* leaf extract. The mixtures were then agitated at 150 rpm at different control times (20 – 150 min). An external magnetic field was applied and the filtrate was analysed for explosive compounds content using HPLC-UV.

4.8.3 The effect of amount of adsorbent

The adsorption was done by transferring 20 mL of 1 mg L^{-1} explosive solutions in a 50 mL container containing coated magnetite ranging between 10 – 120 mg in mass. The optimized sample pH and contact time were utilized. After agitation, a magnetic field was used to separate the magnetite from solution. The solution was then analysed for the content of NAECs from which the adsorbed amount was calculated.

4.8.4 The effect of initial concentration

The adsorption experiments were done by agitating 20 mL of explosive compound solutions containing various initial concentrations (0.2 – 6 mg L^{-1}) in a 50 mL container comprising of the *Moringa* coated magnetic NPs. The pH of the solutions and the mass of the adsorbent were set to those found in the previous optimization studies. Agitation was done at optimum time at 1500 rpm and the adsorbent was separated from the supernatant using a magnetic field. The solution was then analysed for the content of NAECs using HPLC-UV.

4.9 Adsorption experiments

Adsorption of the nitrobenzene (NB), 2,6-dinitrotoluene (2,6-DNT), 1,3-dinitrobenzene (1,3-DNB), 3-nitrobenzene (3-NT), 2-nitrotoluene (2-NT) and 4-nitrotoluene (4-NT) by magnetic NPs coated with *Moringa oleifera* extracts was performed in batch mode. The experiments were done by weighing the nano-adsorbent (uncoated or coated magnetite) in a 50 mL Falcon tube containing 20 mL of the standard solution. The contents were shaken at 150 rpm for a specific period of time. Centrifugation was done for 15 min at 2000 rpm before filtering the contents for analysis using with HPLC–UV.

4.10 Method validation

The magnetite NPs coated with *Moringa* was the method developed in this study and in order to determine the performance of this method, detection limits and linearity were calculated. Calibration curves were done for all the six explosive compounds by measuring standard solutions at a concentration range of 5 – 1500 µg L^{-1} under optimized HPLC-UV conditions.

LODs and LOQs were determined using the S/N ratio method. The S/N ratio for LOD and LOQ is 3 and 10, respectively. All experiments were done in triplicates and the standard deviation (SD) values were used as error bars in all the graphs. The SD (or RSD) was used to represent the level of error in all reported values

4.11 Extraction efficiency

Recovery in extraction was calculated as the fraction of analytes that can be extracted from the sample using the synthesized nano-adsorbent. It refers to the ratio of the amount of explosive compounds in the acceptor phase to the amount of the explosive compounds that was in the sample before extraction was done. Extraction efficiencies were therefore calculated as the ratio of the concentration of each explosive compound before and after extraction using Equation 16.

$$\%E = \frac{(C_0 - C_e)}{C_0} \times 100, \qquad (16)$$

CHAPTER V: RESULTS AND DISCUSSION

Results on the preparation of magnetic nanoparticles, its coating with *Moringa. oleifera* extracts as well as its characterization are discussed in this chapter. Optimum values for an efficient adsorption of explosive compounds are discussed. The end of the chapter discusses the kinetic and adsorption isotherms as well as the method validation results.

5 Results and discussion

5.1 Synthesis and characterization of magnetic NPs coated with *Moringa oleifera* extracts

5.1.1 Synthesis of magnetic nanoparticles

The co-precipitation of ferrous and ferric solutions in the presence of NaOH produced a black magnetite which could be collected by an external magnetic field. Figure 8 shows the magnetic nanoparticles when exposed to a magnetic field.

Figure 8: The response of the Fe_3O_4 due to a magnetic field.

The synthesis of magnetite was done in an aqueous environment, hence there is a possibility that the hydroxyl ions or water adsorbed on the surface of the magnetite. This process is shown by the illustration in Figure 9. When magnetite nanoparticles are in a basic aqueous solution, the uncoated Fe_3O_4 would absorb OH^- on the surface. This process leads to an OH^- rich surface.

According to a study by Betancur *et al.* (2004) on the effect of water concentration on the magnetic properties, the magnetic response on a magnetite decreases with increasing water concentration. The adsorption of the hydroxyl ion produces more vacancies in the nanoparticles, hence the magnetic interactions are better than when there is water on the surface.

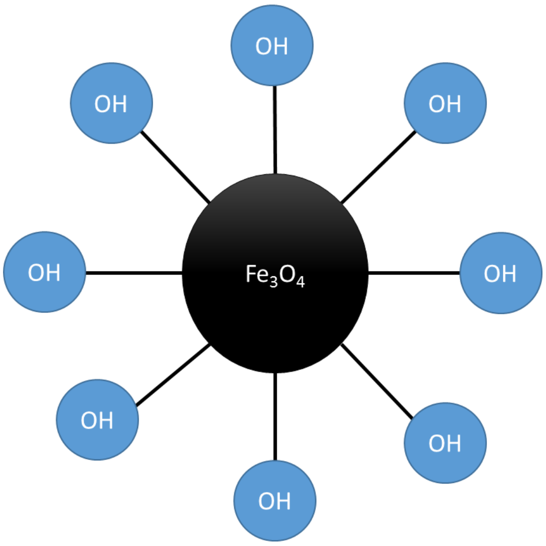


Figure 9: Adsorbed hydroxyl ions on the surface of the magnetite

Powder X-ray diffraction analysis

The diffraction patterns of the magnetite and the *M. oleifera* coated magnetite are shown in Figure 10. It can be seen that there is diffraction peaks that occur at $2\theta = 21.3°$, 35.1°, 41.4°, 50.6°, 63.0°, 67.3° and 74.1°. These peaks correspond to the spinel structure of general magnetite diffraction pattern. Figure 10 shows that there are no other diffraction peaks matching that of the other iron oxide NPs such as hematite and maghemite. This means that the synthesized particles are pure magnetite, however, there were some impurities shown with peaks visible at $2\theta > 63.0°$. The PXRD pattern of the *M. oleifera* coated magnetite is similar to that of the uncoated magnetite. This indicates that the modification process with the *M. oleifera* extract did not alter the core structure of the magnetite.

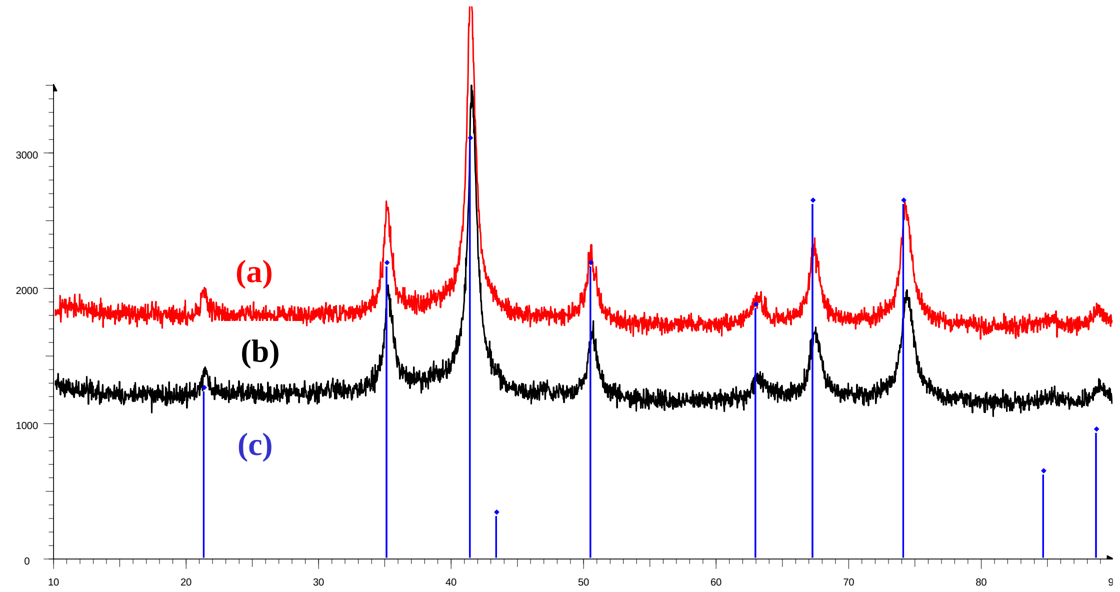


Figure 10: The PXRD pattern of the (a) synthesized magnetite, (b) synthesized magnetite coated with *M. oleifera* extract and (c) the standard XRD pattern for magnetite

The Debye-Scherrer equation (Equation 15), was used to estimate the size of the magnetic NPs. The crystalline size of the magnetic nanoparticles was found to be 12.14 nm, as calculated from the Debye-Scherrer equation. The particle size of the magnetic nanoparticles after coating with the *M. oleifera* leaf and seed extracts, as calculated from the Scherrer equation was found to be 13.37 and 14.28 nm, respectively (see Appendix). This means that the coating increased the particle size of the magnetite by 1.23 and 2.14 nm and that the coating procedure was successful. In studies by Giri *et al.* (2011), Petcharoen and Sirivat (2012), the particle size of desiccated magnetite samples was found to be 7.53 nm and 16.8 nm, respectively.

5.1.2 Modification of magnetite with *M. oleifera* leaf and seed extracts

The magnetic NPs coated with *M. oleifera* leaf extract produced a black powder. The yield % of the reaction was found to be 89.21 ± 2.51. When the magnetic nanoparticles were coated with *M. oleifera* seed oil extract the yield % of the reaction was found to be 67.53 ± 1.89. This reaction was less efficient compared to coating with the leaf extract.

The low yield was due to difference in polarity between the magnetite and the *Moringa* seed oil. The magnetite after synthesis has a polar surface due to the hydroxyl groups on its surface as shown in Figure 9, while the seed oil is non-polar. The reaction produced a black paste instead of a powder after drying. There have been studies where magnetite has been successfully modified with silica (Girginova *et al.*, 2010), aminosilane (Ma *et al.*, 2003) and 3-aminopropyltriethoxysilane (Shen *et al.*, 2004) to which in all cases a black powder was produced. The magnetic response of the modified magnetite was tested using a magnet's magnetic field. The response of the modified nanoparticles to the magnet was significant, indicating that the coating barely affected its magnetism.

FTIR analysis

The FTIR spectra of the synthesized magnetite, the coated magnetite with *M. oleifera* leaf extract and the *M. oleifera* leaf extract is shown in Figure 11. The peak at 565 cm^{-1} for the uncoated Fe_3O_4 can be assigned to Fe–O vibrations. A broad peak at 3289 cm^{-1} is due to the OH groups on the surface of the magnetic nanoparticles. The peak at 1347 cm^{-1} indicates the existence of a C–H group.

The FTIR spectrum of *M. oleifera* leaf extract shows a presence of a broad peak at 3233 cm^{-1} which can be allocated to the stretch of the water molecules. The water stretch for the *M. oleifera* leaf extracts due to water used in the extraction process using PHWE. This broad peak for the extract may have overlapped with other peaks such as the one for the –OH group from the carboxylic acid. The sharp band present at 1580 cm^{-1} for the *M. oleifera* extract can be assigned to the C=C stretch for aromatics. There is a band with a medium intensity at 1397 cm^{-1}, which can be assigned to C–H bend for $-CH_3$ in the extract. The sharp peak at 1045 cm^{-1} can be assigned to the $-O-CH_3$ group.

The peak at 561 cm^{-1} for the coated Fe_3O_4 indicates the Fe–O stretch. New functional groups were observed for the coated magnetite with *M. oleifera* leaf extract. The peak at 1079 cm^{-1} can be assigned to C–O group from ethanol. The medium peak at 1389 cm^{-1} for the coated magnetite indicates the presence of the $-CH_3$ group. A striking feature on the spectrum of the coated magnetite compared to the uncoated magnetite is the presence of the C=C stretch at 1605 cm^{-1}. The broad band at 3229 cm^{-1} indicates the existence of water molecules or –OH groups on the surface.

There is a general shift in the wavenumber for the functional groups due to samples being in different media. The relative transmittance weaken when the *M. oleifera* seed oil extract is coated on Fe_3O_4 because the functional groups are interacting with the magnetite leading to a lesser vibrational frequency. This indicates that there coating procedure was successful. (Ma *et al.*, 2003; Gotić and Musić, 2007; Klokkenburg, Hilhorst and Erne, 2007) assigned the Fe–O vibration peaks for magnetite at 404 and 586; 590 and 635; 357 and 570 cm^{-1}, respectively. Another diffraction peak at around 300 – 400 cm^{-1} was not indicated in this study due to working range of 500 to 4000 cm^{-1}. According to Sharma and Paliwal (2013) the broad peak in the range of 2400 – 3400 cm^{-1} can be assigned to the –OH group. Sharma and Paliwal (2013) performed a study on *M. oleifera* pods and the FTIR spectra of the saponins correspond to the functional groups identified.

