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Synthesis and Functionalisation of Nanomaterials for Biomedical Diagnostic Applications

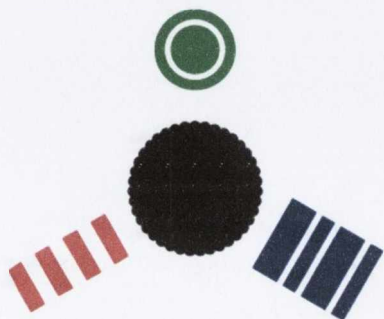


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Under the supervision of Prof. Yurii Gun'ko

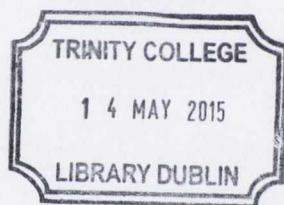
Materials Research Group, TCD

September 2014



NAMDIATREAM

**NANOTECHNOLOGICAL TOOLKITS
FOR MULTI-MODAL DISEASE DIAGNOSTICS
AND TREATMENT MONITORING**



Thesis 10589



DECLARATION

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"If I have seen further it is by standing on the shoulders of ye Giants"

Isaac Newton

This work would not have been possible without the many amazing people I have met over the past ten years of my studies in science. The process of completing this research has instilled a profound sense of gratitude and humility in me; not only due to the incredible kindness of the people around me, but also the vast wealth of knowledge available to us today.

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I have been lucky to be surrounded by such positive, inspiring people, and small or large, they have all contributed to this work in some way.

Summary

This thesis explores synthesis and functionalisation of a variety of nanomaterials for the purpose of biological detection with a potential focus on medical diagnostics. Early diagnosis is the key to disease prevention, improves patient outcome and reduces the need for aggressive therapies for diseases such as cancer. Therefore we aim to push the boundaries of current detection systems and create new materials for more sophisticated, targeted applications. Using visible detection, magnetic sample recovery, and flow detection systems we hope to create fast, cheap, non-invasive detection technologies. This should improve patient compliance, increase voluntary testing and reduce the incidence of advanced stages of disease in the population. Thus, the main goal of this work is to develop new magnetic or multimodal magnetic/plasmonic/fluorescent nanomaterials of various shapes by various synthetic strategies for biomedical applications, in particular for new, improved medical diagnostic techniques, and the detection of biomarkers for disease at early stages.

Chapter 1 of the thesis provides an introduction to nanomaterials, including their synthesis, functionalisation, properties and biomedical applications. Special attention is paid to unique properties, which are typical only attributed to nanomaterials, such as superparamagnetism, plasmon resonance properties and size dependent photoluminescence of quantum confined nanostructures. Various synthetic approaches and functionalisation strategies are discussed in detail. Also, biomedical applications of nanomaterials are also reviewed and summarised.

In Chapter 2, synthesis, biofunctionalisation and characterisation of Fe_3O_4 nanoparticles is described. These well-established nanoparticles were selected to act as a benchmark for characterisation and detection of subsequent more complex nanomaterials prepared in this work, such as bioconjugated metal, alloy, segmented and silica nanowires. Other techniques were also applied to synthesis of magnetically loaded silica nanowires using anodic alumina membrane templates with micron range length and sub-micron width. These wires were easily detectable by scattering techniques, and interesting comparisons were found between nanoparticle and nanowires.

Chapter 3 is dedicated to the preparation of sub-micron nickel particles and their functionalisation for potential biodetection systems. These particles have a diameter smaller than the wavelength of light (particle size <200 nm), and are smaller than current state of the art bead technologies used for flow detection systems, which are generally several

microns in diameter. However, due to their high density and refractive properties, scattering of light from these particles can be achieved and therefore visible detection. This smaller size increases their surface area, hence improves molecular attachment to the surface. It also creates a larger relative change in diameter and signal intensity between bare particles and bioconjugated samples, further enhancing signal detection. These particles have been coated in a novel way and bioconjugated to proteins which are widely used in the field of biomedical research. Their magnetic properties are also greatly improved compared with current state of the art. In addition, these particles possess a special property of soft magnetic behaviour, which allow them to act similarly to small superparamagnetic nanoparticles, whereby they can be magnetised in the presence of a magnetic field, but retain no magnetisation in its absence, thus greatly improving their solvent stability which is crucial for these types of flow detection systems. They can be synthesised in high yields in the multiple gram scale and have the potential for commercial exploitation

In Chapter 4, we present the preparation and characterisation of a variety of novel anisotropic nanomaterials by an organic phase hot injection approach. These materials include iron binary alloy materials of FePt NWs, FePd₃@Fe₃O₄ nanostructures, FeNi, FeCu, and sulphur-based semi-conductors of CuS, Cu₂S, Bi₂S₃ and Ni₃S₄ nanoplates, Fe₃S₄ nanoflakes, and FePt@Au tail-head heterostructures. These materials exhibit various properties such as superparamagnetism, plasmon resonance and fluorescence. We expect to use them to further enhance the sensitivity and selectivity of detection of biomarkers for disease using flow detection systems, by exploiting their ability to scatter light but also interact differentially with different frequencies of the electromagnetic spectrum. These materials have a high surface to volume ratio and due to their unusual shapes and flat surfaces, they can interact differently with biomolecules, which are also in the nanometre size range. Therefore, these anisotropic materials may also improve the binding strength and efficiency to biomolecules. In addition, we have developed a novel transfer route from organic to aqueous phase which improves biocompatibility, allows for further bioconjugation and opens the door to bioapplications for many new materials not previously achievable.

Chapter 5 presents an overall summary and conclusion of the work with suggestions for future work. Chapter 6 of the thesis provides full experimental details on the synthesis and functionalisation of nanomaterials and description of various instrumental characterisation techniques used in this work. Overall this work has made a contribution to the further development of the synthesis and functionalisation approaches of various nanomaterials to be used for potential biomedical applications.

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LIST OF ABBREVIATIONS

acac - acetylacetonate	MeOH - methanol
AcN - Acetonitrile	miRNA – micro ribonucleic acid
APTS – 3-aminopropyltriethoxysilane	MNP – magnetic nanoparticle
Ar. - aryl	MRI – magnetic resonance imaging
Asym. – asymmetric	MTMS – 3-mercaptopropyltrimethoxysilane
4-ATP – 4-aminothiophenol	MUO – 11-mercapto-1-undecanol
BC – biomolecular capture	MUA – 11-mercapto-1-undecanoic acid
bco – body centred orthorhombic	NMR – nuclear magnetic resonance spectroscopy
Bnd. - bending	NP - nanoparticle
Cel – cesium iodide	NW – nanowire
CBS - Citrate Buffer Solution	OA – oleic acid
CD4-PE – human CD4 antibody-R-phycoerythrin	OAc - acetate
DDT - dodecathiol	OAm - oleylamine
Def. - deformation	OOP – out of plane
DRIR – diffuse reflectance infrared spectroscopy	ortho – orthorhombic
EDC – 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide	PBS – phosphate buffer solution
EtOAc – ethyl acetate	PEG – poly(ethylene glycol)
EtOH – ethanol	PTFE – polytetrafluoroethylene (Teflon)
FACS – fluorescence-activated cell sorting	QD – quantum dot
FC – flow cytometry	Rck. - rocking
fcc – face centred cubic	RBF – round-bottomed flask
fct – face centred tetragonal	SPIO – superparamagnetic iron oxide
Fmoc - fluorenylmethyloxycarbonyl	SPPS – solid phase peptide synthesis
FTIR – fourier transform infrared spectroscopy	Str. – stretching
GNP – grapheme nanoplatelet	Sym. - symmetric
hex. – hexagonal close packing	T - tesla
HI – hot injection	<i>t</i> -Bu – <i>tert</i> -butyl
HRTEM – high resolution transmission electron microscopy	TEOS - tetraethylorthosilicate
IP – in plane	tet. – tetrahedral close packing
iPrOH - isopropanol	TGA – thermogravimetric analysis
IR – infrared spectroscopy	THF - tetrahydrofuran
mal - maleimide	UV- <i>vis</i> – ultraviolet-visible absorbance spectroscopy
MBA – 4-mercaptobenzoic acid	X-ray CT – X-ray-computed tomography
	XRD – x-ray diffraction spectroscopy
	VSM – vibrating sample magnetometry
	Wag. - wagging

LIST OF PRODUCTS AND INTERMEDIATES

#	Description	#	Description
1	Fe ₃ O ₄	23	CoNi@PEG@G'@IgG-488 10 μm NWs I
2	Fe ₃ O ₄ @NH ₂	24	CoNi@PEG@G'@IgG-488 10 μm NWs II
3	Fe ₃ O ₄ @COOH	25	CoNi@PEG@G'@IgG-488 10 μm NWs III
4	Fe ₃ O ₄ @mal	26	CoNi@PEG@G'@IgG-488 10 μm NWs IV
5	Fe ₃ O ₄ @G'	27	[CoNi Au CoNi Au CoNi]@OH NWs
6	Fe ₃ O ₄ @G'@IgG-488	28	[CoNi Au CoNi Au CoNi]@PEG-mal NWs I
7	CoFe ₂ O ₄	29	[CoNi Au CoNi Au CoNi]@PEG-mal NWs II
8	CoFe ₂ O ₄ @SiO ₂	30	[CoNi Au CoNi Au CoNi]@G'@IgG-488 NWs
9	CoFe ₂ O ₄ @SiO ₂ @NH ₂	31	Ni I
10	CoFe ₂ O ₄ @SiO ₂ @mal	32	Ni II
11	CoFe ₂ O ₄ @SiO ₂ @CD4-PE	33	Ni III
12	Co@SiO ₂	34	Ni IV
13	Co@SiO ₂ @NH ₂	35	Ni@Au-OH I
14	Co@Au@SiO ₂	36	Ni@Au-OH II
15	Co@PEG-NH ₂	37	Ni@Au-OH III
16	Co@PEG-mal	38	Ni@GNP
17	Co@PEG-COOH	39	Ni@GNP@NH ₂ I
18	Co@PEG-COOH/mal	40	Ni@GNP@NH ₂ II
19	CoNi@PEG-mal 2 μm NWs	41	Ni@GNP@NH ₂ III
20	CoNi@PEG-mal 5 μm NWs	42	Ni@GNP@NH ₂ IV
21	CoNi@PEG-mal 10 μm NWs I	43	Ni@GNP@NH ₂ V
22	CoNi@PEG-mal 10 μm NWs II		

LIST OF PRODUCTS AND INTERMEDIATES

#	Description	#	Description
44	Ni@GNP@COOH I	66	FeNi
45	Ni@GNP@COOH II	67	FeCu
46	Ni@GNP@PEG-mal	68	FeCu
47	Ni@GNP@mal	69	CoPt
48	Ni@GNP@G'	70	CoPt
49	Co I	71	FeCo
50	Co II	72	FeCo
51	Co III	73	FeCo
52	CoPt	74	FeAu
53	Co@Pt	75	MnBi
54	Ni@Pt-COOH	76	Cu ₂ S
55	FePt	77	CuS
56	FePd ₃ @Fe ₃ O ₄	78	Bi ₂ S ₃
57	FePd ₃ @Fe ₃ O ₄	79	NiS
58	FePd ₃ @Fe ₃ O ₄	80	NiS
59	FePd ₃ @Fe ₃ O ₄	81	FeS
60	FeNi	82	FeS
61	FeNi	83	FeS
62	FeNi	84	MnS
63	FeNi	85	FePt@Au
64	FeNi	86	FePt@Au
65	FeNi	87	Fe ₆₅ Pt ₃₅ @PEG-NH ₂

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CHAPTER 1 – INTRODUCTION

1.1 INTRODUCTION TO NANOMATERIALS

1.1.1 Role of Nanomaterials

Nanoparticles have been used for a broad range of biomedical applications including cell labelling, drug delivery, hyperthermia treatment and MRI contrast enhancement.¹⁻⁵ The main focus of this research is the development of new anisotropic nanoparticles and their composites to be used as diagnostic tools in biomedical research. These anisotropic nanostructures possess the unique advantage of the large surface area and small size characteristic of nanoparticles, coupled with the high aspect ratio. Structures whose sizes approach the visible range of the electromagnetic spectrum (380-760 nm) can be detected by optical measurements, such as flow cytometry or optical microscopy. The combination of these properties will be used to push the boundaries of detection on current state of the art technologies, and combine existing technologies in new ways.

Over recent years the development of nanomaterials have opened up new perspectives in all fields of science, especially in biology and medicine. Due to their small size and unique physical properties nanoparticles can directly interact with various biological molecules and living cells that can be used for important applications in toxicology, diagnostic and therapy.^{6, 7} In addition, a large component of biochemistry relies on structural chemistry and supramolecular conformation. The activity that many proteins and nucleic acids exhibit is due to their structural conformation and dimensions.⁸ Hence, shape is very important when dealing with biomedical systems. The control of nanomaterial shapes, which are in a similar region of size to many biomolecules, adds a new layer of complexity to the design and interaction of bionanosystems. The consequences of this are still only in the early stages of discovery. **(Figure 1.1)**

Nanowires and other high aspect ratio structures, such as nanoplates, can demonstrate unique magnetic behaviour (e.g. superparamagnetism), providing the structure has a sufficiently narrow depth or width. High aspect ratio structures may also show varying responses to biological systems relative to their spherical counterparts in properties such as biocompatibility, biodistribution, cell penetration and flow rates, for example. Due to this vast range of opportunities and possibilities, the study of anisotropic magnetic structures is a very interesting and exciting field of research.

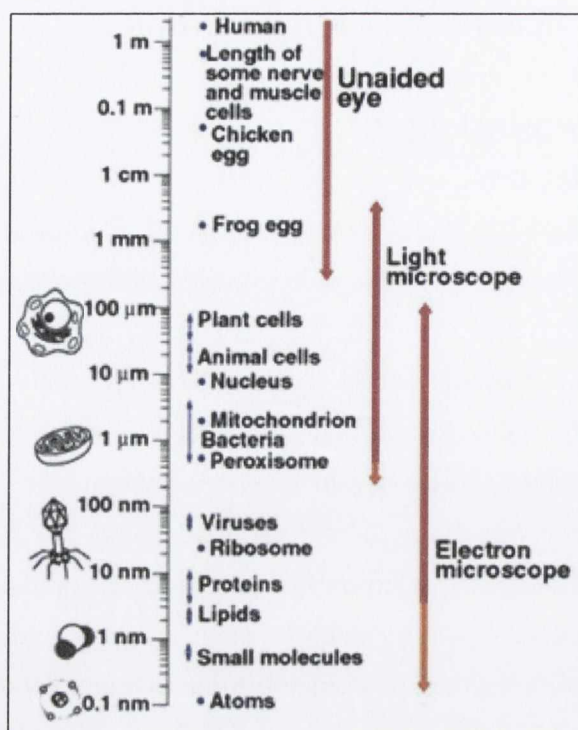


Figure 1.1: The Scale of Life and Microscopic Measurements⁹

Aside from magnetic properties, plasmonic properties in metallic materials is of particular interest.⁵ In addition to classical metal nanoparticles such as Au, Ag or Cu, recently, surface plasmon resonance has also been reported for a number of metallic, magnetic nanoparticles, including FePt,¹⁰ and Ni,¹¹ opening the door to both magnetic and plasmonic properties within single crystal particles. A useful and exciting new emergent technology in recent years is infrared-based surface plasmon enhancement.^{12, 13} With plasmonic materials, such as FePt, Ni, Au and Ag, IR and Raman spectroscopic signals can be greatly enhanced. This technology can be used to detect small molecules at the surface of nanoparticles, and has many potential detection applications in biomedical research and diagnostics, such as protein, peptide, sugar and nucleotide biomarker detection, for example. In this case, IR and Raman techniques could quickly and easily monitor differences in signals between targeting molecules at the surface, and composites with the complimentary antigen also bound. Plasmon resonance can also be used to enhance fluorescent signals.^{14, 15} This enhancement is particularly useful for biological systems, in which biomolecules are generally present in very small concentrations.

To create highly functional nanocomposites, for complex biomedical applications, it is necessary to create multimodal nanomaterials with several functional characteristics. These nanoparticles can be conjugated to biological moieties of interest using orthogonal

ligation strategies by functionalising the nanoparticle surfaces with various organic groups, which can be used for discrete conjugation reactions. These particles can then be used for applications such as cancer cell detection by flow cytometry and cancer serum biomarker assays. In the following section, the properties of some relevant nanomaterials are considered.

1.1.2 Magnetic Nanomaterials

Magnetism involves the alignment of spin orbitals within a material to produce an overall effect of synchronised spins in a particular direction. Due to the Pauli exclusion principal, whereby filled orbitals have opposite spins, which cancel each other out, this phenomenon is only seen in materials with orbitals containing unpaired electrons. Magnetic materials can be of three main types, paramagnetic, ferromagnetic, and ferrimagnetic. **(Figure 1.2)** Paramagnetic materials (soft magnetic materials), have randomly orientated spins across the crystal structure. However, in the presence of a magnetic field, produced by either a permanently magnetic material, or electromagnetic field, their spins synchronise with the direction of the field and hence, become attracted to it.

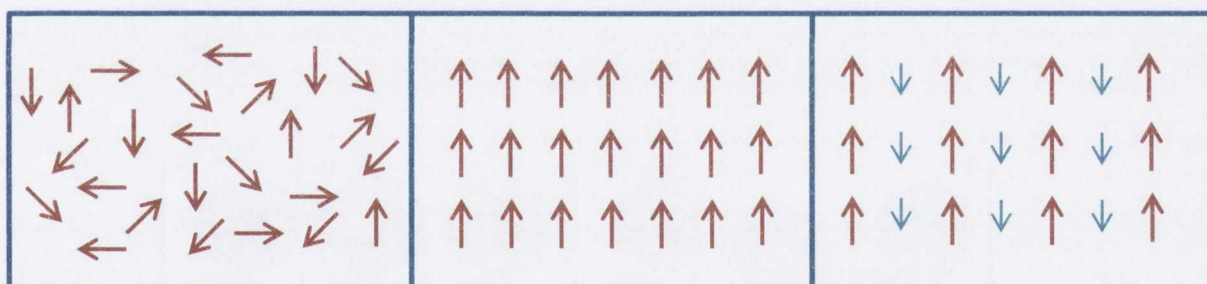


Figure 1.2: Electronic alignment of spin orbitals within magnetic materials, paramagnetic (left), ferromagnetic (middle) and ferrimagnetic (right)

Both ferromagnetic and ferrimagnetic materials retain permanent magnetism. Ferromagnetism, which is more common, involves the alignment of all free spin orbitals in a single direction, with spins parallel to each other. In this way, it allows the material to reduce the energy of spin alignments across the whole crystal. This permanent magnetic field in a particular direction can then induce an effect in neighbouring objects which are susceptible to magnetic field spin alignment.

Ferrimagnetism involves the alignment of spins parallel to each other but in opposite directions in neighbouring atoms. In these materials one direction creates an overall stronger net magnetic field; hence the material has an overall permanent magnetic field,

despite the cancellation from the neighbouring parallel opposing field. Ferrites are an example of this type of spin alignment due to the mixed valence state of the Fe atoms in the crystal.¹⁶

For hard magnetic materials, each material will have its own unique magnetic properties, such as saturation magnetisation and coercivity. In addition, surface defects and impurities can change these values. Each material will also have a Curie temperature, where kinetic effects overtake the propensity for electronic alignment, inducing disorder in the spins by thermal and vibrational energy.

Hard magnetic particles which are sufficiently small in size, such as in the nanoscale region, can demonstrate a unique magnetic property, known as superparamagnetism. In this case, Brownian motion dominates in the absence of a magnetic field. The crystals retain their permanent magnetisation, however the particles spin in random directions, creating an overall net cancelation in the magnetic field for the material. However, in the presence of a magnetic field, all particle domains will align in the direction of the field, inducing a field in the material also. Practically and observationally, these materials are similar to soft magnetic materials, whereby they are only magnetically responsive in the presence of a magnetic field, however, the induced effect for these materials is normally larger than those for paramagnetic (soft) materials. **(Figure 1.3)**



Figure 1.3: Schematic of superparamagnetic materials, with magnetic domains (orange) and their alignment in the absence of (left) and presence of (right) a magnetic field

Thus, superparamagnetism is a form of magnetism found in small nanoparticles made from ferromagnetic materials. These particles can then be magnetised in the presence

of a magnetic field, to perform functions at specific times in targeted locations. This is a valuable property for biomedical applications as for example it allows the particles to form a magnetic fluid without magnetic interaction between nanoparticles until a magnetic force is applied. This increases the biocompatibility and dispersibility of the NPs by reducing clumping of the particles and other non-specific magnetic interactions such as attraction to metallic objects.

In modern technology, hard magnets are useful for a range of applications, particularly in electronics and memory devices for computing applications.¹⁷ For bioapplications, paramagnetic and superparamagnetic nanomaterials are considerably more useful, as these systems generally requires solution phase dispersion of the material.¹⁸⁻²⁰ Hence, in the absence of a magnetic field they retain no overall magnetism or attraction to each other, reducing aggregation, clumping and sedimentation. Magnetism can then be induced for targeted applications by an external magnetic field, when needed/desired. Examples of applications where targeted, induced magnetisation is useful are (i) sample retrieval from solution, post-reaction or experiment, (ii) attraction and collection of magnetic materials to a particular location, for example for targeted treatment of cancer (iii) magnetic thermal induction for hyperthermia treatment *in vivo* or *in vitro* biological systems, which can destroy cells or organisms in a disease state (iv) magnetic resonance imaging *in vivo*, which will show the location of the magnetic material within the biological system (v) magnetic induction at the site of a sensor for magnetic detection, particularly in flow detection systems where general solvent stability and dispersibility of the particles are needed to perform the experiments.

1.1.3 Plasmonic Nanomaterials

Plasmons represent the oscillation of the free electron gas within a conducting nano-sized material. The free electron gas consists of the valence band electrons within the conducting metal material. Oscillations occur, in order to cancel the effect and presence of an external electrical field. **(Figure 1.4)** They oscillate at a frequency that cancels the effect of the field, which is quantised and specific to the size, shape and elemental composition of the material. In addition, surface plasmons can occur due to the small electrical field at the surface caused by the difference in dielectric constants between the bulk material and environment. Here, the plasmons are localised at the surface and their oscillations generally occur at lower frequency, in the visible region of light.²¹

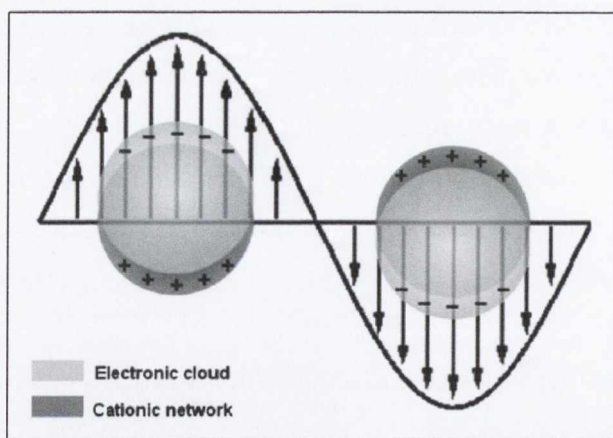


Figure 1.4: Electronic cloud oscillation in metal nanoparticles under the effect of an electromagnetic wave²²

Plasmons can then interact with photons, to produce plasma polaritons. The plasmon oscillations can interact with light of the same or smaller wavelength. Hence, light frequencies below (wavelength above) the plasmon frequency are transmitted and absorbed through the material, generally with energy dissipation, conduction or transformation. Conversely, light frequencies above (wavelength below) the plasmon resonance are reflected.

Metals and semiconductors generally have plasmons in the UV spectral region, hence visible light is reflected and the substance appears shiny. However, surface plasmons can also arise, with different quantised energies that can interact with visible light, creating various colours. As mentioned above, surface plasmons are generally smaller and of a lower wavelength than the overall crystal plasmon, and are therefore useful for biodetection studies due to changes in the colour of the material. However, due to the high surface to volume ratio in nano and sub-micron materials, these surface plasmons have increasing contribution and relevance to the electronic properties of the material overall.

Surface plasmon resonance can also lead to enhancement in signal detection in the infrared region of molecules near the surface, due to its increased relative reflectance at these wavelengths. This is useful in FTIR and Raman spectroscopy and can be used to characterise the environment around the particles and ligands attached to the surface of plasmonic nanomaterials. Paramagnetic conducting metals can also be plasmonic, due to the freedom of valence electrons to oscillate in the absence of magnetic field alignment. In this research, this has shown particular relevance to soft magnetic nickel particles.

Electromagnetic absorption and plasmon resonance frequencies are also related to the more general principles of Rayleigh scattering and absorption for various molecules and particles smaller than the wavelength of light and Mie scattering for sub-micron particles in the region of and larger than the wavelength of light (>400 nm). The extent of scattering is relative to the refractive index of the material and increases for higher density materials. Also absorption increases towards higher frequencies of energy, similarly to plasmonic interactions. This property of increased scattering for materials with higher optical density was employed for enhanced detection of smaller materials by FACS analysis.

1.1.4 Semiconducting Nanomaterials

In semiconductors, the electrons are more restricted in their movement and oscillations, due to the ionic nature of the bonding in the salt crystal structure. Here the electrons require extra energy to be excited from their ground state into the free electron gas. This excitation can then result in conduction of electrons through the material, through p and d orbitals of the cationic metal element. At frequencies higher (wavelength lower) than the excitation wavelength, transmission, absorption and conduction can therefore occur.

However, unlike conducting materials, the valence electron ground state is much more stable, hence around the excitation frequency, which is directly related to the energy difference between the ground and excited state orbitals, the electrons have a stronger tendency to return to their ground state, instead of gradual dissipation and transmission of the energy through the material. This results in a quantised packet of energy being released from the excited electron, in the form of a photon. Hence, the material appears fluorescent. This phenomenon is particularly relevant for small, quantumly confined semiconducting particles known as quantum dots (QDs).²³⁻³²

Larger particles show emission of lower frequency (larger wavelength) as a result. In addition, surface defects can help the energy dissipate in non-radiative forms. Doping with conducting metals can decrease the energy gap between the ground state and conducting state also, due to an increased propensity for electrons to flow between the atoms. Hence, plasmonic activity can also be seen in semiconductors, when there is some degree of freedom in the movement of electrons through the material. This is particularly well documented for Cu₂S in the literature.^{24, 33, 34}

1.2 PREPARATION OF NANOMATERIALS

1.2.1. Main strategies for synthesis of colloidal nanomaterials

There are many strategies towards the synthesis of nanoparticles, this work deals mainly with wet chemical techniques. There are four main types of solution phase synthesis of nanoparticles. These are co-precipitation, thermal decomposition and/or reduction, micelle synthesis and hydrothermal synthesis.¹⁹

(i) Co-precipitation reactions are generally performed under mild aqueous conditions (20 - 90°C) with simple procedures and high yields.³⁵ One example of aqueous phase synthesis is the preparation of magnetic iron oxide nanoparticles by the co-precipitation of iron (II) and (III) salts in aqueous solution under basic conditions is shown in **Figure 1.5**. This reaction is well documented,³⁶ very robust, and produces small, superparamagnetic nanoparticles which are stable under many conditions, hence can be used for a broad range of applications.

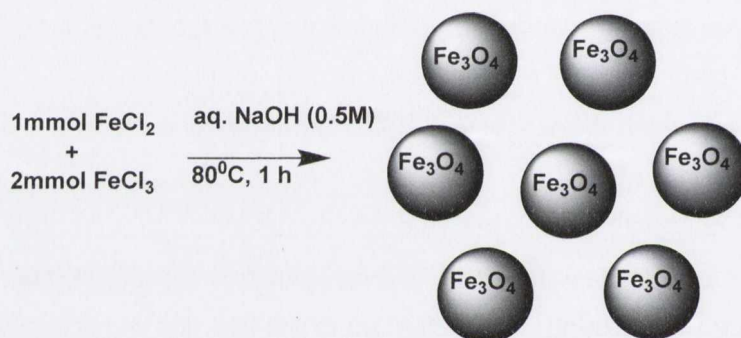


Figure 1.5: Magnetite synthesis by co-precipitation of iron salts.

One drawback to the coprecipitation method, however, is that size and shape is not easily controllable without the addition of surfactants³⁷ or template-assisted methods, such as the doped silica nanowires produced here.³⁸ Hence, other methods have also been explored.

(ii) Thermal decomposition involves organic phase decomposition and/or reduction of metallo-organic compounds or metal salts at high temperature (100°C-320°C) usually containing some kind of surfactant stabiliser.³⁹⁻⁴¹ This method is performed under inert conditions and can involve quite complicated procedures, however the resulting particles generally have excellent size distribution, also morphology can be controlled, and yields are generally high. This method is particularly useful for metal and metal alloy nanoparticles as it minimises oxidation of the crystals during synthesis. It is also useful for the formation of anisotropic nanostructures as outlined in later sections.

(iii) Micelle synthesis involves a two-phase synthesis under mild conditions, where one solvent exists as microdroplets within the other. These droplets promote good dispersion and size distribution of the growing nanoparticles, however yields in this reaction type are generally very low due to high dilution and the procedure can be complicated.⁴²

(iv) Hydrothermal synthesis is performed under aqueous conditions at high temperature and pressure. This synthetic strategy is simple to carry out and produces particles with a narrow size distribution.⁴³ However, similar to micelle syntheses up-scaling to larger quantities can be problematic. Hence, micelle and hydrothermal methods were not carried out in this research, as the particles here are intended for future bioapplications which generally require larger quantities if they are to become viable technologies.

This research is particularly focused on nanowires and other anisotropic materials, therefore the following sections consider the main approaches to the synthesis of these nanomaterials in more detail.

1.2.2. Template-Assisted Nanowire Synthesis

Recently nanowires deserved particular interest as potential materials for medical diagnostics due to their high aspect ratio.⁴⁴⁻⁴⁶ The shape of nanomaterials can drastically change their properties, such as magnetism, conductivity and viscosity. It can also change the way they interact with different systems. For example it may change the way they flow through a detection device, or attach to another surface. Nanowires of controllable length and diameter can be produced by infiltration of a sol-gel before its gelation into anodic alumina membranes. In addition, nanoparticles can be embedded in these wires by dispersing them in the sol-gel solution to be infiltrated.³⁸ This work has been performed previously within our group for both ferrite loaded polystyrene and silica nanowires.^{47, 48} Below is a schematic of the infiltration process. **(Figure 1.6)**

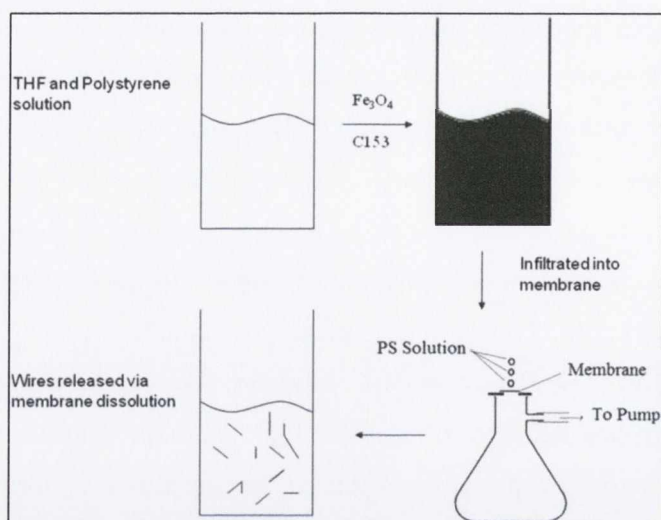


Figure 1.6: Schematic of sol gel formation and membrane infiltration to produce MNP-loaded polystyrene NWs⁴⁷

In this research, an acid-catalysed sol-gel embedded with cobalt ferrite nanoparticles was prepared, by a novel, modified approach. A broad range of concentrations of NPs can be infiltrated into silica wires using this method, providing they are stable towards the reaction conditions, dispersible in the solvent system and small enough to infiltrate the alumina membrane. These wires can then be functionalised by similar methods to those performed on magnetite, for specific conjugation of biomolecules. Additionally, metal, alloy and segmented nanowires were produced in a collaboration with Prof. Coey's group in the School of Physic, TCD.^{49, 50}

Metal wires, supplied through a collaboration with Physics, TCD, were also synthesised using AAO membranes by template synthesis, in this case however, a process of electrochemical deposition was used with metal atoms drawn into the membrane pores using a current and attraction of the ions to a gold plate at the base of the membrane, with subsequent reduction of the ions to their metallic forms by a redox reaction.⁵¹ (Figure 1.7)

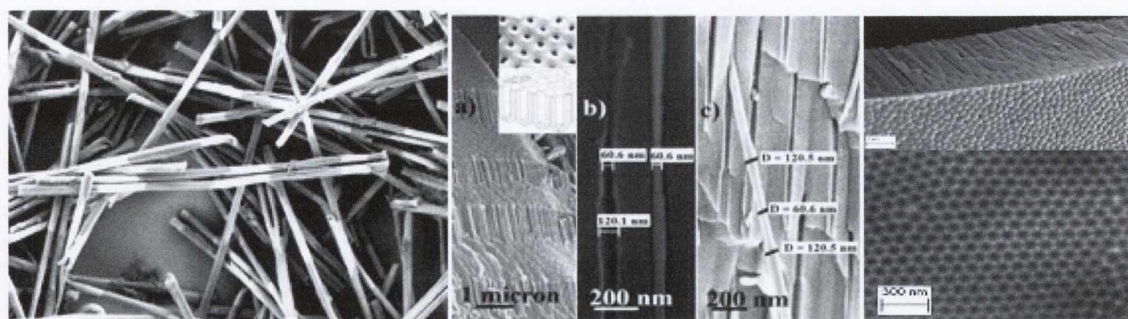


Figure 1.7: Template-grown four-segment Co-Ni magnetic barcode nanowires with gold spacer segments (left). Modulated-diameter nanowires grown in closed pores are shown on the right.⁵¹

After this fabrication, the wires are isolated by dissolving the membrane in aqueous NaOH. This process occurs prior to passivating the surface with an organic stabiliser; hence a cobalt oxide layer is produced. This layer can then be used to functionalise the wires in many different ways using the surface hydroxyl groups as performed and detailed later in this work.

1.2.3 Surfactant-Assisted Synthesis of Anisotropic Nanomaterials

One alternative to template-assisted anisotropic nanostructure synthesis is organic phase reaction in the presence of certain surfactants. This surfactant-assisted growth occurs by preferential growth along a certain face of the growing crystal. The surfactant promotes this process, by steric hindrance along the length of the wires, and increased points of access at the ends of the wires due to the conformation of the surfactant chain.⁵²⁻⁵⁵ (**Figure 1.8**) Along the length of the wire or anisotropic structure, neighbouring alkyl chains are stacked much more efficiently than at the ends, hence surfactant tunnelling occurs.

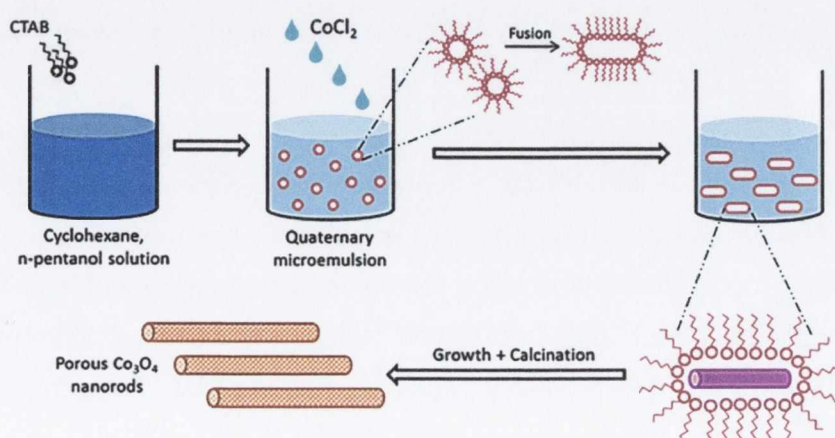


Figure 1.8: Surfactant-assisted synthesis of Co_3O_4 nanorods.⁵⁴

Oleylamine has been used in the synthesis of nanowires with a number of compositions and sizes in the literature, including Au, FePt, and CoPt,^{39, 41, 56} This method was adapted and extended in this research for a wide range of different materials and shapes. Unlike template-assisted nanowire synthesis, these organic phase reactions provide the possibility of up-scaling to considerably larger quantities. In addition it is a useful way to exclude water and oxygen from the reaction to avoid oxidation of the metallic crystals. However, it is important to note that morphology and size of the resulting particles depends on many factors, including the organometallic precursor used, temperature, time, concentration, and the types and ratios of surfactant ligands.

In addition, high surface to volume ratio creates the maximum reactivity for the system, with the added benefit of increased length along one or more axis allowing for enhanced detection capabilities, due to their ability to interact with different ranges of electromagnetic radiation, compared to their smaller spherical counterparts. The aim here is to push the boundaries of typically micro-based detection systems using these characteristics.

1.3 FUNCTIONALISATION AND BIOCONJUGATION OF NANOMATERIALS

1.3.1 Introduction

The chemical functionalisation of nanomaterials is of particular importance for the successful development of their applications. The ever increasing complexity and diversity of nanoparticulate systems emerging in the fields of chemistry, biology and physics inspires the necessity for standardised, straightforward procedures which can be adopted by scientists from across the disciplines to obtain reproducible results. Because their properties differ from those of their bulk counterparts and can be selected through control of particle size, morphology and surface functionalisation, nanoparticulate materials offer a wide range of exciting potential applications, ranging from optics and electronics to medical devices, including drug delivery and disease diagnostics.

In order to build sophisticated, multifunctional nanostructures for specific technologies and applications it is crucial to develop approaches which are applicable across a range of systems and a range of reactions which can be used discretely in tandem. This section briefly reviews procedures and calculations which can be used in many different arrangements for controlled, highly specific nanoparticle functionalisation and conjugation.

Due to their ease of handling and broad chemical stability, surface functionalisation procedures were also optimised using Fe_3O_4 MNPs. Here, they were used as a benchmark, for comparison against more complex systems, such as anisotropic nanostructure detection, and also as a benchmark for characterisation of nanomaterial surfaces. These particles also have a high surface to volume ratio; hence organic ligands on the surface have a relatively high contribution to the overall mass of the nanomaterial. This allows for easier detection of functional groups at the surface. Therefore, surface characterisation techniques were also optimised using this ideal system.

This section deals firstly with the installation of a primary functionality, which is directly bound to the nanoparticle crystal. The goal of primary functionalisation is to achieve the most stable bonding to the nanoparticle surface with the possibility of further modification. Secondly, it describes conjugation and modification reactions to alter this primary functionality for targeted applications.

1.3.2 Surface Silanisation

Silanisation procedures can be used to functionalise the surface of an oxide-based nanoparticle possessing surface hydroxyl groups. The list to which this system applies is not exhaustive but includes silicon dioxide^{38, 57}, titanium dioxide⁵⁸, various ferrite nanoparticles^{59, 60, 61}, ZnO, Al_2O_3 , and CeO_2 ^{62, 63}. These reactions have also been validated experimentally for oxidised surfaces of polymer, metal and metal alloy nanoparticles.⁶⁴ To date 3-aminopropyltriethoxysilane (APTS) is one of the most extensively used trialkoxysilanes for surface functionalisation.^{59, 62, 65-67} However, a similar approach can be used with many trialkoxy silanes to install various functionalities. Furthermore, it can be used on various oxide or hydroxylated nanoparticle surfaces, providing there is sufficient physical difference between the product and starting materials to achieve purification, for example magnetisation, size or charge,^{28, 39, 47, 57, 58, 68-81} A generalised mechanism for this reaction is shown in **Figure 1.9** below.

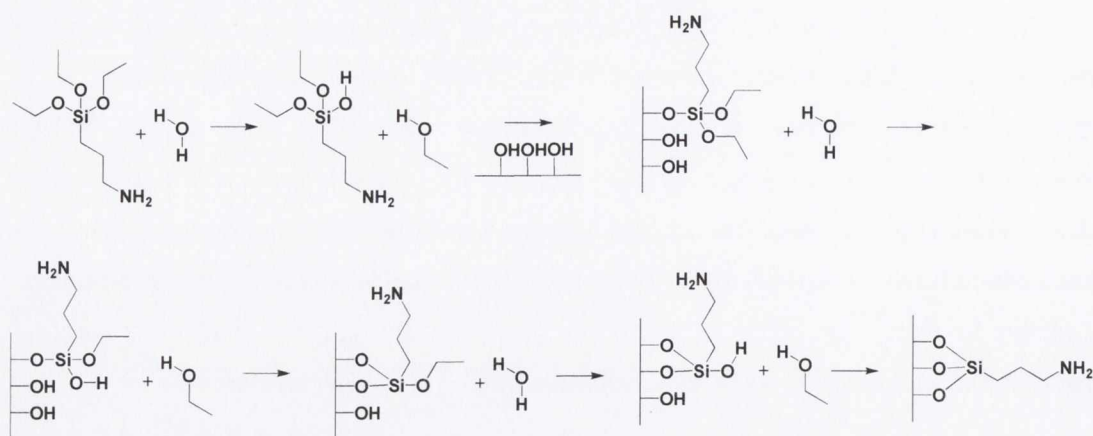


Figure 1.9: Mechanism of surface silanisation using APTS.

This method requires very mild conditions and can be performed in a variety of solvents. Furthermore, it is possible to calculate the exact number of silane groups which have been installed on each particle, which corresponds to the moles of functional groups available for additional reactions per gram particles.^{60, 82} The extent of silane binding to the surface can be calculated by a series of simple calculations. It has been shown that the surface coverage for a single silane is 0.4 nm^2 and the extent of coverage is consistently around 70%.^{60, 82} Hence, using density, mass and volume; the number of surface groups per gram particles can be found. For the theoretical calculation of silane/functional group molarities, equations from **Table 1.10** on the following page can be used.⁸³

These approaches differ from the standard ligand exchange reactions used for QDs, Au, Ag and other metal-based particles synthesized in the organic phase.²⁶ Particularly thiol exchange reactions have been used quite extensively in the literature, which rely on metal-sulfur bonds with non-oxidised surface metal atoms, whereas this silane chemistry addresses those surfaces which are oxidised, and therefore unavailable to organic ligand binding through sigma or pi coordination to the metal.

Table 1.10: Equations for calculating the theoretical molarity of surface silane/functional groups

N_A is Avogadro's constant: $6.022 \times 10^{23} \text{ mol}^{-1}$, Fe_{EDX} is the relative proportion of iron found by EDX, Si_{EDX} is the relative proportion of silicon found by EDX, $\#Si_{NP-EDX}$ is the actual number of silanes per nanoparticle, $\#NP_{sample}$ is the number of nanoparticles per sample, M_{sample} is the mass of the sample, $\#Si_{sample}$ is the number of silanes per mass of the sample, $N_{Si-sample}$ is the moles of silane per mass of the sample, N_A is Avogadro's constant: $6.022 \times 10^{23} \text{ mol}^{-1}$

Value	Description	Equations for Nanoparticles	Equations for Nanowires
V_{NP}	volume per nanoparticle	$4/3\pi r^3$	$\text{Length} \times \pi r^2$
SA_{NP}	surface area per nanoparticle	$4\pi r^2$	$(\text{Length} \times 2\pi r) + 4\pi r^2$
D	density	Literature Bulk Value	Literature Bulk Value
M_{NP}	mass per nanoparticle	$V_{NP} \times D_{NP}$	$V_{NP} \times D_{NP}$
SA_{silane}	surface area per silane	0.4 nm^2	0.4 nm^2
$\#Si_{NP}$	number of silanes per nanoparticle	$(SA_{NP} \div SA_{silane}) \times 0.7$	$(SA_{NP} \div SA_{silane}) \times 0.7$
$\#NP_{sample}$	number of particles per sample	$M_{sample} \div M_{NP}$	$M_{sample} \div M_{NP}$
$\#Si_{sample}$	number of silanes per sample	$\#NP_{sample} \times \#Si_{NP}$	$\#NP_{sample} \times \#Si_{NP}$
$N_{Si-sample}$	moles of silane per sample	$\#Si_{sample} \div N_A$	$\#Si_{sample} \div N_A$
N_{Fe-NP}	Moles of iron per nanoparticle	$(M_{NP} \times 0.725)/55.84 \text{ gmol}^{-1}$	
$\#Fe_{NP}$	Number of iron atoms per nanoparticle	$N_{Fe-NP} \times N_A$	
$\#Si_{NP-T}$	Theoretical maximum number of silane groups per particle	SA_{NP} / SA_{silane}	
Si_{ratio}	Extent of silane binding ($0 \leq x \leq 1$),	$(\#Fe_{NP} / \#Si_{NP-T}) / (Fe_{EDX} / Si_{EDX})$	
$\#Si_{NP-EDX}$	umber of silane groups per particle.	$\#Si_{NP-T} \times Si_{ratio}$	
NP_{sample}	Number of nanoparticles per sample	$M_{sample} \div M_{NP}$	
$\#Si_{sample}$	Number of silane groups per sample	$NP_{sample} \times \#Si_{NP-EDX}$	
$N_{Si-sample}$	Moles of silane per sample	$\#Si_{sample} \div N_A$	

1.3.3. Metal Surface Ligand Exchange

For non-oxidised surfaces on metals, such as those produced in organic phase reactions, other ligands must be used which have an affinity for the specific elements involved to produce stable surfaces and passivate them from further reaction, growth or oxidation. Many metals have an affinity for amino and carboxylic acid groups, which are present during reaction as stabilisers, these can then be replaced with a more stable and higher affinity binding group, which is then difficult to displace.⁸⁴ Here thiol ligands are used due to its affinity for both Au and Pt. For these purposes, this process can be conceptually compared to surface silanisation reaction, whereby both achieve a stable functional monolayer. Once the thiol ligand has been installed, these particles can then undergo the types of functional group modifications and conjugation reactions mentioned below. A multidentate approach can also be used to ligand exchange, whereby the combined effect of several groups from the same molecule/moiety creates an overall stronger binding, such as the graphene coating used here.

1.3.4. Surface Group Modification

Once the primary functionality has been bound to the surface, it can be further modified to attach or generate a diverse range of organic groups, such as amines, carboxylates, azides, alkynes, maleimides and thiols for a variety of applications, including improvement of solvent stability or conjugation to biomolecules, polymers, fluorophores and other nanoparticles. There are many non-competing chemical reactions which can be used to produce multiple nanocomponents in a controlled manner through reliable, robust reactions, such as amide coupling, 'Click' chemistry and amine/azide conversion, and nucleophilic addition. These methods can also be extended to other oxide-based nanoparticles, or other solid supports. In addition they can also be modified to produce multifunctional particles using equal ratios of different linker moieties. In this case, it is then possible to install multiple surface functionalities, using orthogonal chemistry.⁸³

EDC has been used extensively in nanotechnology as an amide coupling agent, due to its solubility in aqueous media, coupling efficiency and relatively cheap cost.^{28, 76, 85} **(Figure 1.11)** For example, this technique has been employed by several groups in order to prepare fluorescent nanoparticles with a covalently attached organic dye, in order to avoid dye leakage.⁸⁶⁻⁸⁸ Also in this manner various multimodal nanoparticles can be prepared. Amide coupling is a robust reaction, however it does have limitations, particularly when conjugating biomolecules to nanoparticles, which contain a diverse range of functional groups. In particular, proteins and peptides contain a number of functional groups, including amines and carboxylic acids, thiols, and hydroxyls. Amide

coupling is therefore not ideal, as the protein will react not just with the nanoparticle surface but also with itself, producing large agglomerates and clumping, it is therefore important to have a collection of reactions which can be used in tandem if constructing more sophisticated bionanocomposites.

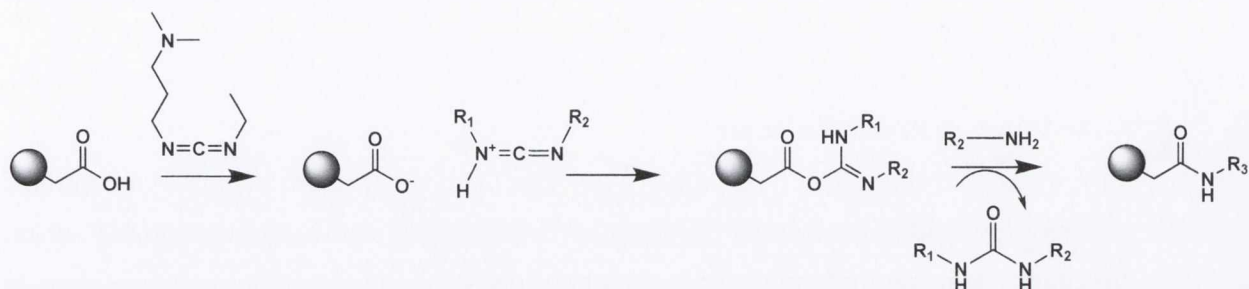


Figure 1.11: Mechanism of EDC activated amide coupling for carboxyl-modified NPs

When modifying functional groups or conjugating various moieties to particles it is essential to know what groups are participating in the reaction to avoid unnecessary side products and to achieve specific and quantitative results. Antibodies, in particular, contain a wide variety of functional groups.

1.3.5. Amine Nucleophilic Ligation

Maleimide-thiol ligation, unlike some other 'Click' reactions, does not require a catalyst, and proceeds stereoselectively by simply controlling the pH.⁸⁹⁻⁹¹ It should be noted that amines can compete in some thiol 'Click' reactions at pH values above their pK_a , and can also be used for protein conjugation.⁹²⁻⁹⁴ However, for the Michael addition the amine participates at a much slower rate. However, due to its relative abundance in biomolecules and ease of addition, amino group maleimide conjugation has also been employed in this work. (**Figure 1.12**)

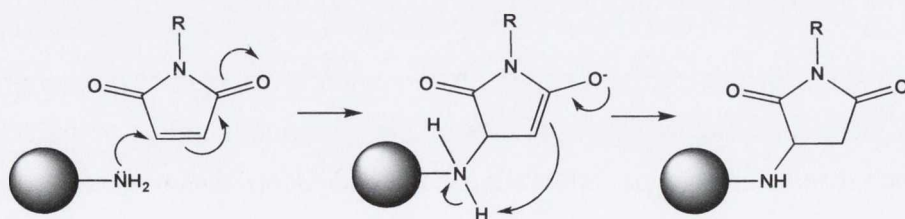


Figure 1.12: Mechanism of amine-maleimide Michael addition for amino-functionalised NPs

α -Haloketones, such as bromoacetic acid or iodoacetamide linkers can also be utilised for thiol ligation through nucleophilic substitution to produce a thioether.^{95, 96} As with the

thiol-maleimide ligation, amines may act as an alternate nucleophilic source in this reaction, displacing the halide to produce an imine.^{97, 98} In addition, conversion/ligation reactions can also be used for multiple functional group installation through simultaneous addition of multiple linker reagents or conjugation to large moieties of interest with minimum modification to the procedures.

1.3.6 Aqueous Phase Transfer

Solvothermal and organic hot injection synthesis facilitates the production of anisotropic structures, provides an arsenal of reactions not available in the aqueous phase, which are generally more uniform and have higher crystallinity due to higher temperature of formation. They are also generally more amenable to upscaling. Hence if organic to aqueous transfer can be achieved, it opens the door to many new areas in diverse applications. Aqueous transfer of nanomaterials is particularly important for biomedical applications as these applications require physiological aqueous media. Aqueous transfer methods can be broadly categorised by ligand exchange, modification or addition.

Ligand exchange relies on the preferential binding of different functional groups to the nanomaterial surface. Here, this is generally metallo-organic coordination, unlike the oxide-silane chemistry described in previous sections. Ligand exchange is particularly useful for Au based particles, as Au-thiol binding is very strong, and comparable to covalent binding. For other metals, however, binding of ligands is more reversible and less stable. Hence, other methods have been developed. The alkyl chain of the surfactant cannot be easily modified, therefore the approach developed here relies on addition to the nanomaterial, encapsulating the particle and surfactant within. This method is an improvement and adaptation of previous methods, including CTAB assisted aqueous transfer,⁹⁹ and polymer assisted aqueous transfer.^{27, 100}

In the CTAB assisted method for transfer, the nanoparticle dispersions are generally very stable and well separated; however, it relies on the permanent charge of the quaternary amine, which is not available for further chemical modification reactions, required in order to conjugate to biomarkers for detection applications. In the polymer assisted method, the particles are also very stable. There are additional functional groups available for conjugation also. However, it was found that the polymer transfer results in agglomerated packets of particles, encapsulated within the polymer. Hence the particles are not well dispersed, and their full surface area not available for conjugation and reaction. Once water soluble and possessing surface organic groups capable of participating in ligation

reactions, these anisotropic nanomaterials can be bioconjugated to biomolecules and fluorescent markers, in a similar manner to methods discussed above.

1.3.7 Biomolecule Conjugation

Protein G' is a recombinant protein, widely used in the biotechnology for attachment to Immunoglobulin G (IgG). IgG then in turn, binds to a number of antibodies, for example HER2, which can then be used for biomedical diagnostics. Protein G' has multiple binding sites, which can bind to IgG. Therefore, regardless of the orientation of Protein G' binding to the NP surface, a site will be available for the correct orientation of IgG, facing outwards from the nanoparticle surface.¹⁰¹ This increases the overall efficacy of the nanobiocomposite for capturing biomarkers of interest, hence reducing costs of expensive bioreagents and also increasing the rate of capture and detection in diagnostic experiments. There are a wide variety of IgG proteins commercially available, which are engineered to bind the complimentary antibodies. Here, IgG proteins will be used, which target cancer biomarker antibodies such as HER2. This system is quite flexible and can be applied to a wide variety of antibodies for various diseases.

1.4. POTENTIAL BIOMEDICAL APPLICATIONS OF NANOMATERIALS

1.4.1 Introduction

Due to their small size, magnetic nanomaterials have a broad range of possibilities in biomedical applications due to their similar size. Also, because they are magnetic, they open up the possibility of target directed applications.¹⁰² This is of great importance, as one major disadvantage of nanoparticle therapy is the problem of getting the particle to the site of interest. Using magnetic nanoparticles, an external magnetic field could be used to direct the particles to the desired area, hold them there until the treatment is complete and finally remove them. Toxicity is of major concern when advocating particles for biomedical use. The important factors which determine the biocompatibility and toxicity of magnetic materials are the nature of the magnetically responsive component and the final size of the particles and their composites. Iron oxide nanoparticles of magnetite or maghemite are by far the most commonly employed for biomedical applications, as there is no risk of further oxidation, which is possible with some other magnetic materials.¹⁰³ For example, magnetite nanoparticles have been found to be physiologically well tolerated, with dextran-magnetite showing no measurable toxicity index LD₅₀.¹⁰⁴

Magnetic nanomaterials have a range of biomedical applications including targeted drug delivery systems, hyperthermia agents and MRI contrast agents. Because nanomaterials

have a size close to biological entities, they are suitable for bio-tagging or labelling. The size and shape of the nanoparticles becomes important when considering cell membrane penetration. In order to interact with a biological target, a biocompatible shell or coating is required around the nanoparticle surface. The choice of shell type may also include fluorescent molecules, thereby conferring fluorescent abilities to the magnetic nanocomposites. Detection techniques must also be considered in nanoparticle design. In order to fully utilise magnetic nanoparticles in biomedicine, it is vital to first investigate the biological tissues in question. Cancerous tissue is of particular interest.

Neoplastic (or tumour) tissue is heterogeneous in nature, with haemorrhaged areas as well as densely vascularised regions present in order to supply the tissue with blood, nutrients and oxygen to sustain angiogenesis (tumour growth).¹⁰⁵ Tumour blood vessels possess several abnormalities including a high proportion of proliferating endothelial cells (responsible for controlling blood pressure, blood clotting, new blood vessel formation and controlled passage of materials),¹⁰⁶ and an increased tortuosity.¹⁰⁷ The combination of abnormalities results in an increase in the permeability of tumour cells. The reported pore cutoff size for transport across tumour membranes is in the region of 380-780 nm.¹⁰⁵

1.4.2 Drug delivery

In order to overcome some of the problems associated with tumour treatment, colloidal nanomaterials could be conjugated with anti-cancer drugs. By careful design and functionality, these nanocomposites can prevent cellular and non-cellular interference with drug activity and increase the cancer cell-selectivity of the drugs while reducing toxicity to normal cells. Nanoparticles may act as vehicles for drug transport while, at the same time, protecting the drug from premature release. The accumulation of intravenously injected nanoparticles relies on diffusion across the leaky, permeable tumour vasculature. The particles may be injected intravenously and transported to the region of interest by the circulatory system.¹⁰³

One major disadvantage with current chemotherapies is their non-specific nature. This leads to deleterious side-effects as they may cause damage not just to cancerous tissue but to the surrounding healthy tissue. The motivation behind magnetic nanomaterials as drug delivery agents is to localise these treatments so that only tissue to be treated is subjected to the drug. For this application to be realised, the size, charge and surface chemistry will play a major role in determining the efficiency of such agents. For example, following administration to the body, larger particles (> 200 nm) are usually sequestered

by the spleen as a result of filtration and are eventually removed by the cells of the phagocyte system, which results in shorter blood circulation times. If the particles are too small (> 10 nm), they have been found to be removed rapidly from the body by the renal system. In order to increase the blood circulation time of these particles, some workers have coated them with polymeric surfactants which minimise the protein adsorption to the particle surface.¹⁰⁸

For example, in order to investigate the uptake of superparamagnetic nanoparticles by human cancer cells, Petri-Fink *et al.*¹⁰⁹ have coated the particles with polyvinyl alcohol (PVA) and some PVA-based copolymers. They have found that cell toxicity is increased for higher polymer concentrations, as with lower concentrations the colloidal stability is sacrificed. Of the modified PVA moieties tested, they found that only the amino derivative was found to interact with the melanoma cells. The authors have postulated that the particles enter the cells by endocytosis.

Bergemann and co-workers¹¹⁰ have prepared negatively charged magnetic nanoparticles to which they have ionically bound drugs, including the anti-cancer drug Epirubicin. The *in vivo* release of the drug was monitored in Sprague-Dawley rats. *In vitro*, a desorption half-life of 30 minutes is reported, while *in vivo* that time is increased to 45 minutes. This may be due to impaired diffusion of Epirubicin through the vascular wall. The first clinical trials of magnetic drug targeting in human patients were reported by Lübke *et al.*¹¹¹ Here, 100 nm particles were coated with the anti-cancer drug Epirubicin in a similar method to Bergemann *et al.*¹¹⁰ At 0.8 T external magnetic field was used to concentrate the drug in the target regions. Patients in the trial had advanced and unsuccessfully pre-treated cancers or sarcomas. It was found that the ferrofluid was directed to the tumours in half the patients tested. It was concluded that transfer of parameters from animal to human trials were not sufficient, as significant physiological changes occur between species and sizes.¹¹²

The efficiency of cobalt ferrite magnetic fluids has also been investigated.¹¹³ In this work, the authors have found that when the particles are coated with polyethylene glycol (PEG) they can remain for longer times in the bloodstreams of Swiss mice than compared to citrate coated particles which are taken up by phagocytic cells.

1.4.3 Magnetic Resonance Imaging (MRI)

Magnetic resonance imaging (MRI) is a diagnostic technique that uses paramagnetic species to enhance proton relaxivity. Beginning as a tomography technique for imaging thin sections of the body, it is now used to give a full 3-D representation of the body. The most common contrast agents currently used are paramagnetic gadolinium ion complexes. Gd^{3+} had seven unpaired electrons and has, therefore, an efficient paramagnetic relaxation mechanism. However, free Gd^{3+} is toxic and gadolinium chelates tend to be non-specific and accumulate rapidly in the liver.¹⁰⁴

Such problems may be overcome with the advent of superparamagnetic iron oxide MR contrast agents. Due to restrictions of size in the extracellular space of 50 nm, it is important to produce monodisperse magnetic nanoparticles of a uniform size with a biocompatible coating. The MRI contrast agents used routinely now are non-selective and their relaxivity is strongly dependent on their particle size. Thus, there is an urgent need and demand for novel magnetic fluids with functionalised nanoparticles of an appropriate size. Because superparamagnetic particles do not retain any residual magnetism after removal of the magnetic field, they are not as susceptible to aggregation as larger multi-domain particles. Magnetic nanoparticles as contrast agents in MR may be used to localise and diagnose brain tumours, cardiac infarcts or liver lesions.¹¹⁰

If superparamagnetic nanoparticles can be functionalised with molecules capable of specific recognition of cell types, they can be localised to tumour cells. Veisheh *et al.*¹¹⁴ have prepared magnetite nanoparticles with a polyethylene glycol coating and have subsequently functionalised these with chlorotoxin (Cltx), a glioma tumour-targeting molecule, and the fluorescent molecule Cy5.5. Cltx is a peptide purified from the venom of the giant Israeli scorpion. They have shown that the nanoparticle-Cltx conjugates target glioma tumour cells and that this internalisation into the cells can be visualised by confocal imaging. The reported T_2 relaxation times (5 ms for the Cltx coated particles, 95 ms without) are promising for glioma detection. They have also demonstrated an affinity of these nanocomposites for glioma cells over healthy tissues.

Tumour specific monoclonal antibodies have also been conjugated to iron oxide nanoparticles in an attempt to provide an alternative contrast agent to paramagnetic gadolinium chelates, which must be administered at high concentrations to detect cancerous cells.¹¹⁵ The mouse antibody L6 has been used since it possesses a surface antigen which is also present in small-cell lung carcinoma, breast carcinoma and colon carcinoma. They found that with osmotic blood brain barrier (BBB) disruption, the

particles can cross the BBB and be used to image the brains of rats. The relaxivities of these agents are 16.5 and 34.8 mmol⁻¹ s⁻¹ for r_1 and r_2 respectively, which is higher than gadopentetate dimeglumine, 4.5 and 5.7 mmol⁻¹ s⁻¹ for r_1 and r_2 respectively. This higher relaxivity shows the potential use of these antibody conjugated nanoparticles.

Dextran coated magnetic nanoparticles have been used to detect liver lesions as they have been found to be specifically absorbed into the reticuloendothelial system in the liver and the spleen.¹¹⁶ Superparamagnetic iron oxide (SPIO) MR contrast imaging agents have been reported to have applications in bowel contrast, liver and spleen imaging, lymph node imaging,^{117, 118} bone marrow imaging, perfusion imaging and MR angiography.¹¹⁹ The primary particle size is 10 nm, while the hydrodynamic radius is 300 nm. Liver and spleen imaging has been accomplished using AMI-25 (Endorem, Guerbet), which is found to rapidly accumulate there upon intravenous injection.

Targeted cancer imaging has been investigated by Choi *et. al.*¹²⁰ using folate treated magnetic nanoparticles. The folate receptor is a protein which is over-expressed in various types of human tumours, where it acts to capture folate to feed rapidly dividing tumour cells. Once the particles were internalised into the cancer cells, it was possible to obtain a tumour image *in vivo*. The resulting T₂-weighted MR images reveal a 38% decrease in the intensity of the tumour tissue owing to the presence of the folate coated nanoparticles. Internalisation was also confirmed using confocal imaging of human carcinoma cells which express the surface receptors for folic acid.

More recently, Prof. Gun'ko his co-workers developed a new magnetic fluid, which is based on flexible linear (quasi one dimensional) assemblies of magnetite nanoparticles.^{40, 70} This nanomaterial was synthesised by co-precipitation in the presence of polysodium-4-styrene sulfonate (PSSS) polyelectrolyte. In this case the negatively charged polyelectrolyte acts as both a stabiliser, where the positively charged iron ions can accumulate before particle precipitation, and as a template for nanowire formation once the particles are formed.

When dried in an external magnetic field, the nanocomposites appear aligned showing parallel linear arrays or “nanowires” where neighboring particles are cross-linked by polyelectrolyte molecules into chains. (**Figure 1.13**) The primary particle size (from 7±1 to 11±1 nm) and the nanowire width were found to change depending on iron/polyelectrolyte ratio. Therefore, the parameters and properties (including relaxivities

and MRI sensitivity) of corresponding magnetic fluids can be controlled by simply changing the ratios of iron salts and polyelectrolyte during the synthetic procedure.

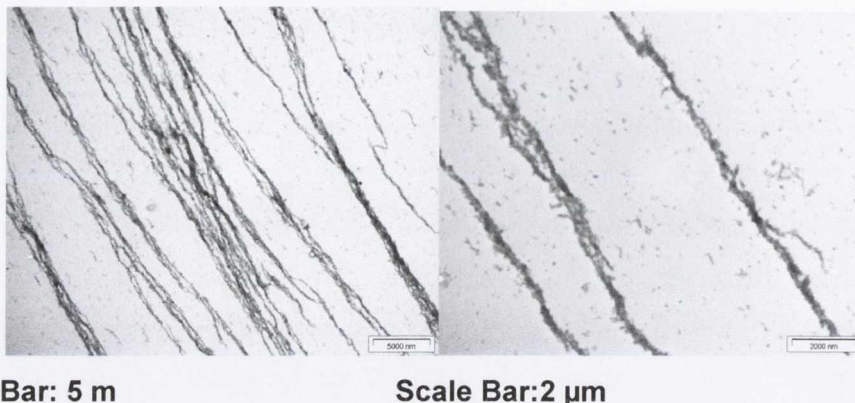


Figure 1.13: TEM images of polyelectrolyte stabilised magnetite linear assemblies aligned into parallel arrays by 1.5 T external magnetic field.⁴⁰

Following research in collaboration with the Institute of Neuroscience in TCD the researchers from the School of Chemistry demonstrated for the first time the utilisation of the new linear one dimensional assemblies of magnetic nanoparticle as in MRI contrast agents in live animals. New magnetic fluids have been injected into live rats in order to study the effect of these contrast agents on the brain, specifically the hippocampus and brain vasculature.

Echo planar image images of the brain after injection have shown a significant darkening as the bolus has reached the brain.⁷⁰ (**Figure 1.14 a, b**) Also in the FLASH scan (**Figures 1.14 c, d**), of the coronal slice a significant darkening of the brain and a great contrast was noticed. In particular, the occlusion of the superior sinus sagittalis vein (SSS) can be clearly seen. No visible signs of any adverse reaction were noted after monitoring the rats for twenty-four hours. This research proved both the biocompatibility of these materials and that the contrast effect is retained in live rats, i.e. that the fluids remain effective, over the timescale of the imaging process. However, while initial results indicate this contrast agent is quickly cleared from the central nervous system, further studies are required to determine localization in tissues over long periods of time.

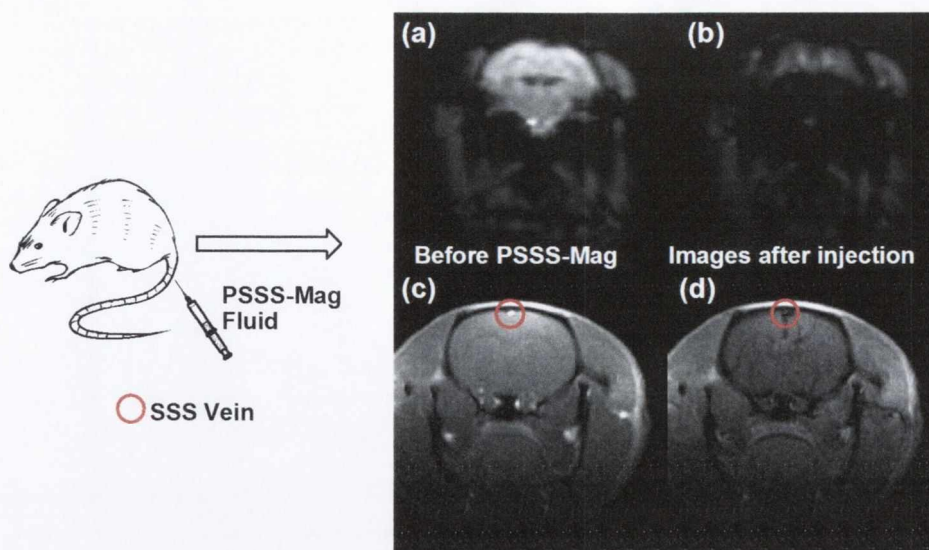


Figure 1.14. Echo planar image (EPI) of mouse brain (a) before and (b) as PSSS-Mag1 passes through; Fast Low Angle Shot (FLASH) image of mouse brain (c) before and (d) as magnetic nanocomposite passes through⁷⁰

Overall MRI has a number of advantages over other instrumental diagnostic methods. MRI can provide excellent, detailed images of soft tissue *in vivo*. MRI is also a functional imaging modality, similar to nuclear medicine techniques. For instance, MRI can be used to measure blood flow in vessels or tissue perfusion or changes in blood oxygenation. In addition, MRI is a dynamic imaging modality. As it is apparently safe, images can be acquired continuously as with ultrasound. This allows dynamic studies to be performed for instance of the beating of the heart, the movement of joints, or the handling of a meal in the gastrointestinal tract, or the central nervous system response to external stimulus. The enormous versatility and flexibility of MRI, and its relative safety and non-invasive nature, has led to an enormous increase in demand for scans over the last decade.¹²¹

1.4.4 X-Ray-Computed Tomography Contrast Agents

X-ray-computed tomography (X-ray CT) can be used as another diagnostic imaging tool, as an alternative to MRI imaging *in vivo*. This technique has the advantage of employing machinery which is cheaper and more readily available in healthcare, as X-ray machines are widely used in hospitals, whereas MRI machines are large costly, and less readily available. Au NPs have been used extensively as potential X-ray contrast agents.¹²²⁻¹²⁵

(Figure 1.15)

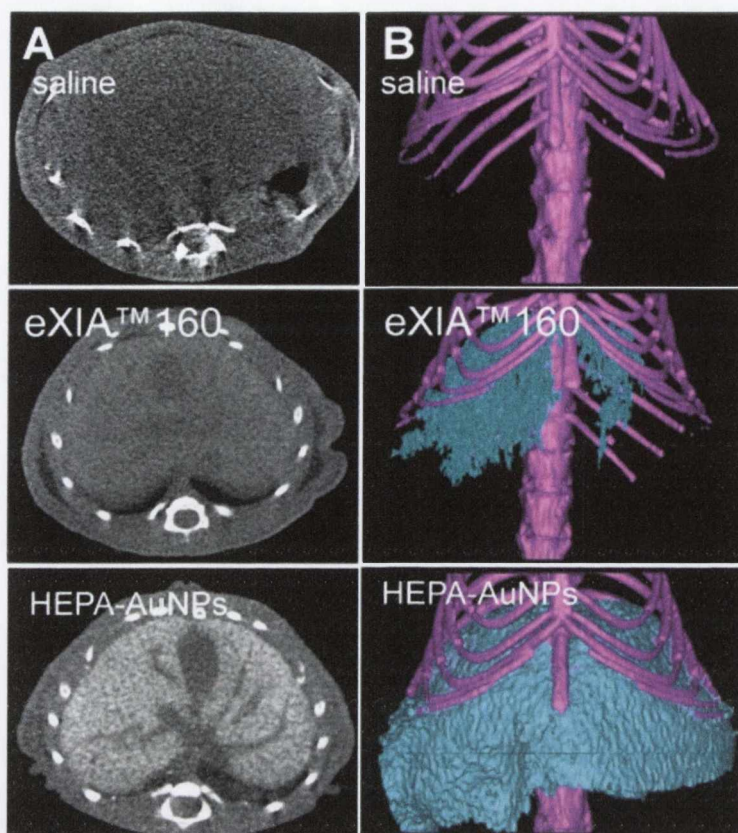


Figure 1.15: (A) Cross-sectional microCT images in livers 2 h postinjection of saline, eXIA 160, an iodine contrast agent (800 mg I kg^{-1}), and heparin-coated AuNPs ($250 \text{ mg Au kg}^{-1}$). (B) Three-dimensional microCT images of livers obtained after 2 h postinjection of saline, eXIA 160, and HEPAuNPs.^{122, 123}

X-ray tomography requires agents to induce enhanced contrast to aid in imaging of the areas of interest. Here, elements which have high X-ray absorptivity are used to create this contrast. Generally, these are elements with high atomic numbers. X-rays are generally used to see contrast between dense bone matter and softer tissue. With the aid of contrast agents bound to target areas of interest, other tissue areas can also be visualised.

Recently, Bi_2S_3 has been shown to have promising results for *in vivo* X-ray CT imaging as a cheaper alternative to Au NPs.¹²⁶ (Figure 1.16) Bi also has a high atomic number, enhancing contrast through increased x-ray absorptivity. Hence, Bi_2S_3 will be explored later in Chapter 4 as part of a series of sulfide-based semiconductor experiments in an anisotropic growth study and also as a potential diagnostic tool for X-ray CT application.

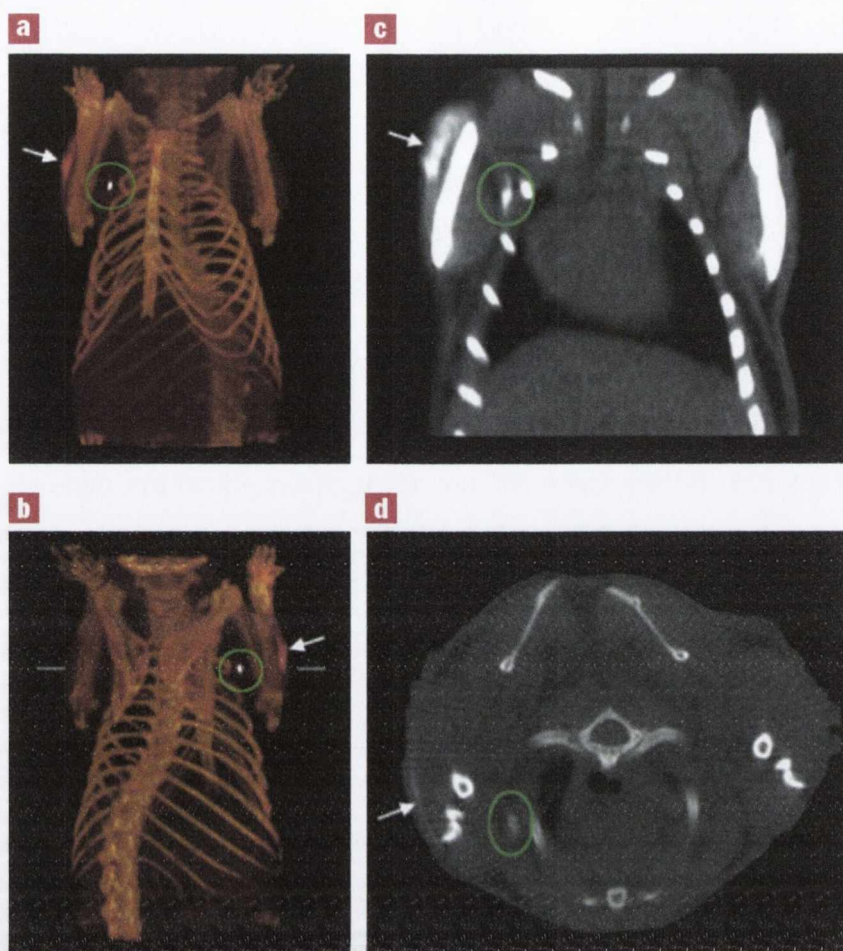


Figure 1.16: Lymph-node imaging. CT imaging of a lymph node of a mouse with the Bi_2S_3 NP imaging agent. a,b, Three-dimensional volume renderings of the CT data set, the length of the reconstruction is 3.8 cm. c, Coronal slice (length of the slice 2.3 cm). d, Transverse slice at the height indicated by the horizontal lines in b. The maximal diameter of the mouse 1.8 cm. The position of the lymph node under the right shoulder is indicated by the ovals, and the injection site is shown by the arrows. Note the lack of contrast in the corresponding contralateral (left shoulder) lymph node¹²⁶

1.4.5 Hyperthermia Therapy

The process of heating certain organs or tissues to temperatures of between 41° and 46°C for cancer treatment is called hyperthermia. Hyperthermia increases the efficiency of radiation and chemotherapy by reducing the viability of cancerous tissues and thereby increasing their sensitivity to treatment. Hyperthermia, however, does not display the more harmful side effects which chemotherapy and radiotherapy do. The first reports of using magnetic nanoparticles for intracellular hyperthermia date back to 1979, when Gordon *et al.* treated Sprague-Dawley rats, which had spontaneous tumours, with ferrofluids.¹²⁷ Spontaneous tumours were chosen because they are most difficult to destroy and they are most closely related to human neoplastic tissue. They proved

cellular uptake of the nanoparticles had been achieved using electron microscopy and also showed that heating occurred once an AC magnetic field was applied.

The general hyperthermia procedure involves the injection of magnetic material into the tissue of interest, followed by the application of an AC magnetic field of the required strength and frequency to cause the particles to heat.¹⁰⁴ The underlying theory supporting the use of magnetic nanoparticles for this purpose is that there will be an induced current flowing through any metallic object once it is subjected to an alternating magnetic field. The amount of current produced depends on the size of the magnetic field and on the size of the object. As the current flows within the material, the metal resists the flow of current and in this way heats up by a process termed inductive heating. If the material is magnetic, this heating effect is even larger. Superparamagnetic or single-domain magnetic particles produce substantially more heat than their bulk counterparts.¹²⁸ In order to be effective, the heat generated by the particles must be at least 42°C for 30 minutes.

1.4.6 Flow Cytometry

Flow cytometers are traditionally used for counting cells based on a scattering technique, and provide a yes/no answer based on fluorescent responses of polymer microspheres or cells bound to fluorescent labels. **(Figure 1.15)**



Figure 1.17: BD Biosciences Flow Cytometer

Here, this cell counting technology is intended to be used instead to count and detect sub-micron particles and nanomaterials. Metallic and metal oxide materials have a higher density and hence higher refractive properties than traditional polymer and cell-based technologies, thus enhancing signal detection. The scattering signal in FACS is

measured according to duration and intensity, which can then be related mathematically to the size, fluorescence intensity and scattering propensity of the material.

Currently there are several technological platforms based on magnetic nanoparticles which are used in biotechnology. One of the most known is Dynabeads® which are magnetic beads used for chemical separation, detection and modification consisting of polystyrene beads with small superparamagnetic metal oxide nanoparticles embedded within. These are 1-5 microns in diameter. Life Technologies also sell non-magnetic analogues of these for FACS. The applications for these beads for are cell separation & expansion, exosome analysis, protein sample prep & isolation, protein-protein interactions, immunoprecipitation, *in vitro* diagnostic (IVD) assay development, nucleic acid isolation, , and original equipment manufacture (OEM). Turbobeads® are cobalt particles, ~40 nm in diameter, produced by combustion of a cobalt precursor. In the last 6 years, Turbobeads® have over 40 publications citing its products. Their applications extend beyond biomedical and include other areas such as catalysis. These applications are also viable for this technology, however, not targeted here.

Turbobead and Dynabead based technologies have already been applied to biomolecule capture for Protein G-IgG pairs.¹²⁹⁻¹³¹ Hence they are a useful benchmark for further studies. New technologies will be developed for biomolecular capture and subsequent detection of these for diseases such as cancer. It is hoped this technology can later be used for capture of serum and urine biomarkers for non-invasive diagnostics of disease. The overall aim is to push the boundaries of current detection capabilities using improved materials and optimising current detection equipment, such as flow cytometers, for the development of new state of the art diagnostic applications.

1.5 AIMS AND OBJECTIVES

The main goal of this work is to develop new nanomaterials of various shapes, sizes and compositions for biomedical applications in particular for new, improved medical diagnostic techniques, and the detection of biomarkers of disease at early stages. Early diagnostics has the potential to improve patient outcome and survival rate, and also eliminate the need for more aggressive treatments. In this work, tailored nanomaterials are synthesised and functionalised mainly for *in vitro* diagnostic testing, such as 'Lab on a Chip' technologies. It is hoped the properties of magnetism, plasmonics and fluorescence characteristics of the various materials will be useful to improve and enhance detection for targeted applications through recognition of specific biomarkers for disease.

The strategy for achieving these aims is firstly to produce nanomaterials by various synthetic strategies and secondly, to functionalise these materials with biomarkers and fluorescent moieties for targeted detection and diagnostics. The aims will be achieved through several means, including:

- (i) Optimisation of surface functionalisation and ligation reactions on a simple Fe_3O_4 model system
- (ii) Application of these methods to Co, CoNi and segmented CoNi|Au|CoNi|Au|CoNi NWs
- (iii) Synthesis of CoFe_2O_4 -doped SiO_2 NWs by a template-assisted method and bioconjugation
- (iv) Synthesis of novel functionalised nickel sub-micron magnetic beads for biomarker separation and flow detection
- (v) Synthesis of novel anisotropic nanostructures, consisting of either Fe-based alloys and sulphur-based semiconductors, by surfactant-assisted growth and subsequent transfer to aqueous media for bioconjugation
- (vi) Testing of the new nanomaterials using flow cytometry studies and integration of these materials and approaches on new systems for biomedical theranostic applications.

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2.1 INTRODUCTION

The work in this chapter was done as part of a collaboration with Physics, TCD, Institute of Molecular Medicine, TCD, BD Biosciences and Toxicology, UCD, among others, under the NAMDIATREAM EU FP7 consortium. The aim of this part of the work is to synthesise nanomaterials and modify the surface of nanomaterials for the purpose of disease diagnostics, using 'Lab on a Chip' technology. Anisotropic nanostructures such as nanowires have been identified as good candidates for these devices as they provide large surface and high aspect ratio. This should enable us to combine the advantages of nanoparticulate properties, such as high surface area and quantum confinement, with the possibility of optical sensing also (400-700 nm), through light scattering. Both nanospheres and nanowires were compared through the course of this work for technology optimisation. Both nanomaterials synthesised here and nanowires, provided by Physics, TCD will be conjugated to polymers and biomolecules for testing by flow cytometry analysis. Some samples will be analysed directly here for their potential bioapplications, whereas others will be sent to biologists for testing.

Previously one of the template methods for the production of ferrite-loaded silica nanowires was developed within our group.¹ This synthesis involves template assisted formation of the wires by infiltration of the AAO membranes with a silica gel dispersion of the ferrite nanoparticles using vacuum assistance. The nanowires have been isolated by dissolving AAO membrane in sodium hydroxide solution. The freestanding magnetically loaded silica nanowires were then functionalised with antibodies and incubated with cancer cells. The incubated cells were analysed by flow cytometry and cancer cell detection was achieved.¹ Other methods were also developed by Chaure *et al* ² in the School of Physics, TCD, using these templates for the electrochemical deposition of magnetic metal, alloy and segmented nanowires. These wires have also been used for similar flow cytometry studies in TCD.³ Here it is hoped to further this work using these silica and metal wires and flow detection technology.

Flow cytometers are traditionally employed for cell counting studies, whereby a fluorescent response indicates a yes/no answer and these signals are counted. Fluorescent polystyrene beads are used in these machines for calibrations and comparisons with cells, such as those provided by Life Technologies.⁴ Here it is hoped to use this machine not for cell counting, but for particle counting and analysis and to

extend these methods to the nanowires themselves; hence, pushing the boundaries of this technology and creating new applications for it.

These nanowires have a similar length to the diameter of cells. Instead of incubating the wires with cells, they can be conjugated to fluorescent antibodies and incubated with serum and liquid based biomarkers. The resulting biocomposite wires can then be analysed by flow cytometry for a scattering and fluorescent response instead of the cell/nanoparticle system. These methods can then be extended to nanowires bioconjugated to biomolecules which are broadly used in research and industry. The products can then be compared against state of the art technologies, to identify their advantages and optimise the technology for this system of materials and conjugates.

In this chapter initially, magnetite nanoparticles are prepared by the co-precipitation technique, which was developed by Massart *et al.*⁵ This reaction is robust, producing small, superparamagnetic nanoparticles, with high peak magnetisation. The particular aim here is to create a standardised system of functionalisation and characterisation for comparison against more complex nanomaterial composites, shapes and structures. These ferrite particles can be modified directly with silanes, in short reaction times, and are easily purified after reaction. The synthesis and characterisation of ferrite nanoparticles has been explored extensively in the literature for a wide range of applications.⁶⁻⁸ Its ease of synthesis, handling and diversity of applications make this material an ideal platform for exploring hydroxyl-based surface functionalisation.

In this work, Fe_3O_4 MNPs will be initially modified with a primary functional monolayer by surface silanisation, to install amino functional groups.⁹ This method involves the reaction of trialkoxysilanes with hydroxyl groups at the surface of nanoparticles and has been widely used to functionalise the surface of silica and magnetite with amino groups using APTS.¹⁰⁻¹³ This silanisation process can be used, not only for magnetite and silica, but also for other ferrites, organic hydroxyl groups and oxidised metal surfaces, such as those on aqueous suspensions of unmodified magnetic nanowires as in the work completed here.

We then use these functionalised surfaces to conjugate biomolecules of interest using strategies such as amide coupling and nucleophilic addition. Surface-activated magnetite particles were prepared to test the efficacy of the flow sensing system, whereby either highly aqueous stable $\text{Fe}_3\text{O}_4@\text{COOH}$ or fluorescent $\text{Fe}_3\text{O}_4@\text{G}'@\text{IgG}$ -488 MNPS were dispersed in aqueous suspensions. These nanoparticles were used as

benchmarks for comparison against more complex anisotropic system of different materials, with similar surface modifications in FACS analysis. Below is a schematic of the surface modification reactions used to prepare $\text{Fe}_3\text{O}_4@\text{COOH}$ and $\text{Fe}_3\text{O}_4@\text{G}'$ MNPS. (Figure 2.1)

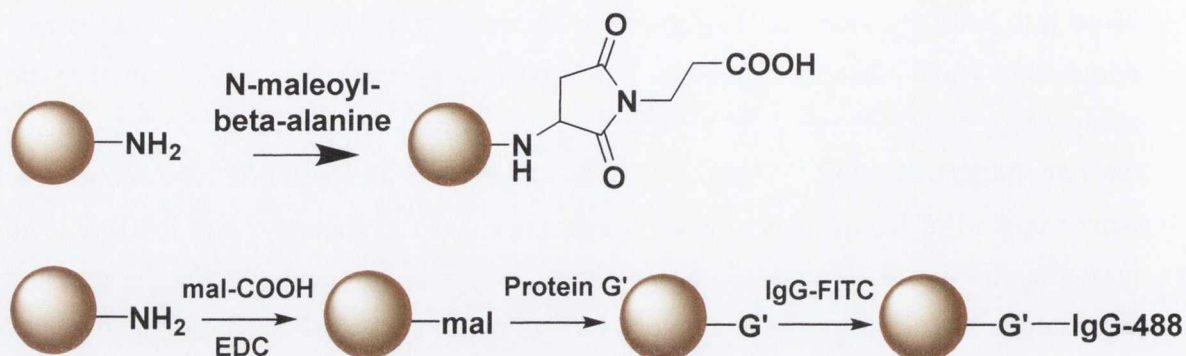


Figure 2.1: Schematic of Fe_3O_4 surface modifications performed for use in FACS analysis

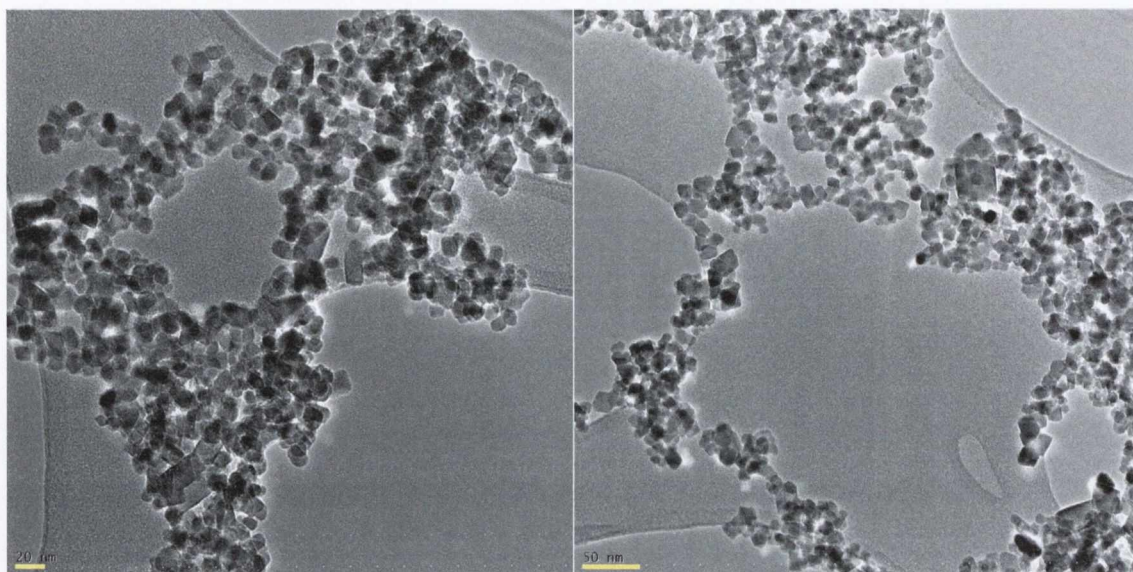
The methods and strategies developed on Fe_3O_4 will then be applied to ferrite-doped silica nanowires, the synthesis for which is also to be optimised here, and will then be applied to metal and segmented barcode magnetic wires provided by Physics, TCD. Here, they are used to PEGylate and further functionalise the surface of doped-silica, cobalt and alloy metal nanowires.

In addition, this oxide layer can first be coated with a layer of silica to enhance the biocompatibility of the NWs, and help reduce further oxidation and degradation. Silica coating with an even, controllable thickness can be achieved by a modified Stöber method,^{14, 15} involving a base catalysed sol-gel with TEOS as the silica precursor, which could then be further functionalised with organic linking groups. Selected bioconjugated wires will then be compared against the model Fe_3O_4 system in FACS analysis.

2.2 FERRITE SYNTHESIS AND SURFACE FUNCTIONALISATION

2.2.1 Preparation and characterisation of Fe_3O_4 (1)

The ferrite NPs synthesised here were produced by previously reported and well documented methods,⁵ involving the co-precipitation of iron salts at basic pH. According to TEM magnetite nanoparticles (**1**) have a mean diameter of 7.5 ± 2 nm. (Figure 2.2)



Scale Bar: 20 nm

Scale Bar: 50 nm

Figure 2.2: TEM images of Fe_3O_4 MNPs (1)

VSM revealed a magnetisation of $76.39 \text{ Am}^2\text{kg}^{-1}$ with no remnant magnetisation at 0 Tesla as shown in **Figure 2.3**. This value is quite close to that of bulk magnetite ($\sim 90 \text{ Am}^2\text{kg}^{-1}$).¹⁶ The small size and high magnetisation, coupled with superparamagnetic behaviour make these ideal NPs for comparison and characterisation of various organic reactions performed at the surface of nanoparticles, such as surface silanisation and amide coupling. In addition they can be produced in larger quantities, than is generally possible for nanoparticle syntheses, with yields of 2-3 g.

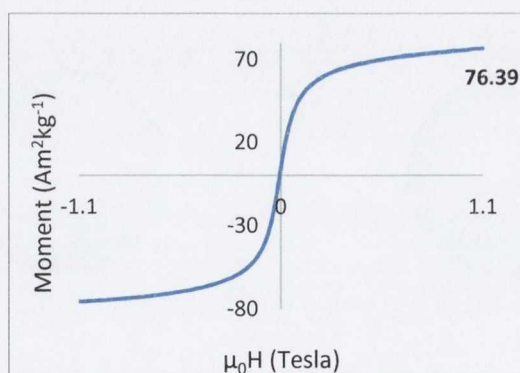


Figure 2.3: VSM curve of Fe_3O_4 MNPs (1)

FTIR revealed characteristic stretching peaks for Fe_3O_4 at 385 (Fe-O), 553 (Fe-O), 1642 (O-H) and 3431 (O-H) cm^{-1} . (**Appendix 2.1.3**)¹⁷ XRD measurements were in agreement with literature values for the face centred cubic structure of Fe_3O_4 with lattice parameters of 30.2 (220), 35.5 (311), 43.2 (400), 53.5 (422), 57.1 (511), 62.7 (440), 74.1 (533), 89.9 (731), 94.7 (800), 105.4 (751), 110.1 (840), 128.4 (844). (**Figure 2.4**)

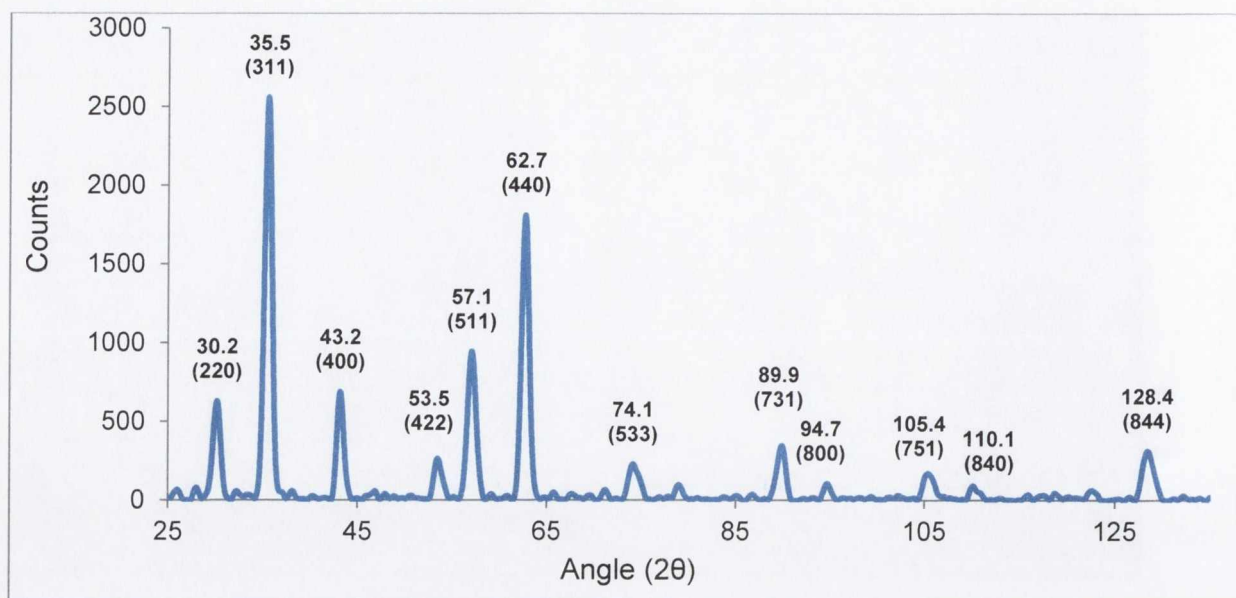


Figure 2.4: XRD pattern for Fe₃O₄ MNPs (1)

2.2.2 Preparation and characterisation of Fe₃O₄@NH₂ (2)

Fe₃O₄ MNPs were chosen as a model system to be modified with functional monolayers using trialkoxysilanes. **(Figure 2.5)** Subsequent experiments will involve more complex materials, and larger particles which may not be so straightforward to characterise, hence it is useful to know what type of signals to expect from the various techniques and also to ensure these experiments are robust and reproducible under the conditions optimised here.

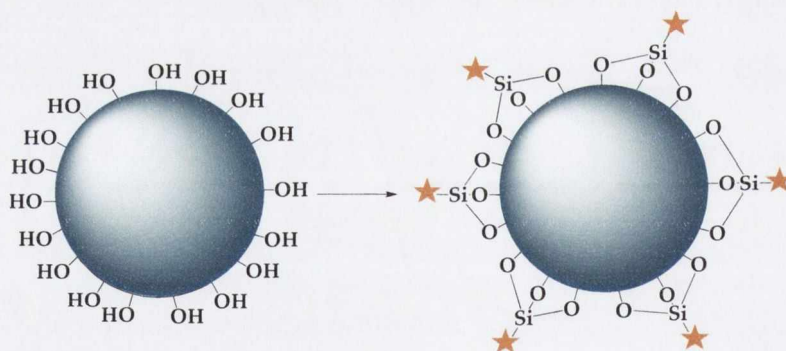


Figure 2.5: Schematic of surface functionalisation by silanisation⁹

This reaction can be used to install any number of functional groups and there are several commercially available. In this work the procedure has been simplified, involving an economy of reagents and mild conditions, which can be easily transferred to different materials, and using different silanes. The reaction uses only residual water in polar solvents as a catalyst for hydrolysis of the trialkoxyl silane. One benefit of this procedure is that it can be performed for the installation of both hydrophilic and hydrophobic

functional moieties, and combinations of the two and several different solvents can be used. Successful installation was achieved for APTS to produce amino-modified MNPs (2) TEM revealed no apparent increase in particle size for this sample. (Figures 2.6) However, surface silicon atoms were detectable by EDX, and hence the extent of functional binding could be calculated. This reaction was repeated several times and yielded almost identical results.

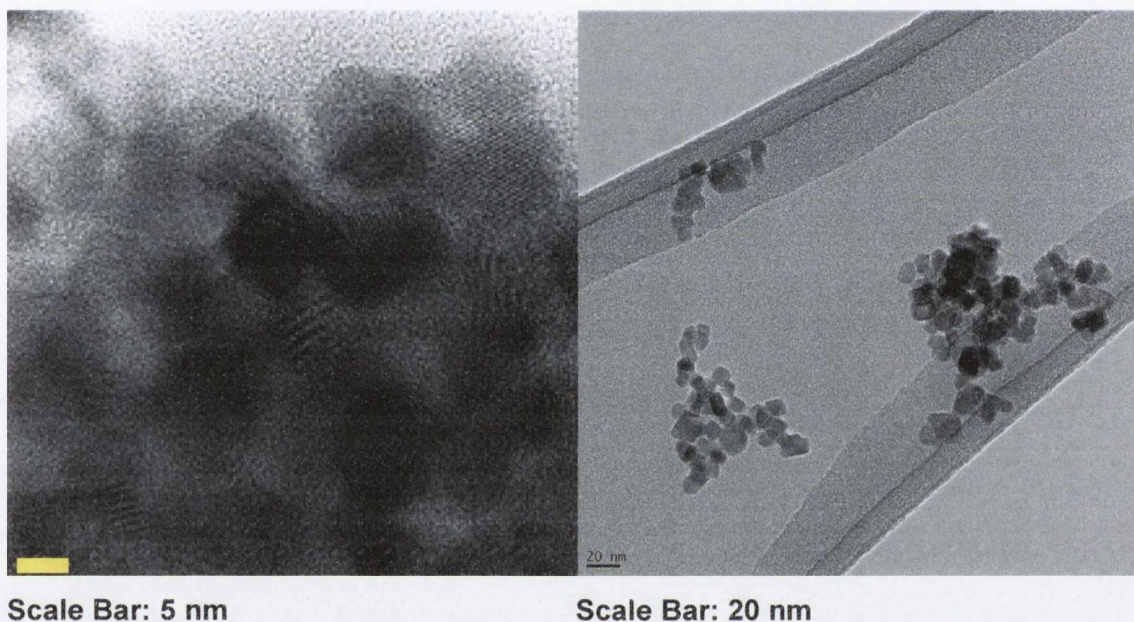


Figure 2.6: TEM images of $\text{Fe}_3\text{O}_4@\text{NH}_2$ (2)

EDX was performed using a FEI Titan TEM. (Figure 2.7) EDX is a spectroscopic technique, providing quantitative information about the binding of surface functional groups. It is important to note that characterisation by EDX can be easily and accurately performed for larger elements, however, it is unreliable for O and elements of lower molecular weights, including C and N. This technique can therefore be useful for silane binding analysis of non-silicon containing nanomaterials. EDX phosphorous measurements have also been used in Chapter 4 for the analysis of PEGylation reactions.

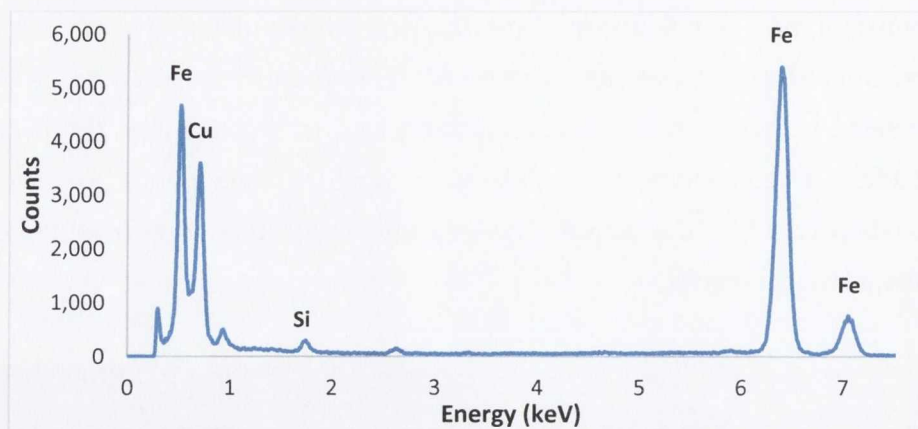


Figure 2.7: EDX spectrum of Fe₃O₄@NH₂ (2)

For silanisation, in each case the extent of functional binding was found to be around 70% coverage, compared to the theoretical maximum based on the coverage required per silane ligand of 0.4 nm².^{18, 19} An example of these calculations for a Fe:Si ratio of 96.5:3.5, found for Fe₃O₄@NH₂ (2) is shown below. (Table 2.8)

TABLE 2.8: Example calculations for surface functional group molarities

Formula	Calculations	Terms
$V_{NP}: (4/3)\pi r^3$	$(4/3) \times 3.14 \times (3.75 \text{ nm})^3 = 220.78 \text{ nm}^3$	V_{NP} is Volume per NP
$SA_{NP}: 4\pi r^2$	$4 \times 3.14 \times (3.75 \text{ nm})^2 = 176.625 \text{ nm}^2$	SA_{NP} is surface area per NP
D: Literature Bulk Value	$5.15 \times 10^{-21} \text{ gnm}^{-3}$	D is the density of the material
$M_{NP}: V_{NP} \times D$	$220.78 \text{ nm}^3 \times 5.15 \times 10^{-21} \text{ gnm}^{-3} = 1.137 \times 10^{-18} \text{ g}$	M_{NP} is the mass per NP
Moles Fe _{NP} : ($M_{NP} \times 0.725$)/55.84 g mol ⁻¹	$(1.137 \times 10^{-18} \text{ g} \times 0.725) / 55.84 \text{ g mol}^{-1} = 1.476 \times 10^{-20} \text{ mol}$	Moles Fe _{NP} is the moles of Fe per NP
Fe _{NP} : Moles Fe _{NP} × N _A	$1.476 \times 10^{-20} \text{ mol} \times 6.022 \times 10^{23} \text{ mol}^{-1} = 8890$	Fe _{NP} is the number of iron atoms per NP
Si _{CALC} : (SA_{NP} / SA_{Silane})	$176.625 \text{ nm}^2 / 0.4 \text{ nm}^2 = 442$	Si _{CALC} is the theoretical maximum number of silanes per NP.
Si _{ratio} : (Fe _{NP} / Si _{CALC}) / (Fe _{EDX} / Si _{EDX})	$(8890 / 442) / (96.5 / 3.5) \times 100 = 73\%$	Si _{ratio} is the extent of silane binding ($0 \leq x \leq 1$)
Si _{EXP} × Si _{CALC} × Si _{ratio}	$442 \times 0.73 = 322.66$	Fe _{EDX} is the relative proportion of Fe found by EDX
NP _{sample} : $M_{sample} \div M_{NP}$	$1 \text{ g} / 1.137 \times 10^{-18} \text{ g} = 8.795 \times 10^{18}$	Si _{EDX} is the relative proportion of Si found by EDX
Si _{sample} : NP _{sample} × Si _{EXP}	$8.795 \times 10^{18} \times 322.66 = 2.838 \times 10^{20}$	Si _{EXP} is the actual number of silanes per NP
Moles _{silane} : Si _{sample} ÷ N _A	$(2.838 \times 10^{20}) / 6.022 \times 10^{23} \text{ mol}^{-1} = 4.7 \times 10^{-4} \text{ mol}$	NP _{sample} is the number of nanoparticles per sample
		M _{sample} is the mass of the sample
		Si _{sample} is the number of silanes per mass of the sample
		Moles _{silane} is the moles of silane/functional groups per mass of the sample
		N _A is Avogadro's constant: $6.022 \times 10^{23} \text{ mol}^{-1}$

Using this technique one can also determine the moles of functional groups per gram of nanoparticles. This number is useful for later conjugation reactions, to estimate the molar equivalents of reagents to be used in the reaction. These calculations can also be

extended to other materials and morphologies by adjusting the geometric equations and densities.

These reactions are very robust and have been validated on small NPs with a variety of compositions; hence it can be assumed that they will react with a similar efficiency on larger particles, such as silica nanowires. Due to the high affinity of Si for O and the consistency in experimental studies of surface coverage, it can be assumed that this silane surface modification will work for the majority of oxide surfaces and RCOH ligands, providing the material surface ligand itself is stable towards degradation.⁹

FTIR for $\text{Fe}_3\text{O}_4@\text{NH}_2$ (**2**) showed relatively good resolution and many of the relevant peaks could be identified. (**Figure 2.9**) The spectrum revealed peaks at 881cm^{-1} (medium, NH_2), 1054 cm^{-1} (medium, Si-O), 1098 cm^{-1} (medium, C-N), 1334 cm^{-1} (medium, Si-C), 1570 cm^{-1} (strong, NH_2), 2865 cm^{-1} (weak, C-H), 2928 cm^{-1} (weak, C-H), and 2971 cm^{-1} (weak, C-H).

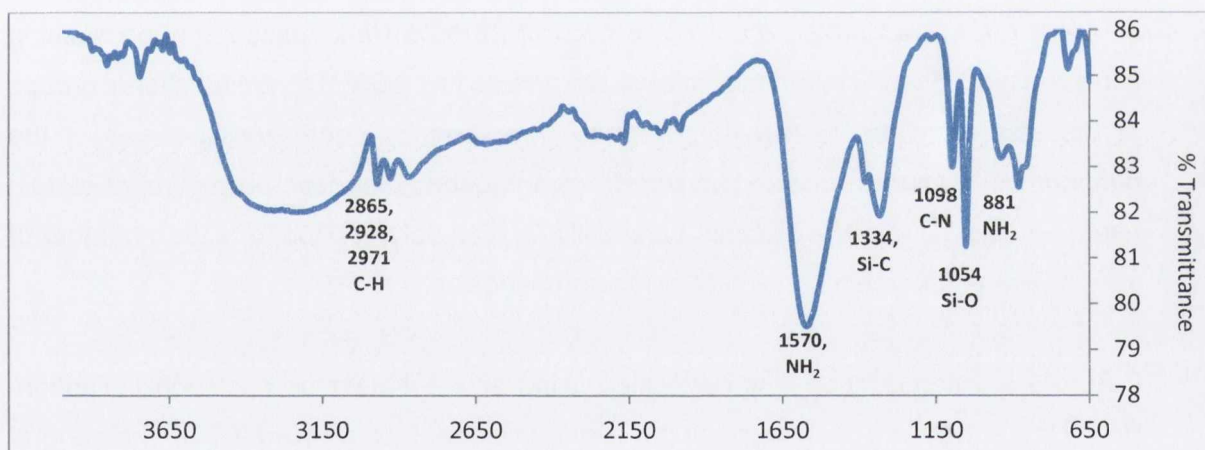


Figure 2.9: FTIR spectrum of $\text{Fe}_3\text{O}_4@\text{NH}_2$ (**2**)

Using FTIR the installation of surface functional groups can be monitored; however this technique is more difficult to perform on larger sized particles (with the small surface area and as result the lower content of functional group), as the peaks for the functional groups become too small for detection in relation to the characteristic peaks of the bulk material. Even on small particles such as $7.5\text{ nm Fe}_3\text{O}_4$, the organic groups only represent about 1% of the total mass. Despite this, FTIR proved to be the most reliable and specific tool for surface characterisation of the various nanomaterials. NMR was not suitable for many samples, due to their magnetic nature.

VSM measurements (**Appendix 2.1.2**) were also performed for $\text{Fe}_3\text{O}_4@\text{NH}_2$ (**2**) magnetisation was found to be $62.0 \text{ Am}^2\text{kg}^{-1}$. This slight decrease in magnetism relative to Fe_3O_4 can be attributed mostly to the increase in mass resulting from the organic surface ligands, and correlates well to the ratio calculated by EDX.

2.2.3 Preparation and characterisation of $\text{Fe}_3\text{O}_4@\text{COOH}$ (**3**)

An approach similar to solid phase stepwise synthesis of peptides and nucleic acids was adopted for functionalization reactions at the surface of these particles. Once the molarity of functional groups was determined, using the calculations outlined above, this was used as a guideline for the molarity of reagents to be added to the reactions. As in solid phase synthesis, an excess of reagents is added, generally 5-10 equivalents, which can be easily removed after reaction by centrifugation, filtration or magnetic separation.

If reactions require forcing conditions these equivalents can be increased or the reaction can be performed multiple times. In addition, these small magnetite particles show similar loading capacity to the standard resins used for automated syntheses. For example, the standard resin used in the Fmoc/tBu strategy of SPPS is Rink amide resin, possessing a loading capacity of $7 \times 10^{-4} \text{ mol linkers}$. Here there are $4\text{-}5 \times 10^{-4} \text{ mol functional groups}$ at the surface. This loading can also be optimised by controlling the size of the nanoparticle supports. Due to the added advantageous magnetic properties of certain nanoparticles, certain MNPs could potentially replace polymer supports for a number of solid phase and combinatorial synthetic approaches.

Carboxyl functionalisation was performed using amine-maleimide nucleophilic ligation. Below is a schematic of changes to the nanoparticle surface (**Figure 2.10**). The general mechanism for Michael addition is shown in Chapter 1.



Figure 2.10: Schematic of carboxyl functionalisation using amino-maleimide 'Click' chemistry

These particles were characterised by FTIR to determine changes to the functionality at the surface, which confirmed the presence of the expected groups. (**Figure 2.11**) With peaks at 385 (Fe-O Str.), 556.5 (Fe-Str.), 864 (C-N-C amide Str.), 1404 (C-O Str. from amide and COOH), (1700 C=O Str. from COOH). TEM showed no visible increase in

size, and VSM showed a peak magnetisation of $59.96 \text{ Am}^2\text{kg}^{-1}$. This decrease is consistent with the increase in surface organic mass. **(Appendix 2.1.2)** These particles were used in later experiments for FACS analysis and comparison, acting as a benchmark for small, aqueous stable, non-protein bound particles.

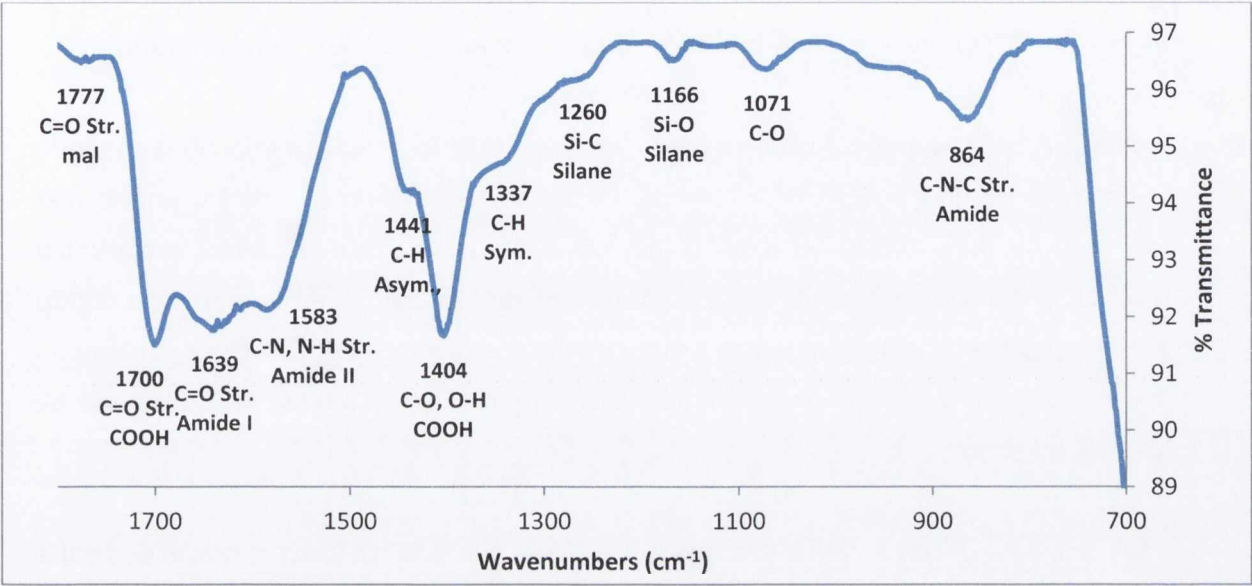


Figure 2.11: FTIR of Fe₃O₄@COOH MNPs

2.2.4 Preparation and characterisation of fluorescent antibody-conjugated Fe₃O₄ MNPs

Bioconjugated magnetite particles were prepared by a stepwise process of silane amino installation, EDC amide maleimide installation, followed by amine-maleimide nucleophilic Protein G' coupling then incubation with a fluorescent IgG protein. Below is a schematic of the functionalisation steps. **(Figure 2.12)**

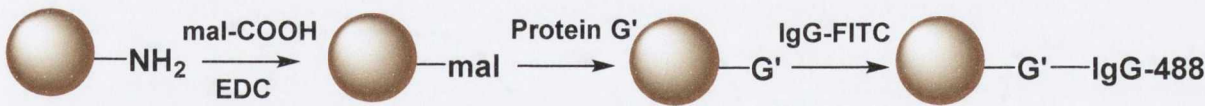


Figure 2.12: Schematic of surface modification steps towards synthesis of fluorescent antibody conjugated MNPs

(i) Fe₃O₄@mal (4)

Fe₃O₄@NH₂ (**2**) was converted to Fe₃O₄@NCO-mal (**4**) by amide coupling with *N*-maleoyl-β-alanine. Below is a schematic of the changes to the nanoparticles surface. **(Figure 2.13)** The full generalised mechanism for EDC-activated amide coupling is shown in Chapter 1.

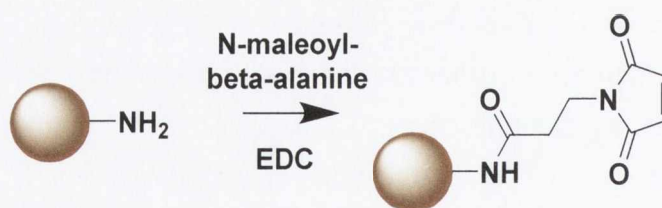


Figure 2.13: Schematic of maleimido functionalisation by EDC amide coupling

In this reaction, the order of addition of reagents is crucial to avoid side reactions between the maleimide ring and surface amines. Hence the carboxy-maleimide ligand is first activated by EDC before addition to the NP suspension. This enhances preferential reaction of the carboxyl group with the amino surface groups. DIEA is normally added to the NP suspension prior to reaction, however here it is omitted, as this reagent enhances the nucleophilicity of the amines making them more likely to attack the maleimide by Michael addition.

FTIR analysis (**Figure 2.14**) revealed a relatively complex spectrum; however peaks relating to both amide bond formation with the NP surface and the new maleimide surface group could be identified quite clearly. In this example good resolution was key to identifying the various peaks. Peaks were observed at 1066.5 cm^{-1} (weak, C-O, C-N), 1356 cm^{-1} (C-H, Def. Sym.), 1401 cm^{-1} (medium, mal C-N-C), 1444.5 cm^{-1} (C-H, Def. Asym.), 1546 cm^{-1} (weak, C=C mal), 1659.5 cm^{-1} (med, C=O Amide I), 1703.5 cm^{-1} (strong, mal C=O Asym.), 1772 cm^{-1} (weak, mal C=O, Sym.), 2902 cm^{-1} (strong, C-H), 2973 cm^{-1} (strong, C-H), 3676 cm^{-1} (medium, Amide).¹⁷ An additional spectral range is shown in the Appendix. (**Appendix 2.1.3**)

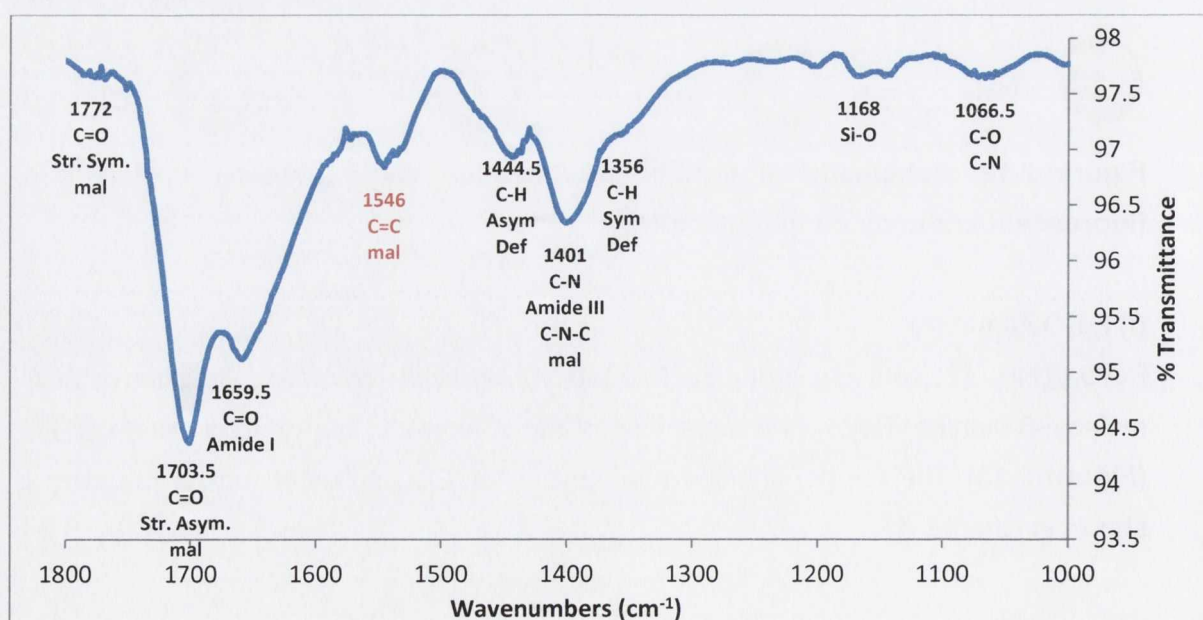


Figure 2.14: FTIR spectrum for Fe₃O₄@NCO-mal (4)

The spectra for $\text{Fe}_3\text{O}_4@\text{COOH}$ (**3**) and $\text{Fe}_3\text{O}_4@\text{mal}$ (**4**) are quite similar due to the similarity in their bonding. However, the maleimide functionalised particles are distinguishable by the C=C stretching vibration at 1546 cm^{-1} in **Figure 2.13** for $\text{Fe}_3\text{O}_4@\text{mal}$. This peak is not observable in **Figure 2.12** for $\text{Fe}_3\text{O}_4@\text{COOH}$. In addition, there is a difference in relative intensity of the peaks, despite the fact they both contain amido bonding. In $\text{Fe}_3\text{O}_4@\text{COOH}$ (**3**) the Amide II N-H peak is more prominent and shifted to the left, as this is a β -amide. Below is a schematic of the differential bonding at the surface of the two functionalised materials. (**Figure 2.15**)

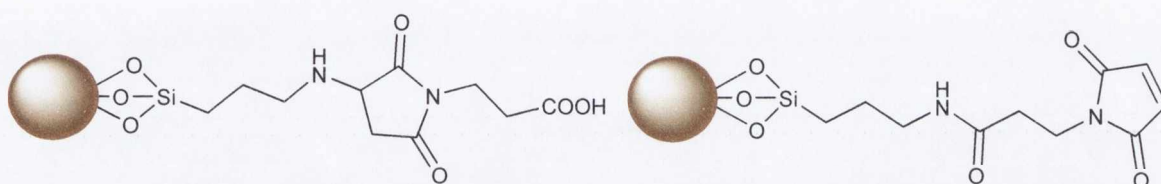


Figure 2.15: Differential bonding in $\text{Fe}_3\text{O}_4@\text{COOH}$ (left) and $\text{Fe}_3\text{O}_4@\text{mal}$ (right)

(ii) $\text{Fe}_3\text{O}_4@\text{G}'$ (**5**)

$\text{Fe}_3\text{O}_4@\text{mal}$ particles (**4**) were conjugated to Protein G' by amino-maleimide ligation, which was performed under sterile conditions. Typically protein have a fairly regular density, hence their size can be calculated from their MW.²⁰ Protein G' has a MW of $20,000\text{ g mol}^{-1}$, hence this corresponds to a diameter of $\sim 2\text{ nm}$.^{21, 22} The amount of G' used was 0.5 equivalents relative to the total nanomaterial surface. This does not represent full surface coverage, however, binding was still detected. FTIR confirmed the presence of three different C=O stretchings corresponding to different bonding modes in the tertiary structure of the protein. Peaks were visible for free (1700 cm^{-1}), alpha helix (1660.5 cm^{-1}) and beta sheet (1641 cm^{-1}) carbonyl Amide I bonds. (**Figure 2.16**)

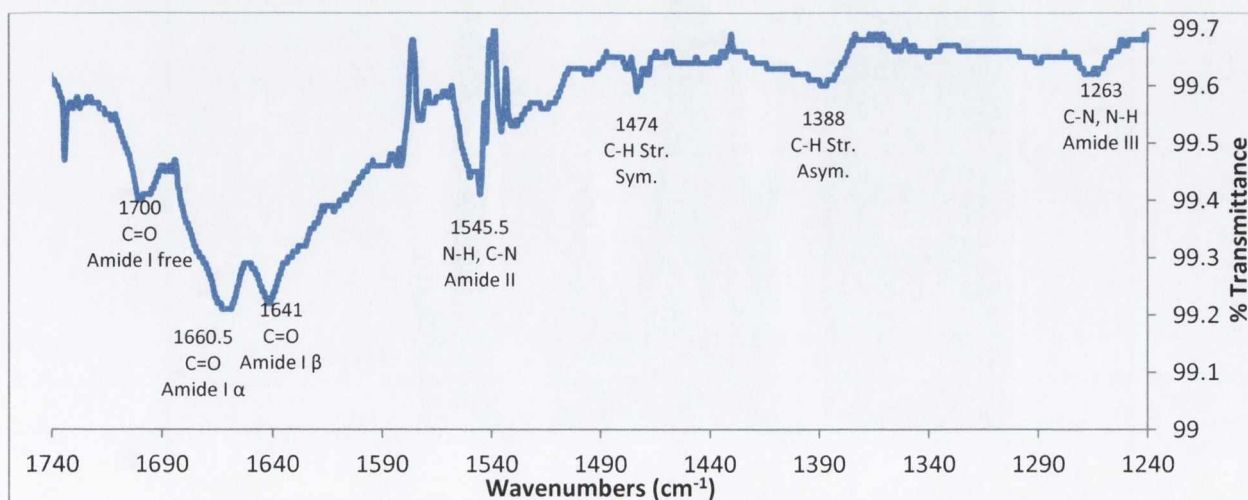


Figure 2.16: FTIR spectrum of $\text{Fe}_3\text{O}_4@\text{G}'$ NPs (5**)**

Protein attachment was not clearly visible from TEM (**Figure 2.17**), however EDX confirmed the attachment of the protein, through the presence of sulfur in the sample, which would not be present otherwise and is due to sulphur containing residues in the protein. (**Figure 2.18**) The ratio of Fe/S was 97.7/0.3. These figures are as expected due to the small contribution of sulphur to the overall protein mass and large contribution of Fe to the nanoparticle core. $\text{Fe}_3\text{O}_4@\text{G}'$ (**5**) was then incubated with IgG-A488 under sterile conditions to produce the final $\text{Fe}_3\text{O}_4@\text{G}'@\text{IgG-A488}$ conjugate (**6**) and subsequently used in FACS experiments.

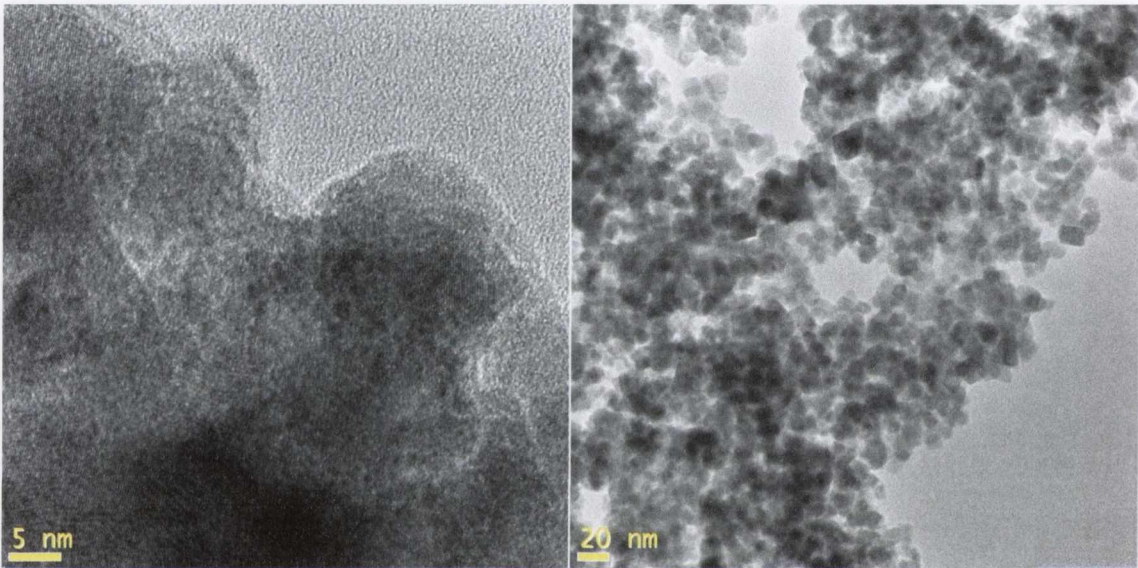


Figure 2.17: TEM images of $\text{Fe}_3\text{O}_4@\text{G}'$ NPs (5)

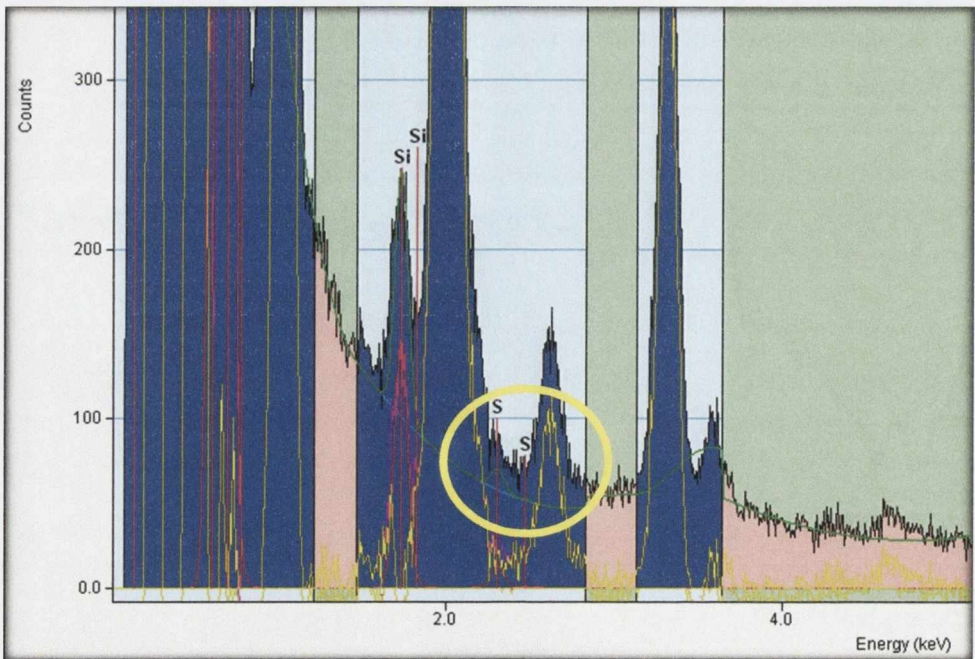
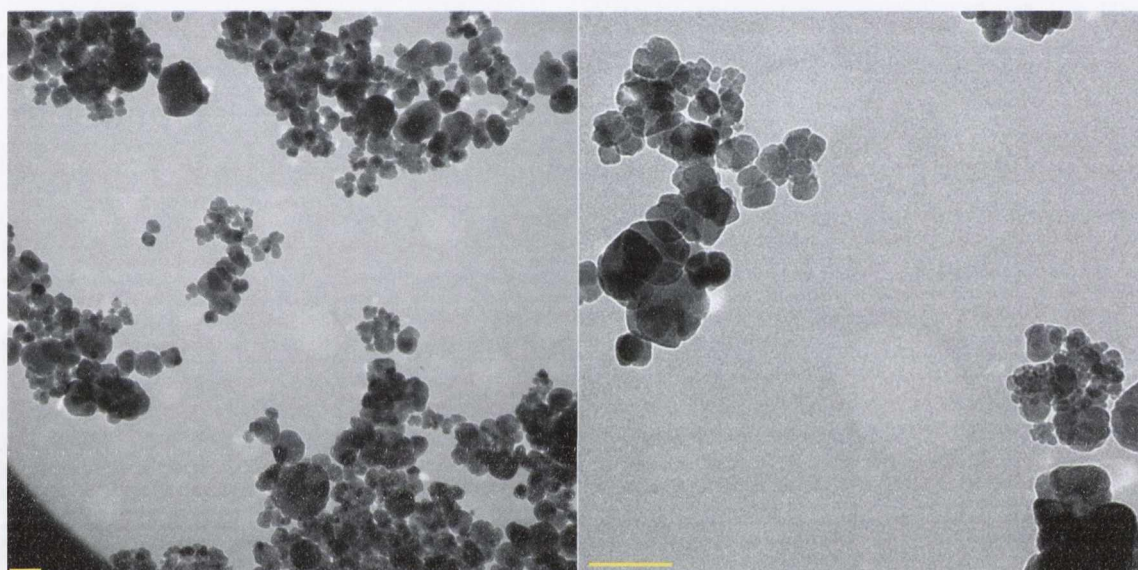


Figure 2.18: EDX spectrum of $\text{Fe}_3\text{O}_4@\text{G}'$ NPs, peak for sulfur circled in yellow (5)

2.3 COBALT FERRITE-DOPED SILICA NANOWIRES

2.3.1 Synthesis Functionalisation and Characterisation CoFe₂O MNP

Next, a series of various nanowires were prepared and functionalised for potential biomedical diagnostic applications and to compare against the Fe₃O₄ NP system. The first of these was cobalt ferrite doped silica nanowires. Here cobalt ferrite was used instead of magnetite due to its higher resistivity to acidic environment, which is a crucial step for the nanowire formation. CoFe₂O₄ MNPs (**7**) were synthesised according to a published procedure.²³ These NPs showed much higher polydispersity in size, with an average diameter of 37 ±23 nm, as shown in TEM. (**Figure 2.19**)



Scale Bar: 100 nm

Scale Bar: 100 nm

Figure 2.19: TEM images of CoFe₂O₄ MNPs (7**)**

These CoFe₂O₄ particles (**7**) exhibited lower magnetisation (42.87 Am²kg⁻¹) than that of Fe₃O₄ (**1**). Also, due to the increase in size, the CoFe₂O₄ NPs produced here are not superparamagnetic and have a considerably high remnant magnetisation (19.985 Am²kg⁻¹) at 0 Tesla. (**Figure 2.20**) This leads to poor solvation of the particles in the absence of sonication, and sedimentation due to clumping. However, as mentioned these NPs exhibited much higher resistivity towards acidic conditions; which is an important part of the silica deposition reaction to produce ferrite-doped silica nanowires. Hence, they were used despite their drawbacks.

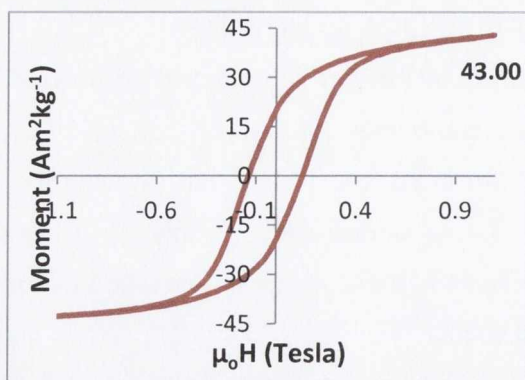


Figure 2.20: VSM curve of CoFe₂O₄ MNPs (7)

The crystal structure was found to be the expected face centred cubic by XRD (**Figure 2.21**), with lattice parameters of 30.9 (220), 35.2 (311), 38.6 (222), 42.9 (400), 53.3 (422), 56.8 (511), 62.5 (440), 74.0 (533), 89.7 (731), 94.5 (800), 105.5 (751), 110.8 (840), 122.4 (931), 128.3 (844), 143.4 (951). IR was also in agreement with literature values with peaks at 403 (Fe-O, Co-O overlapping), 589 (Fe-O, Co-O overlapping) and 3406.5 (O-H) cm⁻¹.¹⁷ (**Appendix 2.2.1**)

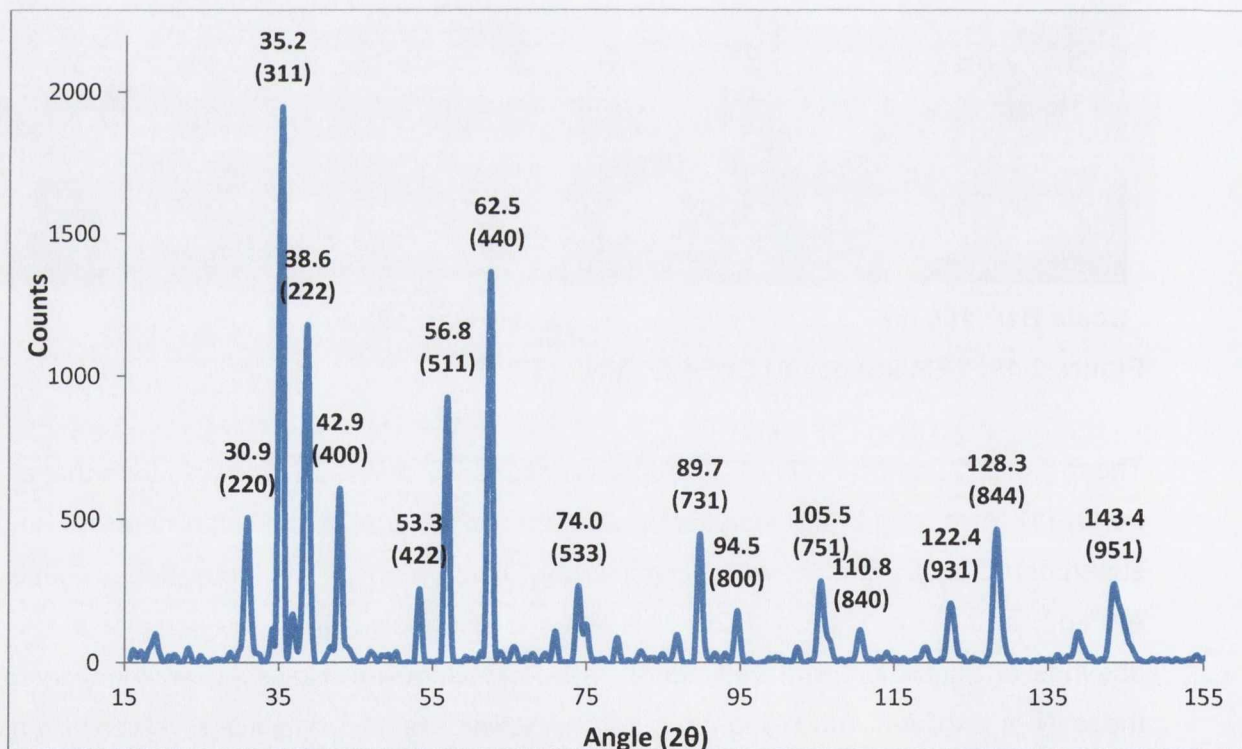


Figure 2.21: XRD pattern of CoFe₂O₄ MNPs (7)

2.3.2 Preparation and characterisation of CoFe₂O₄@SiO₂ NWs

The cobalt ferrite doped nanowires (**8**) were synthesised by a dispersion of the CoFe₃O₄ MNPs (**7**) within the silica gel which was produced from TEOS.¹ The mixture was passed through the alumina templates before solidification of silica gel. These were allowed to

dry overnight before membrane dissolution in NaOH solution. The wires produced had reasonable magnetisation ($2.08 \text{ Am}^2\text{kg}^{-1}$), indicating incorporation of the MNPs into the wires. (Figure 2.22)

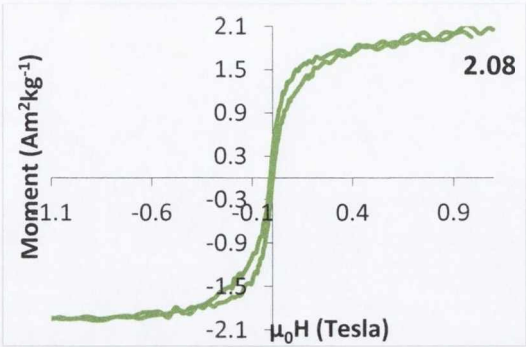
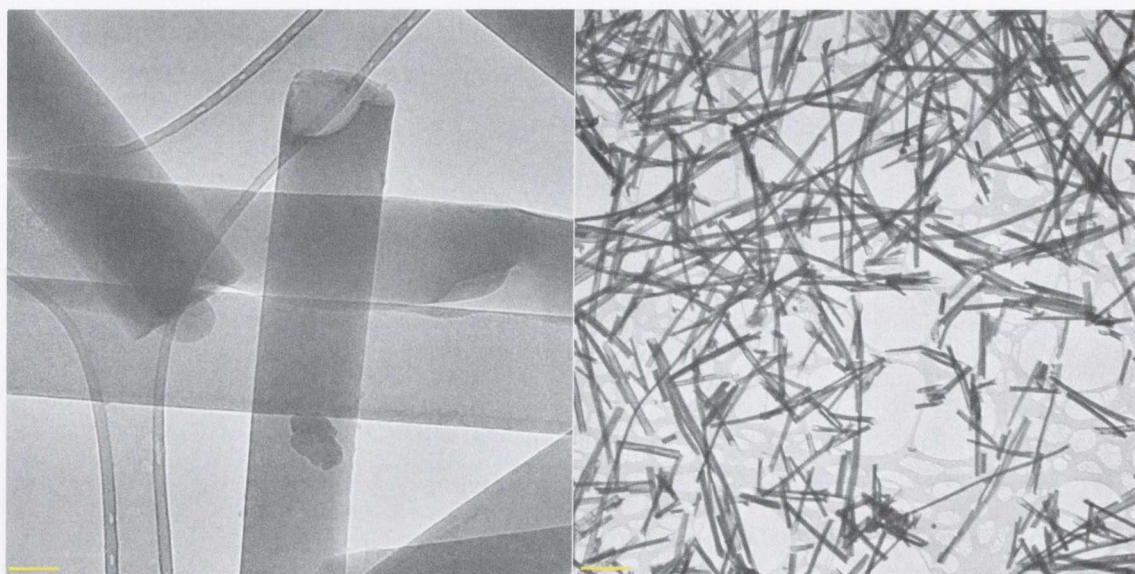


Figure 2.22: VSM curve of CoFe_2O_4 -doped SiO_2 NWs (8)

This relatively low rate of incorporation is most likely due to the high polydispersity in size of the CoFe_2O_4 MNPs (7) and also high remnant magnetisation ($19.985 \text{ Am}^2\text{kg}^{-1}$). In general, larger particles have a lower rate of infiltration of the membranes, particularly if clumping due to magnetic attraction is also a factor. However, this value was still an improvement on previously reported methods using Fe_3O_4 instead of CoFe_2O_4 . This may be due to the increased acidic resistance of the particles. In addition, this magnetisation is still within a useful range for bioapplications. Hence, they were used for further experiments.

2.3.3 Preparation and characterisation of fluorescent antibody-conjugated cobalt ferrite-doped silica nanowires ($\text{CoFe}_2\text{O}_4@ \text{SiO}_2@ \text{CD4-PE}$ NWs)

APTS functionalisation of these wires, to produce $\text{CoFe}_2\text{O}_4@ \text{SiO}_2@ \text{NH}_2$ (9) is very straightforward, however, it is difficult to characterise these surface functionalities. For larger sized nanoparticles, such as these (200 nm diameter x 5 μm length), the mass percent of surface organic groups to total particle is very small. Hence the silica peaks mask the smaller organic peaks in FTIR spectra. However there are slight changes in the spectrum, with a small increase at 2889 cm^{-1} (C-H Str.) and 1585.5 cm^{-1} (N-H Scr.) (Appendix 2.2.2) In TEM there was no apparent change in the structures of the NWs. (Figure 2.23) However due to the robustness and reproducibility of the APTS reaction in previous experiments, combined with the indication of binding to the surface from FTIR, further reactions were continued.

