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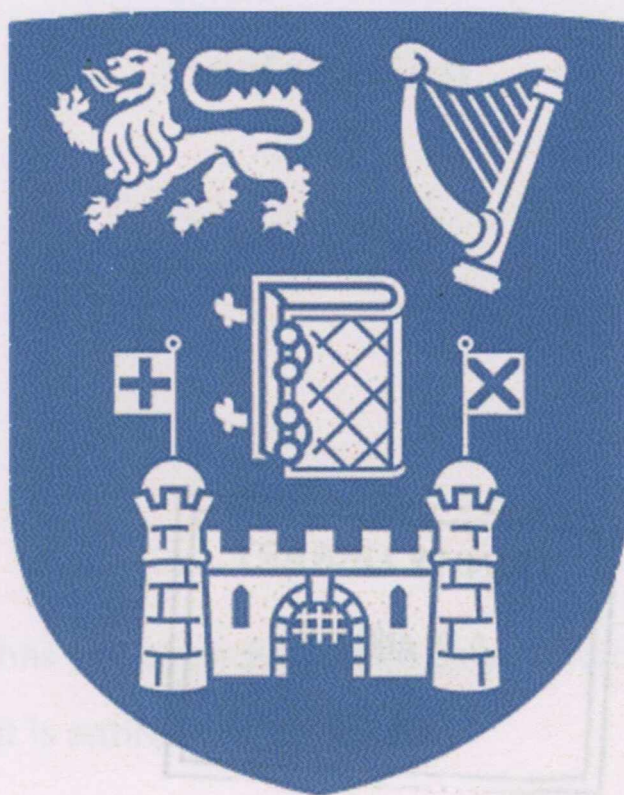
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Development of New Magnetic Nanostructures

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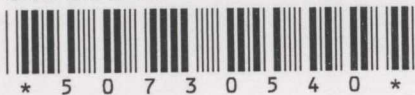


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"Science, my lad, is made up of mistakes, but they are mistakes which it is useful to make, because they lead little by little to the truth."

Jules Verne

A Journey to the Center of the Earth

Summary

The main goal of this project is to develop a series of magnetic nanomaterial composites to be used in catalysis, enantiomeric separation or biomedical applications. The design of new magnetic nanostructures is also discussed. The work involves the synthesis and characterization of core-shell magnetic nanoparticles (CoFe_2O_4 and Fe_3O_4) coated with different materials, such as silica, titania, ceria, CoFe_2O_4 nanoparticles stabilized with amino acids and other nanocomposites.

Chapter 1 provides a brief literature survey and background information related to magnetic nanoparticles describing their properties as well as their main applications and techniques used to synthesis these materials. The chapter concludes with the aims and motivations in the development of new magnetic nanocomposites.

Chapter 2 presents all experimental procedures and techniques used for the synthesis and characterization of the materials. Description of main used characterization techniques, such as Transmission electron microscopy, FTIR, UV-vis, Raman and CD spectroscopy, X-ray diffraction, magnetization measurements are also provided.

Chapter 3 is dedicated to the preparation and investigation of different magnetic core-shell nanomaterials with potential catalytic activity. These new catalytic materials supported on magnetic nanoparticles give the advantage of being easily removed with the help of an external magnetic field. The resulting nanoparticles have been characterised and tested for catalytic activity in the degradation of methylene blue dye.

Chapter 4 presents the development of new magnetic nanostructures synthesised using the co-precipitation method. Amino acid have been used as stabilisers in the synthesis of the nanoparticles and the influence of different parameters, such as concentration of the ligand, temperature, enantiomer and time have been investigated.

Chapter 5 describes the investigation of magnetic nanoparticles stabilised with amino acid and with DNA as potential materials for the enantiomeric separation of racemic mixtures. CD spectrometry has been used to elucidate the chirality of the resultant product of the enantiomeric separation. In addition, silica coated magnetic nanoparti-

Summary

cles have been synthesised and used in the recovery of DNA as an alternative for established protocols for the purifications of DNA.

Chapter 6 describes the development of new yolk-shell multimodal magnetic nanocomposites. The material is based in a magnetic core and a silica shell with a hollow void between them. The hollowing of the silica shell has been demonstrated to occur due to slow etching by hydroxyl groups trapped inside the silica matrix. The loading and release of coumarine derivatives and ZnS quantum dots inside the hollow yolk-shell nanostructures resulted in the development of new multimodal nanocomposites, which have demonstrated exciting properties to be used in related biological applications.

Conclusions of this work, preliminary studies and an overview of planned future research are discussed in chapter 7.

Overall, a series of new nanostructures based on magnetic nanoparticles, including nanoparticles, core-shell nanostructures and Yolk-shell nanocomposites have been prepared and characterised. We believe that this research has made a contribution to further development of magnetic nanocomposite materials which may have a range of potential applications in fields of nano and bio-technology, materials science, catalysis and others.

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Abbreviations

°C	Degrees Celsius
a.u.	Arbitrary units
APTES	Aminopropyltriethoxysilane
B ₀	Applied magnetic field
cm ⁻¹	wavenumbers
CoFe ₂ O ₄	Cobalt ferrite
D-Asp. Acid	D-Aspartic acid
D-Cys	D-Cysteine
D-Glu	D-Glutamic Acid
DMF	Dimethylformamide
DNA	Deoxyribose Nucleic Acid
D-Pen	D-penicillamine
DRIFT	Diffuse Reflectance Infrared Fourier Transform
DTES	Dodecyltriethoxysilane
EDX	Energy-dispersive X-ray Spectroscopy
EtOH	Ethanol
Fe ₃ O ₄	Magnetite
FTIR	Fourier Transform Infrared Spectroscopy
g	grams
H	applied magnetic field
HMT	Hexamethylenetetramine
JCPDS	Joint Committee on Powder Diffraction Studies
L-Ala	L-Alanine
L-Asp Acid	L-Aspartic Acid
LbL	layer-by-layer
L-Cys	L-Cysteine
L-Pen	L-Penicillamine
M	Magnetization
M ₀	Longitudinal magnetization
MB	Methylene blue

Abbreviations

mL	millilitres
mM	milimolar
MP	Millipore
MRI	Magnetic Resonance Imaging
Ms	Saturation magnetization
NH ₄ OH	Ammonium hydroxide solution
nm	nanometres
QD	Quantum Dot
SEM	Scanning Electron Microscopy
SPR	Surface plasmon resonance
STEM	Scanning Transmission Electron Microscopy
T	Tesla
TEM	Transmission Electron Microscopy
TEOS	Tetraethyl orthosilicate
TGA	Thermogravimetric analysis
UV	Ultraviolet
VSM	Vibrating Sample Magnetometer
XRD	X-Ray Diffraction

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Chapter 1. Introduction

1.1 General Introduction

In 1959 the Nobel prize winner Prof. Richard P. Feynman gave a lecture, “There’s plenty of room at the bottom”, that has become an important document in the history of this field.^[1] In this talk, he described the possibilities that could rise if scientists could understand the principles that govern single atoms and molecules at the nanometre scale.

More than fifty years after that lecture, nanoscience and nanotechnology have drawn a lot of attention from a considerable number of scientists, that has led to an extensive development of this area in the last few decades. This has been mostly due to the increase in the different methods of synthesis and the availability of tools for the manipulation and characterization of nanomaterials.

However, what is nanoscience? Well, nowadays scientists are still struggling trying to find the most accurate definition, although people have been working in this field for many hundreds of years without even knowing about nanomaterials. We can find an example of this looking at stained glasses in churches, which are made of different size of silver and gold nanoparticles that were used to create different colours.

Nanotechnology is a research field associated with the development of nanomaterials; a nanomaterial can be described as one with at least one dimension within the nanometre range (between 1 and 100 nm). To put this in a perspective, the size of the smallest atom, hydrogen, is about one-tenth of a nanometre (10^{-9} m). The range of wavelengths in the electro-magnetic spectrum that the human eye can detect is 390-700 nm, visible light. Figure 1.1 below demonstrates the relative scale of nanomaterials compared to real world objects.

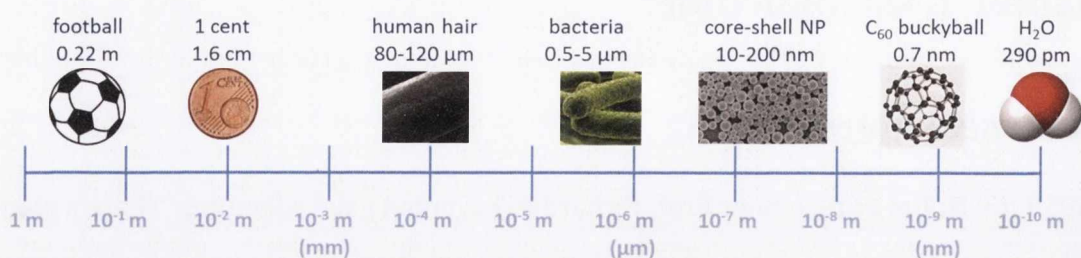


Figure 1.1: Scale to put the length of a nanometre in perspective. Ranging from 1 m to 100 pm illustrated by the size of a football (0.2 m) down to the size of a C₆₀ or water molecule (1 nm and 300 pm respectively). For comparison, the world is approximately one hundred million times larger than a football, which is in turn one hundred million times larger than a buckyball. The length scale of interest for nanoscience and nanotechnologies is from 100 nm down to the atomic scale (approx. 0.2 nm). [Adapted from: The Royal Society & The Royal Academy of Engineering. Nanoscience and nanotechnologies. Chapter 2. (2004) <http://www.nanotec.org.uk/finalReport.htm>]

Nanotechnology designs materials with different properties from those in the bulk state. This means that nanomaterials at the atomic level follow the rules of the quantum mechanics leaving behind their classical physical properties. This opens a new variety of possibilities for researches in the development of new materials.

In practice, nanotechnology combines chemistry, material science, engineering and physics to build new materials, which have potential applications in medicine, data storage, photonics, environmental science and many others.

1.2 Magnetic Nanomaterials

Magnetic nanoparticles (MNPs) have become of great importance for a variety of applications, not only for the general miniaturization of devices but also for the possibility of their manipulation using an external magnetic field. These nanoparticles have found a vast range of applications in many different disciplines including biomedical applications, such as contrast agents in magnetic resonance imaging (MRI), drug delivery systems, biological labels and hyperthermia agents, as well as magnetic fluids, materials for data storage and catalysis.^[2-6]

1.2.1 Magnetism

Magnetism was first documented in the 6th century, when the Greek philosopher Thales of Miletus noticed the attraction of iron to lodestone (magnetite).^[7] Magnetism is defined by both intrinsic and extrinsic properties. Intrinsic properties are saturation magnetization (M_s), Curie temperature (T_c) or anisotropy and are related to the crystallographic structure. Coercivity and remanence, on the other hand, are extrinsic properties and are related to the morphology of the crystal.^[8]

The magnetic moment of an atom or molecule can be associated with electron motion, in other words, the magnetic moment is given by the orientation of the electron spin. Each electron is associated with a magnetic moment generated from these components that combine and give overall resultant magnetic moment, μ , in the material. This magnetic moment is described through μ and B , with μ for the magnetic moment, as mentioned before, and B measuring the applied magnetic field, is equal to the unpaired spins (Equation 1).^[9]

Equation 1

$$\tau = \mu \times B$$

In the case of oxides of magnetic elements with unpaired electrons (Iron, Cobalt and Nickel) the 3d electrons are localized and the magnetic moment is defined by Hund's rules.^[10-11]

In the case of bulk materials, electrons placed on the external shell are implicated in bonding interactions. This can be explained by the Pauli exclusion principal, which says that two electrons occupying a single bonding orbital must have opposite spins; these electrons are non-magnetic because they cancel out net contribution. Nanomaterials have a much larger surface area and the coordination is considerably lower than in the bulk, which leads to a higher availability of individual electrons to contribute to magnetism.

M-H hysteresis loops, as seen below (Figure 1.2), are obtained by monitoring the volume-averaged magnetization M as a function of the external magnetic field (H). Saturation magnetisation (M_s) refers to the applied magnetic field at which all particles

undergo alignment. This is when individual moments of single domain nanoparticles interact with each other and with an external applied magnetic field. In the case of materials that retain some magnetism after removing of the applied field, this remaining magnetism is known as remanent magnetisation (M_r). Finally, the field required to bring the magnetization back to zero is named coercivity of the material (H_c).

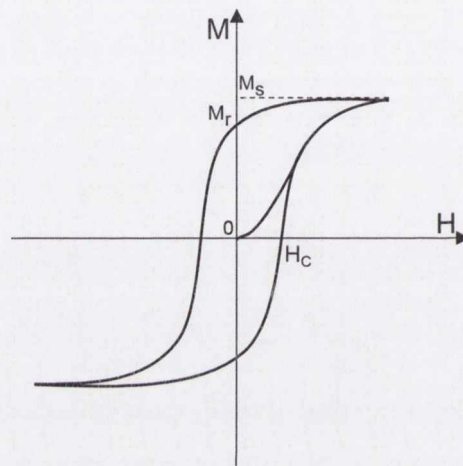


Figure 1.2: A typical hysteresis curve of a ferromagnetic material. On the vertical axis is presented the magnetization (M) and the horizontal axis the magnetic field intensity (H). M_s is saturation magnetization, M_r the remnant magnetization and H_c is the coercive force.

Magnetic anisotropy is the energy of a magnetic solid, which depends on the orientation of the magnetisation with respect to the crystal axes. Soft magnets are characterized by a very low anisotropy; permanent magnets need a high magnetic anisotropy in order to keep the magnetisation in a desired direction.

The electrostatic interaction strongly depends on the orientations of the orbitals of the electrons in this crystalline field. The magnetic moment and the electron orbitals are related by the fact that a favoured orientation of the orbital corresponds to a favoured orientation of the magnetic moment.

A domain is a group of spins, which are separated by domain walls, all pointing in the same direction and acting cooperatively. The main characteristic of these walls are the width and the energy, both associated with their formation. Adjacent domains differ in

their spin orientation. Under the presence of an external magnetic field, all the domains align in the same direction reaching the point of saturation magnetisation. Single domain particles are uniformly magnetised with all spin aligned in the same direction. The absence of the domain wall in a single domain particles leads to the reversal of the magnetisation by spin rotation. In other words, magnetic nanoparticles are superparamagnetic when they are magnetised in the presence of a magnetic field but the magnetisation returns to zero as a result of the thermal fluctuation after removing the field.

Nanoparticles show this superparamagnetic behaviour below a certain critical size. This type of magnetism is expected in small ferromagnetic nanoparticles, and it that depends not only on the material crystalline structure and composition, but also and most importantly on the size, which can occur in a size range between 5nm and 100nm.^[12]

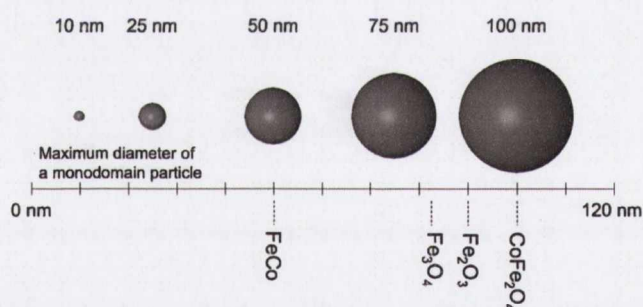


Figure 1.3: Frontier MNPs size from multi-domain to single domain for different crystalline structure and composition.^[12]

Superparamagnetic NPs have an oriented moment when an external magnetic field H is applied so their magnetic moment starts aligning along the applied field but they lose this property once the field is removed. Thus, superparamagnetic NPs do not exhibit remanence or hysteresis.^[13]

1.2.2 Classification of Magnetic Materials

Materials may be classified by their response to an external magnetic field as diamagnetic (opposed to an external magnetic field), paramagnetic (magnetized in the direction and proportional to an external magnetic field) or ferromagnetic (magnetized by orders of magnitude larger than the applied field).

For diamagnetic materials, electrons move around the nucleus and rotate around their axis, which creates a magnetic moment. Diamagnetism occurs in materials with completely full electron shell whereby the moments cancel each other out. Materials with unpaired electrons in their electronic shell are paramagnetic.

Since the magnetic moment comes from the interaction between electrons, this cooperation of electrons gives rise to ferromagnetic or antiferromagnetic behaviour.

Ferromagnetic behaviour occurs when all magnetic moments are parallel and in the same orientation resulting in a permanent magnetic field (Figure 1.4). In antiferromagnetic materials, the moments are aligned in opposite directions resulting in a zero magnetic field. Materials are only antiferromagnetic below their corresponding Néel Temperature (T_N). Ferrimagnetic behaviour occurs when all moments are aligned in opposite directions but their magnetization is no longer equal and do not cancel each other completely.

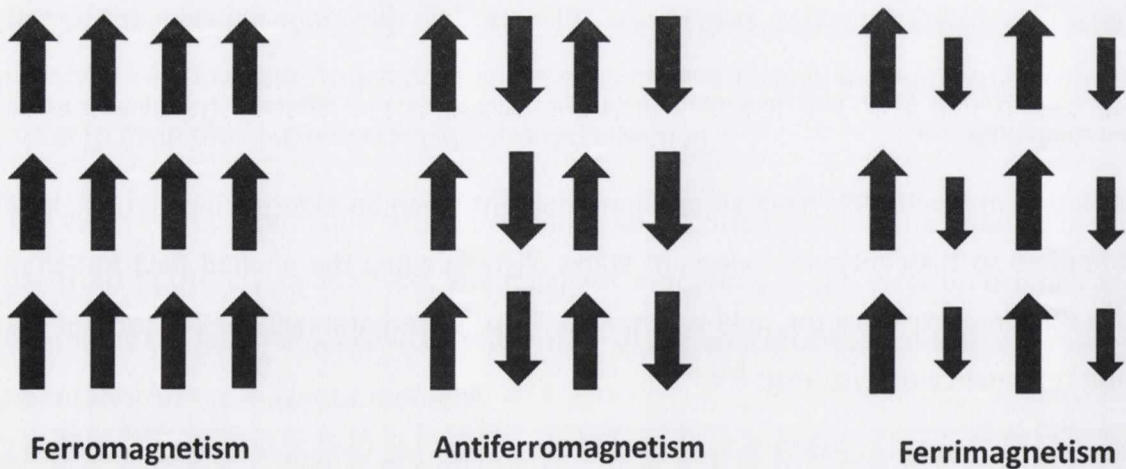


Figure 1.4: Models to explain the different orientations of magnetic moments in the case of ferromagnetism, antiferromagnetism and ferrimagnetism.

1.2.3 Structure

The inverse spinel structure of ferrite was first resolved in 1915 by Bragg and Nishikawa.^[14-15] Ferrite structure is based on a face-centred cubic (FFC) crystal of oxygen atoms where one-eighth tetrahedral and half of the octahedral sites are occupied by cations. This structure consists in three layers geometrically ordered and distinct from each other. The third layer (C) is placed in the octahedral holes created from the pile of layers A and B. The octahedral and tetrahedral holes alternate throughout the structure. If the divalent ions occupy only tetrahedral sites, then the spinel is called direct (Figure 1.5), while if they occupy only octahedral sites, the spinel is inverse.^[16]

Magnetite (Fe_3O_4), maghemite ($\gamma\text{-Fe}_2\text{O}_3$) and cobalt ferrite (CoFe_2O_4) are members of the ferrite family that is denominated with the general formula AFe_2O_4 where A also can be Mn, Ni, Cu or Zn. All of them adopt the inverse spinel structure where half of the trivalent ions occupy the tetrahedral, A, sites and the other half together with the divalent ions occupy the octahedral sites (Figure 1.5).^[17]

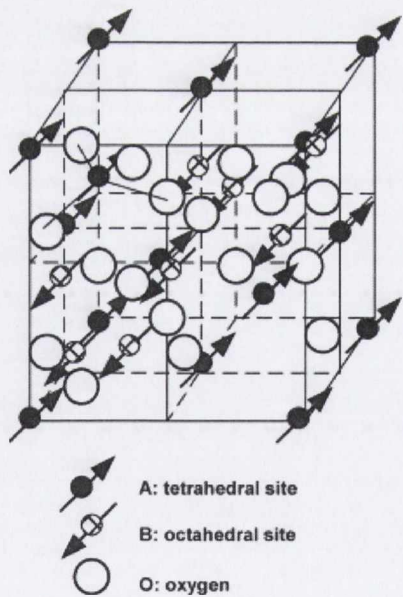


Figure 1.5: Schematic spinel cell structure.^[18]

Transition metal oxides such as ferrites are particularly useful as they combine interesting magnetic and electrical properties with stability against oxidation. For example,

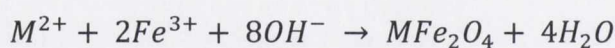
cobalt ferrite has a large anisotropy, high coercivity, moderate saturation magnetization, chemical and thermal stability and mechanical hardness.

1.3 Synthesis of Magnetic Nanoparticles (MNPs)

Several approaches have been developed for the synthesis of magnetic NPs. The method of synthesis applied plays a key role that will influence the properties of the NP. By adjusting or changing the synthesis parameters, the size and shape of the NPs can be tuned.^[19] Herein, a brief summary of the most used routes of the synthesis of magnetic NPs is presented.

1.3.1 Co-precipitation

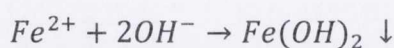
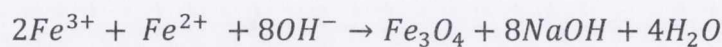
Co-precipitation method was first used by Massart in 1981 to prepare Fe_3O_4 NPs.^[20] This route is a simple and convenient method to prepare metal oxides particles in an aqueous media at different temperatures. Metal oxides based NPs are prepared using stoichiometric mixtures of appropriate metal salts, such as M^{2+} and Fe^{3+} , where M^{2+} correspond to a divalent metal (Fe^{2+} , Co^{2+} or Mn^{2+}). The addition of a base leads to the precipitation of the iron oxide based ferrites. In order to avoid the early oxidation of the divalent metal the use of an inert environment is recommended.^[21]



Scheme 1.1: Co-precipitation scheme to produce iron oxides, where M is the divalent metal ion, (Fe^{2+} , Co^{2+} , Mn^{2+}).

The size, the shape or even the magnetic properties of the material can be modified by varying the pH, salt concentration, temperature, stirring speed, etc. The complete formation of the metal oxide NPs occurs at pH between 8 and 14. Riaz *et al.*^[22] have demonstrated that by varying the pH during the formation of Fe_3O_4 the size and in the saturation magnetization of the NPs can be modified. However, major limitations of the co-precipitation method are still associated with the control of the particle size and shape.

In order to overcome these limitations an intense research work has been carried out by adjusting and improving this method in order to obtain a narrow size distribution and a well-defined shape of synthesized NPs. The effect of the temperature on the size and crystallinity of the MNPs has been studied by Kim *et al.* [23] The researchers showed that the average size of the NPs increases as the reaction temperature increases. This increase in the temperature also leads to a better crystallinity of the material.



Scheme 1.2: General scheme for a co-precipitation reaction.

This technique allows the production of a large amount of magnetic nanoparticles that can be easily stabilized with surfactants or ligands to yield a stable suspension by generating steric or electrostatic repulsions between nanoparticles. The use of the stabilisers also enables us to avoid the agglomeration of the nanoparticles due to their magnetic properties.

1.3.2 Thermal Decomposition

Magnetic NPs can also be synthesized through the thermal decomposition of organo-metallic compounds in high-boiling organic solvents in presence of stabilising surfactants (e.g. oleic acid). Using this method it is possible to achieve high control over size, shape and crystallinity of the synthesized NPs. [24] One of the most used protocols was developed by Sun *et al.* in 2002. [25] The production of Fe_3O_4 nanoparticles is achieved by heating a mixture of iron (III) acetylacetonate ($Fe(acac)_3$), 1,2-hexadecanediol, oleic acid and oleyamine in phenyl ether to 265 °C. The average size of the magnetic nanoparticles obtained is 4 nm (Figure 1.6) and the protocol can be extended to others metal precursors.

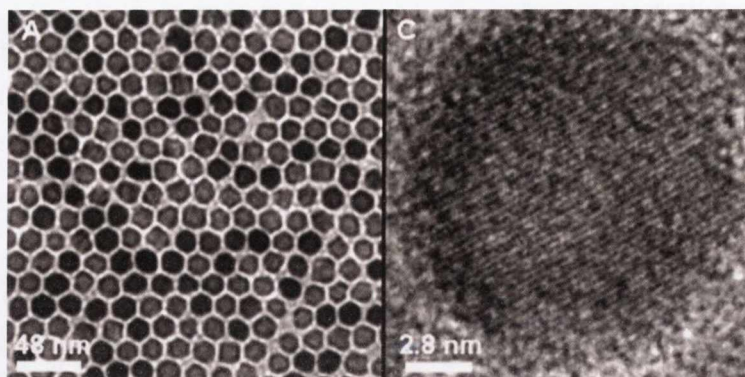


Figure 1.6: TEM images of Fe_3O_4 nanoparticles prepared via thermal decomposition of $\text{Fe}(\text{acac})_3$ in phenyl ether in the presence of 1,2-hexadecanediol, oleic acid and oleylamine.^[25]

Other groups have used the same protocol and the effects of the temperature,^[26] solvent,^[26-28] aging time,^[29] different capping agents,^[26, 30] and concentration of the reactants^[31] have been studied.

In order to obtain water-soluble superparamagnetic MnFe_2O_4 NPs for biological applications the generally used nonpolar high-boiling-point solvents were replaced with water-soluble tetraethylene glycol molecules (TEG). In fact, this procedure avoids the problems related to non-polar solvents and offers the advantage of further functionalisation of the obtained particles according to their future applications.^[32]

1.3.3 Hydrothermal Synthesis

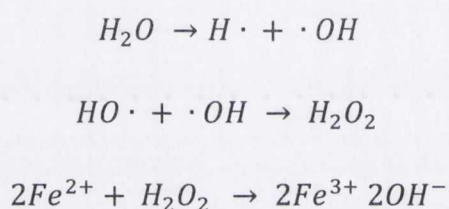
Hydrothermal synthesis is a process that utilizes single or heterogeneous phase reactions in aqueous media at high temperature ($T > 25^\circ\text{C}$) and high pressure ($P > 100$ kPa) conditions to grow materials directly from solution. Synthesis is usually controlled by the internal pressure, which corresponds to the saturated vapour pressure of the solution at the specified temperature and composition of the hydrothermal solution. The reaction time is drastically reduced and the final product normally possess a high crystallinity.^[33]

Li *et al.* have shown that CoFe_2O_4 prepared under this synthesis have a high saturation magnetization value (50 emu/g), superparamagnetic behaviour with negligible coercivity and narrow size distribution. The simple synthesis route used in this work is expected to be a general approach for the preparation of binary and ternary metal oxide, especially spinel ferrite.^[34]

1.3.4 Sonochemistry

Sonochemistry approach arises from the acoustic cavitation phenomenon, which is the formation, growth and collapse of bubbles in liquid medium. Bubble collapse induced by cavitation producing temperatures near 5000°C, pressures of about 500 atmospheres and heating and cooling rates greater than 10⁹ K/s.^[35]

Molecules present within the cavitation bubbles are decomposed and generate several reactive radicals. H[·] and OH[·] are produced when the reaction is carried out in water then the oxidant media generated could lead the oxidation of the metal ion.^[36]



Scheme 1.3: General sonochemical reactions of the formation of radicals and posterior oxidation of metal ion.

One of the most important features of this method is the reduced reaction time from a few hours to a few minutes compared to the conventional co-precipitation method. For example, Liu *et al.* prepared Fe nanoparticles by a sonochemistry method via decomposition of iron pentacarbonyl within minutes. This new approach led to the formation of ultrafine iron particles with an average particle size of only a few nm. Besides, it is possible to control the particles size and the saturation magnetization (M_s) by the power of the sonicator.^[37]

1.3.5 Microemulsion Synthesis

Microemulsions are transparent, isotropic and thermodynamically stable synthesis mediums. The microemulsion based technique permits control of particles properties such as size, geometry, morphology, homogeneity and surface area. In this type of the synthesis, the solution contains at least three components, a polar phase (water), a nonpolar phase (oil) and a surfactant with an amphiphilic behaviour.^[38-39]

The role of the surfactant molecules is to separate polar and non-polar domains forming an interfacial layer. This interfacial layer forms different microstructures ranging from droplets of oil dispersed in a continuous water phase (o/w-microemulsion) or a bicontinuous “sponge” phase to water droplets dispersed in a continuous oil phase (w/o-microemulsion).^[40]

The effects of surfactant structures, such as the charge of polar head group, the hydrophobic chain length and the hydrocarbon parts of polar head group, can play an important role on the lattice defect of the material and stoichiometry during the reaction. These characteristics are critical in the control of the nucleation and growth of the nanocrystal affecting the lattice defects on the particles in structure and composition.^[41] However, one of the disadvantages of this methods is that large volume of solvent is needed to provide a sufficient amount of product.^[42]

1.4 Functionalization of Magnetic Nanoparticles

Due to their dipolar magnetic attraction, unmodified magnetic NPs are inclined to aggregate into clusters that diminish the advantage of the specific properties of single domain nanoparticles. The aggregation associated with magnetic NPs can be avoided by creating an electrostatic double layer using surfactant stabilizers or by growing a shell of a different material around them. Various strategies have been developed for the synthesis of well-dispersed MNPs and to overcome the problem derived from their aggregation. Some of these strategies are briefly discussed below.

Surfactants are used to stabilize magnetic NPs in solution. Surfactant properties can directly affect the particles behaviour in solution. Depending on the compound used as surfactant, the particles surface can become oil-soluble with hydrophobic group, water-soluble, with hydrophilic groups or amphiphilic, with both hydrophobic and hydrophilic groups.

In the past decade, the frequent routes to stabilize magnetic nanoparticles were the use of surfactants such as citric acid ^[43-44] or vitamins, ^[45] and fatty acid salts. For example, Shen *et al.*^[46] have established a method to obtain Fe₃O₄ with different shapes,

nanospheres, nanoneedles and nanotubes, varying the amount of sodium dodecyl sulfate (SDS) and adjusting the irradiation time of the visible light.

Researchers have also developed many approaches for stabilization of magnetic Fe_3O_4 NPs by using polysaccharides as stabilizers during the synthesis. The use of polysaccharides offers the advantage to improve the biocompatibility and biodegradability of the synthesized particles.^[47] Polysaccharides can also form hybrids with metal ions due to their high number of coordinating functional groups (hydroxyl and glucoside groups). They also present interesting dynamic supramolecular associations facilitated by inter- and intra-molecular hydrogen bonding, which can act as templates for the growth of the nanoparticles.^[48]

The use of polymers enables to functionalize and stabilise magnetic nanoparticles and provide them with desired properties for application in different fields. For example, Poly(acrylic acid) (poly(AA))-grafted magnetite NPs were synthesized by a surface-initiated atom transfer radical polymerization (ATRP) of t-butyl acrylate. These polymer-grafted magnetite nanoparticles serve as both steric and electrostatic stabilizers and as a platform for conjugating folic acid, a cancer cell-targeting agent. Poly(AA)-grafted MNPs provided a platform for an efficient surface immobilization of various functional molecules such as DNA, drugs, protein and fluorescent molecules due to its carboxylic acid-enriched surfaces.^[49]

1.5 Core-shell Magnetic Nanostructures

Magnetic NPs coated with inorganic materials have attracted a lot of attention and there is a great variety of compounds that can be used to coat magnetic NPs. These types of core-shell magnetic nanostructures with various functional properties and functionalization are very important for a wide range of applications in biotechnology and nanotechnology.^[50]

1.5.1 Silica Coating

Silica is a typical inorganic material for coatings and formation of core-shell nanostructures. It has been extensively studied and used due to its numerous advantages. Silica

is a non-toxic and biocompatible material and has been widely applied to improve the chemical stability of NPs in a basic environment and to prevent their aggregation. In addition, silica is a material that can be easily functionalised using standard organosilicon chemistry approaches.^[51]

One of the most used techniques for silica shell growth is the well-known Stöber method, which is based on hydrolysis and condensation of tetraethyl ortho-silicate (TEOS) using either a base or an acid in a bulk ethanol solution.^[52] The size or thickness of the shell can be tuned by changing the experimental conditions such as temperature, time or TEOS concentration.^[53]

This silica surface is normally terminated by silanol group (-SiOH) which facilitate the modification of the surface with many different functional groups, such as amines, thiols, and carboxylic acid groups and allowing easy covalent binding of various molecules to the MNPs.^[54] These covalent modifications of the particle surface are usually an advantage used in biomedical applications. Silica coated NPs have been conjugated to many molecules, such as drugs,^[55-57] enzymes,^[58] proteins^[59] or antibodies.^[60]

1.5.2 Carbon Coating

Carbon-coated nanomaterials have also received a lot of attention in recent years due to their thermal and chemical stability and biocompatibility. A carbon layer, hence a protective shell against oxidation, can also preserve the magnetic core from an acidic and oxidising environment enabling to avoid the acid ions pass through it and from being oxidized under an oxygen atmosphere.

Removal of heavy metals or organic pollutants from water is one of the applications explored for carbon coated MNPs. Sun et al.^[61] have reported the efficiency of removing methyl orange by carbon-encapsulated MNPs. MNPs compose of a Fe core and a 15 nm thin carbon layer showed an efficient absorption Cr (VI) of around a 96 wt%.^[62] Carbon coated magnetic NPs can also be further functionalized with the catalytically active Pt species by electrostatic interaction in alkaline solution. The catalytic activity of these nanostructures has been tested for the hydrogenation of aromatic nitro-

compounds showing yields of over 95% without decrease in the conversion efficiency and selectivity after reusing the catalyst 10 times.^[63]

1.5.3 Metal Oxides and Other Inorganic Coatings

Titanium oxide (TiO_2) is one of the most interesting inorganic materials due to its wide range of applications. It has been widely used as a support in heterogeneous catalysis, as a photocatalyst, a photoanode in solar cells, a material for the production of hydrogen, a gas sensor, a white pigment, etc.^[64]

This material has also been investigated as a shell for magnetic nanostructures in order to develop a magnetically recoverable photocatalyst. Different type of MNPs have been synthesized and coated afterwards with TiO_2 in a multistep procedure. Those materials have shown interesting properties as photocatalyst ^[65] and in magnetic and electromagnetic wave absorption applications.^[66]

Similar to many metal oxides, the properties of TiO_2 are defined by its structure. TiO_2 crystallizes in three different phases, rutile (tetrahedral), anatase (tetrahedral) and brookite (orthorhombic). Rutile is the most stable phase while anatase and brookite are the metastable phases. Anatase, however, is the phase that normally has a higher photocatalytic activity when compared to rutile, although for certain photocatalytic reactions a mixture of rutile and anatase has been shown to have the greatest activity.^[67]

TiO_2 is a semiconductor with band gaps of 3.2, 3.02, and 2.96 eV for anatase, rutile and brookite phases respectively.^[68]

The photocatalytic mechanism involves a creation of an electron-hole pair on the surface of TiO_2 by the absorption of a photon which energy must be the same or larger than the band gap of the material. An electron is promoted to the conduction band (CB) while a positive hole is formed in the valence band (VB). Excited-state electrons and holes can recombine and dissipate the input energy as heat, get trapped in metastable surface states, or react with an electron donor or electron acceptor adsorbed on the semiconductor surface or within the surrounding electrical particles. After re-

acting with water, these holes can produce hydroxyl radical with a high redox potential (Figure 1.7).^[69-70]

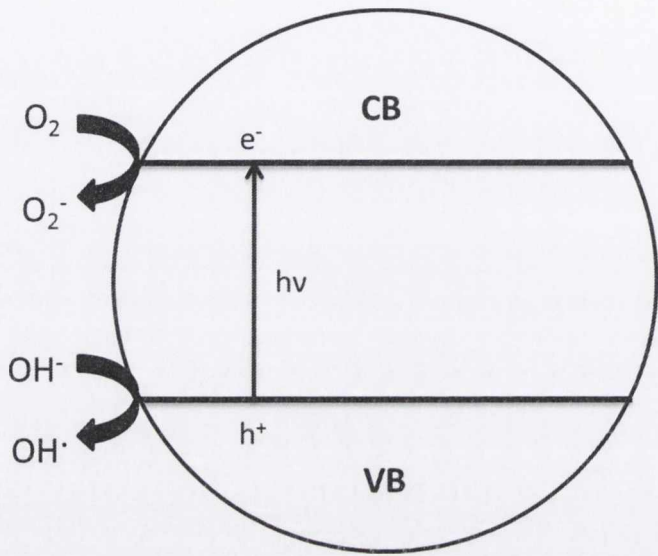
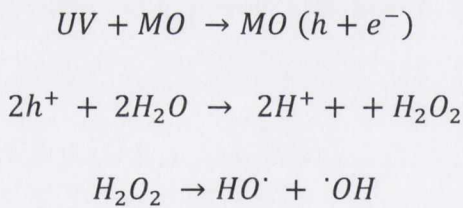
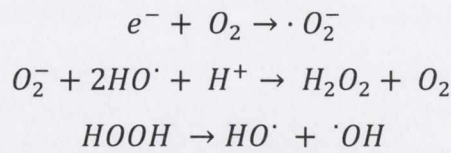


Figure 1.7: General working scheme of a semiconductor material.

Oxidative reactions:



Reductive reactions:



Scheme 1.4: General scheme for photocatalytic redox process where MO represents the metal oxide.^[71]

Thus, TiO_2 is an attractive coating for magnetic nanoparticles as the corresponding core-shell nanostructures can be separated easily by using an external magnetic field and repeatedly used as a catalyst for various photooxidation reactions.

Another important inorganic shell, which is used to functionalize magnetic NPs is ZnO. ZnO has good biocompatibility and biodegradability, which makes it a promising candidate for biomedical applications. ZnO also possesses interesting optical properties (e.g., fluorescence). The combination of these properties resulted in magnetic core-shell nanoparticles which have been studied for multimodal MRI/fluorescent imaging.^[72] ZnO coated magnetic NPs have been tested for enzyme immobilization. The use of magnetic NPs to recover the enzymes immobilized on the Nps by applying a magnetic field makes this process faster and easier.^[73] $\gamma\text{-Fe}_2\text{O}_3/\text{ZnO}$ has been investigated as a photocatalyst for the degradation of different organic dyes including methylene blue, Rhodamine-B, and methyl orange.^[74]

There are a considerable number of publications which report inorganic compounds as coatings. The interest in developing multimodal NPs leads to the introduction of new materials as a shell due to their specific properties (please see below multimodal fluorescent magnetic nanoparticles). Briefly, CdS, CdSe, ZnS and Se have been widely investigated as a shell material to produce two in one fluorescent-magnetic nanoparticles for fluorescence-based imaging and MRI for detection and diagnosis.^[75-80] In_2O_3 coating has been used for ultrasensitive toxic gas detectors, solar cells or transparent conductors.^[81] As semiconductor, PbS and PbSe can be included in the same range of applications and have also been synthesized as a coating on magnetic NPs.^[82]

1.5.4 Coatings Using Metal Nanoparticles

Gold (Au) coated magnetic NPs have attracted the attention of many researchers due to the fact that they are the non-toxic, non-immunogenic and the gold coating is easy to functionalise. These well-known properties of Au coatings make them good candidates for drug delivery systems, adding this advantage to the magnetic properties of the core.^[83-84]

The presence of Au shell on the surface facilitates the preparation of nanocomposites for biomedical applications, for example, the conjugation of thiolate derivatives of bio-

logical molecules to the surface,^[85] the functionalization with cisplatin, which is a chemotherapeutic drug for the treatment of solid tumours,^[86] or conjugation with antibodies for the detection of foodborne pathogens.^[87]

Gold coated magnetic NPs have been investigated also as immunosensors by the combination of a polyvinylalcohol (PVA) modified screen-printed carbon electrode with a $\text{Fe}_3\text{O}_4@\text{Au}$ composite as an electrochemical label in order to detect digoxin, a cardiac glycoside which can indicate an increased risk of toxicity. This assay is based on the immunochemical reaction between free digoxin in samples and immobilized digoxin on the $\text{Fe}_3\text{O}_4@\text{Au}$ nanoparticles surface for the limited antibody sites in solution.^[88]

Silver (Ag) metal has been widely recognized as an antibacterial agent for a long time although it is toxic to healthy cells and its penetration through the bacterial biofilm is too low. It was found that silver shell-coated superparamagnetic NPs with ligand gaps exhibit strong antimicrobial characteristics against bacteria while maintaining remarkable compatibility with the cells. As a result, these NPs can be used as multimodal agents; due to the magnetic core they can penetrate in the bacterial biofilms when an external field is applied, this is something that silver particles cannot do itself.^[89]

Wang and *et al.* demonstrated that MZF@Ag (Ag-Coated MnZn Ferrite) NPs are highly effective for inactivating bacterial growth in both saline solutions and blood platelets medium. This work introduced an important application for these NPs. Indeed, the particles can be used to inactivate bacteria in blood before transfusion in order to ensure blood safety.^[90]

Palladium (Pd) coating has been investigated as catalysts supported on magnetic NPs following the interest of developing the area of heterogeneous catalysis.^[91-92] Some of the advantages of using a heterogeneous catalyst are that it can be recovered easily from the reaction media and can be removed and recycled several times.^[93]

Pd NPs have been incorporated to the synthesis with CoFe_2O_4 NPs to develop an heterogeneous catalyst for Suzuki coupling reaction.^[94] Fe_3O_4 has also been used as a support for this type of catalyst which was applied for first time to hydrogenation and Heck reactions with both a high activity and durability.^[95] This type of catalyst has

been tested for one-pot reductive amination reaction under a hydrogen atmosphere, even at room temperature with high activity after eight cycles.^[96]

1.6 Multimodal Fluorescent Magnetic Nanoparticles

Despite on many problems related to the development of multimodal fluorescent magnetic nanoparticles important advances have been achieved in this field. It is important to improve the material, in which the combination of magnetic nanoparticles and fluorescent entities enables the expansion of the scope in the use of biomedical applications. During the last few years, many groups have tried to overcome the problem of quenching of the fluorescent molecule when it is in contact with the magnetic core. This problem is related to the transfer of energy and electrons between the fluorophore and the metal oxide nanoparticles when they in contact.

Some of the approaches to develop these multimodal fluorescent-magnetic composites are represented in Figure 1.8. One of the strategies to avoid the quenching of the fluorescent component is to use a layer of a different material between them or an alternative molecule as a spacer. For example, the coating of the magnetic core with silica shell is one of the most straightforward approaches. After that, silica can be easily functionalised with fluorescent molecules. Zhang *et al.* fabricated fluorescent mesoporous silica coated superparamagnetic iron oxide nanoparticles (fmSiO₄@SPION_s) to be used as contrast agent in MR imaging.^[97] Silica coated MnFe₂O₄ nanoparticles were functionalised with rhodamine isothiocyanate (RITC) to fabricate a multimodal NP with positive results as a MRI contrast agent in cancer diagnostics.^[98] Fluorescein isothiocyanate (FITC) molecules were embedded in the silica shell coating the Fe₃O₄ nanoparticles. This material was tested as a sensor for the detection and evaluation of food allergens.^[99]

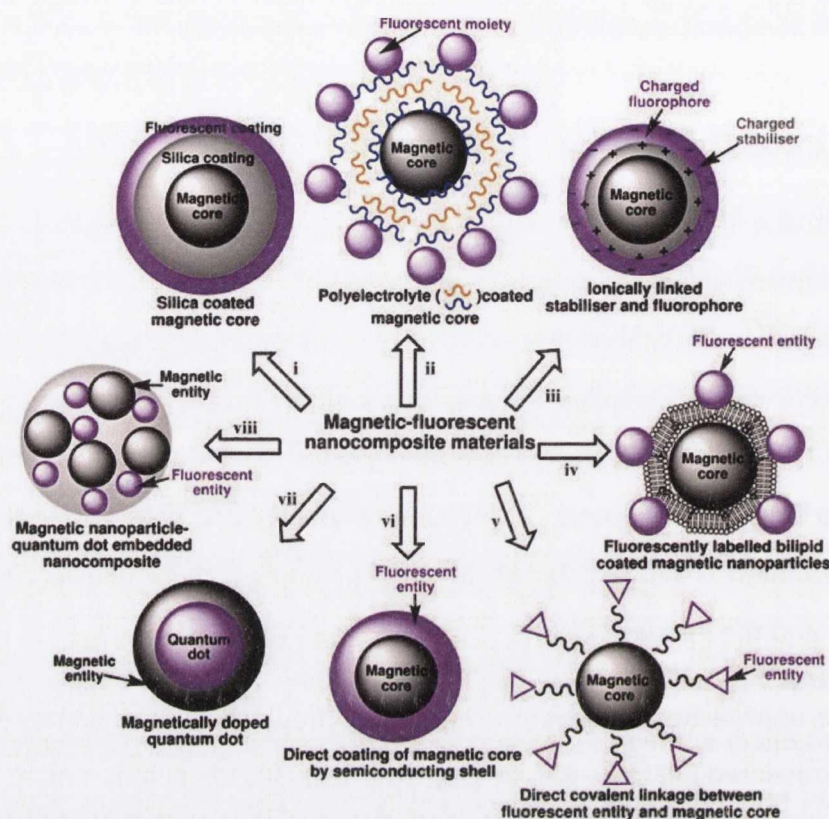


Figure 1.8: Different types of magnetic-fluorescent nanocomposites.^[100]

The combination of polymer or polyelectrolytes functionalised magnetic nanoparticles with fluorescent molecules has also been investigated by many groups. The polymer attached to the magnetic core creates a space with respect to the fluorophore, eliminating the possibility of quenching. The accumulation of different charged layers on the surface of the nanoparticles results in coating known as layer-by-layer (LbL) technique. The possibility of accumulating a different number of layers facilitates the synthesis of a shell of a chosen thickness.^[101] The layer-by-layer technique was also chosen to prepare peptide-fluorescent-magnetic nanocomposites with potential applications for cancer diagnostic. $\text{Fe}_3\text{O}_4@\text{PEI}@\text{Cy5.5}@\text{PEG}@\text{HCBP-1}$ nanoparticles, where Cy5.5 is a fluorescent dye and HCBP-1 a peptide, were tested in the recognition of lung cancer stem cells showing efficient recognition and that the nanocomposite could be used as a imaging agent in tumour detection.^[102]

Wan *et al.* fabricated a nanocomposite with properties for the detection of toxic substances present in biological systems.^[103] Complex entities such as Poly-(isobutylene-alt-maleic anhydride)-functionalized near-infrared (NIR) IR-820 dye conjugated to Fe_3O_4 magnetic nanoparticles were prepared by Yen *et al.*^[104] The material is water soluble and showed to preserve its photochemical properties whilst giving excellent results in the studies of cytotoxicity. Many other examples of this strategy can be found in the literature. Polyethylene glycol has also been chosen for many investigations for the functionalization of nanoparticles with fluorescent entities.^[105-106]

Fluorescent linked lipid-coated magnetic nanoparticles consist of a layer of amphiphilic lipid molecules surrounding the magnetic core. The functionality of the lipid heads allows the attachment of other functional groups. Lipid-modified Poly(amido)amine (PAMAM) dendrimers were attached to iron oxide nanoparticles following a single step route that allows to obtain stable nanoparticles in water. PAMAM-coated nanoparticles can be conjugated by different fluorescent molecules, such as rhodamine and fluorescein.^[107]

Fluorescent molecules can also be directly linked to the magnetic core if the spacer is long enough to avoid the quenching of the magnetic core. Different molecules can be linked to the magnetic nanoparticles and act as stabilisers. Functional group of the free end of the molecular will permit the convenient functionalization. For example, citric acid functionalised Fe_3O_4 magnetic nanorings (NRs) were linked to polyethylenimine PEI capped CdSe/ZnS QDs.^[108]

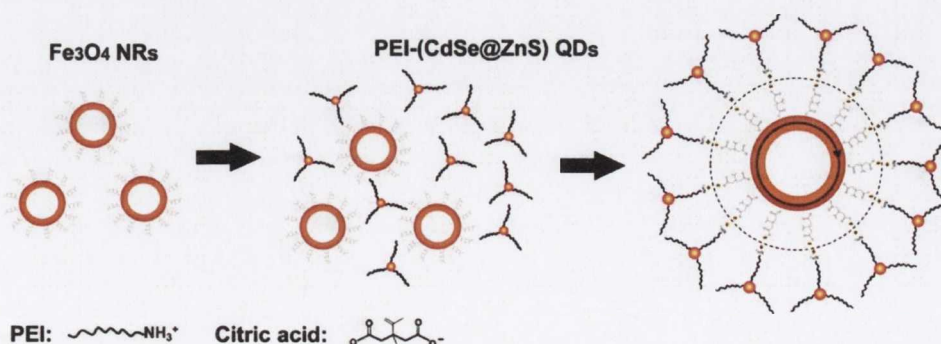


Figure 1.9: Synthesis of Fe_3O_4 NRs capped with PEI-(CdSe@ZnS) QDs.^[108]

Fluorescent magnetic nanocomposites can also be prepared by coating directly the magnetic core using fluorescent semiconductors such as quantum dots (QD). Rapid methods can be carried out by a simple preparation, for example, Mn-doped ZnS QDs and Fe_3O_4 were conjugated by the condensation reaction of the functional groups present on both nanoparticles. Carboxylic groups present on the thiolglycolic functionalised QDs reacted with the amine groups of the APTES functionalised magnetic nanoparticles.^[109]

QDs and magnetic nanoparticles can be embedded into the same matrix to form a larger nanocomposite. By tuning the ratio of QDs (CdSe-ZnS) per magnetic nanoparticles (MnFe_2O_4) in the nanocomposites prepared by Di Corato *et al.* the luminescence can be adjusted. QDs were incorporated to the surface of the magnetic nanoparticles by an amphiphilic polymer (poly-(maleic anhydride-alt-1-octadecene)). QDs and magnetic nanoparticles were mixed together with the polymer in tetrahydrofuran (THF). The surface of the nanocomposites were further functionalised with folic acid to enhance their uptake by cancer cells.^[110]

1.7 Applications of Magnetic Nanoparticles

1.7.1 MRI Contrast Agents

Magnetic resonance imaging technique involves the imaging of protons present in the soft tissues in the human body composed of proteins, water or lipids. Proton spins align under the presence of an applied magnetic field, B_0 . These protons are perturbed from B_0 when a transverse radiofrequency pulse is applied. Subsequently, protons return to their original state through a process of relaxation. These processes are known as T_1 (longitudinal relaxation) and T_2 (transverse relaxation time) and generate a MR image. Difference in relaxations is related to the density or the chemical and physical nature of the tissues under study.^[111] Commonly, paramagnetic complexes, such as Gd-DTPA (gadolinium diethylene triamine pentaacetic acid), are used as T_1 contrast agents and MNPs are selected as T_2 contrast agents.

The acting mechanism of T_2 contrast agents is as follows: both relaxation time, T_1 and T_2 , can be shortened by the use of a magnetic contrast agent. The coating of the nano-

particles can cause a delay in the shortening of the relaxation time but the distance between the protons and the contrast agent has to be minimized. Generally, T_1 uses paramagnetic complexes, such as gadolinium diethylene triamine pentaacetic acid (Gd-DTPA) as contrast agents.^[112-113]

Magnetic field gradient arise when T_2 is shortened due to the difference in the susceptibility between the magnetic material and the medium around them. This gradient causes the diffusion of the protons that leads to a decrease in the relaxation time of the protons. In this case, superparamagnetic nanoparticles are useful as negative contrast agents (T_2).^[114]

The development of superparamagnetic nanoparticles is important due to that they do not retain any residual magnetism after removal of the magnetic field, which avoids the aggregation of the nanoparticles within the tissue. Fluids of superparamagnetic nanoparticles provide an ideal material to use as magnetic resonance contrast agent. By controlling the size of the colloidal nanoparticles, the influence in the increase of the relaxation time can be reduced.^[115-117]

1.7.2 Drug Delivery Agents

In the last decades, magnetic nanoparticles have become an important advanced tool for applications such as drug delivery. Magnetic nanoparticles can be manipulated with an external magnetic field and drive to the desired target area, i.e. cancerous tissue, where the drug can be released and act directly. This advance in medicine enables to reduce the drug dose and potential elimination of side effects.^[118] The success of this therapy strongly depends on the selection of the targeting moiety to have specific affinity of binding to the cell surface receptors.^[119]

The high surface area of the nanoparticles allows a large amount of drug to be adsorbed. However, magnetic nanoparticles used in drug delivery must be functionalised with groups that can assure their biocompatibility.^[120] For example, magnetic nanoparticles functionalised with silica have demonstrated to perform effectively as a drug delivery vehicles to cancer cells. At the same time, this nanomaterial showed the inhibition of growth of the cancer cells.^[121] Organic polymers are other alternative for the biocompatible functionalisation of the magnetic nanoparticles.^[98, 122]

1.7.3 Magnetic Hyperthermia

Back in 1957, Gilchrist et al. carried out investigations using magnetic nanoparticles in hyperthermia. The experiment involved heating of tissue samples containing $\text{Y-Fe}_2\text{O}_3$ nanoparticles in a range of 20-100 nm in size and exposed to a 1.2 MHz magnetic field.^[123] Thus, magnetic hyperthermia is heating of the nanoparticles previously placed in a targeted tissue by applying an AC magnetic field. Consequently, the heat is applied directly to the diseased tissue surrounding the magnetic material and the cancer is destroyed if the temperature can be held for 30 min at 42°C. This technique gives the possibility of treating cancer cells directly by heating while the healthy tissues are kept untouched. The principles of hyperthermia are also based on the fact that cancer cells are more sensitive to the changes in temperature than healthy cells. Magnetic materials are chosen for this technique because they are able to absorb the energy from the AC magnetic field to convert it in heat and the effect of the resultant heating is higher than for other materials. Another advantage of using magnetic nanoparticles is that they can be targeted to the cancer cells magnetically or using biomarkers to ensure that only the intended target tissue is heated.^[124]

1.7.4 Magnetic Separation in Biotechnology

Magnetic nanoparticles with the specific functionalization allow the isolation of biomolecules of interest in a broad range of applications related to biotechnology and biomedicine. The separation of these molecules using magnetic nanoparticles facilitates the removal, reduces times and costs as well as the separation and concentration of the material of interest from bulk solutions.

In the past few decades, this field has grown and magnetic nanoparticles have been investigated for separation and/or purification of different biological molecules such as proteins^[125-127], DNA^[128-130], or removing bacterial from blood.^[131]

Cell separation is similar to protein or DNA purification technique. In this case, this method offers a rapid quantification, high cell recovery when compared to the conventional method such as centrifugation and the advantage of isolating specific cells from the medium due to the specific functionalization of the nanoparticles. Magnetic separation of red blood cells and lymphoid cells was used by Molday *et al.* in 1977.^[132]

Since then, many researchers have been improving this applications in order to facilitate the isolation of cells. Metter *et al.* performed the magnetic separation of islet cells using three different types of superparamagnetic iron oxide nanoparticles (SPIONs), dextran coated SPION, siloxane-coated SPION and heparin-coated SPION.^[133] Huang *et al.* reported the preparation of multifunctional “smart” particles with topographic, magnetic, cell targeting and stimulus-responsive properties, using a “live template” strategy, improving the capture efficiency of target cells and cell release.^[134]

1.7.5 Catalysis

Homogeneous catalysis has been intensely studied and well developed due to the efficiency and selectivity of the catalyst. However, there is a difficulty of removing the catalyst completely from the reaction media and recycling it. Heterogeneous catalysis, in contrast, can be recovered easily from the reaction media, but the lack of selectivity is a parameter that needs to be addressed. Nowadays, it is necessary to design new environmentally friendly catalysts, which can be removed and recycled several times, in order to promote green chemistry and reduce costs.

MNPs have emerged as an important tool in this field due to their easy manipulation, the possibility to remove them from the solution using magnetic separation and their functionalization using different approaches with compounds known for their catalytic properties. They have opened a new window of opportunities for catalysis.

Bare (uncoated) MNPs have been studied for many different catalytic reactions such as CO₂ reduction, water splitting and a broad range of organic reactions. It has been demonstrated that iron oxides nanoparticles can catalyse oxidation ^[135-136] and coupling reactions.^[137-138] The use of spinel ferrite structures with different metals partially replacing the iron, cobalt for example, gives the nanoparticles more chemical and thermal stability while keeping the magnetic properties. This type of bare cobalt ferrite nanoparticles have shown catalytic activity for the oxidation of alkenes to ketones or epoxides ^[139] and for aerobic oxidation of cyclohexane.^[140]

Using coated nanomaterials with catalytic properties can solve the problem of aggregation of naked MNPs. This approach also opens up a new research area in heteroge-

neous chiral catalysis. In the past few decades using magnetic nanoparticles many advances have also been achieved in asymmetric catalysis^[141-142] and in enantioselective separation.^[143-145]

1.7.6 Magnetic Data Storage

Oberling Smith described the principles of magnetic recording in 1878 but it was Valdemar Poulsen twenty years later, in 1898, who demonstrated these principles.^[146] The first magnetic hard drive capable of storage 5 MB with a recording density of 2 kbit in⁻² was built by IBM in 1956.^[147] Last decades, the demand to improve the capacity of storage and reduce the cost has led to the development of considerably smaller, faster and increasing number of gigabytes devices.

In order to accomplish this demand of devices with that high recording density, the size of the magnets used must be reduced their dimensions. Magnetic nanoparticles such as CoFe₂O₄ or CoO·Fe₂O₃, with large magnetocrystalline anisotropy have been studied as the alternative for the development of recording materials.^[148] Doped BaFe₁₂O₉ has been used in commercial tape drives and enabled to increase the recording capacity to 29.5 Gb in⁻².^[149]

Ferro- and ferromagnetic materials are employed in magnetic storage because they do not need the presence of an external magnet to act as individual magnets. Sun *et al.* reported the fabrication of a polymer mediated nanoparticle assembly (PEI/FePt) with high control in the thickness and dimension of the nanomaterial. The addition of different layers during the preparation transforms the assembly from superparamagnetic to ferromagnetic resulting in a suitable material for recording devices.^[150-151]

1.8 Aims and Objectives of the Work

The main aim of this project is to develop new magnetic core-shell nanomaterials, which could be potentially used in catalytic and/or biological applications.

In summary, the major scientific and technical objectives of this work are to:

- Prepare magnetic iron oxide and cobalt ferrite based nanoparticles and nanocomposites by co-precipitation techniques.

- Produce new core-shell nanostructures with magnetic iron oxide or cobalt ferrite core and catalytic metal oxide shell (TiO_2 , CeO_2 , etc.).
- Test the catalytic activities of these particles in selected organic oxidation reactions.
- Prepare magnetic cobalt ferrite nanoparticles stabilised with amino acids and investigate the influence of the effects on the structural and magnetic properties of the material.
- Evaluate these new synthesised amino acid stabilised cobalt ferrite nanoparticles as potential agents for enantiomeric separation and chiral catalysts.
- Prepare DNA stabilised iron oxide nanoparticles and evaluate them as potential agents for enantiomeric separation.
- Prepare silica-coated nanobeads and compare their efficiency for DNA separation and purification with current commercially available protocols.
- Prepare multimodal magnetic nanostructures based in silica coated magnetic nanoparticles for potential biomedical applications.
- Characterise the nanoparticles and nanocomposites using different instrumental techniques. (e.g. TEM, VSM, FTIR and Raman spectroscopy, XRD)

Due to their controllable size and magnetic properties, these nanoparticles and nanocomposites could find many uses in catalysis and biotechnology (e.g. as antibacterial agents or magnetic separation). Overall, the successful realisation of this project should result in the development of new magnetic nanomaterials with a broad range of potential applications.

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Chapter 2. Experimental

2.1 Starting Materials and General Lab Equipment

All chemicals were purchased from Sigma-Aldrich unless stated otherwise. Unless specified otherwise, all syntheses were carried out in water from a Millipore filtration system operating at 18 MΩ (referred to as Millipore (MP) water).

