



DEVELOPMENT OF SILVER-MAGNETITE NANOCOMPOSITES FOR CYSTEINE SENSING AND DYE DEGRADATION

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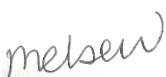
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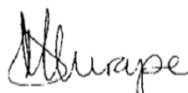
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A Dissertation Submitted to the Faculty of Science in Partial Fulfilment of the Requirements
for the Award of the Degree in Master of Science in Physics of Botswana International
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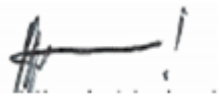
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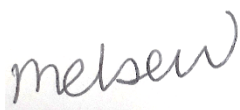
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CERTIFICATION

The undersigned certifies that he has read and hereby recommends for acceptance by the Faculty of Science a dissertation titled: **DEVELOPMENT OF SILVER-MAGNETITE NANOCOMPOSITES FOR CYSTEINE SENSING AND DYE DEGRADATION** in fulfilment of the requirements for the degree of Master of Science in Physics (MSc Physics) of the Botswana International University of Science and Technology.

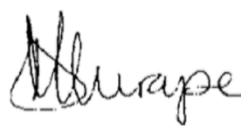


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With my utmost inner self, I am gracefully thankful to my father, our living God for I understand that without his grace and eternal wisdom, I can never achieve anything that is meaningful. I am also thankful to my family and friends for their emotional support. You guys have been great.

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Lastly, my appreciation goes to the staff members from our Department of Physics and Astronomy, that of Biology and Chemistry, especially the technicians, for their support and assistance with regards to some of the equipment and chemical reagents I needed.

DEDICATION

This research work is dedicated to my family, friends and siblings before our living God.

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ABBREVIATIONS

A	Absorbance
C	Concentration
COD	Crystallography open database
DAP	Diamine phenazine
eV	Electron volts
ϵ_0	permittivity of free space
EDS	Energy dispersive spectroscopy
EG	Ethylene glycol
HRP	Horse radish peroxidase
IR	infrared radiation
kV	kilo volts
MB	Methylene Blue
NS	nanostructures
OPD	o-Phenylene diamine
TMB	3, 3', 5, 5' tetramethylbenzidine
R6G	Rhodamine 6G
SEM	scanning electron microscope
SPR	surface plasmon resonance
TEM	transmission electron microscope
XRD	X-ray diffractometer

LIST OF PUBLICATIONS

Publications from the Dissertation.

1. **Micode Mazhani**, Melisew Tadele Alula, Davison Murape, Development of a cysteine sensor based on the peroxidase-like activity of AgNPs@ Fe₃O₄ core-shell nanostructures, *Analytica Chimica Acta* 1107 (2020) 193-202.
2. **Micode Mazhani**, Melisew Tadele Alula, Davison Murape, SERS assisted monitoring of catalytic reduction reaction using silver-magnetic nanocomposites. Under review in *Materials Chemistry and Physics* journal.

Conference Presentations

3. **Micode Mazhani**, Melisew Tadele Alula and Davison Murape. Enhanced Peroxidase-like Activity of Silver Magnetite Core-Shell Nanocomposites for Sensitive Detection of L-cysteine. BIUST Research and Innovation Symposium 2019, 11th- 14th June 2019. Palapye, Botswana.

ABSTRACT

The development of easily recoverable and re-usable catalytic nanostructures exhibiting high performances using simple and cheap procedures for applications in bio-sensing and wastewater treatment technologies is currently a prominent area of research. Noble-metal inclusive magnetic nano-catalysts in particular, have of recent gathered interest owing to their ease of separation from reaction products, environmental benignity and improved activities through synergistic qualities and photo-thermal effects. Herein, silver-magnetite core-shell nanocomposites ($\text{Fe}_3\text{O}_4@\text{AgNPs}$) displaying enhanced peroxidase-like catalytic properties were successfully synthesised via a facile one step solvo-thermal procedure. Governed by Michaelis-Menten kinetics, the nanocomposites' capacity to oxidize the colourless o-phenylenediamine to the yellow coloured 2, 3-diaminophenazine in the presence of hydrogen peroxide progressively improved relative to Fe_3O_4 alone with increasing precursor AgNO_3 to an optimum of 50 mM. Following a study and reaction optimisation conditions on the inhibitory behaviour of cysteine; a biomarker for Alzheimer and other neurological conditions, towards the peroxidase-like catalytic activity of these nanocomposites, a considerably cheap, selective and highly sensitive detection method for this amino acid was developed. With a low limit of detection of 87 nM, the formed cysteine sensor proved to be superior to some of the existing sensors.

A simple silver mirror reaction was also employed to fabricate nano-silver decorated magnetite ($\text{Ag}@\text{Fe}_3\text{O}_4$) free of the often activity limiting and environmentally unfriendly organic reagents. The nano-sized magnetite supports were synthesised using the precipitation method. Following synthesis optimisation procedures, these composites were found to effectively catalyse the reduction of 4-nitrophenol to 4-aminophenol by sodium borohydride more than 8 times repeatedly. With a normalised activity parameter k of $1.44 \times 10^{-3} \text{ L} \cdot \text{s}^{-1} \text{ mg}^{-1}$, the synthesised catalyst performed better than some reported nanostructures. Furthermore, the catalytic degradation methylene blue and Rhodamine 6G which are some of the many water pollutant dyes in textile industry effluents was achieved by these nanocomposites. Also, owing to their SERS property, $\text{Ag}@\text{Fe}_3\text{O}_4$ particles were efficient in real-time monitoring the catalytic degradation of p-Nitrothiophenol by NaBH_4 . Characterisation of all the synthesised nanocomposites in this work was carried out via transmission electron microscopy, scanning electron microscopy, X-ray diffraction, Thermogravimetric analysis and surface-enhanced infrared absorption spectroscopy.

CHAPTER 1: INTRODUCTION

The obligation to develop new and improved materials capable of meeting the ever-growing demands of humanity both for existential sustenance and a better standard of life has continually been a challenge for the science and technology community. Dating back to the bronze age and later on the industrial revolution, efforts were being made to improve the shortcomings of old materials along with their corresponding technological applications. Nowadays, immense attention is centred around developing materials with nanometric dimensions thanks to the plethora of unique and functional properties they exhibit in comparison to both their atomic and/or bulky selves [1]. The utilisation of these particles has emerged as a promising strategy to resolve an array of technological impasses incurred in various branches of scientific research including in gene therapy, cancer detection, the treatment of AIDS and photovoltaics [2-5].

Fundamentally, nanomaterials are engineered assemblies with at least one dimension scaled on a range from 1 to 100 nm [6]. These materials are often further classified based on whether they are one dimensional (1D), two-dimensional (2D) or three dimensional (3D). The source of interest on them spawns from the so called quantum size effects which in essence influences their optical, electrical and magnetic properties [7,8]. Also, the enlarged specific area that these particles have, coupled with their high surface potential has inspired technological advances based on molecule to nanoparticle interactions such as in biomedical applications and catalysis [9,10]. Catalysis in particular is considered a central discipline in the field of science and industry. It has been a core technology for many centres of the global economy including petroleum refining, synthesis of fertilizers, pharmaceuticals and in environmental remediation [11-14].

1.1 Catalysis

The concept of catalysis dates back over 180 years ago when it was first introduced by a Swedish chemist named Jöns Jacob Berzelius [15]. In principle, it involves the use of foreign agents, organic and/or inorganic, in minute concentrations to accelerate the transformation rate of chemical species. By offering a low energy pathway to reactions which would otherwise require a significantly larger injection of energy to overcome the state transition barrier, catalysts provide a mechanism to selectively make products not only at a low cost but also at high turnover frequencies without themselves getting used up [16]. In this manner, an

array of products indispensable to humans including medicines, fine chemicals, polymers, fuels, lubricants and a myriad of other value added substances have been feasible to produce [17]. In fact, it has been reported that at-least 80% of industrial chemical products go through a catalytic process at some stage in their synthesis [18]. Although catalysis is famously known in the context of producing things, its application has over the years grown versatile in that nowadays it can also be employed advantageously to solve other different but critical global issues. Among these are the remediation of polluted wastewaters and the development of simple but highly sensitive and selective biosensors [16].

It is of current understanding that with the rise in industrialisation for both developing and well developed countries, the escalating discharge of environmentally unfriendly substances including synthetic dyes, heavy metals, pesticides, herbicides and more is a major concern [19,20]. In addition to the irreparable damage they exert on the available aquatic ecosystems, the persistence and resistance towards biodegradation some of these compounds exhibit is especially concerning with regards to humanity's health. These compounds eventually make their way up the food chain bringing with them numerous medical disorders such as cramps, hypertension, cancer and many more [21]. Several methods including carbon hybrid membranes technology, physical adsorption, ion exchange, ultra-filtration and biological treatments have thus far been devised in an effort to remove man-made dyes in wastewaters [22,23]. Most of them however, are largely considered unreliable owing to a number of drawbacks including sludge production, high energy demands, expensive equipment and slow performances [24].

Catalytic processes such as photocatalytic oxidation, wet oxidation, catalytic wet ozonation and catalytic wet peroxide oxidation have on the other hand emerged as fast, easy to handle and effective procedures requiring only a low energy input to degrade pollutant dyes [25]. At times, instead of just the removal of these dyes from an aqueous solution, catalysis is often used to turn them into industrially useful compounds. e.g., conversion of 4-nitrophenol to 4-aminophenol, [26,27]. Similarly through catalysis, the field of bio-sensing has been boosted thanks to the revolutionary discovery made over a decade ago that some inorganic nanocrystals exhibit peroxidase-like activities [28]. In place of typical peroxidases like horse radish peroxidase, these nanostructures act as bio-recognition sensing elements and as they interact with different amounts of certain analytes (amino acids and heavy metals), varying degrees in product colour intensities are observed during catalytic oxidations of peroxidase substrates such as o-Phenylene diamine (OPD) and 3,3', 5,5' tetramethylbenzidine (TMB.

Features such as their high catalytic activity, low cost, high stability, easy mass production, and tuneable activity have been especially appealing to researchers seeking to develop cheaper but highly sensitive sensors to determine concentrations of important biochemical compounds in analyses of human urine and blood [10]. As is with wastewater treatment, the need for cheap and simple but highly effective catalysts, benign to the environment for selective and improved performances is imperative.

Traditionally, catalysts are categorized into three groups namely; enzymes, homogenous and heterogeneous catalysts. Enzymes (organic molecules) are generally the most efficient and greenest catalysts found in nature, however, their stability and performances are highly sensitive to environmental changes and this limits the scope of their application. Moreover, the laborious preparation they require, high cost and recycling difficulty has encouraged researchers to consider other alternatives especially in cases where large scale production projects are involved [29]. Heterogeneous catalysts on the other hand are solids and unlike their homogenous counterparts, they catalyse reactions with reagents in a different phase to themselves. The primary advantage they have is that as they are in a solid phase, they can be recovered with relative ease from the gas and/or liquid reactants and products and recycled [30]. This is of great significance particularly when expensive precious metals such as Au, Pt, Rh and others are employed as catalysts. Also to their merit, they can withstand severe environmental conditions including temperatures as high as 1600 K and 350 atm of pressure [31]. In terms of catalytic performances however, typical bulk sized heterogeneous catalysts are lacking pound for pound in comparison to homogenous ones due to the inferior degree of interaction they have with the reagent phase. Nevertheless, the deployment of heterogeneous catalysts in relevant applications is widely practiced and recommended given that they are in alignment with the unyielding environmental and economic pressures focused on the utilisation of clean and selective chemical processes [32,33].

In consideration of their demand, efforts to improve the activity of heterogeneous catalysts, metal and metal oxides in particular, has been of focus for researchers. In their study to understand the fundamental principles governing the structure-performance relationship, previous researchers revealed that the catalytic activity of solid catalysts originates from the coordinatively unsaturated atoms found on their surfaces [31]. Because of their high surface energy, these atoms have the thermodynamic tendency to bind with peripheral molecules thus facilitating catalytic reactions [32]. It is of note however, that in order to obtain maximal activity, the interaction between the reagents and the catalyst should be in accordance with

the Sabatier's principle i.e. neither too strong nor too weak [32]. Another one of their revelations is that catalytic reactions comprise of a series of key elementary steps namely; the adsorption of reactants, diffusion of intermediates and also the desorption of products and all involve the transfer of an electron between the catalyst surface and the reactive species [31]. Based on this knowledge, it became clear that the alteration of the spatial and energy distribution of valence electrons at the catalyst surface can be used to control key catalytic properties such as chemisorption and the activation energy barrier which in-turn determines the reactivity and selectivity of a catalyst. As one of the alternatives, researchers tried to influence and adjust the surface structures and electronic states of catalytic materials by mixing them with additives to enhance their catalytic performances [32,34].

1.2 Nano-catalysis

Meanwhile, the implementation of nanotechnology in catalysis has emerged as the most effective and convenient way to maximise both the activity and selectivity of heterogeneous catalysts [35]. By downsizing the catalytic materials into nano-sized entities, the surface density of catalytically active sites including edges, corners, kinks and vacancies is increased thanks to the enlarged surface area [31,36]. In addition, entirely new but catalytically influential electronic structures emanating from the confinement of electrons can be achieved in accordance with one's wishes simply by modulating the particle sizes [37]. As an example of breakthroughs made through nanotechnology, gold, a material previously assumed to be hopeless as a catalyst given its inert nature in bulk sizes, was observed on the contrary, to be highly effective in catalysing the carbon monoxide oxidation and propylene epoxidation reactions when fashioned in nano-form [38]. Although particle size is generally of prime importance when designing and optimising heterogeneous catalysts for specified reactions, as depicted by varying Turnover frequency trends in response to catalytic particle's diameters as depicted in **Figure 1.1**, other factors including nano-crystalline geometry and composition of a catalyst have been found to be important too [35].

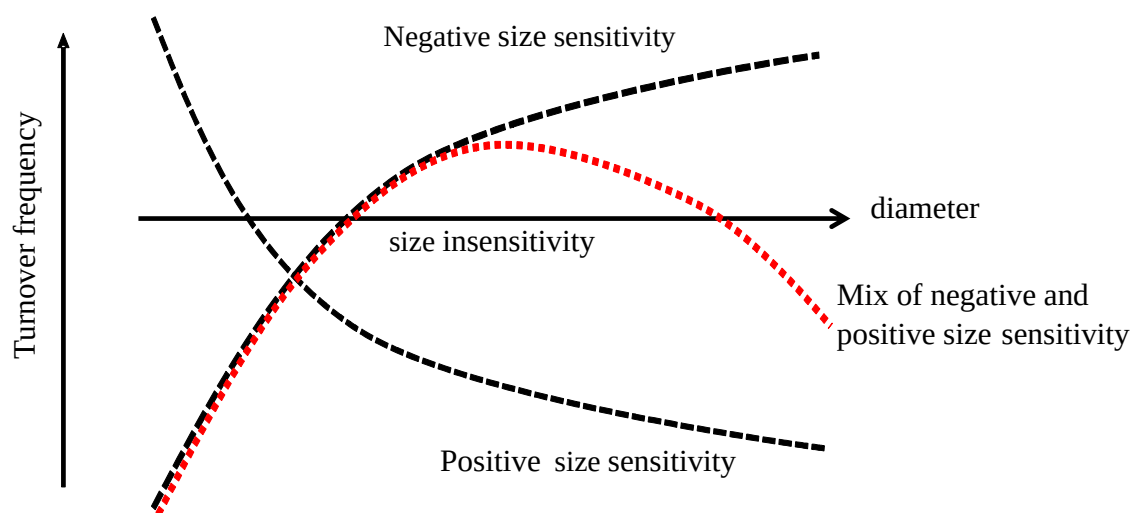


Figure 1.1: Trends describing various relationships between nano-catalyst sizes and turnover frequency for a given combination of reaction and nanoparticle catalyst [39].

To date, many procedures offering the capability to control the abovementioned factors when synthesising catalytic nanoparticles have been formulated. From the highly preferred wet chemical methods such as solvothermal/hydrothermal to expensive high vacuum thin film deposition techniques such as RF sputtering, single material nano-catalysts particularly those of noble metals synthesised in this way have shown some success catalytically. However, the need to develop nanocomposites (combinations of more than one nanomaterials) of these materials as a way to counter many of their individual shortcomings such as easy oxidation, agglomeration, poor stability and at times poor recoverability became apparent [32, 40, 41].

Hybrid core-shell nanostructures of metal and metal oxides, popularly synthesised using solvothermal procedures, and their inverted forms as illustrated in **Figure 1.2**, are among the most researched on architectural conformations nanocomposites have. When used as catalysts, these nanocomposites typically display improved activities in comparison to their single material counterparts. For core-shell nanocomposites, the outer metal oxide layer serves to sustain the activity of the catalyst by preventing the migration, leakage and aggregation of active sites on the encapsulated catalytic core [42,43]. On the other hand, the surface embedded metal on metal oxide nanostructures as depicted in **Figure 1.2(b)** ensures that a good spatial distribution of the attached catalytic phase (noble metal) is maintained [44]. This is vital in facilitating improved activity in that a better contact between the reagents and the catalytic nano-metal is achieved.

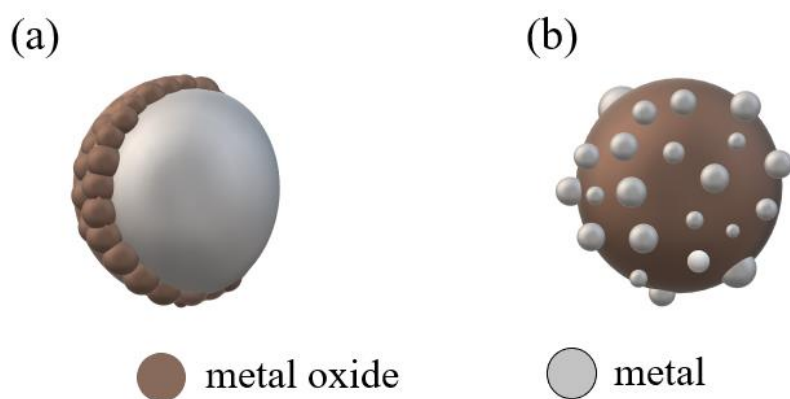


Figure 1.2: Illustrations of **(a)** metal/metal oxide core-shell and **(b)** surface embedded metal on metal oxide nanostructures.

The inclusion of magnetic materials, usually magnetite and cobalt, in any of the aforementioned nanocomposite configurations has helped address the problem of catalyst recovery and recyclability. In their presence, the catalytic nanocomposite can be extracted from the reaction mixture simply by introducing an external magnetic field [45]. This is considered to be a monumentally cheaper and simple alternative to the likes of centrifugation and filtration [46]. Up to now, a lot of research on magnetically recoverable nanocomposites in relation to environmental remediation and bio-sensing, has been biased towards gold based nanocomposites [46,47]. However, given that gold is generally expensive, cheaper but similarly effective elements such as silver need to be exploited more. Also, simple synthesis methods free of environmentally unfriendly compounds need to be used in fabricating high performance nanocatalysts active at room temperature. In reference to the severe need of highly specific enzyme mimics, the same efforts are demanded in order to generate simple and affordable sensors for crucial physiological analytes.

In this work, we present two simple, low cost yet scalable synthesis procedures of silver-magnetite core-shell nanocomposites ($\text{Fe}_3\text{O}_4@\text{AgNPs}$) and silver-decorated magnetic nanoparticles ($\text{Ag}@\text{Fe}_3\text{O}_4$), combining the magnetic and catalytic properties of Fe_3O_4 and Ag.

1.3 Statement of the problem

The global human population is expected to exceed 9 billion by year 2050 and based on current trends, a wide array of medical conditions are expected to increase in incidence [48]. Of interest, it has been projected that Alzheimer disease is set to affect 131.5 million people worldwide by then [49]. Parkinson's which is similarly a neurodegenerative disease, has also shown increments in incidence over the past decade in some countries [50]. It is as such

imperative that measures are taken to help abate both the impending threat they pose to human life and also the potential socioeconomic burden. Interestingly, for both these diseases, changes in physiological cysteine concentrations have been linked with its prevalence [51]. In consideration to this, there is a need to develop cheap, highly sensitive and fast cysteine biosensors to help discern them early so as to allow better and quicker medical choices to be made. To date, magnetite nanoparticles as enzyme mimics have been used to develop many biosensors [10]. Their capacity for easy recovery thus recyclability is very appealing. Nevertheless, improvements on their sensitivity and selectivity are still highly desired.

It has also been reported that over 10 000 different kinds of synthetic dyes including Rhodamine 6G and methylene blue, are produced worldwide in textile industries and a significant fraction of the net 700 000 tons of annual production goes down the drain into water catchment areas [45,52]. Considering the scarcity of water in many countries in part due to its pollution by these dyes, their toxic nature and the dire shortage of medical resources in some areas of the world, it is critical that techniques to manage dye polluted wastewaters are devised. Silver nanoparticles have been widely investigated as potential catalysts to degrade these dyes along with other environment polluting organic waste compounds [53]. It is nonetheless understood that silver deposits are rare, as such, it is important that at the very least, high performance catalysts involving minimal loads of this metal are used and their sustainability is reserved by facilitating efficient catalyst recovery and recyclability. In addition, the designed silver based catalysts should be functional at room temperatures so as to avoid the need for heating.