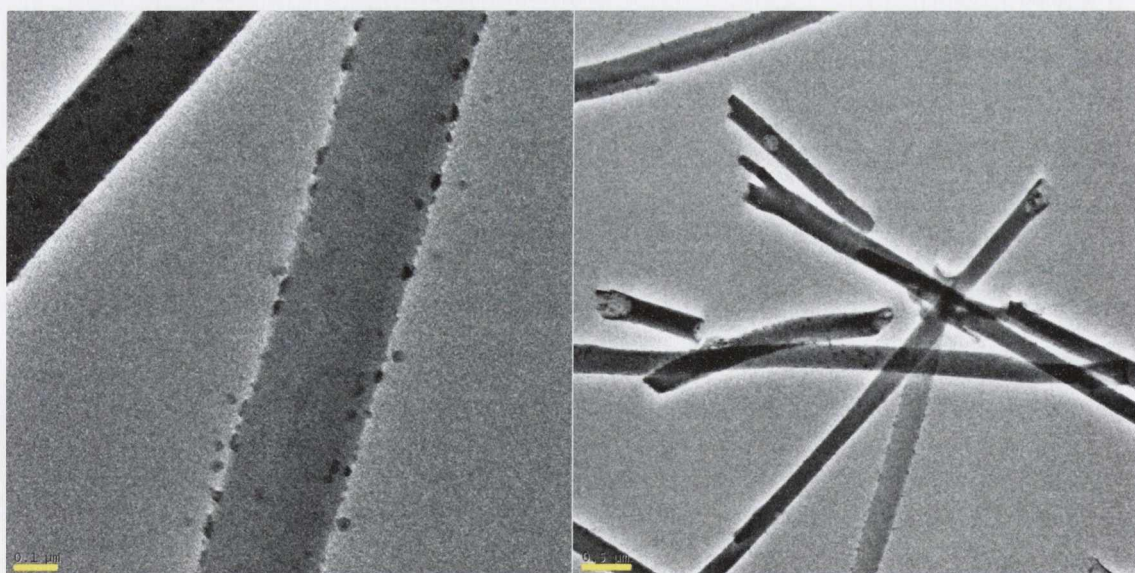


Scale Bar: 100 nm

Scale Bar: 2 μ m

Figure 2.23: TEM images of CoFe_2O_4 -doped $\text{SiO}_2@\text{NH}_2$ NWs (9)

Once modified with amino groups (9), an amide coupling reaction was performed to install a maleimide group (10) for conjugation to the antibody CD4-PE (11), by nucleophilic addition with cysteine and amino residues on the protein surface. Protein attachment is clearly visible by TEM, as shown in **Figure 2.24**. In addition, the NW product has a pink colour, due to the presence of fluorescent antibodies. The sample was then sent to BD Biosciences in California for testing. Results are pending



Scale Bar: 100 nm

Scale Bar: 200 nm

Figure 2.24: TEM images of CoFe_2O_4 -doped SiO_2 NWs functionalised with CD4-PE fluorescent antibodies (11)

2.4 SIILICA-COATED METAL NANOWIRES

2.4.1 Preparation and characterisation of Co@SiO₂@NH₂ (13)

Next, a series of metal nanowires were received by Physics, TCD. These were prepared by a template-assisted electrochemical deposition as previously described. The first of these nanowires to be received were cobalt nanowires with a dimension of 200 nm diameter and 10 μm length. These were coated with silica to improve their biocompatibility, then functionalised with amino groups for further conjugation. Silica coating (12) was achieved by a modified Stöber method.^{14, 24} (Figure 2.25)

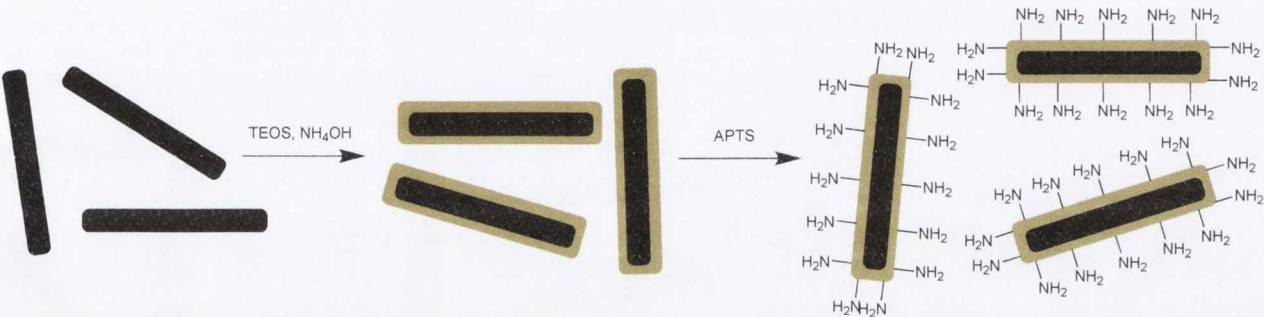


Figure 2.25: Schematic of Co NW silica coating and amino functionalisation

This reaction was performed at high dilution to reduce clumping of the nanowires, which leads to encapsulation of multiple wires within single silica shells. TEM revealed an even well distributed coating of these wires, with an average thickness of 150 nm. These wires were then functionalised using APTS to produce Co@SiO₂@NH₂ NWs (13) (Figure 2.26) by a similar procedure to Fe₃O₄ aminosilane modification. The wires were then sent to the Institute of Molecular Medicine (IMM), TCD for flow cytometry studies and to UCD for toxicology studies.

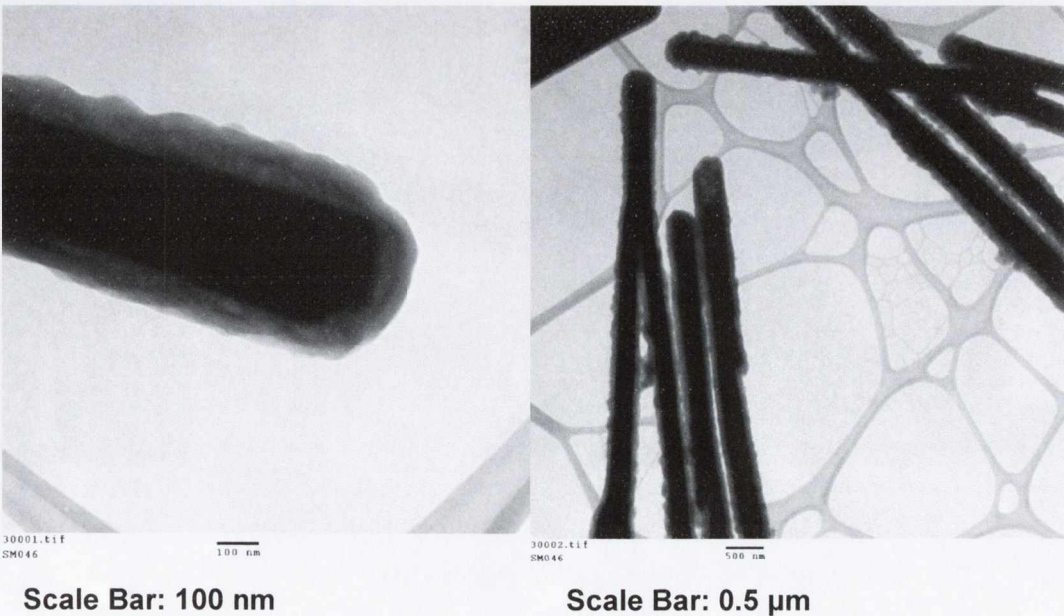


Figure 2.26: TEM images of Co@SiO₂@NH₂ NWs (13)

2.4.2 Preparation and characterisation of Co@Au@SiO₂@NH₂ NWs

Gold-coated cobalt nanowires (Co@Au NWs) were also coated with silica. (14) (Figure 2.27) The initial as-synthesised wires, also provided by Prof. Coey's group, School of Physics, TCD, were surface stabilised by K⁺CN⁻, which was used as part of the gold deposition process. It was hoped that Au would improve both scattering properties and biocompatibility of the wires. For silica coating, the non-oxidised gold surface of these wires was first primed by thiol exchange with thioglycerol, to install surface hydroxyl groups for TEOS attachment and deposition. Silica coating was then performed in a similar manner to Co@SiO₂ NWs. (12)

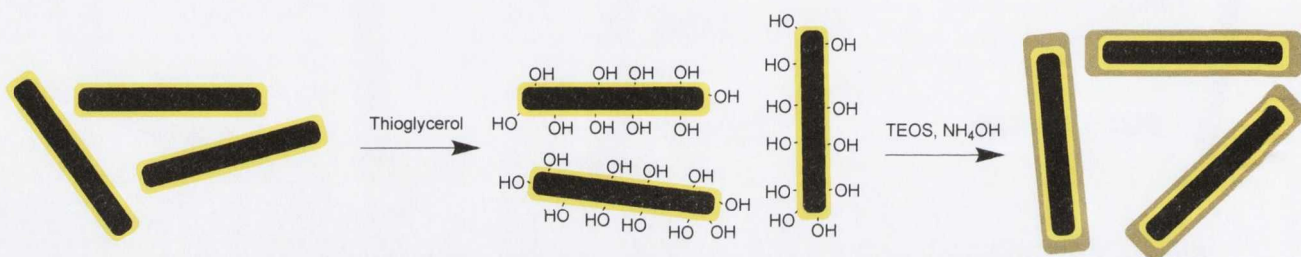
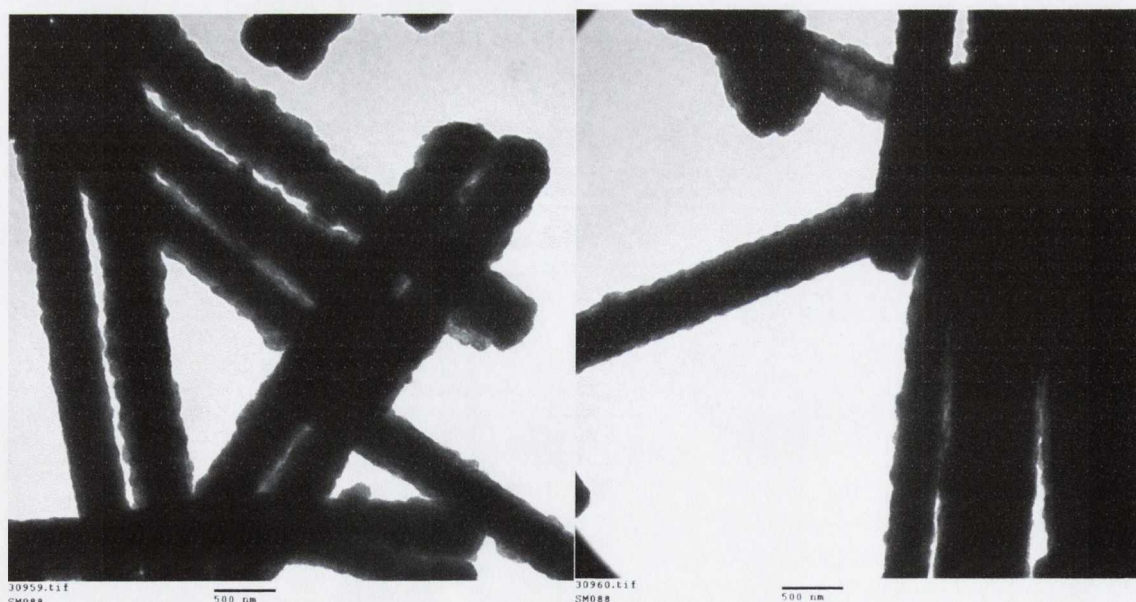


Figure 2.27: Schematic of Co@Au NW surface hydroxylation and silica coating

TEM revealed a relatively even distribution for the silica coating, with an average thickness of 150 nm, as shown below. (Figure 2.28) Samples were then submitted to IMM, TCD for biological testing and UCD for toxicology testing.



Scale Bar: 0.5 μ m

Scale Bar: 0.5 μ m

Figure 2.28: TEM images of Co@Au@SiO₂ NWs (14)

2.5 PEGYLATED METAL NANOWIRES

2.5.1 Cobalt Nanowire PEGylation (15-18)

The Co NWs were also modified with a variety of functionalities using PEG-trialkoxysilanes. PEG is known to improve biocompatibility and bioavailability of nanomaterials. In addition, these heterobifunctional PEGs provided a new surface linking group for further reactions. These methods are similar to magnetite APTS functionalisation, as they utilise hydroxyl groups at the nanoparticle surface to attach the silane. Below is a schematic of the reaction with the new functional moiety represented as a red star. **(Figure 2.29)**

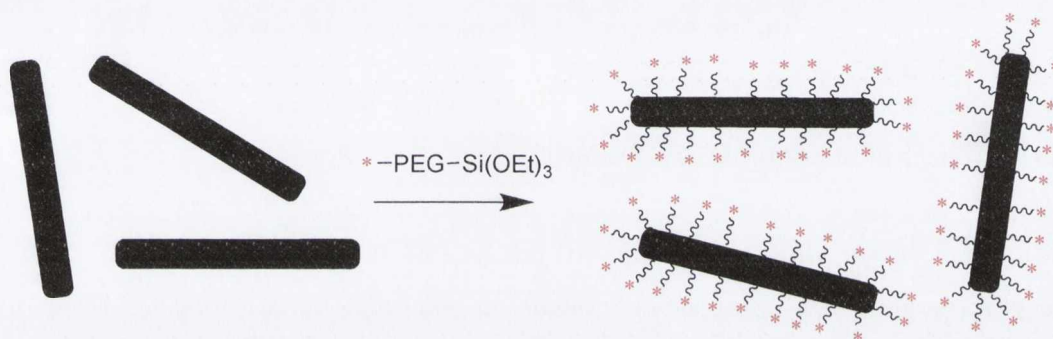
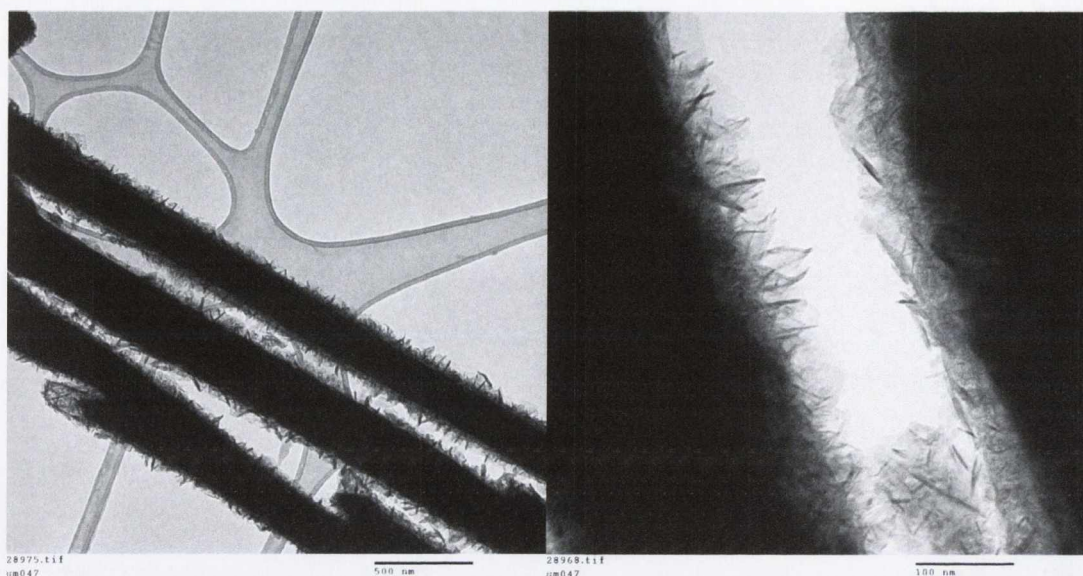


Figure 2.29: Schematic of cobalt NW PEGylation by silanisation

This method was used to produce an amino-PEG **(15)**, maleimido-PEG **(16)** carboxy-PEG **(17)**, and a heterobifunctional-PEG surface, consisting of both surface carboxyl and maleimido groups **(18)**. Bi-functionalised wires can be used to install two distinct moieties of interest, such as a fluorescent marker and targeting agent, for more complex multimodal applications. In each case the PEGylated surface produced mean thickness of 150 nm with a distinct, fluffy morphology. **(Figure 2.30)** These wires were then sent to IMM, TCD for flow cytometry studies, and UCD for toxicological studies.

Overall, for each of the coated cobalt nanowire samples prepared here, toxicological and flow cytometry studies revealed a large degree of clumping for the nanowires in studies and low biocompatibility. This is due to the ferromagnetic behaviour of the cobalt core, which imparts permanent magnetisation to the wires. Even surface modification was only achieved due to constant sonication during the reactions. In the absence of sonication these wires cannot remain in suspension very long, and precipitate in a matter of seconds to minutes. Hence other alloy materials were developed to replace these, with soft magnetic behaviour, which would more readily stay in suspension for flow and biological studies.



Scale Bar: 0.5 μm

Scale Bar: 100 nm

Figure 2.30: TEM images of Co@PEG-NH₂ (15)

2.5.2 Cobalt-Nickel Alloy Nanowire PEGylation (19-22)

CoNi was identified as a good alloy candidate for electrochemical deposition techniques using AAO membranes. These wires were synthesised by Prof. Coey's group in a number of lengths, namely 2 μm , 5 μm and 10 μm in length (unpublished work). PEGylation was identified as the simplest and most effective surface coating strategy. Unlike silica coating, it also provided an already installed functional group in a one-pot reaction.

The cobalt-nickel alloy wires were PEGylated, with a silane heterobifunctional PEG. Upon analysis of the wires by TEM, some samples showed a large amount of fragmentation, with degradation due to oxidation; particularly those with shorter lengths. The 2 μm wires had poor integrity and seemed to be hollow inside, as indicated by the differences in contrast, and therefore density, by TEM. **(Figure 2.31)** The wires were initially characterised by SEM in Physics, prior to reaction. However, this technique did not show the internal integrity of the wires. There was also considerable clumping of the wires in these samples. This could be due to their ferromagnetism, however it could also be in part due to incomplete dissolution of the membranes.

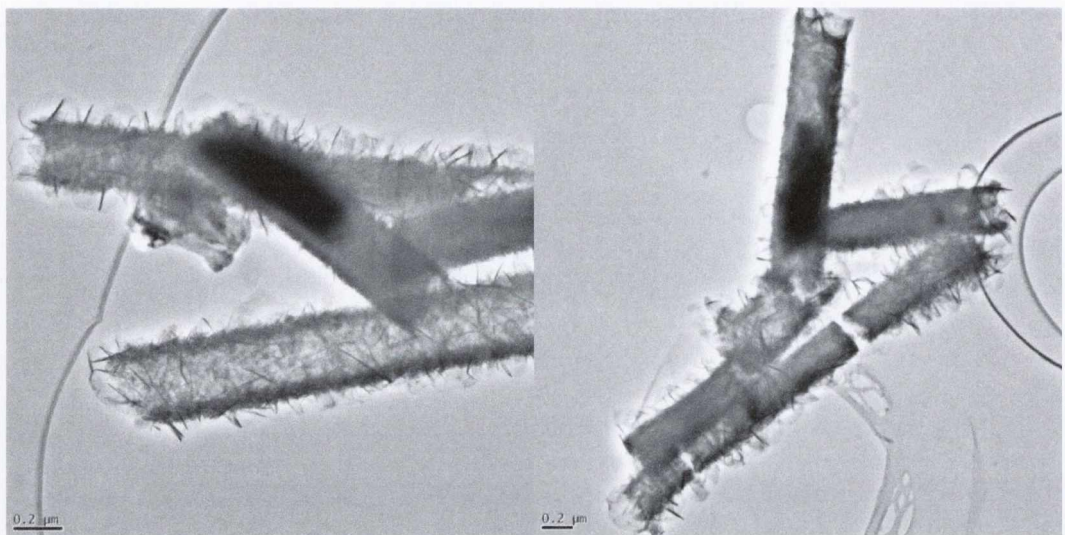


Figure 2.31: TEM images of 2 μm CoNi@PEG-mal NWs (19)

Additionally, there was a presence of some remaining alumina indicated by Al and Si peaks in EDX analysis. **(Figure 2.32)** The expected ratio of oxygen to other elements in these samples was much higher, indicating oxidation of the metal wires. There is no dense core to the wires, hence, this most likely occurred during the membrane dissolution, due to porosity in the wires, which allowed the NaOH solution to infiltrate through the wires, instead of just etching at the surface.

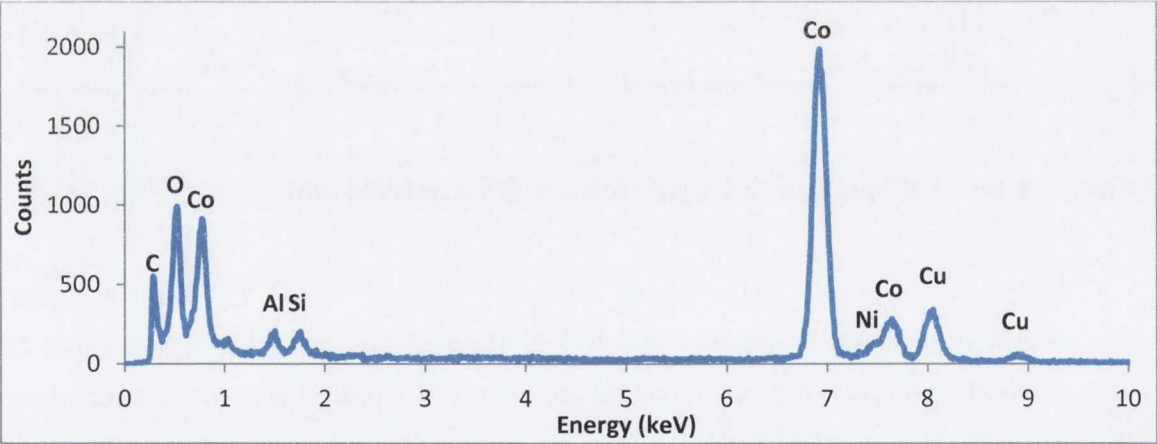


Figure 2.32: EDX Spectrum of 2 μm CoNi@PEG-mal NWs (19)

The CoNi@PEG-mal 5 μm wires **(20)** also showed signs of degradation by TEM analysis and oxidation with alumina traces still present, as established by EDX. **(Figure 2.33, 2.34)** However in this case there was some evidence of a denser core remaining in some of the wires.

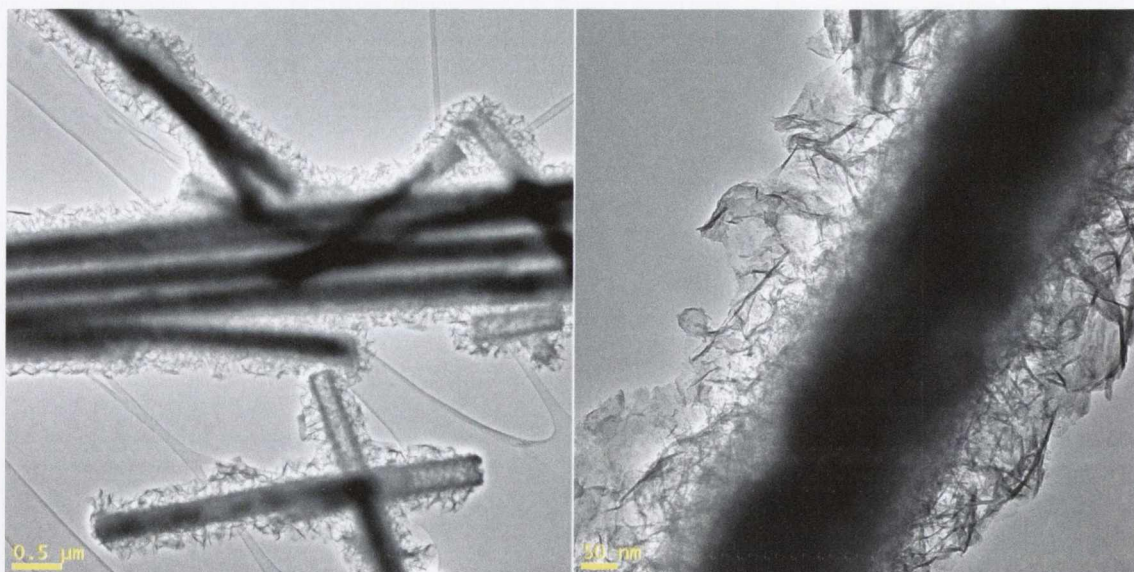


Figure 2.33: TEM images of 5 μm CoNi@PEG-mal NWs (20)

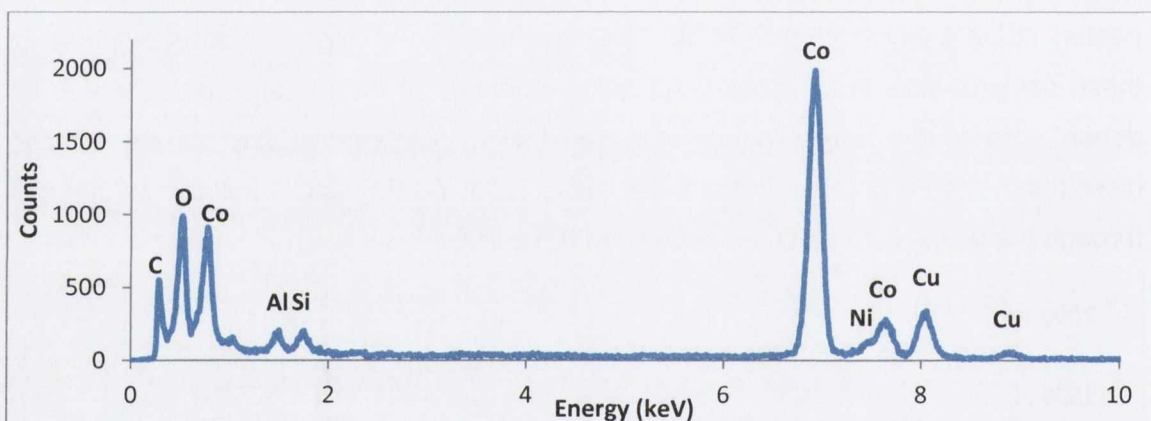


Figure 2.34: EDX spectrum of 5 μm CoNi@PEG-mal NWs (20)

Two samples of 10 μm length nanowires were also received from Physics and were also functionalised similarly to above. TEM studies showed that these CoNi@PEG-mal 10 μm wires (21, 22) had the most density and integrity, compared with the shorter wires. (Figure 2.35, Figure 2.36) Due to this trend of increasing density with length, it was assumed that this may be due to the experimental conditions in the wire formation and rate of deposition in the syntheses. Hence the 10 μm length samples were used for further studies.

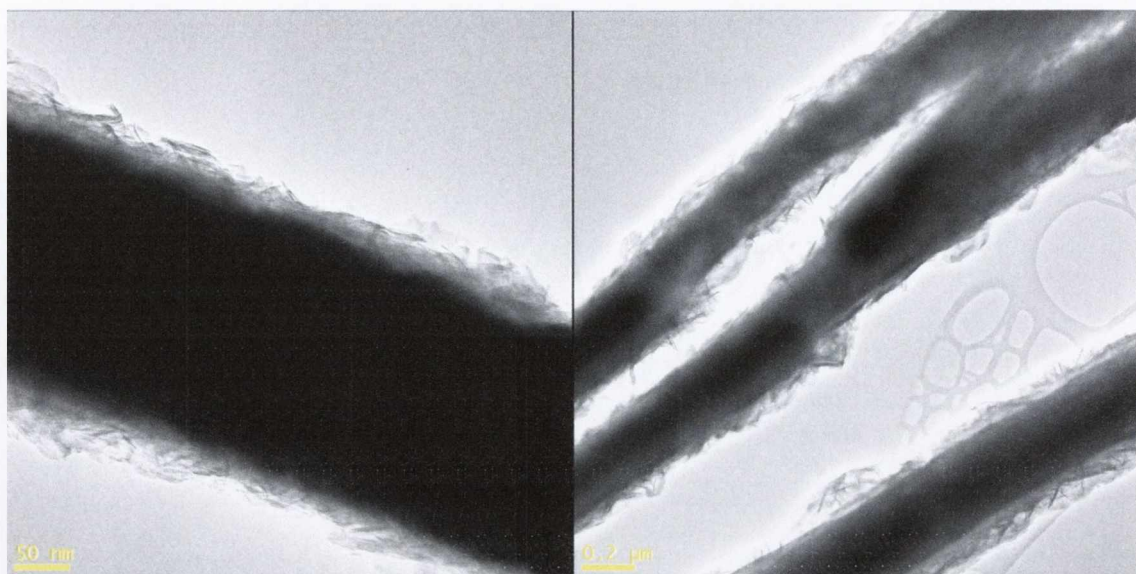


Figure 2.35: TEM images of 10 μm CoNi@PEG-mal NWs (21)

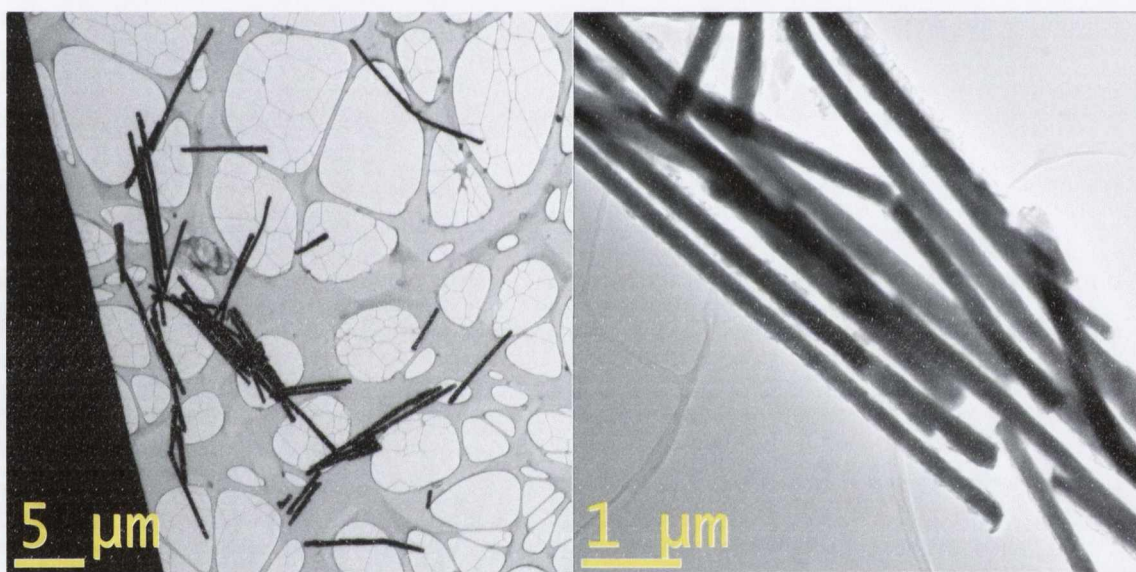


Figure 2.36: TEM images of 10 μm CoNi@PEG-mal NWs (22)

EDX showed a ratio of $\text{Co}_{95.8}\text{Ni}_{4.2}$ for (21), with 4% Al relative to the wires. EDX revealed a ratio of $\text{Co}_{95.1}\text{Ni}_{4.9}$ for (22), with less alumina present compared to the previous sample, with 0.95% Al relative to the wires. (**Appendix 2.4.5**). The difference in Al content of the two samples is most likely due to experimental differences in membrane dissolution. The samples showed very similar ratios of Co to Ni in the final sample. Despite the small inclusion of Ni into the wires, these showed better stability in solution than the previous pure cobalt nanowire samples.

FTIR on (21) showed a considerable degree of alumina on the surface of the particles, as expected from EDX analysis. However, PEG functionality was also successfully

identified (1101 cm^{-1} , C-O-C str.), although contributing a much smaller proportion to the overall mass of the nanowire. (Figure 2.37)

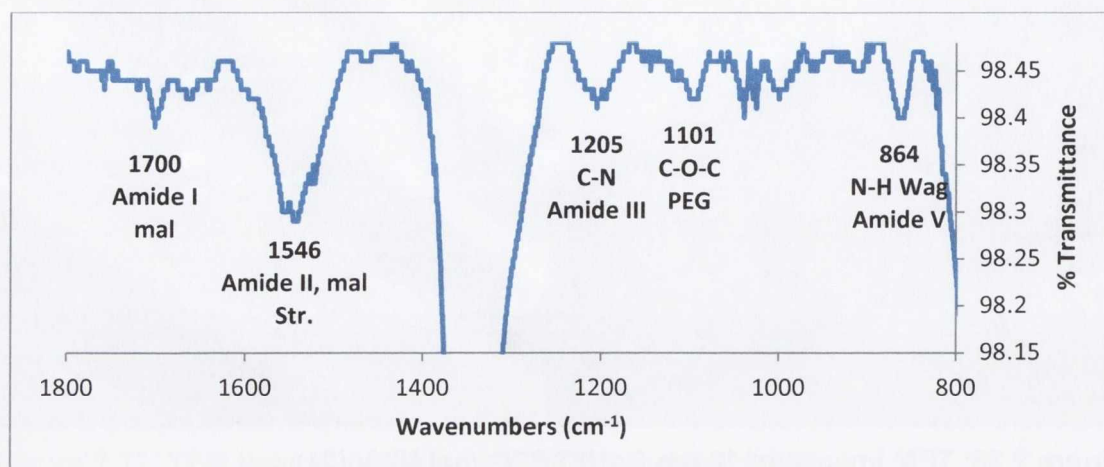


Figure 2.37: FTIR spectrum of 10 μm CoNi@PEG@mal NWs (21)

FTIR analysis on the second CoNi@PEG-mal sample (22) also revealed a presence of alumina, but some PEG was also detectable. (1099 cm^{-1} , C-O-C Str.) (Figure 2.38) In both cases the % transmittance was about 0.5% as expected due to the relative mass contribution of the PEG at the surface. FTIR for other spectral regions for these CoNi@PEG-mal NWs can be found in the Appendix (Appendix 2.4)

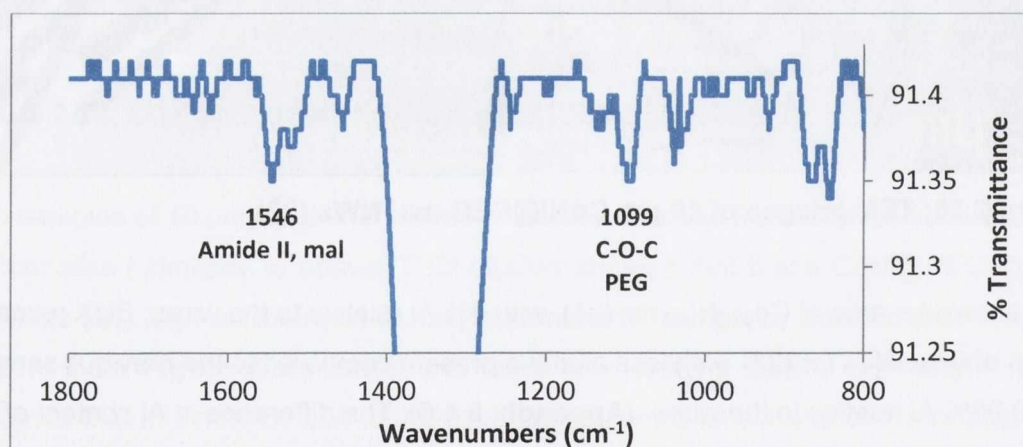


Figure 2.38: FTIR spectrum of 10 μm CoNi@PEG@mal NWs (22)

A series of experiments were performed on these PEGylated NWs, as discussed later, to determine the optimised conditions for protein binding with these wires, to produce CoNi@PEG@G'@IgG-488 (23-26) and subsequent FACS analysis.

2.6 PEGYLATED COBALT-NICKEL/GOLD SEGMENTED BARCODE NANOWIRES

Barcode NWs, consisting of five segments with alternating CoNi alloy and Au spacers were also obtained from Physics, TCD, by a method modified from a published procedure involving Co with Cu spacers.²⁵ These were first treated with 11-mercaptoundecanol, to provide a hydroxylated surface along the full lengths of the wires to produce [CoNi|Au|CoNi|Au|CoNi]@OH (27). The wires were then PEGylated using silane chemistry, to provide maleimide functionality at the surface. TEM of the PEGylated samples (28, 29) are shown below. (Figure 2.39, 2.40)

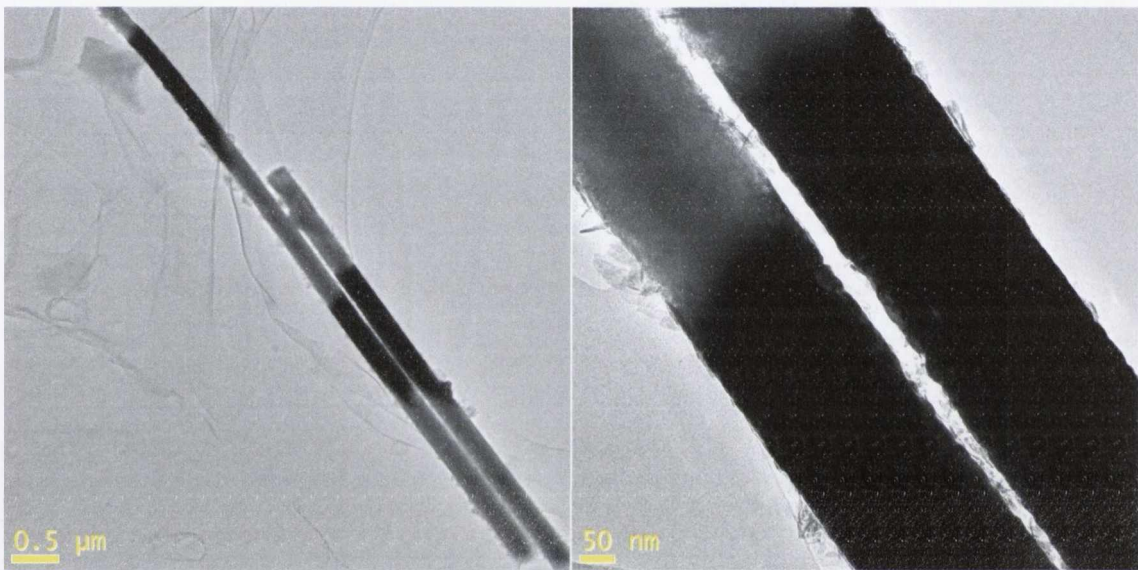


Figure 2.39: TEM images of Co₉₂Ni₈|Au₁₂@PEG-mal NWs (28)

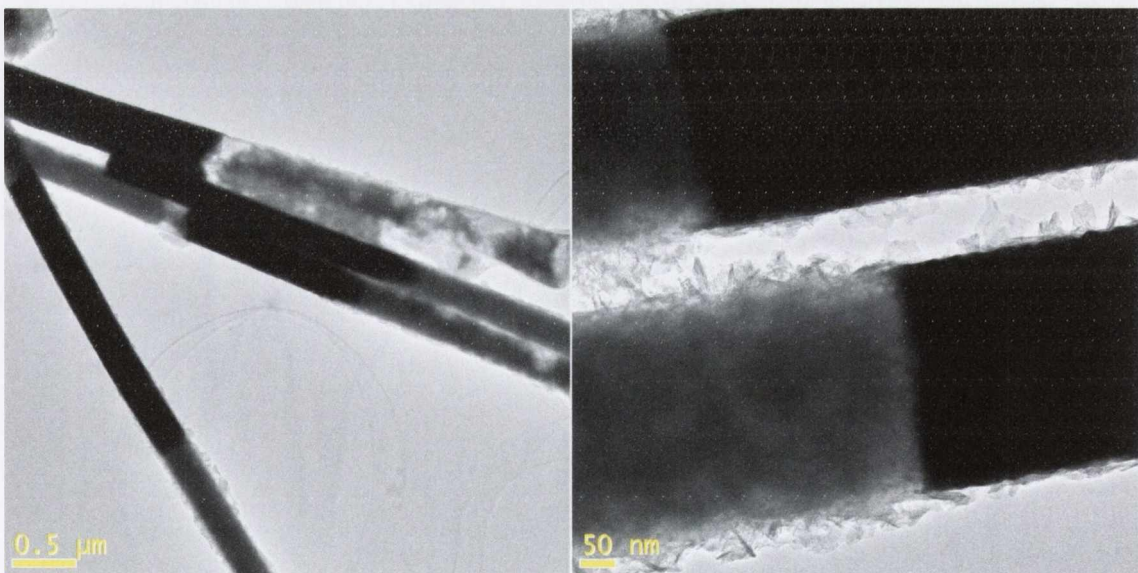


Figure 2.40: TEM images of Co_{90.5}Ni_{9.5}|Au₂₃@PEG-mal NWs (29)

EDX revealed quite similar ratios of Co/Ni/Au for the two samples, with $\text{Co}_{92}\text{Ni}_8|\text{Au}_{12}$ for **(28)** and $\text{Co}_{90.5}\text{Ni}_{9.5}|\text{Au}_{23}$ for **(29)**, indicating good reproducibility in the deposition method. **(Appendix 2.5.2)**

FTIR was performed on the intermediate $[\text{CoNi}|\text{Au}|\text{CoNi}|\text{Au}|\text{CoNi}]@OH$ NWs **(27)** also, which were treated with a heterobifunctional linker containing a thiol and hydroxyl end, to react with the Au portion of the NW surface, and obtain a fully hydroxylated surface for silanisation. **(Figure 2.41)** The bending vibration for O-H groups at the surface was visible in the spectrum.

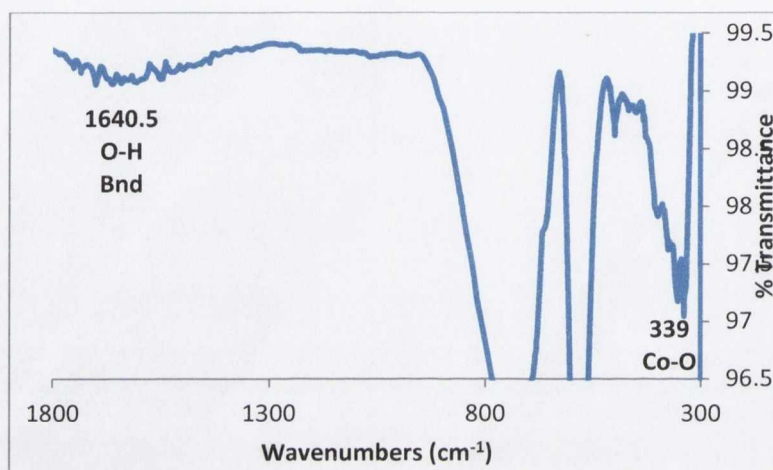


Figure 2.41: FTIR spectrum for $\text{CoNi}|\text{Au}|\text{CoNi}|\text{Au}|\text{CoNi}@OH$ NWs (27)

Subsequent FTIR on the PEGylated sample revealed a new peak, characteristic of C-O-C stretching in the PEG backbone and the disappearance of the peak corresponding to surface hydroxyl groups. **(Figure 2.42, Appendix 2.5.2)**

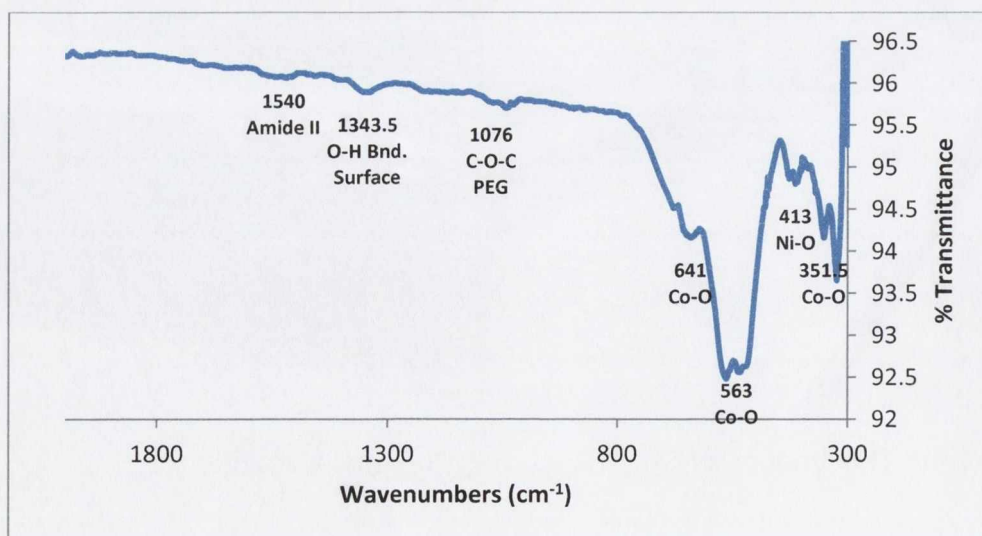


Figure 2.42: FTIR spectrum for $\text{CoNi}|\text{Au}|\text{CoNi}|\text{Au}|\text{CoNi}@PEG\text{-mal}$ NWs (29)

2.7 BIOCONJUGATION AND FACS ANALYSIS

2.7.1 Fe₃O₄ Bioconjugation and FACS analysis

The magnetic nanoparticle bioconjugates were analysed by FACS. Fe₃O₄@COOH (3) was also used as a benchmark reference. (Figure 2.43) The graphs are represented as intensity of detection at 488 nm (FITC channel, X-axis) versus side scattering area (SSC-A, Y-axis). SSC-A is a combination of the scattering width and length of detection time, which correlates to the scattering propensity of the material and size respectively. The regions are separated into quadrants which are manually defined here, based on the occupation of the benchmark versus the bioconjugated materials. These areas would differ for different materials as the intensity of detection of these particles would be related to the distinct properties of other materials, based on their size, shape, viscosity and refractive index, for example.

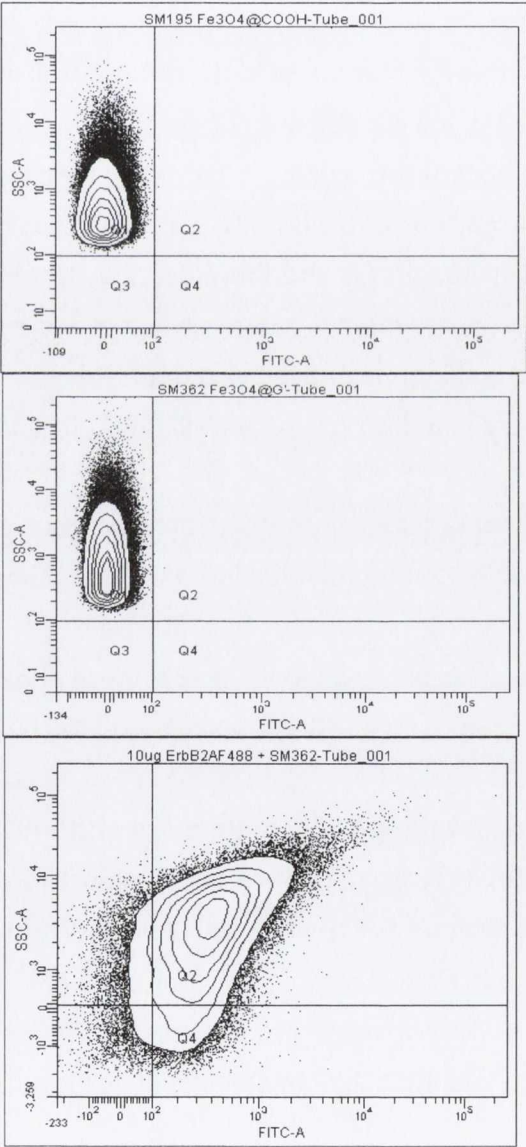


Figure 2.43: FACS analysis comparing Fe₃O₄@COOH (3, top), Fe₃O₄@G' (5, bottom) and Fe₃O₄@G'@IgG-488 (6, right)

As can be seen from the graphs, the $\text{Fe}_3\text{O}_4@\text{COOH}$ and $\text{Fe}_3\text{O}_4@\text{G}'$ particles fall within a lower intensity region for FITC channel detection, due to their lack of fluorescence. It can also be seen that the population is elongated along the SSC-A axis for the $\text{Fe}_3\text{O}_4@\text{G}'$ particles, due to their increase in diameter from the protein conjugation. This increases the scattering length of the particles. This detection is significant as it is the first step towards measuring difference between antigen-bound and unbound particles. As a small nanoparticle, with an average diameter of 7.5 nm, Fe_3O_4 particles represent a model system for studying the detection of increase in particle diameter for scattering events. The difference in size for larger particles should be significantly lower.

$\text{Fe}_3\text{O}_4@\text{G}'@\text{IgG-A488}$ (**6**) showed a significant population in a higher intensity region for 488 nm wavelength detection (80.9 %), indicating the successful binding of the fluorescent immunoglobulin binding protein. It was later realised, upon examining the experimental conditions, that for 10 mg Fe_3O_4 , 2 mg Protein G' would be required, based on theoretical calculations of the surface area of the sample and surface coverage of the protein, excluding its hydrodynamic radius. In the experiment here, only 1 mg of protein was used, which theoretically represents only ~50 % of maximum surface coverage. Hence, it is possible that protein G' and therefore IgG-488 binding was incomplete. Taking into account the hydrodynamic radius and non-close packing addition to the surface, 80.9 % IgG-488 binding may have been the maximum possible, as the surface cover for the IgG protein is ~46 nm², compared with ~10 nm² for Protein G'.

The population in the higher region of fluorescent detection but lower region of scattering detection (Q4) are presumed to be unbound fluorescent proteins, which have a smaller diameter and lower scattering propensity than the particles themselves, but higher intensity detection at the emission wavelength of 488 nm. In addition this lower scattering region was not occupied before IgG-488 incubation and attachment.

2.7.2 Cobalt-Nickel Alloy Nanowire Bioconjugation and FACS Analysis

The $\text{CoNi}@\text{PEG-mal}$ 10 μm NWs (**21**) were conjugated to Protein G', then IgG-488 fluorescent antibodies to perform FACS analysis. (**23-26**) (**Figure 2.44**) In this case, the FACS detection results are plotted with fluorescent detection intensity versus particle count. Here, this was used to optimise the process of Protein G' binding by varying concentration and time to find the optimum conditions, followed by IgG-488 incubation.

The concentration was varied between 10 μg and 100 μg per 0.1 mg NWs, and the time was varied between 2 and 24 h. It was found that for 0.1 mg NWs, 100 μg Protein G' over 2 h gave the optimum results **(25)** with only a 1% increase in binding over 24 h. Hence it was concluded that increasing time was an unnecessary addition.

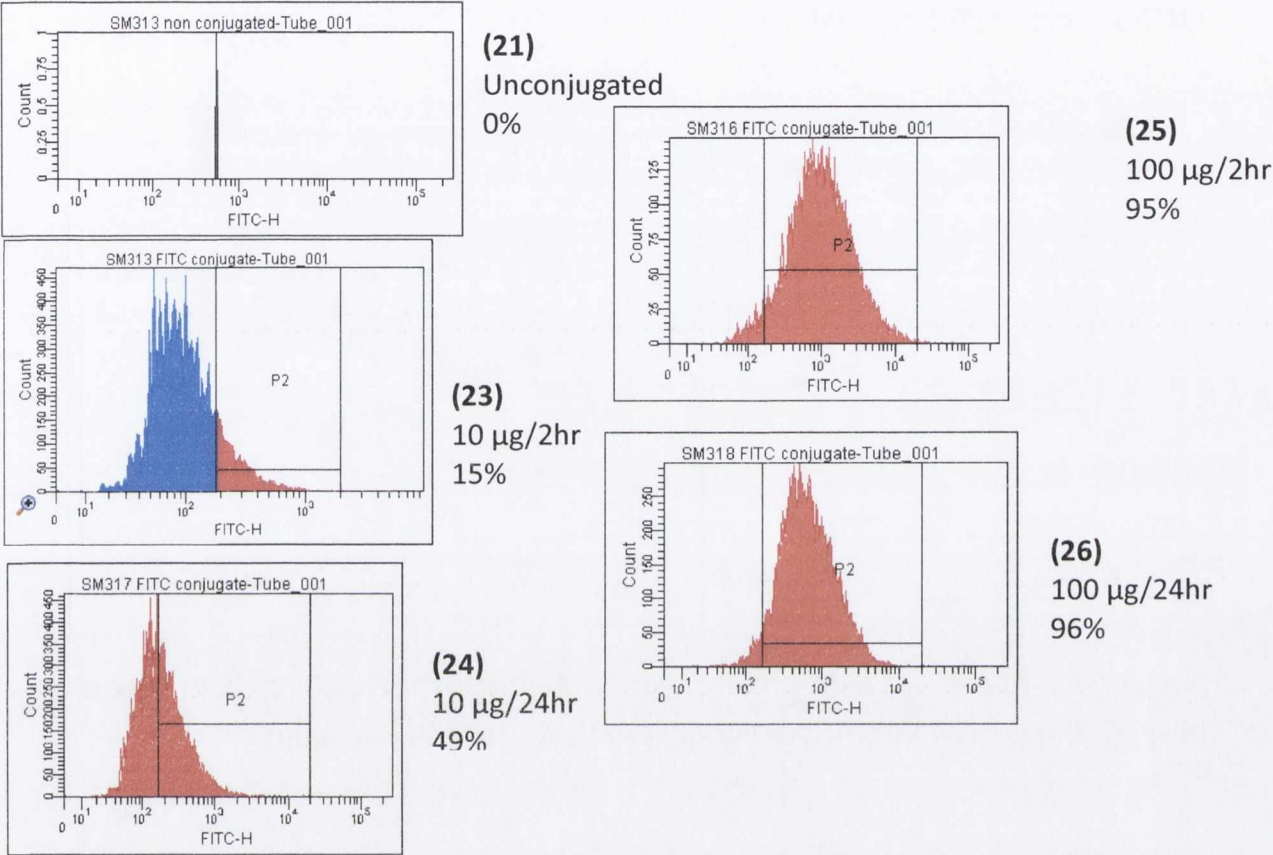


Figure 2.44: FACS analysis of CoNi@PEG@mal NWs (19) versus CoNi@PEG@G'@IgG-488 NW (23-26)

Assuming a cylinder of radius 100 nm (diameter 200 nm) and length of 10 μm for the sample, the maximum surface coverage for the sample was calculated to be $\sim 1.5 \mu\text{g}$ for 0.1 mg of NWs. Here the optimum conditions were found at 100 μg of protein over 2 h. This is much larger than should be required. This is therefore attributed to an uneven surface from alumina and oxidation, and also decreased density in the wires from degradation.

2.7.3 CoNi/Au Segmented Barcode Nanowire Bioconjugation and FACS Analysis

CoNi|Au|CoNi|Au|CoNi@PEG-mal NWs **(28)** were also used for bioconjugation to Protein G' and incubation with IgG-488. **(30)** Similar experimental procedures were used to the optimum procedures found for CoNi NWs. FTIR additionally confirmed the

presence of the protein on the surface. **(Appendix 2.5.3)** In FACS analysis, for the graphs of SSC-A versus FITC-A, as shown below, the non-fluorescent Protein G' coupled nanowires occupy a longer scattering area than seen for the small NPs before. This is as expected due to the relative increase in size and length of the wires to particles. The wires also fell below a similar intensity region in fluorescent detection as seen in the FITC-A channel. **(Figure 2.45)**

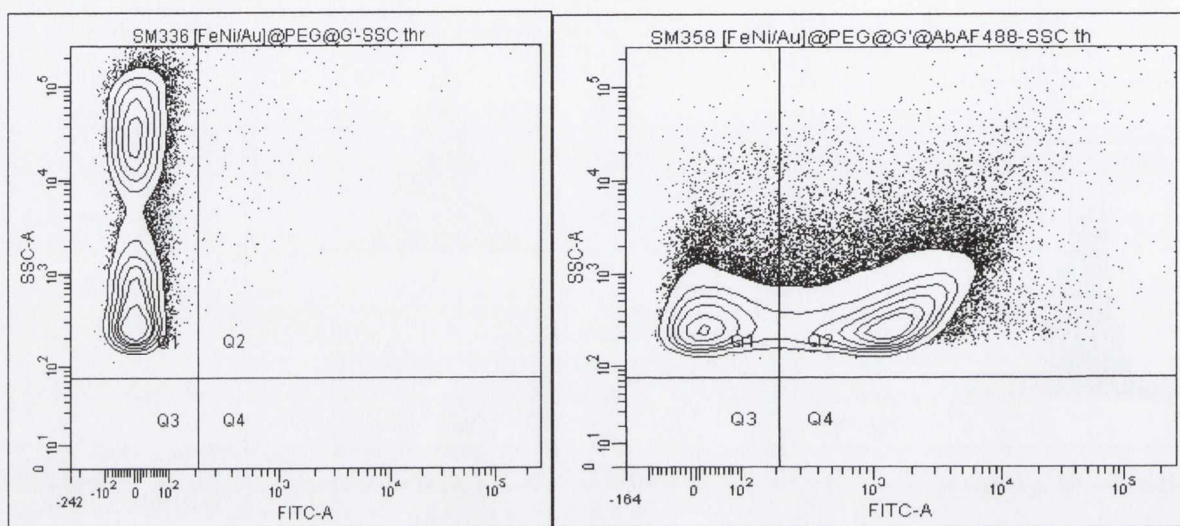


Figure 2.45: FACS analysis of CoNi|Au|CoNi|Au|CoNi@PEG-mal (28, left) versus CoNi|Au|CoNi|Au|CoNi@PEG@G'@IgG-488 Barcode NWs (30, right)

For this experiment, FACS analysis showed ~65% binding for the fluorescent IgG-488 **(30)**, as determined by increase in detection intensity in the 488 nm region. The lower extent of binding is most likely due to incomplete coupling at the surface. There several factors which could account for this decrease in binding, including the increased surface area due to lower integrity and breakage of the wires, or incomplete functionalization and therefore attachment at the Au portion of the surface. However, this result is still a significant achievement.

Future experiments will involve optimisation of protein binding using similar method to those performed for CoNi@PEG@G'@IgG-488 **(23-26)** experiments described above, in the hope to achieve maximum protein binding, and also differentiation between nanowires bound and unbound to non-fluorescent proteins, through differences in scattering alone.

2.8 CONCLUSIONS

Thus, in this part of the work both nanoparticles and nanowires were synthesised from aqueous media, using co-precipitation and sol gel template assisted syntheses. These, along with template-assisted electrochemically deposited nanowires, provided by Physics, TCD, were then functionalised and bioconjugated for the purpose of biomarker detection by FACS analysis.

The optimised procedures developed on Fe_3O_4 proved very useful as the particles are stable towards a wide variety of conditions, surface binding of the silane is strong, and since the particles are small, surface functional groups present a higher mass contribution than larger particles, thus making them more straightforward to characterise and signals attributed to organic surface functionality were easier to detect.

The surface functionalisation methods developed here can be applied to any number of nanomaterials for the purpose of bioconjugation and bioapplications. Surface silanisation is a robust reaction, with a trialkoxy silane hydrolysing under mild condition to attach to a hydroxylated surface. In this work, the method was optimised from previously reported procedures to provide milder reaction conditions, without the aid of an acid or base catalyst, using only residual water in polar solvents as the catalyst for silane hydrolysis. Here it has been applied to various surfaces, using a variety of silane linkers.

For conjugation strategies, amines have predominantly been used here, due to their flexibility and effectiveness in these types of reactions. Either amide coupling with a carboxylic acid, or amine nucleophilic addition to a maleimide can be applied to amino surface groups, to provide new surface functionality. Use of these reactions can avoid many unwanted side reactions when dealing with sensitive and complex biological moieties. These methods are also mild and can be applied to many other nanomaterial systems, possessing surface amino groups. Carboxylated surfaces have also proved useful here, to act as a highly aqueous stable benchmark for comparison against bioconjugated materials.

For primary and secondary surface functionalization, EDX and FTIR proved to be quite useful techniques for characterising these samples. EDX provided quantitative information about the elemental composition of the sample, and FTIR provided qualitative information about the nature of the groups on the surface of the particles. The ability to calculate the molarity of functional groups per mass of sample was also described and applied and is also very useful. It can be used then to control reaction

conditions and molar equivalents of reagents for later conjugation reactions, so that maximum and complete coupling to the surface can be achieved.

Once procedures were optimised on Fe_3O_4 NPs, the methods were applied to nanowire systems. One interesting achievement in this work was the development of a modified procedure for the production of ferrite-doped silica wires with magnetisation values higher than previously reported. This is due to the inclusion of CoFe_2O_4 in place of Fe_3O_4 . These ferrite-doped silica wires were then functionalised, and also metal nanowires were silica coated or PEGylated. Fe_3O_4 MNPs, $\text{CoFe}_2\text{O}_4@\text{SiO}_2$ NWs, CoNi NWs and segmented CoNi|Au|CoNi|Au|CoNi NWs were also bound to fluorescent proteins.

Aqueous stable benchmarks and bioanalogues of selected products were prepared, and they were then tested using flow cytometry detection techniques. Successful binding of the fluorescent markers to the nanomaterial surfaces was observed by fluorescent detection in FACS. The population of conjugated versus unconjugated material could also be ascertained by monitoring the detection intensity at visible wavelengths. It was also shown that FACS technology could detect a difference in small nanoparticles either bound or unbound to non-fluorescent proteins. This represents a significant achievement towards the detection of small biomolecules in solution by targeted capture of biomarkers. This methodology could later be applied to clinical samples of serum and urine for non-invasive diagnostic techniques.

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3.1 INTRODUCTION

The main aim of this part of our work was to prepare and functionalise nickel sub-micron spheres for the purpose of cancer and disease diagnostics, using flow cytometry techniques. These structures can also be used for other biomedical applications such as biomolecule magnetic separation.¹⁻⁴ A schematic of the performed reactions is shown in **Figure 3.1**.

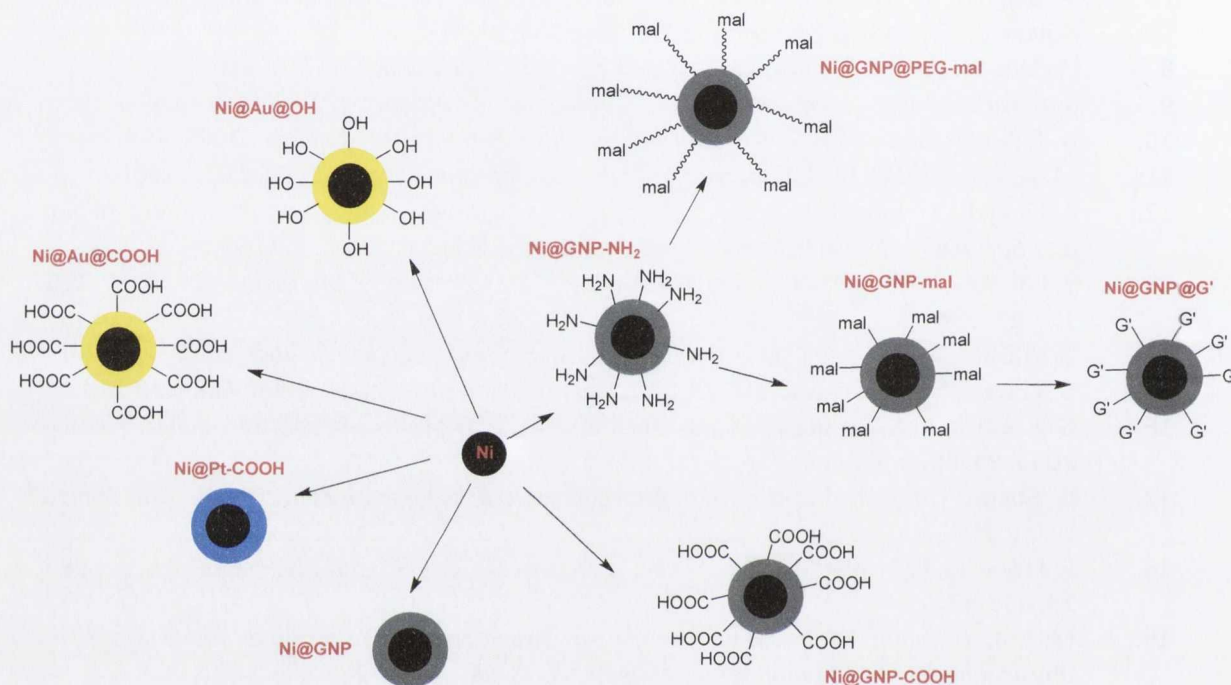


Figure 3.1 Schematic of coating and functionalisation reactions performed with nickel sub-micron particles

The problems it is hoped to address with our technology are (i) reduced size (ii) increased surface area (iii) improved optical detection limits, (iv) solvent stability, (v) uniformity, (vi) enhanced magnetic properties, and (vii) ease of handling. Nickel has enhanced optical density relative to the compositions of Dynabeads®, MagSense beads, Miltenyi beads and other magnetic polymer-based beads traditionally used for flow cytometry and magnetic separation.⁵⁻⁷ For example, Dynabeads, predominantly consisting of polystyrene, have a density of 1.05 g cm^{-3} compared to 8.91 g cm^{-3} for nickel.⁸ It is anticipated that this will reduce the minimum size required for sensing due to enhanced scattering. Hence they would have greater sensitivity in detection, due to increased surface area and also solvent stability. Increased surface area also leads to improved biomolecular capture in separation techniques.

Nickel is a paramagnetic material in bulk form, hence should have low or no coercivity in the absence of a magnetic field. It therefore behaves similarly to superparamagnetic materials, which are ideal for solution phase, flow, and biomedical applications. This soft magnetic behaviour further increases solvent stability, particularly relative to permanently magnetic materials, such as Turbobeeds[®].³ They are also expected to have higher saturation magnetisation than Dynabeads[®], leading to improved ease of handling, reducing reaction times, and is also increasing yields. The saturation magnetisation range for Dynabeads is 10 – 20 Am²kg⁻¹.^{9, 10}

Nickel particles were coated with gold by a galvanic replacement reaction, for enhanced surface stability. In addition, this work provides a new method of graphene coating which is also pre-functionalised with groups for bioconjugation. Graphene coatings by other methods can require harsh reaction conditions, like combustion,^{11, 12} and extra steps to install such functional groups at the surface.¹³ Furthermore, other methods, such as those for Turbobeard[®] production, may provide a mixture of graphene and amorphous carbon shells, which are fused together and not easily separable.³

3.2 SYNTHESIS AND CHARACTERISATION OF CORE NICKEL PARTICLES

Nickel core particles were produced by an organic phase synthesis. At present in our laboratory these particles can be synthesised in gram scale per day. These initial Ni particles were synthesised by organic phase thermal decomposition and reduction of nickel acetate salts (Ni(OAc)₂·4H₂O). The salts were present in high concentration in solution, as particle size is a function of concentration, with lower concentrations producing smaller particles, and also smaller yields. **(Figure 3.2)** In terms of solution phase particle syntheses, these reactions produce relatively high yields, with an average yield of about 1-3 g. This lends to the possibility of upscaling, commercialisation and industrial scale manufacture for potential biomedical applications.

In these reactions, oleylamine acts as both the solvent and surface stabilising agent. The reactions were generally performed at a temperature of 250°C. At this temperature, hydrocarbons begin to decompose, forming a mixture of more complex hydrocarbons, including amorphous carbon.^{12, 14} In initial experiments, an oxidised surface on the nickel particles was produced by introduction on aqueous miscible polar solvents during work-up.

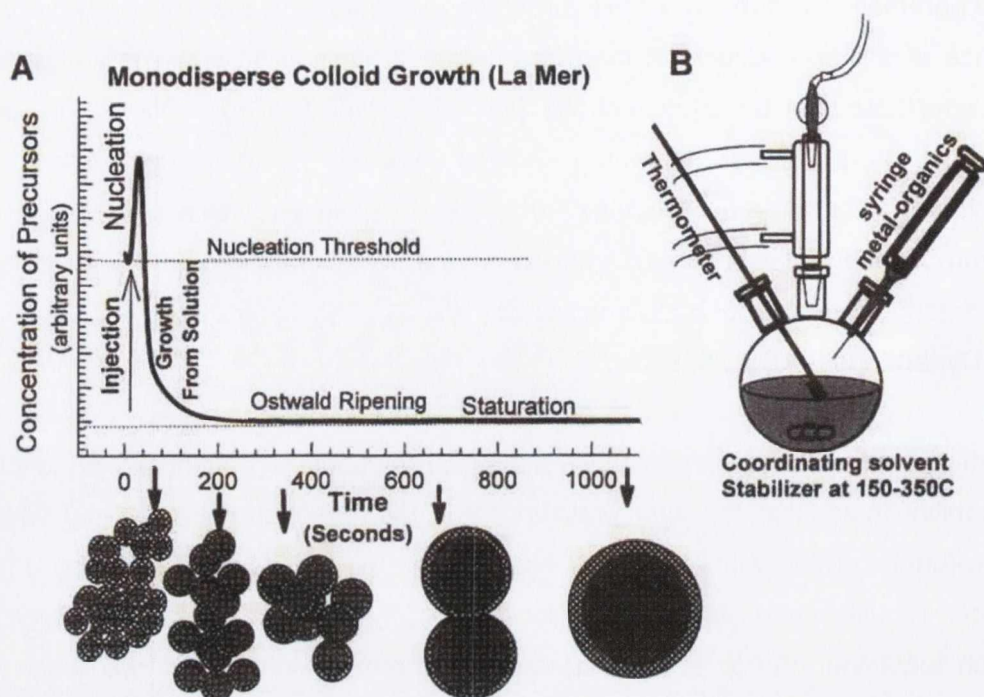


Figure 3.2: Process of crystal nucleation and growth by hot injection method (La Mer Diagram)¹⁵

These core nickel particles were synthesised and the composition and size of particles formed under different conditions were analysed and optimised. The reactions were performed with a variety of concentrations, with the temperature kept constant at 250°C. The temperature was kept to this minimum to avoid formation of amorphous carbon and to control the growth of the particles. This is also the decomposition temperature for the precursor, nickel acetate.

The concentration was varied to find the optimum concentration for large yields in the reaction, without compromising the uniformity of the particles. In these reactions it was found that lower concentrations led to smaller particles, with higher uniformity. However, these particles also increased in magnetic coercivity, as they approached single domain crystallinity. In addition it was found that the larger particles, which approached optical range, were useful for biological sensing applications, as discussed later. **Figures 3.3-3.6** display TEM images of the Ni particles formed under different conditions.

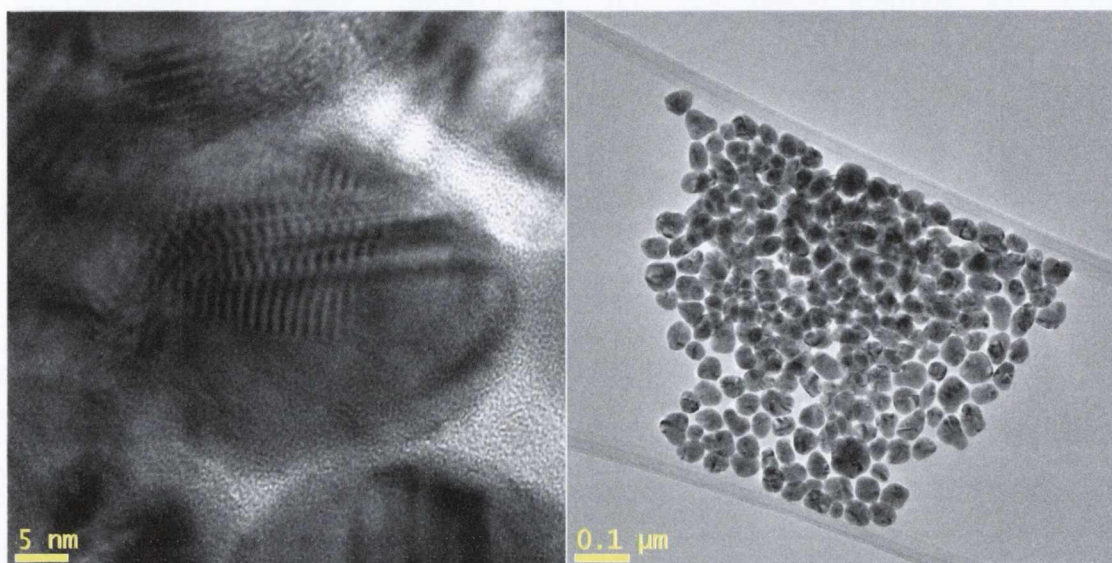
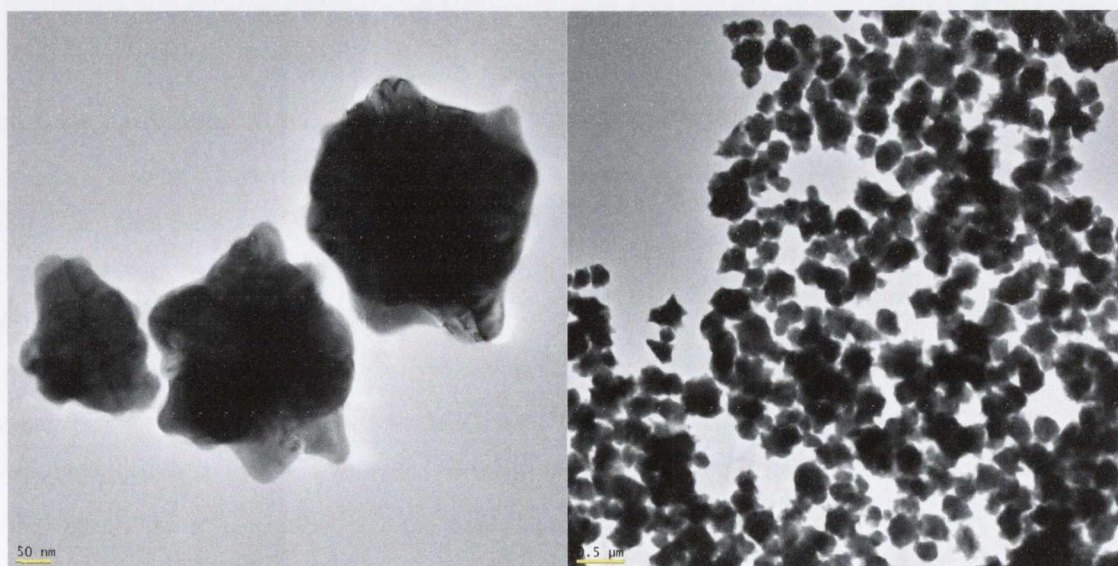


Figure 3.3: TEM images of Ni particles prepared using 20 mM Ni, 100 min, Size 44 ± 7.9 nm (31)

From TEM data it can be seen that an increase in size for the particles prepared at higher concentrations and for longer times, compared with the 20 mM sample (31). However, there is not an absolute trend of increasing size with concentration and time, due to other contributing factors. There is a small reduction in size from 3 h to 24 h for the highest concentration reactions (400 mM). There are a number of reasons this may have occurred; perhaps through Ostwald ripening, as previously described in the La Mer Diagram, which would even and smooth the surfaces, creating particles of a smaller average size. It may also be simply, normal statistical and experimental variation between experiments, with a maximum width of 160-180 nm for particles formed under these conditions.



Scale Bar: 50 nm

Scale Bar: 500 nm

Figure 3.4: TEM images of Ni particles prepared using 200 mM Ni, 3 h, size 254 ± 78.8 nm (32)

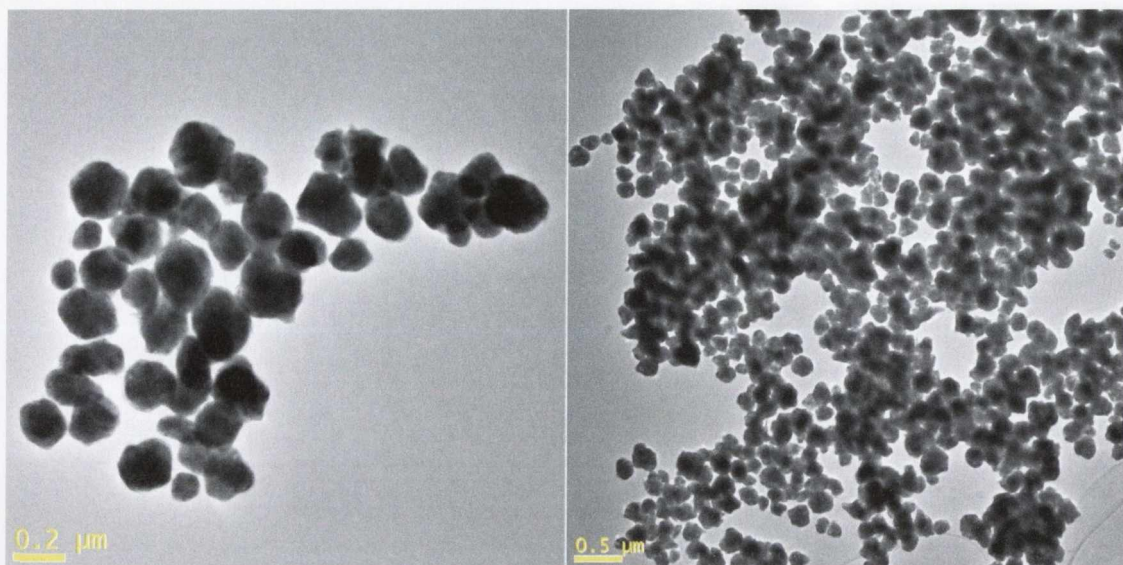


Figure 3.5: TEM images of Ni particles prepared using 400 mM Ni, 3 h, size 180 ± 44.2 nm (33)

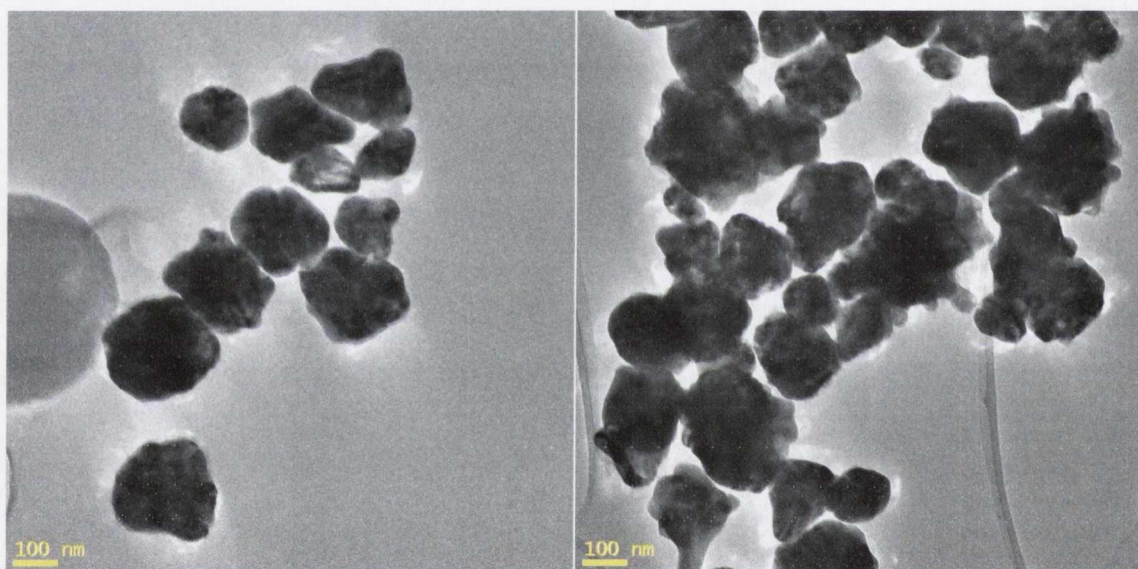
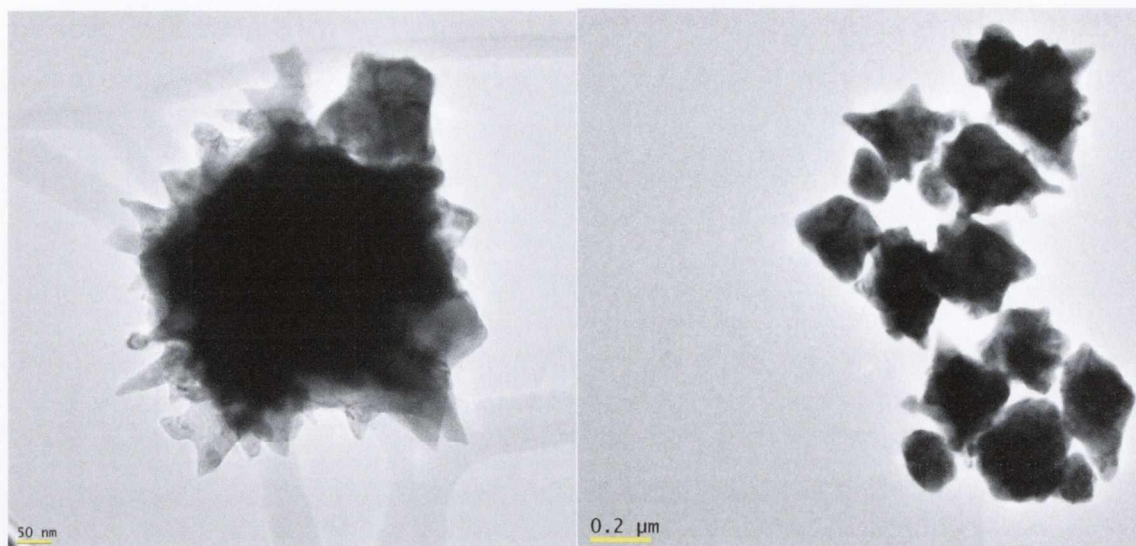


Figure 3.6: TEM images of Ni particles prepared using 400 mM, 24 h, size 163 ± 43.9 nm (34)

Interestingly, sample **(32)** (200 mM, 3 h) produced a larger particle size than 400 mM experiments, despite the smaller concentration. This particular sample was subject to extensive washings in an attempt to strip the surface of ligands and oxidise it. From TEM it can be seen that the particle morphology is much more irregular with pod-like arms extending from the core. **(Figure 3.7)** This is most likely due to etching and oxide formation. In addition, particles sizes were measured manually, using Image J software, which leads to an extra factor of variability and error in the measurements. Sizes were taken as the average diameter, with the extended arms taken as part of the overall diameter.



Scale Bar: 50 nm

Scale Bar: 200 nm

Figure 3.7: TEM images of Ni particles after extensive washing in aqueous miscible polar solvents, with uneven, etched surface (32)

FTIR analysis supported the observations made by TEM. For **(31)**, the characteristic peaks for OAm ligand coordination were observed, with high intensity for aliphatic carbon peaks at 2926 and 2853.5 cm^{-1} . (**Figure 3.8**)

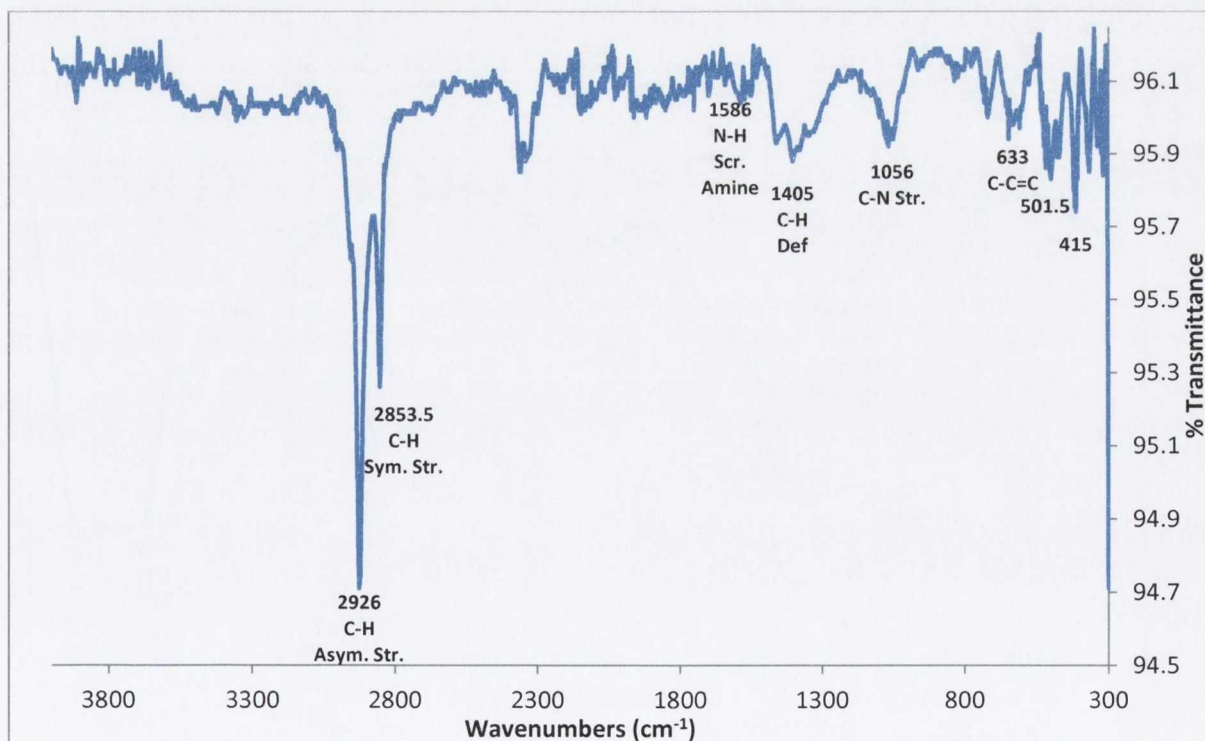


Figure 3.8: FTIR spectrum of Ni particles prepared using 20 mM Ni, 100 min (31)

For the sample which underwent extensive washing steps (**32**), FTIR also revealed a presence of nickel oxide, (**Figure 3.9 and Appendix 3.1.4**) with Ni bonded to a mix of nitrogens from OAm and oxygen from oxidation. This supports the theory of ligand stripping and oxidation that was indicated by the morphology studies by TEM.

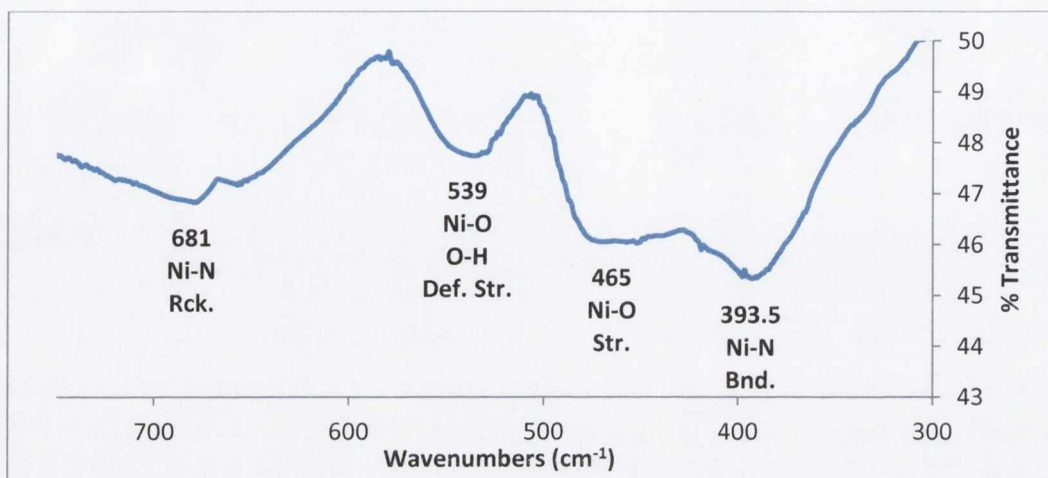


Figure 3.9: FTIR spectrum of Ni particles prepared using 200 mM Ni, 3 h (**32**)

In comparison, (**33**) sample showed the characteristic peaks of aliphatic ligands, due to OAm coordinated to the surface as a stabiliser, with peaks at 384 (Ni-N Str.), 443 (C-H Rck.), 617 (-C=C- Wag.), 668.5 (Ni-N Rck.), 1053 (C-N Str.), 1341 (C-H Sym. Def.), 1454.5 (C-H Asym. Def.), 1626 (-C=C- Asym. Def.), 2965 (C-H Str.) cm^{-1} . (**Figure 3.10**)

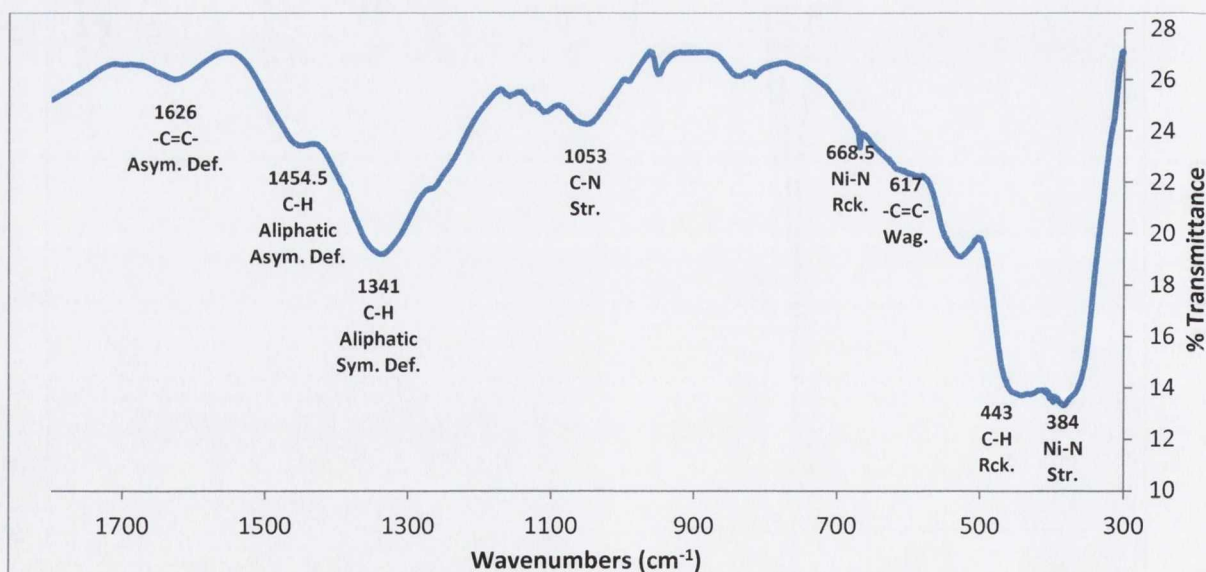


Figure 3.10: FTIR spectrum of Ni particles prepared using, 400 mM Ni, 3 h (**33**)

The sample prepared over 24 h, **(34)** showed quite a different FTIR spectrum with characteristic peaks of carboxylated graphitic carbon (GO) at the surface. **(Figure 3.11)** Aliphatic carbons breakdown around the reaction temperature (250°C). Also nickel may catalyse the formation of grapheme oxide formed from the surfactant and the acetate salt from the nickel salt precursor. Peaks corresponding to GO were seen at 1149.5 (C-O Str.), 1217.5 (C-OH Str.), 1585.5 (C=C Str.), 1737 (C=O Str.) and 3658 (O-H Ar. Str.) cm^{-1} . Additional spectral regions for the various Ni samples are included in the Appendix. **(Appendix 3.1.4)**

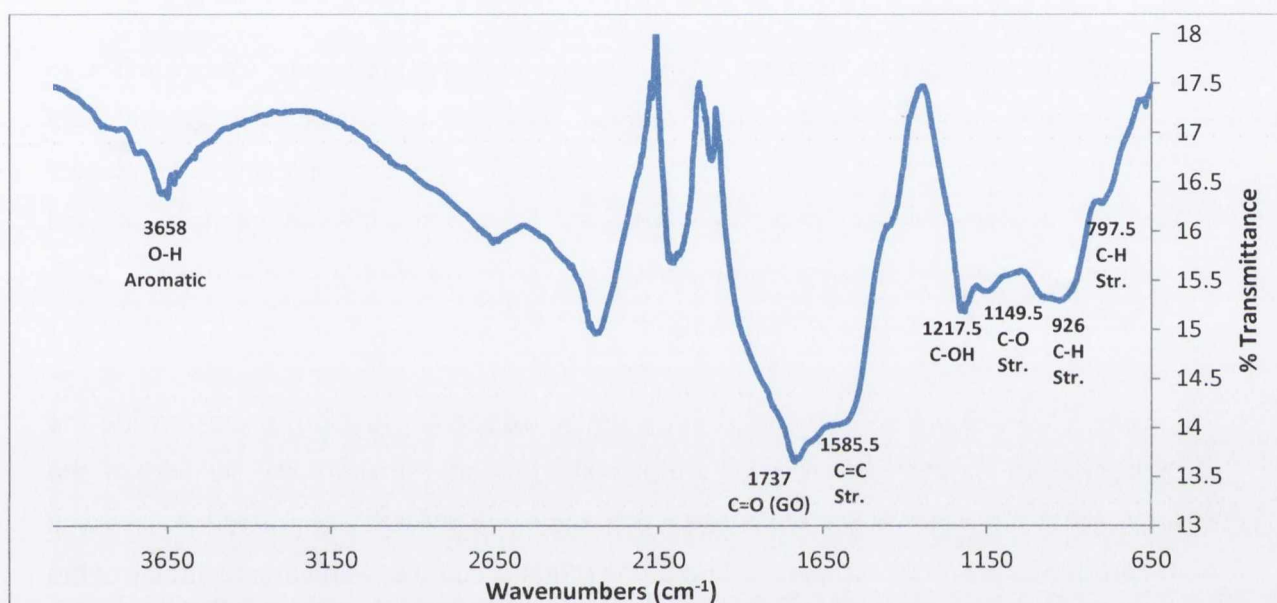


Figure 3.11: FTIR spectrum of Ni particles prepared using 400 mM Ni, 24 h (34)

VSM analysis was performed also, and showed that for larger particles the magnetic behaviour was more similar to bulk, having lower coercivity. All samples had high peak magnetisation, between 40-54 $\text{Am}^2\text{kg}^{-1}$. **(Appendix 3.1.2)** This type of magnetic behaviour is very useful for biodetection and separation applications as it is high, increasing efficiency in particle recovery and ease of handling, due to soft magnetic behaviour. Therefore, magnetism can be induced at the desired time, without magnetic clumping during experimental procedures.

Nickel is also a plasmonic material; hence it absorbs certain wavelengths in the UV-vis region. The particular absorption profile for a nickel material depends on its specific size and shape. Due to the large size of these particles, a broad absorbance across the visible region of the spectrum can be seen, in each of the samples. This absorbance is also due to light scattering in addition to absorbance. **(Figure 3.12, Appendix 3.1.5)**

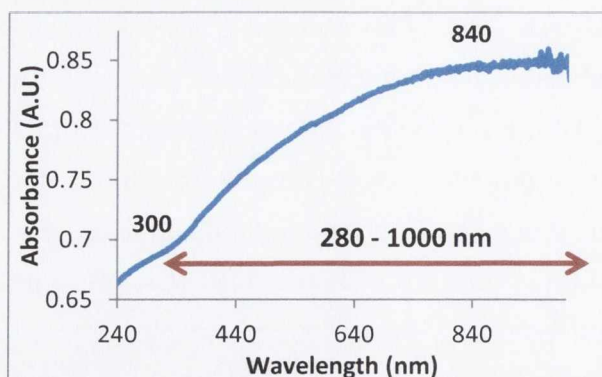


Figure 3.12: UV-vis absorption spectrum of Ni particles in CBS pH 6.5 (32)

Overall, no increase in reaction efficiency or magnetic properties was found with increasing reaction time. Also, the reaction could be performed at relatively high concentration to increase yields from the reactions. (1-3 g) Increasing time did, however lead to a greater amount of amorphous and graphitic carbon at the surface, as mentioned above. Hence, future reactions were performed under the shorter timeframes (2 – 3 h), to avoid unnecessary side reactions at the surface.

Initially it was expected that ligand stripping by extensive washing would provide the hydroxylated surface required for surface silanisation reactions as outlined in the previous chapter. However this proved problematic and difficult to characterise. This was in part due to the more complex nature of the metallic surface, with varying affinity of the different ligands to the nickel by coordination chemistry, but also due to the complex mix of ligands at the surface arising from the surfactant, the acetate salt, and breakdown products from the decomposition/reduction process. In addition, their larger size relative to Fe_3O_4 MNPs, provided a much lower surface to volume ratio. Hence the overall mass contribution of the surface was lower and detectability by FTIR proved limited. Under these conditions, it is difficult to unequivocally assign the peaks at the surface by FTIR.

Therefore, other surface coating reactions were developed to replace the oxidation process, in order to produce a coating which was uniform and could be more easily characterised. The main two outlined here are gold coating and graphene nanoplatelet coating. Both of these coating were performed *in situ* to avoid any side reactions or complications due to new chemicals being introduced into the system. Gold coating takes advantage of Au-S chemistry, which forms a strong bond, similar to covalent binding strength. Unlike Ni single bonds which are considerably weaker. Graphene coating takes advantage of the multidentate ligand approach, where the sheets wrap around the particles, coordinating through many pi bonds with the aromatic rings of the structure.

These particles were used in later experiments for comparison in FACs analysis, as detail later in this chapter.

3.3 SYNTHESIS AND CHARACTERISATION OF GOLD-COATED NICKEL PARTICLES

Gold coating was achieved by injection of a gold salt, at slightly elevated temperatures ($\sim 40^{\circ}\text{C}$), while sonicating.¹⁶ Due to the difference in redox potential of Ni and Au, the spheres were coated in Au by galvanic replacement.^{17, 18} E_0 for the reaction $\text{AuCl}_4^- + 3\text{e}^- \leftrightarrow \text{Au} + 4\text{Cl}^-$ is 1 V, whereas E_0 for the reaction $\text{Ni}^{2+} + 2\text{e}^- \leftrightarrow \text{Ni}$ is -0.26 V. From TEM it can be seen that this forms a raspberry-like structure, where the coating is a dense cluster of small Au NPs. This slightly uneven coating is partly due to surface tension and lattice mismatching of the Ni and Au crystals, but also due to the speed of formation of Au seeds. It may be possible to optimise this process by tuning the reaction conditions, by either slowing the seeding process, or by increasing the shell thickness. In the case of increasing shell thickness however, magnetisation decreases drastically as shell thickness increases. This is due to the high density and elemental mass of Au relative to Ni, which contributes non-magnetic mass to the overall sample. Thiol exchange was also performed *in situ* for these reactions to avoid unnecessary side reactions.¹⁹

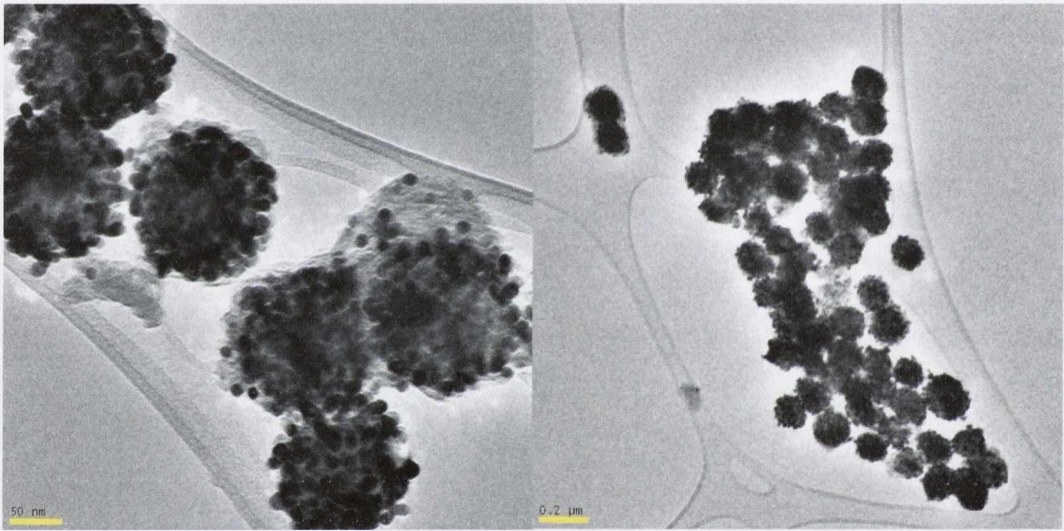
Here, a heterobifunctional linear alkane, 1-mercapto-11-undecanol (MUO) or aromatic bifunctional thiol acid (MBA), was used to install hydroxyl groups at the surface. Once a hydroxy-functionalised surface was obtained, the particles could be further functionalised by silane chemistry (work not shown here). In addition, gold forms very weak bonds with oxygen, so there was less interference from side reactions, which are common with Ni, Co, and Fe chemistry, which form similar bond strengths with a variety of ligands. Ni spheres were also coated with Pt by a similar process of galvanic exchange.^{17, 18, 20}

Also Co and CoPt, Co@Pt and Ni@Pt sub-micron particles were prepared, results of which are included in the appendix (**Appendix 3.5**), however these other samples require further optimisation and characterisation, hence are not discussed further here. In the gold-coating reactions two different precursors were used to prepare the core nickel particles. These particles were then coated with gold *in situ*. Below is a table summarising the reaction conditions and results for the Au coated nickel particles. (**Table 3.13**)

Table 3.13: Summary of reaction conditions and results for gold-coated nickel particles

Sample	Conc. Ni/Au (mM)	Time	Temp (°C)	Size (nm)	Ni/Au start	Ni/Au finish	Mag (Am ² kg ⁻¹) (Peak/Res.)	Coercivity (mT)
35 NiCl ₂	50/12.5	5 min/ 12 h	300/100	132.7 ±12.6	4/1	45/55	6.3/0.97	7.5
36 NiCl ₂	50/16.67	20 min/2 h	300/100	127.4 ±14.7	3/1	73/27	1.29/0.5	16.75
37 Ni(OAc) ₂	400/12.5	3 h/3 h	250/40	173.0 ±50.7	32/1	96.5/3.5	45.1/9.1	10.5

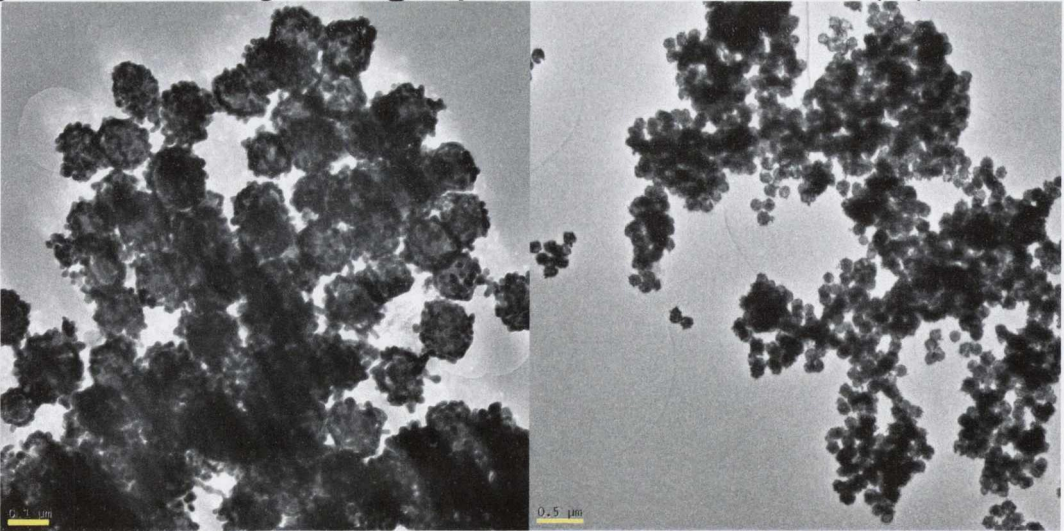
The samples produced by NiCl₂.H₂O were smaller with lower polydispersity. This was due to lower reaction concentrations. From TEM it can also be seen that the gold coating consists of packed clusters of small Au NPs at the surface. (Figures 3.14-3.16).



Scale Bar: 50 nm

Scale Bar: 200 nm

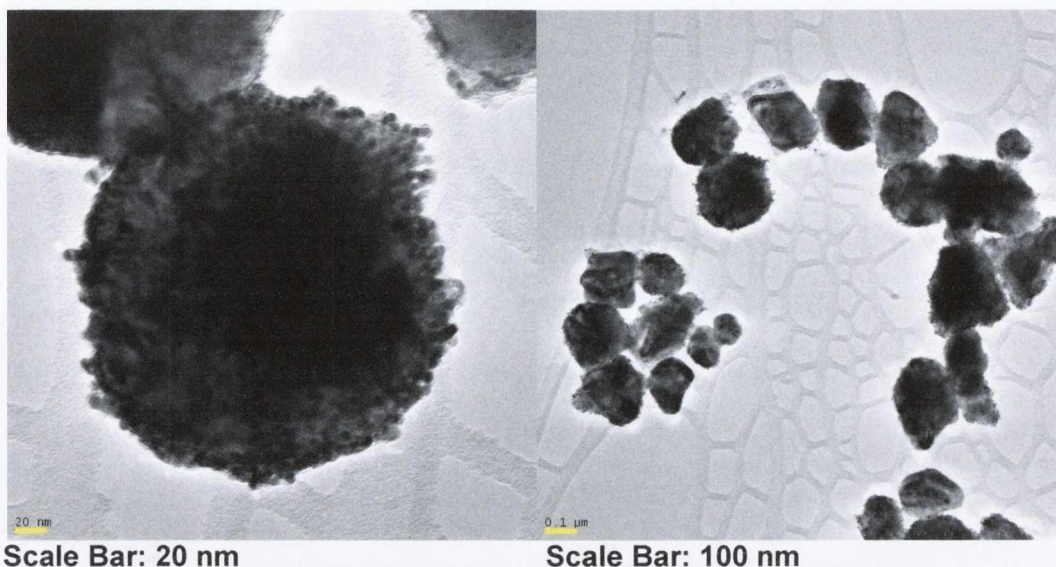
Figure 3.14: TEM images of Ni@Au particles size 132.7 ± 12.6 nm (35)



Scale Bar: 100 nm

Scale Bar: 500 nm

Figure 3.15: TEM images of Ni@Au particles size 127.4 ± 14.7 nm (36)



Scale Bar: 20 nm Scale Bar: 100 nm
Figure 3.16: TEM images of Ni@Au particles size 173 ± 50.7 nm (37)

The ratio of nickel to gold was varied for these reactions, and was not quantitatively retained in the product. EDX showed the ratio of nickel to gold in the product. In **(35)**, the ratio was higher (45/55) than that of **(36)** (73/27), despite having less gold added to the reaction in the latter. This is due to the longer reaction times, as the gold deposits on the surface by galvanic replacement of nickel. **(Appendix 3.4.2)** Hence, it can be assumed that not all gold was reduced in the reaction over 2 h at 40°C.

XRD analysis, revealed a pattern for Ni fcc and Au fcc in **(36)** and **(37)**, with a higher relative intensity for Au in the sample containing a higher gold content. Interestingly, the sample containing 55 % Au, **(35)** exhibited only the pattern for Au fcc and not Ni. **(Appendix 3.2.3)**

The relative content of Au also affected their peak magnetisation, as the amount of gold needed to cover the smaller Ni particle surfaces was greater. The sample prepared using $\text{Ni}(\text{OAc})_2$ produced much larger particles **(37)**, with lower surface area to be covered by gold and this in turn produced considerably higher magnetic behaviour, with peak magnetisation of $45.1 \text{ Am}^2\text{kg}^{-1}$, compared with $6.10 \text{ Am}^2\text{kg}^{-1}$ for **(35)** and $1.29 \text{ Am}^2\text{kg}^{-1}$ for **(36)** prepared using NiCl_2 . **(Figure 3.17)** This sample also had a much higher Ni/Au ratio (96.5/3.5). **(Appendix 3.4.2)**

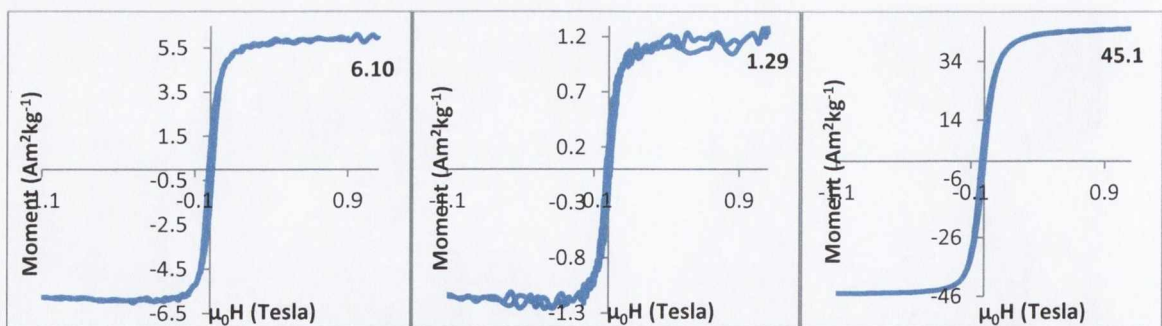


Figure 3.17: VSM curves for Ni@Au samples 35 (left), 36 (middle) and 37 (right)

FTIR for these particles showed quite good resolution in terms of surface characterisation. This is most likely due to the plasmonic enhancement effect of Au at the surface. Sample (35) was functionalised with 11-mercapto-1-undecanol. Producing hydroxyl aliphatic ligands at the surface with the thiol bound to Au. Characteristic peaks for the ligand were seen at 1017 (C-O-H), 1347 (C-H Def), 1443 (C-H Def.), 1583 (N-H Scr.), 2907 (C-H Str.), 2991 (C-H Str.) and 3183 (O-H Str.) cm⁻¹. (**Figure 3.18**)

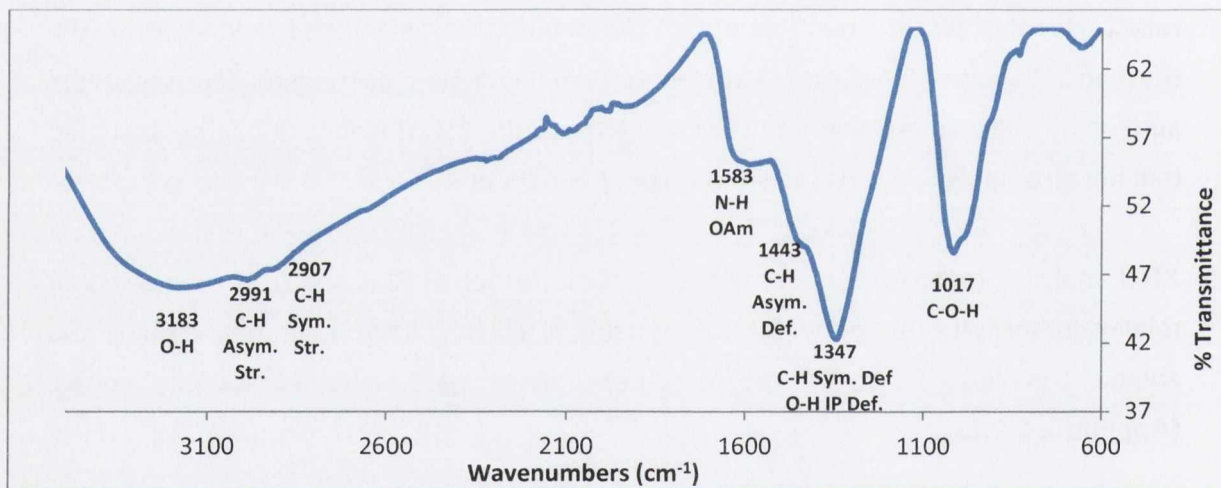


Figure 3.18: FTIR spectrum of Ni@Au@OH particles functionalised with 11-MUO (35)

Larger Ni@Au particles formed using, 400/12 mM, Ni(OAc)₂/Au formed over 3h/3h, 250/40⁰C were also functionalised with 11-MUO and exhibited the characteristic peaks in FTIR. (**Figure 3.19**) These in fact displayed excellent resolution and peak intensity, even when compared with the smaller samples, which should have a higher surface to volume ratio. The contribution of gold at the surface and its particular size, shape and alignment may have added to this peak enhancement.

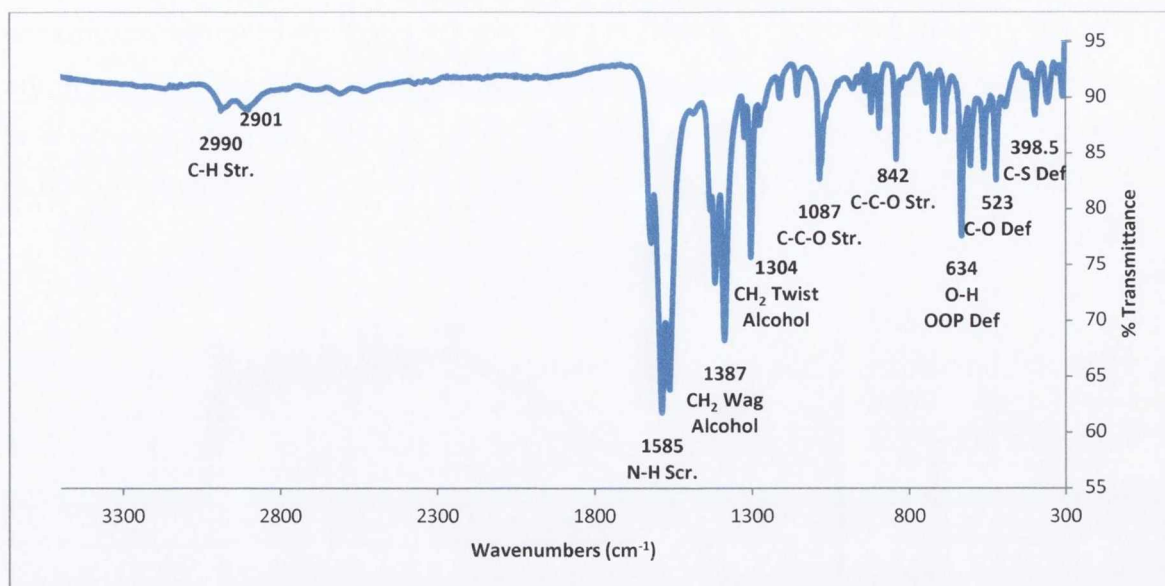


Figure 3.19: FTIR spectrum of Ni@Au@OH particles functionalised with 11-MUO (37)

Ni@Au particles (**36**) were functionalised with mercaptobenzoic acid (MBA) In the FTIR spectrum. resolution for these peaks was slightly lower than for the other two samples. However characteristic peaks for this ligand were identified at 668 (C-S Str.), 987 (OH-H OOP Def.) 1130 (C-O Str.), 1307.5 (C-H Sym. Def.), 1493.5 (C-O Str., O-H Def. overlapping), 1717 (C=O Str.) cm^{-1} . (**Figure 3.20**) Additional spectral regions for these Ni@Au samples is contained within the appendix. (**Appendix 3.2.4**)

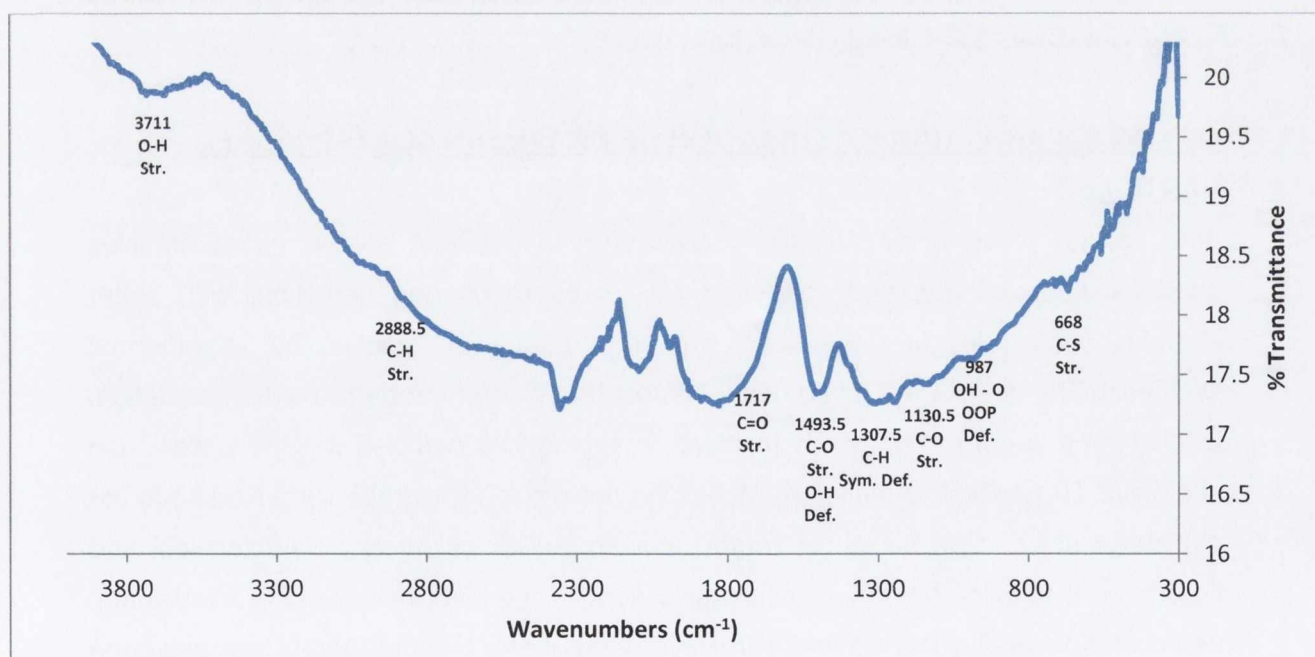


Figure 3.20: FTIR spectrum of Ni@Au@COOH particles functionalised with MBA (36)

UV-vis spectroscopy for these Ni@Au samples revealed broad absorbance across the this region of the spectrum, similar to Ni core particles. They also exhibit scattering, due

to their increased size relative to NPs. In this case, they had an enhanced absorbance in the region of 500-600 nm, (**Figure 3.21, Appendix 3.2.5**) which corresponds to the plasmonic absorption of Au at the surface. As noted by TEM this surface consists of small Au NPs fused together. This maintains their small size, necessary for quantum confinement.

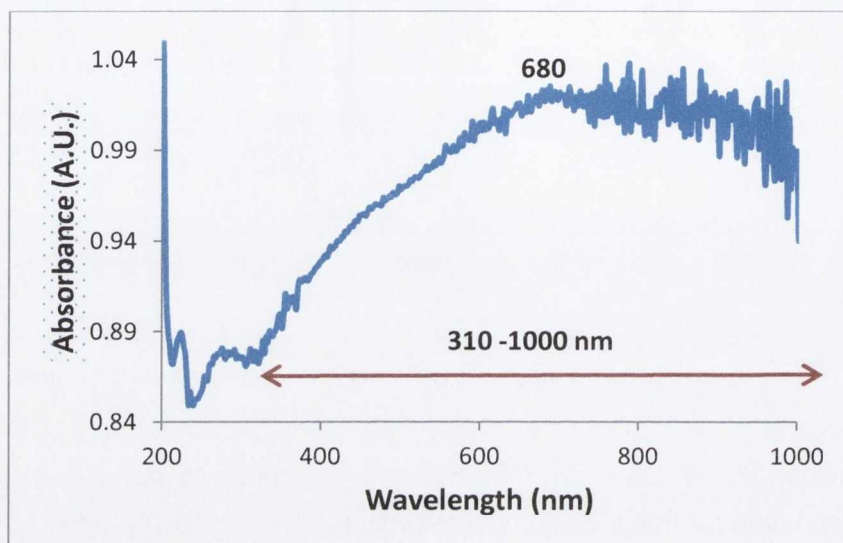


Figure 3.21: Example of the UV-vis absorbance profile and scattering produced across the visible region

These particles were also used in later experiments to compare against Ni core particles in FACS analysis as outlined later in this chapter.

3.4 SYNTHESIS AND CHARACTERISATION OF GRAPHENE COATED NICKEL PARTICLES

This section presents functionalised graphene nanoplatelet coated nickel particles (Ni@GNP) as an improved candidate for bioseparation and detection, with better characteristics than current state of the art, produced in solution by solvothermal synthesis. The development of a novel approach to protect the particles from oxidation, leaching, or degradation, which involves wrapping the particles in GNPs was also achieved. This collective multidentate binding results in an overall strong binding to the particles, which can then be further modified with polymers, biomolecules and fluorophores to tailor them to specific application. These tests included characterisation, and detection studies using FACS. Preliminary testing for bioapplications has also been performed, results of which are included and have been promising.

For GNP-coating, GNPs dispersed in NMP were added to the Ni spheres prior to work up, while sonicating. All reactions were performed at 250°C, without stirring for Ni core

formation, and at 40°C with sonication for GNP coating. Graphene nanoplatelet dispersions were prepared and exfoliated by sonicating a mixture of GNPs in NMP with a concentration of 1 mgmL⁻¹ for 3 days. It was found that the reaction could be tuned to produce individual particles wrapped in GNPs by slow, drop-wise addition of the graphene. The graphene nanoplatelets had an average size of ~3 µm² which worked well with this particular size of particle. Smaller particles would potentially either cover the flat sheets or multiple particles would be encapsulated within them by this approach. The GNPs, obtained by a commercially available source, were also available prefunctionalised with various groups. In this work the particles were therefore coated with pure graphene, and amino and carboxyl functionalised GNPs. **Table 3.22** summarises the various reaction conditions for the production of Ni@GNP, Ni@GNP-NH₂ and Ni@GNP-COOH sub-micron particles and the subsequent results of characterisation.

Table 3.22: Summary of Ni@GNP reaction conditions and results

Sample	Ni Rxn Time	Ni conc. (mM) Ni/GNP (w/w)	Size (nm)	Mag. (Am ² kg ⁻¹) (Peak/Res.)	Coercivity (mT)
38 Ni@GNP	1.5 h	320 400/1	149.98 ± 68.60	53.56/5.97	8.5
39 Ni@GNP@NH ₂	2 h	100 100/1	178.04 ± 21.75	48.75/5.03	10.5
40 Ni@GNP@NH ₂	3 h	400 800/1	193.28 ± 39.92	49.17/7.58	12.5
41 (rapid) Ni@GNP@NH ₂	3 h	400 400/1	184.56 ± 46.48	44.71/3.54	11
42 Ni@GNP@NH ₂	3 h	400 400/1	102.81 ± 18.90	44.50/5.81	9
43 Ni@GNP@NH ₂	3 h	400 200/1	153.19 ± 46.16	43.90/6.9	9.5
44 (rapid) Ni@GNP@COOH	3 h	400 400/1	122.08 ± 33.18	40.81/4.09	8
45 Ni@GNP@COOH	1.5 h	320 400/1	156.04 ± 47.82	54.41/6.41	13.5

Ni@GNP-NH₂ is useful for bioconjugation reactions, with the amino group capable of participating in either amide coupling or nucleophilic addition reactions, similar to those discussed in Chapter 2 involving magnetite and other nanomaterials. These groups were then available for later conjugation reactions, such as binding to PEG. Amino-

functionalised particles are typically an intermediate in biological studies, as these groups do not provide an optimum solvent stability, unless protonated in acidic pH. Hence, Ni@GNP-COOH is also a useful benchmark for studies due to the high aqueous stability of particles possessing this surface functionality, similar to experiments with Fe₃O₄.

In two initial reactions **41** (Ni@GNP-NH₂), **44** (Ni@GNP-COOH), it was found that rapid addition of the GNP dispersion lead to encapsulation of multiple particles within the graphene sheets. Below are images showing the carbon sheet draped across the surface of multiple particles from these experiments. **(Figure 3.23)** All subsequent reactions involved the drop-wise addition of the 1 mgmL⁻¹ suspension over 20-30 min in order to encapsulate single particles within separate graphene sheets. It is also worth noting that the morphology of the GNPs is smoother for the amino functionalised GNPs, compared with the carboxyl functionalised. This is attributed to H-bonding interactions in the graphene oxide sample.

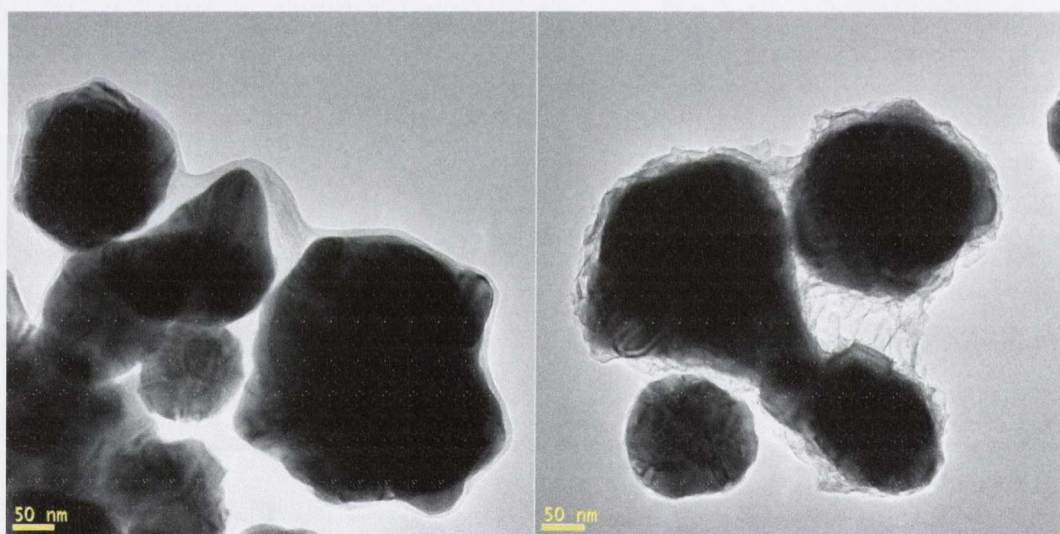


Figure 3.23: TEM of GNPs wrapped around several particles due to the rapid addition of GNPs to the Ni particles, with GNP-NH₂ (41, left) and GNP-COOH (44, right)

Another experiment was performed using unfunctionalised GNPs (**38**) for a shorter timeframe. The purpose here was for it to be used as a benchmark against the functionalised GNP-coated particles in characterisation. In retrospect, this sample produced the sample with most polydispersity in size. **(Figure 3.24)** However as can be seen from TEM, these particles are much smoother and rounder than uncoated particles produced previously. The graphene coating is also visible around the particles, which

provides protection from degradation and etching. The polydispersity in size for this sample could be due to the high concentration coupled with short reaction conditions, leading to less maturity and uniformity of the sample by Ostwald ripening and growth over time.

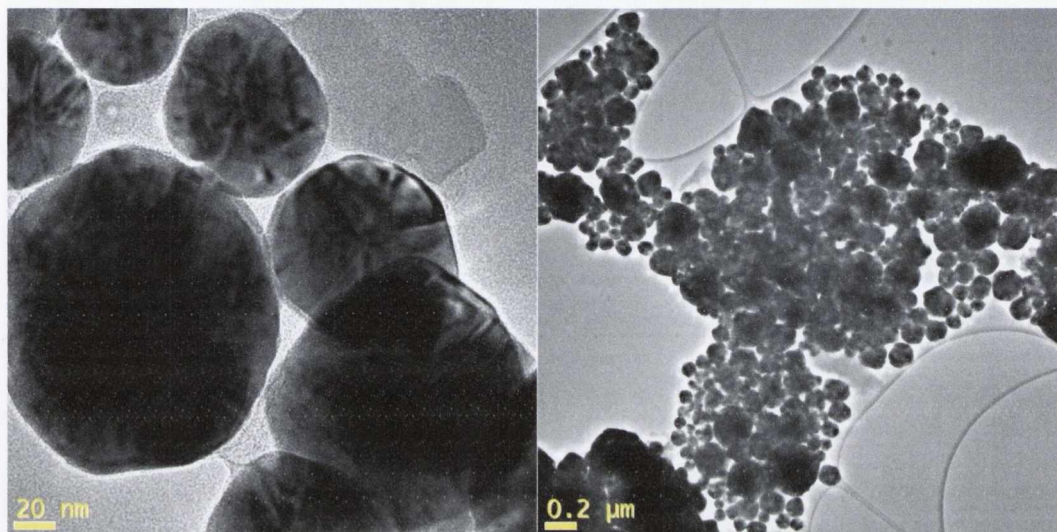


Figure 3.24: TEM images of Ni@GNP, prepared with 320 mM Ni(OAc)₂, 1.5h, 400/1 (w/w) Ni/GNP overnight, size 149.98 ± 68.60 nm (38)

All samples prepared, regardless of time, concentration were in the region of 100-200 nm. Below are TEM images of each of the samples prepared using pre-functionalised GNPs. (**Figures 3.25-3.31**) In the samples with very thin, smooth and uniform graphene coating, the shell is barely visible by TEM, as expected.