Two centrifuges were used throughout. A Hettich Zentrifugen Universal 32 was used for large volumes of non-magnetic materials. For small volumes and higher speed required to wash metal nanoparticles, a Hermle Z233 M-2 was used.

For ultrasonic agitation, a Wise Clean WUC-A03H ultrasonic bath was used and operated at 40 Hz.

2.2 Experimental Procedures

2.2.1 Experimental for Chapter 3

2.2.1.1 Preparation of Cobalt Ferrite Nanoparticles in Aqueous Phase

Cobalt nitrate ($\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) (0.6 g, 0.002 mol) and iron (II) chloride ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$) (0.8 g, 0.004 mol) hydrates were dissolved in 100 mL degassed water in a 250 mL round bottomed flask. The mixture was then heated in an oil bath to 80 °C and then an excess of ammonia was added (up to the pH to 11) resulting in a black precipitate. After that, the mixture was stirred for 1 hour, it was allowed to cool down to room temperature. The dark brown product was collected by magnetic separation (1.2T permanent magnet). The product was then washed with Millipore water several times until a neutral pH was obtained.

2.2.1.2 Preparation of Cobalt Ferrite Nanoparticles Stabilised with Citric Acid

Magnetic nanoparticles (CoFe_2O_4 or Fe_3O_4) (0.225 g) were placed in a 250 mL RBF, 80 mL of degassed water was added. The solution was sonicated in a sonic bath for approximately 30 minutes to disperse the nanoparticles. A solution of citric acid in water was prepared by dissolving citric acid pellets (0.55 g, 0.003 mol) in 120 mL of degassed water. This solution was added to the solution of the magnetic NPs above and the resulting solution was sonicated for 1 h. Tetramethylammonium hydroxide (25 v/v%) was then added dropwise until pH neutral and particles were stored in the stabilised suspension. The mixture was used to prepare core-shell NPs.

2.2.1.3 Preparation of $\text{CoFe}_2\text{O}_4@\text{SiO}_2$ Core-shell Nanoparticles

In a 250 mL RBF, 25 mL of the citric acid stabilised NP solution was mixed with 25 mL ammonium hydroxide in 100 mL of ethanol. Tetraethyl orthosilicate (TEOS) solution (400 μL) was added to this solution. Then the mixture was sonicated on ice for 1 h. The resultant NPs were extracted using magnetic separation (1.2 T magnet) and were washed several times with EtOH. The particles were dried by rotary evaporation.

2.2.1.4 Preparation of $\text{CoFe}_2\text{O}_4@\text{TiO}_2$ Nanoparticles

The TiO_2 coatings were prepared via sol-gel hydrolysis precipitation of titanium isopropoxide ($\text{Ti}(\text{OC}_3\text{H}_7)_4$) followed by the calcination treatment. A defined amount of modified-cobalt ferrite nanoparticles (200 mg) was dispersed in a mixture of water-ethanol with a molar ratio of 1:10. Aqueous HCl (3.0 M) was used to adjust the pH to 5-6. The suspension was sonicated for 30 min and subsequently vigorously stirred for another 8 h to ensure the complete coating. The precipitate was separated from the mother liquor by centrifugation and then re-suspended in ethanol. This washing process was repeated five times. The resulting material was dried and then calcinated at 450 °C for 20 min.

2.2.1.5 Preparation of $\text{CoFe}_2\text{O}_4@\text{SiO}_2@\text{TiO}_2$ Nanoparticles

$\text{CoFe}_2\text{O}_4@\text{SiO}_2$ (0.0495 g) was dispersed in 100 mL EtOH by sonication. Titanium (IV) butoxide ($\text{Ti}(\text{OBu})_4$) (0.4 mL) was added via a syringe and the solution was sonicated for approximately 5 min. The mixture was allowed to stir at room temperature for 24 h. The product was extracted by magnetic separation (1.2 T) and washed with EtOH (3 x 20 mL). The solvent was removed by rotary evaporation. The resulting $\text{CoFe}_2\text{O}_4@\text{SiO}_2@\text{TiO}_2$ nanoparticles were calcinated at 600 °C for 1 h resulting in the formation of a crystalline anatase TiO_2 shell.

2.2.1.6 Photocatalytic Activity Testing of TiO_2 Coating

In a quartz cuvette 10 μL of methylene blue (1 mM) was mixed with 3 mL Millipore water. To this mixture, a few $\text{CoFe}_2\text{O}_4@\text{SiO}_2@\text{TiO}_2$ milligrams (Approx. 5 mg) of nanoparticles were added and the mixture was allowed to stir in the dark for 1 h. A UV-Vis spectrum was recorded for the sample after incubation at a time t_0 . The solution was then placed under a UV light (264 nm) and allowed to stir for 10 min before collecting another UV-Vis spectrum. The procedure was repeated every 10 min for 1 h. The Millipore water was used for the baseline correction.

2.2.1.7 Preparation of $\text{CoFe}_2\text{O}_4@\text{CeO}_2$ Nanoparticles

50 mg of CoFe_2O_4 were dispersed in 100 mL of Millipore water. 2.2 g (0.05 mol) of $\text{Ce}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$ were added to the solution and it was sonicated for 30 min. 7 g (0.5 mol) of hexamethylenetetramine (HMT) were dissolved in 100 mL and both solutions were mixed together. Finally, 2 mL of a solution of 0.5 g of acrylamide in 20 mL of DMF was added and the final solution was allowed to stir overnight. The resultant nanoparticles were washed 3 times with water and dry at 60 °C by rotary evaporation. The sample was calcinated in the oven at 500 °C for 1 h.

2.2.1.8 Preparation of $\text{CoFe}_2\text{O}_4@\text{D-Pen}@\text{SiO}_2$ Nanoparticles

A defined amount of $\text{CoFe}_2\text{O}_4@\text{D-Pen}$ (100 mg) NPs was dispersed in 100 mL of EtOH for 15 minutes using an ultrasonic bath. After this, 200 μL of TEOS (tetraethyl orthosilicate) were added and the mixture was sonicated for 30 minutes in an ice bath (0 °C).

NH₄OH (1.5 mL) was added and the mixture was sonicated for 2 hours in ice bath. The resultant NPs were isolated using magnetic separation and washed 2 or 3 times with EtOH. The NPs were stored in EtOH.

2.2.1.9 Preparation of CoFe₂O₄@D-Pen@SiO₂@TiO₂ Nanoparticles

CoFe₂O₄@D-Pen@SiO₂ (5 mL) was dispersed in 100 mL EtOH by sonication. 1 mL of Titanium (IV) butoxide (Ti(OBu)₄) was added via a syringe and the solution was sonicated for approximately 5 min. The mixture was allowed to stir at room temperature for 24 h. The product was isolated by magnetic separation and washed three times with EtOH. The rest of the solvent was removed by rotary evaporation.

2.2.1.10 Preparation of Ag/TiO₂ Coated CoFe₂O₄@D-Pen@SiO₂ Nanoparticles

12 mL of EtOH, 2.5 mL of Millipore water and 25 mg of CoFe₂O₄@D-Pen@SiO₂@TiO₂ NPs were mixed in a 100 mL RBF and dispersed for 30 minutes in a sonic bath. 0.2 g (0.001 mol) of Silver Nitrate (AgNO₃) was added to the solution, which was sonicated for 15 minutes. 0.170 mL of N-N-Dimethylformamide (DMF) were added in the RBF and then sonicated for 15 minutes. The solution was stirred for 24 hours. The resultant NPs were washed three times with EtOH and dried under vacuum. The resultant product was calcinated at 600 °C for 1 hour.

2.2.1.11 Preparation of Au/TiO₂ Coated CoFe₂O₄@D-Pen@SiO₂ Nanoparticles

8 mL of Ti(OBu)₄, 80 mL of absolute EtOH and 2 mL of CoFe₂O₄@D-Pen@SiO₂ NPs were mixed in a 100 mL RBF and sonicated for 30 minutes. In another RBF, 80 mL of EtOH, 5 mL of Gold (III) chloride hydrate (HAuCl₄) solution and 15 mL of acetic acid were stirred for 1 hour. Gold salt solution was added to the solution containing the NPs dropwise. The mixture was stirred overnight. The resultant NPs were washed three times with EtOH and dried under vacuum.

2.2.1.12 Photocatalytic Activity Testing

In a quartz cuvette, 150 μL of a solution of MB (1mM) was mixed with a 9 mL of water. Few milligrams (5-10 mg) of nanoparticles were added to the mixture and the solution was allowed to stir in darkness for 1 hour. UV-Vis spectrum was recorded for the sample after incubation at a time t_0 . After allowing the adsorption-desorption of the dye on to the surface of the nanoparticles the solution was placed under UV light (264 nm), daylight or darkness according to the experiment requirements. The solution was stirred and UV-Vis spectra were recorded every 10 min until the conclusion of the experiment. MP water was used for the baseline correction.

2.2.2 Experimental for Chapter 4

2.2.2.1 Preparation of CoFe_2O_4 Stabilized with Chiral Ligands

MNPs stabilized with chiral ligands were prepared by the co-precipitation route previously described by Moloney *et al.*^[1] with some modifications. In a 250 mL round bottomed flask, 190 mL of H_2O was heated up to 80 °C and degassed under Argon. Simultaneously, $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (0.6 g, 0.002 mol) was dissolved in a sample tube containing 5 mL of Millipore water and $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (0.8g, 0.004) was dissolved in a separate sample tube containing 5 mL of Millipore water. A defined amount of amino acid was added to each of these solutions (Table 2.1). The solutions were then combined and added into the 190 mL of degassed Millipore water. The resulting solution was continuously degassed with argon for another 1 hour. The pH was adjusted to 11 using a solution of NH_4OH (0.88 M).

The above synthesis was repeated replacing D-Pen with one of the following ligands: D-Aspartic Acid, L-Aspartic Acid, D-Cysteine, L-Cysteine or D-Glutamic Acid to yield the corresponding stabilised CoFe_2O_4 particles.

Table 2.1: Amino acids stabilized CoFe₂O₄ nanoparticles experiments.

Sample	Ligand (mmol)	Ligand (mg)
DP1	0.17	25
DP2	0.34	50
DP3	0.68	100
DC1	0.17	20
DC2	0.34	40
LC1	0.17	20
LC2	0.34	40
AA1	0.17	22
AA2	0.17	22
GA1	0.17	25

2.2.2.2 Preparation of Cobalt Ferrite Nanoparticles Stabilised by L-Alanine

In a 250 mL round bottomed flask, 190 mL of MP H₂O was heated up to 80 °C and degassed under Argon. Simultaneously, Co(NO₃)₂·6H₂O (0.6 g, 0.002 mol) was dissolved in a sample tube containing 5 mL of MP water and FeCl₂·4H₂O (0.8g, 0.004) was dissolved in a separate sample tube containing 5 mL of MP water. A defined amount of L-Alanine was added to each of these solutions (Table 2.2). The solutions were then combined and added into the RBF with 190 mL of degassed MP water. The resulting solution was kept under argon for another 1 hour. Then the pH was adjusted to 11 using a solution of NH₄OH (0.88 M).

Table 2.2: L-Alanine stabilized CoFe₂O₄ nanoparticles experiments.

Sample	L-Alanine (mmol)	L-Alanine (mg)
AL01	0.34	30
AL02	0.68	60
AL03	1.35	120
AL04	2.70	240
AL05	5.40	480
AL06	10.8	960
AL07	21.6	1920

2.2.3 Experimental for Chapter 5

2.2.3.1 Preparation of OH and NH₂ Functionalized Co@C/Fe@C Beads

Co@C/Fe@C nanobeads have been supplied by Functional Material Laboratory (FML), ETH (Zurich) in collaboration with Prof. Wendelin Stark and Dr. Robert Grass.

Functionalization of Co@C/Fe@C nanobeads was carried out according to the described reaction with some modifications.^[2] 5g of Co@C/Fe@C nanobeads were dispersed in 100 mL of H₂O using an ultrasonic bath. 2-(4-aminophenyl) methanol/ 4-(Aminomethyl) aniline were added to the solution. Sodium nitrite (0.9 g, 13 mmol) and HCl (2mL, 37%) were added to this solution to transform the organic compound into the corresponding diazonium salt. The mixture was sonicated for 30 min at low temperature ($\approx 0^{\circ}\text{C}$). The material was recovered from the reaction mixture with the help of an external magnet and washed with water (3x 30mL) and acetone (2x30 mL). After the last washing, the particles were dried under vacuum.

2.2.3.2 Preparation of Co@C@SiO₂ (TEOS/DTES)

2 mg of the above prepared 2-(4-aminophenyl) methanol functionalized Co@C nanobeads were dispersed in 10 mL of EtOH. The mixture was dispersed by the use of an

ultrasonic bath for 10 min. To this mixture, 2.5 mL of NH_4OH and 40 μL of TEOS were added and the resultant solution was kept sonicating at 0°C overnight. The resultant material was washed with EtOH (3x20 mL). After the last washing, the black material was stored in 20 mL of EtOH.

Silica coating of 2-(4-aminophenyl) methanol functionalized Co@C with dodecyltriethoxysilane (DTES) used as silica precursor was carried out in the following reaction similar to the previous one with some modifications. 1 g of nanobeads was dispersed in 200 mL in ethanol. To this dispersion, 2 mL of DTES and 2 mL of NH_4OH were added. The reaction was kept in an ultrasonic bath overnight and the resultant material was washed with EtOH (3 x 20 mL). After washings, the particles were storage in 20 mL of EtOH.

2.2.3.3 Preparation of Fe@C@SiO₂ Nanoparticles

25 mg of 4-(Aminomethyl) aniline functionalized Fe@C were dispersed in 20 mL of EtOH with the help of an ultrasonic bath. After 30 min, 800 μL of TEOS were added and the solution was sonicated for another 30 min. After that, 5 mL of NH_4OH were added and the mixture was sonicated for 3 hours at 0°C . The resultant material was washed with EtOH (3 x 20 mL) and stored in 20 mL of the same solvent.

2.2.3.4 Preparation of DNA Stabilised Magnetite Nanoparticles

Magnetite nanoparticles stabilised by denatured herring sperm DNA were prepared according to published procedure.^[3] Herring sperm DNA (0.06 g, mol) was dissolved in 100 mL of deoxygenated MP water and solution was heated to 80°C with vigorous stirring for 15 minutes to denature the strands. $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (1.08 g, 4 mmol) and $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (0.41 g, 2 mmol) were added to the heated degassed DNA solution. NH_4OH (0.88 M) was added dropwise until a pH of 11-12 was achieved. The reaction mixture was stirred for 2 hours at 80°C and a dark brown precipitate was isolated by magnetic separation and washed several times with EtOH. The product was dried by rotary evaporation.

2.2.3.5 Enantiomeric Separation Tests Using DNA Stabilised Magnetite Nanoparticles

D-Penicillamine (0.003 g, mol) and *L*-Penicillamine (0.003 g, mol) were dissolved in 40 mL MP water under stirring. DNA stabilised magnetite (0.005 g), as prepared above, was added to 10 mL of MP water and stirred for 30 minutes. Then the NPs were isolated by magnetic separation and solution was analysed by CD. This washing step was carried out 3 times before dispersing washed NPs in 10 mL *D*-/*L*-Penicillamine mixture. The NPs were isolated again by magnetic separation and CD spectra were recorded for the solution.

2.2.3.6 Enantiomeric Separation Tests Using Chiral Ligand Stabilized Magnetite

D-Penicillamine (0.003 g, mol) and *L*-Penicillamine (0.003 g, mol) were dissolved in 40 mL MP water under stirring. Chiral ligand (*D*-Pen and *D*-/*L*-Cysteine) stabilised magnetite (0.010 g) was added to the mixture and stirred for 30 minutes. Then the NPs were isolated by magnetic separation and solution was analysed by CD.

2.2.3.7 *D*-penicillamine and *L*-penicillamine Interaction Test Using Chiral Ligand Stabilized Magnetite

Two different solutions of *D*-Penicillamine (0.003 g, mol) and *L*-Penicillamine (0.003 g, mol) in 40 mL MP water under stirring were prepared. Chiral ligand (*D*- and *L*-Cysteine) stabilised magnetite (0.010 g) was added to these separated solutions and stirred for 30 minutes. Then the NPs were isolated by magnetic separation and solution was analysed by CD.

2.2.4 Experimental for Chapter 6

2.2.4.1 Preparation of Fe_3O_4 Nanoparticles

Magnetite nanoparticles were prepared according to the modified published procedure.^[4] Na_2CO_3 (5.29 g, 0.05 mol) was dissolved in 200 ml of degassed MP water and the solution was heated to 90° C. $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (6.75 g, 25 mmol) and $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (2.48 g,

12.5 mmol) were dissolved in 50 ml of degassed MP water. The iron chloride solution was added dropwise to the heated sodium carbonate solution over a period of 30 minutes with vigorous stirring. Once all the iron chloride solution had been added, the reaction mixture was stirred at 90° C for 1 hour. The resulting dark brown precipitated nanoparticles were isolated from the solution by magnetic separation and washed several times with MP water until it was pH neutral. The final washing was carried out in EtOH and the product (2.10 g, 73%) was dried using a rotary evaporator.

2.2.4.2 Preparation of Citric Acid Stabilised Fe₃O₄ Nanoparticles

Fe₃O₄ NPs (0.225 g, 0.97 mmol) as prepared above were dispersed in 100 mL of MP water by ultrasonication for 30 minutes. Citric acid pellets (0.55 g, 2.9 mmol) were dissolved separately in 100 mL Millipore water. The acidic solution was added to the NP suspension and the mixture was sonicated for 1 hour. Tetramethylammonium hydroxide (25 v/v%) was then added dropwise until pH neutral and particles were stored in the stabilised suspension.

2.2.4.3 Preparation of Fe₃O₄@SiO₂ Nanoparticles

Silica coated magnetite nanoparticles of varied shell thickness were prepared following a modified published procedure.^[5] In a 250 mL RBF, as prepared above 10 mL of citric acid stabilised magnetite nanoparticles were added to 100 mL of EtOH and the mixture was sonicated for 10 minutes. Tetraethyl orthosilicate (TEOS) solution of a known volume (200 µL/50 µL) was added with sonication for a further 10 minutes on ice. NH₄OH (2 mL, 0.8M) was then added dropwise and the reaction mixture was sonicated on ice for 2 hours. The product was extracted and washed several times with EtOH using magnetic separation, and stored in a minimum volume of EtOH.

2.2.4.4 Preparation of Fe₃O₄@SiO₂ Nanorattles

1 mL of coated magnetite NP solution as prepared above (thick silica shell) was taken and the NPs were isolated by magnetic separation and washed twice with MP water. 7 mL of MP water was added to the coated nanoparticles in a sample tube and NPs were

shaken to disperse. Particles were stored in the MP water over a period of 3 weeks with regular manual shaking and sonication to re-disperse.

2.2.4.5 Preparation of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ -Coumarin Nanocomposites

0.4 g of coumarin-3 carboxylic acid (2 mmol) was dissolved in 10 mL of absolute EtOH. To this mixture 0.5 mL of (3-Aminopropyl) triethoxysilane (APTES) were added. The final solution was stirred for 12 hours and the resultant mixture was stored in darkness.

The $\text{Fe}_3\text{O}_4@\text{SiO}_2$ nanoparticles containing coumarin were synthesized as in the previous procedure. In a 250 mL RBF, 10 mL of citric acid stabilised magnetite nanoparticles as prepared above was added to 100 mL of EtOH and the mixture was sonicated for 10 minutes. 2 mL of the solution containing coumarin-3-carboxylic acid was added and the mixture was kept sonicating for 1h. After the time, 200 μL of tetraethyl orthosilicate (TEOS) was added with sonication for a further 10 minutes in an ice bath. NH_4OH (2 mL, 0.88 M) was then added dropwise and the reaction mixture was sonicated in ice bath for 2 hours. Resultant magnetic material was washed several times and recovered with external permanent magnet. The final product was stored in a minimum volume of EtOH.

2.2.4.6 Preparation of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ -Coumarin Nanorattles

1 mL of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ -Coumarin nanorattles dispersed in EtOH was taken and NPs were isolated by magnetic separation and washed twice with MP water. 10 mL of Millipore water were added to the coated nanoparticles in a sample tube and the NPs were shaken to disperse. The particles were stored in MP water with regular manual shaking and sonication to re-disperse.

2.2.4.7 Preparation of $\text{Fe}_3\text{O}_4@\text{ZnS:Mn}@\text{SiO}_2$ Nanocomposites

ZnS:Mn QDs have been prepared and provided by a fellow lab group member.

0.5 mL of zinc sulfide QDs solution was taken and dispersed in 100 mL of EtOH by sonication, followed by the addition of 10 mL of citric acid stabilised magnetic NP solution. Then TEOS (200 μL) was added and mixture was sonicated in ice bath for 10 minutes.

NH₄OH (2 mL, 0.88 M) was then added dropwise and the reaction mixture was sonicated in ice bath for 2 hours. The product was isolated using magnetic separation and washed several times with EtOH, and stored in a minimum volume of EtOH.

2.2.4.8 Investigation of the Etching of Fe₃O₄@ZnS:Mn@SiO₂ Nanorattles

1 mg of the Fe₃O₄@ZnS:Mn@SiO₂ product was taken, and stored with regular redispersion in 7 mL of MP water. After 2 weeks, the magnetic product was extracted by magnetic separation and UV-Vis and PL measurements were performed on the supernatant and the precipitate.

2.2.4.9 Investigation of Release of ZnS:Mn QDs from Fe₃O₄@SiO₂ Nanorattles

After 2 weeks of storage in MP water, 3 mL of the prepared above quantum dot loaded nanorattle sample was taken and sonicated for 90 minutes in a sample tube. The sample was then placed on a magnet to isolate the NPs and UV-Vis and photoluminescence spectra were collected for the supernatant and precipitate.

2.3 Characterisation Techniques

2.3.1 Transmission Electron Microscopy (TEM)

Max Knoll and Ernst Ruska in 1931 developed the first transmission electron microscope. TEM shares many similarities with a traditional light microscope, the main difference between them is that an electron beam is used to visualise the sample instead of a light source, which is the case of a traditional optical microscope. The use of high-energy electrons provides information on a specimen such as morphology (size, shape and distribution of the particles), composition, surface features, and crystallographic information.

A source of electrons, usually a thermionic gun fitted with a tungsten filament cathode, produces a flow of electrons, which are accelerated providing a stream of electrons towards the sample. Two condenser lenses focus the electrons into a thin beam.

The first lens determines the spot size and the second lens allows the spot to be pointed onto the sample. Upon illumination of the sample with the electron beam, only the electrons blocked by the sample pass through to a phosphor image screen. This generates light that allows the user to view the image.

Depending of the type of interaction of the electrons with the sample different kind of chemical or physical information can be obtained. Unscattered transmitted electrons are those, which pass through the sample without interaction. Elastic scattering electrons interact with the sample resulting in a deviation from their original path. Their interaction causes no loss of energy and can give rise to diffraction patterns. Inelastic interaction between the primary electrons and the sample lead to a loss of energy to the surroundings along with a slightly deviation and deflection in their path. The magnitude of the energy loss will depend on the element the electrons have interacted with. The energy lost can excite vibrations at the surface of the sample and the resulting energy loss peaks can be observed. This technique is called Electron Energy Loss Spectroscopy (EELS).

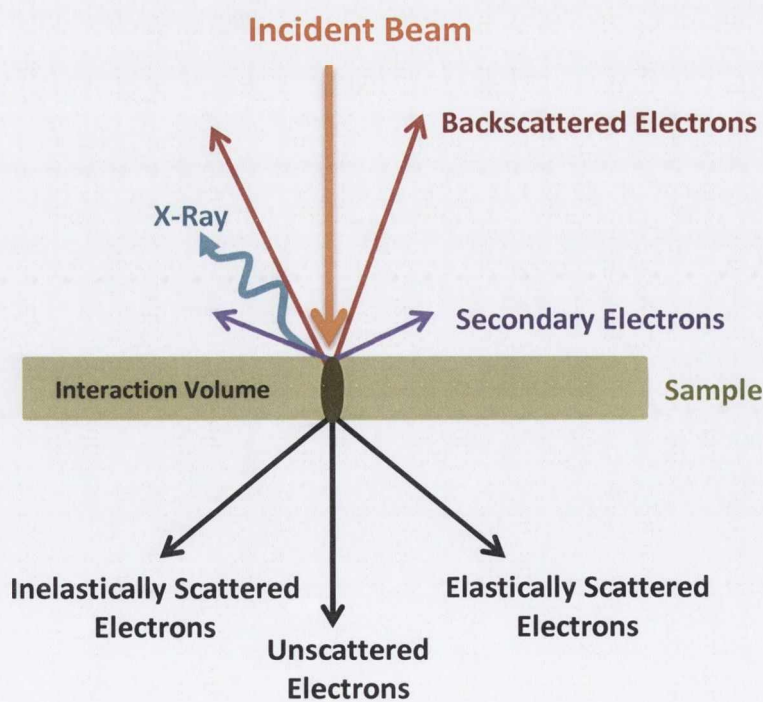


Figure 2.1: Electron interaction events in electron microscopy.

Scanning Transmission Electron Microscopy (STEM) differs from conventional TEM by focusing the electron beam into a narrow spot, which is scanned over the sample in a raster. STEM microscopes can be equipped with different detectors such as Bright Field (BF) or High Angle Annular Dark Field (HAADF) detectors. BF detectors collect the transmitted electrons and hence the holes in the sample appear bright. On the other hand, HAADF detectors collect inelastic scattered electrons and exclude transmitted electrons, making the holes in the sample appear dark. The HAADF detector tends to be very sensitive to variations in the atomic number of atoms in a sample, which lead to variations in image contrast.

In our work, transmission electron microscopy (TEM) images were taken on a FEI Titan Themis 200 series operating at 300 keV and with Jeol JEM-2100, 200kV LaB₆ instruments. Samples for TEM were prepared by deposition and drying of a drop (10 μ L) of the material dispersed in an appropriate solvent (water, ethanol, isopropanol) onto a carbon coated 400 mesh copper grid.

2.3.2 X-Ray Diffraction (XRD)

X-Ray diffraction can provide information on the molecular structure of a crystalline material. It involves the interaction of the electromagnetic radiation with a wavelength (λ) of approximately 0.1 nm with the atoms in the material. Crystals can diffract X-rays because the distances between the atoms are comparable with the wavelength of the radiation. A diffraction pattern results from the variation in the intensities caused by regions of constructive and destructive interference.^[6] Bragg demonstrated that scattering which forms the pattern can be visualised as the reflexion from Miller indices. Miller indices refer to the series of parallel lattice planes within a crystalline material. The X-ray pattern shows the diffraction intensity against the Bragg angle (θ). Each atom plane produces a series of n reflections, depending on the interference conditions. This relation is shown in Bragg's equation where λ is the wavelength of the x-ray and d is the spacing between planes in the crystalline phase.

$$n\lambda = 2d\sin\theta$$

From the pattern, the interlayer spaces (d_{hkl}) can be calculated using the Bragg equation. Each compound has a characteristic set of d values and intensities, which allows the identification of the material.

The half widths of the reflections are inversely proportional to the crystalline sizes and directly proportional to the concentration and magnitude of structural defects. The cell constants, crystal system and space group of the specimen may be determined from the powder diffraction pattern. For a cubic crystal system:

$$\frac{1}{d} = \frac{h^2 + k^2 + l^2}{a^2}$$

Where a is the cell constant.

In order to produce an XRD pattern a monochromatic beam of X-rays is required. These X-rays are produced by bombarding an anode of a pure metal with high-energy electrons in vacuum. Usually, copper metal is used for this purpose and the wavelength produced is 1.5406 Å. The resulting diffraction pattern can be identified by comparison of the peaks and their relative intensities with standard references provided by the Joint Committee on Powder Diffraction Standards (JCPDS) database.

In our work, XRD was performed using three different devices through the project. Samples were deposited as powder on the XRD slide with a thin layer of vacuum grease. The measurements were performed using a Siemens-500 X-Ray diffractometer, which uses Cu cathode $K_{\alpha 1}$ with a wavelength of 0.15406 nm under 12-hour scan time.

Bruker D2 Phaser was also used, this diffractometer also uses a Cu cathode with a wavelength of 1.5406 Å. Scans were run for 2 hours.

PANalytical X'Pert Powder diffractometer was used with help of Dr. Sarah McCharthy. The XRD patterns were measured over 3-6 hours for angles 10 – 155 (2θ). Sample

preparation involved the redispersion of the material in Isopropanol. The solution was then deposited on a glass slide and allowed to dry on air.

2.3.3 Vibrating Sample Magnetometer (VSM)

A Vibrating sample magnetometer (VSM) was invented by Simon Foner at Lincoln Laboratory MIT.^[7] VSM is one method of determining the magnetic properties of bulk and nanomaterials. The hysteresis loop gives information about the characteristics of any magnetic material. When a sample is exposed to an external magnetic field (H), a magnetic moment is induced in that sample. Mechanical vibration causes a magnetic flux, which induces a voltage in the sensing coils. This voltage is proportional to the magnetic moment of the sample.^[8] The type of magnetisation that a sample possesses is plot in the form of a characteristic hysteresis loop. The parameters of this hysteresis loop allow the material to be characterised in terms of its saturation magnetisation (M_s), its remanence (M_r) and coercivity (H_c). Superparamagnetic materials general at 0 T and no coercivity at 0 Am^2/kg .

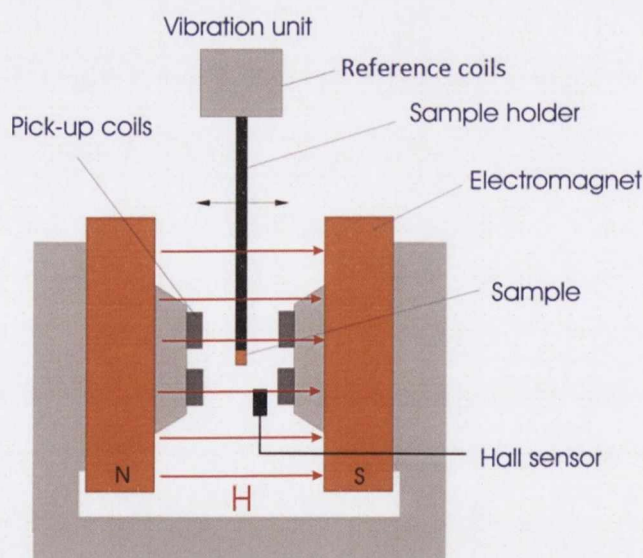


Figure 2.2: Diagram of a Vibrating sample magnetometer.^[9]

In our work, magnetization measurements were carried out using a homemade vibrating sample magnetometer (VSM) at room temperature with a field applied up to 1T.

The dried sample was weighed, wrapped in paper and placed in a hexagonal PVC holder. The sample was set within a uniform magnetic field and mechanically vibrated between a series of coils. A changing magnetic flux was generated which induced a voltage in the sensing coils. The amplitude of the sinusoidal voltage is proportional to the magnetic moment of the sample. The VSM was calibrated using a nickel sample of known mass. Nickel is a ferromagnetic material with a magnetic moment of 55.4 Am²/kg at 1T at room temperature.

2.3.4 Infrared Spectroscopy

Infrared spectroscopy is based on the interaction of the materials with electromagnetic radiation in the wavelength range approximately 1-1000 μm (10 to 10000 cm^{-1}).^[10] These interactions cause the excitation of the vibrational or rotational states of the molecules, which can be detected because of the absorption or transmittance of the infrared radiation. When the charges of the atoms in a molecule are asymmetrically distributed or the vibration of the atoms results in an asymmetry in the charge distribution, a vibrating or rotating atomic group can be associated with the motion of an electric charge. Electromagnetic radiation of a particular frequency can only be observed by a molecule if there is a change in the dipole moment associated with the vibrational excitation of that particular atomic group. Vibrations with no change in the dipole moment are infrared inactive.

A Perkin Elmer Spectrum One FT-IR spectrophotometer was used to record IR spectra (400-4000 cm^{-1}) by diffuse reflectance of samples in CsI.

FT-IR spectra were recorded using a Bruker Tensor II, Mid-Range Extended, with UATR attachment (200-4000 cm^{-1}). Few milligrams of sample were deposited on the hole and pressed to get the spectra.

2.3.5 Raman Spectroscopy

In 1928, Sir Chandrasekhara Venkata Raman discovered “a new type of secondary radiation” known today as the Raman effect. In 1930, Sir C.V. Raman was awarded with the Nobel Prize in Physics. The Raman spectroscopy technique is based on the interaction of electromagnetic radiation with a molecule. When electromagnetic radiation

interacts with molecules, it can promote three events: absorption, emission or scattering. When the energy of the photon involved is the same than the difference in energy between two energy levels of the molecule, absorption or emission take place. Scattering happens as a halfway effect between both ($\approx 10^{-14}$ s) of the interaction of the photon with the molecule, where the photon does not have the same energy than the difference between two energy levels of the molecule. There are different types of scattering processes: Rayleigh scattering, which causes very small frequency changes, and inelastic Raman scattering, which involves energy transfer between the incident photon and the molecules (Stokes or anti-Stokes shifts).^[11]

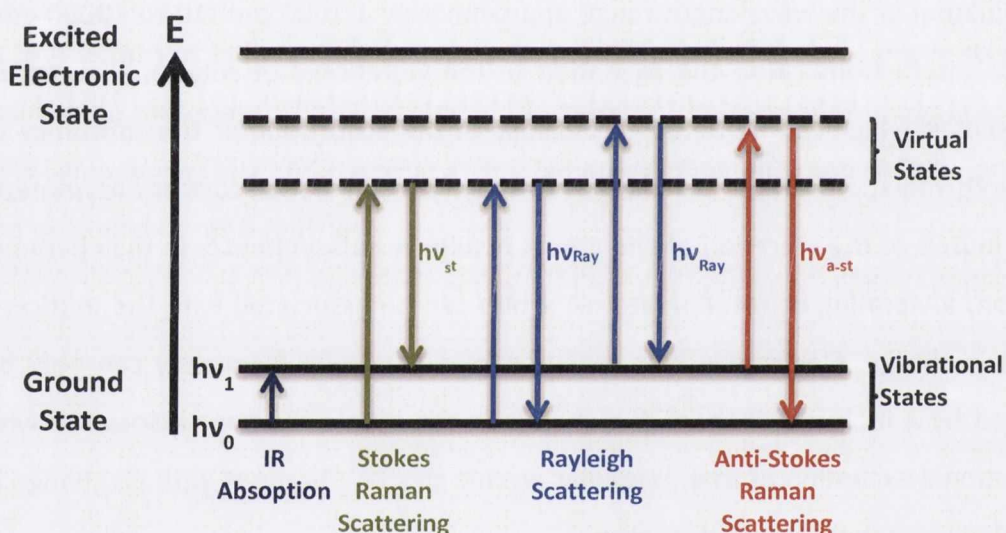


Figure 2.3: Energy level diagram showing the different states involved in Raman process.

The different type of scattering are described in Figure 2.3, where a photon with initial energy ($h\nu_0$) may interact with a molecule and proceed with decreased energy (Raman Stokes scattering, $h\nu_{st}$) or increased energy (Raman anti-Stokes scattering, $h\nu_{a-st}$). Alternatively, the energy can remain unchanged (Rayleigh scattering, $h\nu_{Ray}$). Rayleigh scattering results in lines of strong intensity in the Raman spectrum, often 10^3 - 10^4 times greater the Stokes and anti-Stokes Raman bands. The intensity of the anti-Stokes shift is lower than that of the Stokes shift, therefore, Stokes shift is generally viewed to be more important in Raman spectroscopy.

The difference in frequency between the exciting radiation and the scattered radiation is characteristic of the molecule under study and is independent of the frequency of the exciting radiation. The polarity of any molecule is characteristic of that molecule, as the electric field of an electromagnetic wave can shift the centre of a negative charge in the molecule, therefore inducing a dipole moment (μ_{ind}), which is proportional to electric field intensity, E .

$$\mu_{\text{ind}} = \alpha E$$

The degree of polarizability (α) of a molecule depends on the mobility of its electrons and is always a non-zero quantity. The polarizability is characteristic of the molecule, function of its shape and size, and may vary with the direction in space, which means that it is anisotropic. The Raman effect is the result of the interaction of photons with electrons, because of this, the change in the polarization of the bonds will have an effect on the Raman intensity and frequency of the band observed.

Raman spectroscopy combined with infrared spectroscopy give complementary information and help to elucidate the vibrational states of a molecule.^[6] A molecule is IR active when the light induces a change in the dipole moment. To be Raman active, the incident light must result in a change in the polarizability of the molecule. If in a molecule there is a centre of symmetry, a vibration active in infrared will be inactive in Raman and vice versa.^[12] If there is no centre of symmetry in a molecule, a number of vibrations may exist in both spectra. This is due to the fact that although infrared spectroscopy and Raman spectroscopy are a result of the same physical phenomenon, the interaction of the incident radiation with the sample is different.

Raman spectra were recorded with help of Dr. Joseph McCarthy and Dr. Joseph Govan (Chemistry) with a Renishaw 1000 micro-Raman system. The excitation wavelength was 514.5 nm from an Ar⁺ ion laser (Laser Physics Reliant 150 Select Multi-Line) with a typical laser power of 3 mW in order to avoid excessive heating. The 100 × magnifying objective of the Leica microscope was capable of focusing the beam into a spot of approximately 1 μm diameter.

2.3.6 Ultraviolet-visible Spectroscopy (UV-Vis)

UV-visible spectrum is the result of the absorption of a beam of incident photons by a sample. The region of the electromagnetic spectrum generally measured for this technique is from 200-900nm (covering both the UV and the visible range). The Beer-Lambert law demonstrates that the absorption of light is directly proportional to the path length of the cell used and the concentration of the sample under investigation.

$$A = \epsilon c l$$

Where ϵ is the extinction coefficient of the substance ($M^{-1} \text{ cm}^{-1}$). Bands in UV-vis spectra correspond to electronic transitions, as absorption of the incident light promotes excitation of the valence electrons in a molecule. The frequency (and the intensity) of the absorbed photon is directly proportional to the energy difference between the ground and excited levels. The wavelength of absorption of radiation is characteristic of the material under investigation.

Uv-vis absorption spectra were recorded using a Varian Cary 60 Uv-Visible spectrophotometer. Samples were measured in a 1 cm length quartz cuvette.

2.3.7 Circular Dichroism (CD) Spectroscopy

Circular dichroism (CD) is the difference in the absorption of left-handed circularly polarised light (L-CPL) and right-handed circularly polarised (R-CPL) light and occurs when a molecule contains one or more chiral chromophores (light-absorbing groups).

If a molecule contains chiral chromophores, one circular polarised light state will be absorbed more than the other and the CD signal will have a positive or negative signal. A CD signal can be positive or negative, depending on whether L-CPL absorbs more than R-CPL or less, which will give a negative signal.

Circular Dichroism spectra were recorded using a JASCO J-810 spectropolarimeter. The scans were conducted in the range of 200-800 nm, with 2-3 accumulations and a scan rate of 100-200 nm min⁻¹. Samples were measured using a 1 cm quartz cuvette.

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Chapter 3. Preparation of Core-Shell CoFe₂O₄ Magnetic Nanostructures for Catalytic Applications

3.1 Introduction

In the last few decades, different applications of magnetic nanoparticles have been studied due to their easy manipulation using an external magnetic field. Among many of these applications, one of the most important is the use of magnetic nanoparticles to support a catalyst material. The magnetic properties of these materials allow their easy recovery and possible recyclability.

The field of heterogeneous catalysis using magnetic nanoparticles has grown considerably recently. In particular, TiO₂ and SiO₂@TiO₂ immobilized on magnetic nanoparticles have been extensively studied.

In the case of the single TiO₂ coating, different magnetic cores have been used as a catalyst support. Among them, Fe₃O₄ is one of the most widely used due to its well establish magnetic advantages, such as large surface area for the adsorption and immobilization of metal and ligands and is easy to synthesized.^[1-3] However, other magnetic compounds have also been studied as a magnetic support in this type of nanostructures. For example, Lee et al. used barium ferrite (BaFe₂O₄) as core and the coating of TiO₂ was prepared by the controlled hydrolysis and condensation of titanium bisammonium lactate dihydroxide (TALH) in the presence of polyethyleneimine (PEI). Further calcination of the material would lead to the formation of anatase TiO₂, the catalytically active phase of TiO₂.^[4] NiFe₂O₄ has also been used as core for the formation of a photocatalyst based on TiO₂. The photocatalytic activity of this material was tested on the oxidation of methyl p-tolyl sulphide.^[5]

Silica coating is added in an attempt to avoid a decrease in the photocatalytic activity of TiO₂ directly supported on magnetic nanoparticles due to an increase in the hole-electron recombination.^[6] Alvarez *et al.* confirmed a better photocatalytic activity of TiO₂@SiO₂@Fe₃O₄ than TiO₂@Fe₃O₄ nanocomposites. After following the same procedure to coat both nanostructure with TiO₂, catalytic studies for the photodegradation

of acetaminophen and other PPcPs (pharmaceutical and personal care products) showed that the material with a protective silica shell was slightly more efficient than the same material without a silica shell.^[7] There are many examples in the literature in which Fe₃O₄ has been used as core for a double SiO₂@TiO₂ coating and the photocatalytic properties of resultant materials have been tested in the degradation of different organic compound or water contaminant such as methylene blue^[8-9] and as catalyst during the synthesis of organic compounds.^[10]

The combination of TiO₂ with noble metals is often applied because the incorporation of these metals can act as electron traps promoting the electron-hole separation enhancing the light absorbance of the catalyst in the visible range and improve the photocatalytic activity of TiO₂ under UV-Vis light.

The incorporation of Ag into a TiO₂ matrix has been investigated using magnetic nanoparticles as a catalyst support. Zhang *et al.* developed the novel three-component Fe₃O₄@TiO₂@Ag composite microspheres by a chemical deposition route. Ag nanoparticles were anchored onto the outer layer of Fe₃O₄@TiO₂ by the tyrosine-reduced method.^[11] Zhao *et al.* also used Fe₃O₄ as a core to synthesised sea urchin-like Fe₃O₄@TiO₂@Ag composites. The catalytic activity and recyclability of the material was determined by the catalytic reduction of ampicillin under UV and visible light.^[12]

Au doped TiO₂ is also an interesting approach to enhance the photocatalytic activity of TiO₂ although only few works have used magnetic nanoparticles in their investigations. Multifunctional microspheres composed of a Fe₃O₄ core, a TiO₂ interlayer and an Au shell with different size ranges and densities of an outer Au shell were prepared by Zhang *et al.* The photocatalytic activity of the material was evaluated by monitoring the degradation rate of Rhodamine 6G under UV lamp. Size and density of the Au shell was evaluated and shown to affect the activity of the material.^[13] Deposition of Au metal nanoparticles was also achieved by Li *et al.* following a facile approach to synthesize Au-decorated Fe₃O₄@mTiO₂ core-shell microspheres. Fe₃O₄@mTiO₂@Au showed a high photocatalytic activity in MB degradation, bactericidal tests and recyclability.^[14]

3.2 Aims of This Chapter

The aim of this part of the work is to prepare and characterize new heterogeneous catalysts using magnetic nanoparticles as support and to facilitate the removal and recovery of the catalyst from the reaction using a magnetic field. The magnetic core used in this work was CoFe₂O₄ (cobalt ferrite). To produce the different catalytic nanocomposites, different approaches such as co-precipitation and sol-gel techniques have been chosen. The photocatalytic activity of the new nanocomposites is to be tested in the degradation of Methylene blue (MB). The new materials are to be characterized by VSM and TEM analysis, XRD patterns, UV-Vis spectroscopy and Raman spectroscopy. This work is expected to contribute to the research on nanoparticle based heterogeneous catalysis.

3.3 Synthesis of CoFe₂O₄@SiO₂@TiO₂ Nanocomposites

3.3.1 Synthesis and Characterisation of CoFe₂O₄ Nanoparticles

Initial CoFe₂O₄ nanoparticles were synthesised using a co-precipitation method. The method is based on the chemical reactions of metal salts mixed in a stoichiometric ratio and carried out in basic aqueous medium, allowing the nucleation and growth of the resulting nanoparticles.^[15] In this particular case, the synthetic procedure involved the precipitation of cobalt (II) nitrate hexahydrate (Co(NO₃)₂·6H₂O) and iron (II) chloride tetrahydrate (FeCl₂·4H₂O) in the presence of ammonium hydroxide at pH between 8 and 14.

The resultant black powder was characterized using Transmission Electron Microscopy (TEM), magnetisation measurements (VSM) and X-ray powder diffraction. The TEM analysis showed CoFe₂O₄ nanoparticles with a size range between 20 and 100 nm (Figure 3.1).

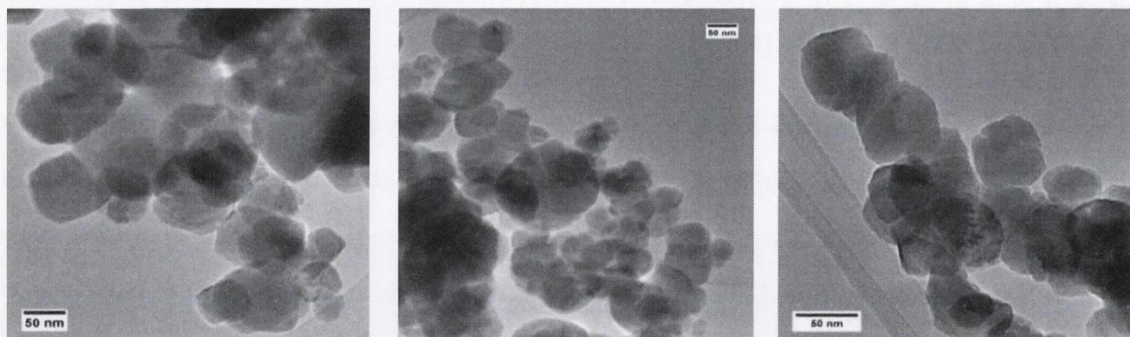


Figure 3.1: TEM images of uncoated CoFe_2O_4 nanoparticles showing their shape, size and morphology.

Vibrating Sample Magnetometer (VSM) was used to determine the magnetic moment of the CoFe_2O_4 nanoparticles. The saturation magnetization of the sample was found to be $86 \text{ Am}^2\text{kg}^{-1}$ (Figure 3.2), higher than that found in the bulk for CoFe_2O_4 at 300K ($80.8 \text{ Am}^2\text{kg}^{-1}$).^[16] The remanent magnetization was found to be $41 \text{ Am}^2\text{kg}^{-1}$.

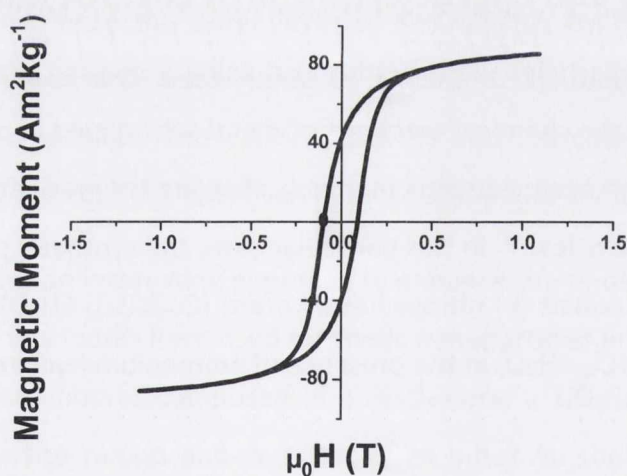


Figure 3.2: VSM Curve of uncoated CoFe_2O_4 nanoparticles.

The X-ray diffraction (XRD) pattern shows below further confirmation of the formation of a CoFe_2O_4 phase (Figure 3.3). The peaks in the diffractogram are confirming the formation a cubic spinel structure in the $\text{Fd}3\text{m}$ spatial group.^[17]

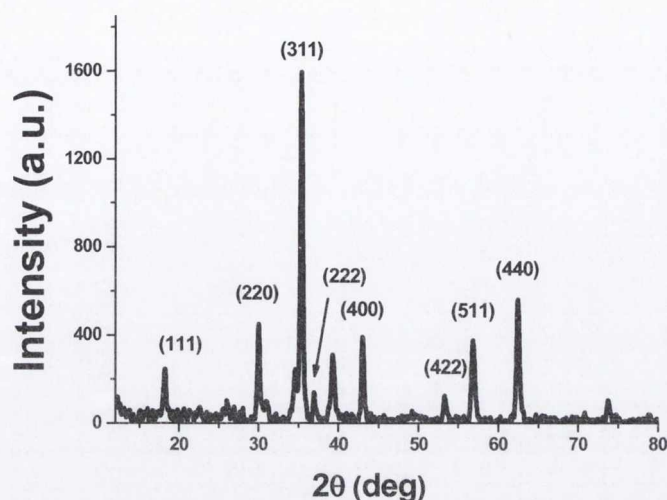


Figure 3.3: XRD of uncoated CoFe₂O₄ nanoparticles.

The cell parameter “a”, which is the lattice constant and it refers to the physical dimension of the unit cell in a crystal lattice, was found to be 8.430 Å which is close to that of bulk cobalt ferrite ($a = 8.395$ Å).^[18]

3.3.2 Synthesis and Characterisation of CoFe₂O₄@SiO₂ Nanoparticles

The silica coating of the cobalt ferrite nanoparticles was produced following the Stöber method as shown in the scheme below. Tetraethylorthosilicate (TEOS) is used as the silica precursor with ammonia solution as the catalyst of the reaction.^[19]

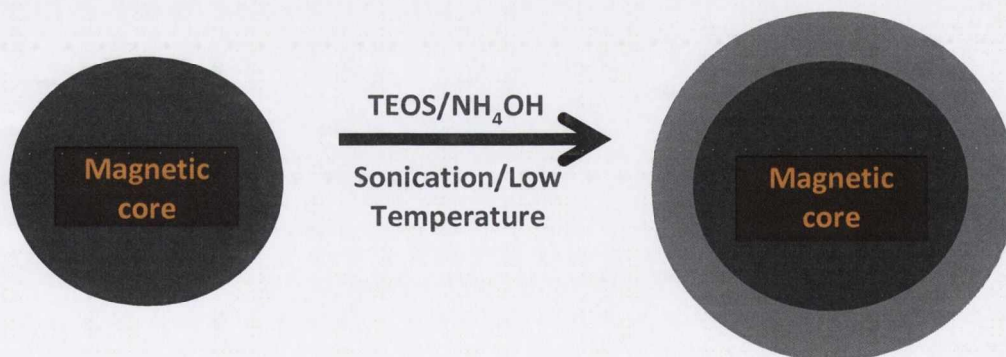


Figure 3.4: Scheme for the generation of CoFe₂O₄@SiO₂ core/shell.

The Stöber method is a well-known procedure to form monodisperse spherical silica nanoparticles based on the hydrolysis and condensation of the silica precursor in alcohol with ammonia acting as catalyst. The preparation of silica coated magnetic nano-

particles follows the same mechanism in which the silica precursor links to the OH groups present on the surface of the magnetic nanoparticles.

According to magnetization studies, the magnetic moment of the sample is reduced to 16 Am²kg⁻¹ compared to 86 Am²kg⁻¹ for the uncoated particles. This drop in magnetic moment was due to the presence of the non-magnetic SiO₂ coating. This significant decrease suggests that a relatively thick coating was formed. The hypothesis was confirmed by TEM analysis (Figure 3.5).

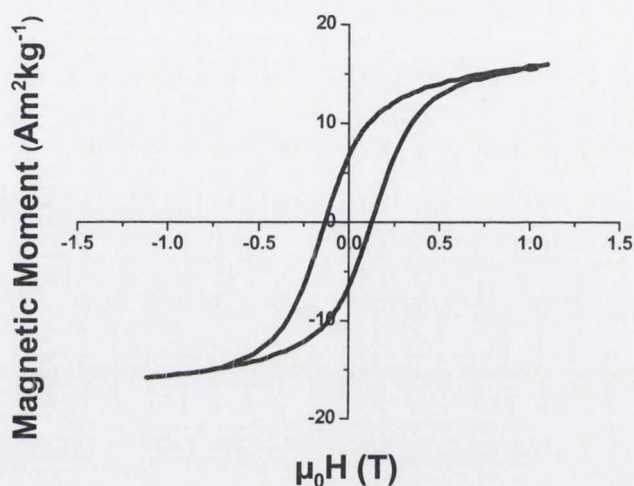


Figure 3.5: VSM curve of uncoated CoFe₂O₄@SiO₂ nanoparticles.

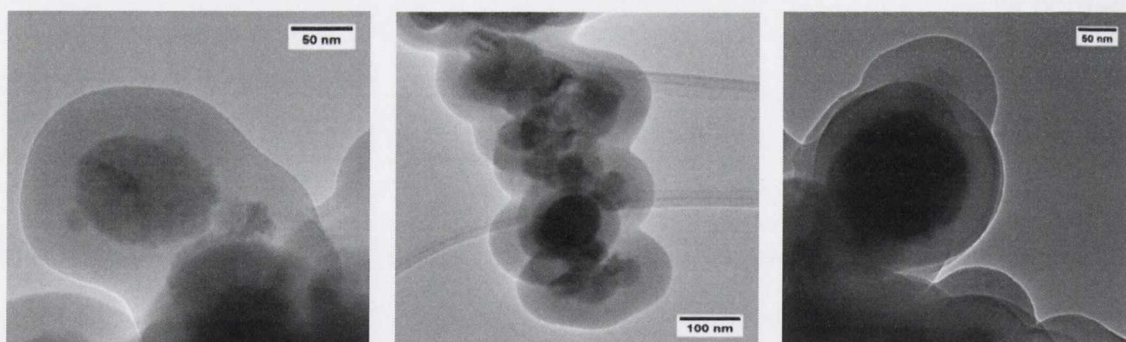


Figure 3.6: TEM images of CoFe₂O₄@SiO₂ coated nanoparticles.

The TEM images show the particles forming aggregates. Additionally a silica coating can be observed with a thickness of around 40 nm. The magnetic core size varies between 40 and 180 nm. The overall particle size ranges between 100 and 200 nm. Although these images do not show an ideal structure of a single core contained within

the silica shell due to the aggregation of the magnetic nanoparticles, they do, however confirm the formation of core-shell silica/cobalt ferrite nanoparticles.

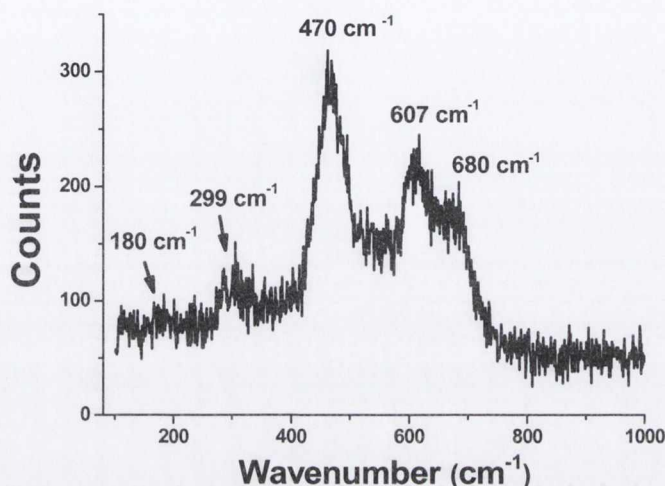


Figure 3.7: Raman spectra of CoFe₂O₄@SiO₂ nanoparticles.

In the Raman spectrum of CoFe₂O₄@SiO₂ NPs (Figure 3.7) there are peaks at 470 cm⁻¹ and 680 cm⁻¹ which indicate the presence of cobalt ferrite. CoFe₂O₄ has an inverse spinel structure with $O_h^7(Fd3m)$ space group. This structure has five active Raman modes ($3T_{2g} + E_g + A_{1g}$) that appear in the spectra. The modes at 607 cm⁻¹ and 680 cm⁻¹ are assigned to the tetrahedral sites; other peaks at 180 cm⁻¹, 299 cm⁻¹ and the 460 cm⁻¹ are distinctive of the octahedral site.^[20]

The broad peak centred around 460 cm⁻¹ might contain the symmetric SiO₂ bending vibration expected at 440 cm⁻¹, however this is unclear due to the large masking caused by the cobalt ferrite peaks. Other weak silica peaks can be observed at 550 cm⁻¹ and 970 cm⁻¹ but these peaks are relatively weak and are difficult to distinguish in the spectra obtained.^[21]

The XRD pattern for SiO₂ coated CoFe₂O₄ NPs is shown in Figure 3.8. As discussed previously, the identified peaks are in agreement with those of CoFe₂O₄ described in the literature. The broad peak at $2\theta = 30^\circ$ indicates the presence of the amorphous SiO₂. When compared to the JCPDS database entry for SiO₂ and the results obtained by Hu *et al.*^[22] the peak at $2\theta = 31^\circ$ aligns with SiO₂ along with the peak at $2\theta \approx 35^\circ$. Both

peaks are highly masked by the underlying CoFe₂O₄ peaks in the same area of the diffractogram.

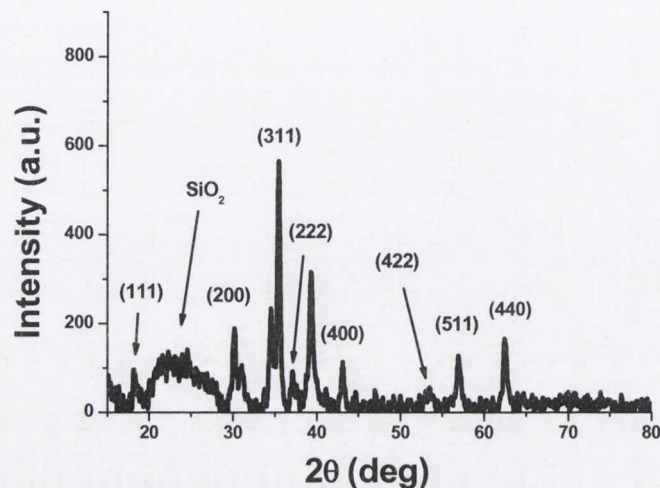


Figure 3.8: XRD pattern of CoFe₂O₄@SiO₂ nanoparticles.

Figure 3.9 shows the XRD patterns of the CoFe₂O₄@SiO₂ nanoparticles and of the same material sintered at 600 °C. This experiment was carried out to show that silica coated nanoparticles were not affected by the heat treatment.

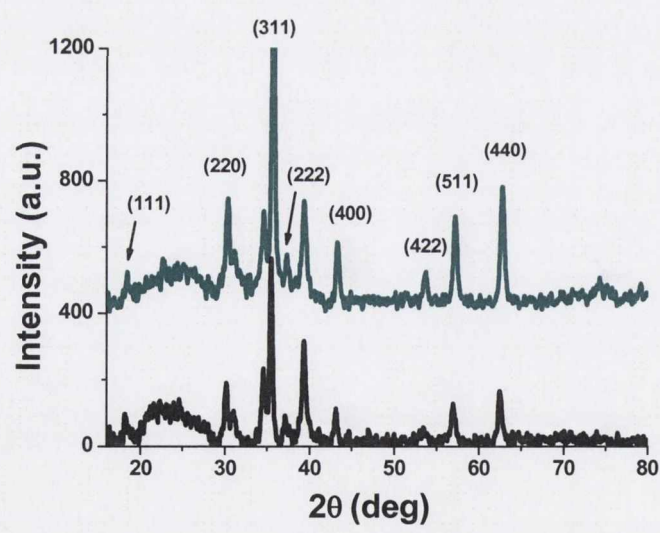


Figure 3.9: XRD pattern of CoFe₂O₄@SiO₂ coated nanoparticles before (black line) and after sintering (green line) at 600 °C.