1.4 Aims and Objectives

The main goal of this thesis was to develop magnetically recoverable nanocomposites having catalytic activity for determination of cysteine by means of peroxidase-like activity and also for the degradation of pollutant molecules. To achieve this aim, the following specific objectives were followed.

- To develop $\text{Fe}_3\text{O}_4@\text{AgNPs}$ with peroxidase-like activity for determination of cysteine.
- To evaluate analytical performance including limit of detection, selectivity, and sensitivity of the sensor in determination of cysteine.
- To synthesize particles with high catalytic performance using silver mirror reaction.

- To evaluate the performance of the catalyst in reduction of 4-nitrophenol and degradation of dye molecules.

1.5 Significance of study

The modulation and design of integrated arrangements of nanomaterials is vital towards obtaining scientific breakthroughs. In this work, facile and environmentally friendly procedures including one with some novelty are utilised in developing a pair of silver-magnetite nanocomposites. Some, based on their enhanced peroxidase-like activity are aimed towards the sensitive and selective detection of cysteine; an amino acid whose quantities in blood plasma is regarded as physiological biomarkers for Alzheimer and Parkinson's diseases and another for the catalytic reduction of 4-nitrophenol and degradation of pollutant dyes [51]. The incorporation of magnetite ensures the recoverability of the nano-catalysts and the silver nanoparticles with their photo-thermal properties originating from their plasmonics facilitates the enhancement in catalytic activity.

1.6 Thesis Layout

The thesis is organized with five chapters.

The first chapter provides a general background on nanotechnology and catalysis, improvements and advances in catalyst design as well as their potential applications in wastewater treatment and bio-sensing. The significance and motivation of this study as well as the aims and objectives are outlined.

Chapter 2 offers an overview on magnetite and silver-nanoparticles with regards to their catalytic capabilities. The mechanisms behind their peroxidase like properties and catalytic pathway in degradation of 4-Nitrophenol are also discussed.

Chapter 3 elaborates on the fabrication of silver-magnetite core-shell nanocomposites for cysteine detection. These particles are studied and optimised with regards to their peroxidase like capabilities and under the best reaction conditions, a sensitive and selective sensor for cysteine is developed.

Chapter 4 presents a procedure to decorate magnetite nanostructures with silver nanoparticles for catalytic degradation of 4-Nitrophenol. With the performances optimised, the particles are further used to investigate their efficacy towards degrading methylene blue and Rhodamine 6G.

Lastly, chapter 5 provides a conclusion of all the obtained results.

CHAPTER 2: BACKGROUND INFORMATION

Nowadays, the integration of magnetite and silver nanostructures in consideration to their applicability in catalytic, antibacterial and plasmonic technologies has been on the rise. This is primarily because of the much desired multifunctional capabilities and synergistic effects with regards to their performances they carry.

2.1 Overview on peroxidases and peroxidase mimetic capabilities of magnetite

Iron oxide nanoparticles have widely been used in many important applications due to their excellent nanoscale physical properties such as magnetism/superparamagnetism [54]. Historically, They have always been assumed to be biologically inert, however, over a decade ago, they were found to possess intrinsic enzyme-like abilities [9]. More specifically, magnetite was discovered to be proficient in mimicking enzymes known as peroxidases. These enzymes are in essence heme proteins responsible for catalysing the oxidation of a large variety of organic substrates in living cells at the expense of hydrogen peroxide [55]. In addition to the regulation of hydrogen peroxide levels in living organisms, they are useful in a many other applications. Lignin peroxidase and manganese peroxidase for example, both associated with lignin degradation, are applicable in bio-pulping and bio-bleaching in the paper industry, and can also be used for the oxidative breakdown of man-made azo dyes [56,57]. Horse radish peroxidase (HRP) has uses in bio-sensing and bioremediation of wastewater, contaminated with phenols, cresols, and chlorinated phenols [56]. As deliberated on previously, these protein-based enzymes encompass many shortcomings such as the high cost, instability and difficulties in recyclability. In consideration to these shortcomings, magnetite nanoparticles are these days considered to be a promising enzyme alternative with regards to some of aforementioned applications.

The mechanism behind the peroxidase mimetic nature of magnetite (Fe_3O_4) is still not well understood, however, Gao *et al.* [9] demonstrated that the Fe^{2+} ion plays a major role. In their study, they showed that upon the addition of NaIO_4 , an oxidizing agent which diminishes the proportion of Fe^{2+} ion on the catalyst, the activity towards the oxidation of 3,3,5,5-tetramethylbenzidine (TMB) decreased dramatically [9]. Chaudhari and co-workers [58] would later on advise that the observed peroxidase-like activity could be explained based on the Fenton-like reaction pathways illustrated in redox equations.



The hydroxyl ions ($\cdot\text{OH}$) generated in accordance with the Fe^{2+} oxidation step would then be used to oxidise relevant substrates just as peroxidases do.

To date, many technological applications utilising the aforementioned understanding, for instance, the oxidation of organic substrates in a bid to reduce their toxicity and/or to produce colour changes for aesthetic purposes in wastewater treatment have been developed [59]. Similarly, biosensors which are analytical devices comprising of a bio-recognition sensing element that produces a signal which is proportionate to the concentration of a measured substance (analyte) have been fabricated. These cheaper but highly sensitive sensors function by exploiting the proportionate trends in colour intensification observed when chromogenic substrates (e.g., TMB, ABTS, and OPD) are catalytically oxidised by magnetite or similarly active nanomaterials (**Table 2.1**) depending on amounts of analytes present. Target analytes commonly analysed are generally amino acids such as cysteine, heavy metal ions (Hg and Pb) and viruses (e.g., Ebola, HIV and so forth) [10,60,61].

Table 2.1: Nanozymes utilised in colometric detection of biomolecules.

	Nanomaterial	Substrate	biomolecule	Ref
1	Fe_3S_4 MNPs	TMB	Glucose	[62]
2	MoS_2 Nano-flakes	ABTS	glucose	[63]
3	CuMnO_2 Nano-flakes	TMB	Cysteine	[64]
4	hemin-graphene hybrid nano-sheets	TMB	cancer cells	[65]
5	Carboxyl-modified graphene oxide	TMB	Glucose	[66]

Despite many of their advantages including the exciting prospect of recyclability given their magnetic properties, nanozymes like magnetite still demand continuous improvements in some aspects. The relatively low catalytic activities they have compared to those of natural

enzymes tend to undesirably prolong the overall biosensor response time [67]. Hormozi Jangi and co-workers [68], have nonetheless suggested that the incorporation of photocatalytic nanomaterials (e.g. silver, gold and copper) to these particles can help improve their catalytic activities. Also, the gap in specificity between nanozymes (low) and natural enzymes (high) such as Horse radish peroxidase can be overcome [67].

2.2 Properties of silver nanoparticles

Silver is a relatively soft, naturally occurring noble metal historically valued for its decorative purposes. With advances in nanotechnology, nanostructures of this metal and its compounds have become fore-front in many applications thanks to multitudes of unique properties they exhibit [69]. One such property which has made silver nanoparticles especially attractive in heterogeneous catalysis is the strong light absorption behaviour they have owing to their surface plasmon resonances (SPR) [70].

Fundamentally, when the electromagnetic radiation consistent with the natural frequency of electrons in the conduction band of a plasmonic nano-material such as silver is incident to that material, a coherent oscillation of those electrons develops [71, 72]. These light induced fluctuations of the surface electron density are known as surface plasmons. Subsequent to their excitation, surface plasmons would then undergo a dephasing process either radiatively by re-emitting a photon or non-radiatively through electron–electron and electron–phonon collisions [73]. Non-radiative energy loss mechanisms are known to generate some thermal energy defined by the correlation below [74]. This thermal energy eventually dissipates from the nanoparticles into the local surroundings [71].

$$Q_d = \frac{1}{2} \varepsilon_r \omega \ln \varepsilon_0 | \mathbf{E} |^2$$

Where Q_d is the heat power volume density, ε_0 and ε_r are the permittivity of the vacuum and relative permittivity of the metal. $\mathbf{E}$ is the induced electric field and ω is the angular frequency.

In the context of catalysis, this localised heating has always brought with it some curiosity as to whether it can augment the catalytic rates of reactions. In their study aimed at discerning this, Gao and co-workers [69] obtained a definitive proof that indeed the photo-thermal effects of nano-silver enhanced the catalytic reduction rate of 4-NP by sodium borohydride by boosting the diffusion rate of peripheral reagent molecules. Furthermore, they noticed that, with a greater intensification of the local electric field, resultant from the generated plasmons,

the reaction constants improved dramatically [69]. This would suggest that in synthesising highly active silver based nano-catalysts, especially where a dielectric support media like polymers and Fe_3O_4 on which they are coated on is involved, attention to the distribution and sizes of silver nanoparticles is necessary since the peripheral electric field depends on them [75].

In addition to the exciting catalytic properties described above, silver nanoparticles have also been extensively exploited in developing sensitive biosensors. These sensors are generally based on variation in plasmon resonant peak intensity when molecules of interest are introduced on their solutions [76,77]. Of recent however, silver particles have been added to the growing list of nanostructures exhibiting enzyme-like activity [78]. Consequentially, they too like magnetite have been used in designing simple and sensitive colorimetric sensors for essential biochemical compounds based on their peroxidase mimetic nature [78]. In consideration of the aforementioned photo-thermal properties of silver nanoparticles, it is a compelling idea that their conjugates with magnetite are synthesised not only to enhance the overall peroxidase-like activity, but also facilitate better specificity in bio-sensing and easier recovery from solutions once they have been used.

2.3 Synthesis methods of nanocomposites

As alluded to earlier on, composites of silver and magnetite nanostructures bring with them a series of useful and multifunctional properties to exploit. From the plasmonic properties of silver which are helpful in enhancing chemical reactions to the ease of catalyst recovery owing to the superparamagnetic nature of magnetite, it is imperative that composites of these materials are synthesized [79,80]. To date, Various synthesis routes such as ion implantation, ion exchange, sol-gel, and others have been used to fabricate nanocomposites of noble metals and inorganic matrices [81]. Wet-chemical synthesis routes, either one step or multistep nonetheless, are used more frequently due to their simplicity, cost effectiveness and nearly hazard free nature. Herein, the solvothermal and silver mirror reaction procedures are discussed.

2.3.1 Solvo-thermal method

Solvothermal synthesis is a facile and prominent method used to fabricate a wide range of nanostructures. Some of the physical features obtainable via this process are sometimes difficult to obtain using other conventional procedures [82]. In its implementation, all reagents (precursors, pH stabilizer, and the organic solvent) are mixed together, transferred

into a Teflon-lined stainless steel autoclave, and heated to desired temperatures, usually above the boiling point of the used solvent [83]. A popular feature about this method is that the crystallization and growth of particles can be controlled easily by modulating a range of parameters including reagents concentrations, reaction temperature, pressure, and synthesis period [84].

By taking advantage of the differences in reduction potentials of metallic precursors, core-shell nanocomposites can be synthesised in a single step reaction using this method [85]. As an example, Zhang and co-workers [85] co-reduced Ag and ferric ions in ethylene glycol at a temperature of 200 °C to generate silver magnetite core-shell nanocomposites (Ag@Fe₃O₄). In detail, the Ag ions are reduced first given their more negative reduction potential, undergo nucleation and eventually form Ag nanoparticles. On these AgNPs surfaces, Fe₃O₄ nanocrystals from the reduced Fe³⁺ grows forming flower like core-shell nanostructures. It is of note that the presence of sodium acetate is critical given that it acts as both the required alkali source and the electrostatic stabilizer [85].

2.3.2 Silver mirror reaction

The silver mirror reaction on the other hand is a simple electro-less procedure historically used to decorate glass surfaces with silver nanostructures to form mirrors [86]. In detail, this procedure entails the formation of a Tollen's reagent first, a solution formed by mixing silver nitrate with ammonia then reducing the formed silver oxide using sodium hydroxide. Thereafter, an aldehyde is introduced to this solution as a reducing agent to convert silver ions into silver atoms eventually forming nanoparticles. In this work, the silver mirror reaction was employed in decorating silver nanoparticles on magnetic nanoparticles.

2.4 Catalytic evaluation of nano-catalysts

The reduction of 4-nitrophenol (4-NP) to 4-aminophenol (4-AP) by NaBH₄ is widely recognized as a benchmark reaction for assessing the catalytic properties of potential catalytic nanostructures [87]. Its simplicity and the ease with which it can be monitored merits this recognition [88]. Upon addition of NaBH₄, the initially colourless 4-NP immediately converts to bright yellow 4-nitrophenolate ions as indicated in **Figure 2.1(a)**. Spectroscopically, this behaviour correlates with an absorbance peak shift from $\lambda = 317$ nm to $\lambda = 400$ nm [88]. This new peak ($\lambda = 400$ nm) has been, by several authors, reported to remain almost unaltered over long durations which suggests that the presence of a catalyst is necessary to facilitate the reduction process [26,27,29]. As would be expected, a significant deterioration of this peak is

observed when a catalyst is added and along with this, there is a development of a peak at $\lambda = 300$ nm, indicating the formation of 4-AP (**Figure 2.1(c)**).

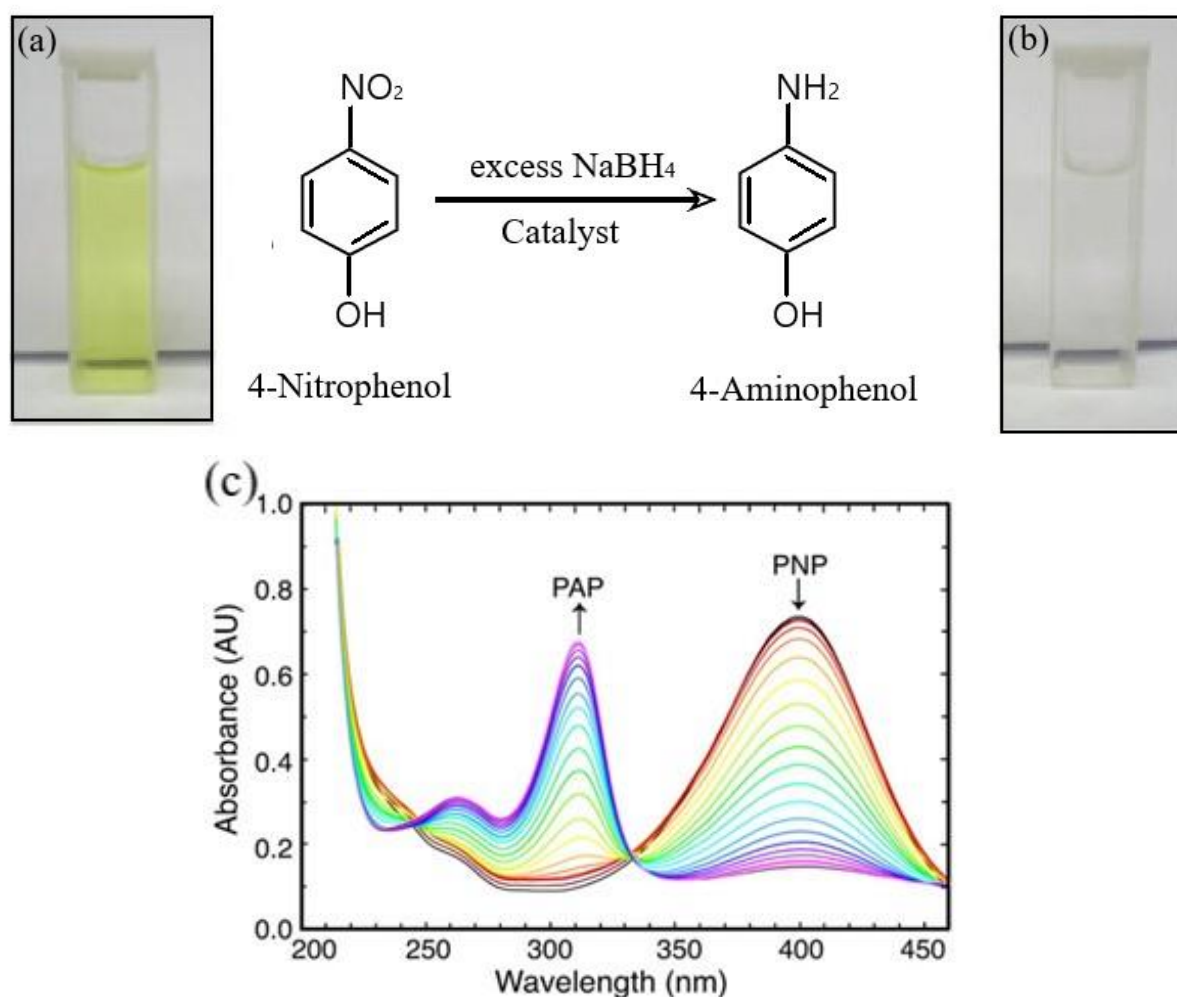


Figure 2.1: Visual transformation of (a) 4-nitrophenate ions to (b) 4-aminophenol under catalysed reduction by sodium borohydride. (c) The corresponding spectrum change during the reaction [47].

From the vanishing intensity of the 4-NP ions, the reaction rates can be calculated assuming the pseudo first order reaction kinetics given by the equation below [87].

$$-\frac{dC_{4NP}}{dt} = k_{app} C_{4NP}$$

Where C_{4NP} is the 4-Nitrophenol concentration and k_{app} is apparent rate constant

It is of emphasis that the used sodium borohydride should be in excess [90].

Gu *et al.* [91] reported that the catalytic mechanistics of this model reaction can be explained based on the Langmuir-Hinshelwood mechanism. Essentially, both the borohydride ions and nitrophenolate ions adsorb on surfaces of an electron relaying catalyst in order to form 4-AP. The 4-AP molecules would then desorb and defuse back into the solution leaving a free active site on which the same process repeats on [90].

2.5 Catalytic degradation of Methylene blue and Rhodamine 6G

Methylene blue (MB) and Rhodamine 6G (R6G) are two of the many coloured organic compounds known as dyes that strongly absorb light in the visible region and can firmly attach to fibres by virtue of chemical and physical bonding [92]. The colour of these dyes is contributed mainly by chromophore groups present in their chemical structures. Although useful in textile, paper leather, food and pharmaceutical industries to name a few, their carcinogenic origins and toxic nature pose a serious threat to human health and the ecosystem as a whole [93]. In light of the ever increasing discharges of effluents containing these dyes, mainly from textile industries into water resources, it is currently a global mandate that ways to purify contaminated waters, also as a way to manage its scarcity, are devised.

The degradation or break down of these dyes chemically into smaller non-toxic molecules via catalytic reduction is one of the standout methods presently utilised [25]. unlike other processes such as reverse osmosis, coagulation, flocculation, ion exchange and activated carbon adsorption, the energy demands are low, equipment is cheaper and performances are faster [8]. It is, however, of emphasis that highly active and recyclable catalysts such as the proposed silver–magnetite nanocomposites are utilised. In this study, the chemical transformation of both MB and R6G during the catalytic reduction by silver decorated magnetite nanostructures was monitored using UV-vis spectrophotometer. Assurances of chemical degradation of the dyes was investigated using the Raman owing to the surface enhanced Raman scattering (SERS) property exhibited by the incorporated silver nanoparticles.

CHAPTER 3: DEVELOPMENT OF A CYSTEINE SENSOR BASED ON THE ENHANCED PEROXIDASE-LIKE ACTIVITY OF SILVER-MAGNETITE CORE-SHELL NANOSTRUCTURES

3.1 Introduction

Cysteine is a semi-essential thiol group functionalised amino acid with uses in a range of fields including the food industry, cosmetics, biochemical research and medicine [94,95]. In biological systems, this compound plays a substantial role in protein secondary structures and also functions by supporting the cross linking of tertiary and/or quaternary structures of many proteins through disulfide bonds [96]. The formation of glutathione, an amino acid responsible for regulation of oxidative stress in cells and the downstream negative effects thought to be associated with it, is highly dependent on cysteine [97]. It has also been established that changes in nominal concentrations of cysteine is related to physiological occurrences such as metabolic disorders, immune dysfunction, cancer and neuro-diseases [98]. On this note, the medical field obliges that fast and sensitive cysteine detection methods are developed to allow informed therapeutic decisions to be made.

To date, a range of analytical techniques for cysteine detection in biological and pharmaceutical samples have been proposed including spectrophotometry [99], liquid chromatography [100], flow-injection [101], fluorescence spectroscopy [102], voltammetry [103], chemiluminescence and mass spectrometry [104,105]. These techniques however, are marred with a series of drawbacks including, instability, laborious sample preparation steps, the need for expensive and sophisticated instrumentation along with highly-trained personnel to operate them. In view of these disadvantages, efforts to develop facile, selective and sensitive detection methods for rapid cysteine detection are continually in demand.