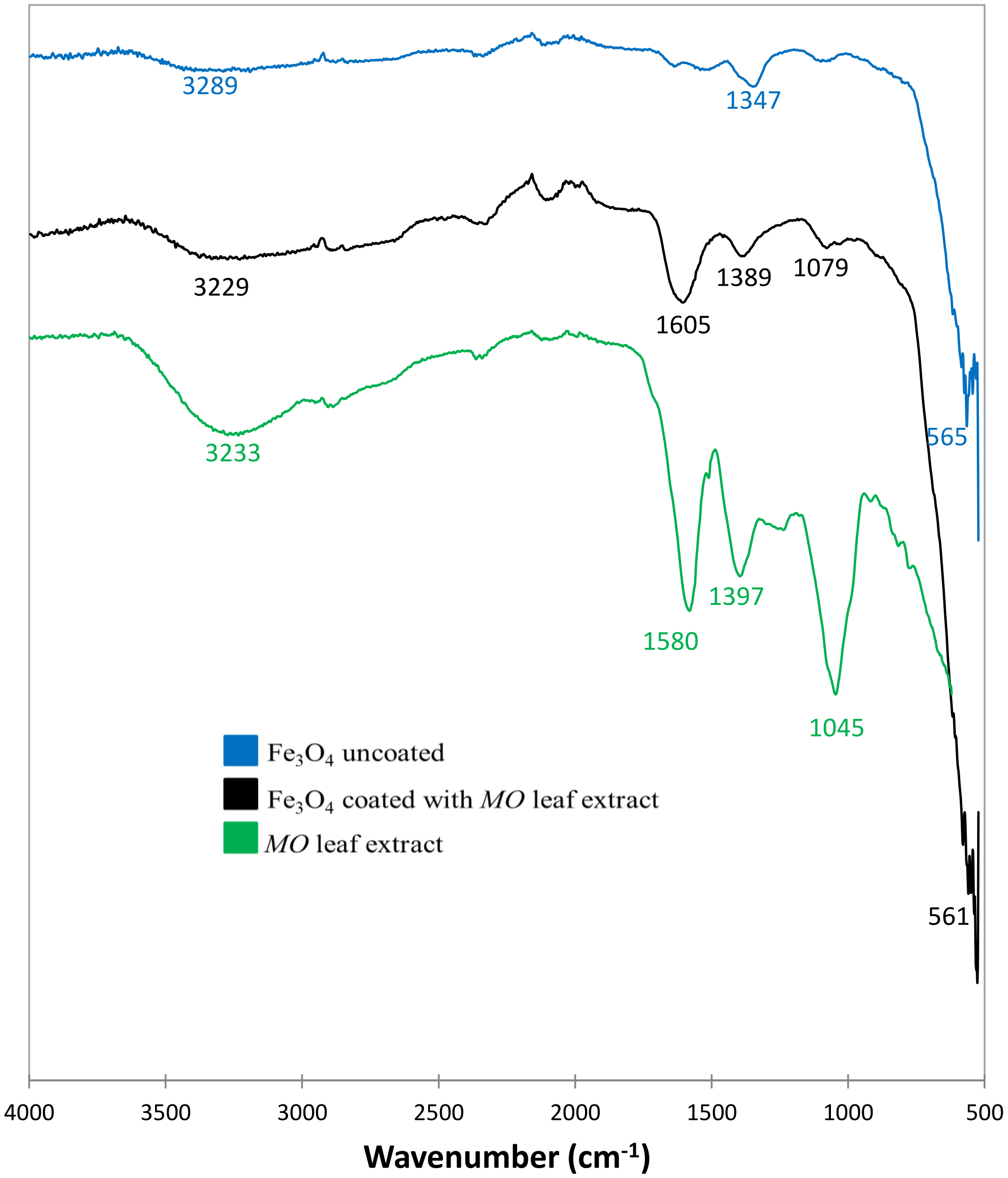


Figure 11: The FTIR spectra of the uncoated Fe_3O_4, coated Fe_3O_4 with *M. olefera* leaf extract and the desiccated *M. oleifera* leaf extract (*MO* – *Moringa oleifera*)

The FTIR spectra of the magnetite, modified magnetite with *M. oleifera* seed oil extract and the *M. oleifera* seed oil extract is shown in Figure 12. The strong peak at 2922 cm^{-1} for the *M. oleifera* seed oil extract can be assigned to the C–H stretch and the strong peak at 2853 cm^{-1} verifies the presence of carboxylic acid OH group. The sharp peaks at 1744 cm^{-1} indicates the presence of the C=O ester/carboxylic group in the seed extract. In the FTIR spectrum, the sharp peak for the $=CH_2$ group can be seen at 1464 cm^{-1}. The weak band at 1376 cm^{-1} can be assigned to the $–CH_3$ group. The peaks at 1161 and 1237 cm^{-1} indicates the existence C–O group of an ester and also >C–O group of an ether, respectively. The C–H bending can be assigned to the strong band at 721 cm^{-1}.

The peak at 555 cm^{-1} for the coated magnetite with *M. oleifera* seed oil extract can be assigned to the Fe–O stretch of Fe_3O_4. The two peaks at 2909 and 2849 cm^{-1} indicate the existence of C–H and carboxylic acid OH group, respectively. The sharp peak at 1778 cm^{-1} can be assigned to the C=O in an ester or carboxylic group in the seed extract. The weak band at 1377 cm^{-1} can be assigned to the $–CH_3$ group. The peak at 1157 cm^{-1} indicates the presence of the C–O group of an ester. The organic functional groups are not present in the uncoated magnetite, hence, are generated due to the coating.

Normally there is a shift in the wavenumber for the functional groups due to samples being in different media. The relative transmittance diminish when the *M. oleifera* seed oil extract is coated on Fe_3O_4 because the functional groups are interacting with the magnetite leading to a lesser vibrational frequency. In studies by Patel and Patel (2011); Sharma and Paliwal (2013) in which *M. oleifera* seed pods and gum were characterized for the same functional groups that include hydroxyls, carboxylic acid, alkynes, alkenes, ethers and esters were found.

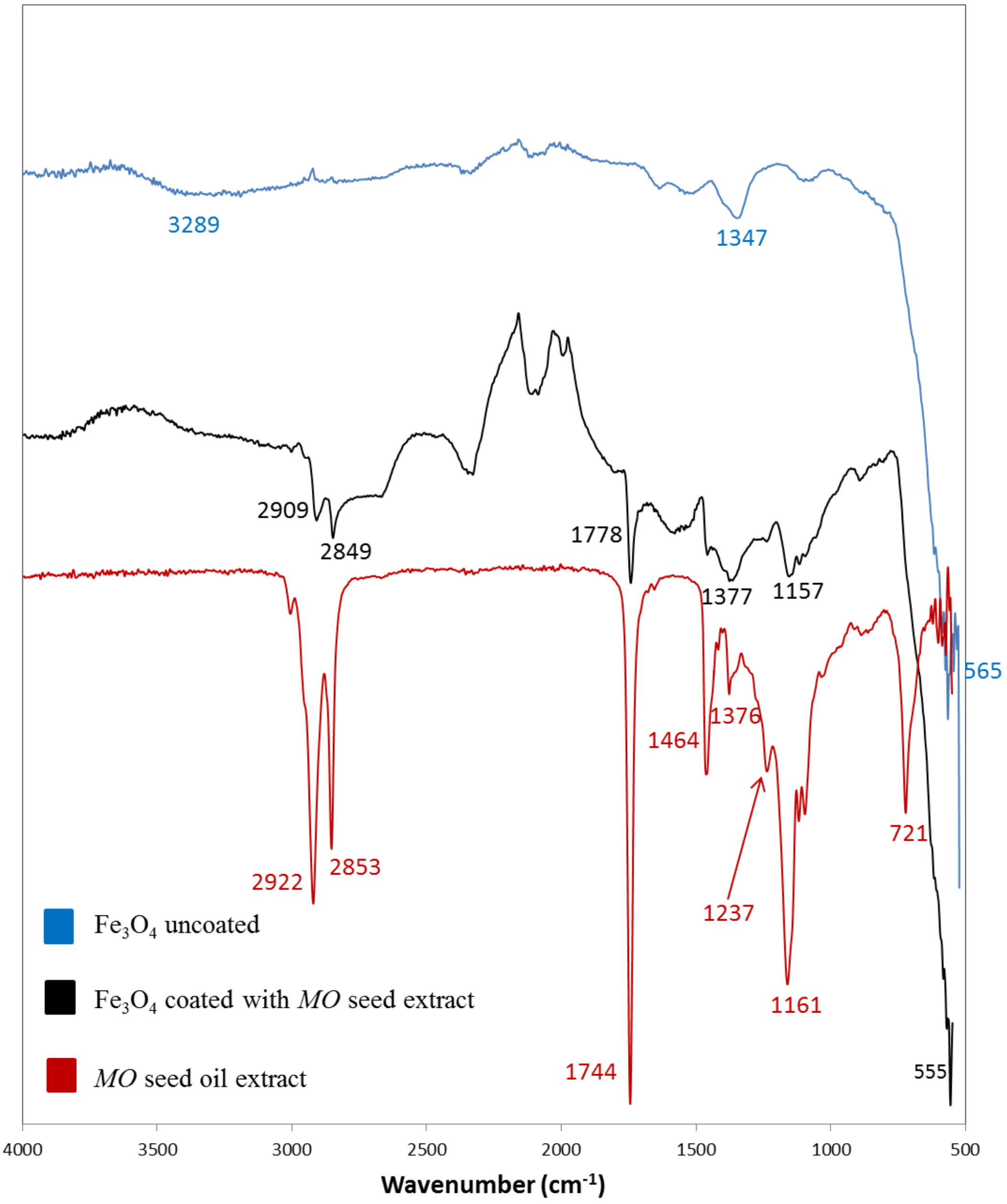


Figure 12: The FTIR spectra of the uncoated Fe_3O_4, coated Fe_3O_4 with *M. oleifera* seed extract and the *M. oleifera* seed oil extract (*MO – Moringa oleifera*)

BET analysis

The BET analysis results for surface area, pore size and volume are shown in Table 5. It is observed that coating with the extracts reduces the surface area of the magnetite to almost half. The coating increases the particle size, hence, the surface area is reduced. The magnetite coated with the seed extract has a slightly higher surface-to-volume ratio than the magnetite coated with the leaf extract indicating that the coating layer of the leaf extract is larger and has a higher particle size. The pore size increases inversely with the increase in surface area. This means that a large coating generates a larger pore size.

Table 5: BET results of the uncoated magnetite and coated magnetite with *M. oleifera* leaf and seed extract

Fe_3O_4 samples	Uncoated Fe_3O_4	Fe_3O_4 – *MO* leaf extract	Fe_3O_4 – *MO* seed extract
Surface Area ($m^2\ g^{-1}$)	75.6439	31.1806	37.3288
Pore Volume ($cm^3\ g^{-1}$)	0.363962	0.216882	0.222424
Pore Size (nm)	19.09011	27.69207	23.90404

MO – Moringa oleifera

Thermal stability using thermogravimetric analysis (TGA)

The thermal stability of the samples was used to clarify the structure of the uncoated and coated Fe_3O_4. Figure 13 and 14 shows the TGA curves of the uncoated and coated Fe_3O_4. An initial decomposition is observed at 80 – 100°C, which can be attributed to loss of water. The effect is more pronounced for uncoated Fe_3O_4 and Fe_3O_4 coated with *M. oleifera* leaf extract. These water molecules are from the surface of the hydroxylated Fe_3O_4 and the *M. oleifera* leaf extract used as the coating. The presence of water molecules in the leaf extract coating is indicative that it is relatively polar. The minimal loss of weight of the *M. oleifera* seed oil coated Fe_3O_4 might be due to the lack of water molecules on the surface, an indication that the seed extract is relatively lipophilic.

The maximal decomposition of the synthesized Fe_3O_4 and Fe_3O_4 coated with *M. oleifera* extracts occurred at a temperature of 220 and 260ºC, respectively. The loss in weight of the uncoated magnetite at 220°C might be attributed to dehydroxylation of hydroxyl groups in the core of the Fe_3O_4. Decomposition of the coated Fe_3O_4 is due to the organic coating of the *M. oleifera* extracts. The decomposition pattern also show that there is further loss of weight of the coated Fe_3O_4 at 300 and 340°C for the leaf extract coating and at 320°C for the seed extract coating. This is indicative of molecules within the extracts that bind more tightly to the Fe_3O_4 nanoparticles. These molecules are different for the leaf and seed oil extracts. Beyond 400°C, both the uncoated and coated magnetite remained stable.

In Figure 14, it can be shown that the remaining weight of the uncoated magnetite was 85%. For the coated magnetite it was 69% for the leaf extract and 75% for the seed extract, respectively. This meant that the other percentage was from water, hydroxyl groups and the organic coating from the *Moringa* extracts that decomposed due to the heating process. Leaf extract coated Fe_3O_4 experienced more loss in weight than the seed oil coated Fe_3O_4 because the coating with the leaf extract produced a larger coating layer. At temperatures above 400°C, there was a total decomposition of the organic surface leaving a very stable Fe_3O_4 core since its decomposition temperature is 2623°C.