3.3.3 Synthesis and Characterisation of $\text{CoFe}_2\text{O}_4@\text{TiO}_2$ Nanoparticles

CoFe_2O_4 nanoparticles were synthesized using the previously described co-precipitation method. The preparation of the TiO_2 shell was prepared by modifying the methods of Wuyou et al.^[23] Firstly, the coated nanoparticles were synthesized by sol-gel hydrolysis precipitation of titanium isopropoxide and treated at high temperature to obtain the anatase phase of TiO_2 . A defined amount of CoFe_2O_4 NPs were dispersed in a mixture of water/ethanol (1:10) and the pH adjusted to 5-6 using 3.0 M HCl.

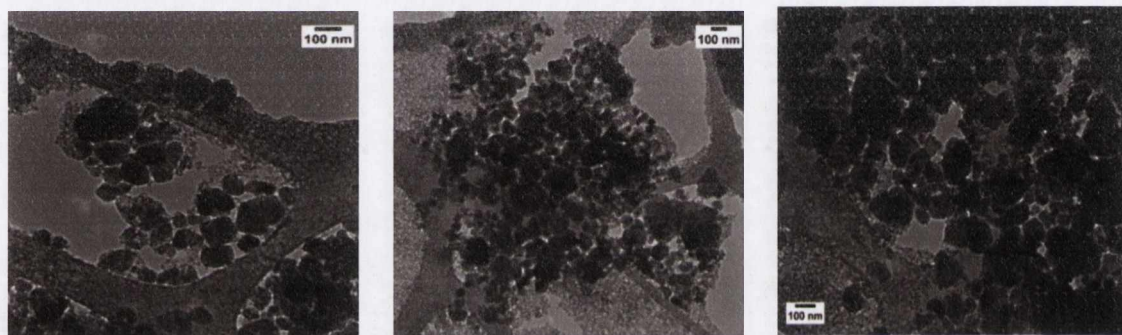


Figure 3.10: TEM images of $\text{CoFe}_2\text{O}_4@\text{TiO}_2$ nanoparticles.

The above figure displays selected TEM images of the sample. The images showed the presence of two different materials in the sample that are not forming a proper core-shell structure. The XRD pattern (Figure 3.11) for the TiO_2 coated nanoparticles display a peak around 25 degrees, which confirm the presence of the TiO_2 in its anatase phase. However, the pattern is dominated by CoFe_2O_4 peaks.^[24]

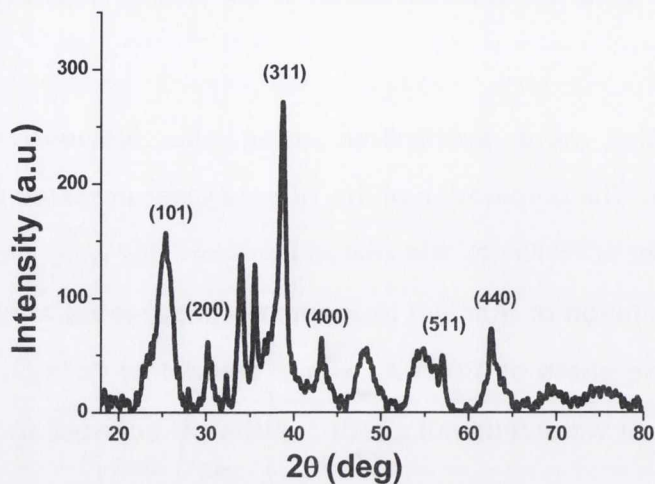


Figure 3.11: XRD pattern of CoFe₂O₄@TiO₂ coated nanoparticles.

VSM shows a drop in the magnetization moment between the uncoated and the coated nanoparticles from 86 to 30 Am²kg⁻¹. This suggests the presence of a non-magnetic coating on the nanoparticles (Figure 3.12).

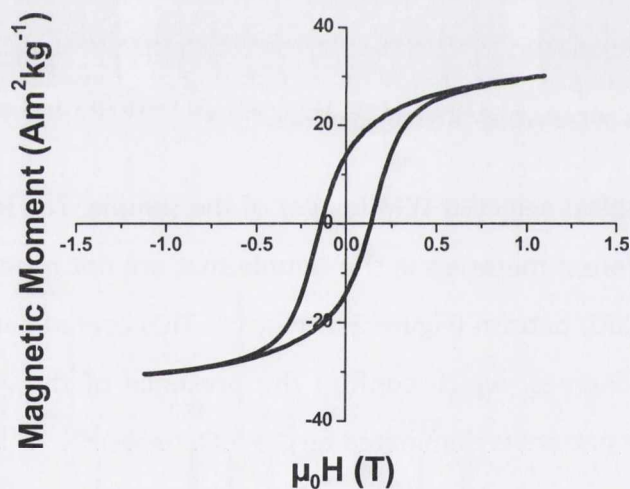


Figure 3.12: VSM curve of CoFe₂O₄@TiO₂ nanoparticles.

Photocatalytic testing was carried out for this sample but very poor or no photocatalytic activity was shown for this material. The direct deposition of TiO₂ on the surface of CoFe₂O₄ NPs can cause this decrease in the activity due to the interaction between the magnetic core and TiO₂ leading to the transfer of charge across the point of contact between materials.^[25] The recombination of electrons between both materials

would lower the oxidising or reducing power of the available electrons and holes and would result in a lower photo activity.^[26]

A silica shell was used in order to avoid this transference of electrons between the metal oxide and the semiconductor.

3.3.4 Synthesis and Characterisation of CoFe₂O₄@SiO₂@TiO₂ Nanoparticles Double Coated Core-Shell Nanostructures

CoFe₂O₄@SiO₂@TiO₂ nanoparticles have been prepared by hydrolysis of the titanium tetrabutoxide precursor in the presence of the silica coated cobalt ferrite nanoparticles following the scheme below (Figure 3.13).

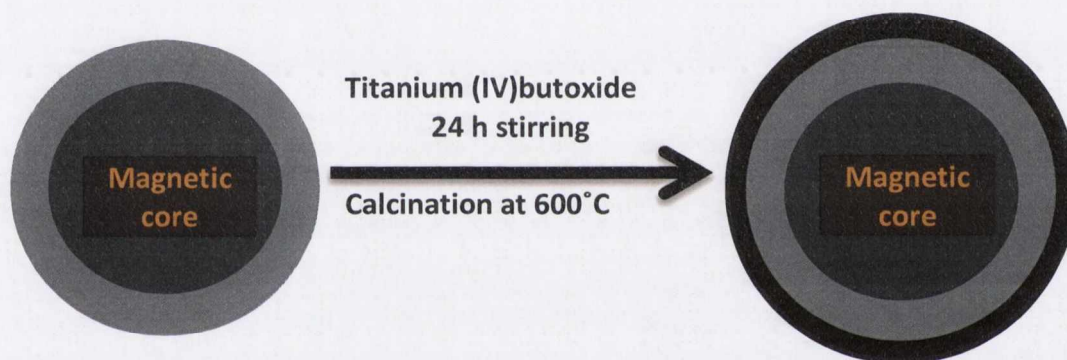


Figure 3.13: Reaction scheme for the coating of CoFe₂O₄@SiO₂ nanoparticles with a TiO₂ coating.

After applying the silica/titania double coating, the magnetic moment of the sample dropped to 12 Am²kg⁻¹ (Figure 3.14) from 16 Am²kg⁻¹ measured for the silica coated and 86 Am²kg⁻¹ for the uncoated CoFe₂O₄ particles (Figure 3.15). This reduction in magnetic moment suggests that the thickness of the non-magnetic coating has increased. This is due to the presence of the TiO₂ shell, which is non-magnetic, resulting in a lower overall magnetic moment. As the drop in magnetic moment from the silica coated to the titania/silica coated particles is not significant, it indicates that the TiO₂ coating is thin in comparison to the SiO₂ coating. This finding was also confirmed by the TEM analysis (Figure 3.16).

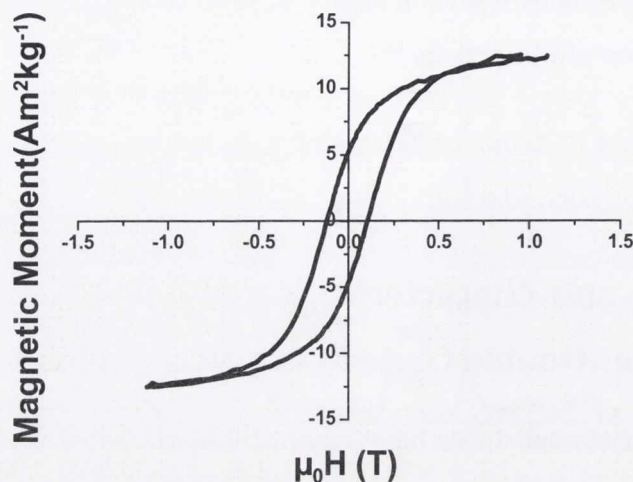


Figure 3.14: VSM curve of CoFe₂O₄@SiO₂@TiO₂ coated nanoparticles.

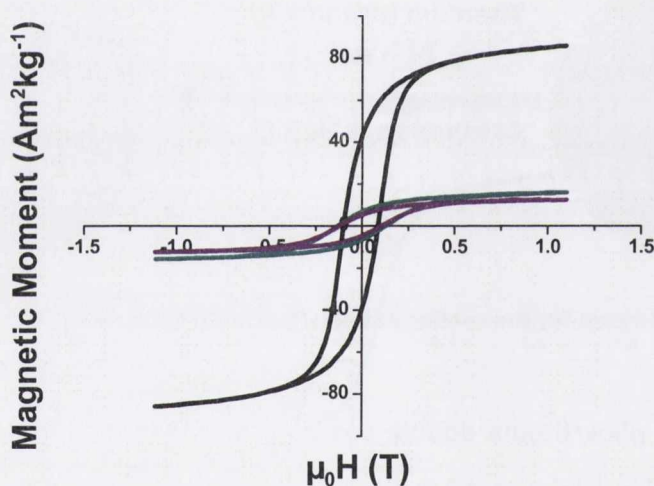


Figure 3.15: Comparison of VSM curves of CoFe₂O₄ (black line) CoFe₂O₄@SiO₂ (green line) and CoFe₂O₄@SiO₂@TiO₂ nanoparticles (purple line).

The TEM images of the double-coated CoFe₂O₄ sample are presented Figure 3.16. The TEM images clearly showed the presence of two types of coating on the CoFe₂O₄ nanoparticles. The dominating coating around the core is the SiO₂ shell of approximately 50 nm thickness. The second coating being TiO₂ covers the silica shell. However, the TiO₂ does not form a uniform solid coating on the SiO₂. Isolated TiO₂ fragments covering the nanoparticle can also be observed in the TEM images. The underlying reason

for this phenomenon is most likely the lattice mismatch between the crystalline SiO_2 and TiO_2 phases.

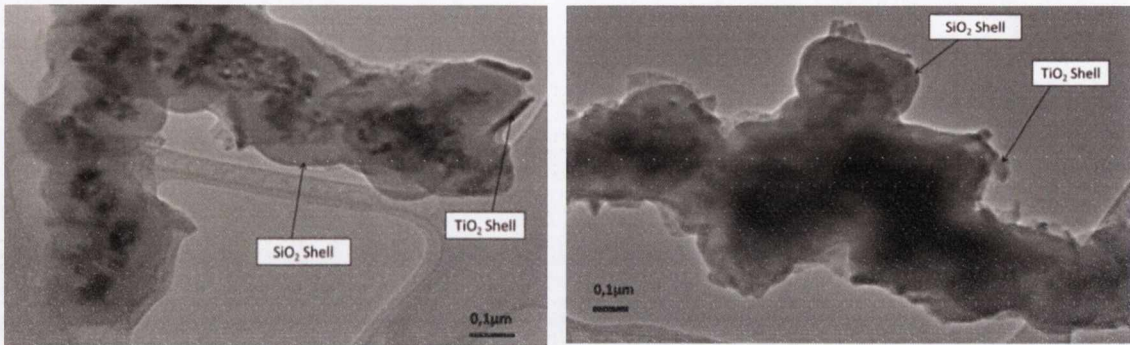


Figure 3.16: TEM images of $\text{CoFe}_2\text{O}_4@ \text{SiO}_2@ \text{TiO}_2$ nanoparticles.

The Energy Dispersive X-ray (EDX) analysis of the $\text{CoFe}_2\text{O}_4@ \text{SiO}_2@ \text{TiO}_2$ sample confirms the presence of elements corresponding to the chemical composition and is further proving the formation of a double layer coating (Figure 3.17).

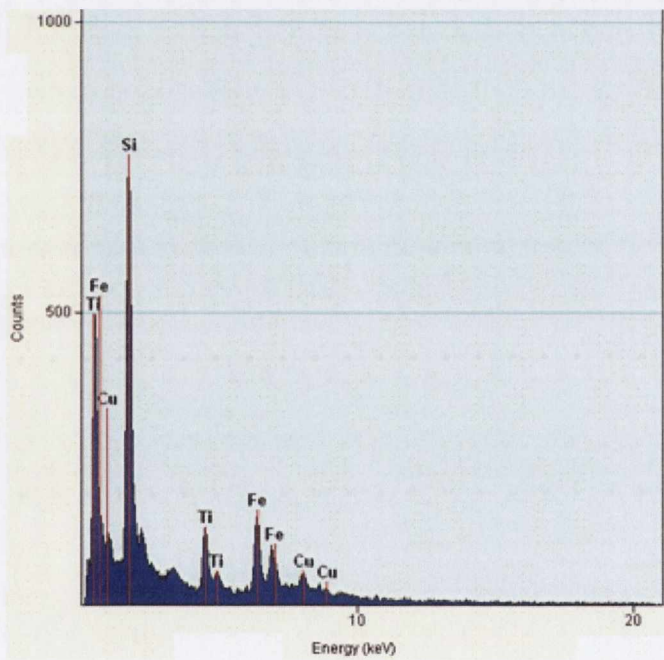


Figure 3.17: Energy Disperse X-Ray (EDX) analysis of $\text{CoFe}_2\text{O}_4@ \text{TiO}_2@ \text{SiO}_2$ nanoparticles.

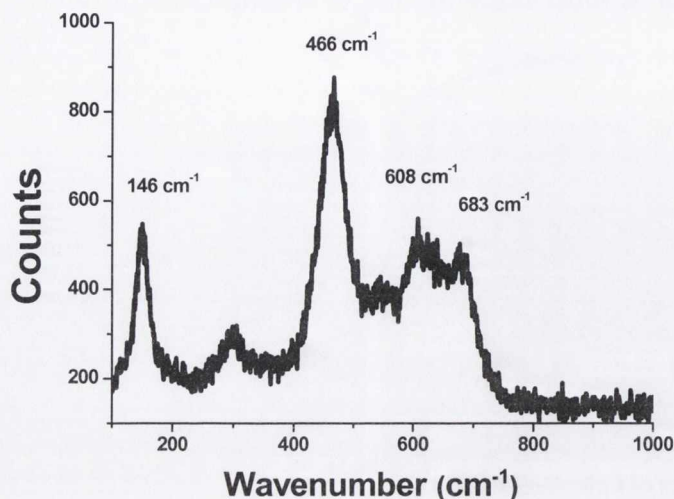


Figure 3.18: Raman spectra for the double-coated $\text{CoFe}_2\text{O}_4@\text{SiO}_2@\text{TiO}_2$ nanoparticles sintered at 600°C for 1 h.

Figure 3.18 presents the Raman spectrum of the double-coated $\text{CoFe}_2\text{O}_4@\text{SiO}_2@\text{TiO}_2$ nanoparticles sintered at 600°C for 1 h. The peak at 146 cm^{-1} is indicative of the presence of TiO_2 in the anatase phase, although the rutile phase also exhibits a peak around this area. Since it is a weak signal, this peak is most likely due to the anatase phase. However, further peaks at 450 cm^{-1} and 614 cm^{-1} would suggest TiO_2 in the rutile phase but these peaks could be masked by the presence of the characteristic peaks of CoFe_2O_4 NPs. However, the hypothetical presence of different phases of TiO_2 could be due most likely to the heat treatment of the nanoparticles. Some of the TiO_2 present may have converted from the amorphous phase to the anatase phase while some may have further converted to the rutile phase resulting in a mixture of phases on the NP surface. This transition originates from the anatase phase being the kinetic product of the heat treatment, whereas the rutile phase is the thermodynamic product. The sintering at 600°C may be too high of a temperature to ensure the sole formation of the anatase phase.^[27]

3.3.5 Catalytic Activity of Double-Coated CoFe₂O₄@SiO₂@TiO₂ Core-Shell Nanoparticles in Methylene Blue

The double-coated CoFe₂O₄@SiO₂@TiO₂ core-shell nanoparticles were tested as photocatalysts in the degradation of methylene blue (MB), which is used as a standard dye to test photocatalytic performance (Figure 3.19). The adsorption of MB onto the surface of TiO₂ is facilitated through the cationic charge present at the amine or sulphur group of the dye interacting with the negative charged TiO₂ coating. The photo-degradation of MB proceeds by the oxidation of the sulphur groups due to the production of photo holes and OH radicals.^[28]

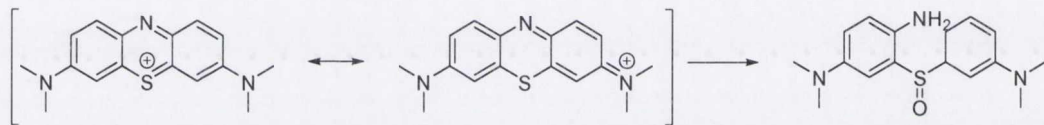


Figure 3.19: Reaction scheme for the photocatalytic oxidation of MB on the surface of CoFe₂O₄@SiO₂@TiO₂ NPs. ^[28]

The CoFe₂O₄@SiO₂@TiO₂ core-shell NPs were sintered at 600 °C and then used for photo-catalytic testing. As soon as the NPs were added to the MB solution, the absorbance intensity at 664 nm decreased. The decrease was more rapid than one in the control sample without the NPs. This describes a clear indication of photo-catalytic activity for these samples.

Figure 3.20 displays the decrease in absorbance of the MB dye over time in the presence of the sintered CoFe₂O₄@SiO₂@TiO₂ NPs under UV illumination (top left) versus a control with no NPs present (top right), a control in the presence of the mono-coated CoFe₂O₄@SiO₂ nanoparticles (bottom left) and a control using the CoFe₂O₄@SiO₂@TiO₂ particles without UV illumination (bottom right). As shown, the rate with which the absorbance decreases in the presence of the illuminated double-coated particles is much higher than those for the different control samples. This shows that the TiO₂ coating is responsible for catalysing the photo-oxidation of the dye molecules. The slight decrease in absorption that can be observed in the different control samples is most likely due to partial bleaching of dye molecules under the UV light.

Another explanation could be the adsorption of dye molecules to the surface of the nanoparticles, especially in the case of the control sample with no UV illumination.

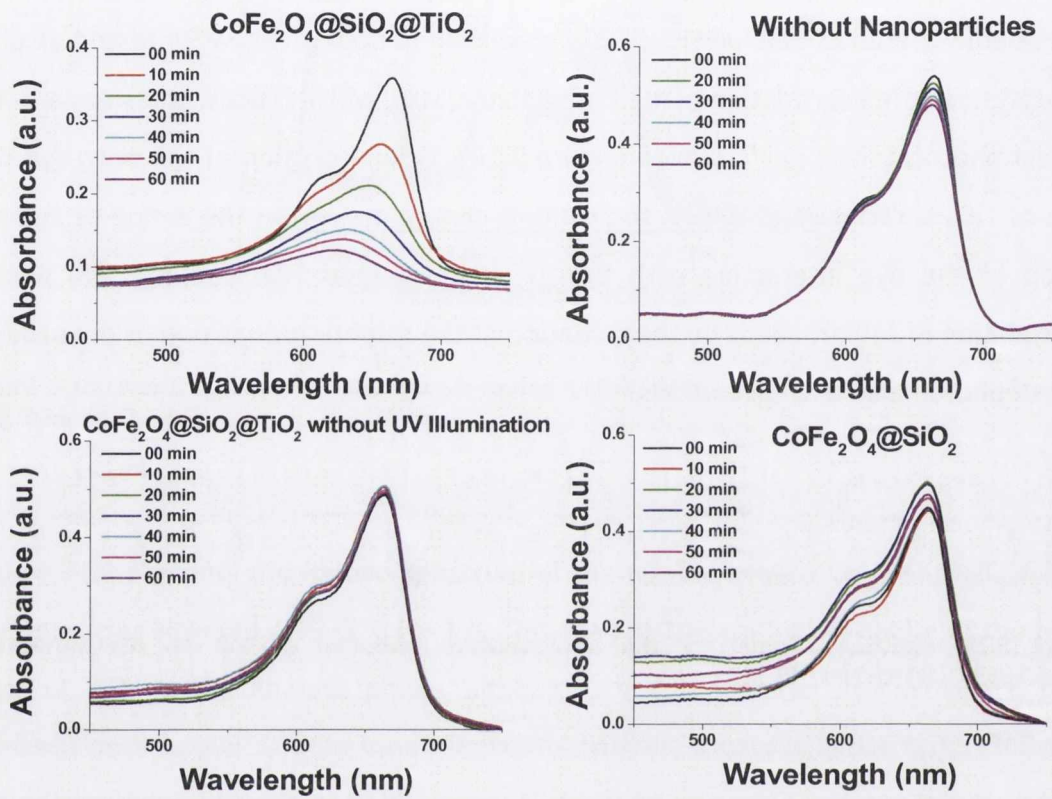


Figure 3.20: Evolution of UV-Vis absorbance spectra of methylene blue in the presence of active photocatalysts and control samples.

The blue shift of the absorption band can be attributed to the less intense colour of MB due to the degradation of part of the auxochromic groups (methylanine or methyl) attached to the chromophoric system.^[29-30]

The following Figure 3.21, on the left, shows the changes in the absorbance maximum of the dye are plotted against the reaction time for the CoFe₂O₄@SiO₂@TiO₂ nanoparticles sintered at 600 °C. When the core-shell nanoparticles are added as a catalyst a pseudo first order reaction can be observed, as visible from plotting of the Ln A_t versus time (Figure 3.21, right). This is in line with previous reports on TiO₂ nanoparticles and their photocatalytic activity.^[23, 31-33] For the control tests, a linear drop of the absorbance over time can be observed. This demonstrates that the bleaching of the dye un-

der UV light is a linear reaction and is independent of the concentration of MB. Additionally, it can be noticed that the $\text{CoFe}_2\text{O}_4@\text{SiO}_2@\text{TiO}_2$ nanoparticles of are completely recoverable from the reaction mixture. This can be done using a simple magnetic separation method based on the use of a permanent magnet as demonstrated in Figure 3.22.

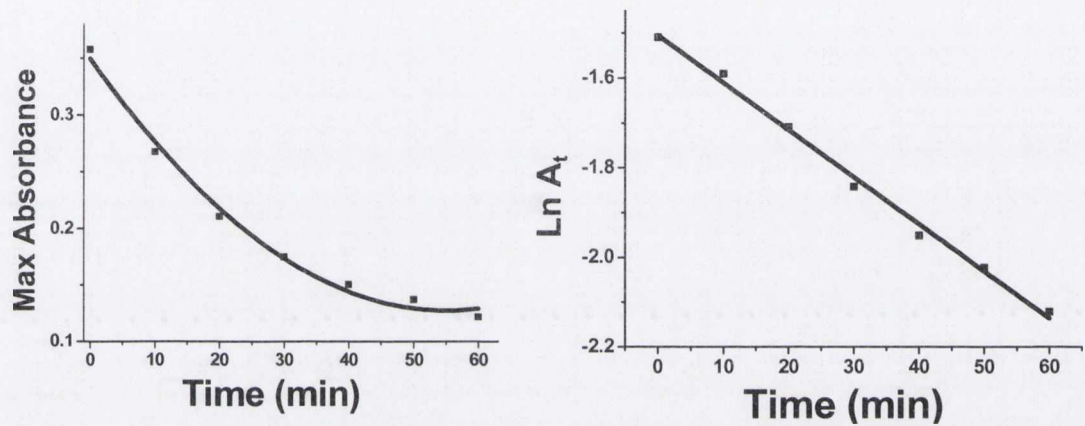


Figure 3.21: Graphs showing the change in main dye absorbance over time for photocatalytic activity (left) and the inverse of the maximum absorbance (right).

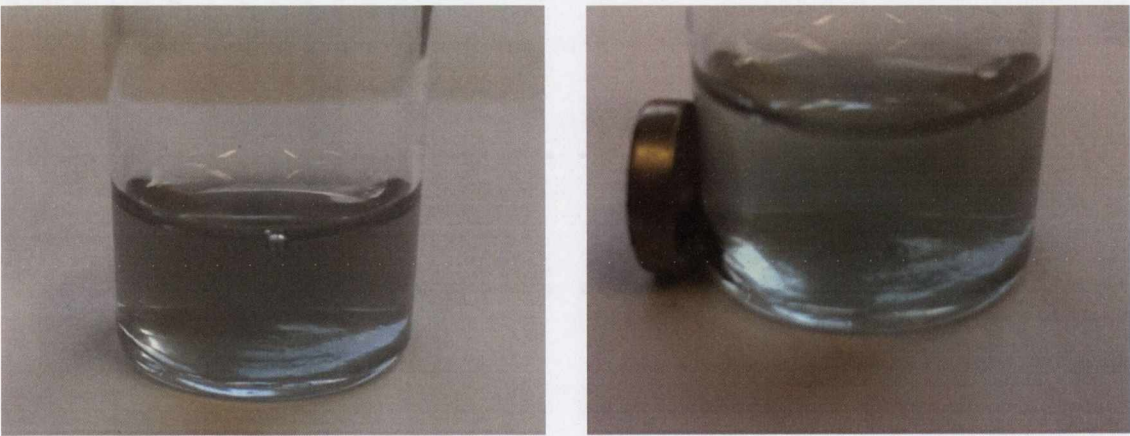


Figure 3.22: Photograph showing the removal of $\text{CoFe}_2\text{O}_4@\text{SiO}_2@\text{TiO}_2$ nanoparticles using a permanent magnet.

3.4 Synthesis and Characterization of CoFe₂O₄@CeO₂ Nanocomposites

3.4.1 Synthesis of CoFe₂O₄@CeO₂ Nanocomposites

CoFe₂O₄ nanoparticles were synthesized using the co-precipitation method mentioned in section 3.1.1. The CeO₂ coating was produced by hydrothermal precipitation of cerium (III) nitrate hexahydrate (Ce(NO₃)₃·6H₂O) with hexamethylenetetramine (HMT) in the presence of the magnetic nanoparticles. A defined amount of cerium salt and HMT were dissolved in 100 mL of water and added to a solution containing 50 mg of CoFe₂O₄ nanoparticles dispersed in 100 mL. The mixture was sonicated for 30 min and allowed to stir overnight. The final product was washed three times with water and dried under vacuum. The resultant grey powder was calcinated at 600 °C for 1 hour.

Figure 3.23 shows TEM images obtained for CoFe₂O₄@CeO₂ nanoparticles. A deposition of CeO₂ can be observed on the surface on the magnetic nanoparticles. Size distribution does not change considerably from that of the original non-coated CoFe₂O₄ nanoparticles that was calculated in the range between 20 and 100 nm. We can also see some raspberry-like structures where the large core CoFe₂O₄ particles are coated by very small (2-4 nm) CeO₂ nanoparticles.

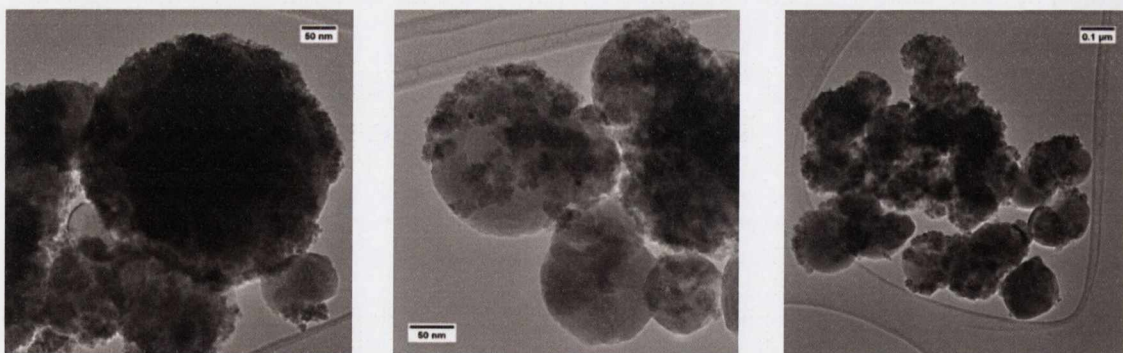


Figure 3.23: TEM images of CoFe₂O₄@CeO₂ nanocomposites.

VSM curves show the influence of the thickness of a non-magnetic deposition on the magnetic moment of the CeO₂ coated NPs. The saturation magnetization of naked nanoparticles was found to be 63 Am²kg⁻¹ and the value obtained after the CeO₂ deposition was very similar with 60 Am²kg⁻¹. The magnetic behaviour of the

nanoparticles does not suffer an significant drop which suggests that the thinkness of the non-magnetic material is not substantial.

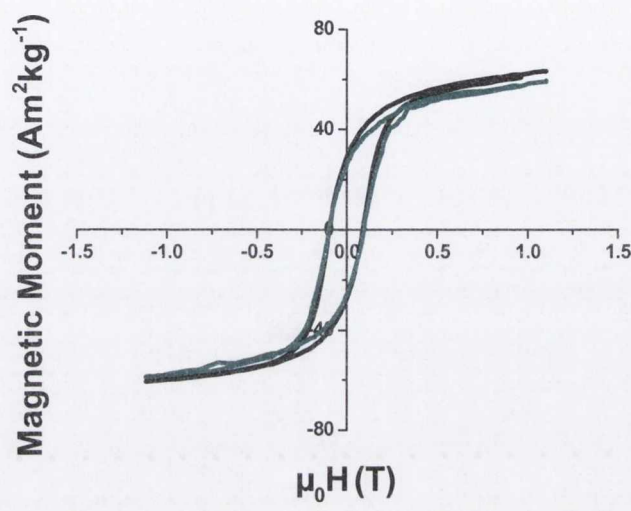


Figure 3.24: VSM curves of CoFe₂O₄ (black) and CoFe₂O₄@CeO₂ (green) nanoparticles.

XRD pattern of CoFe₂O₄@CeO₂ NPs (Figure 3.25), exhibits the characteristics peaks for the spinel structure of cobalt ferrite at (111), (220), (311), (222), (400), (422), (511) and (440). In addition, the formation of a face-centered cubic phase (FCC) from ceria was observed at (111), (200), (220), (311) and (222).^[34]

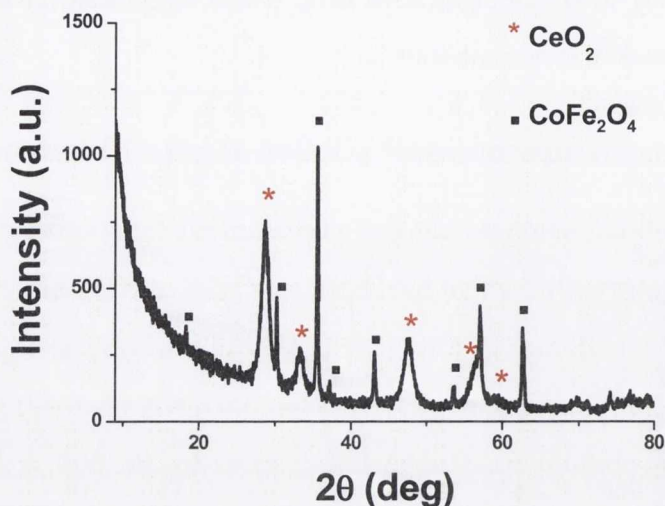


Figure 3.25: XRD pattern of $\text{CoFe}_2\text{O}_4@ \text{CeO}_2$ nanoparticles.

Figure 3.26 shows the Raman spectrum of $\text{CoFe}_2\text{O}_4@ \text{CeO}_2$ NPs. There are peaks at 469 cm^{-1} and 680 cm^{-1} that indicate the presence of cobalt ferrite. The broad peak centred around 469 cm^{-1} might contain the Raman active mode F2g of the cubic fluorite type lattice for CeO_2 . However this is unclear due to the large masking caused by the cobalt ferrite peaks.^[35]

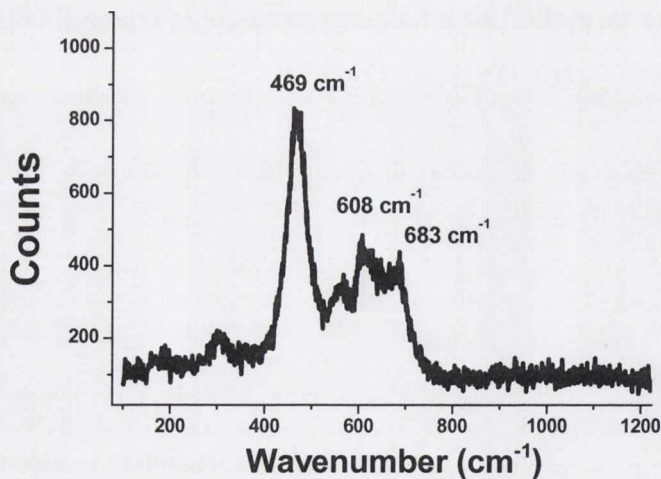


Figure 3.26: Raman spectra of $\text{CoFe}_2\text{O}_4@ \text{CeO}_2$ nanoparticles.

3.4.2 Catalytic Activity of CoFe₂O₄@CeO₂ Nanoparticles

CoFe₂O₄@CeO₂ nanoparticles as synthesized were used in the catalytic testing of the degradation of methylene blue. The catalytic activity test was carried out both under UV and under daylight radiation. The concentrations of MB were calculated from the UV-vis absorption studies using Beer-Lambert’s law at the maximum wavelength (λ_{max} = 664 nm).

Figure 3.27 presents the UV-Vis spectrum of the changes in MB concentrations under UV radiation over 1 hour in the presence of CoFe₂O₄@CeO₂ NPs. From the spectrum it can be seen that the main adsorption peak of the dye in UV-Vis region decreases confirming the degradation of the dye caused by the catalytic activity of the NPs.

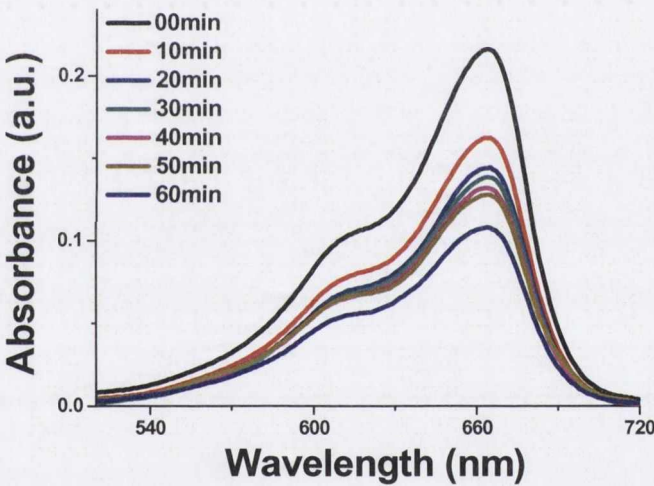


Figure 3.27: UV-Vis Spectrum to show the photo-catalytic activity of CoFe₂O₄@CeO₂ nanoparticles under UV radiation.

The changes in absorbance of the dye versus the reaction time for CoFe₂O₄@CeO₂ NPs are presented in Figure 3.28, left. When plotting the $\ln (A_t/A_0)$ versus the time it can be seen that the reaction follows pseudo first order kinetics, where $\ln (A_t/A_0) = k_t$, with k as the apparent rate constant (min^{-1}), A_t the absorbance of dye at time (t) and A_0 is the initial absorbance, (Figure 3.28, right).^[36] Using Beer-Lambert’s law, the concentration can be substituted by the absorbance of the dye at the peak absorption (664 nm).

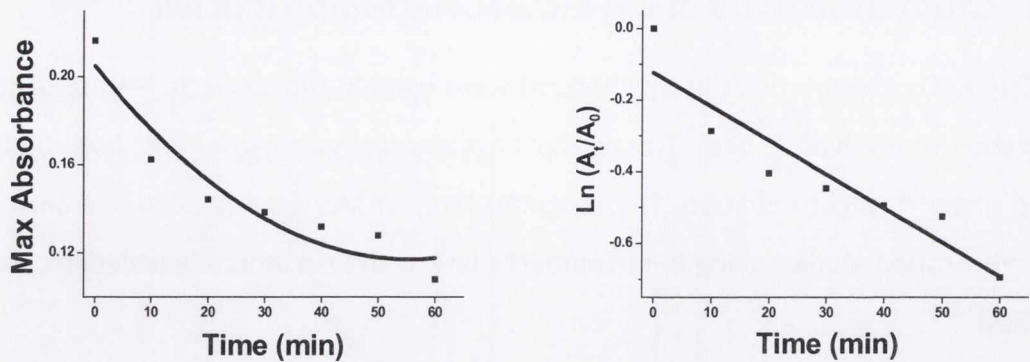


Figure 3.28: Graphs showing the change in absorbance of the dye over time to prove catalytic activity (left) and the natural log of the maximum absorbance (right) for CoFe₂O₄@CeO₂ nanoparticles under UV light.

The same procedure as previously described was followed to test the photocatalytic activity of the material under day light radiation. UV-Vis spectra were recorded every 10 min to follow the degradation of the dye in the presence of the CoFe₂O₄@CeO₂ nanoparticles whilst being expose to daylight. Figure 3.29 shows the drop in the maximum absorbance over time.

CeO₂ is an n-type semiconductor, with a larger number of electrons than holes, whose bandgap has been reported to be close to 2.76 eV, although this can vary depending on the method of preparation. Catalytic properties can be attributed to oxygen vacancies caused by the formation of Ce³⁺ ions defects sites in the grain boundaries. This phenomenon can lead to a decrease in the band gap.^[37]

However, despite the reasonable band gap, the catalytic activity for CeO₂ coated nanoparticles is low, which can be caused by a poor adsorption of the dye. Nevertheless, there is no need for an extra light source for catalytic activity of ceria coted nanoparticles, as was the case for the CoFe₂O₄@SiO₂@TiO₂ NPs.^[38]

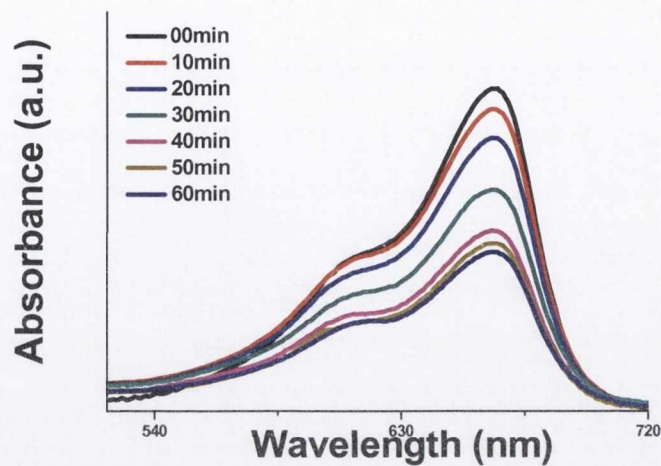


Figure 3.29: Evolution of UV-Vis spectra of dye in the presence of CoFe₂O₄@CeO₂ nanoparticles under daylight radiation.

Kinetics calculations for CoFe₂O₄@CeO₂ NPs show similar results to those already mentioned in previous experiment. The degradation of MB in the presence of CoFe₂O₄@CeO₂ nanoparticles follows a pseudo first order reaction (Figure 3.30).

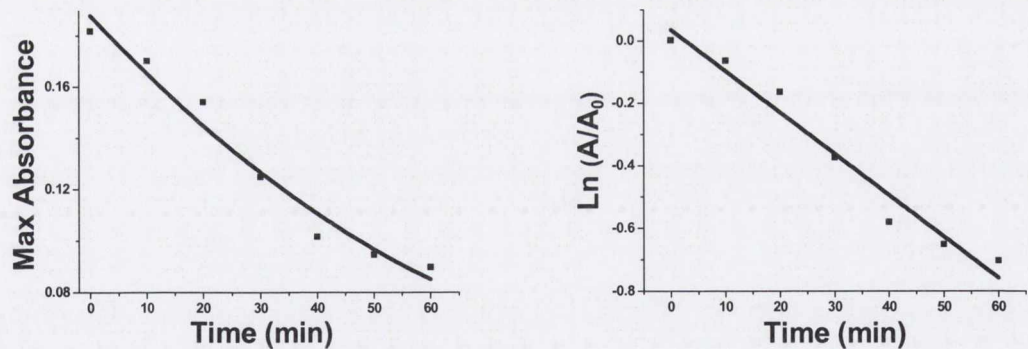


Figure 3.30: Graphs showing the change in main dye absorbance over time for photocatalytic activity (left) and the natural log of the maximum absorbance (right) for CoFe₂O₄@CeO₃ nanoparticles under solar light.

3.5 Synthesis and Characterization of Ag coated CoFe₂O₄@D-Pen@SiO₂-TiO₂ Nanoparticles.

CoFe₂O₄@D-Pen@SiO₂ NPs were synthesized following the Stöber method.^[19] As mentioned in previous sections, this method involves the condensation and polymerization of TEOS using NH₄OH as the catalyst of the reaction. In this case, the magnetic core employed was CoFe₂O₄@D-Pen. These nanoparticles were synthesized using a coprecipitation method with D-Pen as stabilizer. (Synthesis and characterization in section 4.3.2)

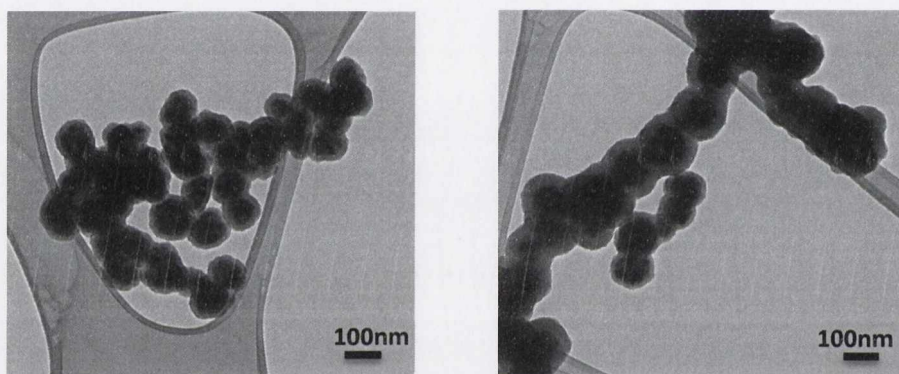


Figure 3.31: TEM images of CoFe₂O₄@D-Pen@SiO₂ NPs.

TEM images show a magnetic core of 70 nm diameter coated with a regular silica shell between 15 and 20 nm in thickness.

VSM in Figure 3.32 shows the magnetic moments of both the magnetic core and the silica coated nanoparticles. The value for CoFe₂O₄@D-pen NPs has been determined to be 62 Am²kg⁻¹. With a magnetic moment of 49 Am²kg⁻¹, there is a reduction in the saturation magnetization for the silica coated nanoparticles due to the presence of the non-magnetic silica coating.

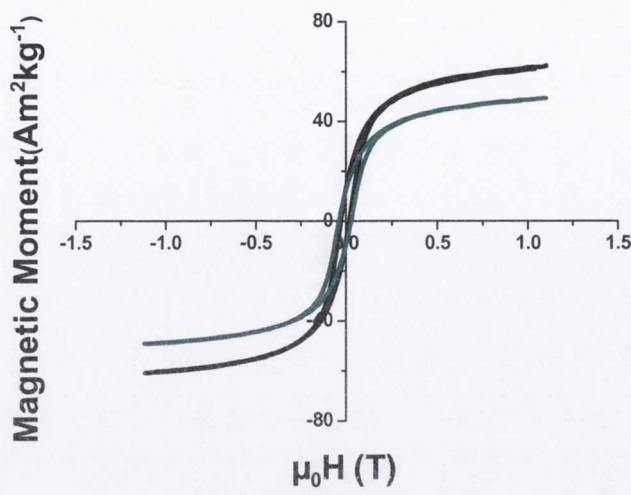


Figure 3.32: VSM curves of CoFe₂O₄@D-pen (black) and CoFe₂O₄@D-pen@SiO₂ NPs (green).

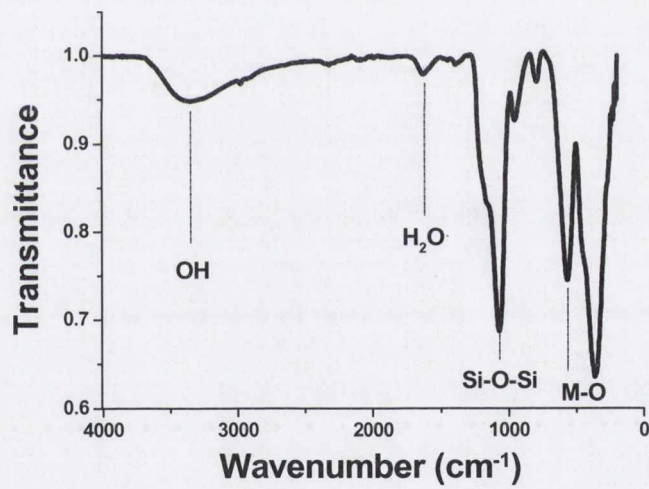


Figure 3.33: FTIR spectrum of CoFe₂O₄@D-Pen@SiO₂ NPs.

The FTIR spectrum of CoFe₂O₄@D-Pen@SiO₂ shows absorption bands which can be assigned to the symmetrical and asymmetrical vibrations of the Si–O–Si bond and the stretching vibration of the hydroxyl group, 800, 950, and 1100 cm⁻¹, respectively. The stretching O–H band for water appears between 3200 cm⁻¹ and 3500 cm⁻¹ and the vibrational stretching mode at 1620 cm⁻¹.^[39] Spinel ferrites have two metal-oxygen bands; the highest one in the range of 600–550 cm⁻¹ corresponds to intrinsic stretching

vibration of the metal at the tetrahedral site, whereas the lower band is assigned to an octahedral metal stretching appearing in the range of 450-385 cm⁻¹. In a spinel ferrite structure, octahedral and tetrahedral site can be occupied by either metal.^[40]

3.5.1 Synthesis and Characterization of Ag coated CoFe₂O₄@D-Pen@SiO₂ nanoparticles

The deposition of Ag on CoFe₂O₄@D-Pen@SiO₂@TiO₂ was carried out in two steps. In the first step, the CoFe₂O₄@D-Pen@SiO₂@TiO₂ nanoparticles have been prepared by hydrolysis of the titanium tetrabutoxide in the presence of CoFe₂O₄@D-Pen@SiO₂ nanoparticles. In the second step, the deposition of Ag on the previously prepared CoFe₂O₄@D-Pen@SiO₂@TiO₂ nanoparticles was achieved by reduction of Ag⁺ ions in N,N-dimethylformamide (DMF). The mixture was stirred for 24 hours. The resultant material was washed three times with ethanol and dried under vacuum. The black powder was calcinated at 600 °C for 1 hour.

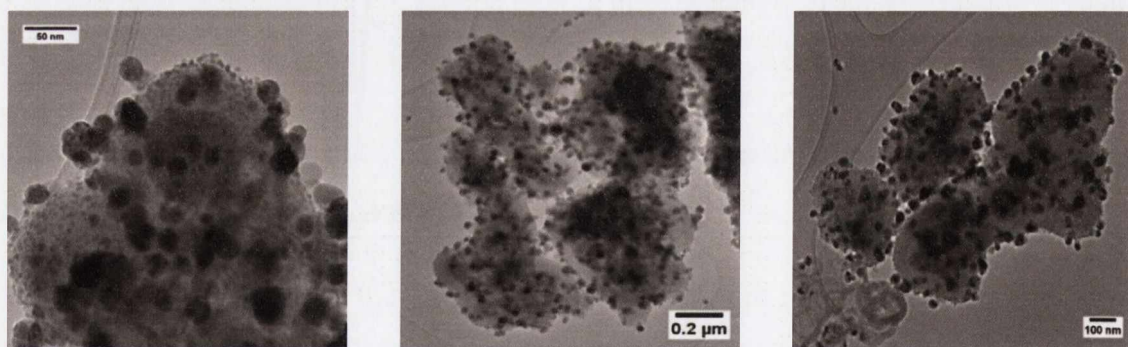


Figure 3.34: TEM images of Ag deposited on CoFe₂O₄@D-Pen@SiO₂@TiO₂ NPs.

TEM images show the formation of the deposition of small silver nanoparticles (15-20 nm) on the surface of CoFe₂O₄@D-Pen@SiO₂@TiO₂ nanocomposites forming raspberry-like nanostructures. Small nanoparticles can also be observed on the silica shell; these dots are due to the nucleation of silver nanoparticles.

Special care has to be put during the synthesis in order to control the size of silver NPs. The rate constant of the catalytic reaction for silver nanoparticles is size dependant and becomes negligible below 2nm or above 50nm.^[41]

Metallic nanoparticles such as Au, Ag and Cu show surface plasmon absorption due to the oscillations of the free conduction band electrons that are induced by the incident electromagnetic radiation.

When one of these metals is deposited on the surface of a semiconductor, the light is absorbed firstly by the plasmonic material. The energy created in the process of the surface plasmon resonance (SPR) excitation is then transferred to the semiconductor. This would result in an enhancement in the photocatalytic activity under visible light for semiconductors, such as TiO₂, with poor activity under visible light.

The presence of metal NPs is confirmed by the observation of the SPR in the visible region of the spectrum (Figure 3.35). This absorption value is affected by the size nanoparticles.^[42] The maximum absorption peak appears around 370 nm. For Ag NPs this broad peak appears between 400-500 nm depending on the size of the nanoparticles. This blue shift can be related to the accumulation of electrons on TiO₂ that are transferred to the metal NPs.^[43]

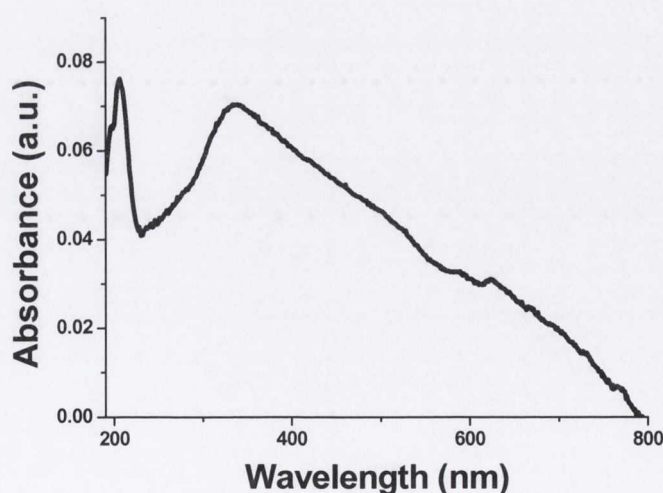


Figure 3.35: Absorption spectrum of Ag coated CoFe₂O₄@D-Pen@SiO₂@TiO₂ NPs.

At the beginning of the photocatalytic process in the presence of TiO₂, electron-hole pairs are formed. The generated holes react with H₂O and OH⁻ groups present on the surface of the semiconductor to produce hydroxyl radicals whilst electrons are attracted to the metal particles due to the difference in the work functions between TiO₂ and metal nanoparticles.^[44] This mechanism hinders the electron-hole recombination process. Metal nanoparticles in the TiO₂ act as an electron sink improving the charge separation and increasing the number of electrons reaching the surface for the reaction to take place.^[45]

3.5.2 Catalytic Activity of Ag Coated CoFe₂O₄@D- Pen@SiO₂@TiO₂ Nanoparticles

Figure 3.36 shows UV-Vis spectra for the degradation of MB using silica coated nanoparticles. This experiment was carried out under UV-Vis light and with the conditions described before allowing the comparison of the results.

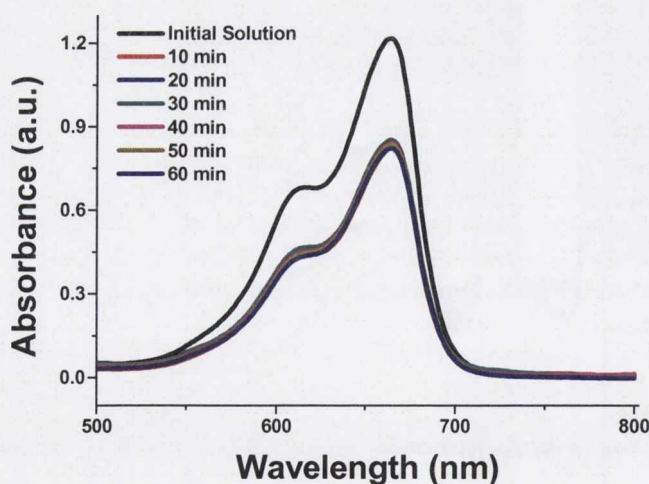


Figure 3.36: Photocatalytic test of CoFe₂O₄@D-Pen@SiO₂ nanoparticles under UV light.

There is a decrease in the absorption after the adsorption of the dye on the surface of the nanoparticles. This can be due to adsorption capacity of porous silica shell on the surface of the nanoparticles.^[46] After reaching the equilibrium, there is no evidence of catalytic activity for this material, as the absorbance remains constant.

Changes in absorption of the MB solution at different times under the effect of daylight using Ag doped nanocomposites are shown in Figure 3.37. No extra light source was used for this experiment. The solution was allowed to stir in the dark in the presence of the nanoparticles to permit the adsorption-desorption of the dye on the surface of the nanoparticles for 1 hour.

Once the equilibrium was reached the changes in absorbance were measured every 10 min. Although slow, some gradual decrease in the absorbance can be observed.

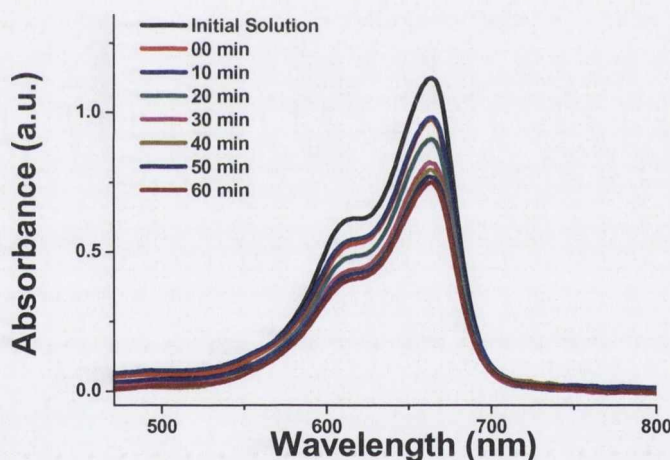


Figure 3.37: Photocatalytic test of Ag/ TiO_2 coated CoFe_2O_4 @D-Pen@ SiO_2 nanocomposites under daylight.

Changes in the absorbance maximum of the dye versus the reaction time for the Ag doped nanocomposites are presented in Figure 3.38. The disappearance of MB follows a pseudo-first order kinetics (Figure 3.38, right).

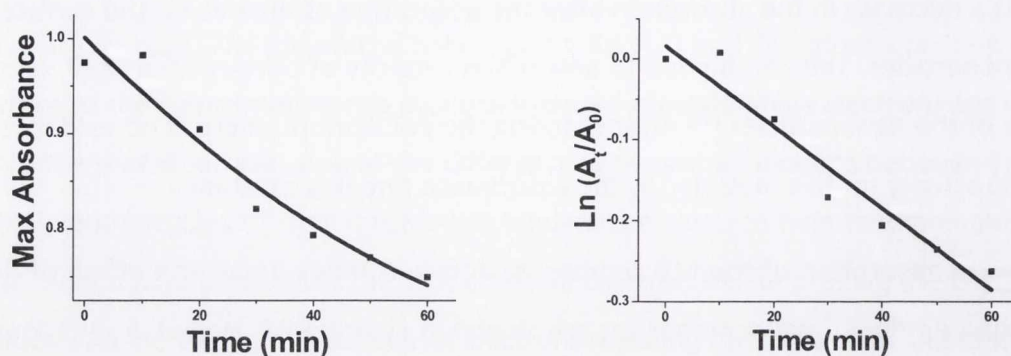


Figure 3.38: Graphs showing the change in main dye absorbance over time for photocatalytic activity (left) and the natural log of the maximum absorbance (right).

In spite of the obvious difference, if these results are compared with those in section 3.3.5 for simple TiO₂ nanocomposites under UV light, it can be seen that Ag-TiO₂ magnetic NPs display a lower photocatalytic activity. After 1 hour of reaction, the degradation of MB using Ag-TiO₂ does not reach the 50% while using TiO₂ is higher than that percentage.

However, the photocatalytic activity of TiO₂-Ag nanocomposites is higher when no UV light source is used. This can be attributed to different factors; the photocatalytic activity of TiO₂-Ag nanocomposites depends on the size and loading amount of Ag nanoparticles. The overloading of Ag ions can lead to a reduction of the active surface area due to the decrease in active catalytic sites on the surface of TiO₂, which would affect the catalytic activity.^[47] Moreover, the size and size distribution of Ag nanoparticles are very important as they affect the maximum of absorbance for these nanoparticles. In our case (Figure 3.35), the sample has quite a broad absorbance spectrum with the maximum around 370 nm. Thus the higher photocatalytic activity of nanoparticles under daylight could be explained by the broad absorbance of the nanoparticles covering the spectral band between 300 and 700 nm.

The enhancement on the photocatalytic activity with a metal is deposited on TiO₂ arises from the difference between the Fermi levels between them. To clarify, Fermi levels of novel metal are lower than that of TiO₂ so excited electrons can be transferred from the conduction band of TiO₂ to Ag deposited on the surface of TiO₂.^[48]

3.5.3 Synthesis and Characterization of Au/TiO₂ Coated CoFe₂O₄@D-Pen@SiO₂ Nanoparticles

The preparation of gold- and TiO₂ coating was performed following a sol-gel method with CoFe₂O₄@D-Pen@SiO₂ nanoparticles as magnetic core. A defined amount of CoFe₂O₄@D-Pen@SiO₂ nanoparticles was dispersed in ethanol and titanium (IV) butoxide (Ti(OBu)₄) was added to the mixture. Separately, 5 mL of a 0.1 M tetrachloroauric acid (HAuCl₄·4H₂O) aqueous solution and 15 mL of acetic acid were dissolved in ethanol and added dropwise to the solution containing magnetic nanoparticles and titanium precursor. The mixture was sonicated for 2 hours and let to stir overnight. The resultant powder was washed with ethanol and dried under vacuum.

TEM images (Figure 3.39) show mainly the formation of magnetic NPs with some coating. Although, it is difficult to distinguish between the silica and the TiO₂ due to the similar contrast in TEM. It may be possible to identify an incomplete TiO₂ coating on the edge of the silica coating. Darker points in the images suggesting more dense material, which can be attributed to Au nanoparticles formed on the surface of the nanocomposites.

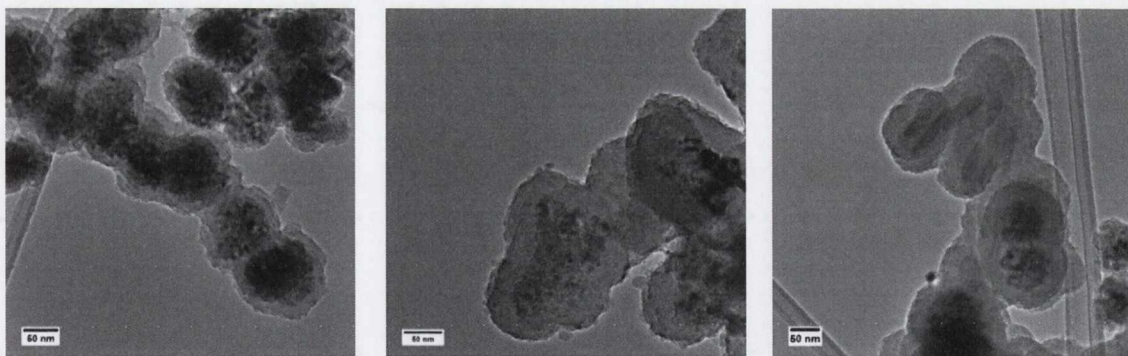


Figure 3.39: TEM images of Au doped /TiO₂ doped CoFe₂O₄@D-Pen@SiO₂ NPs.