Over the recent years, the spectrophotometric analysis of enzyme-catalysed colorimetric reactions has been employed as a simple and facile alternative for detection of a variety of not just cysteine but other analytes as well [94]. Horse-radish peroxidase (HRP) in particular, is a common enzyme used for this technology as it known to catalyse the hydrogen peroxide driven oxidation of chromogenic substrates such as o-phenylenediamine (OPD) and 3, 3', 5, 5' -tetramethylbenzidine (TMB) [106,107]. When molecules of interest including amino acids like cysteine to peptides, nucleotides and even heavy metals are present in this reaction system under specific conditions, they are known to be capable of either suppression or promotion of the reaction rate [108]. Depending on the correlation between the analyte quantity (concentration) and its degree of reaction interference, bio-sensors have been

constructed [108]. Enzymes like HRP nonetheless, are known to be unstable in harsh environmental conditions with respect to temperature and pH. Their laborious preparation, high cost and difficulty of recycling has also prompted researchers to seek for other more manageable enzyme-like alternatives [109].

In 2007, Gao *et al.* [9] discovered that iron oxide nanostructures (NSs), particularly Fe₃O₄, possess intrinsic enzyme mimetic capabilities. This discovery proved to be the much needed breakthrough for the aforementioned technology. Further research revealed that other NSs including metals, metal oxides and metal-carbon compound nanomaterials also portrayed the same property [109]. “Nanozymes” as these nanomaterials came to be known [10], along with Fe₃O₄ comprises of fullerenes [110], AuNPs [111], AgNPs [112], metal oxides such as TiO₂ and CeO₂ [113], V₂O₅ [114] and more. The large surface area to volume ratio that these particles have owing to their nanometric scale, not only renders them better than natural enzymes in efficiency but there is also plenty of room for surface functionalisation which helps tune their catalytic selectivity [109]. Moreover, their cyclic action and the possibility for recovery without significant loss in subsequent cycles of catalytic action makes them truly unique structures [10]. Nanozymes are also inherently robust, stable in harsh conditions unlike natural enzymes and are easy to mass-produce with a simple scale-up [115]. It is based on these benefits that nanozymes are considered promising candidates for analytical applications.

Sensing platforms employing the enzyme mimetic capabilities of Fe₃O₄ have been reported by many researchers. Wang *et al.* [116] used Fe₃O₄ nanoparticles synthesized via the co-precipitation method to make a colorimetric sensor of hydrogen peroxide and glucose. Chen *et al.* [94] likewise used Fe₃O₄ to construct a sensitive and selective colorimetric cysteine sensor with a detection limit of 0.028 µM. In their work, they proposed that the strong complexation capability of the thiol group in cysteine molecules towards Fe³⁺ could be the influential factor in its detection mechanism [94]. Jiang and co-workers [78], on the other hand fabricated a nano-silver based biosensor for glucose after their study revealed that silver-chitosan nanocomposites possessed intrinsic peroxidase like behaviour towards OPD in the presence of hydrogen peroxide. With both Fe₃O₄ and Ag having proven to exhibit peroxidase mimetic activities, it is of interest to study this property for conjugates of these nanomaterials.

In general, interest in research based on properties arising from nanostructures synthesized by integrating multiple materials has been on the rise in recent years. This is due to the multi-

functionalities which are subject to their constituent components. Furthermore, synergistic effects associated with these hybrid nanostructures along with novel qualities characteristic only to the combined state of different nanomaterials have been observed [117,118]. As an example, nanocomposites of Fe_3O_4 and noble metals have been found to combine magnetic, plasmonic and catalytic properties [85,119]. The magnetic recoverability of Fe_3O_4 -based NS in particular, is of prime importance in catalysis economic-wise in that it offers the potential for low cost recycling. In this study, a simple one step solvo-thermal procedure with some slight modifications has been employed to synthesise magnetically recoverable Ag- Fe_3O_4 core-shell NSs showing enhanced peroxidase-like activity [24]. With the catalytic performance conditions optimised, a sensitive and selective colometric cysteine sensor based on the improved capacity for the nanocomposites to catalyse the H_2O_2 driven oxidation of OPD was developed.

3.2 Experimental

3.2.1 Chemicals

The used $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$, di-sodium phosphate, hydrogen peroxide (34.5-35.5 %), ethylene glycol (EG), o-phenylenediamine (OPD), L-cysteine and silver nitrate were all purchased from Sigma Aldrich. Potential interference molecules of analytical grade namely; arginine, glycine, DL phenol-aniline, lysine, uric acid and creatinine were also obtained from Sigma Aldrich. Ascorbic acid and glucose were supplied by Rochelle chemicals, sodium Acetate and ethanol absoluto by Merck and citric acid was purchased from Minema. All solutions were prepared via dissolution in deionised water (18.2 M Ω). The used glassware was thoroughly washed with nitric acid, rinsed with deionised water and dried in an oven at 50 °C. All chemicals were used as purchased without further purification.

3.2.2 Instrumentation

A series of techniques were used in characterising the synthesised nanocomposites. The morphological properties were studied using the JEOL JSM 7100F field emission scanning electron microscope (FESEM), operating at an accelerating voltage of a 10-15 kV and a current of 2-6 mA. The elemental and chemical composition data was obtained from Energy dispersive spectroscopy (EDS) measurements equipped to the same machine. A Bruker model D8 advance powder diffractometer (Bruker, AXS GmbH, Karlsruhe, Germany), equipped with a $\text{CuK}\alpha$ radiation source (accelerating voltage 40 kV and a 40 mA current) and

a LynxEye XE energy-dispersive strip detector was used to generate X-ray diffraction (XRD) patterns of the nanocomposites on a 2θ range from 5° - 90° .

The core-shell structure of the nanocomposites, was revealed using the (JEOL JEM-200 EX) transmission electron microscope (TEM). IR measurements via the attenuated total reflectance (ATR) mode of a Bruker FTIR spectrometer were collected. The time resolved optical absorbances for monitoring catalytic reaction progress were measured using the Evolution 201 UV-Visible spectrophotometer (scanning rate 1 nm on a range of 250-800 nm).

3.2.3 Synthesis of magnetite coated nano-silver

A simple one step solvo-thermal procedure was used to synthesize magnetite coated silver nanoparticles [85]. Briefly, 1.5 g of $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ was dissolved in 40 mL of EG along with AgNO_3 with a final concentration of 20 mM using magnetic stirrer. Sodium acetate (3.6 g) was transferred to the resultant reddish solution and stirred vigorously until a clear orange suspension was observed. The solution was then transferred to a 100 mL teflon-lined stainless steel autoclave and immediately kept in an oven at 200°C for 10 hrs. Thereafter, it was allowed to cool naturally to room temperature. The synthesized black powder was transferred into a clean beaker and decanted from the formed supernatant magnetically. Following this, the particles were washed thoroughly with distilled water (5 times) and absolute ethanol (2 times). The cleaned particles were allowed to dry in an oven at 60°C for 6 hrs, crushed into a fine powder and stored for further use.

3.2.4 Preparation of solutions

A stock solution of 15 mM OPD was prepared using 0.0811 g of the powder. The volumetric flask (100 mL) carrying this solution was wrapped in aluminium foil and stored in a dark place before use. Buffers were prepared from 250 mL stock solutions of 0.2 M di-sodium phosphate (3.55 g) and 0.1 M Citric acid (5.25 g). H_2O_2 was initially used as purchased (9.8 M). Starting with this stock solution, the progressively more dilute solutions of H_2O_2 required were prepared by continually adding relevant volumes of deionised waters to the previously prepared solution. Appropriate masses of interference molecules were also weighed accordingly and dissolved in 250 ml volumetric flasks.

3.2.5 Evaluation of peroxidase-like activity

The chromogenic substrate *o*-phenylenediamine (OPD) is frequently used as a substrate for the study of enzymatic activity as well as quantification of vital organic substances ranging

from amino acids to reducing sugars. Typically, hydrogen peroxide, a powerful oxidant, acts on the colourless OPD in the presence of an enzyme to convert it to mainly 2,3 diaminophenazine (DAP) which is both colourful and fluorescent [120]. The reaction kinetics facilitated by enzymes of interest can therefore be studied either fluorometrically or spectrophotometrically following the abovementioned chemical reaction [120]. The peroxidase mimetic activity of synthesized ($\text{Fe}_3\text{O}_4@\text{AgNPs}$) nanocomposites was studied based on this reaction. A series of experiments were undertaken in an effort to determine the optimum conditions for developing a cysteine sensor based on its tendency to inhibit the oxidation of OPD.

On every occasion, fresh OPD solutions were prepared and employed for all the experiments. For a typical peroxidase activity measurement, 0.5 ml of deionised water was pipetted into a 2 mL Eppendorf tube containing 0.400 mg (unless stated otherwise) of the prepared $\text{Fe}_3\text{O}_4@\text{AgNPs}$ catalyst (weighed using a TGA machine). To this mixture, 0.750 mL each of the phosphate-citrate buffer and 15 mM OPD were then pipetted into, followed by the addition 4 μL of 34.5% hydrogen peroxide. The mixture was then gently shaken for 30 seconds and within minutes, the originally colourless solution turned yellow. After certain reaction intervals, the reaction mixtures were transferred into a quartz cuvette and UV-VIS absorption spectrophotometry measurements were taken on a range from 300-600 nm. To prevent $\text{Fe}_3\text{O}_4@\text{AgNPs}$ from transferring into the cuvette, a magnet was used to trap them inside the Eppendorf tube. A similar approach was carried out for Fe_3O_4 alone and this was used as the control to test the catalytic potential of Ag.

3.2.6 Optimisation of parameters

The catalytic property of metallic nanoparticles for a specific reaction is intrinsically dependent on different factors. It is therefore important to optimise the relevant parameters in order to obtain the optimum catalytic performance. Herein, concentrations of AgNO_3 solution (used to produce the nanocomposites), pH of the reaction mixture and catalyst loading were investigated systematically.

3.2.7 Optimization of silver nitrate concentration

In fabrication of $\text{Fe}_3\text{O}_4@\text{Ag}$ core-shell nanocomposites, varying AgNO_3 masses were weighed into the precursors mixture to give final solution concentrations of 20, 40, 50 and 60 mM. The resulting nanocomposites were subjected to peroxidase-like activity tests as described in **section 3.2.5**.

3.2.8 Study of pH of buffer solutions on peroxidase activity

Phosphate-citrate buffer solutions with pH values ranging from 4-8 were prepared and their influence on the peroxidase-like activity was investigated whilst keeping the mass of the composite constant at 0.100 mg.

3.2.9 Optimisation of catalyst loading

Following the abovementioned optimisations, the influence of varying catalyst loadings was also investigated in the same manner i.e. all reaction conditions were maintained except the catalyst mass. The buffer pH used was 7.4 (optimum).

3.2.10 Steady-state kinetics

As typical of catalysts in general, the steady-state kinetics experiments were performed on the basis of the catalytic activity of Fe₃O₄@Ag nanocomposites. In detail, varying concentrations of OPD and H₂O₂ were assessed as described in **section 3.2.5** under optimum conditions with respect to pH (7.4) and catalyst loading (0.200 mg). Given that changes in absorbances were to be correlated with reaction time, reagent mixtures on a set of Eppendorf tubes were made for measurements to be made at specific times. Plots of initial reaction rate against substrate concentration were also made. The kinetic parameters K_{max} and V_{max} were calculated based on the Lineweaver-Burk plot, which could be given as follows;

$$\frac{1}{\vartheta} = \frac{K_{max}}{V_{max}} \frac{1}{S} + \frac{1}{V_{max}}$$

where ϑ is the reaction velocity and S represents the substrate concentration. K_{max} is considered to be a measure of how well a substrate complexes with a given enzyme, i.e. its binding affinity. A low K_{max} value indicates a large binding affinity whereas a large one suggests that the enzyme does not bind as efficiently with the substrate. V_{max} on the other hand represents the maximal rate of the reaction.

3.2.11 Cysteine detection

Inhibition test

The inhibition effect of cysteine on the kinetics of catalysed OPD oxidation by hydrogen peroxide was determined in the presence and absence of cysteine. In a typical reaction mixture, 0.5 mL of 60 μ M cysteine (or deionized water) solutions was pipetted into Eppendorf tubes containing the optimised catalyst mass (0.200 mg). This was followed by the addition of 0.750 mL each of the pH 7.4 buffer and OPD (9 mM). Spectrophotometric

measurements were taken every 5 minutes and continued up to the 20th minute. In comparison to the spectrum obtained for reaction without cysteine (just deionised water instead), a reaction duration which depicted the most absorbance differential at spectra peaks between the two reactions was determined. This duration was regarded as the optimum incubation time.

Effect of pH

The inhibitory effect of cysteine on oxidation of OPD is highly dependent on the pH of the solution, therefore, buffer solutions with a range of pH from 5.0 to 7.4 were investigated. For a typical measurement, 0.5 mL of 60 μ M cysteine was pipetted into an Eppendorf tube consisting of optimised catalyst loading and from then, 0.75 mL each of 9 mM OPD and a buffer solution with a pH of interest from 5.0-7.4. To this mixture, 4 μ L of optimised H_2O_2 concentration was added and after an incubation period of 5 minutes, absorbance measurements were done. Absorbance differences of the mixture with and without cysteine were used to determine the optimum pH for detection of cysteine. A pH of 6 was selected for developing a cysteine quantification profile as it offered the largest absorbance differential (ΔA) after a 5-minute reaction time.

Sensitivity in determination of cysteine

The sensitivity of the sensor was evaluated by taking a series of cysteine concentrations ranging from 0- 500 μ M. The inhibitory effect of cysteine solutions on the catalytic oxidation of OPD was evaluated based on the optimized conditions; pH 6, catalyst mass 0.200 mg, 15 mM OPD and 40 μ M H_2O_2 . Absorbance measurements were taken 5 minutes after the addition of H_2O_2 . From the obtained spectra, absorbance differentials at 416 nm were calculated and plotted against cysteine concentrations.

Selectivity in determination of cysteine

A study on the influence of possible interference molecules for situations where cysteine is detected in real samples was carried out. A range of plasma compounds namely; arginine, glycine, lysine, DL-phenolalanine, Ascorbic acid, glucose, creatinine and uric acid were studied as to whether they can in a way affect the progress of catalysed OPD oxidation. In doing so, the same peroxidase activity procedure described in **section 3.2.5** was followed but instead of cysteine, 0.496 mL of the interfering molecule with a molarity of 20 μ M was added.

3.3 Results and discussion

3.3.1 Characterization of nanocomposites

The identification of the as-synthesised samples with regards to their composition and phase purity was carried out by taking X-ray diffraction measurements on a 2θ range of $25-85^\circ$. The obtained XRD diffractograms for both Fe_3O_4 nanoparticles and $\text{Fe}_3\text{O}_4@\text{AgNPs}$ nanocomposites prepared by varying AgNO_3 concentrations from 20 mM to 60 mM are as illustrated in **Figure 3.1**.

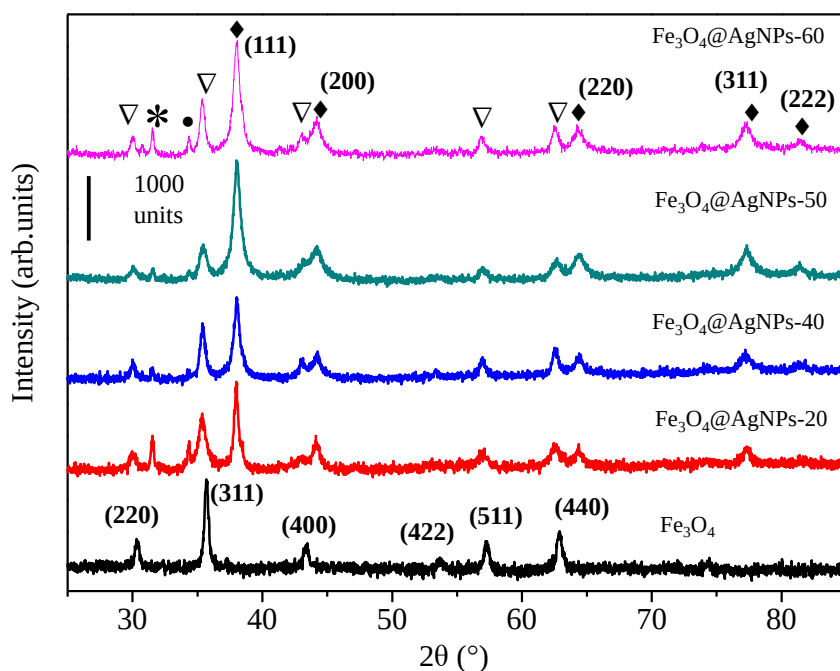


Figure 3.1: XRD diffractograms for (a) Fe_3O_4 and $\text{Fe}_3\text{O}_4@\text{AgNPs}$ fabricated from 50 mM AgNO_3 . (b) Diffractograms for all synthesised nanozymes (0-60 mM AgNO_3). ∇ is for Ag and $\blacklozenge$ for Fe_3O_4 peaks. (*) and ($\bullet$) may attribute to the existence of Ag_2O and AgO phases.

In Analysing the pattern for the bare Fe_3O_4 nanoparticles, it was evident that the obtained series of diffraction peaks positioned at 30.32° , 35.67° , 43.47° , 53.67° , 57.22° , 62.91° and 74.45° matched those of face-centred cubic (fcc) Fe_3O_4 nanocrystals (JCPDS card COD 9005812). In the case of $\text{Fe}_3\text{O}_4@\text{AgNPs}$ -60 however, the acquired pattern resembles a composite of two Bragg reflections with one matching that of the aforementioned Fe_3O_4 . The other new peaks at $2\theta = 37.99^\circ$, 44.22° , 64.53° , 77.32° and 81.40° could be indexed to (111), (200), (220), (311) and (222) lattice planes of an fcc Ag crystal (JCPDS card COD 9013045). This superposition of XRD diffractograms appeared consistently for all particles formed in the presence of AgNO_3 suggesting that indeed nanocomposites of Ag and Fe_3O_4 were

successfully made. The additional peaks at Bragg angles 31.52° and 34.42° labelled as (*) and (•) are thought to be due to the existence of Ag_2O and AgO phases [121].

Given their relatively more intense diffraction peaks, it is clear that AgNPs displayed a superior level of crystallinity to Fe_3O_4 . Their mean crystallite sizes were estimated via line broadening analysis by applying the Debye–Scherrer formula. The general trend is that as more AgNO_3 is used, smaller crystallites form. Particles formed from 20 mM AgNO_3 presented crystalline sizes of 25.59 nm, 18.40 nm (40 mM), 15.10 nm (50 mM) and 15.70 nm (60 mM). Bare Fe_3O_4 particles on the other hand exhibited crystallite sizes of 25.02 nm.

The internal structures of the $\text{Fe}_3\text{O}_4@\text{AgNPs}$ composites were obtained using a TEM as presented in **Figure 3.2 (a-d)** below.

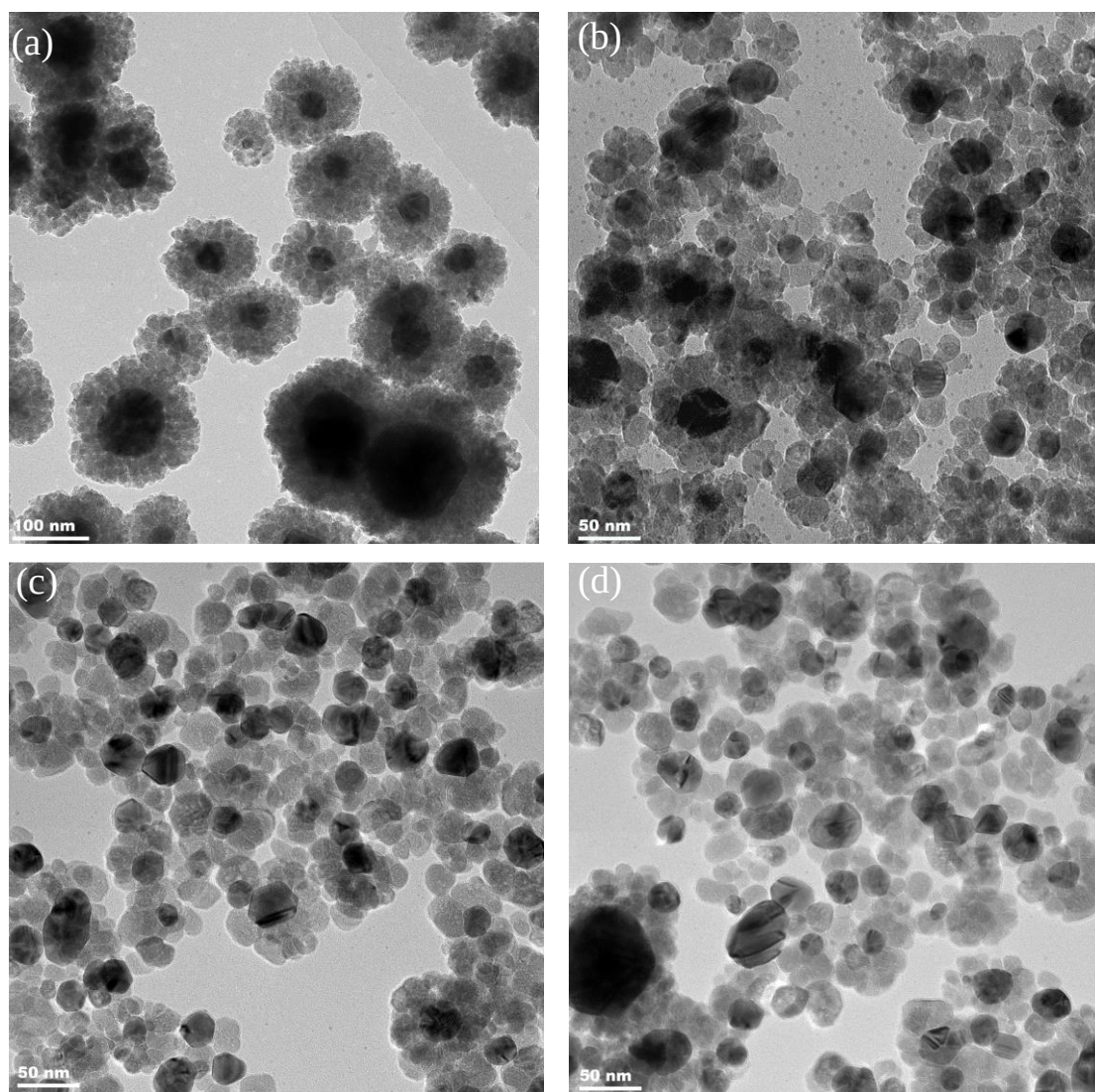


Figure 3.2: TEM images of solvo-thermally synthesized $\text{Fe}_3\text{O}_4@\text{AgNPs}$ in silver nitrate precursor solutions of (a) 20 mM (b) 40 mM (c) 50 mM and (d) 60 mM.