The TGA results are an indication that the *M. oleifera* extracts coating remains attached and stable on the surface of the nanoparticles up to 260°C. The functionalized nano-adsorbent can therefore be applied successfully under all environmental conditions without problems of disintegration.

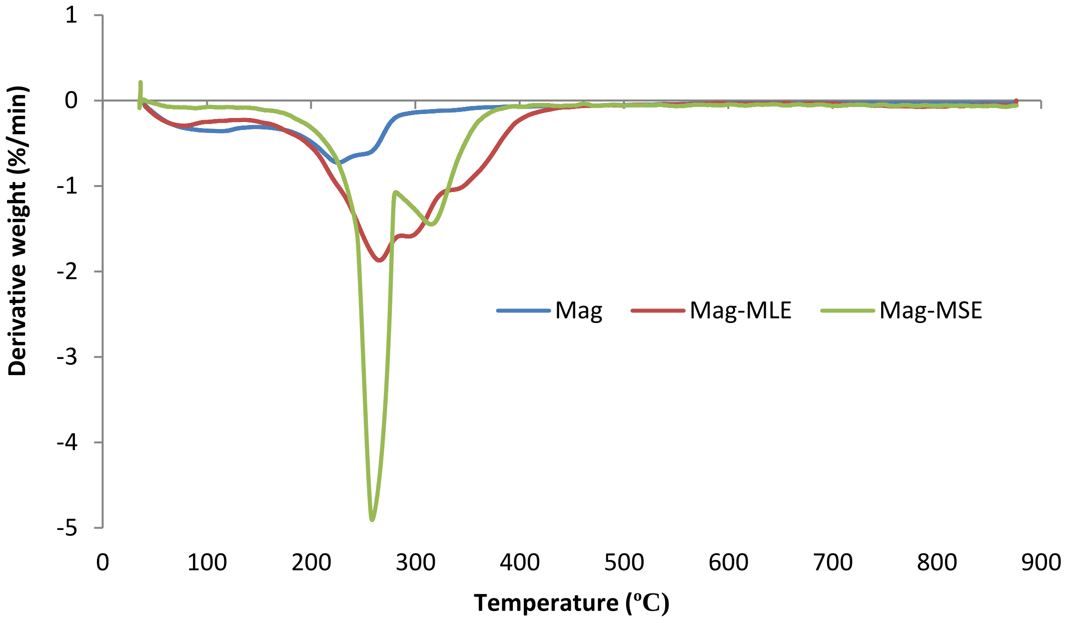


Figure 13: The first derivative weight of the *M. oleifera* coated magnetic nanoparticles and uncoated magnetic nanoparticles (Keys: Mag – Uncoated Fe_3O_4; Mag-MLE – Fe_3O_4 coated with *M. oleifera* leaf extract; Mag-MSE – Fe_3O_4 coated with *M. oleifera* seed extract)

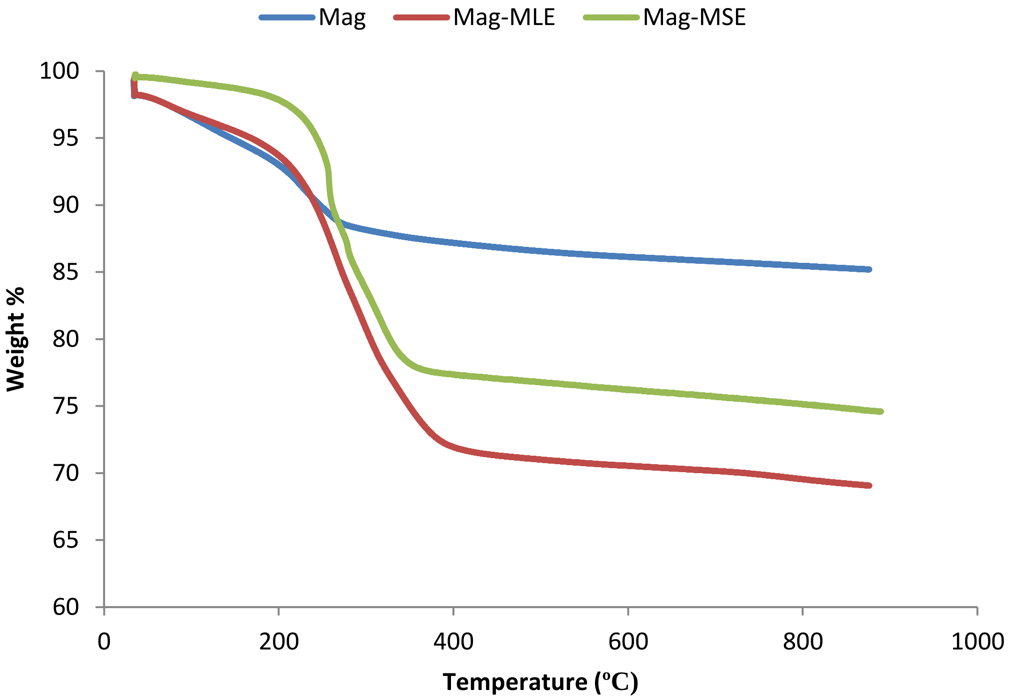


Figure 14: Thermo-gravimetric analysis of magnetic nanoparticles (Keys: Mag – Uncoated Fe_3O_4; Mag-MLE – Fe_3O_4 coated with *M. oleifera* leaf extract; Mag-MSE – Fe_3O_4 coated with *M. oleifera* seed extract)

5.2 Characterization of *M. oleifera* extracts using GC-MS

Gas chromatography-time-of-flight mass spectrometry (GC-TOF/MS) was used to identify some organic compounds in the *M. oleifera* extracts that are known to have high affinity for nanoparticles. One of the significant compounds detected in both extracts was oleic acid as shown in Table 6. Oleic acid has been used in many studies during synthesis of magnetic NPs to inhibit the degree of aggregation and decrease oxidation in magnetic NPs (Liang *et al.*, 2014; Shete *et al.*, 2015; Celis *et al.*, 2017). Aggregation causes the magnetite NPs to lose their monodispersity while oxidation of Fe^{2+} has been known to decrease the degree of magnetism of Fe_3O_4 (Rafiee *et al.*, 2014). Oleic acid is preferred as a coating agent mainly because it is non-toxic and readily accessible (Tadmor *et al.*, 2000; Wu *et al.*, 2004; Li *et al.*, 2010). It has a high affinity for magnetic NPs through its carboxyl (–COOH) group that interacts with the iron cations. The flexible hydrocarbon tail helps to stabilize the interaction by enclosing the NPs. This inhibits the aggregation of the particles.

Generally, it was observed that *M. oleifera* seed extract contained more than six times the amount of oleic acid than the *M. oleifera* leaf extract, as represented by the peak areas in Table 6. In addition, cis-vaccenic acid was also detected in the *M. oleifera* seed extract. This indicates that the seed extract inhibit aggregation of the NPs better as compared to the leaf extract. The coating of magnetite with *M. oleifera* seed extract enhanced monodispersion and surface area. This is also supported by the BET surface area results in Table 5.

Figure 15 shows the similarity in the structures of oleic acid and cis–vaccenic acid. According to Hainfeld (2011), amphipathic molecules such as, oleic acids, cis-vaccenic acid and other moieties having the same chemical structure can be utilized for solubilizing NPs in hydrophilic media. Solubilizing can mean coating of the NPs by direct interaction leading to the surface of the NP covered in the amphipathic or hydrophobic moiety. Even though studies on cis-vaccenic acids are not found, it has a similar chemical structure and formula as the oleic acid and can possibly be used for effective coating of magnetic NPs.

Different tocopherols were also identified in *M. oleifera* seed extracts. The three tocopherol found are given in Table 6. Tocopherols (also generally named as vitamin E) are a group of lipid compounds which are found mostly in fruits, nuts, oils and vegetables (Bramley *et al.*, 2000). Sterols were also detected in the *M. oleifera* seed extract, mainly stigmasterol and β-sitosterol. Previous studies have shown that these two compounds contribute the highest compositions of sterols in *Moringa* seed oil. For example, Panfili *et al.* (2003) have found that stigmasterol and β-sitosterol contribute 19.0% and 46.65% of the total sterols in *Moringa* seed oil. The tocopherols and sterols are responsible for the hydrophobic/lipophilic nature of the surface of the seed coated magnetite. The lack of water molecules on the *M. oleifera* seed extract is also seen in the TGA result at the initial decomposition at 80 – 100°C (Figure 14). There were many other compounds found the *M. oleifera* samples (see Appendices, Figure A1 and A2), however, those mentioned were the major organic compounds found and other peaks were interference (from the column material – siloxane) in the GC-MS instrument.

Table 6: Characterization of *Moringa* extracts using GC-MS

Moringa oleifera leaf extract

Name	Retention time (min)	Area	Weight	Formula
Oleic acid	14.13	4.49×10^7	282	$C_{18}H_{34}O_2$

Moringa oleifera seed extract

Name	Retention time (min)	Area	Weight	Formula
Oleic acid	12.59	2.92×10^8	282	$C_{18}H_{32}O_2$
cis–Vaccenic acid	17.30	1.02×10^8	282	$C_{18}H_{32}O_2$
δ–Tocopherol	18.48	2.14×10^7	402	$C_{27}H_{46}O_2$
γ–Tocopherol	19.33	1.53×10^7	416	$C_{28}H_{48}O_2$
α–Tocopherol	20.16	6.20×10^7	430	$C_{29}H_{50}O_2$
Stigmasterol	21.53	2.34×10^8	412	$C_{29}H_{48}O$
β–Sitosterol	22.41	3.46×10^8	414	$C_{29}H_{50}O$

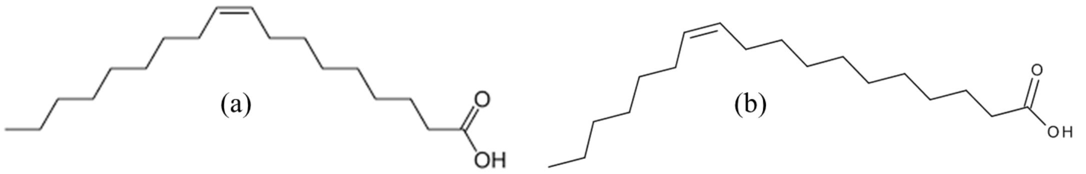


Figure 15: Structures of (a) oleic acid and (b) cis–vaccenic acid

5.3 Optimization of the coating procedure

5.3.1 BET analysis

The difference in the surface area, pore volume and size of the samples are shown in Table 7. Under the experimental conditions specified, it was observed that the surface area decreased with increased coating time. The pore volume also decreased with coating time. This indicates that as more time is given for coating, the more organic capping groups are attached to the magnetite. Thus, increase in the thickness leads to particle size of the magnetite NP to becoming larger. This causes the surface area to a decrease. Similar observations have been made by Raj and Viswanathan (2009) & Shen *et al.* (2009).

Table 7: BET analysis of magnetite at different coating times at 0, 4, 6 and 8 h

Coating time	0 h	4 h	6 h	8 h
Surface Area (m^2 g^{-1})	75.6439	36.62563	31.1806	27.63257
Pore Volume (cm^3 g^{-1})	0.363962	0.192537	0.216882	0.180787
Pore Size (nm)	19.09011	24.65532	27.69207	27.24541

5.3.2 Thermo-gravimetric analysis (TGA)

Figure 16 shows the TGA spectra of magnetite NPs after coating with *M. oleifera* leaf extract at various coating times. It was observed that at the last temperature recorded (>1000°C), the remaining weight of magnetic nanoparticles was 85.2, 71.3, 69.1 and

67.9% for 0, 4, 6 and 8 h, respectively. The lost percentages were from the decomposed organic surface coating agent from *M. oleifera* or water and hydroxyl groups in the case of the uncoated Fe_3O_4. The % weight loss increase with increased coating time because more organic extract continues to accumulate on the surface of the NPs. However, after 6 h the increase is insignificant with the recorded weight dropping from 69.1% to 67.9% after 8 h of coating time. Thus in this study, the coating time of 6 h was used for the coating of magnetite NPs with *Moringa* extracts.

Similar coating times have been reported by Ma *et al.* (2003) who coated their magnetite NPs with amino silane for 7 h. Better coating times of 4 h, 2½ h and 30 min have been reported (Madrakian *et al.*, 2012; Tavengwa *et al.*, 2017).