3.5.4 Catalytic activity of Au doped /TiO₂ doped CoFe₂O₄@D-Pen@SiO₂ nanoparticles

The same protocol described previously was followed to test the photocatalytic activity of Au coated CoFe₂O₄@D-Pen@SiO₂@TiO₂ nanoparticles. MB was used as a dye to

test the efficiency of the material. Two different tests were carried out; one under UV light and one using solar light.

After 140 min, the absorbance decreased by around 35% when the experiment was carried out under UV radiation (Figure 3.40, left). In the case of the experiment carried out under daylight for 110 min, the absorbance decreased by around 32% (Figure 3.40, right).

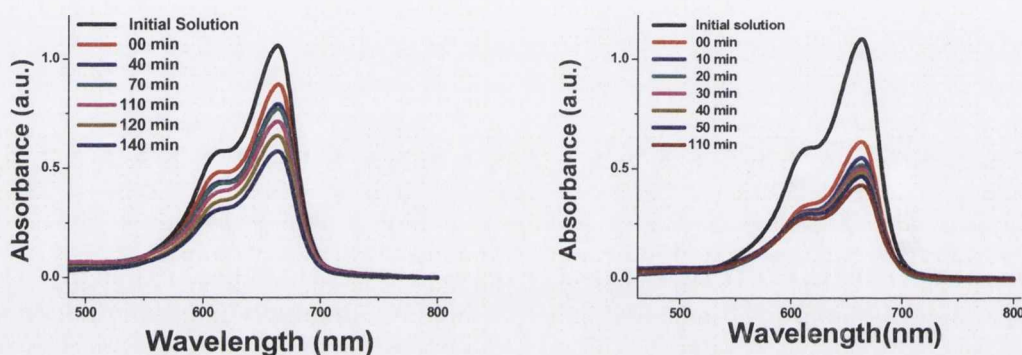


Figure 3.40: Photocatalytic test of Au/TiO_2 coated $\text{CoFe}_2\text{O}_4@\text{D-Pen}@\text{SiO}_2$ under UV light (left) and solar light (right).

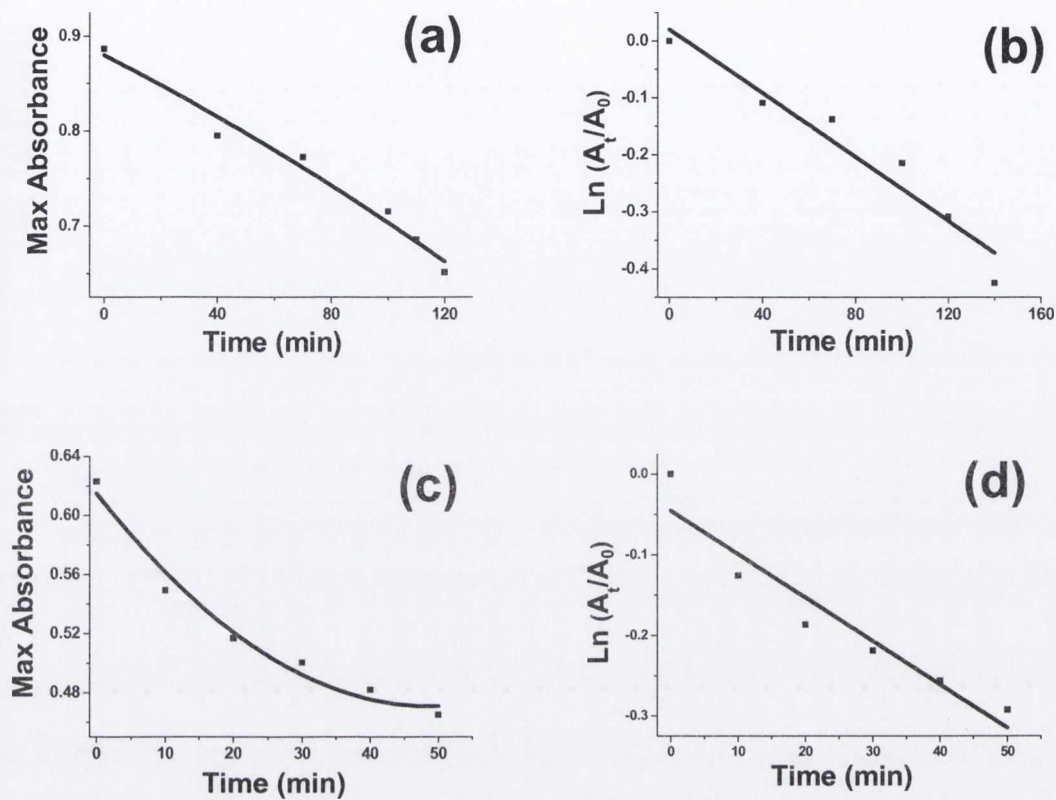


Figure 3.41: Graphs showing the change of maximum absorbance over time for photocatalytic activity of Au doped /TiO₂ doped CoFe₂O₄@D-Pen@SiO₂ (a) and the $\ln(A_t/A_0)$ under UV light (b). Maximum absorbance over time for photocatalytic activity of Au doped /TiO₂ doped CoFe₂O₄@D-Pen@SiO₂ (c) and $\ln(A_t/A_0)$ under daylight (d).

Au/TiO₂ coated CoFe₂O₄@D-Pen@SiO₂ nanocomposites completes the work carried out in TiO₂ coating, broadening the range of reaction to be carried out due to it is not necessary the use of extra light source for the catalyst to perform catalytic activity.

3.6 Conclusions

In conclusion, this work has resulted in the development of various cobalt ferrite nanocomposites, which were prepared by co-precipitation and sol-gel techniques. Although the SiO₂ coating is relatively easy to produce, the complete TiO₂ coating on silica is much more challenging to achieve. The double layer coated CoFe₂O₄@SiO₂@TiO₂ nanostructures demonstrated promising properties. The TEM, EDX, VSM, XRD and Raman spectroscopy confirm the presence of TiO₂ layer, although its thickness is very thin. Despite the additional non-magnetic coatings result in a lower value of the magnetic moment, the particles can still be recovered from the reaction by magnetic separation. The production of photocatalytic active anatase/rutile phases on the surface of

the nano-structures after the sintering of CoFe₂O₄@SiO₂@TiO₂ at 600 °C enables efficient photocatalytic oxidation of methylene blue under UV light.

CoFe₂O₄@CeO₂ nanocomposites were prepared and the presence of crystalline cubic phase of CeO₂ was confirmed by XRD. TEM images showed that the coating consisted in a deposition of CeO₂ nanoparticles on the surface of cobalt oxide magnetic nanoparticles. Catalytic activity of the nanocomposites was confirmed by the oxidation of methylene blue under UV light.

Ag deposited on CoFe₂O₄@D-Pen@SiO₂-TiO₂ nanoparticles was carried out by the reduction of Ag⁺ ions in DMF. Deposition of Ag nanoparticles was confirmed by TEM images. Catalytic studies of methylene blue were performed with promising results.

Au/TiO₂ coated CoFe₂O₄@D-Pen@SiO₂ has been shown to catalyse the oxidation of methylene blue under different conditions. This surpasses the limitations offered by CoFe₂O₄@SiO₂@TiO₂ nanostructures in which it is necessary the use of UV light to activated the photocatalyst.

Overall, the retention of magnetism in these core-shell nanostructures is of particular importance allowing the magnetic recovery and re-use of the catalyst.

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Chapter 4. Amino Acids Stabilised Cobalt Ferrite Nanoparticles

4.1 Introduction

Properties of nanomaterials are different from those of corresponding bulk materials and have been shown to be strongly size and shape dependent. The interest in achieving identical physical and chemical properties has led to an increasing effort to develop synthetic protocols to produce monodispersed and controlled size materials. This is essential to understand and investigate the relationship between properties and particles sizes.^[1-3]

Moreover, the assembly of individual nanoparticles (NPs) into ordered building blocks has also received attention due to their potential biological or magnetic recording applications. The structural characteristics of these nanostructures determine their physical properties, which are directly related to their morphologies. In the case of magnetic materials, nanoparticles show superparamagnetic behaviour at room temperature below a certain size. This property makes them suitable for an increasing number of biomedical applications, as they lose the magnetization in the absence of an applied magnetic field.^[4-5] Nevertheless, the small size of the NPs (around 10 nm) becomes a disadvantage due to the difficulty to separate them effectively from the solution in the presence of a moderate magnetic field causing some limitation in the use of these materials. The strategy to overcome this inconvenience is to form nanoclusters, which are composed of small magnetic NPs maintaining the superparamagnetic behaviour.

The research in this field of new materials mainly studies the growth mechanism to allow predictions towards their physical properties. In some classic theories, crystal growth is thought to occur atom by atom with the dissolution of the less stable phase in a more stable one. The coarsening of small crystals into larger ones is the last stage of the growing process, which would involve the continuous absorption and dissolution of the species present in the reaction.^[6-7]

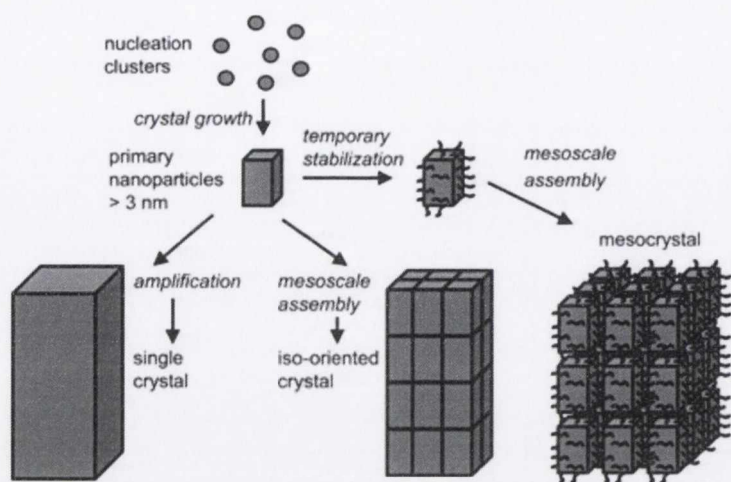


Figure 4.1: Overview to explain crystal growth from nucleation clusters *via* primary nanoparticles towards the formation of single mesocrystals.^[8]

Similar to the classical theory, another mechanism illustrates the aggregation of particles until a more stable phase is achieved. Unstable nuclei will combine with already formed stable nanoparticles in the reaction. These primary nanoparticles will arrange in an oriented crystal, and subsequently, the NPs can fuse and form a single crystal. The incorporation of organic molecules into the reaction can lead to the formation of a mesocrystal, which is a colloidal crystal form. As a result, the mesoscopic transformation of NPs assemblies is followed by fusion to finally give a single crystal.^[9-10]

The formation of nanoclusters is the aggregation of individual crystals into a single crystal or iso-oriented crystal led by self-assembly or fusion, which occurs in order to minimize the surface free energy of the individual NPs. This is described by a different mechanism of crystal growth. The “oriented attachment” model was presented by Penn and Banfield in 1998.^[11] This kind of crystal growth also involves a crystallographic orientation derived from the crystal phase of the individual NPs, which form the final structure. The resultant single crystal material does not have defects.

Although the production of a single crystal by oriented attachment can be achieved by using organic ligands or without any ligand, in the case of the formation of magnetic NPs (MNPs) the use of organic ligands is more common.

Self-assembly of MNPs has been widely investigated to develop new bottom-up approaches. Surfactant or organic ligand assisted methods have been developed to produce these structures. Iso-oriented CoFe_2O_4 NPs assemblies made up of small primary NPs were synthesised by Cannas *et al.* following a micellar method. Primary CoFe_2O_4 NPs of about 7 nm assemble themselves in aggregates of 50-60 nm in diameter, this aggregation is facilitated by the interaction of the polar heads of the surfactant, sodium dodecyl sulphate (SDS).^[12]

Bao *et al.*^[13] developed a shape and structure controlled synthesis of CoFe_2O_4 superparamagnetic nanostructures with spherical and cubic morphology. The self-assembled nanostructures are composed of smaller spherical or rod-like CoFe_2O_4 NPs. The procedure consisted of a variation in the conditions of a previously developed protocol for the synthesis of monodisperse nanocrystals in a high-boiling point solvent following a thermolysis route.^[14-15] Using an ascorbic acid hydrothermal method, Xiong *et al.* fabricated CoFe_2O_4 flower-like microspheres. The as-prepared material showed great performance as anode materials for lithium-ion batteries. Due to their large surface area, there is sufficient contact between active material and the electrolyte enhancing the electrochemical activity.^[16]

Other protocols used in the synthesis of nanostructured crystals based on CoFe_2O_4 are, for example, the combination of the sol-gel and hydrothermal method assisted by citric acid as a ligand.^[17] The co-precipitation method assisted by oleic acid and fatty acids also lead to the formation of CoFe_2O_4 assemblies. By changing the concentration of oleic acid, the size of the spheres can be controlled, showing that is a critical parameter during their formation.^[18]

Among the spinel ferrite materials, cobalt ferrite (CoFe_2O_4) has been investigated because of its outstanding properties such as mechanical hardness, moderate saturation magnetization ($M_s \approx 80 \text{ emu/g}$), magneto crystalline anisotropy ($(K_1 = 270 \times 10^3 \text{ J m}^{-3}, K_2 = 300 \text{ J m}^{-3})$) and chemical stability.^[19] Due to these properties, CoFe_2O_4 is an excellent material for data storage, recording media, lithium-ion batteries or even biomedical applications.^[20-23]

4.2 Aims of This Chapter

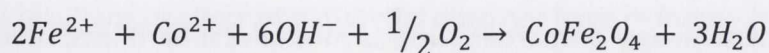
The main aims of this chapter are the development and characterization of new MNPs synthesized in the presence of different amino acid as stabilizers. The effect of the different ligands and the concentration of ligand on the size, shape and properties of the material are to be studied. The materials are to be characterized by VSM and TEM analysis, XRD patterns and FTIR spectroscopy. This work is expected to develop a new strategy and straightforward synthesis of magnetic materials that could be used in a wide range of applications.

4.3 Preparation and Characterisation of Magnetic Nanoparticles Stabilised by Chiral Ligands

4.3.1 Synthesis of Magnetic Nanoparticles Stabilised by Chiral Ligands

The preparation of CoFe_2O_4 stabilised by different chiral ligands was performed following a modified co-precipitation method according to the procedure described by Biswal *et al.*^[24] The stoichiometric reaction of iron (II) chloride and cobalt (III) nitrate is performed in water in the presence of the ligands. The subsequent addition of a base, ammonium hydroxide in this case, leads to the formation of a black precipitate, magnetic cobalt ferrite. The precipitate was washed with ethanol until a neutral pH was achieved and dried to yield a black powder.

As shown in Scheme 4.1 below, the presence of the nitrate anions from the cobalt (II) nitrate salt creates an oxidant environment which leads to the oxidation of Fe^{2+} to Fe^{3+} giving $\text{Co}^{(2+)}\text{Fe}^{(3+)}_2\text{O}_4$ NPs as the final product.



Scheme 4.1: Reaction scheme showing the preparation of cobalt ferrite nanoparticles using co-precipitation.^[25]

The different ligands used for the preparation of these materials were D-Penicillamine (D-Pen), D- and L-Cysteine (D- and L-Cys), D- and L-Aspartic Acid (D- and L-Asp Acid), D-

Glutamic Acid (D-Glu Acid) and L-Alanine (L-Ala). These ligands were incorporated in the reaction mixture by adding a stoichiometric amount to the initial metal salts solutions; the metal salts were each dissolved in 10 mL of Millipore water and a defined amount of the ligand was added to each solution. The mixture was then transferred into a RBF (Round-Bottom Flask) with 180 mL of Millipore water, and heated to the required temperature for the different experiments.

4.3.2 CoFe₂O₄ Stabilised by D-Penicillamine

Penicillamine (Pen) is a thiol amino acid and a strong chelating agent that can react with heavy metal ions. Pen presents D- and L-enantiomer forms with different biological behaviour. However, only the D-enantiomer is used in biological applications due to the high toxicity of the L-enantiomer.^[26]

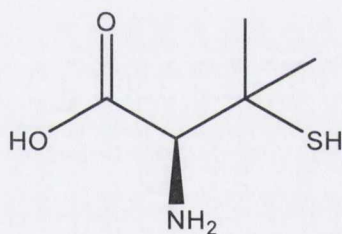


Figure 4.2: Molecular structure of D-Penicillamine.

4.3.2.1 Concentration Effects on CoFe₂O₄ Stabilised by D-Penicillamine

Following the co-precipitation method mentioned above, different experiments were carried out for 1 hour by varying the concentration of the chiral ligand in order to understand its effect on the size, shape and properties of the NPs. D-Pen was used as a ligand in the preparation of magnetic NPs.

Table 4.1: Displays the concentration of ligand used for the different experiments.

Sample	Ligand Conc. (mM)	Lattice param- eter “a” (Å)	M _s (Am ² kg ⁻¹)	d _{Core} (nm)
DP1	0.85	8.416	52.3	4.7 ± 1.1
DP2	1.7	8.382	53.2	----
DP3	3.4	8.443	9.51	3.8 ± 0.9

The X-ray diffraction pattern of the powder of the samples synthesized with a varying concentration of D-Pen identified the final product as CoFe₂O₄ with an inverse spinel structure (Figure 4.3). The main characteristic peaks at (111), (220), (311), (222), (400), (422), (511) and (440) can be observed in the XRD pattern of the sample.^[27] Table 4.1 shows the lattice parameter, a, calculated for the different samples. The values are in line with those of the known bulk material (a = 8.395 Å).^[28]

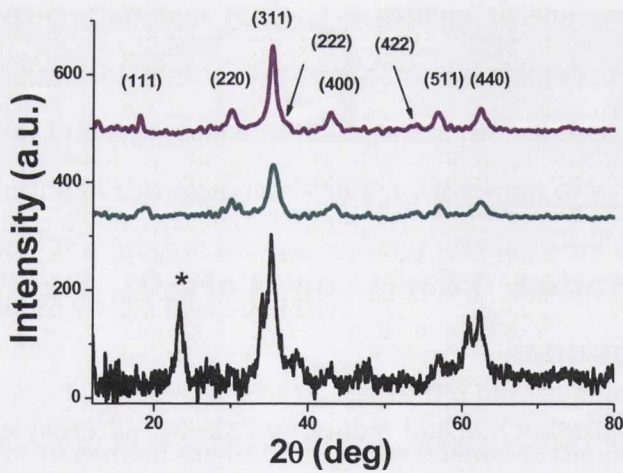


Figure 4.3: XRD patterns of DP1 (purple), DP2 (green) and DP3 (black).

Although the XRD pattern for the sample synthesized with a higher concentration of ligand (DP3) has the main peaks identifying CoFe₂O₄, it also shows a peak at 23°. This peak can be attributed to the presence of other oxides such as hematite (α-Fe₂O₃) indicating that the reaction has also resulted in some amount of the side product.^[29] Dif-

ferent studies in the formation and crystallization of CoFe_2O_4 have demonstrated the presence of these oxides in the first stages of the reactions.^[30]

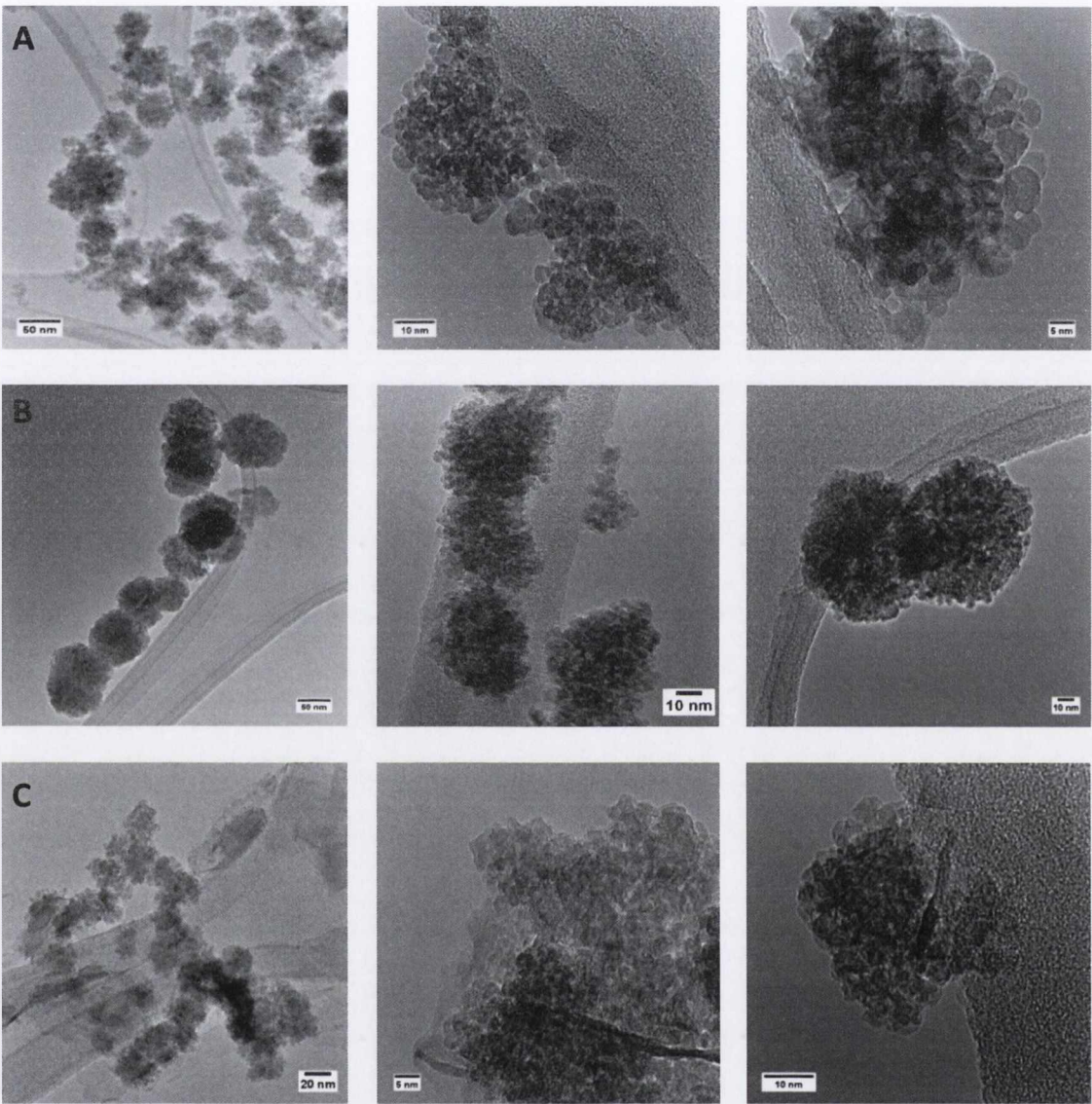


Figure 4.4: TEM images of CoFe_2O_4 @D-Pen nanoparticles synthesized at different reaction times: DP1 (a), DP2 (b) and DP3 (c).

TEM images of CoFe_2O_4 synthesized with 0.85 mM D-Pen (DP1) are shown in Figure 4.4 (a). The histograms with the size distribution of nanoparticles observed in the TEM images are shown in Figure 4.1. The average size was determined by analysing 100 nanoparticles and it was found to be 4.7 ± 1.1 nm for DP1 and 3.8 ± 0.9 nm for DP2. In the case of the experiment carried out with 3.4 mM ligand (DP3), the aggregation of nanoparticles and limited resolution in the images did not allow the determination of the particle size. The TEM images showed spherical nanoparticles with a high degree of

aggregation, leading to the formation of spherical, well define clusters and assemblies of smaller nanoparticles.

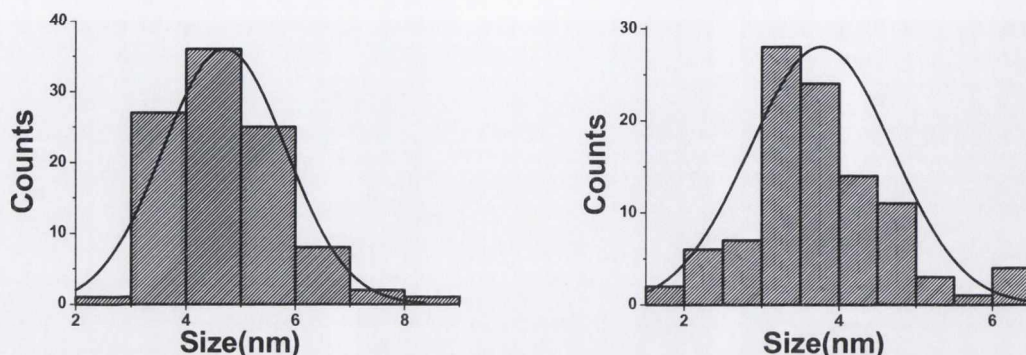


Figure 4.5: Particle size distribution histograms estimated for DP1 (left) and DP2 (right).

The magnetic nanoparticles prepared with the D-Pen ligand demonstrated superparamagnetic properties at room temperature (300 K) because of the absence of coercivity and remanence. Generally, when the size of nanoparticles is smaller than the superparamagnetic critical size, above the blocking temperature, the anisotropy is overcome by its thermal fluctuation leading to the rotation of the moments among the different easy directions and superparamagnetism is exhibited. In other words, the hysteresis loop disappears and the magnetic direction of the nanoparticles follows the direction of the applied magnetic field.^[31]

The magnetization curve for the sample with lower concentration of ligand (DP1) displays a saturation magnetization of $52.3 \text{ Am}^2\text{kg}^{-1}$. This value is in the same range as the magnetization saturation determined for the sample synthesized using the double amount of the ligand, DP2 ($53.2 \text{ Am}^2\text{kg}^{-1}$). In the case of the sample synthesized with 3.4 mM of D-Pen (DP3), the saturation magnetization exhibits a significant reduction of nearly 80% (Figure 4.6).

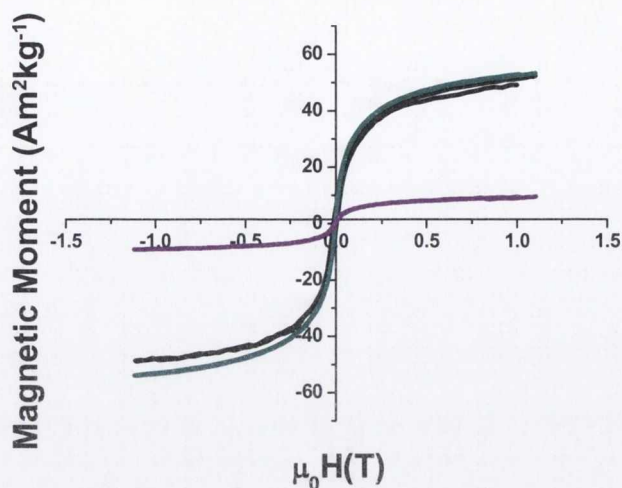


Figure 4.6: VSM curves of $\text{CoFe}_2\text{O}_4@\text{D-Pen}$: DP1 (black), DP2 (green) and DP3 (purple).

Ms for the different experiments are displayed in Table 4.1 and Figure 4.6 shows the magnetic curves. The saturation magnetization for the samples prepared with 0.85 mM ligand (DP1) revealed to have similar magnetization moment. In the case of the sample prepared with 3.4 mM ligand the saturation magnetization was $9.51 \text{ Am}^2\text{kg}^{-1}$, which is a considerable decrease compared to the other samples. These values differ from those obtained for nanoparticles synthesized without of any ligand ($78 \text{ Am}^2\text{kg}^{-1}$) and from those of the bulk material of CoFe_2O_4 ($80 \text{ Am}^2\text{kg}^{-1}$).

This is in line with previous publications, which confirm that the presence of organic ligands during the synthesis of the magnetic material can affect the magnetic properties through ligand-metal bonding. This effect becomes predominant due to the large surface area to volume ratio of the metal atoms in the clusters. It has been shown that the use of π -acceptor ligands, such as carbon monoxide, can cause a reduction of the saturation magnetization. The usage of σ -donor ligands, such as amines, should not affect the magnetic properties.^[32] Additionally, the adsorption of ligand during the formation of the material decreases the saturation magnetization and the coercivity of the magnetic material.^[33]

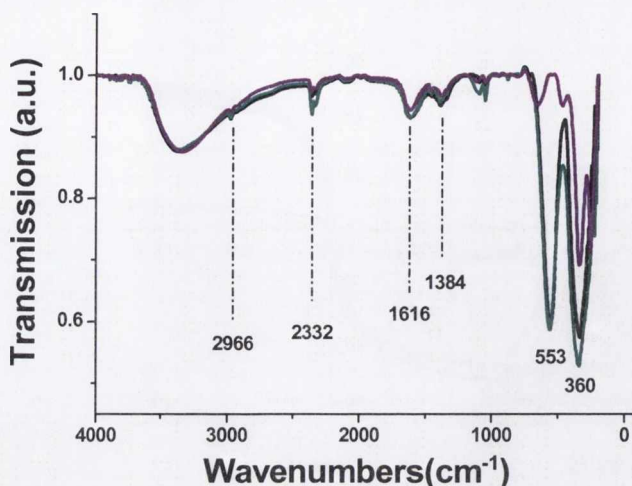


Figure 4.7: FTIR spectra of CoFe_2O_4 @D-pen nanoparticles, DP1 (black), DP2 (green) and DP3 (purple).

The presence of anionic D-pen for all the samples could be confirmed by the FTIR spectrum of CoFe_2O_4 @D-pen nanoparticles (Figure 4.7) because the peak around 2332 cm^{-1} is assigned to the aliphatic C–H bond stretching^[34] Bands in the region between 1300 and 1500 cm^{-1} are complicated to assign due to the possible presence of different modes, such as symmetrical stretching mode of CH_2 groups among other.^[35] The band at 1600 cm^{-1} and the broad peak around 3400 cm^{-1} correspond to the stretching vibrations of hydroxyl groups placed at the surfaces.^[36] The peak at 1600 cm^{-1} could also correspond to COO^- asymmetric stretching for anionic form of D-pen^[37] The small peak appearing around 2900 cm^{-1} can be assigned to the thiol groups.^[38]

Spinel ferrites have two metal-oxygen bands. The highest one in the range of 600 – 550 cm^{-1} corresponds to intrinsic stretching vibration of the metal at the tetrahedral site; whereas the lowest band is assigned to an octahedral metal stretching appears in the range of 450 – 385 cm^{-1} . In a spinel ferrite structure, octahedral and tetrahedral site can be occupied by either metal.^[39]

4.3.2.2 Time Effect on CoFe_2O_4 Stabilised by D-Penicillamine

In order to understand the mechanism on the formation of the spherical aggregates, the synthesis of CoFe_2O_4 @D-Pen with 1.7 mM ligand (DP2) was followed over time.

TEM images of the nanomaterials at the different time points during their preparation are shown in Figure 4.9 and Figure 4.9. After 20 minutes, chiral ligand stabilized nanoparticles have already formed and the formation of spherical aggregates has begun.

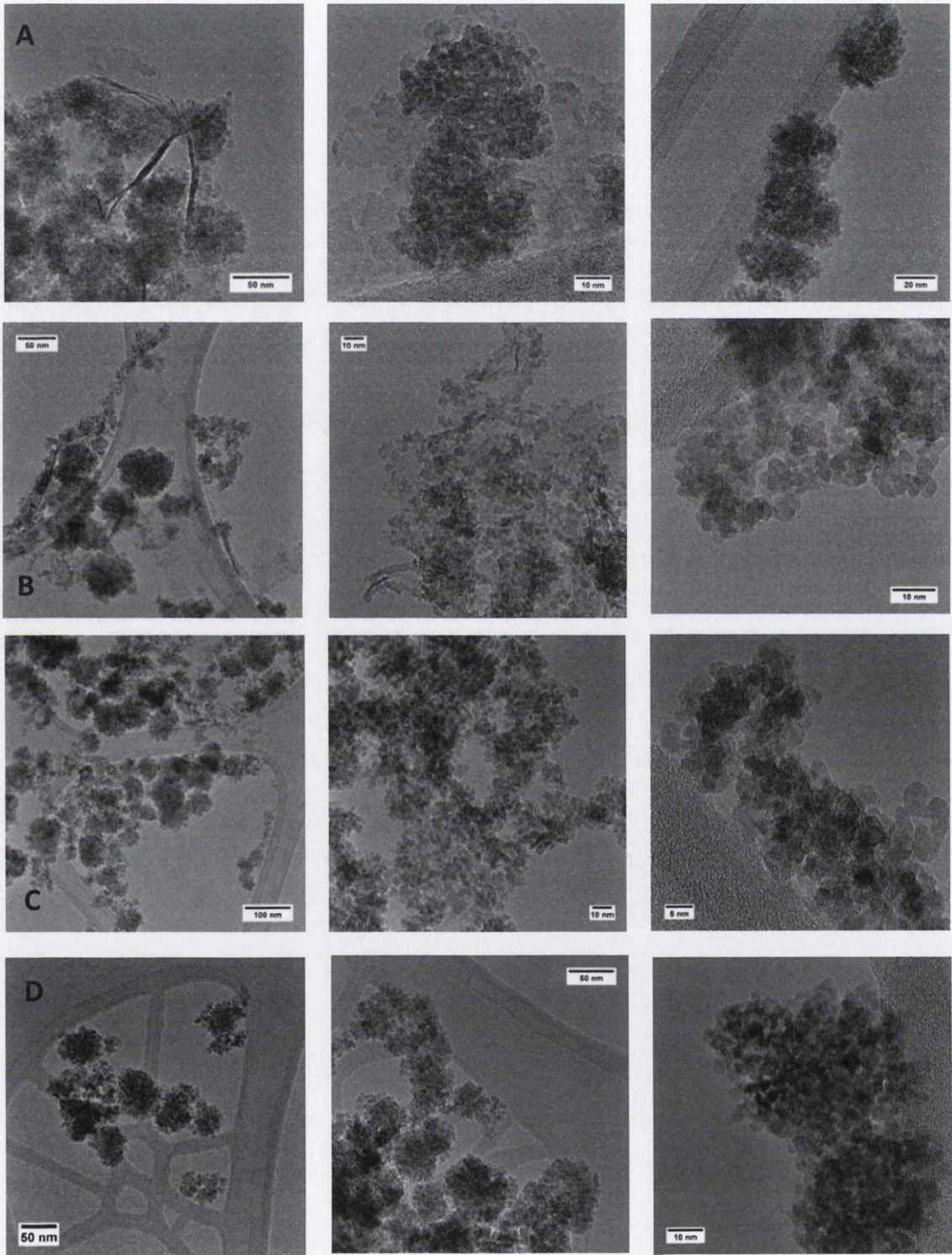


Figure 4.8: TEM images of $\text{CoFe}_2\text{O}_4@D\text{-Pen}$ nanoparticles at 20 (a), 30 (b), 40 (c), 50 (d).

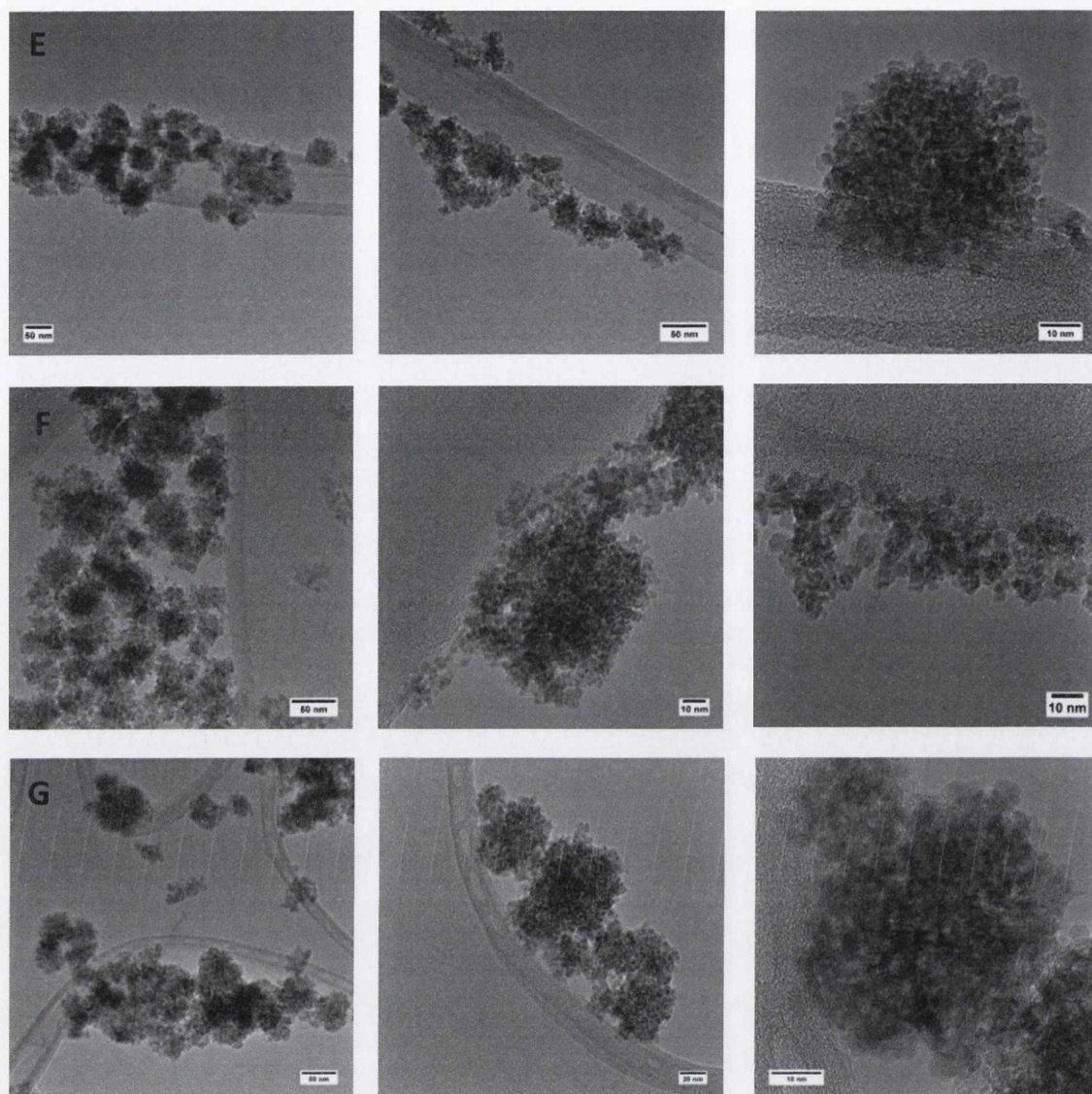


Figure 4.9: TEM images of $\text{CoFe}_2\text{O}_4@\text{D-Pen}$ nanoparticles at 70 (e), 90 (f) and 120 min (g).

Size distribution studies of the nanoparticles were performed for selected time points. These studies gave information about the crystal growth during the reaction.

During the course of the reaction, the size of the nanoparticles increased gradually from 3.6 ± 0.7 nm, after 20 min, to 4.2 ± 0.8 nm after 90 min. The size average was calculated as described before, using an average over 100 nanoparticles for each sample. Due to the aggregation of the nanoparticles, this was not possible in all cases.

The increase in size of the nanoparticles after 90 min of reaction is not significant. This might be due to the fact that, the nucleation and the growth of the nanoparticles occurs within the first stage of the reaction.

Table 4.2: Average size of CoFe₂O₄@D-Pen nanoparticles at different times.

Time (min)	Diameter (nm)
20	3.6 ± 0.7
30	3.9 ± 0.9
40	3.8 ± 0.7
50	4.2 ± 0.8
60	4.5 ± 0.9
70	4.1 ± 0.8
90	4.2 ± 0.8

The relationship between the nucleation step and the growth step during the synthesis is directly related to the resultant particle size. In a normal reaction, without the presence of any stabiliser, nucleation and growth take place almost simultaneously when the reagents are mixed and are heated up. Further growth is related to an increase in the temperature and the time during the process where smaller nanoparticles dissolve and deposit on bigger nanoparticles, leading to what is called Ostwald ripening or coarsening.^[40] On the other hand, as it has been demonstrated before, the use of chelating agent or additives to the reaction during synthesis has been shown to affect the growth of the nanoparticles yielding a material smaller in size.^[41]

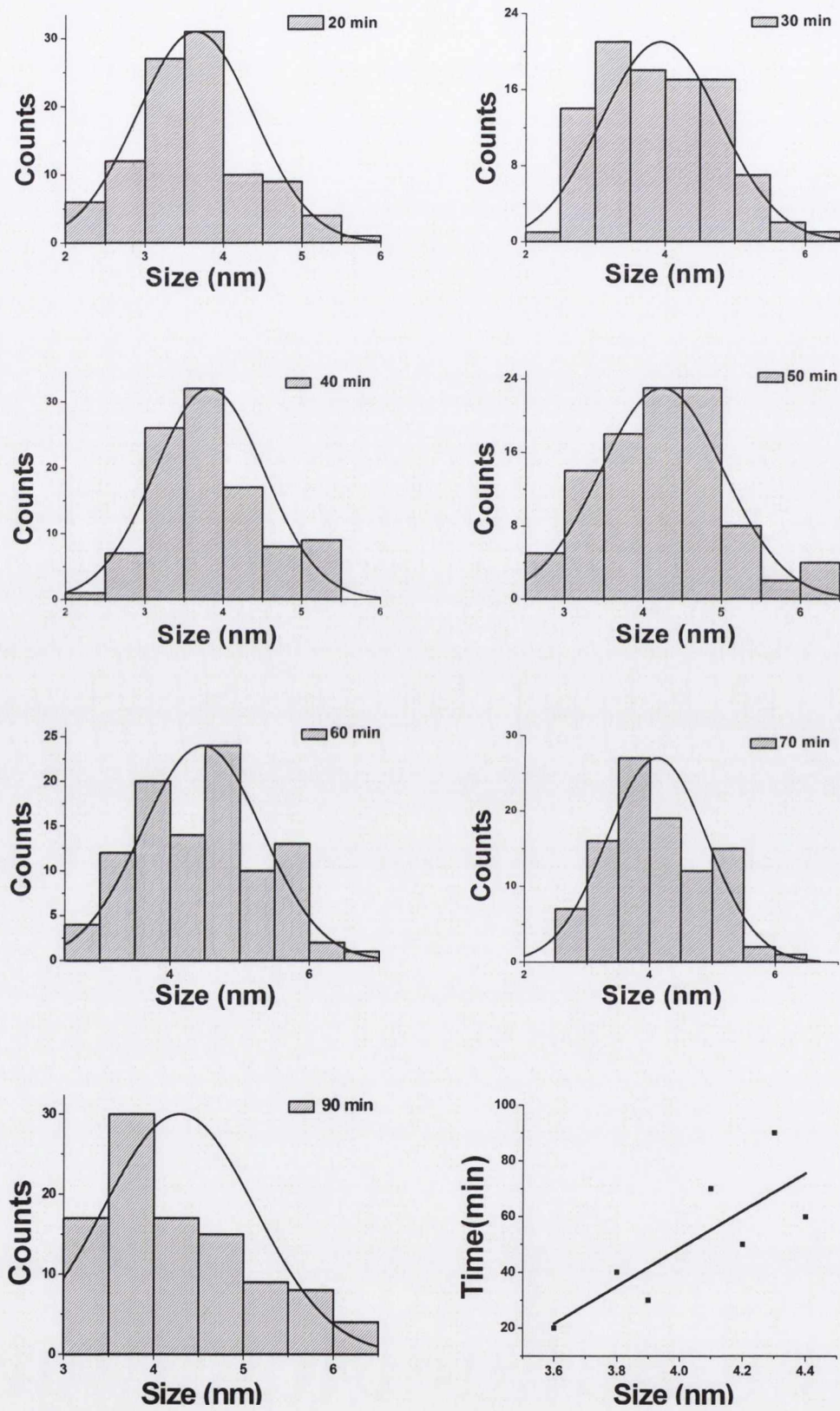


Figure 4.10: Particle size distribution histograms estimated for the formation of $\text{CoFe}_2\text{O}_4@\text{D-Pen}$ over various time points.

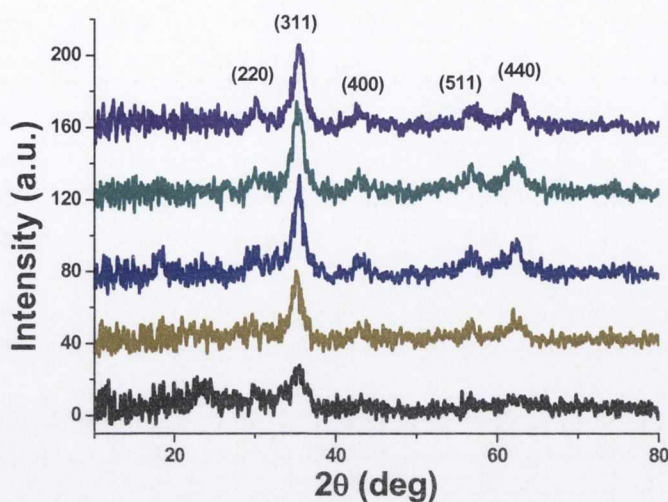


Figure 4.11: XRD pattern of CoFe₂O₄@D-Pen nanoparticles at different times, 20 min (black), 30 min (gold), 40 min (blue), 50 min (green) and 60 min (purple).

The X-ray diffraction pattern in Figure 4.11 shows graphs at different times. The formation of the spinel structure can be observed since early stages during the reaction (20 min) for the presence of the more intense peaks of the structure. However, peaks get sharper as the reaction goes on, which is indicative of better crystallinity in the nanoparticles.

Magnetization measurements showed the increase in the saturation magnetization and the remanent magnetization for longer reaction times. Superparamagnetic behaviour was shown for all the experiments. Although it has been shown that CoFe₂O₄ nanoparticles are superparamagnetic in the range of 10-30 nm at room temperature it can be assumed that the critical size lies below 10 nm.

Figure 4.12 shows the changes in the magnetization moment of the nanoparticles over time. M_s is 24 Am²kg⁻¹ after 20 min reaction, decreasing after 60 min (53 Am²kg⁻¹) and 80 min reaction (44 Am²kg⁻¹).

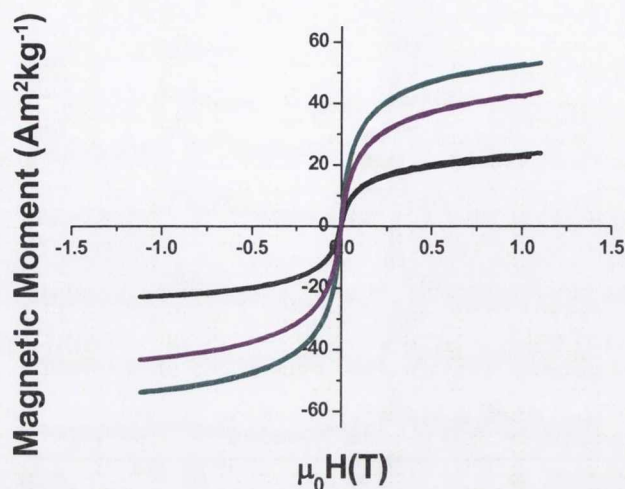


Figure 4.12: VSM curves of CoFe₂O₄@D-Pen nanoparticles at different times: 20 min (black), 60 min (green) and 80 min (purple).

A higher M_s for samples prepared for longer periods is related to the difference in crystallinity. Those samples with higher M_s have a higher crystallinity, which means that the non-magnetic surface layer on the nanoparticles is thinner and has less superparamagnetic relaxation.^[42]

4.3.2.3 Temperature Effect on CoFe₂O₄ Nanoparticles Stabilised by D-Penicillamine

Further experiments were performed to synthesise the nanoparticles using the conditions described but varying the temperature during the reaction. The duration of the reactions was 1 hour. TEM imaging was used to characterize the materials.

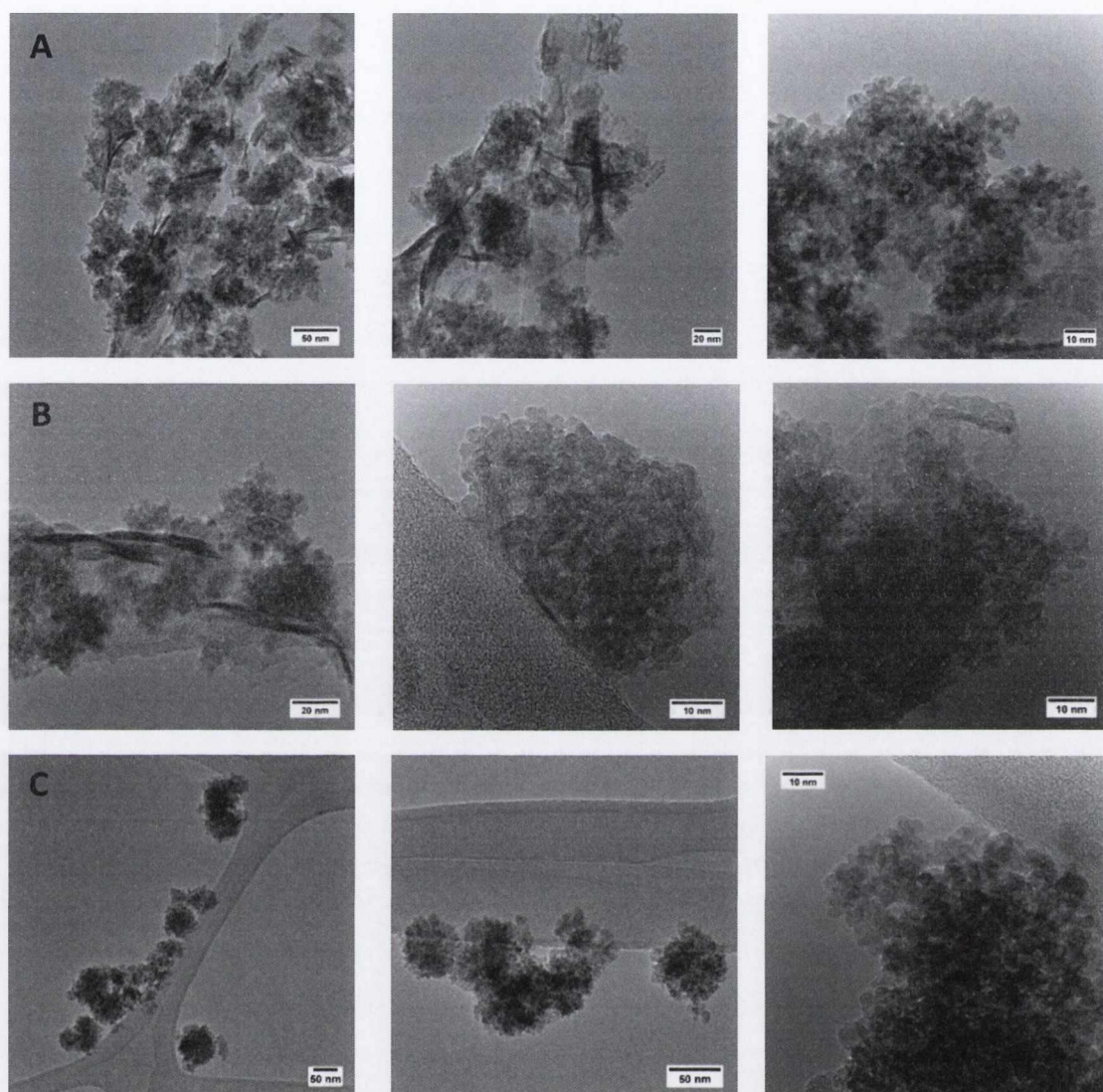


Figure 4.13: TEM images of $\text{CoFe}_2\text{O}_4@\text{D-Pen}$ nanoparticles at 50 (a), 60 (b) and 90 °C (c).

TEM studies showed that the average size of the nanoparticles is not affected by the reaction temperature. A small increase in size with higher temperatures was observed, but it was not significant and within the experimental error. This small increase could be attributed to a possible error during the measurement of the nanoparticles or due to the difficulty associated with the high grade of aggregation for some samples.

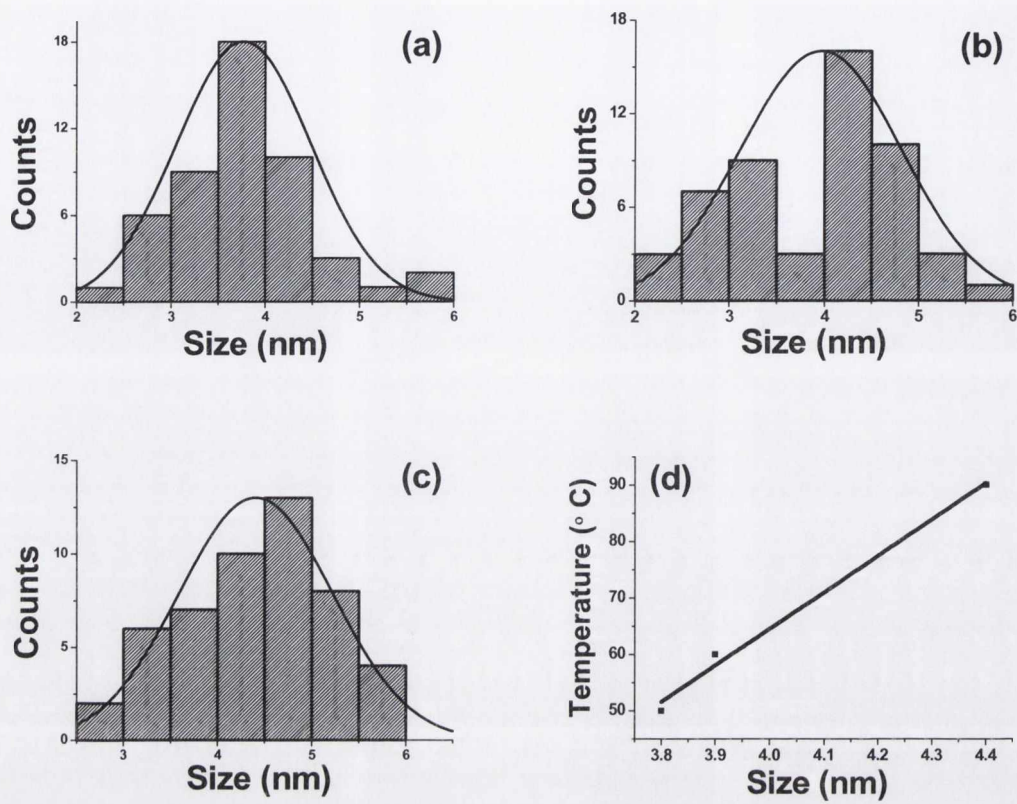


Figure 4.14: Particle size distribution histograms estimated for the formation of $\text{CoFe}_2\text{O}_4@\text{D-Pen}$ at different temperatures: 50 (a), 60 (b), 90 °C (c) and evolution of particle size with temperature (d).

Table 4.3: Size distribution of $\text{CoFe}_2\text{O}_4@\text{D-Pen}$ at different temperatures.

Sample	Temperature (°C)	Diameter (nm)
DP4	50	3.8 ± 0.7
DP5	60	3.9 ± 0.8
DP6	90	4.4 ± 0.8

Figure 4.15 displays XRD patterns of the CoFe_2O_4 powders synthesized at different temperatures. The patterns are in good agreement for those characteristic for the spinel crystal structure. However, there is an increase in the sharpness of the peaks as the temperature of the reaction is increased. As mentioned before, additional peaks around 23° are present in the XRD patterns for lower temperature samples (50 and

60 °C). These peaks can be explained by the presence of hematite ($\alpha\text{-Fe}_2\text{O}_3$) that forms as a side product.^[29]

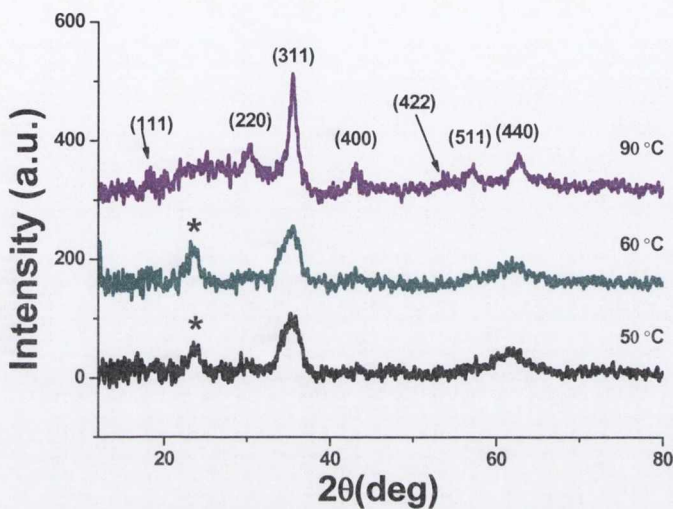


Figure 4.15: XRD pattern of DP4 (black), DP5 (green) and DP6 (purple).

Table 4.4: Ms of $\text{CoFe}_2\text{O}_4\text{@D-Pen}$ NPs at different temperatures.

Temperature (°C)	Ms ($\text{Am}^2\text{kg}^{-1}$)
50	14.7
60	17.6
80	53.1
90	51.3

The analysis of the magnetic curves demonstrated that the temperature of the reaction affects also the magnetization moment. For the experiments carried out at low temperatures (50 and 60 °C) the saturation magnetization is similar (14.7 and $17.6 \text{ Am}^2\text{kg}^{-1}$), these values show that they have a weak response to an external magnetic field which can be related with the poor crystallinity of the material as is shown in the XRD graphs.^[43] VSM curves show that these materials have superparamagnetic behaviour.

However, when the temperature was increased, the magnetization of the samples also increased keeping the superparamagnetic regime, which is an indication that variation of the temperature does not result in an increase in the size of the nanoparticles.

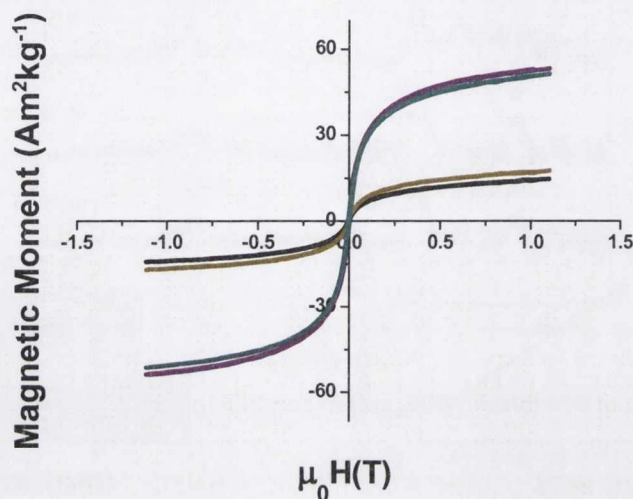


Figure 4.16: VSM curves of CoFe_2O_4 @D-Pen nanoparticles synthesized at different temperatures; DP4 (black), DP3 (gold), DP2 (purple) and DP6 (green).

It has already been demonstrated that an increase in the temperature of the reaction leads to a selective dissolution of Co (II) ions, producing non-stoichiometric CoFe_2O_4 . In addition, the presence of poorly crystalline iron oxides at low temperature can cause a drop in the magnetic properties.^[44]

4.3.2.4 Formation of Aggregates of CoFe_2O_4 @D-Penicillamine Nanoparticles

In addition to controlling the size of nanostructures when using organic ligands or surfactants during the synthesis of nanostructures it is also interesting to note the influence on the self-assembly of the particles. As it has been mentioned before, uniform and large CoFe_2O_4 aggregates, consisting of smaller magnetic nanoparticles, with spherical shape of around 50 nm can be observed in TEM images. This reveals that single nanoparticles merge into assemblies to form aggregates with spherical shape.