The typical apparent contrast between the nano-silver rich phase and that of magnetite in Fe₃O₄@AgNPs composites can easily be identified. It is understood that since Ag atoms have a relatively higher electron density compared to Fe₃O₄, fewer beam electrons are transmitted through them, making the AgNP segment appear darker than that of Fe₃O₄ [85]. The distinct nature with which these segments exist stem from the varying kinetics associated with their formation. According to Zhang *et al.* [85] the reduction potential of Ag⁺ in reference to the standard hydrogen electrode (SHE) is higher than that of Fe³⁺, ($E_{\text{Ag}^+/\text{Ag}}^0 = 0.799 \text{ eV}$ vs $E_{\text{Fe}^{3+}/\text{Fe}^{2+}} = 0.77 \text{ eV}$). Therefore, when a suitable reducing agent is used, AgNPs seeds form faster than the conversion of Fe³⁺ to Fe²⁺. In consequence, the latter formed Fe₃O₄ nanostructures are deposited on the AgNPs surface resulting in either flower-like or heterodimer arrays [85].

Particles made from 20 mM AgNO₃ (**Figure 3.2 (a)**) presented a proper core-shell structure but with increasing silver nitrate (40 to 60 mM), a structural divergence from the flower-like orientation was observed. Nanostructures of Ag partially covered by Fe₃O₄ were observed and their regularity improved with increasing AgNO₃ concentration (**Figure 3.2 (b-d)**). According to Oscar *et al.* [122] the final conformation of these nanostructures is governed by the polar solvent concentration with respect to Ag seed population along with the sizes of the Ag seeds themselves. When Fe₃O₄ nanoparticles start to nucleate on the Ag seeds surface, a charge in the polarized plane at the interface is induced. Polar solvents like EG would then provide the necessary compensation to this electron deficiency instead of delocalised Ag electrons and in doing so, nucleation in multiple facets is facilitated leading to formation of flower like shaped Fe₃O₄@AgNPs [122]. An increasing AgNO₃ concentration is likely to generate large quantities of Ag seeds which in-turn reduces their relative share of the polar solvent (EG) resulting in formation partially Fe₃O₄ covered AgNPs observed in **Figure 3.2 (b-d)**. Zhang *et al.* [123] also obtained similar structural observations. They concluded that the absence of a typical surfactant like poly-vinylpyrrolidone (PVP), which would have helped provide compensation for the aforementioned electron deficiency, impaired the multifaceted association of Fe₃O₄ on Ag surface leading to formation of Fe₃O₄@AgNPs heterodimers [123].

SEM images of the nanocomposites given in **Figure 3.3(a-d)** reveal that the prepared particles are generally spherical in shape, validating observations made via TEM. These particles presented a very narrow particles size distribution. In addition, the obtained EDS

data given in **Table 3.1** illustrates a proportional increase in Ag content with respect to the input precursor AgNO_3 concentration. Herein, the purity of the samples was also confirmed as no foreign elements were detected.

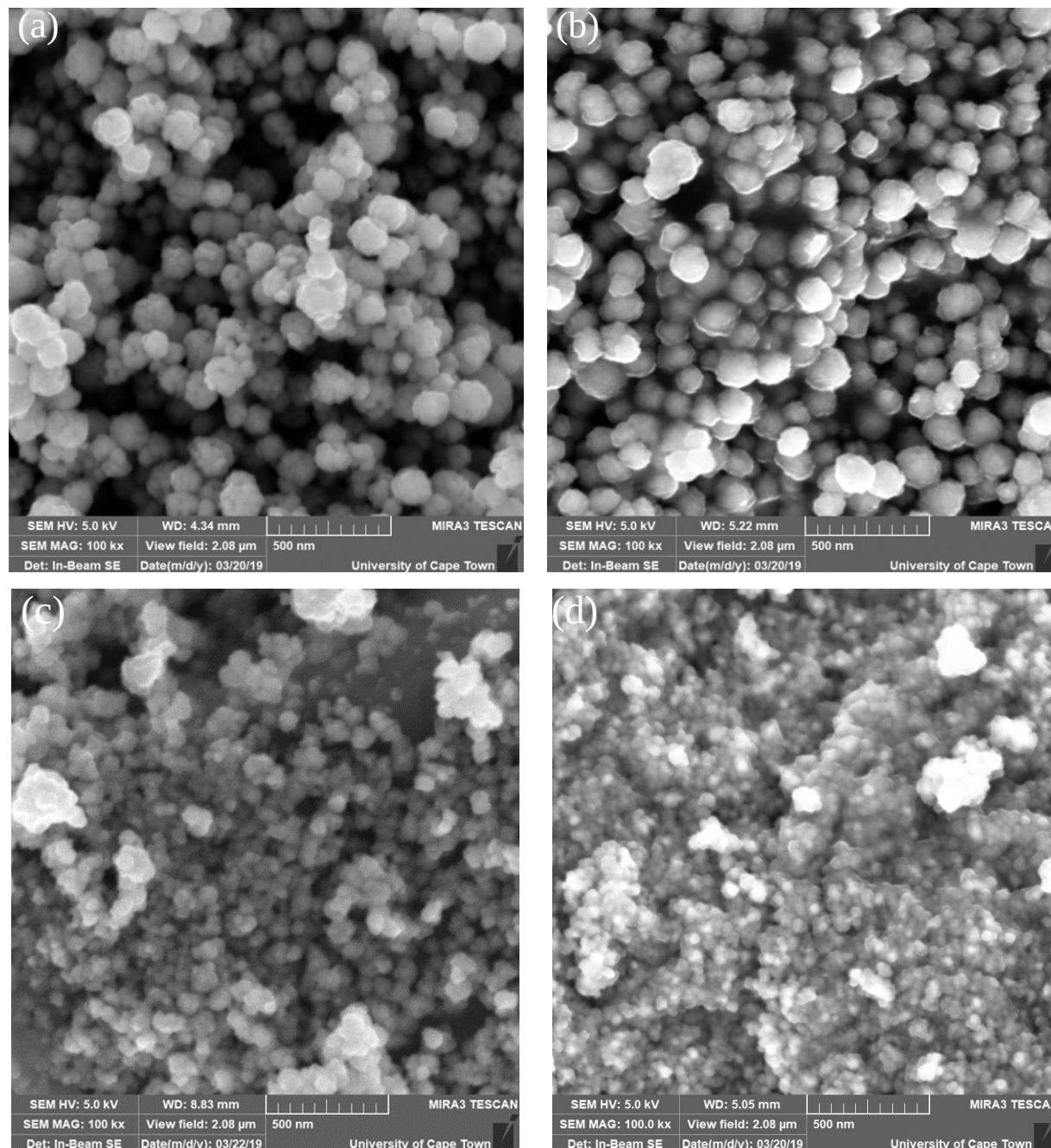


Figure 3.3: SEM images of solvothermally fabricated (a) Fe_3O_4 , and $\text{Fe}_3\text{O}_4@AgNPs$ synthesized in AgNO_3 concentrations of b) 20 mM (c) 40 mM and (d) 50 mM.

Table 3.1: Energy dispersive spectroscopy (EDS) derived elemental weight percentages on synthesised nanozymes.

	Precursor AgNO ₃ molarity (mM)	%O	%Fe	%Ag	Total
1	0	42.94	57.06	0.00	100.00
2	20	64.40	27.03	8.56	100.00
3	40	65.24	21.80	12.96	100.00
4	50	57.65	21.54	20.81	100.00
5	60	36.02	27.73	36.25	100.00

It is understood that the collective oscillatory excitation of free electrons on metallic surfaces particularly silver and other noble metals as described in **section 2.22**, gives rise to a large enhancement of the local electromagnetic field (EM). These enhanced EM can amplify the vibrational signals of surface adsorbed molecules and those in close proximity. Surface-enhanced infrared absorption spectroscopy (SEIRA) is a technique which utilises this concept to detect organic molecules. It has also been reported that along with the aforementioned EM mechanism for signal enhancement, a chemical enhancement mechanism exists too although its influence is dwarfed by the latter [124,125]. Herein, the presence of AgNPs on the fabricated Fe₃O₄@Ag nanocomposites was further confirmed indirectly by running IR tests in ATR mode after adsorbing methanolic p-NTP (1 mM) over a 1 hr duration on their surfaces.

In contrast to the unenhanced IR spectrum observed for the dried mixture of p-NTP and bare magnetite nanostructures, vivid vibrational peaks owing to the SEIRA effect can easily be identified in **Figure 3.4** for all Ag-containing samples. The absence of a S–H mode at 2549 cm⁻¹ however, suggests that the detected signals are not of p-NTP. Instead, it resembles that of bis(4-nitrophenyl) disulfide, a dimer of p-NTP [29]. The strong absorption peak around 1326 cm⁻¹ is in agreement with the literature and it is typically ascribed to the symmetric stretching of the NO₂ group [29]. Other additional strong bands at 779 and 1626 cm⁻¹ that may attributed to the C-H bending and benzene ring breathing respectively [29].

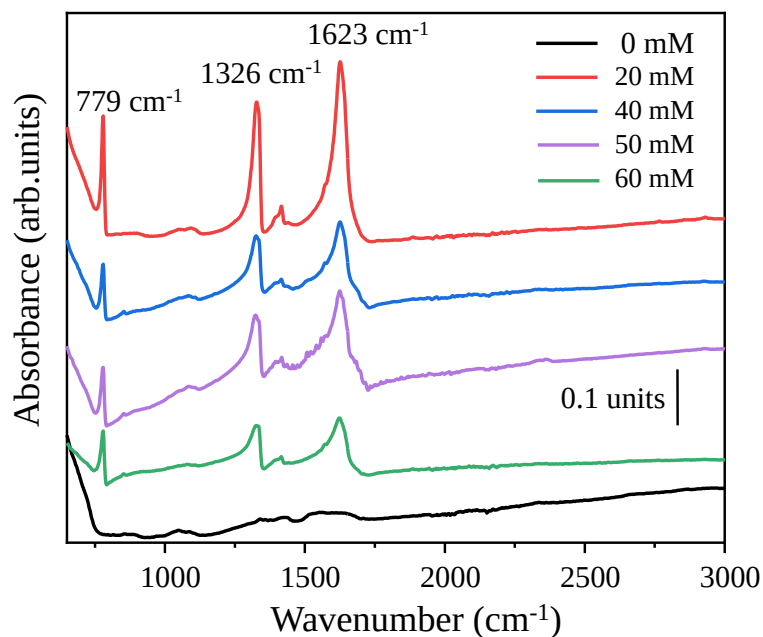


Figure 3.4: SEIRA spectra of Ag adsorbed pNTP in (bis(4-nitrophenyl) disulfide form for all $\text{Fe}_3\text{O}_4@\text{AgNPs}$ synthesised with silver concentration from (0-60) mM.

3.3.2 Peroxidase Activity Analysis

The intrinsic peroxidase like activity of Fe_3O_4 and $\text{Fe}_3\text{O}_4@\text{Ag}$ nanoparticles was confirmed via their ability to oxidise OPD in the presence of H_2O_2 . With a phosphate-citrate buffer of pH 6.2 included, the transformation of a previously colourless reaction mixture to an orange oxidised form of OPD, i.e., diaminophenazine (DAP) with an absorption peak at 416 nm was observed. It was however, evident, as indicated in **Figure 3.5(b)** that $\text{Fe}_3\text{O}_4@\text{AgNPs}$ synthesised from 20 mM AgNO_3 exhibited superior catalytic activity in comparison to bare Fe_3O_4 nanoparticles. This enhancement in activity is tentatively linked with the development of a special electronic structure at the interfaces between the Fe_3O_4 aggregates and the noble metals such as silver and gold nanoparticles [126,127]. Fundamentally, the catalytic activity of the Fe_3O_4 nanoparticles is driven by the transfer of electrons between pairs of different oxidation states of the metal ions i.e. $\text{Fe}^{2+}/\text{Fe}^{3+}$ [9]. The partial electron transfers from Fe_3O_4 to Ag as it is for Fe_3O_4 to Au at their nanoscale interfaces is thought to enhance the overall catalytic capability by stabilising the generated $\cdot\text{OH}$ radicals [128,129]. This phenomenon has also been observed in noble metal based multicomponent nano-catalysts including PtPd- Fe_3O_4 [129]. In addition to the abovementioned peroxidase-like capability of Fe_3O_4 , Ag nanoparticles have also been shown to inherently possess the same property [78]. Furthermore, the experimental work was done in the presence of some internal laboratory lighting, therefore, there is potential for localized heating as a result of the thermal dissipation

owing to localised surface plasmon resonance (LSPR) relaxation in AgNPs [130]. A combination of all these factors could constitute the observed heightening in peroxidase-like activity by $\text{Fe}_3\text{O}_4@\text{Ag}$ nanocomposites.

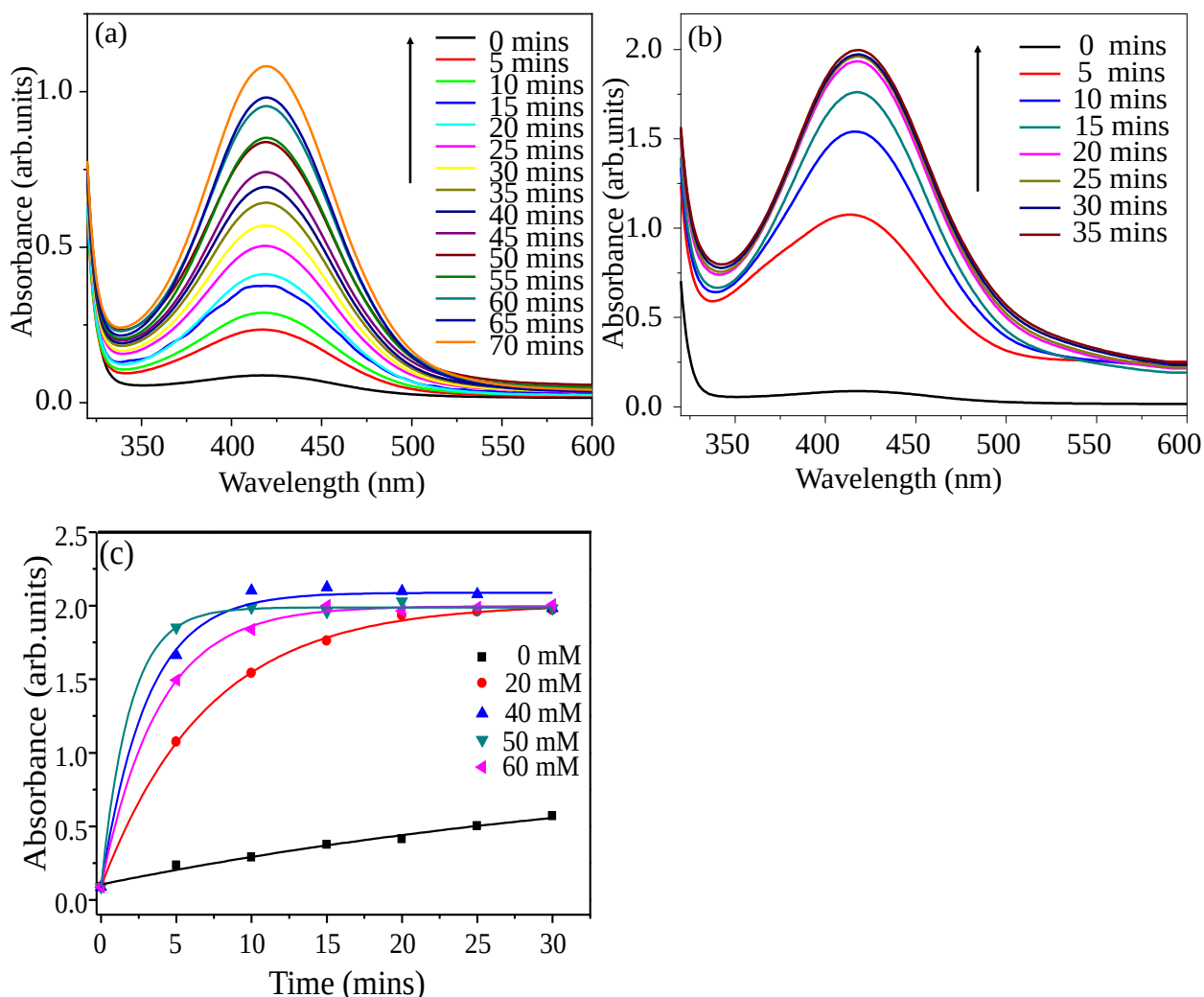


Figure 3.5: Time dependent absorption spectra evolution for H_2O_2 (92.3 Mm) oxidised OPD (15mM) catalysed by (a) Fe_3O_4 and (b) $\text{Fe}_3\text{O}_4@\text{AgNPs-20}$. (c) Reaction progress under catalysis of $\text{Fe}_3\text{O}_4@\text{AgNPs}$ synthesized from varying AgNO_3 concentrations.

Having determined that the presence of AgNPs improves the overall peroxidase like activity, nanocomposites offering optimal performance with respect to the precursor AgNO_3 concentration were sought out. A catalytic activity study of the synthesized particles revealed that those fabricated from a reaction solution consisting of 50 mM AgNO_3 demonstrated the fastest catalytic efficacy (**Figure 3.5(c)**). This behaviour may be accounted for by the possible increase in contact area on AgNPs for OPD- H_2O_2 substrates as more AgNPs facets became exposed given the observed deterioration in flower-like structural integrity of $\text{Fe}_3\text{O}_4@\text{Ag}$ nanocomposites (**Figure 3.2(b-d)**). Additionally, the reduced surface coverage by

the Fe_3O_4 shell on the Ag core leaves room for improved mass transfer to and from the Ag facets. Even though particles with a more exposed Ag surface structure appeared to offer better catalytic performance, nanocomposites made from 60 mM AgNO_3 were found to be marginally worse off to those of 50 mM. It should be noted that although the generation of exposed AgNPs facets enhances the catalytic activity, it is also a trade-off for the formation of Ag/ Fe_3O_4 interfaces which have also been proposed to be vital for catalytic activity enhancement, as such particles made from 60 mM may have exceeded the necessary balance between these factors.