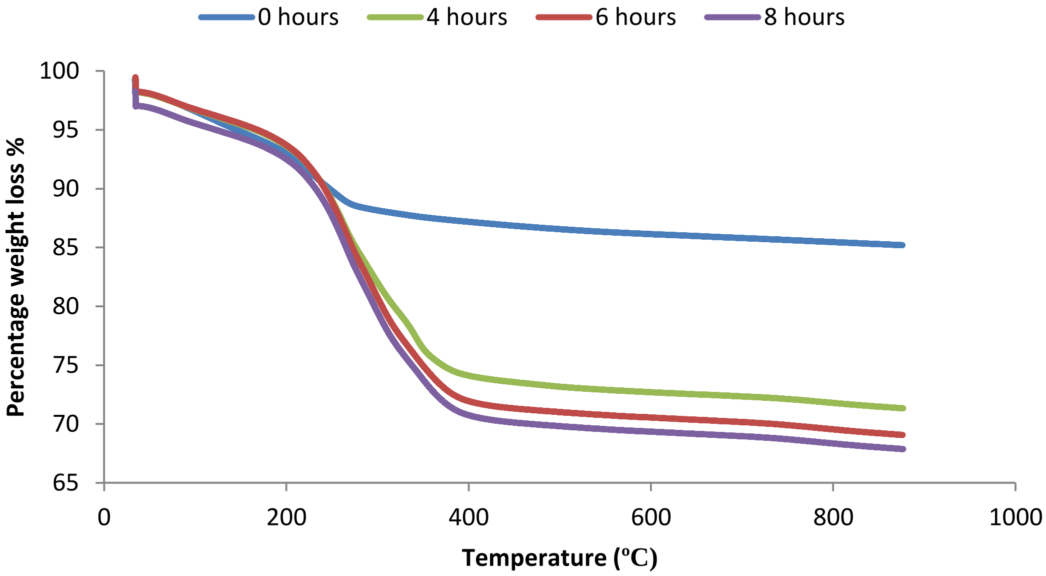


Figure 16: Thermo-gravimetric analysis of the magnetic nanoparticles at different coating times: 0, 4, 6 and 8 h

5.4 Optimization parameters for the extraction of nitroaromatic explosive compounds

5.4.1 The effect of sample pH

The results of the effect of sample pH on the removal of NAECs are represented in Figure 17. It is observed that the maximum adsorption transpired at pH 8. The adsorbent was prepared under alkaline conditions which implies that it is stable under these conditions. Under extreme basic conditions ($pH \geq 9$), the surface of the nano-adsorbent is expected to be more negatively charged. Efficiency is affected by the repulsive interaction between the surface of the NPs and the explosives due to the presence of the delocalized electrons on the $–NO_2$ group. Under acidic conditions ($pH < 7$), the stability of the coated nano-adsorbent is affected which might result in the degradation of the organic coating. The reduced stability of the nano-adsorbent might affect its stability. pH 8 therefore appears as a compromise between the effects of stability (acidic conditions) and surface charge (basic conditions) of the NPs. Similar studies have found the pH to be neutral (Tavengwa, 2013; Chen and Chen, 2015). In a study by Tavengwa *et al.* (2016), pH of 6 was taken as a compromise.

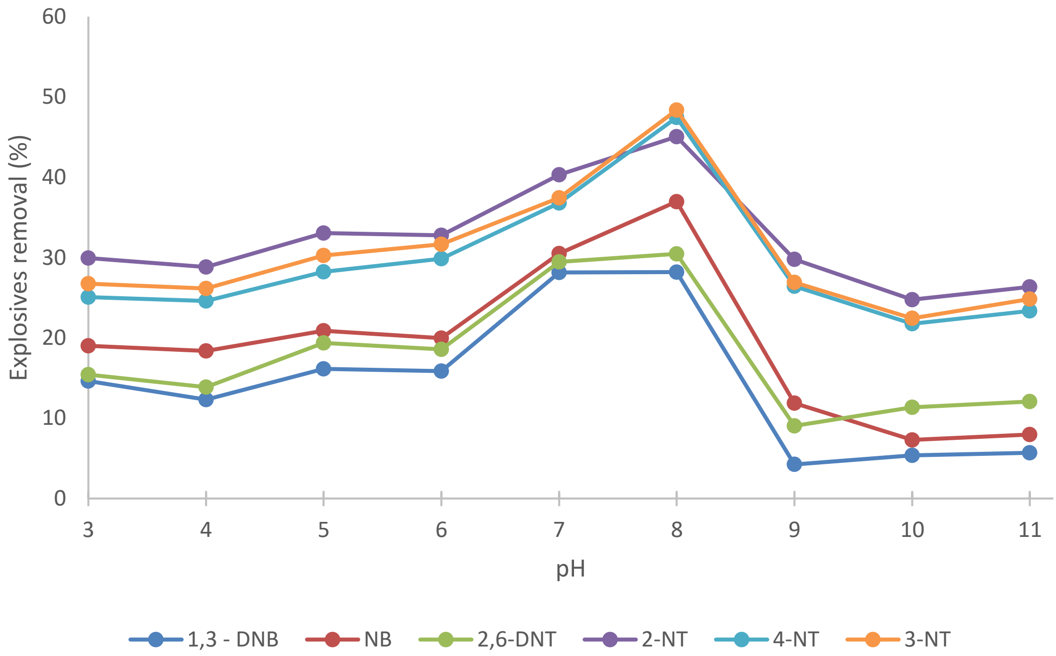


Figure 17: Effect of pH on the removal of NAECs (n = 3; SD < 0.080). Optimization conditions: Coated magnetite amount, 50 mg; Explosives concentration, 1 mg L^{-1}; Sample volume, 20 mL; Contact time, 40 min; Temperature, room temperature; Shaking speed, 150 rpm

5.4.2 The effect of contact time

Figure 18 shows the correlation between contact time and the % removal of explosive compounds by the nano-adsorbent. The increase in time from 20 to 150 min lead to the increase in extraction efficiency of the NAECs until equilibrium was reached. The optimum contact time was taken as 120 min because after this time no significant removal was observed. Hence, the agitation time of 120 min was selected for further experiments. Longer extraction times for NAECs have been reported in previous studies where a bamboo charcoal with $ZnCl_2$ (Fu *et al.*, 2015) and vermicompost-biochar (Yang *et al.*, 2015) were used as adsorbents and the optimal time for sorption was found at 3 and 24 h, respectively. There is a radical increase between 60 – 100 min for 2,6-DNT and 4-NT, however, this increase cannot be clearly explained. Nevertheless, it can be assumed that the differences in selectivity plays a role.

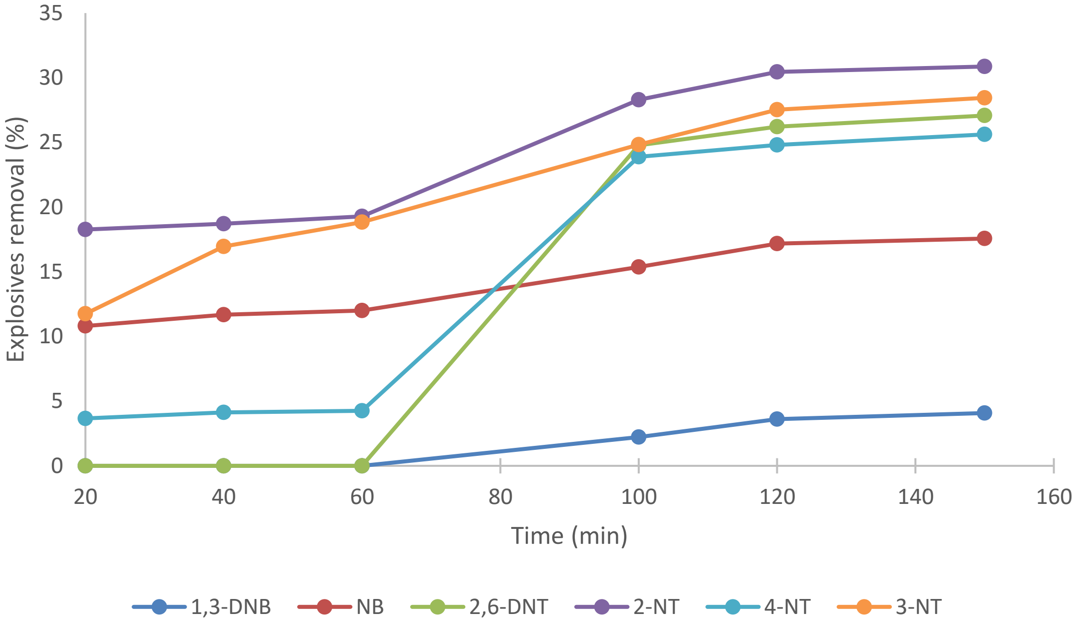


Figure 18: Effect of contact time on the removal of NAECs (n = 3; SD < 0.072). Optimization conditions: Coated magnetite amount, 50 mg; Explosives concentration, 1 mg L^{-1}; Sample volume, 20 mL; Sample pH, pH 8; Temperature, room temperature; Shaking speed, 150 rpm.

5.4.3 The effect of amount of adsorbent

Figure 19 demonstrates how the amount of NAECs adsorbed is dependent on the mass of the adsorbent used. It is observed that with the increasing quantity of the magnetite adsorbent, the extent of removal of explosive compounds increased gradually to nearly a consistent value. The increased availability of the magnetite adsorbent lead to a greater possibility of surface contact between the adsorbent and the explosive compounds. For most of the NAECs the maximum adsorption capacity was found when the mass of the coated magnetic NPs was roughly at 100 mg and this mass was taken as the optimum quantity.

In a study by Madrakian *et al.* (2012) an optimum 20 mg of the magnetic NPs coated with tea waste was found for the adsorption of organic dyes. A study by Tavengwa *et al.* (2017) found 100 mg of the magnetic polymer to be effective for the removal explosive compounds.

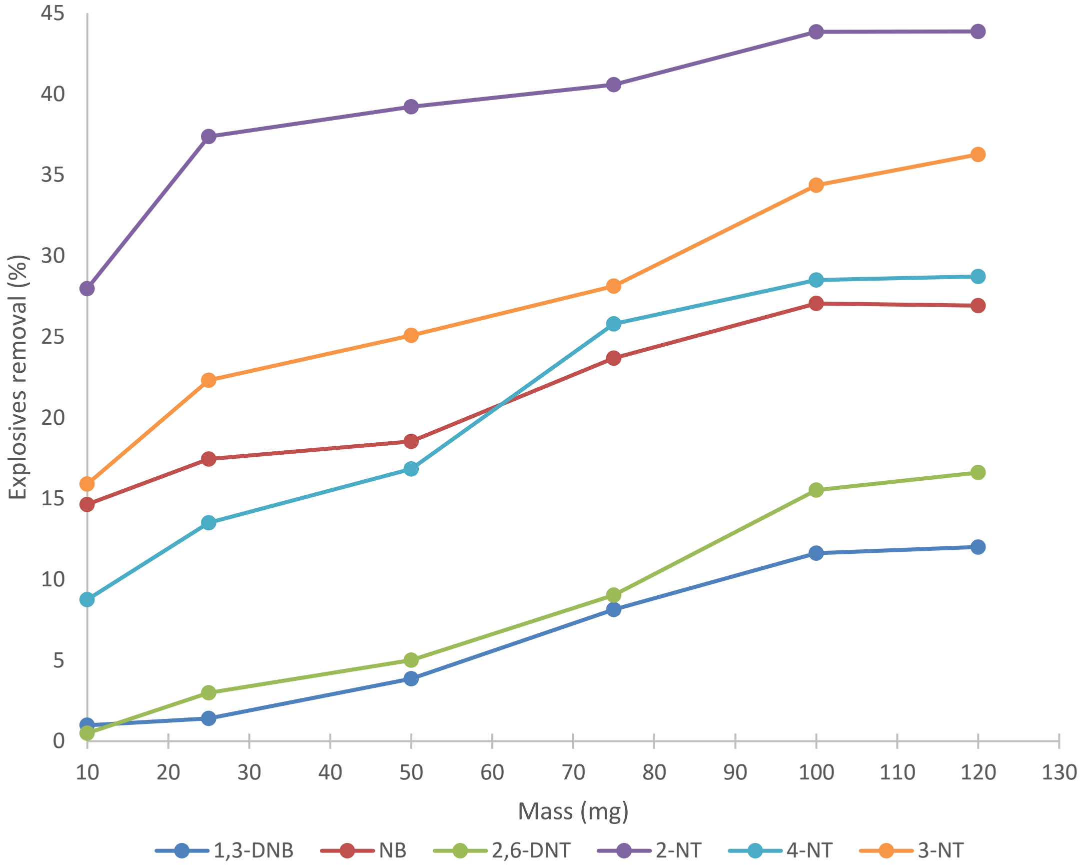


Figure 19: The removal of explosive compounds obtained by varying the mass of the adsorbent at the range of 10 – 120 mg (n = 3; SD < 0.074). Optimization conditions: Explosives concentration, 1 mg L^{-1}; Sample volume, 20 mL; Sample pH, pH 8; Temperature, room temperature; Time, 120; Shaking speed, 150 rpm.

5.4.4 The effect of initial concentration

The dependence of the adsorption capacity of NAECs due to the variation in the initial concentration is illustrate in Figure 20. An increasing trend is also observed from this experiment. The adsorption capacity increased with the increase in the concentration of the NAECs. This trend is due to the increasing number of the explosive compounds in the solution which competed for the fixed number of binding sites on the coated magnetic NPs. The constant loading of the explosive compounds per quantity of the magnetic NP practically increased in a linearly fashion as the initial concentration of the NAECs increased in the solution. The sites on the magnetic NPs were saturated for almost all the NAECs after 4 mg L^{-1} except NB which kept increasing linearly. NB is the smallest compound out of all the explosive compounds and this could mean it binds to those vacant sites on the coated magnetite NPs which cannot be occupied by other larger explosives. At saturation point, the nano-adsorbent has reached its maximum adsorption capacity, therefore, there is minimal increase in the amount of adsorbed NAECs. It was also observed that the binding efficiency of the coated magnetic NPs was expectedly greater than that of the uncoated magnetic NPs. The loading of NAECs per unit mass of the magnetic NPs increased upon increasing the initial concentration of NAECs in solution up to a maximum adsorption capacity of between 0.45 and 0.76 mg g^{-1}.