If the process is followed over time, it can be noted that at the first stage small and individual nanoparticles are formed. As the reaction proceeds, these particles merge into ordered nanostructures (Figure 4.17). The mechanism that describes this phenomenon is known as oriented attachment (Scheme 4.2). The free energy accumulated on the surface of randomly oriented nanoparticles drives to an aligned growth with a particular orientation of the crystal in order to reduce this energy. This approach is in agreement with previous publications.^[45-46] From the XRD and TEM data, a possible interpretation is that the nanoparticles fuse together from single nanoparticles and align alongside the preferential crystallographic orientation, as can be observed in some of the TEM images from the lattice fringes shared by the NPs.

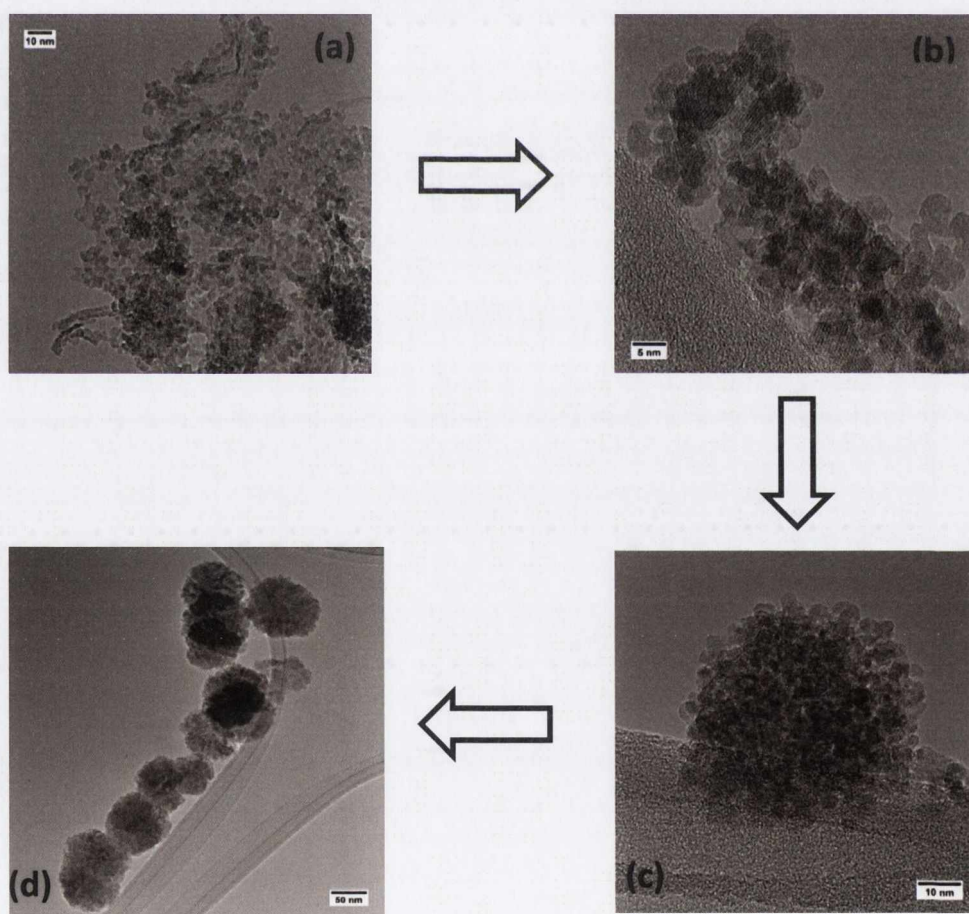
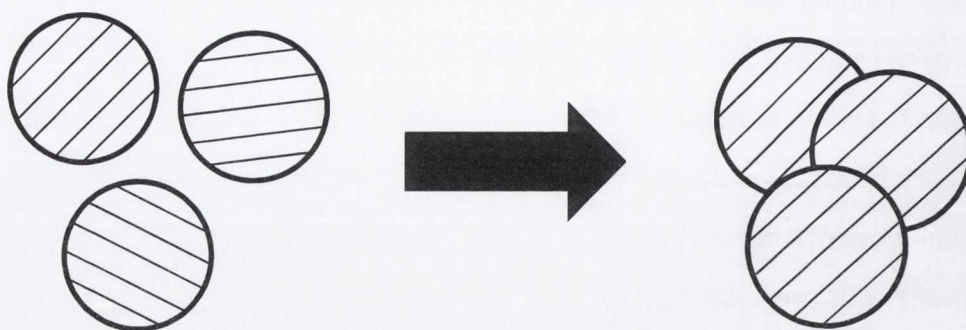


Figure 4.17: Formation of nanoclusters. MNPs after 20 min (a), 40 min (b) and 70 min (c). Image d shows an overview of the material at lower magnification.



Scheme 4.2: Scheme showing the mechanism of oriented attachment of magnetic nanoparticles.

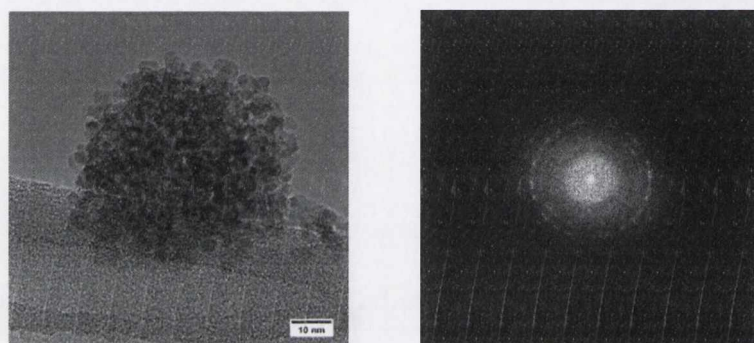


Figure 4.18: CoFe_2O_4 @D-Pen NPs after 70 min reaction forming a nanocluster (left) and FFT of the material (right).

Figure 4.18 shows an individual nanocluster formed by the aggregation of smaller individual nanoparticles. The figure on the right shows the FFT (Fast Fourier transform) of the image on the left. Bright rings confirm the crystallinity of the material formed.

4.4 CoFe_2O_4 Stabilised by D and L-Cysteine

As with penicillamine, cysteine is a thiol-containing amino acid with important biological functions. It has been widely used as capping agent in the functionalization of nanomaterials. Particularly cysteine easily binds to metals ions via its thiols groups. Cysteine can be found in two enantiomeric forms, D- and L-cysteine.

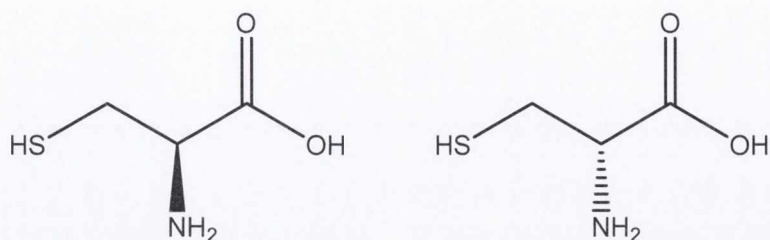


Figure 4.19: Molecular structure of L-Cysteine (left) and D-Cysteine (right).

The same procedure as applied in previous synthesis was followed in the synthesis of CoFe_2O_4 with D-Cys and L-Cys.

Table 4.5: shows the concentration of ligand used in each experiment.

Ligand	Concentration (mM)
D-Cys (DC1)	0.85
D-Cys (DC2)	1.70
L-Cys (LC1)	0.85
L-Cys (LC2)	1.70

TEM images of the sample synthesized with lower concentration of D-Cys showed the aggregation of small nanoparticles into assemblies; similar to the results in previous experiments with D-Pen as ligand. The aggregation and reduced size of the nanoparticles in this experiment made it impossible to calculate the average size distribution.

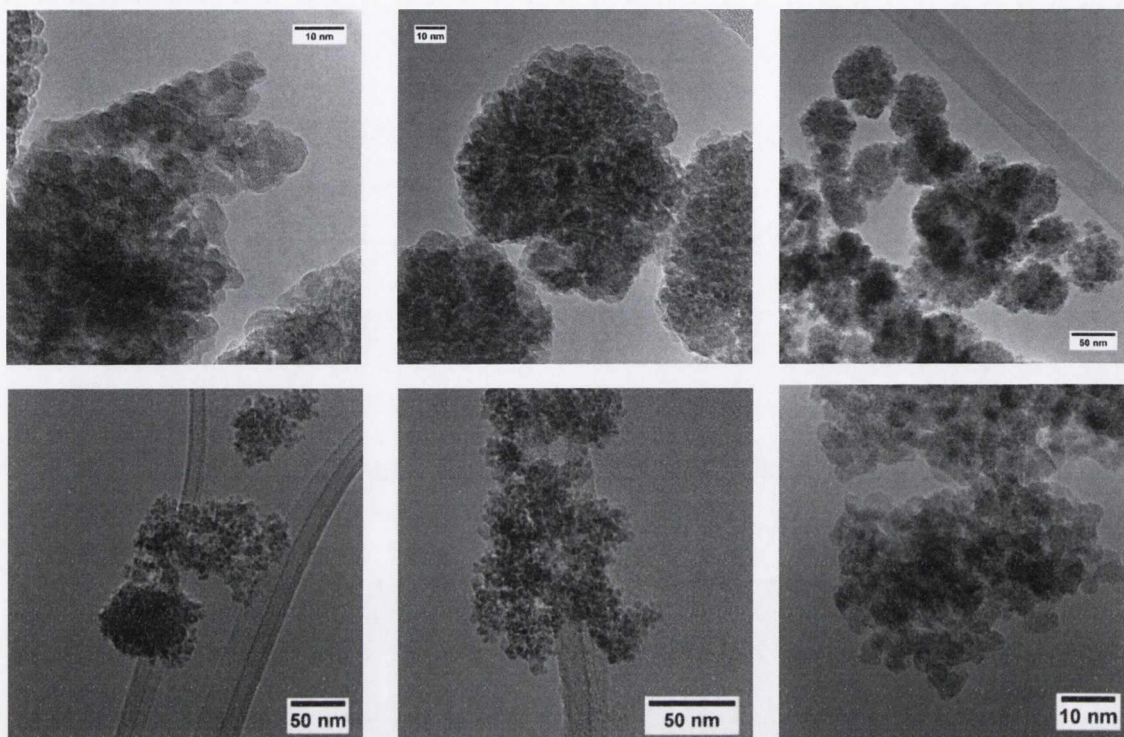


Figure 4.20: TEM images of $\text{CoFe}_2\text{O}_4@D\text{-Cys}$ (DC1, top) and $\text{CoFe}_2\text{O}_4@D\text{-Cys}$ (DC2, bottom).

Fortunately, the size could be determined for the sample synthesized with higher concentration of D-Cys. The result of the investigation showed that the average size in this case was 3.6 ± 0.8 nm after the analysis using over 100 nanoparticles. The size of the nanoparticles in both approaches is similar, indicating that the concentration does not influence size.

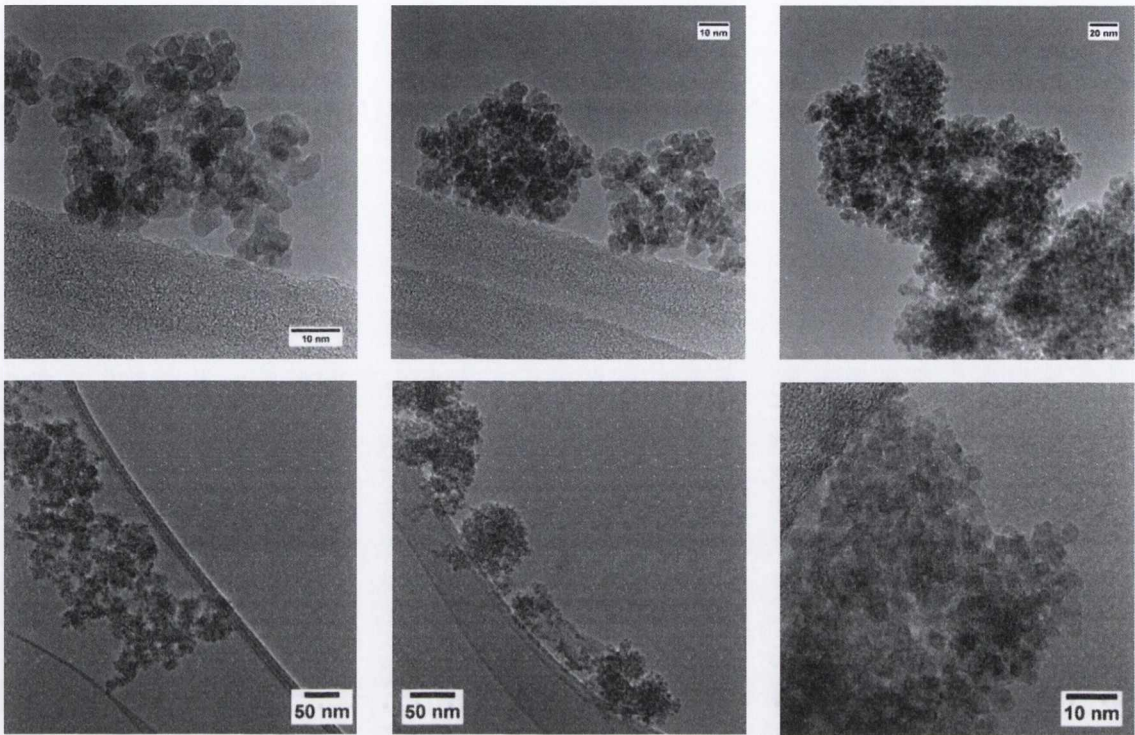


Figure 4.21: TEM images CoFe_2O_4 stabilized with low concentration L-Cys (LC1, top) and L-Cys (LC2, bottom).

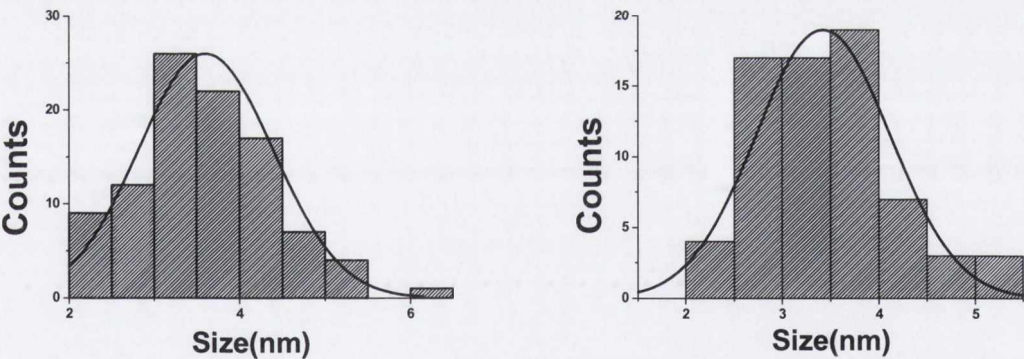


Figure 4.22: Particle size distribution histograms estimated for the formation of $\text{CoFe}_2\text{O}_4@D\text{-Cys}$ (DC2, left) and $\text{CoFe}_2\text{O}_4@L\text{-Cys}$ (LC2, right).

Similar conclusions can be raised when the L-enantiomer is used in the synthesis of the NPs. The morphology and the average size of the material are in the same range than those calculated for the D-enantiomer, as would be expected.

The saturation magnetization for various batches of nanoparticles is displayed in Table 4.6. Higher values of M_s are measured for those samples, which contain a lower concentration of D- or L-Cys. Thus, the increased in the amount of chiral ligand used

leads to a decrease in the magnetization of the material. This is in agreement with the results obtained when D-Pen is used as ligand.

Table 4.6: Ms Values of CoFe₂O₄@D- and L-Cys nanoparticles.

Experiment	Ms (Am ² kg ⁻¹)
D-Cys (DC1)	60.1
D-Cys (DC2)	48.1
L-Cys (LC1)	56.1
L-Cys (LC2)	41.9

Magnetic curves for all the cysteine stabilised nanoparticle samples showed a superparamagnetic behaviour and a Ms lower than that obtained for the material synthesized in the absence of the ligand (Figure 4.23). The hysteresis loop is related to the size of the nanoparticles, whereby if the size of the nanoparticles decreases to a point to reach the single domain regime when they become superparamagnetic.

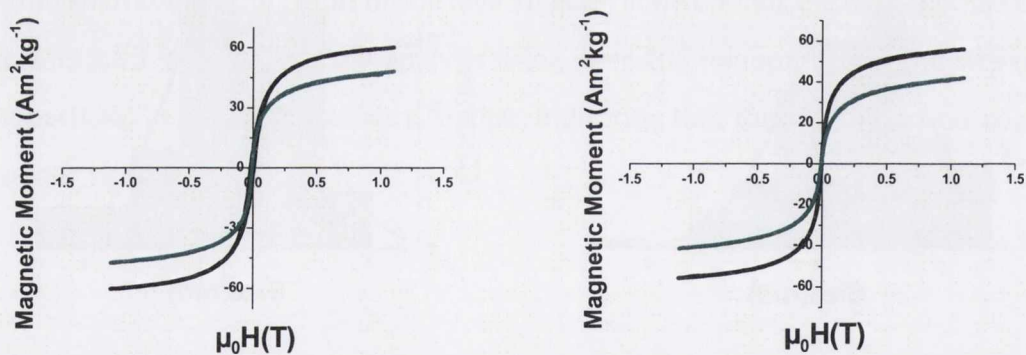


Figure 4.23: VSM curves of CoFe₂O₄@D-Cys nanoparticles (left), DC1 (black) and DC2 (green). VSM curves of CoFe₂O₄@L-Cys nanoparticles (right), LC1 (black) and LC2 (green).

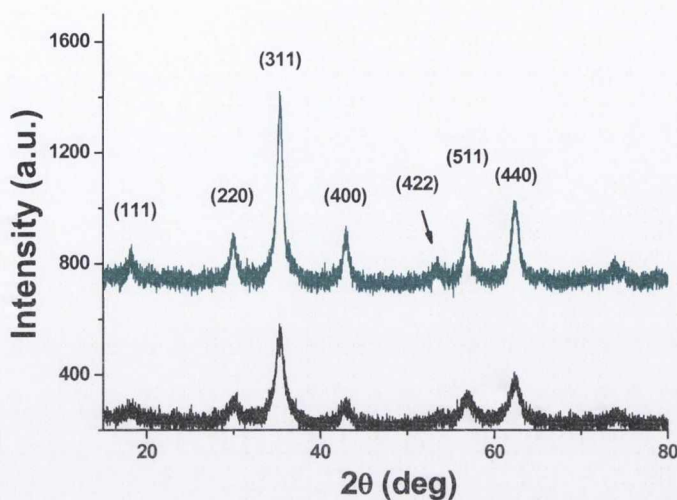


Figure 4.24: XRD spectrum of CoFe_2O_4 @D-Cys nanoparticles (green) and CoFe_2O_4 @L-Cys (black) synthesized with low concentration of amino acid.

XRD patterns performed for the samples prepared with a lower amount of amino acid are shown in Figure 4.24. The peaks corresponding to the spinel structure for CoFe_2O_4 are present and no impurity can be detected.

Figure 4.25 shows the FTIR spectrum of CoFe_2O_4 stabilized with D- and L-Cys. As in previous experiments carried out with D-Pen, similar peaks appear when Cys is used as a ligand. This could be expected since both ligands are very similar in their structure.

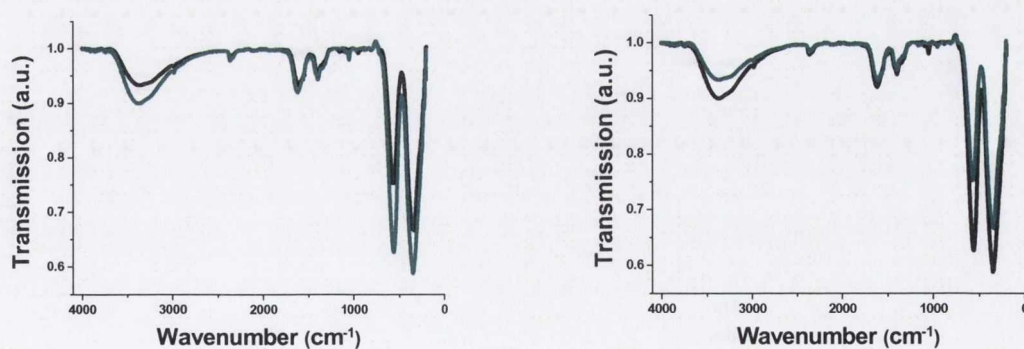


Figure 4.25: VSM curves of CoFe_2O_4 @D-Cys (left, DC1 black line and DC2 green line) and CoFe_2O_4 @L-Cys nanoparticles (right, LC1 black line and LC2 green line).

Thus, no difference can be noted among any of the experiments by varying either the enantiomer or the concentration of ligand. It can be concluded that changing the

amount of ligand or the enantiomer does not affect the functionality at the surface of the nanoparticles.

The material obtained from the synthesis of CoFe_2O_4 NPs assisted with D- and L- Cys produced material similar to that produced following the same synthesis as in previous section using D-Pen as stabilizer. The average size of individual NPs is in the same range below 5 nm exhibiting superparamagnetic behaviour. However, despite the fact that some of the images show a high level of agglomeration in TEM the shape of the nanoclusters is not as regular as in the case of $\text{CoFe}_2\text{O}_4@\text{D-Pen}$.

4.5 CoFe_2O_4 Stabilised by D-Aspartic Acid

Aspartic acid is one of two acidic amino acids that were studied, the other one, Glutamic acid, will be presented in next section. Both amino acids have been intensely studied before and demonstrated to participate in the chelation of metals. [31, 47]

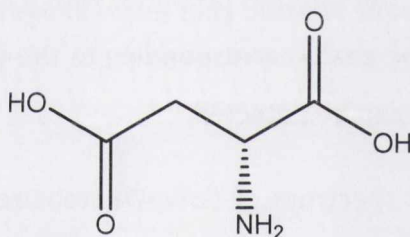


Figure 4.26: Molecular structure of D-Aspartic acid.

The magnetic nanoparticles were synthesised and characterized by the same methods as previously noted and similarly, the product obtained was a dark brown powder.

TEM images of D- and L-Asp acid stabilised CoFe_2O_4 nanoparticles revealed the size of the particles to be within the narrow range 3.7 ± 0.9 nm and 3.6 ± 1.4 nm, respectively with similar granular morphologies (Figure 4.27). However, large clusters of aggregates in the TEM images of CoFe_2O_4 nanoparticles with D- and L-Asp acid were again formed.

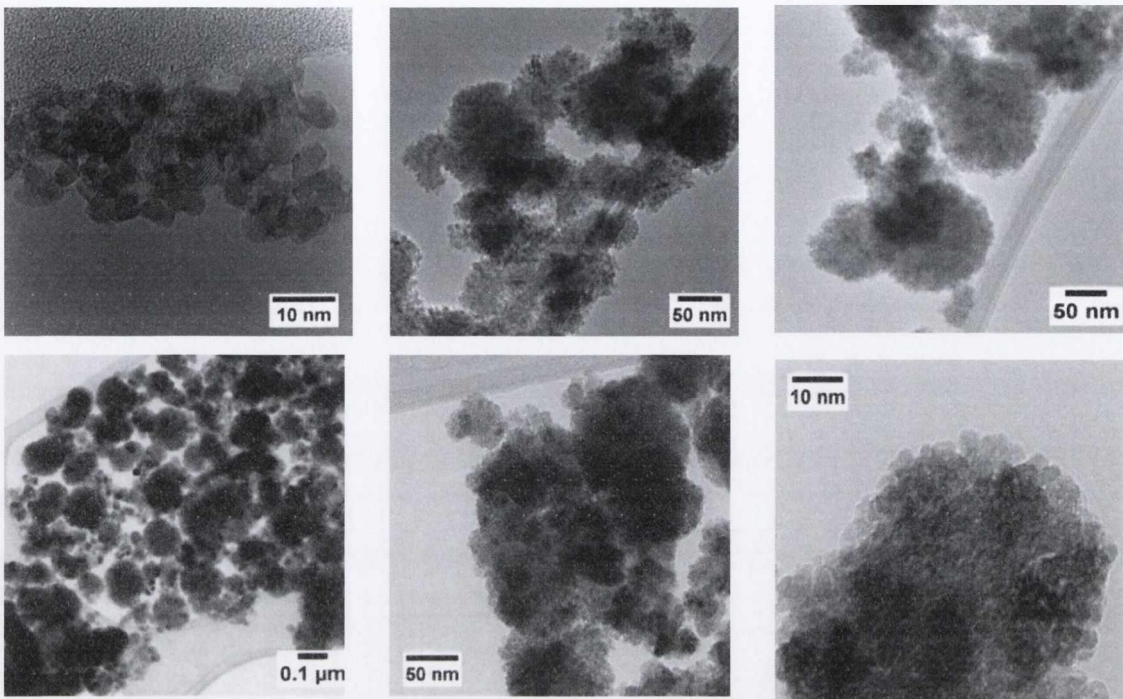


Figure 4.27: TEM images of CoFe₂O₄@D-Asp Acid (AA1, top) and CoFe₂O₄@L-Asp Acid nanoparticles (AA2, bottom).

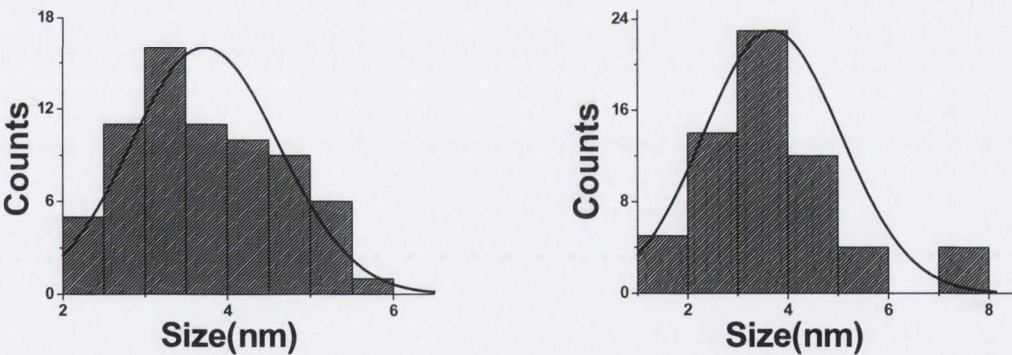


Figure 4.28: Particle size distribution histograms estimated for the formation of CoFe₂O₄@D-Asp Acid AA1 (left) and CoFe₂O₄@L-Asp Acid nanoparticles AA2 (right).

VSM curves showed saturation magnetization of CoFe₂O₄@D-Asp acid and CoFe₂O₄@L-Asp to be 61.8 and 68.5 Am²kg⁻¹, respectively (Figure 4.29), with differences owed probably to experimental error. The curves are almost identical and exhibit small hysteresis loops and remanence values of 13.3 and 19.2 Am²kg⁻¹ respectively

indicating that the particles are ferromagnetic, although very close to the superparamagnetic regime.

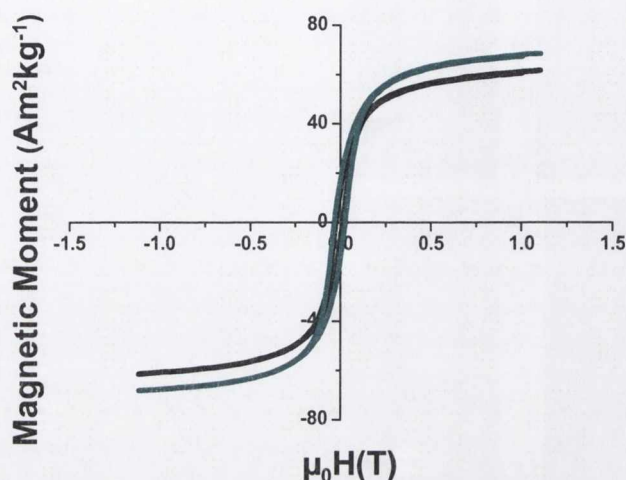


Figure 4.29: VSM curves of $\text{CoFe}_2\text{O}_4@D\text{-Asp Acid}$ (black) and $\text{CoFe}_2\text{O}_4@D\text{-Asp Acid nanoparticles}$ (green).

XRD of D- and L-Asp Acid stabilised CoFe_2O_4 nanoparticles (Figure 4.30) revealed the following peaks which are characteristic of FCC cobalt ferrite lattice parameters: (111), (220), (311), (222), (400), (422), (511), and (440).

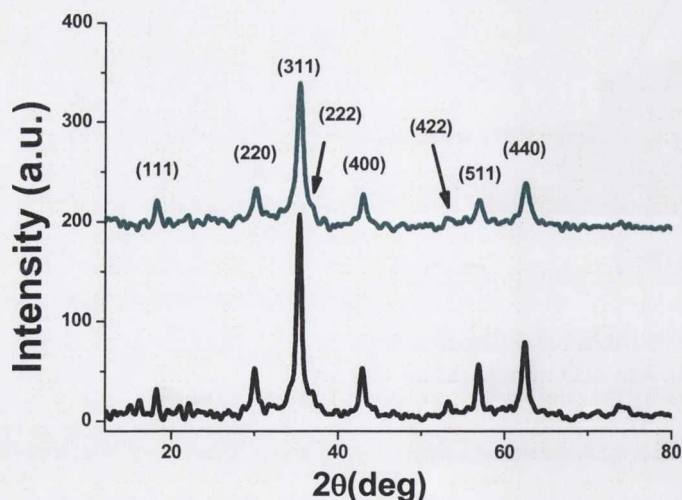


Figure 4.30: XRD patterns of $\text{CoFe}_2\text{O}_4@D\text{-Asp Acid}$ (green) and $\text{CoFe}_2\text{O}_4@L\text{-Asp Acid nanoparticles}$ (black).

As shown in previous experiments, FTIR measurements (Figure 4.31) were carried out and no change could be observed from the other spectra already explained. Therefore,

this can confirm that by changing the amino acid during the synthesis the functionalization of the NPs is not affected.

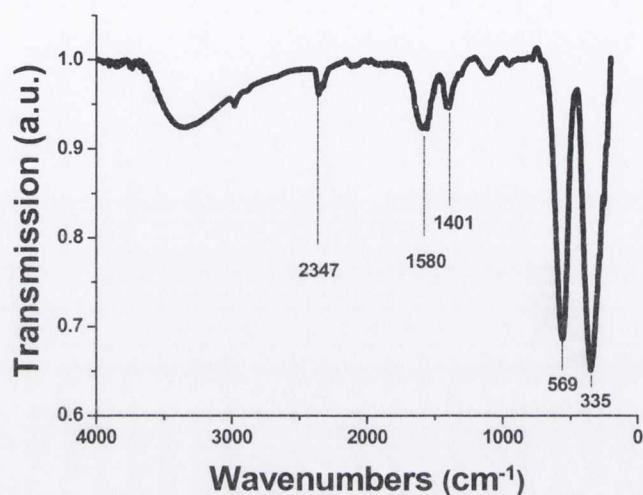


Figure 4.31: FTIR spectrum of CoFe_2O_4 @D-Asp Acid nanoparticles.

As with previous experiments, the use of aspartic acid as a ligand during the synthesis of MNPs shows to have an influence in the morphology of the material. CoFe_2O_4 NPs were obtained with an average size close to 4 nm. Individual NPs form larger clusters with a size between 60 and 100 nm. Despite the size, the nanoclusters formed preserved the superparamagnetic behaviour that would correspond typical of magnetic material with smaller size.

4.6 CoFe₂O₄ Stabilised by D-Glutamic Acid

D-Glutamic acid displays interesting properties as a weak chelating agent and can be used in chemistry in the design of new materials.

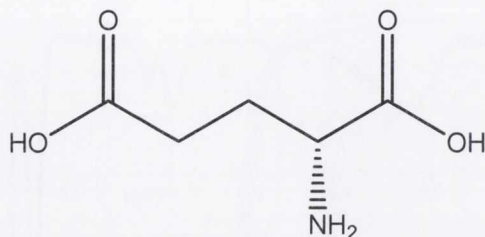


Figure 4.32: Molecular structure of structure of D-Glutamic acid.

TEM analysis of D-Glutamic Acid stabilised CoFe₂O₄ (Figure 4.33) demonstrated that the particles exhibit similar nanocrystalline morphologies and a narrow size distribution ranging between 3-5 nm. However, the presence of large clusters of aggregates in the TEM images of CoFe₂O₄@D-Glu Acid made difficult to measure exact particle sizes, although based on other similarities it can be assumed that they have approximately the same sizes than those in the sample carried out using aspartic acid as a ligand.

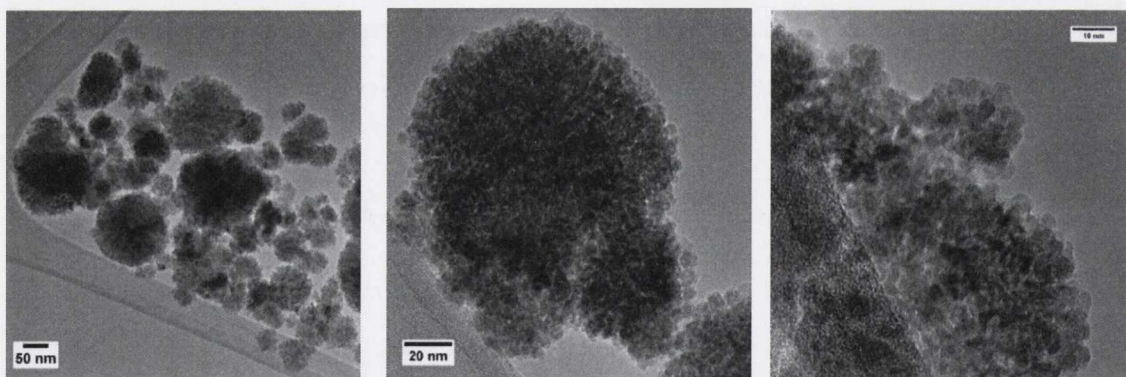


Figure 4.33: TEM images of CoFe₂O₄@D-Glu Acid nanoparticles.

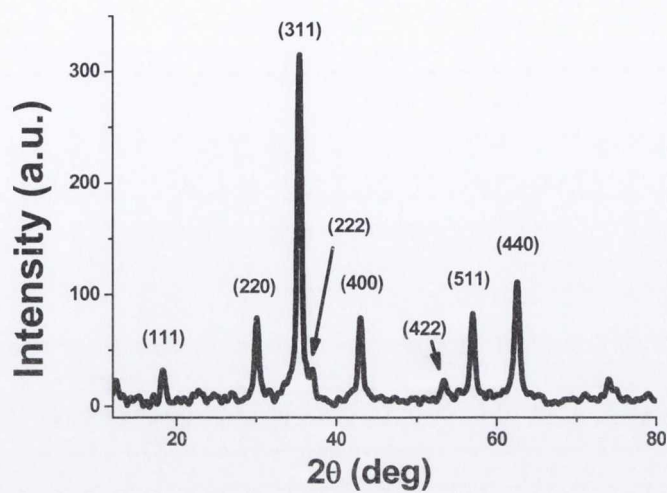


Figure 4.34: XRD pattern of CoFe₂O₄@D-Glu Acid nanoparticles.

The formation of the spinel structure owed to CoFe₂O₄ is confirmed in XRD pattern (Figure 4.34). Characteristic peaks can be observed at (111), (220), (311), (222), (400), (511) and (440) can be observed. The small size and high crystallinity of nanoparticles resulted in the nearly superparamagnetic behaviour, which was confirmed by VSM (Figure 4.34). The saturation magnetization and remanence were found to be 63.2 and 13.4 Am²kg⁻¹ respectively.

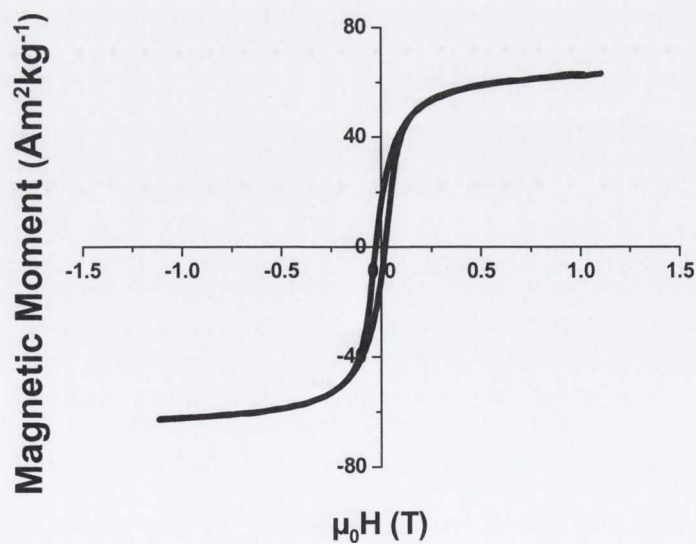


Figure 4.35: VSM curve of CoFe₂O₄@D-Glu Acid nanoparticles

Figure 4.36 shows FTIR spectra of CoFe_2O_4 @D-Glu Acid nanoparticles. No variation can be observed in the peaks from those already studied previously.

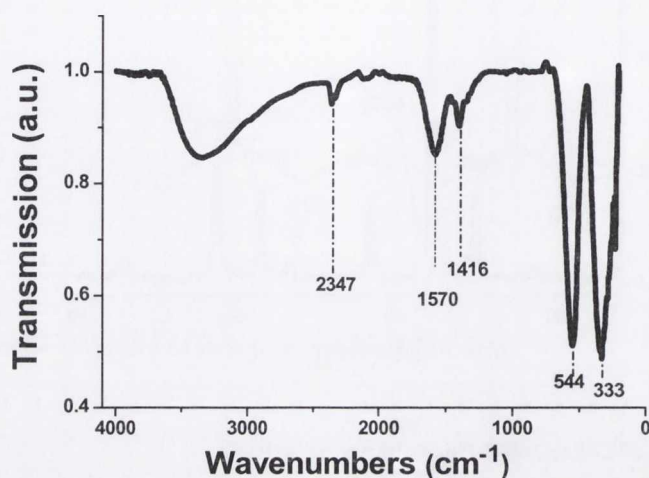


Figure 4.36: FTIR spectrum of CoFe_2O_4 @D-Glu Acid nanoparticles.

As already explained for the synthesis of CoFe_2O_4 carried out with aspartic acid, the results obtained in this experiment are in the same line. Restriction during the growth of the NPs is achieved obtaining individual NPs with an average size below 4 nm. The formation of larger clusters with uniform sizes varying between 50 and 100 nm. Nearly superparamagnetic material is obtained with a very narrow hysteresis loop.

To conclude, the usage of amino acids such as for the synthesis of magnetic nanoparticles leads to the generation of small nanoparticles (3-5 nm) at early stages of the reactions. These nanoparticles form large clusters through oriented attachment. Additionally, the reduction in the size leads the nanoparticles to reach the superparamagnetic regime.

The characteristics, narrow size distribution, structure and superparamagnetic properties make these nanostructures interesting candidates to be used in biomedical applications among others. The simplicity of the synthesis and the usage of chemicals with relatively low toxicity provide a suitable strategy in the preparation of magnetic nanoclusters.

4.7 CoFe₂O₄ Stabilized by L-Alanine

L-alanine is the smaller molecule among the amino acids and a building block of more complex amino acids.

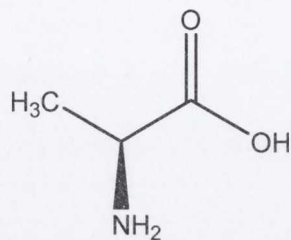


Figure 4.37: Molecular structure of L-Alanine.

Samples of CoFe₂O₄@L-Ala nanoparticles were prepared as in the previous experiments in this chapter. The set of experiment was performed by varying the concentration of ligand. The aim was to study the influence of the ligand on the size, shape and properties of the nanomaterial.

Table 4.7: Alanine stabilized CoFe₂O₄ nanoparticles experiments and average size.

Sample	L-Alanine (mM)	Size (nm)	Ms (Am ² kg ⁻¹)
AL01	1.70	120.5 ± 41.1	82
AL02	3.40	81.2 ± 31.6	68
AL03	6.80	69.2 ± 14.2	78
AL04	13.6	43.2 ± 11.3	65
AL05	27.1	55.7 ± 9.10	79
AL06	54.0	17.1 ± 4.8	48
AL07	108	11.3 ± 2.4	50

TEM images recorded for experiment AL01 (Figure 4.38), showed square shaped nanoparticles with an average size of 120 ± 44.1 nm. Size distribution was calculated for all the samples taking an average of around 200 nanoparticles.

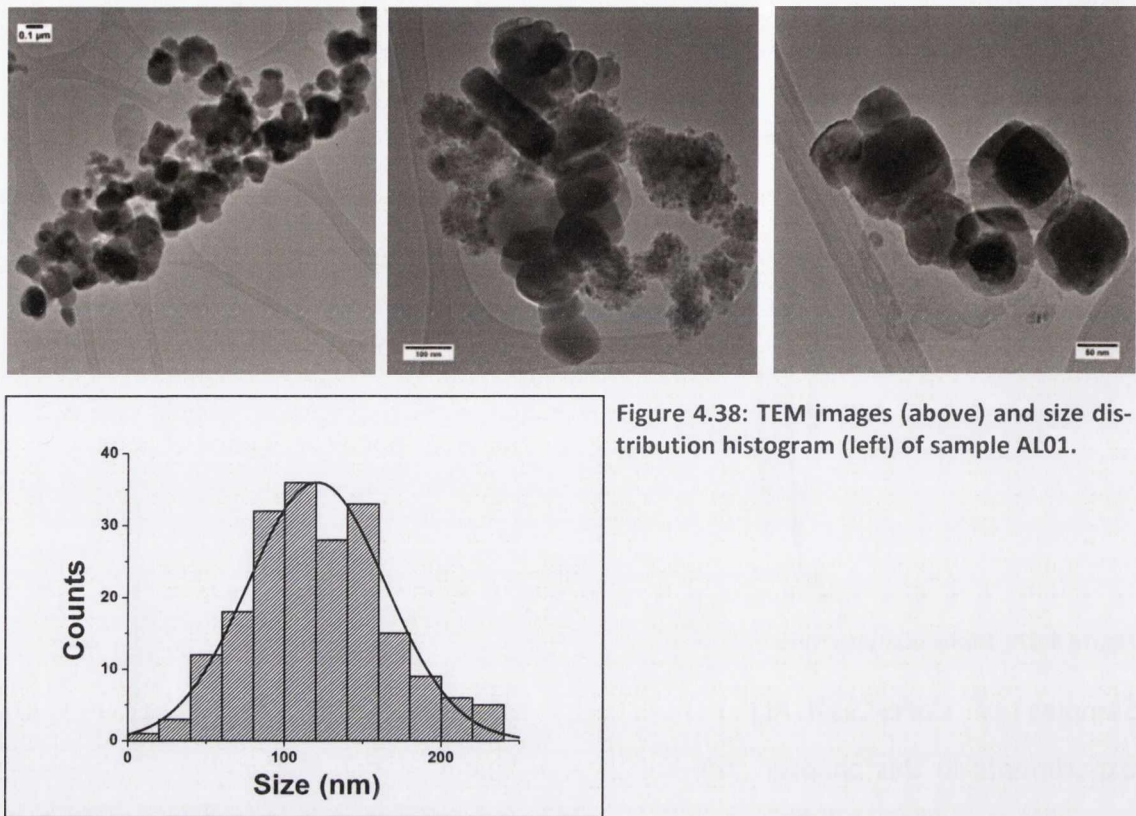


Figure 4.38: TEM images (above) and size distribution histogram (left) of sample AL01.

Magnetization moment for the sample AL01 (1.70 mM ligand) is shown in Figure 4.39. The VSM curve is a typical hysteresis loop for a ferromagnetic material with a saturation magnetization of $82 \text{ Am}^2\text{kg}^{-1}$ and a remanent magnetization of $40 \text{ Am}^2\text{kg}^{-1}$.

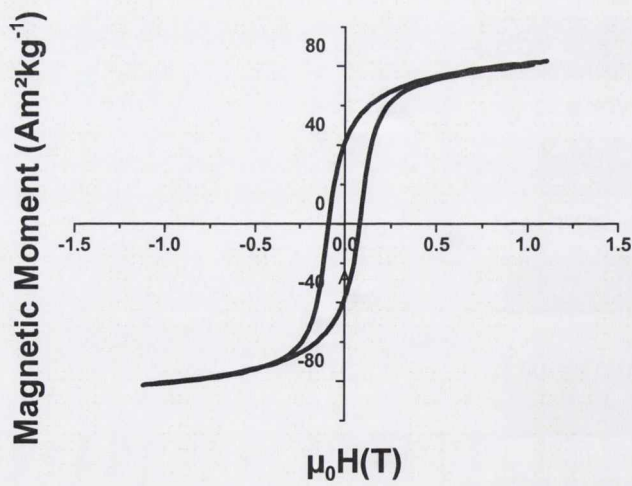


Figure 4.39: VSM curve of $\text{CoFe}_2\text{O}_4\text{@L-Ala}$ (AL01).

The X-ray diffraction pattern of the obtained material is shown in Figure 4.40. The diffraction pattern confirms the formation of CoFe_2O_4 with the expected inverse spinel structure without the presence of impurity. The lattice parameter was found to be 8.404 Å.

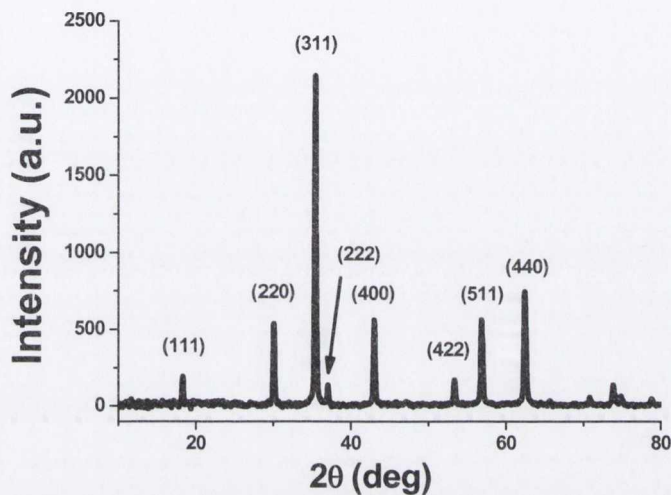


Figure 4.40: XRD pattern of CoFe_2O_4 @L-Ala (AL01).

For the experiment carried out with 3.40 mM of L-Alanine, (AL02), the TEM images show the formation of near squared nanoparticles with some aggregation. The average size distribution for this sample was found to be 81.2 ± 31.6 nm.

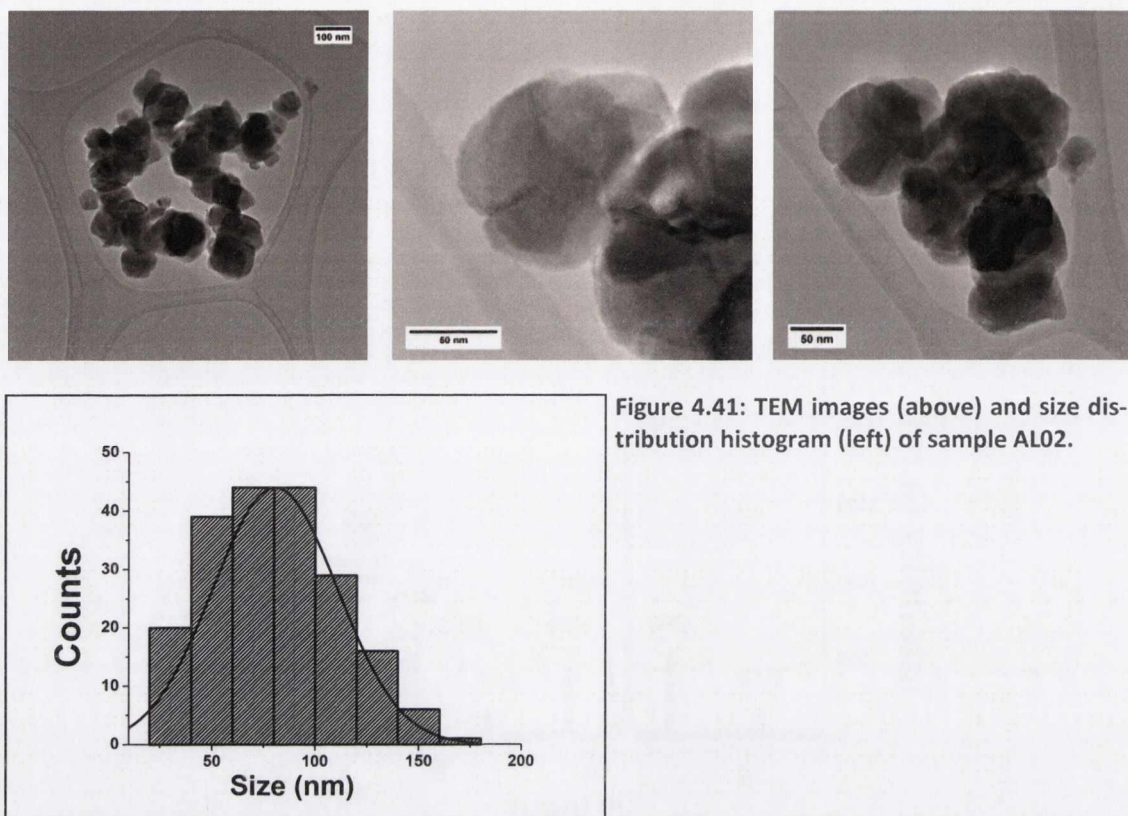


Figure 4.41: TEM images (above) and size distribution histogram (left) of sample AL02.

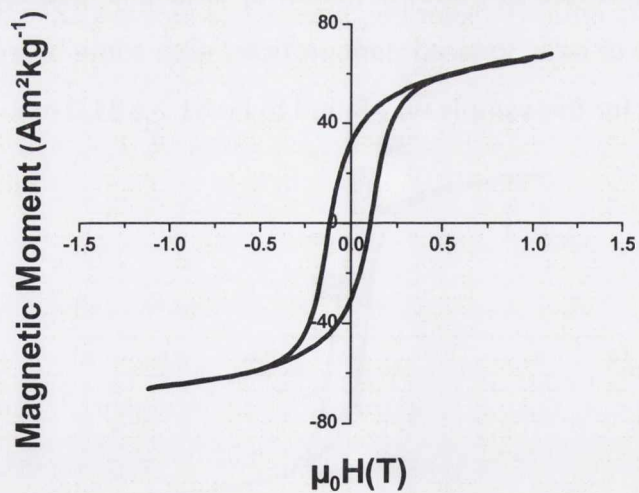


Figure 4.42: VSM curve of $\text{CoFe}_2\text{O}_4@\text{L-Ala}$ (AL02).

Magnetic measurements showed a saturation magnetization of $68 \text{ Am}^2\text{kg}^{-1}$ and a remanent magnetization of $31 \text{ Am}^2\text{kg}^{-1}$. This modest decrease in the M_s is a result of the increase in the concentration of ligand.

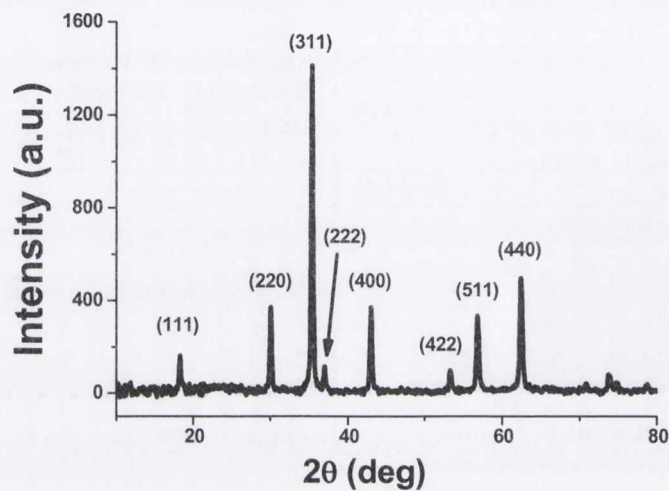


Figure 4.43: XRD pattern of $\text{CoFe}_2\text{O}_4@\text{L-Ala}$ (AL02).

The formation of a spinel ferrite structure was confirmed by X-ray diffraction pattern. It was found that the increase in the concentration of ligand does not affect the crystallinity of the material; the lattice parameter calculated for this sample was $8.404 \text{ \AA}$.

TEM images of the product from the experiment AL03 (6.80 mM ligand) showed the same aggregation of nanoparticles with some displaying a hexagonal shape. The average size calculated in this experiment was found to be $69 \pm 14 \text{ nm}$.

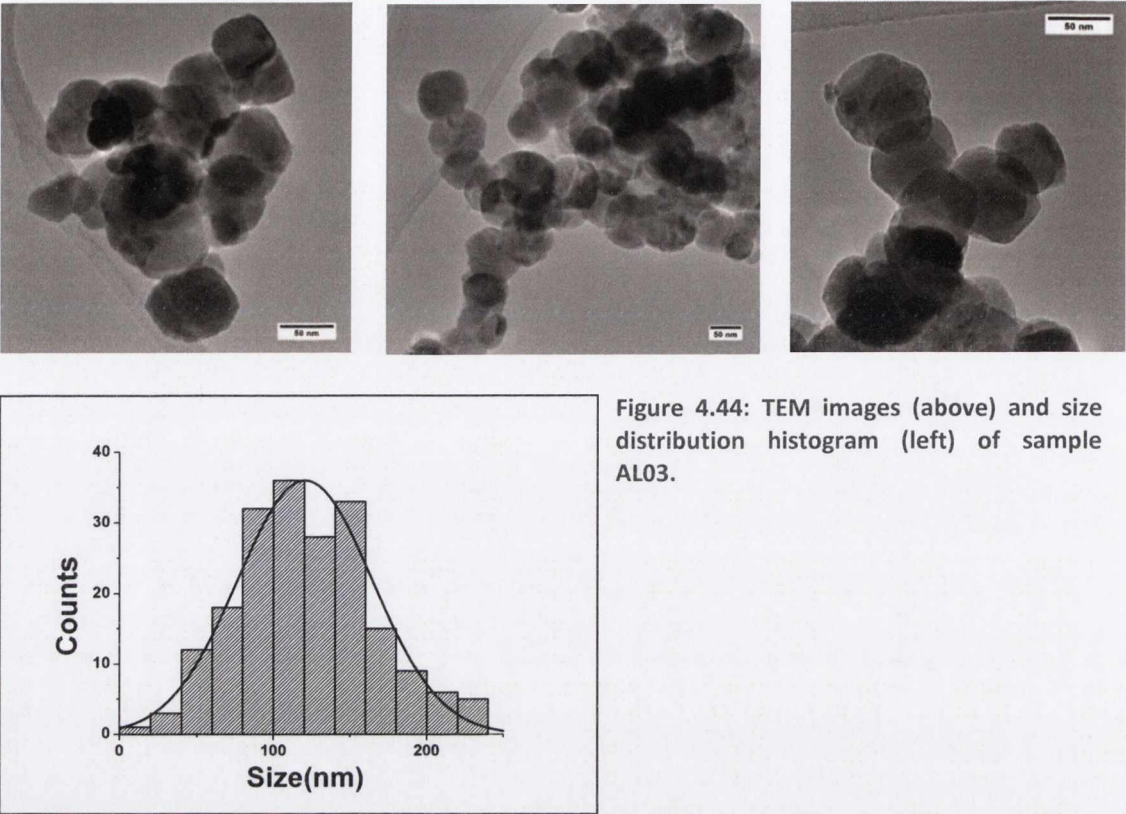


Figure 4.44: TEM images (above) and size distribution histogram (left) of sample AL03.

Magnetic measurements showed a curve typical for a ferromagnetic material. Saturation magnetization for this experiment was $78 \text{ Am}^2\text{kg}^{-1}$ with a remanent magnetization of $38 \text{ Am}^2\text{kg}^{-1}$. Compared to the sample AL01, there is a slight reduction in the magnetization moment of the nanoparticles, but the values are within the same range.

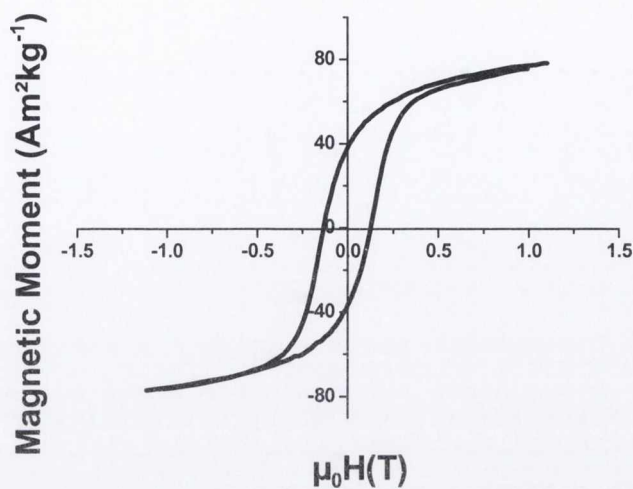


Figure 4.45: VSM curve of $\text{CoFe}_2\text{O}_4@\text{L-Ala}$ (AL03).

The formation of the inverse spinel structure of CoFe_2O_4 was confirmed by X-ray diffraction pattern, no peak for impurity was found. A parameter of the lattice, which did not change as in previous experiments, was 8.404 Å.

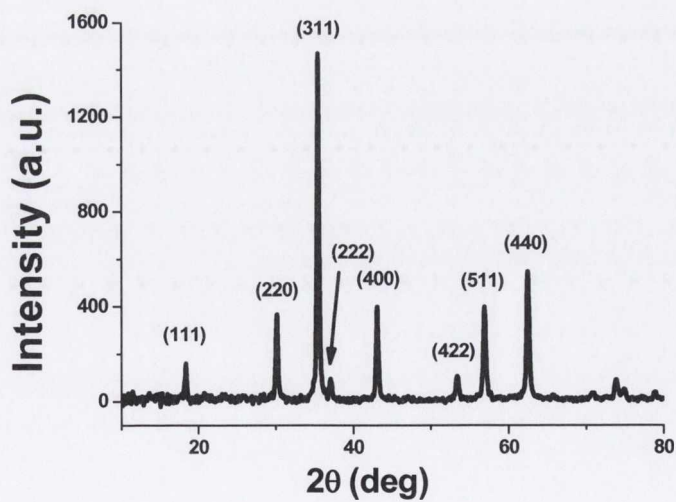


Figure 4.46: XRD pattern of $\text{CoFe}_2\text{O}_4@\text{L-Ala}$ (AL03).