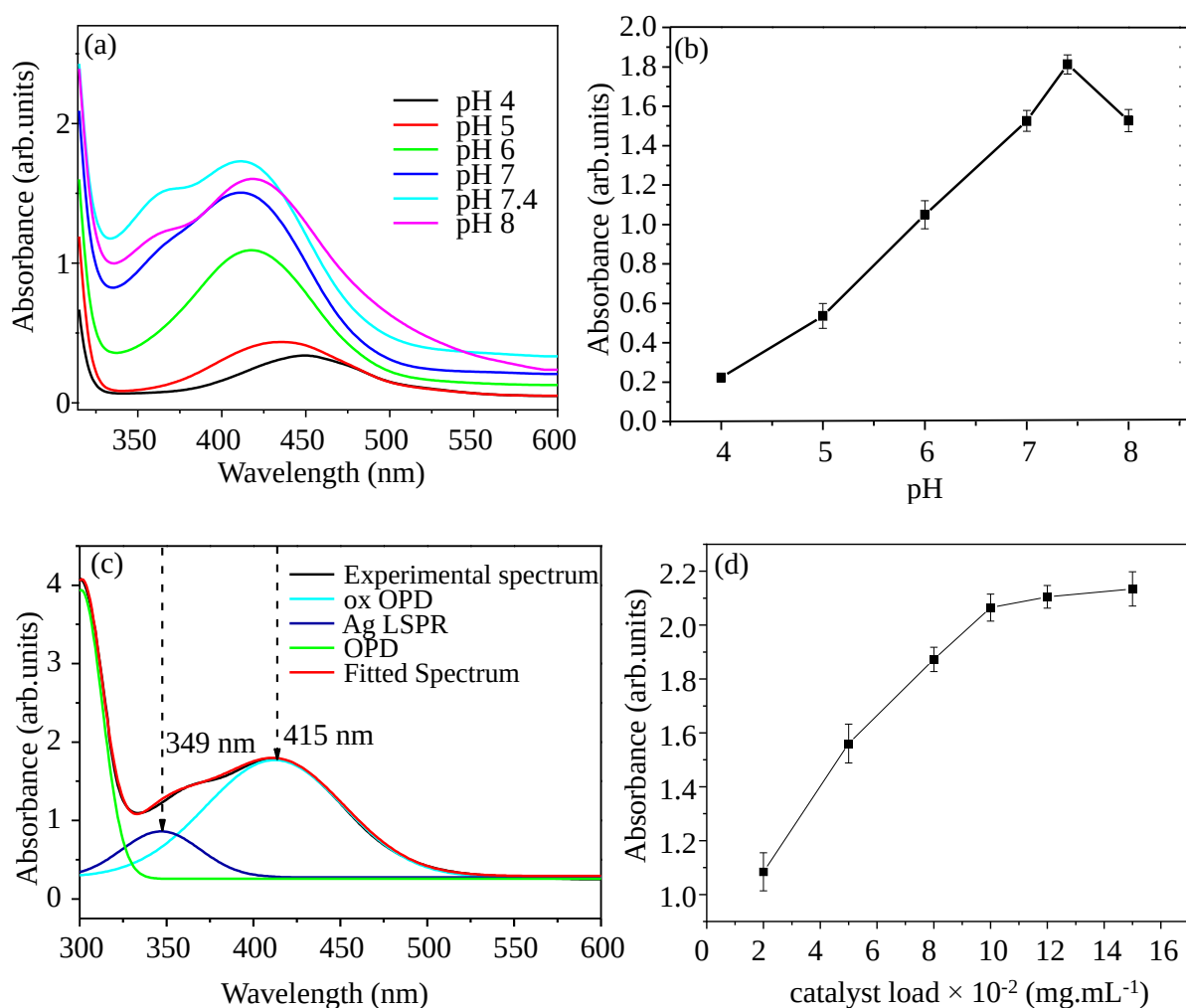


Figure 3.6: (a) Sample absorption spectra of a 5-minute-long catalysed OPD (15 mM) oxidation by H_2O_2 (91.8 mM) under varying pH conditions. (b) corresponding maximum Absorbance plotted against Ph. (c) decomposition of a typical absorption spectra for the kinetically superior pH condition [7.4]. (d) influence of catalyst load on solution absorbance after a reaction time of 10 minutes at pH 7.4.

Similar to typical nanozymes and peroxidases, the peroxidase-like activity of the $\text{Fe}_3\text{O}_4@\text{AgNPs}$ was found to depend on the pH, concentrations of H_2O_2 and OPD as well as the catalyst loading. A 5-minute-long incubation period of the reaction mixture in 0.100 mg of AgNO_3 -optimised- $\text{Fe}_3\text{O}_4@\text{AgNPs}$ was carried out at varying pH conditions (4-8). The OPD oxidation rate was found to increase with pH and the optimal activity was obtained at pH 7.4. Since H_2O_2 tends to decompose into O_2 and H_2O instead of hydroxyl radicals in basic conditions, even more so when a noble metal catalyst is present, the subsequent decline in activity at pH 8 may have been caused by this behaviour [36]. Contrary to its detrimental effect under more basic conditions, the same production of O_2 could also have improved the conversion rate at pH 7.4 through bubbling thus facilitating a better solution mixing rate.

Furthermore, the appearance of a higher order LSPR peak at 349 nm (**Figure 3.6(c)**) can be accounted for by the presence of suspended AgNPs owing to O_2 bubbles. The maximum absorption peak of oxidised OPD was found to shift depending on the reaction pH as illustrated in **Figure 3.6(a)**. This suggested that oligomers with different makeups (constitutions), molar masses, levels of oxidation were being formed [37]. Catalyst loadings ranging from 0.010-0.150 mg. mL^{-1} (**Figure 3.6(d)**) were subjected to the reaction system at a pH of 7.4. As more active sites are made available with more of the catalyst, an increase in activity was observed prior to 0.100 mg. mL^{-1} loading. For loadings in excess of the 0.100 mg. mL^{-1} , saturation in catalytic activity was obtained. This indicated that 0.100 mg. mL^{-1} was the optimum catalytic amount.

Figure 3.7(a) illustrates the effect of OPD concentration on $\text{Fe}_3\text{O}_4@\text{AgNPs}$ performance. With the optimum loading of 0.100 mg. mL^{-1} employed in the reaction mixture at a pH of 7.4 and the H_2O_2 concentration maintained at (4 μL of 35%), a stagnation in absorbances at ~ 2.0 for 9 mM OPD concentrations and beyond after a 10-minute incubation period was observed. This substrate concentration independence is characteristic to a change in enzymatic reaction kinetics from 1st order to zero order. In detail, the catalyst is considered to be working at full capacity after this transition. Repeating the same procedure with OPD concentration set at 9 mM revealed the optimum H_2O_2 molarity to be 40 mM **Figure 3.7(b)** concentrations in excess of 40 mM were proved to be indifferent.

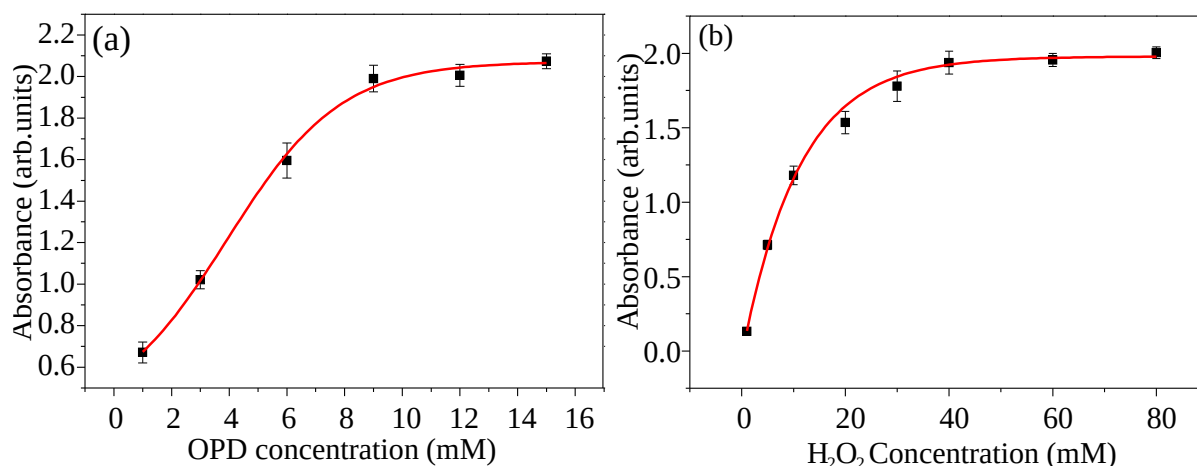


Figure 3.7: Absorbance variation to determine the effect initial concentration of **(a)** OPD and **(b)** H_2O_2 has on catalytic activity of $\text{Fe}_3\text{O}_4@\text{AgNPs}$.

The steady state kinetics study as described in **section 3.2.10** for both OPD and H_2O_2 revealed that the catalytic conversion mechanism closely resembles the Michaelis-Menten model (**Figure 3.8(a&c)**). Lineweaver-plots in **Figure 3.8(b&d)** were used to determine the Michaelis constants (K_m). Since low values of these constants are indicative of stronger binding affinity of substrates towards a catalyst, the small K_m value (28.0 mM) for H_2O_2 acquired in this work in comparison to that obtained by Gao *et al.*[9] for bare Fe_3O_4 (154 mM) suggests that AgNPs improve the overall complexation of H_2O_2 with the nano-catalyst. Other kinetic parameters are as presented in **Table 3.2** below. Of a few other nanozymes of which catalytic studies was carried out using OPD, copper based carbon dots were found to show better affinities to it with a K_m value of 0.548 mM [131].

Table 3.2: Michaelis-Menten kinetic parameters for the silver-magnetite core-shell catalyst

Item		OPD	Hydrogen Peroxide
1	Kmax (mM)	2.91	28.0
2	Vmax (mM.min ⁻¹)	0.0366	0.526

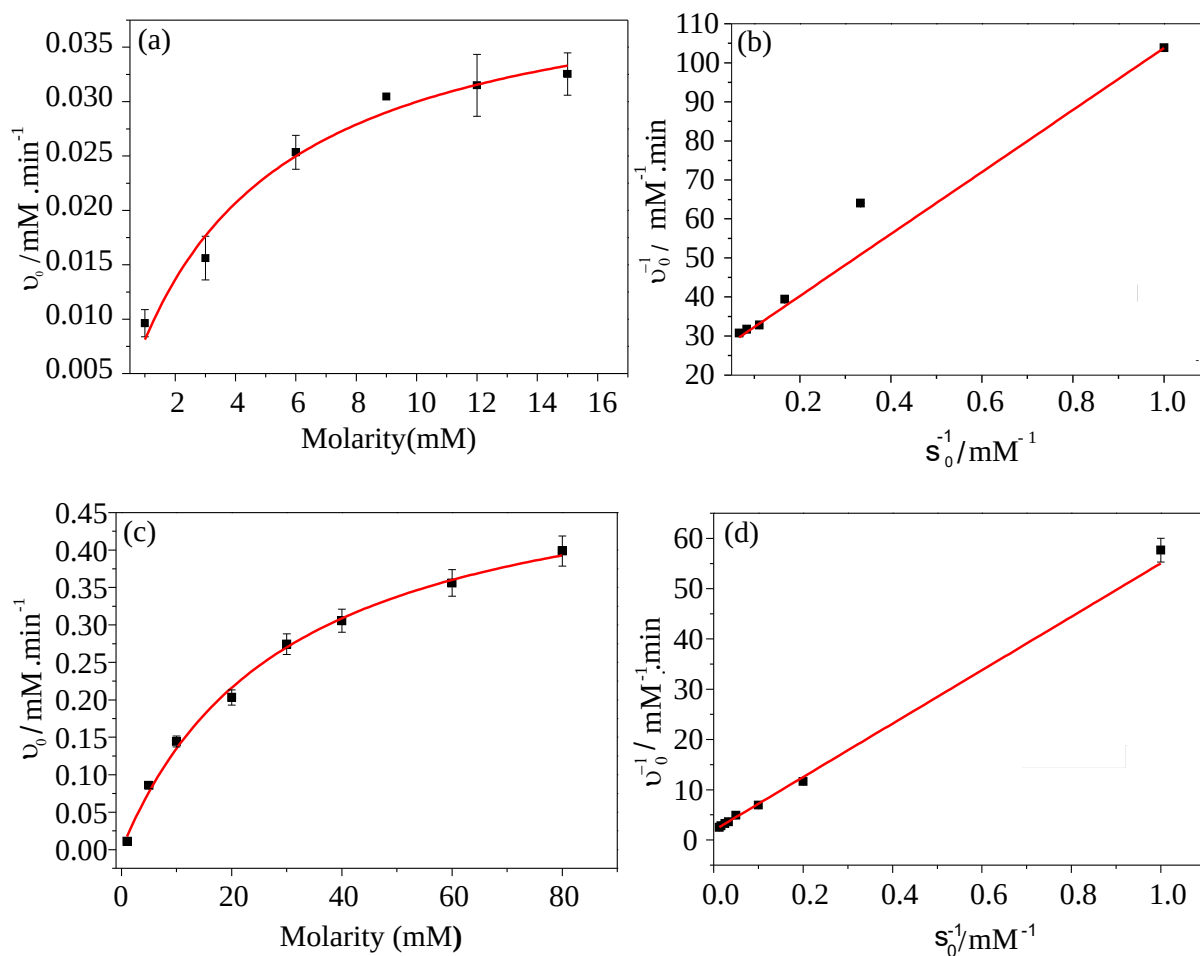


Figure 3.8: (a) The influence of OPD concentration on the initial reaction velocities of $Fe_3O_4@AgNPs$ -catalysed oxidation of OPD (b) corresponding Lineweaver-Burk plot for graph.(c) initial OPD oxidation rate dependence on the original concentration of H_2O_2 .(d) Lineweaver-Burk plot for the synthesised $Fe_3O_4@AgNPs$ with respect to H_2O_2 as a substrate.

3.3.3 Cysteine detection

It is thus far acknowledged that the peroxidase-like activity of nanozymes is facilitated by substrate oxidising reactive oxygen species (ROS) generated via the decomposition of H_2O_2 . The Introduction of 60 μM L-cysteine into the reaction mixture of ($Fe_3O_4@AgNPs$ + OPD and H_2O_2) however, was found to temporarily deter the progress in peroxidase-like activity of $Fe_3O_4@AgNPs$ (**Figure 3.9(a)**). Through weak electrostatic interactions, polarised zwitterionic molecules such as cysteine are known to adsorb on metal oxide surfaces including Fe_3O_4 nanostructures resulting in inhibition of the aforementioned peroxidase activity [132]. Furthermore, cysteine molecules also comprise of thiol functional groups which are also prone to adsorption on AgNPs, as such this provides a further boost to the net inhibition.

Following an incubation period of 10 minutes, the absorbance differential ΔA of a 60 μM cysteine inclusive reaction mixture relative to the control was found to be very low under optimum conditions. With an understanding that the binding of polar amino acids on nanozymes is pH dependent, an optimisation procedure providing results in **Figure 3.9(b)** revealed that even though it is at the expense of nanozyme activity, a reaction system at pH 6 offered the best platform for developing a cysteine sensor owing to its relatively large ΔA . Under this condition, a concentration response profile in **Figure 3.9(c)** was generated and its corresponding curve was also plotted (**Figure 3.10(a)**). On evidence, this curve presents a gradual increase in ΔA as L-cysteine dosages are increased from 0-500 μM . Crucially, a linear correlation with a regression coefficient R^2 of 0.9966 was obtained on a 1-20 μM range, suggesting that this system could be used within this range to detect cysteine (**Figure 3.10(b)**). The detection limit of this sensor was found to be 87 nM at a signal ratio (S/N) of 3.

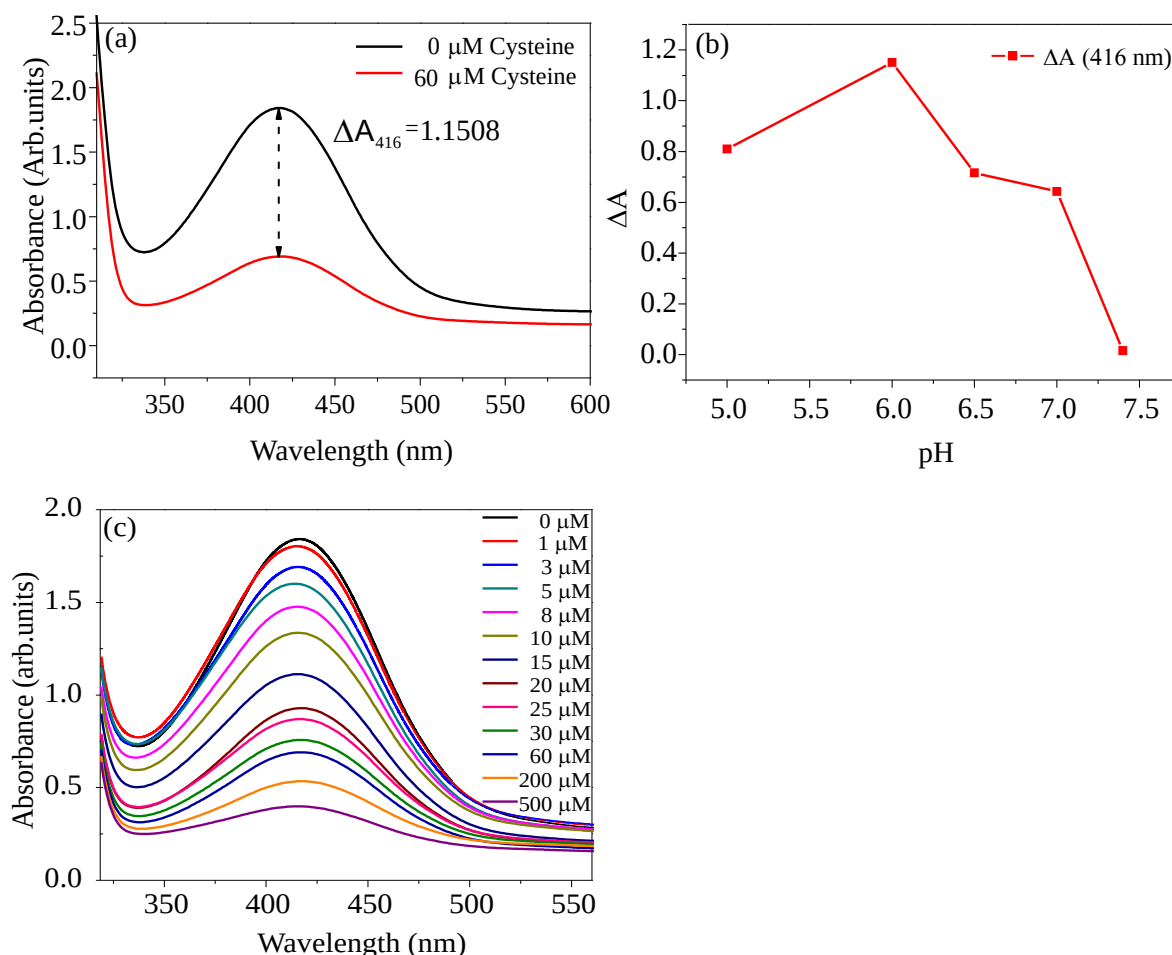


Figure 3.9: (a) Absorbance differential spectra for 60 μM cysteine $\text{Fe}_3\text{O}_4@\text{AgNPs}$ catalysed OPD oxidation after a 10 minute of incubation. (b) Inhibition capacity of cysteine at different pH conditions. (c) Absorption spectra of oxidised OPD as a function of cysteine concentrations.

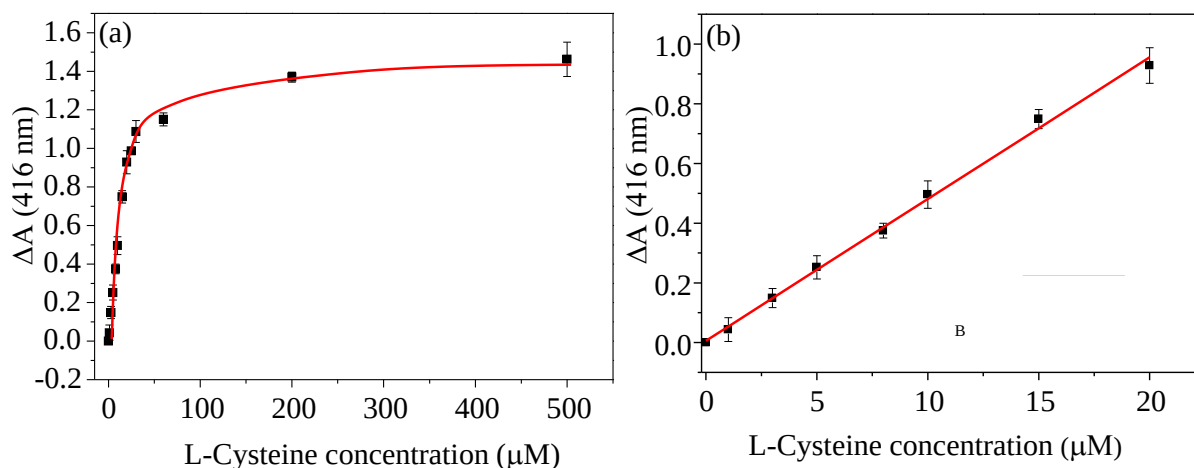


Figure 3.10: (a) DAP Absorbance differentials as a function of cysteine concentration for catalysed OPD oxidation over 10 minutes of reaction time. **(b)** linear calibration of ΔA with respect to cysteine concentration.

In a comparative study presented in Table 3.3 below, the $Fe_3O_4@AgNPs$ synthesized in this work proved superior to a number of nanozymes used in colorimetric sensors for L-cysteine based on either the limit of detection (LOD) or the detection range.

Table 3.3: Limits of detection (LOD) and detection ranges of L-Cysteine for various nanozymes based sensors.