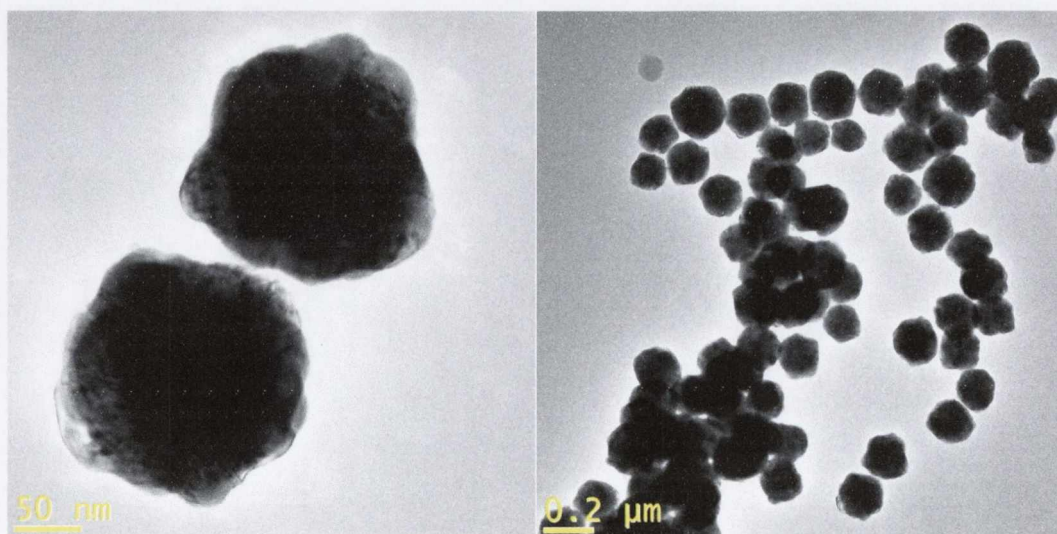


Figure 3.25: TEM images of Ni@GNP-NH₂ particles, size 178.04 ± 21.75 nm (39)

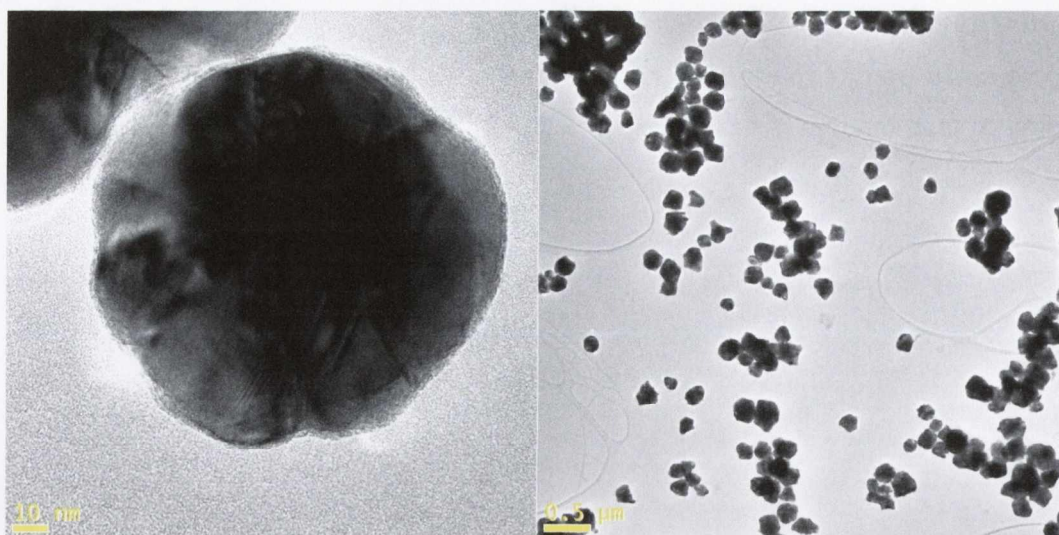


Figure 3.26: TEM images of Ni@GNP-NH₂ particles, size 193.28 ± 39.92 nm (40)

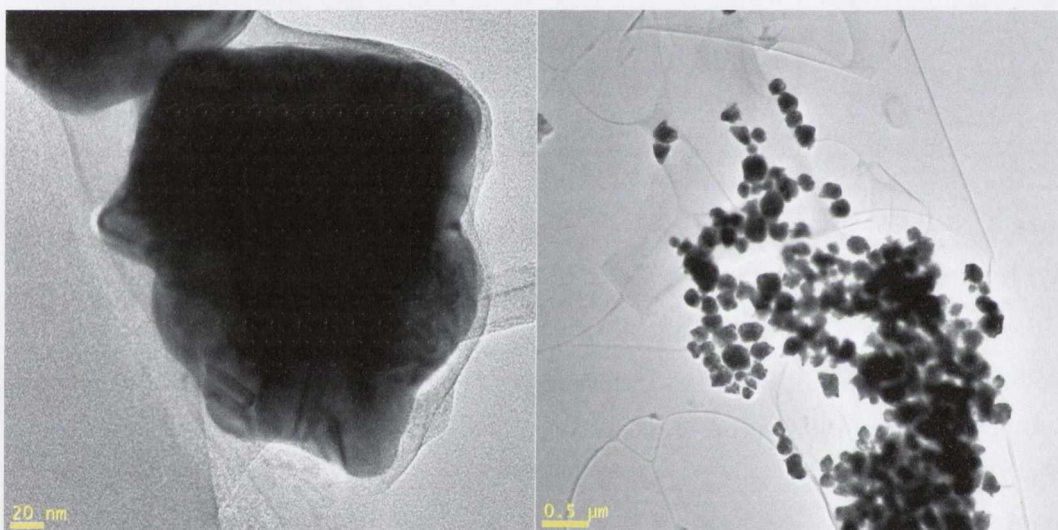


Figure 3.27: TEM images of Ni@GNP-NH₂ particles, size 184.56 ± 46.48 nm (41)

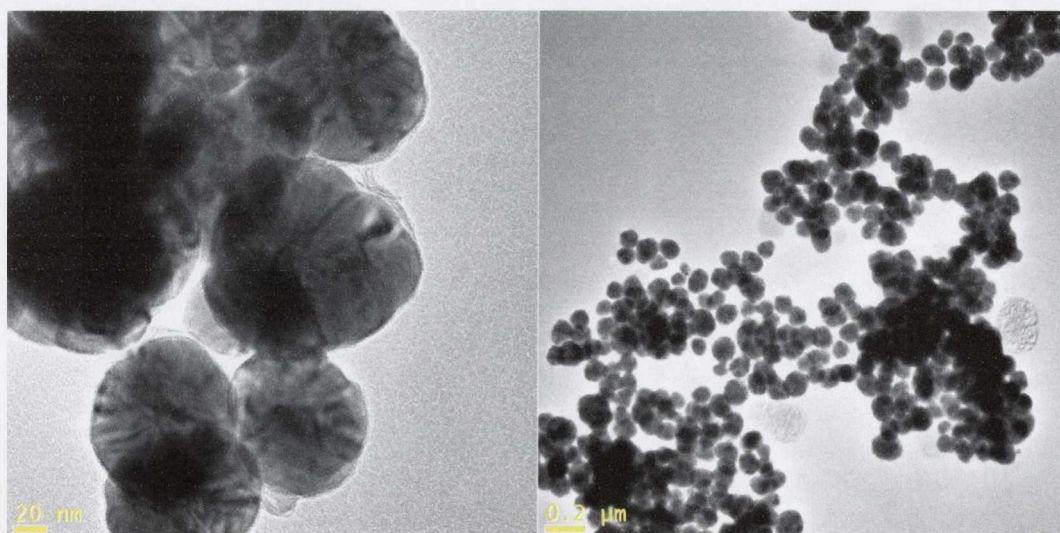


Figure 3.28: TEM images of Ni@GNP-NH₂ particles, size 102.81 ± 18.90 nm (42)

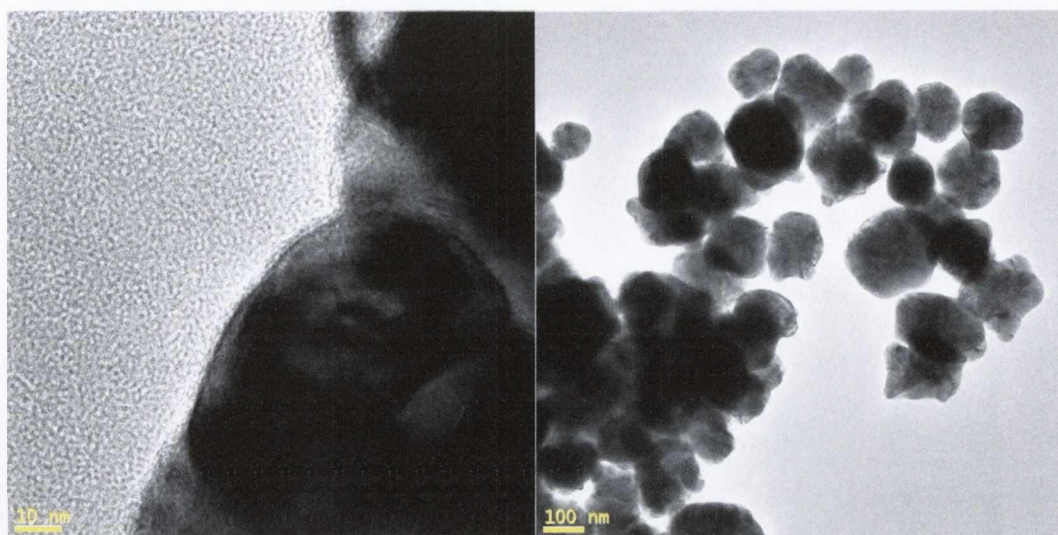


Figure 3.29: TEM images of Ni@GNP-NH₂ particles, size 153.19 ± 46.16 nm (43)

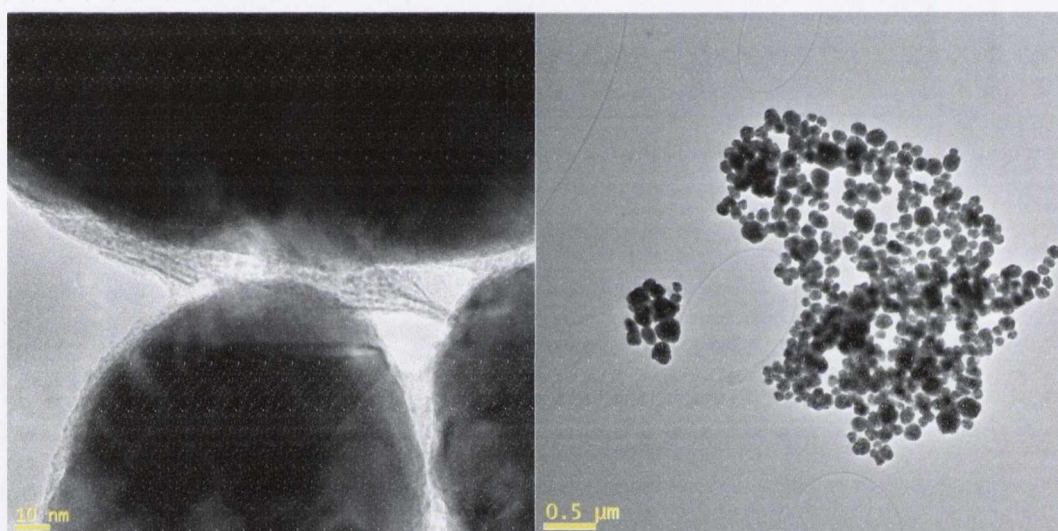


Figure 3.30: TEM images of Ni@GNP@COOH particles, size 122.08 ± 33.18 nm (44)

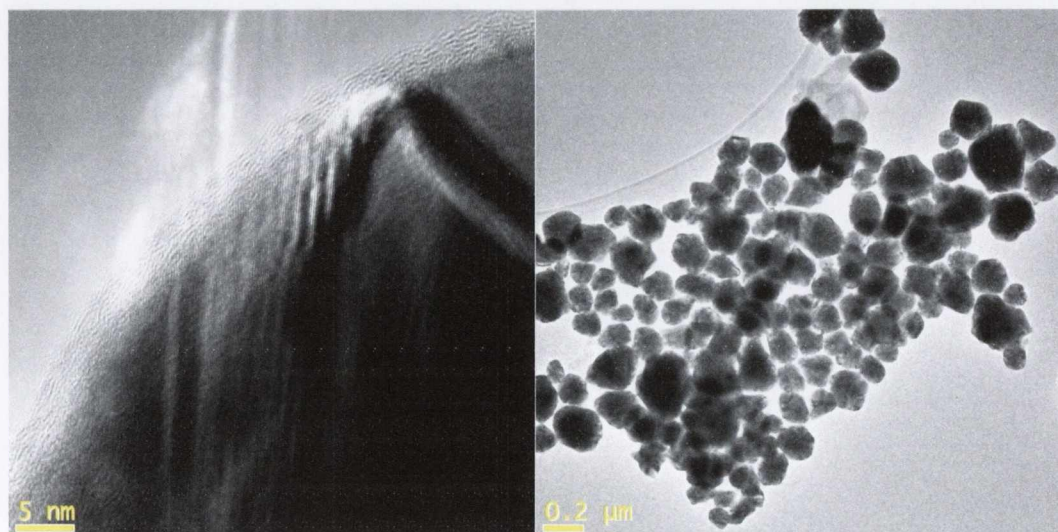


Figure 3.31: TEM images of Ni@GNP-COOH particles, size 156.04 ± 47.82 nm (45)

All samples showed the characteristic pattern for the Ni fcc crystal structure by XRD, and no peaks for graphite were present, indicating the carbon was present as a thin graphene layer. Below is a sample pattern from (38). (Figure 3.32) All XRD patterns are shown in the appendix for this series of products. (Appendix 3.3.2)

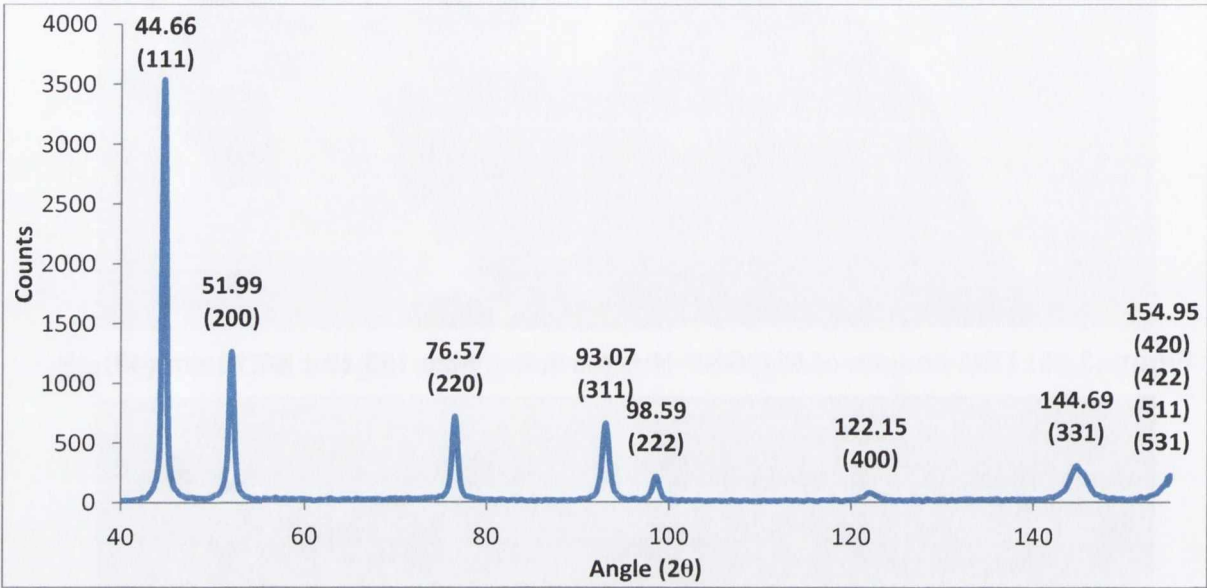


Figure 3.32: XRD pattern for Ni@GNP showing Ni fcc crystal structure (38)

FTIR confirmed the presence of the graphene at the surface through the organic binding sites attached across the sheet, in the first case consisting of amine bonds, bound to the sheet. (Figure 3.33) For GNP-COOH the graphene shell could be identified from the newly installed C-O bondings and their characteristic peaks. FTIR spectra for all samples prepared are contained within the appendix. (Figure 3.34, Appendix 3.3.3)

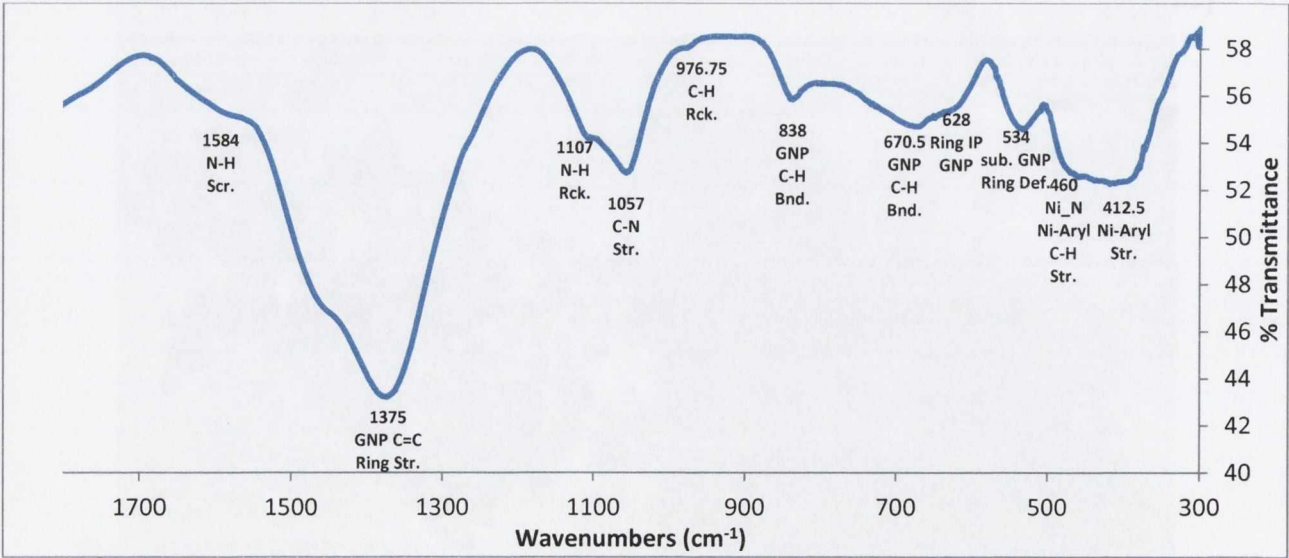


Figure 3.33: FTIR spectrum showing amino-graphene bonding on Ni particles (42)

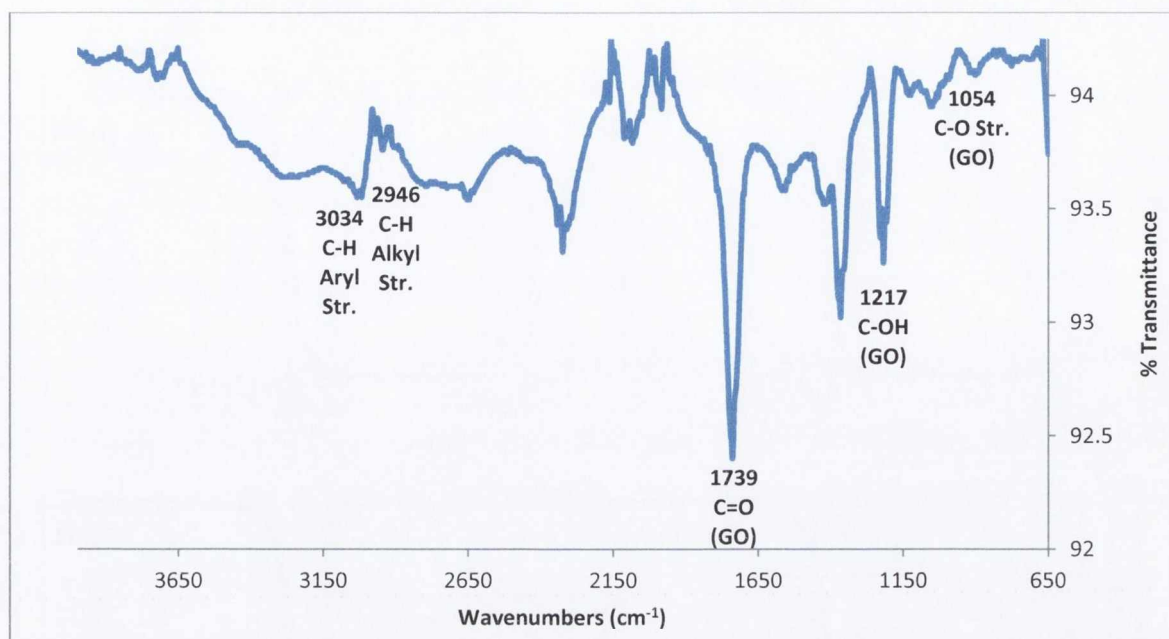


Figure 3.34: FTIR spectrum showing carboxy-graphene bonding on Ni particles (44)

VSM for all samples showed excellent, soft magnetic behaviour with peak magnetisations ranging from 40.81-54.41 Am²kg⁻¹ (**Figure 3.35**)

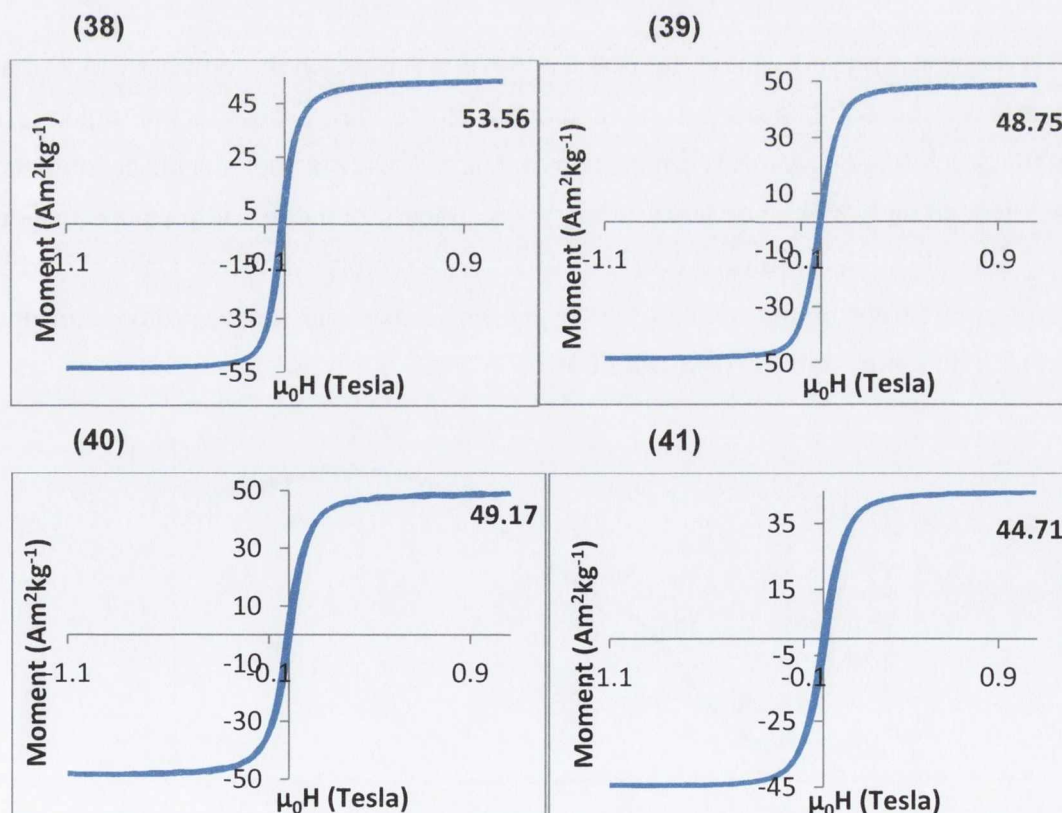


Figure 3.35: VSM curves for Ni@GNP-X samples

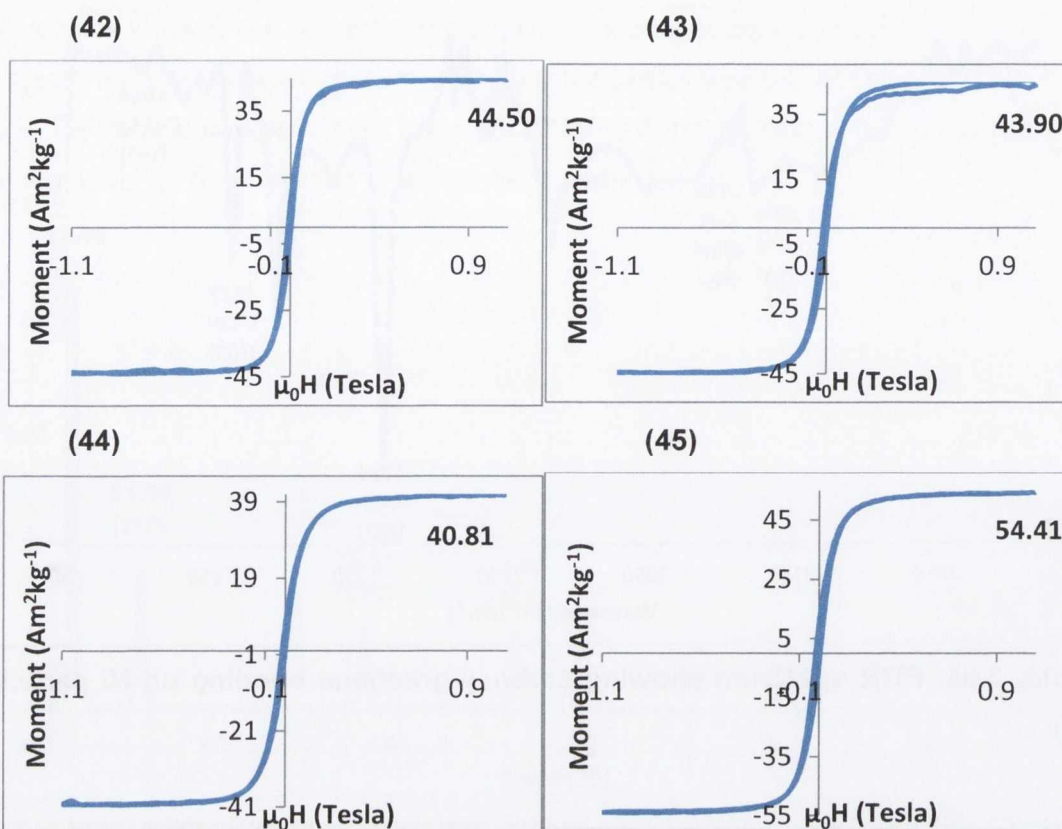


Figure 3.35: VSM curves for Ni@GNP-X samples (continued)

UV-vis analysis showed similar spectra for these samples when compared to Ni bare particles, with broad absorbance and scattering across the visible spectral region. This scattering, due to their metallic composition and high density is useful in optical detection methods such as FACS bioanalysis. Absorption spectra for these samples are included in the appendix. **(Appendix 3.3.4)** Below is an example of these UV-vis studies, performed in citrate buffer solution (CBS) pH 6.5 to promote amino protonation, thus enhancing aqueous stability. **(Figure 3.36)**

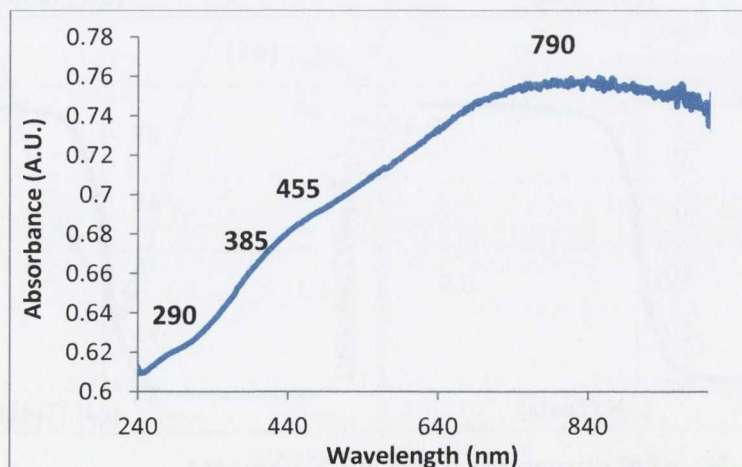


Figure 3.36: UV-vis absorption for Ni@GNP-NH₂ particles in CBS pH 6.5 (39)

3.5 BIOCONJUGATION AND BIOLOGICAL APPLICATIONS

4.5.1 Ni Core Particle FACS Analysis

FACS analysis of the Ni sub-micron particles (32) showed promising results, with the particles detectable with good intensity and low polydispersity for the bare particles. (Figure 3.37) Intensity and dispersion are indicated by the narrow width and sharpness of the peak in the scattering detection channel on the X-axis versus particle count on the Y-axis.

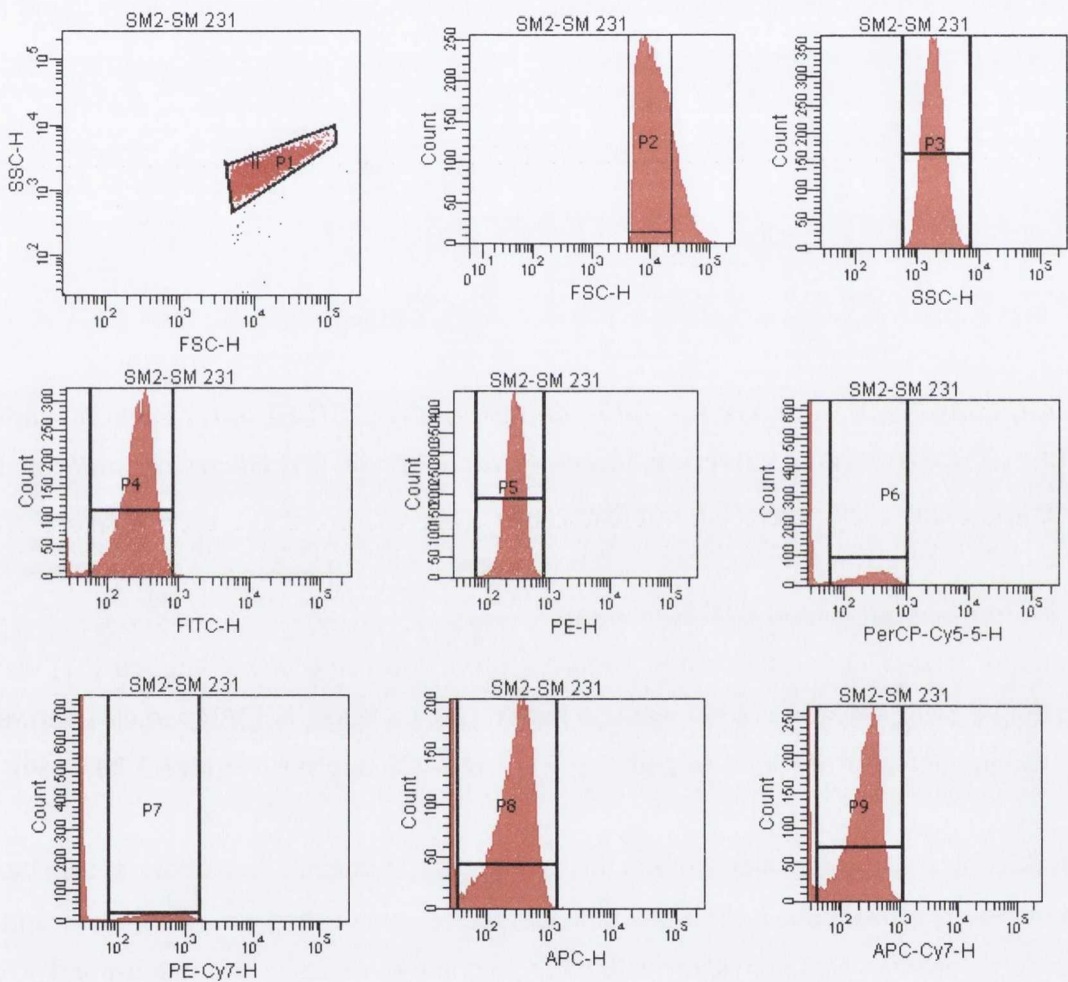


Figure 3.37: FACS analysis for count versus wavelength intensity for various wavelengths on sub-micron Ni particles, Size = 254 ± 78.8 nm (32)

Interestingly, there was no signal detected in the PerCP-CY5 and PE-Y7 channels. PerCP-Cy5 corresponds to excitation at 488 nm and emission at 695 nm and PE-CY7 corresponds to excitation at 650 nm and emission at 795 nm. The particles were also shown to absorb most strongly by UV-vis spectroscopy in the far visible to NIR region, as highlighted previously. Hence, future studies will involve fluorescent detection in these

spectral regions (695 and 796 nm emission) as FACS analysis detected no events for these excitation/emission combinations. In this way it may be easier to detect a difference between samples bound and unbound to fluorescent biomarkers. However, it should be taken into consideration that this is also the region of greatest absorption for the particles, as measured by UV-vis spectroscopy.

The small, concentrated detection area for SSC-H and FSC-H versus detection intensity at PE-H (565 excitation, 578 emission) indicates a narrow size distribution also. **(Figure 3.38)** Scattering for these particles was an order of magnitude greater than found for Fe_3O_4 by SSC and FITC-H in chapter 2.

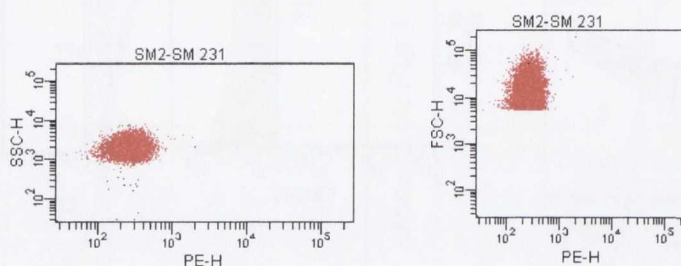


Figure 3.38: FACS analysis for side scatter height (SSC-H) and forward scatter height (FSC-H) versus detection intensity for 578 nm (PE-H) on sub-micron Ni particles, Size = 254 nm $\pm$ 78.8 nm (32)

4.5.2 Gold-Coated Nickel FACS Analysis

The larger, sub-micron gold-coated particles, produced using nickel acetate **(37)** were also tested by flow cytometry. Similarly to bare nickel particles, Ni@Au particles showed excellent signal intensity and low polydispersity in FACS analysis **(Figure 3.39, 3.40)**

Both FSC and SSC intensities were in the same order of magnitude as before, with bare Ni particles. **(Figure 3.39, 3.40)** It was anticipated the gold coating would enhance signal detection; however, was no discernable difference in the results between bare and gold-coated particles, despite the high density and refractive properties of gold. However it is worth noting that these Ni@Au particles are slightly smaller (173 nm) compared to the bare Ni particles (254 nm). This may account for the similar scattering results and future work will investigate various size comparisons. Since gold coating did not significantly enhance the efficiency of particle detection, it was concluded that gold is an unnecessary addition to the surface of these particles, and further studies were conducted with an alternative coating, consisting of the cheaper, more novel graphene nanoplatelets, as outlined in the following section.

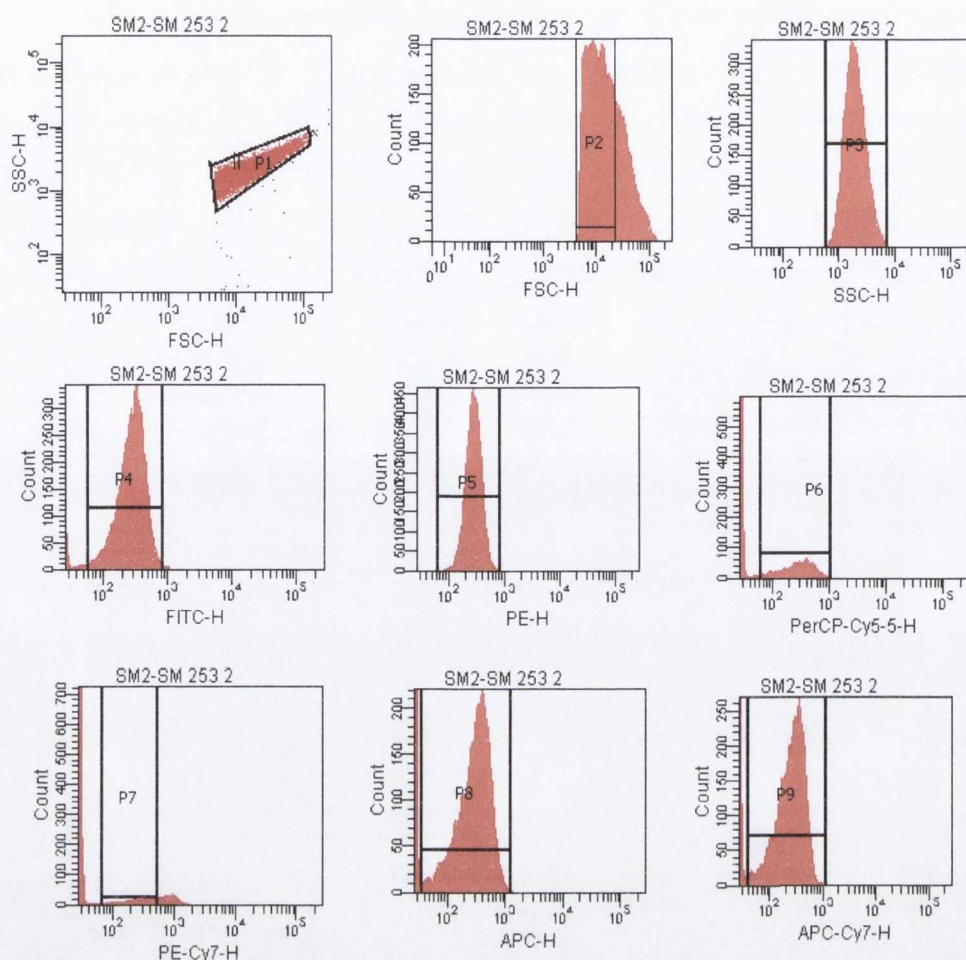


Figure 3.39: FACS analysis for count versus detection intensity for various wavelengths on sub-micron Ni@Au particles, Size = 173 ± 50.7 nm (37)

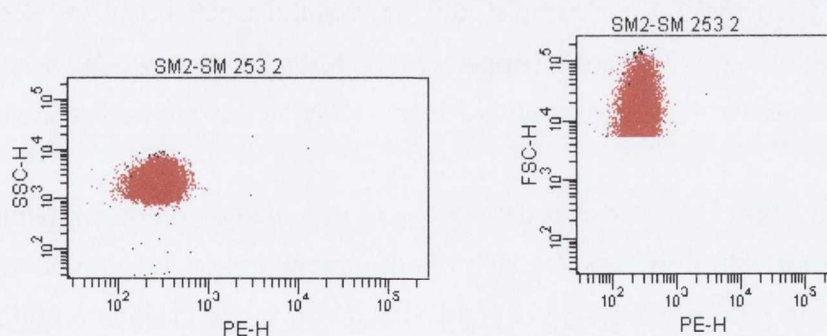


Figure 3.40: FACS analysis for side scatter height (SSC-H) and forward scatter height (FSC-H) versus detection intensity for 578 nm (PE-H) on sub-micron Ni@Au particles, Size = 173 ± 50.7 nm (37)

4.5.3 Graphene-Coated Nickel Bioconjugation Reactions

Nickel@GNP-NH₂ particles (**40**) were functionalised with PEG-mal by amide chemistry, to produce Ni@GNP@PEG-mal. (**46**) This PEG linkers could potentially provide enhanced aqueous stability and also improved biocompatibility. From TEM analysis there is a notable change in morphology at the surface of the PEGylated particles. (**Figure 3.41**). We can see fluffy material of less density extending from their surfaces, confirming the presence of the polymer, relative to the previously smooth GNP-NH₂ surface coating seen before.

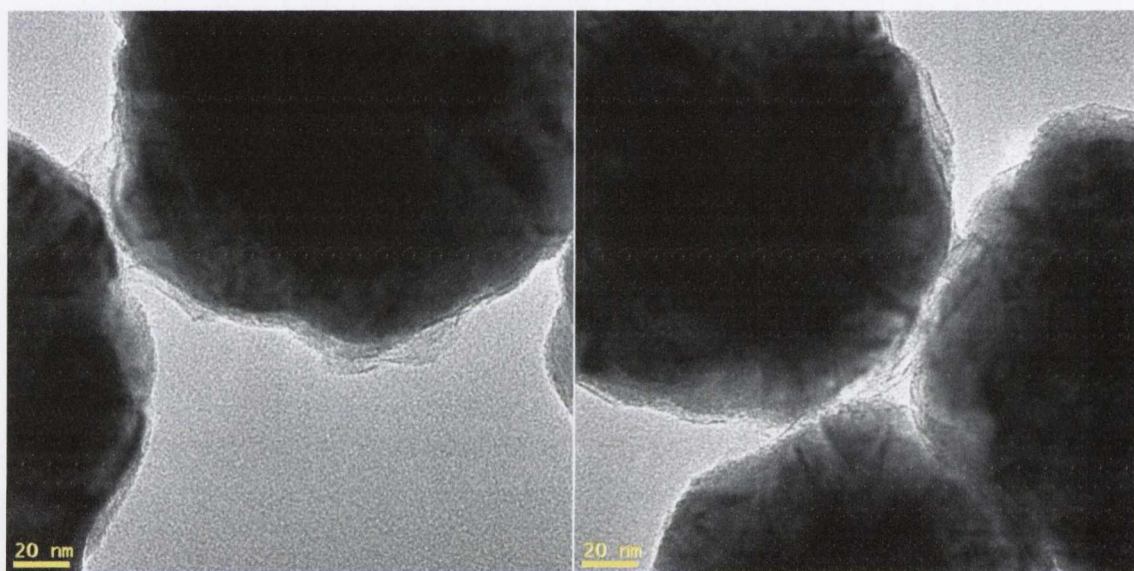


Figure 3.41: TEM images of Ni@GNP@PEG-mal particles (46)

FTIR of the Ni@GNP@PEG-mal sample confirms that the functionality has successfully been installed on the surface. (**Appendix 3.4.2**) This provides a protective, biocompatible layer for the particles and is used widely across biomedical research.

However, it was later concluded that differences in size based on antigen binding could be more easily detected on samples with direct attachment to the graphene surface. Hence, maleimide groups were also directly conjugated to the graphene surface using *N*-maleoyl- β -alanine. FTIR confirmed the presence of the maleimide functionality. (**Figure 3.42**)

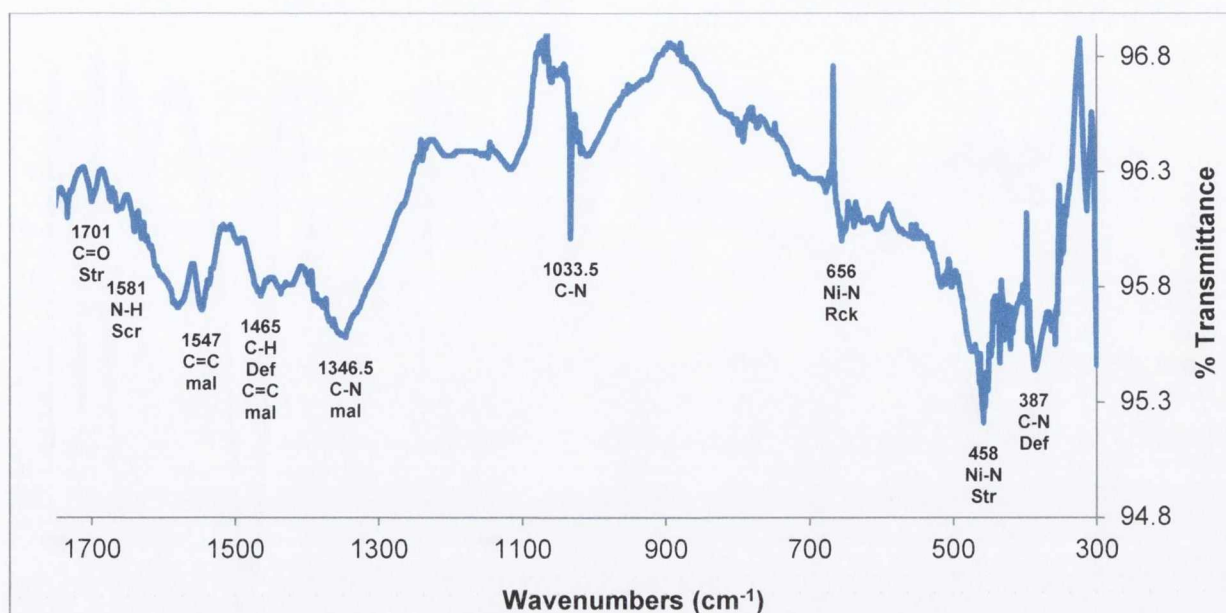


Figure 3.42: FTIR spectrum of Ni@GNP@mal particles (47)

Ni@GNP@mal (**47**) was then used for conjugation to Protein G' (**48**) for biomarker capture and detection studies. This was also characterised by FTIR, (**Figure 3.43**) Installation of the protein on the surface can be clearly identified by the appearance of a new peak at 1634 cm^{-1} , attributed to Amide I C=O stretching in the α helix and β sheets conformational structure of the protein. Samples have been sent to Thermo Fisher Scientific for biological testing. Results are pending and this technology is in the process of commercial exploitation.

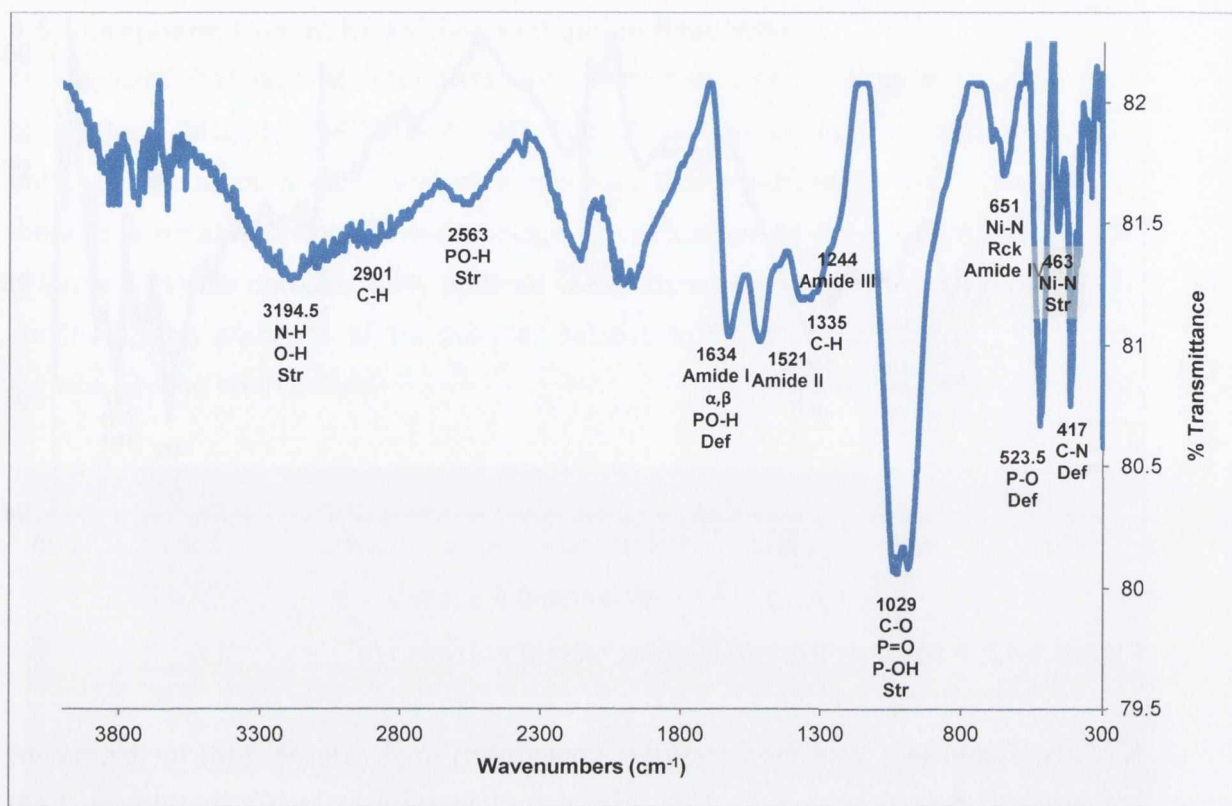


Figure 3.43: FTIR spectrum of Ni@GNP@G' particles (48)

3.6 CONCLUSIONS

Thus, new graphene-coated nickel sub-micron particle (Ni@GNP-X) materials and technologies have been developed here, which showed particular promise and have many potential applications. A novel approach to protect the particles from oxidation, leaching, or degradation was also presented, which involves wrapping the particles in functionalised graphene nanoplatelets. These particles are coated with graphene in a novel way, which has not been previously described, and can only be performed on particles in the size range presented here, using these experimental conditions and this particular size of GNPs. This provides a stable, uniform coating, and additionally possesses organic groups which can be used for conjugation strategies. **(Figure 3.44)**

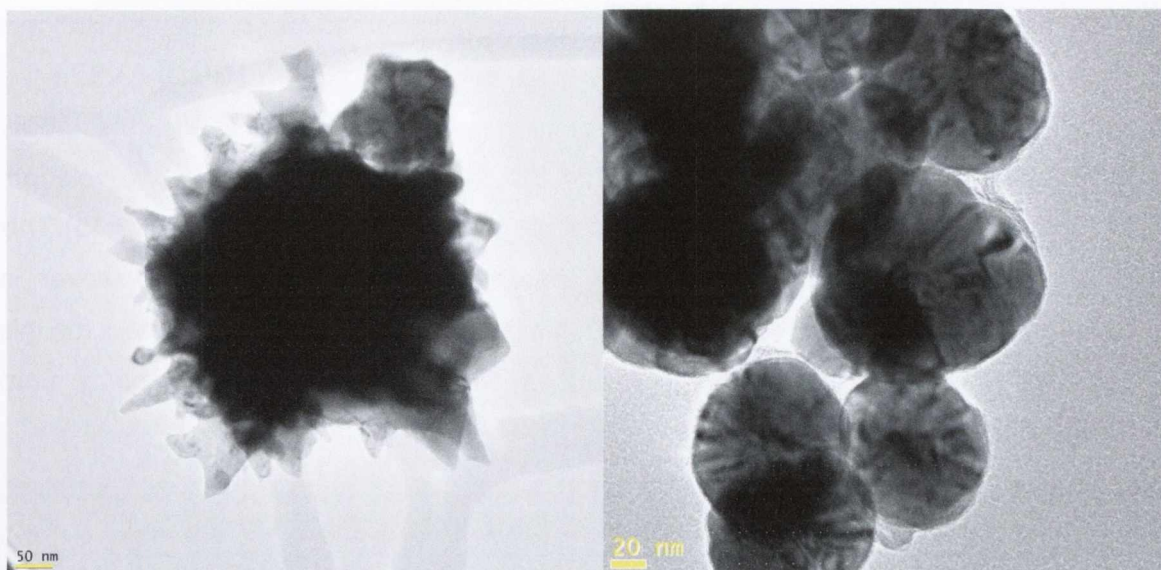


Figure 3.44: Bare nickel particles oxidised by extensive washing (left) (32) and graphene-coated nickel (right) (42)

Due to their larger size, they exhibit some properties of bulk. In this case they are composed of a nickel core, which is a soft magnetic material. These types of material have low or no coercivity in the absence of a magnetic field. Hence they do not agglomerate in solution. This is ideal for biological applications as good dispersibility is a key consideration, especially for flow techniques such as flow cytometry used here.

Once purified, these particles can then be used for the development of biocomposites, using a variety of conjugation strategies which have already been optimised within our lab, including amide coupling, nucleophilic addition, 'Click' reactions, nitrile reduction and oxidation, diazotransfer, and azide reduction. Future work will involve targeting them specifically for biomedical applications such as cell surface and serum biomarker detection using flow cytometry (FACS) systems and also biomolecule capture (BC) and separation. Due to their size, magnetic properties, stability and uniformity they are ideal candidates for these applications.

They have several key advantages over the existing biodetection technologies in current state of the art. Compared to Dynabeads® they have higher magnetisation, smaller size, higher surface area, higher optical density, improved detection, and resistance to degradation. Compared to Turbobeads® they have soft magnetic behaviour, pre-installed functional groups for conjugation, are well separated, uniform, require mild reaction conditions, and also nickel is less toxic and cheaper than cobalt, (nickel: ~16,500USD/tonne, cobalt: ~30,500USD/tonne) (www.mineralprices.com)

They can then be conjugated to biomolecules and fluorophores for specific applications, and also several different functionalities can be installed on a single particle *via* sequential, orthogonal modifications.²¹ Another consequence of their size is detectability in the visible spectral region. Despite the fact that they are slightly below the wavelength of light in diameter (~100-200 nm) they are still detectable optically. **(Figure 3.45)** This is due to their high density and refractive properties, as they are metallic. However, in terms of flow cytometry, they are much smaller than traditional beads used for this application, providing a much higher surface area for bioconjugation, and a more efficient, economic process.

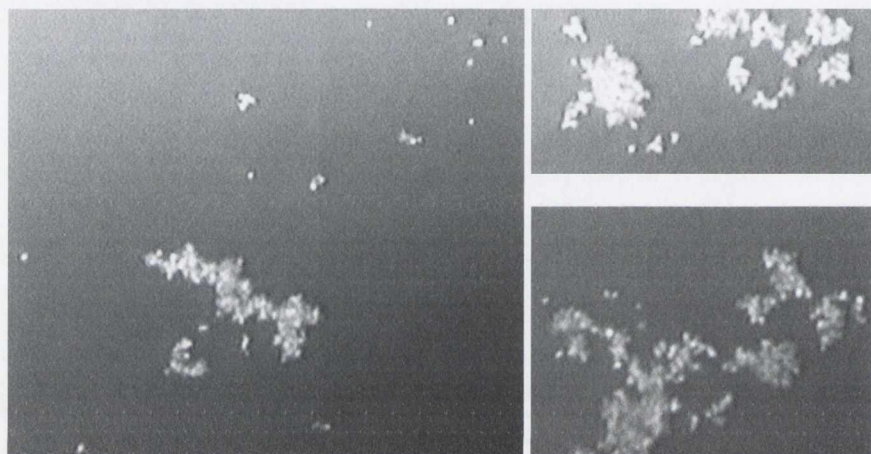


Figure 3.45: Nickel sub-micron particles under bright field microscope, magnification with 150x objective (32)

The range of bioconjugates and their optimised preparation produced in this part of the work is expected to have broad applications in the biomedical and chemical industry, in the areas of basic biomedical science, nanotechnology, with special relevance to diagnostics, manufacturing competitiveness, processing technologies. This should lead to improvements in biomolecule capture and detection, and hence in diagnostics, using biomarkers for disease. Future work will also develop an up-scaling process that should lead to further commercialisation of the new graphene-coated nickel sub-micron particle (Ni@GNP-X) and should enable the development of various applications of these materials in biotechnology.

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4.1 INTRODUCTION

Anisotropic nanomaterials have been gaining considerable momentum in recent years in the area of biomedical research. This is due to advances in the synthesis of novel materials, and their surface modifications specifically targeted for various applications. The field of nanotechnology is very large and diverse, as it is fundamentally a function of size, and can therefore be applied to any conceivable material, which can be reliably prepared in the nanoscale. This function of size also represents a level of control over a system down to the molecular and supramolecular scale. We are still opening avenues towards new applications through the preparation of nanoparticles made of previous unachievable materials consisting of metals, alloys, oxides, semiconductors etc. The possibility of high aspect ratio, anisotropic materials of different sizes and shapes, and also hybrids of these materials, opens even more possibilities and potential applications, as they impart new properties on the materials. **Figure 4.1** contains a schematic of the mechanisms of crystal growth for different anisotropic shapes, dependent on addition to various facets of the growing crystal.^{1, 2}

The field of magnetic nanomaterials has attracted a considerable attention in electronic, computing, energy and biomedical research areas, among others.³⁻⁶ Magnetic properties in nanomaterials are very useful for a number of reasons. They can be used for many applications including biomedical and biosensing research. Another key advantage of magnetic nanomaterials is their response to a magnetic field, particularly for soft and superparamagnetic materials, which allow us to initiate a controlled response at the desired time, and enable us to manipulate and modify our detection systems in a more precise manner. Magnetism also allows for greater ease of handling and sample recovery, compared with non-magnetic particulate solids. They can be selectively retrieved from solutions more easily, yet are still readily dispersible in solvents. This increases their amenability to industrial scale applications, automation, and cost-effectiveness. Hence, magnetic materials can improve the overall effectiveness of diagnostic and detection systems, along with other applications.

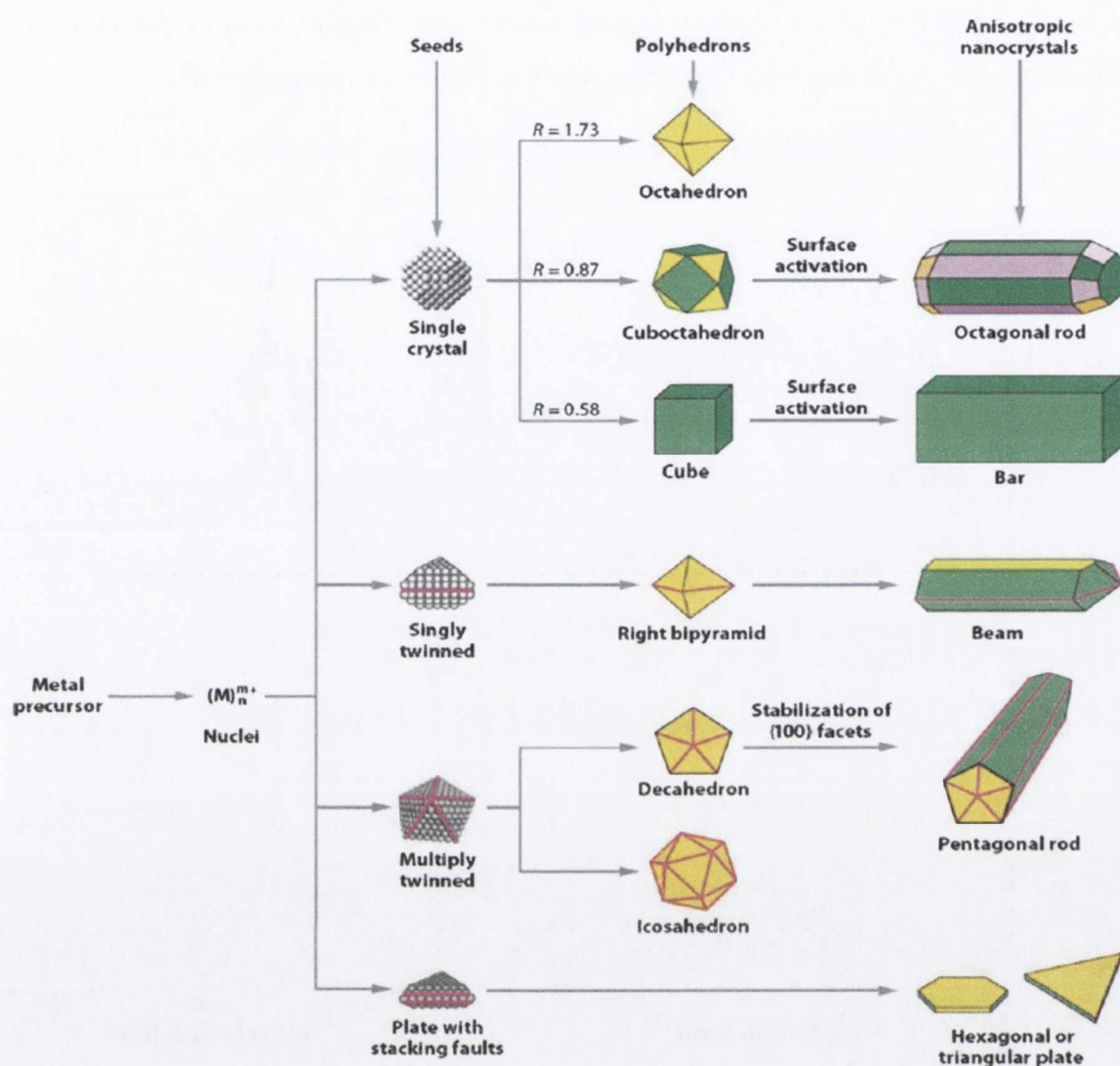


Figure 4.1: A schematic illustrating various stages of the reaction that leads to the formation of noble-metal nanoparticles with different shapes. After the formation of nuclei (small clusters), they become seeds with a single-crystal, singly twinned, or multiply twinned structure. Stacking faults in the seeds results in plate-like structures. Green, orange, and purple represent the (100), (111), and (110) facets, respectively. The parameter R is defined as the ratio between the growth rates along the (100) and (111) directions. Twin planes are delineated in the drawing with magenta lines.^{1, 2}

Plasmonic and fluorescent materials can potentially allow us to achieve more enhanced detection by producing electromagnetic signals in the visible or near visible region of the spectrum. We can pair these signals to markers of interest by attaching them in some way to a target moiety. There are many examples in the literature of how these materials have led to enhanced detection and also new discoveries and insights into the systems being studied.^{7, 8} Plasmonic materials can also enhance signals close to their surfaces, which allow us to detect multiple unknown elements and local environment within a system. It has also been shown that structural anisotropy affects plasmon resonance

due to interaction of the electromagnetic waves with different axes of the structure, allowing us to achieve more specific and complex analysis.² (Figure 4.2)

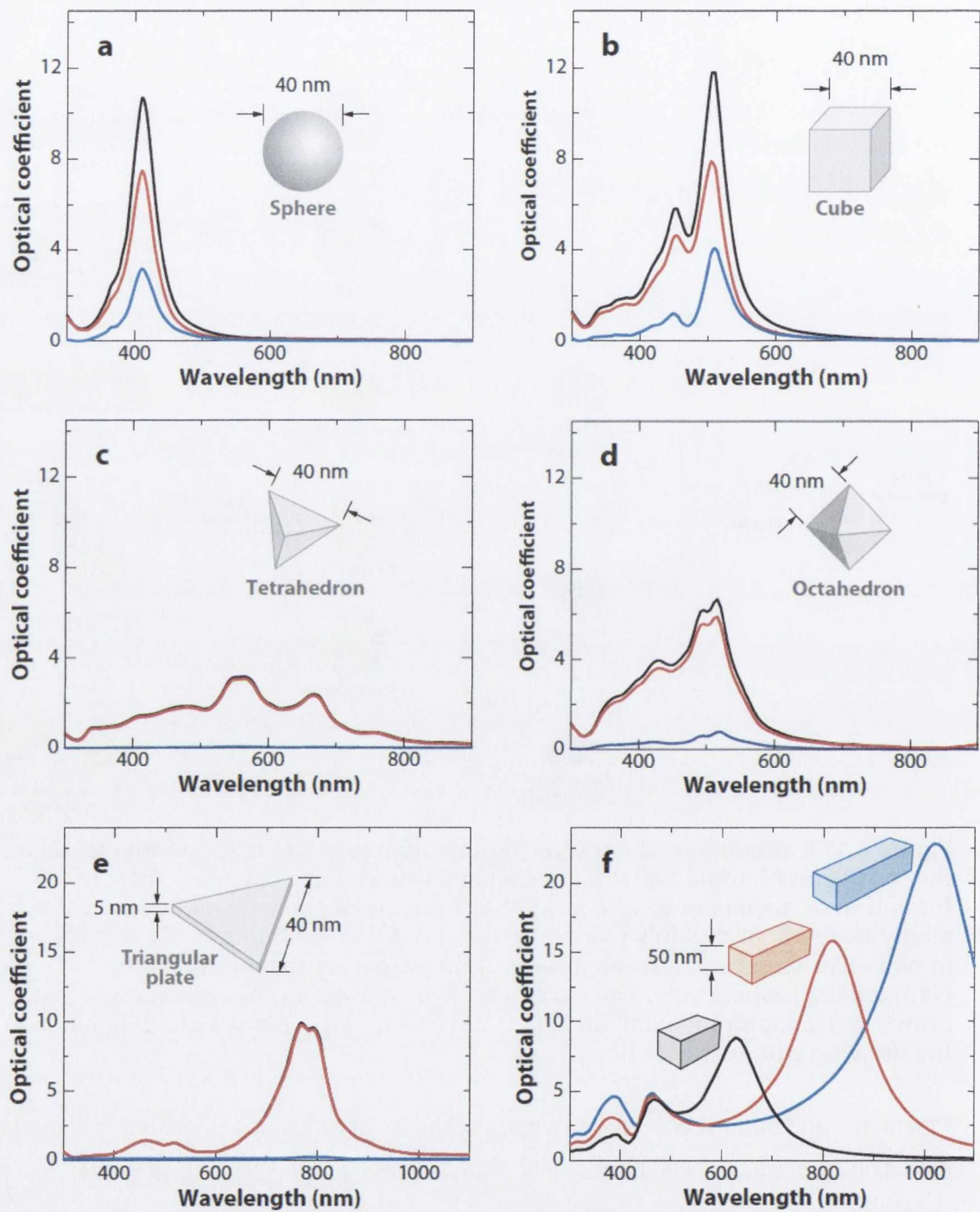


Figure 4.2: Extinction (*black*), absorption (*red*), and scattering (*blue*) spectra calculated for Ag nanoparticles of different shapes: (a) a sphere displaying a single dipole resonance peak and (b) a cube, (c) a tetrahedron, (d) an octahedron, and (e) a triangular plate. (f) Extinction spectra of rectangular bars with aspect ratios of 2 (*black*), 3 (*red*), and 4 (*blue*). Note that the nonspherical particles typically exhibit multiple, red-shifted resonance peaks²

As nanomaterials are within the same size range of many biomolecules, such as proteins, nucleic acids, glycolipids etc., they may specifically bind to the biomolecules which perform all the many complex functions required in biological systems. For biomolecules, their functions are intrinsically linked to their shape. For example, enzymes perform catalytic reactions, by the fitting of a target molecule into an active site which has been arranged to provide the correct amino acid bases, which specific organic groups, surrounding the target compound to perform a chemical reaction. Small changes in the active site, or in the structure of a molecule can drastically affect this function.⁹ The function of nucleic acids also depends on their structural conformation. Hence, it stands to reason, that changes in the shape of nanomaterials could also change their dynamics and interactions with biological systems. It is hoped here, by fine-tuning the shape of particles, and opening the doors to new avenues of research, more effective diagnostic tools can be created.

The main aim of this part of the project is to produce anisotropic nanomaterials for improved biomarker detection systems. It is hoped to use magnetic properties to improve sample handling and recovery of these particles, and plasmonic and fluorescent properties to potentially enhance signal detection. Anisotropic nanomaterials have the advantage of an increased surface area relative to their spherical counterparts of a similar diameter, with the added advantages of properties such as high aspect ratio and differential electromagnetic interaction along their axes. Hence, interesting variations in their properties based on differences in their sizes and shapes is also expected.

In this part of the work a range of new anisotropic nanomaterials were produced including FePt nanowires, FePd@Fe₃O₄ nanoclusters, FeNi, FeCu, CuS, Bi₂S₃, and NiS nanoplates, and also Fe₃S₄ nanobelts, as summarised in **Figure 4.3**. The synthetic route adopted was performed by a hot injection approach, involving the surfactant oleylamine, which acts as solvent, reducing agent and surface stabiliser. CoPt, FeCo, FeAu, MnBi and MnS anisotropic nanostructures were also produced, however not discussed here, as these experiments require further optimisation and characterisation, results of which are included in the appendix. (**Appendix 4.11**)

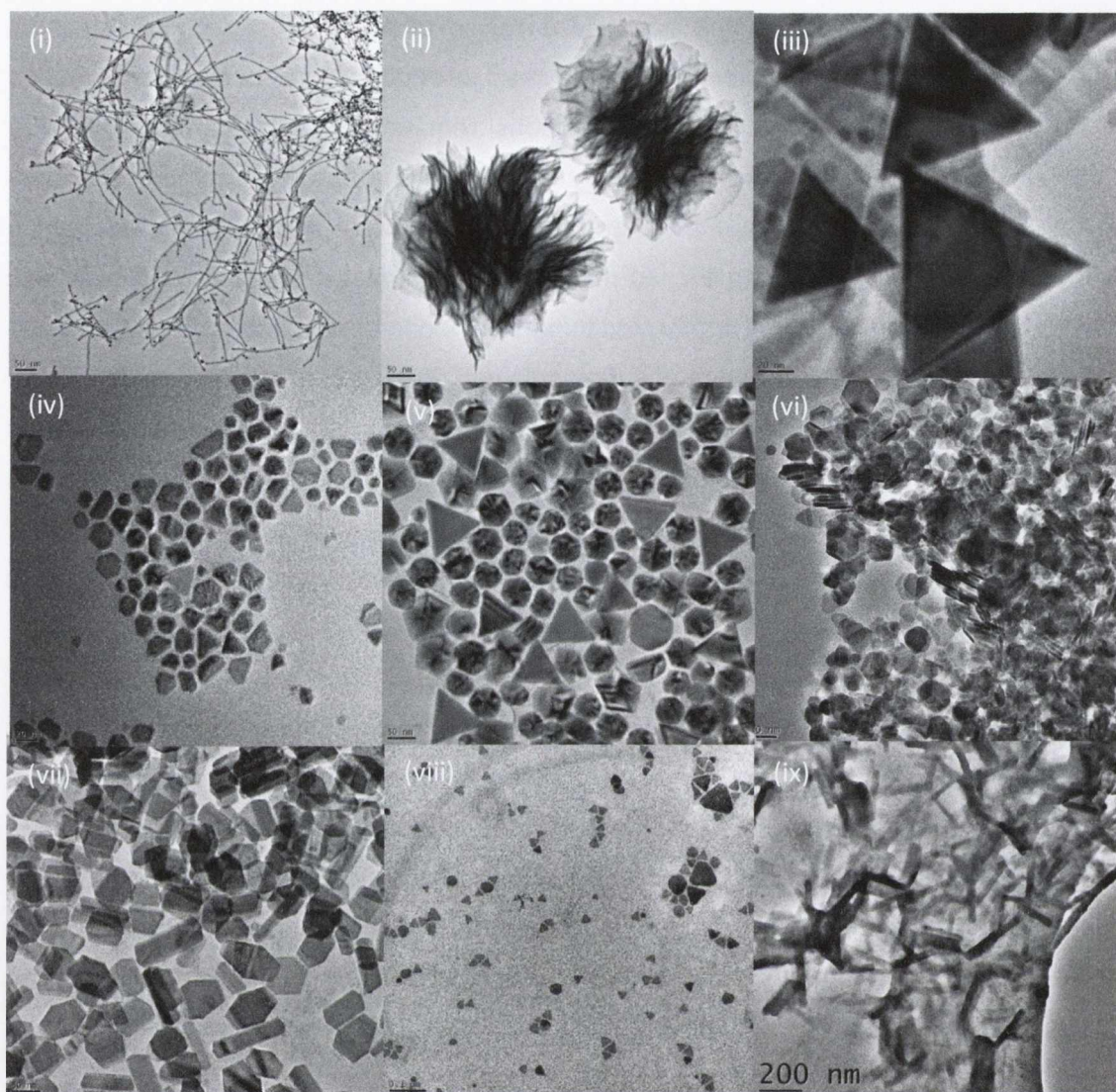


Figure 4.3: TEM image examples of anisotropic nanomaterials produced in this work with (i) $\text{Fe}_{65}\text{Pt}_{35}$, (ii) $\text{FePd}_3/\text{Fe}_3\text{O}_4$, (iii) $\text{Fe}_5\text{Ni}_{95}$, (iv) $\text{Fe}_{25}\text{Ni}_{75}$, (v) $\text{Fe}_4\text{Cu}_{96}$, (vi) CuS , (vii) Bi_2S_3 , (viii) Ni_3S_4 , and (ix) Fe_3S_4

This method involves an organic phase thermal decomposition and reduction route in the surfactant, and can be used to prepare a variety of anisotropic nanoparticles consisting of different materials and shapes. Anisotropic growth is believed to be guided by the surfactant, which creates a tunnelling effect due to differences in stacking and alignment of neighbouring alkyl chains. The surfactant promotes this process, by steric hindrance along the length of the crystal, and increased points of access at the corners of the crystal due to the conformation of the surfactant chain. Along the length of the structure, neighbouring alkyl chains are stacked much more efficiently than at the ends, hence surfactant tunnelling occurs. Binding strength of the surfactant coordinating group to the surface of the growing crystal is also important and amino groups have been used

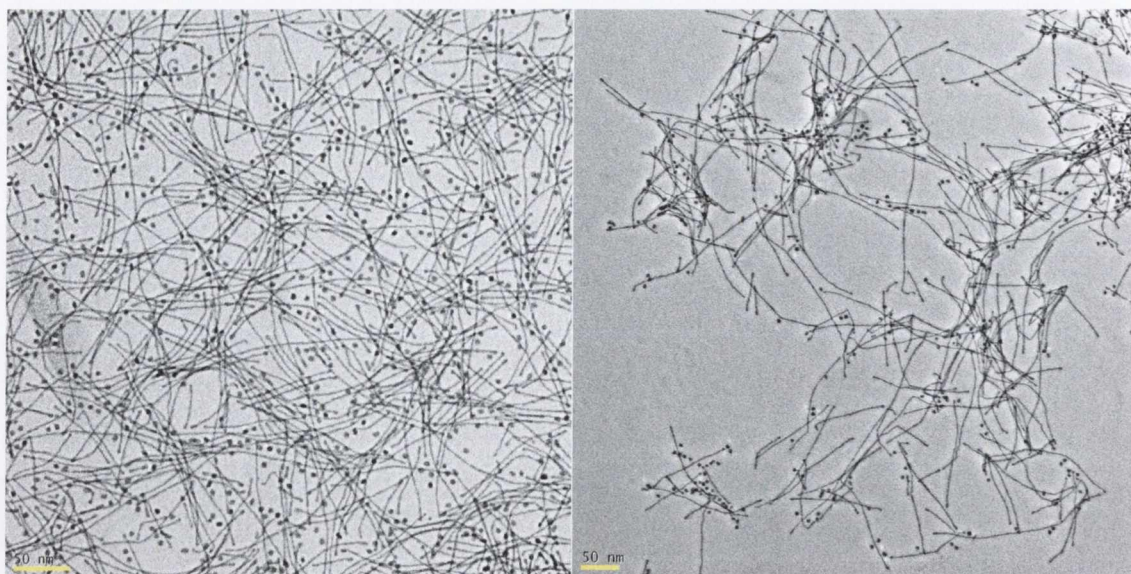
in a number of different methods to promote anisotropic growth.^{10, 11} Hence, temperature is also a key consideration, as this affects the binding strength of the coordinating ligand.

Oleylamine has been used in the synthesis of anisotropic nanostructures with a number of compositions and sizes in the literature, including Au, FePt, CoPt and CuS.¹²⁻¹⁶ Unlike template-assisted nanowire synthesis, these hot injection organic phase reactions provide the possibility of up-scaling to considerably larger quantities. In addition it is a useful way to exclude water and oxygen from the reaction to avoid oxidation of the metallic crystals. However, it is important to note that morphology and size of the resulting particles depends on many factors, including the organometallic precursor used, temperature, time, concentration, and the types and ratios of surfactant ligands.

Although some other methods exist in the literature for anisotropic nanocrystal growth¹⁶⁻¹⁸ this method unifies many different materials, metals, semiconductors and metal oxides of different shapes, under one reliable organic phase hot injection route which uses an economy of reagents, and similar reaction conditions in each case. It is predicted that this method could also be used to create many other anisotropic materials not shown here, providing they fulfil the criteria of the reactions outlined above.

4.2 IRON-PLATINUM NANOWIRES (FePt NWs)

FePt nanowires **(55)** were synthesised, according to a modified technique published in the literature.^{13, 14} The iron precursor $\text{Fe}(\text{CO})_5$ was used in liquid form, which lends to ease of handling, and allows for direct hot injection without the need to disperse the $\text{Fe}(\text{CO})_5$ precursor in solution. This in turn led to monodisperse nanowires from the reaction, with good uniformity and high yields, however some initial seed nanoparticle were still present in the sample. From TEM analysis it can be seen that the NWs formed are very uniform in diameter (~ 1.5 nm) with lengths in the region of 150- 500 nm. It has been shown in the literature that addition of oleic acid to the reaction, and varying the ratio of oleylamine to oleic acid, does result in a change of length in the wires.¹³ Here, pure OAm was used to obtain the maximum aspect ratio and length for the NWs. **(Figure 4.4)**



Scale Bar: 50 nm

Scale Bar: 50 nm

Figure 4.4: TEM images of FePt NWs formed over 30 min (55)

From XRD it can be seen that $\text{Fe}_{65}\text{Pt}_{35}$ crystals with fcc lattice were formed. **(Figure 4.5)** The products correlate well to the amount of precursors added to the reaction, minus the amount of Fe which would be oxidised in the galvanic replacement reaction. This also corresponds well to the ratio of 70/30 for Fe/Pt found by EDX **(Appendix 4.1)** $\text{Fe}_{65}\text{Pt}_{35}$ possesses a disordered fcc phase.¹¹ It was noted that this structure grows preferentially in the (111) direction due to reduced surface tension from close packing, as indicated by the increased relative intensity of the (111) parameter in the XRD pattern. This type of growth also aids in anisotropic crystal growth. Hence for future experiments with other materials, ratios for the chemical precursors were picked which could form close packing crystal structures for that particular alloy in question.

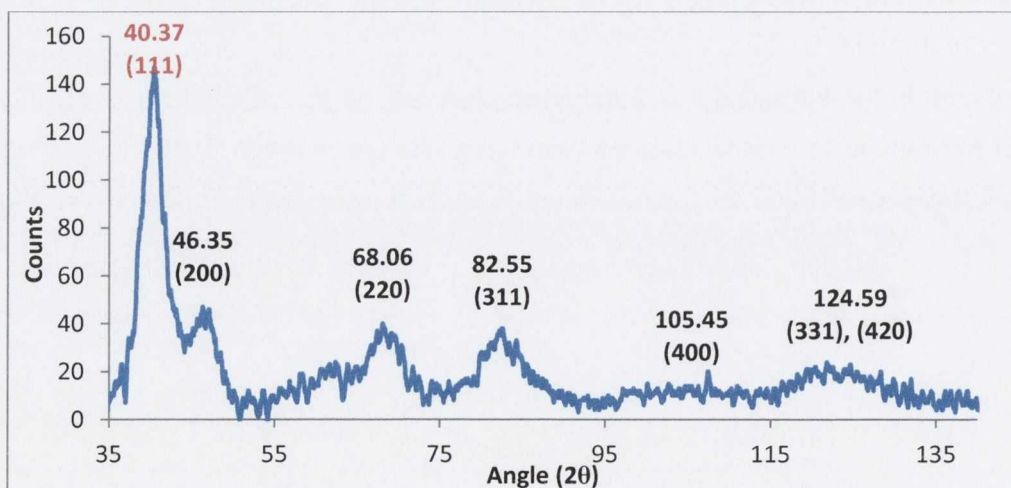


Figure 4.5: XRD pattern for FePt NWs showing a $\text{Fe}_{65}\text{Pt}_{35}$ fcc crystal structure

VSM results showed a low magnetic moment for this sample, with a peak magnetisation of just $0.43 \text{ Am}^2\text{kg}^{-1}$. (**Figure 4.6**) This result is expected, due to the disordered phase of the crystal. Annealing would be required to create higher crystallinity and improved magnetisation properties in the sample. Unfortunately, this annealing process would also change the morphology of the sample and destroy the associated anisotropic properties, and potentially create a fused network with amorphous carbon, rendering the product unusable for bioapplications.

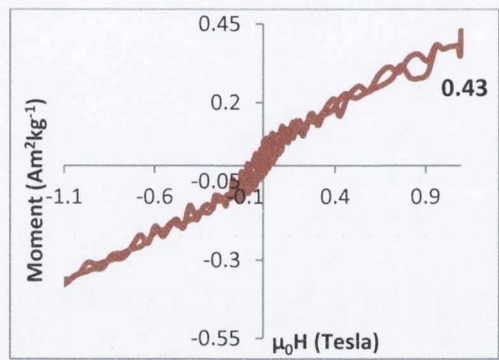


Figure 4.6: VSM curve of FePt NWs

FTIR confirmed the presence of the OAm ligand on the surface for the reaction over 30 min, with coordination of the amine to both platinum (574 cm^{-1}) and iron (332.5 cm^{-1}) at the surface. (**Figure 4.7**) Other spectral regions of this sample are shown in the appendix. (**Appendix 4.1**)

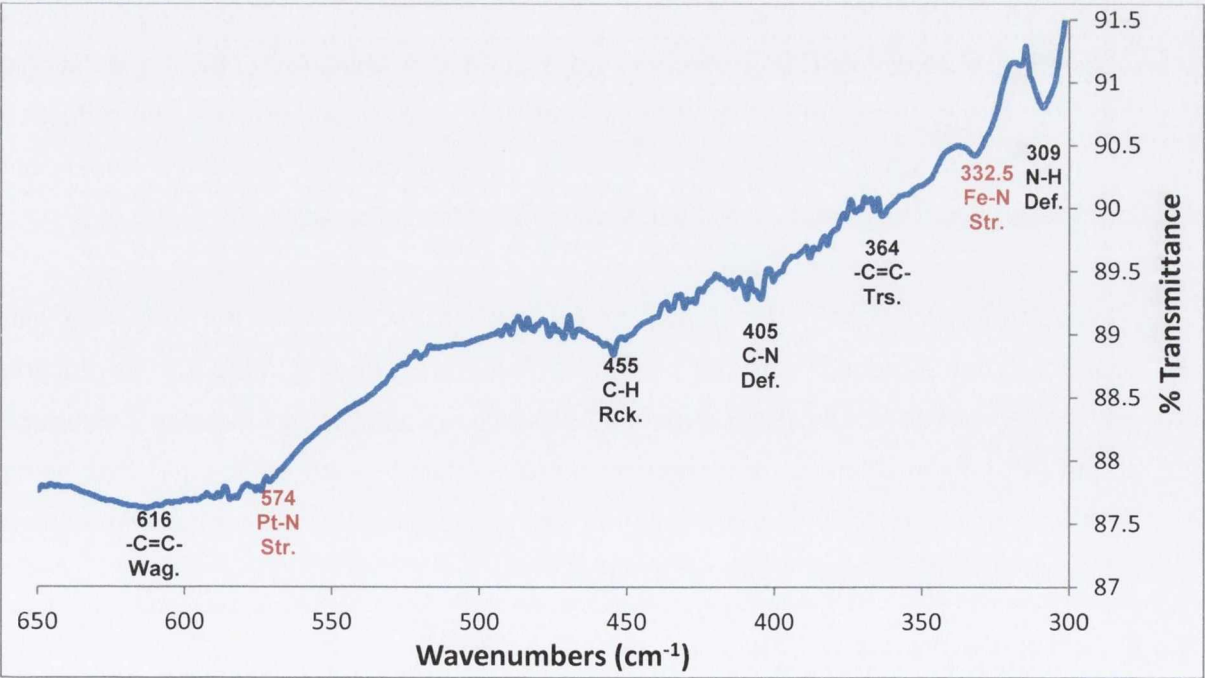


Figure 4.7: FTIR spectrum of FePt NWs stabilised with OAm, with peaks for metal-ligand binding shown in red

UV-vis spectroscopy also revealed plasmonic behaviour for these wires, with an absorption shoulder at 345 nm tailing off into the visible region. The peak at 275 nm is due to OAm. **(Figure 4.8)** This profile of increasing absorption towards UV wavelengths is characteristic of conducting materials, as higher energy electromagnetic waves are transmitted through the material. Hence, they could be potentially used for dual sensing using magnetic and plasmonic detection. Also, fluorescence studies should employ markers which emit in the lower spectral region towards far visible and infrared (450 – 100 nm).

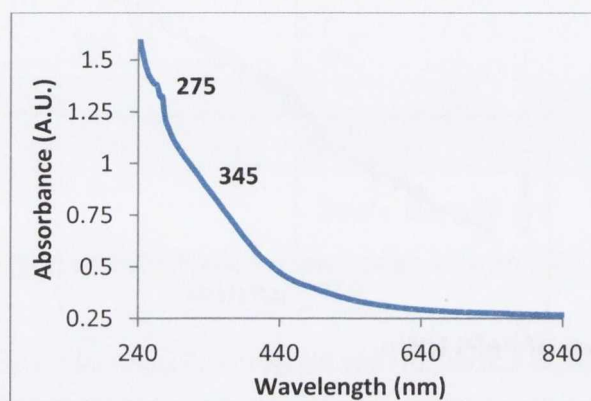


Figure 4.8: UV-vis absorption profile for Fe₆₅Pt₃₅ NWs in hexane

From this reaction, a theory was hypothesised that the mechanism proceeded *via* a decomposition/galvanic replacement mechanism, whereby the Fe(0) precursor was first decomposed, then this zero valency metal reduced the other salt precursor due to their difference in electrochemical potential. The process of iron alloy formation involves addition of a zero valent iron precursor to a surfactant solution containing a cationic metallo-organic precursor of the other material at a specific temperature. The reduction of the other precursor is achieved *via* electron transfer from iron; hence it is dependent on differences in the standard reduction potential of the materials.

The standard reduction potential (E^0) for the reaction $\text{Fe}^{2+} + 2 e \rightleftharpoons \text{Fe}$ is -0.447V with respect to the standard hydrogen electrode. The corresponding value for the reaction $\text{Pt}^{2+} + 2 e \rightleftharpoons \text{Pt}$ is +1.18V. Hence reduced Fe atoms in solution will transfer 2 electrons to the Pt^{2+} ions in solution. New reactions were designed based on this principle, using elements which would form alloys with iron and possessing a precursor material with an electrochemical potential higher than that of Fe^{2+} .

The reaction for production of FePt NWs has been previously described and is well documented,^{13, 14} hence further optimisation of this reaction was not performed. However

FePt NWs were used for further experiments discussed later in the formation of novel FePt-Au heterostructures and in a new aqueous transfer reaction involving a heterobifunctional PEG for further bioconjugation, detection and biosensing applications. These experimental conditions also formed the basis of new experiments for the productions of novel anisotropic materials of a variety of different alloys, oxides and semiconductors, in the hope of finding materials with magnetic, plasmonic and fluorescent properties which could enhance detection and diagnostics for clinically relevant biomarkers, as outlined in following sections.

CoPt, FeCo and FeAu nanowires were also prepared, results of which are included in the Appendix. However, these samples were not uniform and required further optimisation and characterisation. Hence, they are not discussed further here. (Appendix 4.11)

4.3 IRON-PALLADIUM/MAGNETITE NANOSTRUCTURES (FePd₃@Fe₃O₄)

In these reactions the temperature was kept at 180°C for 20-30mins. The concentration and ratio of Fe and Pd precursor ratio was varied and then the change in composition and other properties were measured. E° for the reaction Pd²⁺ + 2 e⁻ ⇌ Pd is +0.951V, which is quite similar to that of FePt. Hence this reaction was performed with all condition remaining similar to that of FePt, replacing Pt(acac)₂ with Pd(OAc)₂. An preliminary reaction was performed under the same conditions to that of FePt NWs, replacing only the metal salt. This yielded a non-magnetic product with very high Pd content. (Figure 4.9) NWs were formed but another material was also noted from TEM analysis, which could not be removed even after extensive washing. Consequently, the amount of Fe was increased in subsequent reactions in an attempt to form NWs with high Fe content.

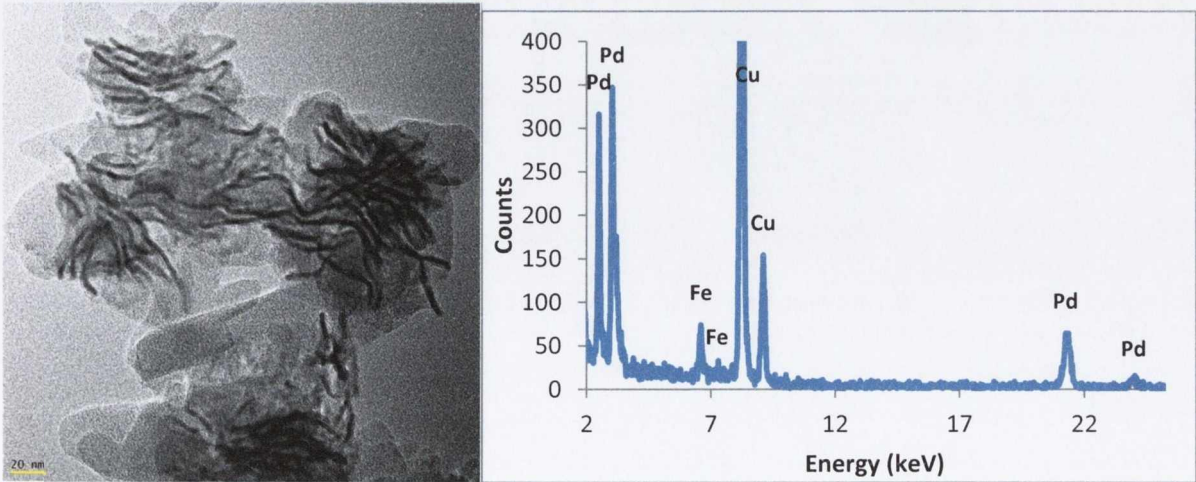


Figure 4.9: TEM images (scale bar: 20 nm) (left) and EDX analysis (right) of Fe₁₇Pd₈₃ nanostructures

Interestingly, in the reactions where higher quantities of $\text{Fe}(\text{CO})_5$ were used this produced a different type of heterostructure, (56-59) with wires fused together into bundles. (Figure 4.10-4.13) These nanostructures have not been previously reported in the literature, and although several hundred nanometres in diameter, still possess nanomaterial characteristics as discussed below. The average diameter of the structures varied 100-200 nm, with the denser core wires having an average width of 1.5 nm and length of ~50 nm, and a shell thickness ~3 nm.

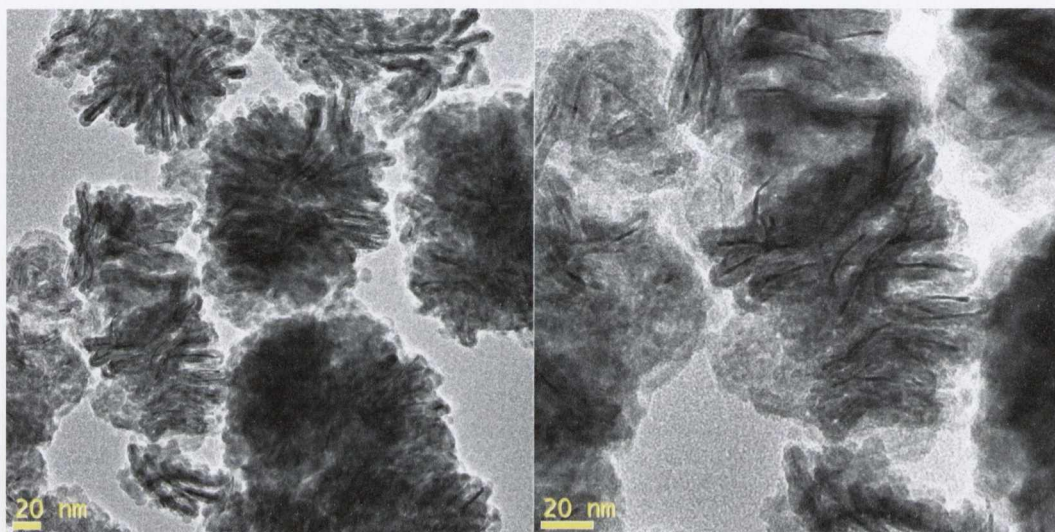


Figure 4.10: TEM images of $\text{FePd}_3@ \text{Fe}_3\text{O}_4$ 150 nm nanostructures formed with a Fe/Pd ratio of 11.5/1 (56)

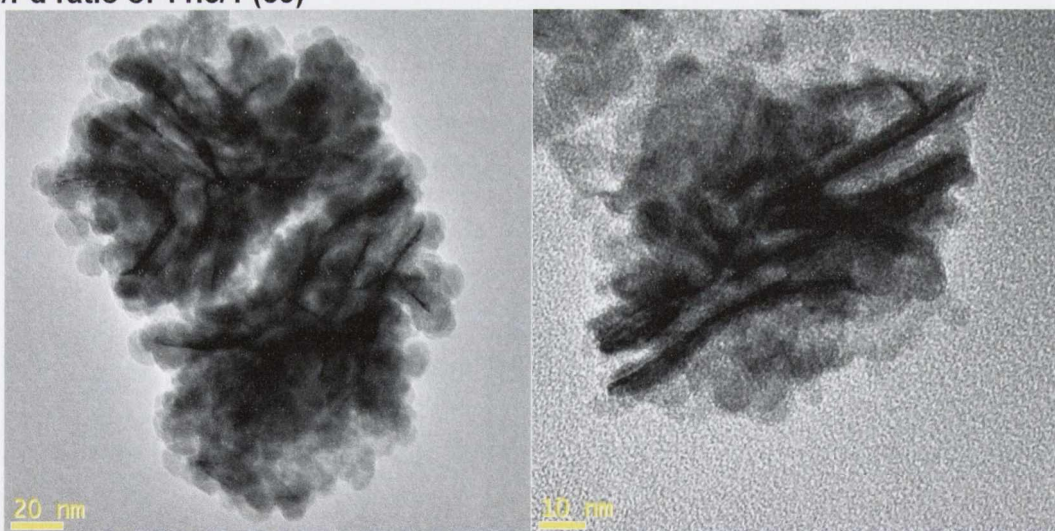


Figure 4.11: TEM images of $\text{FePd}_3@ \text{Fe}_3\text{O}_4$ 170 nm nanostructures formed with a Fe/Pd ratio of 7.66/1 (57)

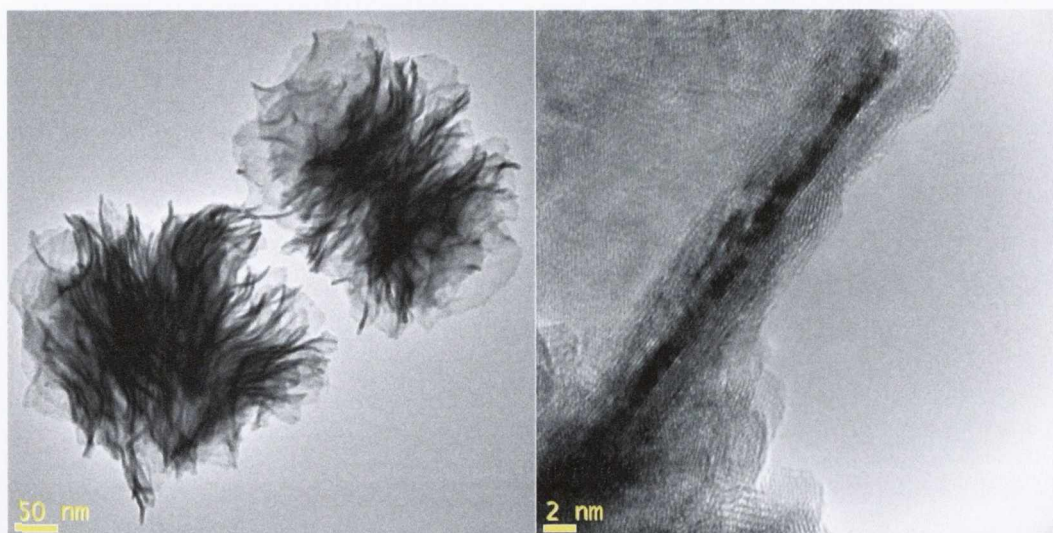


Figure 4.12: TEM images of $\text{FePd}_3@\text{Fe}_3\text{O}_4$ 220nm nanostructures formed with a Fe/Pd ratio of 3.83/1 (58)

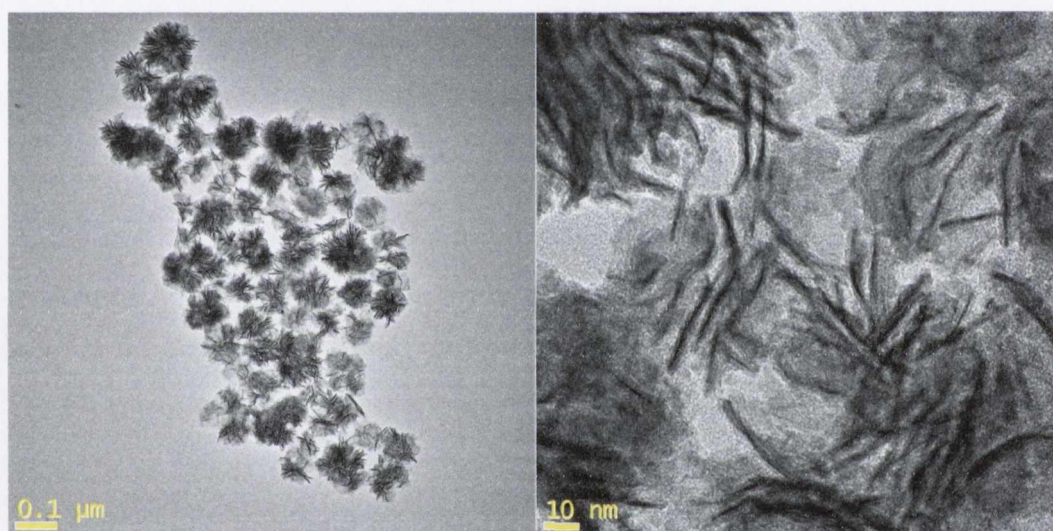


Figure 4.13: TEM images of $\text{FePd}_3@\text{Fe}_3\text{O}_4$ 100 nm nanostructures formed with a Fe/Pd ratio of 2.3/1 (59)

For the sample with lowest Fe content (**59**), the individual nanowires were more distinct and separated, and some wires were observed independent of the aggregates. It is predicted that with much lower Fe content, NWs could be formed; however these present the same problem as seen with FePt and CoPt samples with the requirement of annealing to increase magnetisation, as indicated by the preliminary experiment.

For all samples, it was noted during sample cleaning and work-up, the products formed smoke upon drying, after washes to remove excess OAm. XRD revealed the presence of both FePd_3 and Fe_3O_4 in the samples. (**Appendix 4.2.3**) Hence Fe_3O_4 in the sample is attributed to excess Fe forming Fe_3O_4 upon combustion of the material under

atmospheric conditions post-reaction. Fe metal is known to be very readily oxidised in atmosphere, particularly with a highly reactive, large surface area. This could also have contributed to fusion of the iron-palladium wires into the larger clusters. The difference in contrast, as shown by TEM, also indicates the FePd₃ is in the core of these composites. The last experiment, with the smallest Fe content, used the same ratio of precursors as used in FePt NW formation, however in the case of FePt, a larger ratio of Fe was incorporated into the alloy crystal (Fe₆₅Pt₃₅). Iron-platinum, however, has more stable crystal structure variations than iron-palladium alloys.

XRD patterns for this series of composites all showed peaks for both fcc Fe₃O₄ and fcc FePd₃ and are included in the appendix. **(Appendix 4.2.3)** As expected, the relative intensity of the FePd₃ peaks increased with decreasing Fe content in the sample. Below is an example of the XRD patterns obtained. **(Figure 4.14)** EDX revealed a decrease in Fe content with decreasing Fe starting ratio as expected. **(Appendix 4.2.2)** However, this decrease was not quantitative. The reactions were all performed over 20-30 min; hence it is likely not all the Fe precursor reacted within this time.

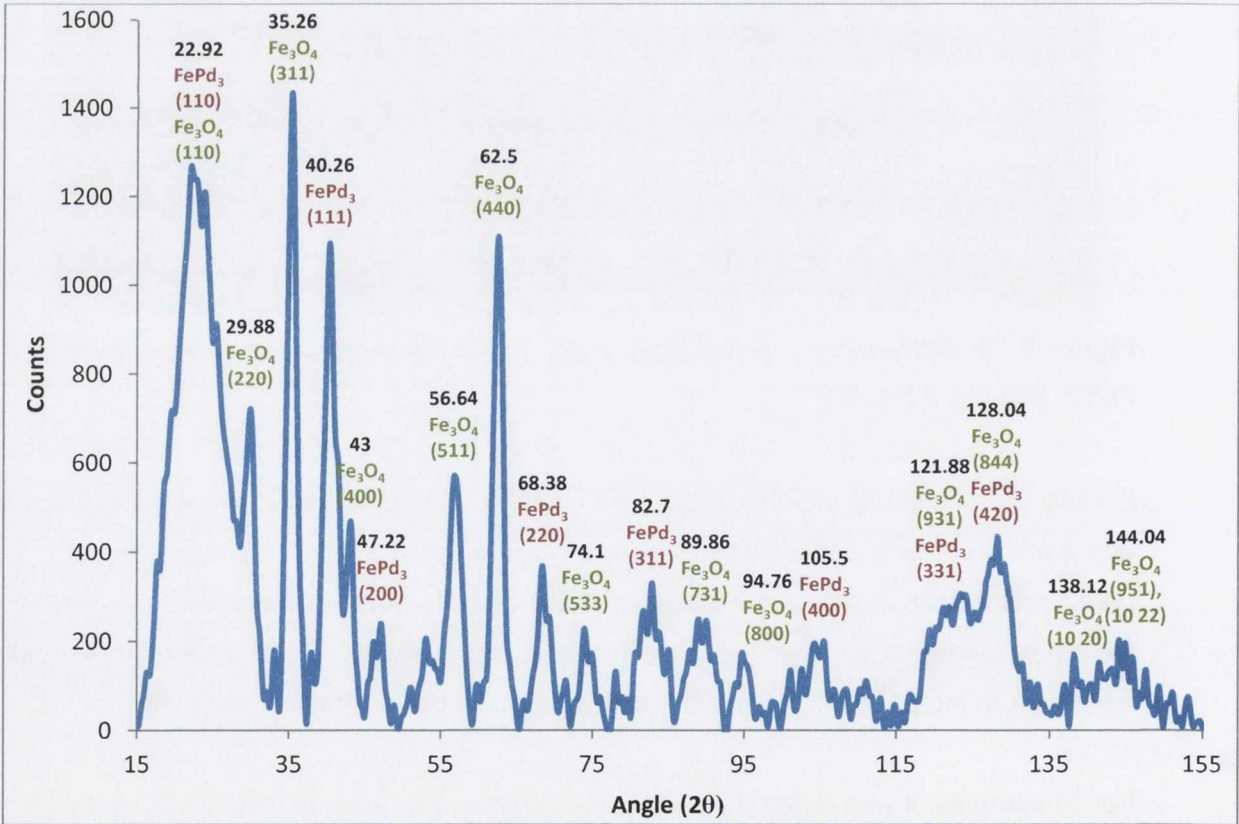


Figure 4.14: XRD pattern for FePd₃@Fe₃O₄ produced using a starting Fe/Pd ratio of 11.5/1 (56)

Larger, micron-length FePd NW syntheses have been reported several times in the literature using AAO template-assisted synthesis, similar to methods used to produce the metal nanowires described in Chapter 2.¹⁹⁻²³ FePd nanoparticles have been produced using an organic phase hot injection route,²⁴ however, to our knowledge, FePd₃ nanowires and FePd₃@Fe₃O₄ nanostructures with the size and morphology shown here and formed using these synthetic methods have not been previously reported.

Despite their sub-micron size, all samples exhibited superparamagnetic behaviour, regardless of starting ratios or peak magnetisation. This is due to the quantum confinement of Fe₃O₄ as it exists as a 2D thin layer around the FePd₃ NW clusters. As expected, VSM showed an overall decreasing peak magnetisation with decreasing Fe content with values ranging from 42.50 - 13.17 Am²kg⁻¹. **(Figure 4.15)** As with FePt NWs the FePd₃ is a disordered phase for fcc and requires annealing to induce ferromagnetism. Hence, the magnetic contribution of the wires to the clusters would not be large. It therefore stands to reason that the magnetism would decrease, as Fe₃O₄ is the main magnetic component. These could be very useful for bioapplications, as previously mentioned. Such magnetic behaviour as seen here is also not usually achievable in this size range for these materials.

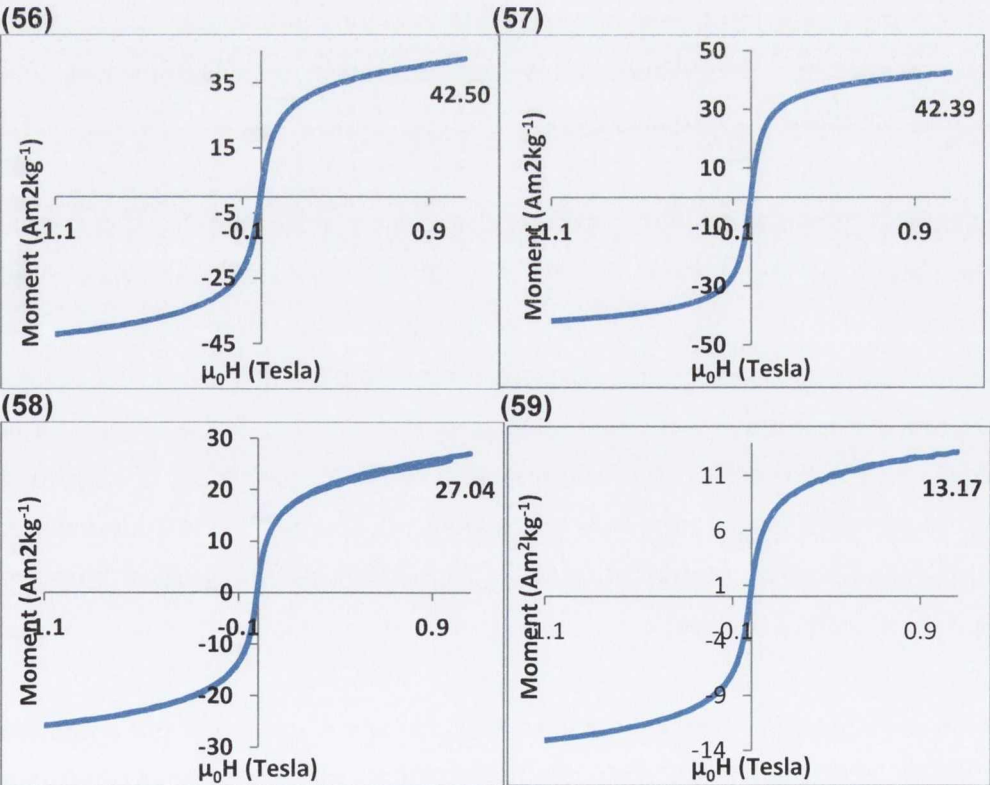


Figure 4.15: VSM curves of FePd₃@Fe₃O₄ samples, with Fe/Pd finishing ratio of (56) 86.4/13.6, (57) 85.93/14.07, (58) 76.07/23.93, (59) 59.78/40.22

FTIR spectra for these compounds showed the expected peaks for Fe-O stretching vibrations characteristic of Fe₃O₄. (**Appendix 4.2.4**). Characteristic peaks for oleylamine coordinated to the surface were also detectable by FTIR, with lower intensity than those of the shell metal oxide peaks, which constitutes a higher mass percentage for the composites.

Due to their size, stability, superparamagnetism, reproducibility in high yields, and also biocompatible Fe₃O₄ coating these composites could also be potentially used as a diagnostic detection beads, similar to Ni@GNP discussed in chapter 3. In addition their cluster shape further enhances surface area for conjugation and surface reactivity. This would prove particularly useful for biomolecular capture studies, with increased binding sites for target markers. Hence future work should involve these studies for bioconjugation, molecular capture, and also fluorescent and flow cytometry detection.

4.4 IRON-NICKEL NANOPATES

FeNi alloys are useful due to their high saturation magnetisation, low coercivity, cheap cost and reasonable biocompatibility. There also the possibility of plasmonic behaviour in these alloys, for high nickel content, soft magnetic compositions. There are already several reports in the literature of FeNi and Ni anisotropic nanostructures, due to these highly useful properties.^{18, 25, 26} However, these reported methods differ from ours, and have not yet been targeted to bioapplications.

The electrochemical potential for the reaction $\text{Ni}^{2+} + 2 \text{e}^- \rightleftharpoons \text{Ni}$ is -0.257V. This is only slightly higher than that of the reaction $\text{Fe}^{2+} + 2 \text{e}^- \rightleftharpoons \text{Fe}$ (-0.447V). However it was found that this difference was enough to initiate the galvanic replacement reaction. The precursor, NiCl₂.6H₂O was used. In other experiments, in preliminary work (not shown here) for the production of the nickel sub-micron sphere in Chapter 3, it was found that this precursor would not react at the temperatures used in this series of reactions (160°C), and much higher temperatures were needed (250°C) when it was added alone. In addition the decomposition temperature for NiCl₂.6H₂O does not have a literature value, with a boiling point of 972.8°C.

Hence it can be deduced that it was indeed the Fe(0) in solution which brought about the reduction of nickel. In addition it was found that this reaction required a longer timeframe to produce fully crystalline, anisotropic structures, relative to FePt₃. This could also be accounted for by the smaller difference in electrochemical potential. These longer reaction times could also contribute to the difference in growth mechanism, with longer

timeframes for crystal formation allow for increased attachment points on the growing crystal.

FeNi nanostructures were prepared with hot injection reactions performed at 160°C, and concentration fixed at either 1.55/1 or 2.3/1 and with time varied. By varying time, the process of anisotropic growth was studied, and the optimum conditions for formation of uniform anisotropic nanostructures were found. It was discovered that the mechanism of growth for FeNi alloys differed from that of FePt and FePd NWs. Here, instead of nanowires nanotriangles and polygons were formed with a flat 2D structure of ~80 nm diameter. **(Figure 4.16)** In this case the product progressed from seeds, to triangle/polygons to icosahedrons through Ostwald ripening and recrystallisation. For the FePt-type growth increasing time did not produce 2D polygon shapes. Even after several days of reaction, the product remained as NWs (work not shown here).

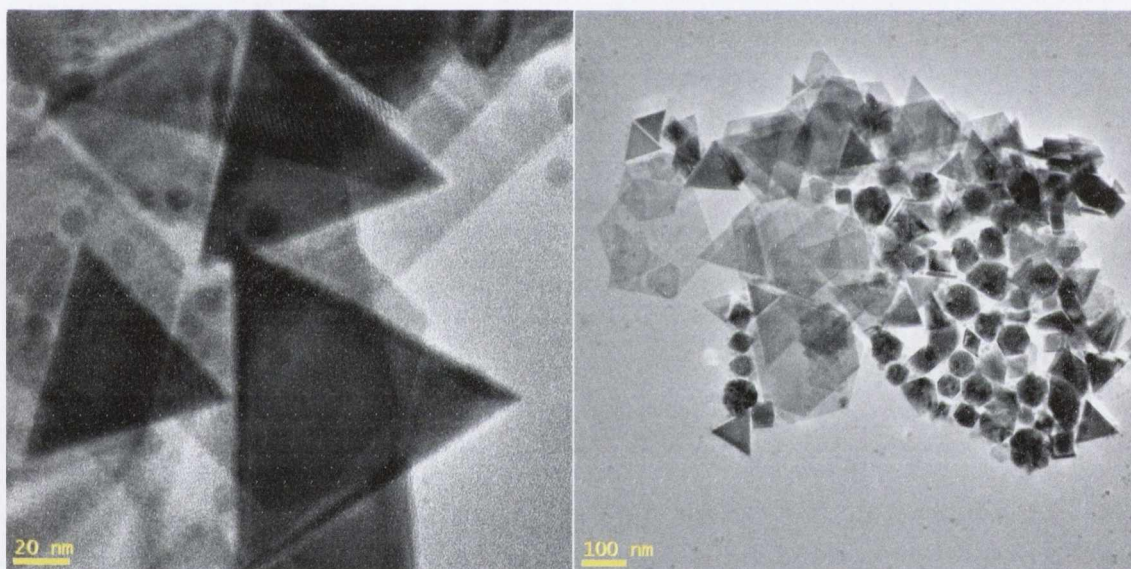


Figure 4.16: TEM images of FeNi nanostructures prepared using a ratio of 1.55/1 Fe/Ni over 4 h (60)

In reactions performed with a 1.55/1 ratio little or no product was obtained for timeframes shorter than 4 h. After this time, anisotropic 2D nanotriangles and hexagons had formed, with high uniformity. EDX showed a ratio of ~5/95 Fe/Ni, hence these nanotriangles are nickel with a small doping of Fe. **(Appendix 4.3.2)** XRD also confirmed the pattern for Ni fcc for this sample. This sample also possesses a high saturation magnetisation ($61.68 \text{ Am}^2\text{kg}^{-1}$), higher than that of bulk nickel ($55.4 \text{ Am}^2\text{kg}^{-1}$).²⁷ This is attributed to the iron doping of the crystal.

With longer reaction times, a new reaction is forming, and a mixture of products after 5 h, **(61)** with excess Fe crystallising into seeds. **(Figure 4.17)** The ratio of Fe/Ni has increased in this reaction to 32/68, as indicated by EDX. **(Appendix 4.3.2)**. Interestingly, the nanoplates are smaller in size (~40 nm). This is most likely due to equilibrium processes in the crystal growth mechanism, and more irregular polygons are observed. XRD confirmed a mixture of products containing the patterns of both $\text{Fe}_{25}\text{Ni}_{75}$ fcc and Fe_2O_3 tet. Peak magnetisation was also considerably lower ($19.78\text{Am}^2\text{kg}^{-1}$) owing to the oxidised iron and polycrystallinity in the sample. **(Appendix 4.3.4)**.

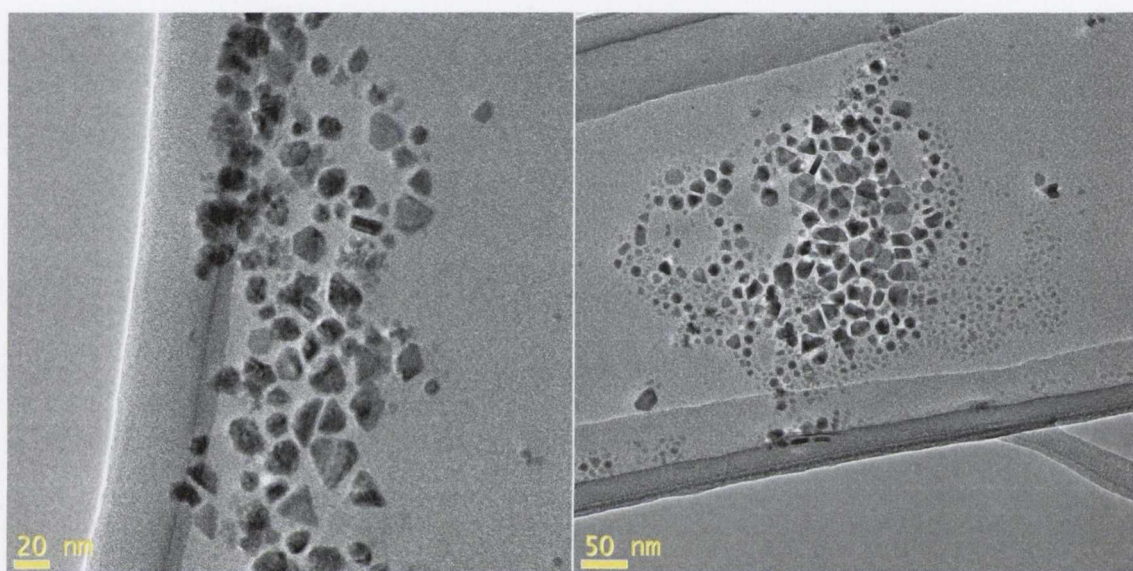


Figure 4.17: TEM images of FeNi nanostructures prepared using a ratio of 1.55/1 Fe/Ni over 5 h (61)

After 24 h **(62)** there is still a mixture of nanostructures present, and the seed particles detected in the 5 h reaction have grown into larger particles. **(Figure 4.18)** EDX for this reaction revealed an overall ratio of 50/50 for this sample, and varied across the sample. XRD, again, confirmed the presence of both $\text{Fe}_{25}\text{Ni}_{75}$ fcc and Fe_2O_3 tet, however the relative intensity for these peaks indicated by a lower contribution of maghemite than seen in the 5 h reaction. This is also indicated in the increase in peak magnetisation ($65.24\text{Am}^2\text{kg}^{-1}$). **(Appendix 4.3.3)**

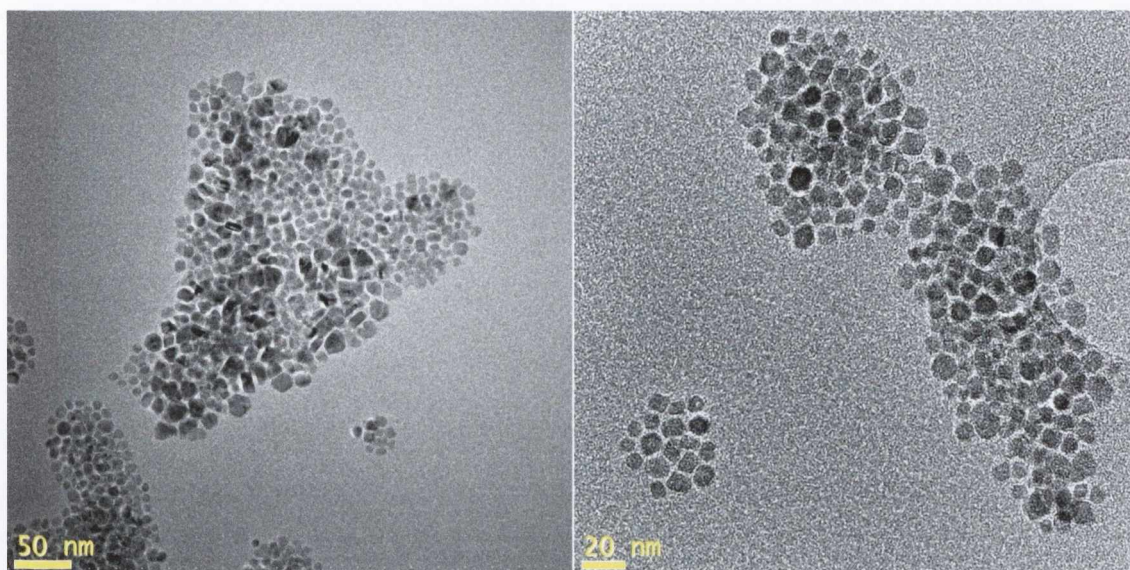


Figure 4.18: TEM images of FeNi nanostructures prepared using a ratio of 1.55/1 Fe/Ni over 24 h (62)

After 5 days **(63)** a more uniform product is seen again, showing anisotropic nanoflakes and icosahedral particles (~ 30 nm). **(Figure 4.19)** EDX showed a ratio of 25/75 Fe/Ni. **(Appendix 4.3.2)** This ratio also corresponds well to the initial starting ratio of 1.55/1, where 1 equivalent of the Fe precursor is used up in the reduction of $\text{Ni(II)Cl}_2 \cdot \text{H}_2\text{O}$. XRD on the product from this reaction confirmed the presence of the $\text{Fe}_{25}\text{Ni}_{75}$ alloy exclusively **(Appendix 4.3.4)** At this point the reaction has gone to completion, with full incorporation of excess Fe(0) into the material. Magnetisation for this sample was also high ($56.57 \text{ Am}^2\text{kg}^{-1}$) and retained no coercivity. **(Appendix 4.3.3)**

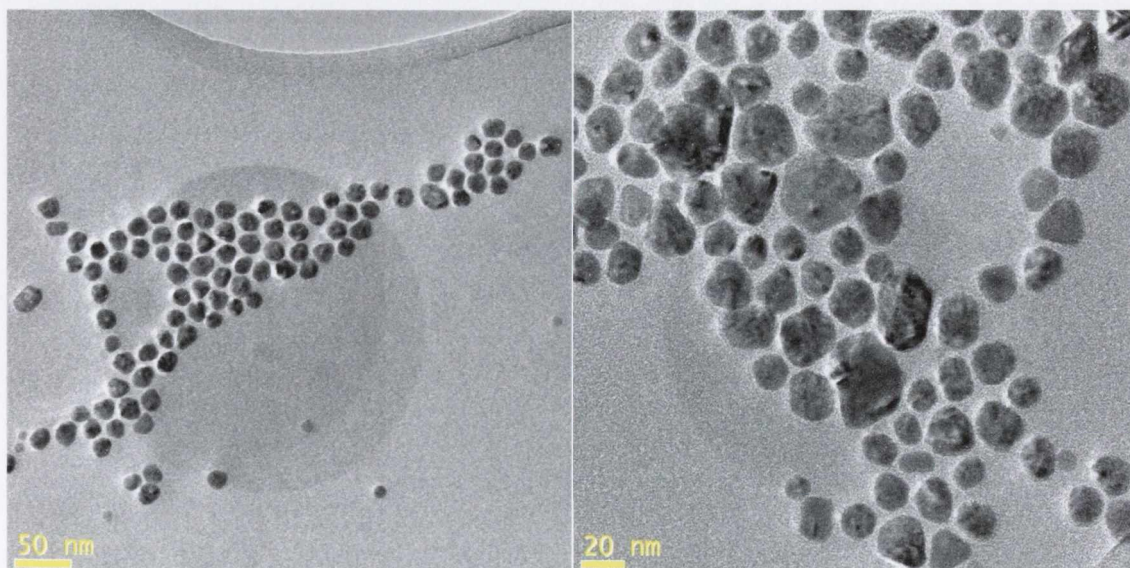


Figure 4.19: TEM images of FeNi nanostructures prepared using a ratio of 1.55/1 Fe/Ni over 5 days (63)

For reactions involving the higher 2.3/1 ratio for Fe/Ni the rate of reaction and extent of Fe incorporation into the crystal differed. After 4 h reaction, the morphology and size of the product is similar to that obtained over 5 days with 1.55/1. **(Figure 4.20)** EDX for this sample shows a ratio of 22.5/77.5 for the sample, close to the ideal ratio of 25/75 and XRD showed the pattern for $\text{Fe}_{25}\text{Ni}_{75}$ fcc crystal structure. **(Appendix 4.3.4)** VSM revealed reasonably high magnetisation, again with no coercivity ($42.91 \text{ Am}^2\text{kg}^{-1}$). **(Appendix 4.3.3)**

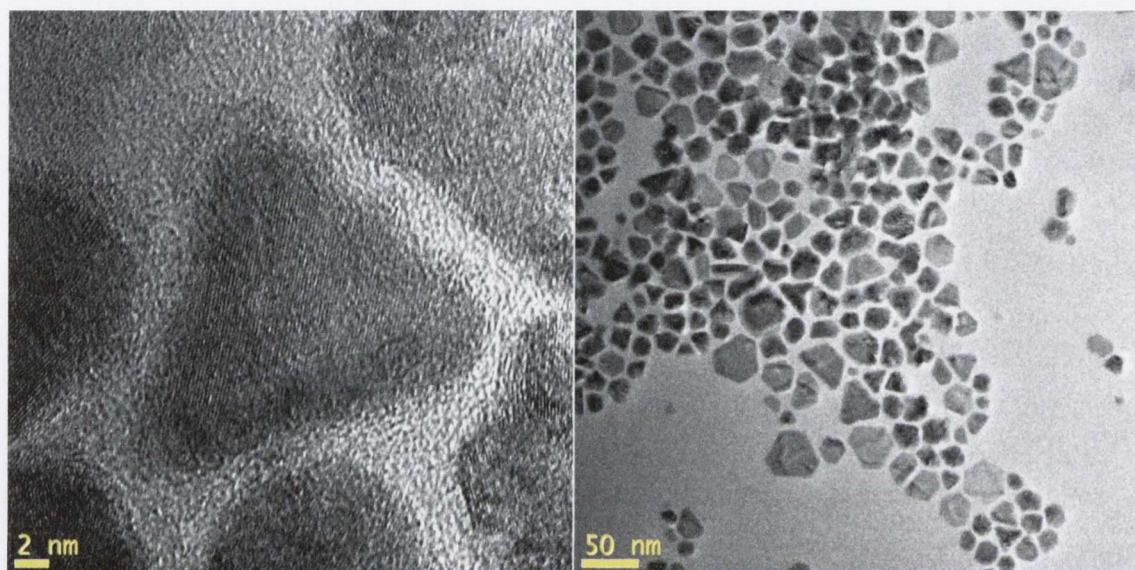


Figure 4.20: TEM images of FeNi nanostructures prepared using a starting ratio of 2.3/1 Fe/Ni after 4 h $\text{Fe}_{22.5}\text{Ni}_{77.5}$ (64)

After 5 h reaction for this 2.3/1 ratio Fe/Ni an increase in width for these nanoflakes ($\sim 50 \text{ nm}$) is seen, and also the presence of smaller nanoparticles. **(65) (Figure 4.21)** EDX showed a ratio of 17.3/82.7 for this reaction. **(Appendix 4.3.2)** XRD showed the patterns for $\text{Fe}_{25}\text{Ni}_{75}$ fcc and Ni hex. This indicates that for this particular ratio of precursors, the rate of $\text{Fe}_{25}\text{Ni}_{75}$ formation is faster than the rate of Ni(II) reduction, as more Ni has been incorporated over this timeframe. **(Appendix 4.3.4)**

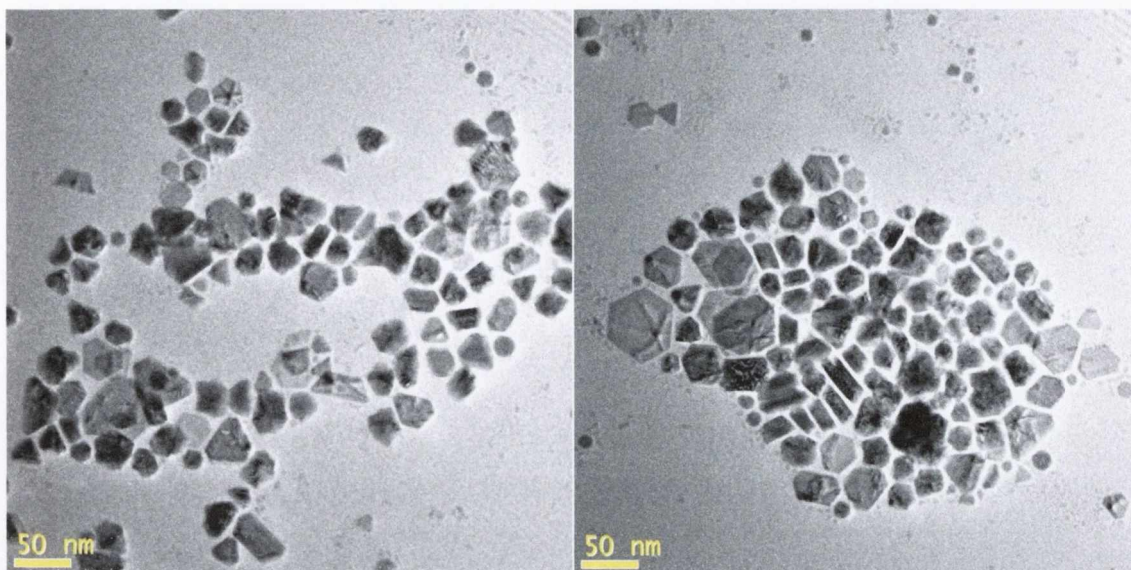


Figure 4.21: TEM images of FeNi nanostructures prepared using a starting ratio of 2.3/1 Fe/Ni after 5 h $\text{Fe}_{17.3}\text{Ni}_{82.7}$ (65)

Similarly to reactions involving the 1.55/1 ratio, after 24 h there is a mixture of products formed for the 2.3/1 ratio. **(Figure 4.22)** In this case, the predominant crystal formed is $\text{Fe}_{50}\text{Ni}_{50}$ as indicated by EDX and XRD with higher incorporation of Fe into the crystals over time. There is also a small presence of oxidised Fe in the form of maghemite (Fe_2O_3 tet). **(Appendix 4.3.4)**. Although not performed, it is anticipated a 5 day reaction would yield a similar progression to that found with the 1.55/1 ratio reaction, and a more uniform $\text{Fe}_{50}\text{Ni}_{50}$ product formed.

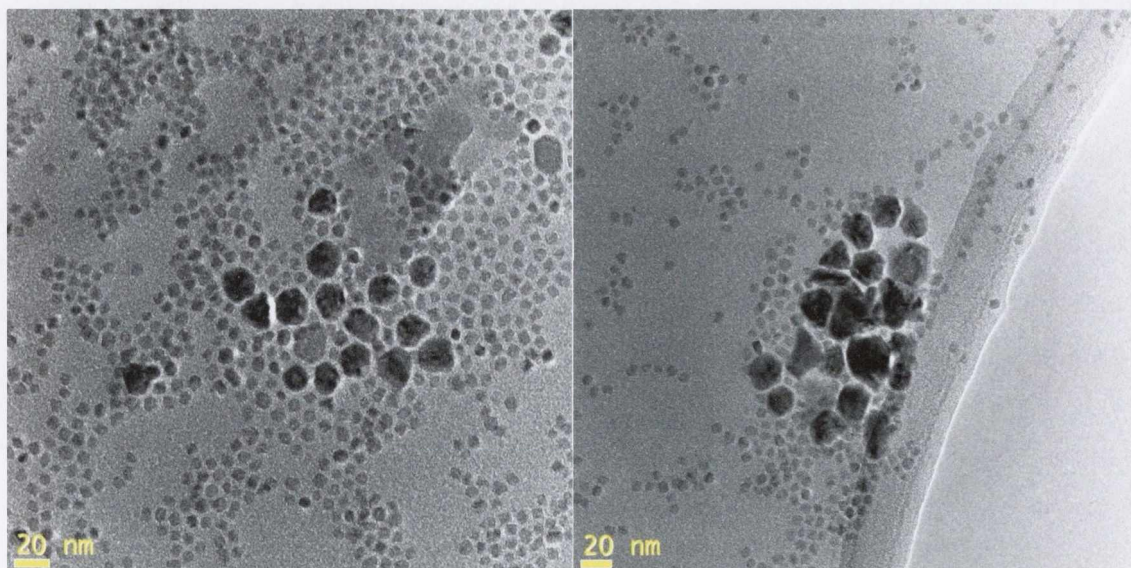


Figure 4.22: TEM images of FeNi nanostructures prepared using a starting ratio of 2.3/1 Fe/Ni after 24 h $\text{Fe}_{50}\text{Ni}_{50}$ (varied) (66)

EDX and XRD revealed a range of alloy compositions were produced, depending on reagent concentrations and reaction lengths. Due to the slower reaction rates and reduction process of this experiment to form the alloy product, the ratios of Fe/Ni were not stoichiometric in the products compared to starting materials. It was found, instead, that the alloy depended on the rate of Fe incorporation relative to the rate of Ni reduction. As expected, higher Fe incorporation was found for increasing Fe concentration. However, in higher concentration experiments, Ni incorporation initially increased due to the competing rate of Ni reduction.

It was found that Fe-doped nickel triangle/hexagon nanosheets could be produced with a ratio of 1.55/1 for Fe/Ni after 4 h reaction at 160°C. It was also found that Fe₂₅Ni₇₅ nanopolygons could be produced with increasing time and/or Fe precursor. The syntheses producing Fe₂₅Ni₇₅ have similar morphology to the particles developed by Chen *et al*²⁸ and McNerny *et al*¹⁸ using acetylacetonate precursors. A synthesis involving nickel formate, oleic acid and oleylamine has also been developed and reported by Leng *et. al.* producing Ni particles with a similar morphology to the first sample, (60) however this involves differing reaction conditions.^{25, 26}

VSM studies revealed good magnetic properties with high peak magnetisation and superparamagnetic behaviour for these samples, without the need for annealing. This is a significant achievement, as they can retain their anisotropic structure, which would otherwise be lost through the annealing process. As a result, these FeNi nanostructures are good candidates for future magnetic based anisotropic biodetection systems.

In each sample, FTIR showed characteristic peaks for the binding of OAm to the surface, with the amine coordinated to both Fe and Ni. (**Appendix 4.3.5**) Also UV-vis spectroscopy revealed broad absorbance in the region of 280-540 nm for each of the samples, increasing towards ultraviolet similar to conductive FePt NWs. (**Figure 4.23**) Hence, these materials can also be detected in the visible region, and fluorophores should be targeted to lower energy region of the visible spectrum. (600 -1000 nm)

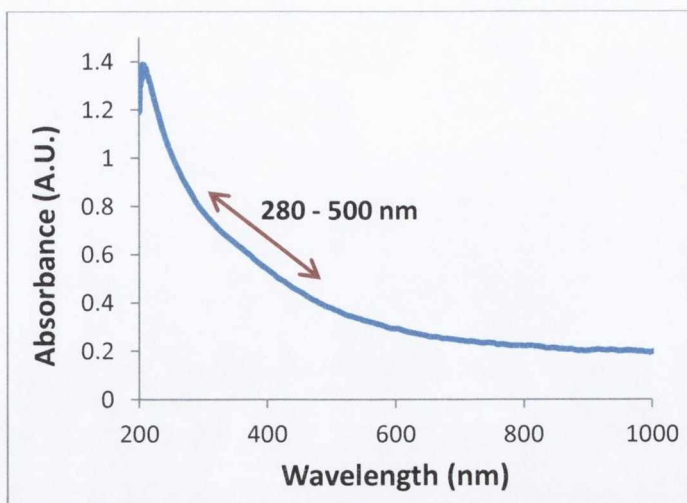


Figure 4.23: UV-vis absorption spectrum of Fe₂₅Ni₇₅ nanoplates in hexane (63)

4.5 IRON-COPPER NANOPLATES

Copper alloys represent a very interesting class of materials due to their plasmonic properties and cheaper cost relative to Au and Ag. Other methods exist in the literature for the production of Cu triangles and hexagons by surfactant assisted hydrothermal routes.²⁹⁻³¹ However, to our knowledge, this is the first reported synthesis of Fe-doped Cu nanopolygons.

Copper is a well-documented plasmonic material. In copper sulphide (CuS) structures both fluorescent and plasmonic behaviour can be observed under the correct conditions.³² Here, for copper-iron alloy, it was hoped to instead achieve both magnetic and plasmonic activity for dual functionality within the material. This could potentially be achieved through soft magnetic behaviour, as the electron gas is free to oscillate when not in the presence of a magnetic field.

Iron-copper alloy experiments were performed using similar conditions to FeNi experiments. E° for the reaction $\text{Cu}^{2+} + 2 e^- \rightleftharpoons \text{Cu}$ is 0.3419V, hence it was anticipated that this reaction would proceed much quicker than that of FeNi. The experiment was therefore performed under the same timeframe as that of Pt, due to the redox potential of the copper precursor. This reaction was successful in producing anisotropic structures for both 2.9/1 **(67)** (**Figure 4.24**) and 23.3/1 **(68)** (**Figure 4.25**). Fe/Cu starting ratios, and both reactions showed similar morphology, with slightly higher uniformity in the 23.3/1 Fe/Cu experiment. They also showed a similar diameter of ~80 nm to FeNi **(60)**

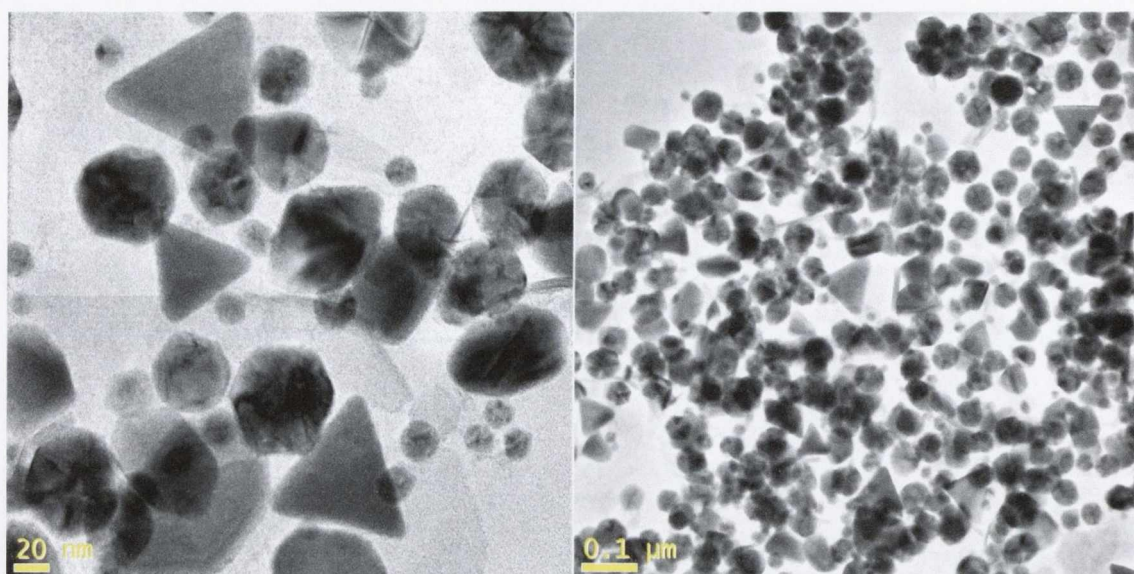


Figure 4.24: TEM images of FeCu nanostructures prepared using a starting ratio of 2.9/1 forming $\text{Fe}_7\text{Cu}_{93}$ (67)

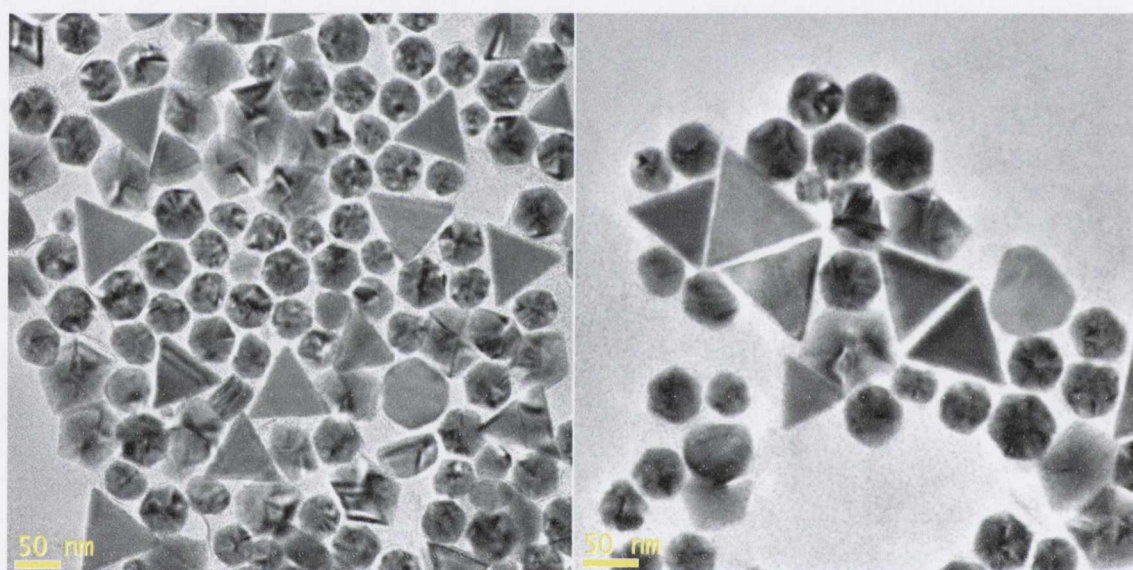


Figure 4.25: TEM images of FeCu nanostructures prepared using a starting ratio of 23.3/1 forming $\text{Fe}_4\text{Cu}_{96}$ (68)

These products are very similar in morphology and size to the FeNi products which contained nanopolygons, where the sample had low Fe incorporation in the product. (60) EDX revealed they also contained a low content of Fe. (Appendix 4.4.2) XRD confirmed the crystal structure for fcc Cu in both samples, with a small presence of CuO for the higher Fe ratio experiment. (Appendix 4.4.3) It was also found that if left too long at 160°C before addition of the Fe precursor, the $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ would begin to react spontaneously, forming nanoparticles. This stands to reason as $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ has a

decomposition temperature of 100°C. Even with the addition of an increased amount of Fe, Fe₄Cu₉₆ was still formed, which actually has a lower Fe content. This would suggest that this particular crystal form is the most stable, kinetic and readily formed product under these conditions.

UV-*vis* spectroscopy revealed plasmonic behaviour with absorbance in the visible region at ~700 nm for both samples. **(Figure 4.26, 4.27)** The first sample with lower Fe ratio, and more irregular morphology exhibited a blue colour to the eye and had multiple absorbance peaks, with absorbance at 500 nm and 365 nm also, due to structural anisotropy in the material and also possibly due to plasmonic interaction from the smaller icosahedral particles in the sample. The second sample exhibited a green colour to the eye due to decreased relative absorbance in this spectral region. This also exhibited an extra plasmon peak at 336 nm due to structural anisotropy. In this case, the additional peak has a higher relative intensity due to higher contribution from the narrow axis. Hence, both of these products can be used for visible detection methods. The shape and differential plasmonic interaction in these samples could potentially be exploited for biomolecule detection and sensing using IR and Raman spectroscopy by plasmon resonance enhancement.

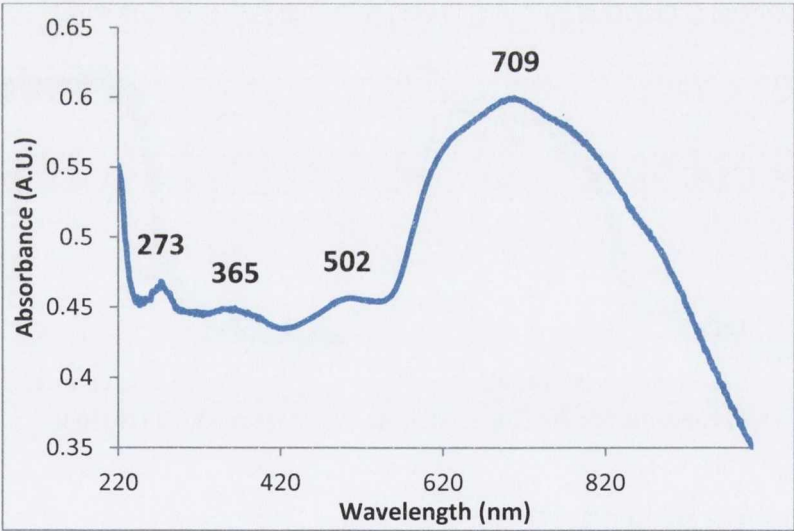


Figure 4.26: UV-*vis* absorption spectrum for FeCu nanostructures prepared using a starting ratio of 2.9/1 in hexane (67)

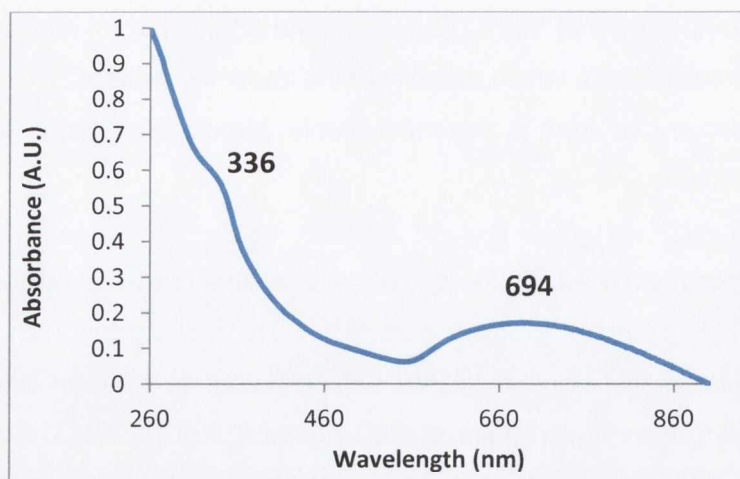


Figure 4.27: UV-vis absorption spectrum for FeCu nanostructures prepared using a starting ratio of 23.3/1 in hexane (68)

VSM analysis showed some magnetic behaviour in these samples. **(Figure 4.28)** This is attributed to the Fe doping in the samples. Interestingly, the sample containing less Fe, produced magnetisation values an order of magnitude higher. This reaction **(68)** involved a higher starting concentration of Fe, which may have led to more efficient and complete incorporation into the structure. FTIR analysis for these samples also showed the presence of the OAm ligand on the surface **(Appendix 4.4.4)** Hence, these novel magnetic, plasmonic nanoplates have great potential for new detection applications.

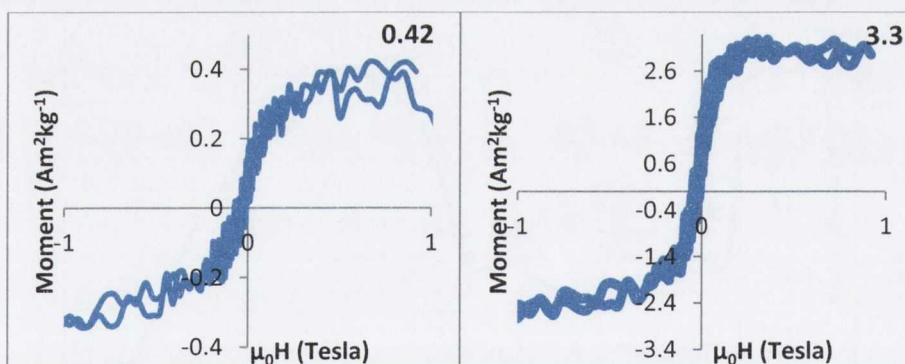


Figure 4.28: VSM curves for FeCu samples 67 (left) and 68 (right)

4.6 COPPER SULFIDE NANOPLATES

A series of sulfide semiconductor nanomaterials were synthesised to test the hypothesis that the reaction conditions, involving time, temperature, and the OAm surfactant contributed to the anisotropic growth of this series of nanomaterials. This series of reactions to produce semiconductor crystals proceeds *via* a slightly different mechanism than the alloy based series, whereby S is first reduced by OAm from its $S(0)$ to $S(-2)$ state, which then either pairs as a salt to the cationic metal or reduces the salt then pairs as a salt crystal.

The process of sulphur-based semi-conductor formation involves, firstly the reduction of zero valent elemental sulphur to S^{2-} . This most likely occurs through harvesting electrons from the amino group on OAm, oxidising it to the primary imine and possibly again to the nitrile to produce H_2S twice, which then reacts with the metal precursor to form the semiconductor salt crystal. Below is a possible mechanism for the sulphur reduction reaction. (**Figure 4.29**)

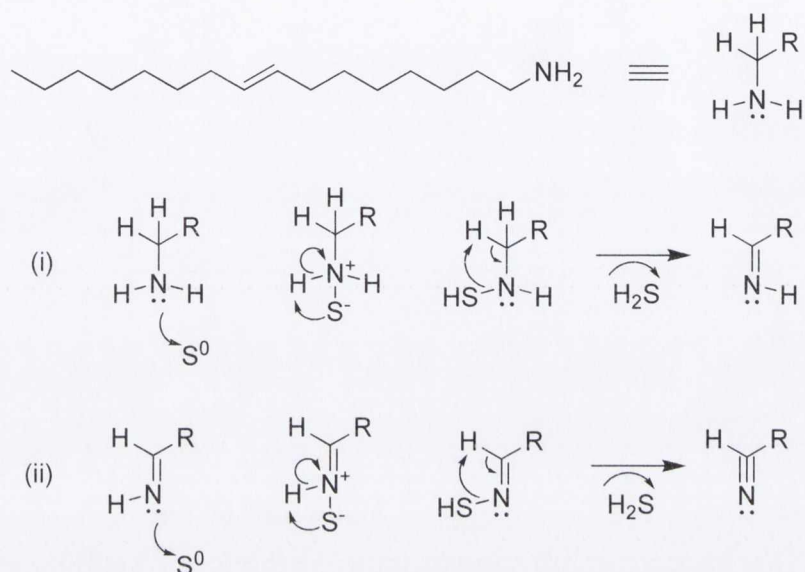


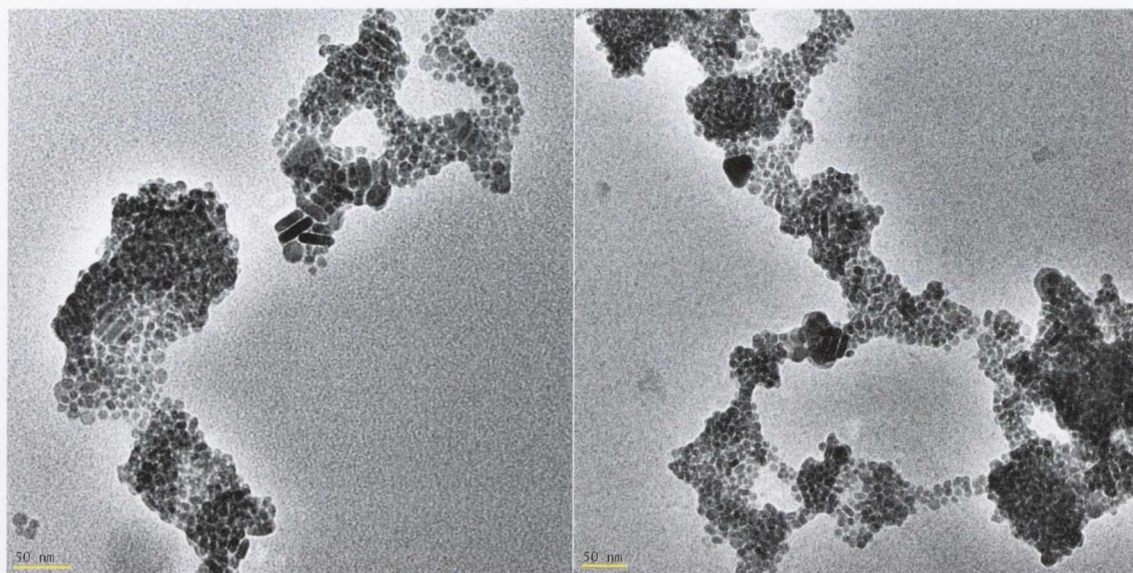
Figure 4.29: Proposed Mechanism for the Reduction of Elemental Sulfur to S^{2-}

This reaction has not been previously reported however oxidative dehydrogenation of amines³³⁻³⁵ oxidation of benzylamine using elemental sulphur³⁶ and general dehydrogenation of organic compounds with sulfur³⁷⁻³⁹ has been previously reported. In this research, a reaction was performed containing only sulphur and OAm. FTIR for this revealed the presence of imine peaks for a reaction in the ratio of 1/4 for S/OAm. (**Appendix 4.11.6**)

Copper sulfide exhibits both fluorescent and plasmonic properties.³² Thus it can be used for visible detection by either fluorescence measurements or absorbance. This material has been widely documented in the literature due to its reduced cost and toxicity relative to other fluorescent semiconductor nanocrystals.^{40, 41}

Here, in the first experiment, elemental sulphur was added to the reaction as solid, to a dispersion of $CuCl_2 \cdot 2H_2O$. In this reaction mostly nanoparticles (~ 10 nm) were formed with a number of rod structures. (**76**) (**Figure 4.30**) All subsequent experiments involving sulfur based materials were performed by the initial dissolution of sulphur, with hot injection of the metal salt precursor at the reaction temperature. This modification was

performed in order to ensure good dissolution of the reagents, pre-reduction of sulphur, create more uniformity in the conditions, and avoid side reactions with competing kinetics.



Scale Bar: 50 nm

Scale Bar: 50 nm

Figure 4.30: TEM images of CuS nanostructures produced by addition of elemental sulfur to a solution of CuCl_2 in OAm (76)

In the second reaction, a solution of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ in NMP was added by hot-injection to a solution of sulphur in OAm at 160°C . This reaction structure avoided the premature decomposition of the copper chloride precursor at 100°C , and allowed time for the pre-reduction of sulphur into S^{2-} . In this reaction, 2D structures were formed, consisting mainly of hexagons with a width of 5 nm and diameter of ~ 60 nm. (77) (Figure 4.31)

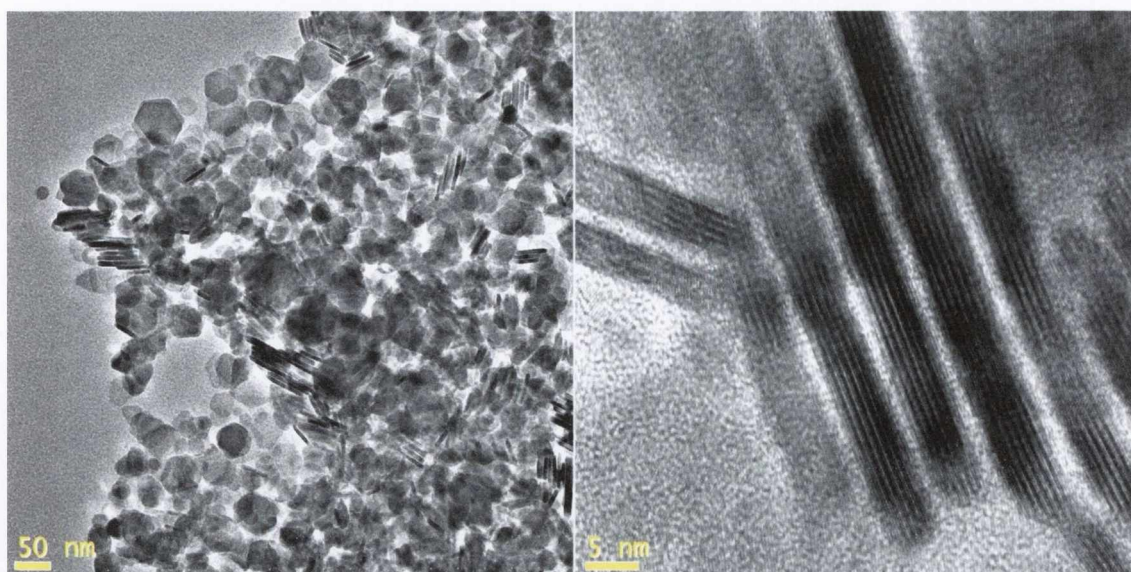


Figure 4.31: TEM images of Cu_2S nanostructures produced by hot injection of CuCl_2 in NMP to a solution of sulfur in OAm (77)

XRD analysis showed two different crystal structures for these compounds. This is most likely due to the order of addition of the reagents in the experiments. In the first reaction where solid elemental sulfur was added to the Cu(II) salt solution, Cu_2S bco nanocrystals were formed. (Figure 4.32) In this reaction, both Cu and S are reduced to form the $(\text{Cu}^+)_2\text{S}^{2-}$ crystal.