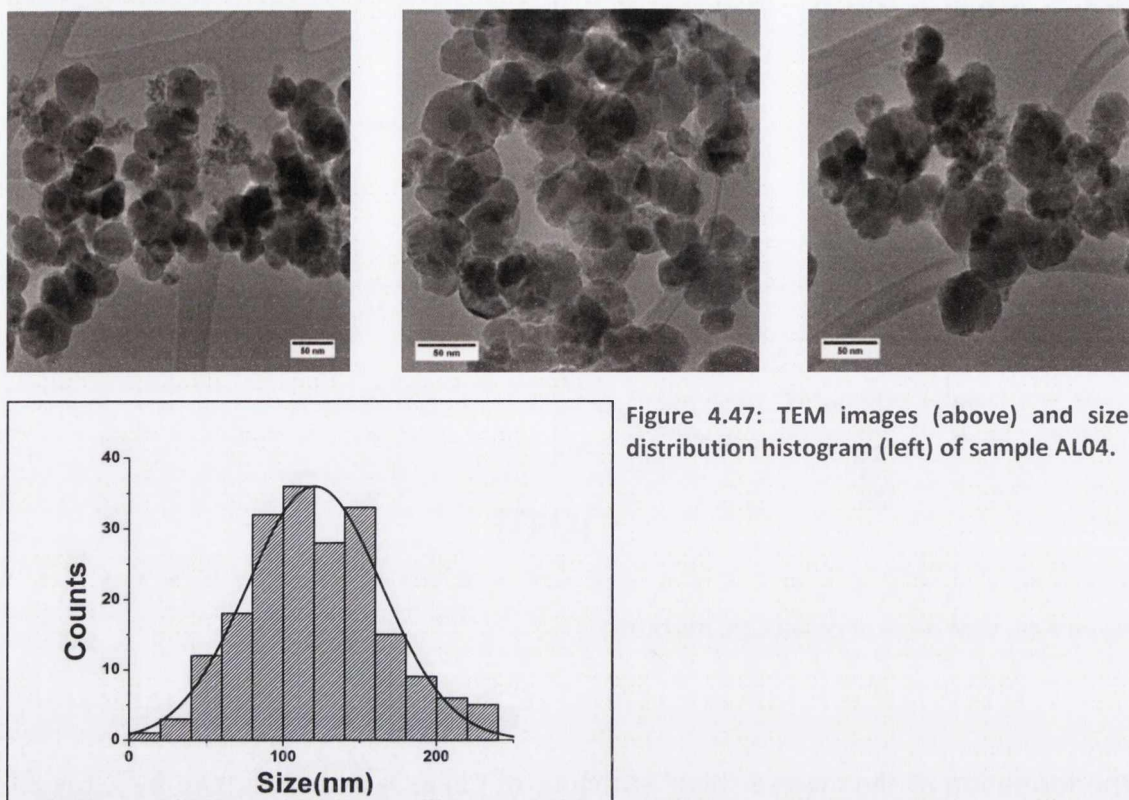


Figure 4.47: TEM images (above) and size distribution histogram (left) of sample AL04.

The size of the nanoparticles in the sample AL04, where 13.6 mM ligand was used, was found to be 43 ± 11 nm. TEM images showed more clearly the formation of nanoplates with a regular hexagonal shape. Comparing these results with the previous ones, it can be observed a clear reduction in the average size of the nanoparticles.

The magnetization curve does not show an evident decrease in in the magnetization that could be related with a reduction of the size of the nanoparticles. Saturation magnetization was found to be $65 \text{ Am}^2\text{kg}^{-1}$ and the remanent magnetization $30 \text{ Am}^2\text{kg}^{-1}$. The reduction from saturation magnetization in sample AL03 is close to 17%. The coercivity obtained from the same graph was 0.200 T.

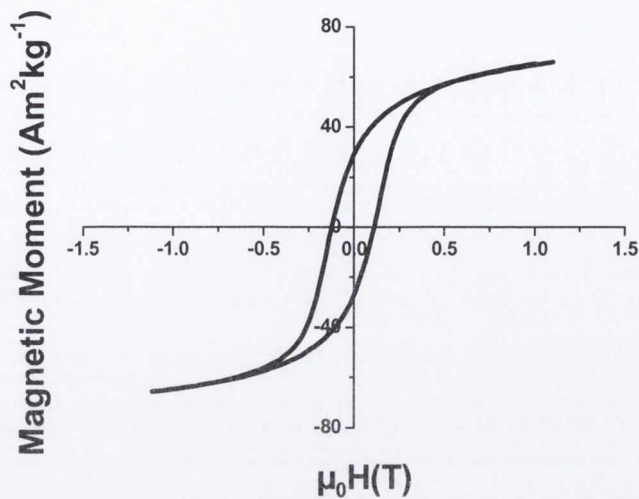


Figure 4.48: VSM curve of $\text{CoFe}_2\text{O}_4@\text{L-Ala}$ (AL04).

The XRD pattern for the sample AL04 shows the formation of spinel ferrite structure without the presence of impurities. Lattice parameter was calculated using the most intense peak (311) and was found to be 8.404 Å.

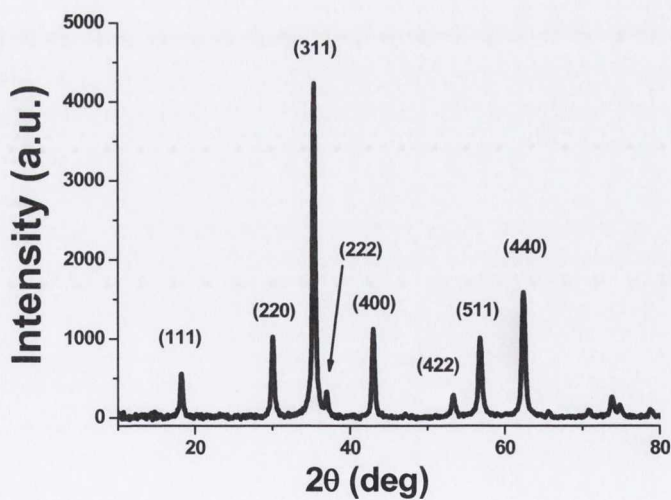


Figure 4.49: XRD pattern of $\text{CoFe}_2\text{O}_4@\text{L-Ala}$ (AL04).

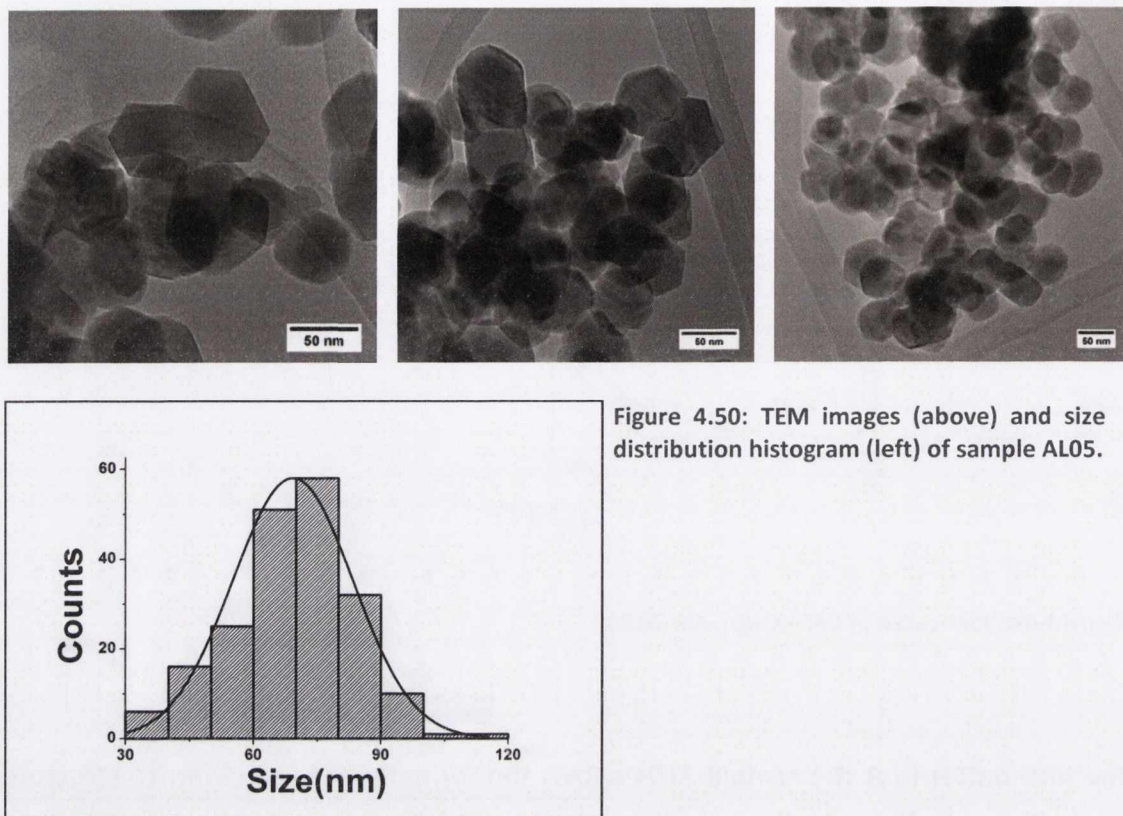


Figure 4.50: TEM images (above) and size distribution histogram (left) of sample AL05.

The increase of the ligand concentration up to 27.1 mM led to the formation of regular nanoplates with a more defined hexagonal shape as can be observed in the TEM images (Figure 4.50). Size distribution calculated for sample AL05 was 56 ± 9 nm; nanoparticles are slightly larger than in the precious experiment with half concentration of ligand (AL04). Magnetization saturation of sample AL05 is $79 \text{ Am}^2\text{kg}^{-1}$ and the remanent magnetization of $43 \text{ Am}^2\text{kg}^{-1}$. The coercivity was 0.100 T.

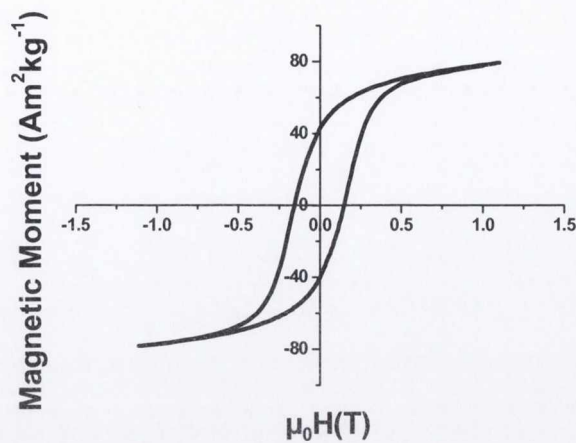


Figure 4.51: VSM curve of $\text{CoFe}_2\text{O}_4@\text{L-Ala}$ (AL05).

XRD pattern for sample AL05 is in agreement with previous experiments and confirms the formation of the crystal structure characteristic of CoFe_2O_4 . No variation is found in the lattice parameter with respect to previous calculations resulting to be 8.404 Å.

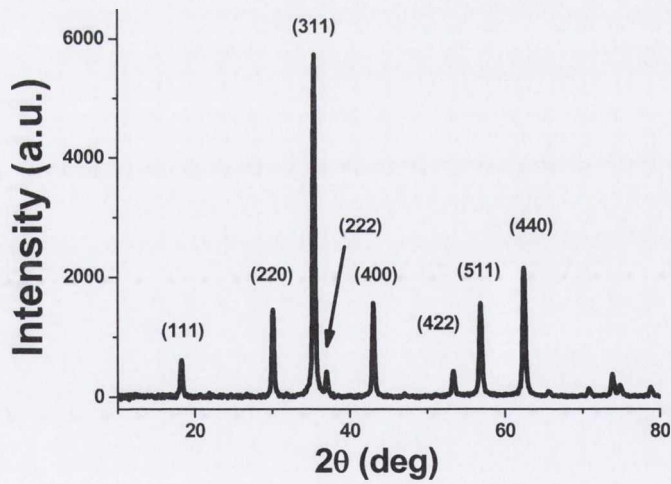


Figure 4.52: XRD pattern of $\text{CoFe}_2\text{O}_4@\text{L-Ala}$ (AL05).

Sample AL06, with 108.8 mM ligand, demonstrated a considerable reduction in the size of the nanoparticles as is shown in TEM images. However, it can be observed that there are two well distinguishable sizes of nanoparticles. The average size was calcu-

lated for larger nanoparticles showing a similar shape to the ones of the other experiments. The size average was 17 ± 5 nm. Hexagonal shape nanoparticles can be seen in the sample.

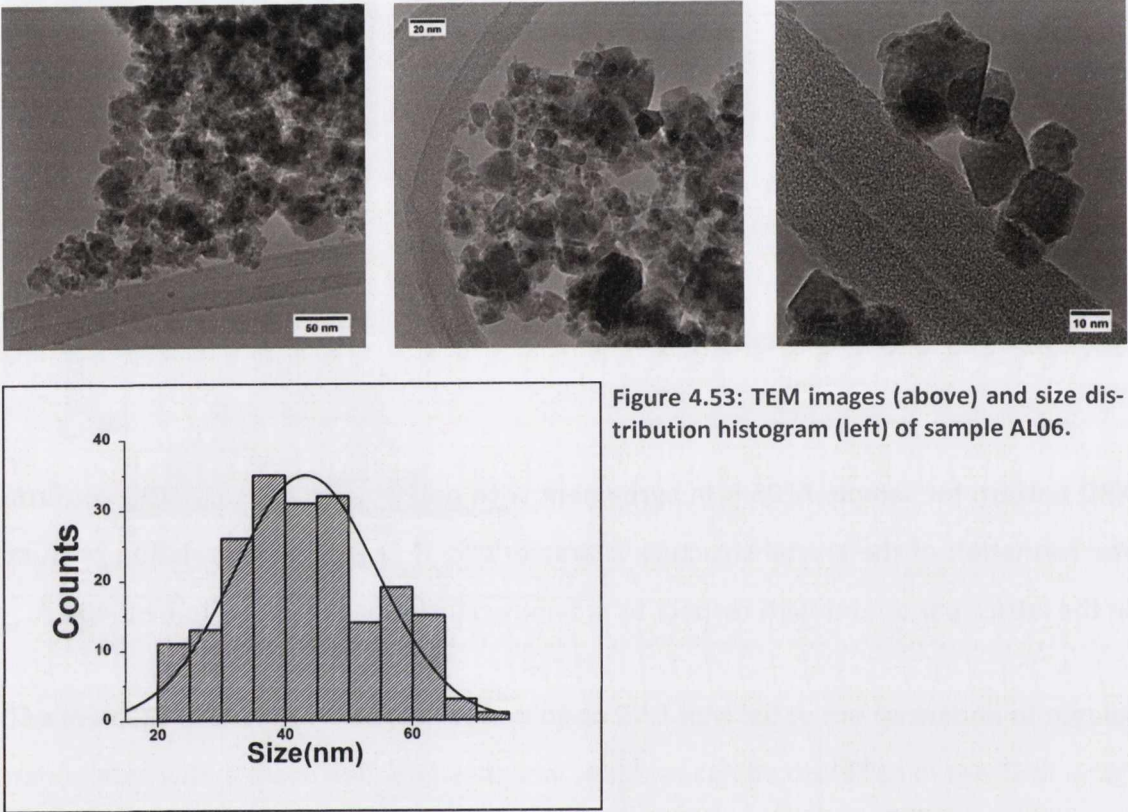


Figure 4.53: TEM images (above) and size distribution histogram (left) of sample AL06.

The VSM curve showed a saturation magnetization of $48 \text{ Am}^2\text{kg}^{-1}$ and a remanent magnetization of $8 \text{ Am}^2\text{kg}^{-1}$. A reduction in the coercivity can be observed in the curve that showed a value of 0.025 T.

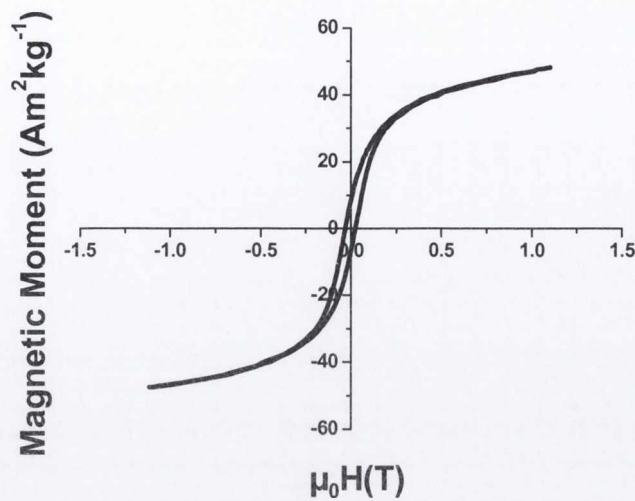


Figure 4.54: VSM curve of CoFe₂O₄@L-Ala (AL06).

XRD showed a broadening in the peaks indicating a reduction in the particle size. The lattice parameter was calculated and found to be 8.363 Å.

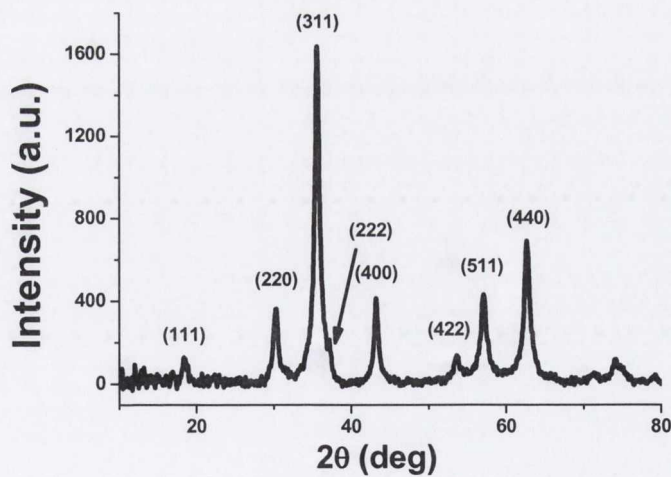


Figure 4.55: XRD pattern of CoFe₂O₄@L-Ala (AL06).

The sample AL07, prepared with 108 mM ligand, showed a further decrease in the size of the nanoparticles. The sample displayed a high degree of aggregation which made

very difficult to calculate the size distribution average, using more than 80 nanoparticles. The nanoparticle size is estimated to 11.3 ± 2.4 nm.

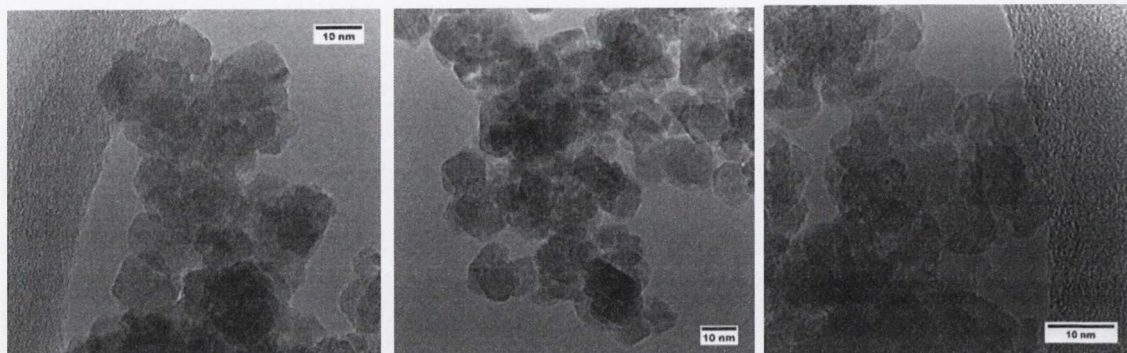


Figure 4.56: TEM images of sample AL07.

With a decrease in the size of the nanoparticles, a decrease in the coercivity of the magnetic material was observed. The VSM curve showed the typical curve for a superparamagnetic material with complete absence of coercivity (no hysteresis loops). M_s for this sample was found to be $50 \text{ Am}^2\text{kg}^{-1}$.

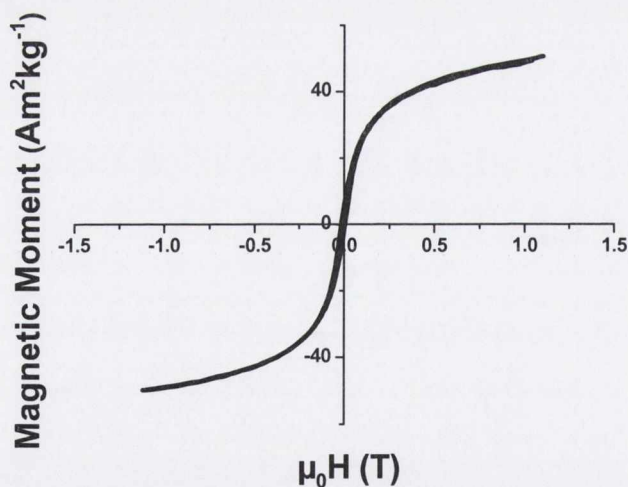


Figure 4.57: VSM curve of CoFe_2O_4 @L-Ala (AL07).

Formation of cobalt ferrite was confirmed by X-ray diffraction pattern. Broad peaks are an indication of the reduction on the size in the nanoparticles compare to precious experiments. The lattice parameter was found to be 8.363 Å for the sample AL07.

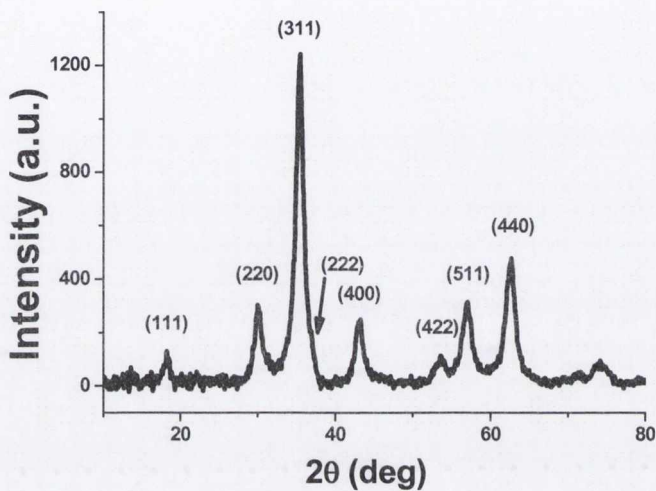


Figure 4.58: XRD pattern of CoFe₂O₄@L-Ala (AL07).

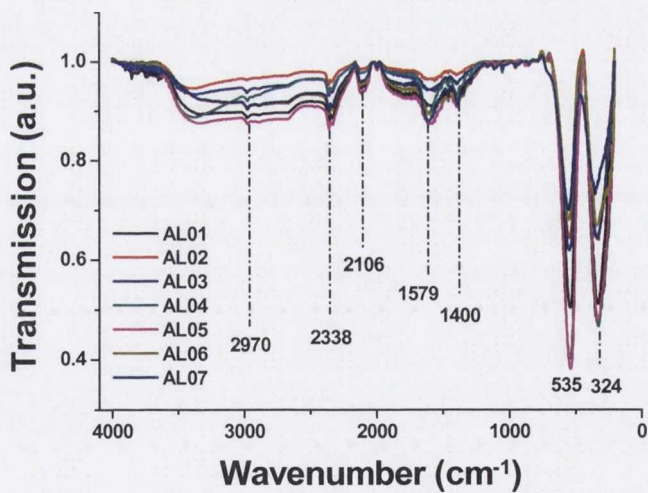


Figure 4.59: FTIR spectra of CoFe₂O₄ stabilised with L-Ala.

Figure 4.59 shows FTIR spectra of CoFe₂O₄ NPs stabilised with L-Ala. As it has been observed on the experiments carried out with other amino acids, the same peaks are present. An increase in the intensity of the band at 2106 cm⁻¹ can be observed. This

band corresponds to the combination of NH_3^+ groups symmetric stretching and bending can be observed.^[48]

The nanocrystal sizes could be controlled by changing the concentration of L-Alanine. Variations in the amount of ligand results in a reduction in the size of the nanoparticles, see Table 4.7. The grain size was initially 120.5 nm (Figure 4.38), where it decreased to 11.3 nm after raising the concentration of ligand (from 1.70 to 108 mM). The decrease in the standard deviation can be considered a narrowing in the size distribution. The strong magnetic interaction between the nanoparticles caused agglomeration. When the degree of agglomeration is reduced, the grains in some samples are nearly hexagonal.

The as-synthesized nanoparticles are ferromagnetic at room temperature with magnetic moment decreasing from $82 \text{ Am}^2\text{kg}^{-1}$ in sample AL01 to $48 \text{ Am}^2\text{kg}^{-1}$ in sample AL06. In addition, a clear decrease in coercivity (from 0.080 to 0.025 T) and M_s (from 40 to $8 \text{ Am}^2\text{kg}^{-1}$) is observed after reducing the particle size.

The ferrite structure is composed of two spinel phases with different cation distribution, which result in a change in the configuration on the surface of the core. Although individually, each phase can exhibit a perfect alignment under the presence of an external magnetic field the spin on the surface of the core are canted leading to a different magnetic behaviour. This effect, called spin-canting, results in a reduction of the magnetic behaviour for smaller particles size.^[49]

The increase in coercivity with increasing volume has been considered due to the increase in magnetic anisotropy since an applied field at a given temperature should be able to overcome the energy barrier and change the orientation of magnetization.

Sample AL07 did not show any coercivity, which is characteristic of superparamagnetic behaviour. The average size in this case agrees with the size range for CoFe_2O_4 in the superparamagnetic regime at room temperature is known to be of 10-30 nm.^[50]

The formation of sample AL06 was followed over time in order to understand the morphology evolution of the nanoparticles. Figure 4.60 shows the TEM images of the nanomaterial produced at different times during the reaction.

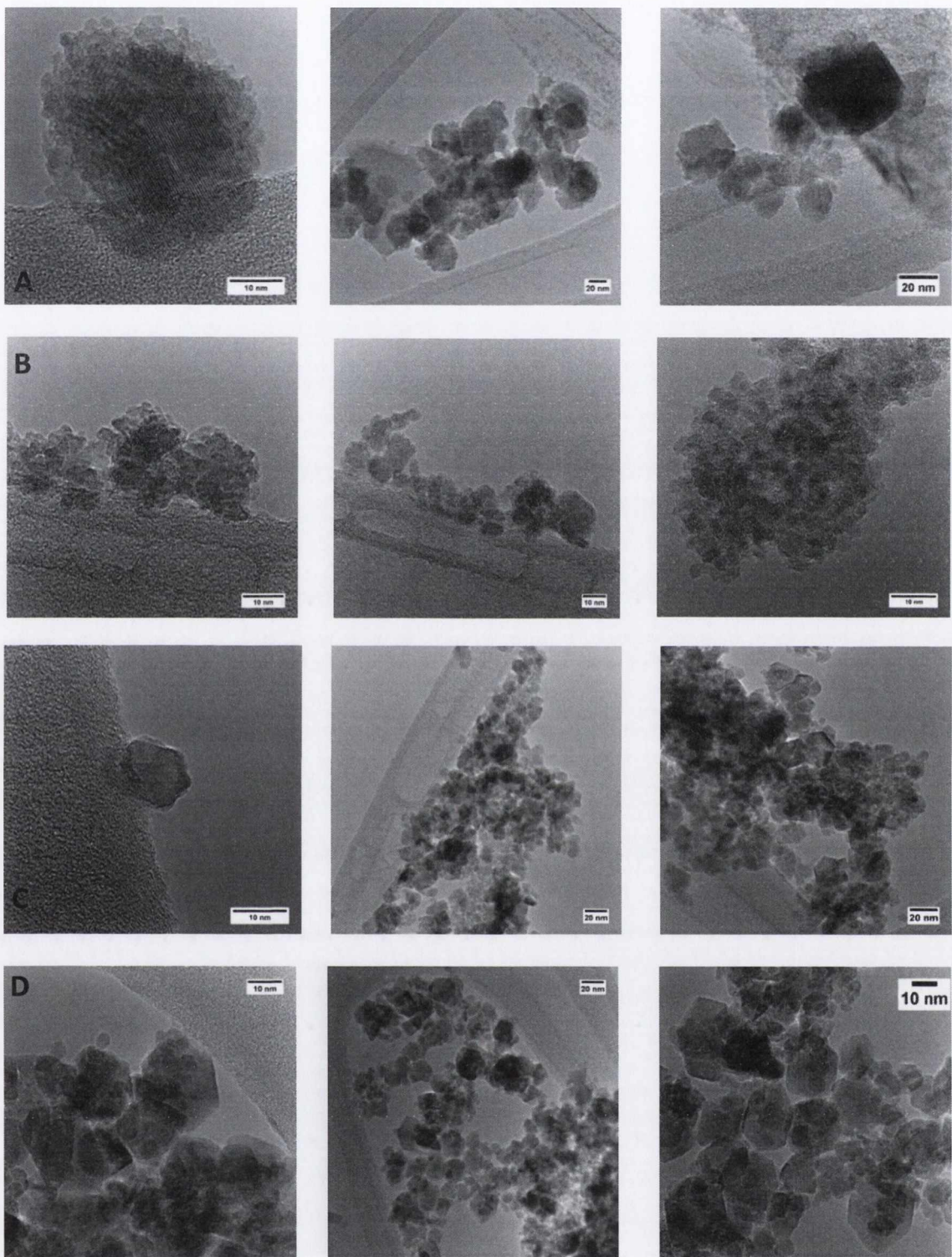


Figure 4.60: TEM images of CoFe_2O_4 @L-alanine at different times; 5 min (a), 10 min (b), 30 min (c) and 2 hours (d).

The evolution of the morphology of nanocrystals depending on the reaction time is clear from the TEM images. After 5 min (Figure 4.60, A), nanoparticles with an average size of 5 nm are already formed and associated into spherical assemblies. These self-assembly structures are also presented after 10 min reaction time. Figure 4.60 (B)

shows the TEM image of nanomaterial after 30 min; hexagonally shaped nanoparticles were formed. The TEM images of the material after the 2 h of reaction have confirmed the production of the hexagon-like nanoparticles along with smaller spherical nanoparticles.

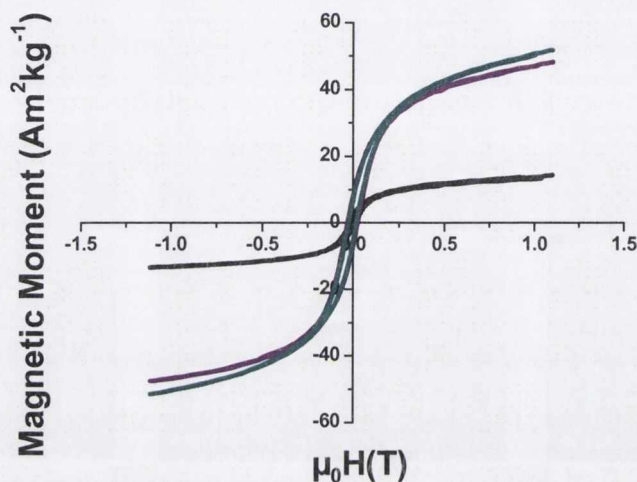


Figure 4.61: VSM curves of $\text{CoFe}_2\text{O}_4@\text{L-alanine}$ at different times; 5 min (black), 60 min (purple) and 2 hours (green).

Figure 4.61 shows the evolution of the magnetic behaviour during the reaction. M_s after 5 min of the reaction was found to be $14 \text{ Am}^2\text{kg}^{-1}$. After 1 h reaction the M_s increased to $48 \text{ Am}^2\text{kg}^{-1}$. This value is similar for that of the material after 90 min, which was found to be $53 \text{ Am}^2\text{kg}^{-1}$. It can be concluded that after 1 h the magnetic nanoparticles have fully formed and longer reaction times do not affect the magnetic properties of the material.

X-ray pattern at different times were collected (Figure 4.62). The main peaks for spinel ferrite structure were observed at any time point during the reaction. At the earliest measurement of 5 min no significant presence of impurities was found.

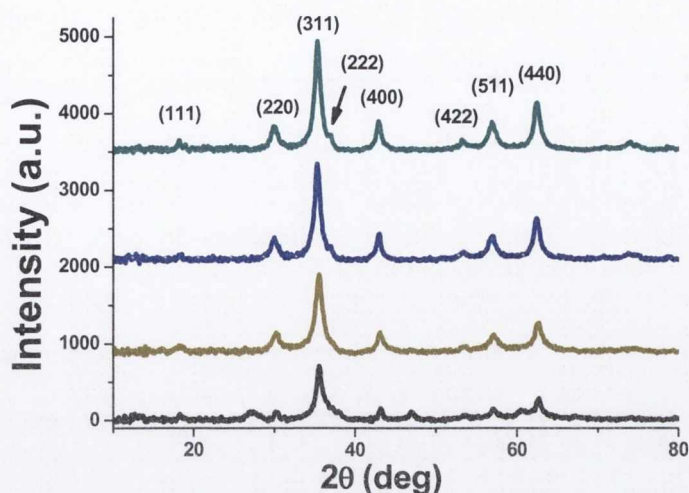


Figure 4.62: XRD patterns of CoFe_2O_4 @L-alanine at different times; 5 min (black), 10 min (yellow), 30 min (blue) and 2 hours (green).

Overall, the usage of L-Ala as stabiliser during the synthesis of CoFe_2O_4 NPs has been shown to affect the size and morphology of the NPs. An increase on the concentration of the ligand leads to the reduction of the grain size. This reduction in the size results in the formation of CoFe_2O_4 with superparamagnetic properties.

4.8 Conclusions

In conclusion, we have developed a new protocol to synthesise a series of CoFe_2O_4 magnetic NPs stabilised with amino acids. The use of amino acids during the formation of the magnetic NPs affects the size of the nanoparticles whilst their magnetic properties remain strong enough that the material can be retrieved by an external magnet. When amino acids such as D-Penicillamine, D- and L- Cysteine, D- and L-Aspartic Acid and D-Glutamic Acid are used the resultant material form clusters (50-70 nm) of smaller individual NPs (3-5nm). The formation of these clusters is driven by an oriented attachment in which smaller NPs merge into larger ones in order to reduce the surface energy and they align following the same crystal orientation.

The size and shape of CoFe_2O_4 NPs can be modified by varying the concentration of stabiliser when L-Alanine is used. By increasing the amount of ligand, the size of the NPs is reduced up to a point that superparamagnetic behaviour in the material is reached. As the size and magnetic properties of the material can be controlled, we be-

lieve that this new protocol is an important approach in the synthesis of magnetic NPs to be able to design of magnetic NPs with interesting applications, such as catalysis, biomedical applications or data storage.

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Chapter 5. Development of Separation Techniques using Magnetic Nanoparticles

5.1 Introduction

Magnetic separation techniques employing magnetic particles coated with different shells and functional groups have been used progressively for biotechnology applications. There is a wide range of separation and isolation procedures used in the areas of biosciences and biotechnology. One of the most essential processes in molecular biology is the isolation and extraction of DNA and other nucleic acids from cells.^[1-2]

There is a fast growing development of new solid supports and/or surface modification protocols to enhance the isolation and/or recovery efficiency of DNA on the carrier surface.^[3] The adsorption of DNA molecules on solid supports is driven through hydrogen-bonding, hydrophobic/hydrophilic, and electrostatic interactions, which are intimately associated with the surface condition of the solid support. MNPs can be surface-modified with functional groups that play an important role in physiological efficiency, for example polyvinyl alcohol,^[4] dextran^[5] and other compounds with different molecular weights.^[6] Another approach is the coating of MNPs with inorganic materials (e.g., silica and gold) to achieve high loading (isolation) efficiency.^[7-8] Among these, silica has been widely selected as one of the most effective solid phases to purify plasmid DNA for its several features of straightforward synthesis, easily modification and biocompatibility.^[9-11] Silica with different morphologies and surface functionalization has been used in DNA isolation. For example, positively charged silica (e.g. functionalised with amino groups) can increase the electrostatic affinity to the negatively charged phosphate backbone of DNA.^[12-13]

Another chemical application is related to the enantiomeric separation or isolation of molecules from a racemic mixture. A racemic mixture is composed of identical amounts of left and right-handed enantiomers or chiral molecules. Chiral molecules are very common in natural and synthetic compounds. They play vital roles in chemical

and biological recognition processes and biopharmaceutical technology, as most of the currently used drugs are carrying chiral entities. Most importantly, the enantiomers can show completely different physiological and biological activities depending on their configurations, as a result a high enantiomeric purity of the compounds is crucial for biopharmaceutical and food industries. Therefore, there is a lot of attention focused on this area.

One of the most widely used methods in production of enantiomerically pure compounds is asymmetric synthesis. However, it is very challenging to achieve high selectivity and enantiomeric excess in many chemical reactions. There are some other techniques used for enantiomeric separation, such as high performance liquid chromatography (HPLC)^[14] or chiral gas chromatography,^[15] enzymatic resolution,^[16] membrane separation^[17-18] or capillary electromigration.^[19] However, most of these techniques are expensive, time consuming or both. Therefore, there is a continuous demand for new efficient methods for the separation of racemic mixtures.

Large surface area, size, biocompatibility, low toxicity and easy manipulation make magnetic nanoparticles good candidates for enantiomeric separation of chiral compounds. To the best of our knowledge, very little work has been performed in this field. Chiral molecules with chiral recognition can be immobilized on the surface of the MNPs and be used for enantiomeric separation in a racemic mixture. Some work has been done using a chiral aromatic amino acids with this purpose.^[20] Another strategy is to bind the chiral selector or amino acid to silica coated MNPs.^[21-22]

5.2 Aims of This Chapter

There are several goals related to this part of the work. One of our main aims is to develop new techniques for DNA separation and purification using silica coated magnetic nanomaterials and permanent magnets.

Another aim is to investigate whether chiral ligand stabilized CoFe_2O_4 nanoparticles can be used for potential magnetic separation of racemic enantiomeric molecules. The

plan is to employ different magnetic NPs stabilized with chiral molecules to test for the enantiomeric magnetic separation of a racemic mixture of D- and L-Pen. In theory, the NPs are expected to show preferential binding to one of the enantiomers. The enantiomeric separation in solutions will be investigated by Circular dichroism (CD) spectroscopy.

5.3 Purification of DNA using Silica Coated Magnetic Nanocomposites

Initial magnetic materials used in this section have been provided by the Functional Materials Laboratory (FML), ETH Zurich. Carbon-coated magnetic nanoparticles were synthesized using flame spray pyrolysis.^[23] The reaction used to produce Co@C and Fe@C nanoparticles involved the combustion of the appropriate metal compound in the presence of organic precursors and fuel.^[24]

5.3.1 Preparation and Characterisation of Co@C@SiO₂ Nanoparticles

The preparation of carbon-coated cobalt and iron nanoparticles functionalized with OH and NH₂ groups was carried out by modifying the method described by Schätz *et al.*^[25] Carbon-coated cobalt nanoparticles were functionalized using 4-(Aminomethyl) aniline in the case of NH₂ functionalization. For NPs functionalized with OH group, 2-(4-aminophenyl) methanol was used. The reactions were carried in water, in the presence of NaNO₂ and HCl. This covalent functionalization on the carbon coated metal nanoparticles facilitates the growth of the silica shell.

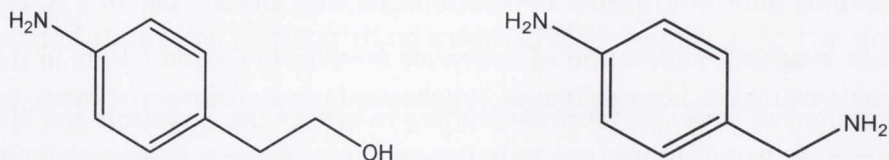
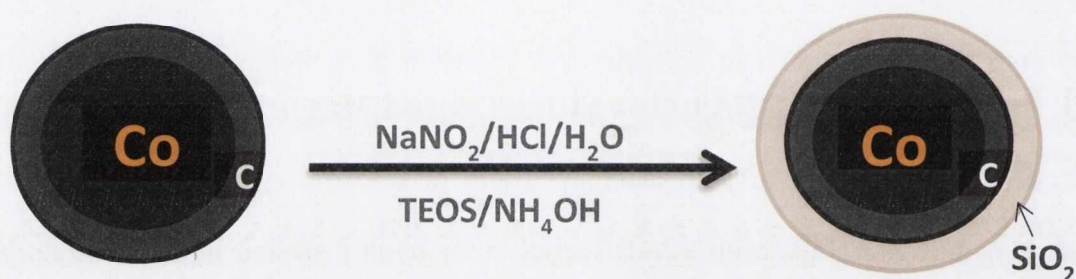


Figure 5.1 : Molecular structure of 2-(4-aminophenyl) methanol (left) and 4 (Aminomethyl) aniline (right) used in the functionalization of Fe@C and Co@C NPs.



Scheme 5.1: Overall reaction scheme of the OH and NH_2 functionalization of nanobeads and further silica coating.

TEM images for the Co@C nanoparticles showed that the material has a broad size distribution (Figure 5.2). Nanoparticle sizes range from 20 nm to 200 nm in the samples. However, the carbon layer it is clearly visible, forming a shell surrounding the magnetic NPs.

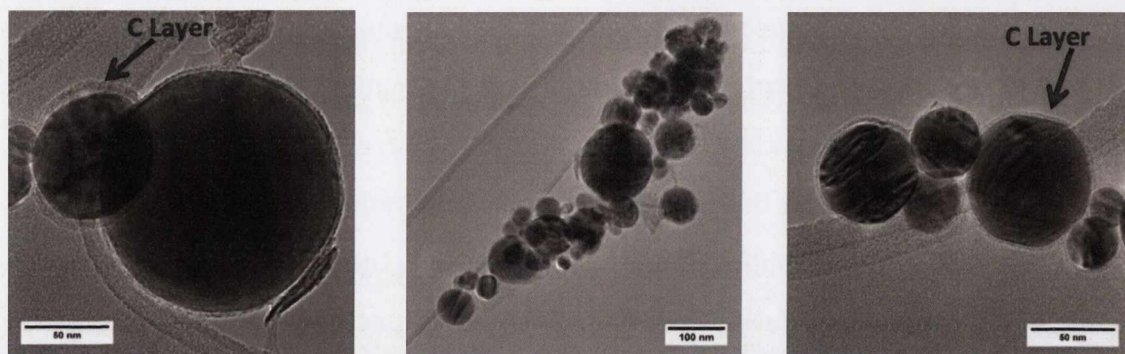


Figure 5.2: TEM images of Co@C nanoparticles.

One of the main characteristics of the nanomaterial is its high stability against oxidation due to the presence of the previously mentioned carbon layer.

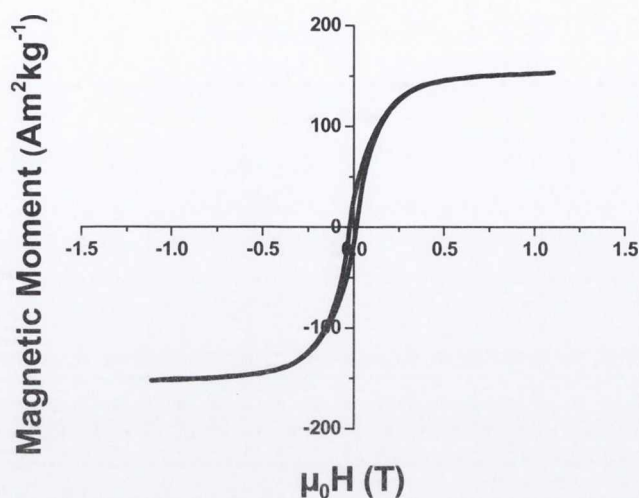


Figure 5.3: VSM curve of carbon coated cobalt nanoparticles.

Another important feature is the high saturation magnetization. The measure saturation magnetization for Co@C nanoparticles is $151 \text{ Am}^2\text{kg}^{-1}$ (Figure 5.3). This value makes the material suitable for further non-magnetic coating whilst an elevated magnetization moment is preserved.

5.3.2 Preparation and Functionalization of Co@C@SiO₂ Nanobeads

Silica coating of NH₂-functionalized Co@C nanobeads was carried out by different approaches in order to optimise their functionality.

The first approach used was the standard Stöber method using TEOS as the silica precursor. This is the most straightforward way to synthesize silica nanoparticles and accepts many different modifications.

A defined amount of Co@C beads were dispersed in 10 mL of ethanol by sonicating the solution for 30 min. This reaction was performed on a small scale with a high concentration of NH₄OH to ensure the condensation and polymerization of the silica precursor. 2.5 mL of NH₄OH was rapidly added to the solution, followed by the addition of 40 μL of TEOS. The reaction was kept in a sonic bath at low temperature ($\approx 0^\circ\text{C}$) over-

night. The product of the reaction was washed with ethanol three times and stored in the same solvent for future use.

STEM analysis of the black magnetic product (Figure 5.4) was performed and the images showed the nanoparticles with high degree of aggregation. Silica NPs and Co@C beads can be observed in the images, although is difficult to see a uniform silica coating surrounding the magnetic NPs.

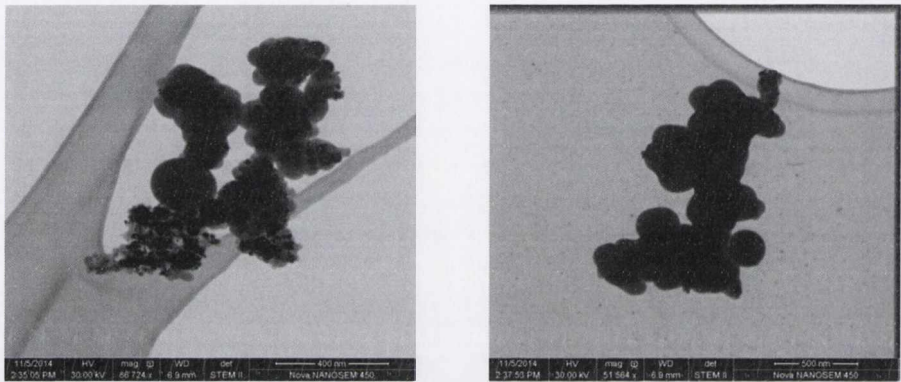


Figure 5.4: STEM images of Co@C@SiO₂ beads, small scale reaction.

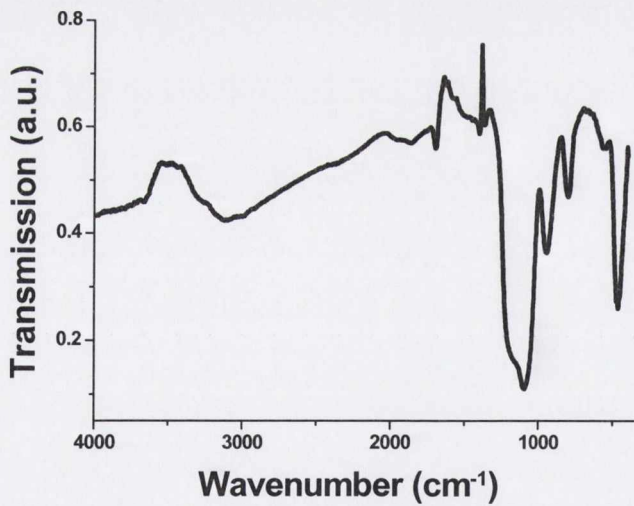


Figure 5.5: FTIR spectrum for Co@C@SiO₂ beads.

FTIR spectrum for the Co@C@SiO₂ synthesis is shown in Figure 5.5. It shows typical bands for the aromatic ring at 1630 cm⁻¹ and C-H stretching vibrations at 1380 cm⁻¹. A broad peak around 3400 cm⁻¹ can be assigned to the OH groups. The presence of silica

was demonstrated by the bands around 800, 950 and 1100 cm^{-1} which are assigned to the symmetrical and asymmetrical vibrations of the Si-O-Si bonds and the stretching vibration of the hydroxyl group, respectively.^[26]

Following the same synthesis on a larger scale, silica coating was produced using dodecyltriethoxysilane (DTES) as the silica precursor. After dispersing the nanoparticles in ethanol in a sonic bath, NH_4OH and DTES were added to the solution. The mixture was sonicated for 2 hours at low temperature ($\approx 0^\circ\text{C}$).

The STEM images of the resulting product are shown in Figure 5.6. It is apparent that only an incomplete silica coating was achieved in the experiment. Single silica NPs can also be observed, as well as free Co@C beads.

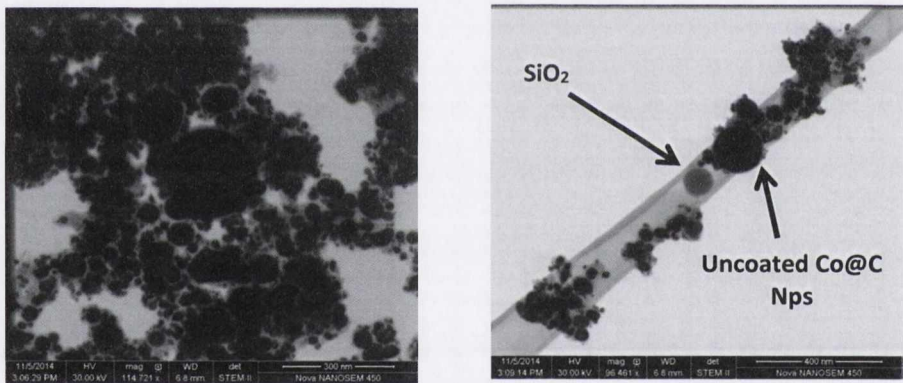


Figure 5.6: STEM images of Co@C@SiO₂ nano beads using n-dodecyltriethoxy silane as silica precursor.

Uncoated metal NPs may cause problems during the application process due to the presence of DNA. The strong interaction of the phosphate backbone with the metal can cause difficulty in the release of the macromolecule of interest.^[27] Therefore, this material was not tested in the purification of DNA.

5.3.3 Preparation and Characterisation of Fe@C@SiO₂ Nanoparticles

Fe@C nanoparticles have similar characteristics to those described for the Co@C nanoparticles presented above. The functionalization of the nanoparticles was carried

out using the same protocol using 2-(4-aminophenyl) methanol to introduce the functional OH groups.

Figure 5.7 shows the TEM images obtained; nanoparticles have different size from around 20 nm to 200 nm. Several carbon layers coating the metal core can be observed.

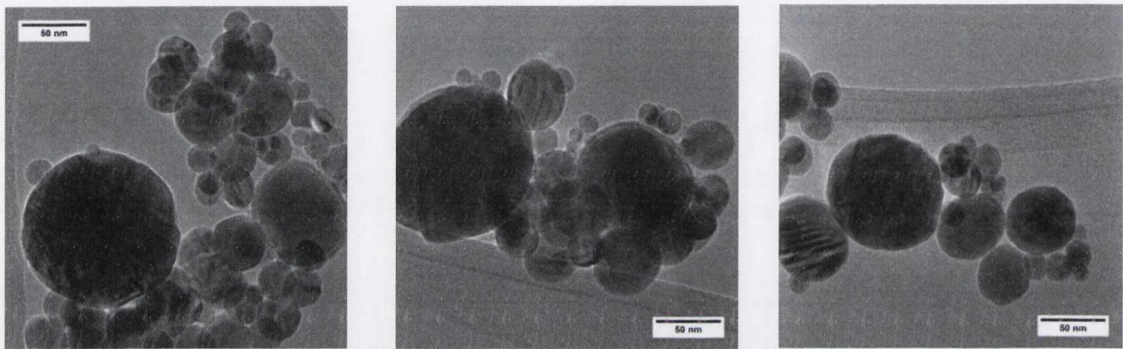


Figure 5.7: TEM images of Fe@C nanoparticles.

The saturation magnetization was found to be $135 \text{ Am}^2\text{kg}^{-1}$ with a low coercivity (Figure 5.8). High saturation magnetization facilitated the retrieving of the nanoparticles from the solution and it is important to assure that an additional non-magnetic coating should not heavily affect magnetisation.

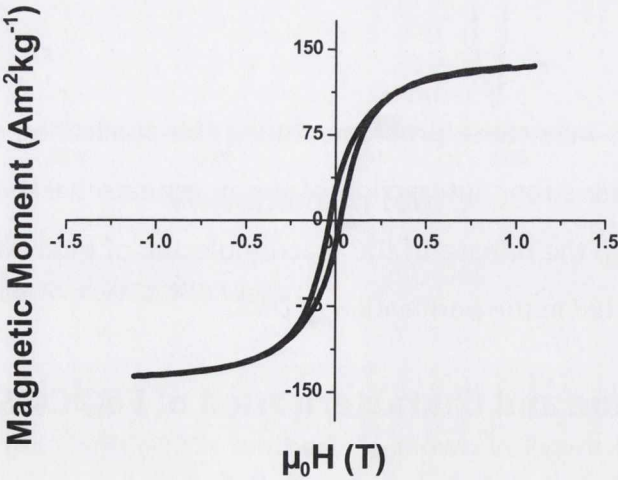


Figure 5.8: VSM curve of Fe@C nanoparticles.

The production of Fe@C@SiO₂ NPs was carried out using the Stöber method, with some modifications. A defined amount of Fe@C beads was dispersed in ethanol, assisted by sonication for 30 min. TEOS was added to the solutions and the mixture was allowed to disperse under sonication for another 30 min. Finally, NH₄OH was added and the synthesis was continued for 2 hours at low temperature ($\approx 0^\circ\text{C}$).

By adding the catalyst (NH₄OH) to the reaction last and after the dispersion of the rest of the reactants, the silica was allowed to assemble on the surface of the nanoparticles, before the formation of the coating.

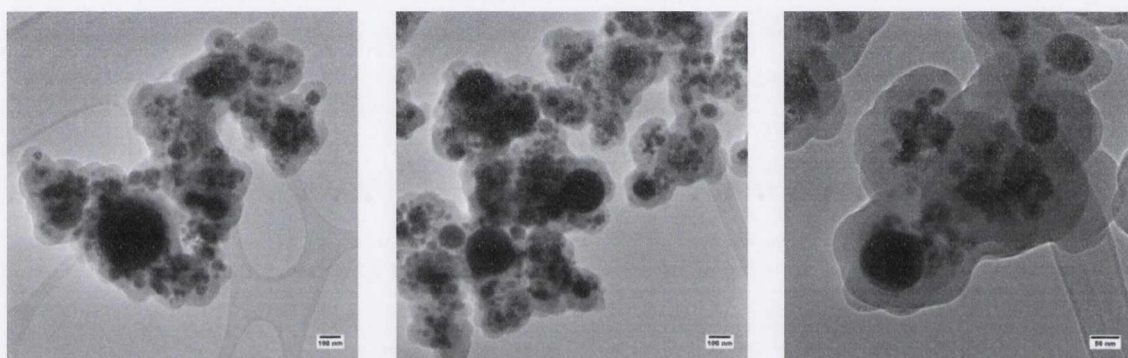


Figure 5.9: TEM images of Fe@C@SiO₂ NPs.

TEM images of Fe@C@SiO₂ NPs (Figure 5.9) confirm the formation of an approximately 50 nm thick silica shell around the magnetic NPs. Magnetic NPs without shell are not observed throughout the sample.

According to VSM data in Figure 5.10, there is a reduction in the M_s for the Fe@C@SiO₂ NPs compared to the M_s of the Fe@C beads without coating. The magnetization moment dropped from $135 \text{ Am}^2\text{kg}^{-1}$ to $68 \text{ Am}^2\text{kg}^{-1}$, indicating the formation of the silica shell has halved the magnetic properties of the material.

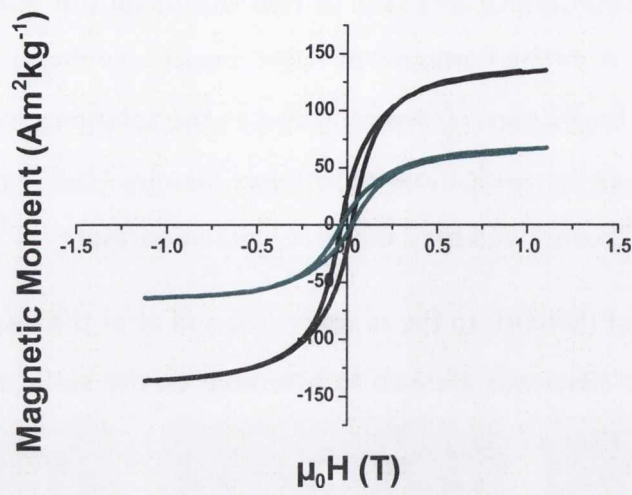


Figure 5.10: VSM curves of Fe@C@OH (black) and Fe@C@SiO₂ NPs (green).

The FTIR spectrum of the Fe@C@SiO₂ NPs (Figure 5.11) shows the characteristic peaks for SiO₂ (800, 950, and 1100 cm^{-1}) assigned to the symmetrical and asymmetrical vibrations of the Si–O–Si bond and the stretching vibration of the hydroxyl group, respectively.^[26]

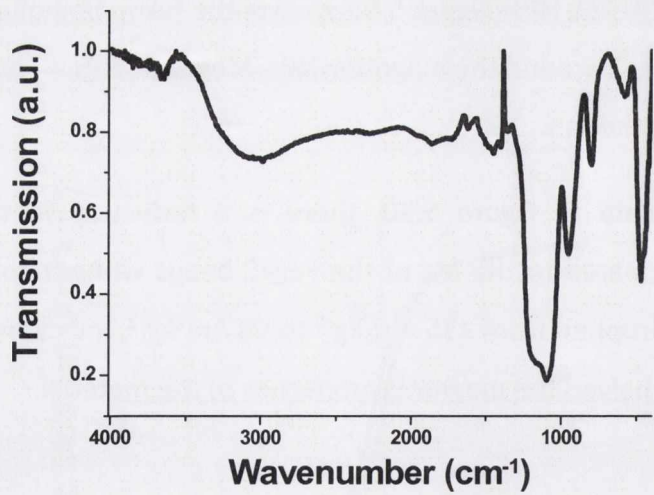
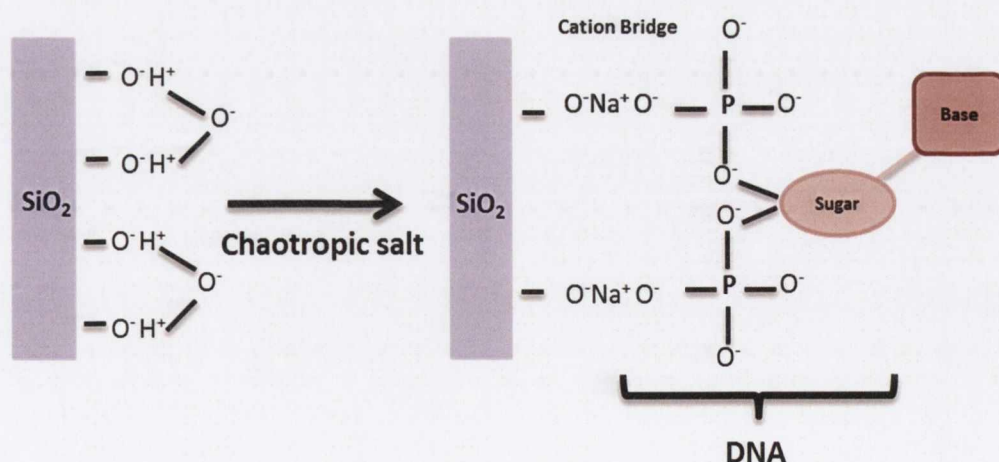


Figure 5.11: FTIR spectrum of Fe@C@SiO₂ nanoparticles.

5.3.4 DNA Extraction using Fe@C@SiO₂ Magnetic Nanoparticles

The DNA extraction technique described here utilizes the commercially available QIAquick[®] PCR Purification Kit in parallel with the Fe@C@SiO₂ nanobeads. QIAquick[®] uses spin columns with a silica-based membrane. These spin columns are originally designed to capture polymerase chain reaction (PCR) products that are larger than 100 base pairs (bp) of DNA and smaller than 10 kilobase pairs (kb = 1000 bp), while at the same time excluding nucleotides, proteins, and salts. DNA used for the experiment was O'RangeRuler 20 bp DNA Ladder with fragments of 20 bp steps between 20-300 bp.

The way DNA binds to silica is based on the action of guanidinium HCl (GuHCl), present in the binding buffer, which acts as a chaotropic agent. A chaotropic agent denatures biomolecules by disrupting hydrogen bonding and the hydration shell around them. This allows positively charged ions to form a salt bridge between the negatively charged silica and the negatively charged DNA backbone in solutions with the high salt concentration. The DNA can then be washed with high salt solutions and ethanol to remove other impurities. Ultimately, DNA can be eluted with low-ionic strength buffer solution or even water (Scheme 5.2).



Scheme 5.2: Schematic presentation of the interaction between SiO₂ and DNA.