	Nanozyme	Range (μM)	Limit Of Detection (μM)	Reference
1	Fe_3O_4 Nanofibers	2–10	0.028	[94]
2	$Gd(OH)_3$ Nano Rods	0.2–75	2.6	[108]
3	FeCo-Carbo nanofibers	1-20	0.15	[133]
4	Fe_3O_4	0 to 50	0.97	[134]
5	$MnCo_2O_4$ Nanofibers	0.5 -10	0.0343	[135]
6	Mo^{6+} -Doped Co_3O_4	0.05 - 10	0.0242	[136]
7	$Fe_3O_4@AgNPs$	2-20	0.087	This work

3.3.4 Selectivity

As previously mentioned, the detection of cysteine in body plasma and or other fluids is undeniably vital. Given that these fluids are usually a concoction of a range of molecules, a selectivity study to determine the influence of potential interference molecules in the devised cysteine detection system is also important. Herein, common amino acids and plasma fluids

listed in **Figure 3.9(a)** were investigated. With optimal conditions for cystiene detection maintained, ΔA values of 20 μM solutions were determined as shown in

Figure 3.11(b). Other than for ascorbic acid and creatinine, all other compounds exhibited ΔA values of insignificance with respect to that of cysteine at the same concentration. This promising degree of selectivity may be attributed to strong binding propensity of thiol groups on ferric irons and nano silver [134].

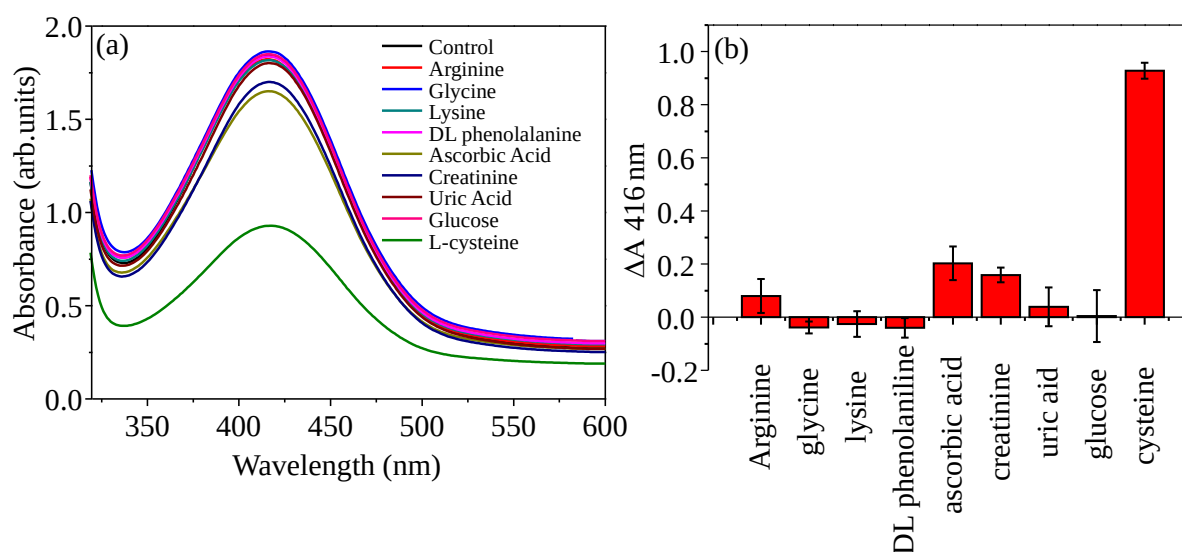


Figure 3.11: (a) Absorbance spectra for detection mixtures in the presence of some 20 μM solutions of potential interference molecules found in body plasma and their respective **(b)** absorbance differentials with respect to the control solution for selectivity analysis.

3.4 Conclusion

The magnetically responsive $\text{Ag}@\text{Fe}_3\text{O}_4$ nanocomposites exhibiting enhanced peroxidase-like activity were fabricated successfully following a simple one-step solvo-thermal process. By increasingly varying the concentration of AgNO_3 used in their synthesis, a transformation from core-shell nanostructure orientation to those where the silver core was sparingly decorated by magnetite particles was observed. These loosely decorated Ag core nanocomposites exhibited a superior behaviour in catalysing the oxidation of OPD in the presence of H_2O_2 at physiological pH conditions. Based on the high catalytic activity of these nano-structures, a simple colorimetric cysteine detector operating at room temperature was developed. The low detection limit (87 nM) and high selectivity exhibited by this sensor makes it a promising candidate for clinical diagnosis, environmental applications.

CHAPTER 4: NANO-SILVER DECORATED MAGNETITE FOR CATALYTIC DEGRADATION OF 4-NITROPHENOL, METHYLENE BLUE AND RHODAMINE 6G.

4.1 Introduction

The utilisation of silver nanoparticles in a variety of fields including medicine, analytical sensing, waste water treatment, biotechnology and food packaging has grown to prominence over the past decade on account to the fascinating optical, antibacterial and electrochemical properties they have [137-140]. Their role in catalysing chemical transformations in such processes as carbon monoxide oxidation, organic synthesis and reductive catalysis of chlorinated or nitrogen containing hydrocarbons is well documented [141-143]. AgNPs however, are prone to aggregation given their high surface energy and in consequence, this lowers their overall intrinsic catalytic efficiency [144]. Also, in nanometric sizes, the recovery of these particles from a reaction solution has been found to be a challenging prospect. These drawbacks have prompted the need to develop innovative approaches as solutions. Among them, the immobilisation of catalytic metal NPs onto solid supports as a way to facilitate stability and nanoparticle dispersion has gained popularity [145]. Additionally, in conjunction with their ability to control particle growth with regard to size and shape, solid supports provide platforms to fabricate hybrid nanostructures with multifunctional properties [146]. It has also been realised that the deposition of AgNPs on suitable matrices such as polymers, graphene sheets, metalorganic frameworks and metal oxides can via synergistic effects enhance the catalytic activity of silver nanocomposites [145,147].

Of recent, Fe_3O_4 nanostructures have undergone an intensive study and found uses in a range of applications including as support media to incorporate noble metals on [147]. Their superparamagnetic nature, unlike other previously mentioned media, allows for the simple and effective separation of catalytic particles from a reaction mixture simply by applying an external magnetic field [148,149]. This mechanism has proven to be particularly important in trivialising the otherwise tedious and time-consuming catalyst recovery process and its eventual re-dispersion in solution when such techniques as centrifugation or filtration are employed. In addition to being environmentally benign, Fe_3O_4 particles are also known to be insoluble in aqueous solutions, a property which helps sustain their stability in solutions during catalytic reactions [145,150]. In view of these qualities, efforts to develop noble-metal decorated Fe_3O_4 nanocomposites including those with Ag have been on the rise, however,

many of the methods that have so far been successful in properly integrating the two have required the use of organic surface modifying compounds as linking agents [150-152]. As an example, Zhang *et al.* [153] declared that for their solvo-thermal synthesis of Ag@Fe₃O₄ heterodimers, the intervention of polyvinylpyrrolidone (PVP) was necessary. Surface modifying thiol-groups played a role towards the fabrication of dye degrading Fe₃O₄/SiO₂-Pr-S-Ag NPs as per Veisi *et al.* [154]. Das and co-workers [155] also employed polypyrrole (PPy) modified mercaptoacetic acid (MAA) in producing Fe₃O₄@PPy-MAA/Ag nanocatalysts for organic pollutants degradation. It is however known that the presence of surfactants not only pose environmental pollution problems but they can also to some extent depress the catalytic activity of silver particles by inhibiting the adsorption of reactants onto the surfaces of the particles [156-158].

In this study, a facile, two-step wet chemistry based procedure, utilising the silver mirror reaction (SMR) in the absence of environmentally hazardous organic chemicals to deposit silver on magnetite, was employed to synthesise silver decorated magnetite (Ag@Fe₃O₄) nanocomposites for applications on the catalytic degradation of typical environmental pollutants including 4-Nitrophenol (4-NP). The remediation of 4-NP from the environment in particular, is currently considered to be of prime importance given its ‘priority pollutant’ status as per the US Environmental Protection Agency and the European Union. In spite of its uses in drug manufacturing, leather darkening, fungicides, insecticides, and dyes, 4-NP is a highly toxic and carcinogenic compound capable of damaging the central nervous system, liver, kidney and blood of humans and animals [159]. As the agricultural and pharmaceuticals industries continue to relentlessly grow due to the ever-increasing demands of the modern society, the discharge of their 4-NP containing effluents particularly into water bodies has progressively attracted a lot of consideration [87,90]. Furthermore, its solubility and high level of stability in water makes this compound a very persistent and difficult pollutant to remove using conventional techniques [90].

As yet, various other methods including sonolysis, extraction and those making use of adsorbents have been proposed to mitigate 4-NP in wastewaters [19,90,159]. These techniques however, carry with them some disadvantages including incomplete 4-NP removal, high energy requirements and also the generation of a toxic sludge which would then demand further processing [8]. In comparison to these, the catalytic reduction of 4-NP into the less toxic 4-AP is a green and generally popular procedure for 4-NP treatment. The produced 4-AP has uses in pharmaceuticals, corrosion inhibition and photographic

development [26,160]. Driven by the abovementioned reasons, a simple to synthesise, magnetically recoverable, recyclable and highly effective nano-catalyst in degradation of 4-NP, Methylene blue (MB) and Rhodamine 6G has been fabricated.

4.2 Experimental

4.2.1 Chemicals

All reagents were of analytical grade and used as received without further purification. $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{Fe}_2(\text{SO}_4)_3$, and NH_4OH (28-30) % were all purchased from Merck. 4-Nitrophenol, Rhodamine 6G and Methylene blue along with AgNO_3 were purchased from Sigma. In preparation of solutions and rinsing steps, Millipore ultrapure double deionized water was used.

4.2.2 Instrumentation

The crystal structure, morphology and elemental composition of the samples were characterized using a Bruker D8 X-ray diffractometer with Ni-filtered $\text{CuK}\alpha$ radiation ($\lambda = 1.5406 \text{ \AA}$) operated at a generator voltage and an emission current of 40 kV and 40 mA, respectively, a field-emission scanning electron microscopy (FESEM) (JEOL JSM-7800F, JEOL Ltd., Tokyo, Japan) and the incorporated Energy Dispersive X-ray spectroscopy (EDS). A Thermogravimetric Analyser (TGA) was also used to study the mass loss of the synthesised nano-catalysts over a heating range from 60-600 °C. The Evolution 201 UV-visible spectrophotometer with a 1-cm path length quartz cuvette was used to monitor changes in solution concentrations for catalytic activity tests over a wavelength range from 250-800 nm. Raman spectra were measured using the Horiba LabRam Raman spectrometer equipped with 785-nm laser line as excitation source (JOBIN–YVON Technology, France) and a CCD detector. The accumulation and exposure period were set at 2 and 5s respectively on a $\times 100$ objective.

4.2.3 Preparation of Silver coated magnetite nanoparticles

4.2.3.1 Synthesis of magnetite

Herein, 10 mL each of 6 mM FeSO_4 and $\text{Fe}_2(\text{SO}_4)_3$ mM solutions were measured into a 50 mL beaker. Under constant magnetic stirring, 0.5 mL of a NH_4OH (28-30) % solution was pipetted into the beaker and the reaction was allowed to progress for 1 minute. Following this period, the magnetic stirrer was immediately removed and the precipitated black product was allowed to age for 2 hrs. To discard the supernatant, the product particles were first trapped

inside the beaker by positioning a strong permanent magnet directly underneath it before pouring off the liquid phase. The precipitate was then washed 4 times with deionized water.

4.2.3.2 Decoration of Silver nanoparticles on magnetite

The silver mirror reaction was employed to synthesise silver coated magnetic nanoparticles Ag@Fe₃O₄. This reaction involves the generation of metallic silver by reducing Ag ions in the Tollen's reagent using an aldehyde. The still-wet magnetic nanoparticles (MNPs) formed previously by following the co-precipitation method as described above were re-dispersed in 6 mL of deionised water. With this mixture evenly distributed, 1 mL each time was pipetted into six 5 mL glass vials. All the water was then poured off from the Fe₃O₄-NPs again with the help of a permanent magnet. To these particles, 3 mL AgNO₃ solutions with different concentrations (20-60) mM were measured into the glass vials. 50 µL of 1 M NaOH was then pipetted into the mixture followed by 100 µL of ammonium hydroxide. The mixture was then gently shaken to speed up the dissolution of the formed Ag₂O precipitate before the glass vials were placed on an ice bath where they would reside for 10 minutes. This habitation period was to enable the formed silver-ammonium complex to adsorb to equilibrium on the Fe₃O₄-NPs. Then after, 900 µL of 0.5 M glucose was then pipetted into the vials which were immediately transferred to a water bath set at 55 °C [138]. The reaction was allowed to progress for a period of interest also depending on the respective optimisation parameter. Once the reaction time had elapsed, the glass vials were immediately transferred to an ice bath to cease the reaction. After 3 minutes, the formed nanoparticles were decanted with the help of a magnet and thoroughly washed 4 times using deionised water. The nanoparticles were stored in an ice bath awaiting measurements for their catalytic efficacy.

4.3 Catalytic reduction of 4-nitrophenol

The catalytic activity of the Ag@Fe₃O₄-NPs was evaluated using the reduction of 4-nitrophenol by NaBH₄ to 4-aminophenol as the model reaction. In a typical reaction, a catalyst batch synthesized as described above was transferred into a 50 mL beaker. Subsequently, a 10 mL aqueous solution of 0.4 mM 4-NP followed by the same volume of a freshly prepared 30 mM NaBH₄ were poured into the catalyst containing beaker. A minute later, following addition of NaBH₄, 2 mL of the reaction mixture was pipetted into a quartz cuvette and absorbance measurements were done of wavelength ranges 250-550 nm. The same procedure was repeated continually in 2 minute intervals and every-time pouring back the measured solution into the reaction mixture. For comparison, the reference measurement (zero) was made on solution consisting of the same reagents but without a catalyst.

4.4 Optimisation

The catalytic performance of metallic nanoparticles towards a specific chemical reaction is usually dependent on a range of factors. Here in, a series of parameters namely; the SMR exposure period, AgNO_3 concentration, catalyst loading, support medium content (Fe_3O_4) and 4-NP concentrations were all investigated for their influence.

4.5 Recyclability

A recyclability study for $\text{Ag@Fe}_3\text{O}_4$ -NPs was carried out following their extraction from the product solution via the application of an external magnetic field. Prior to their introduction to each of the 7 subsequent reaction cycles, the particles were rinsed 3 three times in deionised water every time.

4.6 Dye degradation

For this study, two water-soluble organic dyes, methylene blue (MB) and Rhodamine 6G were selected as substrates to test the applicability of $\text{Ag@Fe}_3\text{O}_4$ -NPs in degrading a variety of other potential pollutant dyes. In a typical degradation process, 10 mL of aqueous a dye solution (0.2 mM) was measured into a beaker containing the $\text{Ag@Fe}_3\text{O}_4$ catalyst. Subsequently, 10 mL of a freshly prepared NaBH_4 solution (15 mM) was also added to the above mixture. The catalytic activity was studied based on the attenuation of UV-vis absorption spectra at λ_{max} of 515 nm and 650 nm for R6G and MB respectively over selected time intervals. The distinction between simple dye discoloration and degradation was carried out by obtaining Raman spectra of each dye whilst adsorbed on the synthesized SERS active $\text{Ag@Fe}_3\text{O}_4$ -NPs before and minutes after the addition of NaBH_4 . Prior to these measurements, 1 mL of each dye at 0.2 mM concentrations was soaked on the nanocomposites for 1 hr, rinsed with distilled water and dried naturally.

4.7 Results and discussion

4.7.1 Characterization of the nanostructures

The surface morphology and composition of the as-prepared nanoparticles was investigated using FESEM and EDS. A close view of the obtained FE-SEM image in **Figure 4.1(a)** reveals that the synthesised Fe_3O_4 NPs are nearly spherical in shape and mostly aggregated. In addition to large surface energies, this aggregation can also be accounted for by inter-particle magnetic interactions [161]. Following the deposition of metallic silver on Fe_3O_4 surfaces, the same nearly spherical shape of the nanocomposite was retained (**Figure 4.1(b)**). The availability of silver was confirmed via EDS analysis as shown on **Table 4.0-1**.

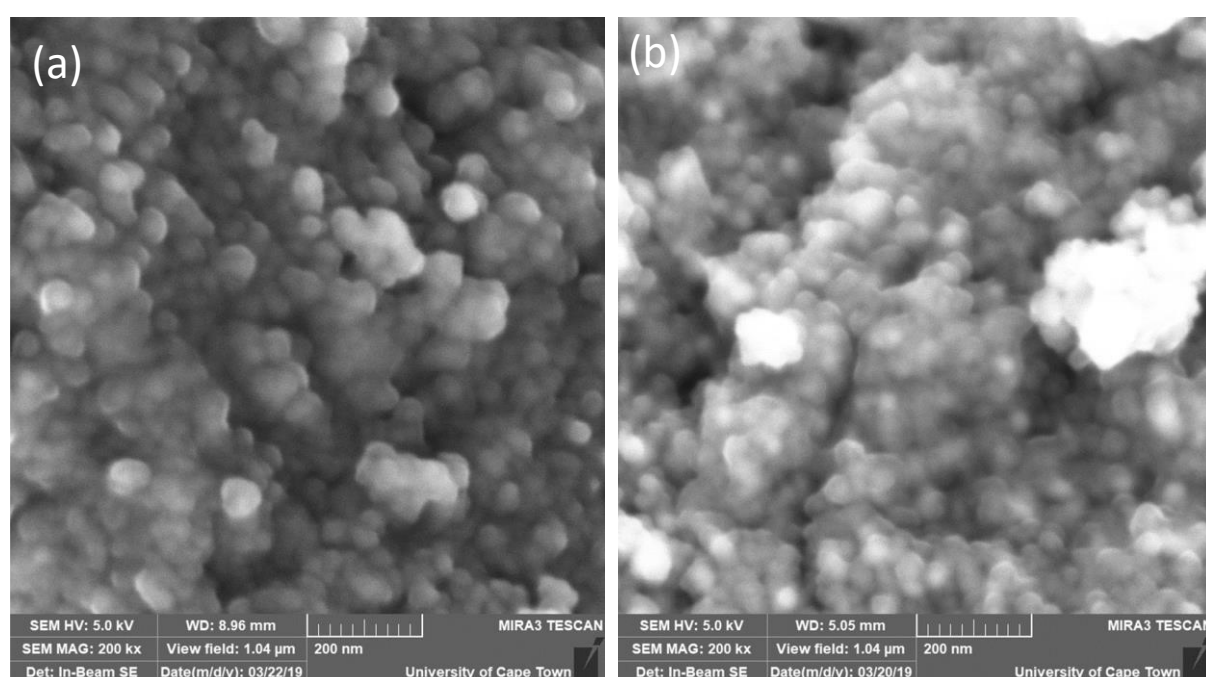


Figure 4.1: SEM micrographs of (a) Fe_3O_4 -NPs and (b) $\text{Ag@Fe}_3\text{O}_4$ -NPs.

Table 4.0-1: Elemental compositions of the synthesised catalysts obtained using the EDS

Item	Sample	Carbon %	Oxygen %	Iron %	Silver %
1	Fe_3O_4	10.59	37.06	52.35	0
2	$\text{Ag@Fe}_3\text{O}_4$	21.37	17.75	8.38	52.50

X-ray diffraction (XRD) patterns for both nanostructures were also obtained to determine their crystalline purities and phases as shown in **Figure 4.2(a)**. In reference to the Fe_3O_4 sample, a pattern comprising of five major diffraction peaks centred at $2\theta = 30.16^\circ$, 36.62° , 43.75° , 57.56° , and 63.43° was obtained. This array is naturally consistent with an fcc crystal structure of Fe_3O_4 (standard card: COD 9005812). Moreover, the absence of any extra peaks, typically of FeO and Fe_2O_3 phases, confirmed the purity of this phase. In relation to the $\text{Ag}@\text{Fe}_3\text{O}_4$ sample, comparatively more intense diffraction peaks in excess to those of Fe_3O_4 are observable at $2\theta = 37.87^\circ$, 44.16° , 64.37° , 77.26° and 81.46° . This suggests that an additional phase in conjunction to that of Fe_3O_4 was present. An array of this nature could be indexed to face centred cubic crystals of silver nanoparticles (JCPDS card COD 9013045). By utilising the Debye-Scherrer formula, the crystalline sizes of bare Fe_3O_4 -NPs and AgNPs were estimated to be 17.16 nm and 27.13 nm respectively.