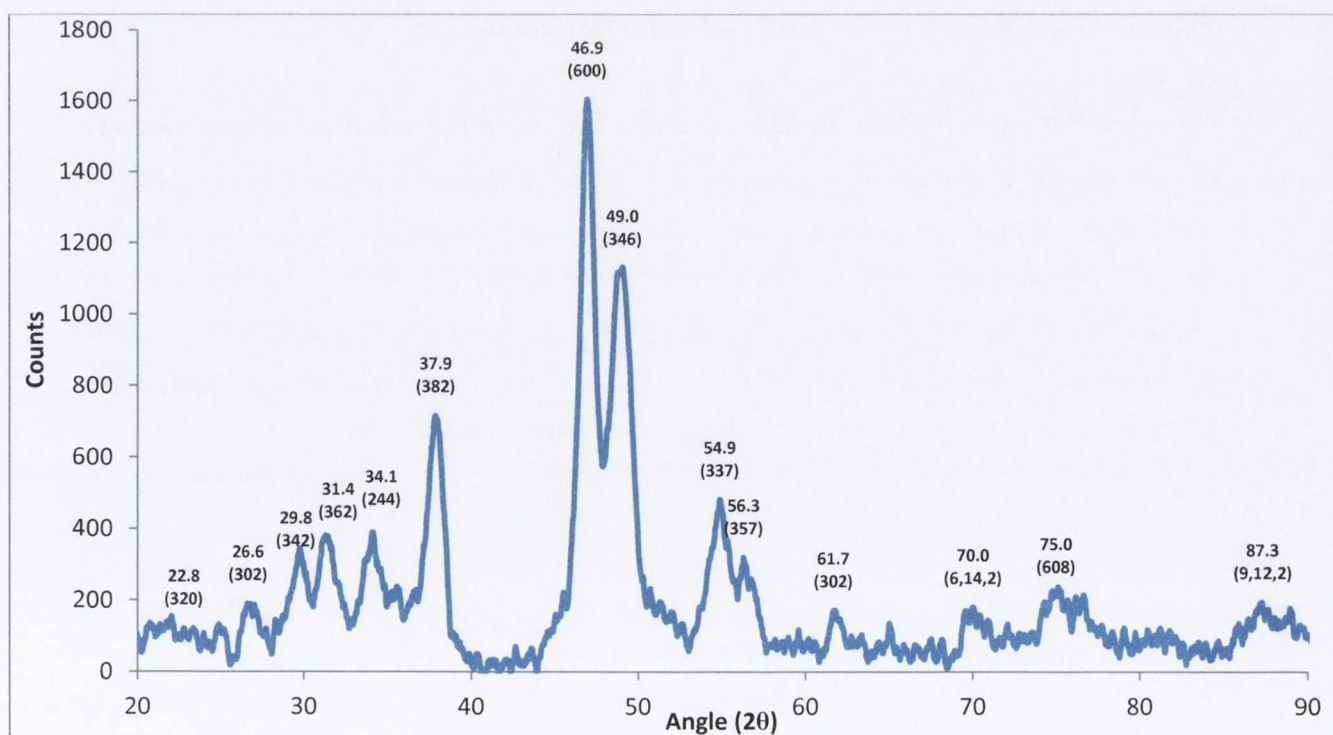


Figure 4.32: XRD pattern for Cu_2S bco nanostructures (76)

In the second reaction, with hot injection of the Cu^{2+} salt into the S-OAm solution, CuS hex nanoplates were formed, as shown by XRD. **(Figure 4.33)** In this reaction, sulfur is pre-reduced by OAm, and pairs to the Cu(II) cation which is not further reduced. The difference in crystal structure between these two reactions highlights the importance of the order of addition of reagents in these reactions, and also the competition between the rates of reduction for the precursors and the crystal formation kinetics.

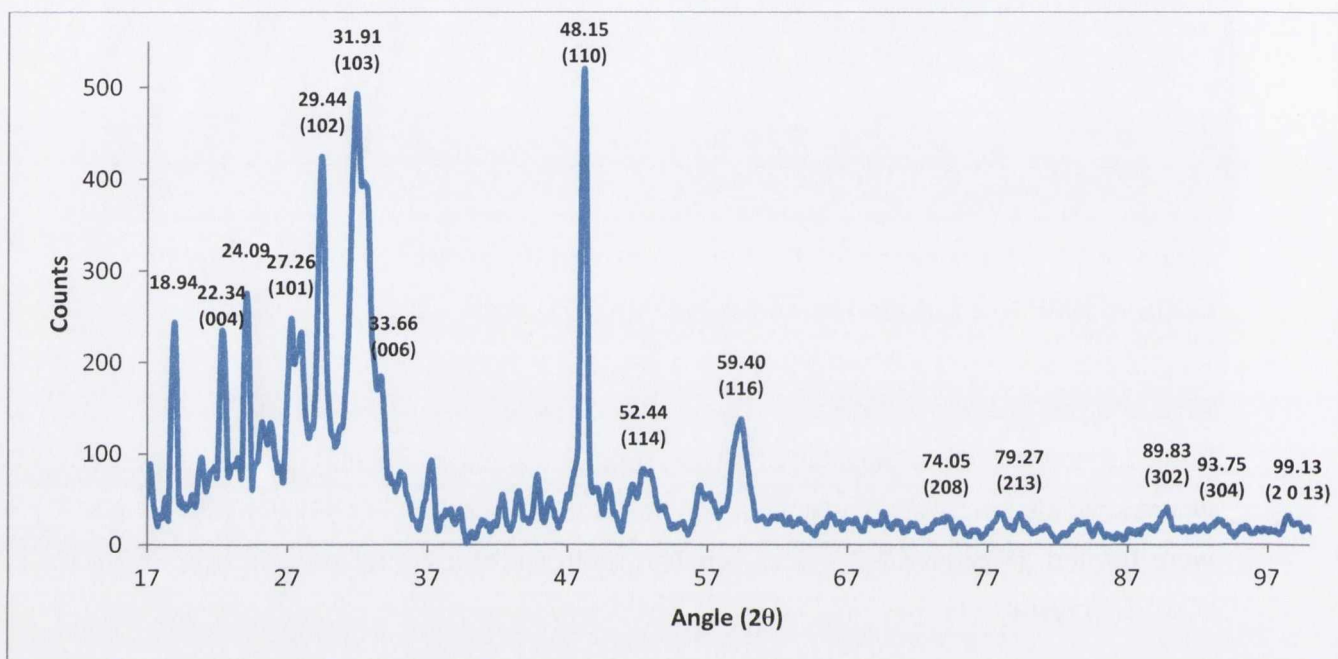


Figure 4.33: XRD pattern for CuS hex nanostructures (77)

UV-vis and PL spectroscopy studies confirmed both fluorescence and plasmonic activity in the samples. **(Figure 4.34, 4.35)** Emission for both of these materials was in the region of 350 nm. One plasmon peak was detected for Cu_2S nanoparticles at 504 nm, and two for CuS nanoplates, owing to their structural anisotropy; the first at 405 nm and the second at 971 nm extending into the IR region. Future work will involve more detailed fluorescence and plasmon analysis. FTIR confirmed the presence of the expected peaks for OAm bonded to the surface in both samples. **(Appendix 4.5.2)**

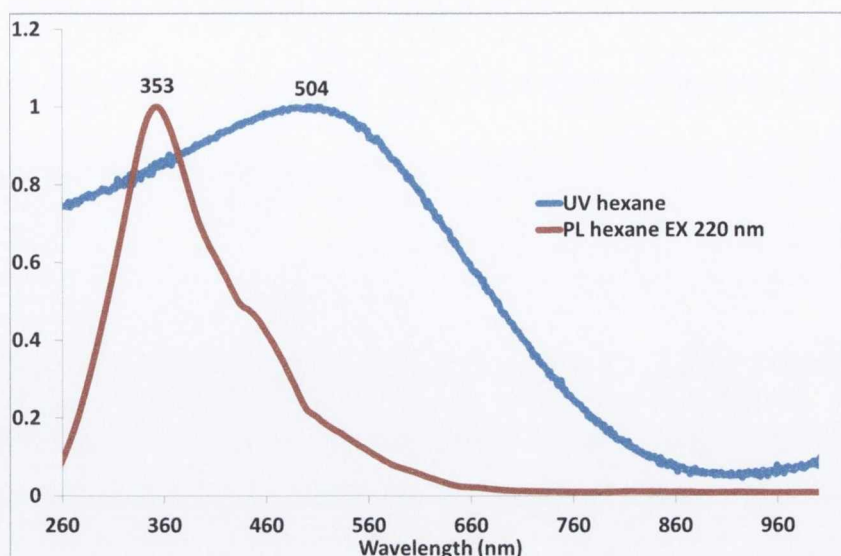


Figure 4.34; UV/PL absorption and emission spectra for Cu₂S in hexane (76)

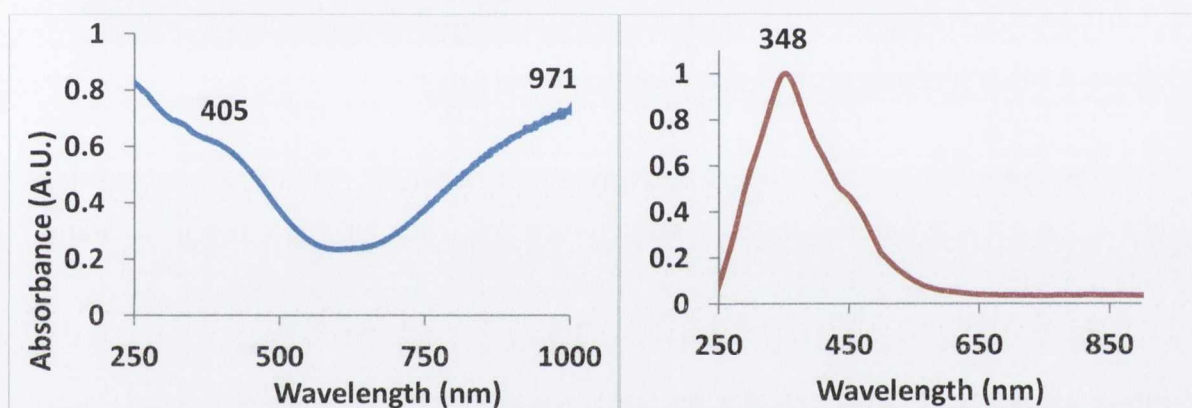


Figure 4.35: UV-vis (left) and PL (right) absorption and emission spectra for CuS nanoplates in hexane (77)

4.7 BISMUTH SULFIDE NANOPLATES

Bi₂S₃ has a low band gap compared to other semiconductors. Hence it has useful conducting properties. It has also been shown to have excellent photocatalytic properties, due to electron transfer.⁴² E° for the reaction $\text{Bi}^{3+} + 2\text{e}^- \rightleftharpoons \text{Bi}^+$ is 0.2V and E° for the reaction $\text{Bi}^{3+} + 3\text{e}^- \rightleftharpoons \text{Bi}$ is 0.308V. Hence, both OAm and Bi³⁺ can potentially reduce S⁰ to S²⁻. Bi₂S₃ forms an fcc crystal structure. Bi(III)Cl₃ was used as the precursor. In this crystal the valency of bismuth should remain as 3+. The ratio of Bi and S precursors used, however, were 2/3 respectively and this ratio is retained in the semiconductor product.

In this reaction hexagonal discs were formed. TEM revealed monodisperse nanopolygon structures. (78) (Figure 4.36) Other methods exist in the literature for the production of Bi₂S₃ nanorods, nanowires, nanoflakes, nanotubes and nanoflowers.⁴²⁻⁴⁸ However, to

our knowledge, this is the first reported synthesis of Bi_2S_3 nanopolygons of this uniformity, size and shape, using this particular method (diameter 60 nm, width 12 nm).

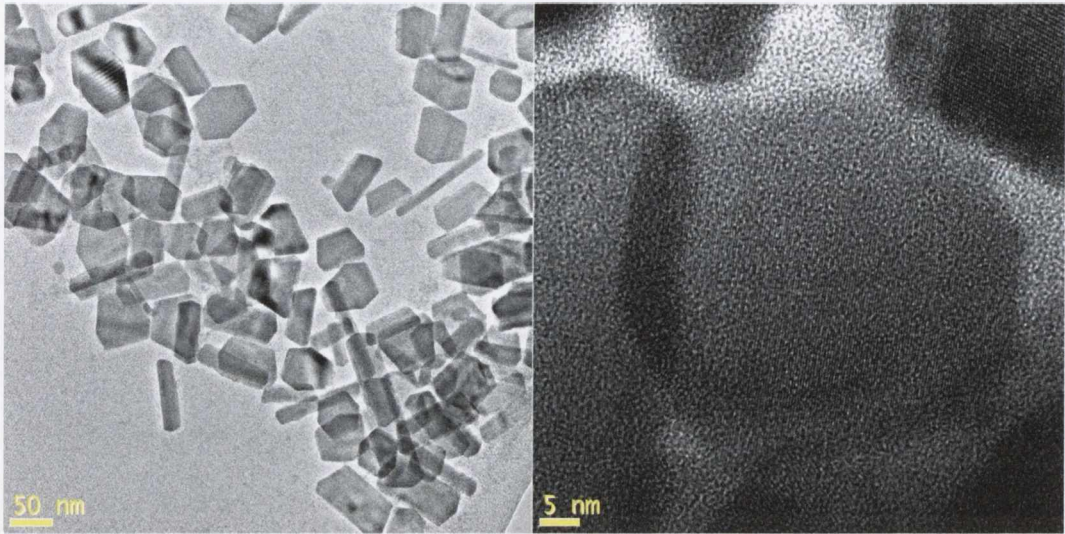


Figure 4.36: TEM images of Bi_2S_3 nanopolygons (78)

EDX showed a ratio of $\text{Bi}_{32.5}\text{S}_{67.5}$. (Appendix 4.6.2) However, the crystal structure for orthorhombic Bi_2S_3 , which is the only structural conformation for this material, is clearly visible by XRD. (Figure 4.37)

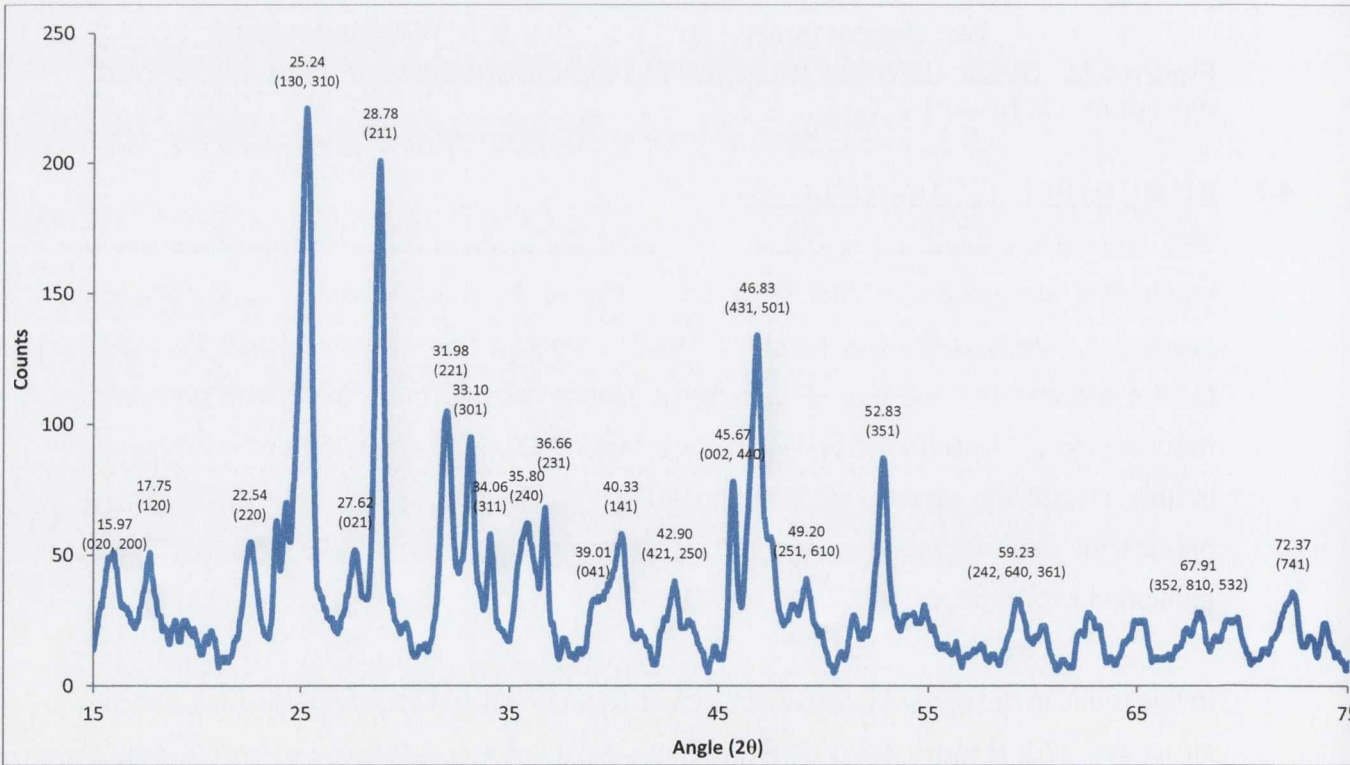


Figure 4.37: XRD pattern for Bi_2S_3 orthorhombic crystal structure (78)

UV-vis and PL spectroscopy revealed a broad absorbance for these nanostructures across the visible spectrum and increasing towards the UV region of the spectrum, with a shoulder absorbance at 400 nm. This could potentially be plasmon resonance absorption, however more detailed further analyses are necessary. Increasing absorbance towards UV is characteristic of a conducting material. The interaction with electromagnetic energy in the visible region allows us to detect these particles by scattering and absorbance measurements also. There is a lower absorbance present towards the red region of the spectrum. This could also allow us to detect moieties at the surface using fluorophores for this region. As with copper sulfide samples; fluorescence in Bi_2S_3 nanoplates exhibited emission in the region of 350 nm. (Figure 4.38) FTIR also showed the expected peaks for OAm coordinated to the surface. (Appendix 4.6.3)

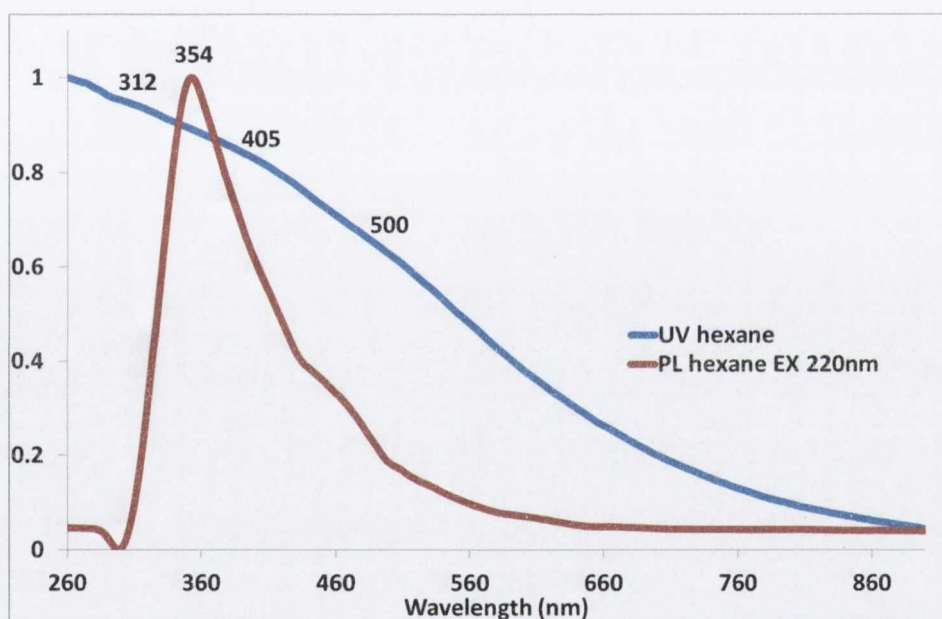


Figure 4.38: Normalised UV/PL absorption and emission spectra for Bi_2S_3 nanoplates in hexane (78)

4.8 NICKEL SULFIDE NANOSTRUCTURES

As a semiconductor, NiS can potentially form fluorescent nanoparticles in sufficiently small, high crystalline samples.^{49, 50} In addition, due to the nickel content, this material could also potentially be magnetised in the presence of a magnetic field, particularly for nickel-rich compositions where there is a greater number of valence electrons free to oscillate/fluctuate and interact with electromagnetic fields; however this magnetisation is generally small for nickel sulfide.⁵¹ It can also display paramagnetic behaviour similar to nickel at room temperature.⁵² Nickel sulfide is also a birefringent material (birefractive). This property could also be exploited for detection applications. In these types of

materials, incident light is split and re-emitted as two different unequal frequencies. This splitting and re-emission could be used to enhance signal detection at those wavelengths, similarly to plasmonic signal enhancement.⁵³

Two experiments were performed with a fixed starting ratio of 1/1.54 for Ni/S and temperature of 160°C, over two timeframes, 30 min **(79)** and 2 h **(80)**. NiS nanopolygons and nanoflakes were prepared with good uniformity in each of the products. The reaction over 30 min produced nanopolygons (~60 nm). **(Figure 4.39)** The reaction for a longer timeframe produced shapes which lost their polygonic nature and became smaller (~ 30 nm) and more irregular in their edges; however they still exhibited anisotropy in their structure. **(Figure 4.40)** This change in structure is most likely due to Ostwald ripening during the reaction. Another synthesis has been recently reported for the synthesis of rods and 3D pyramids, using OAm, OA, HDD, DDT and two salt precursors NiCl_2 and $\text{Ni}(\text{acac})_2$.⁵³ However, these differ to the methods and products reported here.

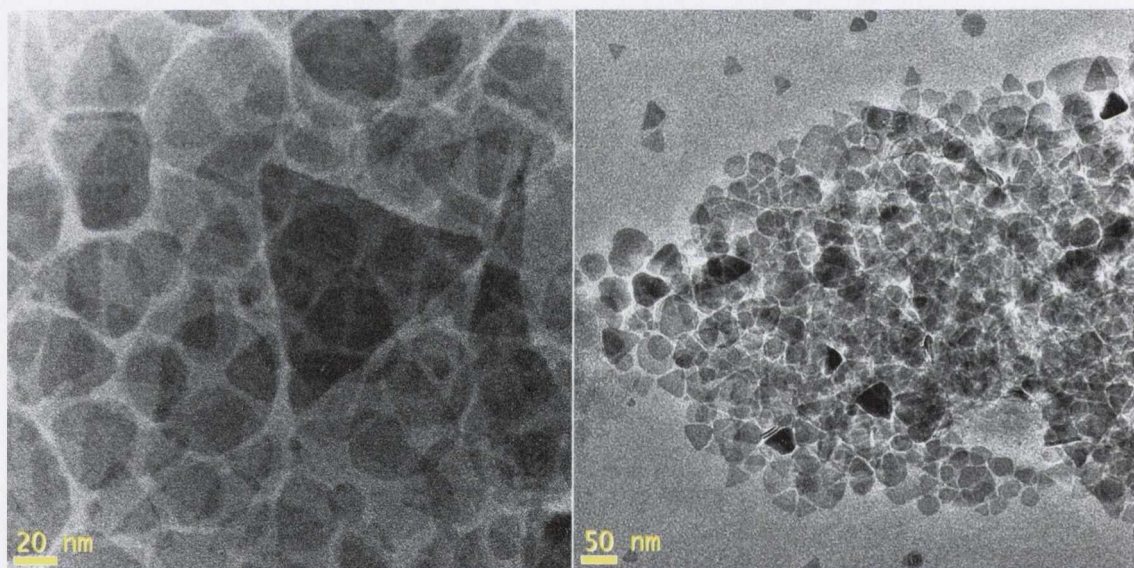


Figure 4.39: TEM images of NiS 60 nm nanostructures formed over 30 min (79)

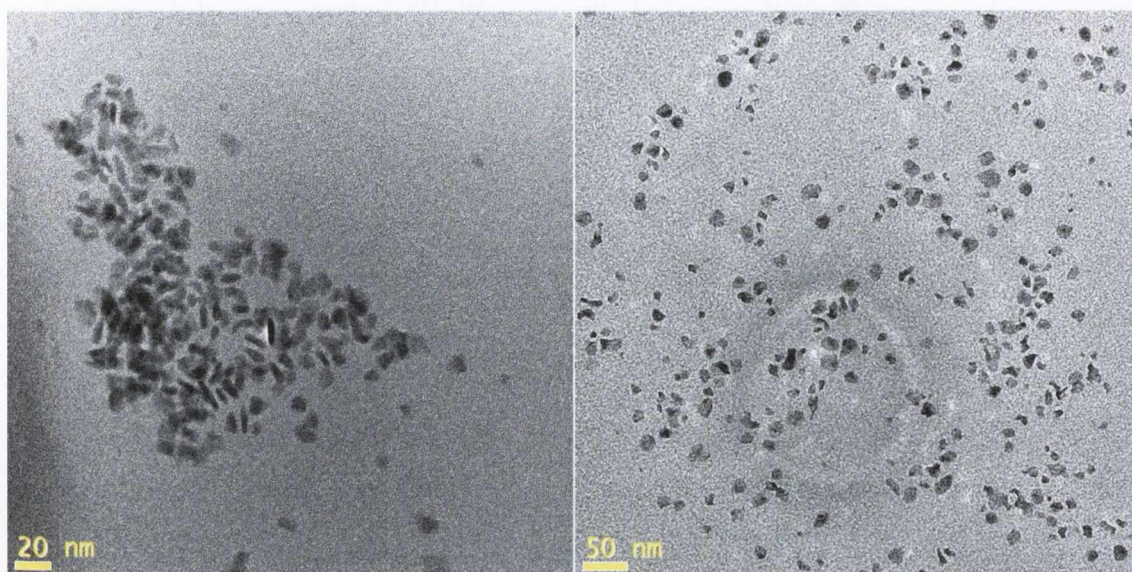


Figure 4.40: TEM images of NiS nanostructures formed over 2 h (80)

EDX showed an elemental ratio of $\sim\text{Ni}_{50}\text{S}_{50}$ for these two experiments. (**Appendix 4.7.2**) XRD showed a Ni_3S_4 fcc crystal structure for these samples. (**Figure 4.41**) There is a difference in the relative intensity profile for these patterns which is most likely due to the differences in their shapes, with the reaction formed over 30 min exhibiting much higher anisotropy in its shape and increased intensity of the (004) parameter. In (80) new lattice parameters are visible corresponding to (111) and (022).

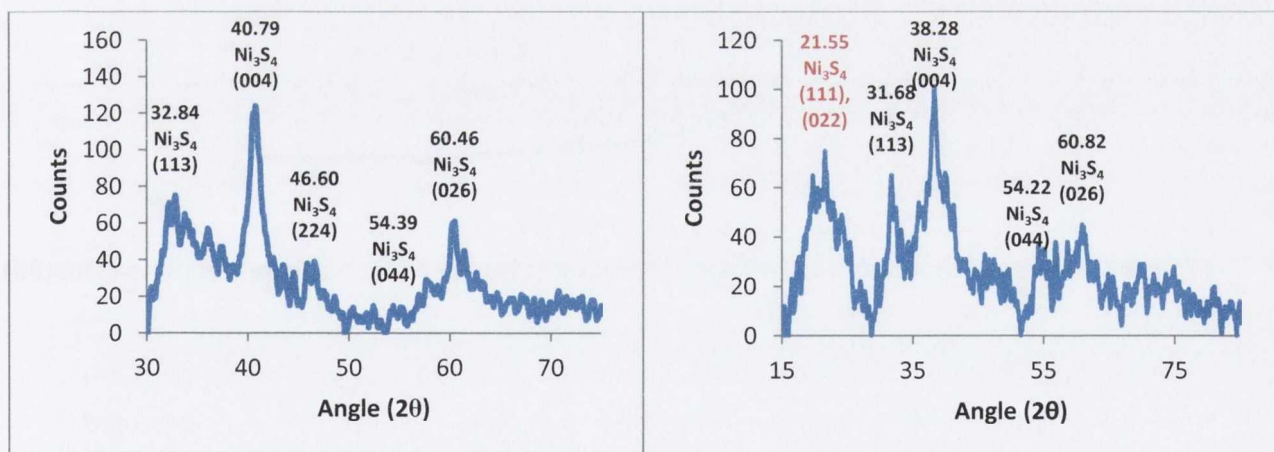


Figure 4.41: XRD patterns for sample 79 (left) and 80 (right) Ni_3S_4 fcc nanostructures

UV-vis for these samples showed an absorbance shoulder at ~ 340 nm for both samples. Emission in these materials is red shifted, approaching visible compared to the other sulfide samples, with emission at 360 nm and 375 nm respectively. (**Figure 4.42, 4.43**). The sample showing higher structural anisotropy (79) also shows an additional

absorbance peak at 300 nm, which may be due to plasmonic or birefringent interactions. Future work will investigate this further.

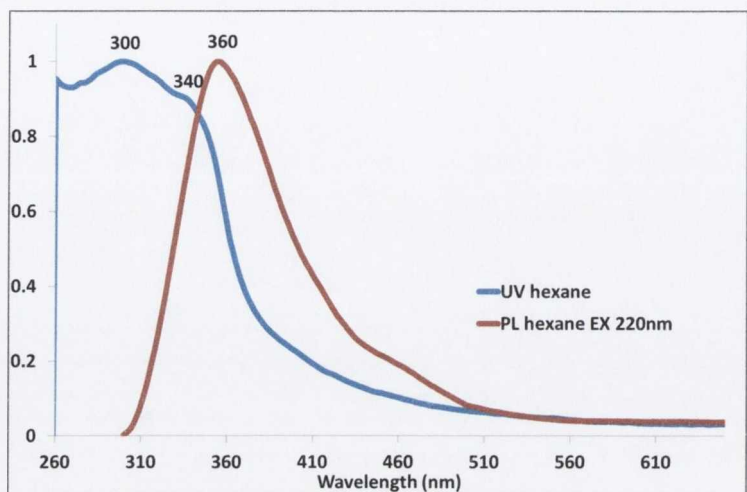


Figure 4.42: Normalised emission/absorption spectra for Ni_3S_4 nanopolygons in hexane (79)

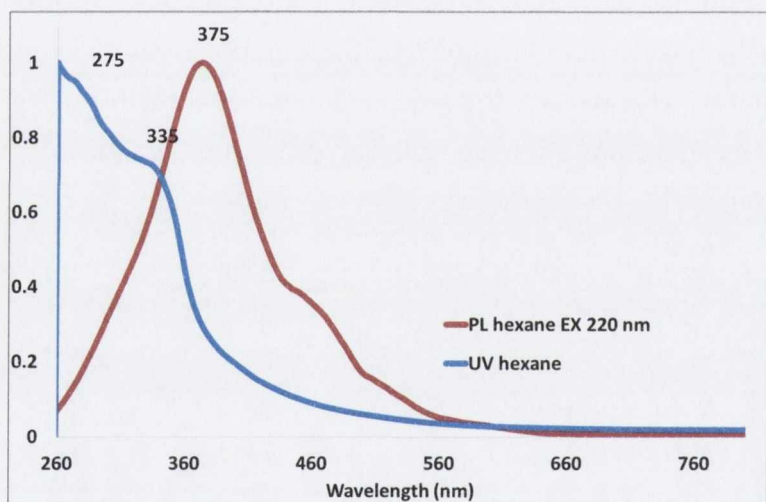


Figure 4.43: Normalised emission/absorption spectra for Ni_3S_4 nanoflakes in hexane (80)

VSM studies revealed some magnetic behaviour in these samples. **(Figure 4.44)** Saturation magnetisation values are low in these samples; however, this is as expected due to the restrictive binding in the ionic semiconductor crystal, leading to reduced spin alignment in the conduction band valence electrons. FTIR analysis confirmed the expected peaks for oleylamine coordination at the surface. **(Appendix 4.7.3)**

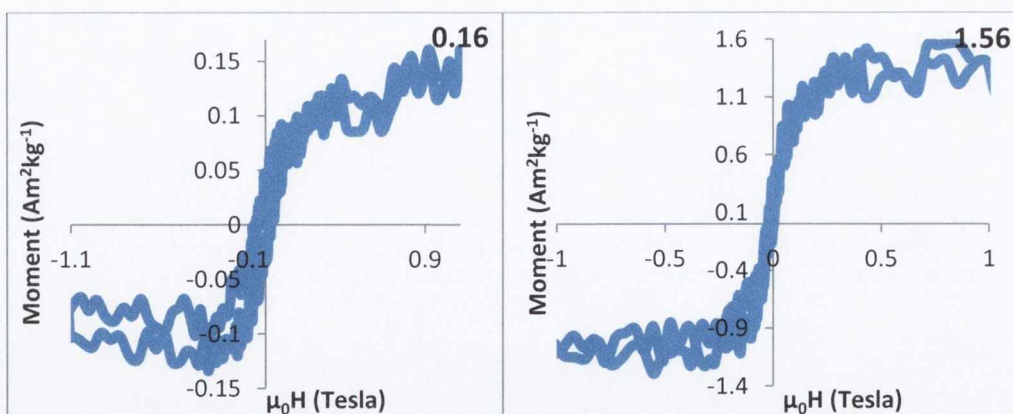


Figure 4.44: VSM curves for Ni_3S_4 nanostructures formed over 30 min (left) and 2 h (right)

4.9 IRON SULFIDE NANOSTRUCTURES

Fe_3S_4 nanosheets have been previously prepared by a hydrothermal route.^{54, 55} Nanoplatelets of iron sulphide have also been produced by organic phase synthesis, however these syntheses did not employ OAm as a stabiliser or reagent.⁵⁶ FeS_2 pyrite nanocubes have been prepared using a mixture of OAm and other solvents.⁵⁷ Like nickel sulfide, iron sulphide can be magnetised in the presence of a magnetic field.⁵⁸ Fe_3S_4 nanoparticles have also been successfully used in cancer hyperthermia treatments due to their magnetic properties.⁵⁹ This material is structurally and electronically similar to Fe_3O_4 . Thus, this material has already proved useful in bioapplications.

FeS experiments were performed using both Fe(II) and Fe(III) salts. The reaction with Fe(II) produced thin nanothreads, **(81)** (Figure 4.45) whereas reactions with Fe(III) produced wider nanobelts over 30 min. **(82)** (Figure 4.46) and nanosheets over 60 min **(83)** (Figure 4.47). Fe_3S_4 fcc, like Fe_3O_4 , has a mixed valence state for Fe. Thus, some Fe(III) must be reduced in the reaction to form this crystal. This could also be a contributing factor in the differences in growth and therefore morphology for reactions of Fe(II) and Fe(III) precursors. An increase in time from 30 min to 1 h also produced wider nanosheets in the form of rectangular flakes, but with otherwise similar morphology to the 30 min sample. These nanobelts also showed an enhanced uniformity in size compared with previously reported similar Fe_3S_4 materials reported in the literature.⁵⁹

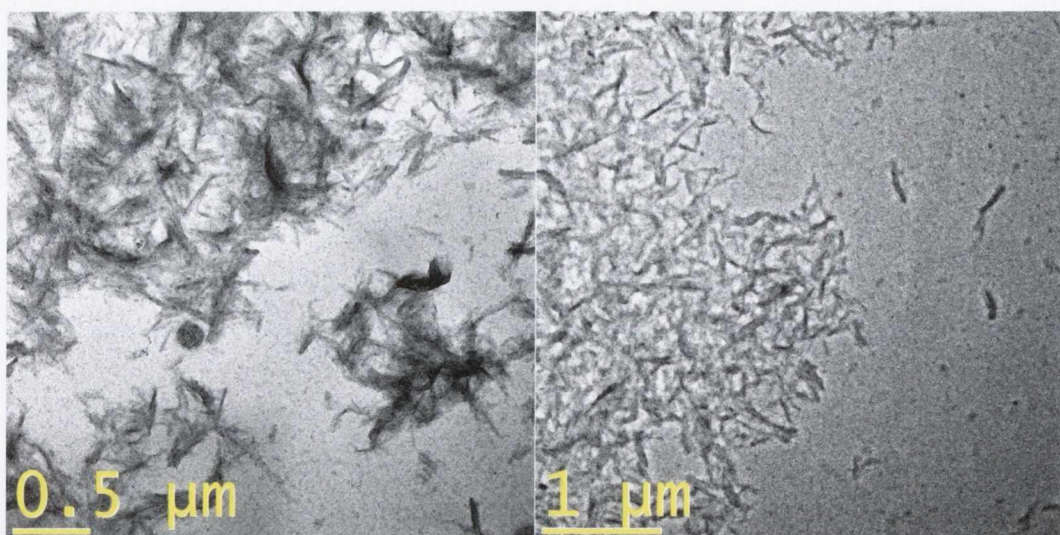


Figure 4.45: TEM images of FeS nanothreads formed using Fe(II) over 30 min (81)

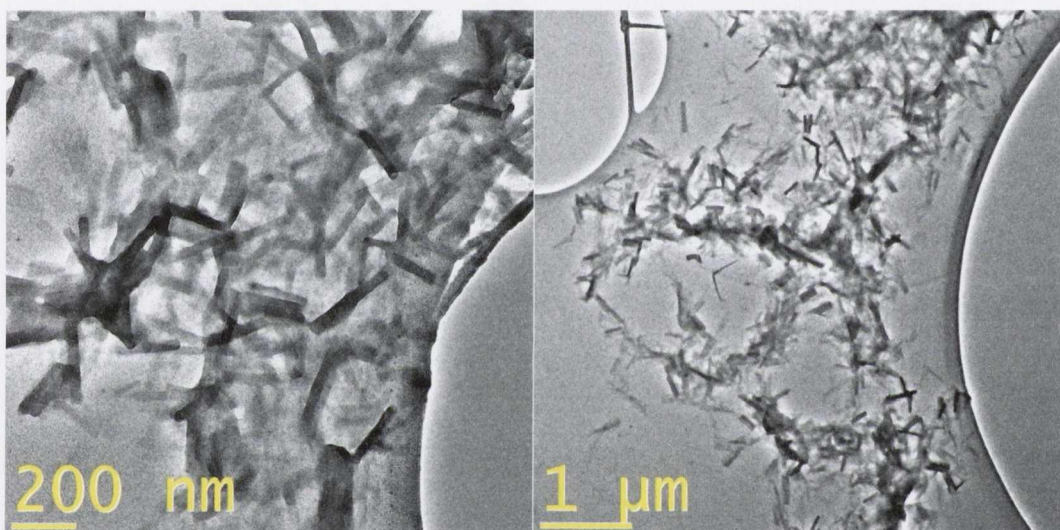


Figure 4.46: TEM images of FeS nanobelts formed using Fe(III) over 30 min (82)

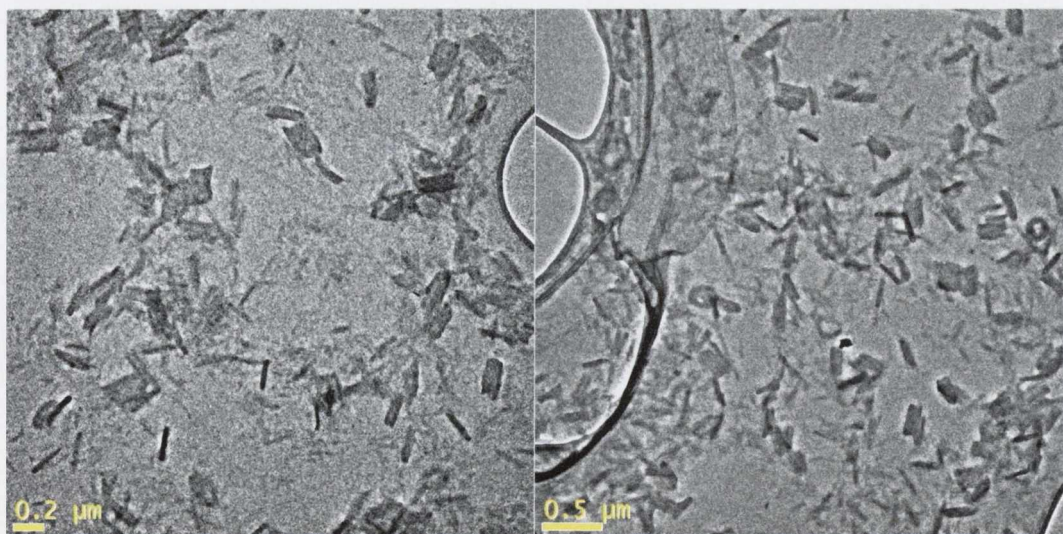


Figure 4.47: TEM images of FeS nanosheets formed using Fe(III) over 60 min (83)

XRD for samples produced with Fe(II) and with Fe(III) over 1 h showed an XRD pattern of Fe₃S₄ fcc. The experiment performed over 30 min with Fe(III) (82) showed very poor crystallinity, and could not be unequivocally assigned due to considerable noise in the pattern and low count rates. However for (81) and (83) the Fe₃S₄ pattern was identified. (Appendix 4.8.3)

UV-vis absorption spectroscopy showed an interesting absorption profile with shoulder peaks observable in each sample along the increasing absorbance towards UV, as shown in the spectra below. For this material, each of the samples also showed emission in the visible region, with peaks at 380 nm (81), 390 nm (82), 405 nm (83). Hence these samples could be used for further fluorescence studies. (Figure 4.48-4.50)

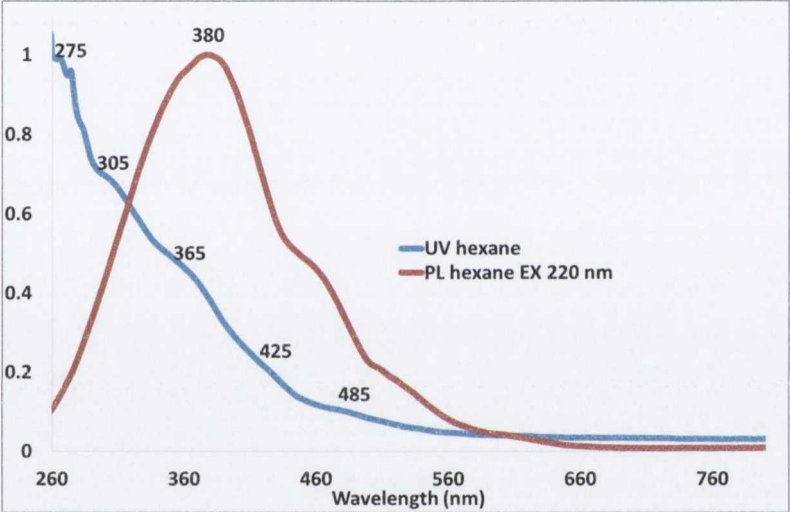


Figure 4.48: UV/PL absorption and emission spectra for FeS nanothreads in hexane formed using Fe(II) over 30 min (81)

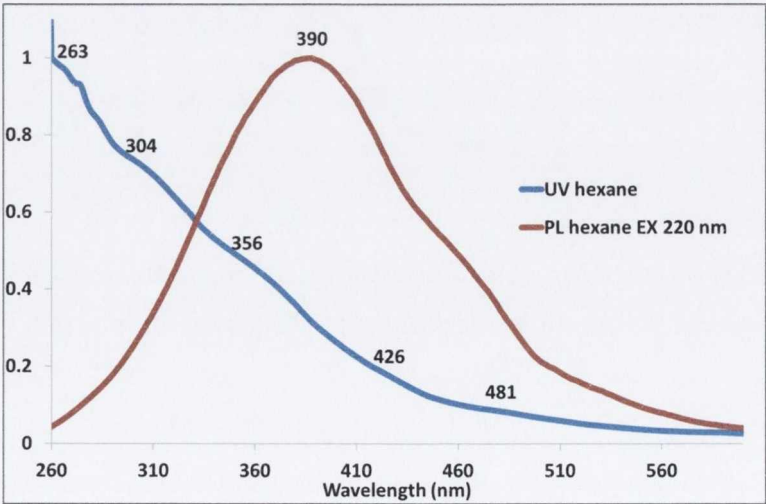


Figure 4.49: UV/PL absorption and emission spectra for FeS nanobelts in hexane formed using Fe(III) over 30 min (82)

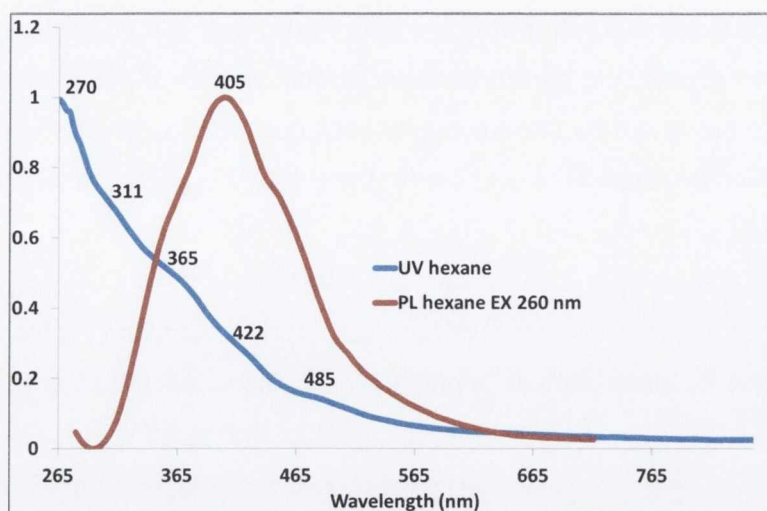


Figure 4.50: UV/PL absorption and emission spectra for FeS nanosheets in hexane formed using Fe(III) over 60 min (83)

As with NiS, VSM studies revealed magnetic behaviour in these products. **(Figure 4.51)** These values are lower than previously reported literature values,⁵⁸ however, as shown in the XRD patterns, the peaks have considerable noise and are broadened hence the crystallinity of these nanobelts could be improved. However, their combined magnetic/fluorescent properties are an advantage. Future experiments should involve reactions at 180°C instead of 160°C and for longer timeframes to help improve this crystallinity. FTIR studies revealed the characteristic peaks for OAm binding to the surface for each of the samples. **(Appendix 4.8.4)**

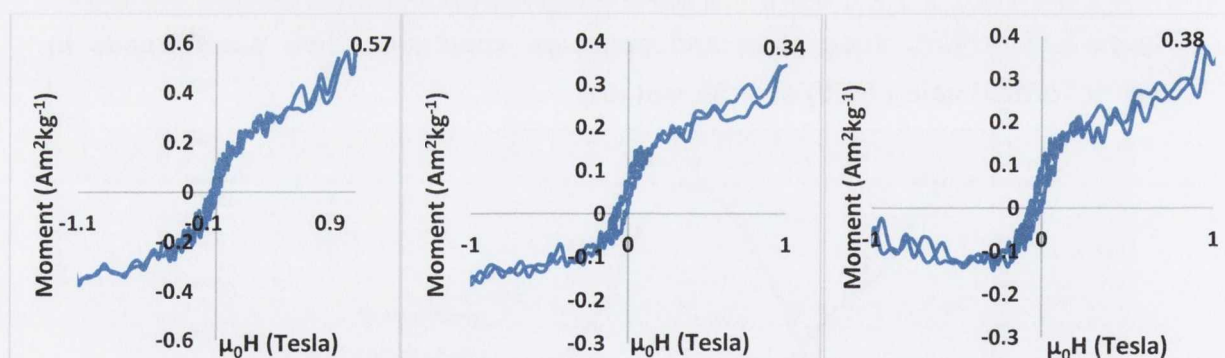


Figure 4.51: VSM spectra for FeS nanostructures formed using Fe(II) over 30 min (81, left), Fe(III) over 30 min (82, middle) and Fe(III) over 60 min (83, right)

In other experiments MnBi and MnS anisotropic nanostructures were also prepared, results of which are included in the Appendix. **(Appendix 4.11)** These experiments require further optimisation, hence are not included in the discussion here. Future work will involve their optimisation, as these materials also have interesting magnetic and conducting properties.⁶⁰⁻⁶³

4.10 IRON-PLATINUM/GOLD COMBINED HETEROSTRUCTURES

FePt-Au tail head nanoparticles were synthesised on a one-pot procedure, with tail elongation then subsequent seeding and growth of the Au head (Figure 4.52). Here the oleylamine surfactant serves to channel the growth of the FePt tail, then provides a preferential attachment site for the Au NP head. These composites were also modified *in situ* by a thiol exchange reaction using dodecanethiol, to produce particles which are stable in organic solvents. This synthesis may also be used to install other functionalities, such as amines, for further surface modification reaction.

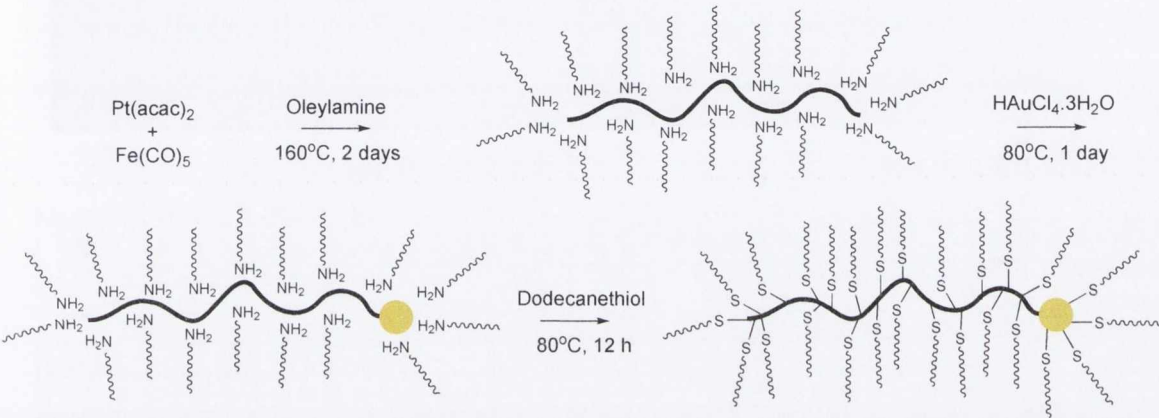
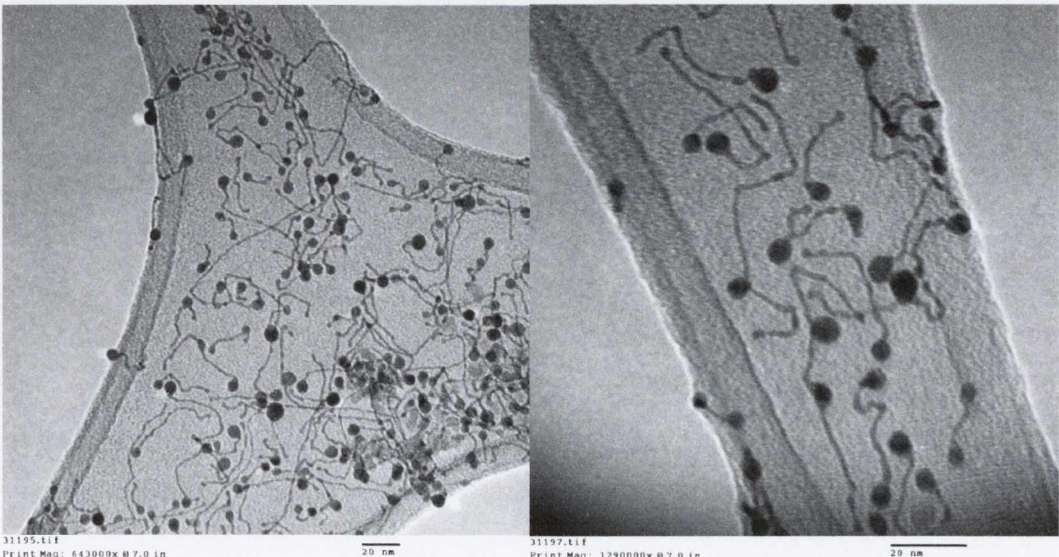


Figure 4.52: Schematic of one-pot FePt@Au synthesis with FePt tail formation, Au head attachment, and thiol exchange reaction

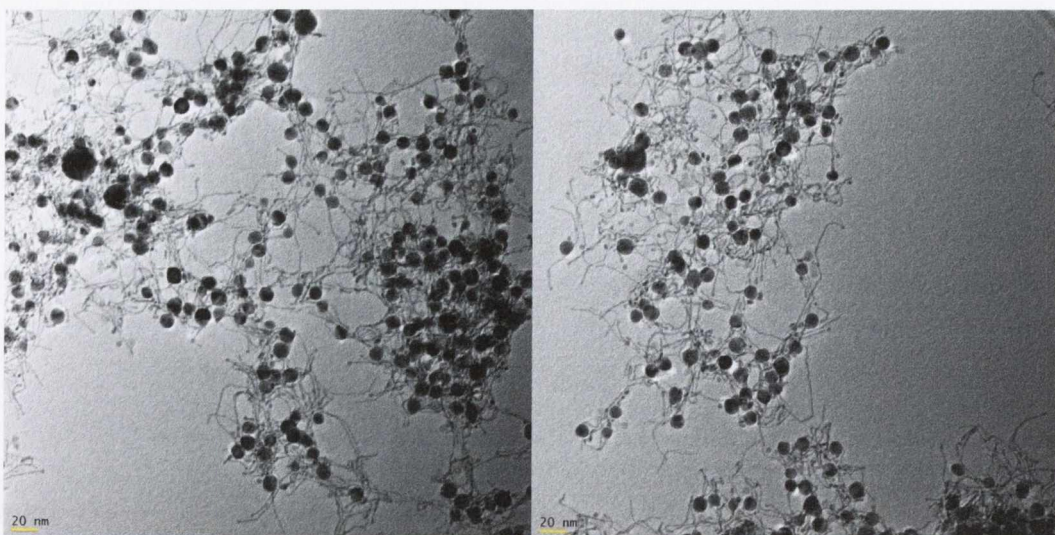
TEM analysis of the first sample (85) revealed the diameters of the FePt wires were 1.5 nm and the Au heads were 5 nm. The length of the tails were 60 ± 20 nm (Figure 4.53). Whereas the second sample, performed over a longer timeframe, (86) the reaction produced a network of FePt NWs and Au NPs. (Figure 4.54)



Scale Bar: 20 nm

Scale Bar: 20 nm

Figure 4.53: TEM images of FePt@Au tail-head nanostructures (85)



Scale Bar: 20 nm

Scale Bar: 20 nm

Figure 4.54: TEM images of FePt@Au nanostructures with FePt-Au wire-particle networks (86)

UV-vis in toluene revealed a plasmon peak for both Au at 520.94.⁶⁴ **(Appendix 4.9.4)** This property may prove useful for detection applications, using FTIR or Raman spectroscopy for the identification of molecules in the local environment of the nanocomposites.

XRD exhibits lattice parameters for both FePt and Au. **(Appendix 4.9.3)** FePt lattice parameters are 22.52 (001), 35.4 (110), 38.3 (111) overlapping, 56.96 (112). Au parameters are 38.3 (111) overlapping. 44.22 (200), 64.48 (220), 77.44 (311), 81.5 (222).⁶⁵ These parameters indicate a fcc structure for FePt and a face centred cubic structure for Au. The prominent 111 peak, which is overlapping for FePt and Au, exhibits a noticeable increase compared to literature values, which indicates preferential growth of the crystal along this face and subsequent anisotropic structure.

Magnetisation was shown to be $14.68 \text{ Am}^2\text{kg}^{-1}$ for the first sample **(85)** and $1.46 \text{ Am}^2\text{kg}^{-1}$ for the second **(86)** by VSM **(Appendix 4.9.2)**. These samples also showed no remnant magnetisation. The magnetisation for these nanocomposites is much lower than that found for Fe_3O_4 MNPs; however it is still within the useful range for a wide variety of applications. This lowering is mostly due to the non-magnetic contribution of Au, which has a high molecular weight and high density, and also the lower magnetisation of the fcc disordered phase FePt crystals, as opposed to the ordered fct phase obtained after annealing above 550°C .^{66, 67}

The combination and fusion of spherical 1D nanomaterials of different materials has been recently reported in Nature Chemistry.⁶⁸ However, the combination of anisotropic materials of different compositions has not been previously reported and represents a new class of more complex, sophisticated nanomaterials, produced in a controlled and reproducible manner. Future studies will involve combination of other materials, including the materials outlined in this chapter. This will open the door to many new and diverse applications.

4.11 PEGYLATION AND AQUEOUS TRANSFER

Anisotropic structures were transferred to aqueous solution using heterobifunctional PEGs, possessing long aliphatic chains for intercalation between oleylamine chains and an ionic phosphate head, attached to a functional group to maintain aqueous solvent stability, by electrostatic interactions, with the additional possibility of further functionalisation. (**Figure 4.55**)

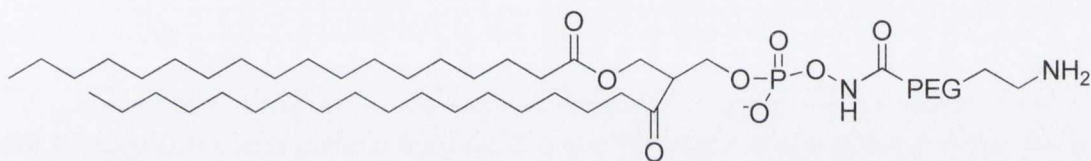


Figure 4.55: Structure of DSPE-PEG-NH₂

The phospholipid ligand was installed by mixing with the particles with it in a mixture of polar solvent and a charged buffer solution. In this way both the aqueous and organic stable component could be dispersed in the same liquid. The organic solution was subsequently evaporated and the complex was formed during this process, due to thermodynamic and repulsion forces.

FePt@PEG-NH₂ (**87**) was therefore successfully prepared and the nanowires transferred to aqueous media using this phospholipid PEG. FTIR analysis shows the characteristic peaks of OAm have been maintained in the product, with additional peaks, corresponding to the newly installed, intercalating PEG phospholipid. (**Figure 4.56**) Additional FTIR spectral regions and the spectrum for the DSPE-PEG-NH₂ ligand are presented in the appendix. (**Appendix 5.10**)

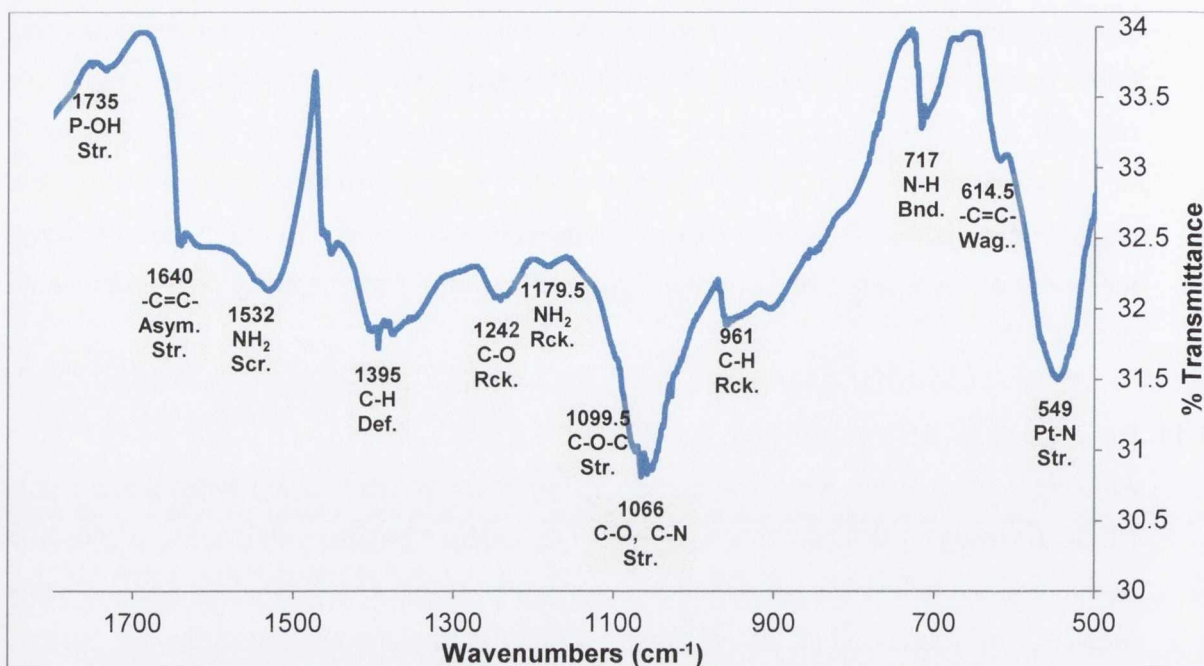


Figure 4.56: FTIR spectrum of FePt@PEG-NH₂ NWs (87)

TEM analysis shows that the wires still remain intact and separated from each other, and have not agglomerated due to ligand stripping during the coating process. **(Figure 4.57)** Due to overlapping in the P and Pt peaks, it is difficult to get an exact and accurate ratio of the phospholipid to nanoparticle ratio. For P, K_{α} is 2.013 keV and for Pt M is 2.048 keV. However, multiple scans were run, and the ratio consistently maintained at about 1/2 for P compared with the combined Fe and Pt metal atoms. In addition the software calculated the atomic ratio based on P K_{α} (2.013 keV), Pt L_{α} (9.441 keV), and Fe K_{α} (6.398 keV), all of which are well separated. **(Appendix 4.10)**

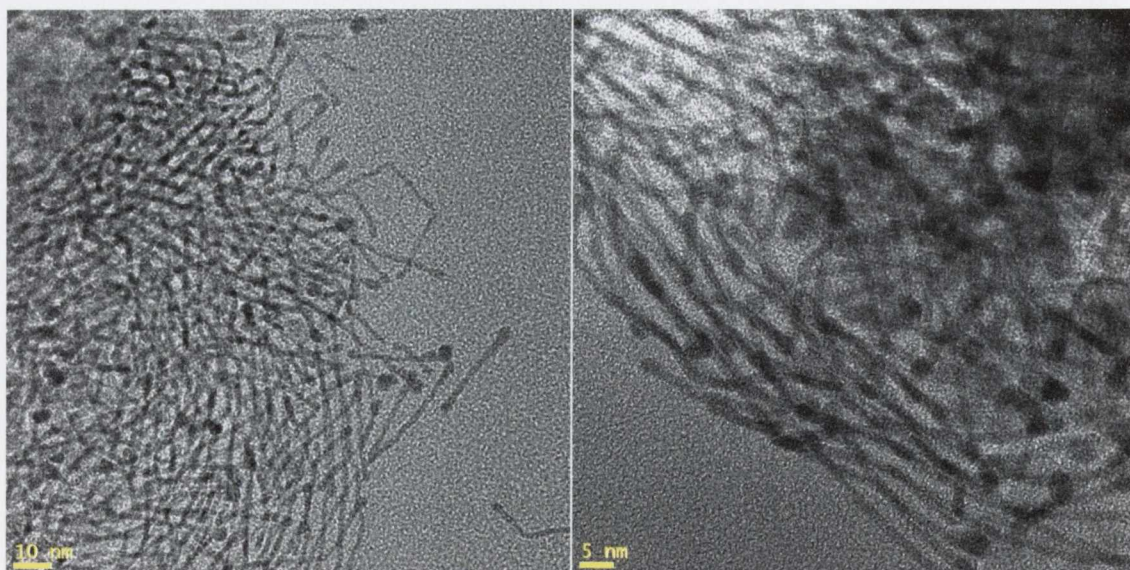


Figure 4.57: TEM images of FePt@PEG-NH₂ NWs, Fe_{67.2}Pt_{32.8}@(PO₃)_{53.8}-PEG-NH₂, (87)

UV-*vis* analysis for these phosphonate-PEG functionalised NWs (**87**) revealed a different absorbance profile than found for the OAm stabilised wires. (**55**) (**Figure 4.58**) Here, the NWs showed a broad peak absorbance in the visible region with a maximum at 430 nm. This absorbance could prove useful for biodetection, as the wires could be identified using excitation and absorbance studies in this spectral region. In addition, fluorescent moieties can be attached which emit in lower spectral region towards red. The wires do not show increasing absorbance towards the UV region. This could be due to the PEG spacer, which would inhibit contact between wires and conduction between them.

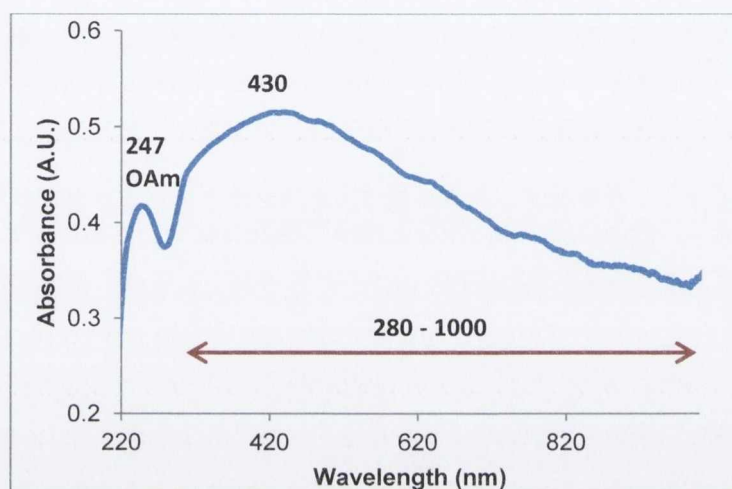


Figure 4.58: UV –*vis* absorption spectrum for FePt@PEG-NH₂ NWs (87**)**

VSM also revealed a reasonable magnetisation for the particles (peak 9 Am²kg⁻¹). Surprisingly, this is higher than that found for the starting FePt NWs. This may be due to loss of excessive OAm in the sample during the aqueous transfer process. Hence, magnetisation values for other samples outlined in previous sections may also be improved by this aqueous transfer method. (**Appendix 5.10**)

This method of PEG coating and aqueous transfer could be applied to any number of different nanomaterials, opening the door to biological studies of new anisotropic nanomaterials, and increasing their variety of possible applications. Future work will involve transfer of other interesting materials produced during this work which are outlined previously in this chapter, such as Fe_xNi_x, Fe₅Cu₉₅ nanotriangles and CuS, NiS, and Bi₂S₃ nanopolygons. These aqueous-stable nanomaterials can then be conjugated to biological molecules.

4.12 CONCLUSIONS

Thus a synthesis for a range of new anisotropic magnetic alloy and sulfide based nanomaterials has been developed here (e.g. FePt, FePd@Fe₃O₄, FeNi, FeCu, CuS, Bi₂S₃, Ni₃S₄, Fe₃S₄ and others) using a hot injection approach in organic solvents. A key advantage of this synthesis is its economy of reagents, which lends to scaling, cost-effectiveness and reproducibility of the reaction. In addition, many of the nanomaterials have demonstrated very interesting optical properties (e.g. plasmon peaks) and magnetic behaviour.

The rates of these reactions correlate well to the differences in standard reduction potential of the precursors used; with materials possessing a smaller difference in potential, requiring longer reaction times. It has been shown that the alloy obtained has a ratio of elements consistent with the amount of precursors added, taking into consideration the amount of the zero-valent precursor consumed in the reduction process. It was also noted experimentally that this process of anisotropic crystal formation works best when reagent starting materials are in the correct ratio to form a standard stable crystal alloy. Materials in unstable or mixed crystalline forms produced either particles or a disordered mix of shapes. This may be due to non-ideal ratios for other crystal compositions. It was also noted that there is a preferential growth in the direction of close-packed crystal faces.

For this series of experiments, the combination of EDX and XRD proved extremely useful in elucidating the composition and crystal structure of the products formed. This information was crucial in designing strategies for the formation of ideal ratios to form standard crystal structures. The elemental ratios in the products, as determined by EDX acted as a guide, compared against starting precursor ratios, in the experimental design. These ratios also acted a guide in determining the crystal structure, as shown by XRD analysis. Apart from crystal structure assignment, XRD also aided in the identification of side products of the reactions.

FePt NWs were successfully synthesised by a modified method reported in the literature.^{13, 14} These wires showed good uniformity in width and length, with only some particles presented. However, these wires showed reasonable low magnetisation, due to their low temperature of formation, which leads to a disordered crystal phase. Annealing, which would destroy the structural anisotropy would be required to improve their magnetic properties, hence other materials, produced by a similar method were explored.

Next FePd alloys were explored. Unlike FePt, which forms a number of stable alloys, FePt, Fe₇₅Pt₂₅, Fe₂₅Pt₇₅, for example. Iron-palladium preferentially formed a FePd₃ fcc crystal structure. After 20 min reaction this is the only alloy formed which indicates it is the kinetic product for this reaction. The iron-palladium alloy retained this ratio regardless of the starting ratio of Fe/Pd. In these reaction Fe formed a shell around the FePd₃ wires, which combusted in air to form FePd₃ NW clusters incased in Fe₃O₄. This shell thickness relied on the starting ratio of Fe/Pd, and magnetisation of the particles increased with increasing Fe content. Like iron-platinum alloys, FePd₃ possesses a disordered fcc phase at low temperature of formation, therefore this also has low intrinsic magnetisation. Hence, the search for anisotropic structures continued.

FeNi anisotropic nanostructures were successfully synthesised with varying ratios of Fe/Ni. At smaller quantities of Fe, Ni fcc nanoplates is the kinetic product. Interestingly, although Fe is the reducing agent in this reaction, over time the re-reduction of Fe by the surfactant OAm was observed and new alloy ratios were formed. In the lower Fe/Ni ratio experiments, the the kinetic product was Fe₅Ni₉₅ and the thermodynamic product after several day's reaction, was Fe₂₅Ni₇₅ nanoplates, due to Fe re-inclusion. With a larger starting ratio of Fe, the kinetic product was Fe₂₅Ni₇₅ nanoplates and longer reaction rates lead to the formation of Fe₅₀Ni₅₀ by a similar process of Fe re-reduction and inclusion. All products from this series of reactions possessed high saturation magnetisation and no coercivity (superparamagnetic). Hence, these iron-nickel alloy nanoplates can be used as synthesised, without the need for annealing. These nanoplates should have broad applications in biomedical research and their suitability should be explored in future work.

Next, FeCu experiments were performed to complete the range of Fe-based alloys and further elucidate the mechanism of the reaction. Here two reactions were performed with high and low concentration of Fe. In this experiment, the same product was formed In each case. With high Cu content and low Fe-doping. Here, Cu fcc is the kinetic product of the reaction. These structure are useful for the plasmonic properties, as indicated by their absorbance in the visible region. The small Fe doping did impart some magnetic properties, which may also be useful in future work, as the particles can therefore be more easily retrieved from suspension by magnetic separation. These materials also showed multiple plasmon peaks due to structural anisotropy. Hence, these Fe-doped Cu nanoplates should be investigated further for biosensing and detection applications.

A series of sulphur based experiments were then performed to prepare semiconductor anisotropic nanomaterials. The purpose here was to prepare either fluorescent or

plasmonic materials, and to test the adaptability and robustness of the OAm-directed synthetic approach for the production of anisotropic materials. The first experiments involved copper sulfide synthesis, which is well documented in the literature and readily forms crystal structures. This material has also been shown to be both fluorescent and plasmonic under the correct conditions of size, shape and purity. Interestingly, both Cu_2S and CuS can be formed from the reaction, as the OAm competes with the reduced S^{2-} for reaction with the Cu^{2+} precursor. It was found that by initial addition of elemental sulphur, and its pre-reduction prior to addition to the metal salt, lead to quantitative inclusion of the sulphur and metal constituents into the ionic crystal. Hence, further experiments followed this methodology for the sake of simplicity and straightforward reaction conditions and results.

Next Bi_2S_3 was prepared by a similar method. This product showed excellent uniformity and crystallinity. Bismuth sulfide forms only one stable crystal structure, hence this was both the kinetic and thermodynamic product of the reaction. Future work for these particles should explore their suitability for *in vivo* X-ray CT diagnostics, in addition to more general molecular biosensing and detection applications.

NiS experiments were then performed. Under varying time, the shape of this material changed by a standard mechanism of ripening. The initial product consisted of nanoplates, which over time became more uniform nanoflakes. These nanostructures exhibited lower crystallinity, however they had interesting optical properties, as shown by UV-vis and PL studies. Full exploration of these optical properties was not within the scope of this research however, this should be explored in more depth in future work. Like FeCu , these also exhibited a small magnetic response, however the main advantage of this material is its unique optico-electronic properties and birefringence nature, useful for detection and sensing applications.

Fe_3S_4 was also synthesis and formed rectangular sheets in these reactions. The mechanism for this reaction is complicated by the mixed valence state in the product. Hence, high crystallinity was not achieved under the reaction conditions reported here. In its pure state, this material behaves similarly to Fe_3O_4 and has good magnetic properties. Here magnetisation values were low, however emission in the visible spectral region was detected by PL spectroscopy. Future work should involve higher temperatures and longer reaction times for this material, as well as further investigation into their optical and magnetic properties.

In addition to producing a library of alloy and semiconductor anisotropic materials, with the possibility of extending the method to other various materials, it has also been shown that these nanomaterials can be effectively transferred from organic into aqueous phase and therefore potentially used for biomedical applications. FePt NWs were transferred into aqueous suspension using a heterobifunctional phospholipid PEG, possessing surface amino groups for further bioconjugation. This can be used for any number of potential bioanalogues for a wide variety of biosensing, detection and diagnostic applications. Overall, this work has opened the door to continued research in various areas of biomedical research. However, it is still necessary to further and fully study the properties and behaviour of these new nanomaterials as well as to develop their specific applications. Their unique combination of properties in the areas of magnetism, plasmonics and fluorescence can then be applied for better sample handling and recovery, and also improved detection and sensing for bioapplications.

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CHAPTER 5 – CONCLUSIONS AND FUTURE WORK

A variety of nanostructures were prepared for potential biomedical applications, particularly for diagnostics and sensing by biomolecular capture of complimentary molecules and detection using fluorescence and FACS. Nanoparticles, sub-micron particles, and a range of anisotropic nanostructures were prepared and functionalised and selected materials were tested for these applications.

5.1 AQUEOUS PHASE SYNTHESIS AND FUNCTIONALISATION

Standardised procedures were developed for the surface modification of a variety of NP surface types. Modifications included primary surface functionalisation by silanisation using APTS and heterobifunctional silane-PEGs. This technique can be transferred to a wide variety of oxidised and hydroxylated surfaces. It is a robust and reproducible reaction and the extent of surface binding is also consistent, between experimental measurements and theoretical calculations. Here the procedure was optimised to be performed with an economy of reagents under mild conditions, using only residual water in polar solvent to catalyse the hydrolysis of the silane precursor for addition to the nanomaterial surfaces. The flexibility of performing this reaction in a variety of polar solvents was also highlighted.

The extent of surface binding is then quantifiable by EDX. This method produced results that closely reflected theoretical calculations for surface binding. These calculations have been outlined in detail, and applied to various materials of different sizes, shapes and core composition, to ascertain the surface group molarity of nanomaterial samples for further reactions. Once the primary functionality has been installed, ligation reactions can be performed to convert surface groups to a variety of other functionalities. These reactions can also be used for the controlled, quantitative addition of biological markers, such as antibodies and fluorescent moieties to the surface of nanomaterials. Surface group molarities can also be calculated.

$\text{Fe}_3\text{O}_4@\text{COOH}$ and $\text{Fe}_3\text{O}_4@\text{G}'@\text{IgG-488}$ MNPs were prepared by sequential reactions with amide coupling and nucleophilic addition reactions. This sequential functionalisations avoided the typical loss of activity, agglomeration and cross-linking generally seen when applying amide coupling directly to antibodies. They were then characterised by a variety of techniques, including FTIR and EDX and then analysed using FACS technology. These particles provided a model system and acted as a benchmark for further experimental design and sample characterisation of more complex

systems, involving a variety of nanowires, which were functionalised in similar manner to the Fe_3O_4 MNPs.

Surface silanisation was also applied to Co, CoNi, and CoNi|Au|CoNi|Au|CoNi NWs for coating with a variety of functional PEGs. This reaction is highly reproducible, and can be used to simultaneously install multiple surface functionalities. In addition, Co and Co@Au NWs were coated with silica by base-catalysed TEOS deposition, then amino-functionalised with APTS. As with the PEGylated NWs, this silica deposition is highly uniform, promotes biocompatibility and reduces degradation of the wires over time.

Larger nanostructures, such as the template-grown metal nanowires, have a considerably lower surface to volume ratio by a factor of ~ 10 -100. Hence the signal is lower. Optimised procedures on Fe_3O_4 for measuring and processing characterisation data were very useful, where signals were difficult to obtain. This also pushed the detection limits of various characterisation and detection techniques. In addition, due to the small sizes of the samples, only limited characterisation was possible.

The Co NWs are quite large, and therefore ferromagnetic, and this leads to poor solvation of the wires. Hence, these products are not ideal for biological assays. However the methods which were used to coat these wires can be adapted to other systems with more suitable properties, such as superparamagnetic/paramagnetic behaviour and reduced size. It was found, however, that CoNi alloy nanowires had more attractive magnetic properties, with lower residual magnetisation due to the 'soft' nickel contribution. It was found CoNi 10 μm wires produced the most uniform sample, hence this was used for further comparisons by FACS.

A modified method for the production of ferrite-doped SiO_2 NWs was also developed here, based on a previously reported method from within our group. In this synthesis, acid catalysis was used for sol gel formation, and CoFe_2O_4 MNPs were embedded within the matrix instead of with Fe_3O_4 MNPs. It was found that these NWs produced a higher magnetic response than previously reported, due to inclusion of the cobalt ferrite particles and their higher resistivity towards acidic conditions. The surface functionalisation strategies which were optimised on Fe_3O_4 were then employed to produce novel fluorescent antibody conjugated CoFe_2O_4 -doped silica NWs. By using a maleimide-thiol nucleophilic addition strategy, these antibodies were installed in a controlled manner, through reaction with cysteine thiols on the protein. This method

avoids the polymerisation and loss of activity as a result of direct EDC coupling of the protein to surface amines, as already mentioned.

This template-assisted method produces wires with very uniform diameters. However there are a number of limitations to the processes of template-assisted nanowire synthesis. The wires which are formed can be very brittle, hence prone to breakage which creates debris and high polydispersity in the lengths of the wires. Uniformity in length can be further promoted by filtration separation techniques, using large pore membranes to exclude fragmented wires. These template-assisted methods also produce relatively small yields of product, hence purification further reduces yields. The possibility of up-scaling is an issue, as is complete characterisation of the product due to limited sample. Future work should involve larger scale batches for more complete studies, which would involve combining multiple batches manually. Although time consuming, this is achievable.

Fe₃O₄ MNPs, CoNi 10 µm NWs, and segmented barcode CoNi|Au|CoNi|Au|CoNi NWs were chosen for comparison by FACS analysis. Samples were prepared of aqueous-stable and fluorescent protein-bound analogues, to compare the difference in scattering response and detection signal at different wavelengths. It was found that binding could be determined using differences in detection intensity at various wavelengths, compared against non-fluorescent bound controls. It was also found that a difference between small nanoparticles bound and unbound to non-fluorescent proteins, such as Protein G', could be determined by this method, by a difference in the scattering height, width and area of the particles, due to their increase in diameter and hydrodynamic radius. The detection of non-fluorescent signals by scattering is a significant result, as in future, it may be applied to clinical samples for antigen detection in serum or urine.

5.2 SUB-MICRON PARTICLE SYNTHESIS AND FUNCTIONALISATION

Ni@GNP-X technologies have many potential applications; however we have targeted them for biomedical applications such as cell surface and serum biomarker detection using flow cytometry systems, and also biomolecule capture and separation. It hoped these particles will compete against current state of the art technologies. Due to their size, magnetic properties, stability and uniformity they are ideal candidates for these applications and have already shown improved properties in comparison to commercially available technologies.

Firstly, sub-micron particles were synthesised by a method involving organic-phase surfactant-assisted thermal decomposition and reduction of a Ni^{2+} salt. The surfactant acts as a reducing agent, donating electrons from the amino group, as a surface stabilising group, also through amino electron donation, and finally as a viscous solution and steric separation agent for a highly concentrated solution. Sub-micron particles were chosen, due to their specific and special properties. These particles are smaller and denser than current state of the art for scattering detection applications. Therefore, not only will they push the boundaries of current detection limits on these systems, they will impart improved properties, such as greater surface area for capture. In addition, the reaction conditions, cheap precursors, and highly concentrated solutions make these reaction very amenable to up-scaling and increase their cost-effectiveness.

Upon characterization of the bare nickel particles, it was discovered that surface characterisation and modification was problematic, due to two discrete factors. The small relative mass contribution ($\sim 0.1\%$) the groups at the surface were difficult to characterise. This was further complicated by the mix of ligands at the surface. This mix of ligands was caused by the formation of amorphous carbon and also graphene oxide in these experiments, by surfactant, ligand and salt decomposition due to high temperature, particularly for reactions over a longer timeframe. Hence in future experiment, reaction times were kept to a minimum (2–3 h).

It was initially hoped that the surface of these particles could be oxidized in polar media by hydrolysis with aqueous residue, in order to perform silanisation experiments similar to Chapter 2. This oxidation was indeed achieved, however it presented the additional problem of particle leaching, and therefore instability and reduced biocompatibility. Silanisation experiments (not included here) were also performed but results were inconclusive. Hence, a more suitable, biocompatible, uniform and characterisable surface was sought.

Next, nickel particles were coated with a gold shell. Gold coating was successful, however, it produced a coating of clustered Au nanoparticles, instead of a thin, uniform shell. As a result, the particles retained the plasmonic properties characteristic of small Au NPs. The benefit of this method is that Au is more easily functionalised than Ni metal, as Au-S forms bonds almost covalent in strength. Each of the sample were functionalised with heterobifunctional linkers by thiol exchange to provide either hydroxylated or carboxylated surfaces. It was found however, that Au contributes a large, dense non-magnetic component, and increasing Au shell drastically reduces saturation

magnetisation. In addition, they presented no detectable benefits in scattering propensity in flow studies. Hence, a new method was devised.

Ni particles were then coated with graphene nanoplatelets. The particles are coated with graphene in a novel way, which has not been previously described and can only be performed on particles in the size range discussed here. The method, like thiol exchange, relies on thermodynamics, with the strongest and most stable coating left on the particles. This requires time for the π system of the graphitic benzene rings to coordinate to the metal surface, then cannot be removed due to the overall collective, multidentate effect. This provides a stable, uniform coating, and also possesses organic groups which can be used for conjugation strategies, as the nanoplatelets are prefuntionalised prior to coating, with linker organic groups. It was also shown that this coating prevented leaching and their surfaces remained smooth and unoxidised under a variety of experimental conditions with extensive washing steps. In addition, graphene is a much cheaper approach to surface coating.

Once graphene coated, they can then be conjugated to biomolecules and fluorophores for specific applications, and also several different functionalities can be installed on a single particle *via* sequential, orthogonal modifications.¹ In this research they were conjugated to PEG-maleimide and a maleimide linker. The Ni@GNP@mal particles were then coated with Protein G'. These particles were sent to a multinational company for comparison against their own technologies, however, future studies should involve 'in house' flow cytometry and biomolecular capture, sensing and detection studies and also integration and comparison with other materials and technologies.

5.3 ANISOTROPIC NANOMATERIAL SYNTHESIS AND FUNCTIONALISATION

In order to achieve anisotropic nanomaterials with a smaller size and the potential for up-scaling to larger quantities, a thermal decomposition/reduction approach was adopted. A variety of materials were synthesised in OAm, which was used as solvent, stabiliser and reducing agent for this reaction. OAm assists in the anisotropic growth of the nanostructures by creating preferential attachment points at the end points of the crystals. Along the length of the growing crystals, the surfactant is more efficiently stacked, preferentially protecting the surface from attack. At either end the surfactant chains are more loosely stacked due to the curvature of the surface. This process is aided by the reduced surface energy of the close packed faces. We can detect preferential growth in these structure, as by the relative intensity of the peaks in diffraction pattern, particularly the (111) lattice parameter in XRD.

This method shows great potential for multiple applications and has been applied in this research to numerous materials. It is anticipated it could be applied to other materials also, providing a generalised simple route to the production of anisotropic nanomaterials, with an economy of reagents. Here, only the two precursors of the binary material (either iron alloy or sulfide semiconductor) and the surfactant is used, with a small amount of polar solvent used in the sulphur-based experiments for hot injection. This economy of reagents leads to cost effectiveness and also reduction of by-products from the reaction. It also reduces the factors which can affect and vary the reaction outcome. Here, we have reduced the reaction factors to a function of time and ratio of precursors at a temperature between 160 – 180°C, which seems to be the ideal temperature for coordination of the amine to metal atoms at the surface. If the temperature is too low, the surface is not penetrable, if too high, kinetics take over from thermodynamic growth, and anisotropic particles may not be formed.

Future work will focus on functionalising these anisotropic nanomaterials for biological applications. For example, these nanomaterials will be used for biosensing and detection in aqueous systems, by initial PEGylation, a reaction and process which was also optimised in this work, for controlled, uniform, and distinct functionalisation. It will also focus on subsequent attachment of these PEGylated, anisotropic nanomaterials to biological moieties, such as, Protein G', IgG-488, Streptavidin, biotin, fluorescent tags and also nucleic acids and peptides for targeted diagnostics. These particles will then be tested for a range of potential biomedical diagnostic applications, such as toxicology, cell uptake, biomarker capture and detection, flow detection, X-ray CT, and MRI.

Overall, in Chapter 4, a variety of novel anisotropic materials were produced, consisting of both Fe-based metal alloys and S-based semiconductors, and also composite tail-head heterostructures of FePt/Au. In addition, preliminary work shows the production of other metal based alloys such as MnBi and also other semiconductors such as NiP (work not shown here).

The FePt/Au nanocomposites are of particular interest, as they open the door to a new level of complexity and control in nanomaterial design. They are both superparamagnetic and possess a plasmon band in the visible spectral region, combining the properties of both FePt and Au. This synthesis provides a starting point to a variety of other syntheses, combining many diverse materials with a variety of physical properties, with different shapes and functions. One important consideration, which was discovered through the course of this research (work not shown here), is that the preliminary wire sample must

be highly uniform, with few NP side products, to achieve good Au head attachment to the wires.

In addition, the ratio of starting materials for these fusion reactions must be optimised for each new material, as the rate of wire growth, and ultimate length will change, depending on the material. Also, the nanocomposite head may show preferential attachment to certain materials over others, depending on the crystal structure affinities and mechanism of growth. It is also possible to mix the two distinct nanomaterials, as opposed to growing one on the other, due to the variable binding strength of the surfactant, which protects the nanoparticle, but allows for preferential attachment under slightly elevated temperatures.

In this chapter EDX and XRD proved the most valuable characterisation techniques, as unlike previous chapters, each new product was composed of a different ratio of varying elemental composition, which was measured by EDX spectroscopy. In addition to elemental composition, new ratios in some cases produced varying crystal structures, as measured by XRD. The combination of these techniques was crucial in determining the compositions of these materials and was by no means an arbitrary undertaking.

For each material, consisting of either iron alloys or sulfide semiconductors, there is a limited number of possible crystal structures for the binary material. Non-ideal elemental ratios in the product, which do not correlate to these ideal crystal structures, result in mixtures of products. The information obtained from these two characterisation technique assisted in designing the experimental conditions used to form the most stable and uniform products. Changes in these ratios was also crucial in determining the preferred products of the reaction based on kinetics and thermodynamics.

Without these studies and corresponding results, an understanding of the mechanisms of anisotropic growth, and also the discrete redox reactions occurring, would have been less clear, and application to new materials would have been more difficult. Further experiments, on different materials utilising this OAm-directed anisotropic growth should also employ EDX and XRD, and considerations such as preferred crystal structure and elemental ratio for a given material must be considered, and precursor ratios selected accordingly. One should also take into account the relative redox potentials of the precursors in the reaction, with regard to reaction rate, and also product change over time due to reinclusion and reduction of constituents, as seen in FeNi experiments.

5.4 FUTURE WORK

In terms of nanomaterial characterisation, FTIR is a relatively uncommon technique, which has not been widely explored. The same holds true for FTIR characterisation of metalo-organic compounds, but to a lesser extent. There are a number of potential reasons for this. IR in terms of organic chemistry, became a complimentary technique, as opposed to primary, with the introduction of NMR, which can provide more precise measurements, due to neighbouring atom resonance coupling. In IR, characteristic peaks reside in areas or bands of the spectrum, which can shift depending on the nature of the material. Hence, it can be more qualitative and a process of elimination than an exact method, particularly without the wealth of characterisation literature available for NMR of organic compounds, or comprehensive treatment in the literature of inorganic compounds and shifting of metal bondings in the far-IR region. In addition, only the fingerprint region of the spectrum is normally characterised. However, for magnetic materials, NMR is not possible, hence alternatives need to be considered.

Generally, bioconjugation to nanomaterials or even surface functionalisation is determined by inference, i.e. through a fluorescent response, adhesion to a cell surface or transfer to different solvent, for example. In this work it was hoped to achieve more comprehensive determination of the nanomaterial surface environment. Through optimisation of FTIR measurement procedures, it was found that one could achieve similar resolution and intensity for FTIR as can be achieved for NMR. Organic functionality at the surface of nanomaterials represents ~0.1-1% total mass contribution, depending on the size and density of the material. In the measurements here, the % transmittance corresponded well to this ratio. In addition, measurement intensity correlates to the quality of contact with the diamond crystal. Measurements can be improved by better contact. Here this was achieved by drying of the sample onto the crystal from a volatile solvent. This achieves better contact than dry solid packing, as during dispersion the particles pack at the surface in a more fluid manner, to achieve optimum contact at the surface, without air packets.

It was also found that for sequential modifications of the surface, which produces an elongating organic chain, the outermost groups, which would be in closest contact with the crystal surface, produced the most intense signals. Hence relative intensity of signals also changes as the surface changes. It was also found that plasmonic surfaces, such as gold-coated nickel particles produced organic signals much higher than expected based on the relative contribution of the organic matter to the overall material mass. This phenomenon is attributed to surface plasmon enhancement. This phenomenon is widely

documented for Raman spectroscopy (SERS), however, to a lesser extent for FTIR. Future work should investigate this further.

In addition to nanomaterial surface characterisation, here an optimised method is also presented for measurement across the full range of 4000 - 300 cm^{-1} by FTIR. Generally, the range of 650 - 300 cm^{-1} cannot be achieved by this method, however, by this procedure, the full range can be achieved. The novelty of this approach allows us to simultaneously measure the organic surface functionality and metal bonding in the crystal structure and observe their relative intensities. It also allows us to simply measure the organo-metallic bonding at the nanomaterial surface. However, since traditional organic characterisation involves only the 4000 - 650 cm^{-1} region and nanomaterial surface characterisation is still in its infancy, again Raman, which widely applied to inorganic materials, would be an invaluable complimentary technique for characterising and confirming FTIR measurements.

Therefore, these optimised procedures for surface characterisation should also be extensively applied to Raman spectroscopy. This technique would provide a wealth of information. Unfortunately this technique was outside the scope of this study. However, Raman would help verify the peak assignments in IR, further elucidating the surface functionalities. Optimisation is not a trivial matter, as standard Raman lasers can be strong enough to burn or oxidise a sample, changing the surface environment or even nanomaterial core.

Patience must also be employed in these techniques, as measurements must run at lower beam intensities and over longer timeframes the generally required to obtain sufficient signal intensity to differentiate noise in the spectrum from the small peaks obtained by organic group which only represent a small contribution to the overall material mass. For perspective, FTIR measurements of organic samples typically take a few seconds, whereas standard measurements on nanomaterials take 5-30 min. Raman measurements may require even longer timeframes. However, although non-trivial in its execution, this work is vital for future research into the more detailed intricacies of nanomaterial surfaces, and also to further concrete the analyses by other methods such as FTIR. Overall, although difficult and sometimes not fully conclusive, these types of characterisations are crucial to avoid the pitfalls of assumption, and as nanomaterials represent the most highly reactive surface systems, representing the highest surface to volume ratio achievable for a solid material, their surface characterisation also deserves particular attention.

Apart from more extensive FTIR and Raman spectroscopy, other surface characterisation techniques should be explored, such as atomic absorption spectroscopy (AAS), which was also outside the scope of this research, but would represent a powerful and useful complimentary tool for the EDX analyses presented here. AAS could also provide further information on the organic contribution to the nanomaterial surfaces, which is not easily obtainable by EDX due to small differences in the relative angles of diffraction of the X-rays for elements such as oxygen and lower atomic numbers.

Future work will also involve conjugation of the optimised nanomaterials produced here to a range of disease targeting biomarkers and subsequent testing for sensing, detection, biomolecular capture and flow systems. The range of biomarkers which could be employed is not exhaustive, but includes antigen-targeting moieties, such as proteins, peptides, nucleic acids, sugars, and glycolipids, for example. Fe₃O₄ MNPs, CoNi|Au|Co|Au|CONi NWs, Ni@GNP particles, FePt NWs, FePd₃@Fe₃O₄ nanoclusters, FeNi, FeCu, CuS, Bi₂S₃ and Ni₃S₄ nanoplates showed particular promise for these bioapplications.

Smaller nanoparticles are of particular interest, as these show the highest relative diameter increase after bioconjugation for scattering analysis. Also plasmonic materials are of interest for their plasmon-enhanced resonance in spectroscopic measurements. FePd₃@Fe₃O₄ nanoclusters should also be compared against the Ni@GNP particles for efficiency studies in biomolecule binding and flow detection, due to their similar size. In terms of biomolecular capture, these FePd₃@Fe₃O₄ particles may even out-perform the Ni-based particles, due to their irregular and therefore higher surface area.

Nanoplates represent another area of interest, due to their enhanced scattering abilities relative to smaller spherical nanomaterials as a result of their widest axis, with the added benefit of retaining properties typically conferred on smaller nanomaterials, due to quantum confinement across their narrowest axis. In addition, for plasmonic materials, they exhibit more complex and interesting absorbance profile. For cell studies, these nanoplates could also be of particular interest, due to the different conformation in which they could bind to or enter a cell surface or cellular components, such as lysozymes, mitochondria or the nucleus. Toxicology studies based on differences in shape for various materials would also be very relevant, as without comprehensive experimental studies, it is unclear which shapes will increase or decrease biocompatibility, bioavailability and suitability for future *in vivo* research.

Other anisotropic alloys and semiconductors studied included CoPt, FeCo, FeAu, MnBi and MnS. **(Appendix 4.11)** Preliminary TEM results confirmed structural anisotropy for these materials; however these experiments require further optimisation and characterisation for better uniformity of the samples and definitive characterisation of their compositions. These optimisations will also be performed in future work, and the optimised materials will then be used for further bioapplications, as part of the series of nanomaterials of different compositions and properties, and of different shapes, to compare their suitability and varying responses for these applications. We will also perform aqueous transfer and PEGylation reactions for the full range of optimised anisotropic structures of different compositions produced here, with subsequent biofunctionalisation, analysis, detection and also toxicology and flow testing.

In addition, a range of experiments will be performed for the production of new heterostructures, involving the anisotropic nanomaterials developed here by fusion of different materials in a controlled manner to produce new hierarchical structures for more complex, targeted applications. As with bioconjugation, the list of potential heterostructures is not exhaustive and can be applied to any conceivable combination of material composites and shapes, under the right experimental conditions. Key considerations in these experiments are relative concentration of the two materials, length and concentration of the reaction (heterostructures versus heteronetworks), compatibility for crystal fusion (lattice matching) and relative redox potentials.

CHAPTER 6 – MATERIALS AND METHODS

Starting Materials:

Co and Co@Au NWs were received from Prof. Coey's research group in the School of Physics, TCD. Heterobifunctional PEGs were purchased from www.nanocs.com, CD4-PE was received from BD Biosciences, GNPs were purchased from www.cheaptubes.com, and all other chemicals were purchased from Sigma-Aldrich. All chemicals were used without further purification, unless specified in the procedure.

METHODS

6.1 AQUEOUS PHASE SYNTHESIS AND FUNCTIONALISATION OF NANOMATERIALS

6.1.1 Carboxyl-Coated Magnetite MNPs

(i) Fe_3O_4 (1):

0.5M NaOH was prepared by dissolving 5 g of NaOH pellets in millipore water (250 mL). This solution was then degassed by bubbling Ar through it for ~30 min. 0.4M HCl was prepared by taking stock 12M HCl (0.8285 mL) and dissolving into 25 mL millipore water (using volumetric flask). This HCl solution was degassed by bubbling Ar through it for 30 min. $\text{FeCl}_3 \cdot \text{H}_2\text{O}$ (6.56 g, 0.024 mol) and $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (2.48 g, 0.012 mol) were then added to the HCl solution and stirred for ~15 min. The NaOH solution was heated to 80°C and the iron solution added to it drop-wise. Black precipitate formed. The reaction mixture was then stirred with heating for 1 h. The resulting black precipitate was washed with millipore water until pH neutral, and then dried under vacuum. Yield 3.067 g. Product characterised by TEM, XRD, VSM, FTIR

FTIR (cm^{-1}): 385, 553, 1643, 3431

(ii) $\text{Fe}_3\text{O}_4@\text{NH}_2$ (2)

Fe_3O_4 (3.1) (1 g) was dispersed in EtOH (50 mL) in a 100 mL RBF. APTS (6 mL) was added and the reaction was sonicated overnight. The product was then washed with EtOH and centrifugation (5 x 30 mL) then dried under vacuum. Product characterised by TEM, EDX, FTIR, and VSM.

FTIR (cm^{-1}): 396, 560, 630, 859, 939, 1058, 1123, 1341, 1583, 2928, 2980, 3433

(iii) $\text{Fe}_3\text{O}_4@\text{COOH}$ (3)

$\text{Fe}_3\text{O}_4@\text{NH}_2$ NPs (100 mg) and DIEA (20 μL) were dispersed in THF (10 mL) with sonication for ~15 min. *N*-maleoyl- β -alanine (20 mg) in THF (10 mL) was then added. The reaction was sonicated for 1 h, and then washed with THF (5 x 20 mL). Product characterised by TEM, FTIR, VSM, and FACS.

FTIR (cm^{-1}): 385, 556.5, 864, 1071, 1166, 1260, 1337, 1404, 1441, 1583, 1639, 1700, 1777, 2978, 3432

6.1.2 Fluorescent Antibody-Conjugated Magnetite MNPs

(i) $\text{Fe}_3\text{O}_4@\text{mal}$ (4)

$\text{Fe}_3\text{O}_4@\text{NH}_2$ (2) (100 mg) was dispersed in THF (10 mL) with sonication for ~15 min. EDC (60 mg) and *N*-maleoyl- β -alanine (20 mg) in THF (10 mL) was then added. The reaction was sonicated for 1 h, and then washed with THF (5 x 20 mL). Product characterised by TEM, FTIR, and VSM.

FTIR (cm^{-1}): 392.5, 559, 867, 1066.5, 1168, 1358, 1401, 1444.5, 1546, 1659.5, 1703.5, 2980, 3402

(ii) $\text{Fe}_3\text{O}_4@\text{G}'$ (5)

Under sterile conditions, $\text{Fe}_3\text{O}_4@\text{mal}$ (4) (10 mg) was dispersed in PBS pH7.4 (20 mL) with sonication for ~30 min. Protein G' (1 mg) in PBS (1 mL) was then added and the reaction agitated on a shaker for 2 h. Washed with PBS (3 x 10 mL). Product characterised by TEM, EDX, FTIR, and FACS.

FTIR (cm^{-1}): 391.5, 440, 871, 993.5, 1096.5, 1263, 1388, 1474, 1495, 1545.5, 1641, 1660.5, 1700, 1734.5, 2948, 2979.5, 3325

(iii) $\text{Fe}_3\text{O}_4@\text{G}'@\text{IgG-488}$ (6)

Under sterile conditions, $\text{Fe}_3\text{O}_4@\text{G}'$ (5) (0.1 mg) in PBS (1 mL) was incubated with IgG-488 (100 μg) in PBS (1 mL) overnight on a shaker at 4°C then washed with PBS (3 x 1 mL) and magnetic separation. Product characterised by FACS.

6.1.3 Fluorescent Antibody-Conjugated Cobalt Ferrite-Doped Silica Nanowires

(i) CoFe_2O_4 (7)

CoFe_2O_4 was produced similar to Fe_3O_4 (1), instead using $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (4.96 g, 0.024 mol) and $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (3.492 g, 0.012 mol), with heating to 90°C and reaction for 2 h. Yield 2.83 g. Product characterised by TEM, XRD, VSM, and FTIR.

FTIR (cm^{-1}): 403, 589, 3406.5

(ii) $\text{CoFe}_2\text{O}_4@\text{SiO}_2$ NWs (8)

TEOS (8.2 mL), EtOH (2.25 mL), H_2O (0.6 mL) and CoFe_2O_4 NPs (7) (20 mg) was made into a solution. HCl (1 drop) was added then the mixture sonicated for 1 h. HCl (3 drops) was then added. Sonication was continued as the sol-gel mixture (3 drops) was extracted and passed through Anodisc 0.1 μm pore 25 mm diameter membranes under vacuum.

The sample was passed through a total of 30 membranes with 3 drops each. The filters were left in an oven overnight, then excess silica was exfoliated from the surface of the membranes with fine grade sandpaper. The membranes were then dissolved with NaOH (20 mL per filter, 0.1 M), with sonication. The wires were then washed by filtration through a nitrocellulose 0.4 μ m pore size 45mm diameter membrane 3 times. Yield 25.7 mg. Product characterised by optical microscope, VSM, and FTIR.
FTIR (cm^{-1}): 795.5, 942.5, 1048, 1646.5, 3369

(iii) $\text{CoFe}_2\text{O}_4@\text{SiO}_2@\text{NH}_2$ NWs (9)

$\text{CoFe}_2\text{O}_4@\text{SiO}_2$ NWs (8) (15.5 mg), were dispersed in EtOH (10 mL) with brief sonication and agitation. Then APTS (2 mL) was added and the mixture agitated for 3 h. The sample was washed with EtOH (1 mL) 3 times with centrifugation then washed by filtration through a PTFE 5 μ m filter 3 times. Yield 11.3 mg. Product characterised by TEM, FTIR
FTIR (cm^{-1}): 454, 551, 685, 800, 937.5, 1076, 1585.5, 1633, 2889, 3354.5

(iv) $\text{CoFe}_2\text{O}_4@\text{SiO}_2@\text{mal}$ NWs (10)

$\text{CoFe}_2\text{O}_4@\text{SiO}_2@\text{NH}_2$ NWs (9) (3.9 mg) was dispersed in THF (1 mL) with sonication. *N*-maleoyl- β -alanine (3.5 mg) and EDC (10 mg) were dissolved in THF (500 μ L), with brief sonication. This solution was added to the nanowire suspension, and the reaction was agitated overnight at room temperature then washed with EtOH (3 x 1 mL) with centrifugation then dried under vacuum. Yield 3.3 mg. Product characterised by optical microscope.

(v) $\text{CoFe}_2\text{O}_4@\text{SiO}_2@\text{CD4-PE}$ NWs (11)

$\text{CoFe}_2\text{O}_4@\text{SiO}_2@\text{mal}$ NWs (10) (3.3 mg) were dispersed in H_2O (1 mL) with sonication. Then CD4-PE (70 μ L, 0.83 gmL^{-1} suspension in H_2O), was added and the reaction agitated for 2 h at room temperature, then washed by filtration through a nitrocellulose 5 μ m pore 45 mm membrane 3 times, with H_2O . Product characterised by TEM.

6.1.4 Silica Coated Metal Nanowires

(i) $\text{Co}@\text{SiO}_2$ NWs (12)

Cobalt NWs (1mL, 1.5 mgmL^{-1} H_2O) was dispersed in H_2O (4.8 mL) and EtOH (20.25 mL) with sonication for 10 min. TEOS (15 μ L) was added, then NH_4OH (1.35 mL) 5 min after. Sonication was continued for 1 h then the product washed with EtOH and magnetic separation (5 x 20 mL). Product characterised by TEM, FTIR
FTIR (cm^{-1}): 379, 500.5, 615, 800, 923, 1091, 1343

(ii) Co@SiO₂@NH₂ NWs (13)

Co@SiO₂ NWs (**12**) (1.5 mg) were dispersed in EtOH (500 μ L), with sonication. APTS (200 μ L) was added and the reaction sonicated for 1 h then washed with EtOH and magnetic separation (5 x 1 mL). Product characterised by TEM, and FTIR.

FTIR (cm⁻¹): 379, 500.5, 615, 800, 923, 1091, 1343, 1584, 2887

6.1.5 Gold/Silica-Coated Cobalt Nanowires

Co@Au NWs (1 mL, 1 mgmL⁻¹) was centrifuged and the water removed. Then EtOH (1 mL) and thioglycerol (200 μ L) was added and the reaction agitated on a shaker overnight. The nanowires were then washed with EtOH (5 x 1 mL) to remove excess thiol. They were then suspended in H₂O (500 μ L), which was added to a mixture of EtOH (10.125 mL) and H₂O (2.4 mL) with sonication for 10 min. Then TEOS (15 μ L) was added and NH₄OH (675 μ L) shortly after. Sonication was continued for 1 h, and then TEOS (15 μ L) was added again. Sonication was continued for a further 30 min, and then the product washed with EtOH and magnetic separation (5 x 10 mL). Product characterised by TEM.

6.1.6 PEGylated Metal Nanowires

(i) Co@PEG-NH₂ NWs (15)

Co NWs (1 mL, 1.5 mgmL⁻¹ H₂O) were centrifuged and the water removed. It was washed once with EtOH (1 mL) then resuspended in EtOH (1 mL). Silane-PEG-NH₂ (0.5 mg, 5 kDa) was added and the mixture sonicated for 3 h. The product was washed by magnetic separation with EtOH (5 x 1 mL). Product characterised by TEM, and FTIR

FTIR (cm⁻¹): 382, 573, 774.5, 892, 1021, 1105.5, 1204, 1300, 1428, 2805, 2922

(ii) Co@PEG-mal NWs (16)

Co NWs (1mg, 1 mgmL⁻¹ H₂O) was centrifuged and the water removed. It was washed once more with EtOH (1 mL) then resuspended in EtOH (1mL). Silane-PEG-maleimide (5kDa, ~0.5 mg) was added and the mixture agitated for 3 h. The product was washed with EtOH using magnetic separation. Product characterised by TEM and FTIR

FTIR (cm⁻¹): 375, 617, 911.5, 998, 1104, 1200, 1303.5, 1425, 1509.5, 2818, 2921

(iii) Co@PEG-COOH NWs (17)

Silane-PEG-COOH (3 mg) was dispersed in EtOH (10 mL) with sonication. To this was added a suspension of Co NWs (1 mL, 30 mgmL⁻¹ H₂O). The reaction was sonicated for 1.5 h, and then washed with EtOH and magnetic separation (5 x 10mL). Product characterised by TEM, and FTIR.

FTIR (cm⁻¹): 339, 572, 776, 907, 993, 1105, 1202, 1300, 1429, 1528.5, 2839, 2922

(iv) Co@PEG-COOH/mal NWs (18)

Silane-PEG-COOH (2.5 mg, 5 kDa) and silane-PEG-mal (2.5 mg, 5kDa) were dispersed in EtOH (10 mL) with sonication. To this was added a suspension of cobalt nanowires (1 mL, 30 mgmL⁻¹ H₂O). The reaction was sonicated for 1.5 h then washed with EtOH and magnetic separation (5 x 10 mL). Product characterised by TEM, FTIR

FTIR (cm⁻¹): 382, 551.5, 775, 907.5, 991, 1105.5, 1202, 1300, 1424, 1510, 2817, 2920

(v) CoNi@PEG-mal (19-22)

CoNi NWs were made into a solution with EtOH (0.5 mgmL⁻¹) and sonicated. silane-PEG-mal (w/w NWs) was made into a solution with EtOH (1 mgmL⁻¹) and sonicated. The PEG solution was then added to the NWs and the reaction sonicated for 1 h. The product was washed with EtOH (3 x 5 mLmg⁻¹). Product characterised by TEM, EDX, and FTIR.

(19) FTIR (cm⁻¹): 419, 578.5, 786, 1048, 1356, 1588.5, 2980

(20) FTIR (cm⁻¹): 362, 517, 983.5, 1103.5, 1350.5, 1551.5, 2933

(21) FTIR (cm⁻¹): 344, 520.5, 575, 740, 864, 1101, 1205, 1353, 1646, 1700, 3386

(22) FTIR (cm⁻¹): 423, 530, 1099, 1353, 1546, 3383

(vi) CoNi@PEG@G'@IgG-488 (23-26)

(i) Under sterile conditions, CoNi@PEG-mal NWs **(21)** (0.1 mg) was dispersed in PBS pH 7.4 (0.5 mL) with vortex agitation. Protein G' (0.01/0.1 mg) was dispersed in PBS pH 7.4 (0.5 mL) with vortex agitation. The protein solution was then added to the NW suspension and agitated at 4°C (2/12 h), then washed with PBS (2 x 0.5 mL). Products characterised by FACS.

(ii) Under sterile conditions, CoNi@PEG@G' NWs (0.1 mg) were dispersed in PBS (0.5 mL). IgG-488 (1 µg) was dispersed in PBS (0.5 mL) then added to the NWs. Agitated at 4°C overnight, then washed with PBS (2 x 0.5 mL). Products characterised by FACS.

6.1.7 PEGylated Segmented Nanowires

(i) CoNi|Au|CoNi|Au|CoNi@OH NWs (27)

[CoNi|Au|CoNi|Au|CoNi] NWs (20 mg) were dispersed in EtOH (20 mL) with vortex agitation. Then 11-MUO (50 mg) in EtOH (10 mL) was added and the reaction agitated overnight on a shaker. Washed with EtOH (3 x 10 mL). Product characterised by TEM, EDX, and FTIR.

FTIR (cm⁻¹): 339, 580, 730, 1640.5

(ii) CoNi|Au|CoNi|Au|CoNi@PEG-mal NWs (28, 29)

CoNi|Au|CoNi|Au|CoNi@OH NWs (**27**) (10 mg) were dispersed in EtOH (10 mL) with vortex agitation. Then Si(OEt)₃-PEG-mal 5K (10 mg) in EtOH/THF/H₂O (100/10/1, 10 mL) was added and the reaction agitated on a shaker for 1 h. Washed with EtOH (3 x 10 mL). Product characterised by TEM and FTIR.

(28) FTIR (cm⁻¹): 351, 582, 735, 1096, 1553

(29) FTIR (cm⁻¹): 351.5, 563, 641, 1076, 1343.5, 1540

(iii) CoNi|Au|CoNi|Au|CoNi@PEG@G'@IgG-A488 (30)

(i) Under sterile conditions, CoNi|Au|CoNi|Au|CoNi@PEG-mal NWs (**28**) (0.1 mg) were dispersed in PBS pH 7.4 (7.5 mL) with vortex agitation. Protein G' (0.1 mg) was dispersed in PBS pH 7.4 (7.5 mL) with vortex agitation. The protein solution was then added to the NW suspension and agitated at 4°C (2 h), then washed with PBS (2 x 15 mL). Product characterised by FACS.

(ii) Under sterile conditions CoNi|Au|CoNi|Au|CoNi@PEG@G' NWs (0.1 mg) were dispersed in PBS (7.5 mL). IgG-488 (1 µg) was also suspended in PBS (7.5 mL). The suspensions were then mixed and the reaction mixture agitated overnight at 4°C on a shaker. Washed with PBS (3 x 5 mL). Product characterised by FTIR and FACS.

FTIR (cm⁻¹): 348.5, 464, 521, 617, 831.5, 916, 1069.5, 1342.5, 1559, 1684.5, 2562, 2841.5, 2991

6.2 SYNTHESIS AND FUNCTIONALISATION OF NICKEL SUB-MICRON PARTICLES

2.2.1 Ni Core Particles (31-34)

Ni(OAc)₂·4H₂O (varied) was added to OAm (varied) and dissolved with stirring. Once degassed, the stirrer bar was removed and the temperature raised to 250°C under Ar. The reaction was continued without stirring at 250°C for varying amounts of time.

Note: For (**31**) stirring was continued throughout the reaction. Products were characterised by TEM, VSM, XRD, FTIR, and UV-vis.

(31) FTIR (cm⁻¹): 368, 415, 501.5, 633, 720.5, 1056, 1335, 1405, 1463, 1586, 2363, 2853.5, 2926

(32) FTIR (cm⁻¹): 393.5, 465, 539, 681, 836.5, 1058, 1358.5, 2924, 3716.5

(33) FTIR (cm⁻¹): 384, 443, 527, 617, 668.5, 843, 951.5, 1053, 1341, 1454.5, 1626, 2363, 2965

(34) FTIR (cm⁻¹): 328, 365, 466, 535.5, 619, 829, 1058, 1337.5, 1440, 1625.5, 2965

Table 6.1: Summary of reaction conditions for the synthesis of nickel particles

Sample	OAm (mL)	Ni(OAc) ₂ (mmol)	Conc. (mM)	Time	Stirring
31	100	2	20	100 min	Y
32	40	8	200	3 h	N
33	40	16	400	3 h	N
34	40	16	400	24 h	N

6.2.2 Gold-Coated Nickel Particles (Ni@Au) (35-37)

(i) Ni@Au@OH (35)

NiCl₂ (2 mmol) was added to OAm (40 mL) and dissolved with stirring. Once degassed, the temperature was raised to 300°C under Ar. The reaction was continued with stirring at 300°C for 5 min. The temperature was then reduced to RT and HAuCl₄.3H₂O (0.5 mmol) was added. The reaction was then stirred at 100°C overnight. 11-MUO (50 mg) was then added and stirring continued at 100°C overnight. Washed with hexane, THF, H₂O. Product characterised by TEM, EDX, VSM, XRD, FTIR, and UV-vis.

FTIR (cm⁻¹): 381.5, 461.5, 475, 553.5, 634.5, 655, 1030, 1357

(ii) Ni@Au@COOH (36)

NiCl₂ (3 mmol) was added to OAm (60 mL) and dissolved with stirring. Once degassed, the temperature was raised to 300°C under Ar. The reaction was continued with stirring at 300°C for 20 min. The temperature was then reduced to RT and HAuCl₄.3H₂O (1 mmol) was added. The reaction was then stirred at 100°C for 2 h. 11-MUA (100 mg) was then added and stirring continued at 100°C overnight. Washed with hexane, THF, H₂O. Product characterised by TEM, EDX, VSM, XRD, FTIR, and UV-vis.

FTIR (cm⁻¹): 472.5, 668, 987, 1130.5, 1307.5, 1493.5, 1717, 2888.5, 3711

(iii) Ni@Au@OH (37)

Ni(OAc)₂.xH₂O (16 mmol) was added to OAm (40 mL) and dissolved with stirring. Once degassed, the temperature was raised to 250°C under Ar, and stirrer bar removed. The reaction was continued without stirring at 250°C for 3 h. The temperature was then reduced to RT, transferred to a sonic bath and HAuCl₄.3H₂O (0.5 mmol) in THF (1 mL) was added while sonicating. After 1 h sonication, 11-MUO (500 mg) was added and sonication continued overnight. Washed with THF, iPrOH, acetone, H₂O. Product characterised by TEM, EDX, VSM, XRD, FTIR, and UV-vis.

FTIR (cm⁻¹): 398.5, 523, 634, 842, 1087, 1304, 1387, 1585, 2901, 2990

Table 6.2: Summary of reaction conditions for the synthesis of gold-coated nickel particles

Sample	OAm (mL)	Ni (mmol)	Conc. (mM)	Au (mmol) Ni/Au ratio	Ligand	Time	Temp (°C)	Stirring
35	40	NiCl ₂ .H ₂ O, 2	50	0.5, solid (RT) 4/1	MUO	5 min/12 h	300/100	Y
36	60	NiCl ₂ .H ₂ O, 3	50	1, solid (RT) 3/1	MUA	20 min/2 h	300/100	Y
37	40	Ni(OAc) ₂ .H ₂ O, 16	400	0.5/THF 32/1	MUO	3 h/3 h	250/40	N/Sonics

6.2.3 Graphene-Coated Nickel Particles (Ni@GNP-x) (38-45)

GNP (150 mg) was dispersed in NMP (150 mL) with sonication for three days to produce a 1 mgmL⁻¹ dispersion. Ni(OAc)₂.xH₂O (varied) was added to OAm (varied) and dissolved with stirring. Once degassed, the stirrer bar was removed and the temperature raised to 250°C under Ar. The reaction was continued without stirring at 250°C for varying amounts of time. The reaction vessel was then transferred to a sonic bath and retained under Ar. The GNP suspension (varied) was then added to the Ni reaction suspension, while sonicating under an Ar flow. The reaction was then sonicated overnight. Products characterised by TEM, VSM, XRD, FTIR, and UV-vis.

(38) FTIR (cm⁻¹): 353, 408.5, 563, 864.5

(39) FTIR (cm⁻¹): 355, 461, 518, 608, 657, 823.5, 992, 1029, 1157, 1346.5, 1439.5, 1546.5, 1611

(40) FTIR (cm⁻¹): 436.5, 680, 846, 1058, 1381.5, 1123, 1471, 1565, 1644

(41) FTIR (cm⁻¹): 482, 544, 626, 669, 847, 915, 1074.5, 1115.5, 1277, 1376, 1549

(42) FTIR (cm⁻¹): 412.5, 460, 534, 628, 670.5, 838, 977, 1057, 1107, 1375, 1584, 2918, 3750

(43) FTIR (cm⁻¹): 338.5, 413, 527, 623.5, 830, 1027, 1260.5, 1313, 1437.5, 1584.5, 2960

(44) FTIR (cm⁻¹): 1054, 1217, 1739. 2946, 3034

(45) FTIR (cm⁻¹): 526.5, 625, 679.5, 725.5, 842, 974, 1069, 1123.5, 1250, 1422, 1571, 1642, 1745

Table 6.3: Summary of reaction conditions for the synthesis of graphene-coated nickel particles

Sample	OAm (mL)	Ni (mmol)	GNP-X (1 mgmL ⁻¹ in NMP)	Ni Rxn Time
38 Ni@GNP	50	16	GNP 10 mL, 20min	1.5 h
39 Ni@GNP@NH ₂	40	4	GNP@NH ₂ 10 mL, 20 min	2 h
40 Ni@GNP@NH ₂	40	16	GNP@NH ₂ 5 mL, 30 min	3 h

41 Ni@GNP@NH ₂	40	16	GNP@NH ₂ 10 mL rapid	3 h
42 Ni@GNP@NH ₂	40	16	GNP@NH ₂ 10 mL, 30 min	3 h
43 Ni@GNP@NH ₂	40	16	GNP@NH ₂ 20 mL, 30 min	3 h
44 Ni@GNP@COOH	40	16	GNP@COOH 10 mL rapid	3 h
45 Ni@GNP@COOH	50	16	GNP@COOH 10 mL 20 min	1.5 h

6.2.4 Graphene-Coated Nickel Particle Bioconjugation Reactions

(i) Ni@GNP@PEG-mal (46)

Ni@GNP-NH₂ (**39**) (100 mg) was dispersed in H₂O (80 mL) with sonication for ~5 min. A solution of HOOC-PEG-mal 5K (35 mg) and EDC (200 mg) in H₂O (70 mL) was then added. Sonication was continued at RT for 24 h, then washed with H₂O (4 x 20 mL). Product characterised by TEM, VSM, and FTIR.

FTIR (cm⁻¹): 309, 328, 370, 399, 500, 595.5, 669, 798, 878.5, 1040, 1159, 1382, 1425, 1483, 1589, 1672, 1715, 2882.5, 2960.5 3665.5

(ii) Ni@GNP@mal (47)

Ni@GNP-NH₂ (**39**) (225 mg) was dispersed in iPrOH (130 mL) with sonication for ~5 min. A solution of N-maleoyl-β-alanine (10 mg) and EDC (20 mg) in H₂O (130 mL) was then added. Sonication was continued at RT for 1 h, then washed with H₂O (4 x 20 mL). Product characterised by TEM, VSM, and FTIR

FTIR (cm⁻¹): 387, 458, 656, 1033.5, 1346.5, 1465, 1581, 1701, 2849, 2919

(iii) Ni@GNP@G' (48)

Ni@GNP@mal (**47**) (100 mg) was dispersed in PBS (200 mL) with agitation. A solution of Protein G' (10 mg, 1mgmL⁻¹ PBS) was then added, and the reaction agitated on a shaker for 1 h. Product characterised by TEM, VSM, and FTIR.

FTIR (cm⁻¹): 417, 463, 523.5, 651, 1029, 1244, 1335, 1521, 1634, 2563, 2901, 3194.5

6.2.5 Other Sub-Micron Particles

(i) Cobalt Particles (49-51)

CoCl₂/Co₂(CO)₈ (1/4 mmol) was added to OAm (20/40 mL) and dissolved with stirring. Once degassed, the stirrer bar was removed and the temperature was raised (180/250°C) under Ar. The reaction was continued without stirring at this temperature for 18-20 h. Product characterised by TEM

Table 6.4: Summary of reaction conditions for the synthesis of cobalt particles

Sample	OAm (mL)	Co (mmol)	Ligand	Time	Temp (°C)	Stirring
49	20	CoCl ₂ , 1	DDT	18 h	180	N
50	20	Co ₂ (CO) ₈ , 1	DDT	18 h	180	N
51	40	CoCl ₂ , 4	OAm	20 h	250	N

(ii) Cobalt-Platinum Alloy Particles (CoPt) (52)

CoCl₂ (0.5 mmol) and Pt(acac)₂ were added to OAm (20 mL) and dissolved with stirring. Once degassed, the temperature raised to 180°C under Ar and the stirrer bar was removed. The reaction was continued without stirring at 180°C for 18 h. The temperature was then reduced to 85°C and DDT (15 mL) was added. The reaction was then stirred at this temp. overnight. Washed with toluene and MeOH. Product characterised by TEM, VSM

(iii) Platinum-Coated Cobalt Particles (Co@Pt) (53)

CoCl₂ (2 mmol) was added to OAm (40 mL) and dissolved with stirring. Once degassed, the temperature was raised to 250°C under Ar. The reaction was continued with stirring at 250°C for 2 h. The temp. was then reduced to RT and Pt(acac)₂ (0.5 mmol) was added. The reaction was then stirred at 180°C for 1 h. The temp was then reduced to RT and MBA (100 mg) was added. The reaction was then stirred at 100°C overnight. Washed with hexane, THF, H₂O. Product characterised by TEM, and FTIR

FTIR (cm⁻¹): 356.5, 430, 517, 554.5, 985.5, 1040.5, 1342, 1399.5, 1550, 2887, 2981, 3328.5

(iv) Platinum-Coated Nickel Particles (Ni@Pt) (54)

NiCl₂ (2 mmol) was added to OAm (40 mL) and dissolved with stirring. Once degassed, the temperature was raised to 300°C under Ar. The reaction was continued with stirring at 300°C for 15 min. The temperature was then reduced to RT and Pt(acac)₂ (0.5 mmol) was added. The reaction was then stirred at 180°C for 1 h. MBA (100 mg) was then added and stirring continued at 100°C overnight. The product was then washed with hexane, THF and H₂O. Product characterised by TEM, VSM, and XRD.

6.3 SYNTHESIS AND FUNCTIONALISATION OF ANISOTROPIC NANOMATERIALS

6.3.1 Iron-Based Alloys

(i) Iron-Platinum (FePt) (55)

OAm (40 mL) was degassed under vacuum at 80°C with stirring, then covered in a blanket of Ar. Pt(acac)₂ (1 mmol) was added and stirring was continued until fully dissolved. The temperature was then raised to 180°C. At 120°C, Fe(CO)₅ (300 µL, 2.3 mmol) was added rapidly. Stirring was continued at 180°C for 30 min. Product characterised by TEM, EDX, VSM, XRD, FTIR, and UV-vis.

FTIR (cm⁻¹): 3321.5, 2957.5, 2919, 2851, 1642, 1546, 1467.5, 1378.5, 1176, 1087.5, 963.5, 720.5, 616, 574, 455, 405, 364, 332.5, 309

(ii) Iron-Palladium/Magnetite (FePd@Fe₃O₄) (56-59)

OAm (varied) was degassed under vacuum at 80°C with stirring, then covered in a blanket of Ar. Pd(OAc)₂ (varied) was added and stirring was continued until fully dissolved. The temperature was then raised to 180°C. At 120°C, Fe(CO)₅ (varied) was added rapidly. Stirring was continued at 180°C for 20-30 min. Below is a summary table of the reaction conditions for different samples. One sample was treated with phosphoric acid, to oxidise the surface for aqueous transfer, the remaining were washed with hexane/acetone (3 x 40 mL). Products characterised by TEM, EDX, VSM, XRD, FTIR, and UV-vis.

(56) FTIR (cm⁻¹) 399, 571.5, 1409, 1529.5, 2846.5, 2907.5

(57) FTIR (cm⁻¹): 402, 583.5, 1425, 1525.5, 2852, 2924

(58) FTIR (cm⁻¹) 611, 1067, 1378, 1520, 2901, 2975

(59) FTIR (cm⁻¹): 438.5, 561, 961.5, 1359, 2845, 2912, 2954

Table 6.5: Summary of reaction conditions for the synthesis of iron-palladium/magnetite particles

Sample	OAm (mL)	Fe(CO) ₅ (mmol)	Pd(OAc) ₂ (mmol)	Conc (mM) (Fe/Pd)	Ratio Fe/Pd	Time (min)	Temp (°C)
56	100	23	2	230/20	11.5/1	30	180
57	50	7.67	1	152/20	7.66/1	30	180
58	50	3.83	1	76/20	3.83/1	30	180
59	50	2.3	1	45.6/20	2.3/1	20	180

(iii) Iron-Nickel (FeNi) (60-66)

OAm (50 mL) was degassed under vacuum at 80°C with stirring, and then covered in a blanket of Ar. $\text{NiCl}_2 \cdot 4\text{H}_2\text{O}$ (130 mg, 1 mmol) was added and stirring was continued until fully dissolved. The temperature was then raised to 160°C. At 160°C, $\text{Fe}(\text{CO})_5$ (150/300 μL , 1.55/2.3 mmol) was added rapidly. Stirring was continued at 160°C for varying amounts of time. **Table 6.6** summarises the reaction conditions. Washed with hexane/acetone (3 x 40 mL). Products characterised by TEM, EDX, VSM, XRD, FTIR, and UV-vis.

(60) FTIR (cm^{-1}): 388.5, 719, 1005.5, 1306.5, 1456, 2851, 2920

(61) FTIR (cm^{-1}): 373, 490.5, 612, 720, 963.5, 1057, 1147, 1323, 1454.5, 1561, 1608, 2849, 2916, 2953, 3002, 3265

(62) FTIR (cm^{-1}): 499, 584, 720, 816, 963, 1058, 1323, 1464, 1578, 1612, 2849, 2918, 2999, 3315

(63) FTIR (cm^{-1}): 323.5, 400, 516, 666, 827, 1050, 1339, 2842, 2905

(64) FTIR (cm^{-1}): 381, 472, 523, 634, 794.5, 833, 922.5, 1016.5, 1339, 1550, 2845.5, 2920, 3170.5

(65) FTIR (cm^{-1}): 357, 668, 1303, 2840

(66) FTIR (cm^{-1}): 403, 606, 708, 831.5, 1074, 1400, 1456, 1565, 2851.5, 2922, 3314

Table 6.6: Summary of reaction conditions for the synthesis of iron-nickel particles

Sample	OAm (mL)	$\text{Fe}(\text{CO})_5$ (mmol)	$\text{NiCl}_2 \cdot \text{H}_2\text{O}$ (mmol)	Conc. (mM) (Fe/Ni)	Time	Temp. (°C)
60	50	1.55	1	30.4/20	4 h	160
61	50	1.55	1	30.4/20	5 h	160
62	50	1.55	1	30.4/20	24 h	160
63	50	1.55	1	30.4/20	5 d	160
64	50	2.3	1	45.6/20	4 h	160
65	50	2.3	1	45.6/20	5 h	160
66	50	2.3	1	45.6/20	24 h	160

(iv) Iron-Copper (FeCu) (67, 68)

$\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ (85/170 mg, 0.5/1 mmol) was added to OAm (50 mL) and degassed under vacuum at 100°C with stirring, and then covered in a blanket of Ar. Stirring was continued until fully dissolved, with some sonication also. The temperature was then raised to 160/180°C, then $\text{Fe}(\text{CO})_5$ (0.275/3 mL, 2.9/23.3 mmol) was added rapidly. Stirring was

continued at 160/180°C for 1 h/30 min. Washed with hexane/acetone (3 x 40 mL). Products characterised by TEM, EDX, VSM, XRD, FTIR, and UV-vis.

(67) FTIR (cm^{-1}): 429, 495, 711, 758, 841, 965.5, 1050, 1348.5, 1376, 1457, 1565, 1665, 2852.5, 2922, 3325.5, 3570

(68) FTIR (cm^{-1}): 472, 615, 721, 847, 963, 1082, 1188, 1377.5, 1460, 1575, 1614, 2850.5, 2922, 2954.5, 3329

6.3.2 Other Alloys

(i) Cobalt-Platinum (CoPt) (69, 70)

OAm (40 mL) was degassed under vacuum at 80°C with stirring, then covered in a blanket of Ar. $\text{Pt}(\text{acac})_2$ (1 mmol) was added and stirring was continued until fully dissolved. The temperature was then raised rapidly. At 120°C, $\text{Co}_2(\text{CO})_8$ (2.3 mmol) in OAm (5 mL) was added rapidly. The temperature was raised further to 160°C and stirring was continued at this temperature for 3 days. The temperature was then reduced and 4-aminothiophenol (100 mg) was added. Stirring was continued overnight at 80°C. Washed with hexane/acetone (3 x 40 mL). Product characterised by TEM, EDX, VSM, and XRD.

(ii) Iron-Cobalt (FeCo) (71-73)

OAm (50 mL) was degassed under vacuum at 80°C with stirring, and then covered in a blanket of Ar. CoCl_2 (130/260 mg, 1/2 mmol) was added and stirring was continued until fully dissolved, with some sonication. The temperature was then raised to 160°C. At 160°C, $\text{Fe}(\text{CO})_5$ (500 μL , 3.88 mmol) was added rapidly. Stirring was continued at 160°C for varying amounts of time. **Table 6.7** summarises the reaction conditions. Washed with hexane/acetone (3 x 40 mL). Products characterised by TEM, EDX, and UV-vis.

Table 6.7: Summary of reaction conditions for the synthesis of iron-cobalt particles

Sample	OAm (mL)	$\text{Fe}(\text{CO})_5$ (mmol)	CoCl_2 (mmol)	Conc. (mM) (Fe/Co)	Time	Temp (°C)
71	50	3.88	1	76.1/20	5 h	160
72	50	3.88	1	76.1/20	19 h	160
73	50	3.88	2	76.1/40	1 h	160

(iii) Iron-Gold (FeAu) (74)

OAm (50 mL) was degassed under vacuum at 80°C with stirring, then covered in a blanket of Ar. $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ (400 mg, 1 mmol) was dispersed in NMP (2 mL). The temperature was then raised to 160°C. At 160°C, $\text{Fe}(\text{CO})_5$ (2.6 mmol) and the Au solution were added rapidly, simultaneously, by hot injection under Ar. Stirring was continued at 180°C for 30 min. Washed with hexane/acetone (3 x 40 mL). Product characterised by TEM, EDX, VSM, XRD, FTIR, and UV-vis.

FTIR (cm^{-1}): 395, 720, 808, 964, 1058, 1307, 1376, 1464, 1556, 1619.5, 2852, 2920, 3005, 3312.5

(iv) Manganese-Bismuth (MnBi) (75)

OAm (50 mL) was degassed under vacuum at 80°C with stirring, then covered in a blanket of Ar. BiCl_3 (315 mg, 1 mmol) was added and stirring continued until dissolved. The temperature was then raised to 160°C. Once at this temperature, a solution of $\text{Mn}_2(\text{CO})_{10}$ (250 mg, 1.2 mmol) in NMP (5 mL) was added by syringe. The reaction was continued at 160°C with stirring for 30 min then cooled to RT. Washed with hexane/acetone (3 x 40 mL). Product characterised by TEM, EDX, VSM, FTIR, and UV-vis.

FTIR (cm^{-1}): 401, 499, 595.5, 957, 1082, 1145, 1383, 1598, 2891.5

6.3.3 Sulfur-Based Semiconductors

(i) Copper Sulfide (CuS) (76, 77)

(a) OAm (40 mL) was degassed under vacuum at 80°C with stirring, then covered in a blanket of Ar. $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ (340 mg, 4 mmol) was added and stirring continued until dissolved. Sulfur (1 mmol) was then added and the temperature was raised to 250°C with stirring. The reaction was continued at 250°C for 15 min then cooled to RT. 4-ATP was then added, and the reaction stirred at 80°C for 3 d. Product characterised by TEM, XRD, FTIR, UV-vis and PL.

FTIR (cm^{-1}): 328, 419.5, 488.5, 610.5, 1106, 1241, 1590.5, 1663, 3260

(b) Sulfur (2 mmol) was added to OAm (50 mL) and degassed under vacuum at 80°C with stirring, then covered in a blanket of Ar. The temperature was then raised to 160°C. Once at this temperature, a solution of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ (170 mg, 1 mmol) in NMP (5 mL) was added by syringe. The reaction was continued at 160°C with stirring for 30 min then cooled to RT. Washed with hexane/acetone (3 x 40 mL). Product characterised by TEM, XRD, FTIR, UV-vis and PL.

FTIR (cm^{-1}): 354, 433, 616, 681, 860.5, 965, 1106, 1373.5, 1454, 1629, 2852, 2914, 2950.5, 3136.5

(ii) Bismuth Sulfide (Bi₂S₃) (78)

Sulfur (2 mmol) was added to OAm (50 mL) and degassed under vacuum at 80°C with stirring, then covered in a blanket of Ar. The temperature was then raised to 160°C. Once at this temperature, a solution of BiCl₃ (410 mg, 1.3 mmol) in NMP (5 mL) was added by syringe. The reaction was continued at 160°C with stirring for 30 min then cooled to RT. Washed with hexane/acetone (3 x 40 mL). Product characterised by TEM, EDX, XRD, FTIR, UV-vis and PL.

FTIR (cm⁻¹): 430, 529, 612, 719.5, 1033, 1160, 1462, 1520, 1613.5, 2850.5, 2919.5, 3097.5, 3260

(iii) Nickel Sulfide (NiS) (79, 80)

Sulfur (2 mmol) was added to OAm (50 mL) and degassed under vacuum at 80°C with stirring, then covered in a blanket of Ar. The temperature was then raised to 160°C. Once at this temperature, a solution of NiCl₂·6H₂O (240 mg, 1 mmol) in NMP (5 mL) was added by syringe. The reaction was continued at 160°C with stirring for 30 min/2 h then cooled to RT. Washed with hexane/acetone (3 x 40 mL). Products characterised by TEM, EDX, XRD, VSM, FTIR, UV-vis and PL.

(79) FTIR (cm⁻¹): 327, 624.25, 720.5, 965.75, 1038.25, 1172.75, 1378.25, 1466, 1559.5, 1658.75, 2853.5, 2923, 3005.75, 3250

(80) FTIR (cm⁻¹): 613, 718.75, 964.5, 1071.25, 1377.5, 1466.25, 1559, 1646, 2850.25, 2916.75, 3327

(iv) Iron Sulfide (FeS) (81 - 83)

Sulfur (2 mmol) was added to OAm (50 mL) and degassed under vacuum at 80°C with stirring, then covered in a blanket of Ar. The temperature was then raised to 160°C. Once at this temperature, a solution of FeCl₂·4H₂O/ FeCl₃·6H₂O (200/270 mg, 1 mmol) in NMP (5 mL) was added by syringe. The reaction was continued at 160°C with stirring for 30/60 min then cooled to RT. Table 6.8 summarises the reaction conditions. Washed with hexane/acetone (3 x 40 mL). Products characterised by TEM, EDX, XRD, FTIR, VSM, UV-vis and PL

(81) FTIR (cm⁻¹): 353.5, 468, 540, 613, 720.5, 964, 1020, 1196, 1300, 1365, 1456, 1550, 1662, 2850, 2919, 3002

(82) FTIR (cm⁻¹): 352, 460, 556, 616, 721, 952.5, 1021, 1161, 1377, 1467, 1546, 1656.5, 2850, 2919

(83) FTIR (cm⁻¹): 360, 466, 1021, 1465.5, 1667, 2852, 2921, 3006

Table 6.8: Summary of reaction conditions for the synthesis of iron sulfide particles

Sample	OAm (mL)	Fe(X)Cl _x .H ₂ O (mmol)	S (mmol)	Conc. (mM) Ratio start (Fe/S)	Ratio finish (Fe/S) EDX/XRD	Time (min)	Temp. (°C)
81	50	1, Fe(II)	2, OAm	26/40 1/1.54	Fe ₄₁ S ₅₉ Fe ₃ S ₄	30	160
82	50	1, Fe(III)	2, OAm	26/40 1/1.54	Fe ₄₀ S ₆₀ -	30	160
83	50	1, Fe(III)	2, OAm	26/40 1/1.54	Fe _{29.42} S _{70.58} Fe ₃ S ₄	60	160

(v) Manganese Sulfide (MnS) (84)

Sulfur (2 mmol) was added to OAm (50 mL) and degassed under vacuum at 80°C with stirring, then covered in a blanket of Ar. The temperature was then raised to 160°C. Once at this temperature, a solution of Mn(acac)₃ (350 mg, 1 mmol) in NMP (5 mL) was added by syringe. The reaction was continued at 160°C with stirring for 30 min then cooled to RT. Washed with hexane/acetone (3 x 40 mL). Product characterised by TEM, EDX, FTIR, UV-vis, PL

FTIR (cm⁻¹): 320, 365, 420, 505.5, 612, 1098.5, 1466, 1661, 2854, 2925.5, 3000

6.3.4 Combined Heterostructures

(i) FePt@Au Tail Head Nanostructures (85)

Pt(acac)₂ (200 mg, 0.5 mmol) was dissolved in OAm (20 mL), then degassed. The temperature was then raised rapidly to 120°C, Fe(CO)₅ (150 µL, 1.55 mmol) was added with brief stirring then the stirrer bar removed. The temperature was raised further to 160°C, and allowed to react for 2 days before cooling to RT. To the solution of FePt NWs (0.5 mmol) in OAm (20 mL) was added a solution of HAuCl₄.3H₂O (200 mg, 0.5 mmol) in toluene/OAm (20/5 mL) with stirring. The temperature was then raised to 85°C and stirred overnight. DDT (20 mL) was then added and stirred again overnight at this temperature before cooling and washing with toluene/MeOH. Product characterised by TEM, XRD, VSM, FTIR, and UV-vis.

(85) FTIR (cm⁻¹): 330, 390.5, 575, 716, 960.5, 1039, 1139, 1439, 1529, 1607.5, 2851, 2916, 3314

(ii) FePt@Au Wire/Particle Networks (86)

Pt(acac)₂ (1 g, 2.5 mmol) was dissolved in OAm (80 mL), then degassed. The temperature was then raised rapidly to 120°C, Fe(CO)₅ (800 µL, 6.13 mmol) was added with stirring. The temperature was raised further to 160°C and then the stirrer bar was

removed. The reaction was continued for 2 days before cooling to 60°C. To the solution of FePt NWs (2.5 mmol) in OAm (80 mL) was added a solution of H₂AuCl₄·3H₂O (1 g, 2.5 mmol) in toluene/OAm (80/20 mL) with stirring. The temperature was then raised to 90°C and stirred for 3 days. 4-ATP (500 mg) was then added and stirred again overnight at this temperature before cooling and washing with toluene/MeOH. Product characterised by TEM, XRD, VSM, FTIR, and UV-vis.

(86) FTIR (cm⁻¹): 378, 518, 596.5, 821, 1034, 1106, 1197, 1311, 1425.5, 1697, 2813.5, 2933.5, 3050

Table 6.9: Summary of reaction conditions for the synthesis of iron-platinum/gold particles

Sample	OAm (mL)	FePt (mmol)	HAuCl ₄ ·H ₂ O (mmol)	Conc. (mM) (FePt/Au)	Ligand	Time	Temp (°C)	Stirring
85	20	0.5	0.5	25/25	DDT	2 d/12 h	160/85	N/Y
86	80	2.5	2.5	31.25/31.25	OAm	2 d/3 d	160/90	N/Y

6.3.5 PEGylation and Aqueous Transfer

FePt@PEG-NH₂ (87)

Fe₆₅Pt₃₅ NWs (**55**) (2.5 mg) was dispersed in iPrOH (25 mL, 1mgmL⁻¹). DSPE-PEG-NH₂ (25 mg) was dispersed in CBS pH6 (25 mL) with sonication. The solutions were mixed and sonicated for ~10 min at 40°C. The iPrOH was then evaporated to leave the CBS/FePt dispersion. Washed with CBS (1 x 25 mL) and H₂O (2 x 25 mL). Product characterised by TEM, EDX, VSM, FTIR, and UV-vis.

FTIR (cm⁻¹): 3193, 2915.5, 2849, 1735, 1640, 1532, 1394, 1242, 1179.5, 1099.5, 1066, 961, 717.3, 614.5, 549, 492.5, 426, 412.5, 405, 394.5, 362.5, 345, 336, 320, 311

6.4 CHARACTERISATION TECHNIQUES

6.4.1 Transmission Electron Microscopy

TEM analyses were performed using a FEI Titan transmission electron microscope. For many of the products described here, TEM is the primary method of characterisation. This method provides size and morphological data for the particles, which is essential to confirm structural anisotropy in the materials. The technique uses an electron beam, in the X-ray spectral region. At this wavelength, the energy can be either diffracted from the material

Contrast in TEM, and hence imaging, relies on scattering of the incident rays. Rayleigh scattering is possible at low magnification, however Bragg scattering is the predominant source of information in TEM. This involves the interaction of incident X-rays with neutrons in the nuclei of materials or unpaired electrons in their shells. Scattering through the multiple structured planes of crystalline materials amplifies scattering at specific angles, hence crystalline materials are more readily detectable by this technique. The angles are determined by the crystal structure and Bragg's law. (Eqn. 6.1). This technique is complimented by XRD.

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

Diffraction contrast is the predominant source of information at low resolution TEM, through interaction with the atomic planes in the crystal and hence diffraction. In HRTEM, phase contrast is also a factor in information processing, due to the interaction of the electron beam with the electrons in the atomic shells. This interaction can be either constructive, whereby they combine to form a higher energy wavelength, or destructive, whereby they cancel each other to reduce the electron energy.

6.4.2 Electron Diffraction X-Ray Spectroscopy

EDX analyses were performed using a FEI Titan transmission electron microscope. EDX provides information on the elemental composition of a material. An electron beam is diffracted by the shell electrons of different elements at distinct angles. A detector monitors this electron diffraction and quantifies the ratio of the various elements detected, by counting the occurrences at distinct energies between 0-20 keV. In this way we can obtain a ratio of elements in the sample.

In this research EDX is used to quantify the degree of binding of silane groups to the surface of particles by comparison with iron atoms in the core of the particle. It is also

used here to determine the ratio of elements within an alloy composite. Generally Cu grids coated with holey carbon for organic nanomaterials or lacey carbon for aqueous nanomaterials were used. In some cases Mo grids were used to detect the presence of Cu in the sample.