The concentration of DNA recovered was quantified by using a Qubit® fluorimeter with a Qubit® dsDNA (high Sensitivity) Assay Kit, which is highly selective for double-stranded DNA (dsDNA). The Fluorimeter measures the fluorescence intensity of a fluorescent dye bound to DNA enabling to determine the concentration. The DNA wavelength of absorbance is 260 nm, (same values are found for RNA), while 280 nm corresponds to proteins. Therefore, UV absorbance methods are not accurate for determining DNA concentrations. The fluorimeter is more sensitive than a UV spectrophotometer and the intensity in fluorescence is directly proportional to the concentration, which increases when the concentration of DNA in the sample increases. The dyes are composed of advanced molecular probe fluorophores. Although these compounds initially have low fluorescence intensity, when they bind to DNA the fluorescence increases by several orders of magnitude. Once standards of known concentration have been measured, the concentration of a sample can be calculated using a calibration curve.

Table 5.1: Composition of buffers used in the DNA extraction protocol.

Buffer	Composition
Buffer PB (Binding buffer)	5 M Gu-HCl 30% isopropanol
Buffer PE (Washing buffer)	10 mM Tris-HCl pH 7.5 80% ethanol
Buffer EB (Elution buffer)	10 mM Tris-Cl, pH 8.5

Different concentrations of DNA were prepared to test the efficiency of the separation by the nanoparticles. During the purification process a set of different volume of NPs (10, 50 and 100 μL) dispersed in ethanol were tested. The concentration of this solution containing the NPs in ethanol was 10 mg mL^{-1} (Suspension of nanoparticles in ethanol). Eppendorf tubes were used to combine the required volume of NPs and 250 μL of binding buffer (buffer PB). The mixture was sonicated for a few minutes to allow the dispersion of the nanoparticles and, in this way, increase the surface area of the beads available to bind to DNA. Aliquots of 50 μL of DNA were added to the mixture and pi-

petted in and out to achieve a homogeneous mixture that would lead to an increase of the DNA bound to the surface of the nanoparticles. Magnetic separation was used to separate the nanoparticles from the solution. The nanoparticles were kept in the Eppendorf whilst the solution was transferred to a spin column. The solution transferred to the spin column was applied to the QIAquick protocol. This was done to track the DNA that had not been bound to the nanoparticles in the previous step. QIAquick column was centrifuged at 13,000 r.p.m for 1 min. The flow through was discarded and the DNA was washed by adding 750 μ L of washing buffer (buffer PE) and centrifuged for 1 min. After flow through removal, the spin column was centrifuged again in order to ensure the complete removing of the solution. The DNA was then eluted from the column with 50 μ L elution buffer (EB buffer).

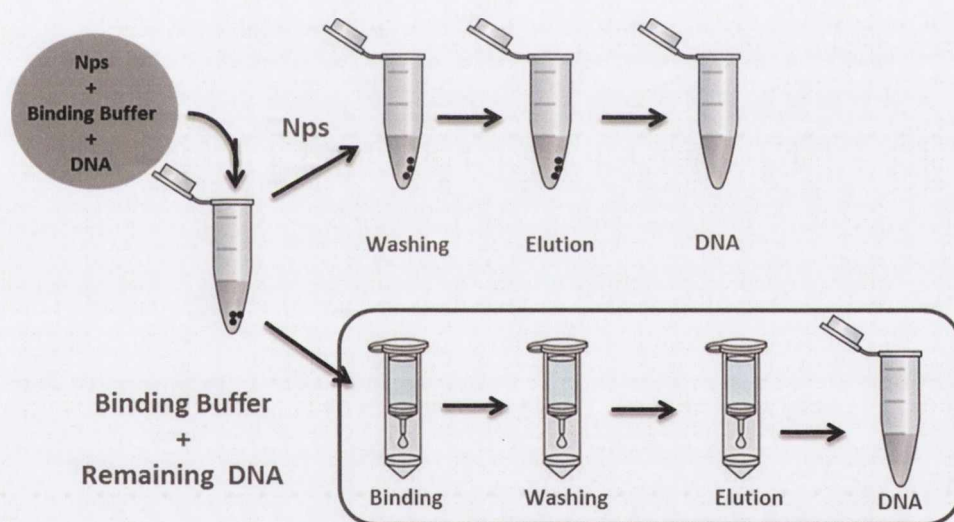


Figure 5.12: Schematic presentation of DNA extraction using magnetic NPs (top) and QIAquick® PCR Purification Kit (bottom).

The same procedure was followed to wash and elute the DNA bound to the Fe@C@SiO₂ NPs (Figure 5.12). The nanoparticles left in the Eppendorf were washed with 750 μ L of PE Buffer and separated from the solution by magnetic separation. PE Buffer contains 80% of ethanol, solvent in which DNA is insoluble. It is important to remove all of the buffer from the NPs by letting them dry completely, to enable the elution of the DNA from the nanoparticles in the next step. The DNA was eluted from the NPs by adding 50 μ L of EB Buffer. To ensure the efficiency of this step, the solution

with the NPs was pipetted in and out in order to disperse the NPs in the solution and produce a homogeneous dispersion. The solution was then let to settle for 5 min and the NPs were removed with a magnet.

The first test was carried out with a 52.1 ng mL⁻¹ solution of DNA. The purification procedure was performed with different concentrations of nanoparticles as described above. The results of these experiments are shown in Table 5.2. One of the experiments was completed with no nanoparticles present to be able to compare the efficiency of the nanoparticles with the commercially available spin columns.

Table 5.2: DNA extraction results using 52.1 ng mL⁻¹ of DNA and a solution NPs with a concentration of 10 mg mL⁻¹.

DNA purified (%)		
NPs (μL)	NPs	Column Extrac-
	Extraction	tion
0	-----	64.3
10	Too low	60.3
50	15.0	41.3
100	25.0	23.6

As it can be deduced from values in the table, some of the DNA binds to the nanoparticles and the remaining DNA in the solution is recovered through the spin columns. Below certain values, the concentration of DNA is not detected by the fluorimeter. In the case of the experiment with a low volume of NPs (10 μL), the DNA recovered is too small to be detected by the apparatus. The rest of DNA is recovered by the spin column and the concentration remaining in the solution is close to the value recovered from the experiment carried out without NPs.

From both experiments with higher concentrations of nanoparticles, similar amounts of DNA were recovered although using 100 μL of nanoparticles seems to be more efficient. Nevertheless, there is still certain amount of DNA recovered by the silica-based spin columns that was not bound to the NPs. There is a significant amount of DNA that has not recovered by either the NPs or the columns. It is also worth mentioning that a small amount of DNA can be lost during the process that is why the percentage recovered is far off 100%. This loss occurs as a portion of DNA does not bind to the silica or due to incomplete elution

Another set of experiments was carried out with a lower concentration of DNA (4.92 ng mL^{-1}). The initial concentration was reduced approximately 10 fold and the final concentration was measured by the fluorimeter (Table 5.3).

Table 5.3: DNA extraction results using 4.92 ng mL^{-1} of DNA and a solution NPs with a concentration of 10 mg mL^{-1} .

DNA purified (%)		
NPs		Column Extrac-
NPs (μL)	Extraction	tion
0	-----	68.7
10	Too low	33.0
50	38.0	36.8
100	23.0	27.4

Similar results were obtained for the experiment in which 10 μL of nanoparticles were used; the amount of DNA recovered was too low to be measured by the device. This result can be expected, but it is also worth mentioning that the percentage of DNA purified by the spin column was too low to compare with the experiment without nanoparticles. It can be also possible that a big amount of DNA was bound to the NPs without being eluted efficiently, which will decrease the amount recovered by the column.

However, when the amount of nanoparticles was increased, the concentration of DNA recovered also increased. In this case, the most efficient concentration used was that of 50 μL of nanoparticles.

5.4 Stabilised Magnetite Nanoparticles for Enantiomeric Separation

Single stranded DNA was used in the synthesis of the nanoparticles. Herring sperm DNA was chosen due to availability and affordability. The co-precipitation technique was used to conjugate the DNA macromolecules *in situ* to the magnetic nanoparticles during their formation. A stoichiometric mixture of $\text{Fe}^{2+}/\text{Fe}^{3+}$ ions (ratio 1:2) was added to a RBF containing single stranded DNA, which was prepared by dissolving the herring sperm DNA in water and heating the solution at 80 °C. After stirring the mixture, nanoparticles were precipitated by adding ammonium hydroxide to reach a pH of approximately 12.

DNA is a remarkably useful and interesting template for preparing assemblies of magnetic nanoparticles. These properties are related to the regular structure of negatively charged phosphate groups along its backbone, which provide a nanosized cross-section to build these assemblies. Both arrangements, single and double stranded herring sperm DNA, have been investigated regarding their ability to act as template for magnetic NPs and both have shown efficiency for this purpose.^[28]

However, in this project single stranded DNA was chosen as a chiral template to produce magnetic nanocomposites for potential enantiomeric separation of racemic mixtures of selected molecules.

An excess of a single stranded DNA was used during the synthesis of the nanocomposites in order to provide extra DNA backbone to wrap around the nanoparticles and to limit the crystal growth after nucleation. This also resulted in the formation of linear assemblies of Fe_3O_4 NPs in aqueous suspension in the presence of an external magnetic field.

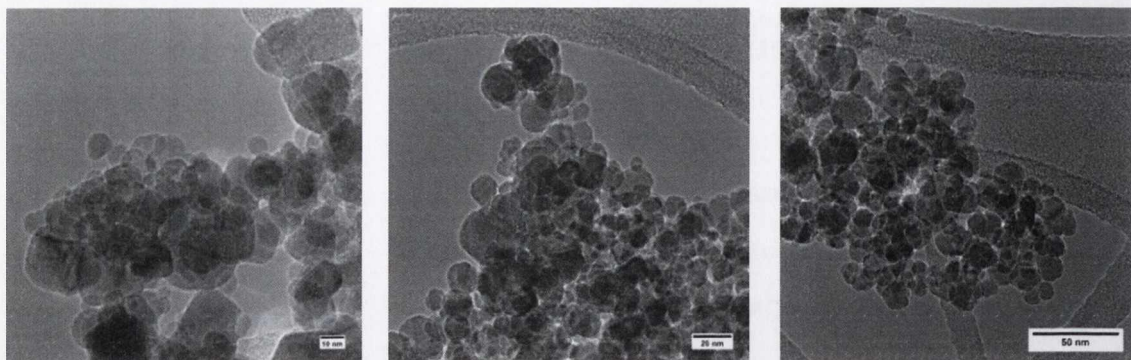


Figure 5.13: TEM images of Fe₃O₄@DNA nanocomposites.

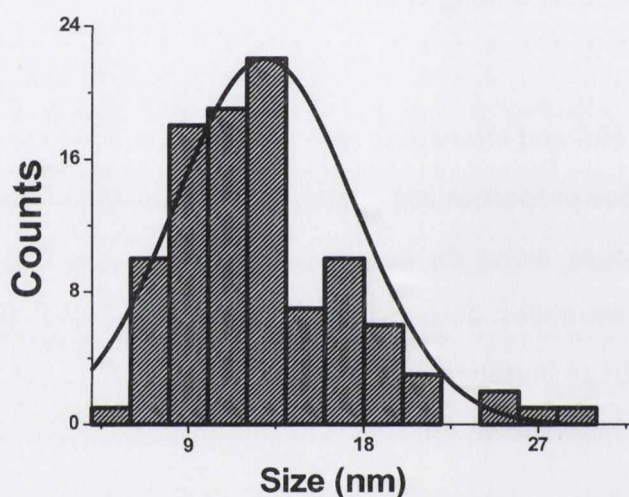


Figure 5.14: Size distribution of Fe₃O₄@DNA nanocomposites.

The TEM images (Figure 5.13) and size distribution analysis of the products (Figure 5.14) showed a broad size distribution of the nanoparticles compared to those synthesised in the absence of DNA (11.7 ± 0.3 nm). However, the average size of Fe₃O₄@DNA nanoparticles was found to be 12.9 ± 4.6 nm. The difference in the distribution of the nanoparticles can be related to the lack of uniformity in the interaction of the nanoparticles with DNA in the solution during the formation of the nanocomposites.

A suspension of the nanocomposites in water was placed on a copper grid and dried in the presence of a 0.5 T magnetic field. The purpose of the experiment was to investigate the crosslinking of magnetic nanoparticles by DNA. TEM images in Figure 5.15 show Fe₃O₄@DNA nanocomposites aligned in the direction of the magnetic field.

Some randomly distributed chains with variable lengths can be observed for the particles grown with denatured herring DNA.

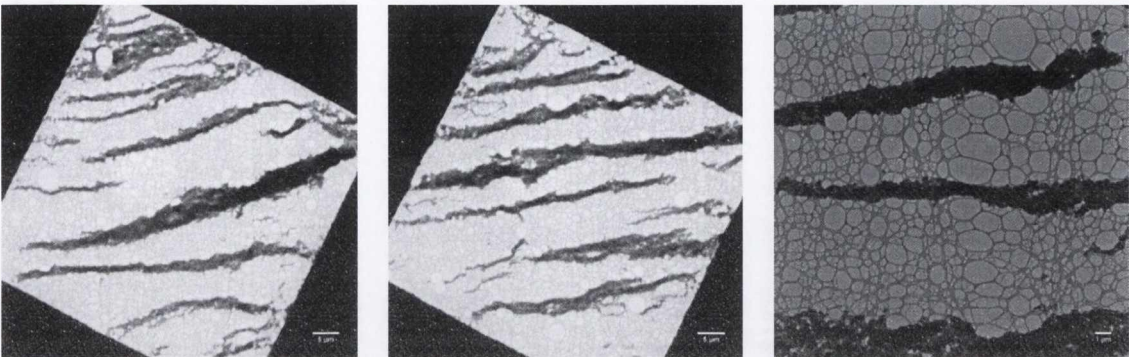


Figure 5.15: TEM images of Fe₃O₄@DNA nanocomposites dried on 0.5 T magnetic field.

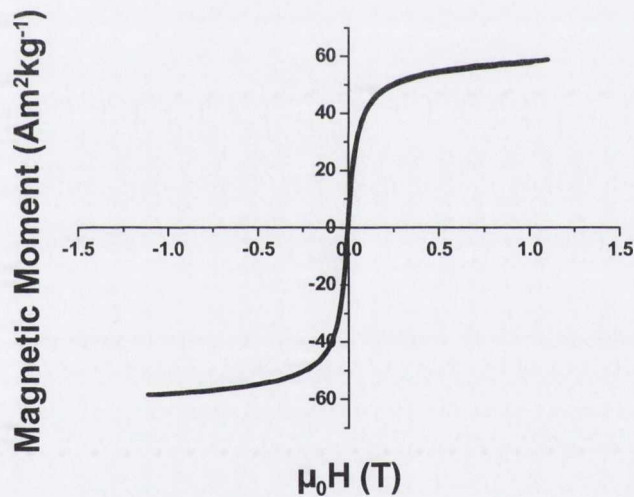


Figure 5.16: VSM curve of Fe₃O₄@DNA nanocomposites.

VSM measurement was performed on the Fe₃O₄@DNA sample and showed a magnetic moment of 59 Am²kg⁻¹. This value is lower than the value obtained for Fe₃O₄ synthesized following the same procedure without the presence of DNA (77 Am²kg⁻¹). A drop in the Ms of the NPs can be attributed to the presence of the DNA acting as ligand, which has been demonstrated to contribute to decrease this value when used in the synthesis of magnetic materials. However, superparamagnetic behaviour in the nano-

particles remained, as the average size calculated previously falls within the critical size required to preserve the single domain regime.

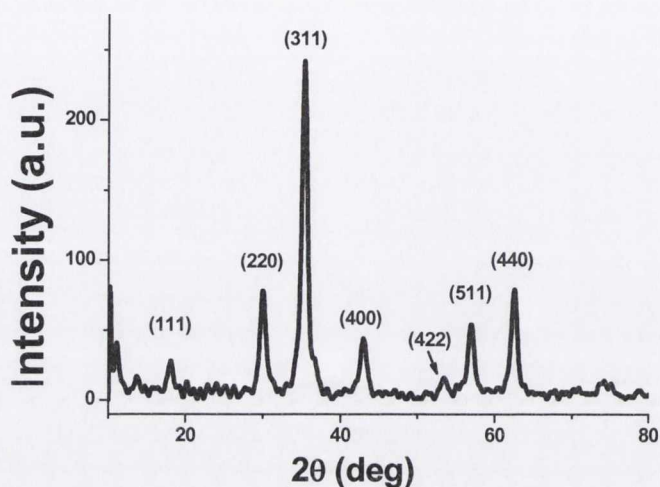


Figure 5.17: XRD pattern of Fe_3O_4 @DNA sample.

The formation of the face-centered cubic phase of Fe_3O_4 was confirmed by the X-ray diffraction pattern (Figure 5.17) without any impurity of maghemite or hematite. Peaks positions are in good agreement with values reported in the literature (JCPDS file No 75-0449).

The FTIR spectrum of the Fe_3O_4 @DNA nanocomposites (Figure 5.18) showed broad bands at 3244 cm^{-1} and 1665 cm^{-1} which can be assigned to the OH groups on the particle surface, associated with water molecules. Phosphate groups attributable to the DNA chain were also identified by IR with bands present at 1211 and 1077 cm^{-1} corresponding to P-O stretching vibrations. Fe-O stretches appear at 580 and 390 cm^{-1} .

1. [29]

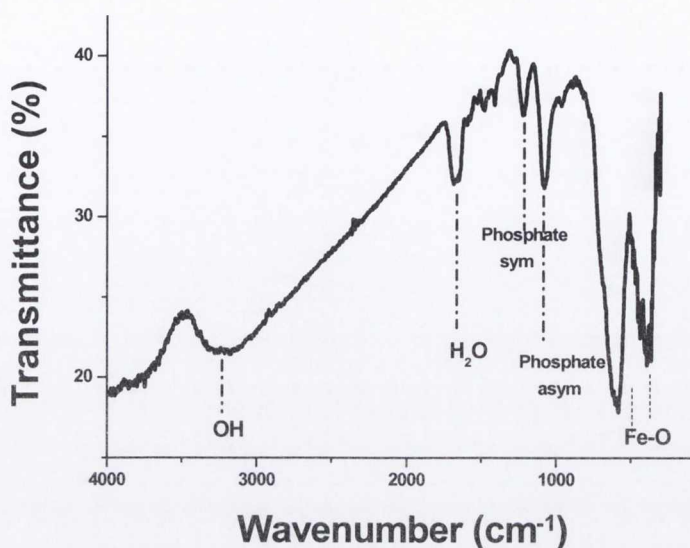


Figure 5.18: FTIR spectrum of $\text{Fe}_3\text{O}_4\text{@DNA}$.

5.4.1 Enantiomeric Separation using $\text{Fe}_3\text{O}_4\text{@DNA}$ Nanocomposites

After the confirmation of DNA being bound to Fe_3O_4 NPs, the nanocomposites were tested as reagents for the enantiomeric separation of a racemic mixture. The racemic mixture was prepared with D- and L-Pen enantiomers. Due to the chiral properties of DNA, the macromolecule bound to Fe_3O_4 may selectively interact with one of the enantiomer in the mixture while having no effect on the other. The use of an external magnet allows magnetic extraction, yielding a purified enantiomeric product. Figure 5.19 shows the CD spectra of D- and L-Pen (left) enantiomers and herring DNA (right) in water. For these ligands, a maximum is observed in the far-UV region while no signal can be detected above that range (280–800 nm).

Initially, DNA stabilised nanoparticles have been carefully washed with water to ensure the removal of any residual unbound DNA from the material. Briefly, magnetic DNA stabilised MNPs were washed with Millipore water 3 times. These washings were collected and monitored by CD (Figure 5.20).

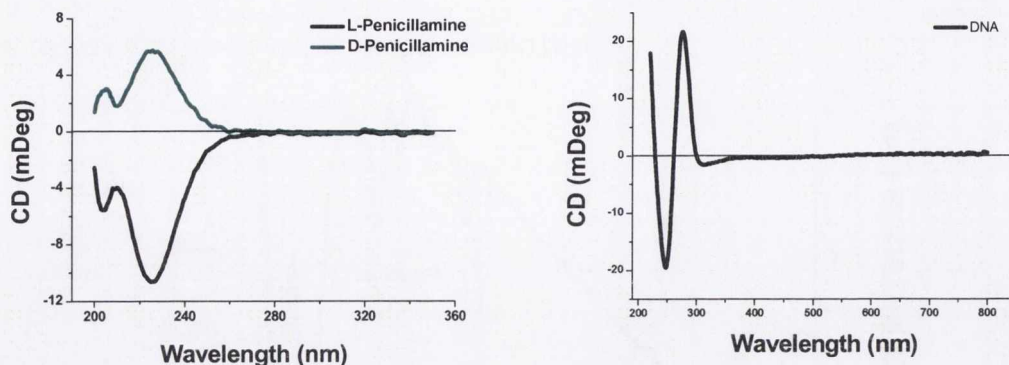


Figure 5.19: CD spectra of D- and L-Penicillamine (left) and DNA (right) in water.

In the CD spectra of the solutions after washing the NPs (Figure 5.20), a broad negative peak around 300 nm can be observed as well as a weaker positive shoulder around 600 nm. This peak disappears after 2 consecutive washings. This latter peak can be attributed to the presence of iron ions complexed with DNA.

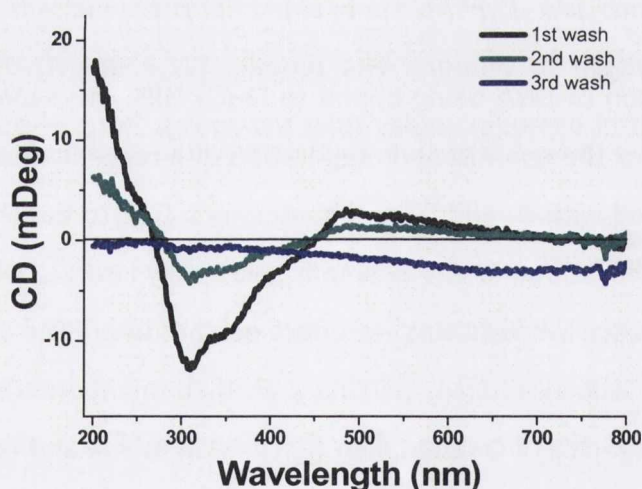


Figure 5.20: CD spectra of the solutions after washing the Fe_3O_4 @DNA NPs in water; first washing (black), second washing (green) and third washing (blue).

The washed DNA stabilised MNPs were used in the enantiomeric separation experiments. The Fe_3O_4 @DNA nanocomposites were added to a racemic Pen solution; the mixture was stirred and after that, the nanoparticles were removed by an external permanent magnet. After being treated with the Fe_3O_4 @DNA nanocomposites the slightly brown resulting solutions of the enantiomeric mixture were analysed by CD

spectroscopy, (Figure 5.21). CD spectra were recorded at different times during the experiment.

The CD spectrum of the original racemic D+L-Pen solution before treatment indicates that the mixture is almost racemic with a small excess of D-Pen present giving a negligible signal at around 232 nm (see Figure 5.19, left). After treatment of the racemic mixture with the DNA stabilised MNPs, a small peak at 233 nm with opposite chirality is detected, which can be attributed to L-Pen. This could be an indication of the selective removal of D-Pen from solution by the magnetic DNA nanocomposites. However, a second negative peak centred around 300 nm is also detected, which could be due to some interaction of the Penicillamine in the solution with iron ions or DNA leaking from the nanocomposites over a longer period of time (see Figure 5.20). It is important to note that this peak become stronger after 60 min of the exposure of the racemic mixture to the DNA stabilised nanoparticles. The interaction of the DNA with the metal ions has been reported before, showing negative and positive peak in the CD spectrum.^[30] A peak due to the base stacking of the DNA usually appears at 275 nm and a peak corresponding to right-handed helicity is visible at 248 nm.^[31] In this work, a positive peak appears around 600 nm and a negative one around 300 nm. Studies in the formation of iron(III) complex with DNA are in agreement with the peak previously given and no shift is observed in the CD spectrum. Due to the difficulty to assign these CD signals further conformational studies are needed in order to identify the origin of these peaks.^[32-33]

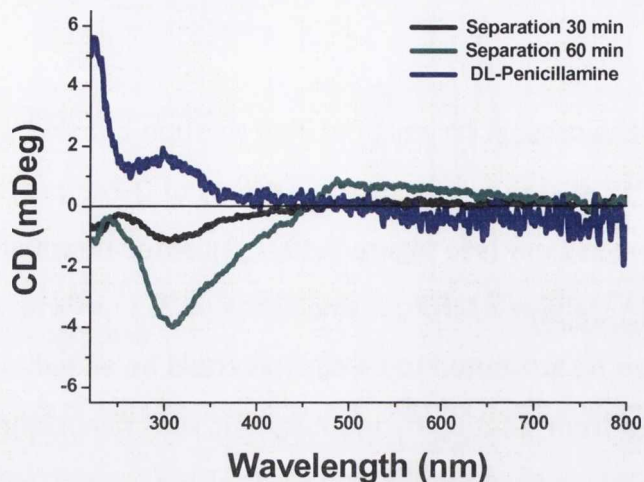


Figure 5.21: Enantiomeric separation of D+L-penicillamine using DNA stabilized MNPs after 30 min (black), after 60 min (green) and original racemic D+L-Penicillamine (blue) mixture used in the reaction.

UV-Visible spectra were recorded for the sample before and after treatment, as well as recording for a sample of herring sperm DNA in water. The spectra confirm the complete removal of unbound DNA before using the MNPs for enantiomeric separation, and show a steady increase in absorption for all samples at 200 nm. The absence of peaks identifying a metal complex could suggest that the signals in the CD spectra are due to a separation of enantiomers. However, further work is needed to confirm this hypothesis.

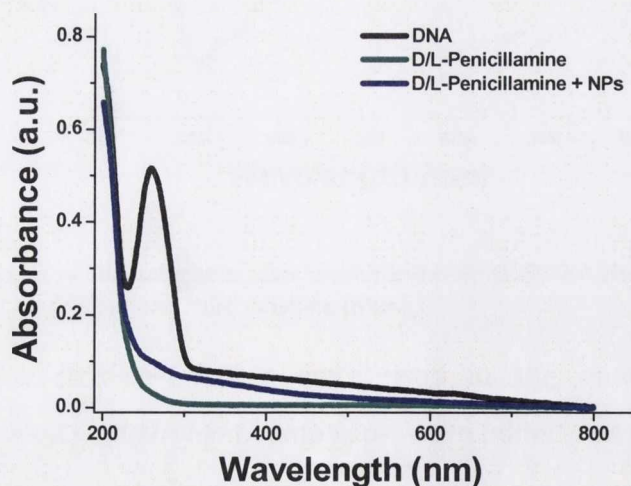


Figure 5.22: UV-Vis spectra of DNA (black), D- and L-penicillamine mixture (green) and mixture D-and L-Penicillamine with magnetic nanoparticles (blue).

5.5 Chiral Ligand Stabilised Cobalt Ferrite Nanoparticles for Enantiomeric Separation

5.5.1 Enantiomeric Separation using D-Penicillamine Stabilised CoFe_2O_4 Nanoparticles

Selected nanoparticles stabilised with chiral ligands such as penicillamine and cysteine (see chapter 4) were used in enantiomeric separation of a racemic mixture of D+L-Pen.

In this experiment, CoFe_2O_4 @D-Pen (the sample DP2, see Chapter 4) was added to a racemic mixture of D- and L-Pen. The mixture was sonicated for 5 min to allow the MNPs to interact with the chiral ligands present in the solution. After that, MNPs were removed from the solution using a magnet and CD spectrum of the resultant solution was recorded.

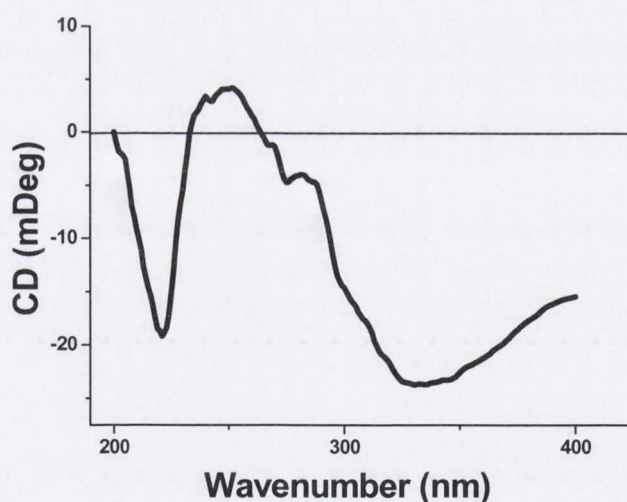


Figure 5.23: CD spectrum of solution after enantiomeric separation racemic mixture of D- and L-Penicillamine using CoFe_2O_4 @D-Pen nanoparticles.

A strong L-Pen signal appears around 220 nm (Figure 5.23). This could be an indication of either D-Pen being selectively removed by enantiomeric separation. In addition, there are strong broad CD signals between 300 and 400 nm that could demonstrate the formation of some new chiral metal complex. After the treatment with the NPs, the solution turned slightly brown. This indicates the presence of metal impurities that

could form a complex with penicillamine in the solution generating the CD signals at higher wavelengths.

In order to avoid potential leaking of chiral stabilisers and check if the particles can keep “chiral memory” after the removing chiral ligands, $\text{CoFe}_2\text{O}_4@D\text{-Pen}$ NPs were calcinated at 250 °C for 1 h. According to TGA (Thermogravimetric Analysis), this is the temperature necessary to decompose and remove the ligands from the NPs (Figure 5.24). The TGA curves exhibits small and continuous weight loss at low temperatures due to solvent evaporations. At 165 °C, the curve shows a decrease in the weight due to the ligand decomposition with a total weight loss of around 2.8% (0.1 mg). A second phase is observed around 600 °C with a weight loss of around 1.8% (0.06 mg). This is a possible indication of the formations of other derivatives of nitrogen or sulphur from penicillamine decomposed at high temperatures. The remaining mass at higher temperatures is due to CoFe_2O_4 core of the NPs.^[34]

After the thermal treatment, the NPs were treated with the enantiomeric mixture as in previous experiments and CD spectrum was recorded. CD spectrum shows a signal between 200 and 240 nm and others of different intensity between 250-300 nm and 300-400 nm (Figure 5.25). These results are in agreement with the initial suggestion that $\text{CoFe}_2\text{O}_4@D\text{-Pen}$ NPs may have preferential binding for one of the enantiomers and that some chirality has been induced on their surface. However, the presence of the other signals at higher wavelength could also indicate that more complex interactions occur in the system.

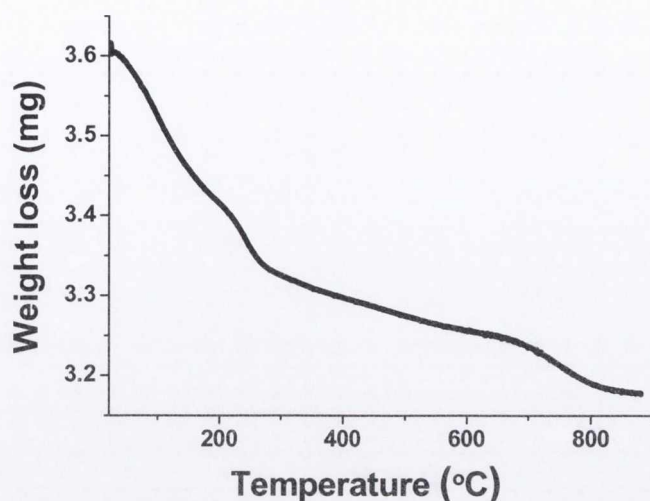


Figure 5.24: TGA curve of CoFe₂O₄@D-Pen NPs.

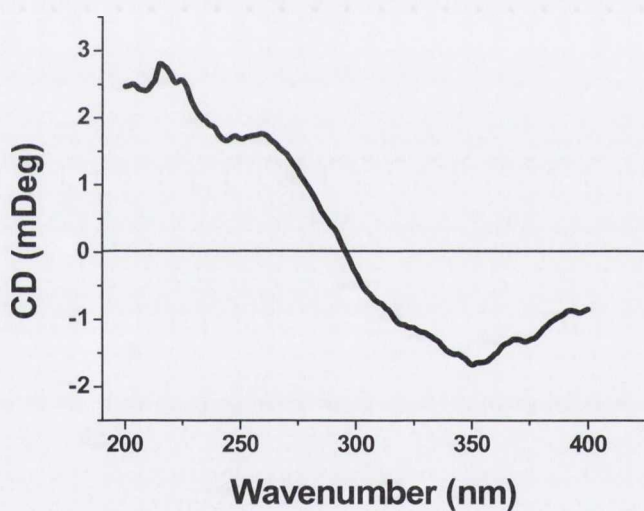


Figure 5.25: CD spectrum of D+L-Pen mixture after the enantiomeric separation using CoFe₂O₄@D-Pen after calcination.

Although there are peaks present in the spectra for both experiments that can be attributed to the enantiomers, suggesting either the removal of one of the enantiomers or the leaking of the ligand from the NPs, it is difficult to be certain. The presence of other species in the solution makes the peaks very complex to be assigned properly. To clarify this, it would be appropriate to perform more sophisticated characterization, such as chiral gas chromatography.

5.5.2 Enantiomeric separation using D- and L-Cysteine stabilised CoFe₂O₄ nanoparticles

Following the same procedure, D- and L-Cysteine (D- and L-Cys) stabilized CoFe₂O₄ were tested for chirality induced in their surface, imprinted after removing the chiral ligand.

A racemic mixture of D+L-Pen was used for the separation. The same experiment was carried out using CoFe₂O₄ NPs synthesized without any chiral ligands. This was to observe the difference in the behaviour of the chiral ligand stabilized CoFe₂O₄ and naked CoFe₂O₄ MNPs. Additionally, two different experiments were performed; using the same components, racemic mixtures and NPs, one of them was stirred and the other was sonicated for the same period. The purpose of this was to verify if one of the processes displays better conditions for the interaction of the MNPs with the solution.

A broad peak appears above 280 nm suggesting the formation of chemical species such as complexes between metal and chiral ligand present in the solution (Figure 5.26). The peak is more intense when sonication is used during the experiment indicating that this procedure enhances the interaction between the ligands present in the solution and metal ions from nanoparticles resulting in the formation of the complexes. However, the peak around 230 nm is in the same region as the peak for L-Pen, which might potentially indicate some removal or binding of D-Pen.

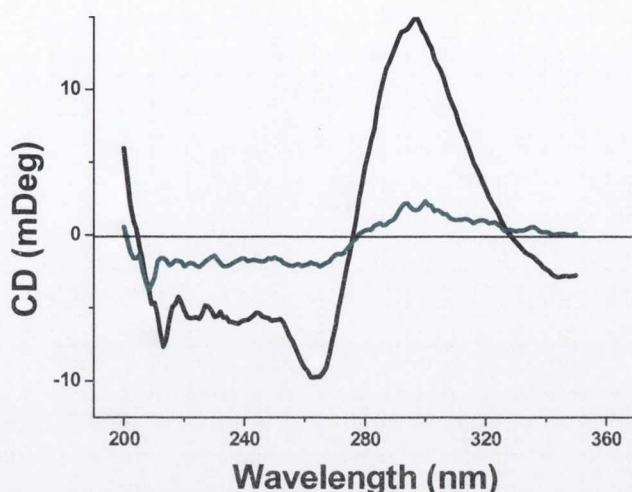


Figure 5.26: CD spectrum of enantiomeric separation of CoFe_2O_4 after sonication (black) and CoFe_2O_4 after stirring (green).

Additionally, a series of experiments using CoFe_2O_4 NPs functionalized with different concentrations of D- or L-Cys during the synthesis were carried out. Synthesis of these nanomaterials has been presented in more detail in Chapter 4. Table 4.4 presents the concentration of chiral ligand used in the synthesis of the MNPs.

Table 5.4: Concentration of ligands used during the synthesis of D- and L-Cysteine stabilised CoFe_2O_4 nanoparticles.

Ligand	Concentration [mM]
D-Cys (DC1)	0.85
D-Cys (DC2)	1.7
L-Cys (LC1)	0.85
L-Cys (LC2)	1.7

The separation experiments were performed in the same way as described above. Racemic mixture containing magnetic nanoparticles were stirred or sonicated and then the NPs were removed by magnetic separation. After that, CD spectra of the resulting solution were recorded.

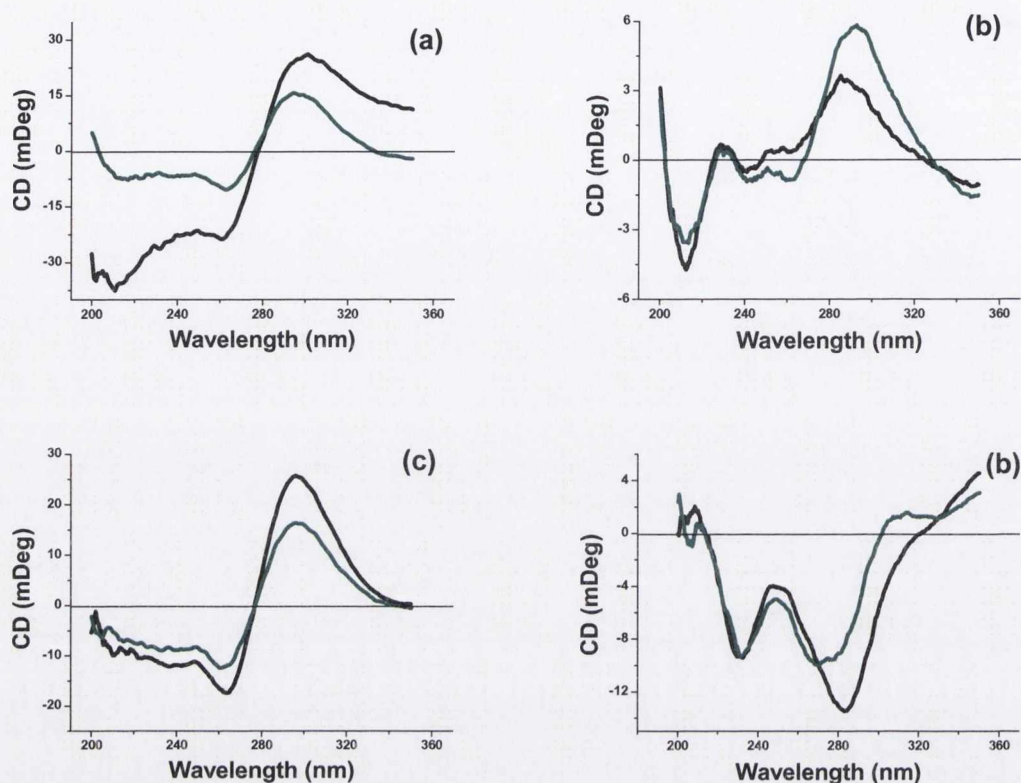


Figure 5.27: CD spectrum the product of the enantiomeric separation using $\text{CoFe}_2\text{O}_4@\text{D-Cys}$, DC1(a), DC2 (b) and $\text{CoFe}_2\text{O}_4@\text{L-Cys}$, LC1(c) and LC2(d) showing those samples stirred (green) versus those sonicated (black).

The CD spectra for the experiments with $\text{CoFe}_2\text{O}_4@\text{D-Cys}$ and $\text{CoFe}_2\text{O}_4@\text{L-Cys}$ are shown in Figure 5.27. The results are very similar to those obtained for uncoated CoFe_2O_4 . A broad peak, probably caused by the formation of cobalt or iron complexes with penicillamine, appears between 280 and 320 nm. Independently of the chiral ligand and present in the MNPs, the peak appears in all the experiments. For the sample LC2 ($\text{CoFe}_2\text{O}_4@\text{L-Cys}$), this peak appears in opposite direction. The intensity of the peak increased when the experiment is performed with the nanoparticles containing less amount of chiral ligand, except in the case of the sample DC2 ($\text{CoFe}_2\text{O}_4@\text{D-Cys}$). This could be due to the fact the less amount of ligand can lead to a higher presence of free metals available to form these compounds while in the case of the nanoparticle synthesized with a higher amount of ligand would protect better the material from leaching. In the case of the spectra for the sample $\text{CoFe}_2\text{O}_4@\text{D-Cys}$, DC2 (Figure 5.27, b) an intense peak appears around 210 nm, this peak could correspond to the presence of L-

Pen and is slightly shifted. Additionally, for the sample $\text{CoFe}_2\text{O}_4\text{@L-Cys}$ (LC2) a weak peak is present around 230 nm, in the same range as the peak for L-Pen.

In the case of the experiment performed using the same material synthesized with different concentration of L-Cys (Figure 5.27, right), behaviour of $\text{CoFe}_2\text{O}_4\text{@L-Cys}$ with less amount of ligand is comparable to the experiment carried out with $\text{CoFe}_2\text{O}_4\text{@D-Cys}$ already explained. When $\text{CoFe}_2\text{O}_4\text{@L-Cys}$ NPs synthesized with high concentration of ligand are used, the broad peak around 280 nm is still present, which would confirm the formation of the chiral metal complexes.

In order to elucidate the origin of the peaks in the spectra, a series of controls were carried out. Figure 5.28 shows the spectra of different experiment in which the precursor metal salts used in the synthesis of the MNPs ($\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$) have been dissolved in a racemic mixture D+L-Pen. In the experiments carried out with $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ a broad peak between 280 and 320 nm is visible, similar to the peak present in the spectra presented above for the experiments in which MNPs were used. This could be an indication of the formation of a cobalt complex in the presence of Co^{2+} ions. However, no CD signal corresponding to the formation of complex is detected in the spectrum when $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ is used, although there is a peak below 240 nm which is in the range of the characteristic peak for L-pen.

An aqueous solution of metal ions and chiral compounds give a circular dichroism signal in the visible range due to the d-d transitions. This is a confirmation of the formation of metal complexes with these optically active ligands.^[35]

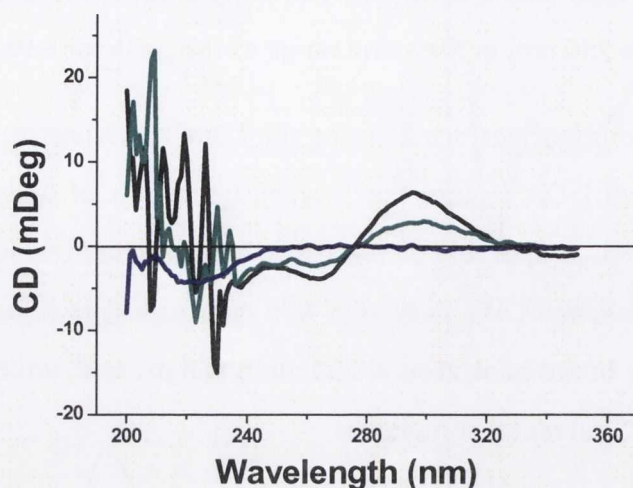


Figure 5.28: CD spectra of metal salts sonicated in a racemic mixture; Co(II) and Fe(II) (black), Co(II) (green) and Fe(II) (blue).

Figure 5.29 shows CD spectra corresponding to the interaction of the L- or D-Cys stabilised CoFe_2O_4 NPs with one of the enantiomers of Penicillamine. A defined amount of NPs was dispersed and sonicated in a solution of one of the enantiomers (D-Pen or L-Pen) and after removing the NPs from the solution, CD spectroscopy was performed.

Solutions of either D- or L-Pen after the treatment with Cys stabilized CoFe_2O_4 NPs show an interaction of the ligands with the NPs causing new changes, which generates new CD signals. Broad peaks between 280 and 320 nm are consistent with the result obtained in previous experiments, which confirms the formation of metals complexes in the presence of the ligands.

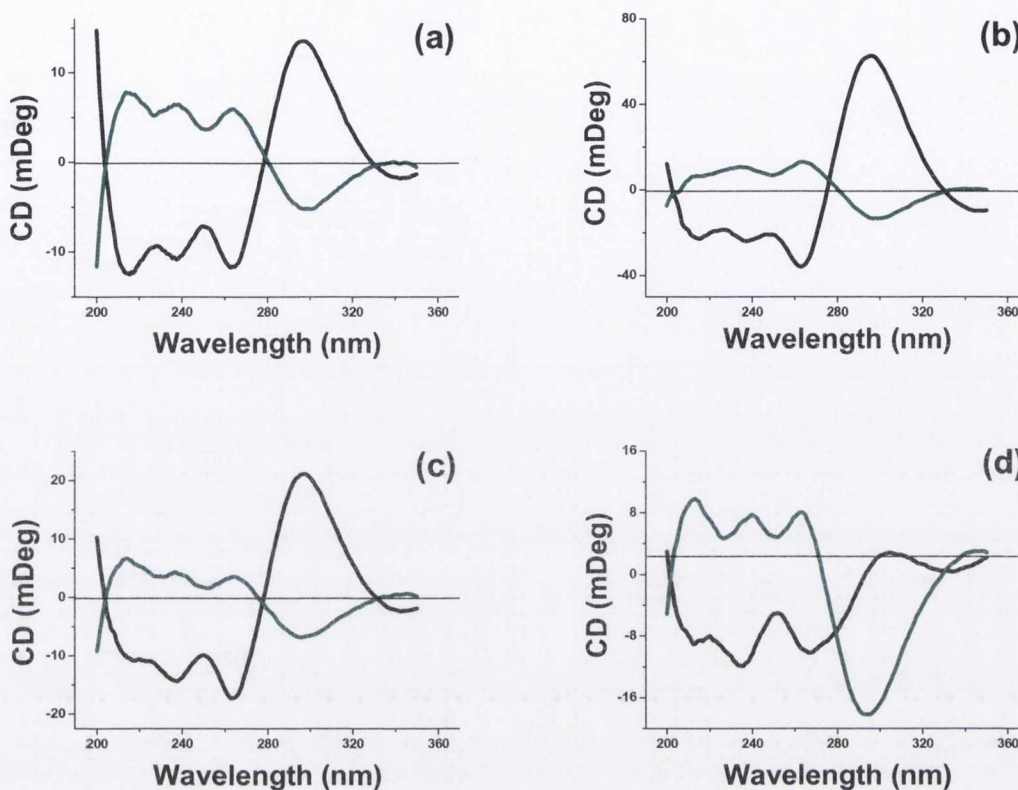


Figure 5.29: CD spectrum of solution after treatment of CoFe₂O₄@D-Cys, DC1 (a), DC2 (b) and CoFe₂O₄@L-Cys, LC1(c) and LC2 (d) with D-Pen (black) and L-Pen (green).

The use of D- and L- Pen as ligand in the solutions after the treatment with the magnetic NPs results in mirror images of the same peaks in all the experiments carried out. These results confirm the formations of new species in the solutions due to the presence of metal ions and the chiral ligands.

5.6 Conclusions

To conclude, core-shell Fe@C@SiO₂ NPs have been synthesized and characterized. This material was tested in DNA purification and compared with commercially available DNA purification kits. As a result, it has been demonstrated that these magnetic nanomaterials can be used in the recovering of DNA with the advantage of being able to recover the material by applying an external magnetic field. This advantage makes the process faster and cheaper. However, further studies are necessary to investigate

the potential up scaling of the process and to improve the recovery to match commercially available kits.

A variety of chirally modified CoFe_2O_4 NPs have been used in the magnetic enantiomeric separation of racemic mixtures using penicillamine and cysteine ligands. The results obtained from the preliminary test were very interesting, although the leaching of Co and Fe ions from the NPs and binding of these ions to chiral ligands was identified as a major problem that needs to be addressed. Therefore, further detailed studies are necessary to confirm if the presence of the chiral ligands could imprint chiral features enabling the enantiomeric recognition, selection and separation of chiral compounds.

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Chapter 6. Magnetic Yolk-Shell Nanocomposites

6.1 Introduction

The development of nanomaterials with a hollow structure have emerged lately as an interesting and useful approach to fabricate new nanostructures because of their variety of possible applications, such as drug storage with controlled release,^[1-3] catalysis,^[4-6] or chemical and biological sensing.^[7-13]

This growing interest is due to their properties which include low density, high surface area and mesoporous structure.^[14] Different materials have been investigated to form these mesoporous structures, such as TiO_2 ,^[15-16] carbon^[17-18] and polymers^[19-21]. Among them, SiO_2 has arisen as one of the most studied due to its biological compatibility, low toxicity and stability.^[22-27]

Nowadays, several different methods have been employed to synthesize these hollow structures. The hard template approach involves four steps: preparation of the template, functionalization or modification of the surface, coating of the template to form a shell and finally the removal of the template to create the inner void. However, within this approach, there are different strategies to form the shell around the previously formed hard template; Caruso *et al.* developed a technique to prepare hollow structures using the Layer-by-Layer assembly (LBL) approach.^[28] This technique involves the layer-by-layer deposition of polymers with opposite charges that are induced by electrostatic interactions.

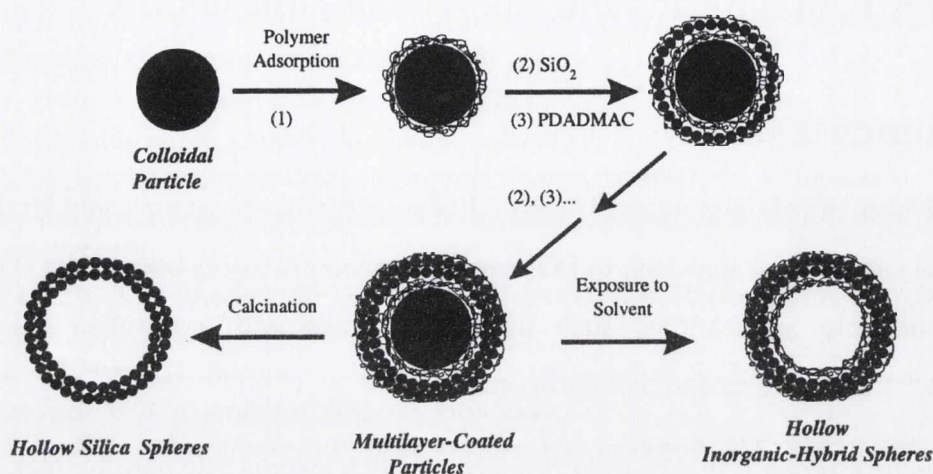


Figure 6.1: Scheme of layer-by-layer techniques for the formation of hollow spheres.^[28]

Another commonly used strategy is the direct chemical deposition of shell materials on the template. The deposition is carried out by precipitation of the materials on the template using their chemical or physical interactions followed by a post-treatment step to compress the shell.^[29-30]

Hollow spheres can also be prepared by chemical adsorption on the surface layer; this method uses a direct adsorption-calcination route. For example, Hyeon *et al.* used this route to form hollow palladium spheres. Pd was deposited on silica spheres, which were then removed by treating the Pd-coated silica spheres with 10 M HF solution.^[31] Alternative routes using hard templates include the nanocasting of a mesoporous shell, which involves the incorporation of a suitable porogen agent during the silica synthesis^[32-33], colloidal crystal and the lox-wax approach. For the latter cases, a sol-gel precursor is infiltrated into a stable colloidal crystal.^[34-35]

Sacrificial templating is a slightly different approach to the one using hard templates. In this case, the template also plays a structural role, determining the shape and the size of the cavity of the resultant material. Kirkendall effect^[36-37] and galvanic replacement are often used in the formation of hollow structures by sacrificial template.^[38-39]

Soft templates are also used to form these hollow structures. Contrary to hard templates, which use solid compounds, soft templates use liquid or gaseous species. As in the hard templates, there are different strategies in the case of soft template. Emulsion droplets used two immiscible liquids, which are stirred making the drops of one of the liquids dispersed into the other.^[40-41] Supramolecular structures form when amphiphilic molecules from the surfactants form an assembly that leads to the generation of micelles or vesicles when their concentration in solution increases.^[42-43] Another interesting strategy is the dispersion of bubbles into a liquid to create emulsions (Figure 6.2). The selected material forms a compact shell of NPs around the bubbles. The forces that drive the formation of that shell can be electrostatic interactions or hydrodynamic conditions. The result can also be affected by the size, shape or other particle properties.^[44-45]

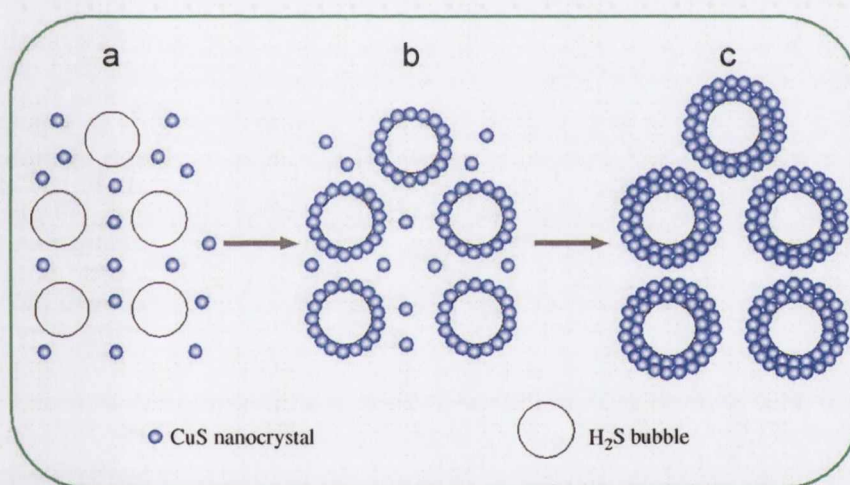


Figure 6.2: Scheme of the formation of CuS hollow microspheres based on the bubble template route.^[44]

Template free methods have also been investigated to develop hollow structures. One of the methods used in this case is Ostwald ripening. The outer layer of the nanostructures crystallises faster due to the contact with the surroundings and as result, the inner material tends to dissolve.^[46-47]

Some of the strategies presented above for the formation of hollow structures can also be used in the preparation of yolk-shell or rattle-like nanostructures. These structures are formed by a solid core and an external shell with a hollow space (void) in between. The incorporation of magnetic nanoparticles to the hollow nanostructures is

particularly attractive as it adds the advantage of magnetic separation and manipulation of the material. In addition, the empty space allows the loading of different compounds such as drugs, catalyst, etc., that could be released on demand by an external magnetic field, which could break the nanorattles.

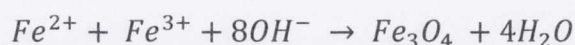
Following different approaches $\text{MNPs}@SiO_2$ have been prepared and tested for different applications. Rattle-type $\text{Fe}_3\text{O}_4@SiO_2$ hollow spheres were loaded with Doxorubicin hydrochloride (DOX), an anticancer drug, and were evaluated for the cytotoxicity in HeLa cells, showing interesting results when compared to free DOX cytotoxicity.^[48] A similar material was prepared and loaded with aspirin showing very positive results for drug release.^[49]

However, these structures have also demonstrated great results in other fields. For example, Au NPs deposited on the surface of Fe_3O_4 MNPs were coated with a hollow porous silica structure. The dimension of the pores made the Au NPs available to carry out the catalytic reduction of 4-nitrophenol and epoxidation of styrene showing promising results.^[50] The adsorption of contaminants in water has been demonstrated by Wang *et al.* using $\text{Y-Fe}_2\text{O}_3@SiO_2\text{--}mSiO_2$ rattle-type nanostructures.^[51] Also, the synthesis of magnetic yolk-shell nanocomposites comprising of magnetic core and silica shell was also previously achieved by removing the silica layer using a hydrothermal treatment.^[52]

The main aim of this chapter is to develop and characterize new $\text{Fe}_3\text{O}_4@SiO_2$ magnetic nanorattle-like structures. In this work, the slow and selective internal etching of mesoporous silica under basic conditions was the procedure chosen to produce these materials. The plan is to use an organic dye and quantum dots (QDs) to extend the multifunctional properties of the nanostructures and potentially use them in biological applications (e.g. biological imaging). The new magnetic nanorattles are to be investigated by FTIR spectroscopy, XRD, and TEM analysis. This work is expected to provide an interesting contribution in the further development of multimodal magnetic nanocomposite material for a range of potential applications.

6.2 Synthesis and Characterisation of Silica Coated Magnetite Nanoparticles

Magnetite nanoparticles were prepared via co-precipitation of Fe^{2+}/Fe^{3+} ions, (ratio 1:2), by reacting a mixture of chloride iron solutions with a base, as it is shown in Scheme 6.1. Upon addition of the iron salt solutions to the basic solution of pH around 12, a black precipitate was formed. The suspension was allowed to stir for one hour. The resultant particles were washed with MP water three times and dried under vacuum.



Scheme 6.1: The reaction scheme for the formation of Fe_3O_4 NPs.

Transmission Electron Microscopy (TEM) images of the obtained powder revealed the spherical shape of the nanoparticles with a high level of aggregation probably due to the magnetism of the materials. The histogram in Figure 6.4 shows that the average size of the nanoparticles is 11.7 ± 0.3 nm. The study is based on a sample of 100 nanoparticles from the TEM images.

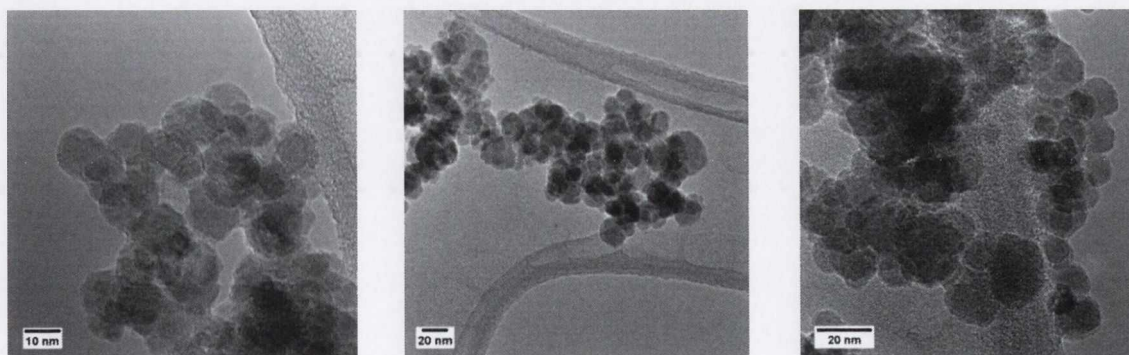


Figure 6.3: TEM images of the Fe_3O_4 nanoparticles.

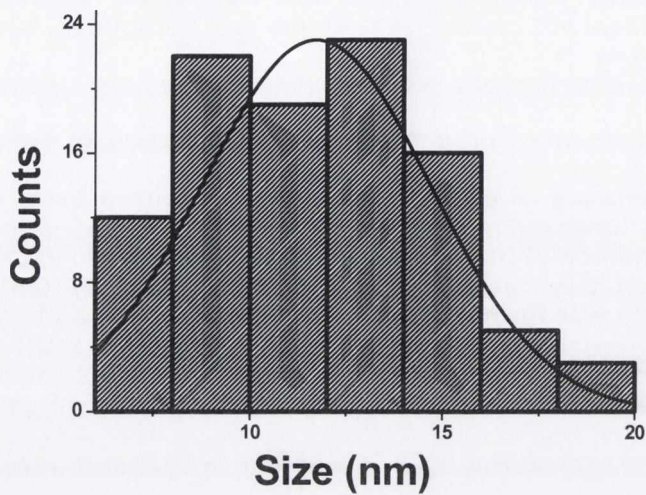


Figure 6.4: Size distribution of the Fe₃O₄ nanoparticles.