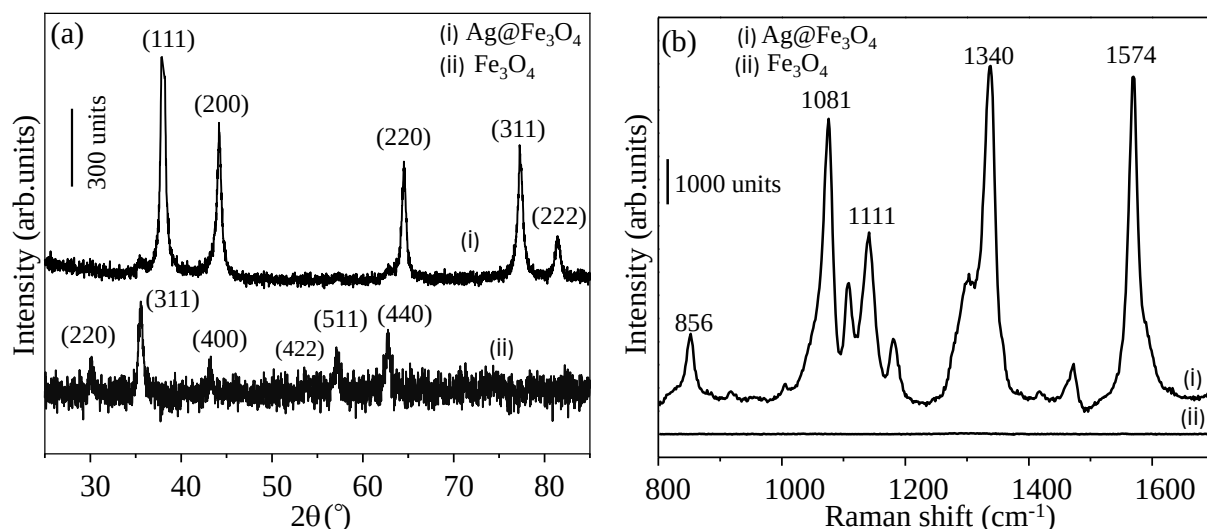


Figure 4.2: (a) XRD patterns of Fe_3O_4 -NPs and $\text{Ag}@\text{Fe}_3\text{O}_4$ nanocomposites synthesised from 40 mM AgNO_3 . (b) SERS signal of p-NTP adsorbed on $\text{Ag}@\text{Fe}_3\text{O}_4$ and Fe_3O_4 .

As a complementary characterisation technique to both EDS and XRD, the surface enhanced Raman enhancement (SERS) effect of silver nanoparticles was exploited to further confirm their presence on the nano-catalyst. Similar to the sierra effect in **section 3.3.1**, SERS is also dependent on the development of plasmonic excitations which arise when light interacts with the conduction band electrons of noble metals, for the enhancement of Raman scattered signals from peripheral Raman active molecules [162]. For this investigation, samples of Fe_3O_4 and $\text{Ag}@\text{Fe}_3\text{O}_4$ nanostructures were soaked on 1 mM of methanolic-4- nitrothiophenol (a common SERS probe molecule) for 1hr to facilitate sufficient surface adsorption.

Following this period, the particles were collected magnetically on a Teflon surface and allowed to dry for a further hour. As shown in **Figure 4.2(b)**, the Raman spectra for both the 4-NTP adsorbed nanoparticles over a 785 nm laser were obtained. In contrast to the non-silver containing Fe_3O_4 / 4-NTP sample where no peaks could be observed, an array of highly intense ones set at 1111 cm^{-1} (vC-S), 1081 cm^{-1} (vC-S), 1340 cm^{-1} (vNO₂) and 1574 cm^{-1} (vC-C) are clearly visible for the supposedly Ag@ Fe_3O_4 /4-NTP sample proving that indeed Ag is present [152]. Of note, these observed enhanced Raman peaks are consistent with those of 4-Nitrophenyl disulphide (NPDS), a dimer structure of 4-NTP [163].

Figure 4.3 shows the thermal responses of Fe_3O_4 and Ag@ Fe_3O_4 nanoparticles upon evaluation using a TGA following a 4 hr long drying period in an oven set at 60 °C. For both nanostructures, no major mass losses were observed over a heating range from 60-600 °C. Nonetheless, the TGA curves demonstrated a slight decline not more than 0.4 % between 100 and 200 °C for both samples. This decline is usually allied with the desorption of surface adsorbed water molecules on the particles [161]. The absence of any considerable mass loss beyond the 200 °C mark suggests that no organic molecules on either Fe_3O_4 or the incorporated AgNPs were attached to their surfaces. In consideration to the similarity in TGA graphs for both samples, it is clear that the adsorbed mass of AgNPs on Fe_3O_4 after the SMR could be estimated as the difference in masses between the Ag@ Fe_3O_4 batch and that of Fe_3O_4 as no other substances but the two were available. Following this procedure, an input of 40 mM AgNO_3 (optimum) was found to deposit (0.0104 ± 0.0014) g of silver from the available 0.0203 g on (0.0034 ± 0.0010) g of the exposed Fe_3O_4 .

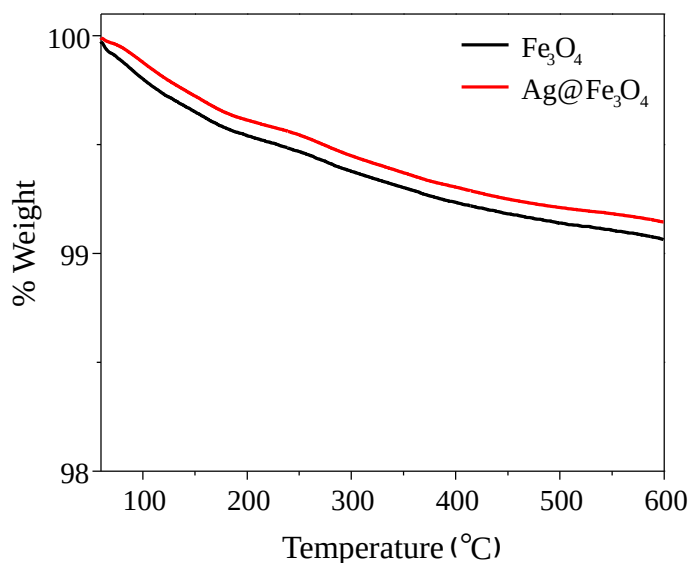


Figure 4.3: TGA curves for Fe₃O₄ and Ag@Fe₃O₄ synthesized from 40 mM AgNO₃.

4.7.2 Catalytic activity

Following the successful fabrication of both Fe₃O₄ and Ag@Fe₃O₄ nanostructures, their catalytic efficacy towards the reduction of 4-NP in the presence of excess NaBH₄ was assessed. UV–vis absorption spectra as illustrated in **Figure 4.4 (a-b)** were recorded for both the nanocatalysts with respect to time to monitor changes in reagent concentrations within the reaction mixtures. Initially, the addition of freshly prepared NaBH₄ solution resulted in the intensification in colour of the originally light yellow 4-NP solution. This change is generally attributed to the formation of 4-nitrophenolate ions with more pronounced π -conjugated donor-acceptor property in alkaline conditions [97]. According to literature, the absorption peak intensity of this solution can remain unaltered for a couple of days in the absence of any catalyst [164].

As the reaction progressed, the yellow 4-NP solution turned colourless and the characteristic 4-nitrophenolate ion absorption peak at 400 nm continuously attenuated. The rate of attenuation for a Ag@Fe₃O₄ nano-catalyst based reaction was largely greater than that of Fe₃O₄ where almost no reaction progress could be observed until 120 minutes had elapsed (**Figure 4.4 (a-b)**). This signalled the potentiality of AgNPs as the catalytic component in the Ag@Fe₃O₄ heterostructures.

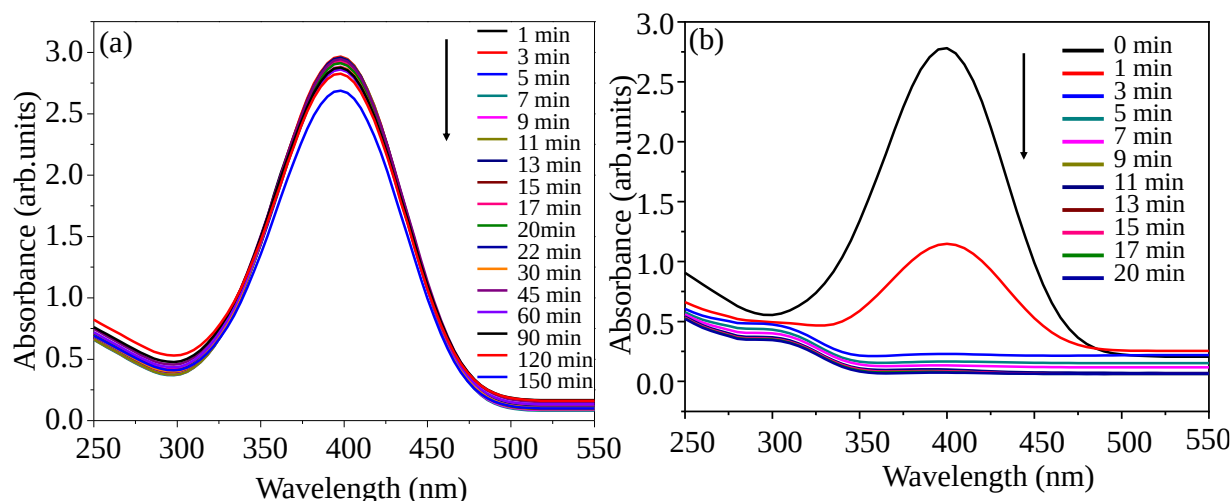


Figure 4.4: Comparison of the NaBH_4 based catalytic reduction of 4NP (0.4 mM) using (a) bare Fe_3O_4 and (b) $\text{Ag}@ \text{Fe}_3\text{O}_4$ synthesised from 20 mM AgNO_3 as catalysts.

4.7.3 Optimisation

It is generally accepted that the catalytic action of AgNPs occurs with them acting as a relay system for the transfer of electrons between substrates [99]. Veisi *et al.* [165] postulated that with both ions adsorbed on AgNPs, the nucleophilic BH_4^- ions donate electrons to the electrophilic 4-NP via AgNPs leading to the formation of 4-AP [53,165]. Based on this understanding, it is mandatory that the surface coverage of AgNPs on Fe_3O_4 during synthesis is maximised in order to fully avail the adsorption sites for optimum performances.

Figure 4.5(a) illustrates a study on the catalytic activity of $\text{Ag}@ \text{Fe}_3\text{O}_4$ as influenced by the SMR progress time during their fabrication. Although the attained discrepancy in activity is low, particles synthesised over a period of 4 and 5 minutes showed a better catalytic performance, with 58 % of 0.4 mM 4-nitrophenolate ions undergoing conversion within the 1st minute. The relatively smaller standard deviation $\sigma = 0.02$ in A/A_0 ratios at $t = 1$ minute presented by the 5-minute synthesis period validated its selection over that of 4 minutes ($\sigma = 0.43$) as optimum.

With the Ag on Fe_3O_4 decoration period now fixed at 5 minutes, sets of $\text{Ag}@ \text{Fe}_3\text{O}_4$ were synthesised by varying AgNO_3 concentrations from 20-60 mM. These particles were also evaluated for their catalytic efficacy as shown in **Figure 4.5(b)**. A 91.9 % conversion of 4-NP to 4-AP within the 1st minute suggested that particles made from 40 mM AgNO_3 presented the best activity. In essence, it is understood that increments in AgNO_3 concentration generally improves the deposition of AgNPs on Fe_3O_4 surfaces. In-turn, the catalytic activity of the nanocomposites also improves but as more of it is used, aggregation of surface AgNPs

becomes apparent [138]. This is believed to be a consequence of increased competition for spaces to adhere onto and eventually, the net surface area to volume ratio of catalytic AgNPs depreciates resulting in reduced activity as observed for Ag@Fe₃O₄ fabricated using AgNO₃ concentrations in excess of 40 mM.

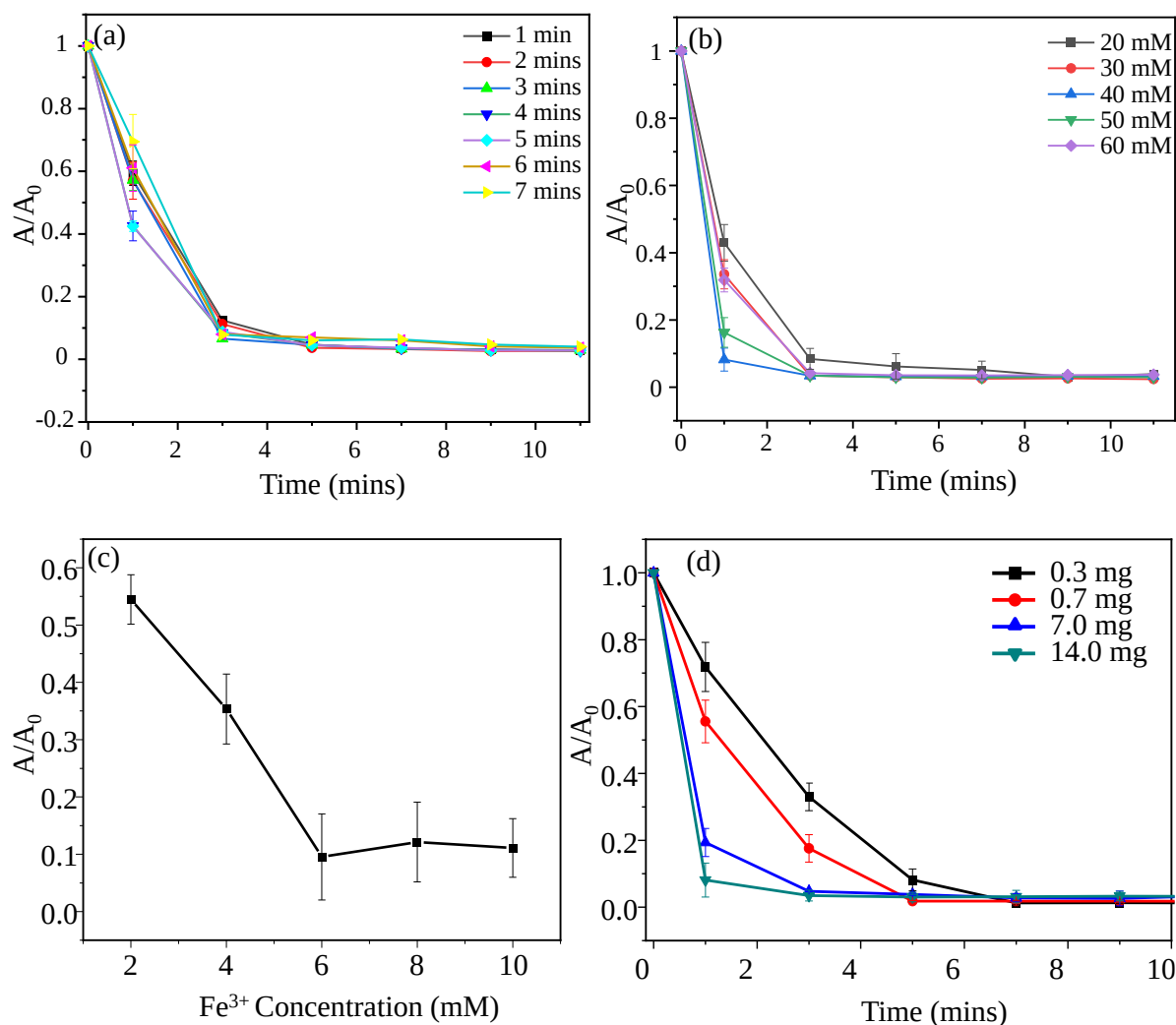


Figure 4.5: The influence of **(a)** SMR exposure time and **(b)** AgNO₃ concentration on the catalytic activity of Ag@Fe₃O₄ in degradation of 4-NP (0.4 mM) by 30 mM NaBH₄. **(c)** The effect of varied Fe₃O₄ amounts (based on the amount of Fe³⁺) used as a Ag substrate (from 40 mM AgNO₃) on the catalytic degradation rate of 4-NP. **(d)** The effect of Ag@Fe₃O₄ catalyst loading on the reaction rate.

To confirm that indeed particles synthesised from 40 mM AgNO₃ performed optimally, varying amounts of the MNPs were coated with Ag as described in **section 4.2.3.2** whilst maintaining the used AgNO₃ concentration at 40 mM, and were evaluated for their catalytic performances. The quantification of these MNPs was based on the stock-solution concentration of Fe³⁺ used during their synthesis. As illustrated in **Figure 4.5(c)**, a ratio of 40

mM: 6 mM for Ag^+ to Fe^{3+} proved indeed to provide the best activity. Increments in Fe_3O_4 content beyond those formed using 6 mM Fe^{3+} exhibited neither a positive nor a detrimental effect. The understanding of this behaviour is that, increasing MNPs simply provides more room to adhere AgNPs onto of which a AgNO_3 greater than 40 mM would be necessary. Another study to investigate the influence of different amounts of $\text{Ag}@ \text{Fe}_3\text{O}_4$ was undertaken and the results are as illustrated in **Figure 4.5(d)**. Essentially, the more catalyst there is, the faster the reaction rate.

Following the optimisations of particle synthesis conditions along with their quantities for enhanced catalytic activity, a study of the reagents concentrations was also investigated. Considering that the concentration of NaBH_4 (30 mM) was much higher than that of 4-NP, the effect of the initial 4-NP concentration was studied by applying pseudo first order kinetics. The apparent rate constants (K_{app}) corresponding to these initial concentrations were calculated based on plots of $\ln (A/A_0)$ against the reaction time t as illustrated in **Figure 4.6(a)**. Generally, the rate constants varied between 1.0 and 4.5 min^{-1} increasing inversely with 4-NP concentration **Figure 4.6(b)**. Saturation levels became evident at 4-NP concentrations of 0.45 mM and in excess. This trend infers that the catalytic action of $\text{Ag}@ \text{Fe}_3\text{O}_4$ is governed by the Langmuir Hinshelwood kinetics [8]. In essence, this deterioration in K_{app} at high concentrations can be traced back to the competition between both reactants for the free sites on the catalyst surface. Given that the number of active sites for a catalyst is fixed and reaction can only take place between the surface adsorbed molecules, a strong surplus of 4-NP leads to a substantial decrease in adsorbed BH_4 ions leading to an overall decrease in activity [91].

Also of interest, a number of research groups have encountered an induction period in their study when catalytically reducing 4-NP. This time is defined as the delay period observed at the start of catalytic reactions. Some researchers have attributed this period as due to reagents still adsorbing onto the catalyst but Menumorov and co-workers [42] argued against this theory, experimentally proving that it relates to a reverse reaction in which the dissolved oxygen converts the product back to the reagent molecules. Herein, this period was not observed. This may be a consequence of high performance nature of the $\text{Ag}@ \text{Fe}_3\text{O}_4$.

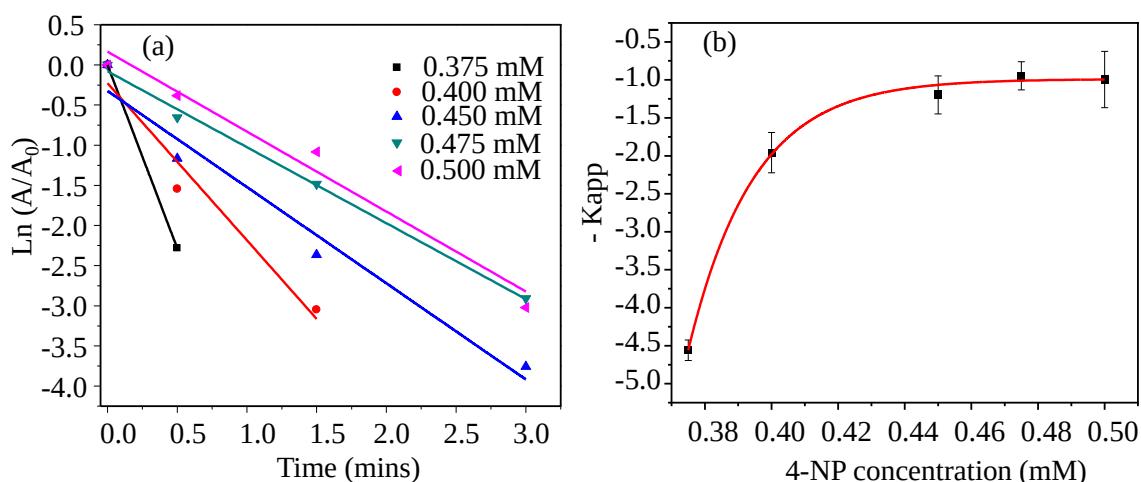


Figure 4.6: (a) Time resolved plots of $\ln(A/A_0)$ to determine the rate constants and (b) illustrate their dependence on initial 4NP concentrations.

Having investigated the catalytic capabilities of $\text{Ag@Fe}_3\text{O}_4$, a comparative study utilising a normalised activity parameter $k = k_{app}/[C_{catalyst}]$ as per Kästner and Thünemann [90] which accounts for the amount of catalyst used in a solution was carried out. As shown in **Table 4.2** below, it is evident that the $\text{Ag@Fe}_3\text{O}_4$ catalyst performs significantly better than some of the noble metal based nano-catalysts.