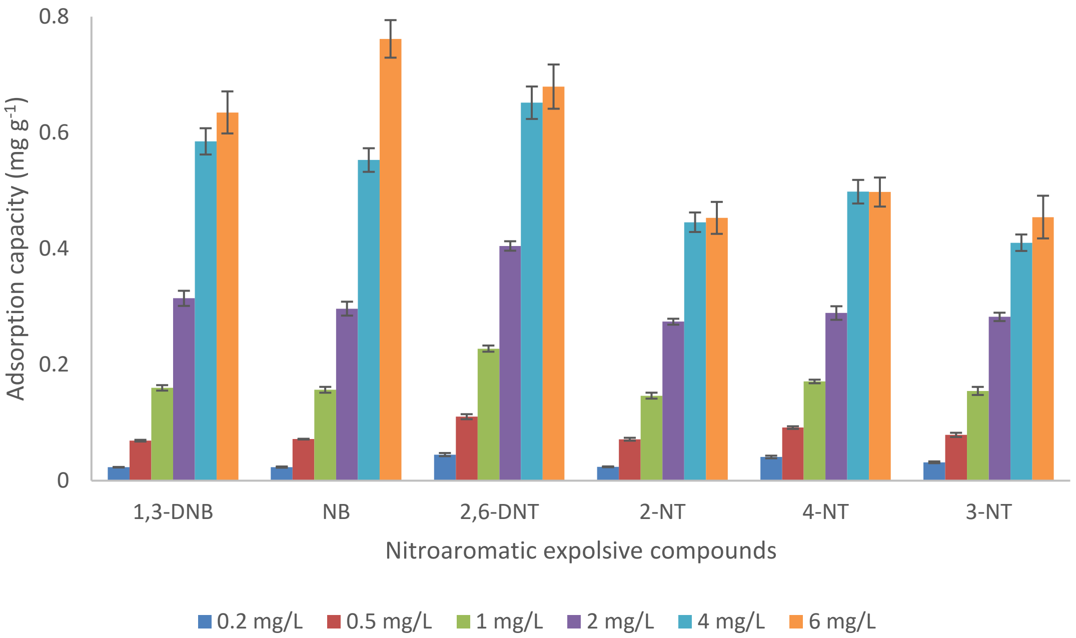


Figure 20: Effect of initial on the removal of NAECs (n = 3; SD < 0.144). Optimization conditions: Explosives concentration, 0.2 – 6 mg L^{-1}; Mass of adsorbent, 100 mg; Sample volume, 20 mL; Sample pH, pH 8; Temperature, room temperature; Time, 120; Shaking speed, 150 rpm.

Under optimized experimental conditions, the maximum adsorption recoveries of the NAECs go up to 46% for 3-NT, 45% for 2-NT, 42% for 4-NT, 32% for NB, 21% for 2,6-DNT and 14% for 1,3-DNB. The NAECs competed for binding sites on the *Moringa* coated magnetic NP when in solution. In almost all the optimization experiments a similar trend in extraction preference was observed. The order of selectivity for the NAECs studied was 2-NT ≈ 3-NT > 4-NT > NB > 2,6-DNT > 1,3-DNB. Similar trends have been reported by other researchers. Kaur *et al.* (2007)’s study investigated the extraction of explosive compounds using SPME-HPLC-UV method. From their findings, the order of selectivity was 4-NT > 2-NT > 3-NT > NB > 1,3-DNB. From Tavengwa *et al.* (2017), the magnetic polymers were used to extract NAECs and the order of selectivity was found as: 2,4-DNT > 2-NT > NB.

5.5 Kinetic modelling: Pseudo-first order and Pseudo-second order

The effect of varying contact time on the adsorption capacity was used to plot the linearized kinetic models. The plots of the linearized pseudo first (Equation 3) and second order (Equation 5) models are shown in Figure 21 and 22, respectively. The coefficient of determination (R^2), the rate constants (k_1 and k_2 corresponding to pseudo 1st and 2nd order models, respectively) and the predicted binding capacity (q_e) for each explosive compound are summarized in Table 8.

Table 8 shows that there is variation in the R^2 value between the pseudo 1st and 2nd order models. The R^2 values of the pseudo 2nd order kinetics shows more linearity than the pseudo 1st order kinetics, indicating that the pseudo 2nd order model explains the mechanism of adsorption in this study. The pseudo 2nd order indicates that there was a chemical interaction (chemisorption) between the *Moringa* coated magnetic NPs and the explosive compounds implying that the nano-adsorbent is selective. Similar results were observed in various studies where magnetite NPs was used as an adsorbent (Wang *et al.*, 2011; Madrakian *et al.*, 2012; Tavengwa *et al.*, 2017).

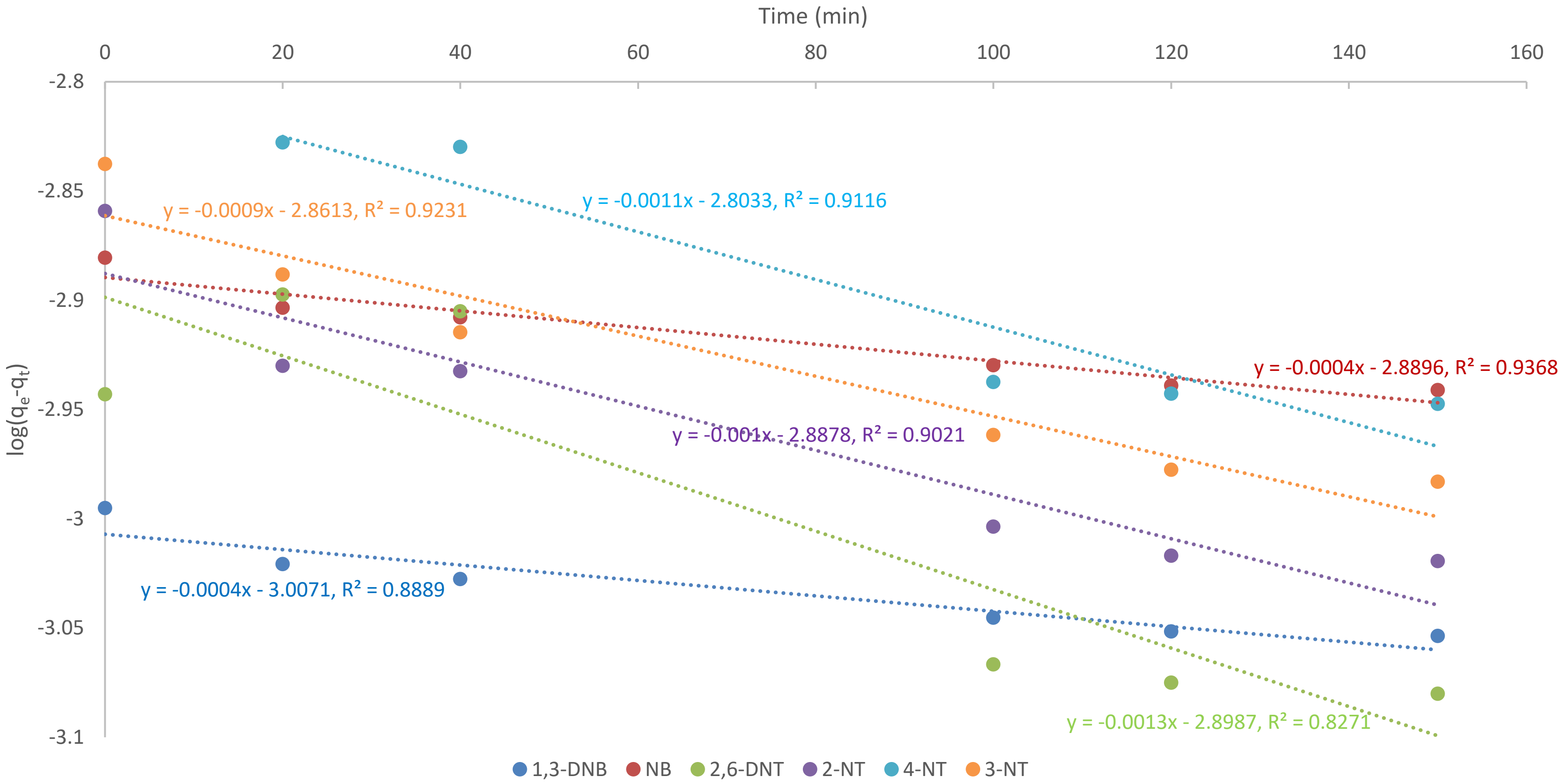


Figure 21**:** The pseudo first order linear plots for the adsorption of NAECs onto the magnetic NPs coated with *M. oleifera*.

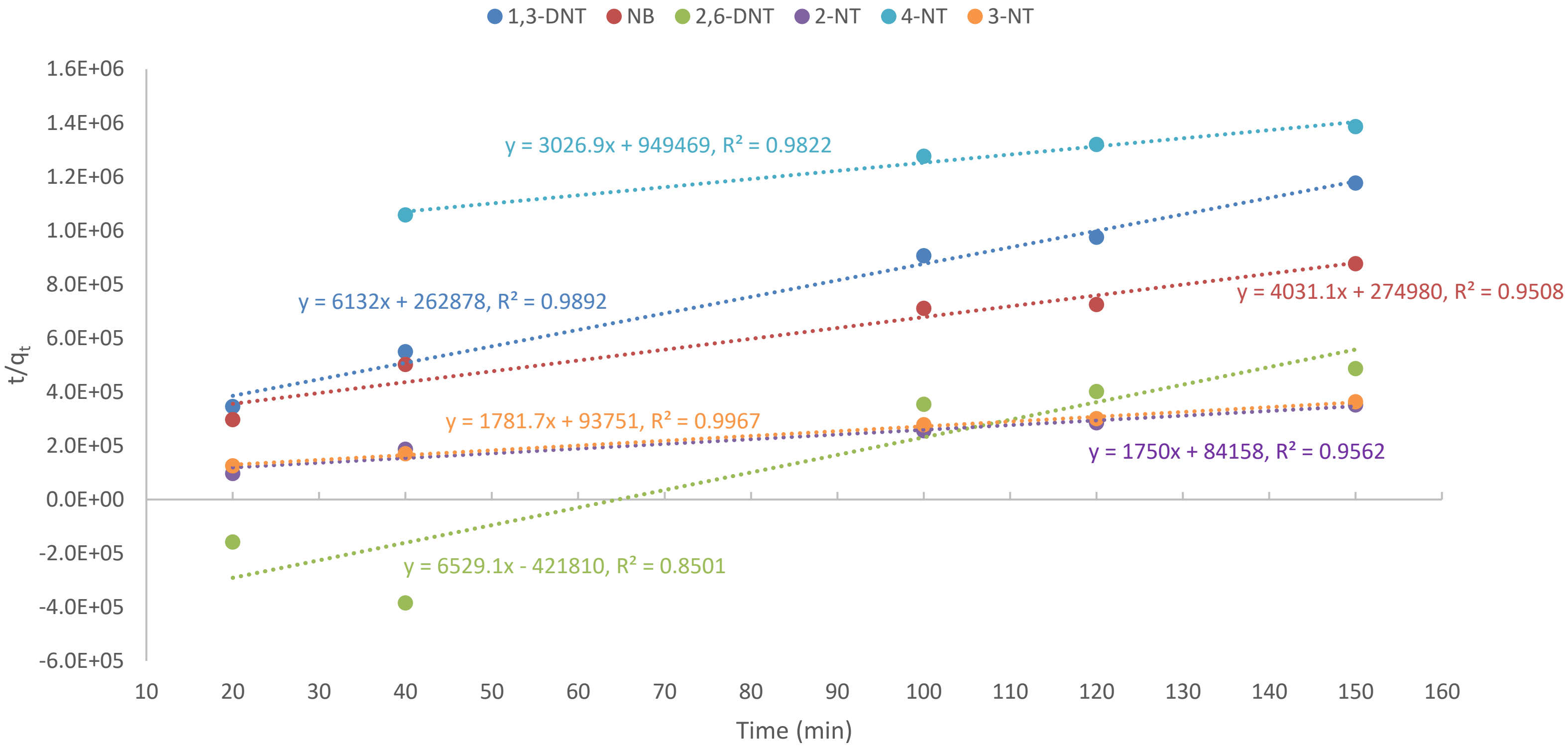


Figure 22**:** The pseudo second order linear plots for the adsorption of NAECs onto the magnetic NPs coated with *M. oleifera*