Characteristic X-rays

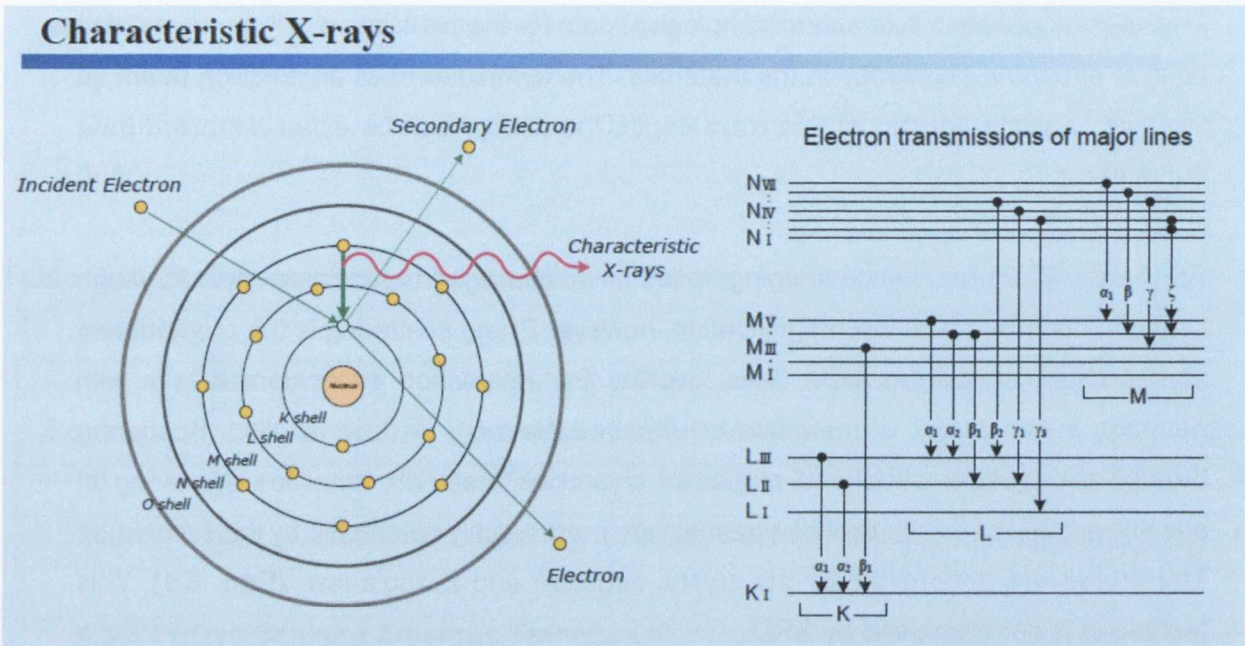


Figure 6.10: Electron transition energies measured by EDX¹

Table 6.11: EDS energies of electron hole pairs for various elements

Z	Element	Kα	Lα	M
6	C	0.277	-	-
8	O	0.525	-	-
14	Si	1.739	-	-
15	P	2.013	-	-
16	S	2.307	-	-
25	Mn	5.894	0.637	-
27	Co	6.924	0.776	-
28	Ni	7.471	0.851	-
29	Cu	8.04	0.93	-
36	Fe	6.398	0.705	-
42	Mo	17.441	2.293	-
46	Pd	-	2.838	-
78	Pt	-	9.441	2.048
79	Au	-	9.712	2.12
83	Bi	-	10.837	2.419

6.4.3 X-ray Diffraction Spectroscopy

XRD analyses were performed using a Siemens D500 diffractometer and PANalytical VRD Diffractometer. XRD is used to determine the crystal structure for a material. The peaks obtained are compared against literature values for a given material, which is unique to that material, based on the unit cell and lattice parameters for the particular crystal structure. These peaks appear at specific angles and correspond to a specific coordinates within the unit cell for that material. Hence, the crystal structure can be determined. X-rays are in the nanometre and sub-nanometre region of the electromagnetic spectrum. Hence they can interact with the lattice planes between neighbouring atoms, which represent the distances between planes in the crystal structure and also bond lengths between individual atoms. This creates a scattering effect which is interpreted and measured using Bragg's law. The scattering angle is inversely proportional to the distance between the atoms.

6.4.4 Vibrating Sample Magnetometry

VSM analyses were performed using a custom made permanent magnet variable flux source vibrating sample magnetometer. VSM provides information on the magnetic characteristics of the material. Magnetisation is recorded in the presence of a magnetic field which varies from 1 Tesla to -1 Tesla, and then the reverse back to 1 Tesla. From the hysteresis loop obtained we can quantify the maximum magnetic moment (magnetisation) of the sample and its residual magnetism in the absence of a magnetic field (remanence). Ideally, for the applications involved in this research, the material should have high magnetisation and low remanence.

6.4.5 Infrared Spectroscopy

Diamond tip FTIR spectrometry was performed using a Perkin Elmer Spectrum One NTS FTIR spectrometer. This was the primary technique used for surface characterisation, and is based on absorbance of energy in the IR spectral region which corresponds to vibrational energies between bonds in the materials.

In early experiments, for bare NP characterisation, diffuse reflectance IR (DRIR) was used with samples ground with Cel salt and measured as powder. DRIR is ideal for samples with a large surface area, such as NPs, and has an extended range of 4000-200 cm^{-1} which is needed for identification of metalo-organic and metal oxide/chalcogenide bonds. Secondly diamond tip FTIR was used for the characterisation of organic groups at the surface of nanoparticles. Dry, neat samples were placed directly on the crystal under high force and backgrounds were run in ambient conditions.

Normally, diamond tip FTIR is only used in the range of 4000-650 cm^{-1} however the spectra show greater resolution and definition than DRIR, which is needed for measuring surface functional groups representing only about 1 % of the overall sample mass.

In later experiments, a new FTIR technique was available, which allowed for the simultaneous measurement of organic, inorganic and metal bonds, though long-range FTIR using a Spectrum 100 FT-IR Spectrometer. This technique greatly improved and enhanced the data processing and output of information, and allowed for relative intensities to be observed also. The procedure was optimised for a range of 4000-300 cm^{-1} , however longer ranges are possible. Here samples were compared against a baseline of ambient conditions and dried onto the crystal from dispersion in a volatile solvent to produce a thin film with optimum contact with the crystal. This proved more effective than dry samples under pressure to form good contact with the crystal.

Successful and complete conversion of functional groups can be assessed using FTIR. These peaks are generally very small, in the 1-10% transmittance range due to their small contribution to the overall mass. Longer scans, over a period of 5-30 min were therefore used to gain optimum resolution and intensity for the peaks. Scans for pure organic compounds normally only require a few seconds.

6.4.6 UV-Vis Absorption Spectroscopy

UV-vis absorption measurements were performed using a Agilent Technologies Cary 60 UV-vis spectrophotometer. Here, UV-vis spectroscopy is used to characterise plasmonic and luminescent semiconducting and metal materials. Electromagnetic energy in this region can interact with the materials in a number of ways, primarily through electron excitation to non-occupied orbitals, scattering of the waves from materials, or through interaction with the electromagnetic field of the particle, either in their body or at their surfaces. For plasmonic absorbance, the location of these peaks differ significantly between nanoparticles of different compositions, morphologies and sizes, however they are characterised by a lack of photoluminescence when excited at the peak absorption wavelengths. This excitation, instead interacts with the local electrical field, which then lead to the interesting properties such as signal amplification seen in infrared, Raman, and photoluminescence by surface enhanced plasmon resonance.

6.4.7 Photoluminescence Spectroscopy

PL characterised was performed primarily for the anisotropic semiconductors prepared here, as these types of materials are well documented for photoluminescent properties in the literature. Measurements were performed using a Horiba Scientific FlouroMax-4

Spectrophotometer using a high energy excitation wavelength for maximum emission detection, over the full spectral region of 240-1100 nm, in order to detect any possible luminescence. However, it should also be noted that OAm, the surface ligand used in these experiments, contains its own intrinsic luminescence, with emission at ~350 nm, which can mask the spectra.

6.4.8 FACS

FACS analysis was performed using a FACS Canto desktop flow cytometer. This technique required aqueous solutions, and relied on scattering of laser excitation from the particles onto a detector. There are two detectors present for either side or forward scattering and information is related to the intensity and duration of the scattered light. Hence it can be related to the size, shape and density of the material when relative, control scans are performed under standardised conditions. Information processing for this technique is much more complex and less defined, due to the large complexity of the systems being measured, including solvent flow and viscosity and differential scattering of the materials, particularly for anisotropic structures and composite and/or bioconjugated materials.

REFERENCES

1. www.jeol.com, *Energy Table for EDS Analysis*.

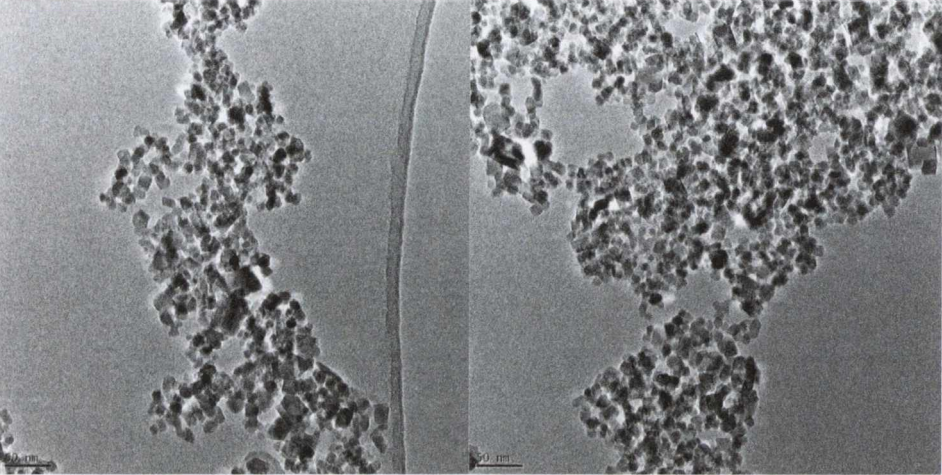
APPENDIX TO CHAPTER 2

APPENDIX 2.1

FERRITE SYNTHESIS AND FUNCTIONALISATION

A.2.1.1 TEM

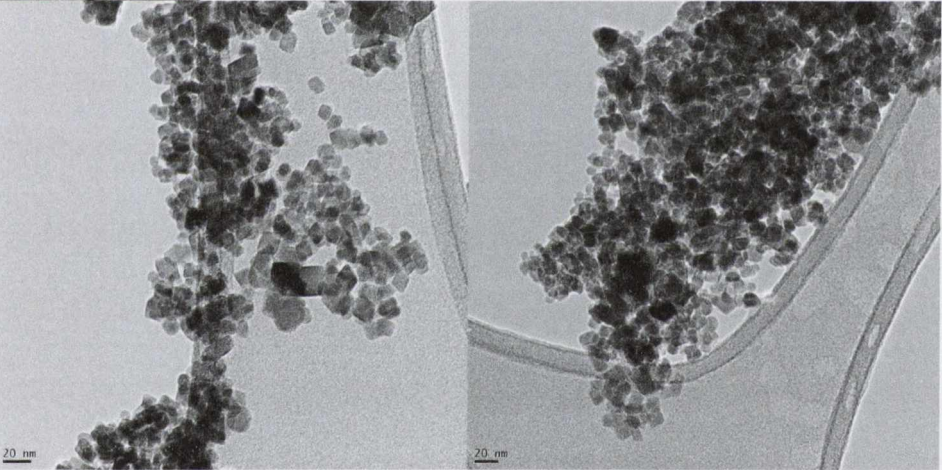
Fe_3O_4 (1)



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Scale Bar: 50 nm

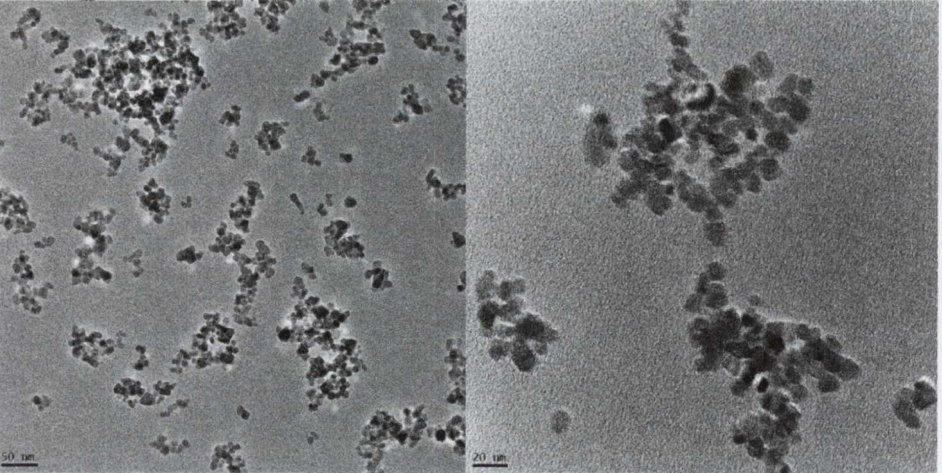
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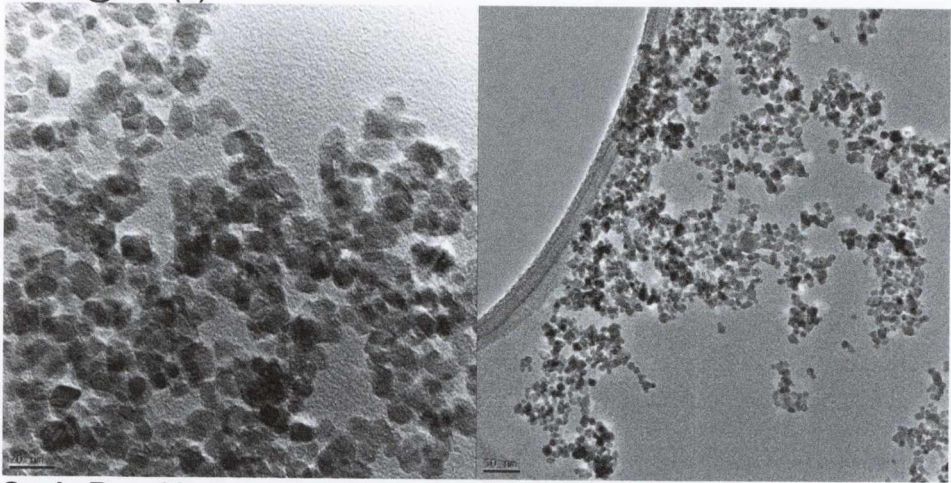
$\text{Fe}_3\text{O}_4@\text{COOH}$ (3)



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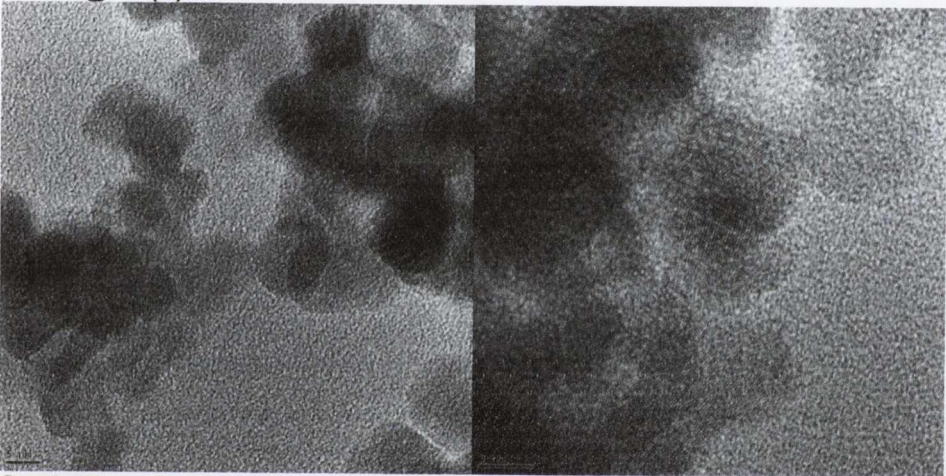
Fe₃O₄@mal (4)



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Fe₃O₄@G' (5)

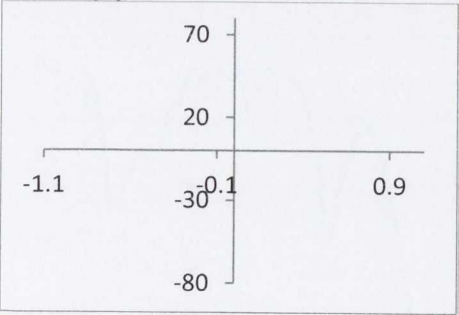


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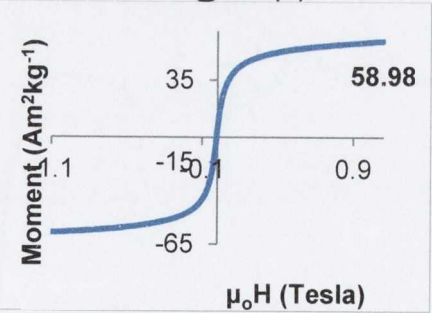
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A.2.1.2 VSM

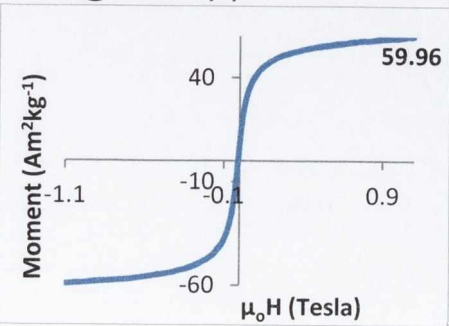
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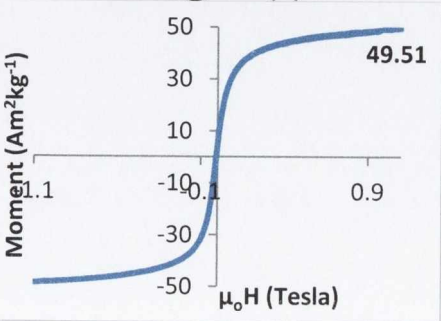
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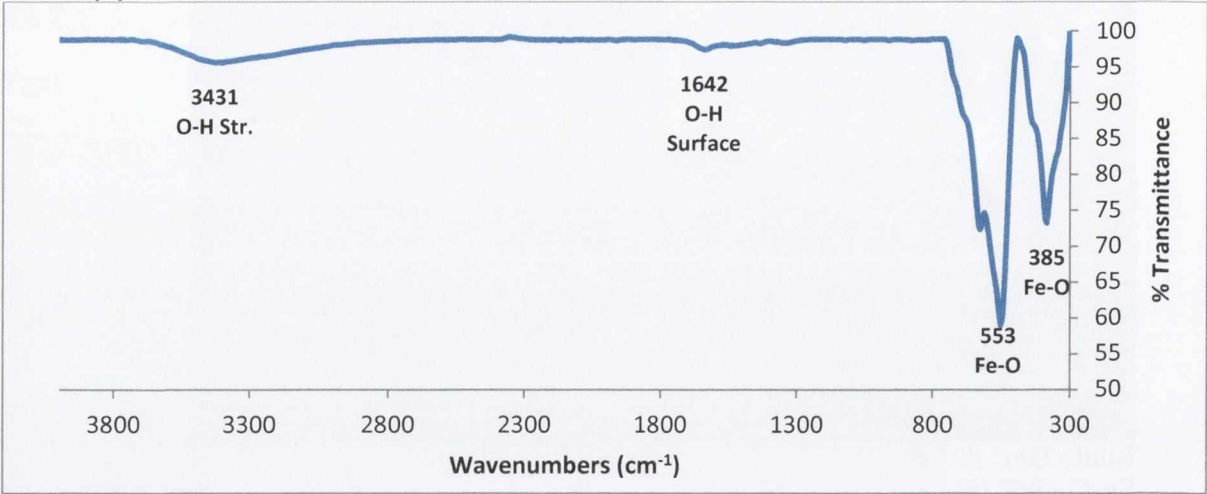
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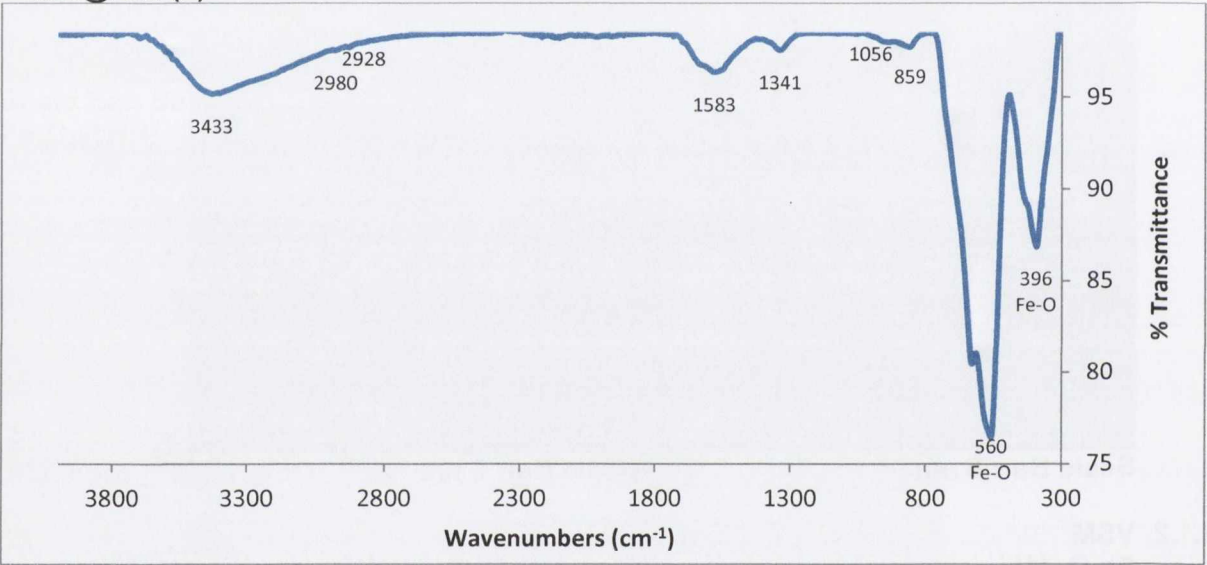
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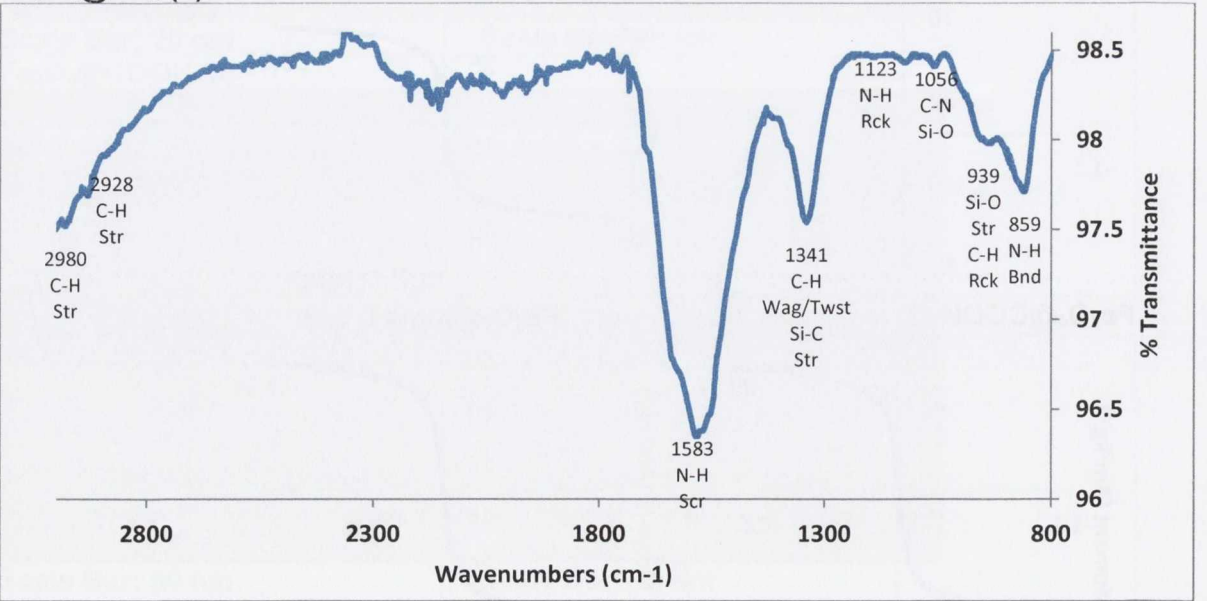
A.2.1.3 FTIR
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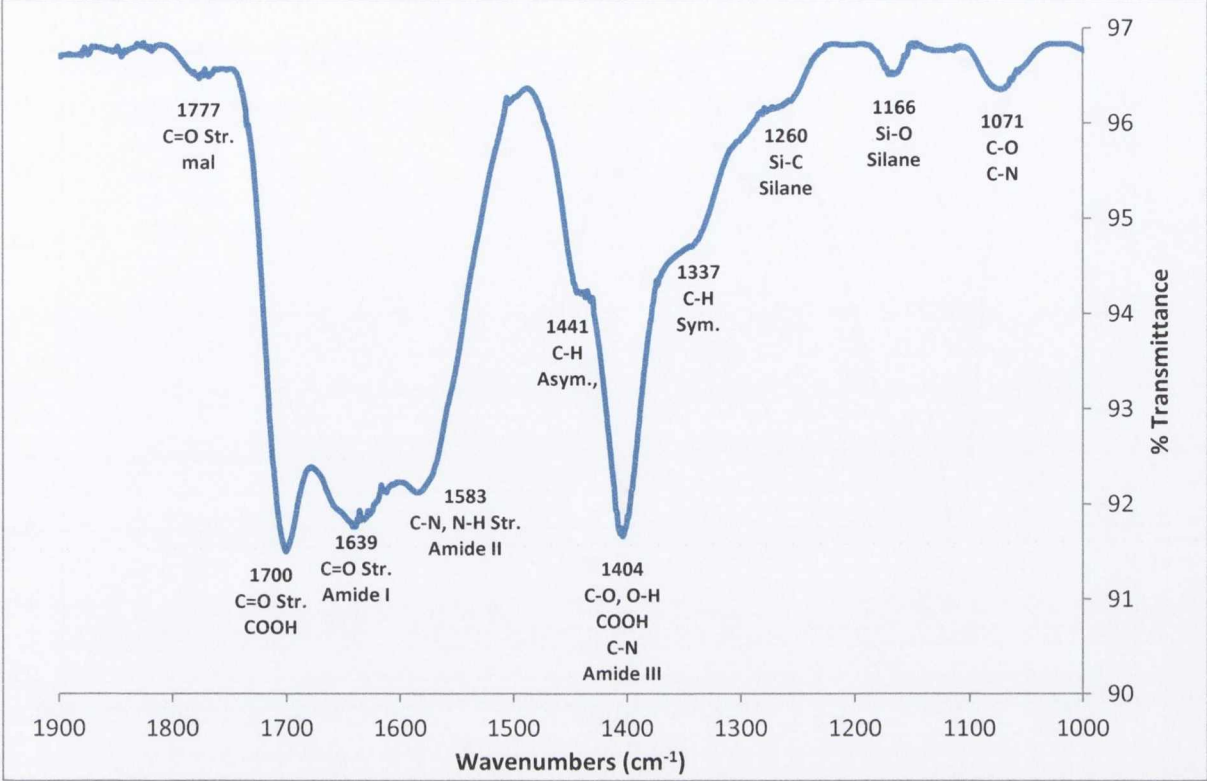
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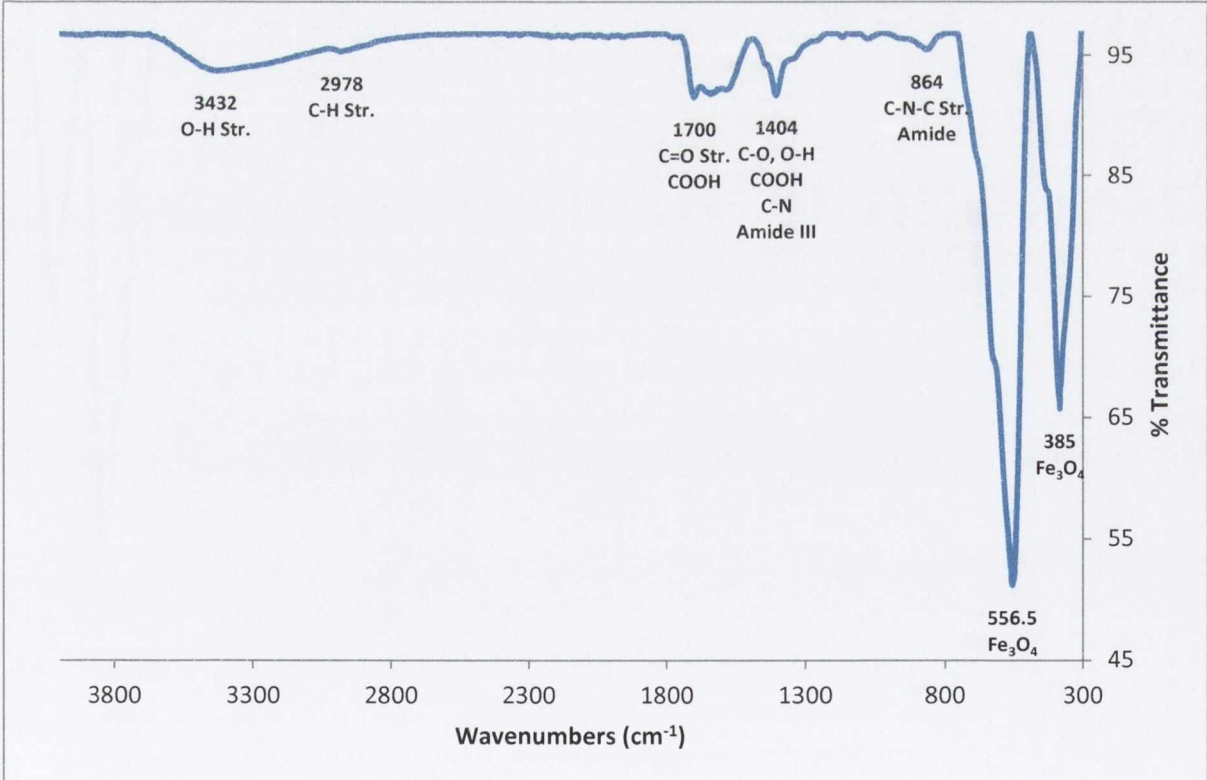
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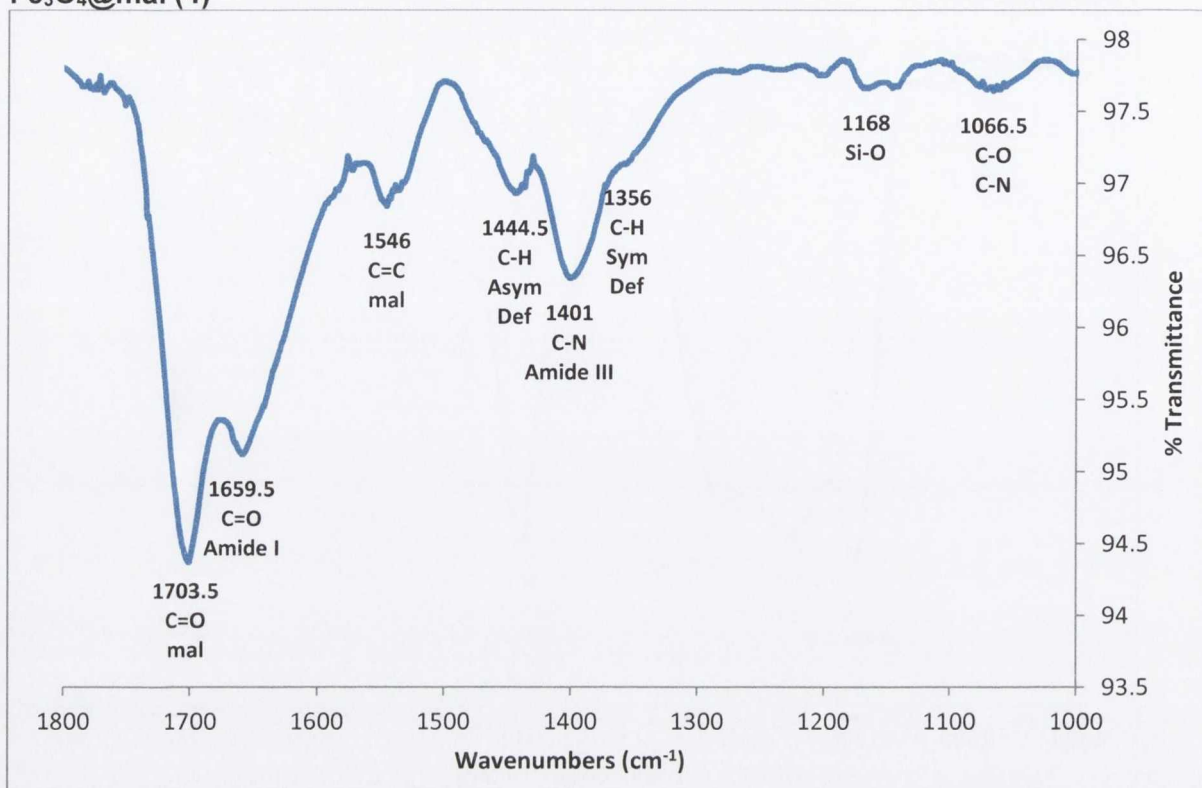
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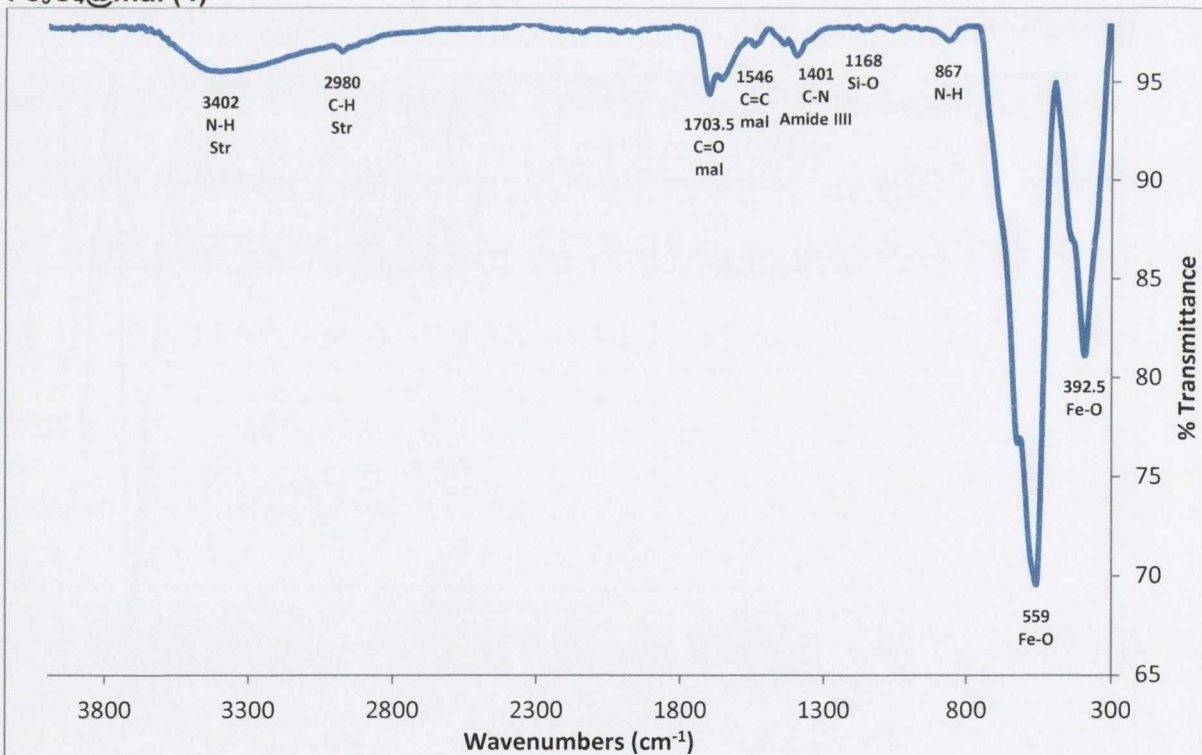
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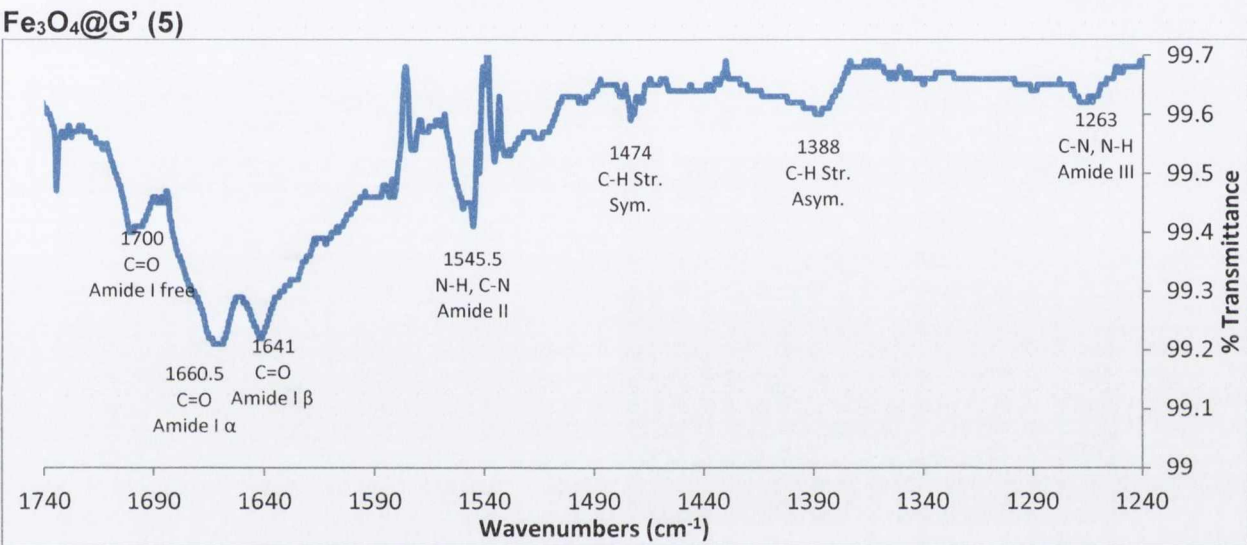
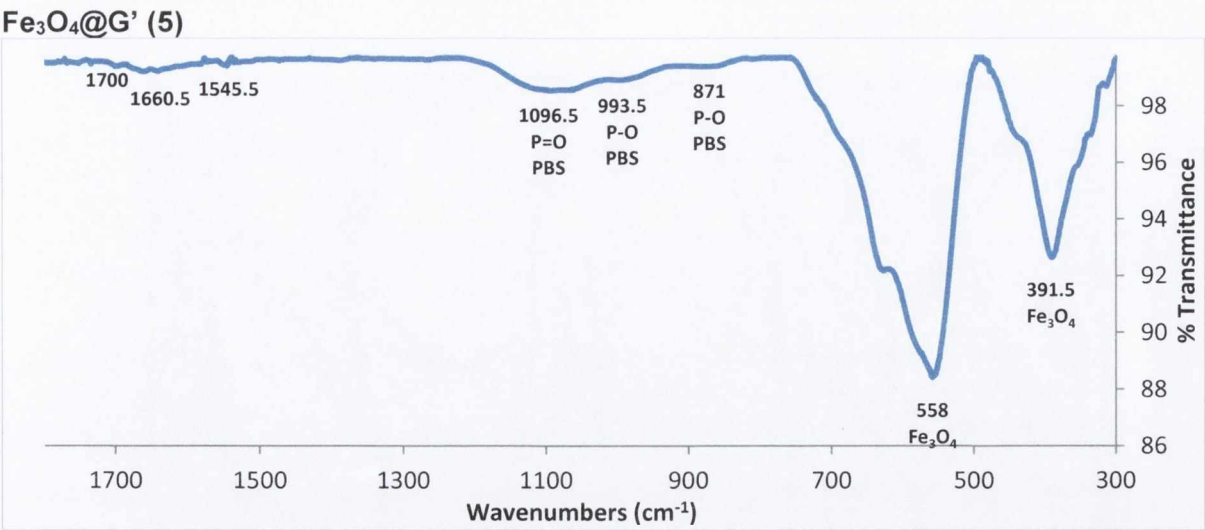


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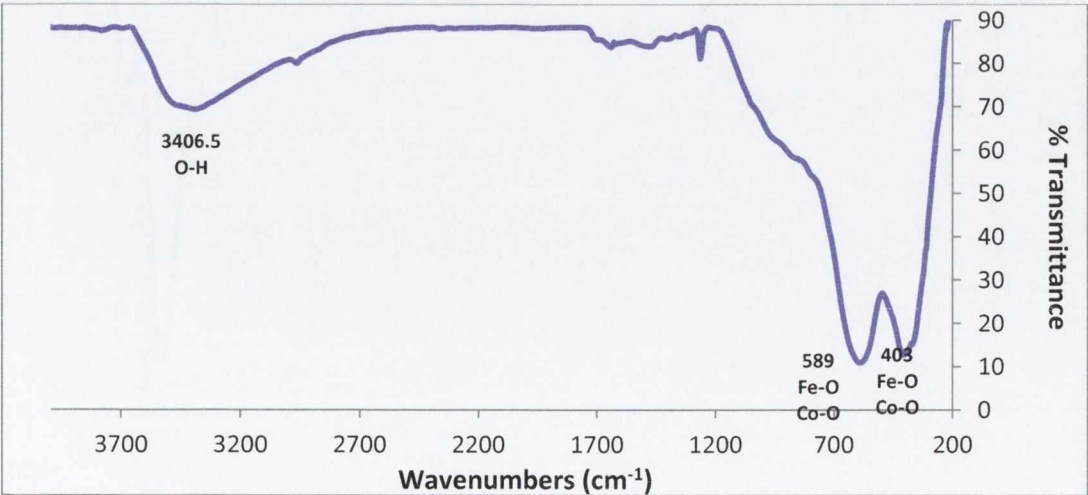
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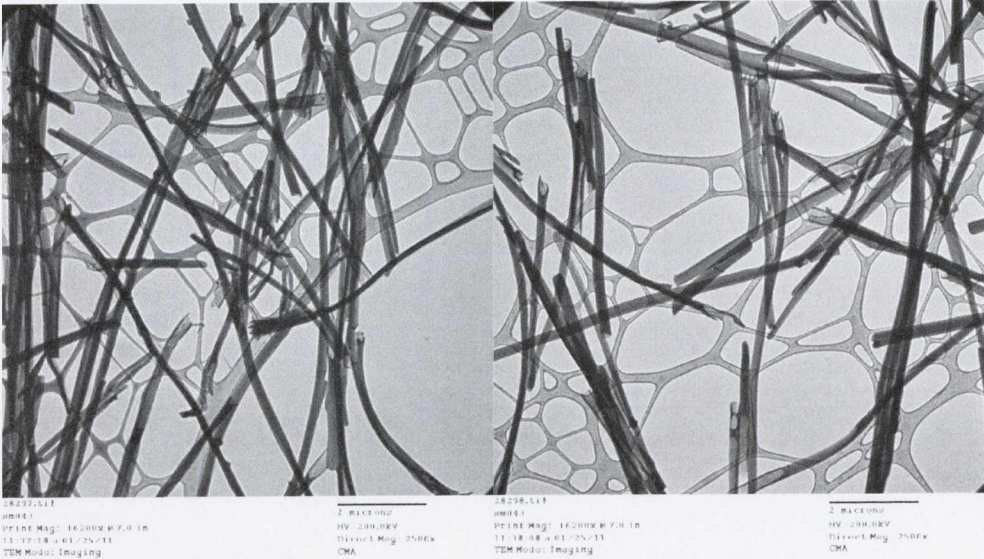
APPENDIX 2.2 COBALT FERRITE-DOPED NANOWIRES

A.2.2.1 CoFe₂O₄ (7) IR



A.2.2.2 CoFe₂O₄@SiO₂ NWs (8)

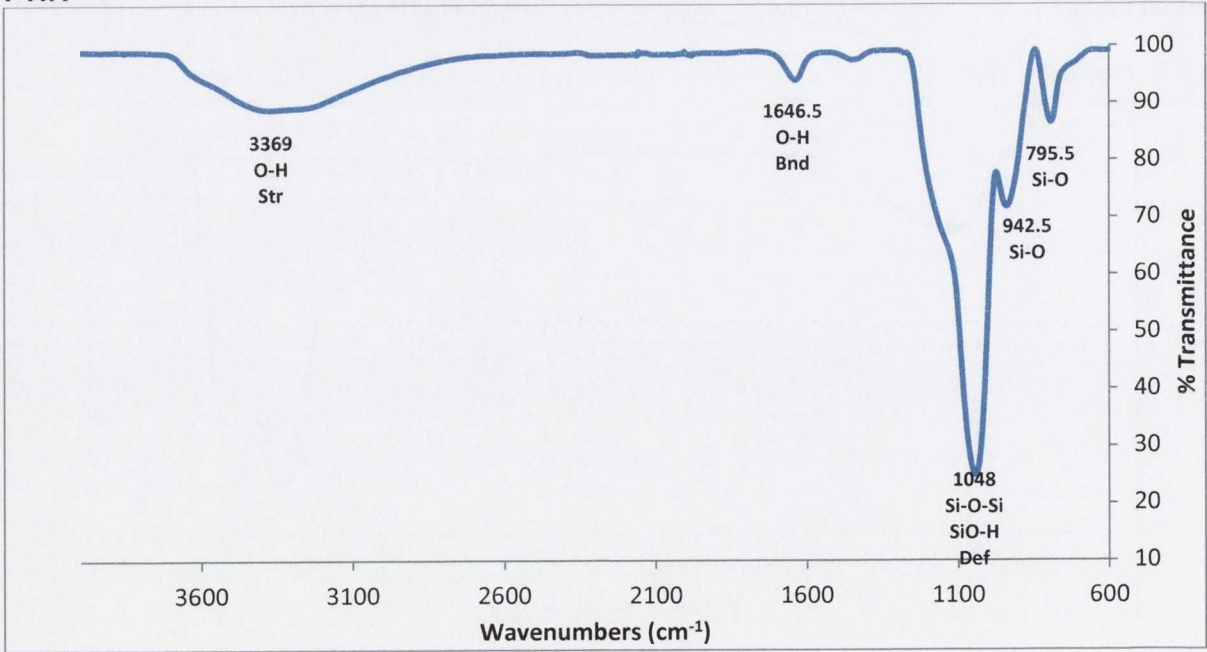
TEM



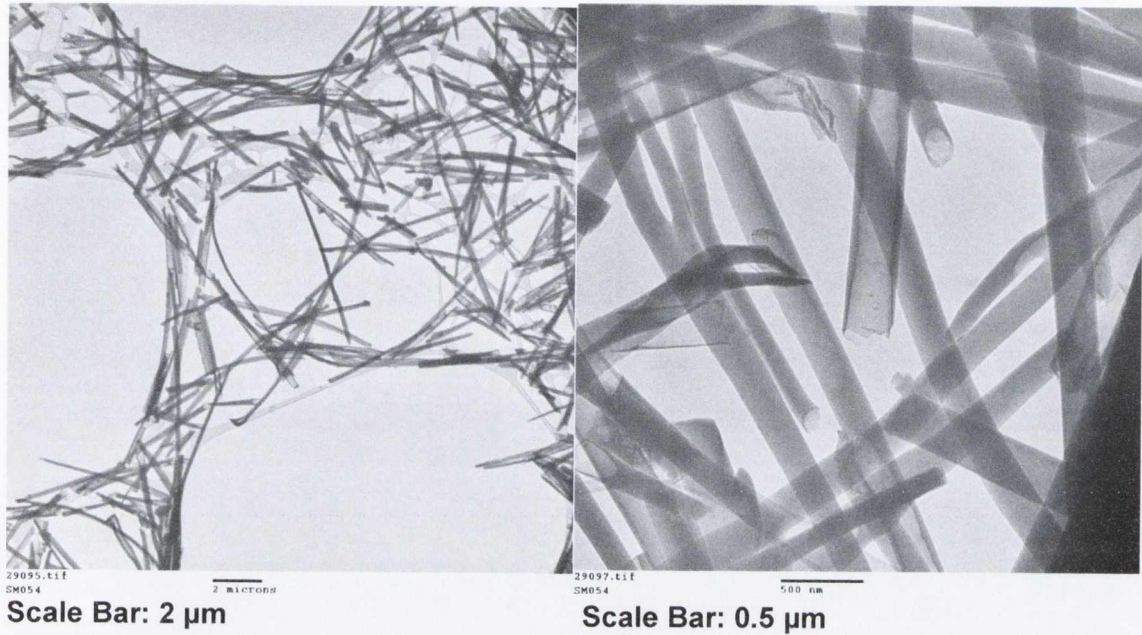
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Optical Microscope



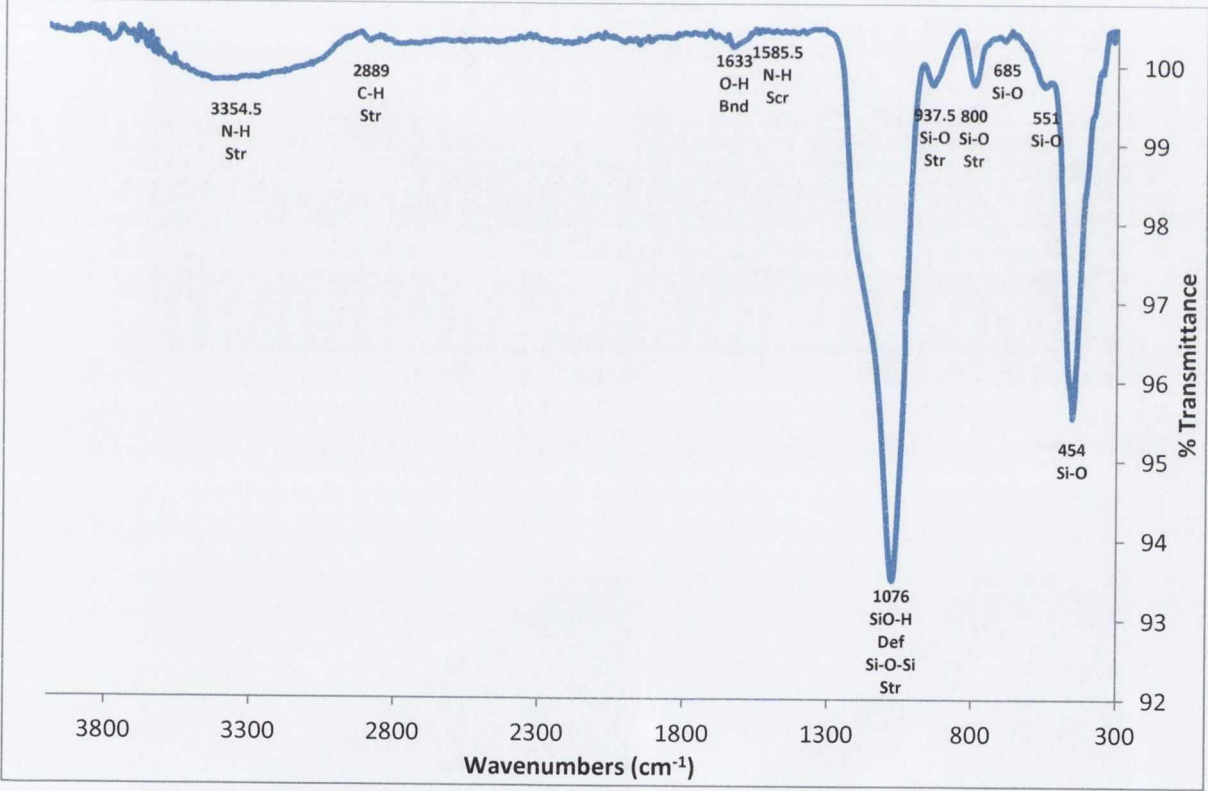
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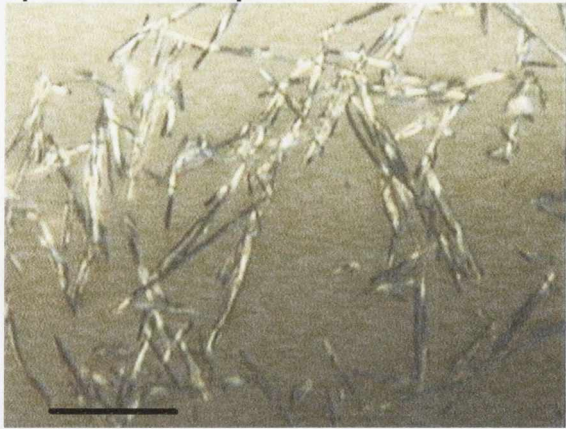
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FTIR

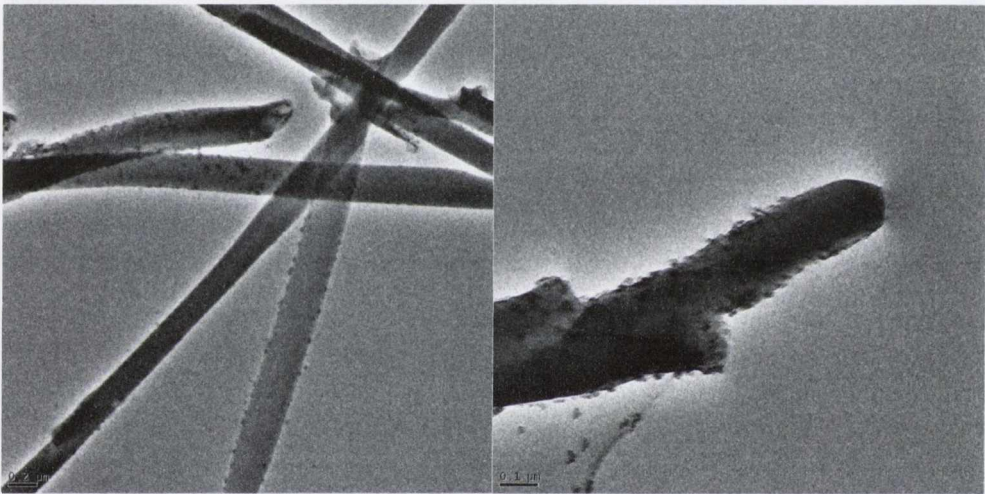


A.2.2.4 $\text{CoFe}_2\text{O}_4@\text{SiO}_2@\text{mal}$ (10)
Optical Microscope



Scale Bar: 3 μm

A.2.2.5 $\text{CoFe}_2\text{O}_4@\text{SiO}_2@\text{CD4-PE}$ (11)
TEM



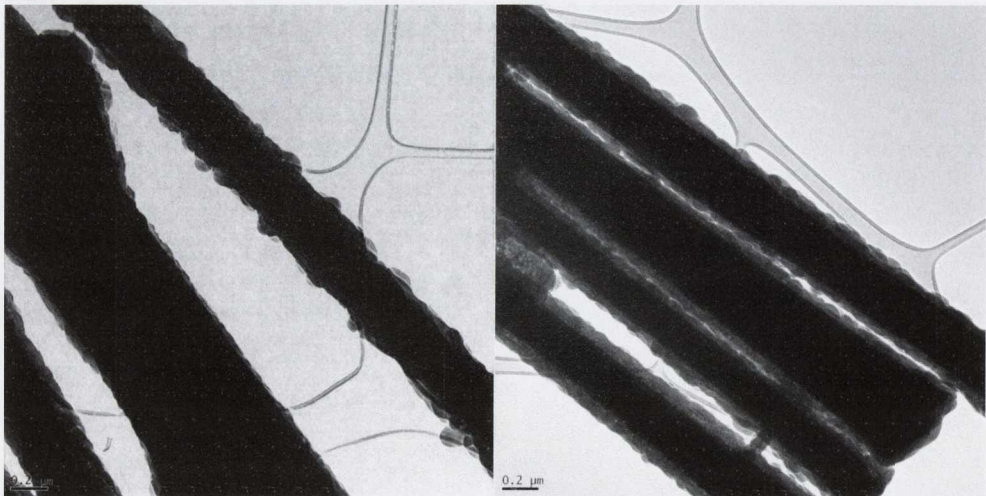
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APPENDIX 2.3

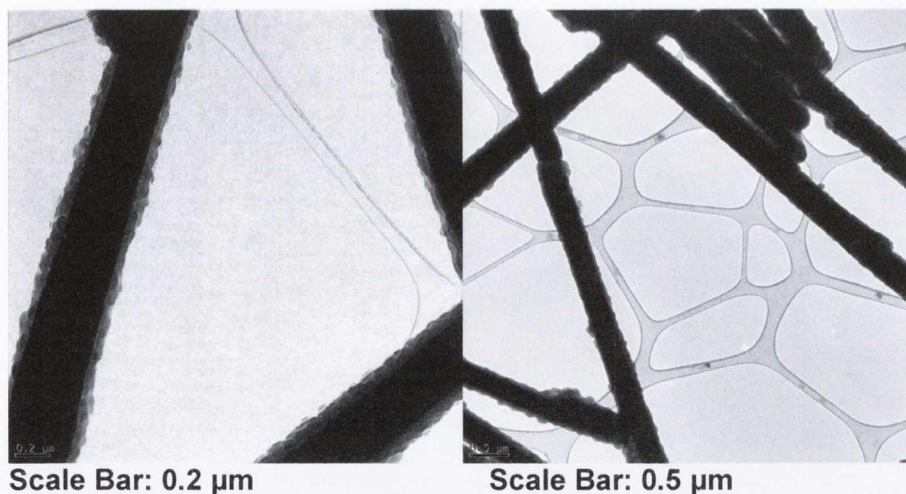
SILICA-COATED METAL NANOWIRES

A.2.3.1 $\text{Co}@\text{SiO}_2$ (12)
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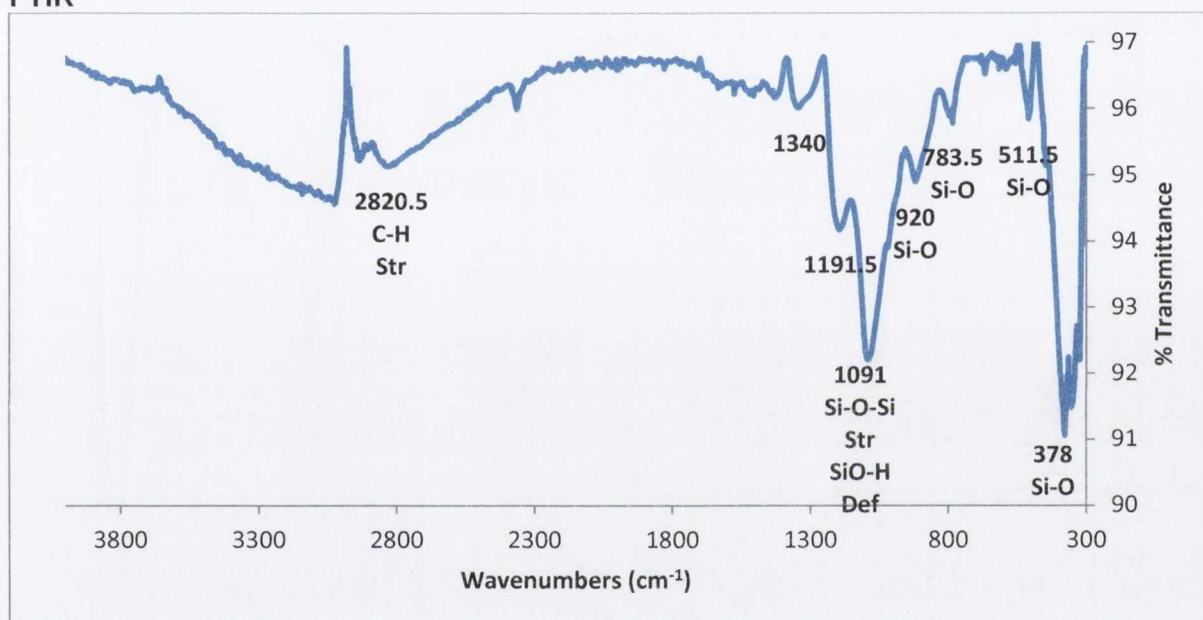


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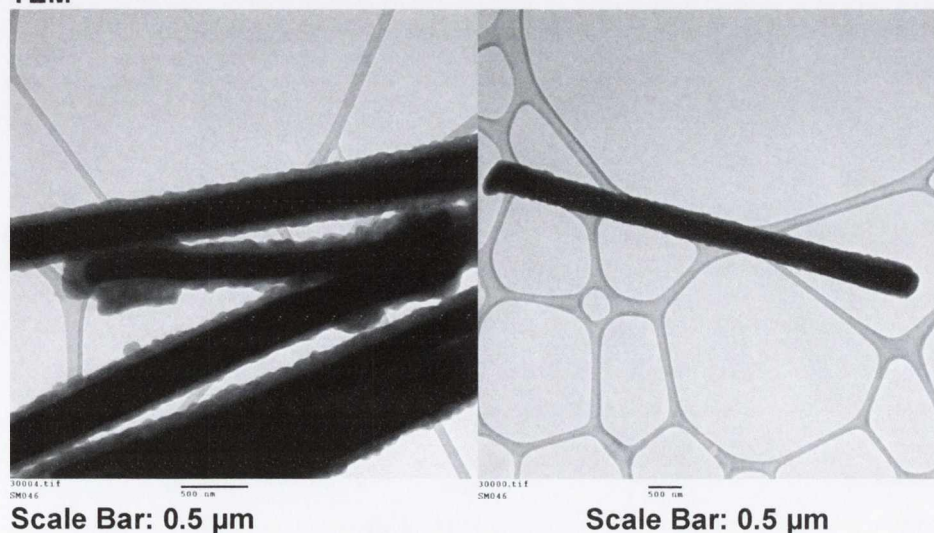


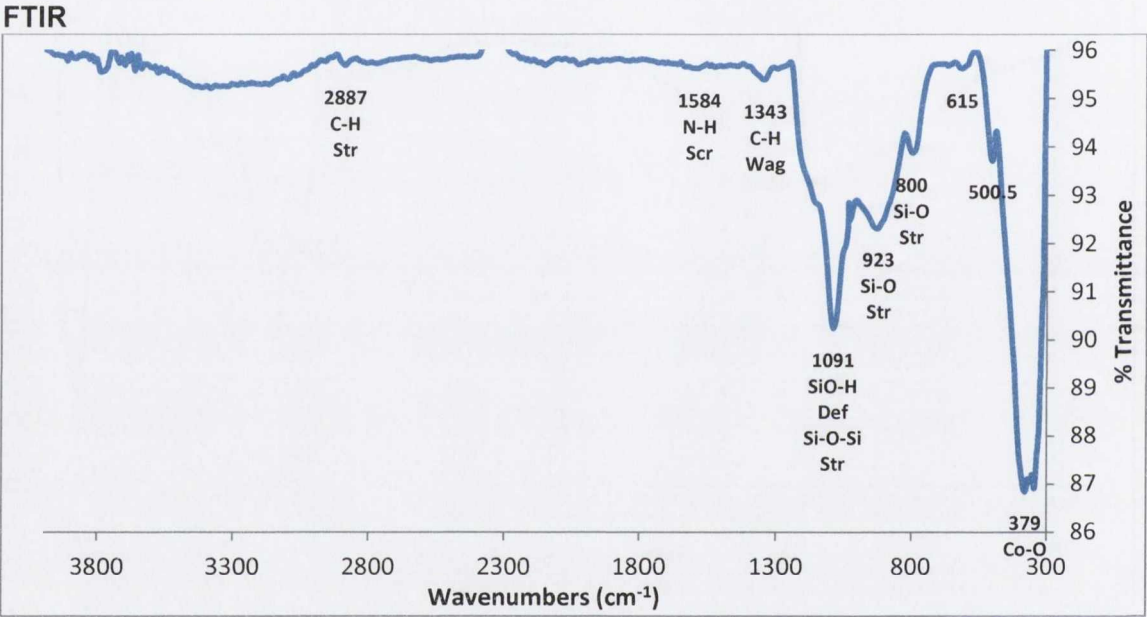
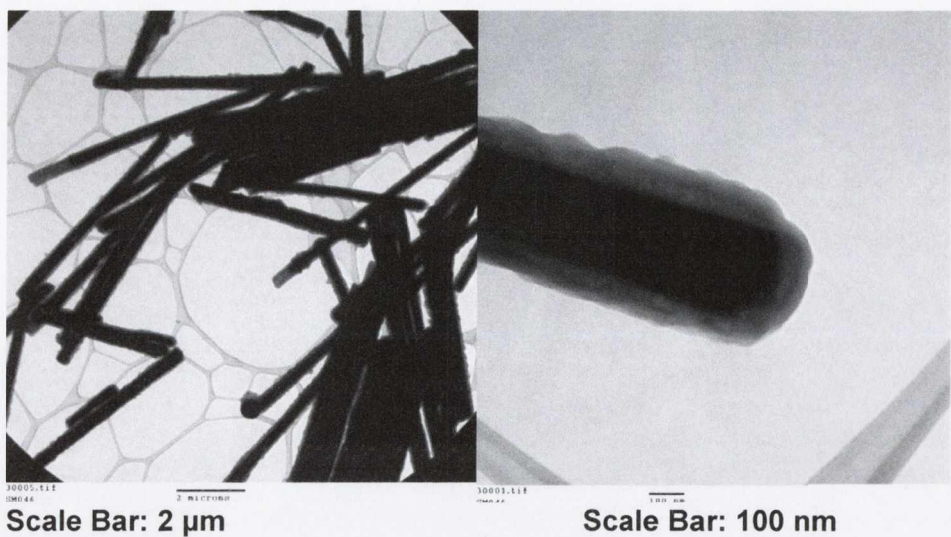
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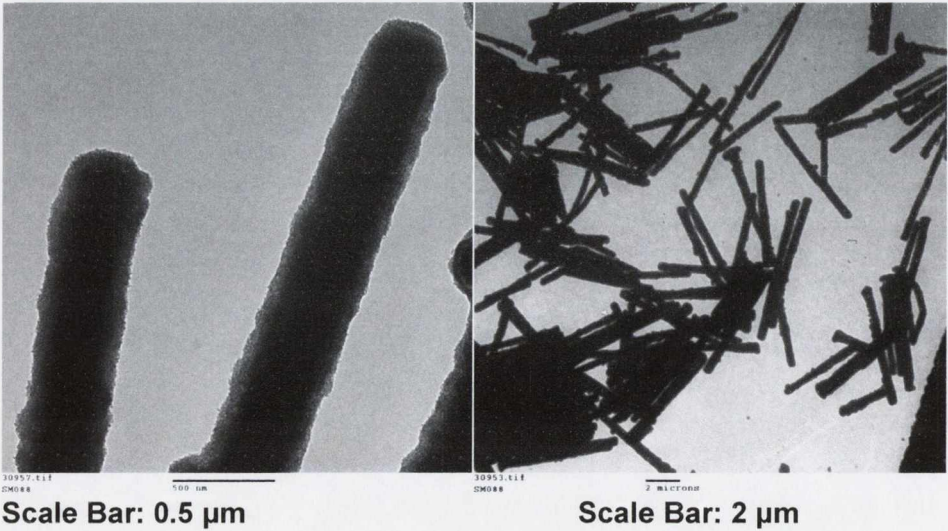
A.2.3.2 Co@SiO₂@NH₂ (13)

TEM

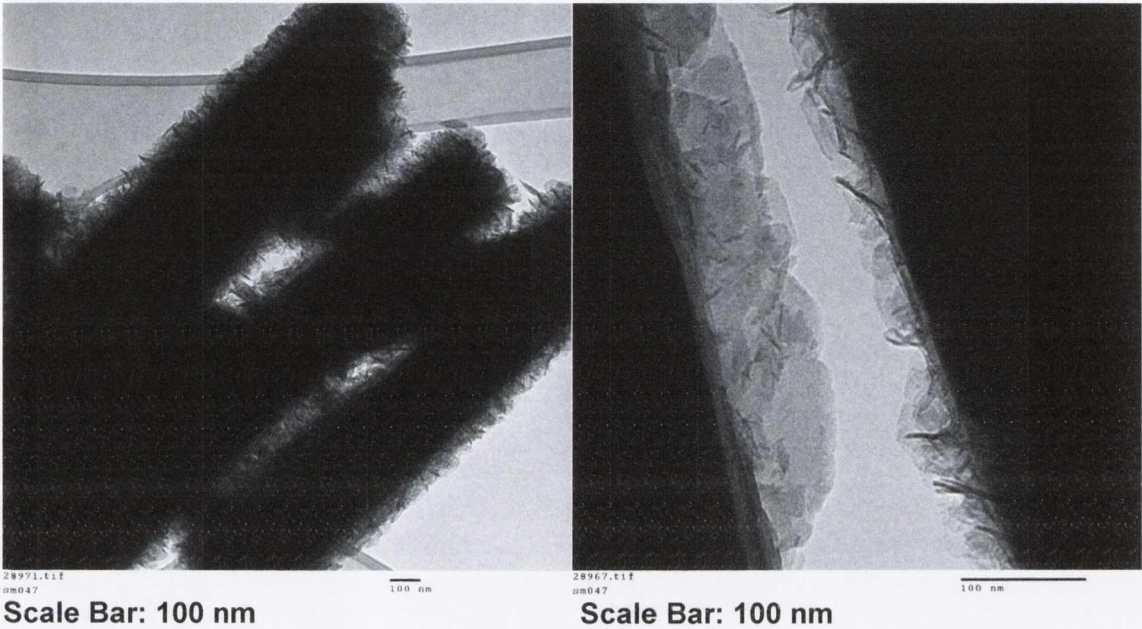




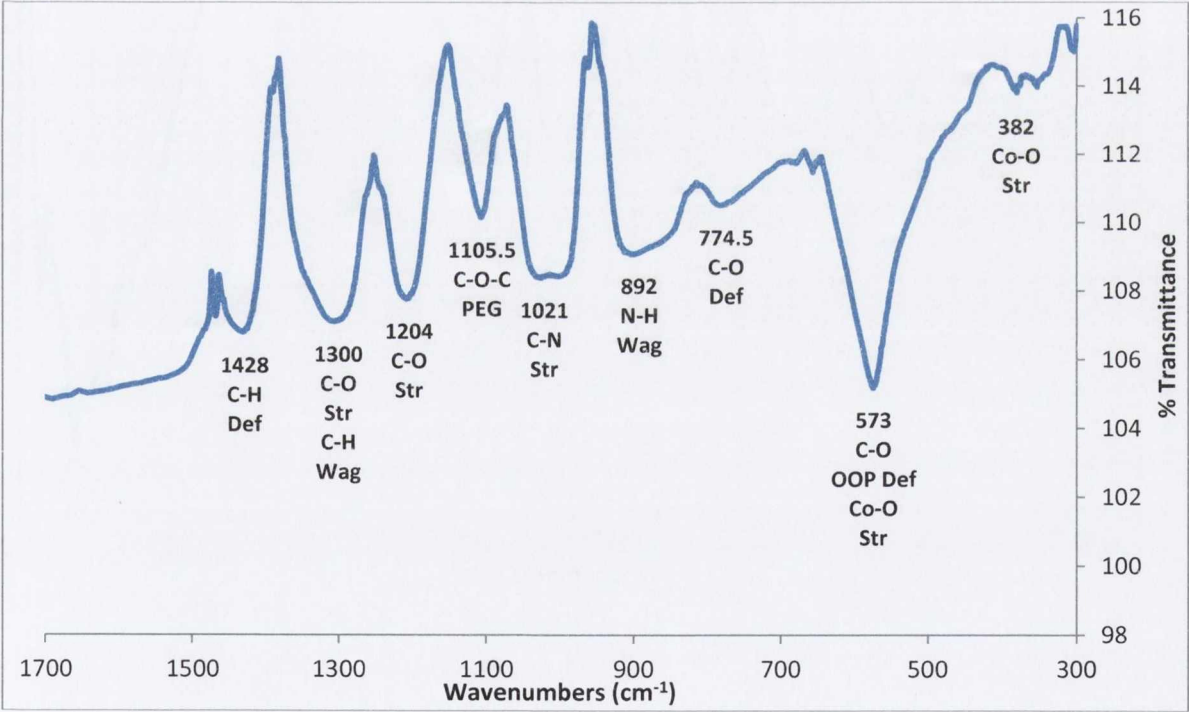
A.2.3.3 Co@Au@SiO₂ (14)
TEM



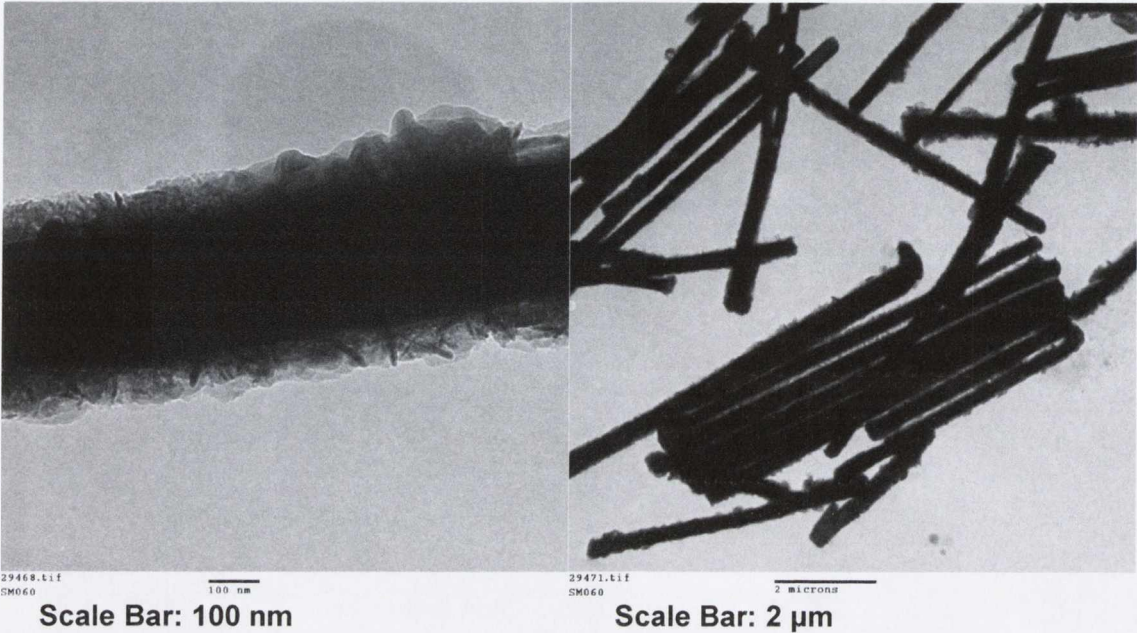
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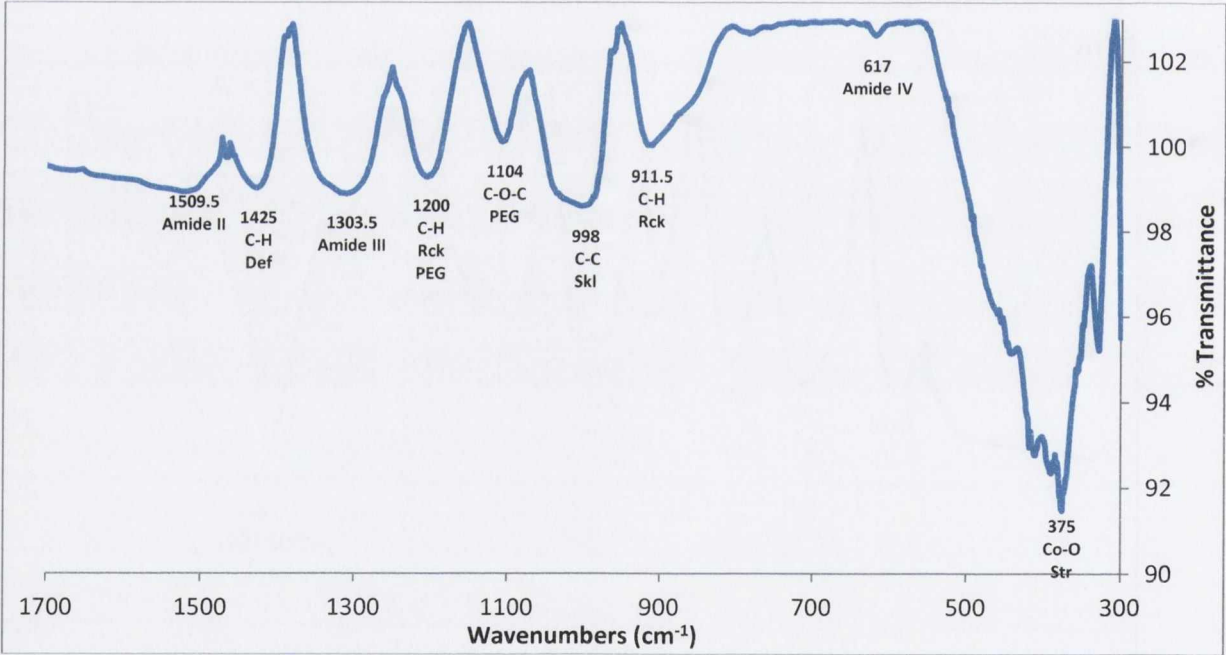
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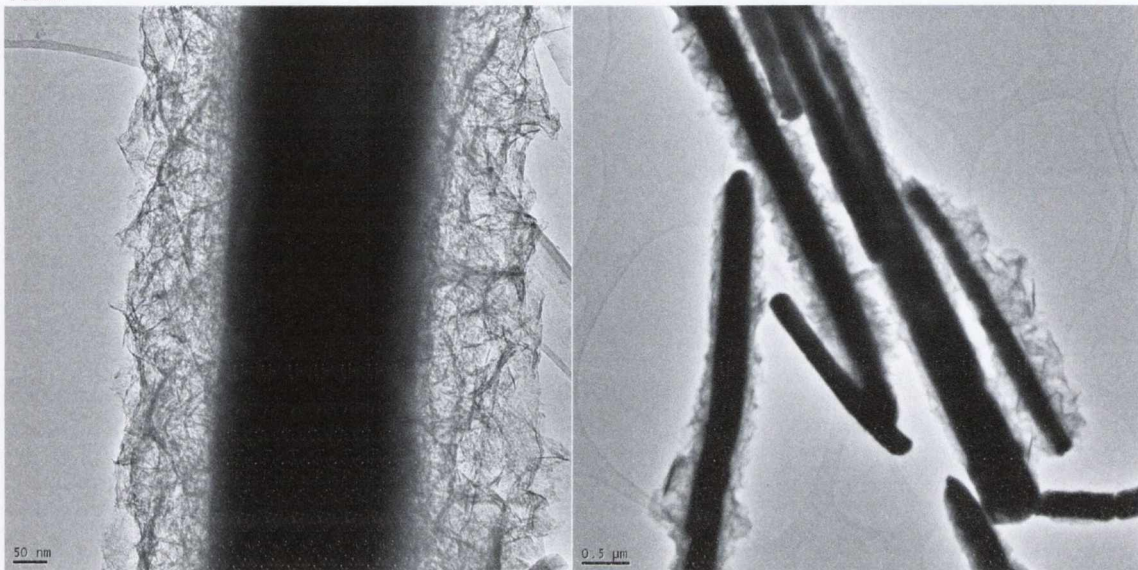
A.2.4.2 Co@PEG-maleimide (16)
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FTIR



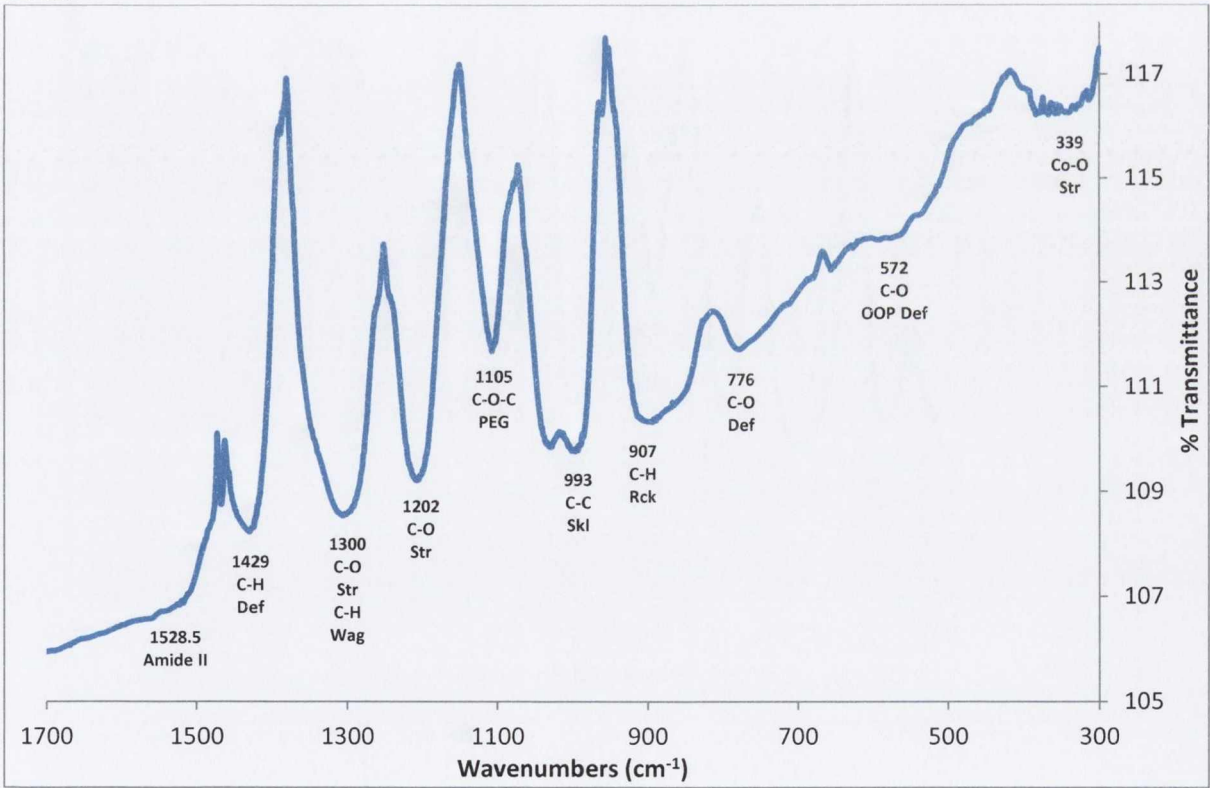
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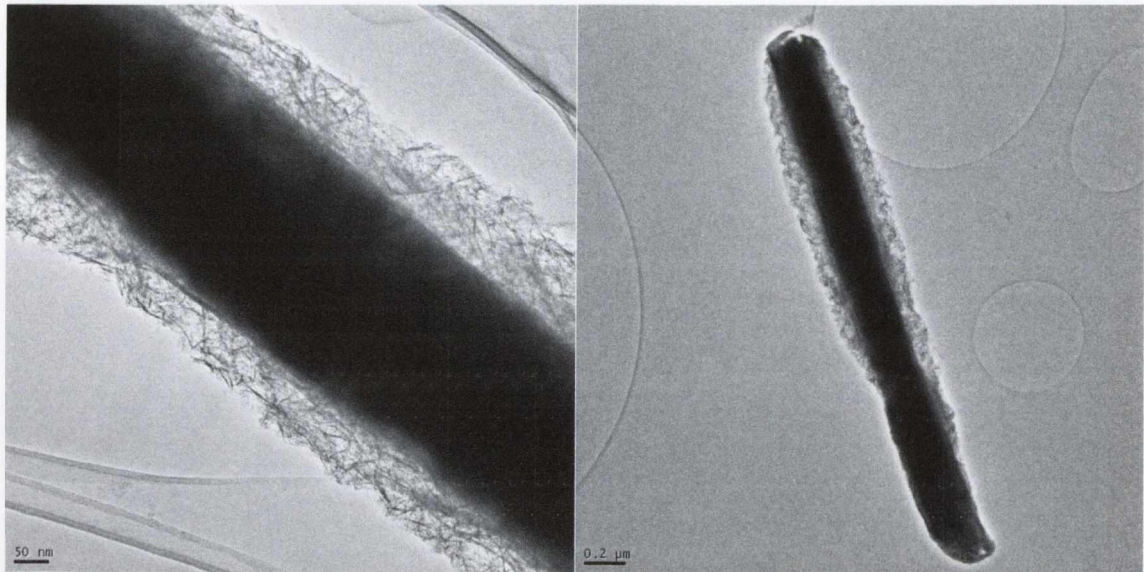
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FTIR



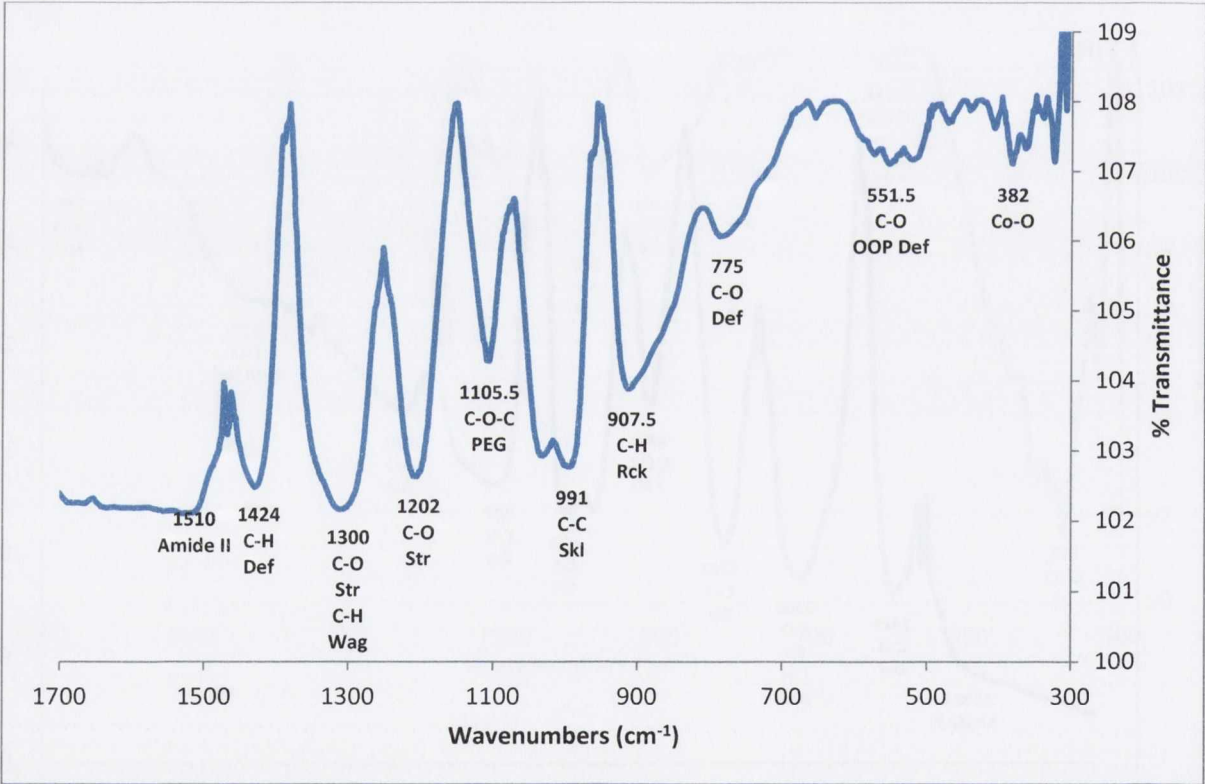
A.2.4.4 Co@PEG-COOH/mal (18)
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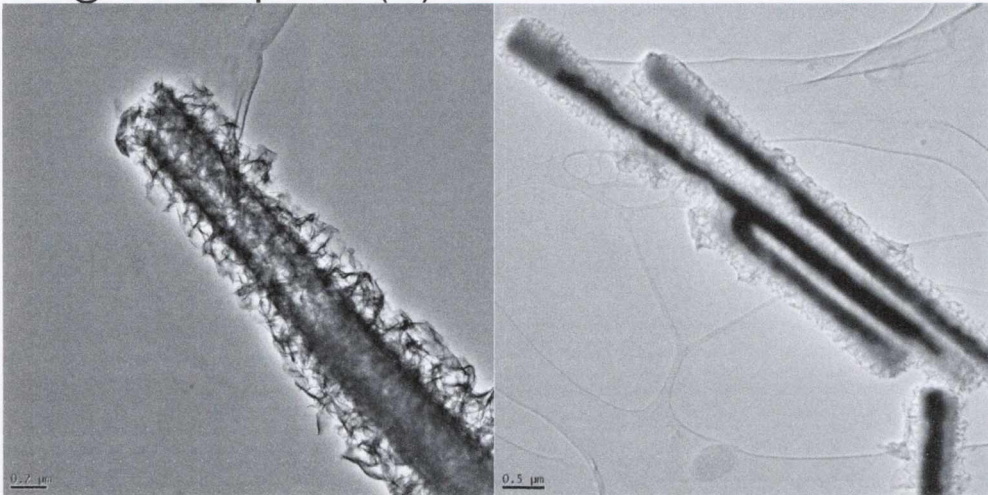
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FTIR



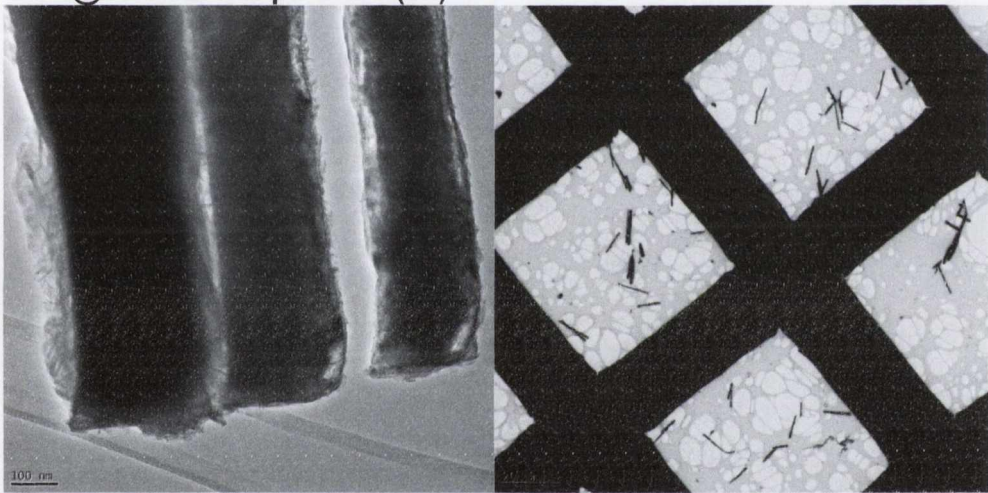
A.2.4.5 CoNi@PEG-mal
TEM
CoNi@PEG-mal 5 μm NWs (20)



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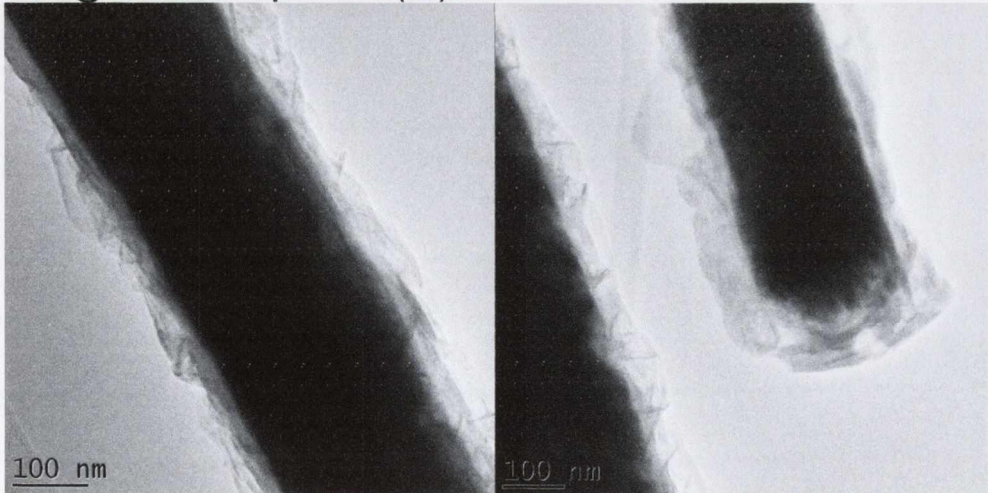
CoNi@PEG-mal 10 μm NWs (21)



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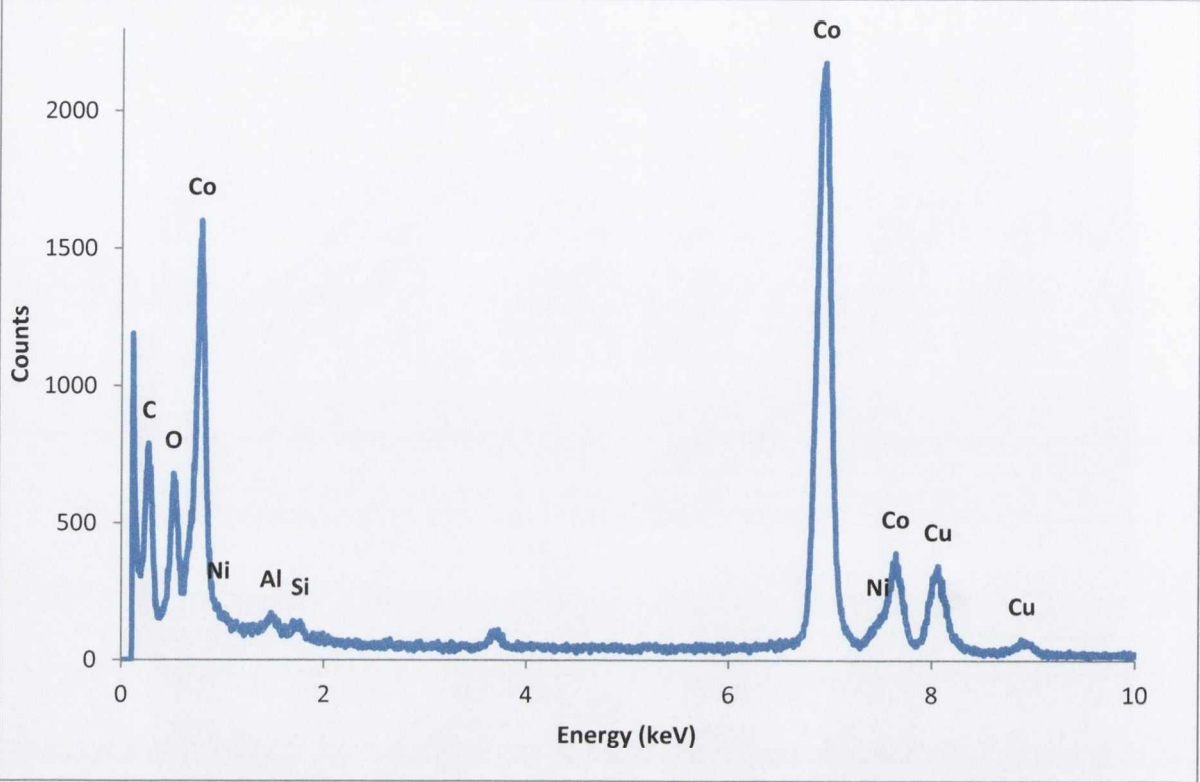
CoNi@PEG-mal 10 μm NWs (22)



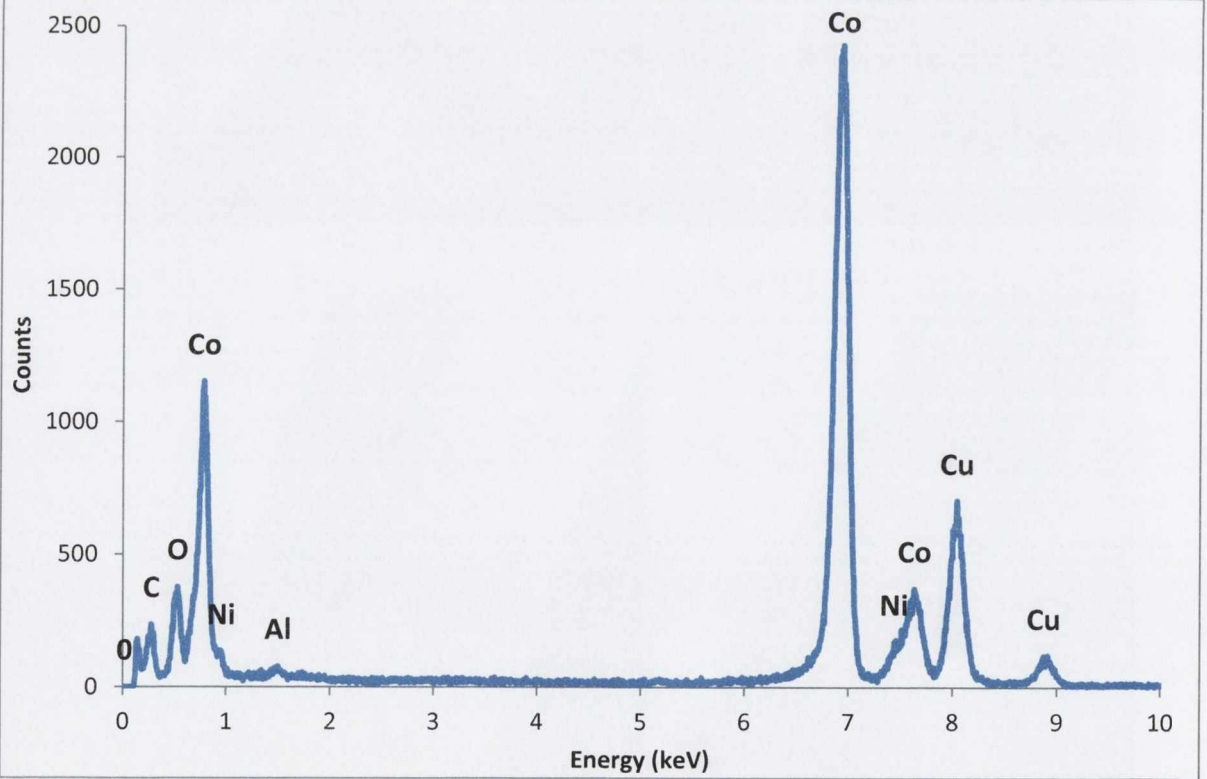
Scale Bar: 100 nm

Scale Bar: 100 nm

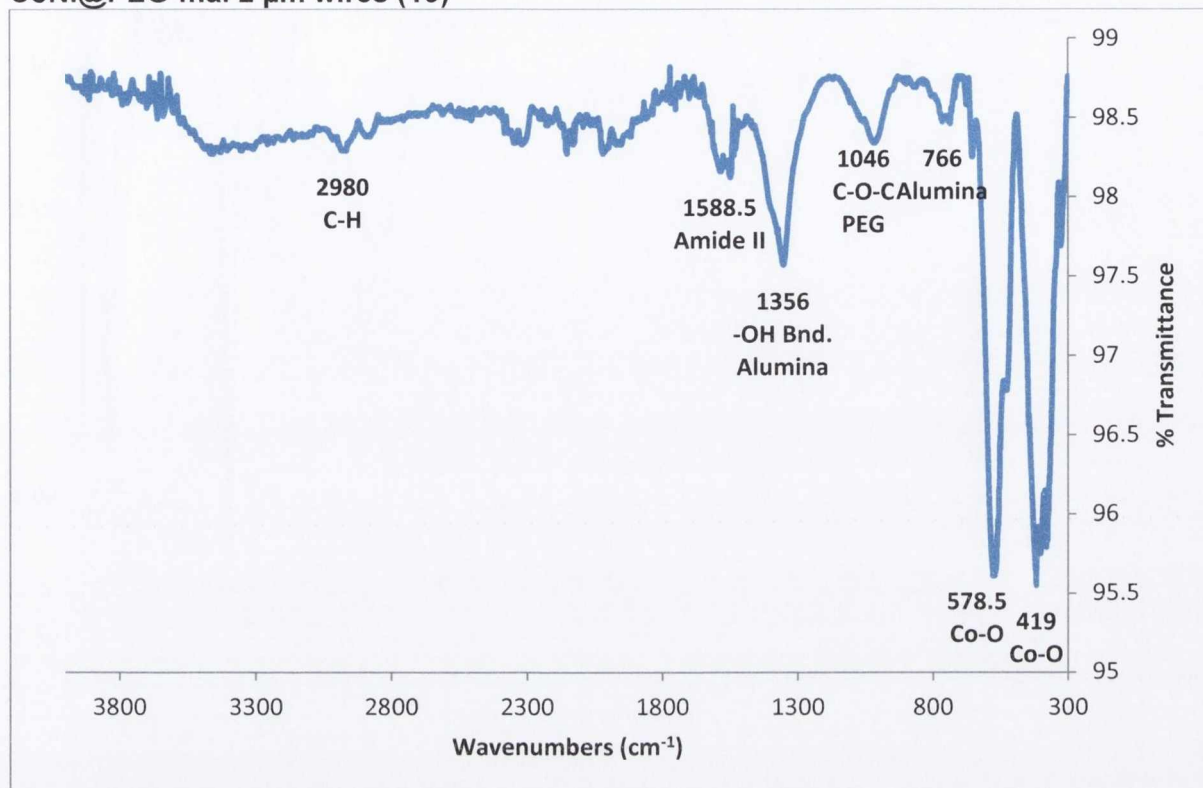
EDX
CoNi@PEG-mal 10 μm wires $\text{Co}_{95.8}\text{Ni}_{4.2}$ (21)



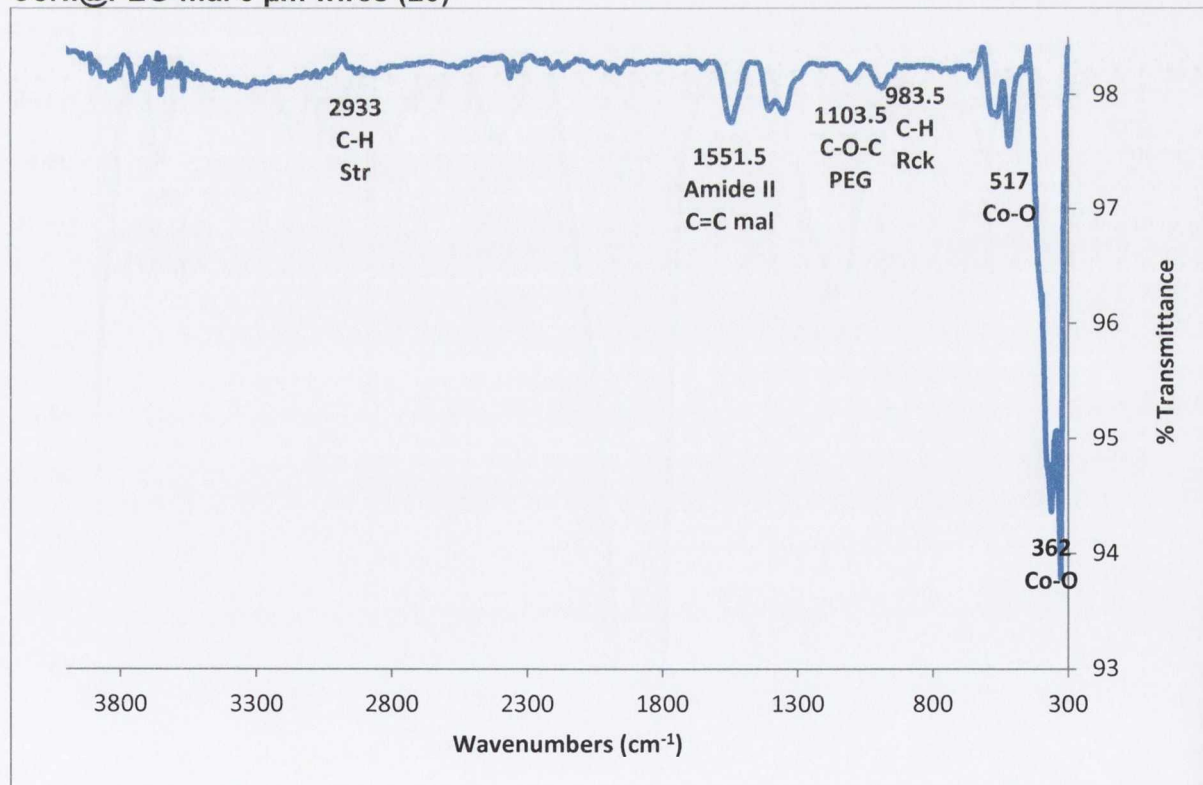
CoNi@PEG-mal 10 μm wires NWs $\text{Co}_{95.1}\text{Ni}_{4.9}$ (22)



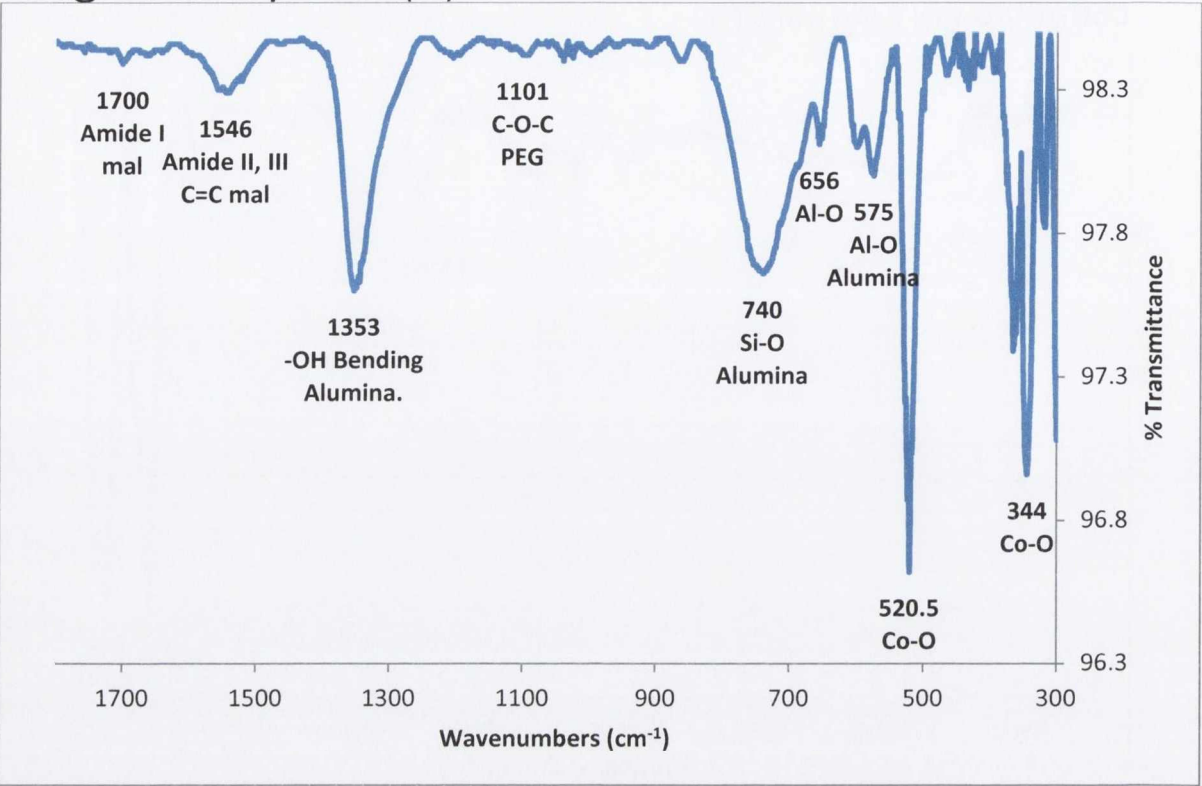
FTIR
CoNi@PEG-mal 2 μm wires (19)



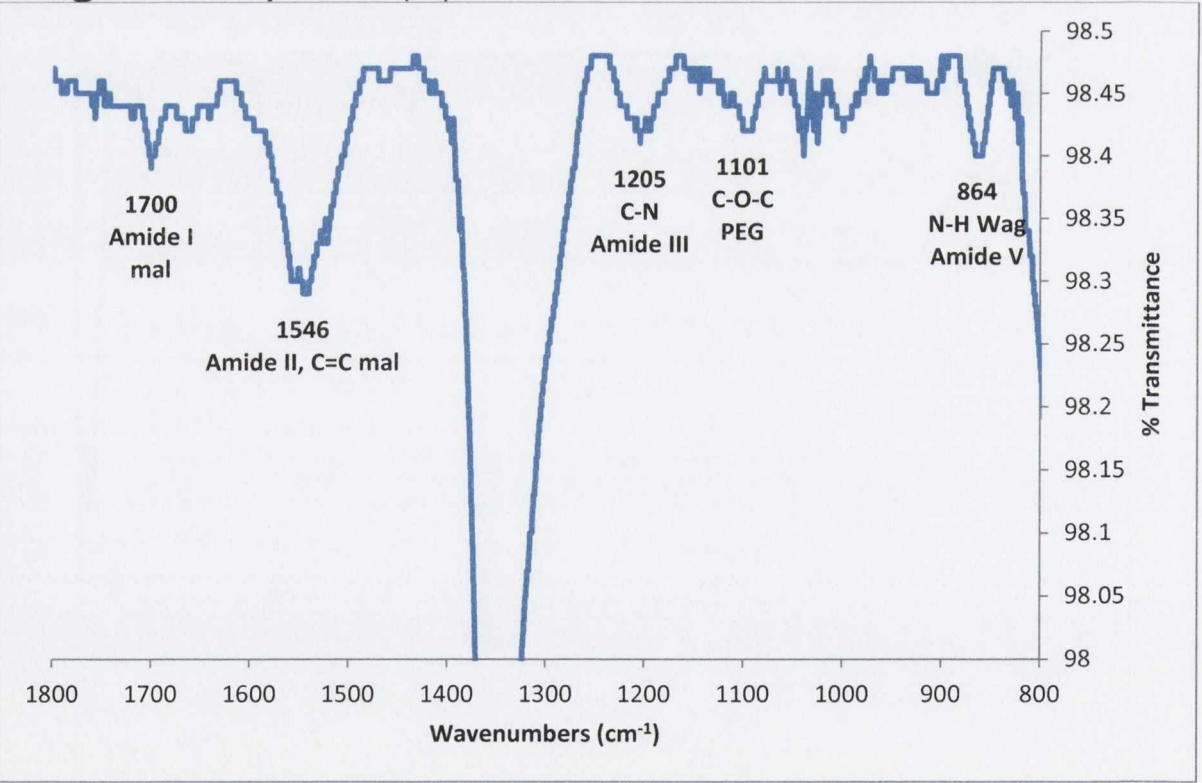
CoNi@PEG-mal 5 μm wires (20)



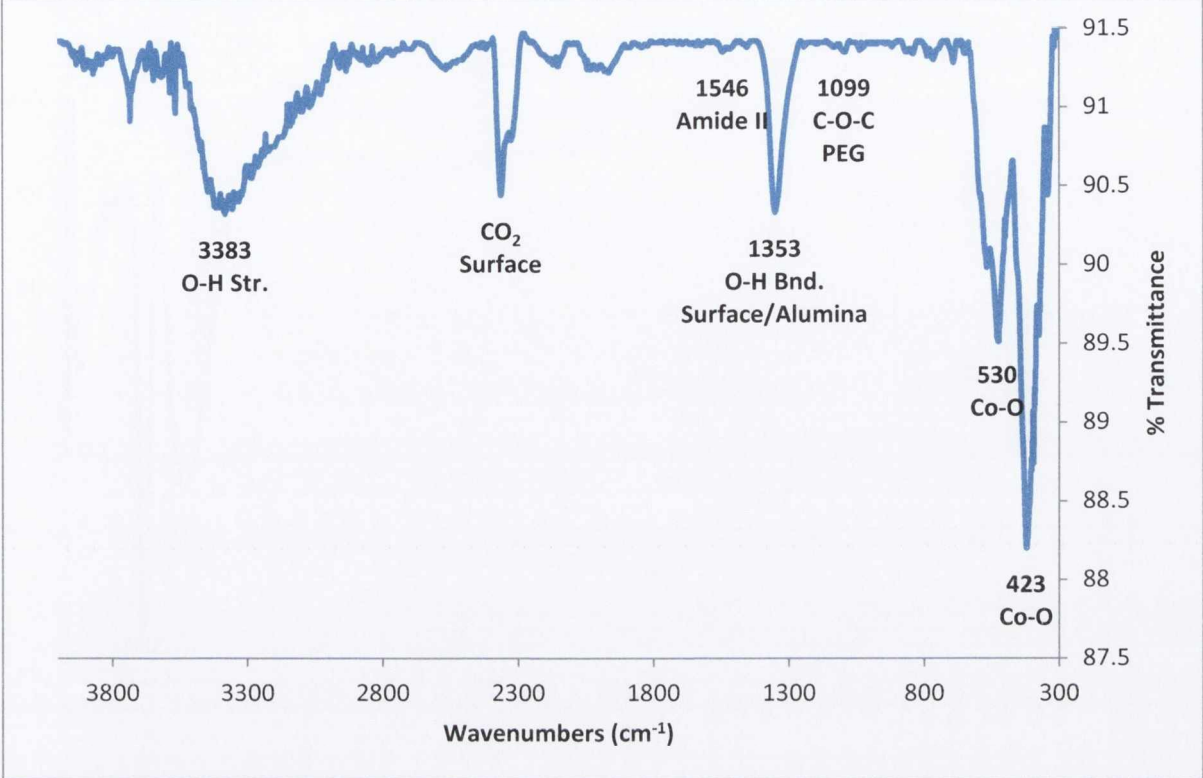
CoNi@PEG-mal 10 μm wires (21)



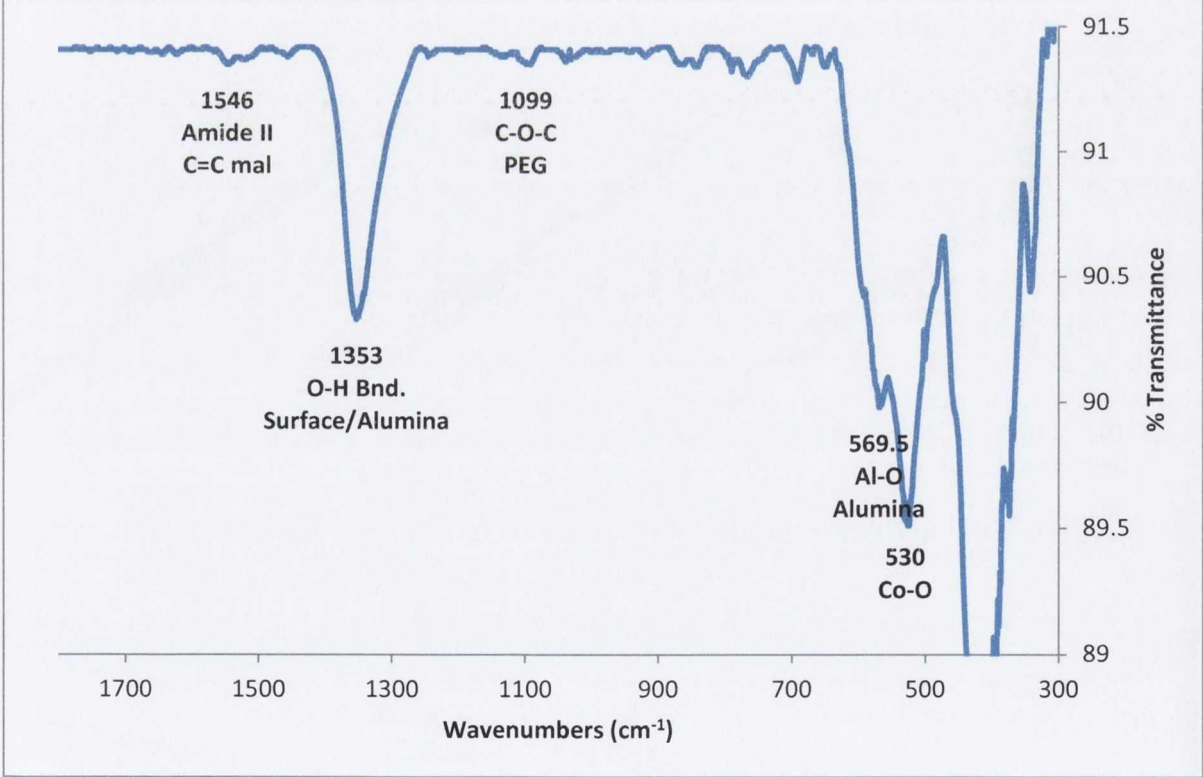
CoNi@PEG-mal 10 μm wires (21)



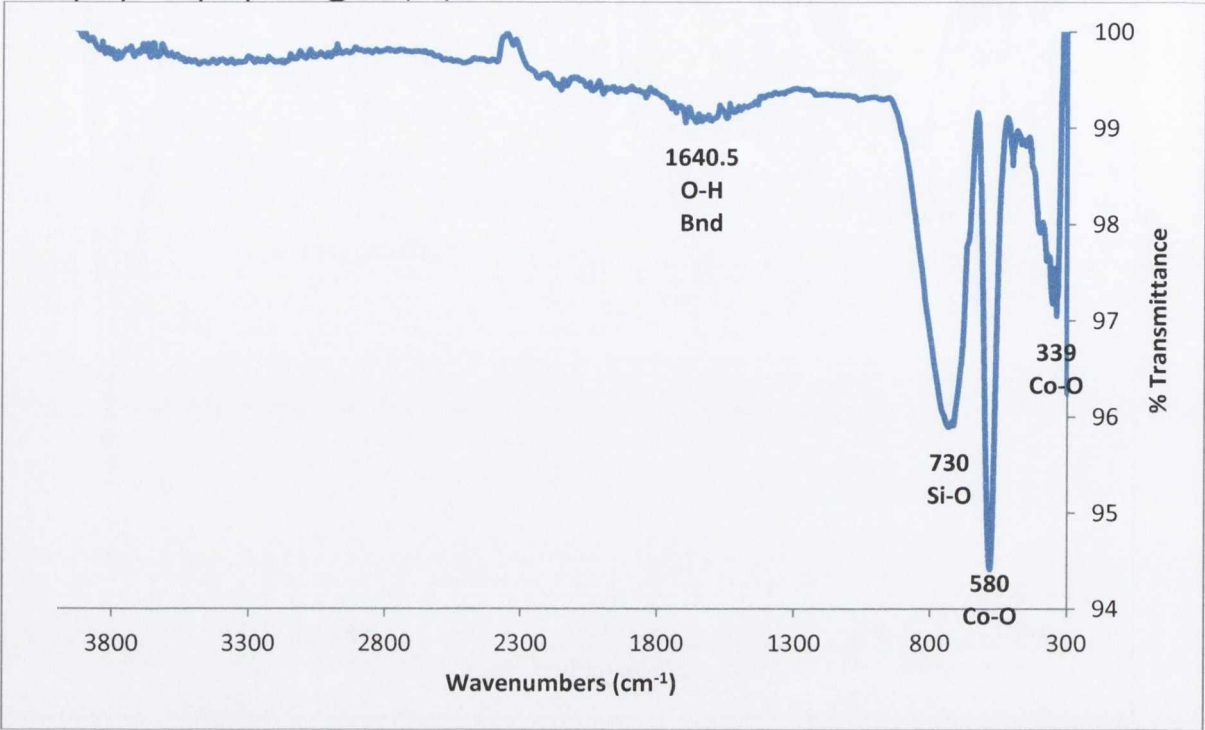
CoNi@PEG-mal 10 μm wires (22)



CoNi@PEG-mal 10 μm wires (22)



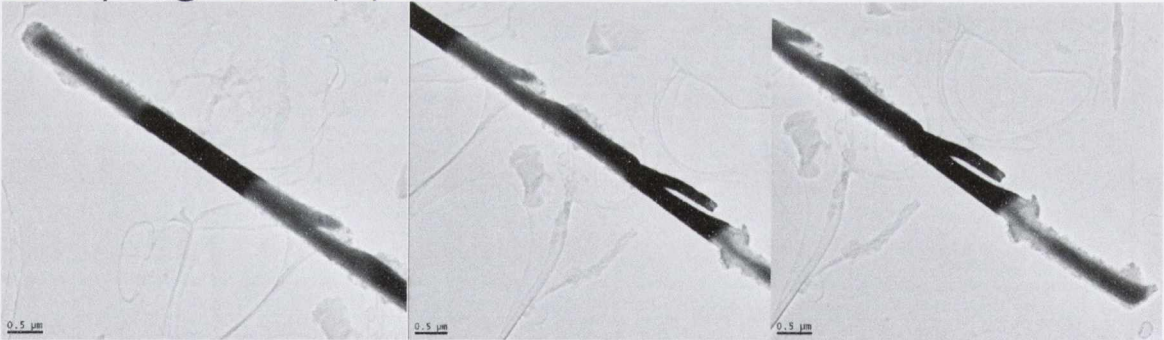
A.2.5.1 CoNi|Au|CoNi|Au|CoNi@OH (27)



A.2.5.2 CoNi|Au|CoNi|Au|CoNi@PEG-mal (28, 29)

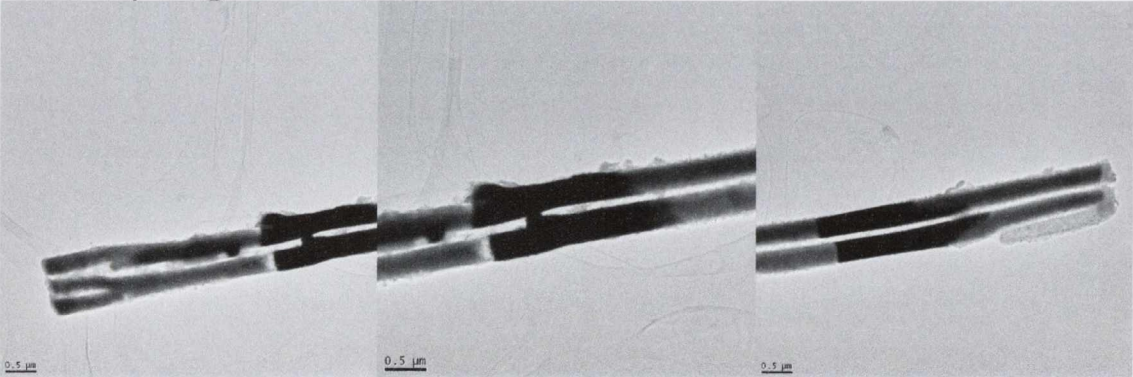
TEM

Co₉₂Ni₈|Au₁₂@PEG-mal (28)



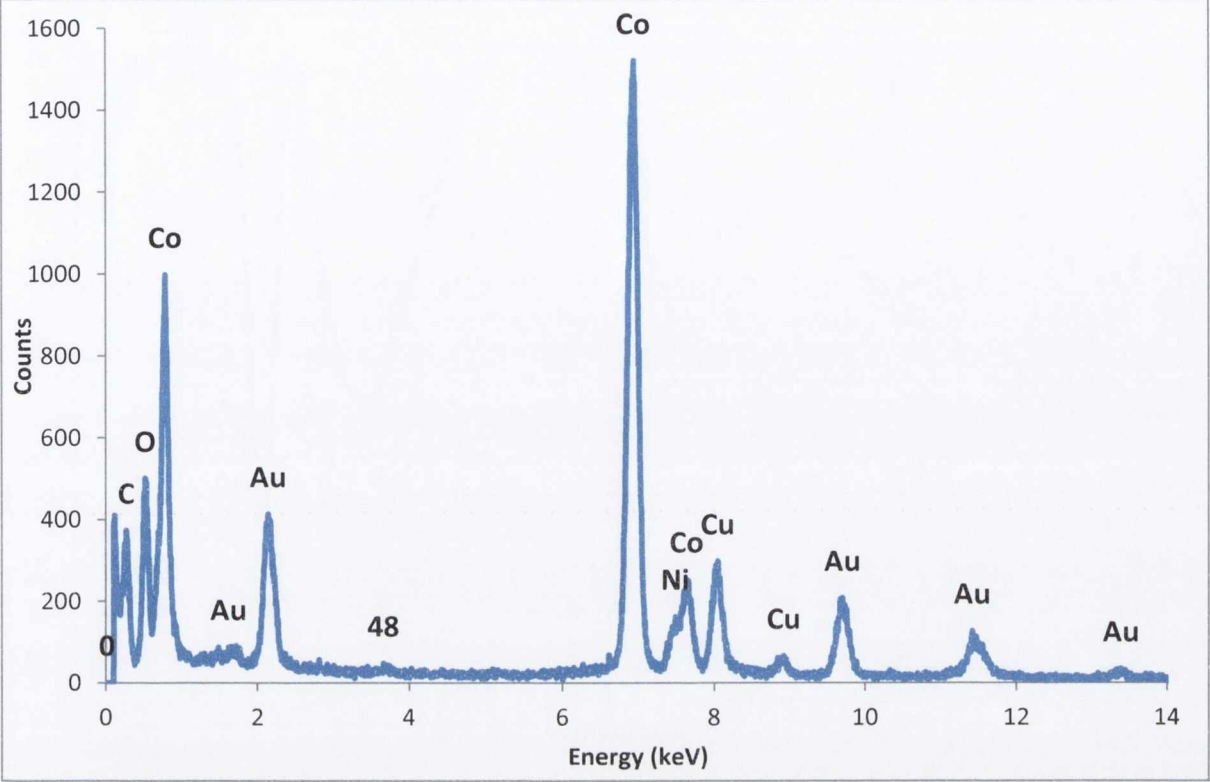
Scale Bar: 500 nm

Co_{90.5}Ni_{9.5}|Au₂₃@PEG-mal (29)

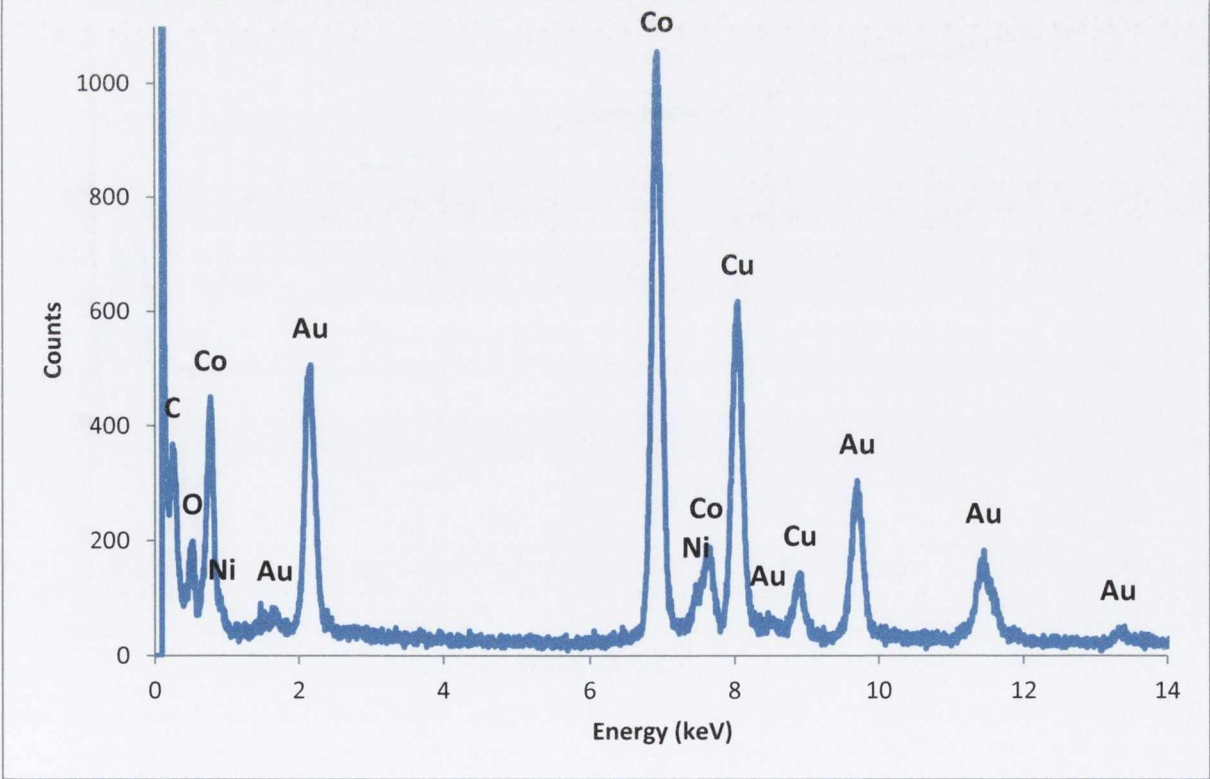


Scale Bar: 500 nm

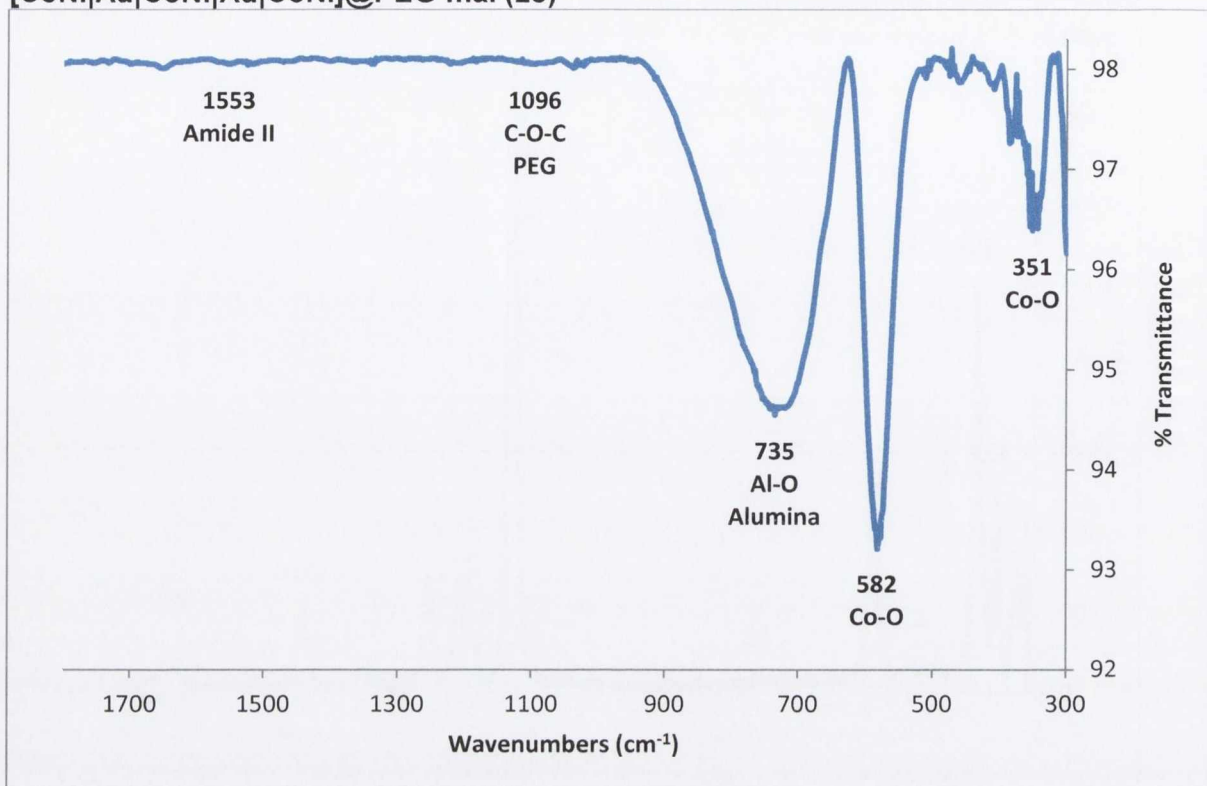
EDX
 $\text{Co}_{92}\text{Ni}_8|\text{Au}_{12}@\text{PEG-mal}$ (28)



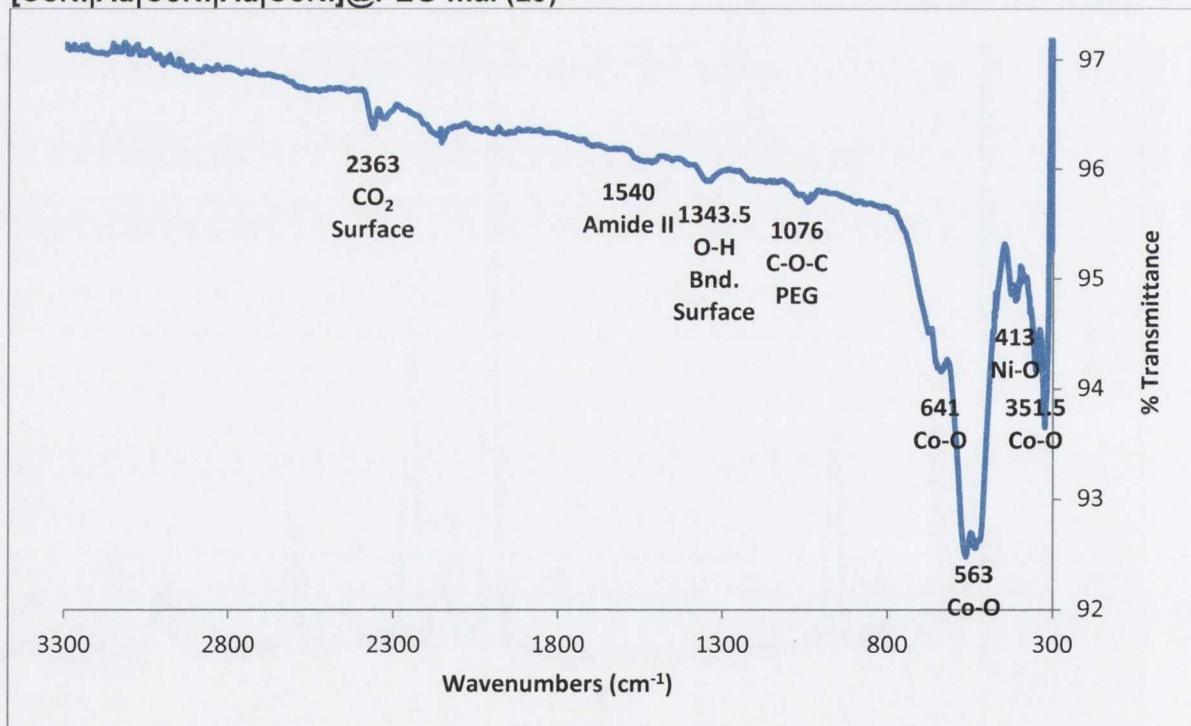
$\text{Co}_{90.5}\text{Ni}_{9.5}|\text{Au}_{23}@\text{PEG-mal}$ (29)



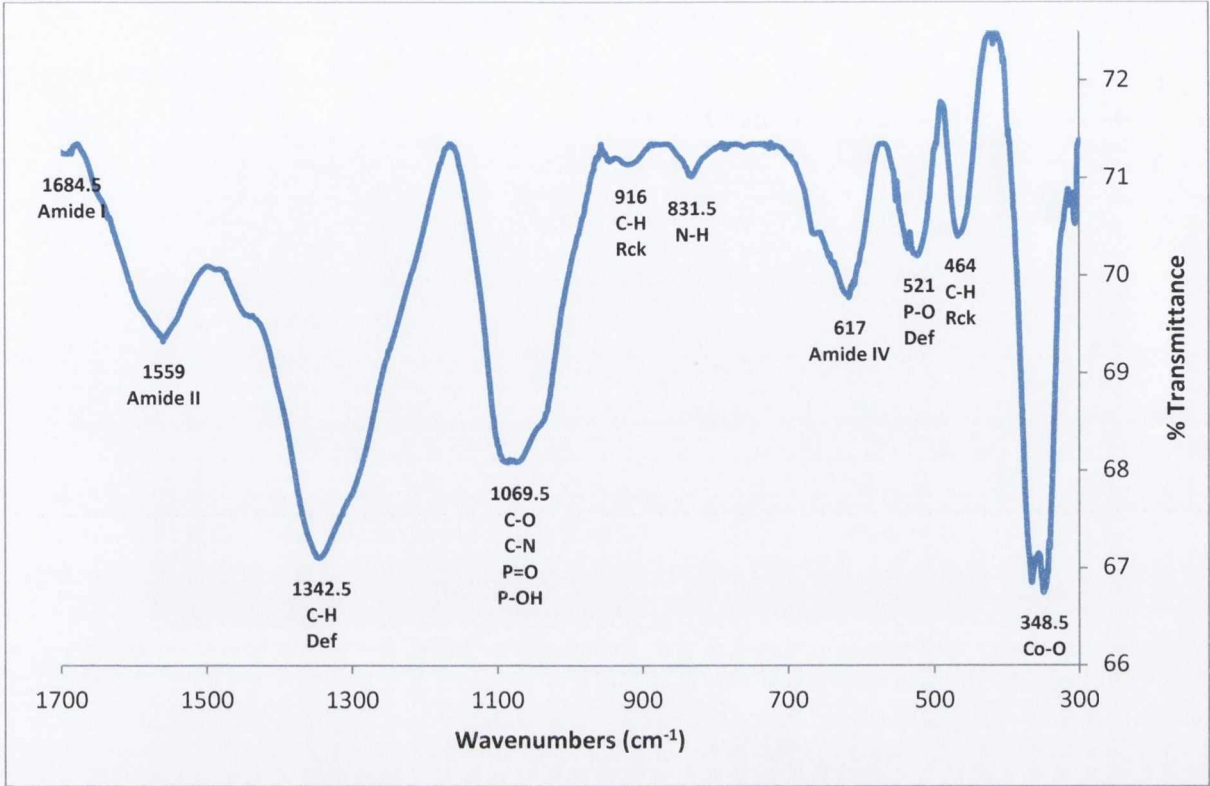
FTIR
[CoNi|Au|CoNi|Au|CoNi]@PEG-mal (28)



[CoNi|Au|CoNi|Au|CoNi]@PEG-mal (29)



A.2.5.3 [CoNi|Au|CoNi|Au|CoNi]@PEG@G'@IgG-488 (30)
FTIR



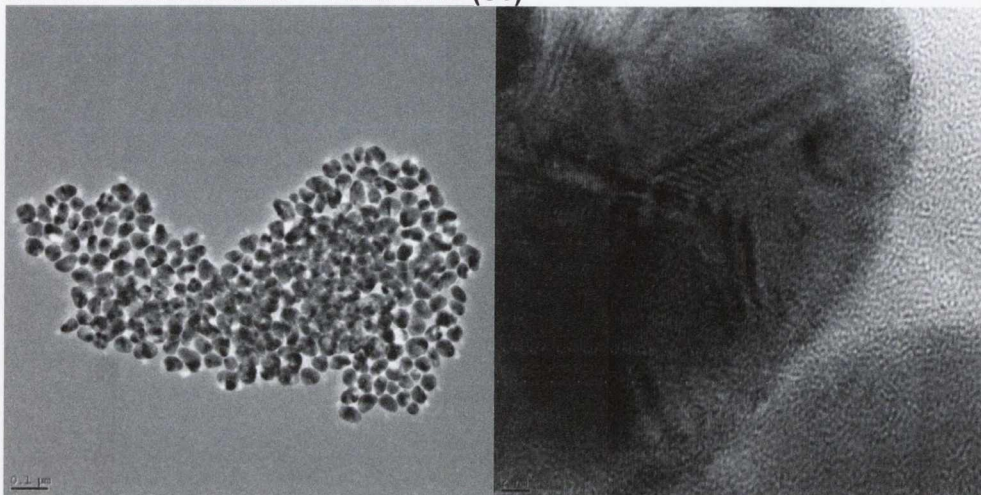
APPENDIX TO CHAPTER 3

APPENDIX 3.1

NICKEL CORE PARTICLES

A.3.1.1 TEM

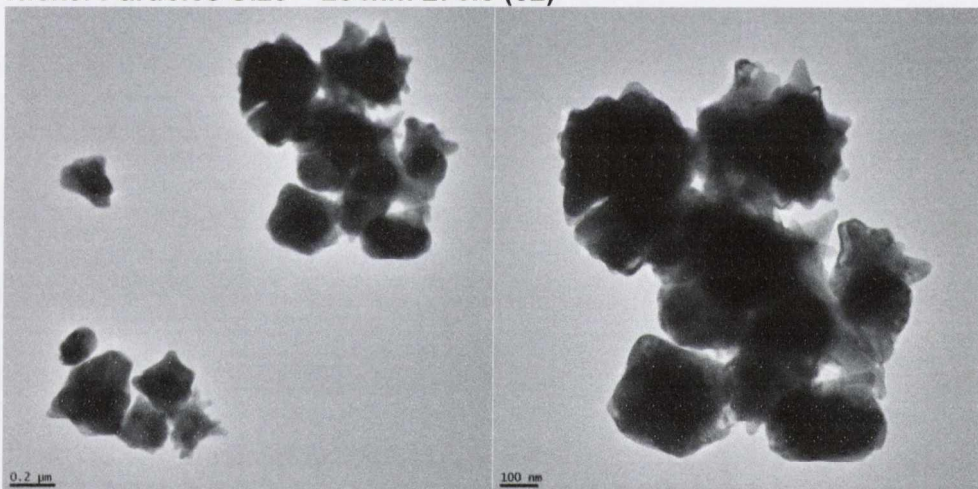
Nickel Particles Size = $44\text{nm} \pm 7.9$ (31)