Table 4.2: Activity comparison between synthesised nanocomposite with other nanocatalysts towards the reduction of 4-nitrophenol.

	Catalyst	(Ag) (mg.L ⁻¹)	K_{app} (s ⁻¹)	(k) (L. s ⁻¹ mg ⁻¹)	References
1	$\text{Ag}_{0.90}\text{Au}_{0.078}\text{Pd}_{0.022}$	167	1.58×10^{-2}	9.46×10^{-4}	[166]
2	micron-SiO ₂ @Ag NPs	107.4	3.56×10^{-3}	3.31×10^{-5}	[167]
3	CTAB-stabilized AuNPs	2.67×10^{-5}	1.26×10^{-3}	4.7×10^{-5}	[168]
4	Ag NPs on Fe_3O_4 @C	611.3	1.70×10^{-2}	2.78×10^{-5}	[169]
5	AgNP-PG-5K composite	0.9	3.94×10^{-3}	1.375×10^{-3}	[170]
6	Ag NPs@PGMA-SH composite	0.9	3.94×10^{-3}	4.38×10^{-3}	[171]
7	$\text{Ag@Fe}_3\text{O}_4$	52.0	7.5×10^{-2}	1.44×10^{-3}	This work

4.7.4 Recyclability

The as synthesised Ag@Fe₃O₄ nanoparticles proved to very effective for the catalytic reduction of 4-NP. Owing to their magnetic property, they could easily be recovered from the product solution. Since the economic value of a heterogeneous catalyst is subject to its recyclability, the recovered Ag@Fe₃O₄ nanocomposites were washed thoroughly with distilled water (×3) and reused 8 times producing a trend illustrated in **Figure 4.7**. It is of note that although not represented in between every cycle, there is a delay period owing to catalyst recovery, washing and reintroduction to a new solution.

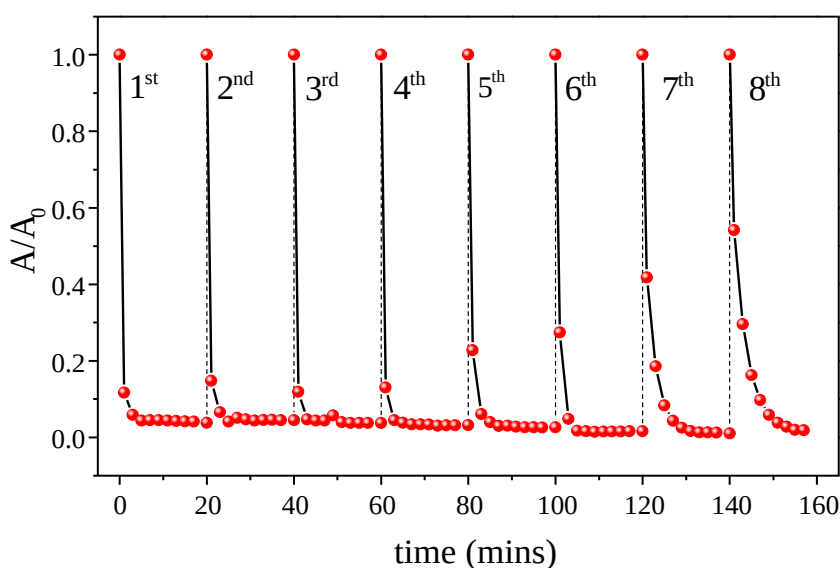


Figure 4.7: Recyclability test cycles for Ag@Fe₃O₄.

Up to four (4) times, the catalyst maintained its level of activity but then-after, a decrease in catalytic efficiency could be observed with every subsequent cycle. This is better than some catalysts in literature as shown on **Table 4.3**. This deterioration may be attributed to the loss of small amounts of the nano-catalysts due to leaching during the washing and recovery processes [172]. In detail, it is understood that dissolved oxygen facilitates leaching of Ag ions [173]. The excessively available NaBH₄ would then reduce these ions back to silver atoms eventually aggregating to form unsupported AgNPs. Since these nanoparticles are unattached to Fe₃O₄, they are not magnetically recoverable, as such, they are highly likely to be poured off with the product solution. The existence of these free AgNPs is possibly signalled by the development of a weak plasmon resonance peak at a wavelength of 395 nm, observable only after all the 4-NP ions have been reduced (**Figure 4.8**) [173,174]. Another reason which could account for the loss in catalytic activity is the slippage of ad-atoms from

the catalytically favourable regions (edges, kinks and corners) to low potential regions during the reaction. Although it is clear that catalytic activity diminished over time, the conversion of 4-NP was maintained above 96 % as calculated in accordance to the equation below [91].

$$\% \text{ degradation} = \left(1 - \frac{A}{A_0}\right) \times 100.$$

Where A and A₀ are the final and initial absorbances.

The calculated % degradation could be claimed to be more given that absorbances of 4-AP and NaBH₄ and some remnant catalyst particles are unaccounted for.

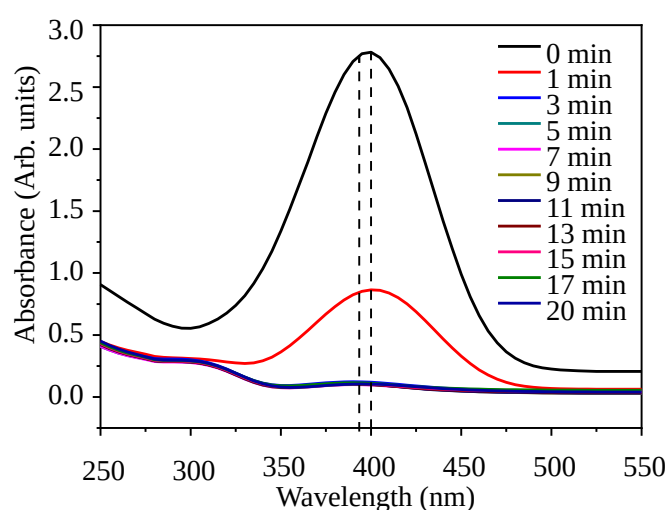


Figure 4.8: The time resolved spectra for the reduction of 4NP by sodium borohydride with Ag@Fe₃O₄ as the catalyst and the remaining Ag LSPR peak after the reaction completion.

Table 4.3: Comparison table for nanocatalysts recyclability towards 4-nitrophenol reduction

	Catalyst	Recyclability (without performance deterioration)	Ref
1	Pd–Co alloy	3	[175]
2	Activated carbon-supported gold nanocatalysts (Au/AC)	1	[176]
3	Ag@Fe ₃ O ₄	4	This work

4.7.5 Dye degradation.

Diversity in applicability of a catalyst towards multiple reactions is largely a desired property. As was with 4-NP reduction, the synthesized Ag@Fe₃O₄ nanoparticles were also effective in catalysing the discoloration of 0.2 mM concentrations of MB and R6G in 10 mL volumes as

shown in **Figure 4.9(b-d)**. Confirmation of this was made in reference to the comparatively slow self-discoloration rate for both the dyes as monitored by their characteristic absorbance peaks at 655 nm for MB and 520 nm for R6G (**Figure 4.9 (a-c)**).

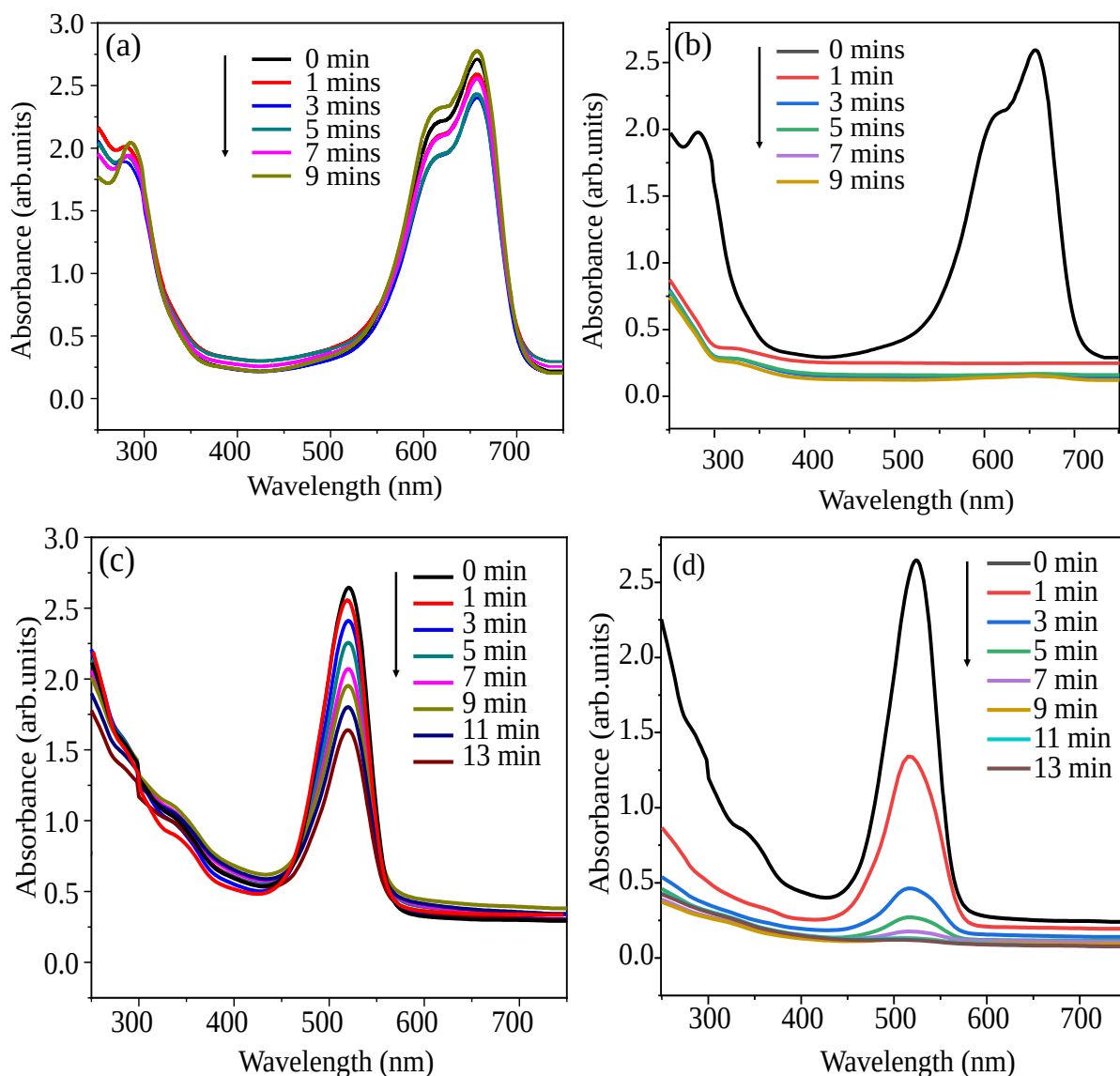


Figure 4.9: UV-vis absorbance variation for the degradation of (a) 0.2 mM MB without Ag@Fe₃O₄ (b) with Ag@Fe₃O₄, and (c) 0.2 mM Rhodamine 6G without Ag@Fe₃O₄ (d) with Ag@Fe₃O₄.

To provide a better understanding on molecular changes involved on the aforementioned catalysed dye transformations, the Raman spectra of both dyes whilst adsorbed on Ag@Fe₃O₄ nanoparticles were measured initially before the addition of 80 μ l of 15 mM NaBH₄ and a few minutes after as shown in **Figure 4.10**. Given the SERS property of the nano-catalyst, a series of bands characteristic to each dye as listed in **Table 4.3** could easily be identified.

Their eventual disappearance 3 and 9 minutes for MB and R6G respectively after the addition of NaBH₄ proves that instead of simple discoloration, the dyes were indeed undergoing molecular degradation.

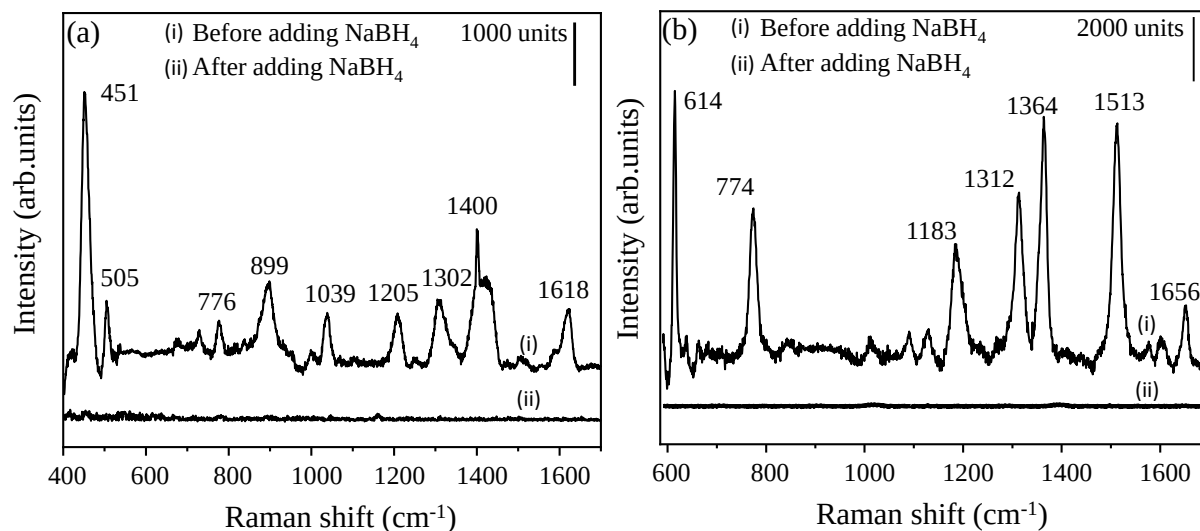


Fig. 4.10. SERS spectra of 0.2 mM **(a)** Methylene Blue and **(b)** Rhodamine 6G adsorbed on AgNPs@Fe₃O₄ surfaces before and after catalytic degradation by NaBH₄ (15 mM).

Table 4.4: SERS bands for Methylene blue and Rhodamine 6G adsorbed on AgNPs@Fe₃O₄ surfaces.

	Methylene blue bands (cm ⁻¹)	Band Assignments [177], [178]	Rhodamine 6G bands (cm ⁻¹)	Band assignments [179]
1	451	δ (C-N-C)	614	α (C-C-C)
2	505	δ (C-N-C)	774	γ (C-C-C)
3	776	β (C-H)	1183	ν_{sym} (C-C)
4	899	β (C-H)	1312	ν_{sym} (C-C)
5	1039	β (C-H)	1364	ν_{sym} (C-C)
6	1215	ν (C-N)	1513	ν_{sym} (C-C)
7	1305	α (C-H)	1656	ν_{sym} (C-C)
8	1400	α (C-N)		
9	1618	ν (C-C) ring		

Abbreviations: ν , stretching; α , in-plane ring deformation; β , in-plane bending; γ , out-of-plane bending; and δ , skeletal deformation.

4.7.6 SERS based monitoring of catalysis

In consideration of the SERS effect exhibited by the AgNPs@Fe₃O₄ particles as shown in **Figure 4.2(b)**, their propensity towards real time monitoring of molecular activity during catalysis via the Raman was also investigated.

Figure 4.11 designates the time dependent evolution of the SERS spectra of 4-NTP functionalised particles following the addition of 80 μ l of 100 mM NaBH₄. Originally, three dominant Raman bands consistent with those of 4-NTP were found at 1082 cm⁻¹, 1347 cm⁻¹ and 1576 cm⁻¹ [180]. As the reaction progressed however, the 1347 cm⁻¹ (blue-shifts to 1336 cm⁻¹) and 1576 cm⁻¹ peak intensities diminished progressively and at the same time, a new peak developed at 1594 cm⁻¹ (benzene ring C=C stretching) [181]. These changes implicated a transformation of 4-NTP molecules to those of 4-ATP. It is worth noting that midway the reaction, peaks characteristic for 4,4-dimercaptoazobenzene (DMAB) molecules at bands 1392 cm⁻¹ [181] and 1437 cm⁻¹ (N=N stretching) became prominent before completely degrading over time [181]. This suggests that the reaction mechanism is a two-step process first involving the transformation of 4-NTP to DMAB then to 4-ATP. Based on this study, it is clear that in addition to being a recyclable catalyst effective in degradation of 4-Nitrophenol, methylene blue and Rhodamine 6G, the synthesized AgNPs@Fe₃O₄ nanocomposites are also a good candidate for use in real time catalysis monitoring.

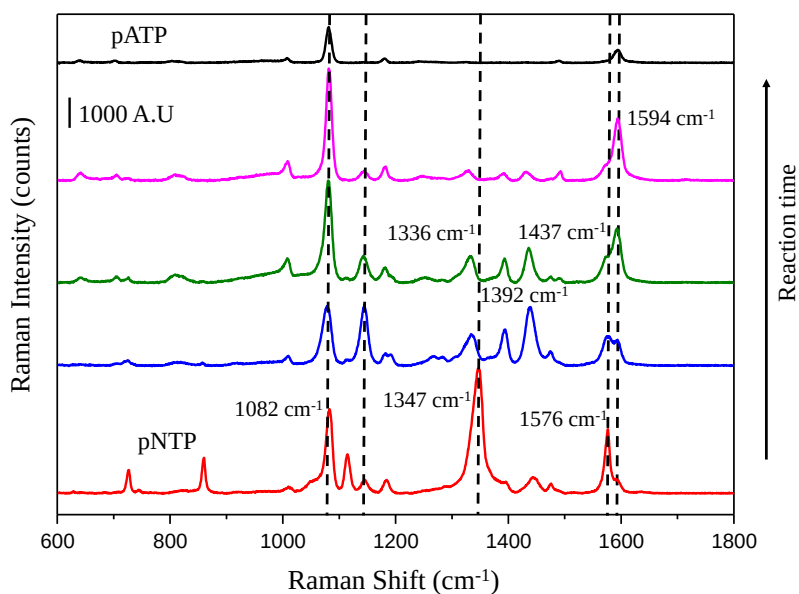


Fig. 4.11. Time dependent SERS spectra for the catalytic degradation of surface adsorbed 1 mM methanolic 4-NTP by NaBH₄ (100 mM).

4.9 Conclusion

In this study, the magnetically recoverable Ag@Fe₃O₄ nanocomposite was successfully synthesised via a simple two step surfactant free procedure. The surface deposition of AgNPs on Fe₃O₄ nanoparticles synthesised following the co-precipitation method was carried out using the silver mirror reaction. The prepared catalyst (Ag@Fe₃O₄) was characterized using the FE-SEM, TEM, EDX, XRD and TGA. Following optimisation procedures with respect to the nano-catalyst synthesis period (5 minutes) and AgNO₃ concentration (40 mM), the evaluation in catalytic activity of these particles at room temperature revealed they are highly efficient in degrading 4-nitrophenol (4-NP), Rhodamine 6G (R6G) and methylene blue (MB) solutions in the presence of the NaBH₄. Furthermore, a recyclability study indicated that the particles can operate more than 8 cycles before reduction in performances significantly. Also, the nanocomposite displayed promising potential as a substrate for SERS based real-time monitoring of molecular surface activity during catalysis.

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CHAPTER 5: CONCLUSION

In this thesis, bi-component nanocomposites comprising of silver and magnetite were successfully synthesised. Following the solvothermal procedure, the first batch of core-shell orientated nanostructures with silver encapsulated by a magnetite outer layer were successfully synthesized. These particles exhibited an improved peroxidase-like activity in comparison to bare magnetite nanostructures when introduced to a solution consisting of a peroxidase substrate o-phenylenediamine and the electron accepting hydrogen peroxide. Further optimisation of the nanocomposites with regards to their activity was carried out by taking different concentrations of AgNO_3 . In doing so, a structural transformation, as determined by a TEM, from a perfectly magnetite enclosed silver core (flower-like) into a heterodimer like orientation was revealed. These heterodimers displayed further improvements in peroxidase-like activity at physiological pH conditions ($\text{pH} = 7.4$). These nanocomposites were also successfully used to design a highly selective, room temperature functional colometric cysteine sensor with a limit of detection as low as 87 nM and a detection linear range of 1-20 μM .

A simple silver mirror reaction was used to synthesize nano-silver decorated magnetic nanoparticles which were used as a catalyst towards the reduction of 4-NP to 4-AP. With sodium borohydride as the reducing agent, these magnetically recoverable nanoparticles proved effective in degrading 4-nitrophenol. To further boost their performances, the reaction conditions for decorating of AgNPs with regards to the degradation of 4-NP was examined by varying the AgNO_3 concentration and reaction time. While the reaction time proved less important, modulations of AgNO_3 concentrations provided drastic performance improvements with 40 mM proving optimal. In addition to their ability to effectively catalyse the degradation both methylene blue and Rhodamine 6G, they also offer potential in real time analysis of molecular transformations during catalysis owing to their SERS property. Furthermore, they could be recycled continuously up to 4 times without any change in their activities.

Of emphasis, the procedures utilised in synthesising both these nanocomposites involved no usage of environmentally hazardous chemical substances.

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