Table 8**:** Kinetic parameters calculated for pseudo first and pseudo second orders for the NAECs at a concentration of 1 mg L^{-1}

NAECs	Pseudo first order			Pseudo second order		
	R^2	k_1 (min^{-1})	q_e (mg g^{-1})	R^2	k_2 (g mg^{-1} min^{-1})	q_e (mg g^{-1})
1,3-DNB	0.8889	8.124×10^{-4}	0.165	0.9892	0.851	0.0274
NB	0.9368	8.803×10^{-4}	0.159	0.9508	0.480	0.0305
2,6-DNT	0.8271	3.080×10^{-3}	0.230	0.8501	0.555	0.0279
2-NT	0.9021	2.329×10^{-3}	0.174	0.9562	0.271	0.0767
4-NT	0.9116	$2.512, \times 10^{-3}$	0.211	0.9822	0.072	0.0443
3-NT	0.9231	2.115×10^{-3}	0.185	0.9967	0.252	0.0753

5.6 Adsorption isotherm modelling

To model the Freundlich and Langmuir adsorption isotherms, the results of the relationship between initial concentration and adsorption capacity (Figure 20) were used. The Freundlich and Langmuir parameters determined from their respective linearized plots are presented in Table 9. Comparing the R^2 values of the two isotherms, it is observed that the Langmuir isotherm demonstrates better linearity than Freundlich isotherm. This indicates that the Langmuir isotherm model fits for the adsorption of NAECs onto the coated magnetic NPs better than the Freundlich model. The Langmuir model indicates that the adsorption of the NAECs onto the nano-adsorbent forms a homogenous surface or a monolayer. This adsorption mechanism is also supported by the kinetic studies which suggest selectivity for NAECs, hence, a homogenous surface is favourable. In addition, the R_L values obtained for all NAECs were found in the range 0.96 – 0.99, confirming that the Langmuir isotherm explains the extent of adsorption of explosive compounds onto the magnetite NPs. It has also been identified in a study by Tavengwa *et al.* (2016) as a model that explained the adsorption of NB onto an adsorbent made from *M. oleifera* seed oil. The value of b for 2,6-DNT is higher than the other NAECs, indicating its affinity of the binding sites on the adsorbent. Nevertheless, all b values obtained were found between 0 and 1, demonstrating that the adsorption process was favourable.

Table 9: Freundlich and Langmuir constants for the adsorption of NAECs onto *M. oleifera* coated magnetic NPs

NAECs	**Freundlich**			**Langmuir**			
	R^2	K_f ($L\,g^{-1}$)	n	R^2	R_L	q_m ($mg\,g^{-1}$)	b ($L\,g^{-1}$)
1,3-DNB	0.852	5.798	0.824	0.994	0.997	0.129	0.128
NB	0.897	12.17	0.833	0.993	0.996	0.317	0.137
2,6-DNT	0.815	6.354	0.544	0.976	0.966	0.025	0.229
2-NT	0.944	19.75	0.843	0.991	0.994	0.510	0.158
4-NT	0.852	5.798	0.824	0.995	0.997	0.103	0.160
3-NT	0.829	78.54	0.771	0.994	0.985	0.399	0.198

5.7 Method validation – LOD and LOQ

Figure 23 below shows one of the chromatograms that were generated and utilized for the calibration **(**standard concentration of 1000 µg L^{-1}**)**. The observed order of elution is 1,3-NB, NB, 2,6-DNT, 2-NT, 4-NT, 3-NT. The polarity of 1,3 DNB is the highest ($XLogP_3$ = 1.5) making it elute faster while 4-NT and 3-NT eluted last due to their low polarity ($XLogP_3$ = 2.4).

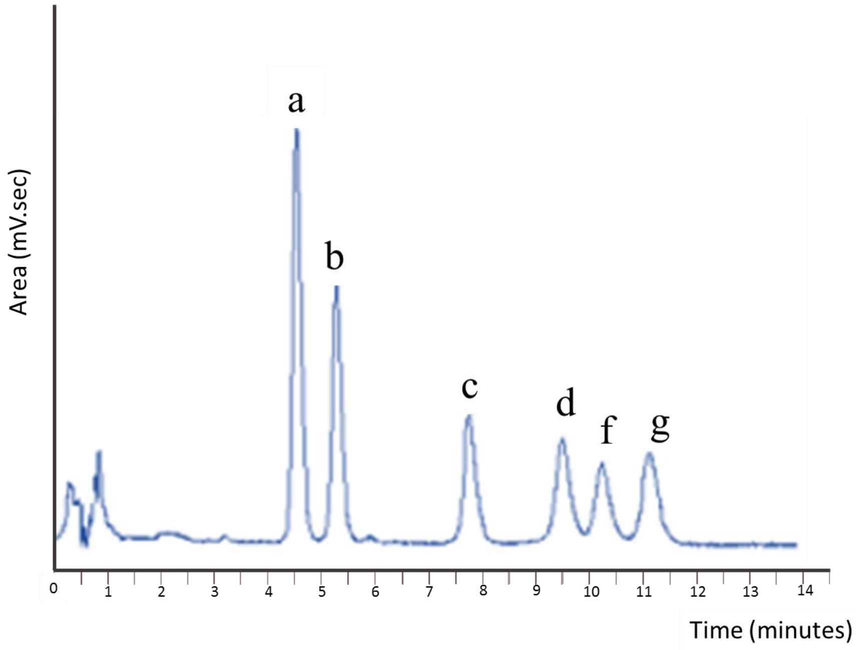


Figure 23: A chromatogram for a 1000 µg L^{-1} standard solution containing nitroaromatic explosive compounds, (a) 1,3-NB; (b) NB; (c) 2,6-DNT; (d) 2-NT; (e) 4-NT; (f) 3-NT

The calibration parameters and method detection limits (MDL) for the NAEC are presented in Table 10. The calibration equations for the nitroaromatic explosives show great linearity with the R^2 values greater than 0.996. It is observed that the method where extraction was performed with the magnetite coated with *Moringa* leaf extract (Mag-MLE) gives a lower LOD for all the explosive compounds compared to the method where extraction was performed with the magnetite coated with *Moringa* seed extract (Mag-MSE). This indicates that the Mag-MLE method is more efficient in detecting explosive compounds in aqueous samples.

Table 10: HPLC-UV calibration parameters of six explosive compounds

NAEC	Retention time (min)	Equation	R^2	MLD (μg L^{-1})			
				Mag-MLE		Mag-MSE	
				LOD	LOQ	LOD	LOQ
1,3-DNB	4.5	$y = 82.678x - 0.2484$	0.9979	0.40	1.35	0.45	1.50
NB	5.4	$y = 186.83x - 0.2\ 722$	0.9995	0.90	3.01	1.06	3.52
2,6-DNT	7.8	$y = 97.75x + 0.0182$	0.9982	0.44	1.45	0.61	2.03
2-NT	9.6	$y = 38.027x + 0.0054$	0.9998	1.03	3.43	1.72	5.72
4-NT	10.1	$y = 15.25x - 0.0451$	0.9964	4.47	14.89	5.66	18.85
3-NT	11.4	$y = 61.345x + 0.0326$	0.9994	0.37	1.25	1.13	3.76

*Each experiment conducted was repeated 3 times (n = 3)

Comparison of the LOD of the current study's methods to those done previously is summarized in Table11. It is seen that the methods developed in this study were more efficient than the other methods except the method developed by (Malik and Rai, 2009), which had a lower detection limits for almost all the explosive compounds. The results suggest that the method can be utilized in real aqueous samples, such as in wastewater treatment for determining the quantity of NAECs.

Table 11: Comparing the limit of detection (µg L^{-1}) of the methods developed and the previous studies on various explosive compounds using HPLC–UV

Extraction method	1,3-DNB	NB	2,6-DNT	2-NT	4-NT	3-NT	Reference
Mag-MLE, reverse phase amide column, .acetonitrile/water	0.40	0.90	0.44	1.03	4.47	0.37	Current study
Mag-MSE, reverse phase amide column, acetonitrile/water	0.45	1.06	0.61	1.72	5.66	1.13	Current study
SPME, Res-Elute CN column and Bodensil C-18 column, isocratic methanol:water	1.3	1.2	1.3	1.8	1.9	1.7	(Furton *et al.*, 2000)
SPME, reverse phase amide column, methanol:water	0.47	0.37	–	0.28	0.47	0.40	(Malik and Rai, 2009)
SPE using carbon nanofibers, reverse phase amide column, acetonitrile/water	3.8	7.1	8.6	9.7	23.1	13.0	(Tavengwa *et al.*, 2016)

Mag – Magnetite MLE – *Moringa* leaf extract MSE – *Moringa* seed extract

CHAPTER VI: CONCLUSIONS AND SUGGESTED FUTURE RESEARCH

This chapter highlights the efficiency of the synthesized *M. oleifera* coated magnetic nanoparticles for the removal of NAECs and their potential application in real samples. Other potential future research to improve the developed method are also highlighted.

6 Conclusions and suggested future work

6.1 Conclusions

The synthesis of magnetite was successfully achieved by co-precipitation using ferric and ferrous salts under alkaline medium. *M. oleifera* extracts were used to coat the magnetite for functionality. Coating was done successfully for both leaf and seed extract. The coated and uncoated magnetic NPs where analysed using PXRD and its pattern indicated that the coating procedure did not alter the core structure of the magnetite. The particle size of the uncoated magnetic NPs was found to be 12.14 nm and this increased by 1.23 and 2.14 nm after coating with *M. oleifera* leaf and seed extracts, respectively.

BET analysis indicated that *M. oleifera* seeds extract has a better capacity for the removal of contaminants when compared to the *M. oleifera* leaf extract. The maximum adsorption of NAECs onto the magnetite NPs was found in the range 14 – 46%. Although the recoveries are low, they were consistent. Kinetic studies revealed that chemisorption interaction occurred between the coated magnetite NPs and the NAECs. Batch adsorption studies indicated the formation of a homogenous surface best describes the adsorption of NAECs on the *Moringa* coated magnetite NPs. These outcomes suggest that the *Moringa* coated magnetite NPs could be used as a sorbent to efficiently extract NAECs from polluted water despite its lower recoveries.

6.2 Suggested future research

- The method developed should be applied to real environmental samples contaminated with NAECs to determine if it is applicable as in this study spiked deionized water samples were used.
- Identify the organic moiety in *M. oleifera* responsible for binding.
- Identify the type of interactions occurring during adsorption so as to increase the extraction recoveries.
- Try to extract NAECs at higher temperatures (e.g. 40°C) and increased stirring speed to improve the extraction recoveries.
- Compare the selectivity in real samples to other sorbents.

- More verification tests on the nanoparticles should be done such as, DLS equipment to determine the zeta potential, transmission electron and scanning electron microscopy for size and morphology.
- Evaluate and characterize the dimeric cationic protein believed to be responsible for coagulation.
- Include a control test where the uncoated nanosorbent is compared to the coated nanosorbent.

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APPENDICES

A.1. Particle size calculation from PXRD analysis using equation 6

<u>Uncoated magnetic NPs</u>

$$\bar{d} = \frac{57.3k\lambda}{\beta cos\theta}$$

$$= \frac{(57.3)(0.9)(1.540598 \times 0.1)}{(0.5 \times 1.6)\ cos 35.1}$$

$$= 12.14\ nm$$

<u>*M. oleifera* leaf extract coated magnetic NPs</u>

$$\bar{d} = \frac{57.3k\lambda}{\beta cos\theta}$$

$$= \frac{(57.3)(0.9)(1.540598 \times 0.1)}{(0.5 \times 1.47)\ cos 35.1}$$

$$= 13.37\ nm$$

<u>*M. oleifera* seed extract coated magnetic NPs</u>

$$\bar{d} = \frac{57.3k\lambda}{\beta cos\theta}$$

$$= \frac{(57.3)(0.9)(1.540598 \times 0.1)}{(0.5 \times 1.36)\ cos 35.1}$$

$$= 14.28\ nm$$

A.2. GC-MS analysis

The Figures below show the total ion chromatogram (TIC) for *M. oleifera* leaf and seeds extract