Scale Bar: 100 nm

Scale Bar: 2 nm

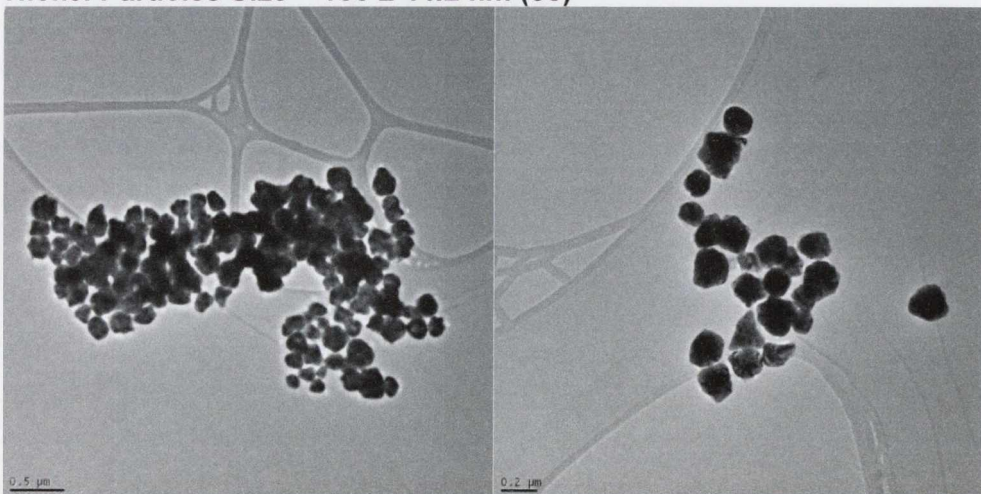
Nickel Particles Size = $254\text{nm} \pm 78.8$ (32)



Scale Bar: 200 nm

Scale Bar: 100 nm

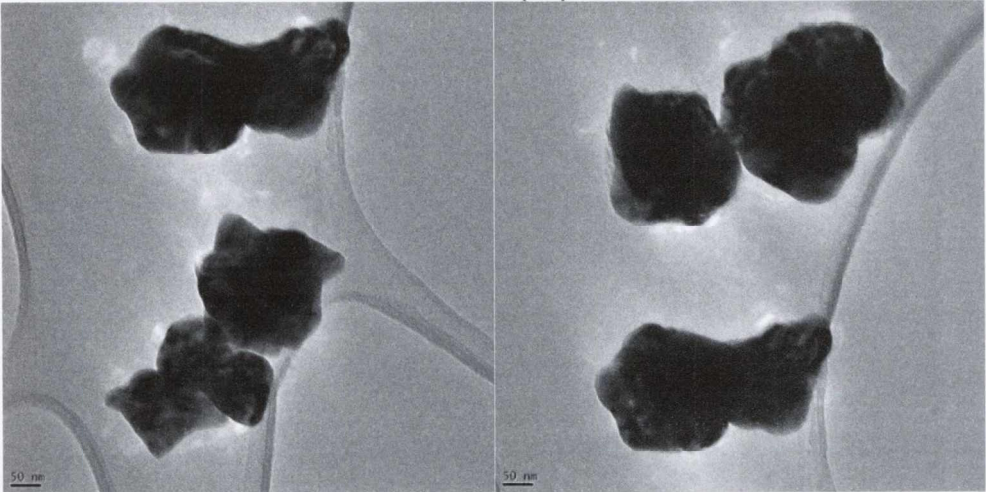
Nickel Particles Size = 180 ± 44.2 nm (33)



Scale Bar: 500 nm

Scale Bar: 200 nm

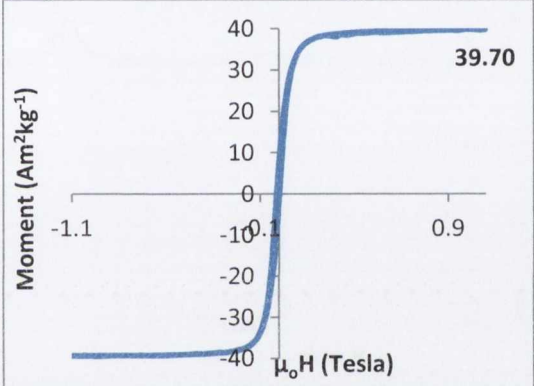
Nickel Particles Size = 163 ± 43.9 nm (34)



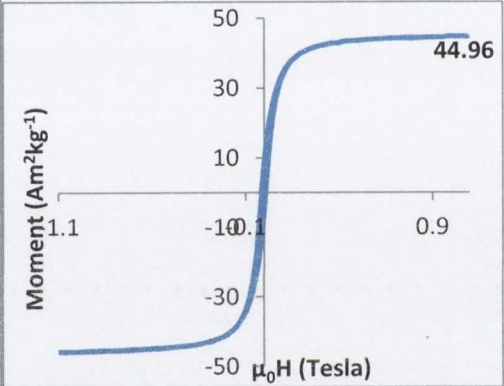
Scale Bar: 50 nm

Scale Bar: 50 nm

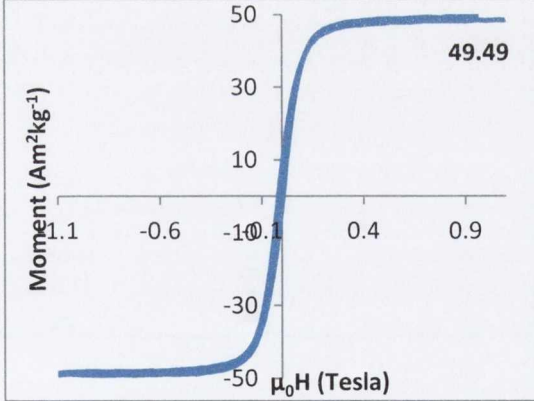
A.3.1.2 VSM
(31)



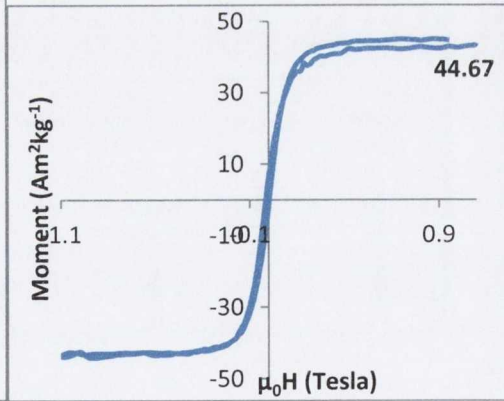
(32)



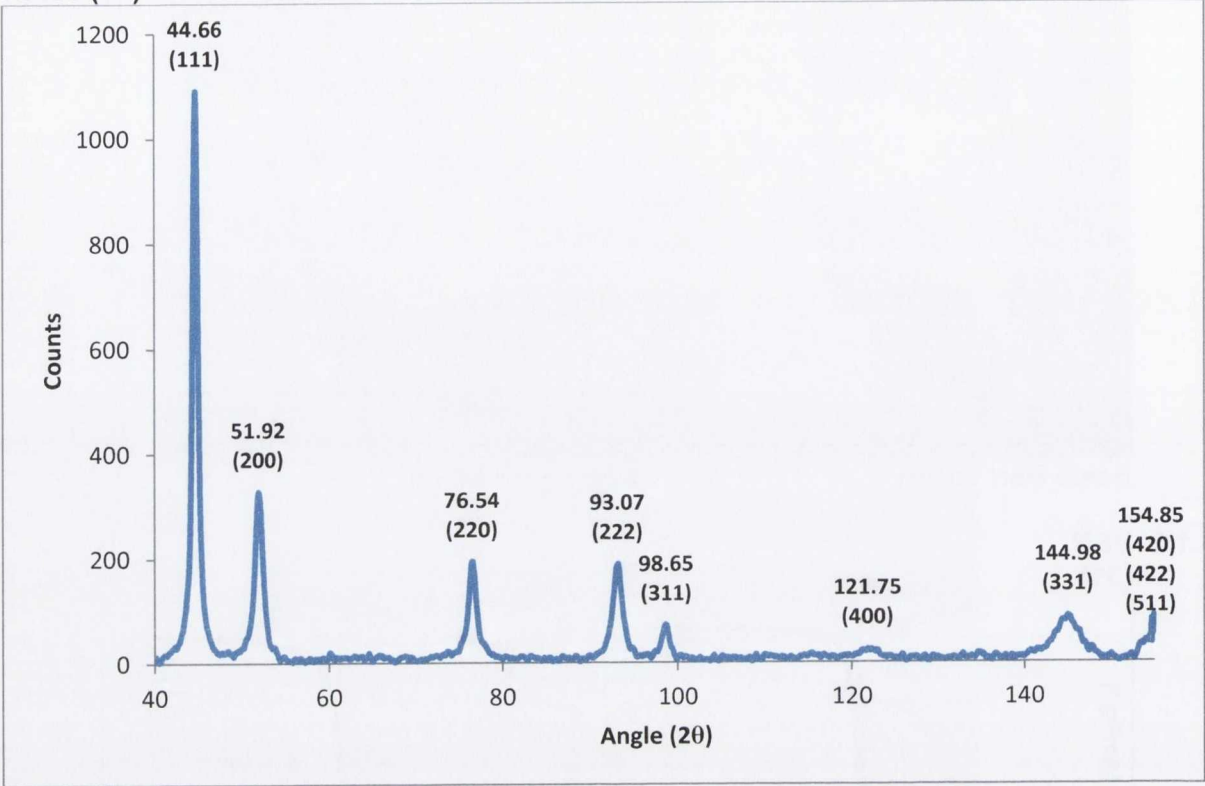
(33)



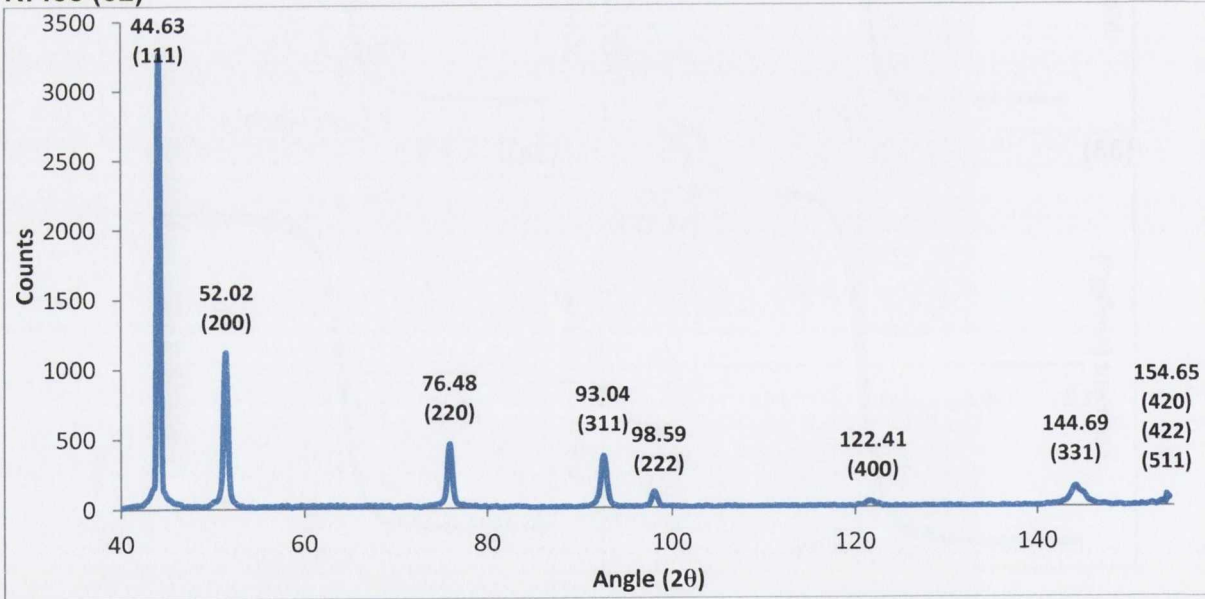
(34)



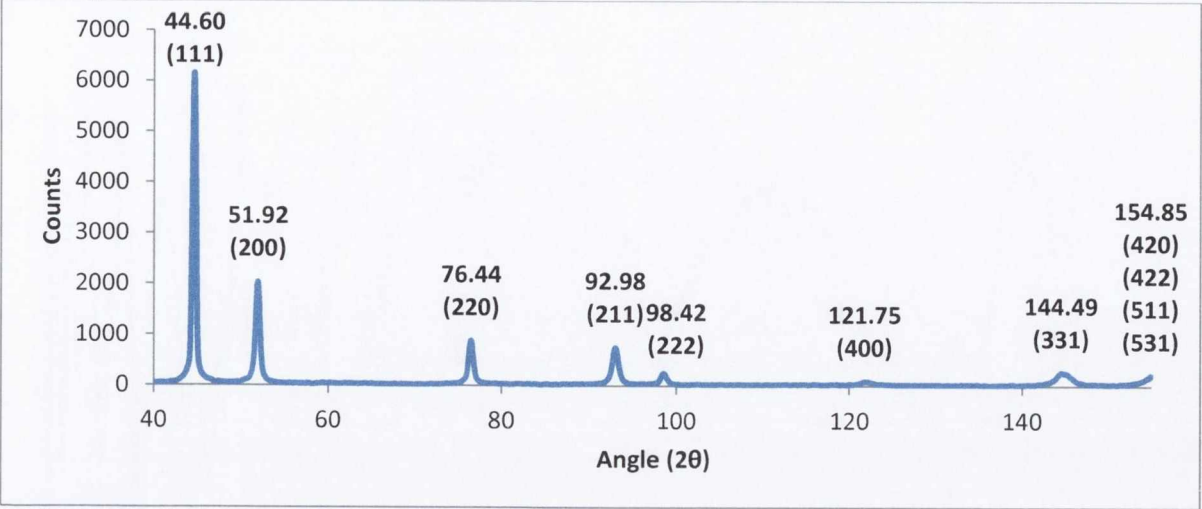
A.3.1.3 XRD
Ni fcc (31)



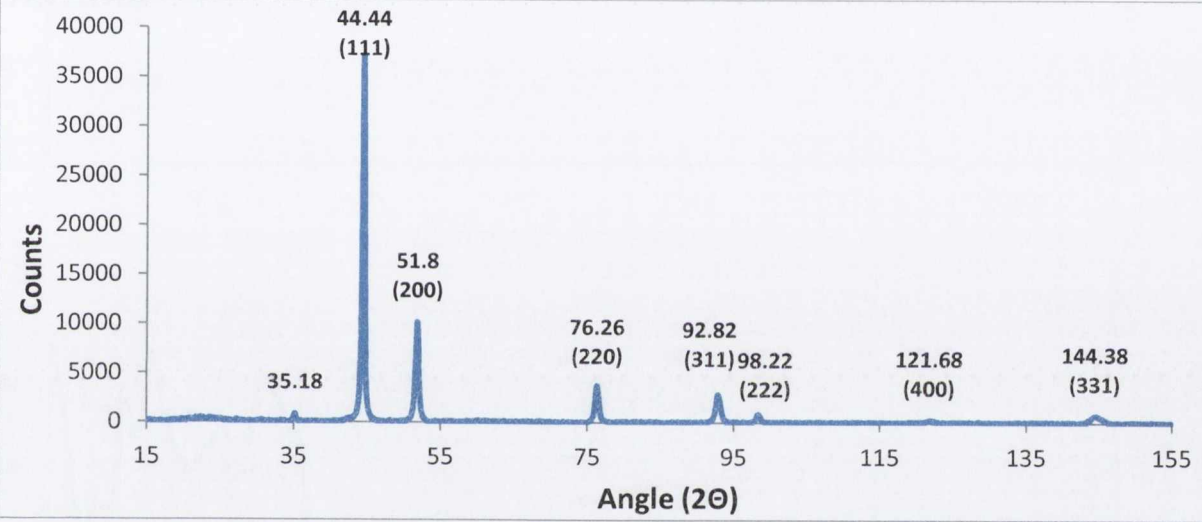
Ni fcc (32)



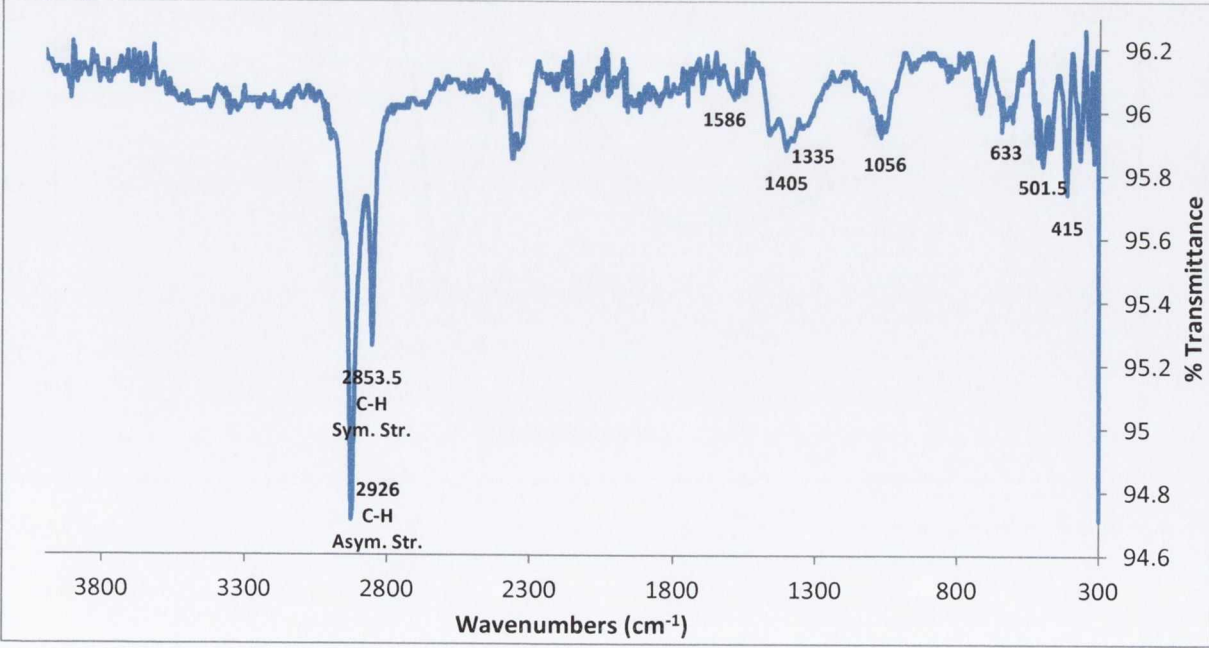
Ni fcc (33)



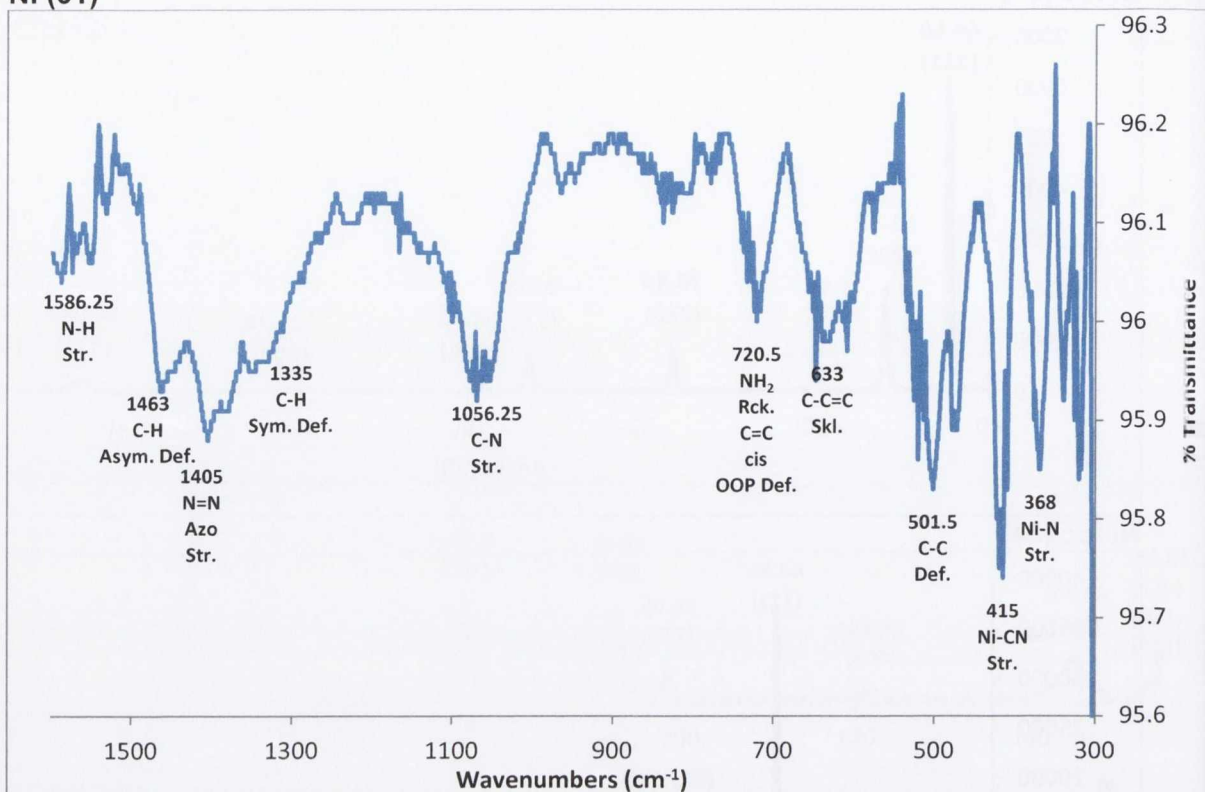
Ni fcc (34)



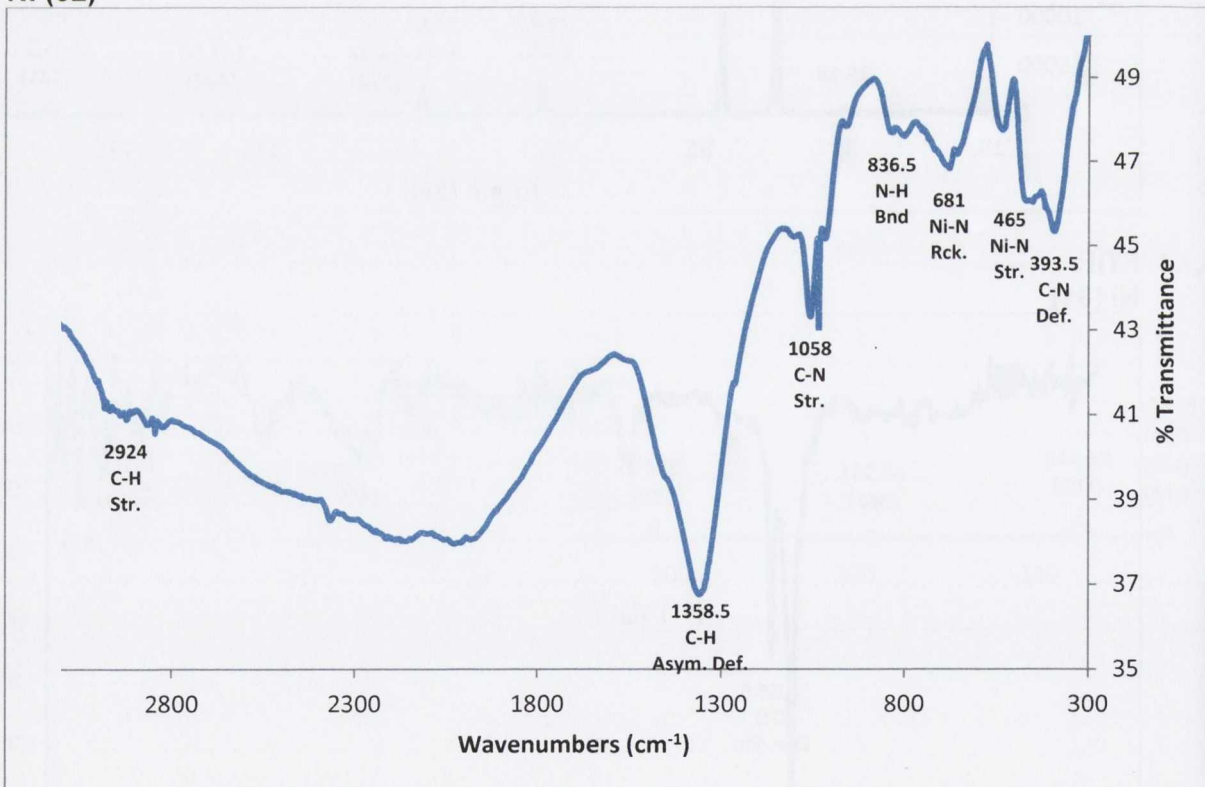
A.3.1.4 FTIR
Ni (31)



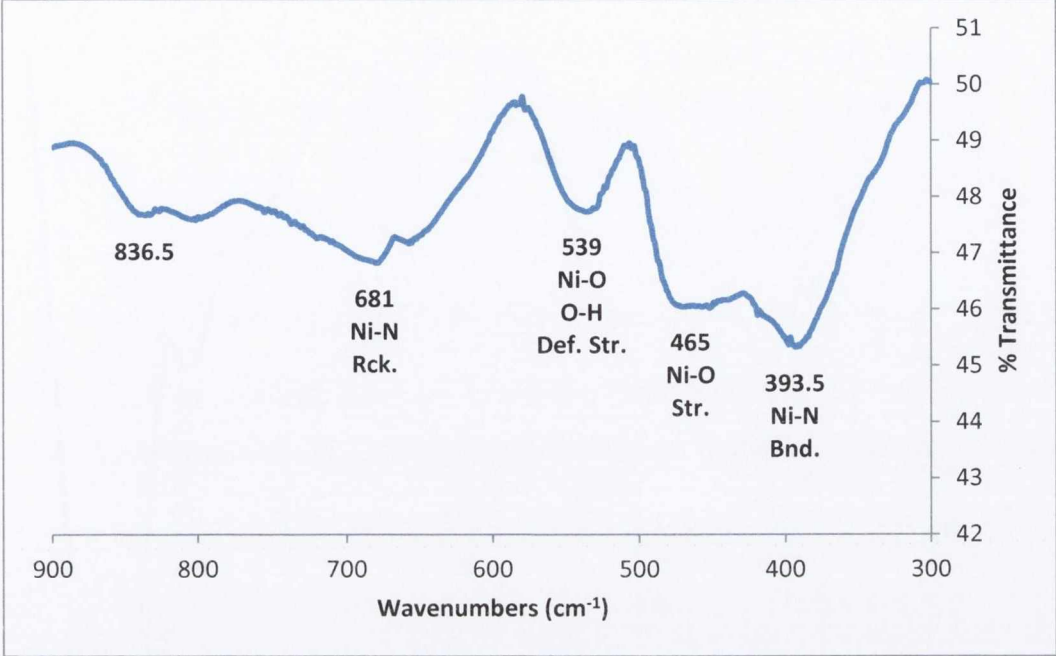
Ni (31)



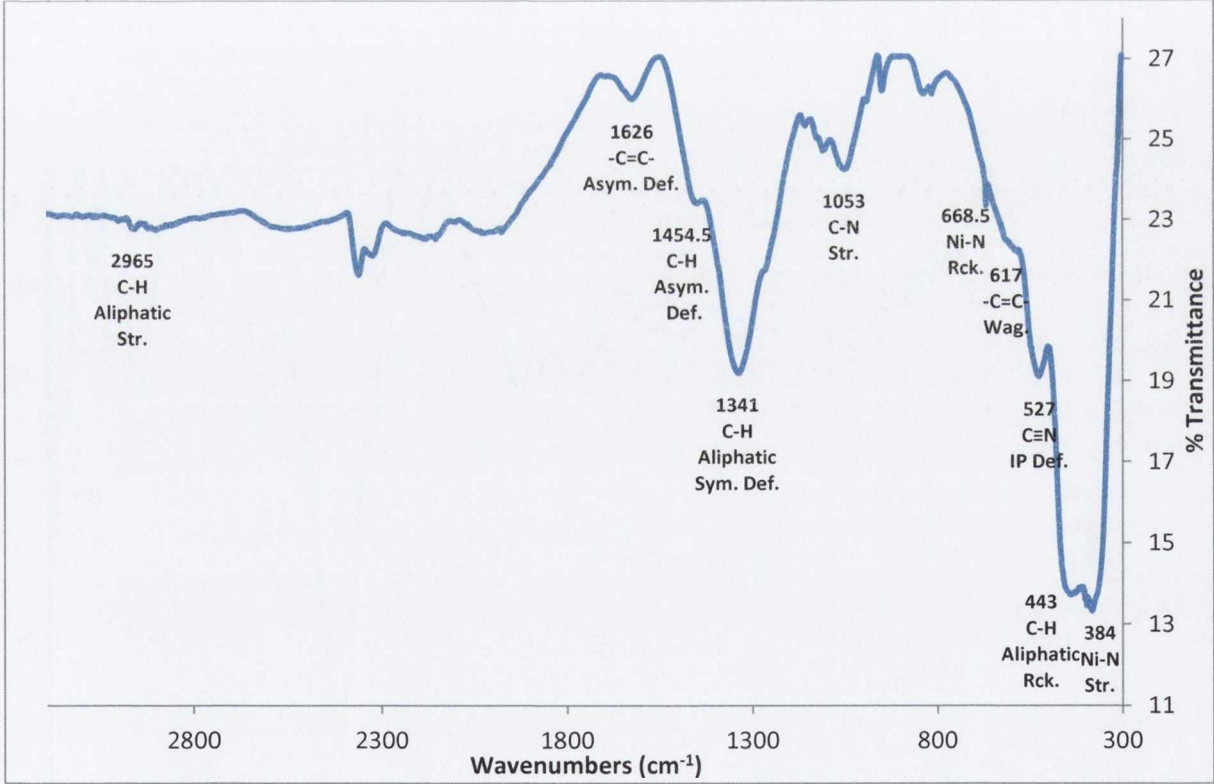
Ni (32)



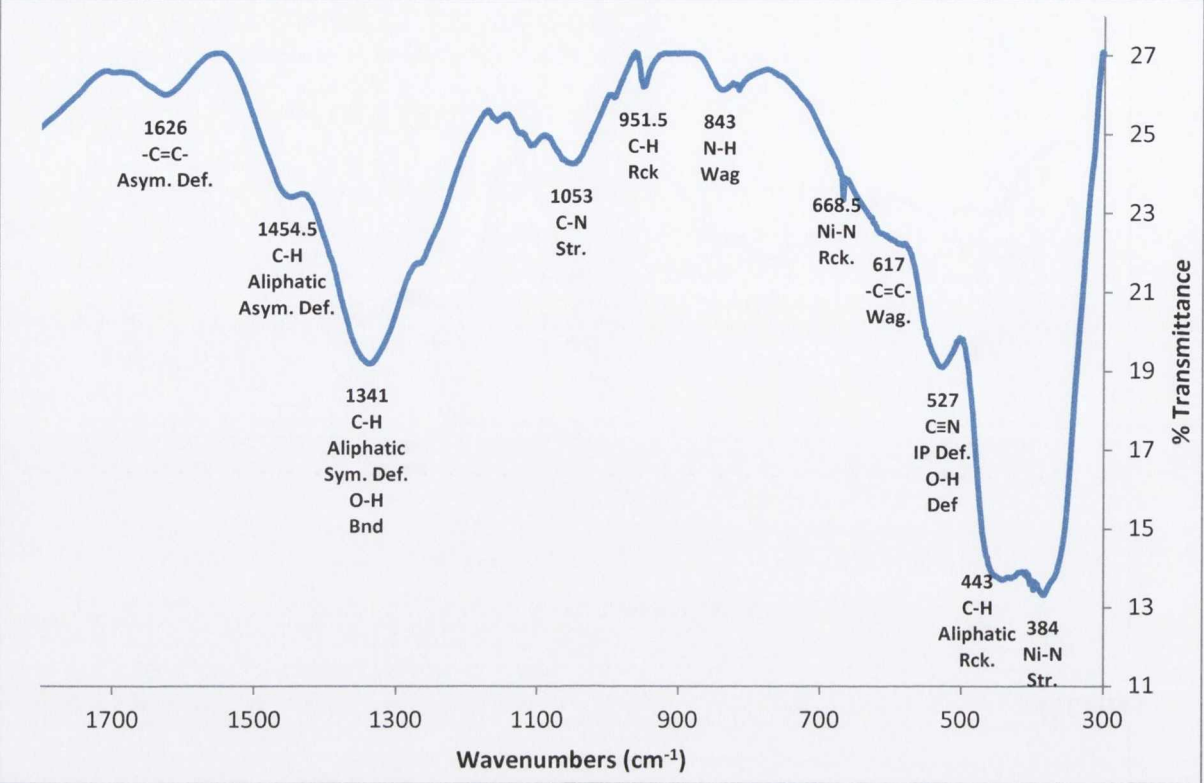
Ni (32)



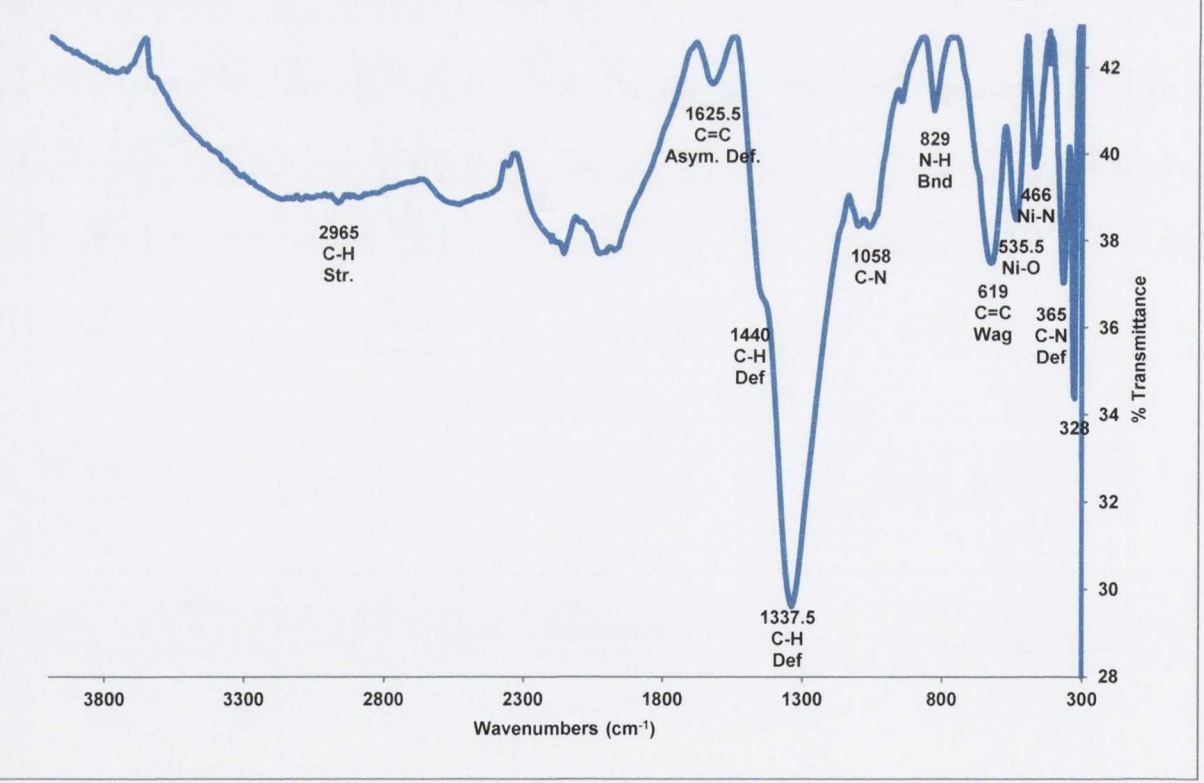
Ni (33)



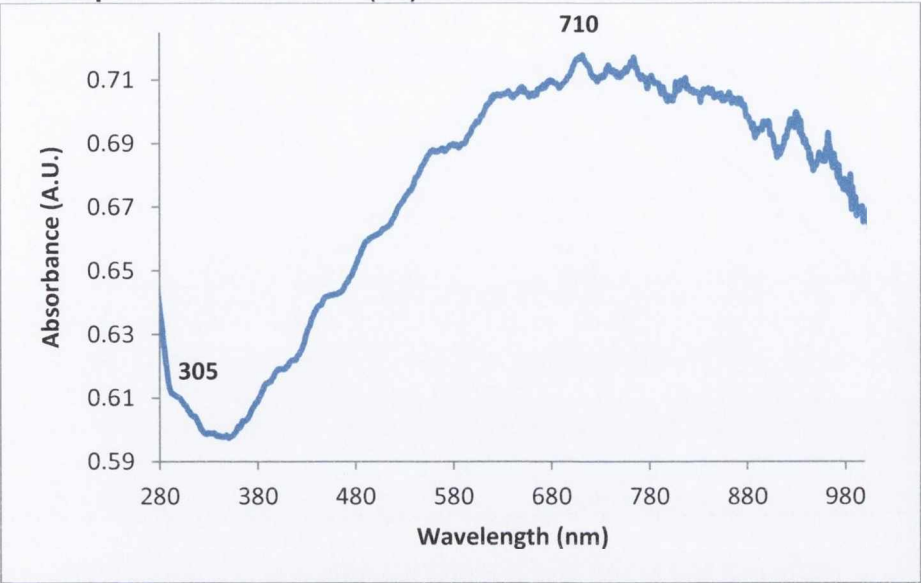
Ni (33)



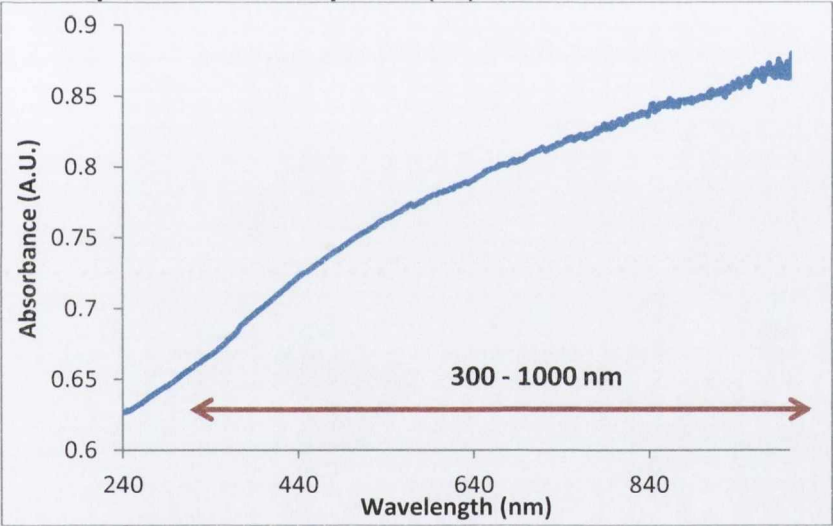
Ni (34)



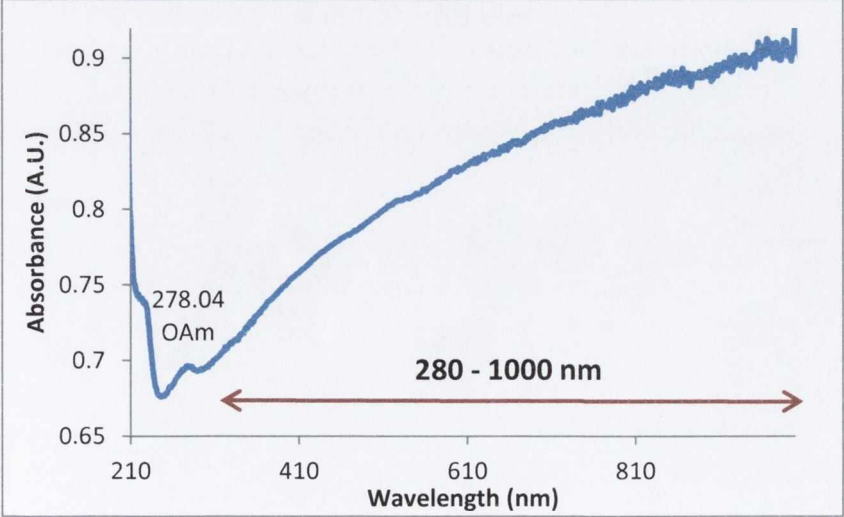
A.3.1.5 UV-vis
Nickel particles in hexane (31)



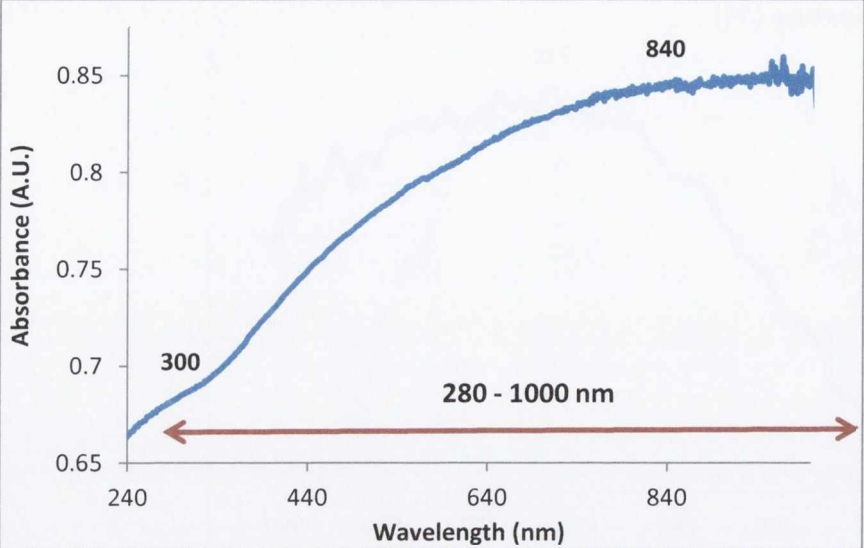
Nickel particles in CBS pH 6.5 (32)



Nickel particles in CBS pH 6.5 (33)



Nickel particles in CBS pH 6.5 (34)

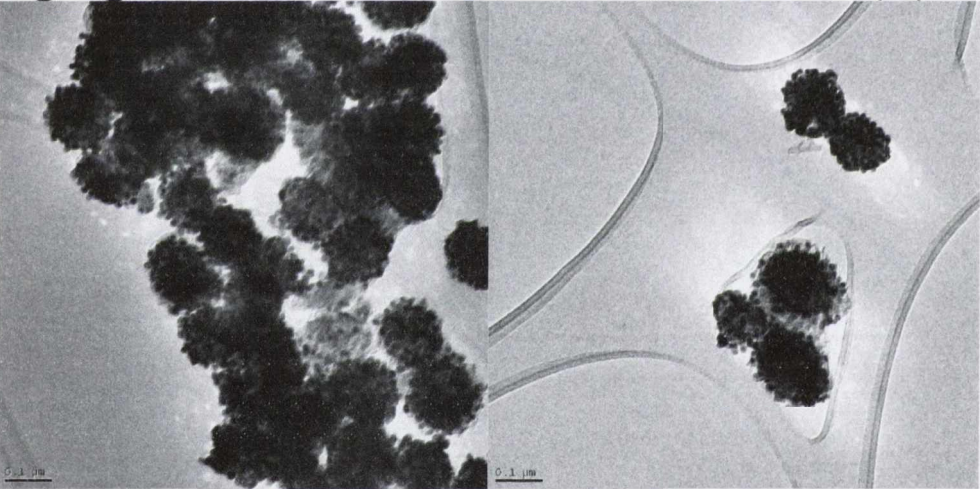


APPENDIX 3.2

GOLD-COATED NICKEL PARTICLES

A.3.2.1 TEM

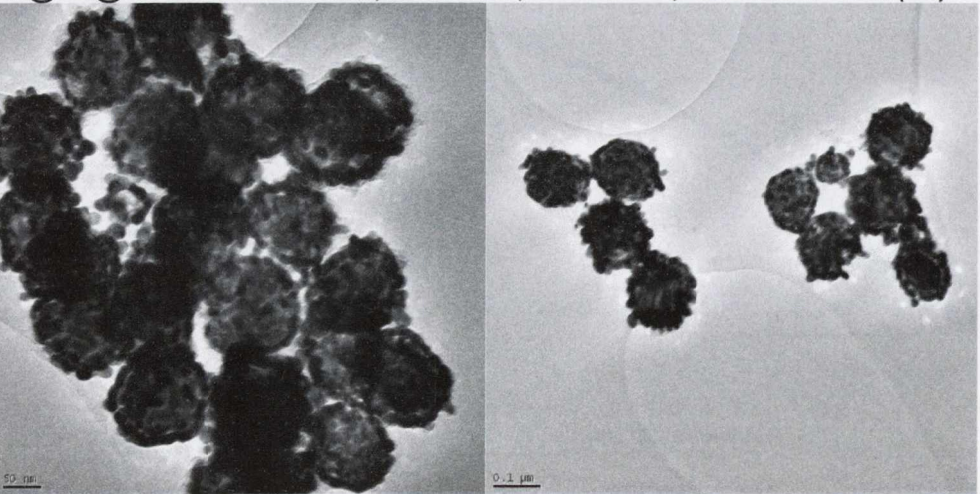
Ni@Au@OH , 50/12.5 mM, 5min/12h, 300/100°C, 132.7±12.6 nm (35)



Scale Bar: 100 nm

Scale Bar: 100 nm

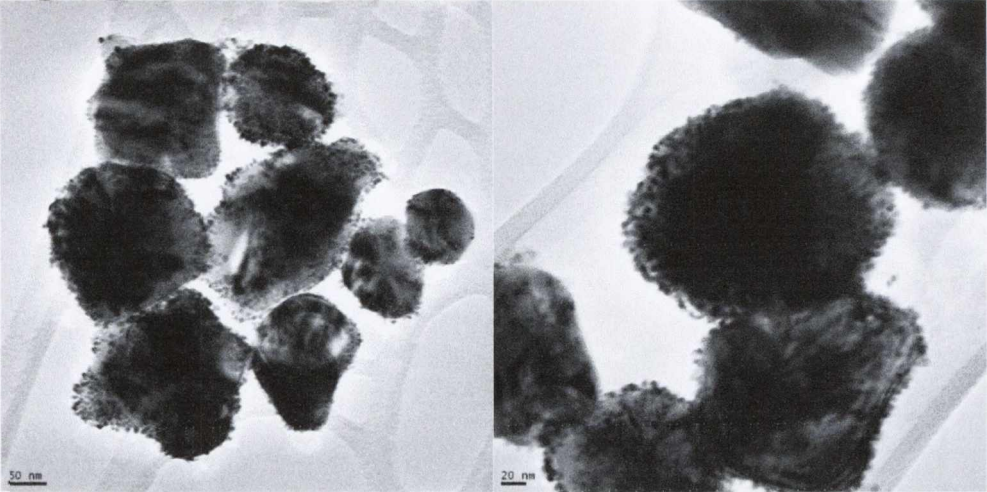
Ni@Au@COOH 50/16.7 mM, 20min/2h, 300/100°C, 127.4±14.7 nm (36)



Scale Bar: 50 nm

Scale Bar: 100 nm

Ni@Au@OH , 400/12 mM, 3h/3h, 250/40°C, 173±50.7 nm (37)

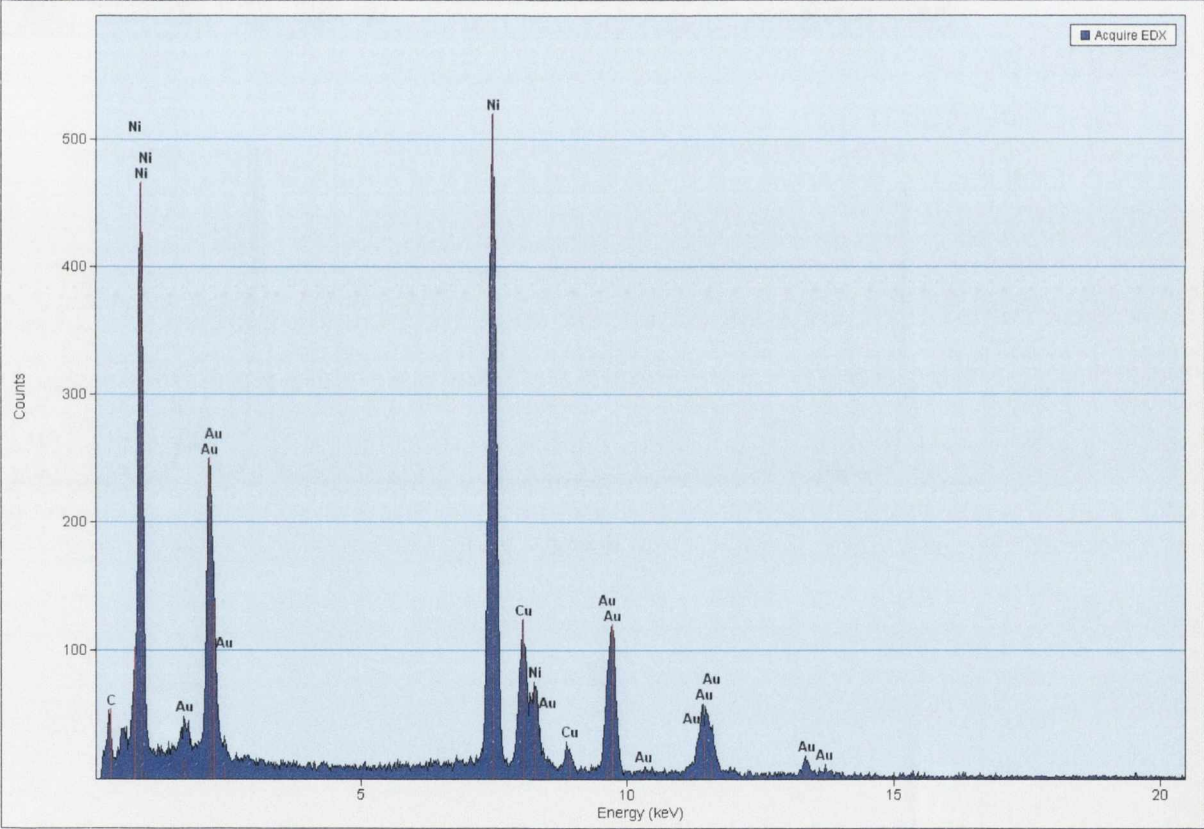


Scale Bar: 50 nm

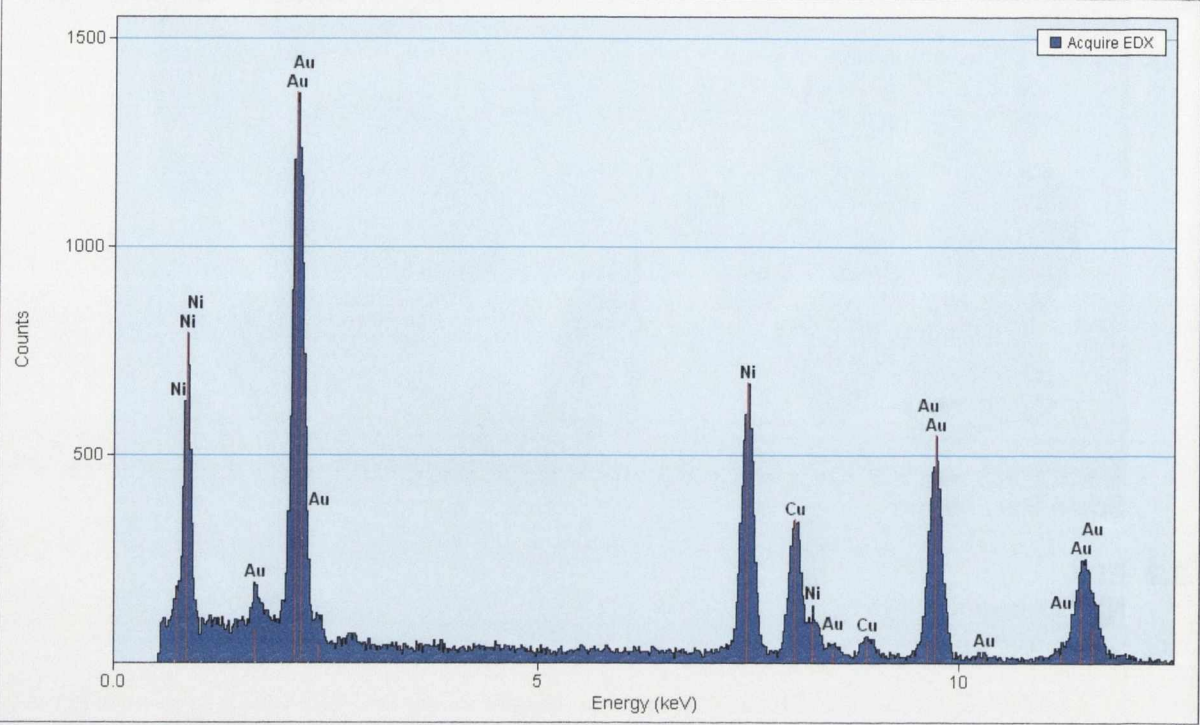
Scale Bar: 20 nm

A.3.2.2 EDX

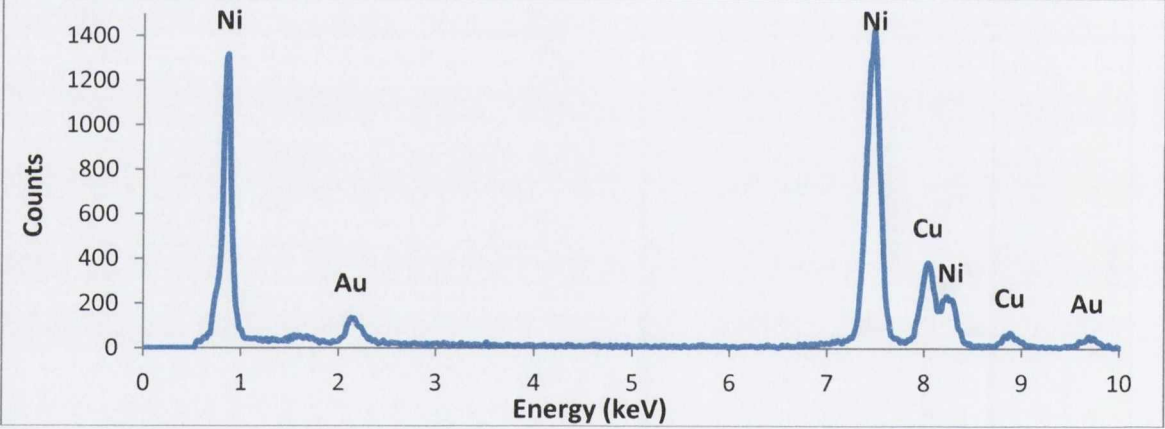
Ni₄₅@Au₅₅@OH (35)



Ni₇₃@Au₂₇@COOH (36)

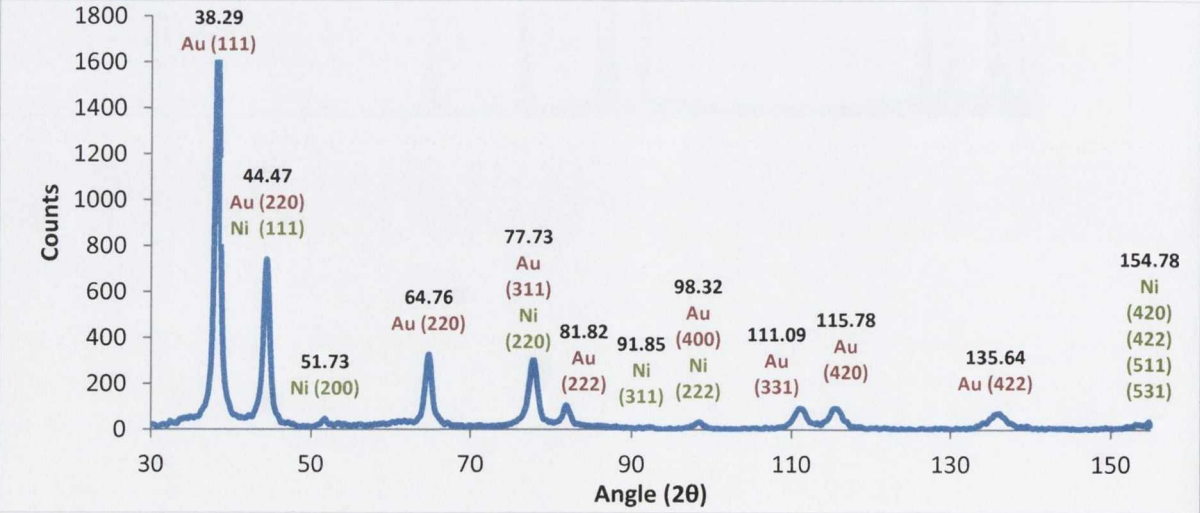


Ni_{96.5}@Au_{3.5}@OH (37)

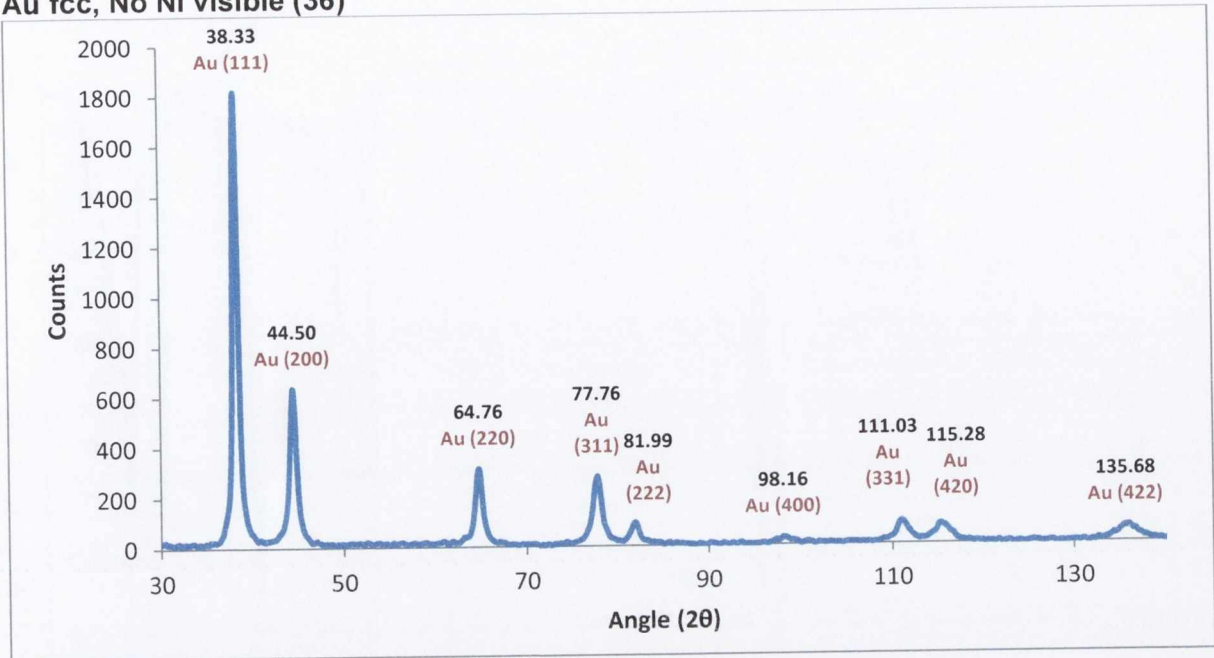


A.3.2.3 XRD

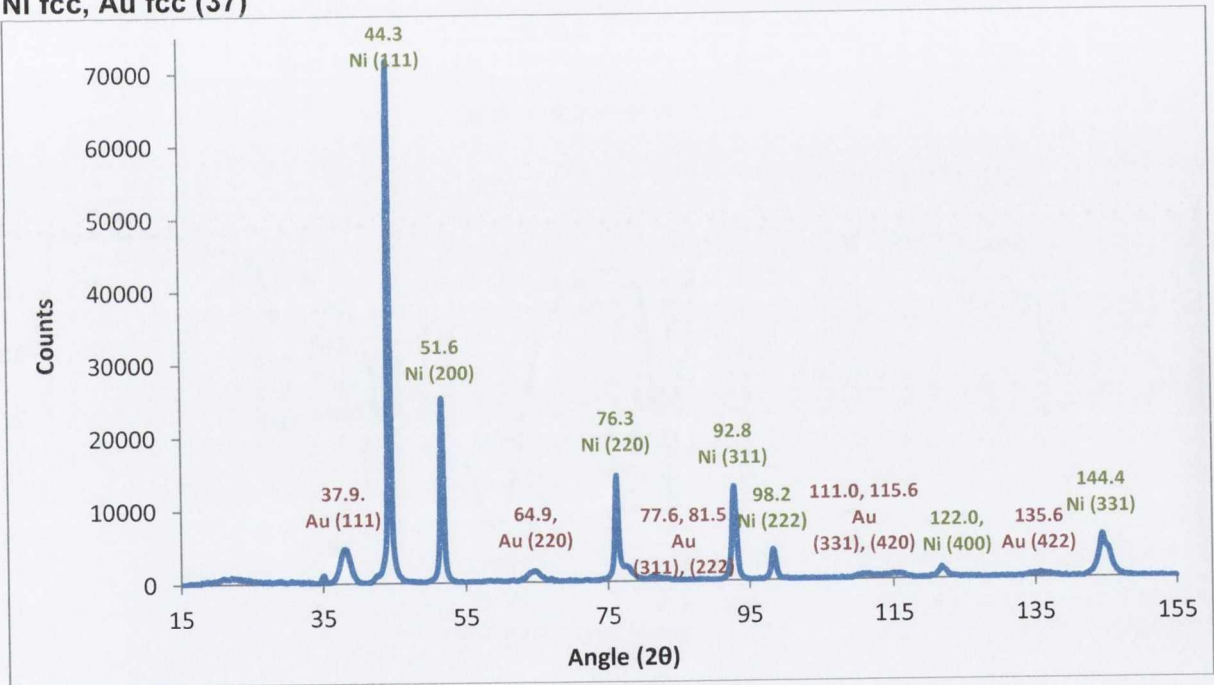
Ni fcc, Au fcc (35)



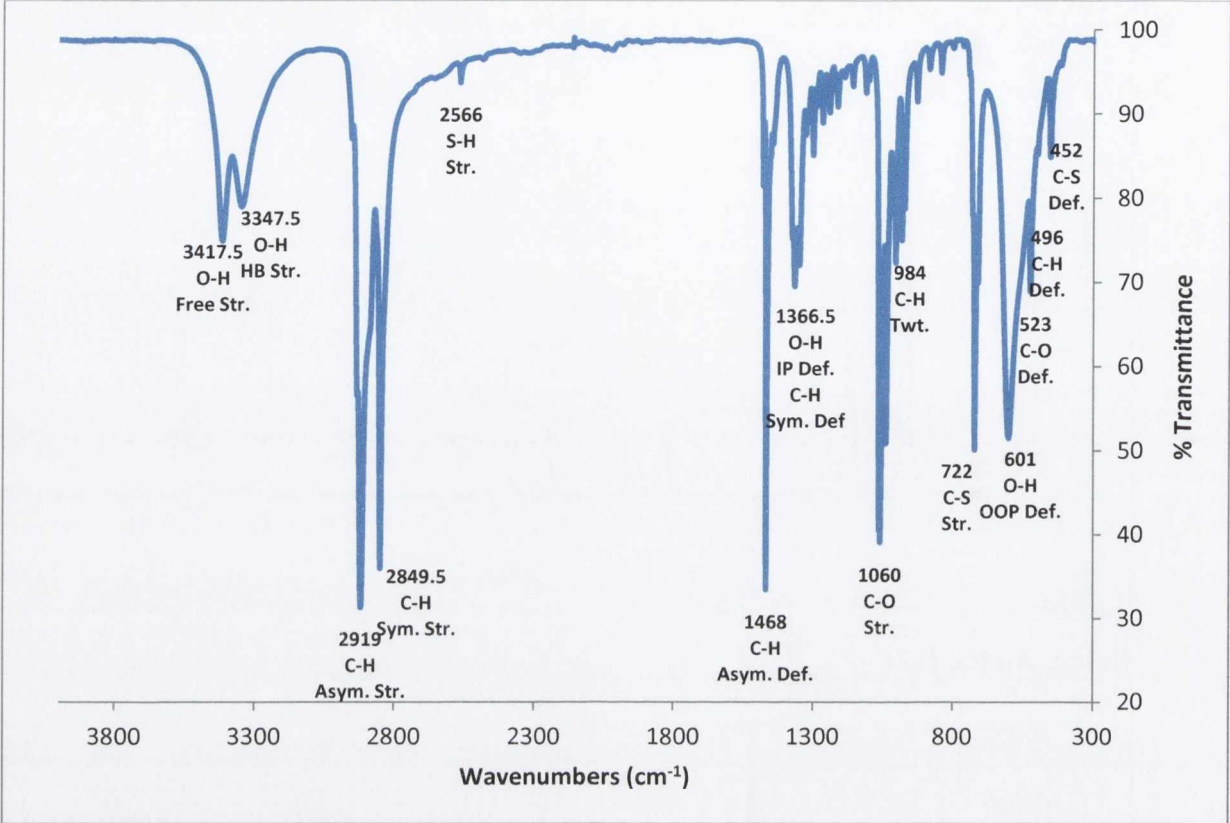
Au fcc, No Ni visible (36)



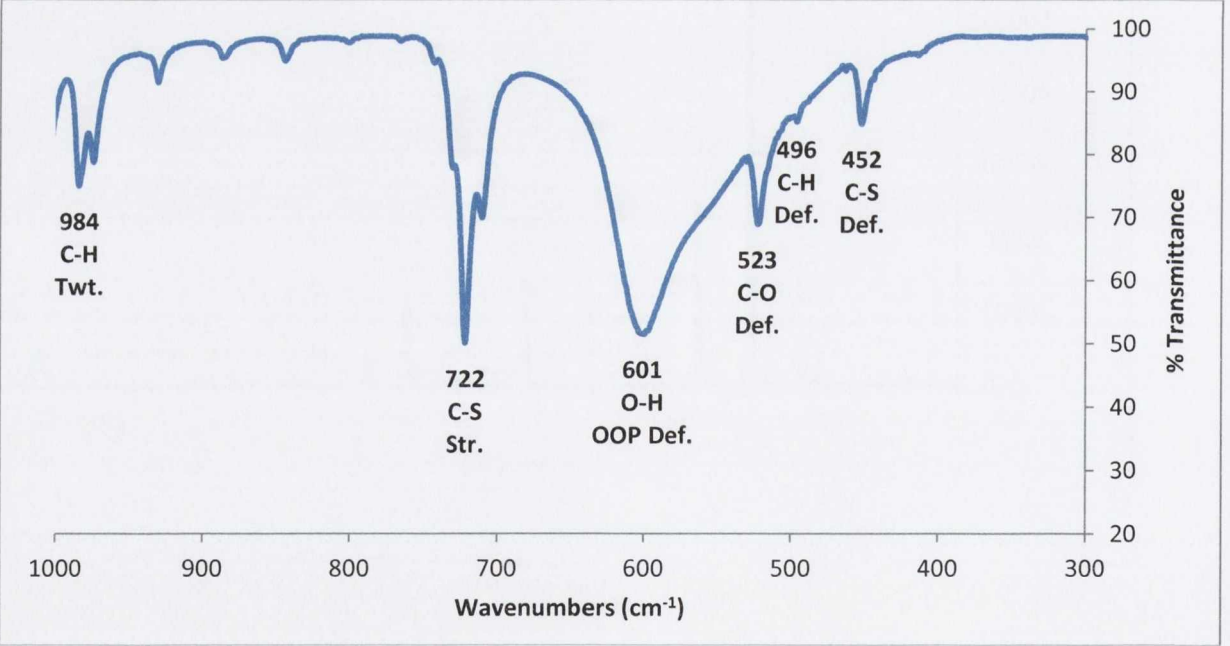
Ni fcc, Au fcc (37)



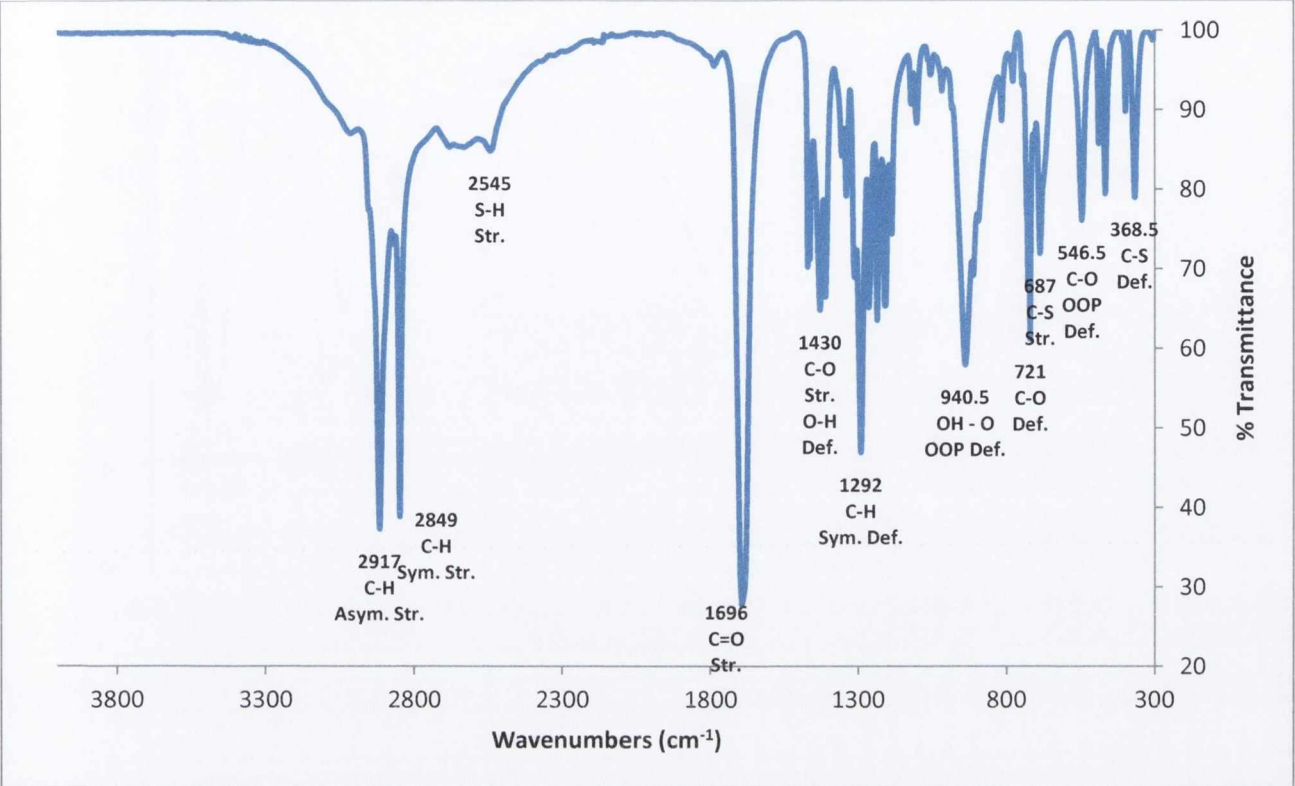
A.3.2.4 FTIR
11-Mercapto-1-undecanol



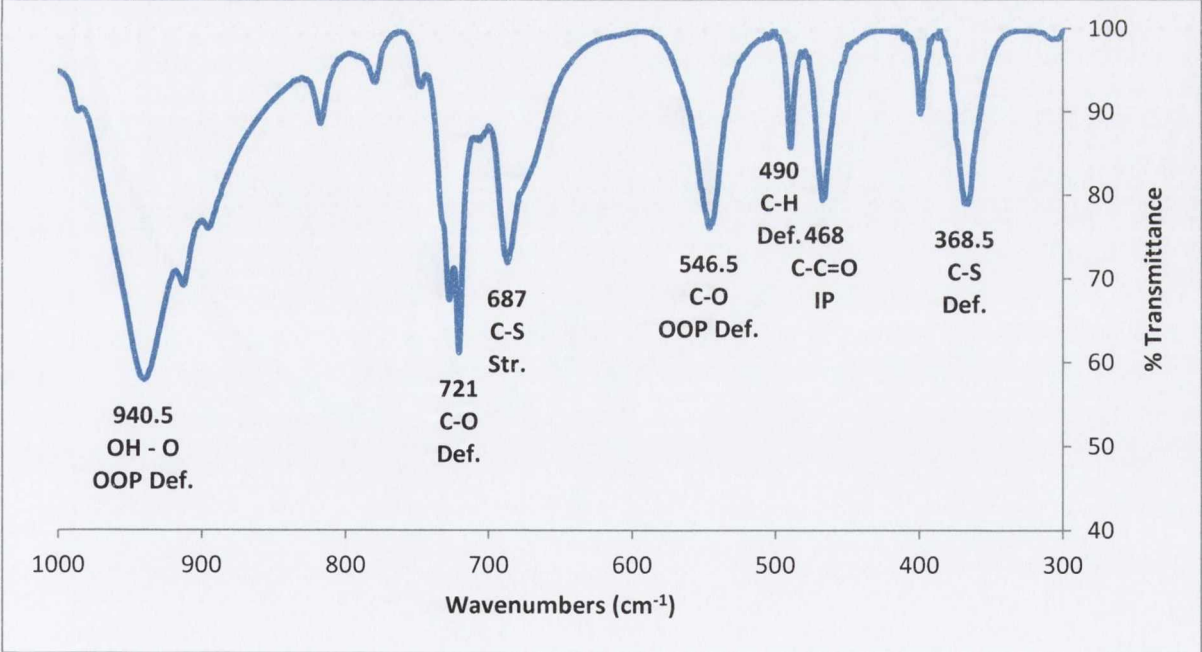
11-Mercapto-1-undecanol



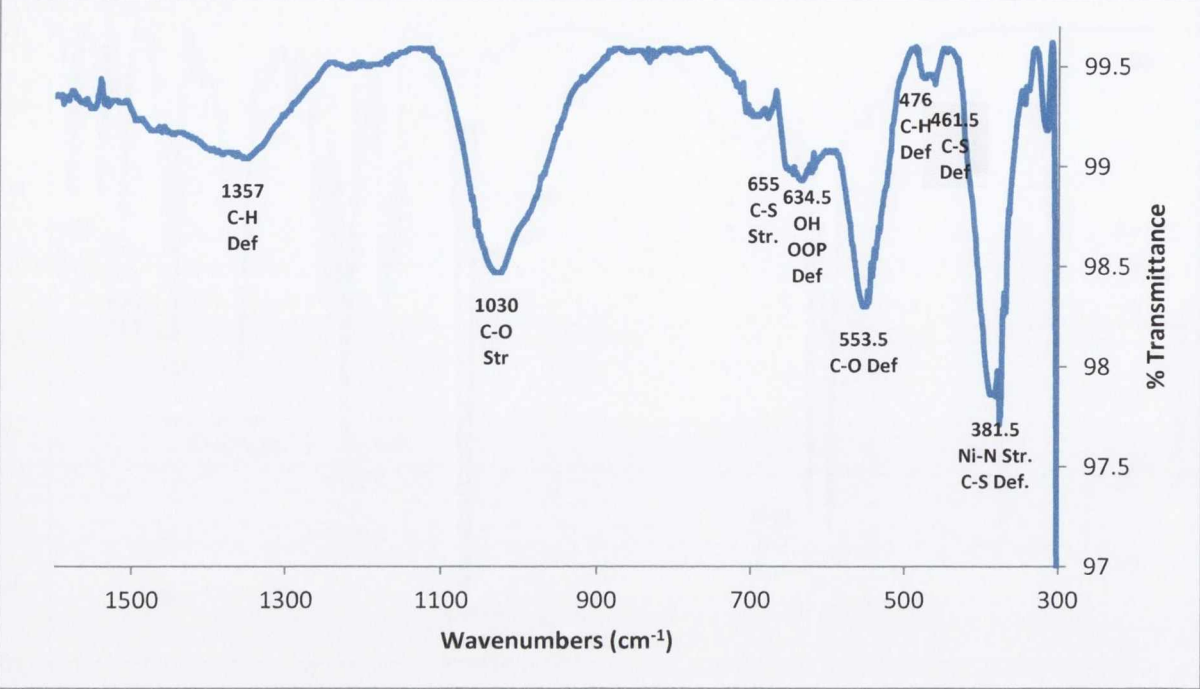
11-Mercapto-1-undecanoic acid



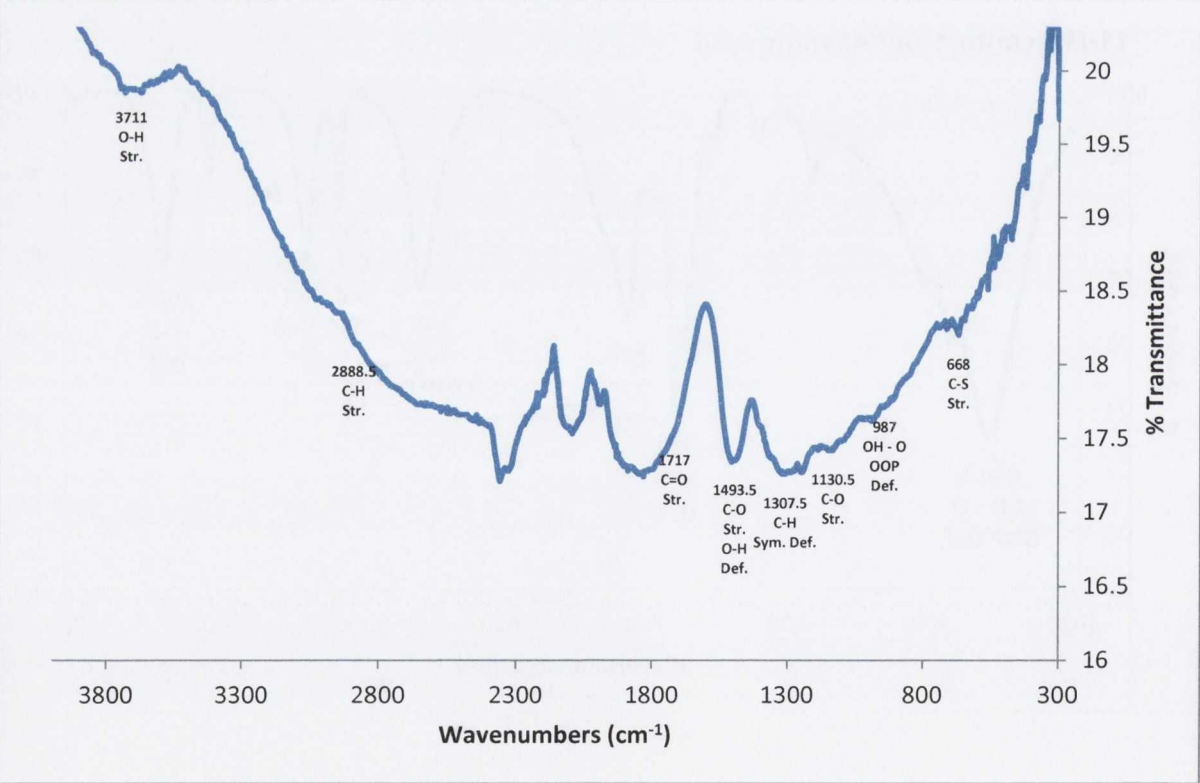
11-Mercapto-1-undecanoic acid



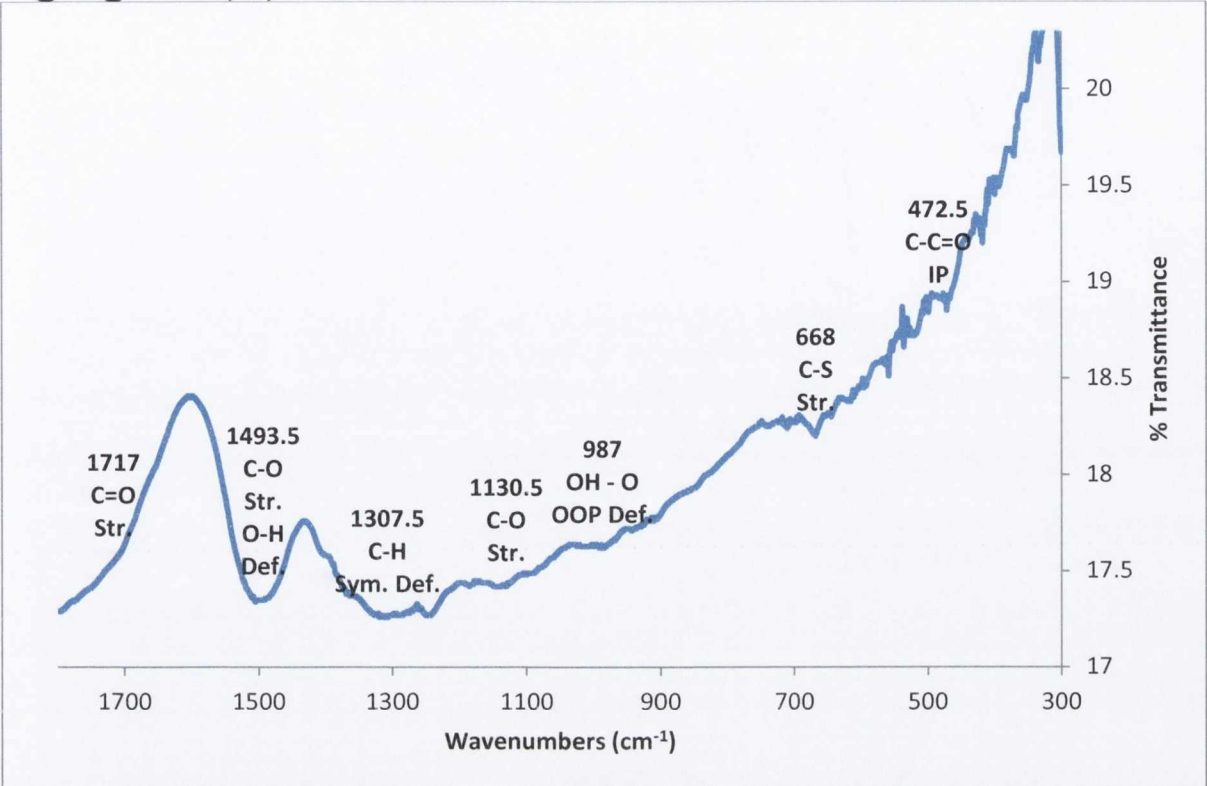
Ni@Au@OH (35)



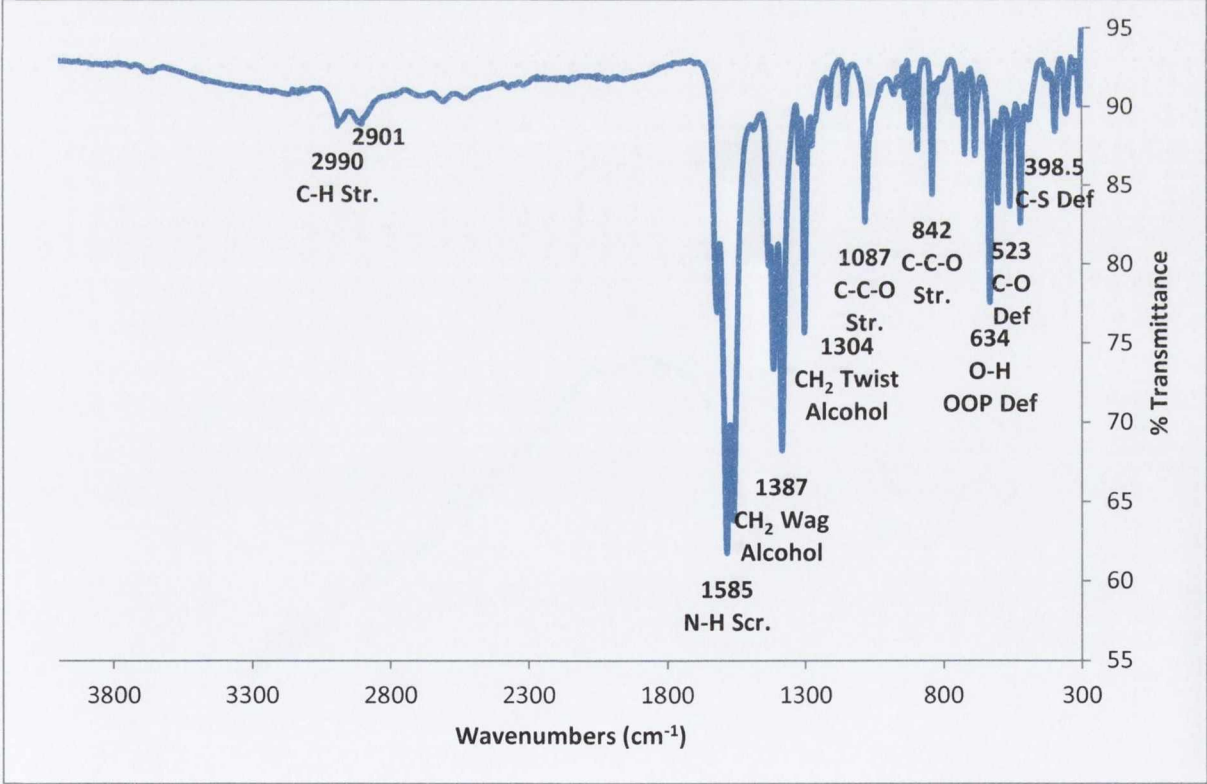
Ni@Au@COOH (36)



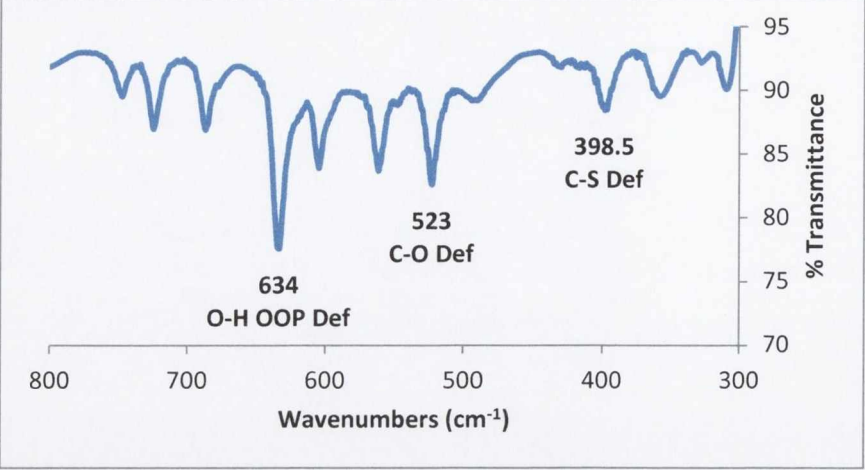
Ni@Au@COOH (36)



Ni@Au@OH (37)

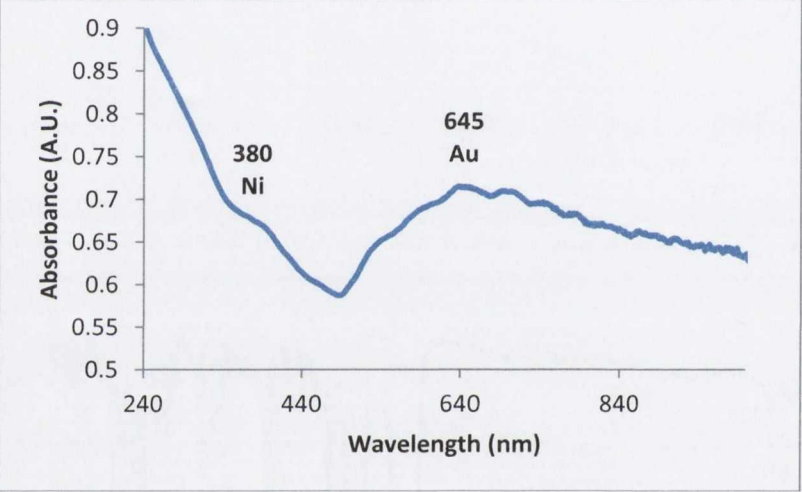


Ni@Au@OH (37)

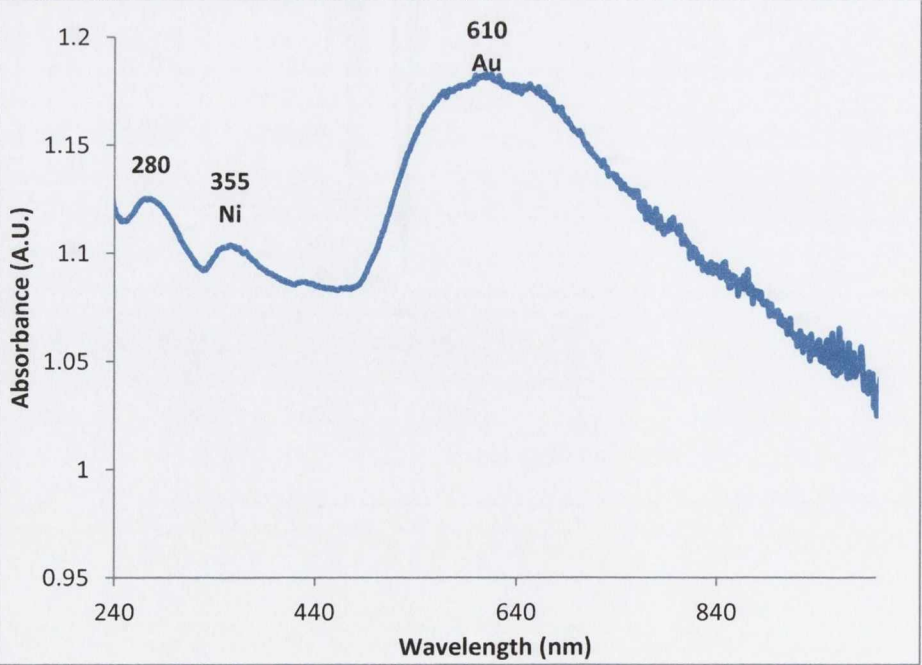


A.3.2.5 UV-vis

Ni@Au@OH in CBS pH 6.5 (35)

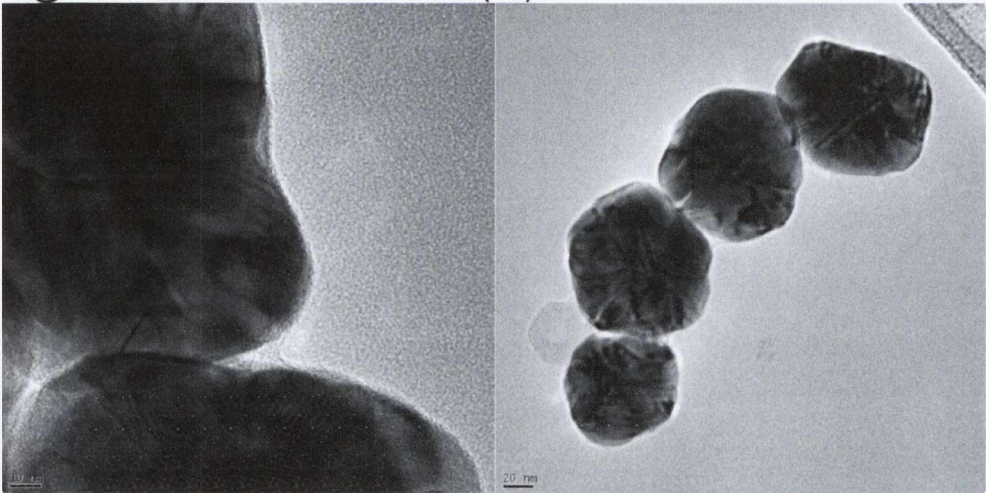


Ni@Au@COOH in CBS pH 6.5 (36)



A.3.3.1 TEM

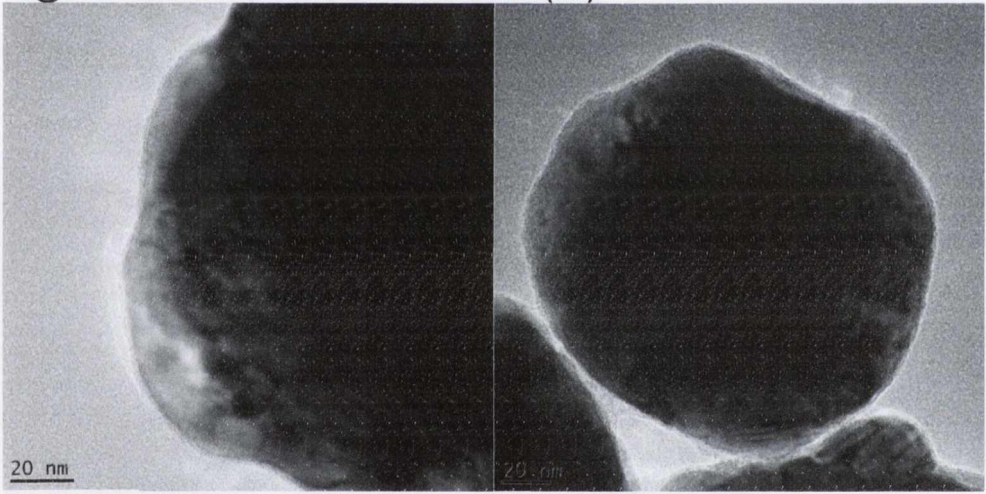
Ni@GNP Size = 149.98 ± 68.60 nm (38)



Scale Bar: 10 nm

Scale Bar: 20 nm

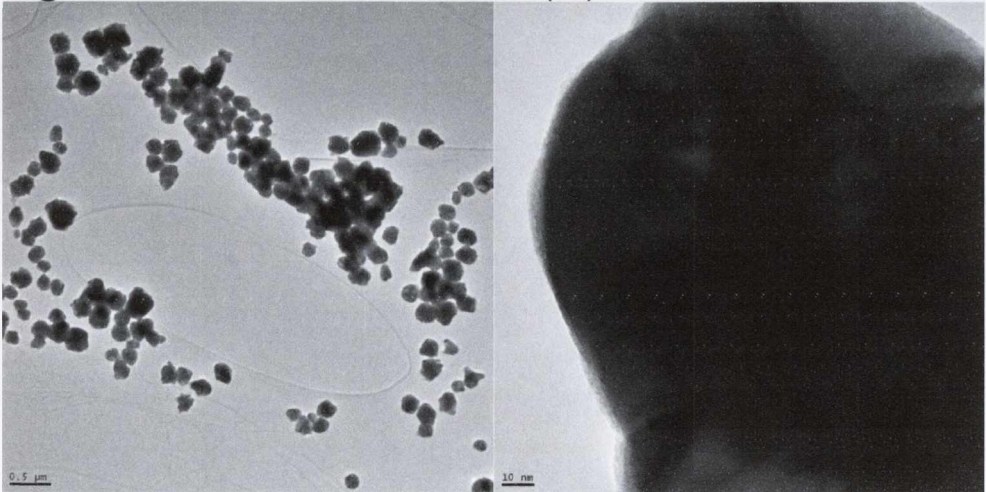
Ni@GNP-NH₂ Size = 178.04 ± 21.75 nm (39)



Scale Bar: 20 nm

Scale Bar: 20 nm

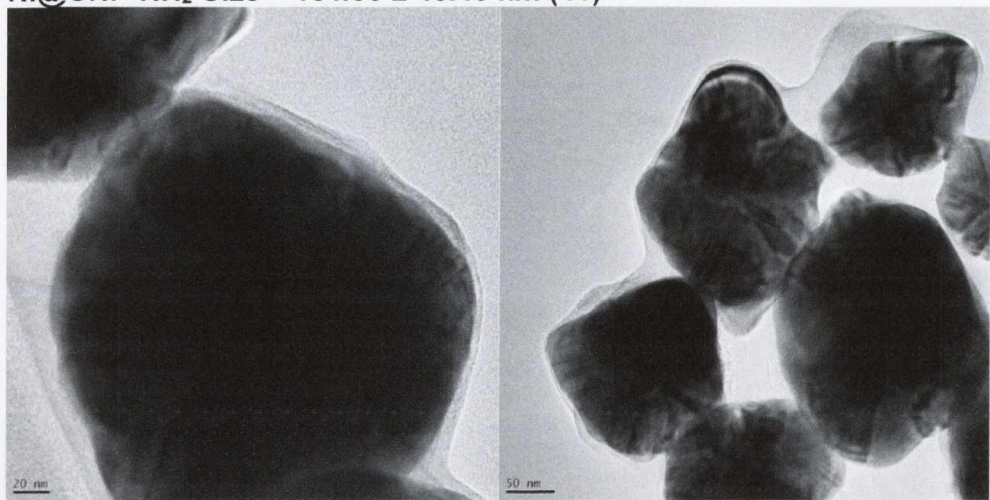
Ni@GNP-NH₂ Size = 193.28 ± 39.92 nm (40)



Scale Bar: 500 nm

Scale Bar: 10 nm

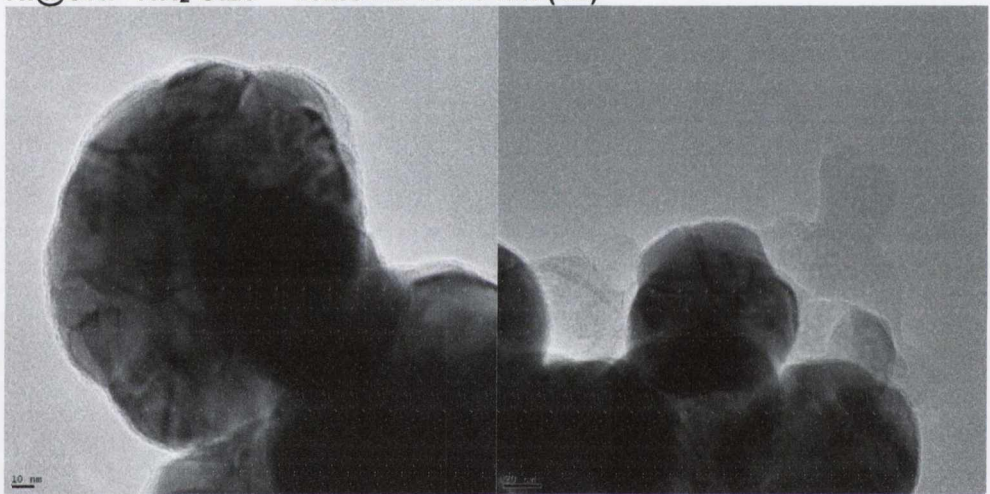
Ni@GNP-NH₂ Size = 184.56 ± 46.48 nm (41)



Scale Bar: 20 nm

Scale Bar: 50 nm

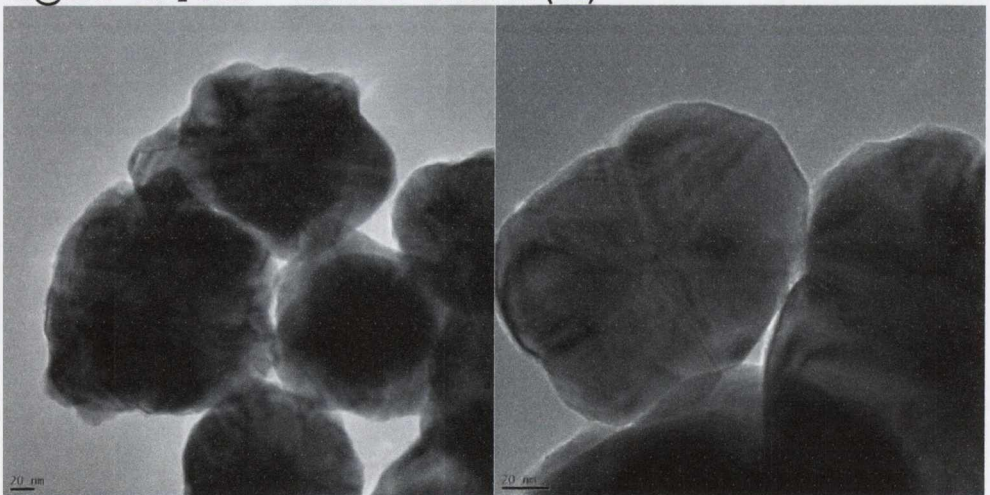
Ni@GNP-NH₂ Size = 102.81 ± 18.90 nm (42)



Scale Bar: 10 nm

Scale Bar: 20 nm

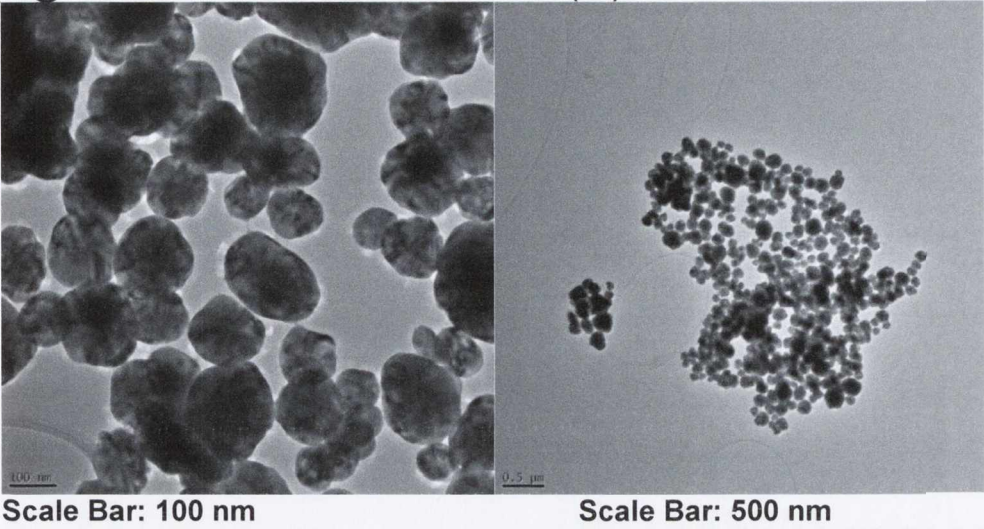
Ni@GNP-NH₂ Size = 153.19 ± 46.16 nm (43)



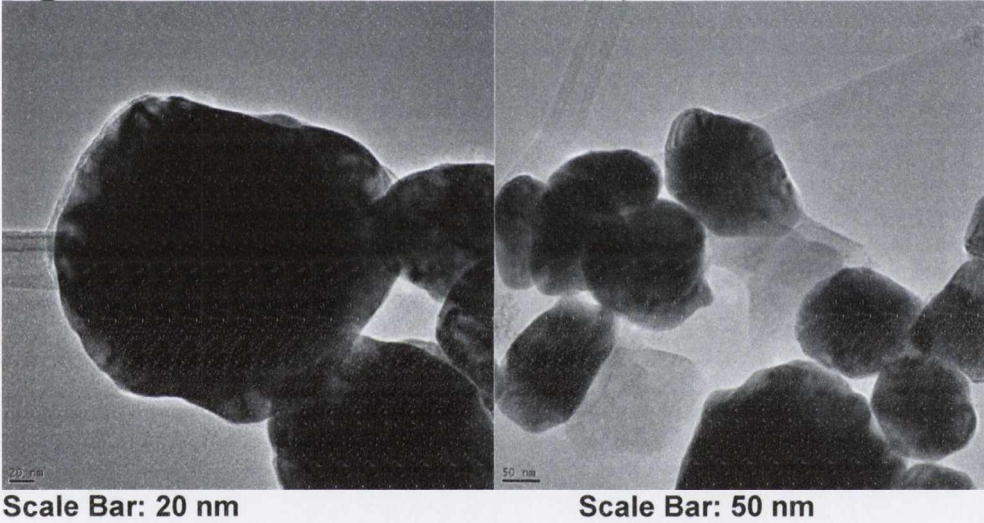
Scale Bar: 20 nm

Scale Bar: 20 nm

Ni@GNP-COOH Size =122.08 ± 33.18 nm (44)

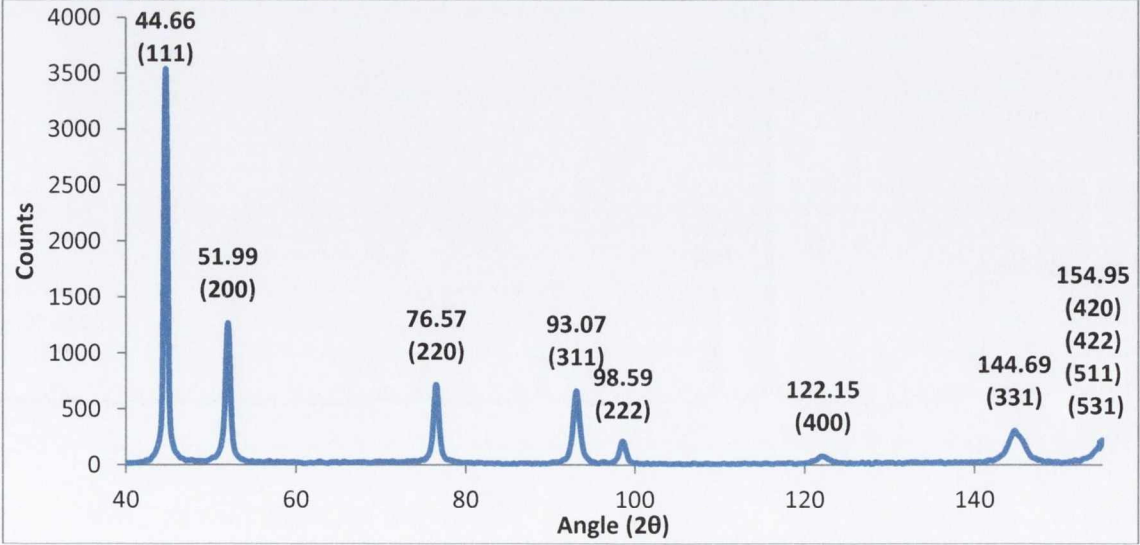


Ni@GNP-COOH Size = 156.04 ± 47.82 nm (45)



A.3.3.2 XRD

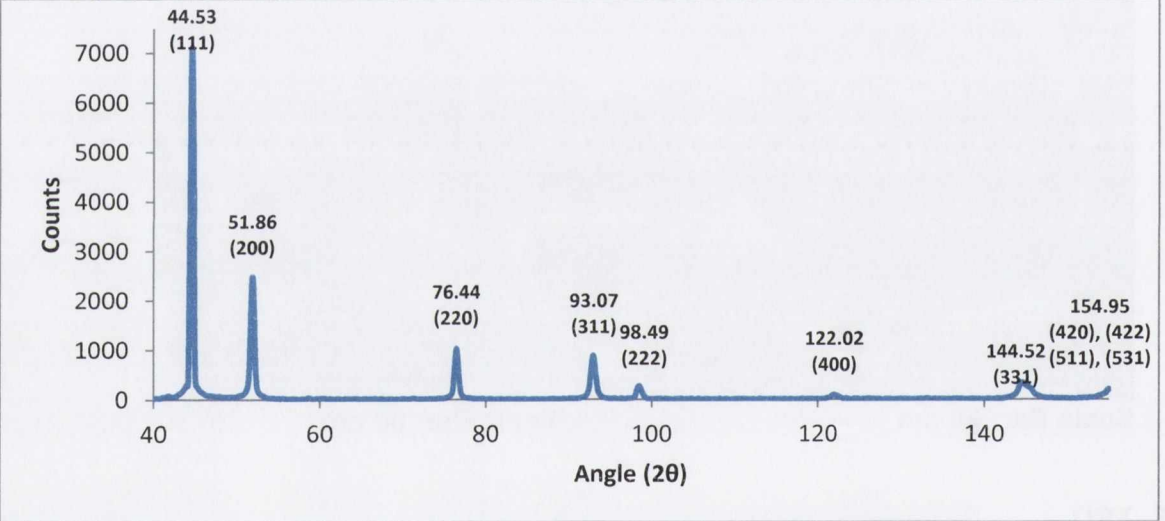
Ni fcc (38)



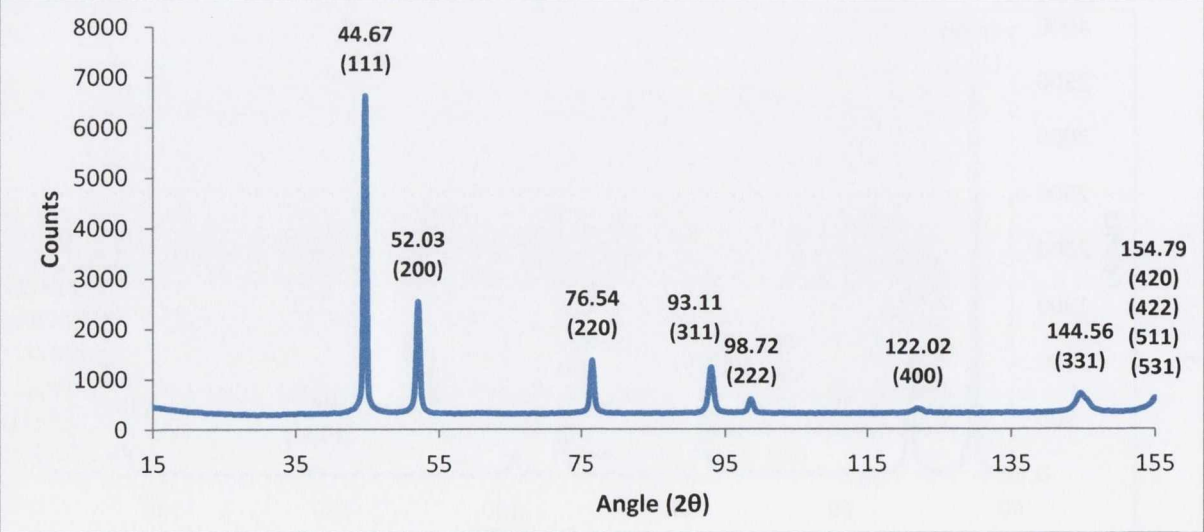
Ni fcc (39)



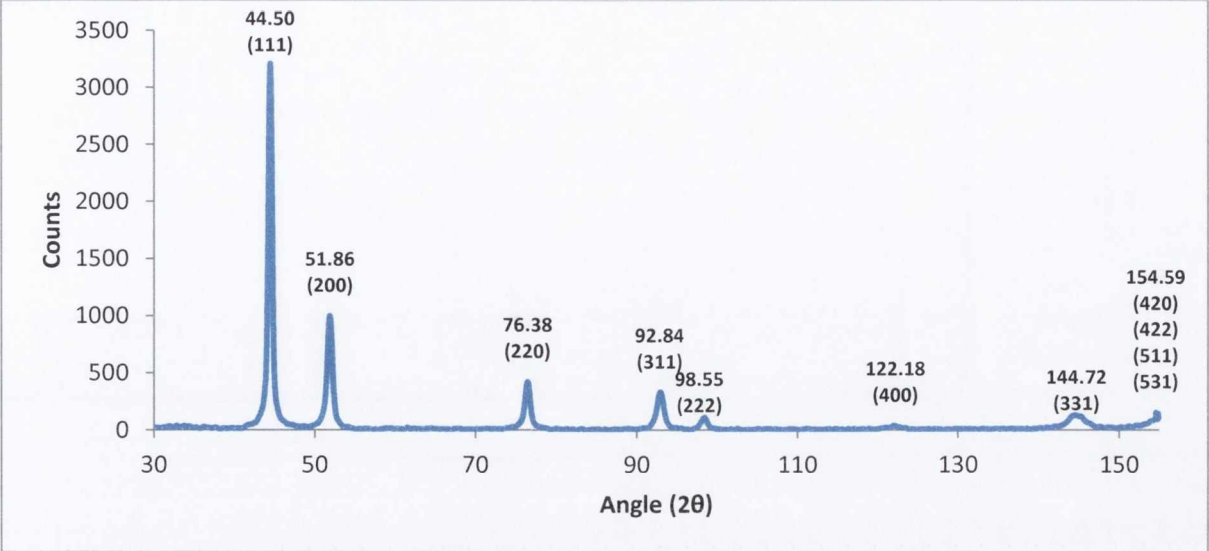
Ni fcc (40)



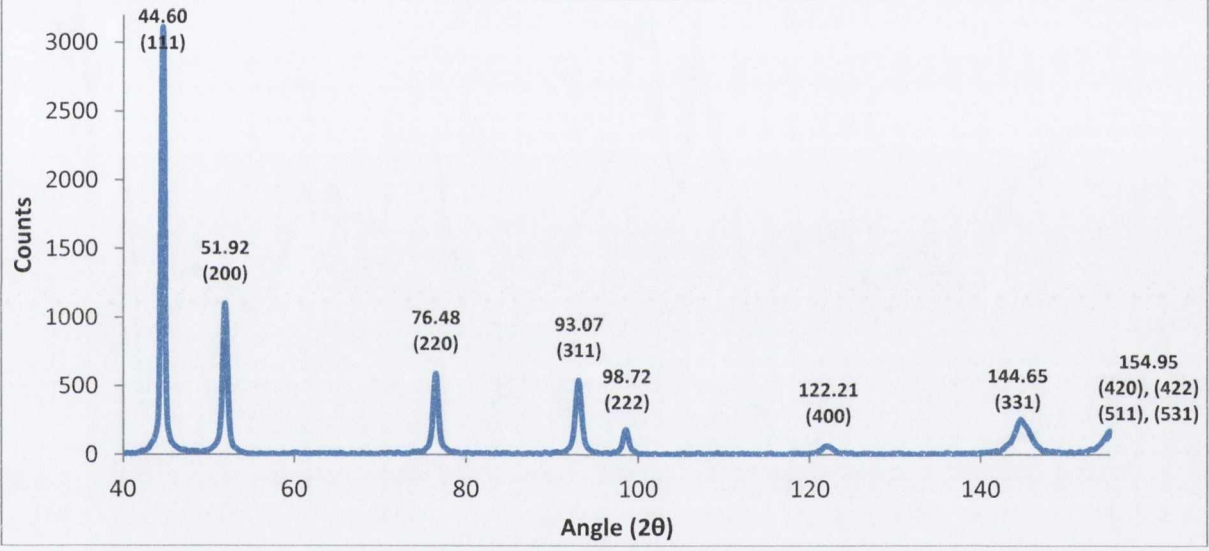
Ni fcc (41)



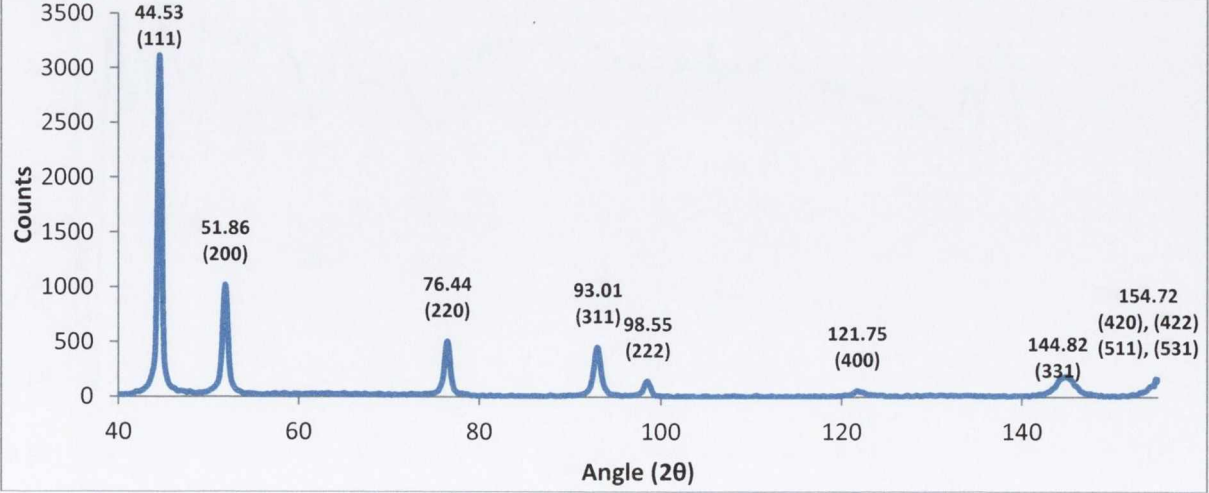
Ni fcc (42)



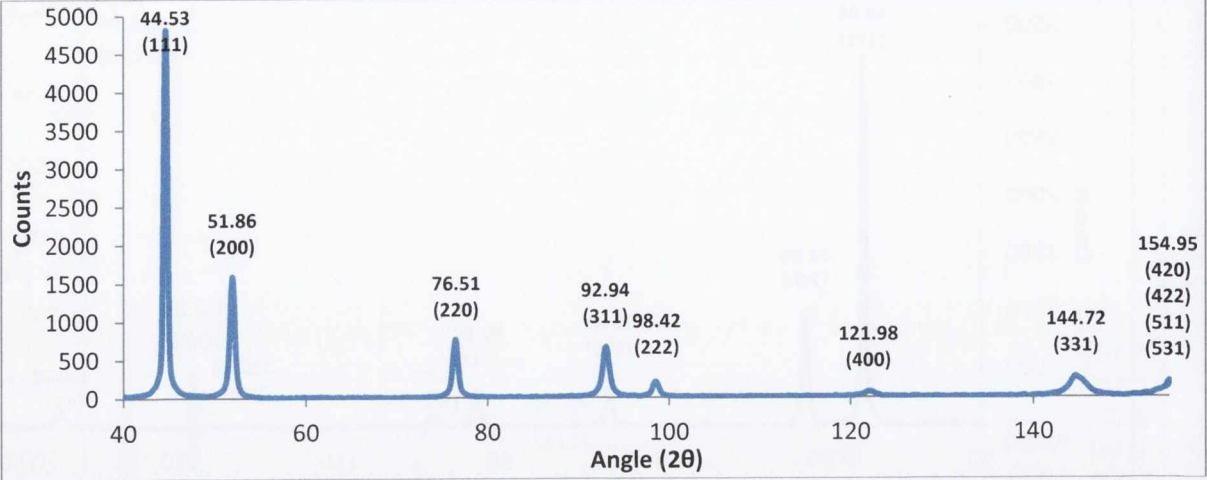
Ni fcc (43)



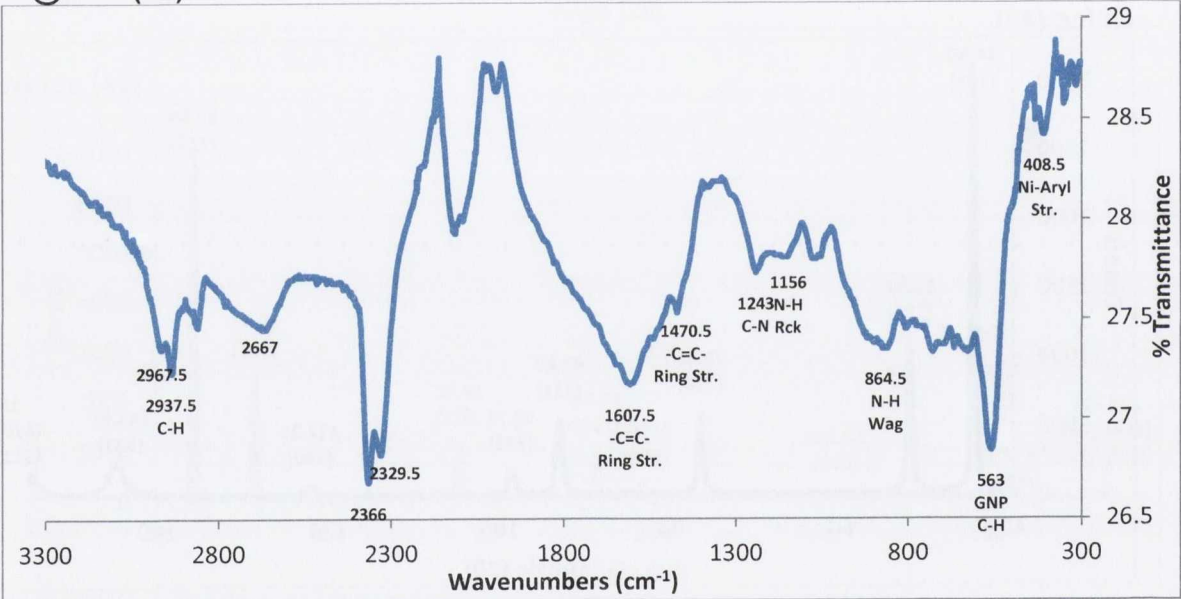
Ni fcc (44)



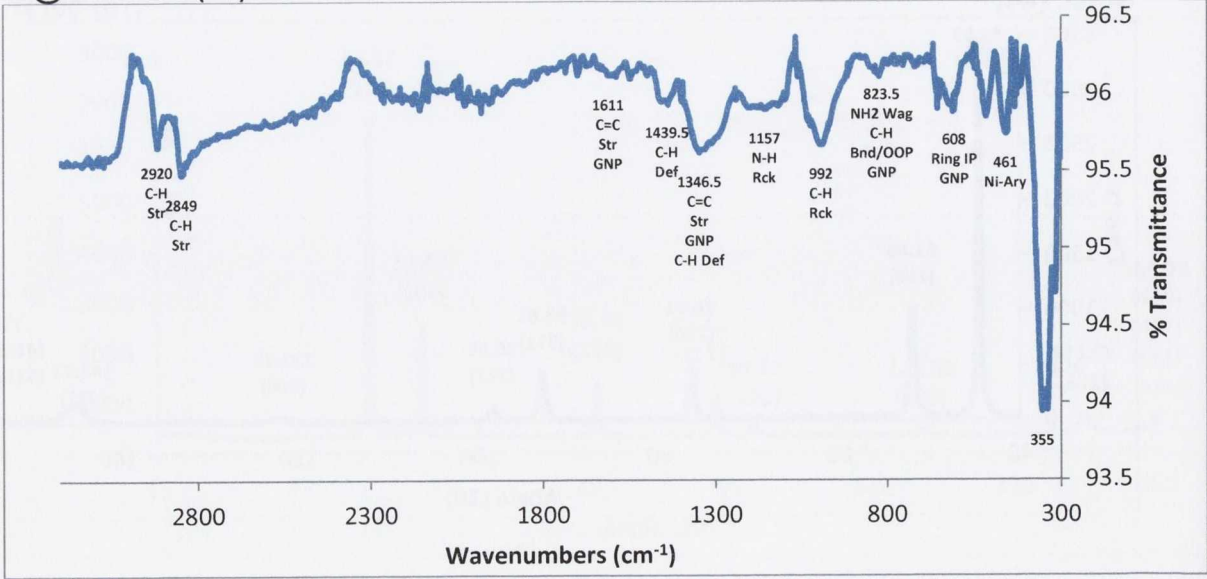
Ni fcc (45)



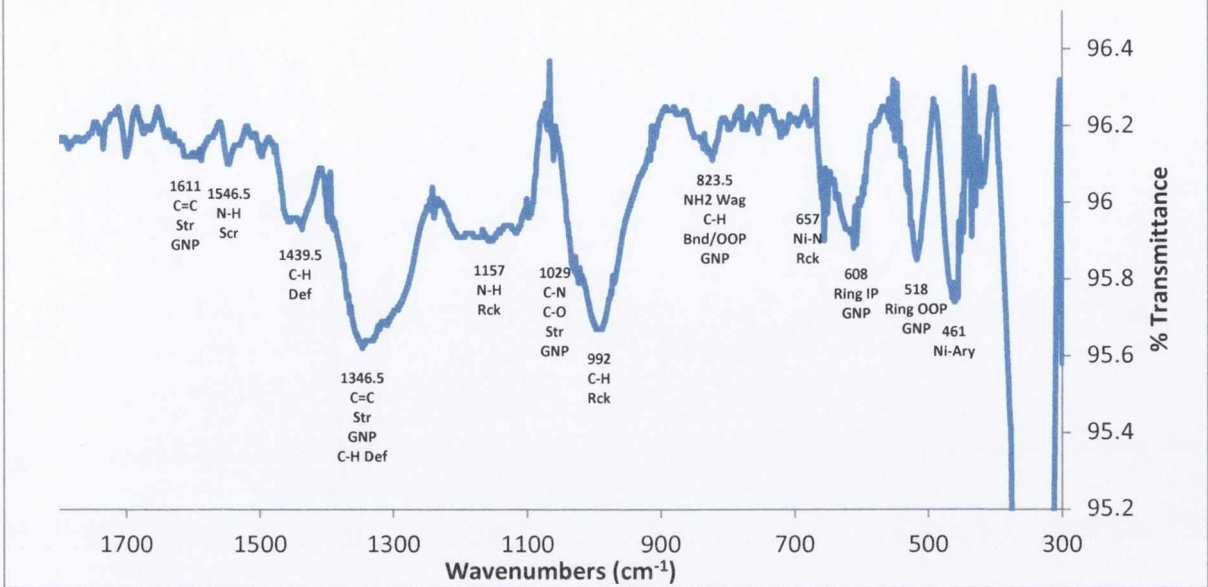
A.3.3.3 FTIR
Ni@GNP (38)



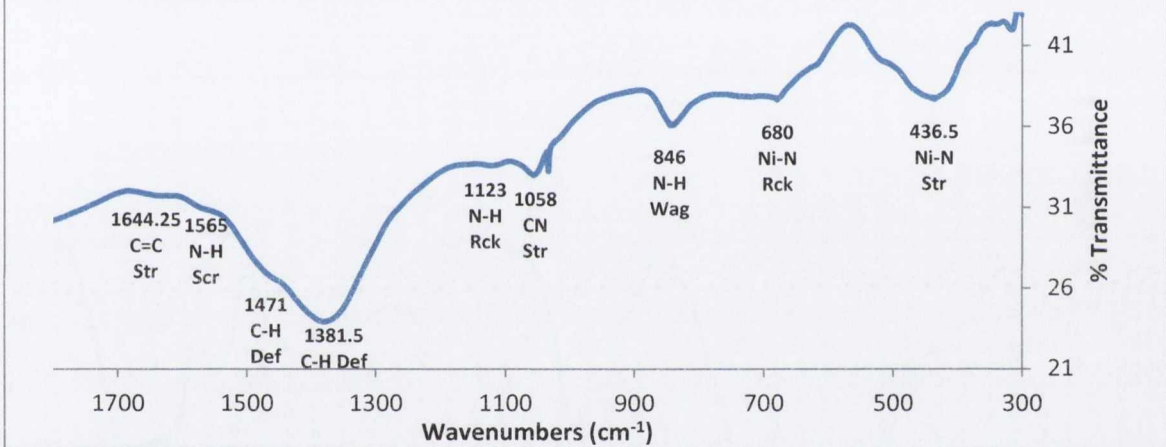
Ni@GNP-NH₂ (39)



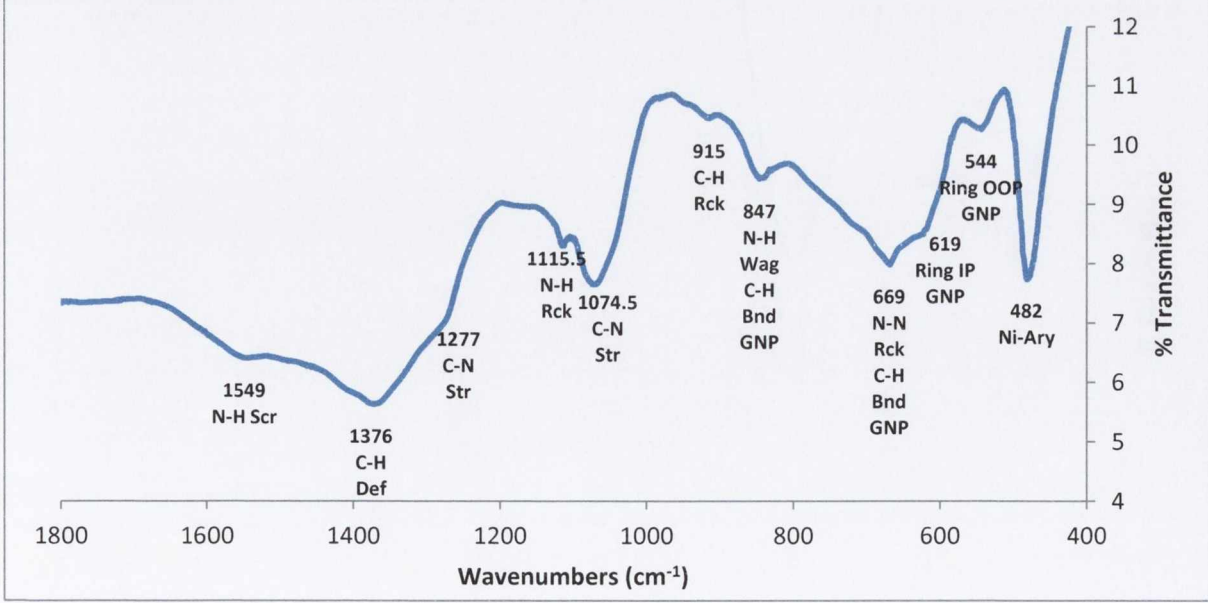
Ni@GNP-NH₂ (39)



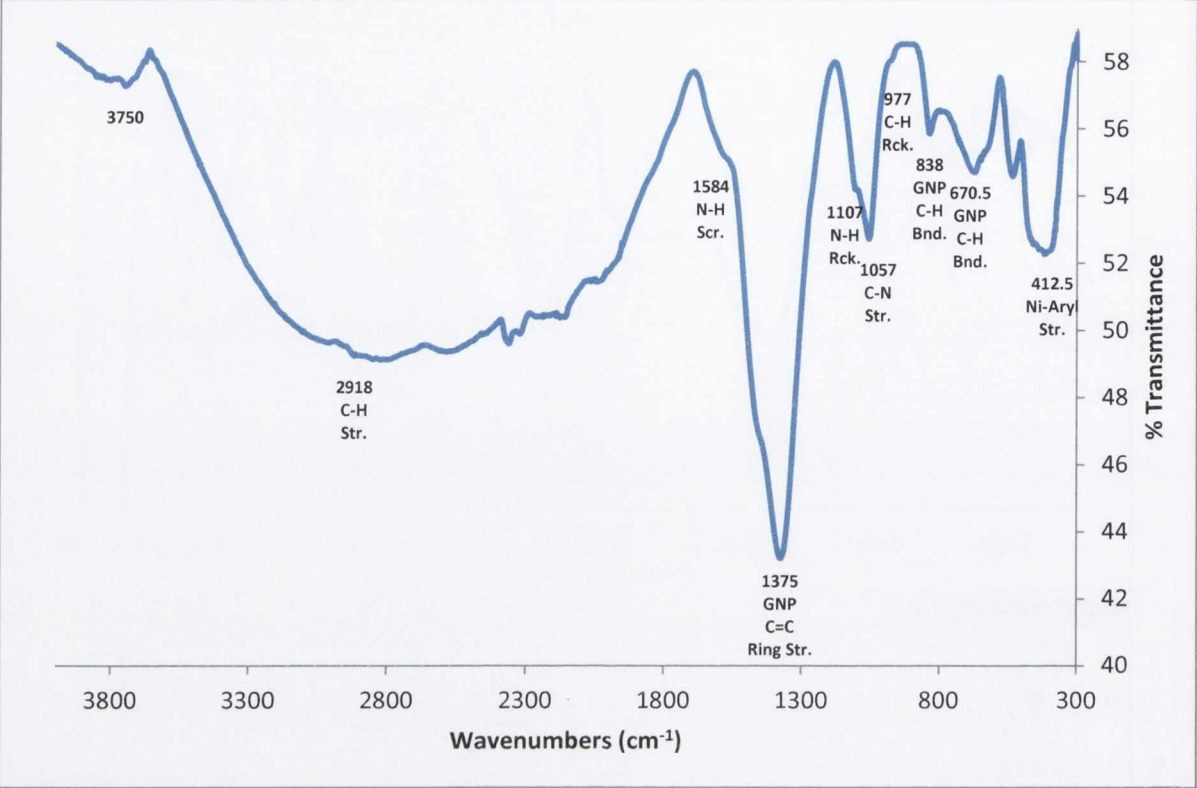
Ni@GNP-NH₂ (40)



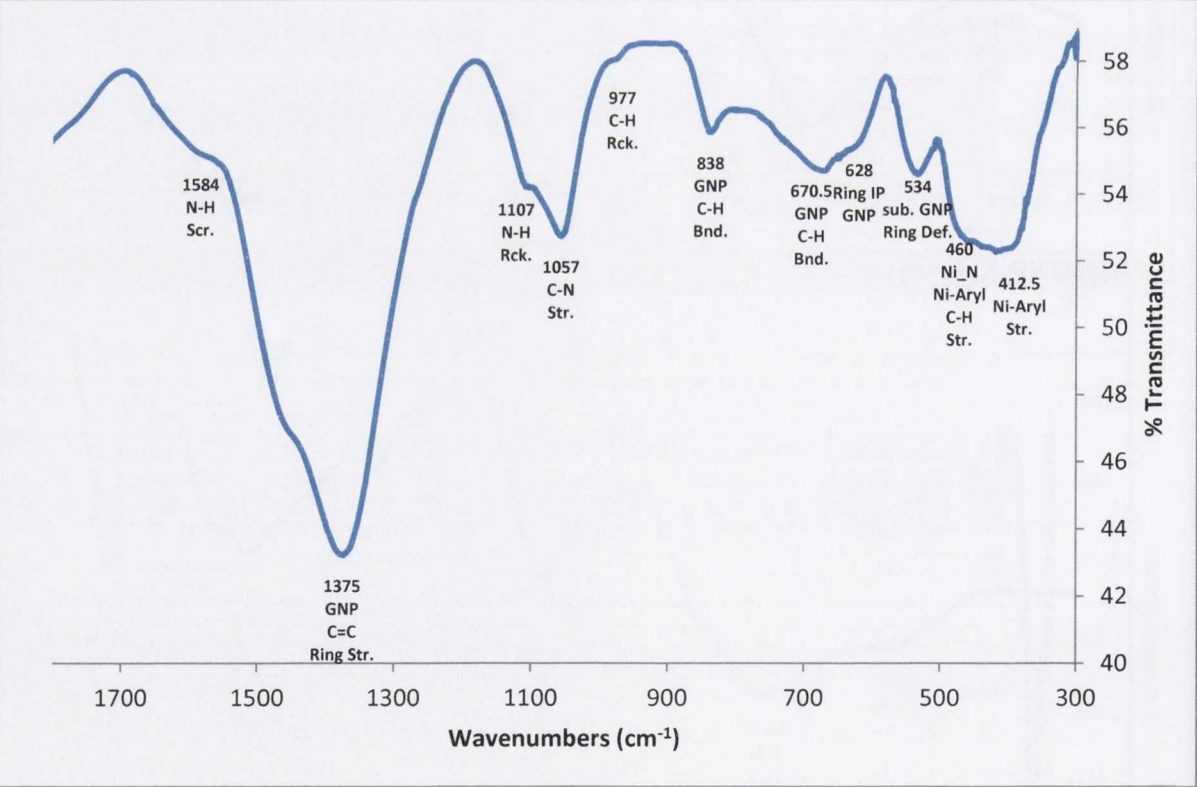
Ni@GNP-NH₂ (41)



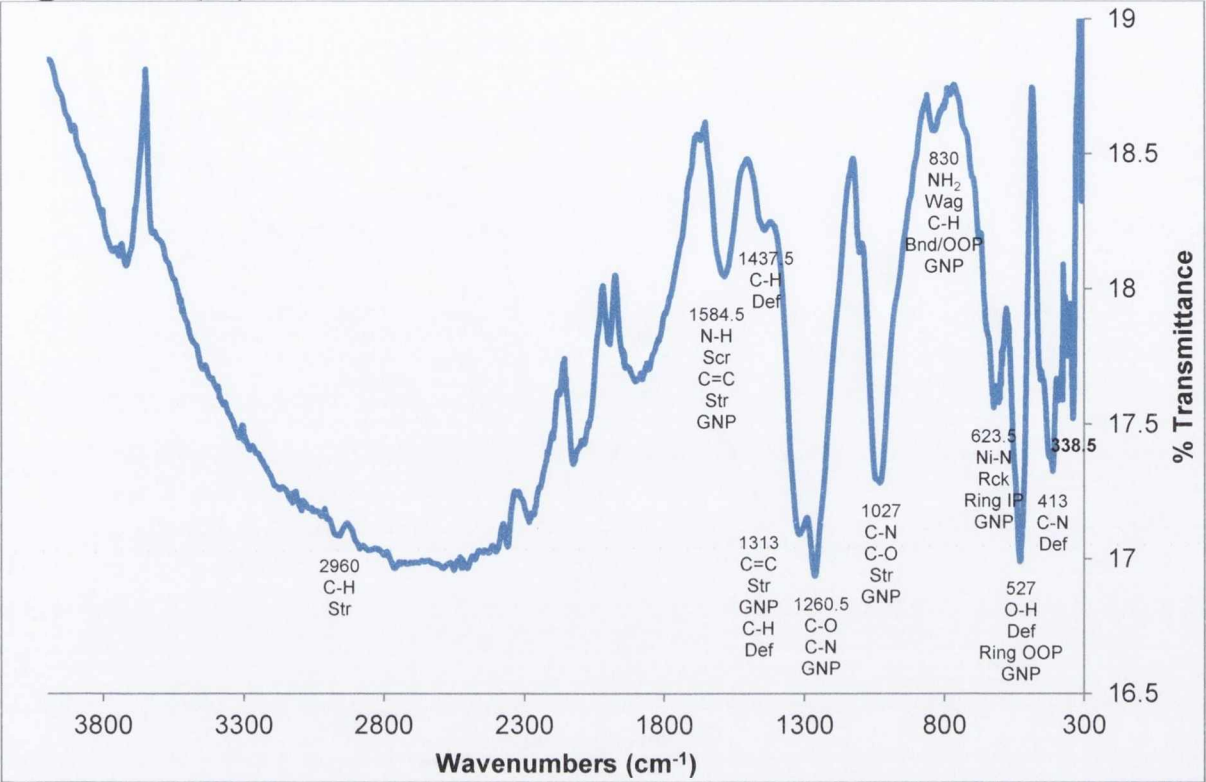
Ni@GNP-NH₂ (42)



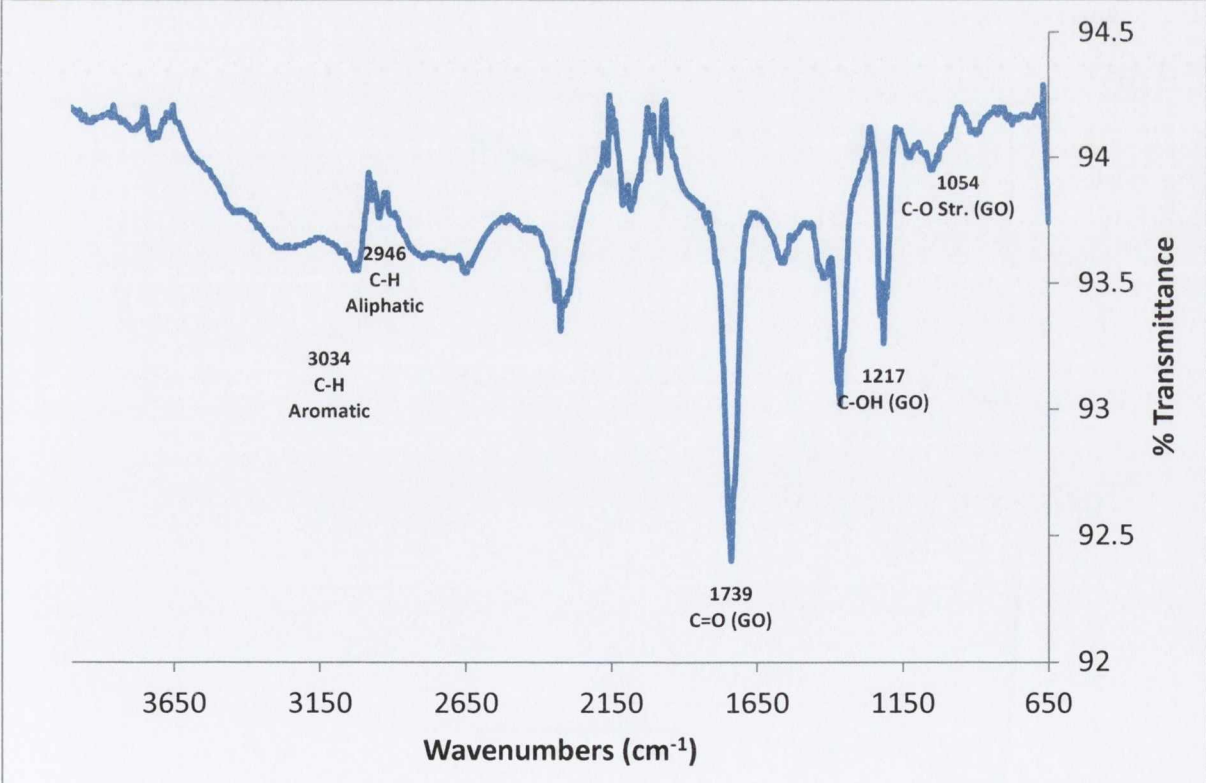
Ni@GNP-NH₂ (42)