Vibrating Sample Magnetometer (VSM) measurement confirmed that the material is mostly superparamagnetic derived from the absence of a hysteresis loop. Saturation magnetization was found to be 77 Am²kg⁻¹, which is lower than the Ms of bulk magnetite (92 Am²kg⁻¹).^[53]

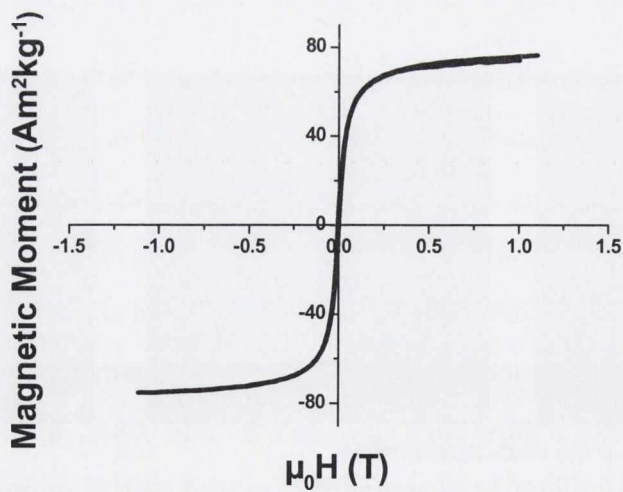


Figure 6.5: VSM curve for the Fe₃O₄ nanoparticles.

The X-ray diffraction pattern collected for the sample confirmed the formation of the cubic phase of Fe_3O_4 without the presence of any impurity (Figure 6.6). Peak positions and Miller indices are in good agreement with values reported in the literature. Those peaks are (111), (220), (311), (222), (422), (511) and (440).^[54]

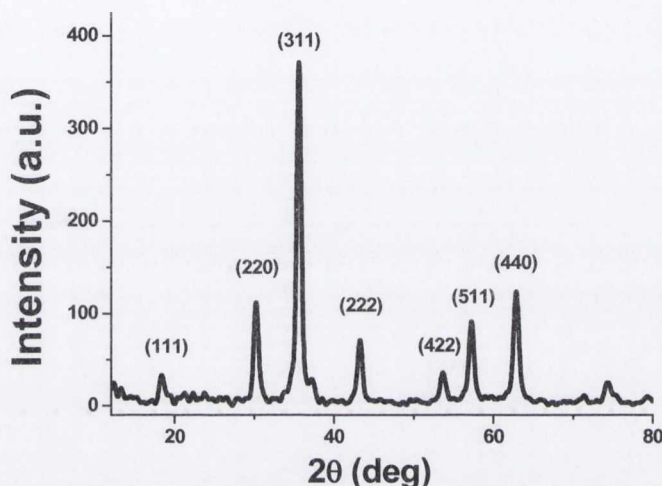


Figure 6.6: XRD pattern of the Fe_3O_4 nanoparticles.

Before coating Fe_3O_4 NPs with a silica shell, the material was functionalized with citric acid. A defined amount of magnetic NPs was dispersed in water and a solution containing citric acid was added. The resultant mixture was sonicated for 1 h, after which the pH was adjusted to neutral using trimethyl ammonium.

The magnetic nanoparticles were then coated with silica using the Stöber method.^[55] Tetraethyl orthosilicate (TEOS) was added to a dispersion of the previously prepared citric acid stabilized Fe_3O_4 nanoparticles in ethanol. Ammonia was used as the catalyst for the reaction. The concentration of TEOS added to the reaction mixture was varied to modify the shell thickness. Two samples of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ nanoparticles with an evident difference in the shell thickness were produced.

Both samples, labelled SH1 (50 μL TEOS) and SH2 (200 μL TEOS), were analysed using TEM and the images are shown in Figure 6.7. The sample prepared with lower concentration of TEOS (top image) resulted in a thinner shell, with a higher degree of aggregation. In contrast, samples prepared with a larger amount of silica precursor showed a thicker shell of silica and individually coated nanoparticles.

An approximate size distribution analysis performed from TEM images showed a size of 28.5 ± 6.3 nm for the thin shelled NPs (SH1) and 111 ± 4 nm for the thick shelled NPs (SH2). The average thickness of the shell was found to be around 5-10 nm for SH1 and 35 nm for SH2 samples.

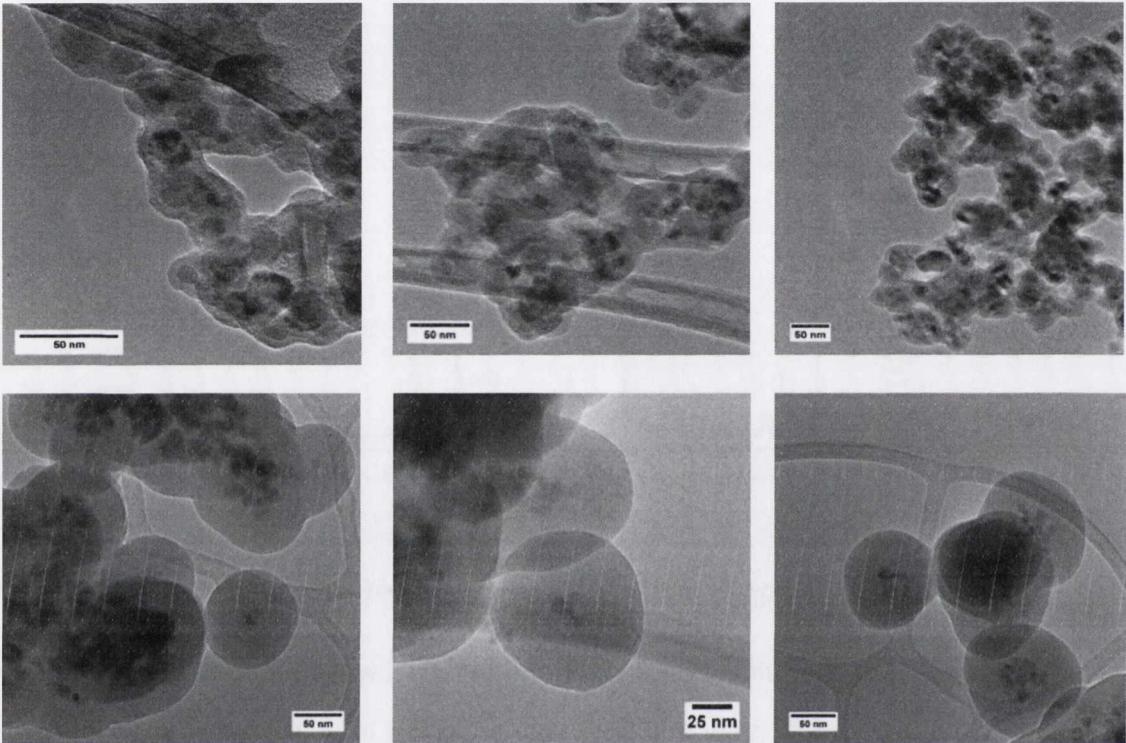


Figure 6.7: TEM images of silica coated Fe_3O_4 nanoparticles: samples SH1 (top images) and SH2 (bottom images).

Changes in the M_s for both samples can be observed in VSM curves (Figure 6.8). The M_s for the nanoparticles with the thinner shell was found to be $47 \text{ Am}^2\text{kg}^{-1}$. The increasing presence of non-magnetic material leads to a decrease in the magnetisation saturation, in the case of the sample prepared with a higher concentration of silica, with a value of $19 \text{ Am}^2\text{kg}^{-1}$.

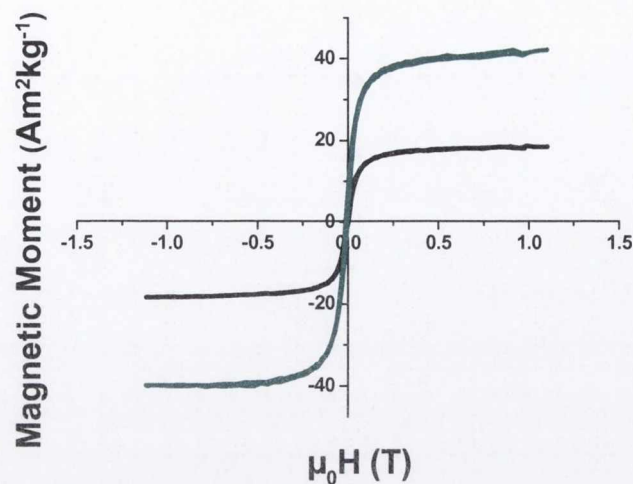


Figure 6.8: VSM curves of the $\text{Fe}_3\text{O}_4@\text{SiO}_2$ NPs: sample SH1 (green) and SH2 (black).

The X-ray diffraction pattern was recorded for the sample with the thick shell. Magnetite retains the same cubic phase, indicating that the magnetic core has not been altered in the process. The broad and unresolved peak between 20° and 30° in the diffractogram confirmed the presence of amorphous silica.^[56]

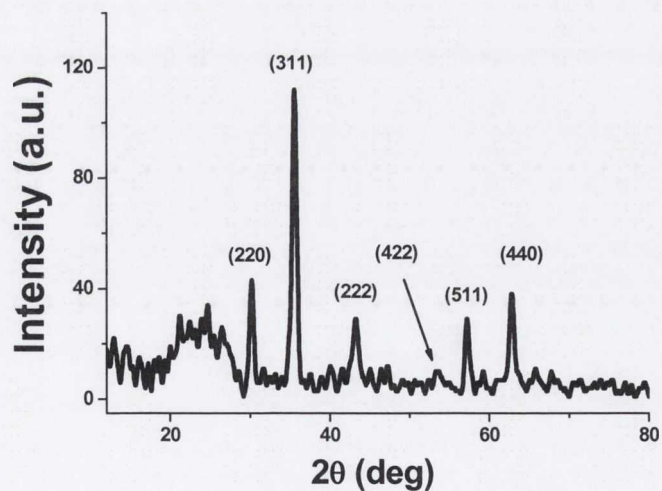


Figure 6.9: XRD pattern for the $\text{Fe}_3\text{O}_4@\text{SiO}_2$ nanoparticles with the thick silica shell.

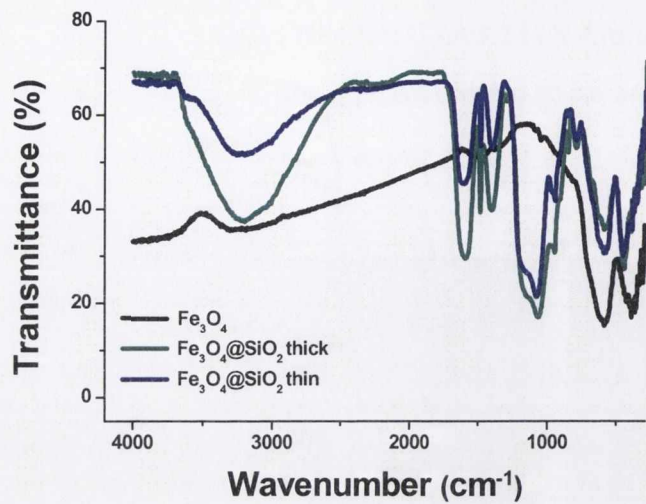


Figure 6.10: FTIR spectra of the Fe₃O₄ NPs (black), SH1 (blue) and SH2 (green).

The FTIR spectroscopy by diffuse reflectance (DRIFT) was carried out from 400 cm⁻¹ to 4000 cm⁻¹ for all samples (Figure 6.10). For magnetite, Fe-O stretches are found at 458 and 588 cm⁻¹.^[57]

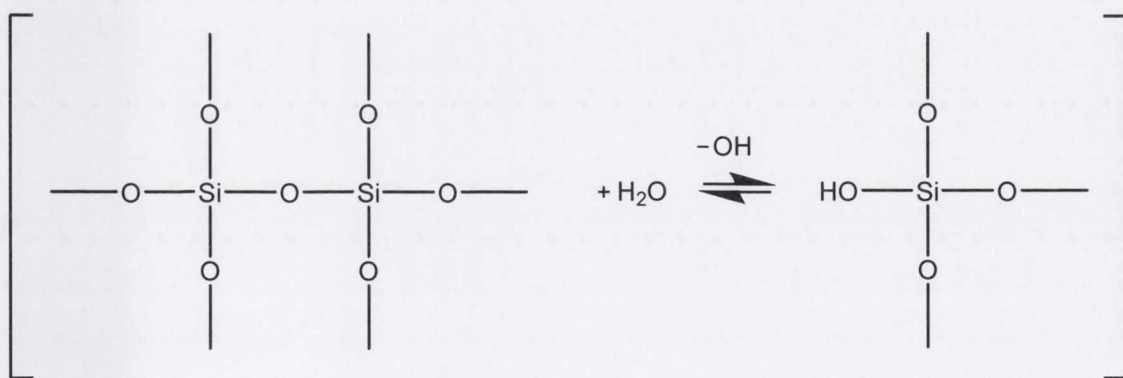
The formation of silica was confirmed by the presence of the asymmetrical stretching vibration mode of Si-O-Si that appears around 1000-1200 cm⁻¹. The band at around 800 cm⁻¹ corresponds to the symmetric stretching of the Si-O-Si group.^[58] The intensity in the asymmtric Si-O-Si peak increases as the concentration of silica present in the samples increases. The peaks near 3200 and 1590 cm⁻¹ correspond to the vibration of H₂O.

6.3 Synthesis and Characterisation of Hollow Magnetite@Silica Nanostructures

A new and simple method for the production of hollow nanostructures composed of magnetic nanoparticles as a core and a silica shell was developed.

The process to prepare these hollow nanostructures involves a slow and selective internal etching of the silica shell after the synthesis of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ nanostructures in water at basic conditions. Previously a similar slow etching approach has been reported for the generation of hollow silica nanospheres.^[59]

In these experiments, the formation of the hollow silica shell is a spontaneous phenomenon that occurs under mildly basic conditions. These basic conditions can be derived from the base-catalysed reaction used to initially produce the silica itself. This means that the residual base (e.g. ammonia), present in the synthesis of the silica shell, remains in the solution even after the purification and can act as an etchant for the silica nanoparticles, causing the structural change. Free hydroxyl groups present in the solution can coordinate to the Si atoms, leading to the cleavage of the Si-O bonds (Scheme 6.2).



Scheme 6.2: Schematic presentation for etching of a silica structure in the presence of base.

In the case of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ nanoparticles, residual base is expected to be present after the purification steps, as it is needed to reach a neutral environment. Mild basic conditions are achieved after the purification by transferring the NPs to water; this leads to

a raise in the pH to 7.5. It is then when OH groups from residual ammonium hydroxide act as selective etchant, cleaving Si-O bonds. The process starts from the formation of initially small voids that grow overtime and join eventually to form a hollow space in the centre.

In these experiments, $\text{Fe}_3\text{O}_4@\text{SiO}_2$ NPs with different shell thicknesses were prepared as described in the previous section and were stored in water and analysed by TEM over a period of one week in the case of the sample with thin shell and three weeks in the case of the sample with thick shell.

TEM images collected for NPs of SH1, with the thin silica shell, showed a complete destruction of the silica coating after the first week (Figure 6.11), fragments of silica linking darker magnetic NPs are present in the images. The results of the experiment depend on the density and size of the silica shell. A less dense shell coating around the magnetic nanoparticles is easier to be etched, thus the reaction will proceed faster.

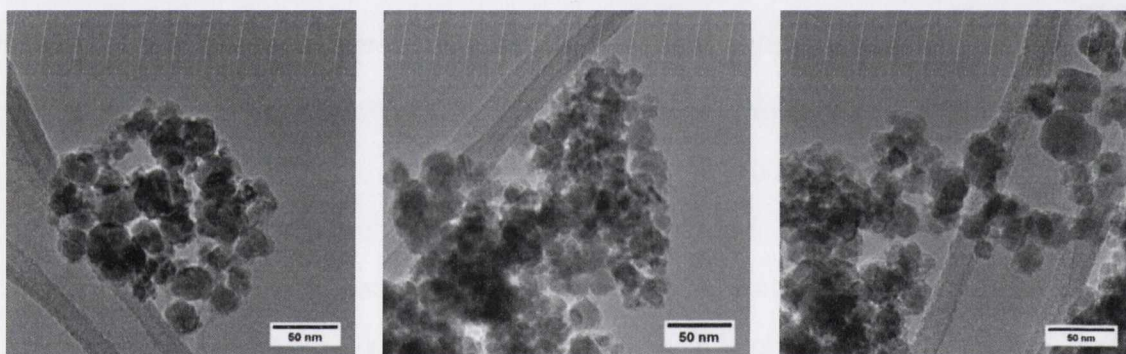


Figure 6.11: TEM images of SH1 stored in water for one week.

In the case of the TEM images of NPs with a thick shell of silica (SH2), small voids and a porous shell can be observed after one week of storage (Figure 6.12,a). Note that the porosity of silica has changed compared to the sample before the storage in water.

After two weeks, the formation of voids close to the centre of the NPs are present, as shown by the TEM images (Figure 6.12, b). The formation of these voids, particularly in the centre of the nanostructure, can be faster due to the presence of OH groups on the magnetite core. Additionally, the small voids observed after one week continued to grow into a more defined cavity between the shell and the core. The initially formed

small pores grew overtime and merged to give larger voids close to the magnetic core.
[60-61]

After three weeks of the storage in water, more aggregated nanoparticles with an evident hollow structure can be observed in TEM images (Figure 6.12, c). The magnetic moment of the nanocomposites increases with the formation of the cavities, the higher degree of aggregation of the nanoparticles is a result of that increase.

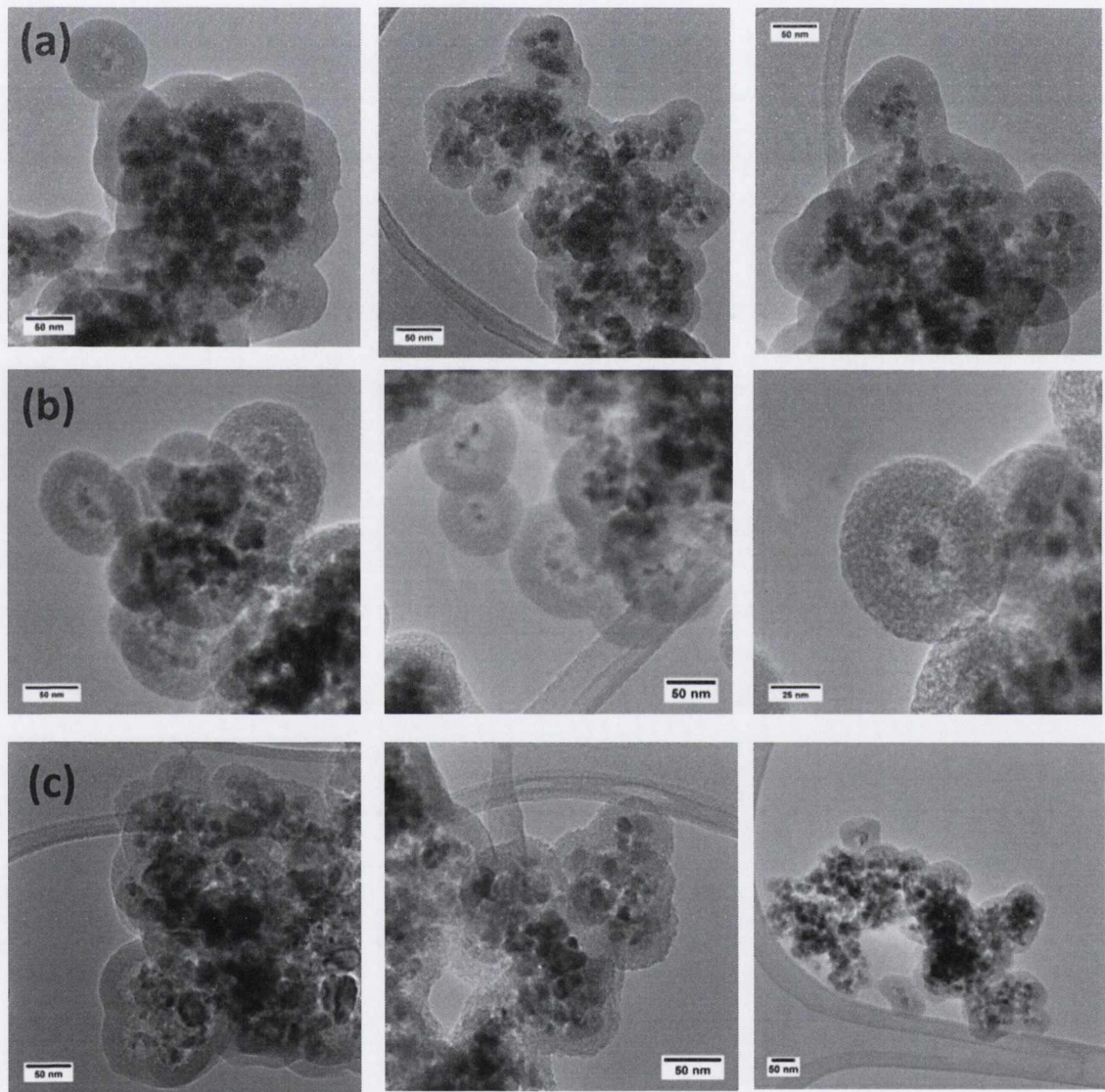


Figure 6.12: TEM images of the sample SH2 being stored in water for 1 week (a), two weeks (b) and three weeks (c).

The average composite size of the hollow Fe₃O₄@SiO₂ nanocomposites (SH2) was found to be 96.2 ± 15.7 nm from the available TEM images. This value is not far from that obtained in the case of Fe₃O₄@SiO₂ nanoparticles before the water treatment,

which was found to be 111 ± 16.4 nm. Both values are very close to each other, so it can be assumed that even if some silica has been removed from the outer layer, the most important etching occurs in the inner layers of the silica shell.

A possible reason that would explain that the etching occurs faster inside the nanoparticles than in outer layers is because the concentration of OH groups inside around the magnetic core is much higher than outside. It also been reported that the growth of the silica nanoparticles is based in the aggregation of smaller nanoparticles. The incorporation of monomer leads to an increase in the size. Thus, this process derives in a more porous material in the internal parts to the nanoparticles, which leads to a faster degradation in that area. [56-57]

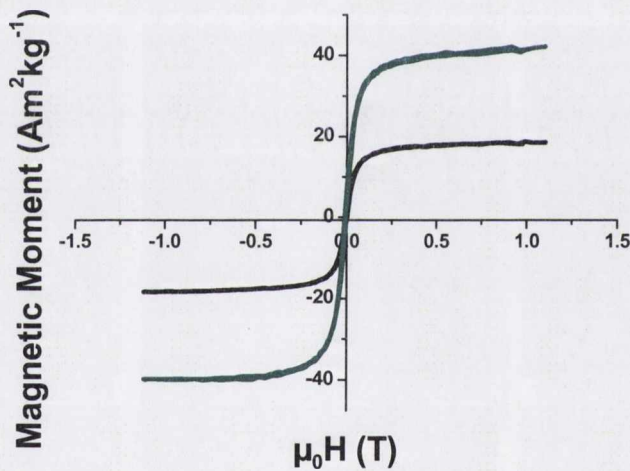


Figure 6.13: VSM curves of Fe₃O₄@SiO₂ (SH₂, black) and Yolk-Shell structure after two weeks in water (green).

The VSM data shown in Figure 6.13 shows the variation in the magnetic moment of Fe₃O₄@SiO₂ and the hollow Fe₃O₄@SiO₂ structures after two weeks in water. The M_s of Fe₃O₄@SiO₂ NPs is, as it has been shown earlier, 19 Am²kg⁻¹. The VSM performed for the sample after two weeks showed the M_s of the material was 43 Am²kg⁻¹. The increase of the magnetic moment of the nanostructures confirmed the removal of part of the non-magnetic silica shell, as it can be observed in the TEM images. It is interest-

ing to note that this value lies between the M_s values of Fe_3O_4 NPs ($76 \text{ Am}^2\text{kg}^{-1}$, Figure 6.5) and $\text{Fe}_3\text{O}_4@\text{SiO}_2$ NPs ($19 \text{ Am}^2\text{kg}^{-1}$).

6.4 Synthesis and Characterisation of Hollow Magnetite@Silica Nanostructures loaded with coumarin

Hollow $\text{Fe}_3\text{O}_4@\text{SiO}_2$ nanostructures were loaded with a coumarin derivative, a yellow dye (Figure 6.14), which was incorporated into the nanostructure before the silica coating.

Coumarin is a benzopyrone, which consist in a benzene ring combined with a lactone ring. Lactone rings contains double bonds that extends the π -system over the molecule.^[62] Coumarin and its derivatives have been investigated for biological applications such as anti-inflammatory or anticoagulants among others.^[63-64]

Here, coumarin has been used to test the functionality of the hollow $\text{Fe}_3\text{O}_4@\text{SiO}_2$ as a potential drug carrier and consequently a drug release system. The use of a dye in this case allows us to track the presence of the compound at different stages of the synthesis by spectroscopy.

Here, coumarin-3 carboxylic acid was treated with (3-Aminopropyl) triethoxysilane (APTES) in a slow reaction stirring for 24 hours, before it was added to a dispersion of Fe_3O_4 NPs in ethanol. The solution was then sonicated for 1 hour to homogenate the mixture and bind dye molecules to the magnetic NPs.

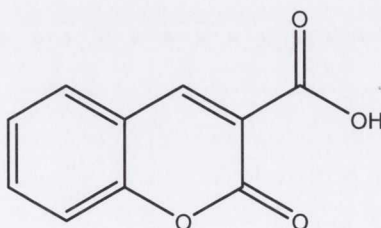


Figure 6.14: Structure of Coumarin-3 carboxylic acid.

After that, the protocol used for the synthesis of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ giving sample SH2 (section 6.2) was followed to grow a silica shell coating on the magnetic nanoparticles.

The changes in the structure and the formation of the hollow structures were followed overtime using TEM analysis. Figure 6.15 shows the TEM images demonstrating the evolution overtime. The $\text{Fe}_3\text{O}_4@\text{SiO}_2$ nanocomposites after the silica coating are shown in Figure 6.15, a. There is an aggregation of small Fe_3O_4 NPs which are coated with a thick shell of silica. The average size of these structures was measured from TEM images ($N = 40$) and found to be 125.9 ± 40.0 nm. The observed broad size distribution of the nanocomposites can be attributed to the poor homogeneity during the reaction.

After this material was transferred to water, in order to change the pH of the solution and start the growth of voids in the structures. The $\text{Fe}_3\text{O}_4@\text{SiO}_2$ nanocomposites were further analysed with TEM. Images in Figure 6.15, b shows the formation of the hollow structures after three days in water.

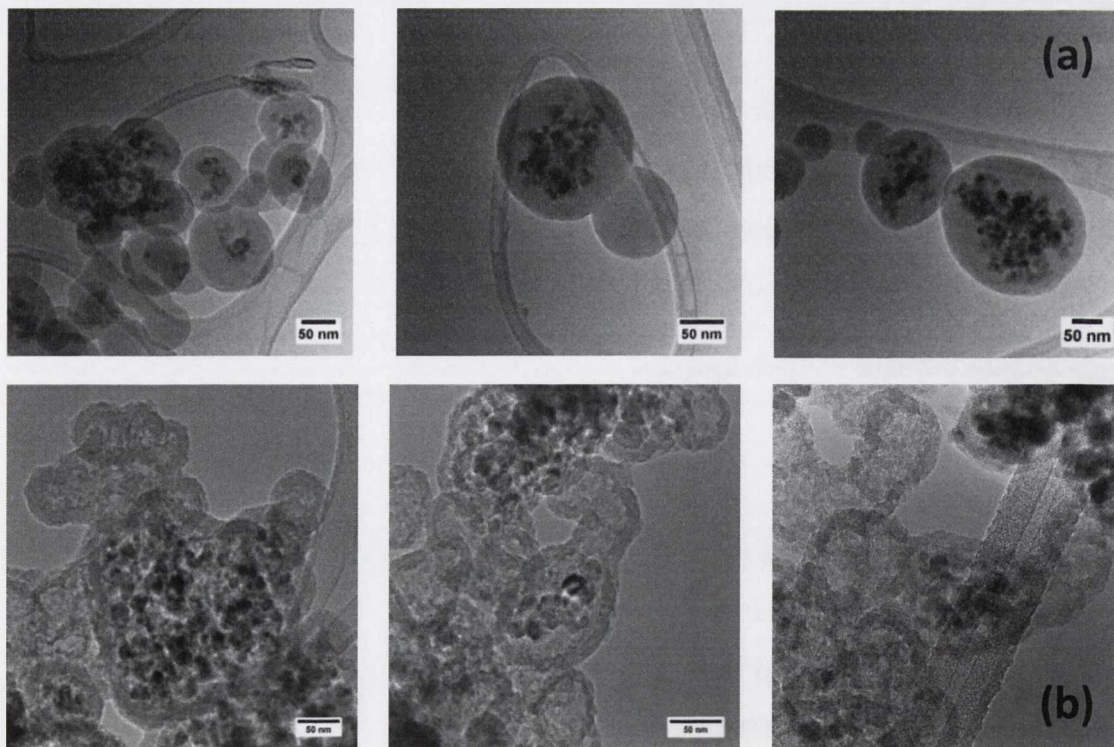


Figure 6.15: TEM images of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ -dye nanocomposites before water storage (a) and after 3 days storage in water (b).

The formation of hollow $\text{Fe}_3\text{O}_4@\text{SiO}_2$ -dye nanocomposites was much faster (3 days) than in the experiment carried out in the absence of dye (1-3 weeks). As the change in the structure of silica is related to the pH, the rate of the hollowing can be affected by the pH inside of the nanostructures. In this case, aside from the residual OH groups from ammonium hydroxide used during the formation of the silica shell; the pH can also be raised due to the presence of NH_2 groups from the residual APTES.

The UV-Vis spectra were measured to track the presence of the dye. After the synthesis of the initial $\text{Fe}_3\text{O}_4@\text{SiO}_2$ nanocomposites, the material was washed three times with ethanol and isolated using an external magnetic field. The UV-Vis spectra of these washings were recorded and are shown in Figure 6.16. The UV-Vis spectra of the solution after finishing the reaction confirmed the presence of dye, which was not incorporated into the nanostructures. Sequential washings reduced the presence of unbound dye in the solution. This simple test proved that dye was not released from the nanocomposites into the solution before transferring the material from ethanol to water.

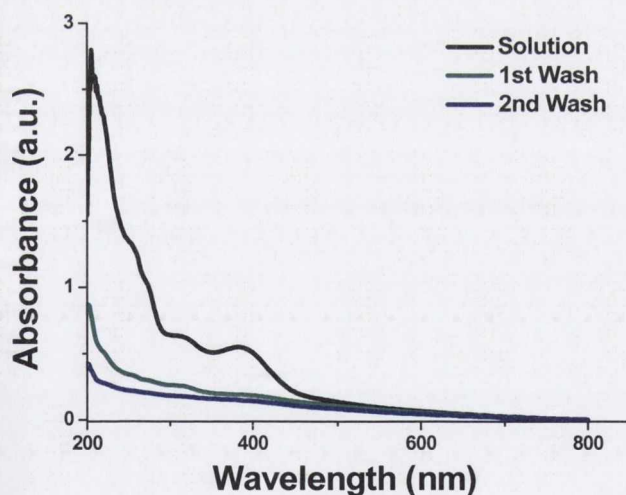


Figure 6.16: UV-VIS spectrum of solution after reaction and formation of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ nanocomposites (black), first washing with ethanol (green) and second washing with ethanol (blue).

After transferring the material from ethanol to water, the solution was monitored with UV-Vis spectroscopy in order to detect any change in its composition. After 3 days in

water, the solution showed the characteristic peaks of the dye. However, no further changes can be observed for the same solution after 8 days of storage.

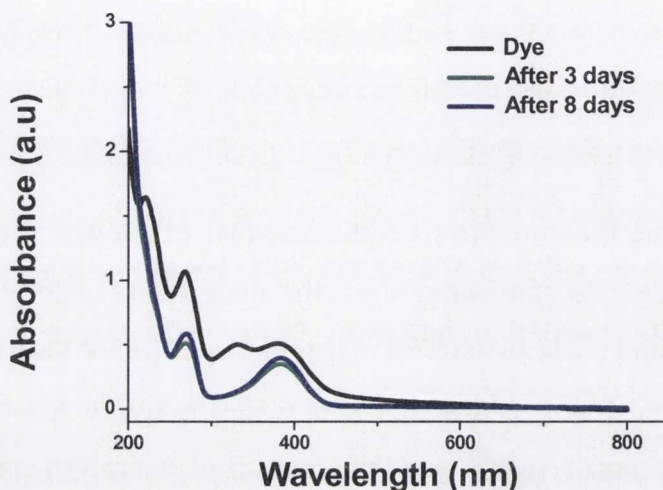


Figure 6.17: UV-Vis Spectrum of the pure dye in water (black), solution after 3 days of storage (green) and solution after 8 days of storage (blue).

An analysis of the material after the release of the dye was carried out using TEM. The images in Figure 6.18 show hollow structures with no evident difference to those before the dye was released. Despite some free Fe_3O_4 NPs being observed, well-defined nanorattles are the dominant species in the sample. It can be concluded that the dye is mostly released through the pores formed after the etching of the silica shell.

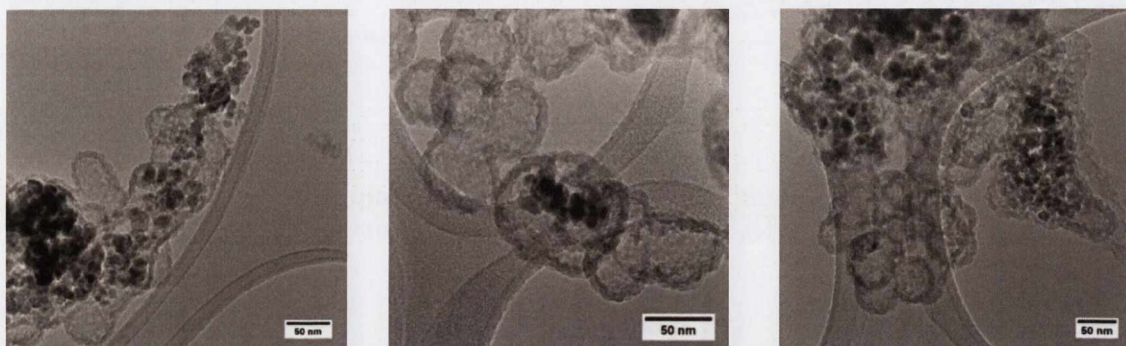


Figure 6.18: TEM images of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ -dye nanorattles after the release of the dye over 8 days.

New hollow $\text{Fe}_3\text{O}_4@\text{SiO}_2$ -dye nanocomposites have been successfully prepared. It was also found that the mesoporous silica etching process facilitates the releases of the dye opening new opportunities for applications of these nanomaterials.

6.5 Synthesis and Characterisation of Loaded Yolk-Shell Magnetite@ZnSQD@Silica Nanostructures

Another experiment carried out, was the synthesis of yolk-shell $\text{Fe}_3\text{O}_4@\text{SiO}_2$ nanoparticles containing ZnS:Mn QDs.^[65] Fluorescent QDs were combined with the same amount of Fe_3O_4 NPs used in experiment SH2 in ethanol. Silica coating was carried out in the same way that the experiment mentioned earlier, using TEOS as the silica precursor. Following the same protocol, the sample was treated with water and stored for two week to selectively etch the silica shell. The purpose of this experiment was to investigate a different application of the hollow structures, such as releasing the encapsulated material by sonication.

Figure 6.19 shows TEM (left) and STEM (right) images of the ZnS:Mn QDs. The average size distribution of the ZnS:Mn QDs is 5 nm, as can be observed in the images. In addition, there is a considerable difference in the contrast compared to the magnetic nanoparticles enabling us to assure the presence of the QDs.

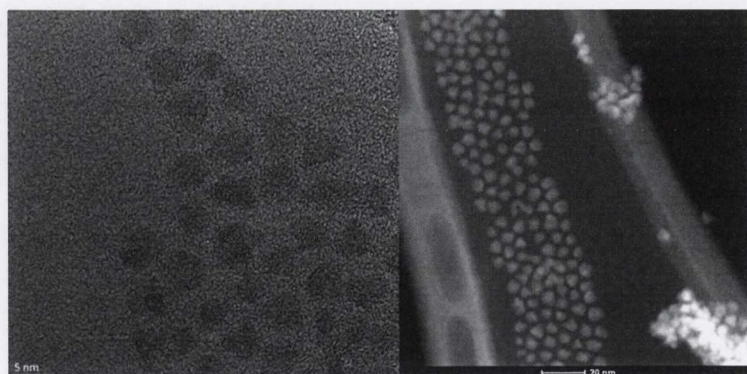


Figure 6.19: TEM (left) and STEM (right) images of ZnS:Mn QDs

The nanocomposite was analysed over time using TEM. The TEM images in Figure 6.20 show the nanocomposites after the coating with the silica. Aggregation of magnetic NPs and QDs inside the silica shell can be observed. Based on the images, it can be

assumed that the smaller (5 nm) particles are ZnS:Mn QDs and the larger particles represent the Fe_3O_4 NPs (11.7 ± 0.3 nm).

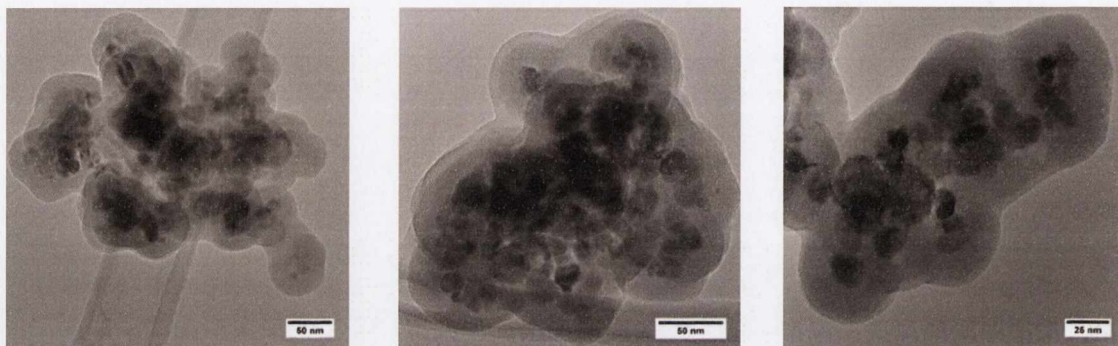


Figure 6.20: TEM images of the $\text{Fe}_3\text{O}_4/\text{ZnS}@ \text{SiO}_2$ nanocomposites.

The TEM images of the nanocomposites after two week of storage in water are shown in Figure 6.21. Complete decomposition of the silica shell is observed although some thinly layered silica fragments remain in the sample. This behaviour has already been observed in the previous experiments and was discussed above. This observation can be connected to the fact that the concentration of NPs (MNPs + QDs) was increased during the reaction, by adding QDs to the solution. As the rest of the reactants were kept in the same proportions to previous experiments, it can be expected that the silica present is no longer sufficient to give a shell with the same thickness than previous experiment. This would result in a thinner shell (15-20 nm) compared to the sample formed in the case of the experiments carried out for the hollow $\text{Fe}_3\text{O}_4@ \text{SiO}_2$ and $\text{Fe}_3\text{O}_4@ \text{SiO}_2$ -dye nanocomposites. However, another more likely reason is that there is a highly basic environment in the inner layer of silica due to the presence of the QDs, which were stored in a basic solution. A higher pH would accelerate the process of etching.

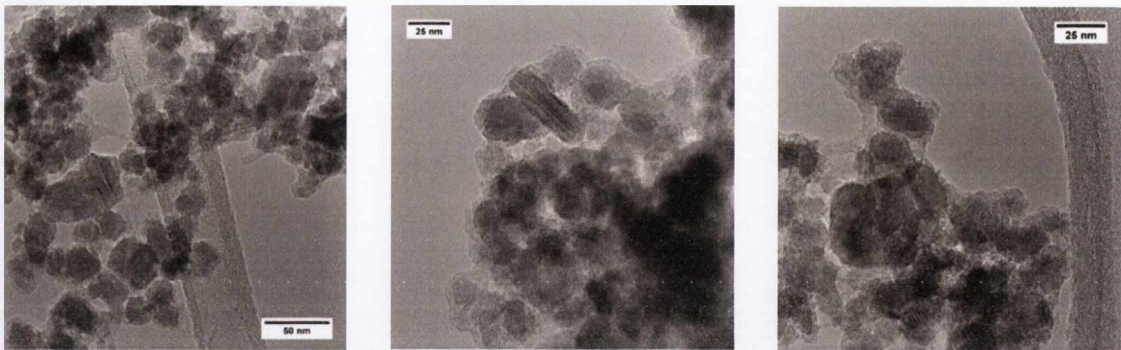


Figure 6.21: TEM images of Fe₃O₄/ZnS:Mn@SiO₂ nanocomposites after two weeks storage in water.

After two weeks of storage in water, the Fe₃O₄/ZnS:Mn@SiO₂ nanocomposites were sonicated for 90 minutes in a sonic bath. A UV-Vis spectrum of the solution before and after sonication was recorded. The MNPs were removed from the solution by magnetic separation before performing the measurements. Figure 6.22, left shows the presence of the ZnS:Mn QDs in solution for the sonicated nanocomposite sample. The signal centred around 300 nm is broad, but the red shift could be explained by the fact that the quantum dots are aggregated in the silica shells and/or have grown due to heating of the sample during the sonication process. The UV-Vis spectrum of the solution before sonication does not show a significant signal; this can be an indication that the leaching of the QDs is induced by sonication. Prevention of the quantum dot quenching could be brought about in the future by employing a double coating of silica in the synthesis; a layer before and after the addition of QDs to separate them from the magnetite NP cores.

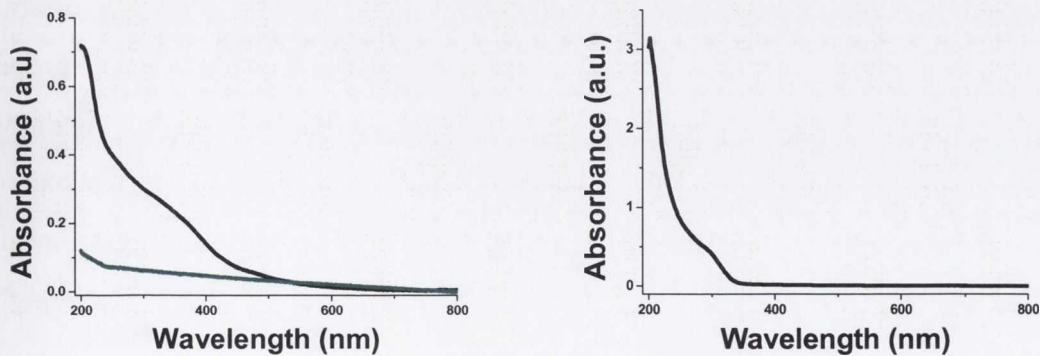


Figure 6.22: UV-Vis spectra of Fe₃O₄/ZnS:Mn@SiO₂ nanocomposites in solution (left) before sonication (green) and after sonication (black) and UV-Vis spectrum of ZnS:Mn QDs (right).

Photoluminescence (PL) measurements were carried out for the nanocomposites before and after sonication. These measurements (not shown here) did not show any luminescence peaks demonstrating the presence of ZnS:Mn QDs, which can be due to the complete quenching of the luminescence, as a result of the presence of the MNPs and for example a partial degradation of the QDs.

6.6 Conclusions

The development of new interesting yolk-shell multimodal magnetic nanocomposites has been demonstrated here. In this case, superparamagnetic magnetite nanoparticles served as the core material for the nanocomposites. Nanoparticles coated with a controlled amount of silicon oxide were used as a starting material to produce yolk-shell magnetic nanostructures by a new and facile method of synthesis. It was found that residual hydroxyl groups trapped inside the silica matrix together with magnetic nanoparticles could etch the silica shell over a 1-3 week period of time, if the nanoparticles were kept in water. It is obvious, that in this case, water molecules can diffuse throughout the silica shell and create a local basic solution inside the silica shell leading to etching of the shell from the inside. This process resulted in hollow yolk-shell nanostructures where magnetite nanoparticles were trapped inside a silica shell. The structures and processes were visualised by TEM. As the prepared yolk-shell structures did not exceed 100 nm in diameter and magnetisation was considerably retained, the thickness of the silicon oxide shell could potentially be increased and the nanocomposites could be stored in a mildly basic medium for a longer period of time, in order to increase the loading capability of the nanocomposite. The loading and release properties of the shell material were demonstrated experimentally using a coumarine derivative and ZnS:Mn quantum dots. This research arguably opens up a new approach to novel yolk-shell magnetic nanomaterials with a range of potential applications, including drug delivery and release systems, catalysis, on demand magnetic triggering of processes, multimodal biological imaging and others.

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Chapter 7. Conclusions, Feasibility Studies and Future Work

7.1 Conclusions

In this work, a number of new magnetic nanoparticles and nanocomposites materials with a broad range of potential applications have been prepared and characterised using various instrumental techniques.

Several new cobalt ferrite based core-shell nanocomposites have been developed using a combination of co-precipitation and sol-gel techniques and catalytic properties of these nanostructures have been studied. The double layer coated $\text{CoFe}_2\text{O}_4@\text{SiO}_2@\text{TiO}_2$ nanocomposites showed photocatalytic activity in the oxidation of methylene blue under UV light. In order to achieve the active photocatalytic phase of TiO_2 , anatase, the sample was sintered at 600 °C. The preparation of $\text{CoFe}_2\text{O}_4@\text{CeO}_2$ nanocomposites involved the deposition of CeO_2 on the surface of cobalt oxide magnetic nanoparticles that was confirmed by TEM and other techniques. Catalytic activities studies carried out with this material showed the degradation of the organic with and without light.

Core-shell nanostructures involving the deposition of metals such as Ag and Au on $\text{CoFe}_2\text{O}_4@\text{D-Pen}@\text{SiO}_2@\text{TiO}_2$ have also been developed. The nanomaterials have been used as catalysts in the degradations of the organic dye, methylene blue, and have demonstrated promising results.

It is important to notice that after coating of magnetic nanoparticles with different materials shells of, the magnetic parameters of resulting nanocomposites remained strong enough to enable to recover them from the reaction using an external magnetic field. Therefore, these nanocomposites can serve as promising magnetically recoverable catalysts.

The use of amino acids as stabilisers during the formation of cobalt oxide magnetic nanoparticles has shown to have pronounced effects on the size, shape and magnetic

properties of the nanomaterial. There was a reduction in the size of the nanoparticles with an increase of the concentration of amino acids, such D-Penicillamine, D- and L-Cysteine, D- and L-Aspartic Acid and D-Glutamic Acid were used in the co-precipitation reaction accompanied by a narrowing of the average size distribution. There was also a significant aggregation and the formation of clusters due to the oriented attachment and magnetic dipole-dipole interactions.

We have also found that the size of cobalt oxide magnetic nanoparticles can be controlled by changing the concentration of L-alanine as a stabiliser during the reaction. By increasing the amount of ligand the size of the NPs is reduced up to a point that superparamagnetic behaviour of the material is reached. We believe that the use of amino acid as stabilisers for ferrite nanoparticles opens up a new strategy to design magnetic nanomaterials of controlled size, shape and aggregation for a broad range of potential applications.

Fe@C@SiO₂ core-shell nanostructures have been prepared and tested in DNA purification. These nanomaterials have also been compared to silica based spin column design for DNA recovery and a commercially available DNA purification kit is used. Results show that Fe@C@SiO₂ core-shell nanostructures can effectively recover DNA in a faster way than the traditional protocols due to the advantage of being quickly removed magnetically from the solution. However, further studies are necessary to investigate the further development and potential up scaling of the process and to improve the recovery to overtake commercially available kits.

Preliminary experiments have also shown interesting results in the enantiomeric separation of racemic mixtures using chiral functionalised cobalt oxide magnetic nanoparticles. Although the possible leaching of metal ions to the solution adds some difficulty to the interpretation of the results, we believed that it should be a promising and useful approach for the separation of enantiomeric compounds. We believe that further detailed studies are necessary to confirm if the presence of the chiral ligands could

imprint chiral features enabling the enantiomeric recognition, selection and separation of chiral compounds.

New yolk-shell multimodal magnetic nanocomposites of silica coated magnetic nanoparticles have been successfully developed in our work. It was found that silica coated magnetic nanoparticles are not stable in aqueous medium and residual hydroxyl groups trapped inside the silica matrix together with magnetic nanoparticles could etch the silica shell over a 1-3 week period of time. This process was found to generate new hollow yolk-shell nanostructures where magnetite nanoparticles were trapped inside a silica shell that was confirmed by TEM. TEM images showed that the prepared yolk-shell structures did not exceed 100 nm in diameter and magnetisation was considerably retained and even increased after removing part of the non-magnetic shell. Thickness of the silica shell can be controlled by the time of the etching process in water. We have also developed new multimodal nanostructures by loading the initial magnetic-silica core-shell nanostructures with coumarine derivatives and ZnS:Mn quantum dots. Then we have demonstrated the release of coumarine derivatives and quantum dots over time period. Properties of the shell have been demonstrated experimentally using Zn:Mn QFs and coumarin. This research opens up a new approach to produce yolk-shell magnetic nanomaterials with a range of potential applications, including new drug delivery and release systems, as catalysis, on demand magnetic triggering of processes, multimodal biological imaging and others.

7.2 Preliminary Studies and Future Work

Future work will include further research and development on the applications of the new synthesised magnetic nanomaterials.

7.2.1 Development of Magnetically Activated Adhesives Based on $\text{CoFe}_2\text{O}_4@\text{CeO}_2$ NPs and Super Glue Formulations

On-demand initiation of chemical reactions using a magnetic stimulus is one of the most challenging and intriguing problems in chemistry. For the first time, we developed on-demand magnetic activation of a polymerisation process using an anaerobic adhesive formulation as an example of our approach toward triggering chemical reac-

tions using an external magnetic field. Our strategy involved the use of a new colloidal system comprising functional methacrylate ester monomers, peroxide and Cu^{II}-salt as polymerisation initiators and magnetic nanoparticles (e.g. CoFe₂O₄) coated with an oxidizing shell (e.g. MnO₂ or CeO₂). This unique combination prevents reduction of the reactive transition metal (Cu^{II}) ion by the metal substrates (steel) to be joined - hence inhibiting a polymerisation reaction and efficiently preventing adhesion. The polymerisation and corresponding adhesion process can be triggered by removal of the magnetic particles using a permanent external magnet, enabling the polymerisation to proceed through Cu^{II}-mediated reduction.

For example, core-shell CoFe₂O₄@CeO₂ nanoparticles can be used in the activation of anaerobic adhesive. In our studies, we used anaerobic adhesive formulation, which composed of a monomer (Triethylene glycol dimethacrylate), an initiator (tert-butyl peroxybenzoate) and a surface activator (copper (II) tetrafluoroborate hydrate). In order to inhibit and delay the polymerization of the mixture of oxidasing CoFe₂O₄@CeO₂ nanoparticles are added. A series of experiments varying the amount of magnetic nanoparticles were carried out and the reaction was followed during time to follow the effect of the nanoparticles in the adhesion time (activation of the glue).

Table 7.1: Reaction conditions using CoFe₂O₄@CeO₂ NPs.

Amount of CoFe ₂ O ₄ @CeO ₂	Control Sample					
	0 g	0.01g	0.025g	0.05g	0.075g	0.1g
Time (s)	20	30	30	50	50	50

Table 7.1 shows the conditions of the reaction using CoFe₂O₄@CeO₂ NPs. It can be observed that the time it take the adhesive to harden increases when the amount of material is higher. The time becomes constant when the amount of magnetic nanoparticles is increased up to 50 seconds, meaning that it is not necessary to add more nanoparticles to force the activation of the glue.

After removing the magnetic nanoparticles from the mixture, the adhesion time remaining constant and in the same value that the control experiment (20s). This is an

indication that there is not oxidising CeO_2 left in the solution after removing the nanoparticles.

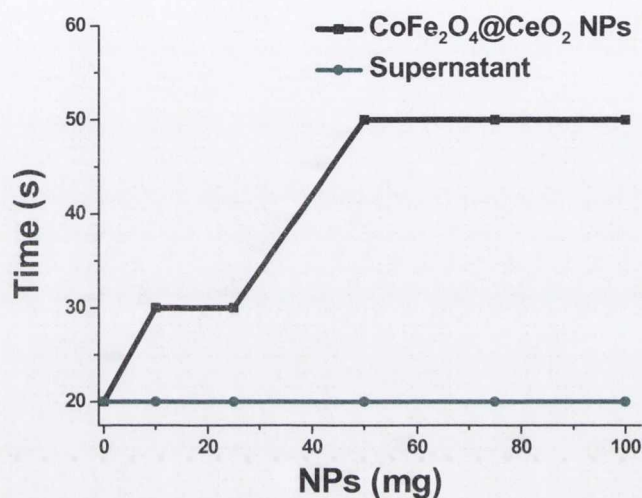


Figure 7.1: Adhesion tests using $\text{CoFe}_2\text{O}_4@\text{CeO}_2$ NPs (black line) and supernatant (green line).

Figure 7.1 Shows the increase of the time of adhesion when the amount of NPs added increases. The time of adhesion remains constant when the magnetic nanoparticles are removed from the solution.

Thus our novel approach potentially enables on-demand magnetically-triggered reaction initiation and holds great potential for a range of important applications in chemistry, materials science and relevant industrial manufacturing. Therefore we plan to develop this method and area in our future work. Our future studies will involve the development of a range of magnetic core-shell nanostructures with oxidising metal oxide shells (e.g. CeO_2 , MnO_2 , OsO_4 etc.) and testing of these nanomaterials in the inhibition and activation by magnetic removal of various polymerisation reactions involving various redox (e.g. $\text{Cu(II)}-\text{Cu(I)}$ redox pair) initiators. We believe that this work could also open up fundamentally new approaches in colloidal chemistry, offering a novel concept of magnetic stabilisation and subsequent destabilisation or activation of

reactions using colloidal systems (potentially including relevant biological colloidal systems).

7.2.2 Development of Amino Acid Stabilised CoFe_2O_4 NPs as Antibacterial Agents

Preliminary studies were performed for some of the samples of cobalt oxide magnetic nanoparticles stabilised with amino acids. The purpose of this experiment was to determine the effect of amino acid stabilized cobalt ferrite (CoFe_2O_4) on the viability of *Escherichia coli* (E coli) bacteria over 3 and 6 hours contact periods. The test was performed using the measurement of antibacterial activity on glass slides (ISO 22196:2011).

Glass slides for coating with amino acid functionalized CoFe_2O_4 NPs were supplied to Airmid Healthgroups Ltd, which has also performed antibacterial tests. Glass slides with no coating, were used as control. The measured area was 25 X 25 mm. The samples were autoclaved at 105 °C for 30 min prior to testing to ensure their sterility. Glass slides were labelled as uncoated, coated with cobalt ferrite (CoFe_2O_4) and four amino acid enantiomers coated of cobalt ferrite (CoFe_2O_4 L-Cysteine, CoFe_2O_4 D-Cysteine, CoFe_2O_4 D-Penicillamine, CoFe_2O_4 L-Penicillamine) nanoparticle samples.

The experimental procedure was performed in accordance with BS ISO 22196:2011 measurement of antibacterial activity with some alterations made to the protocol due to the dimensions of the samples. Two main alterations to the standard were the surface area used (25 X 25 mm rather than 50 X 50 mm as specified in the standard) and a contact time of 3 hours or 6 hours rather than the 24 hours in the standard. The different contact times were performed on separated days. The method was as follows: *E coli* ATCC8739 was grown in Tryptic Soy Broth (TSB) overnight at 37°C aerobic conditions and was adjusted to a concentration of 8×10^5 cells/mL in 1/500 Nutrient Broth. The adjustment in cellular concentration was calculated from previously performing serial dilutions of an overnight culture and correlating the bacterial concentration

against the level of absorbance at an optical density of 600 nm using a spectrometer.

A 150 μ L aliquot of adjusted bacterial suspension was placed on the surface of the treated and untreated specimens under sterile conditions in a laminar flow cabinet. The treated and untreated specimens were immediately transferred to a thermostatically controlled incubator at 35 °C and high relative humidity and were incubated for 3 hours and 6 hours. The results of antibacterial tests are summarised in Tables 2 and 3.

Table 7.2: Antibacterial activity values of coating after 3 hours incubation.

	Sample Description	Ln ₁₀	Antibacterial Activity R
U _t	Control	4.98	N/A
A _t	CoFe ₂ O ₄	4.49	0.49
	CoFe ₂ O ₄ L-Cys	4	1.47
	CoFe ₂ O ₄ D-Cys	2.66	2.32
	CoFe ₂ O ₄ L -Pen	3.38	1.60
	CoFe ₂ O ₄ D-Pen	3.03	1.95

Table 7.3: Antibacterial activity values of coating after 6 hours incubation.

	Sample Description	Ln ₁₀	Antibacterial Activity R
U _t	Control	5.22	N/A
A _t	CoFe ₂ O ₄	4.03	0.95
	CoFe ₂ O ₄ L-Cys	3	2.32
	CoFe ₂ O ₄ D-Cys	1.56	3.42
	CoFe ₂ O ₄ L -Pen	2.03	2.95
	CoFe ₂ O ₄ D-Pen	1.93	3.05

Table 7.2 and Table 7.3 show the antibacterial activity of coated nanoparticles after 3 and 6 hours incubation respectively. The calculations were carried out following the equation:

$$R = (U_t - U_0) - (A_t - U_0) = U_t - A_t$$

Where:

R is the antibacterial activity

U_0 is the average of the common logarithm of the number of viable bacteria in cell/cm² recovered from the untreated test specimens immediately after inoculation.

U_t is the average of the common logarithm of the number of viable bacteria in cell/cm² recovered from the treated test specimens after incubation.

A_t is the average of the common logarithm of the number of viable bacteria in cell/cm² recovered from the treated test specimens after incubation.

The data obtained in these studies demonstrated the cobalt ferrite coatings submitted for testing showed antimicrobial activity properties with some enantiomers displaying higher levels of activity than others. The antibacterial activity of CoFe₂O₄ D-Cysteine and CoFe₂O₄ D-Pen nanoparticles was higher than corresponding samples stabilised by L- enantiomers and significantly higher than activity of uncoated CoFe₂O₄ nanoparticles. It was also noted that the antimicrobial activity values increased as the contact time with the bacterial inoculum increased from 3 hours to 6 hours. All criteria for the fulfilment of a valid test were met where applicable. Thus these feasibility studies have demonstrated that amino acid stabilised ferrite nanoparticles can be promising antibacterial agents. Most importantly our preliminary testing of these nanomaterials clearly demonstrated enantiomeric selectivity in the toxicity for biological species. Therefore our future work will involve detailed antibacterial studies of all our amino acid stabilised magnetic nanoparticles with particular attention to be paid to various D- and L – enantiomer stabilised nanomaterials. We believe that this research would allow us to design and fabricate new types of antibacterial materials for potential pro-

duction of antibacterial powders, paints and coatings. Moreover, magnetic modality of these materials might enable to design new processing techniques as well as ability to selectively isolate dangerous bacterial species using a magnetic separation.

7.2.3 Further Development of Yolk-Shell Magnetic Nanostructures for Biomedical Applications

Finally one more of our future objectives is to further develop new hybrid silicon dioxide based magnetic hollow rattle-like or yolk-like nanostructures. We plan to prepare a range of new magnetic nanorattles based with the loading of new drugs and carry out drug delivery and drug release biological tests in cells in order to demonstrate the ability of the drug to be selectively released in a cell cultures media. Particular attention will also be paid to further development of multimodal magnetic nanorattle-like structures which magnetic nanoparticles will be trapped in the hollow silica shell together with quantum dots and or plasmonic nanoparticles. To the best of our knowledge these multimodal systems have never been studied. Meanwhile, these nanomaterials could open up an access to novel materials, which can change their properties (e.g. colour, emission etc.) by the swipe of a magnet. Finally, magnetic nanorattle like structures can be potentially used as nanoreactors to study various processes and catalytic systems. The presence of magnetic nanoparticles in the core of the nanostructures would facilitate the recovery and re-use of the catalytic nanostructures by a simple magnetic separation. Moreover the use of an external variable magnetic field could facilitate the stirring and convection of the reaction mixtures inside the hollow silica vesicles.