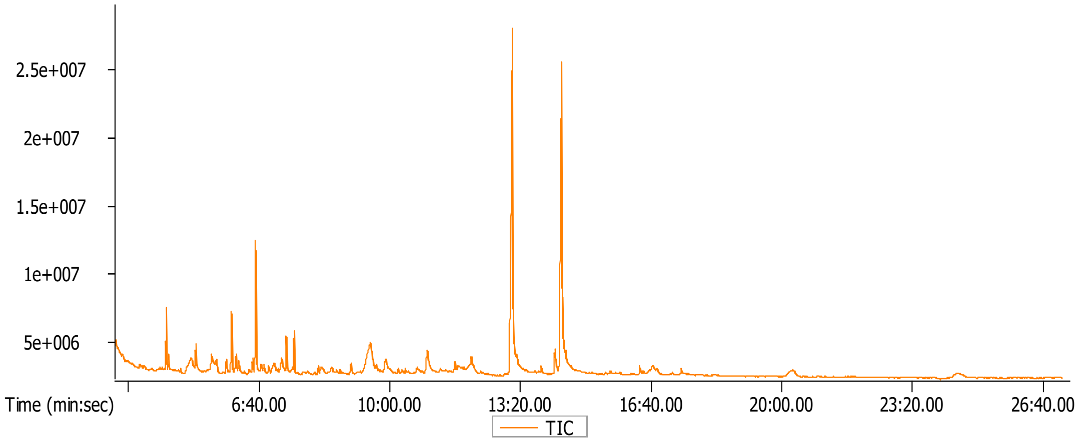


Figure A1: The chromatogram of *M. oleifera* leaf extract

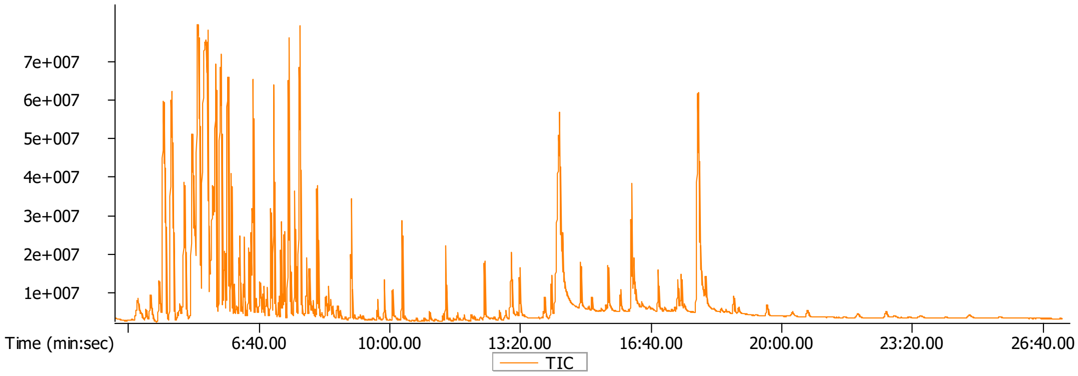
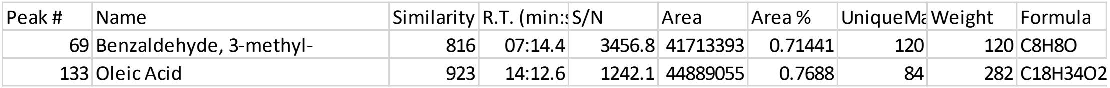


Figure A2: The chromatogram of *M. oleifera* seeds extract

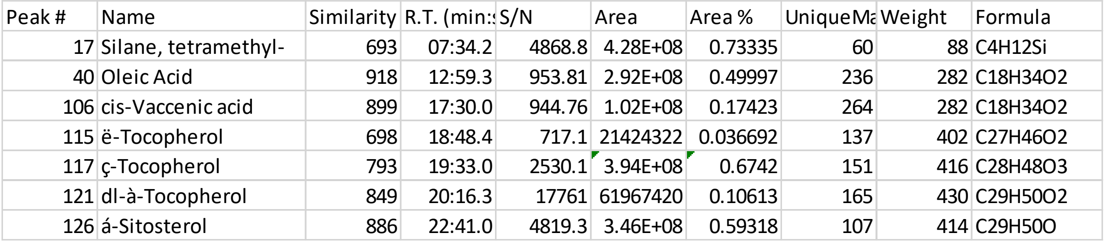
Tables below show the major compounds that were identified by GC-MS for *M. oleifera* leaf and seeds extract

Table A1: Compounds identified from *M. oleifera* leaf extract by GC-MS



Peak #	Name	Similarity	R.T. (min:s	S/N	Area	Area %	UniqueMa	Weight	Formula
69	Benzaldehyde, 3-methyl-	816	07:14.4	3456.8	41713393	0.71441	120	120	C8H8O
133	Oleic Acid	923	14:12.6	1242.1	44889055	0.7688	84	282	C18H34O2

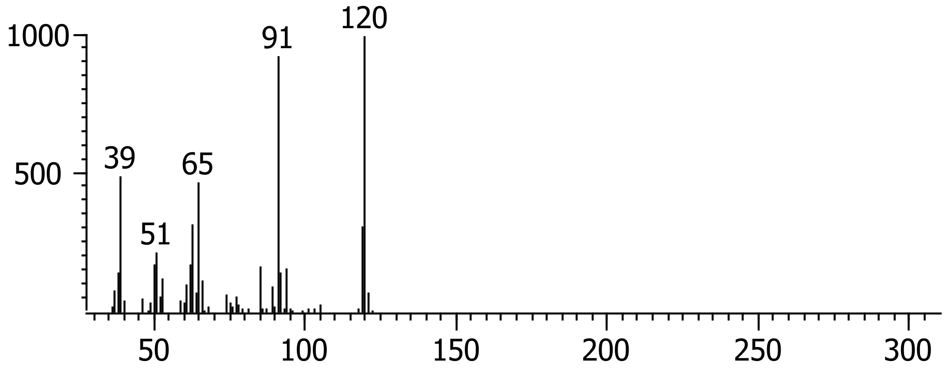
Table A2: Compounds identified from *M. oleifera* seeds extract by GC-MS



Peak #	Name	Similarity	R.T. (min:s	S/N	Area	Area %	UniqueMa	Weight	Formula
17	Silane, tetramethyl-	693	07:34.2	4868.8	4.28E+08	0.73335	60	88	C4H12Si
40	Oleic Acid	918	12:59.3	953.81	2.92E+08	0.49997	236	282	C18H34O2
106	cis-Vaccenic acid	899	17:30.0	944.76	1.02E+08	0.17423	264	282	C18H34O2
115	ë-Tocopherol	698	18:48.4	717.1	21424322	0.036692	137	402	C27H46O2
117	ç-Tocopherol	793	19:33.0	2530.1	3.94E+08	0.6742	151	416	C28H48O3
121	dl-à-Tocopherol	849	20:16.3	17761	61967420	0.10613	165	430	C29H50O2
126	á-Sitosterol	886	22:41.0	4819.3	3.46E+08	0.59318	107	414	C29H50O

Mass spectra for the compounds in *M. oleifera* leaf extract by GC-MS. These compounds are compared with those found in the GC-MS library.

Peak True - sample "M.O leaf:2", peak 69, at 7:14.40 min:sec



Library Hit - similarity 816, "Benzaldehyde, 3-methyl-"

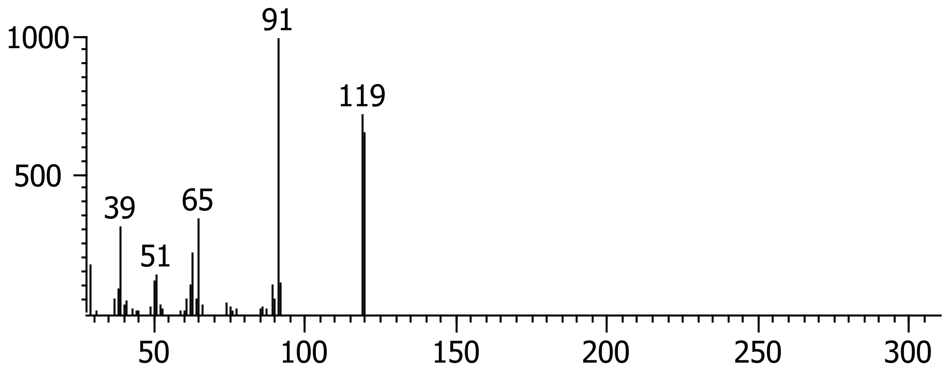
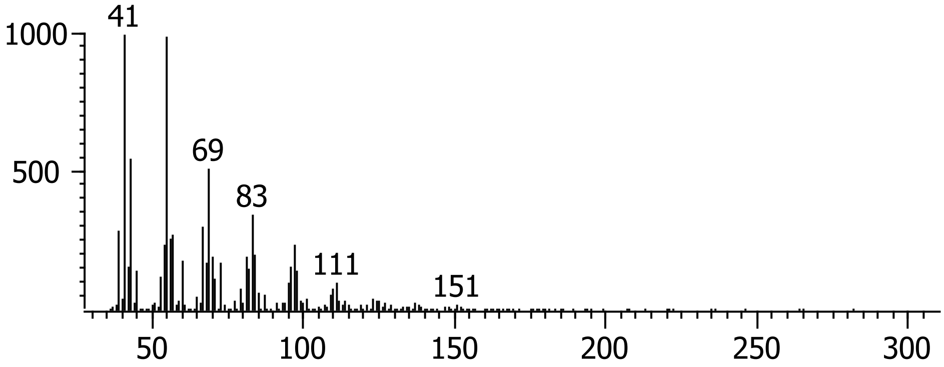


Figure A3: Mass spectrum of benzaldehyde

Peak True - sample "M.O leaf:2", peak 133, at 14:12.60 min:sec



Library Hit - similarity 923, "Oleic Acid"

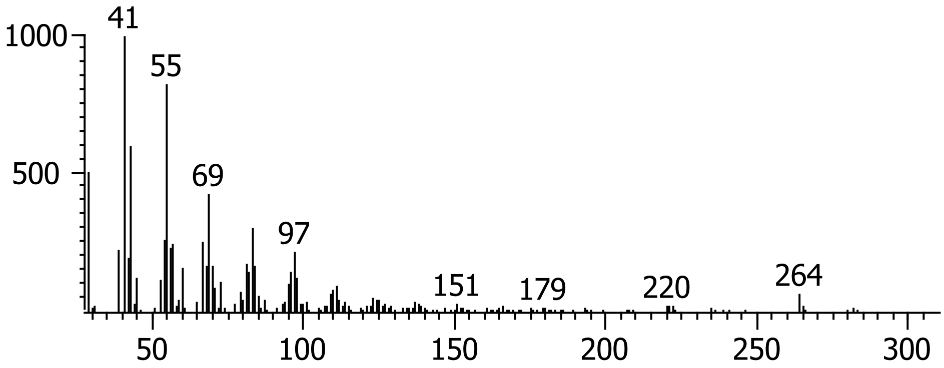
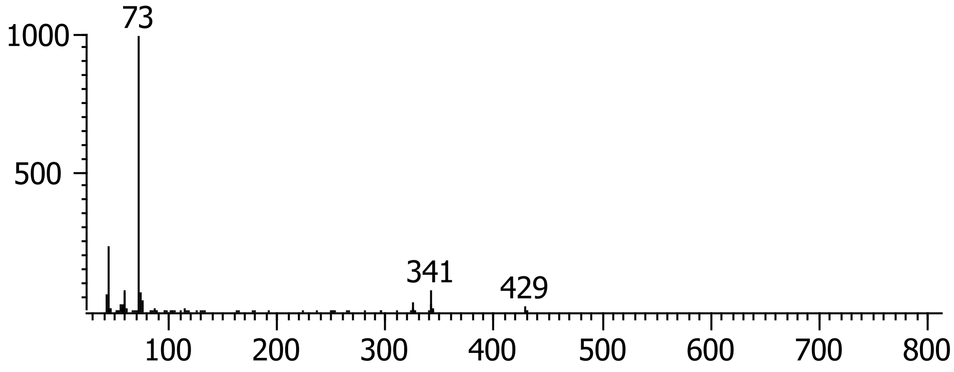


Figure A4: Mass spectrum of oleic acid

Mass spectra for the compounds in *M. oleifera* seeds extract by GC-MS. These compounds are compared with those found in the GC-MS library.