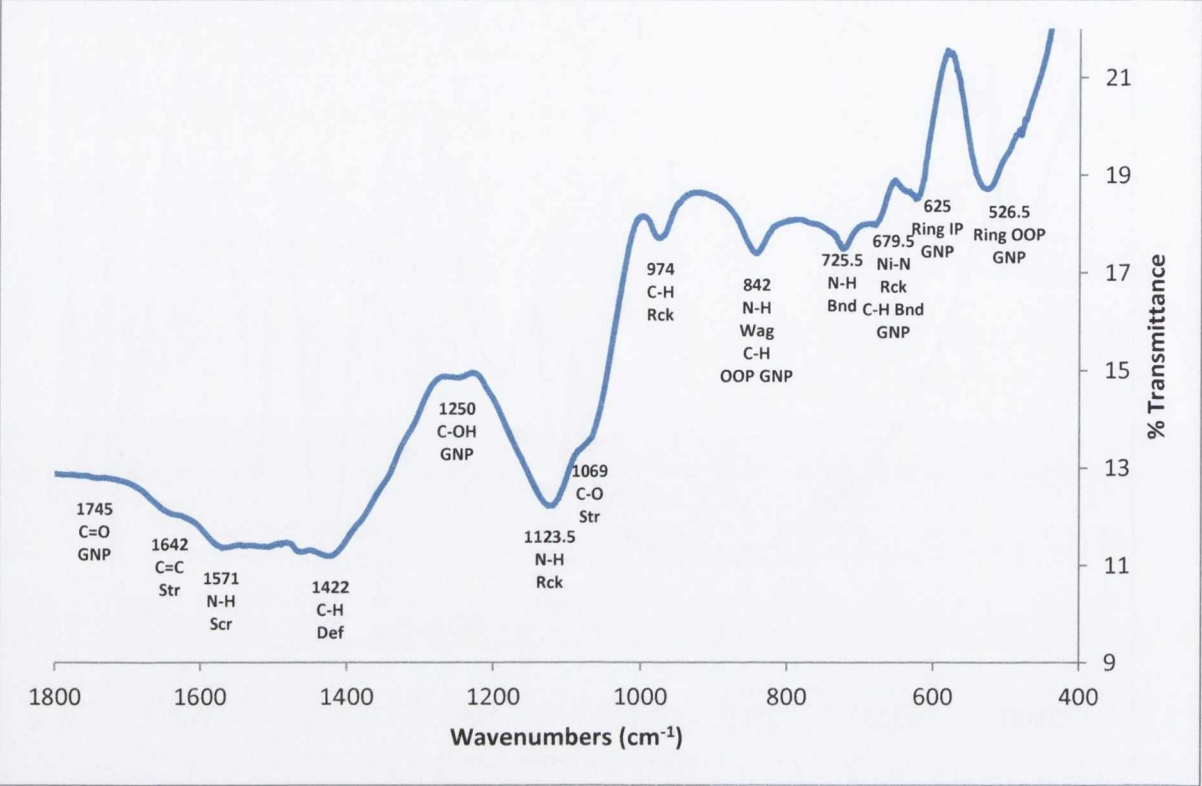
Ni@GNP-NH₂ (43)



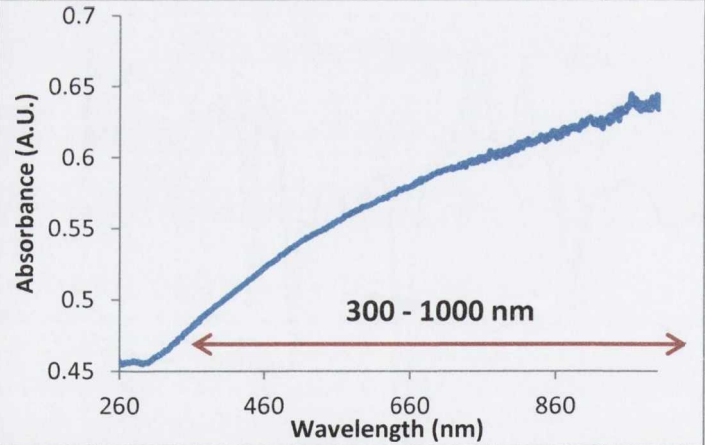
Ni@GNP-COOH (44)



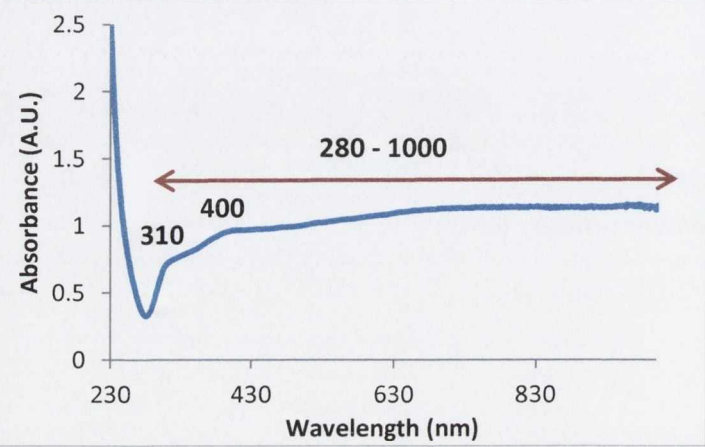
Ni@GNP-COOH (45)



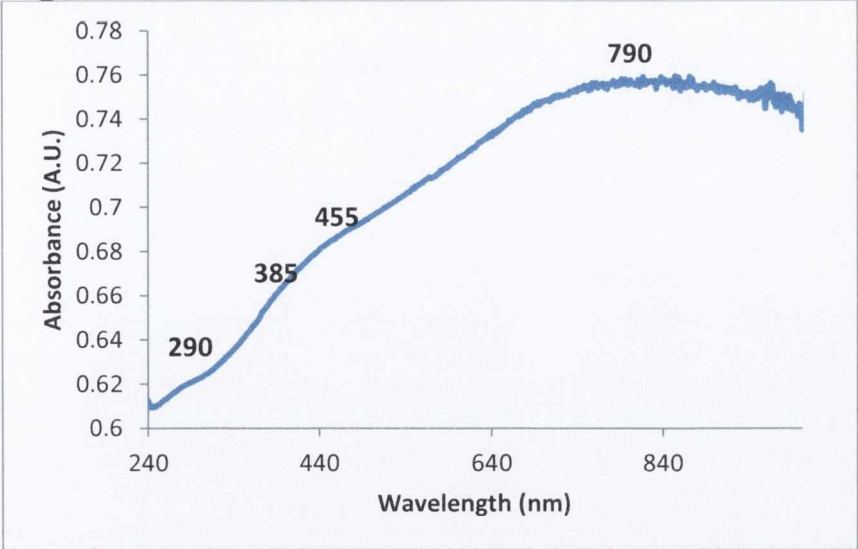
A.3.3.4 UV-vis
Ni@GNP in hexane (38)



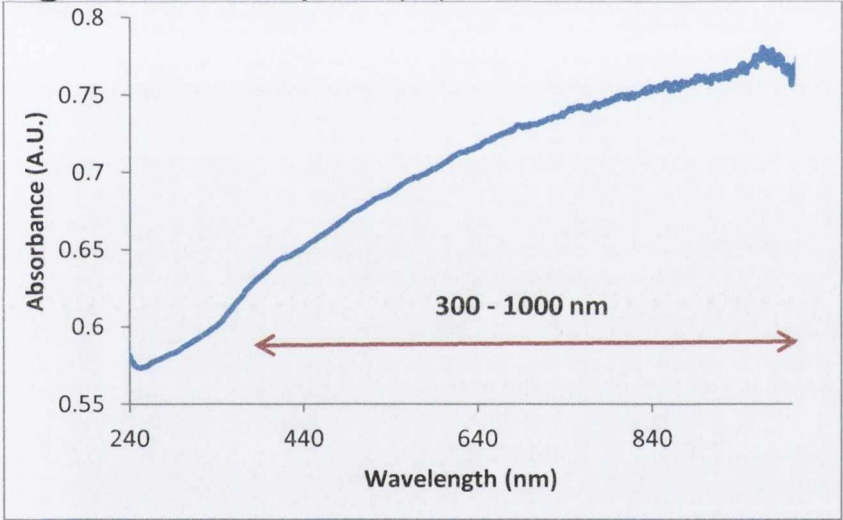
Ni@GNP-NH₂ in H₂O (39)



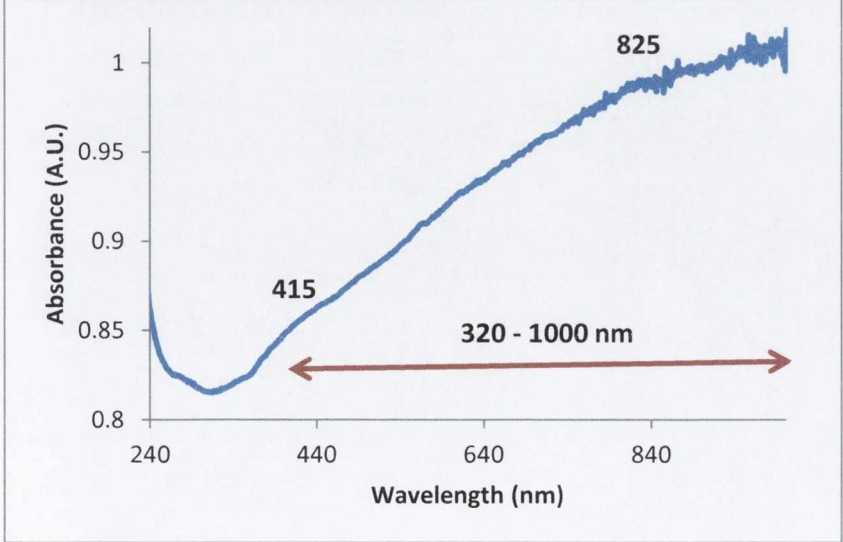
Ni@GNP-NH₂ in CBS pH 6.5 (39)



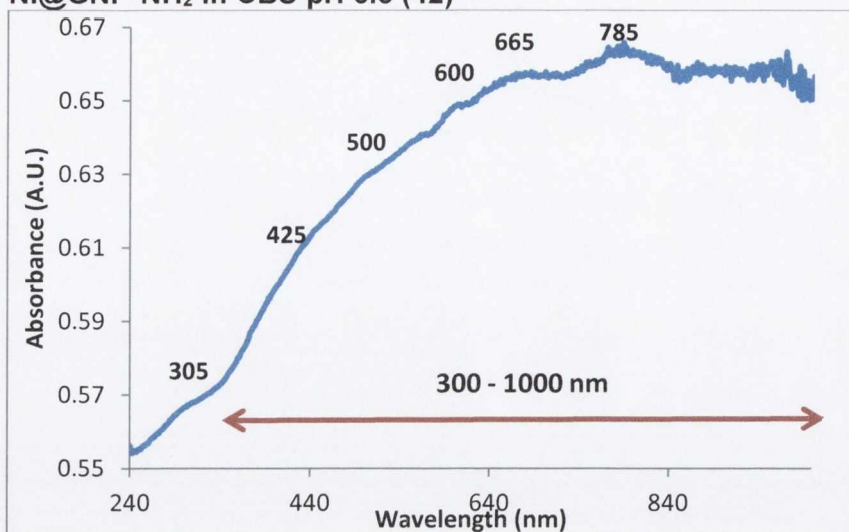
Ni@GNP-NH₂ in CBS pH 6.5 (40)



Ni@GNP-NH₂ in CBS pH6.5 (41)



Ni@GNP-NH₂ in CBS pH 6.5 (42)

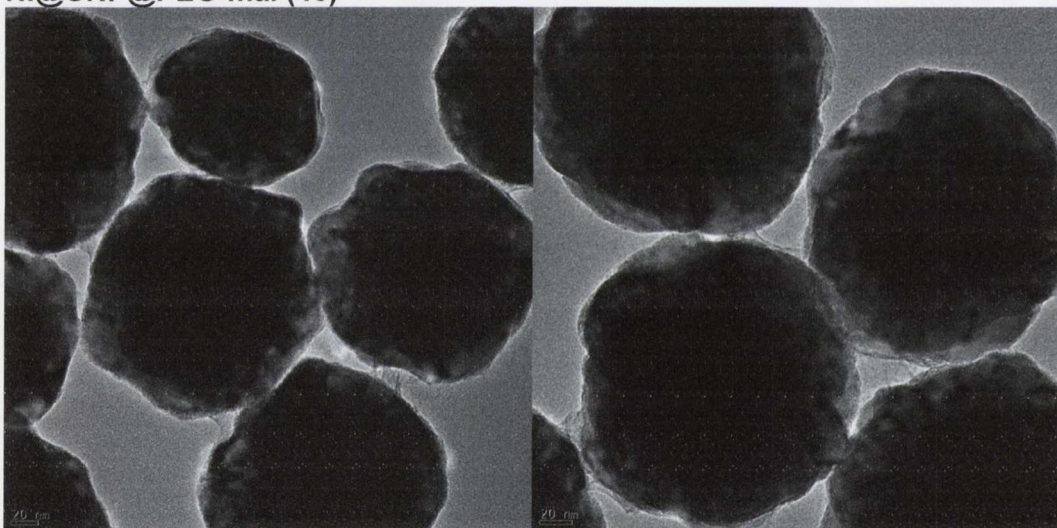


APPENDIX 3.4

BIOCONJUGATION OF GRAPHENE-COATED NICKEL PARTICLES

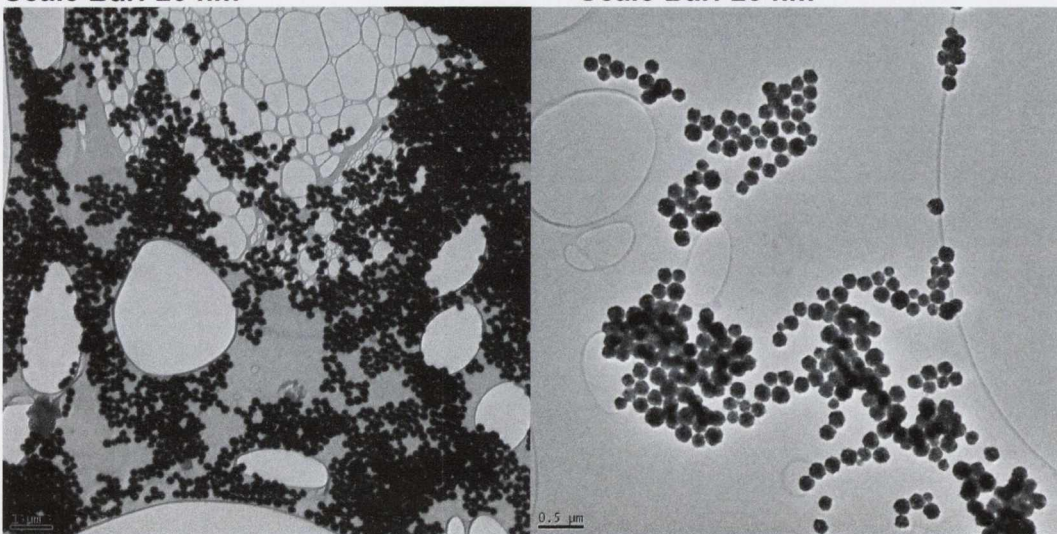
A.3.4.1 TEM

Ni@GNP@PEG-mal (46)



Scale Bar: 20 nm

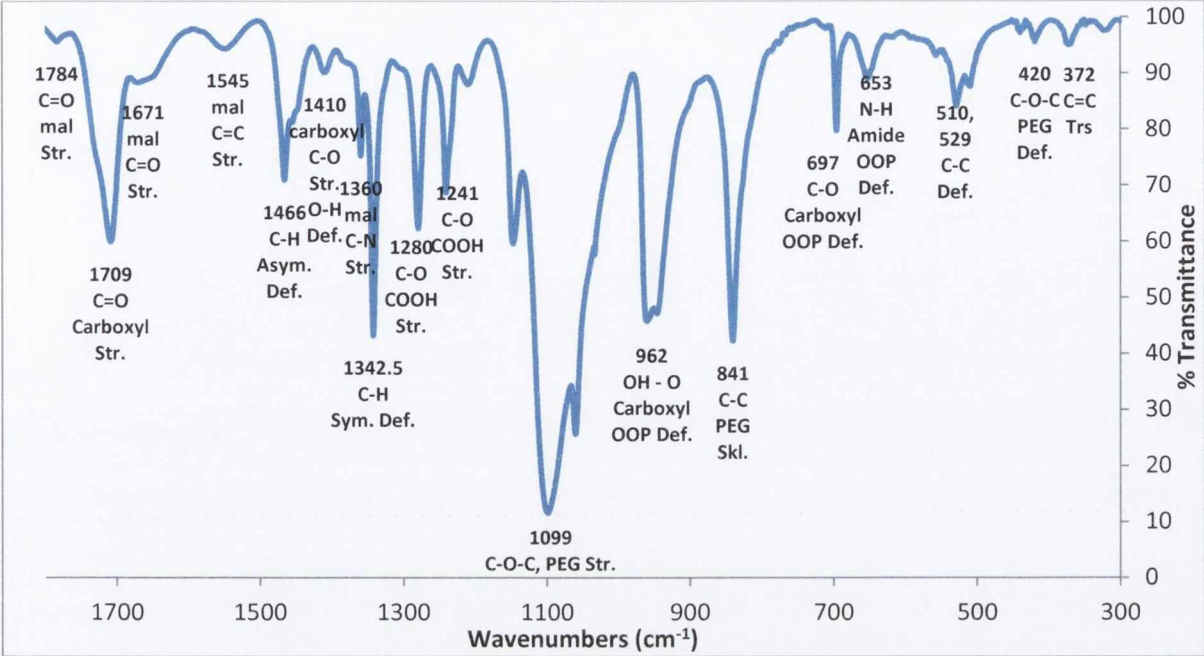
Scale Bar: 20 nm



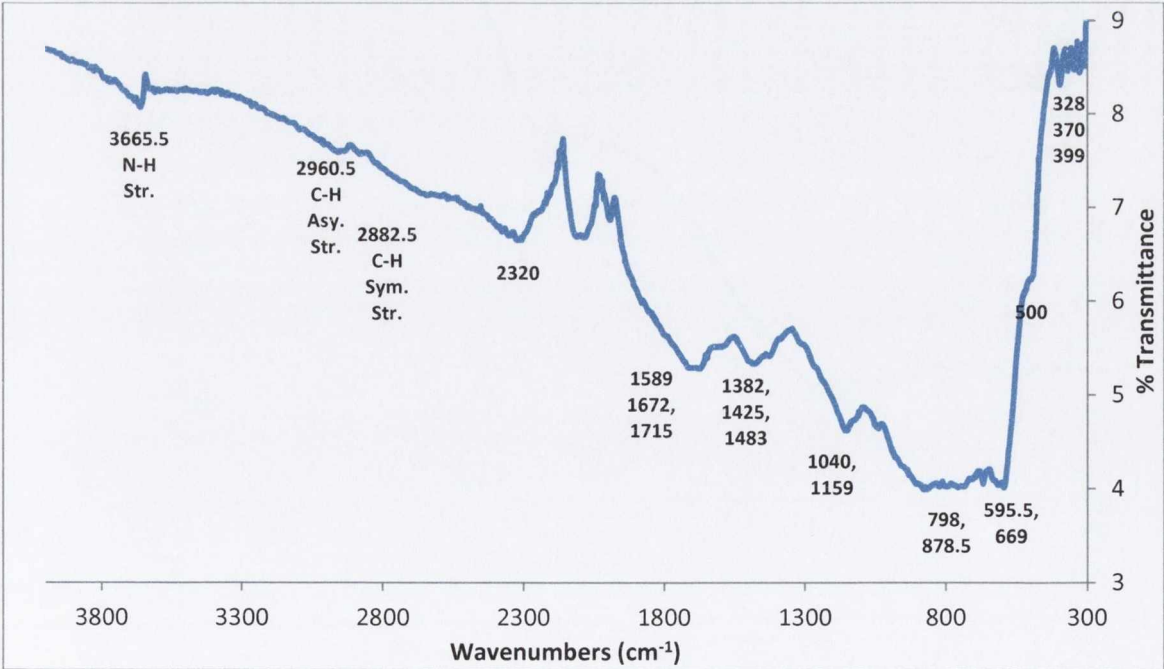
Scale Bar: 1 μm

Scale Bar: 500 nm

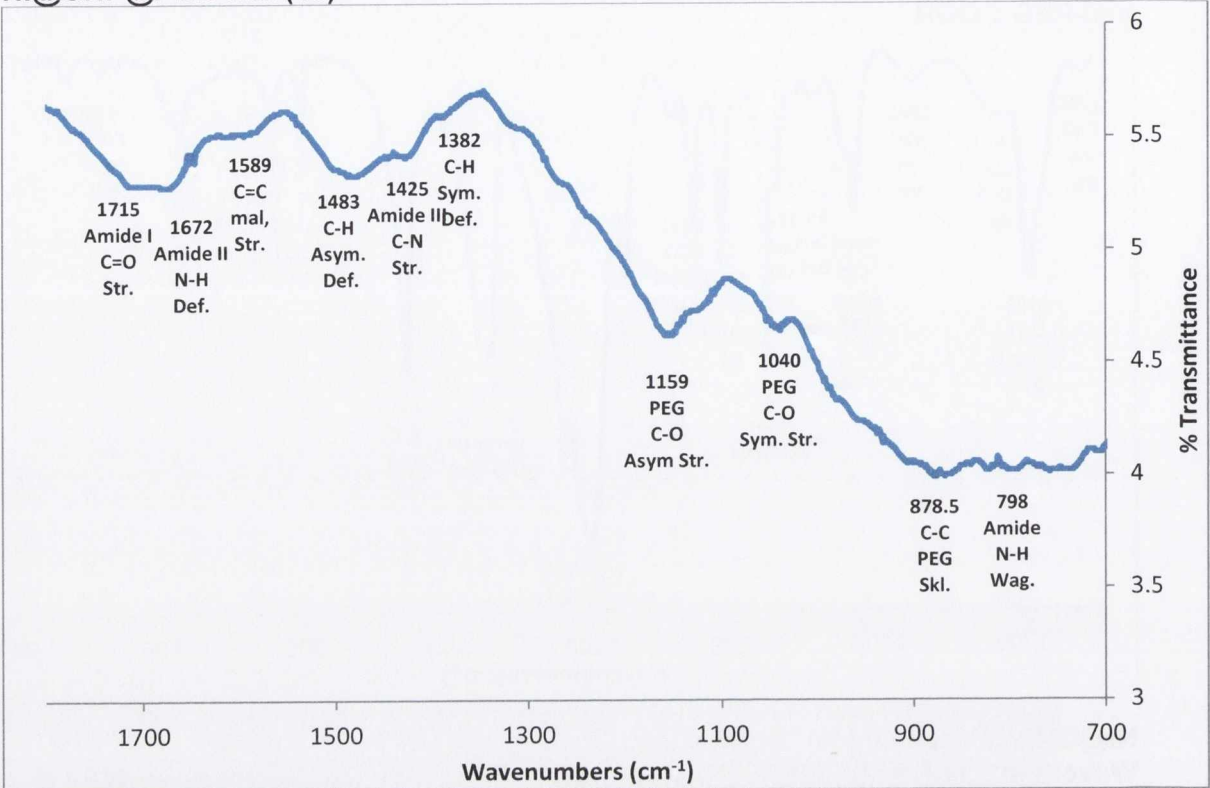
A.3.4.2 FTIR
mal-PEG-COOH



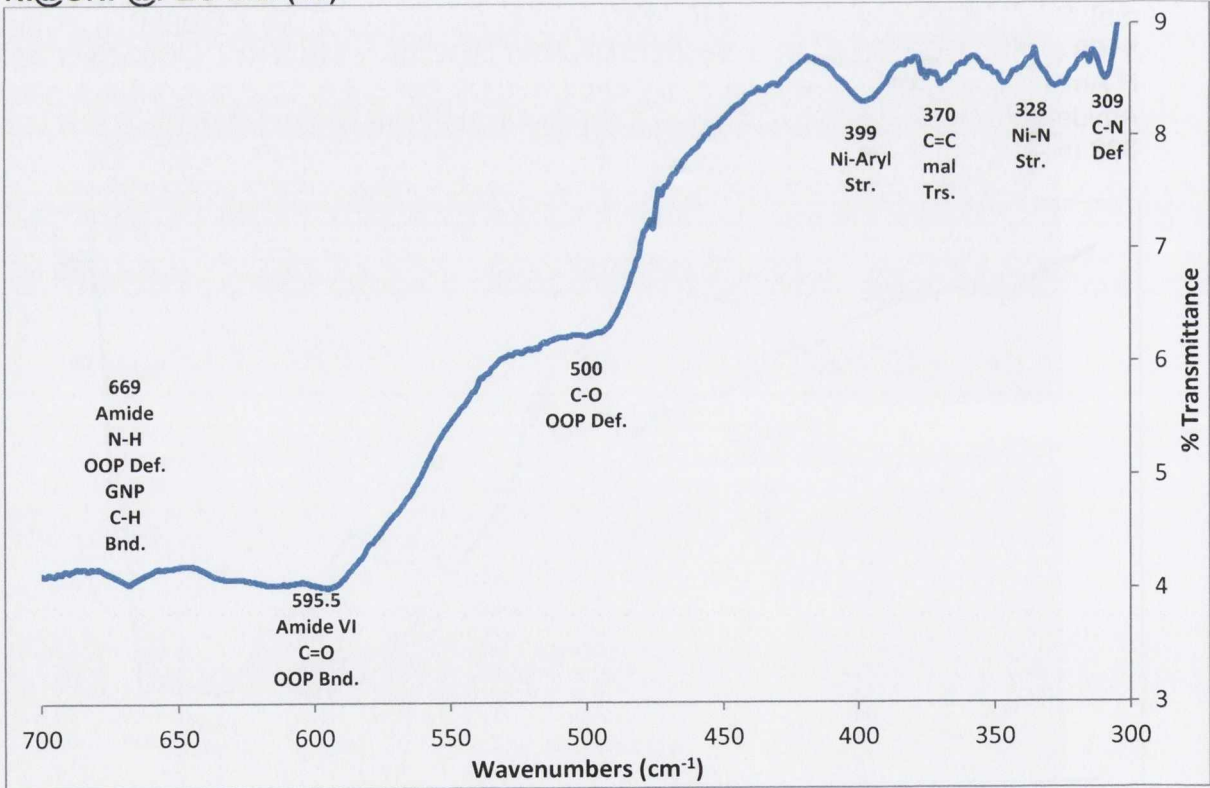
Ni@GNP@PEG-mal (46)
Wavenumbers (cm⁻¹): 3665.5 (N-H, Str.), 2960.5 (C-H aliphatic, Asym. Str.), 2882 (C-H aliphatic, Sym. Str.), 1715 (C=O Amide I, Str.), 1672 (N-H Amide II, mal, Def.), 1589 (C=C mal, Str.), 1482.5 (C-H, Asym. Def.), 1425.5 (C-N Amide III, mal, Str.) 1382 (C-H, Sym. Def.), 1159 (C-O-C PEG, Asym.Str.), 1040 (C-OC PEG, Sym Str.), 878.5 (C-C PEG, Skl.), 798 (N-H Amide, Wag.), 669 (Overlapping, N-H Amide, OOP Def. | C-H GNP, Bnd.) 595.5 (C=O Amide VI, OOP Bnd.), 500 (C-O PEG, OOP Def.), 399 (Ni-Aryl, Str.), 370 (C=C mal, Trs.), 328 (Ni-N, Str.)



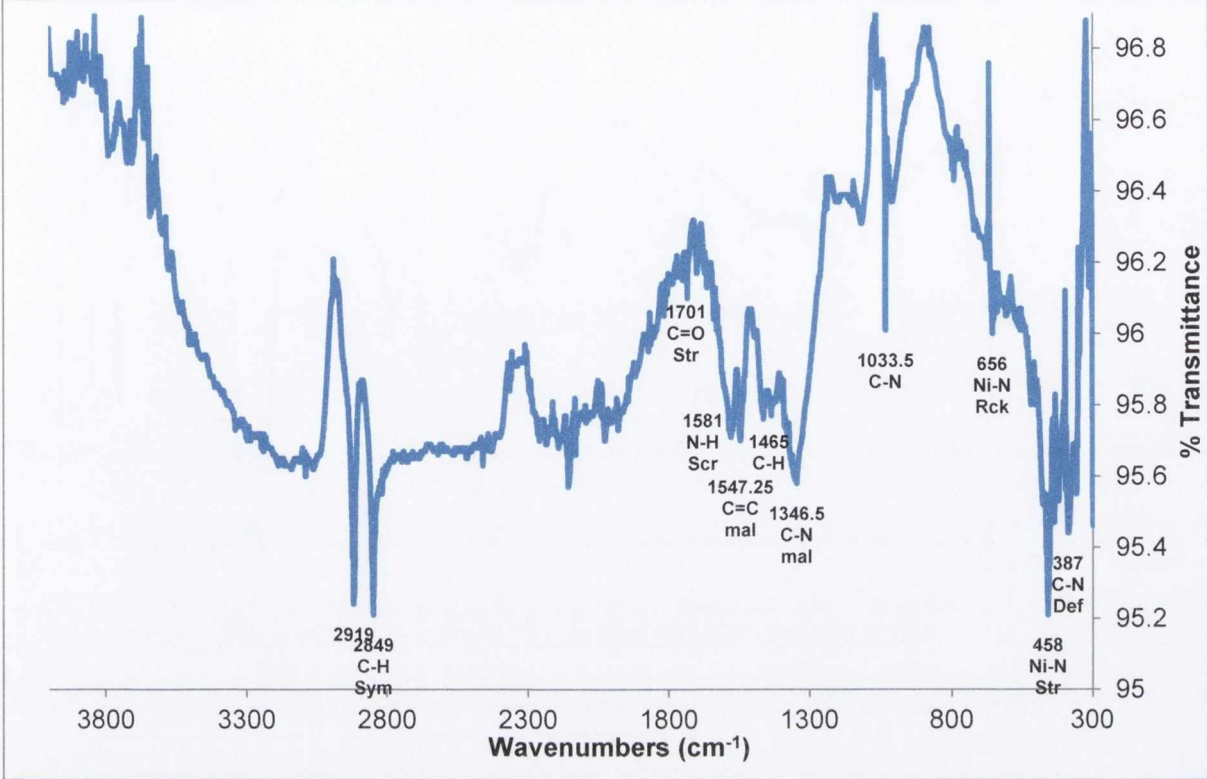
Ni@GNP@PEG-mal (46)



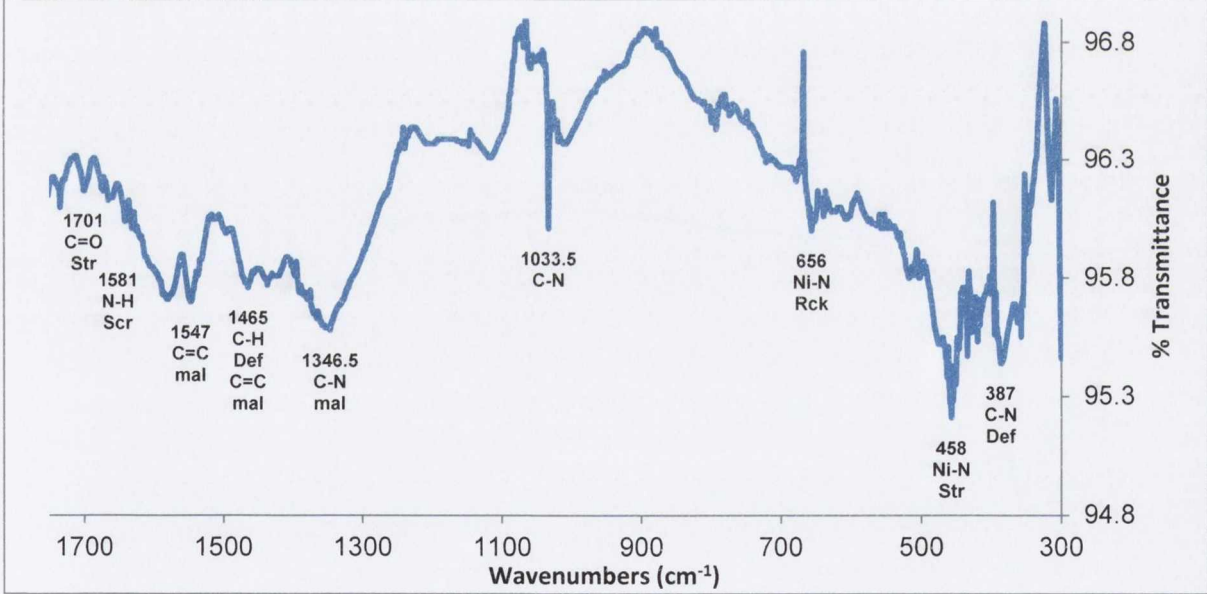
Ni@GNP@PEG-mal (46)



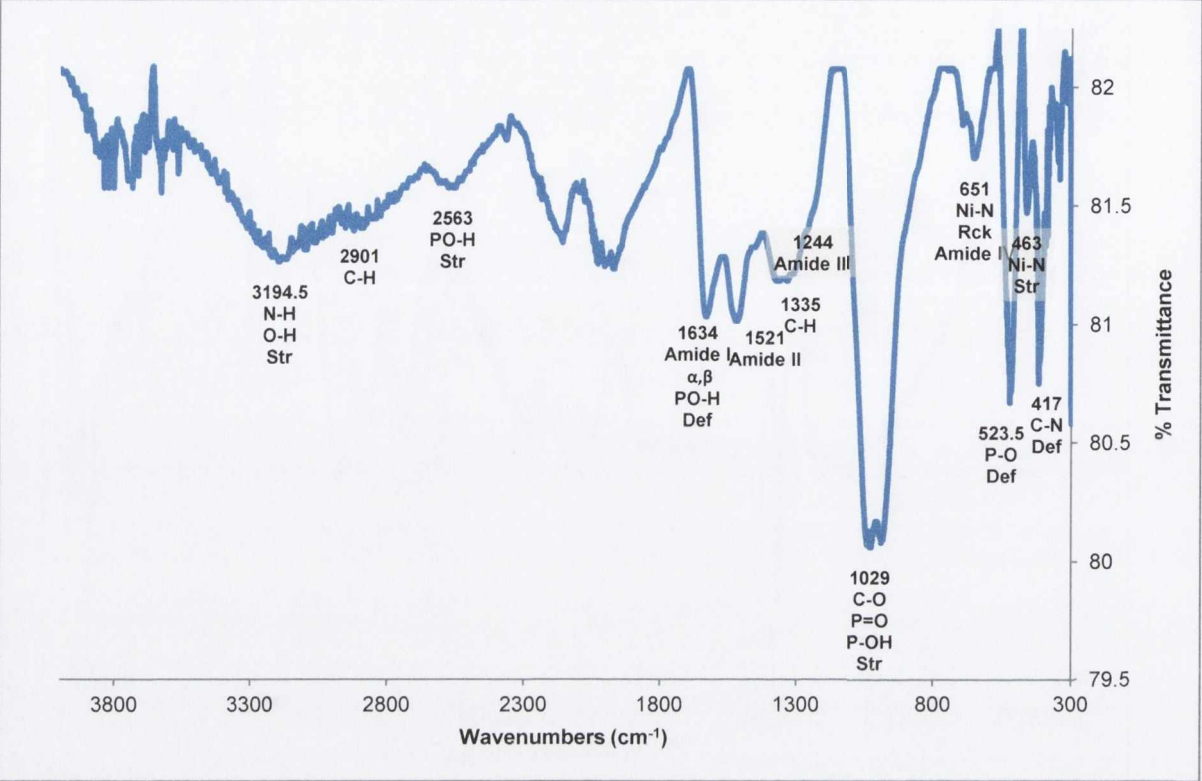
Ni@GNP@mal (47)



Ni@GNP@mal (47)

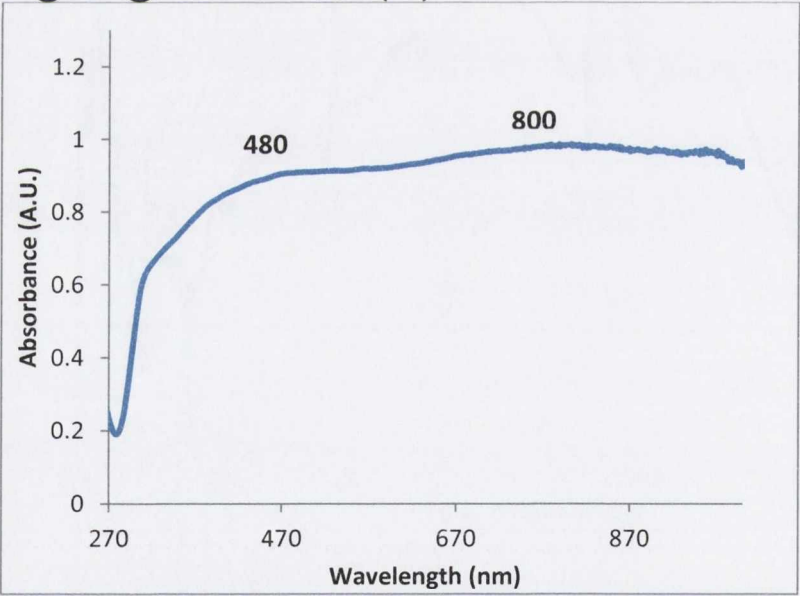


Ni@GNP@G' (48)



A.3.4.3 UV-vis

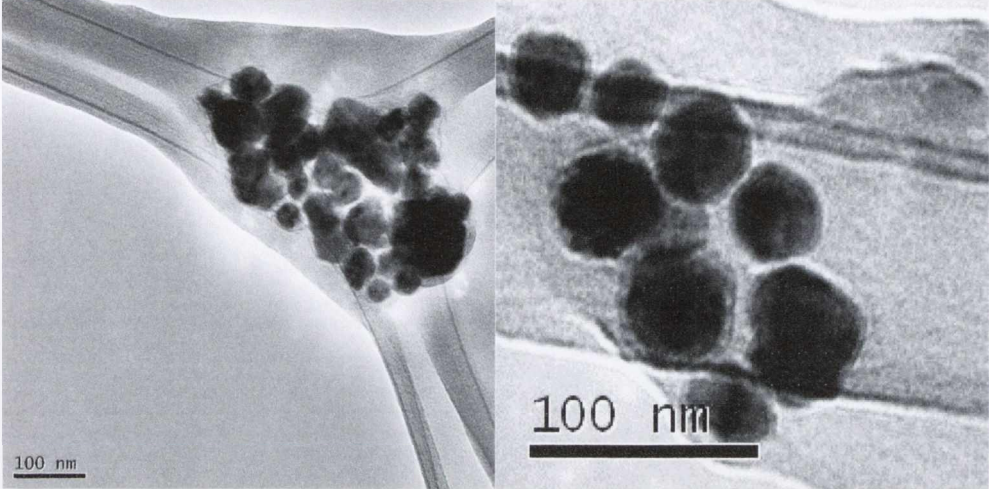
Ni@GNP@PEG-mal in H₂O (36)



A.3.5.1 - Cobalt Sub-Micron Particles

TEM

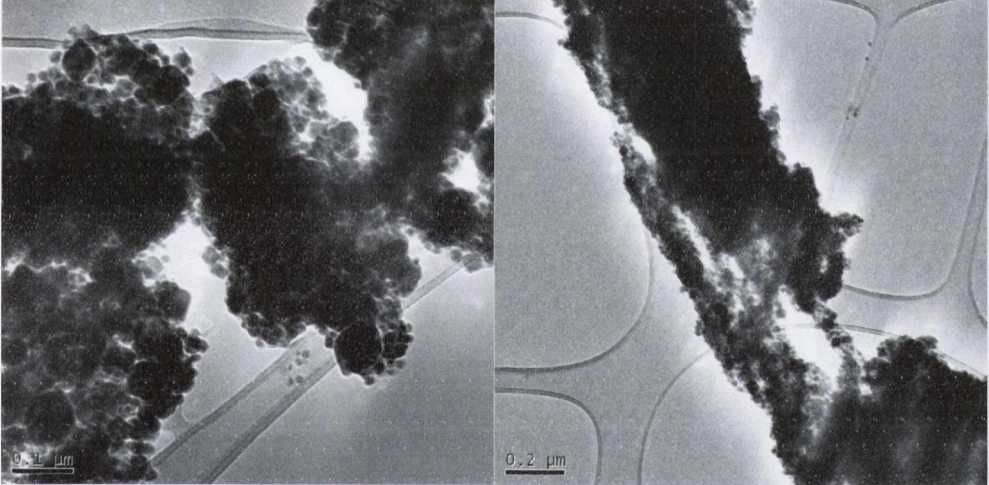
Cobalt Particles (49)



Scale Bar: 100 nm

Scale Bar: 100 nm

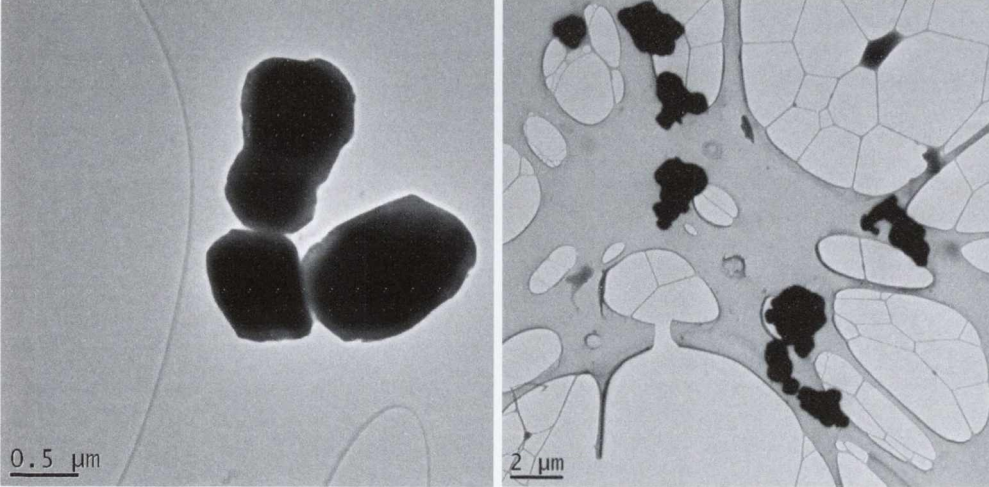
Cobalt Particles (50)



Scale Bar: 100 nm

Scale Bar: 200 nm

Cobalt Particles (51)

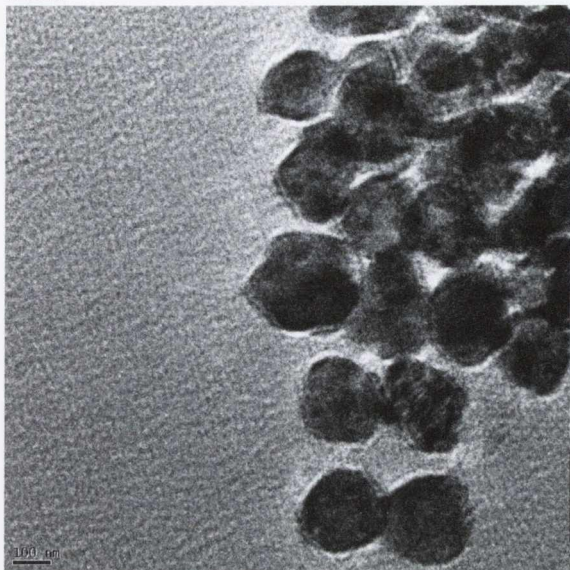


Scale Bar: 500 nm

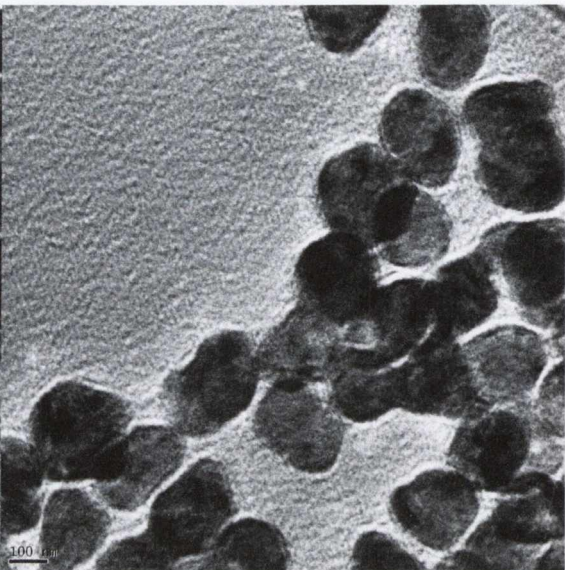
Scale Bar: 2 μm

A.3.5.2 Cobalt-Platinum Alloy Particles (52)

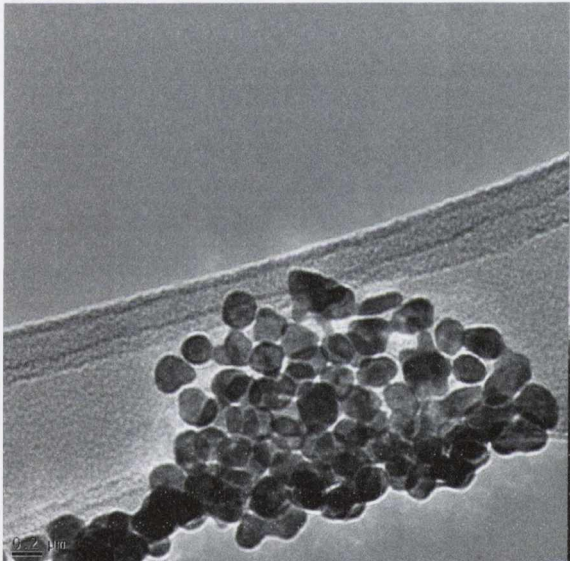
TEM



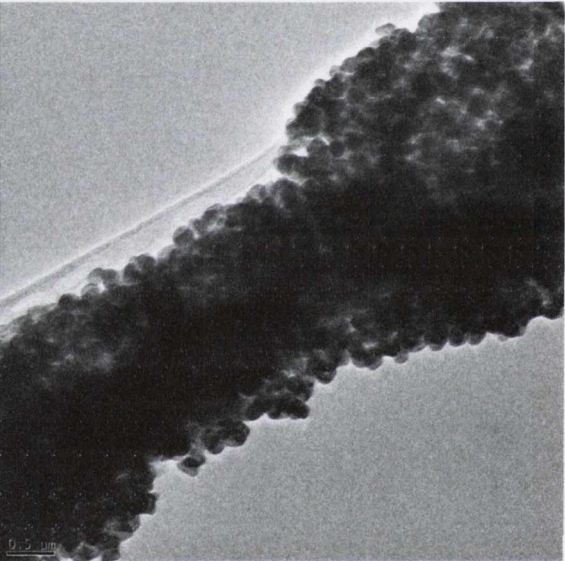
Scale Bar: 100 nm



Scale Bar: 100 nm

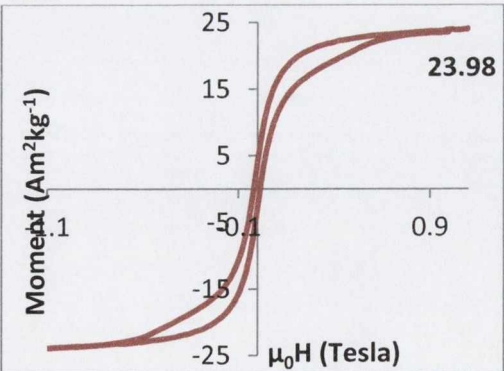


Scale Bar: 200 nm



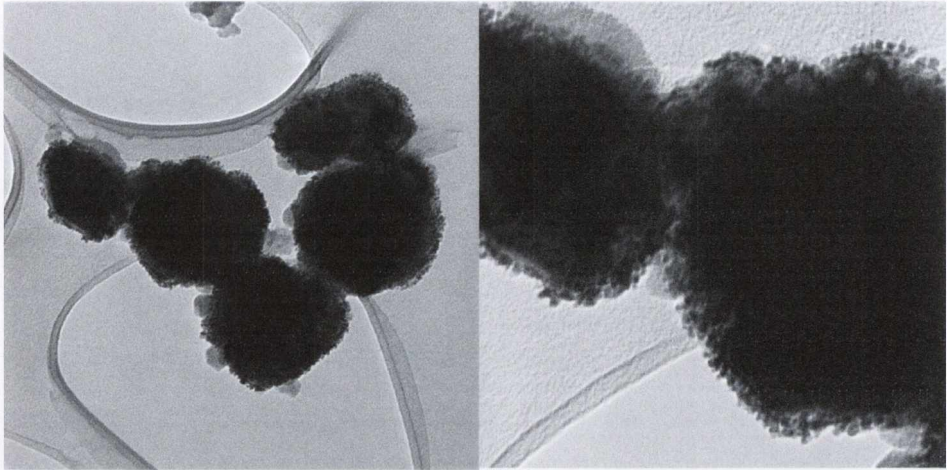
Scale Bar: 500 nm

VSM



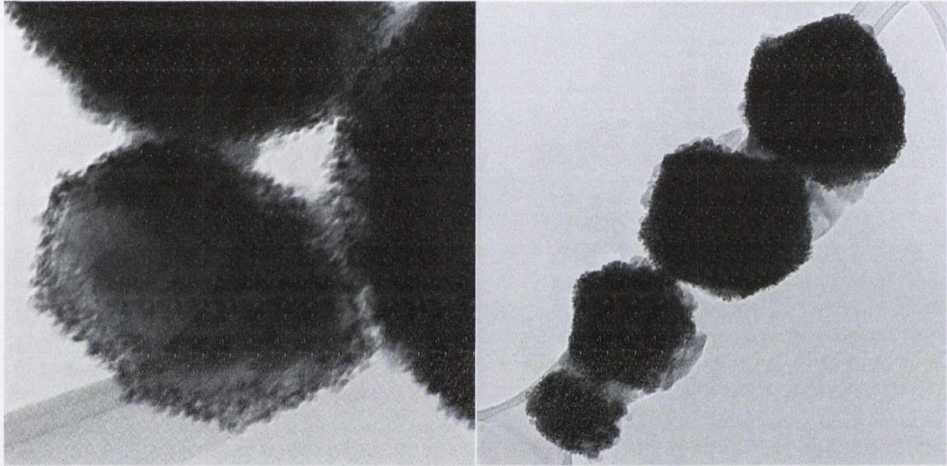
A.3.5.3 Platinum-Coated Cobalt Particles (53)

TEM



Scale Bar: 100 nm

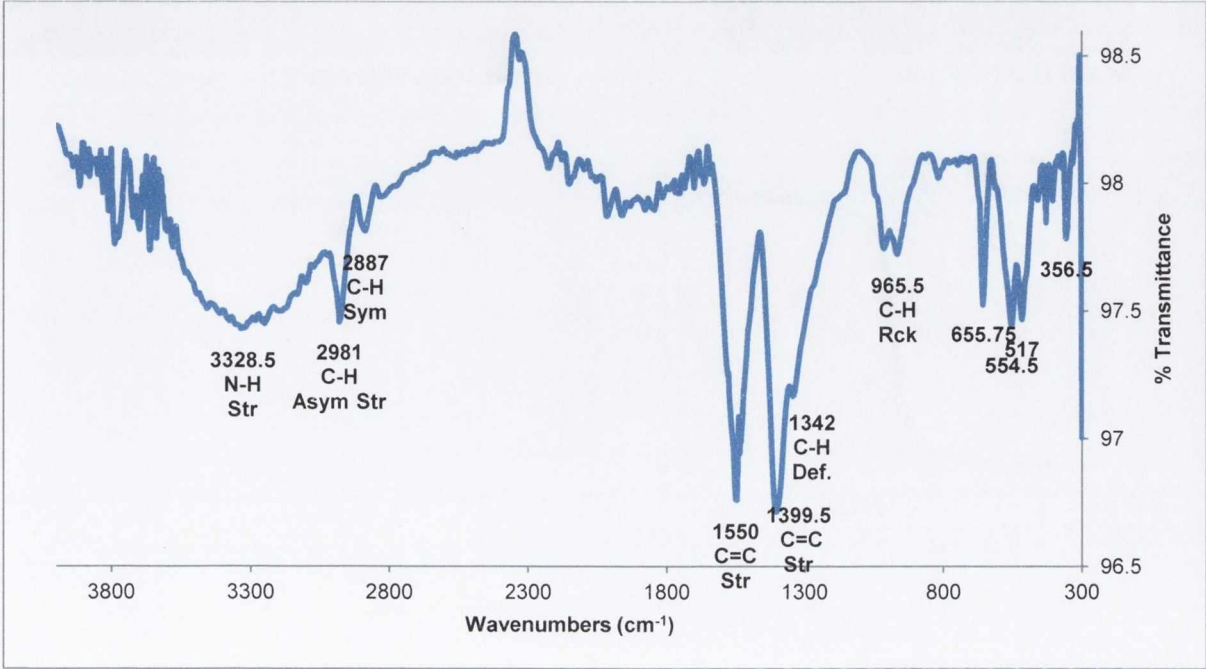
Scale Bar: 20 nm



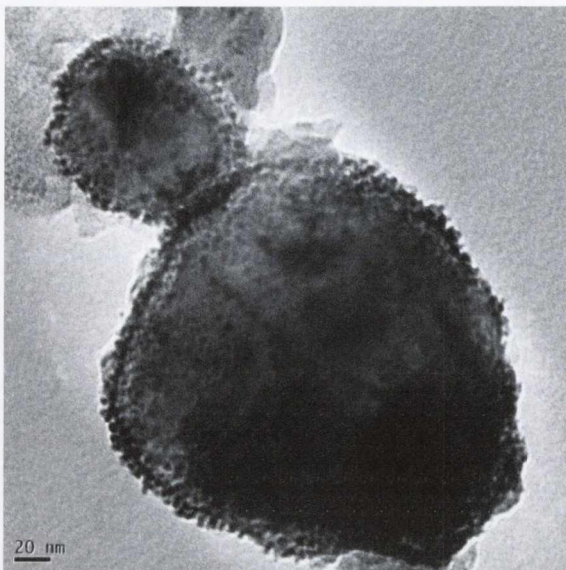
Scale Bar: 20 nm

Scale Bar: 100 nm

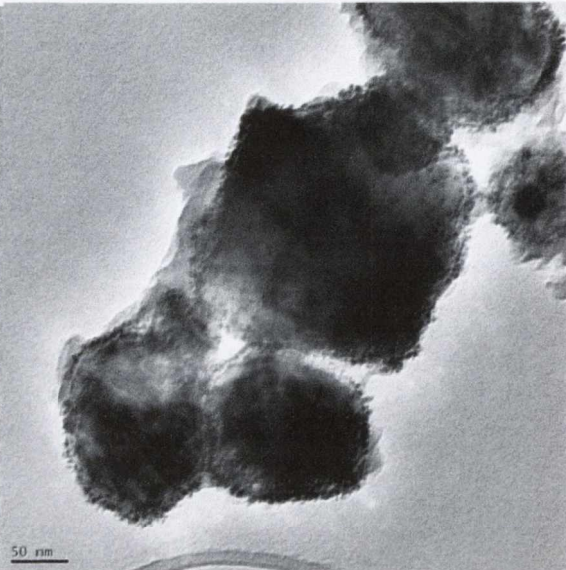
FTIR



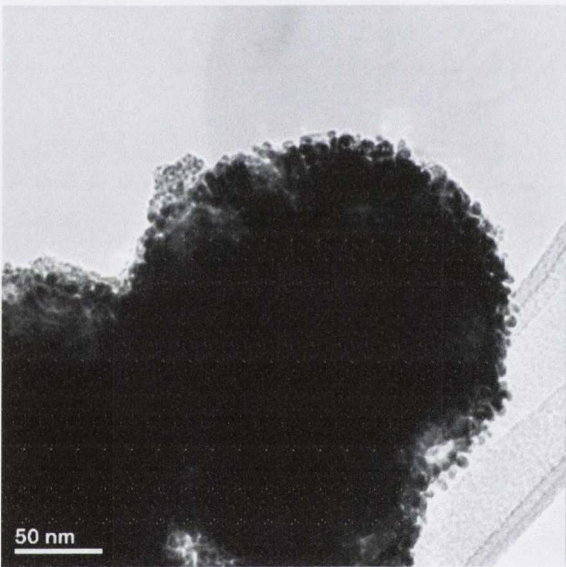
A.3.5.4 Platinum-Coated Nickel Particles (54)
TEM



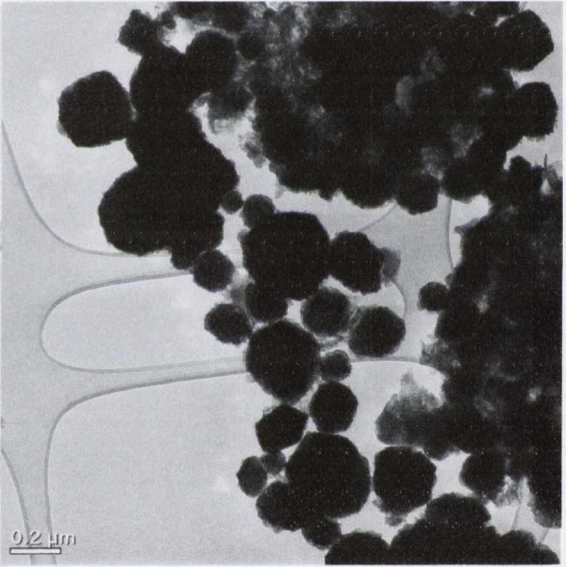
Scale Bar: 20 nm



Scale Bar: 50 nm

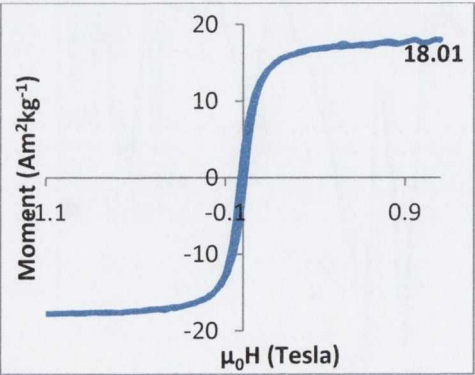


Scale Bar: 50 nm

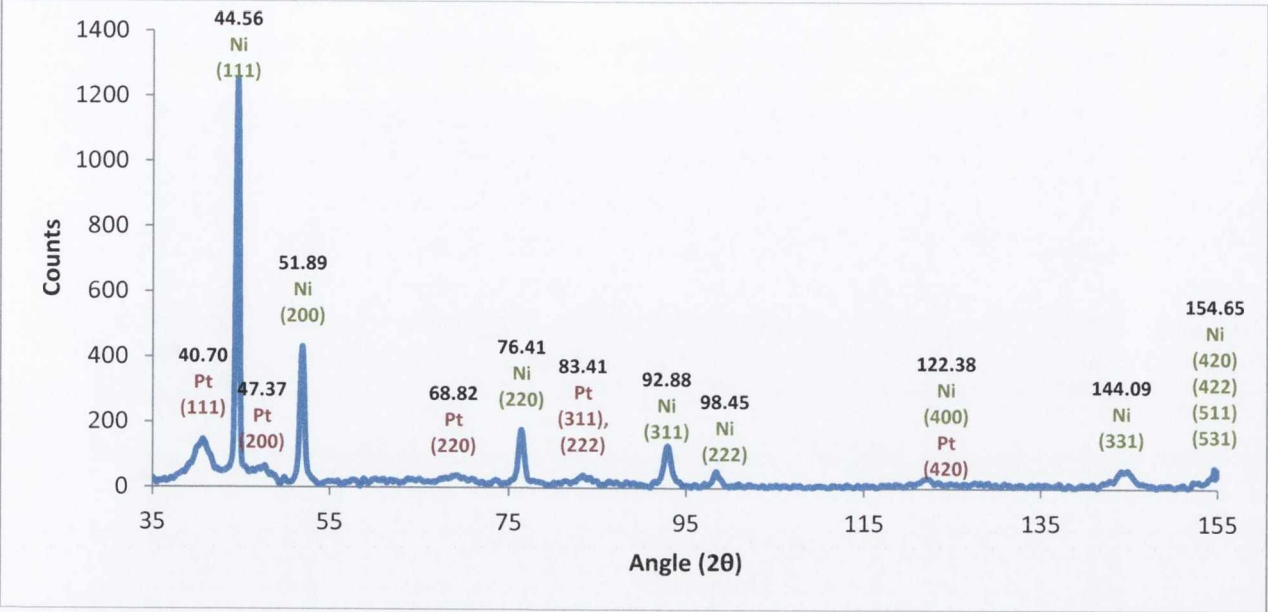


Scale Bar: 200 nm

VSM



XRD Ni fcc Pt fcc (54)

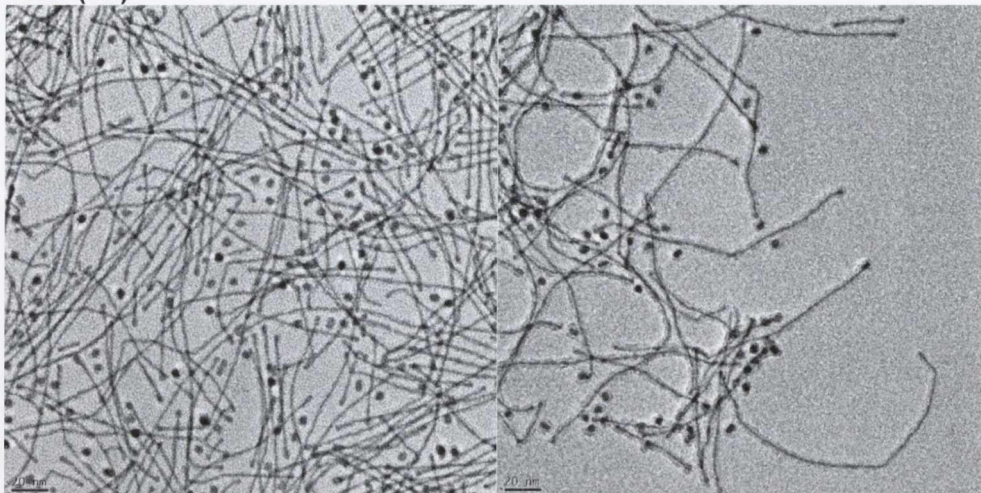


APPENDIX TO CHAPTER 4

APPENDIX 4.1

IRON-PLATINUM ALLOY NANOWIRES (55)

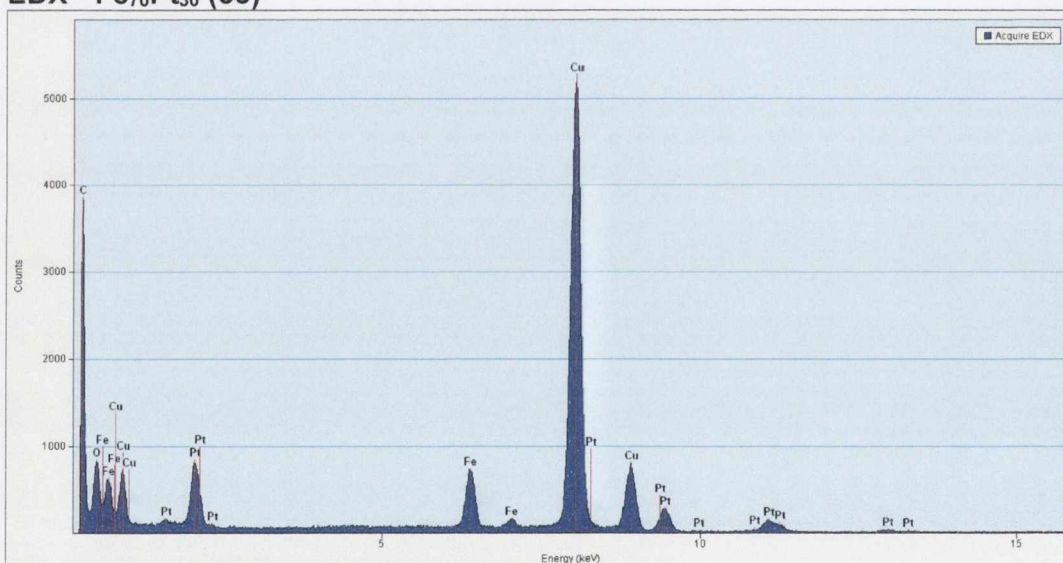
TEM (55)



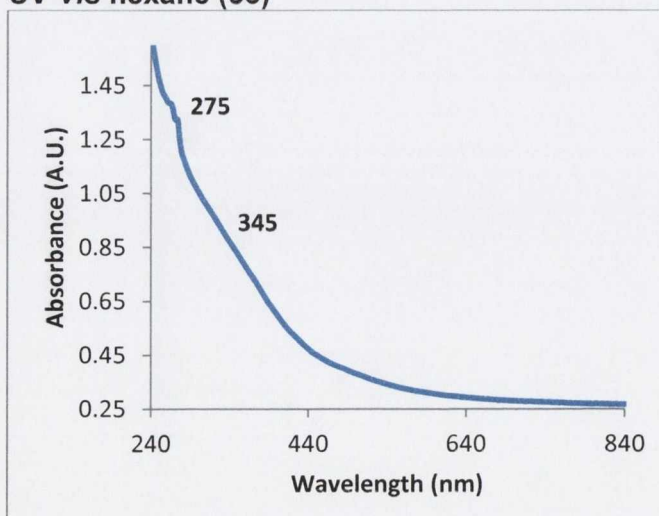
Scale Bar: 20 nm

Scale Bar: 20 nm

EDX - Fe₇₀Pt₃₀ (55)

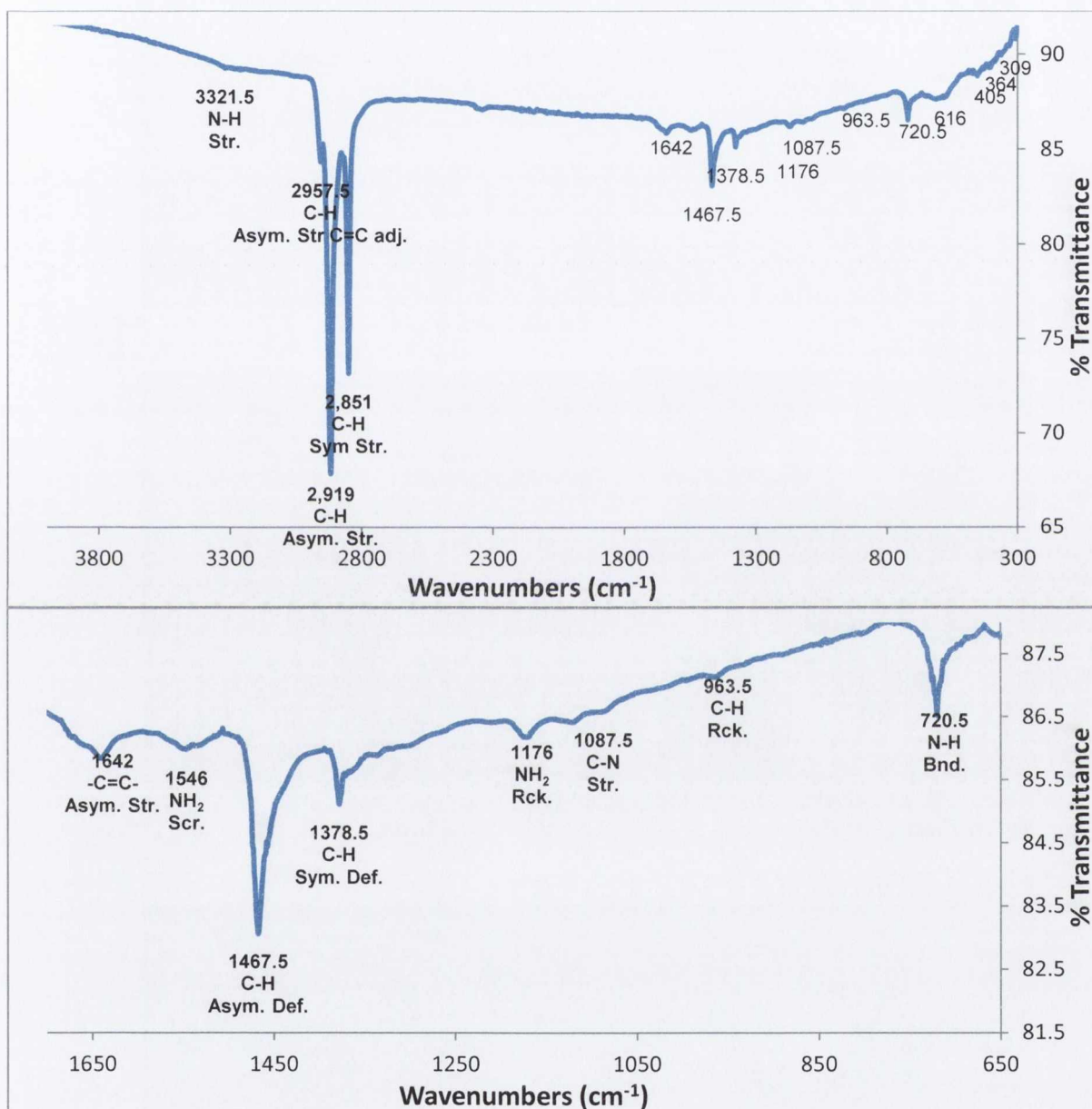


UV-vis hexane (55)



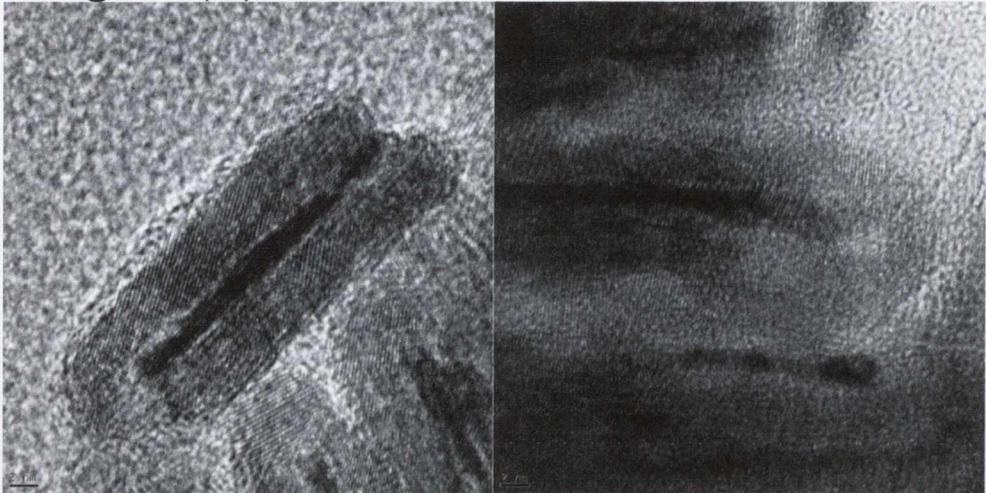
FTIR (55)

(cm^{-1}): **3321.5** (N-H, amine stretching), **2957.5** (C-H aliphatic adjacent to olefin, Asym. Str.), **2919** (C-H aliphatic, Asym. Str.), **2851** (C-H aliphatic, Sym. Str.), **1642** (-C=C-, Aym. Str.), **1546** (N-H amine, Scr.) **1467.5** (C-H aliphatic, Asym. Def.), **1378.5** (C-H aliphatic, Sym. Def.), **1176** (NH_2 amine, Rock.), **1087.5** (C-N amine, Str.), **963.5** (C-H amino adj.aliphatic, Rock.), **720.5** (N-H amine, Bnd.), **616** (-C=C-, Wag.), **574** (Pt-N, Str.), **455** (C-H aliphatic, Rock.), **405** (C-N amine, Def.) **364** (-C=C-, Tors.), **332.5** (Fe-N, Str.) , **309** (N-H, Def.)



A.4.2.1 - TEM

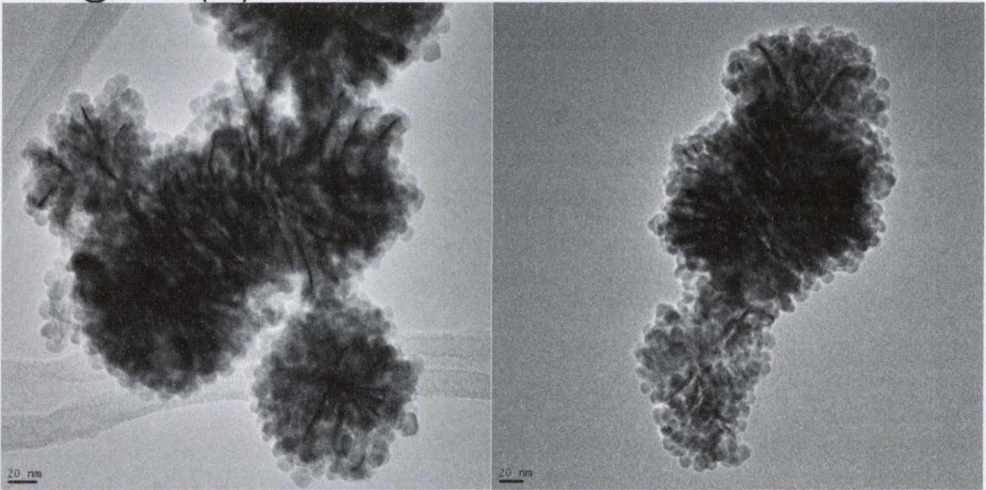
$\text{Fe}_3\text{O}_4@\text{FePd}_3$ (56)



Scale Bar: 2 nm

Scale Bar: 2 nm

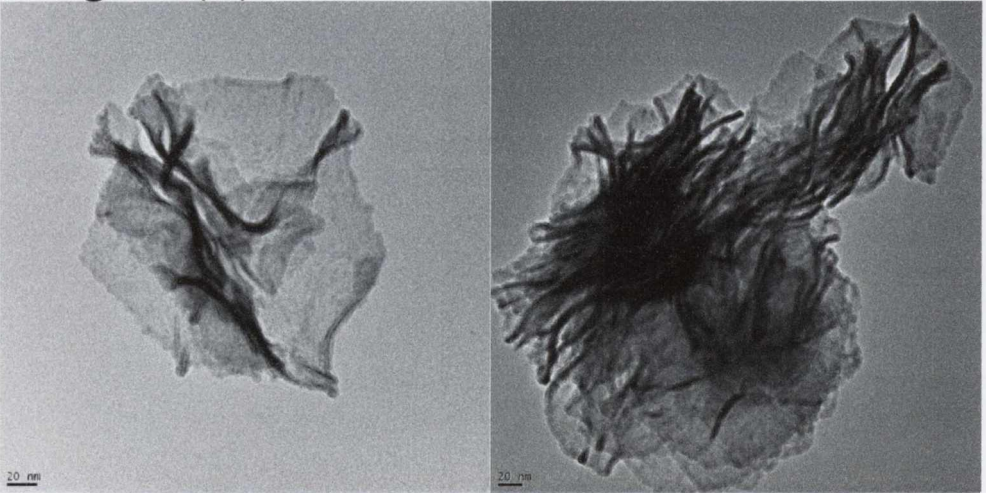
$\text{Fe}_3\text{O}_4@\text{FePd}_3$ (57)



Scale Bar: 20 nm

Scale Bar: 20 nm

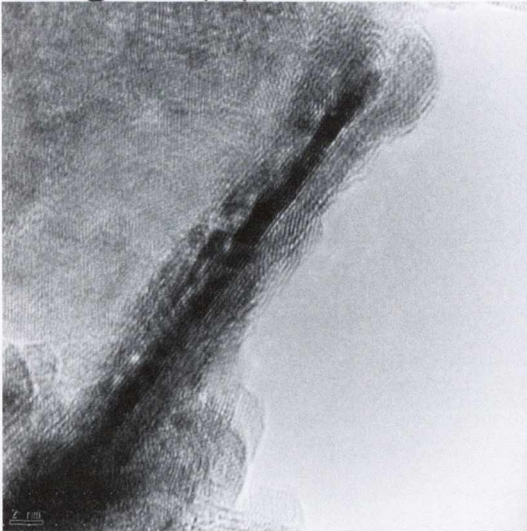
$\text{Fe}_3\text{O}_4@\text{FePd}_3$ (58)



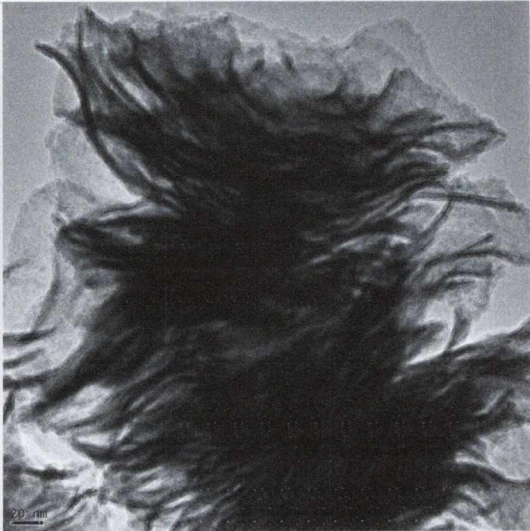
Scale Bar: 20 nm

Scale Bar: 20 nm

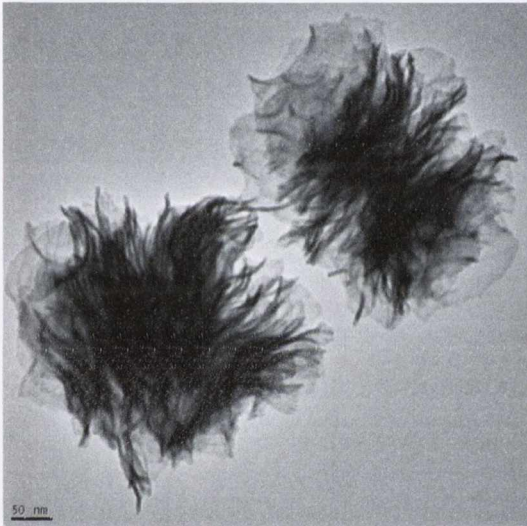
Fe₃O₄@FePd₃ (58)



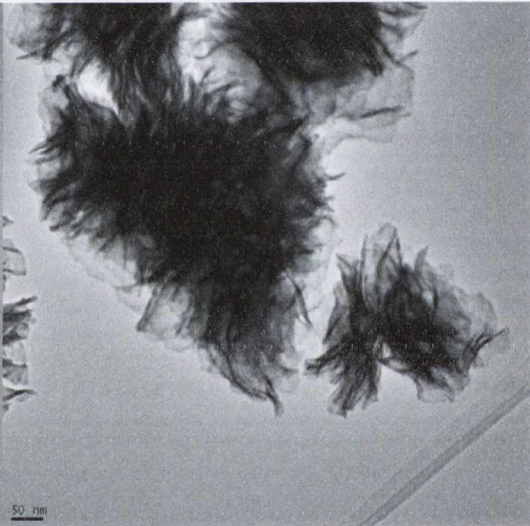
Scale Bar: 2 nm



Scale Bar: 20 nm

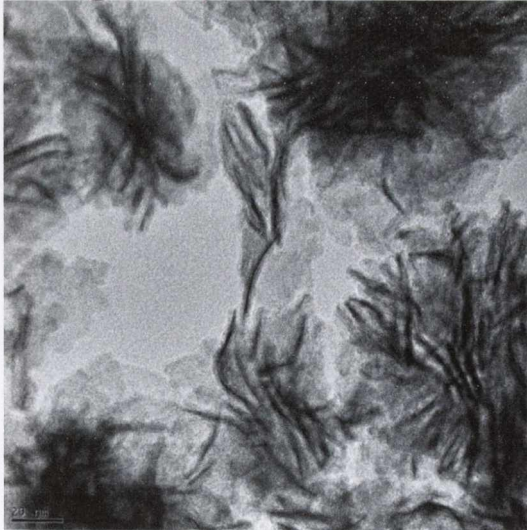


Scale Bar: 50 nm

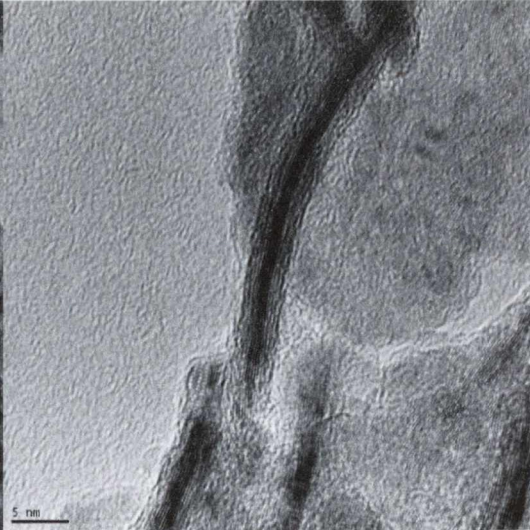


Scale Bar: 50 nm

Fe₃O₄@FePd₃ (59)

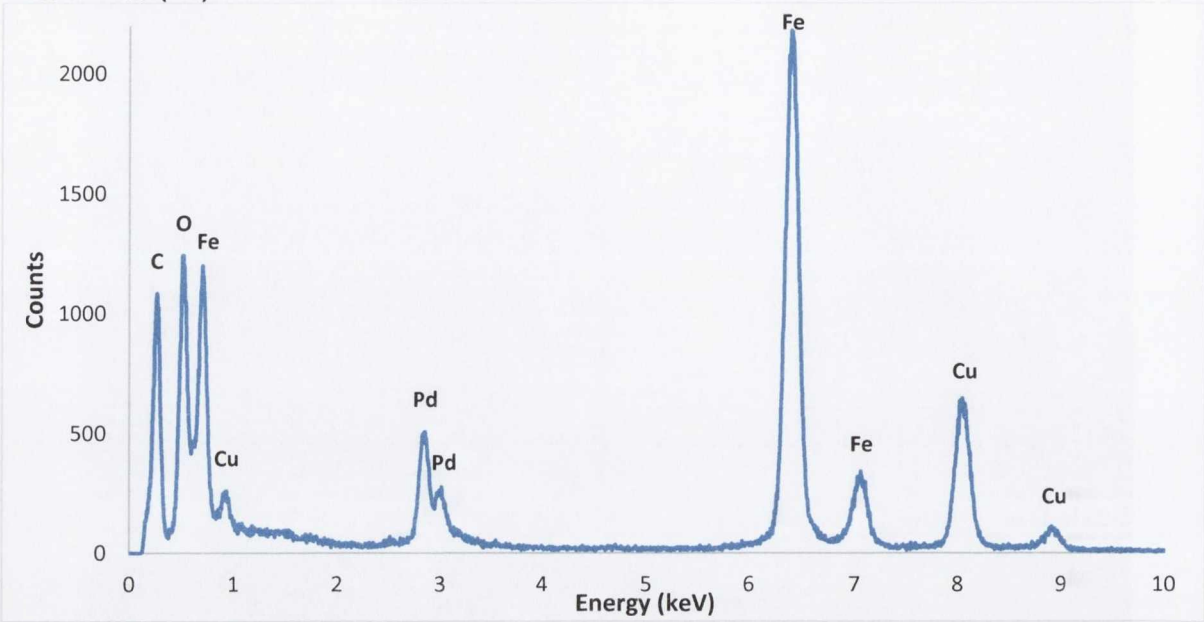


Scale Bar: 20 nm

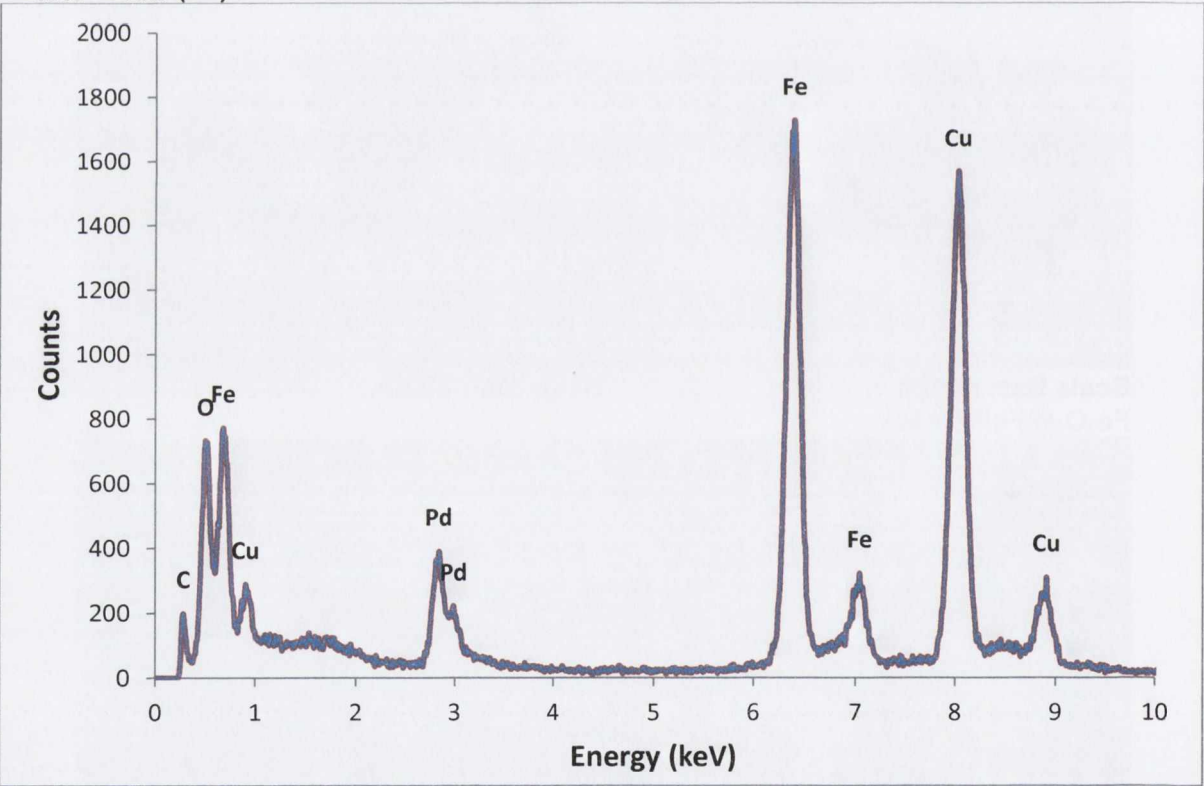


Scale Bar: 5 nm

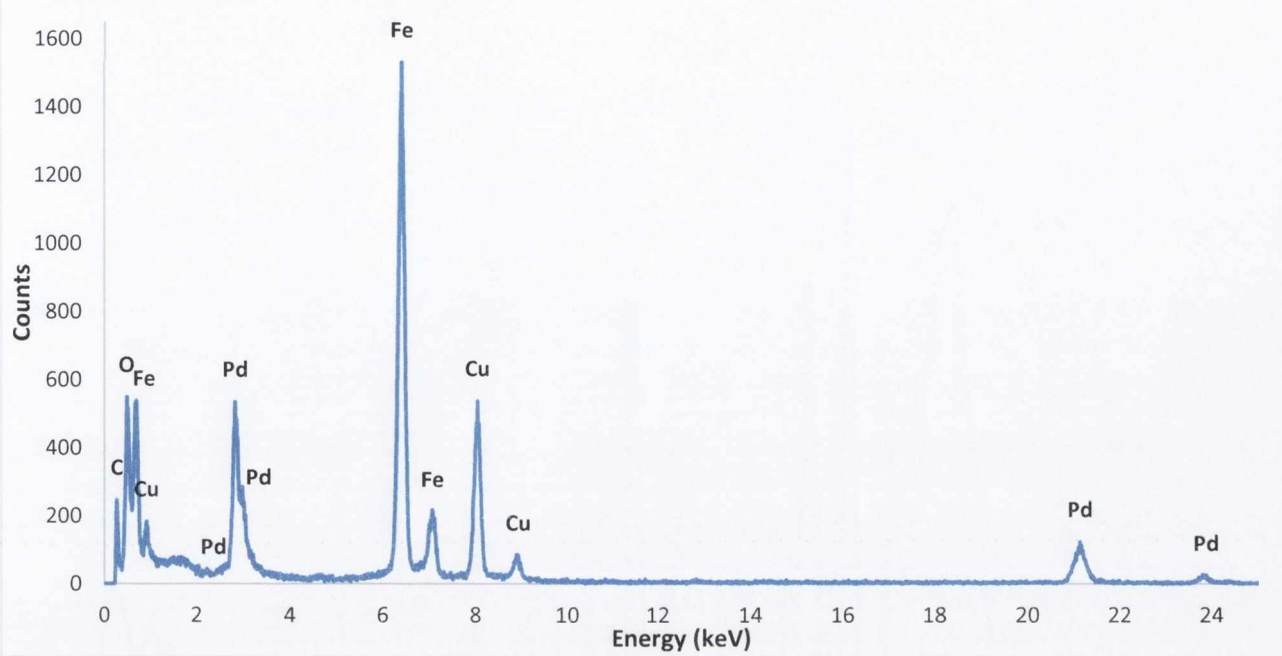
A.4.2.2 EDX
 $\text{Fe}_{86.4}\text{Pd}_{13.6}$ (56)



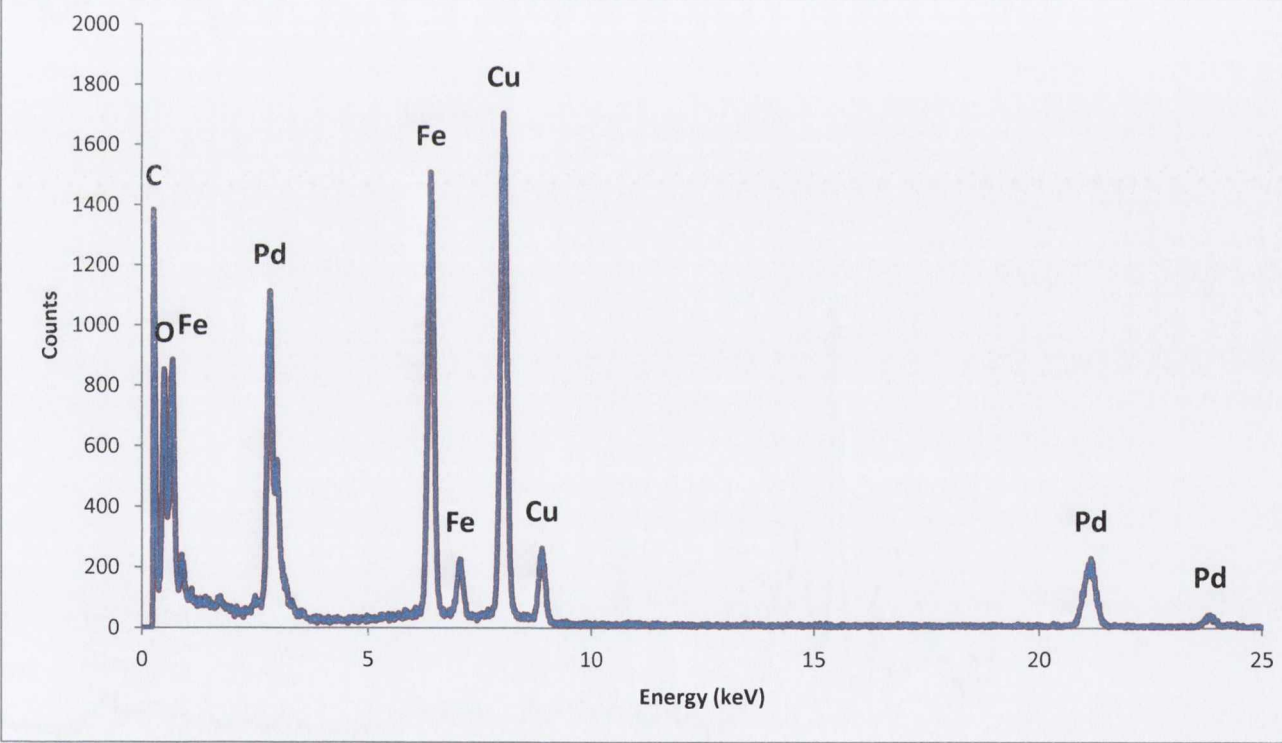
$\text{Fe}_{85.93}\text{Pd}_{14.06}$ (57)



Fe_{76.07}Pd_{23.93} (58)

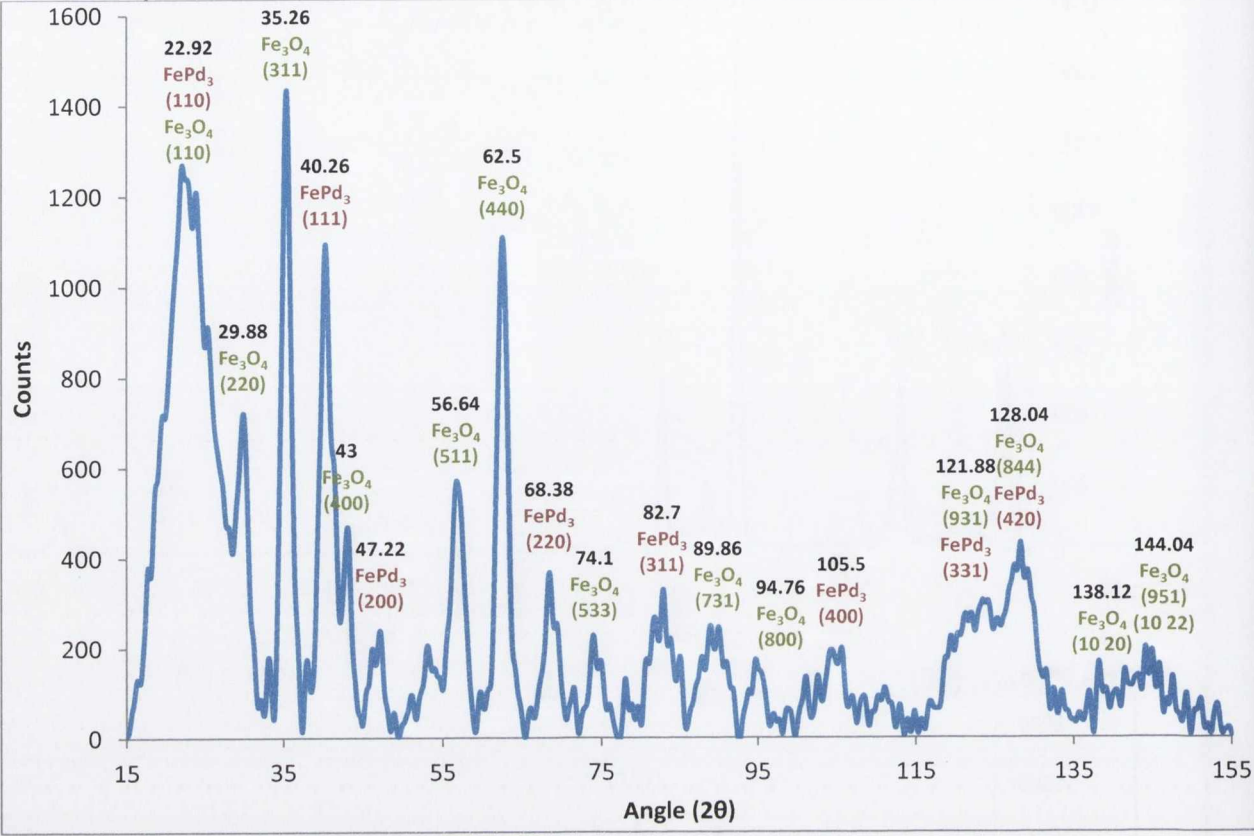


Fe_{59.78}Pd_{40.22} (59)

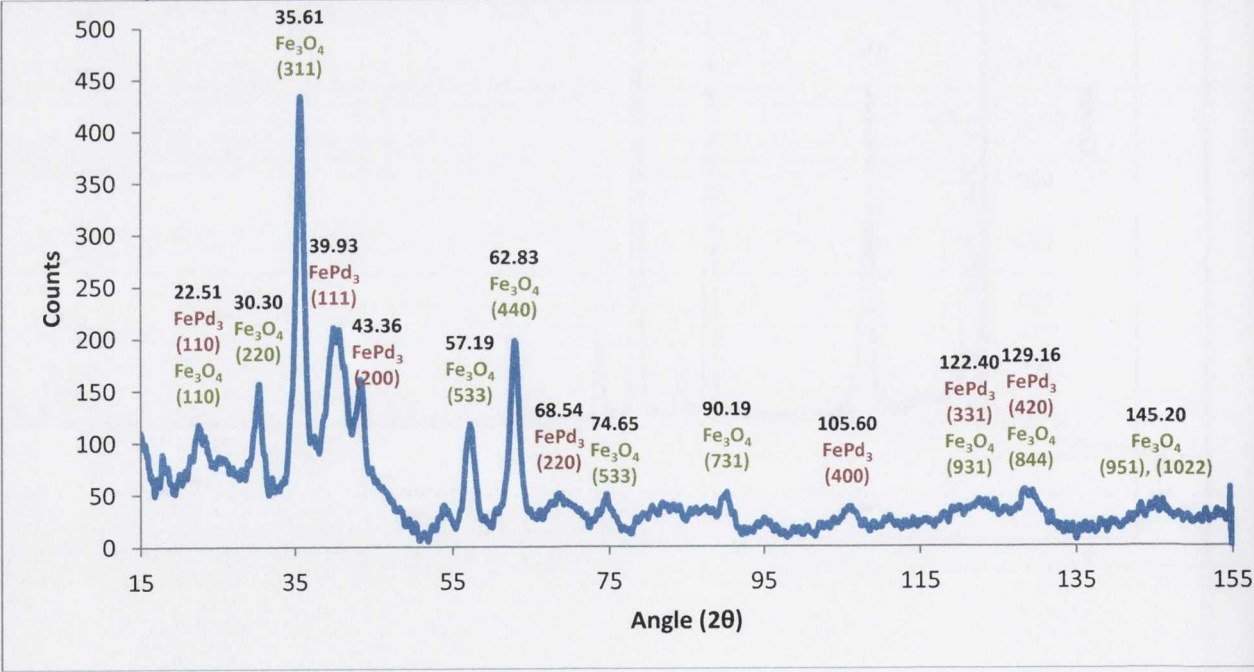


A.4.2.3 XRD

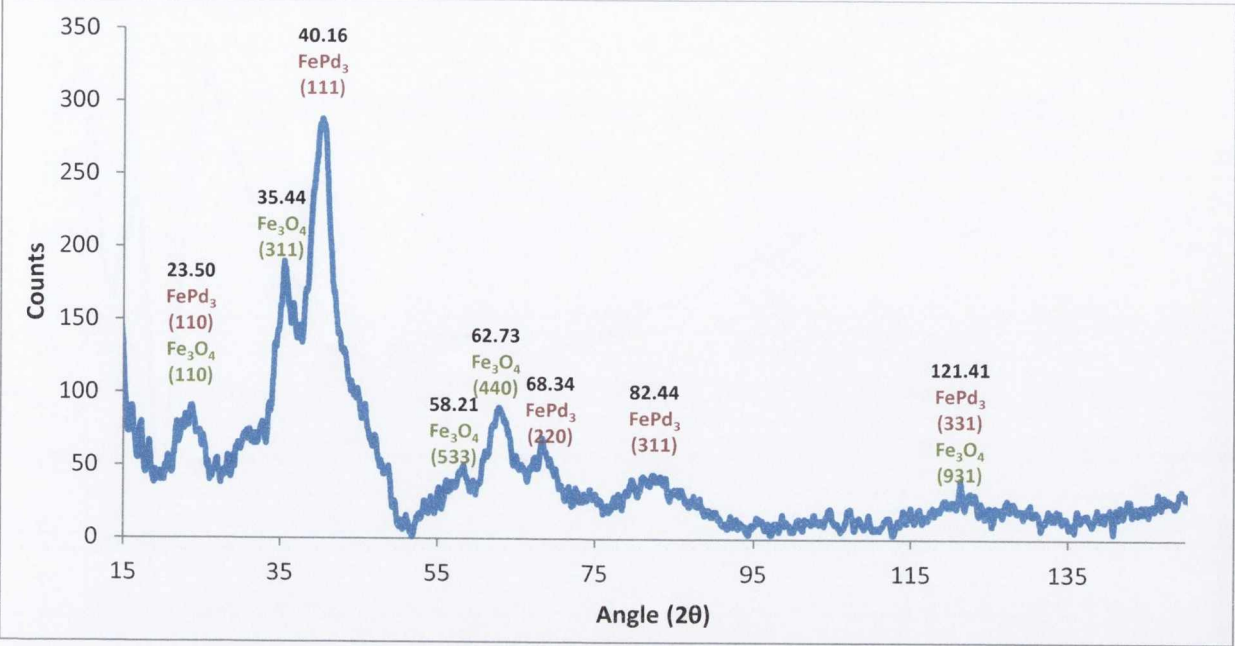
FePd₃ fcc | Fe₃O₄ fcc (56)



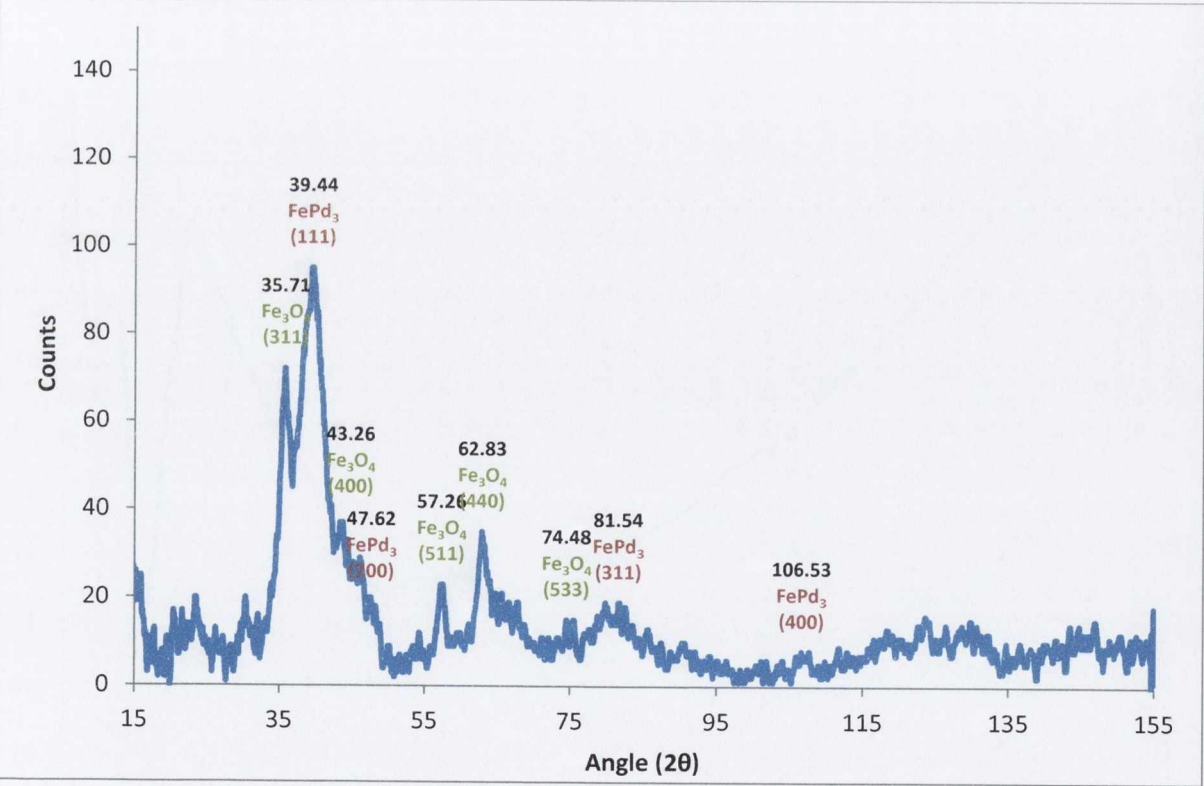
FePd₃ fcc | Fe₃O₄ fcc (57)



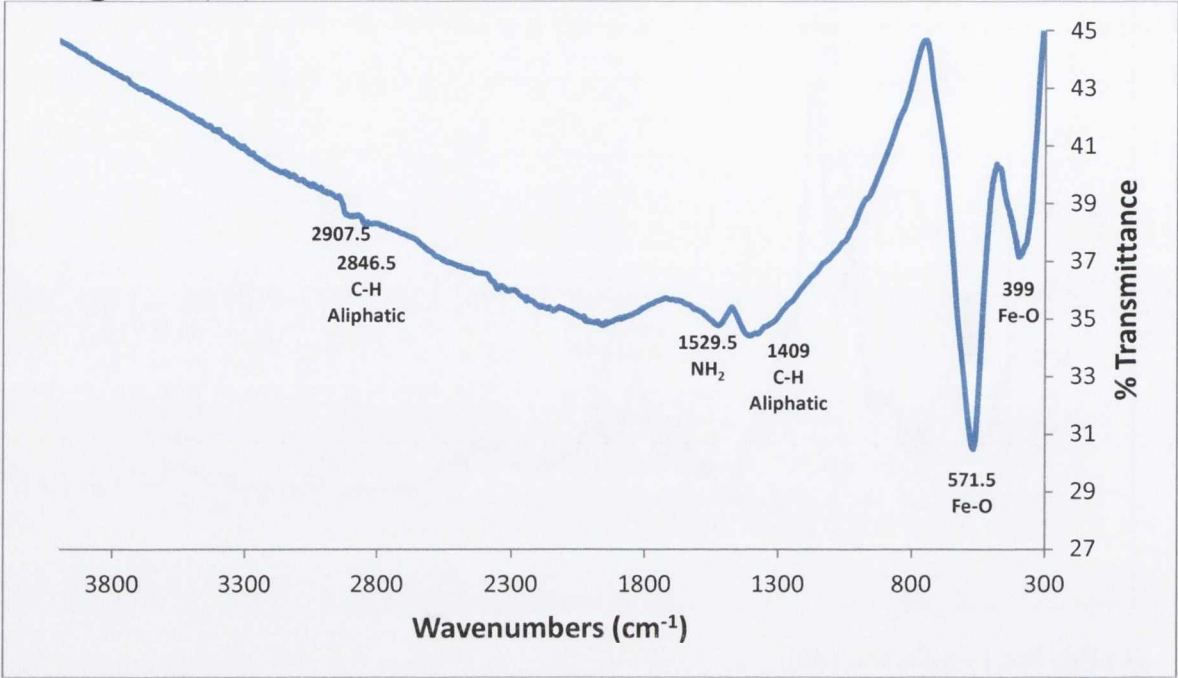
FePd₃ fcc | Fe₃O₄ fcc (58)



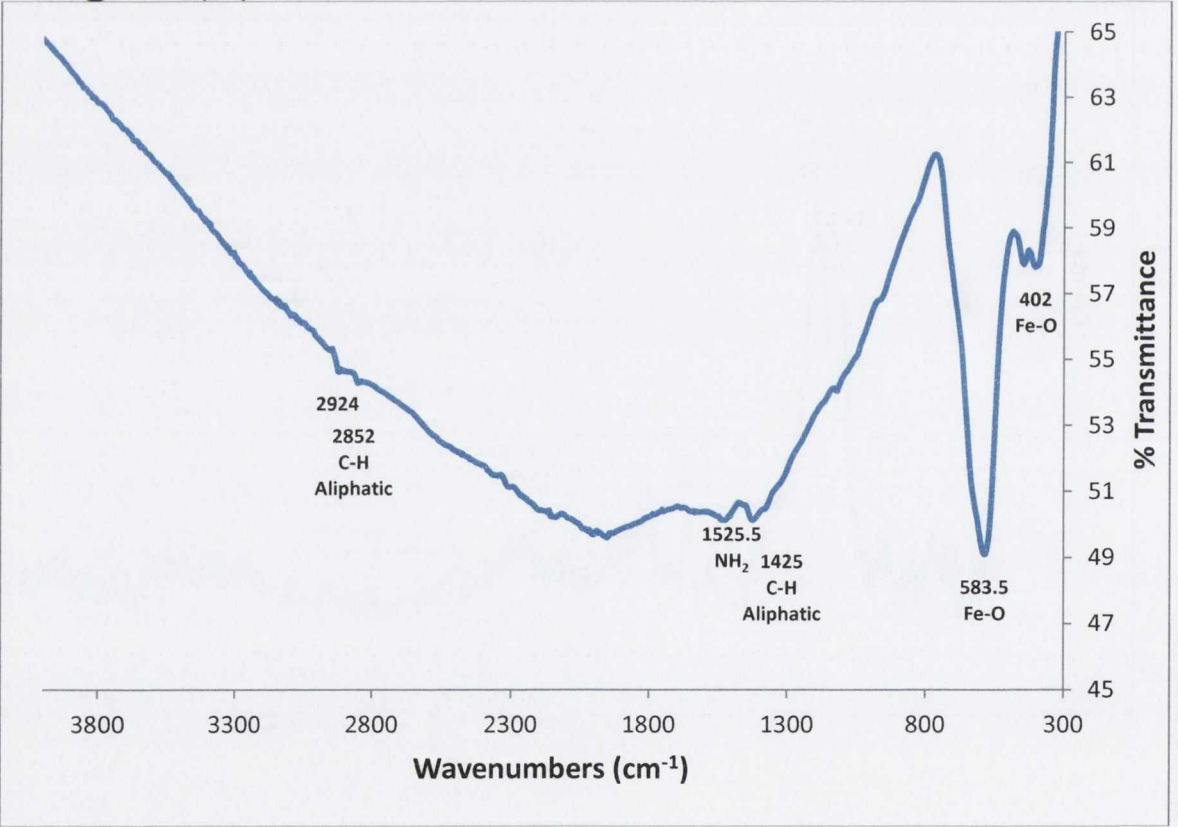
FePd₃ fcc | Fe₃O₄ fcc (59)



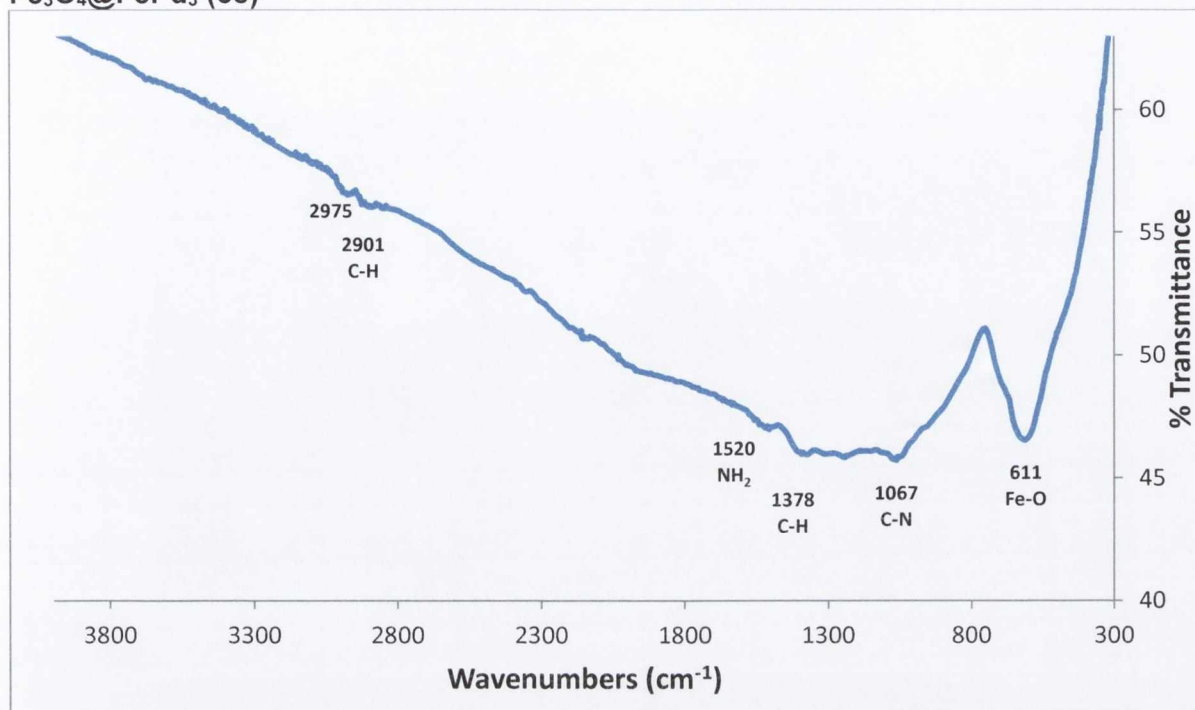
A.4.2.4 FTIR
 $\text{Fe}_3\text{O}_4@\text{FePd}_3$ (56)



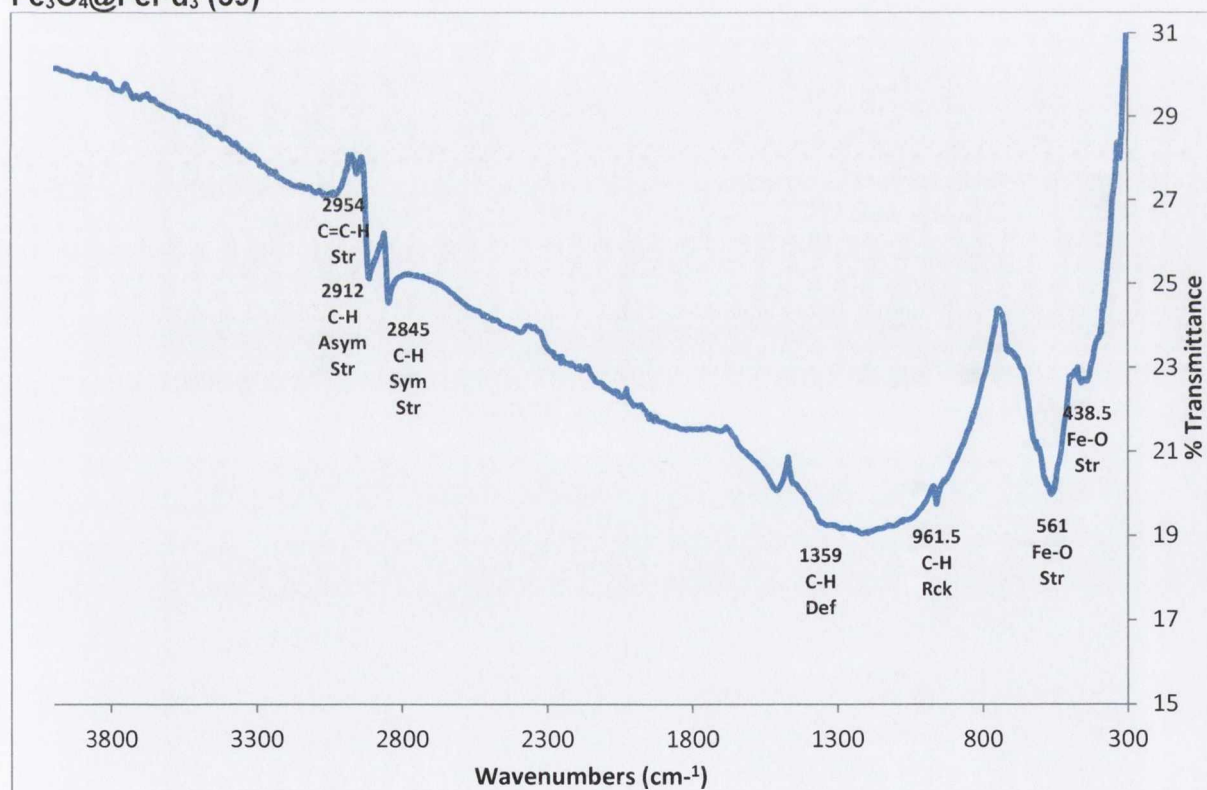
$\text{Fe}_3\text{O}_4@\text{FePd}_3$ (57)



Fe₃O₄@FePd₃ (58)

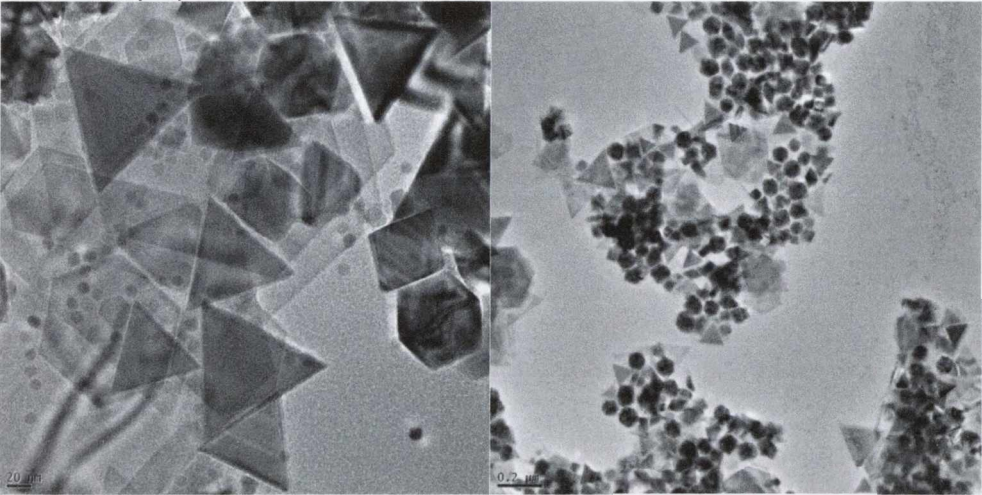


Fe₃O₄@FePd₃ (59)



A.4.3.1 - TEM

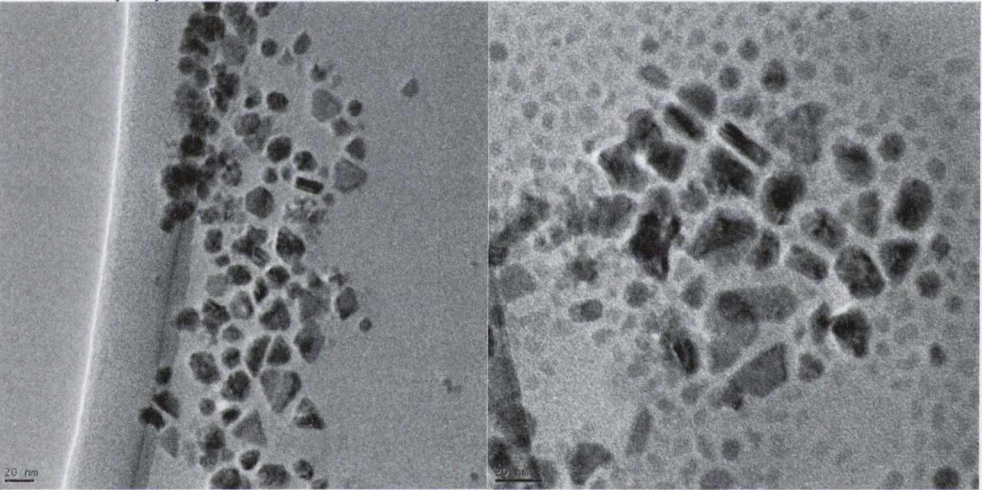
$\text{Fe}_{5.4}\text{Ni}_{94.6}$ (60)



Scale Bar: 20 nm

Scale Bar: 200 nm

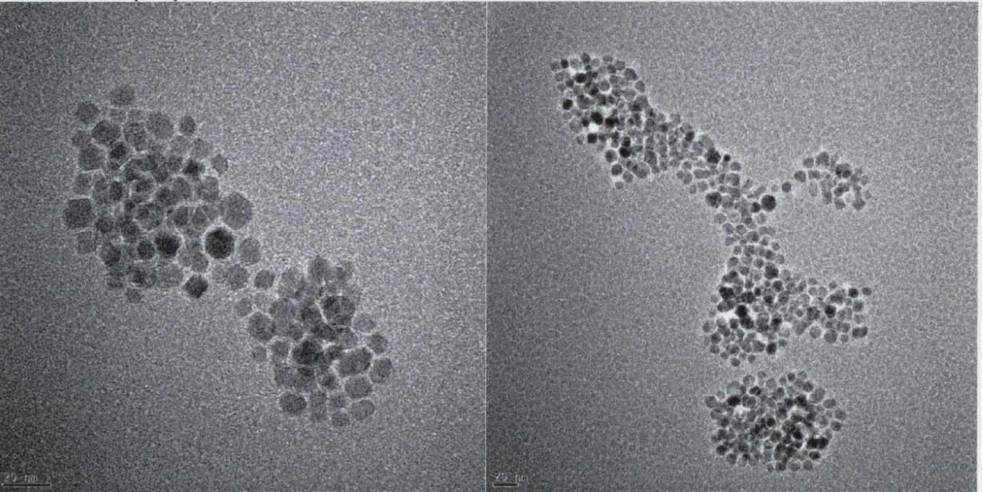
$\text{Fe}_{32}\text{Ni}_{68}$ (61)



Scale Bar: 20 nm

Scale Bar: 20 nm

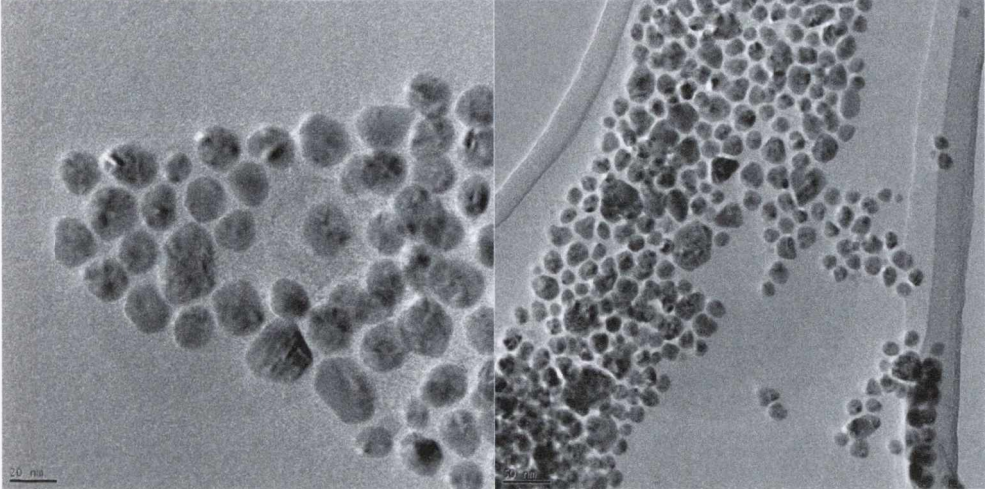
$\text{Fe}_{50x}\text{Ni}_{50x}$ (62)



Scale Bar: 20 nm

Scale Bar: nm

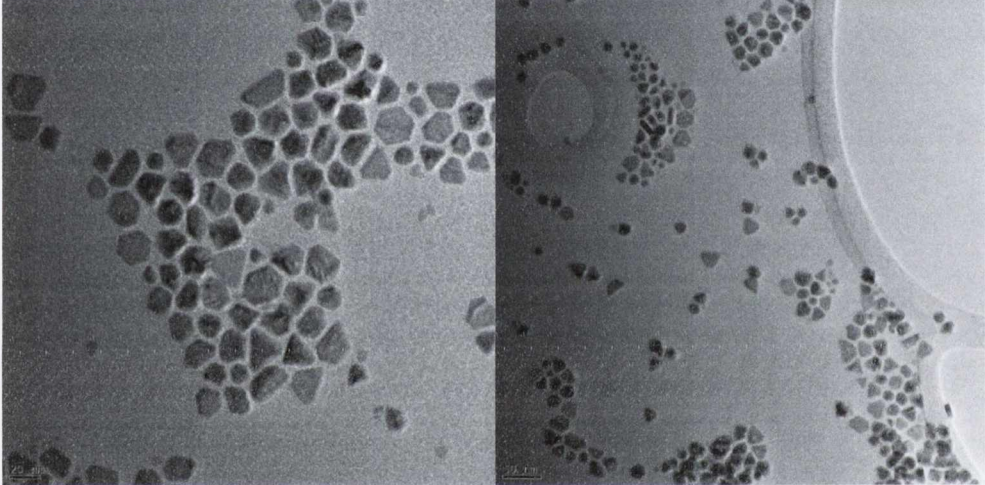
Fe₂₄Ni₇₆ (63)



Scale Bar: 20 nm

Scale Bar: 50 nm

Fe_{22.5}Ni_{77.5} (64)



Scale Bar: 20 nm

Scale Bar: 50 nm

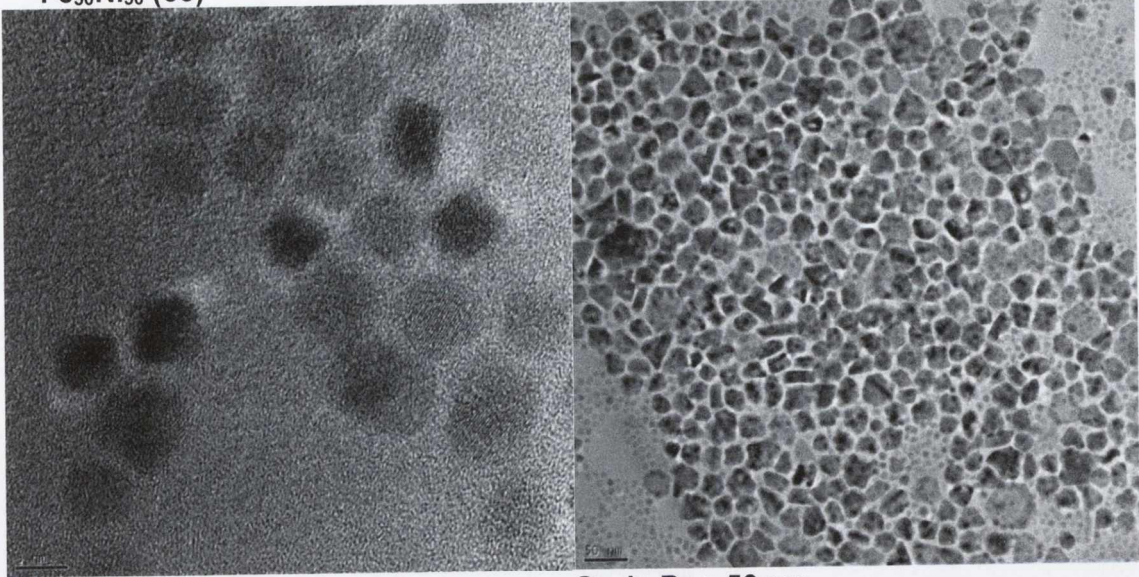
Fe_{17.3}Ni_{82.7} (65)



Scale Bar: 2 nm

Scale Bar: 2 nm

Fe₅₀Ni₅₀ (66)

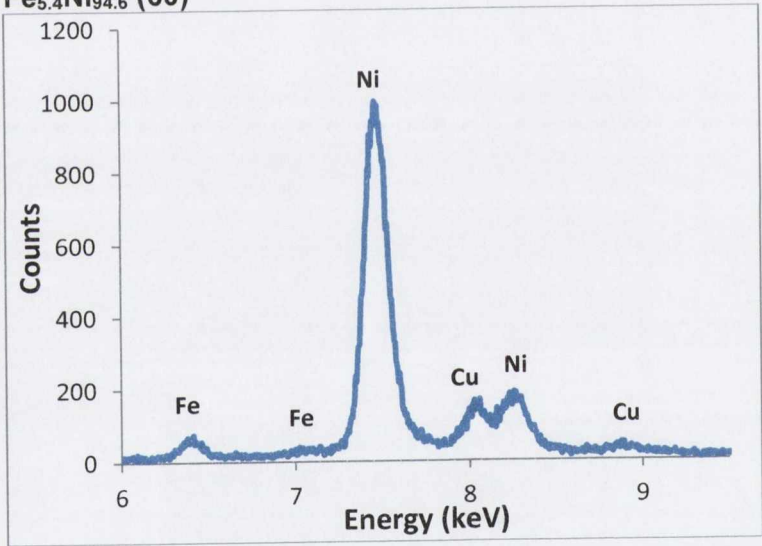


Scale Bar: 5 nm

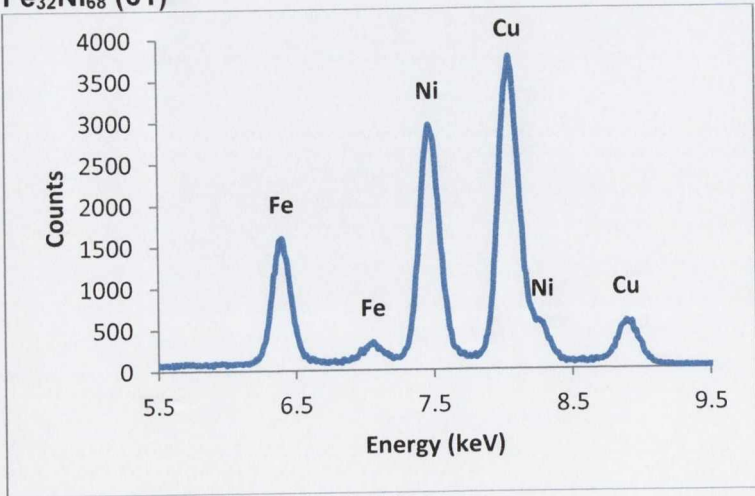
Scale Bar: 50 nm

A.4.3.2 - EDX

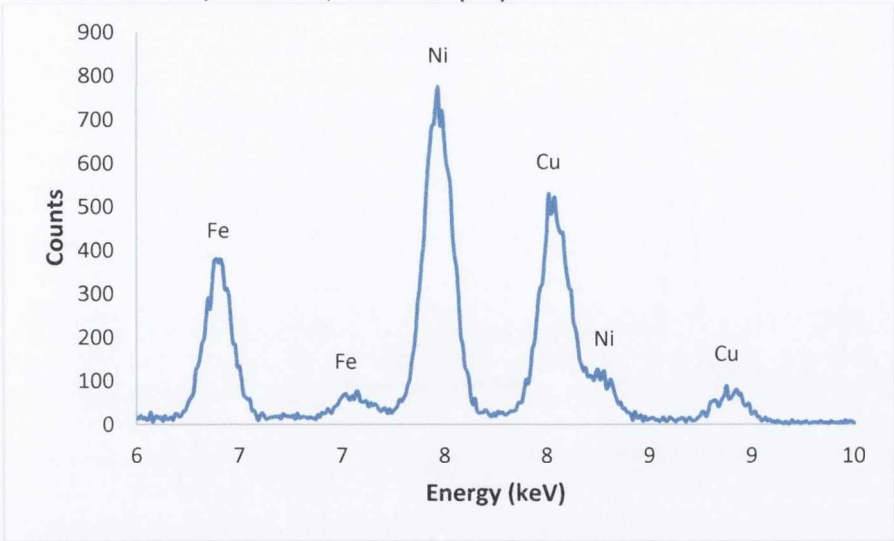
Fe_{5.4}Ni_{94.6} (60)



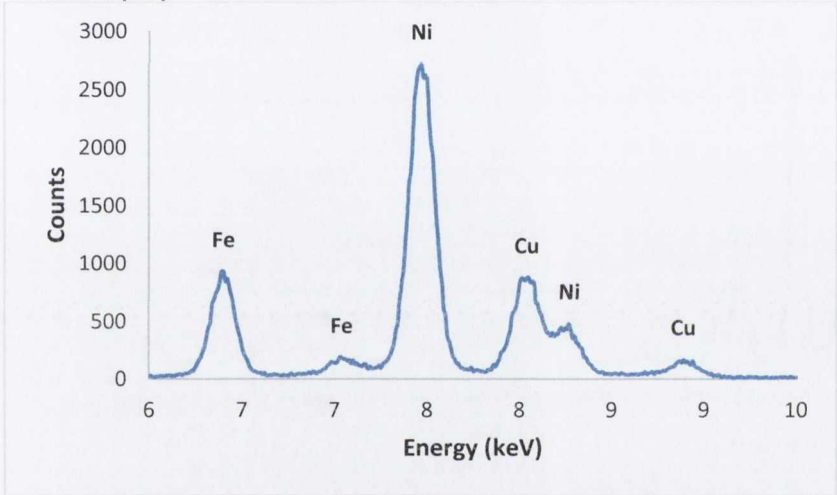
Fe₃₂Ni₆₈ (61)



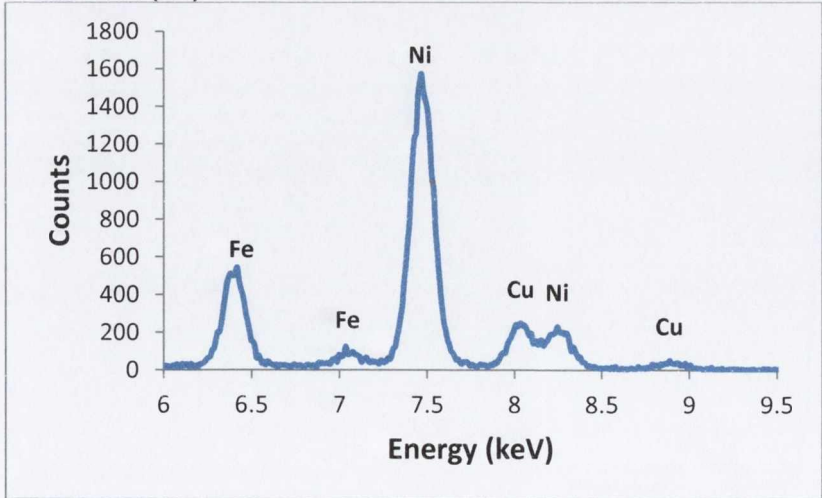
varied Fe₅₀Ni₅₀, Fe₄₀Ni₆₀, Fe₃₀Ni₇₀ (62)



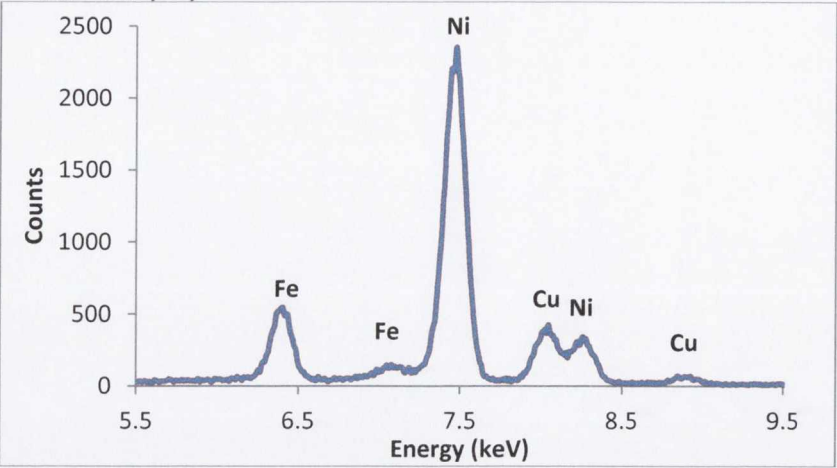
Fe₂₄Ni₇₆ (63)



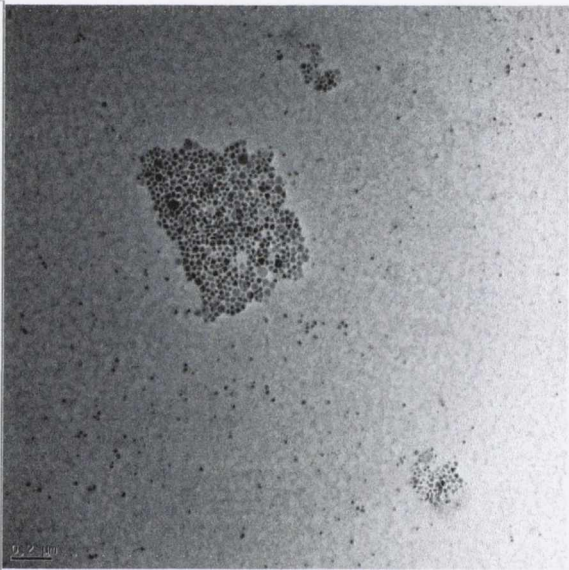
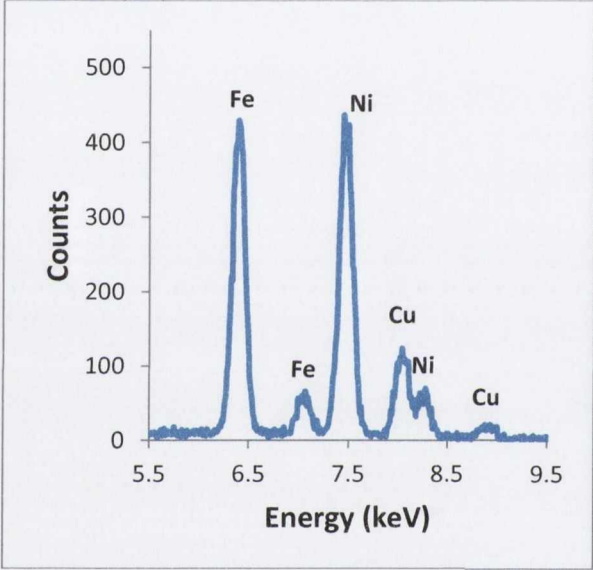
Fe_{22.5}Ni_{77.5} (64)



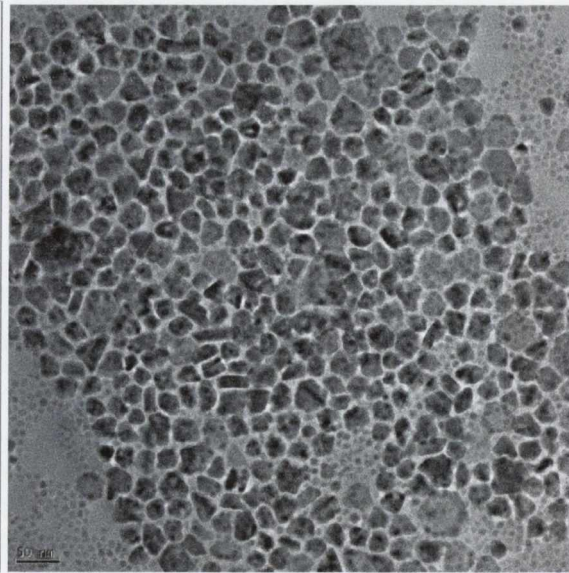
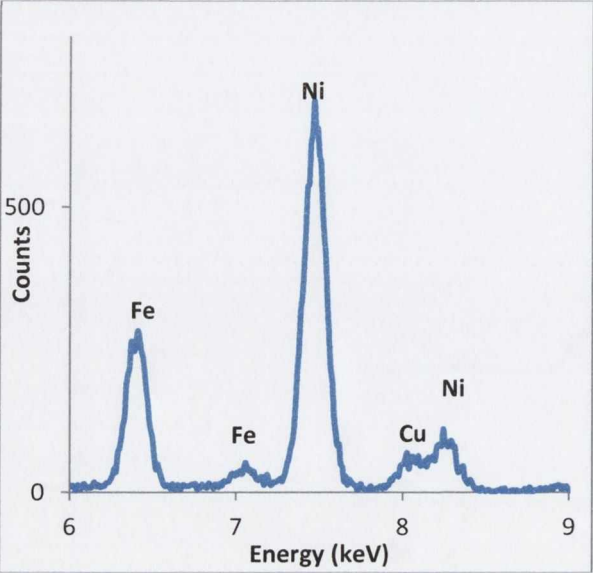
Fe_{17.3}Ni_{82.7} (65)



Fe₅₀Ni₅₀ (66)

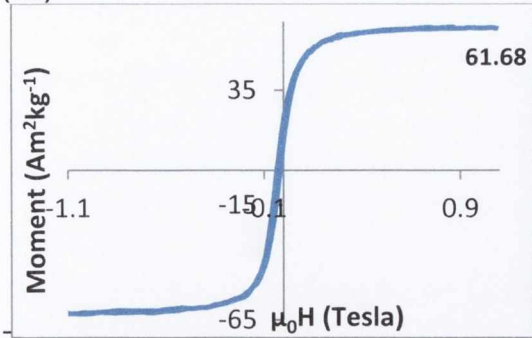


Fe₂₆Ni₇₄ (66)

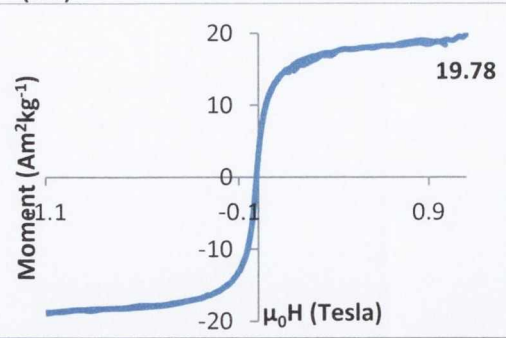


A.4.3.3 VSM

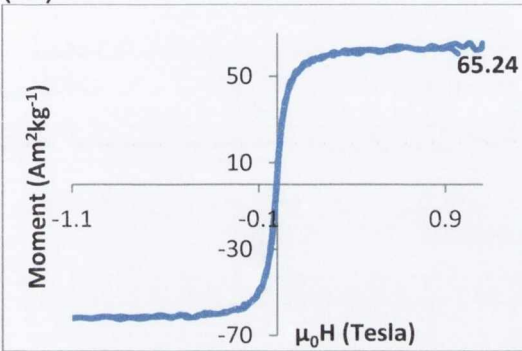
(60)



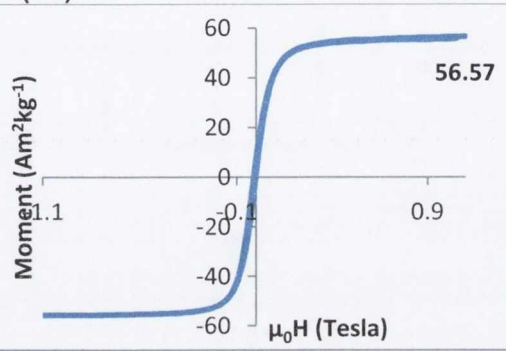
(61)



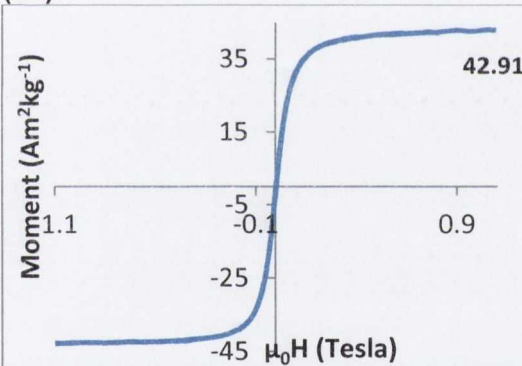
(62)



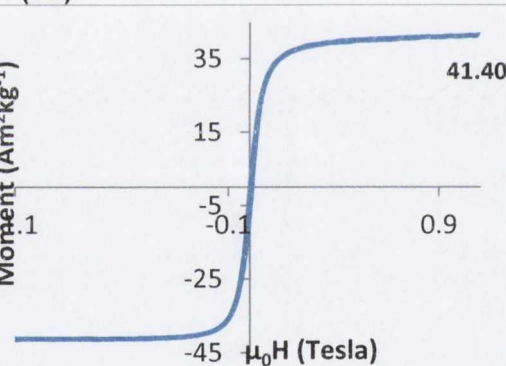
(63)