Peak True - sample "M.O seed nf:1", peak 17, at 7:34.20 min:sec



Library Hit - similarity 693, "Silane, tetramethyl-"

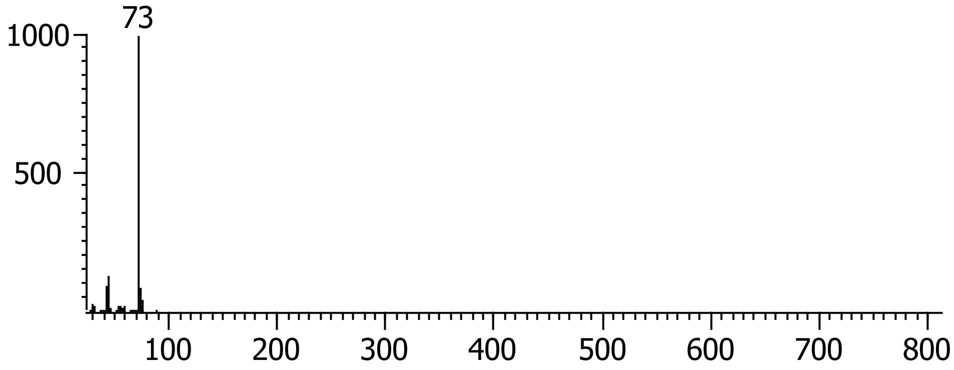
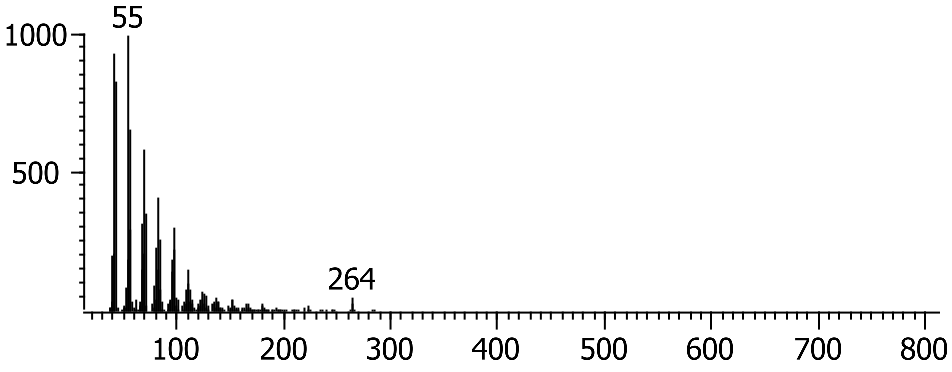


Figure A5: Mass spectrum of silane

Peak True - sample "M.O seed nf:1", peak 106, at 17:30.00 min:sec



Library Hit - similarity 899, "cis-Vaccenic acid"

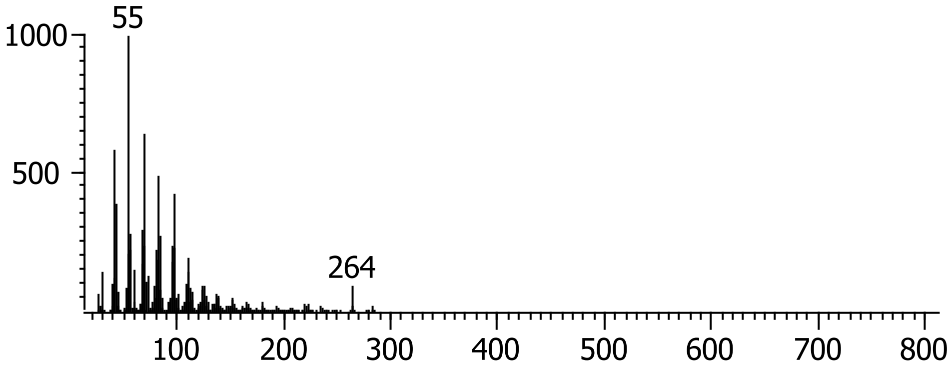
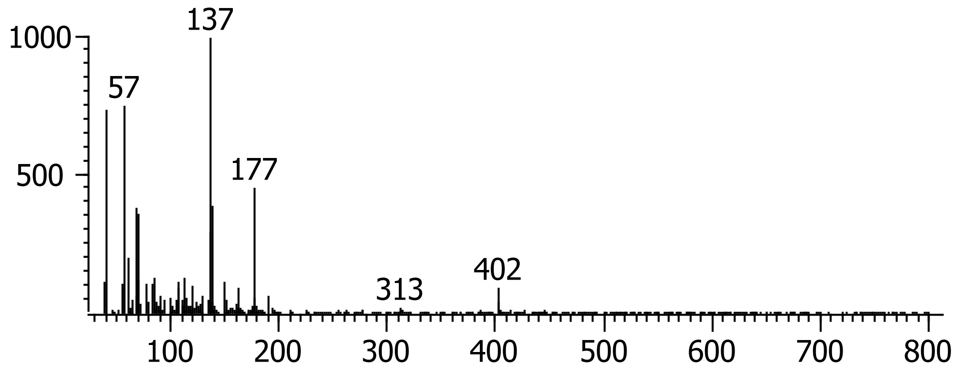


Figure A6: Mass spectrum of cis-Vaccenic acid

Peak True - sample "M.O seed nf:1", peak 115, at 18:48.40 min:sec



Library Hit - similarity 698, "ë-Tocopherol"

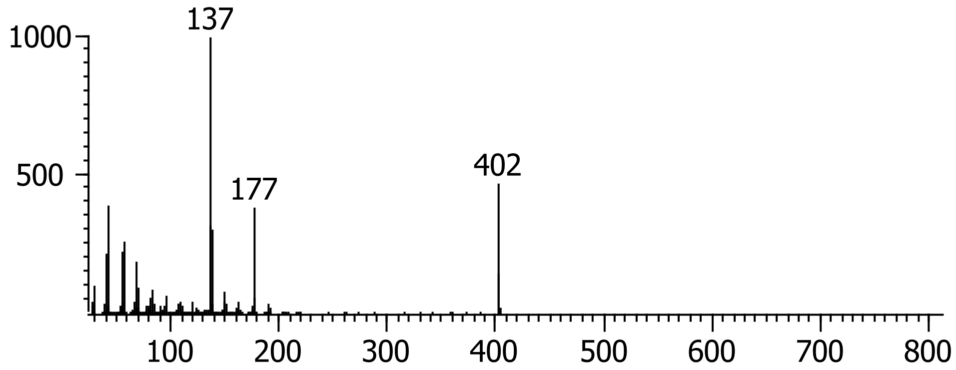
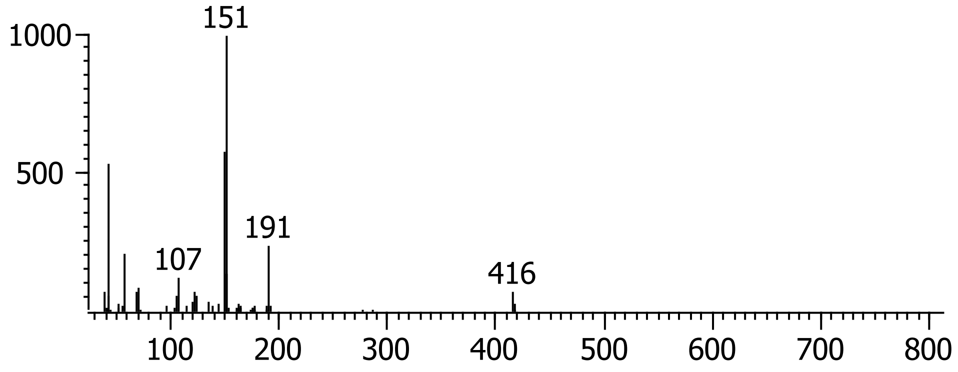


Figure A7: Mass spectrum of δ – Tocopherol

Peak True - sample "M.O seed nf:1", peak 117, at 19:27.00 min:sec



Library Hit - similarity 744, "ç-Tocopherol"

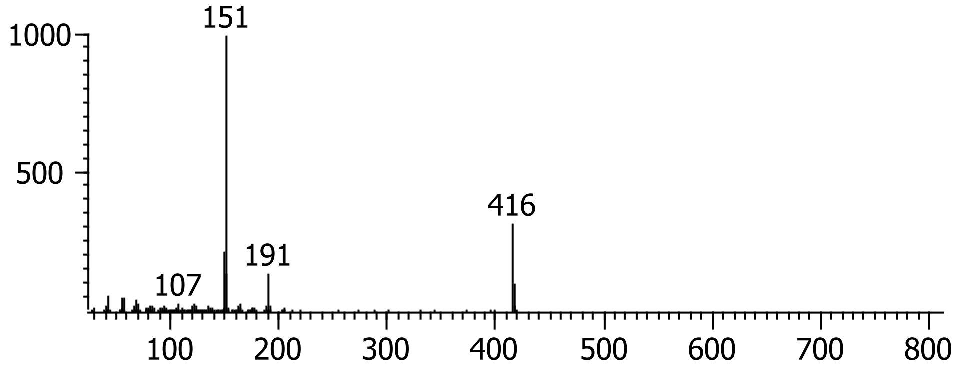
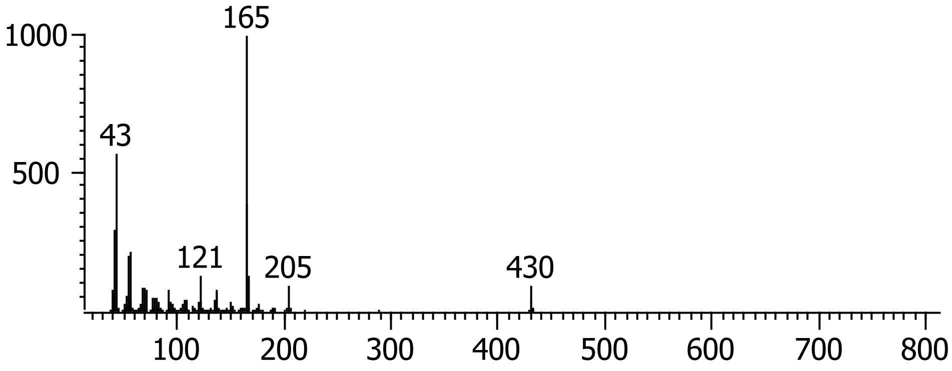


Figure A8: Mass spectrum of γ – Tocopherol

Peak True - sample "M.O seed wf:1", peak 304, at 20:16.40 min:sec



Library Hit - similarity 846, "dl-à-Tocopherol"

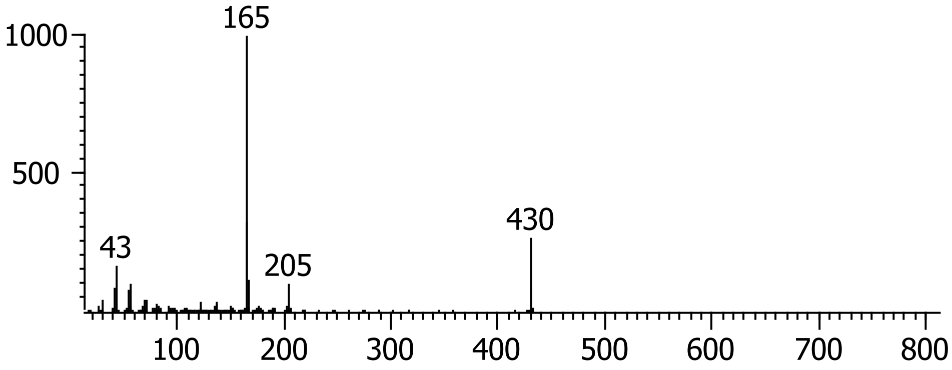
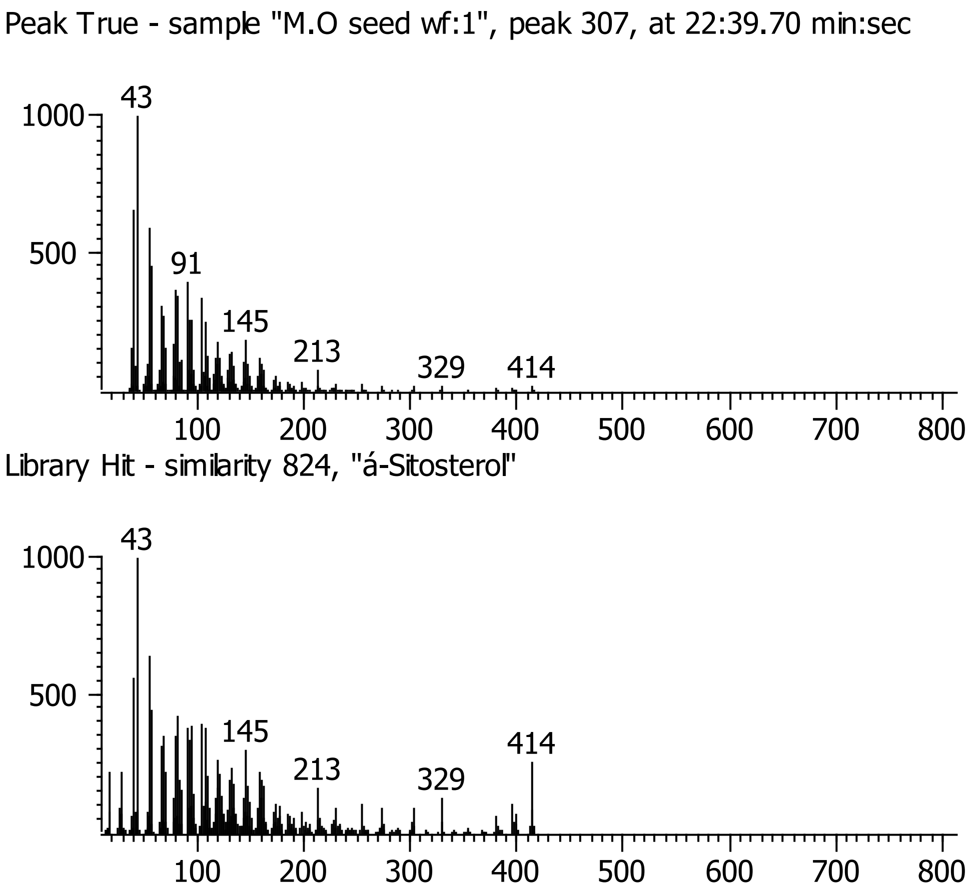


Figure A9: Mass spectrum of α – Tocopherol


Peak True - sample "M.O seed wf:1", peak 307, at 22:39.70 min:sec

Library Hit - similarity 824, "á-Sitosterol"

Figure A10: Mass spectrum of β – Sitosterol