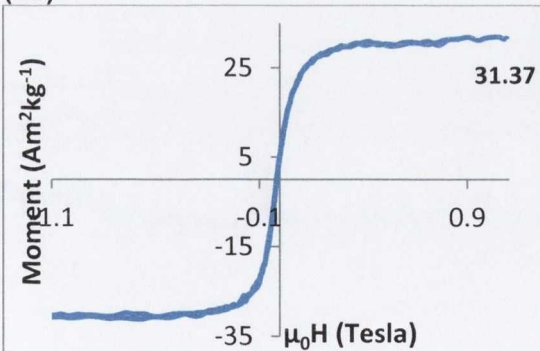
(64)



(65)

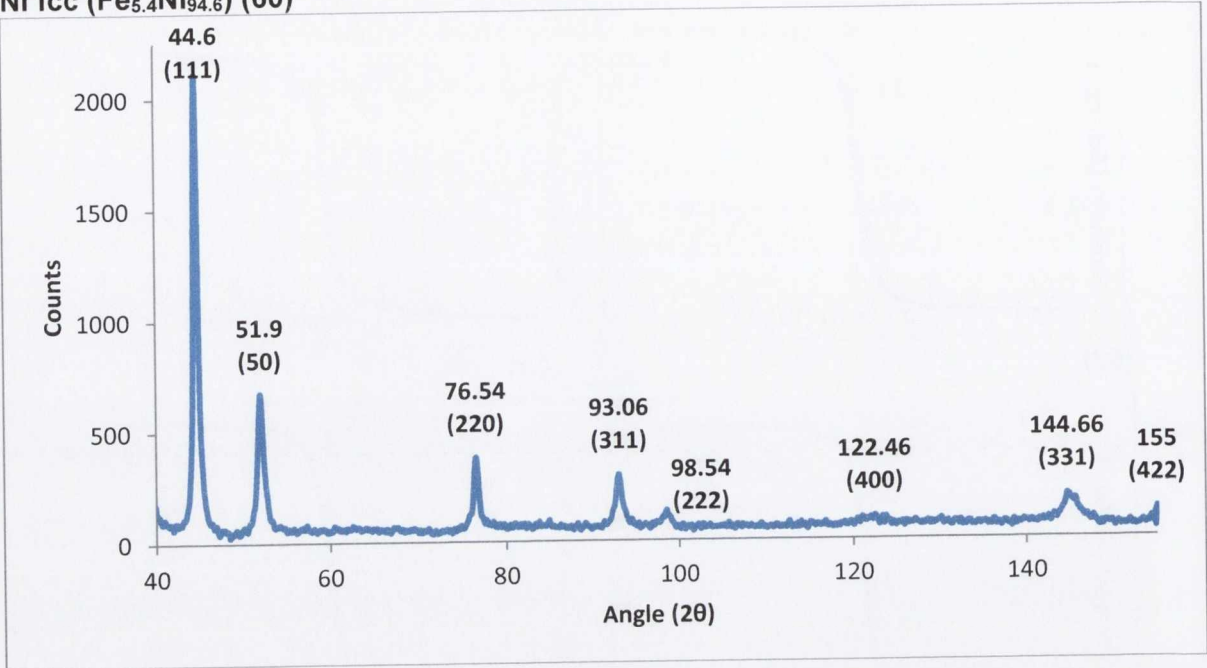


(66)

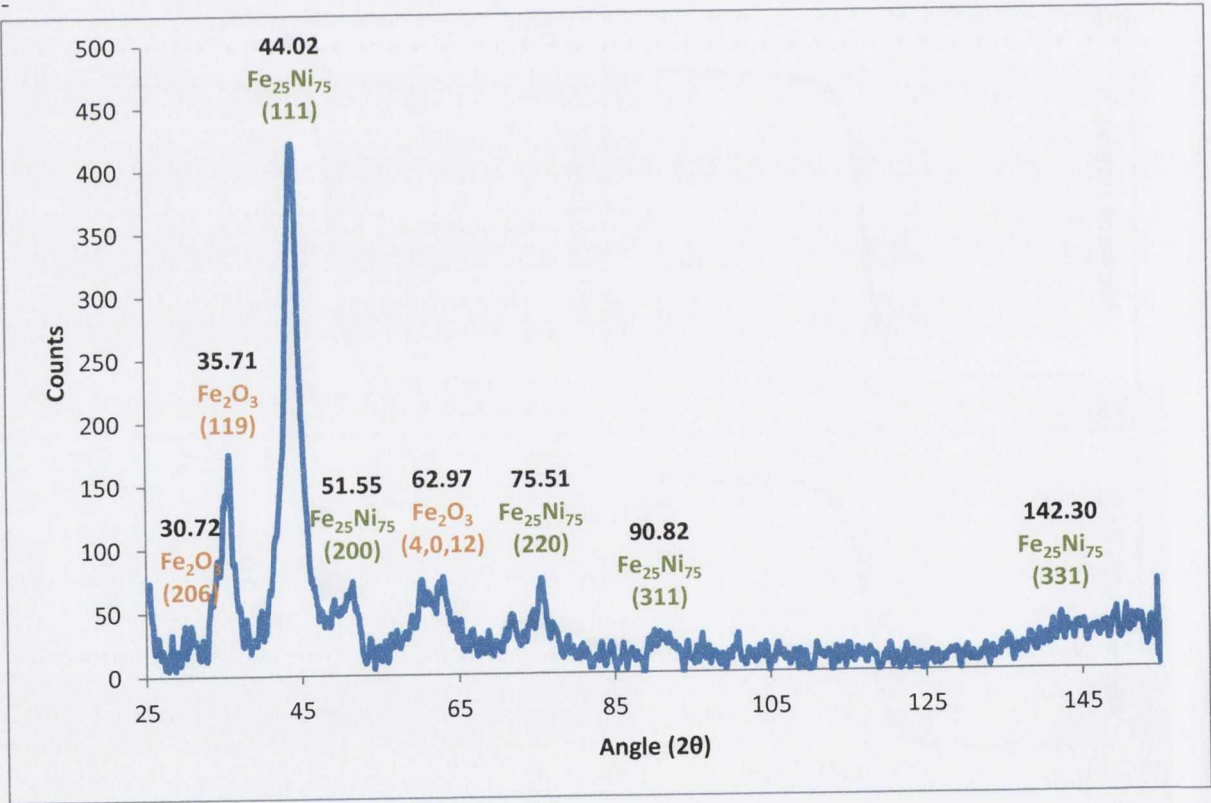


A.4.3.4 XRD

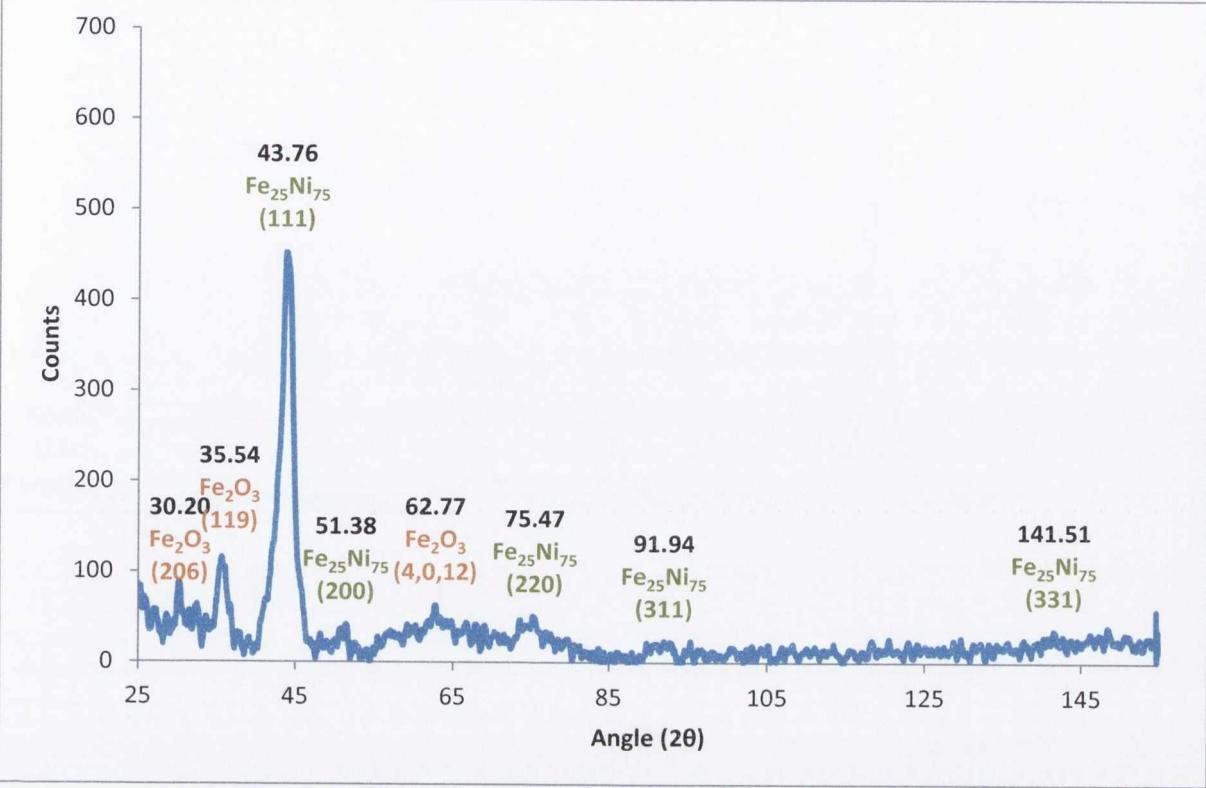
Ni fcc ($\text{Fe}_{5.4}\text{Ni}_{94.6}$) (60)



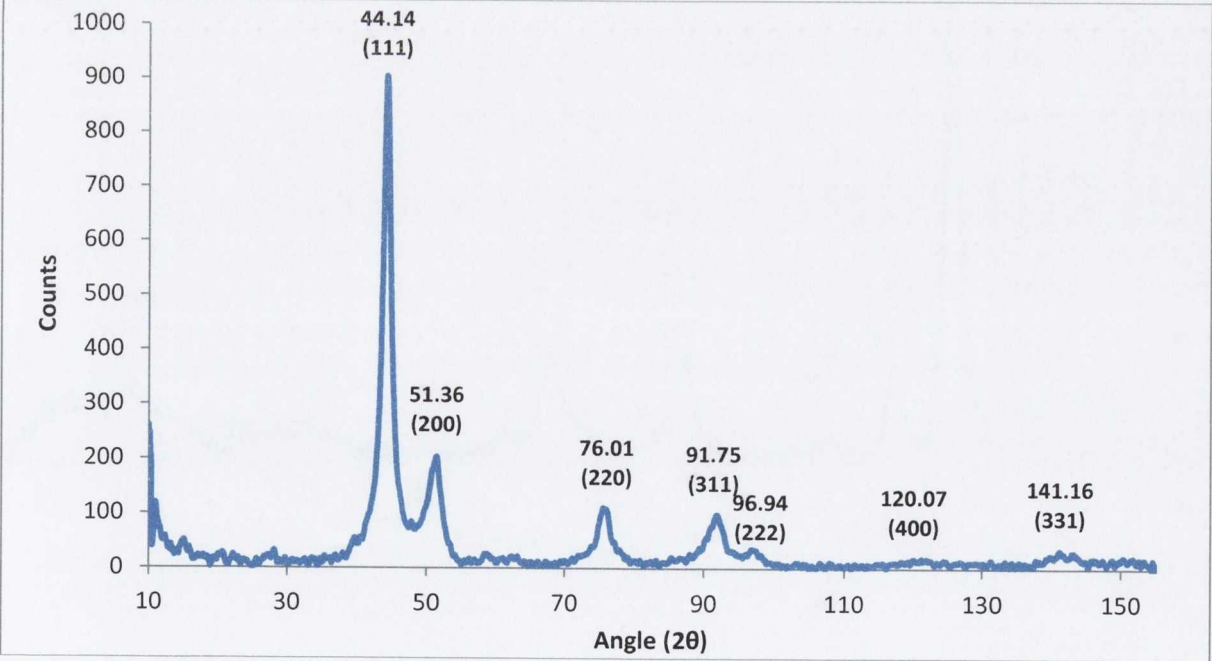
$\text{Fe}_{25}\text{Ni}_{75}$ fcc | Fe_2O_3 tet (61)



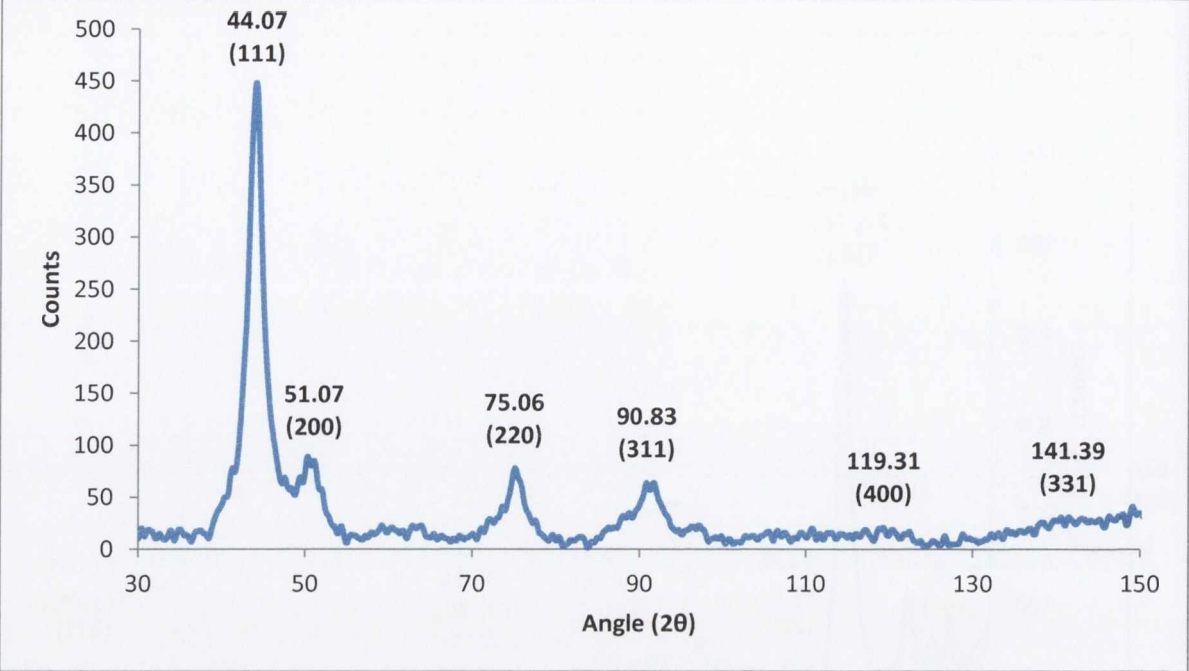
Fe₂₅Ni₇₅ | Fe₂O₃ tet (62)



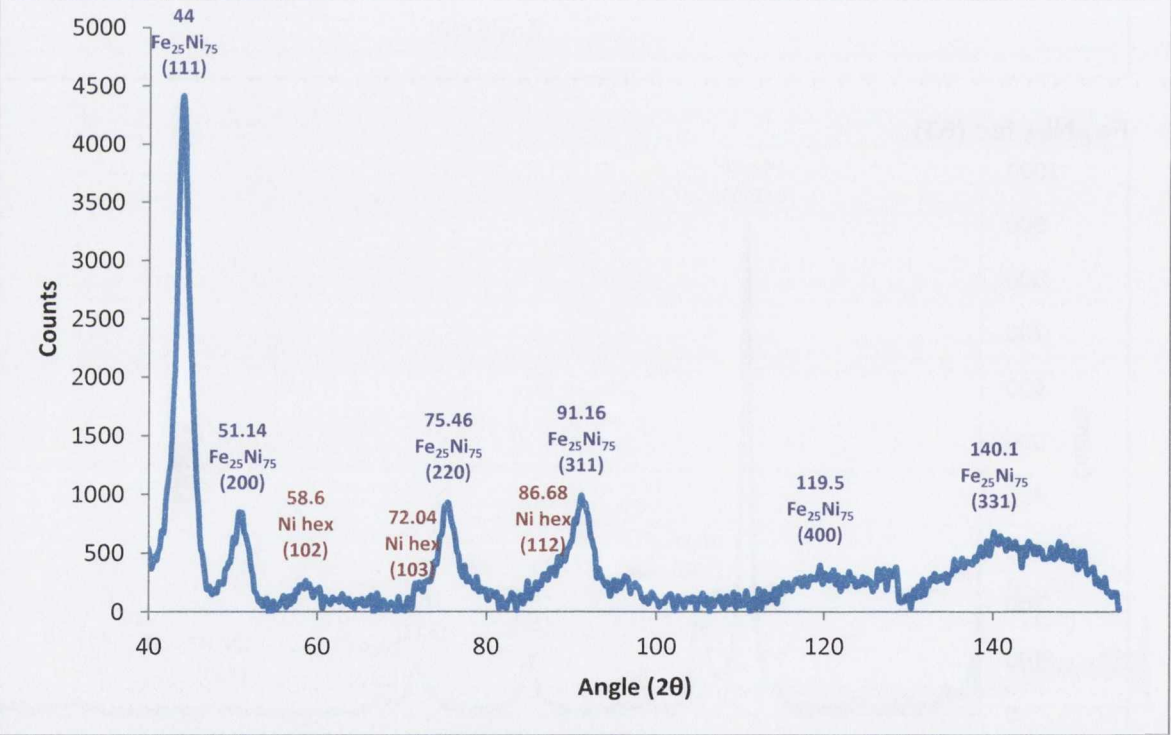
Fe₂₅Ni₇₅ fcc (63)



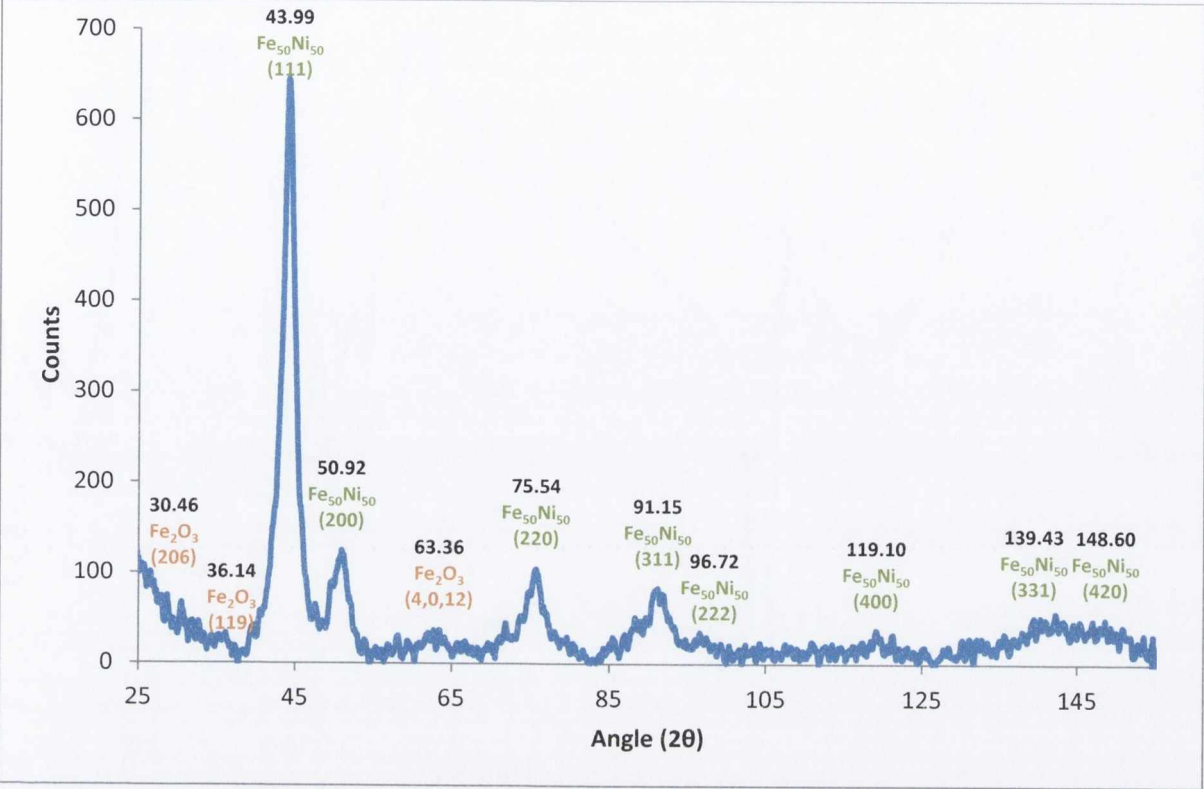
Fe₂₅Ni₇₅ fcc (Fe_{22.5}Ni_{77.5}) (64)



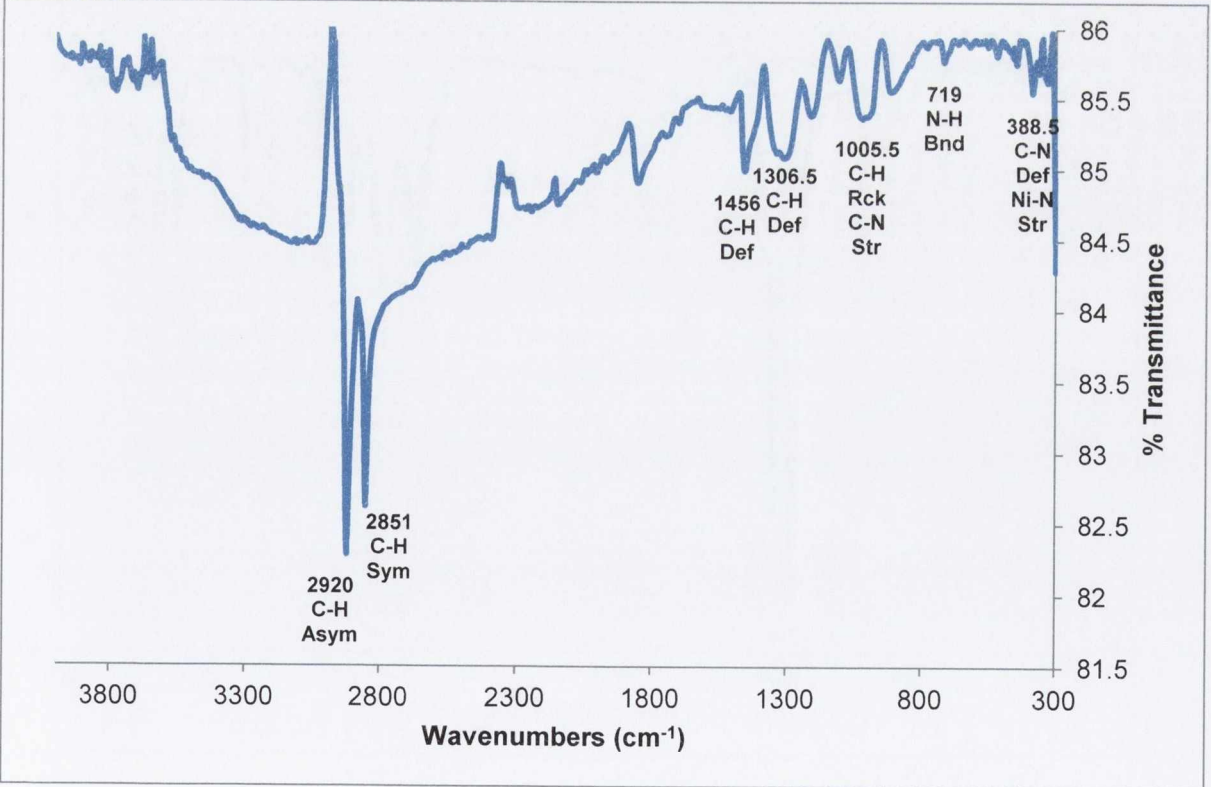
Fe₂₅Ni₇₅ fcc | Ni hex (65)



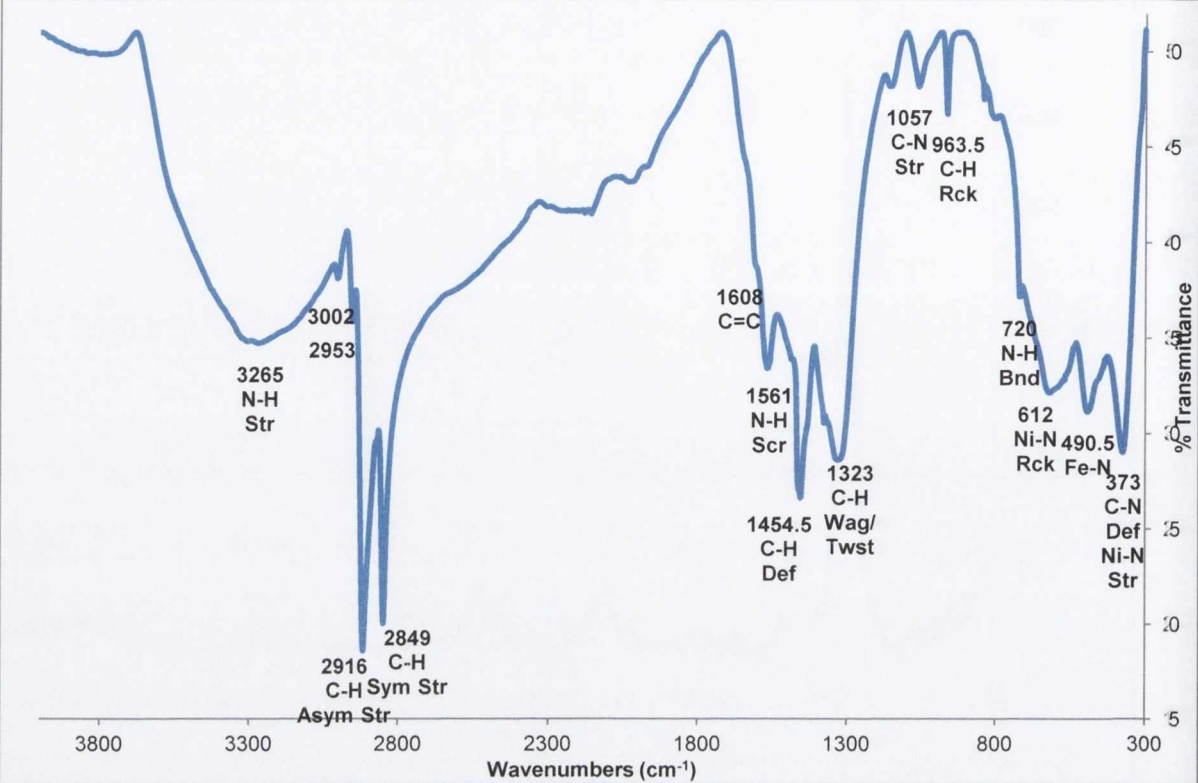
FeNi fcc | Fe₂O₃ tet (66)



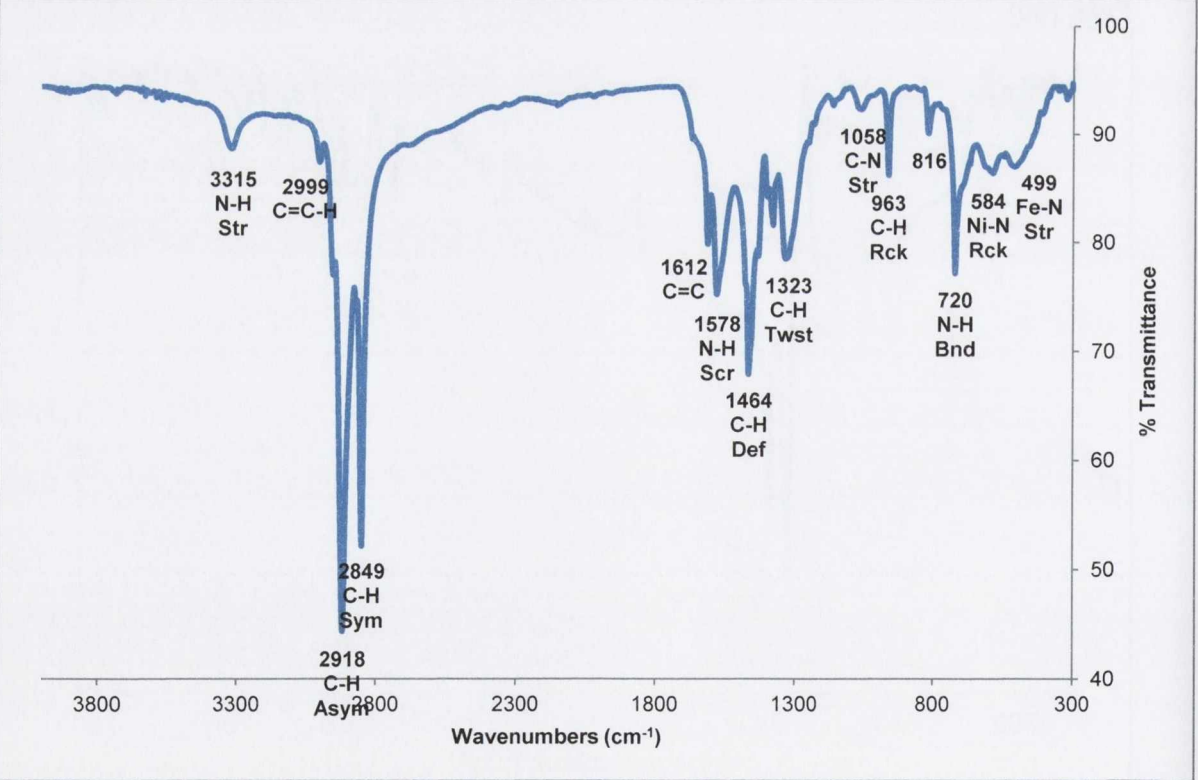
A.4.3.5 FTIR
FeNi (60)



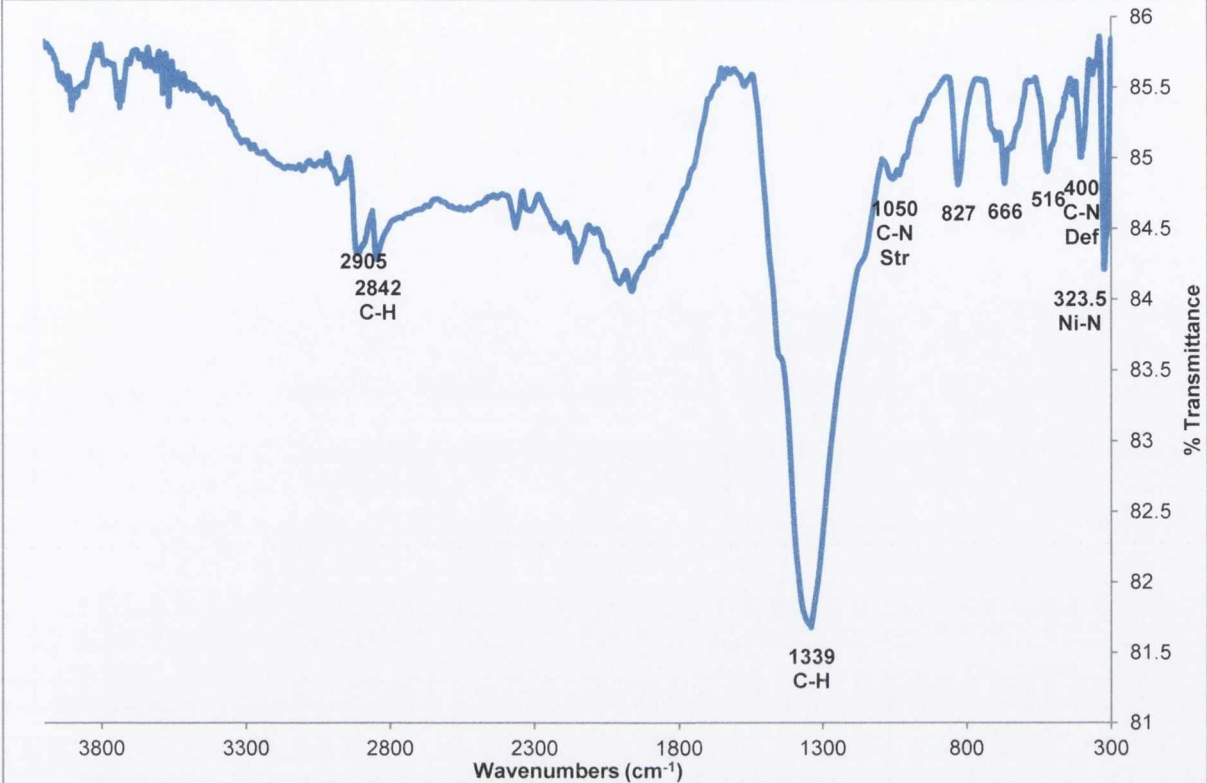
FeNi (61)



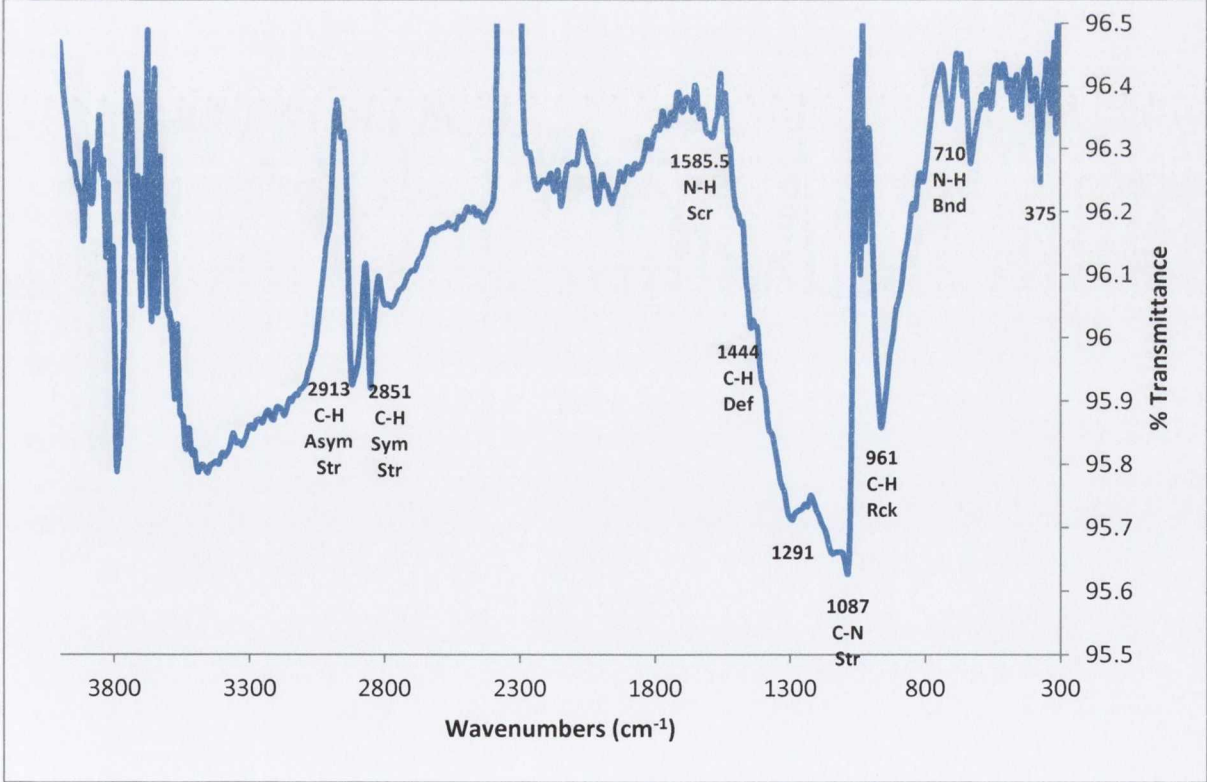
FeNi (62)



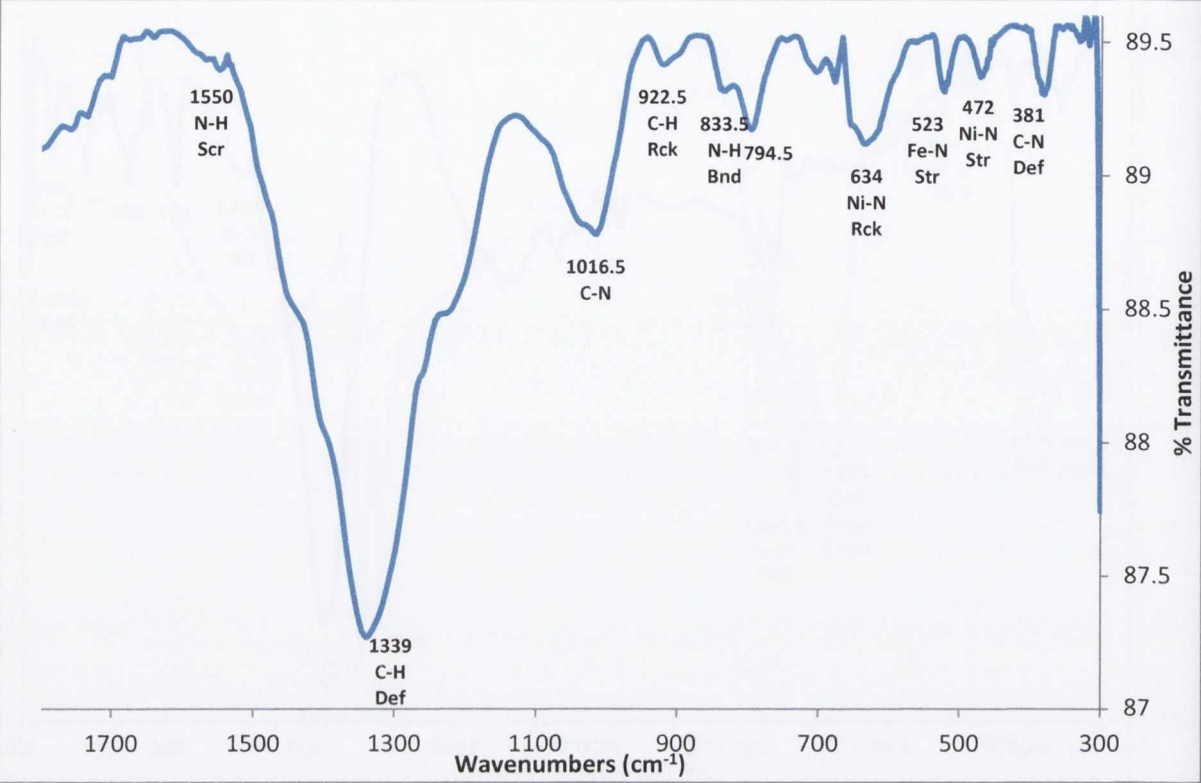
FeNi (63)



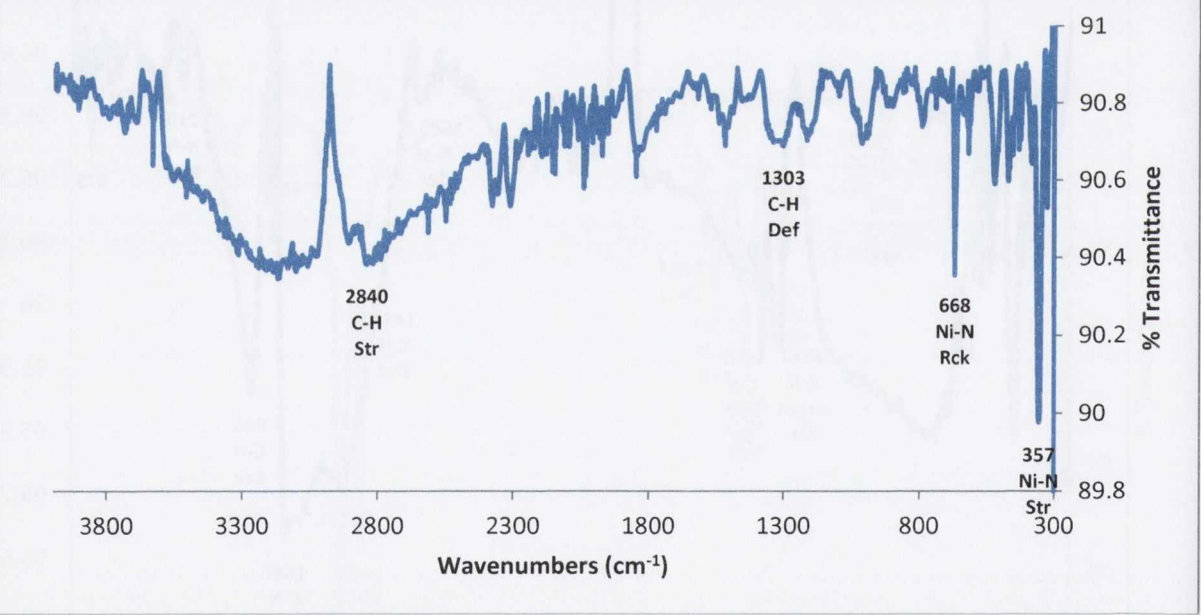
FeNi (63)

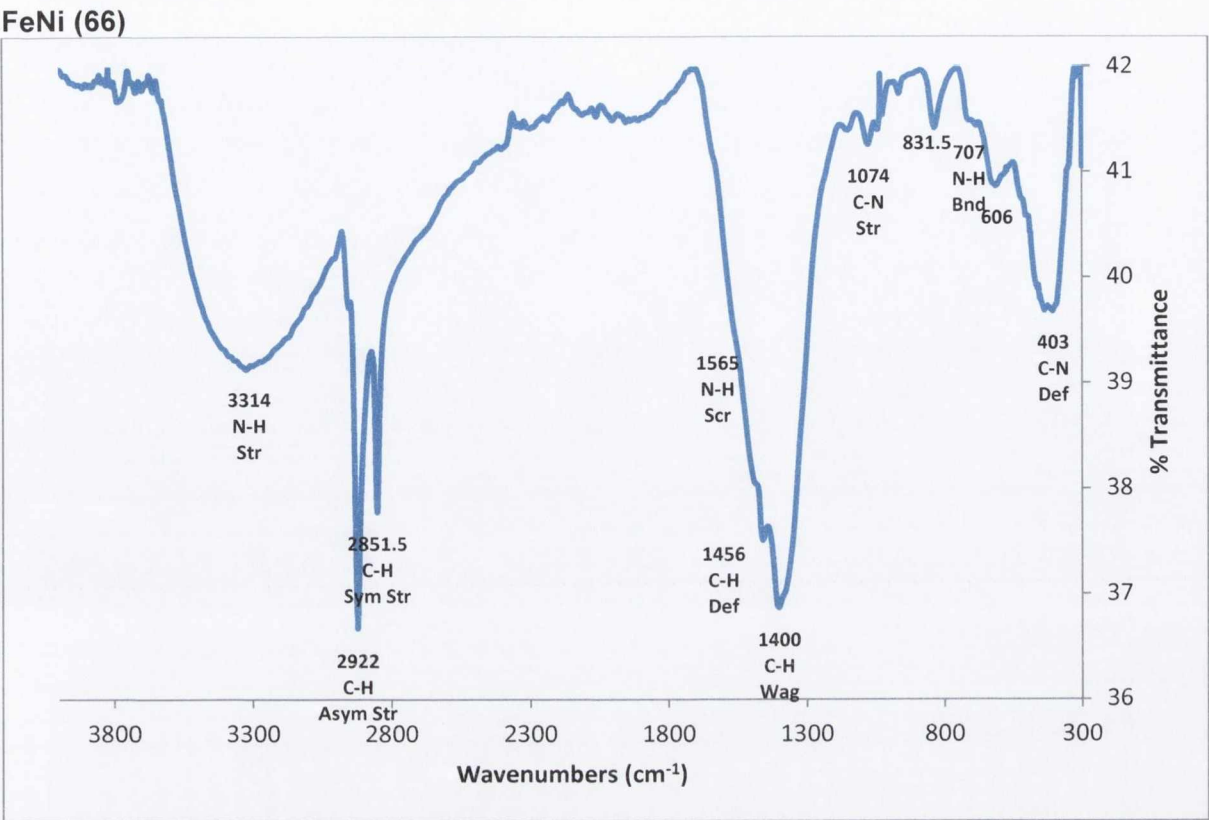


FeNi (64)



FeNi (65)



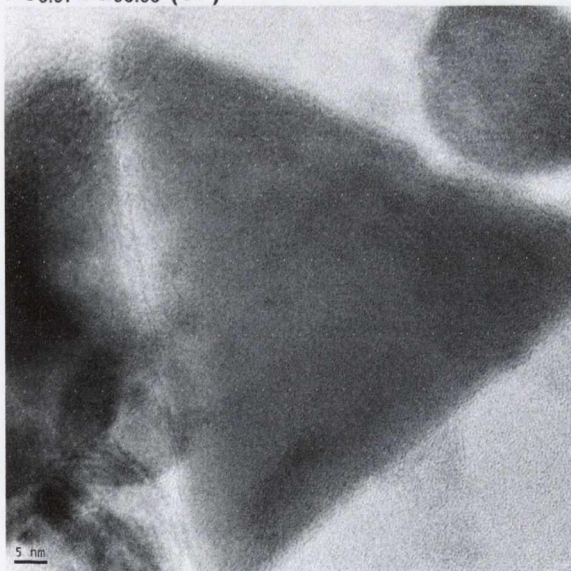


APPENDIX 4.4

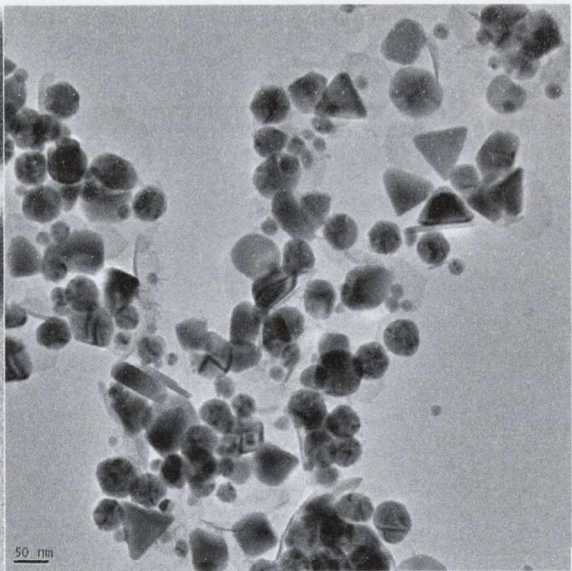
IRON-COPPER ALLOY NANOSTRUCTURES

A.4.4.1 TEM

$\text{Fe}_{6.97}\text{Cu}_{93.03}$ (67)

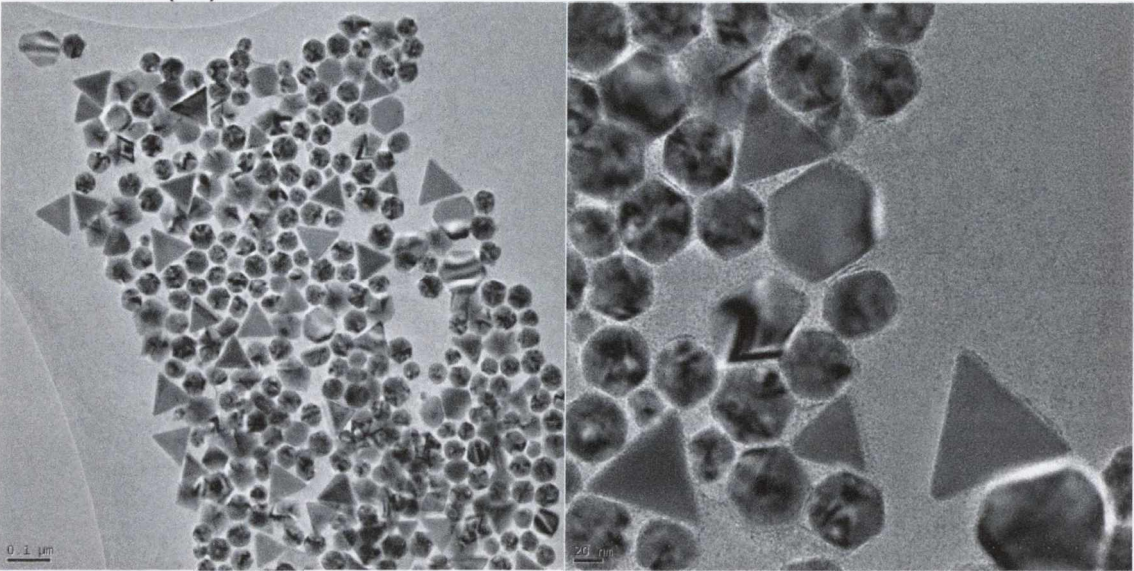


Scale Bar: 5 nm



Scale Bar: 50 nm

Fe_{4.18}Cu_{95.82} (68)

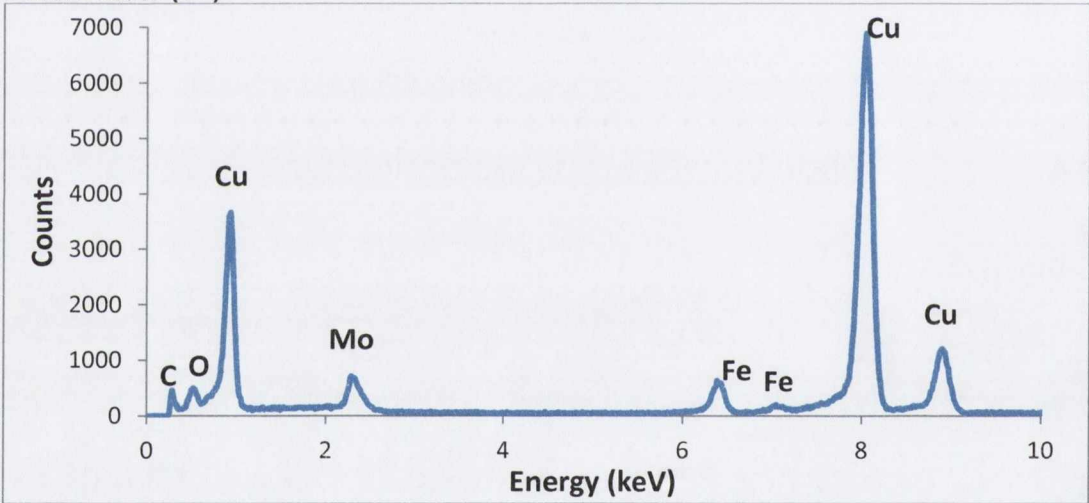


Scale Bar: 100 nm

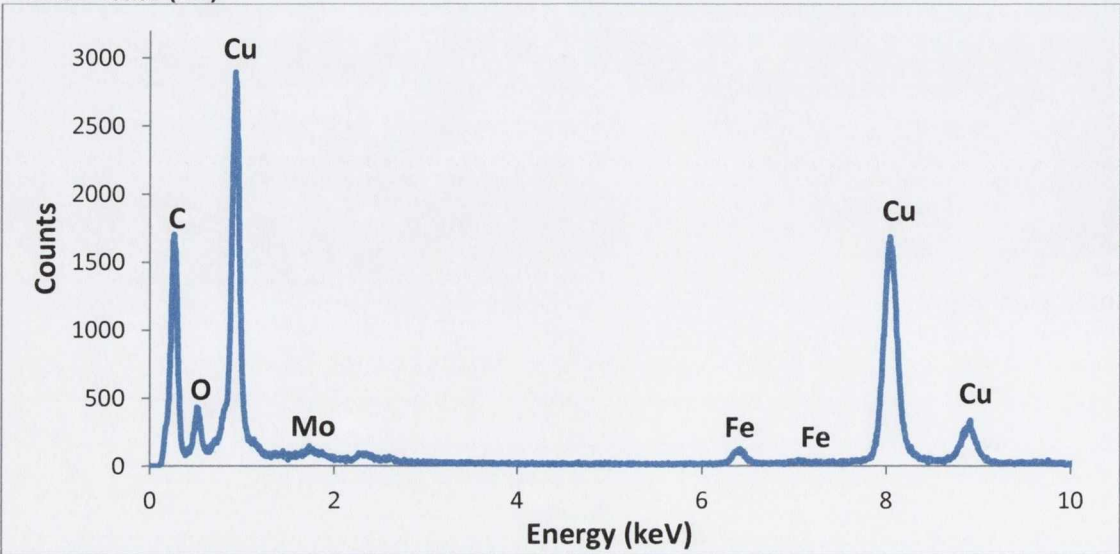
Scale Bar: 20 nm

A.4.4.2 EDX

Fe_{6.97}Cu_{93.03} (67)

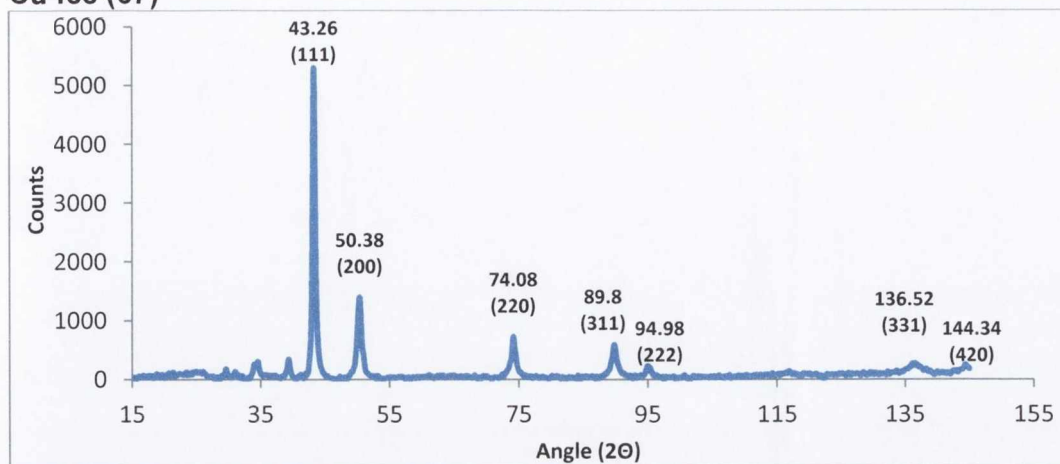


Fe_{4.18}Cu_{95.82} (68)

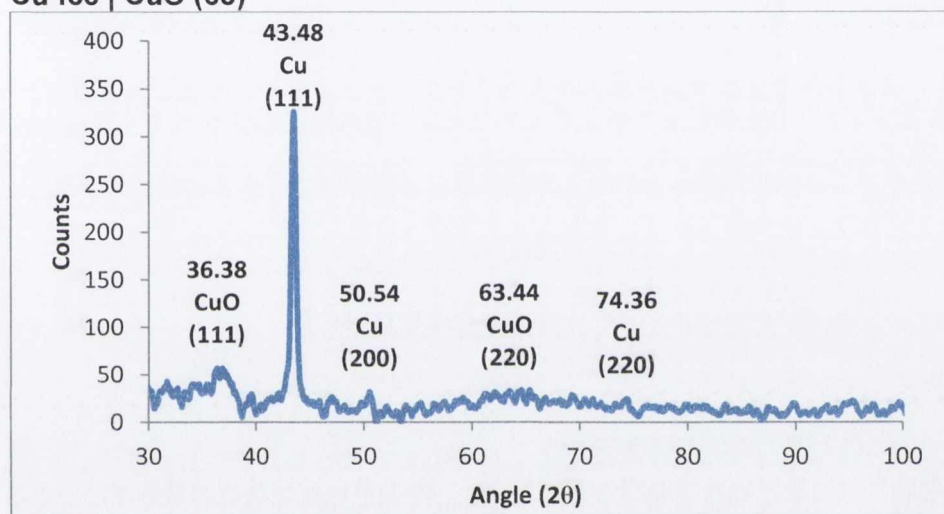


A.4.4.3 XRD

Cu fcc (67)

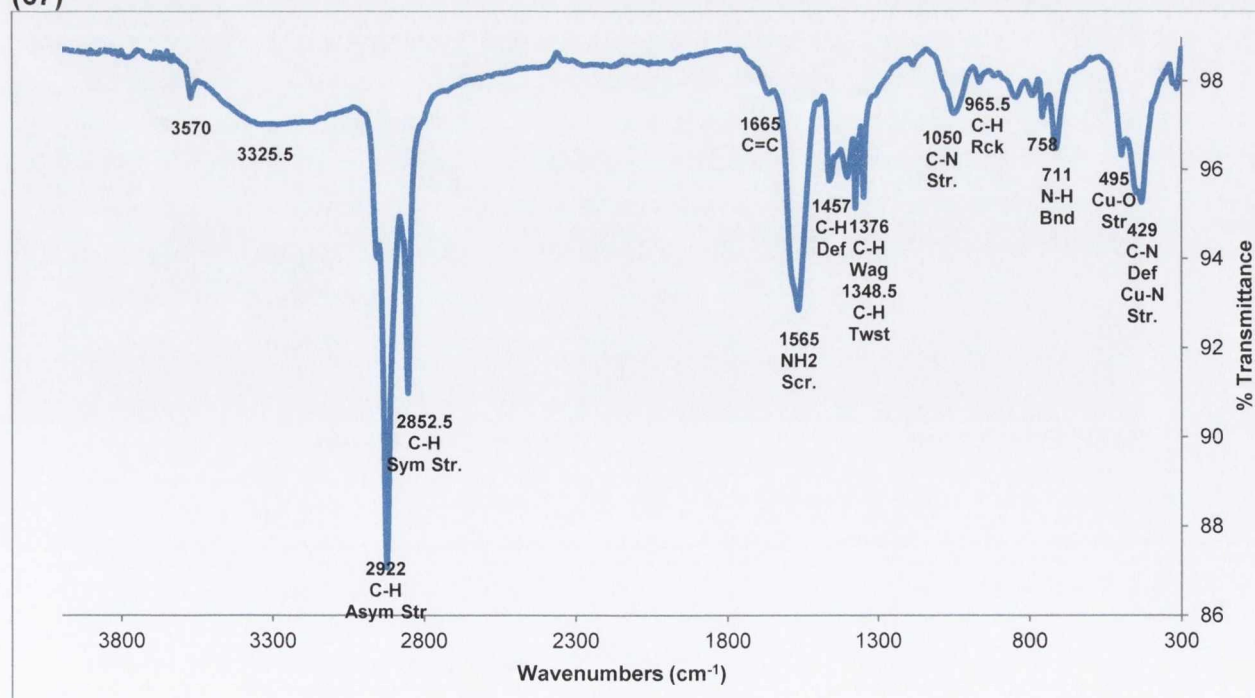


Cu fcc | CuO (68)

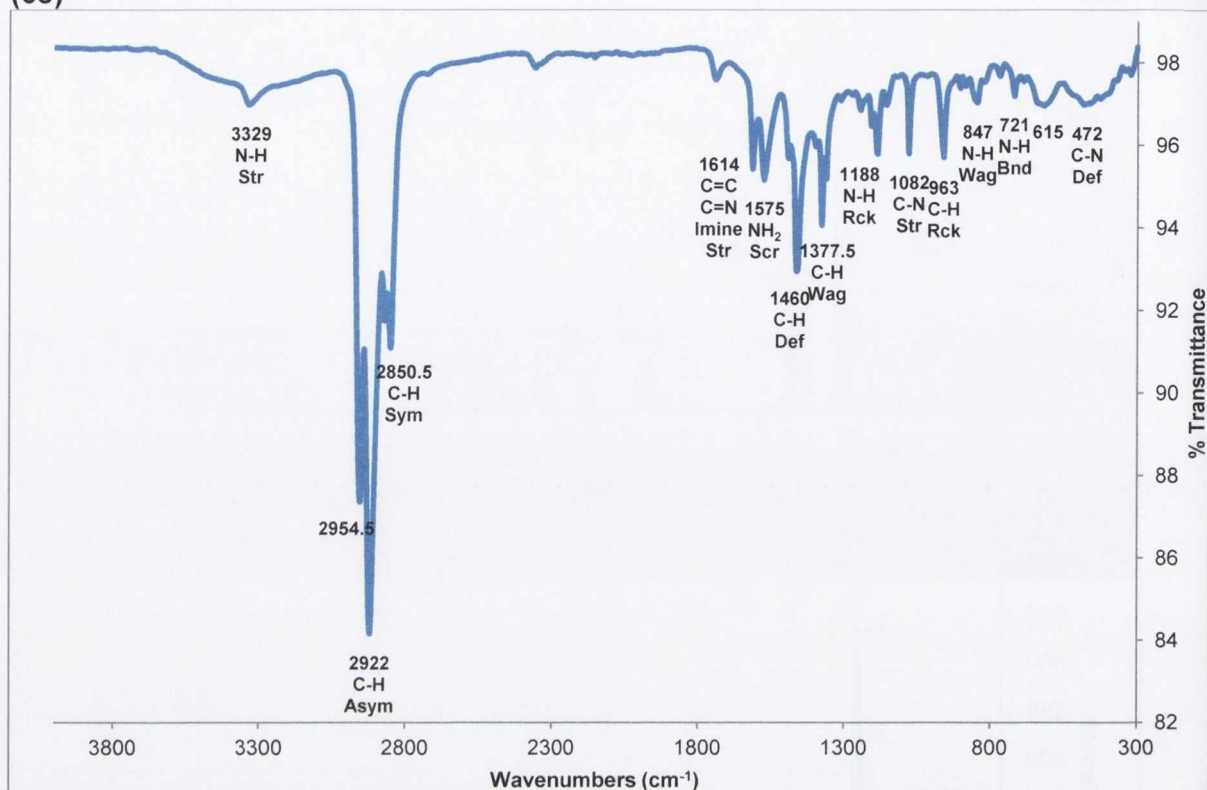


A.4.4.4 FTIR

(67)



(68)

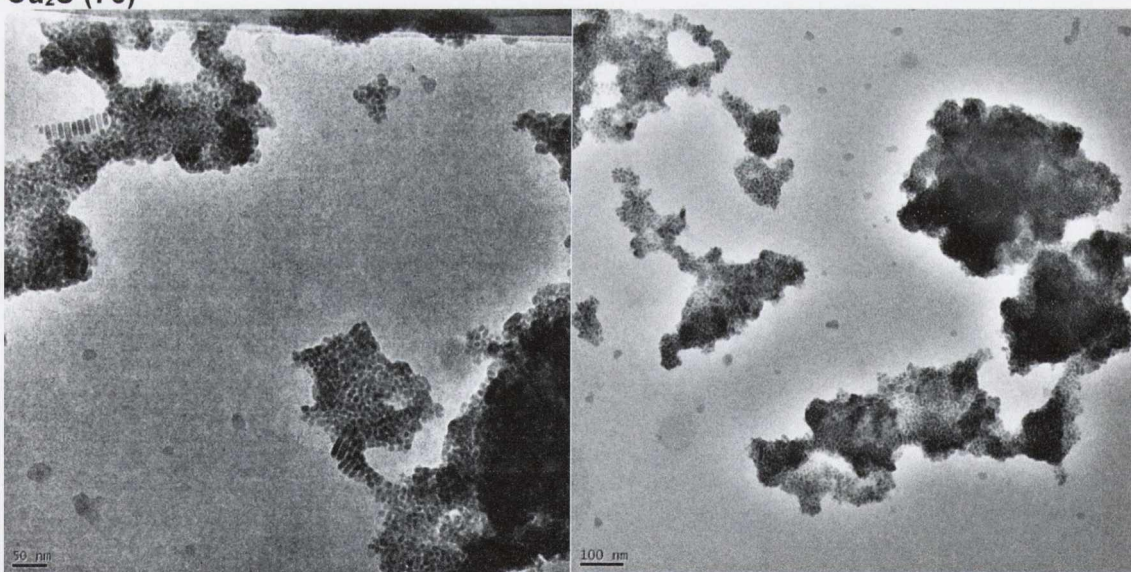


APPENDIX 4.5

COPPER SULFIDE NANOSTRUCTURES

A.4.5.1 - TEM

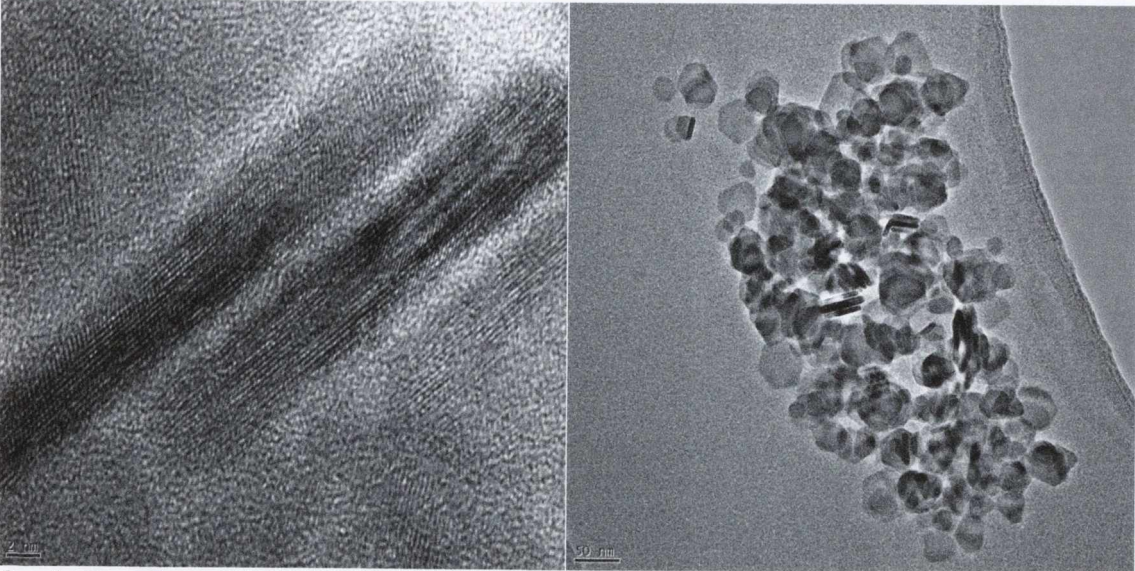
Cu_2S (76)



Scale Bar: 50 nm

Scale Bar: 100 nm

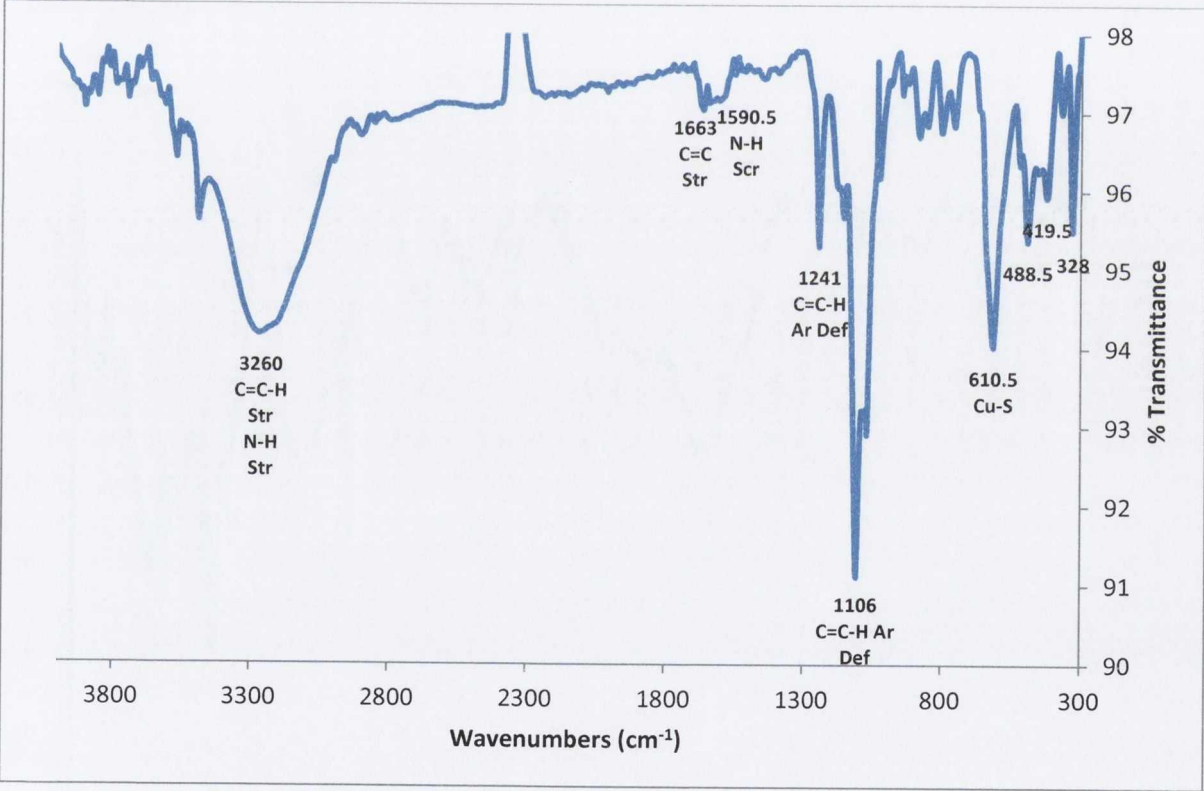
CuS (77)



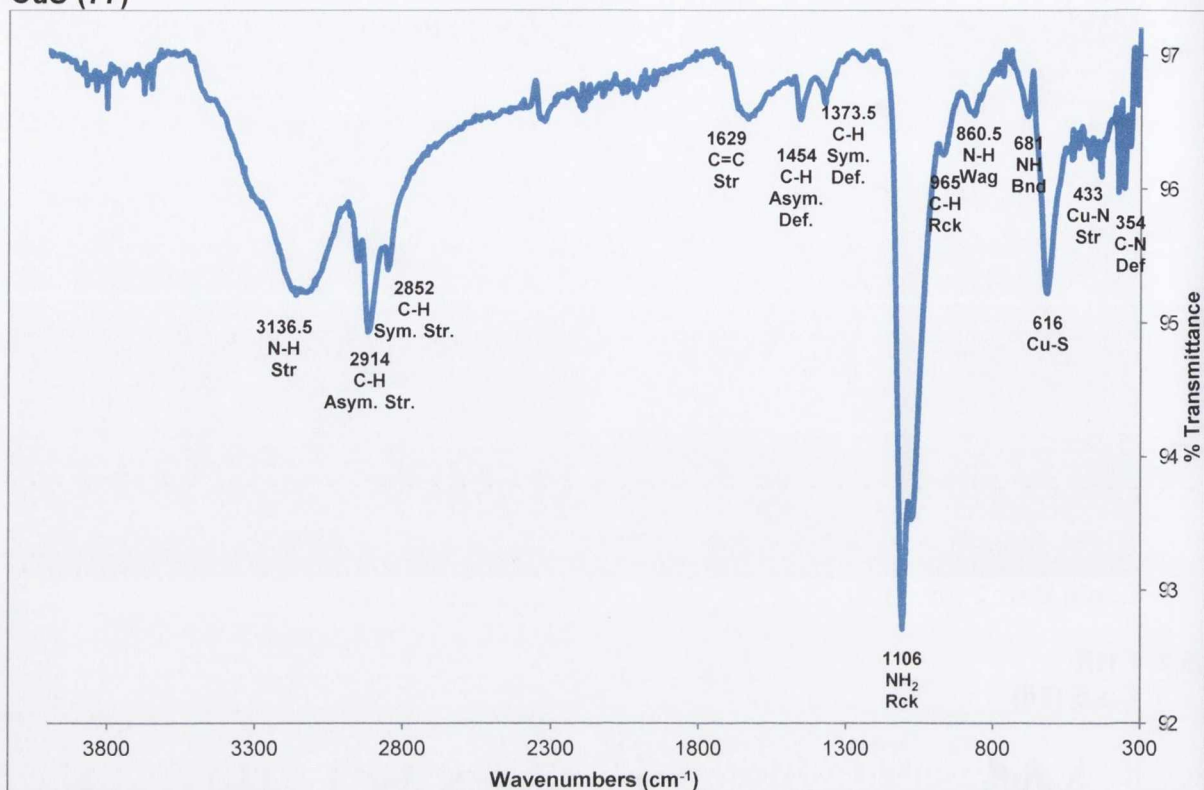
Scale Bar: 2 nm

Scale Bar: 50 nm

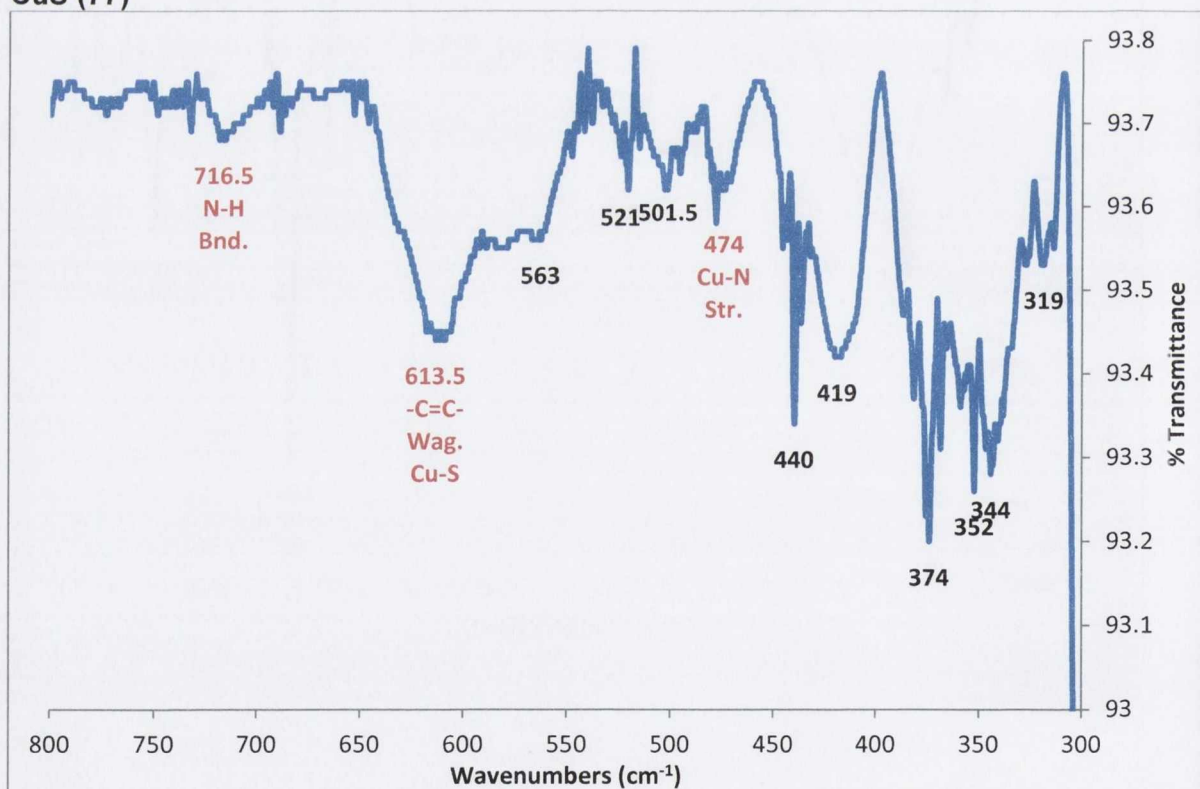
A.4.5.2 FTIR
Cu₂S (76)



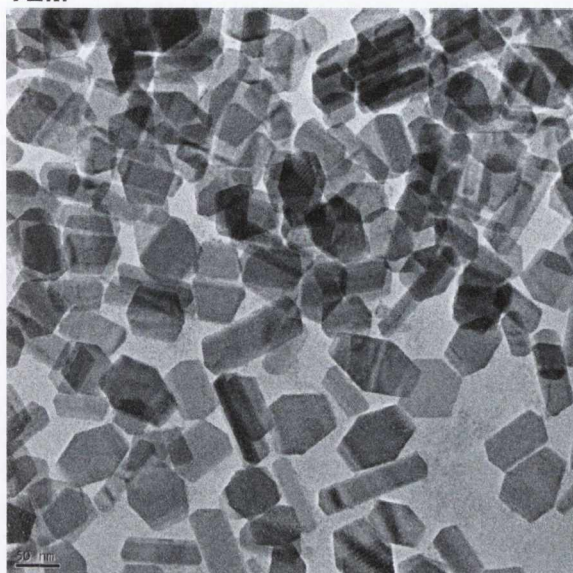
CuS (77)



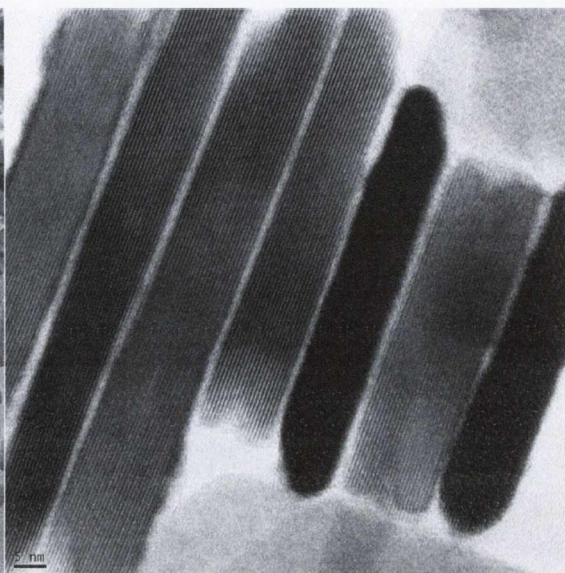
CuS (77)



A.4.6.1 TEM

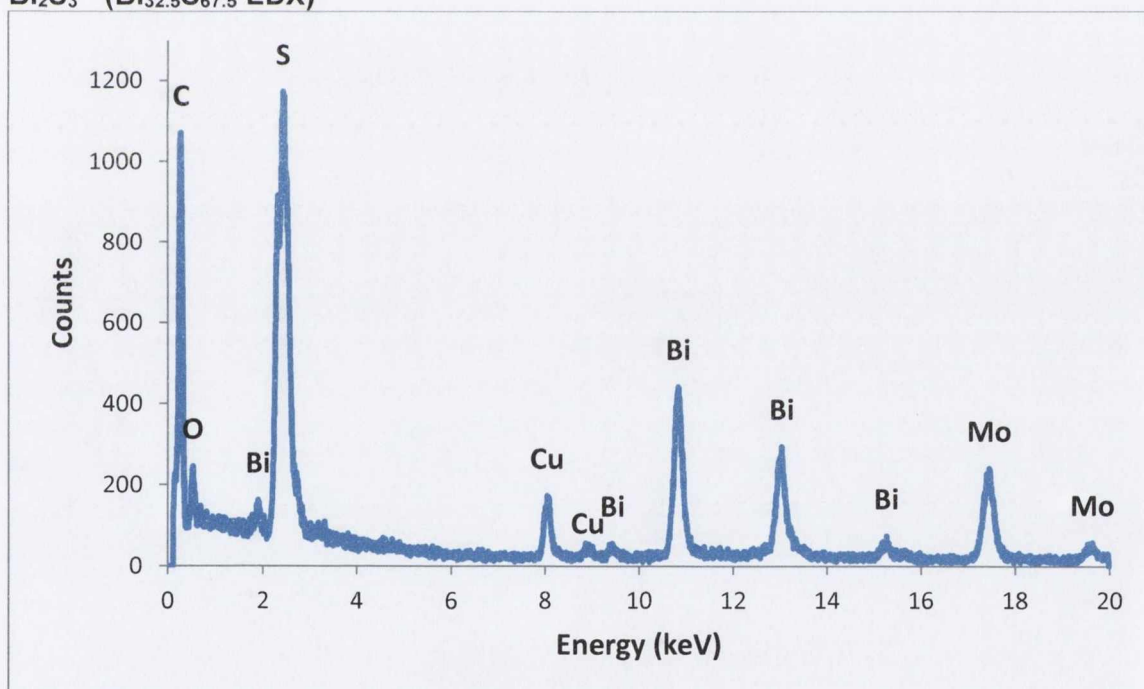


Scale Bar: 50 nm

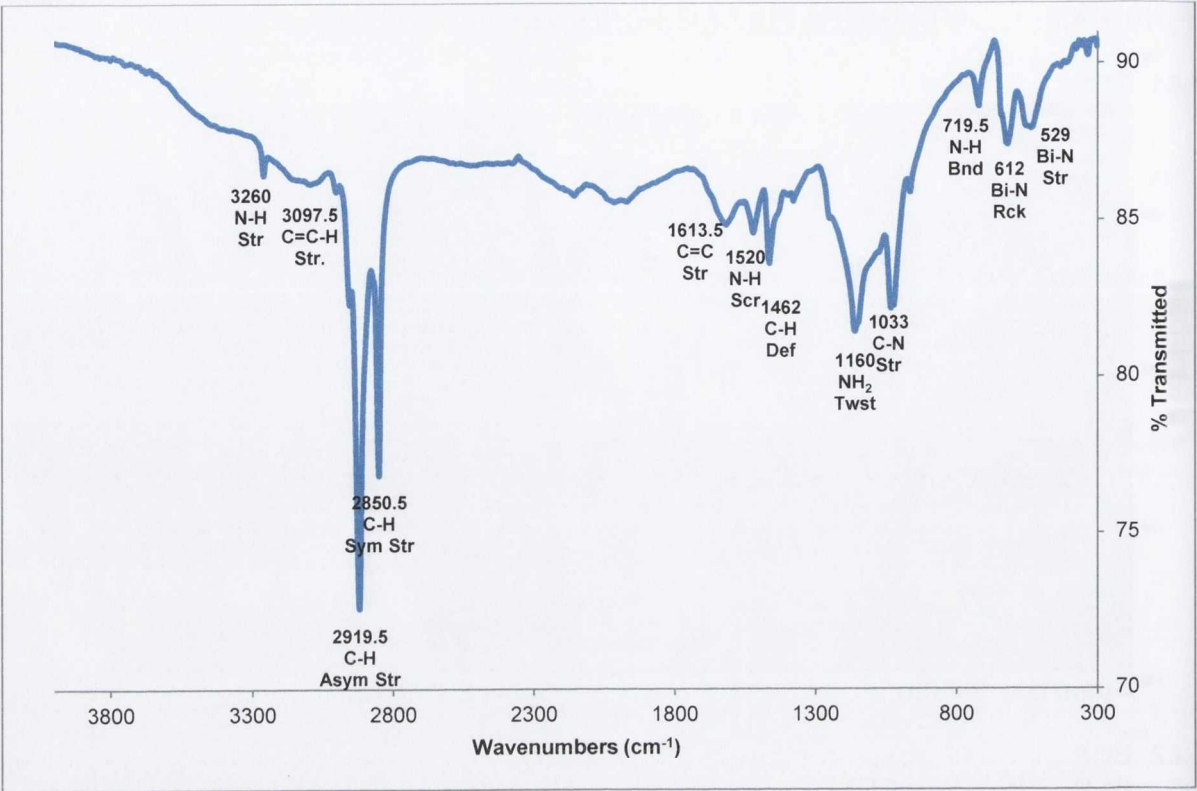


Scale Bar: 5 nm

A.4.6.2 EDX

 Bi_2S_3 ($\text{Bi}_{32.5}\text{S}_{67.5}$ EDX)

A.4.6.3 FTIR

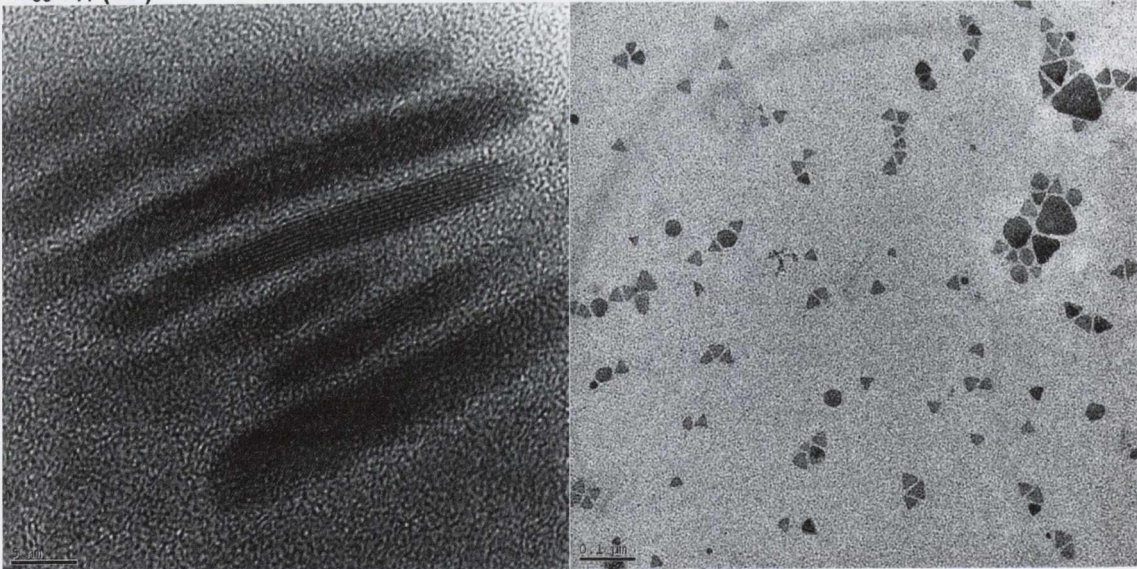


APPENDIX 4.7

NICKEL SULFIDE NANOSTRUCTURES

A.4.7.1 TEM

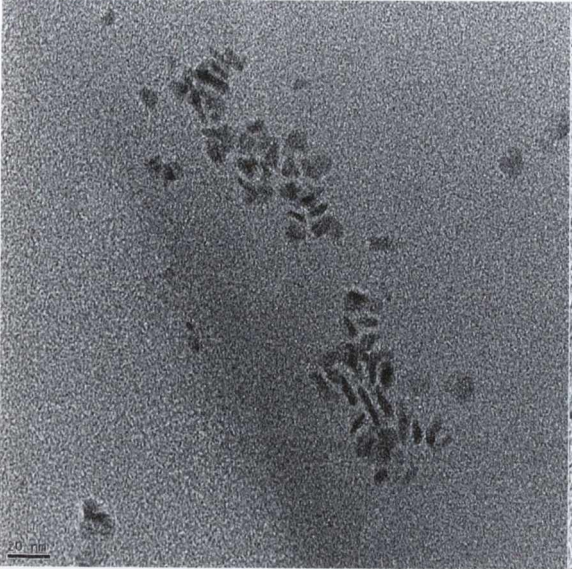
Ni₅₆S₄₄ (79)



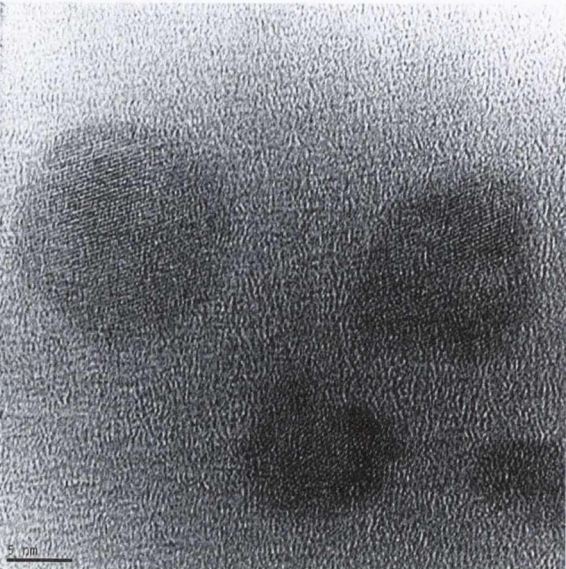
Scale Bar: 5 nm

Scale Bar: 100 nm

Ni₅₂S₄₈ (80)



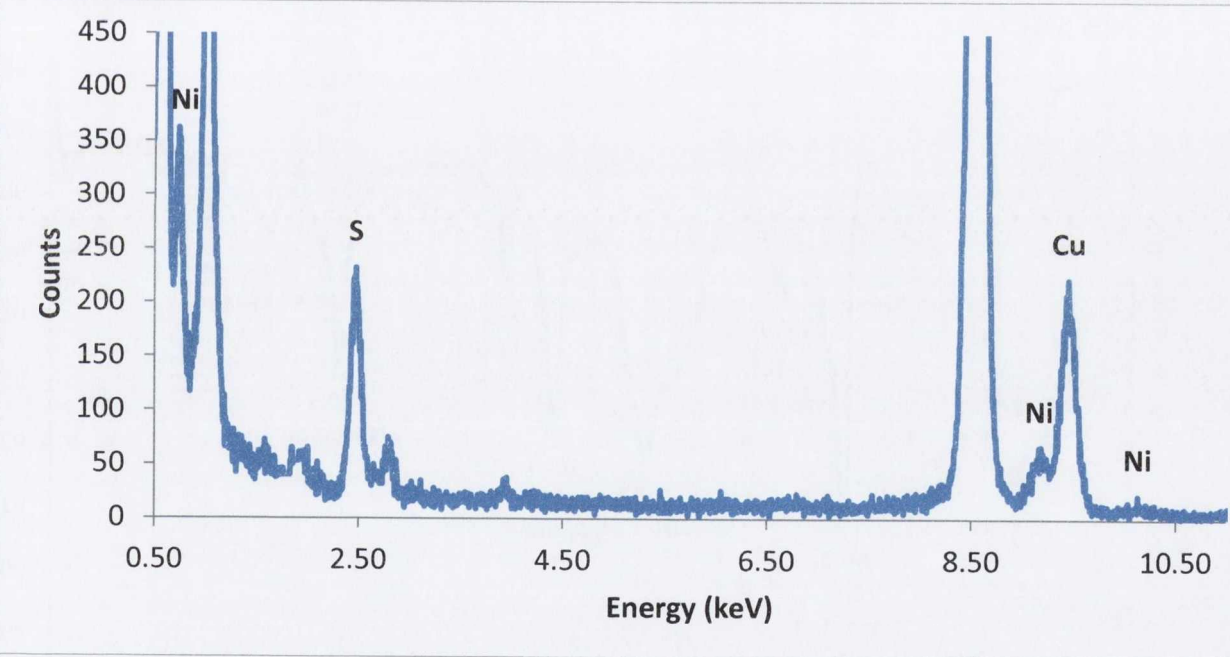
Scale Bar: 20 nm



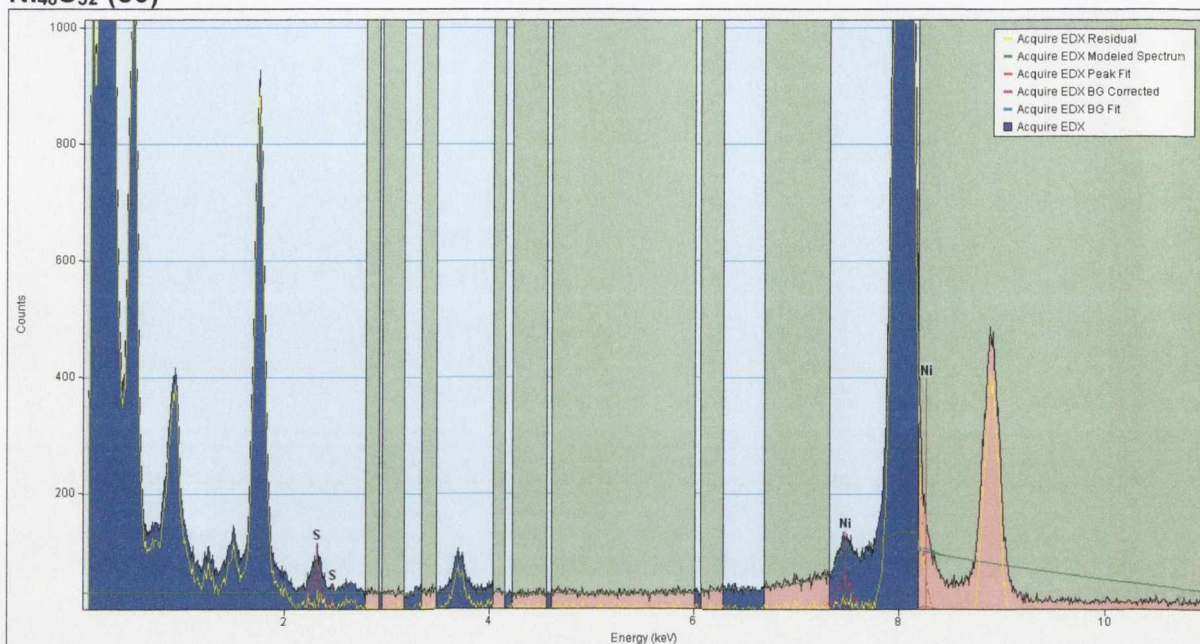
Scale Bar: 5 nm

A.4.7.2 EDX (fcc = Ni₂S₃, NiS₂)

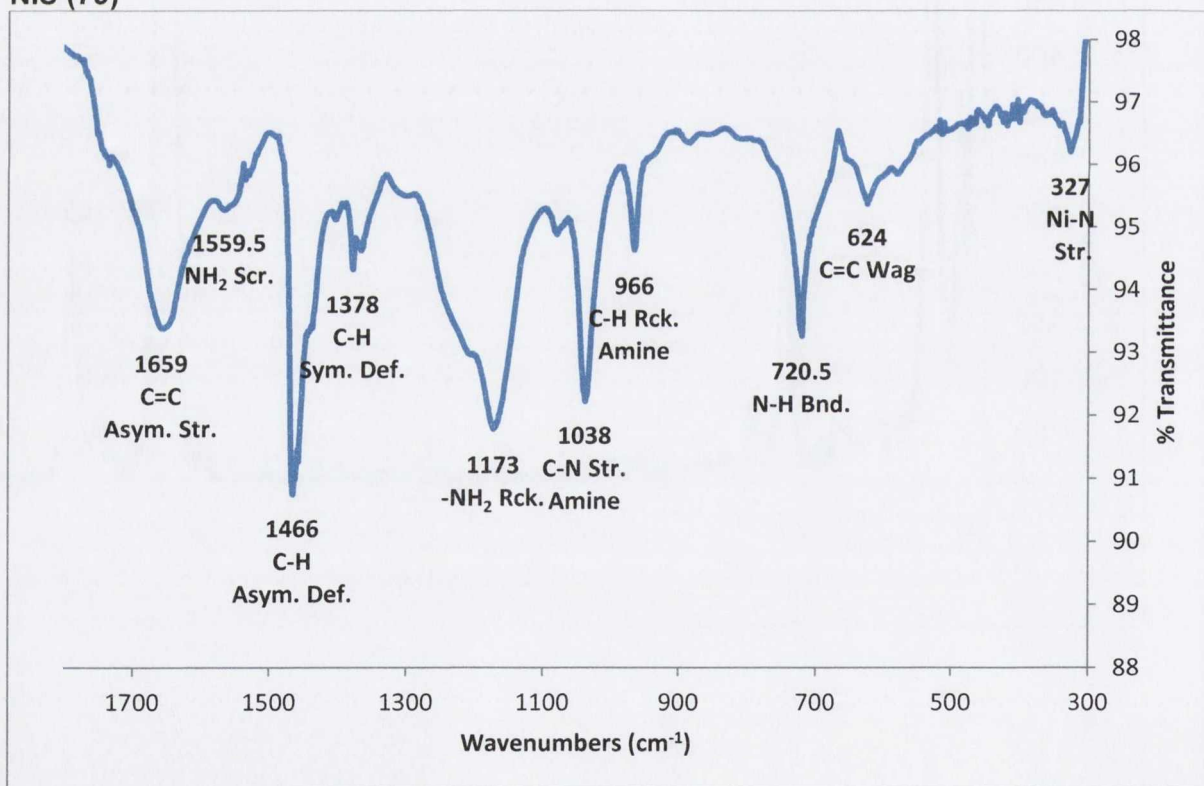
Ni₅₆S₄₄ (79)



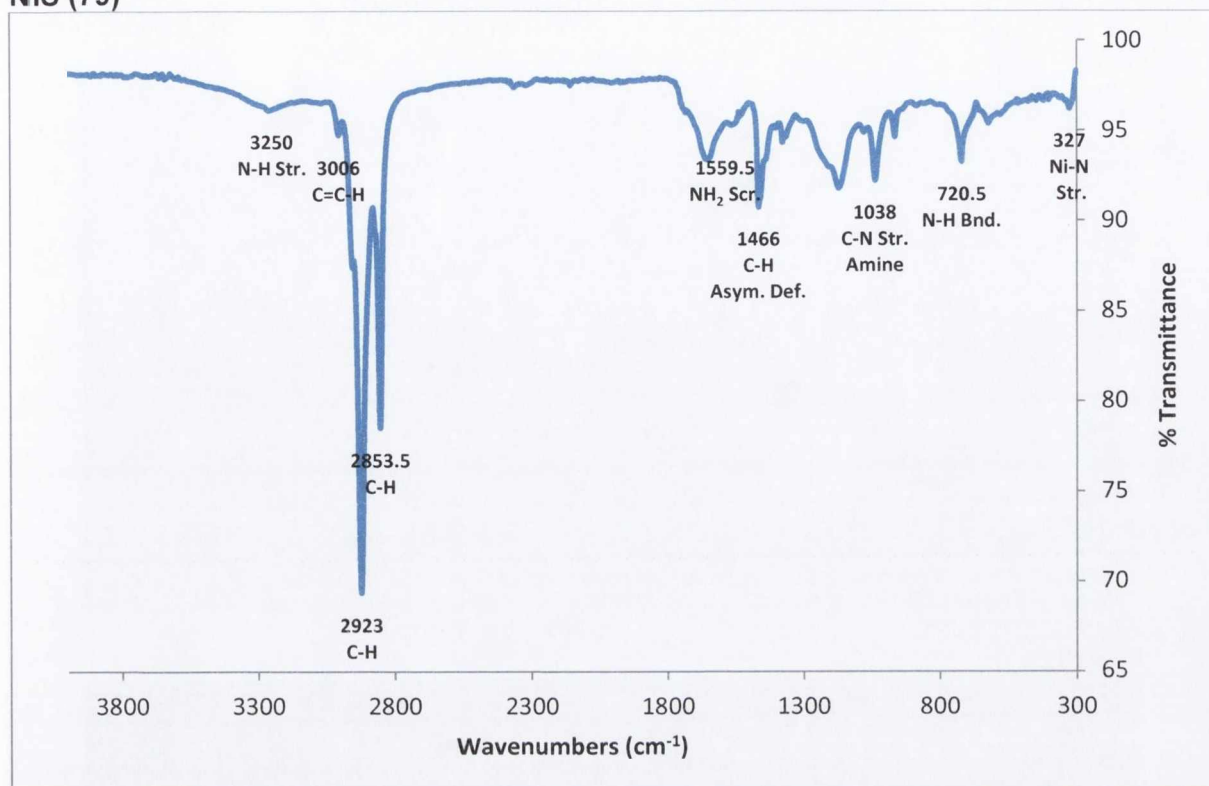
Ni₄₈S₅₂ (80)



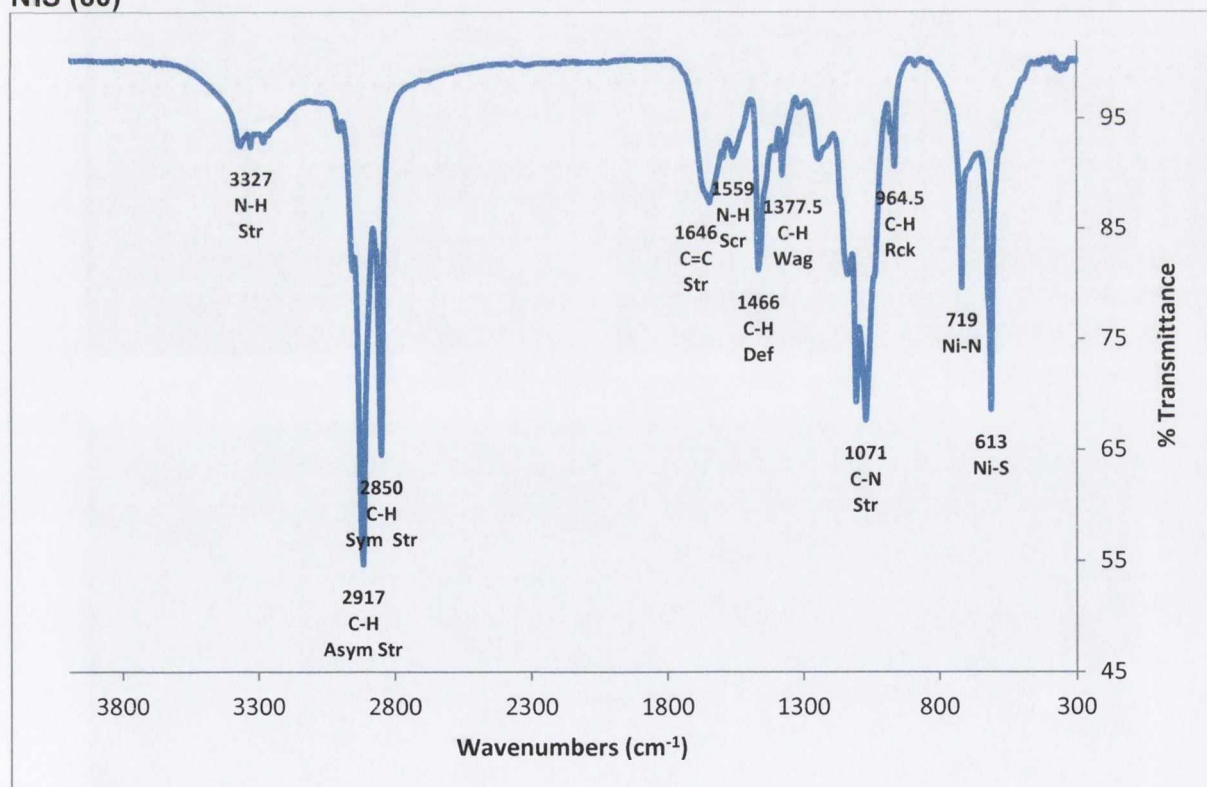
A.4.7.3 FTIR NiS (79)

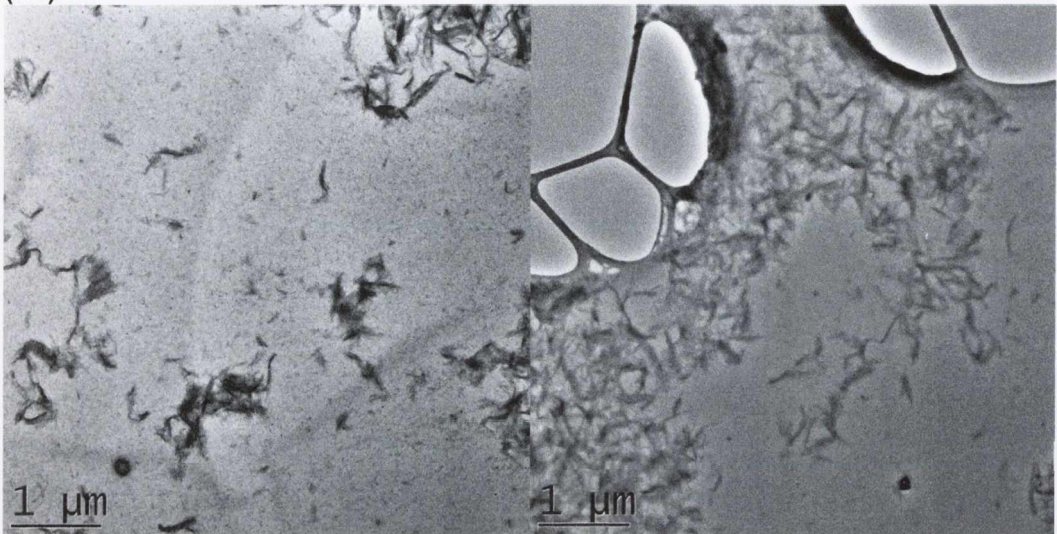


NiS (79)



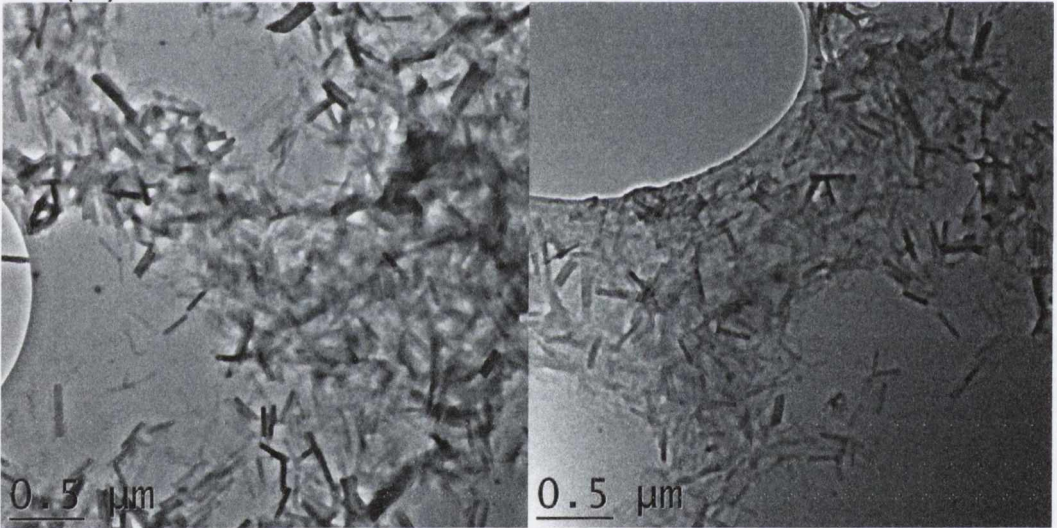
NiS (80)





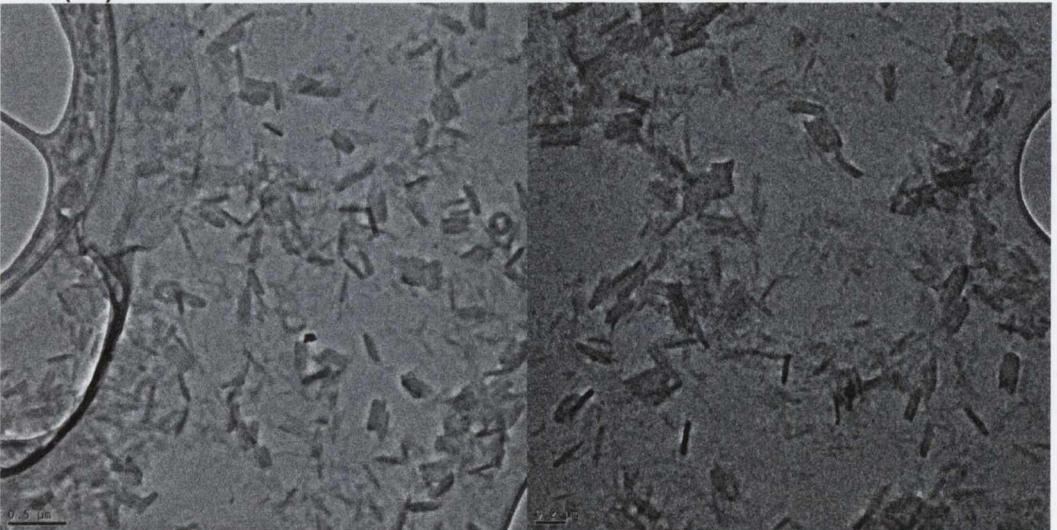
Scale Bar: 1 μm
FeS (82)

Scale Bar: 1 μm



Scale Bar: 500 nm
FeS (83)

Scale Bar: 500 nm

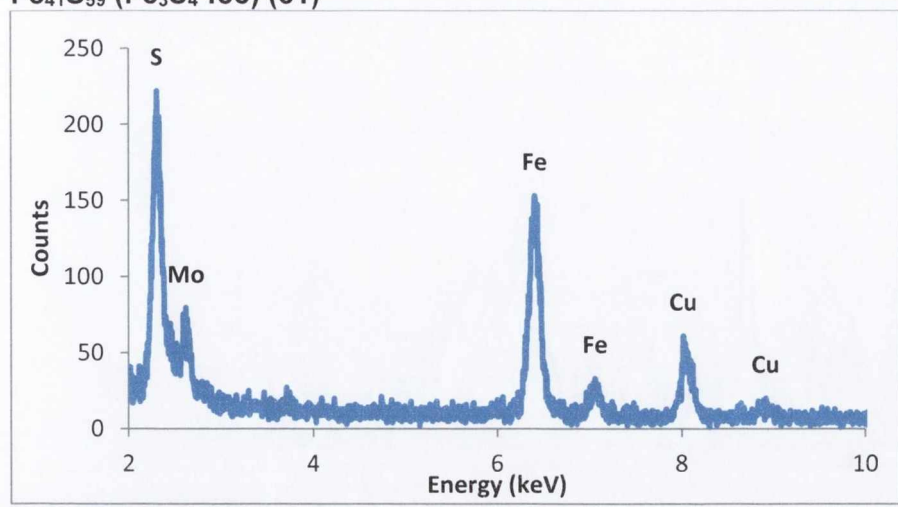


Scale Bar: 500 nm

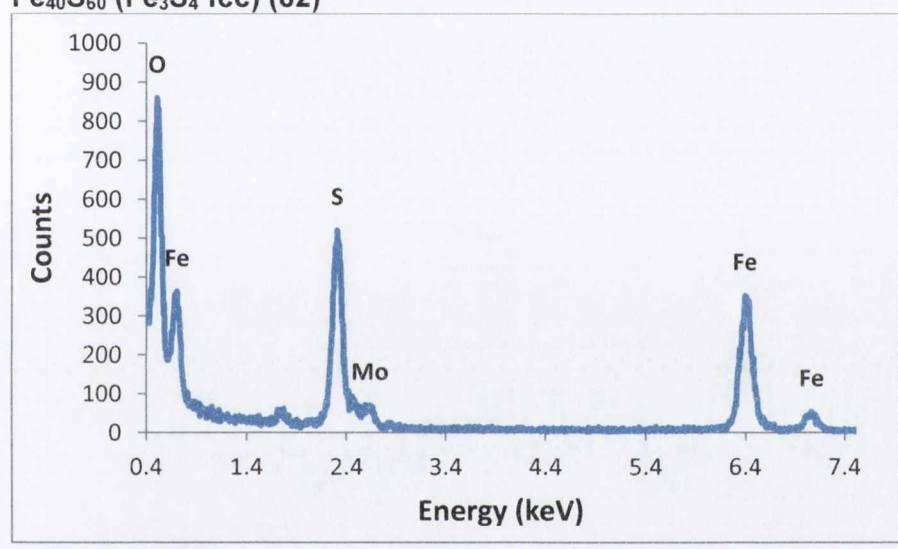
Scale Bar: 200 nm

A.4.8.2 - EDX

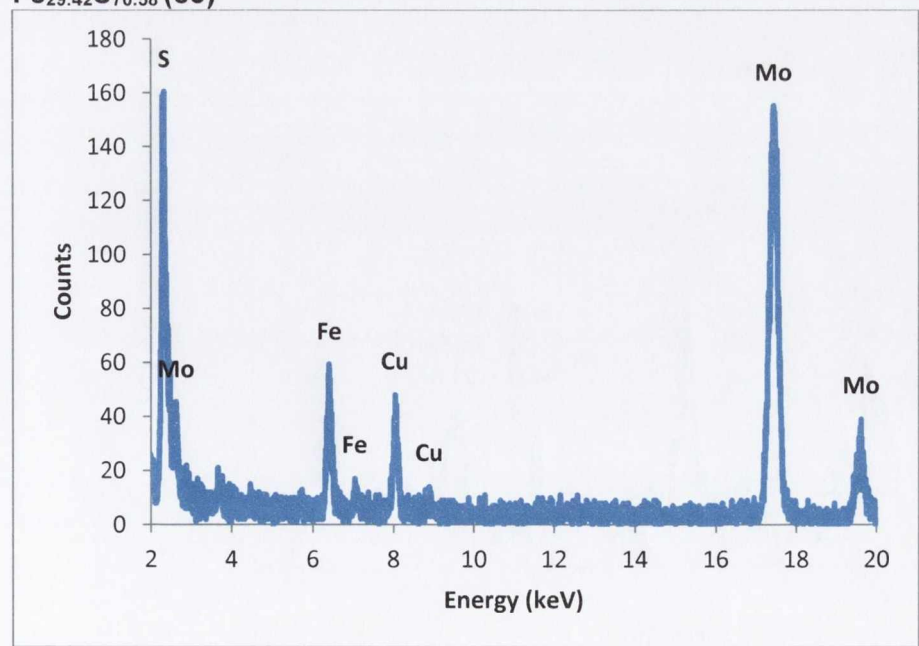
Fe₄₁S₅₉ (Fe₃S₄ fcc) (81)



Fe₄₀S₆₀ (Fe₃S₄ fcc) (82)

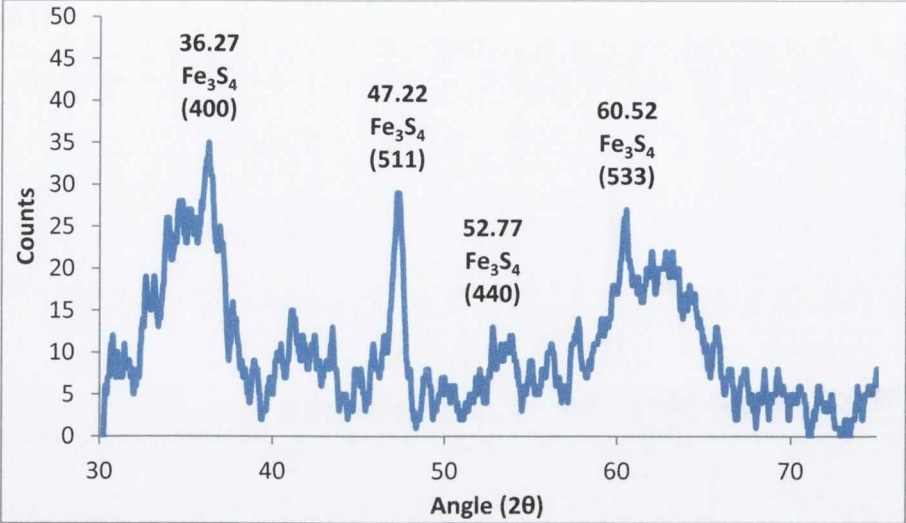


Fe_{29.42}S_{70.58} (83)

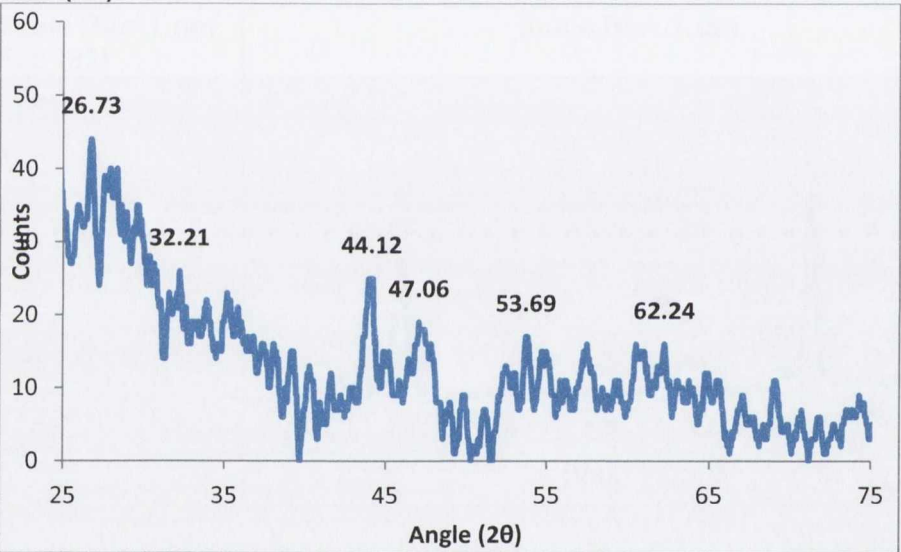


A.4.8.3 - XRD

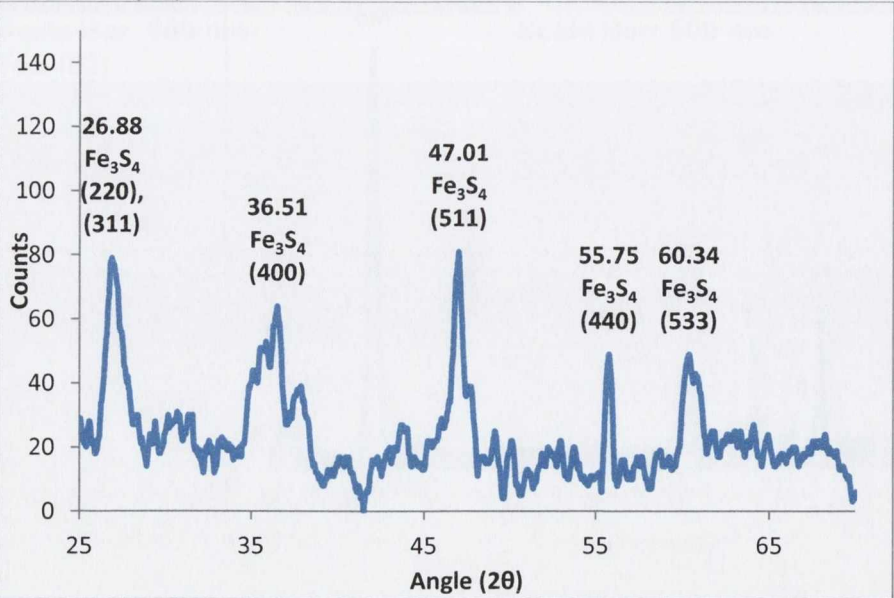
Fe₃S₄ fcc (81)



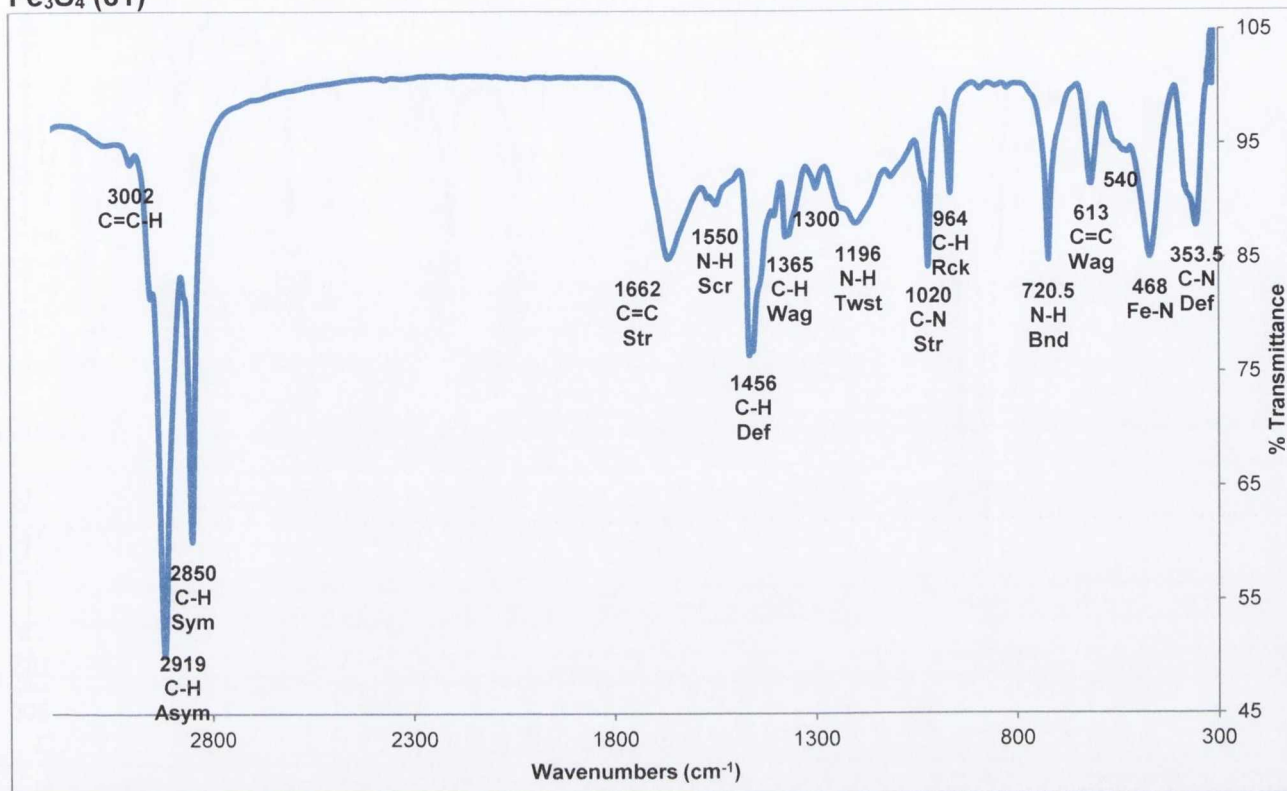
FeS (82)



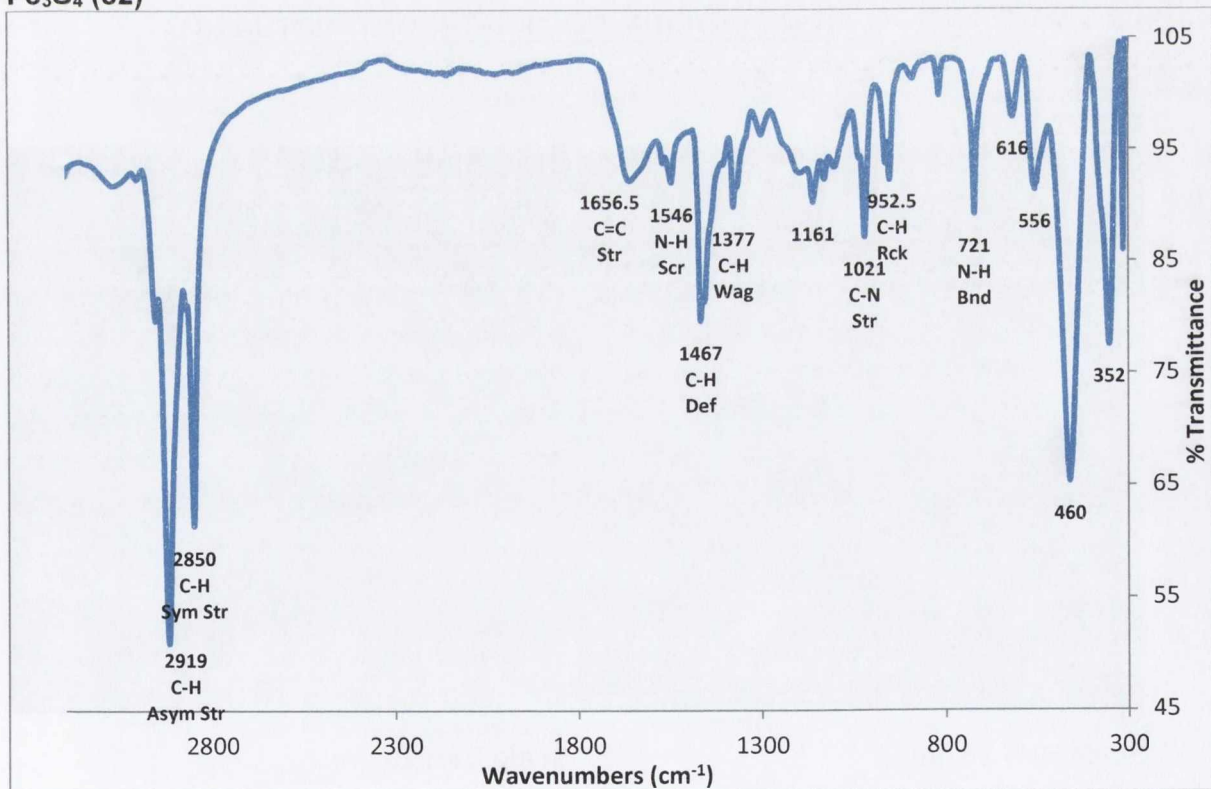
Fe₃S₄ fcc (83)



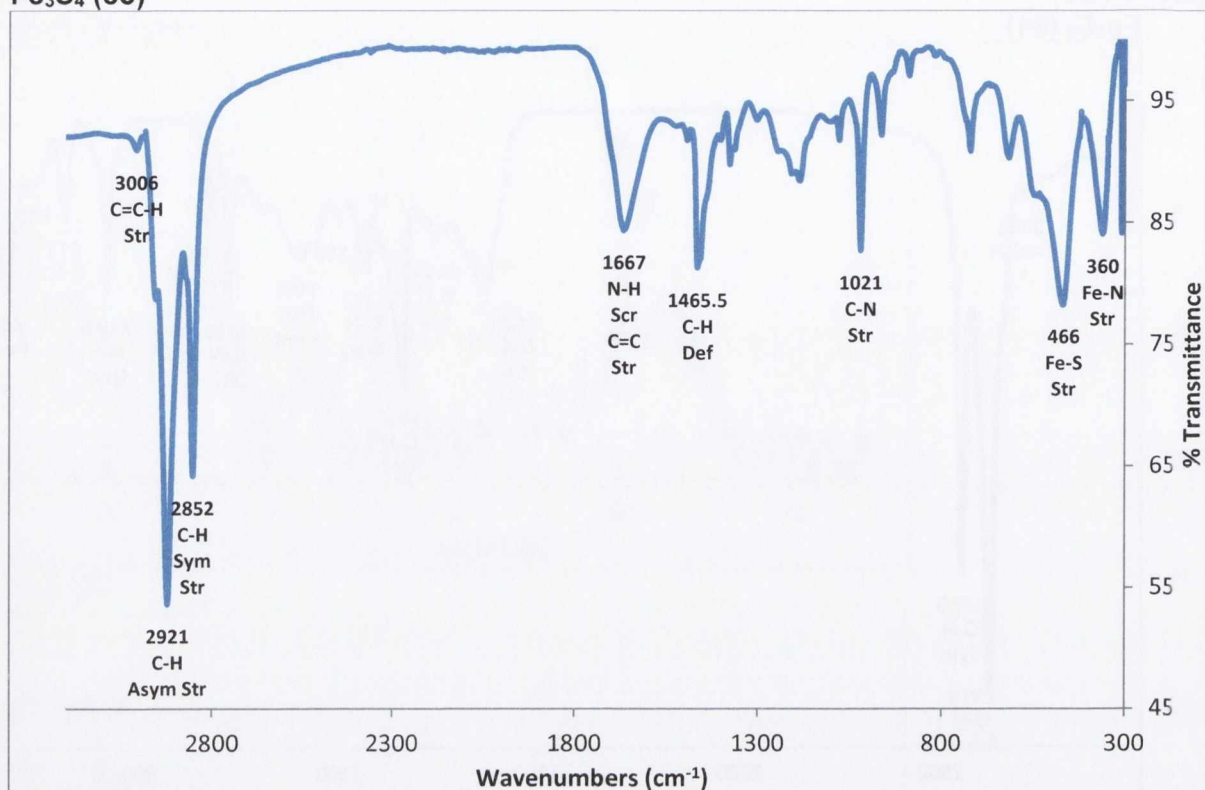
4.8.4 - FTIR Fe₃S₄ (81)



Fe₃S₄ (82)



Fe₃S₄ (83)

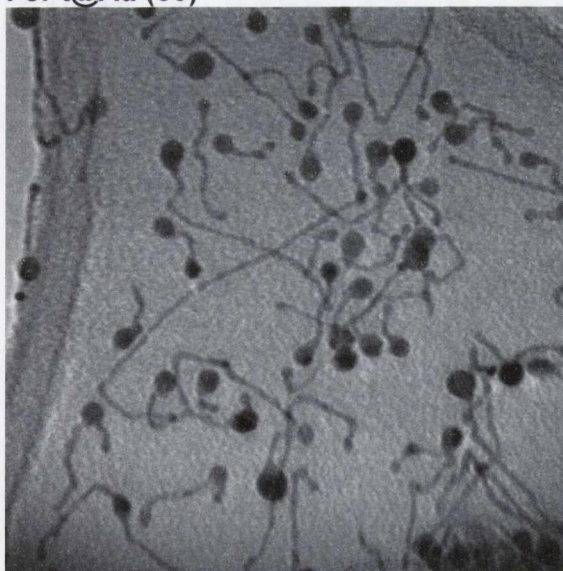


APPENDIX 4.9

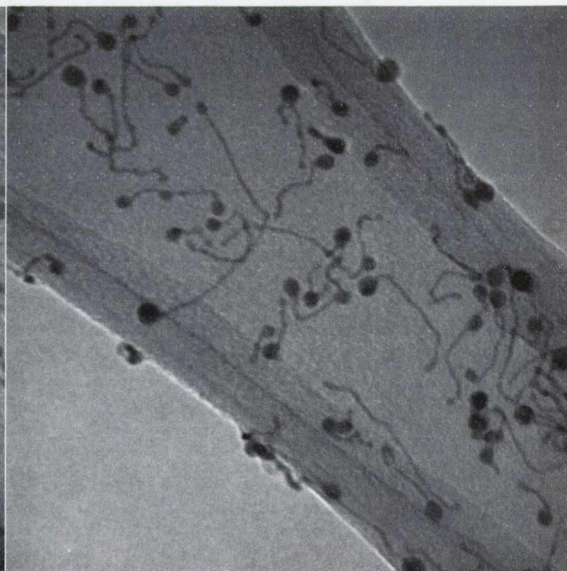
IRON-PLATINUM/GOLD HETEROSTRUCTURES

A.4.9.1 - TEM

FePt@Au (85)

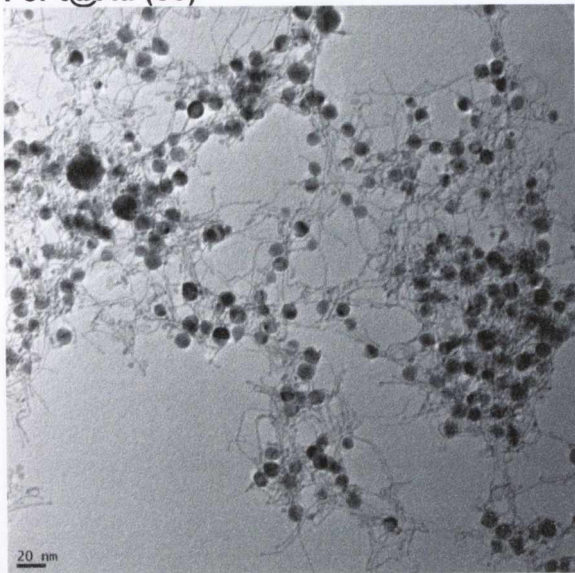


31196.tif
Print Mag: 1290000x @ 7.0 in
Scale Bar: 20 nm

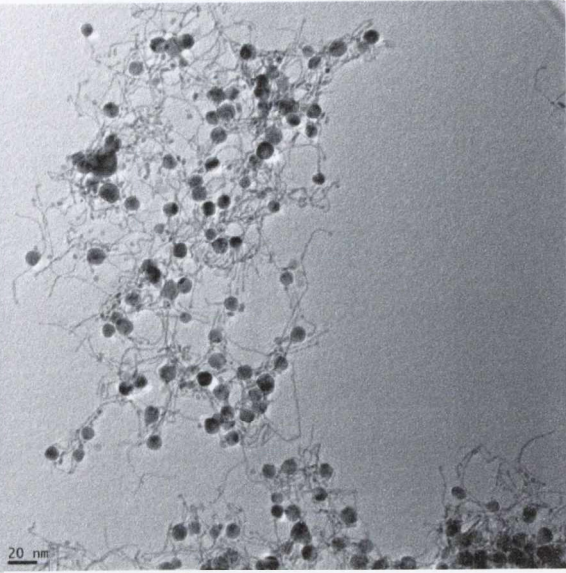


31199.tif
Print Mag: 975000x @ 7.0 in
Scale Bar: 20 nm

FePt@Au (86)

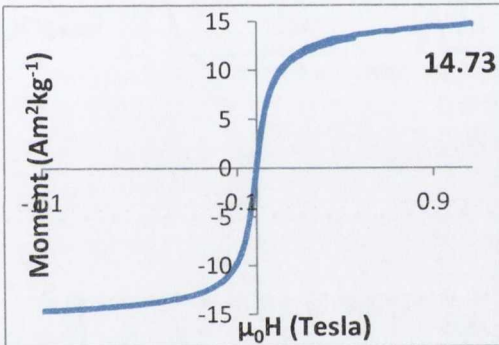


Scale Bar: 20 nm

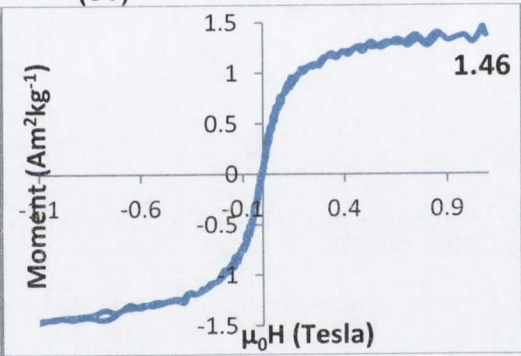


Scale Bar: 20 nm

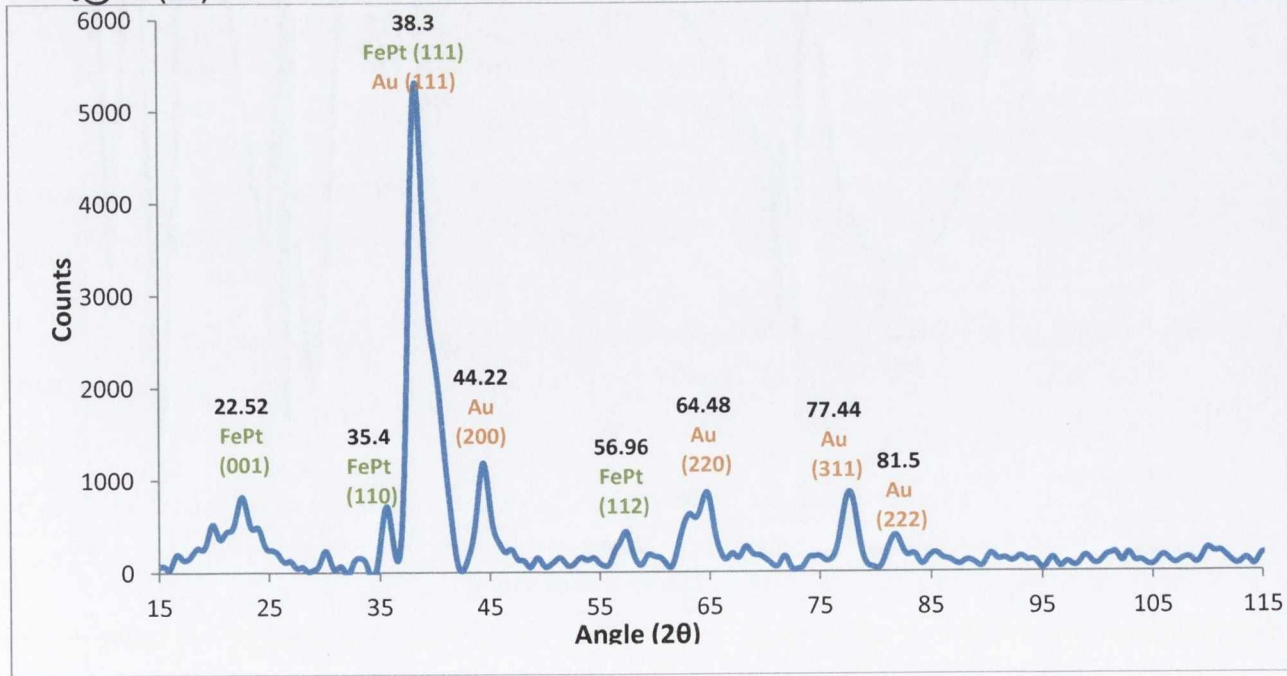
A.4.9.2 VSM
(85)



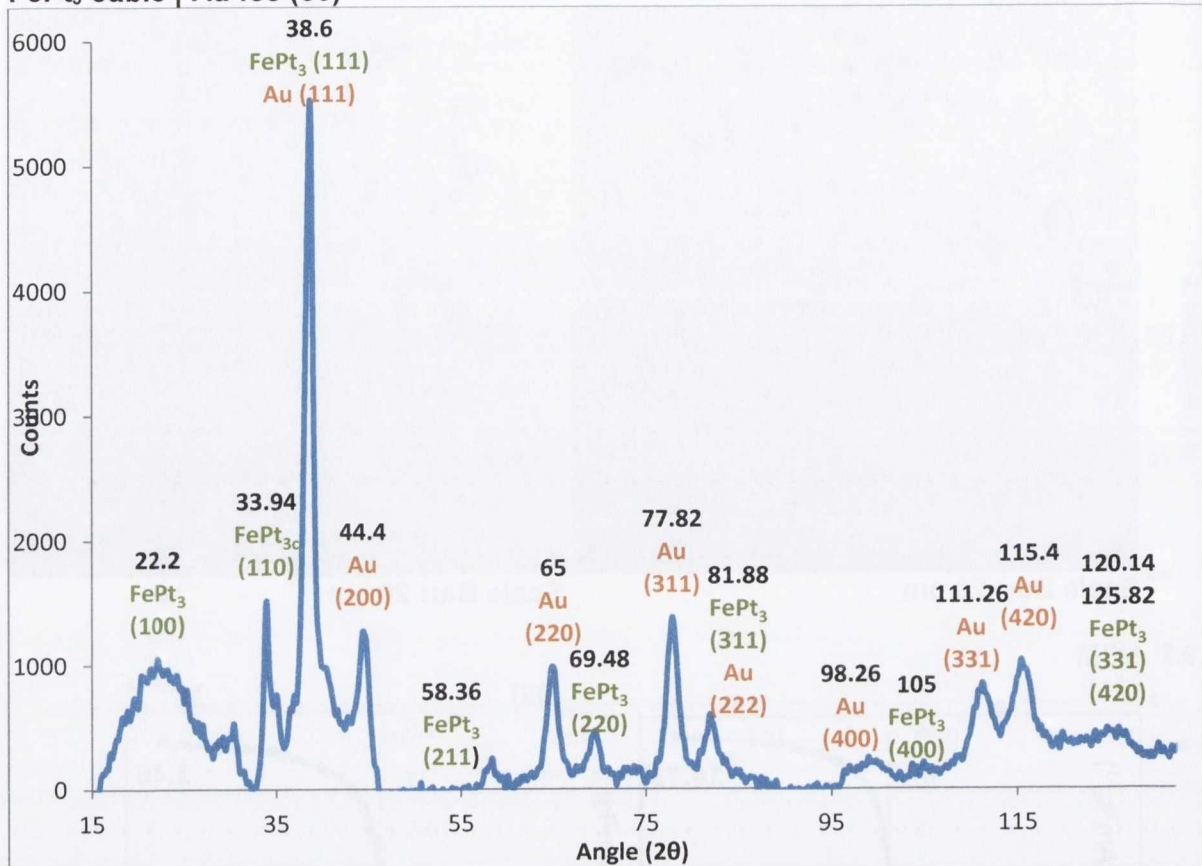
(86)



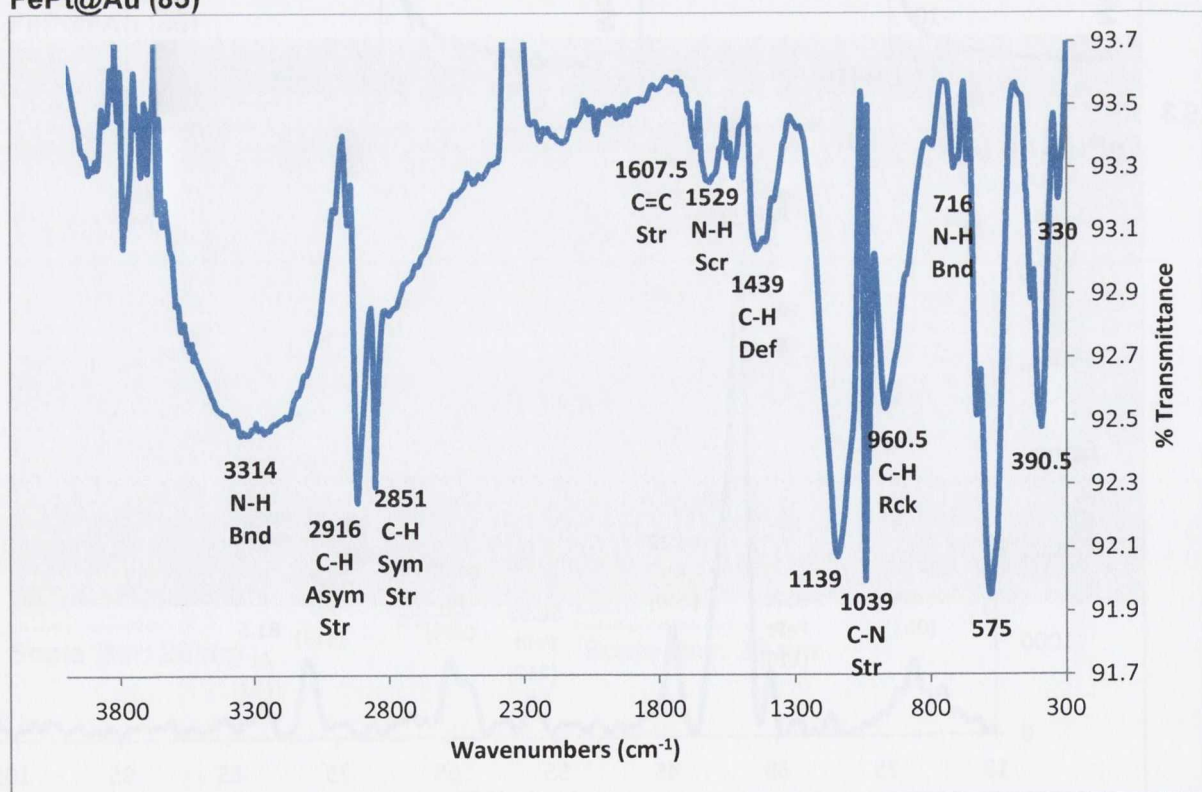
A.4.9.3 XRD
FePt₃@Au (85)



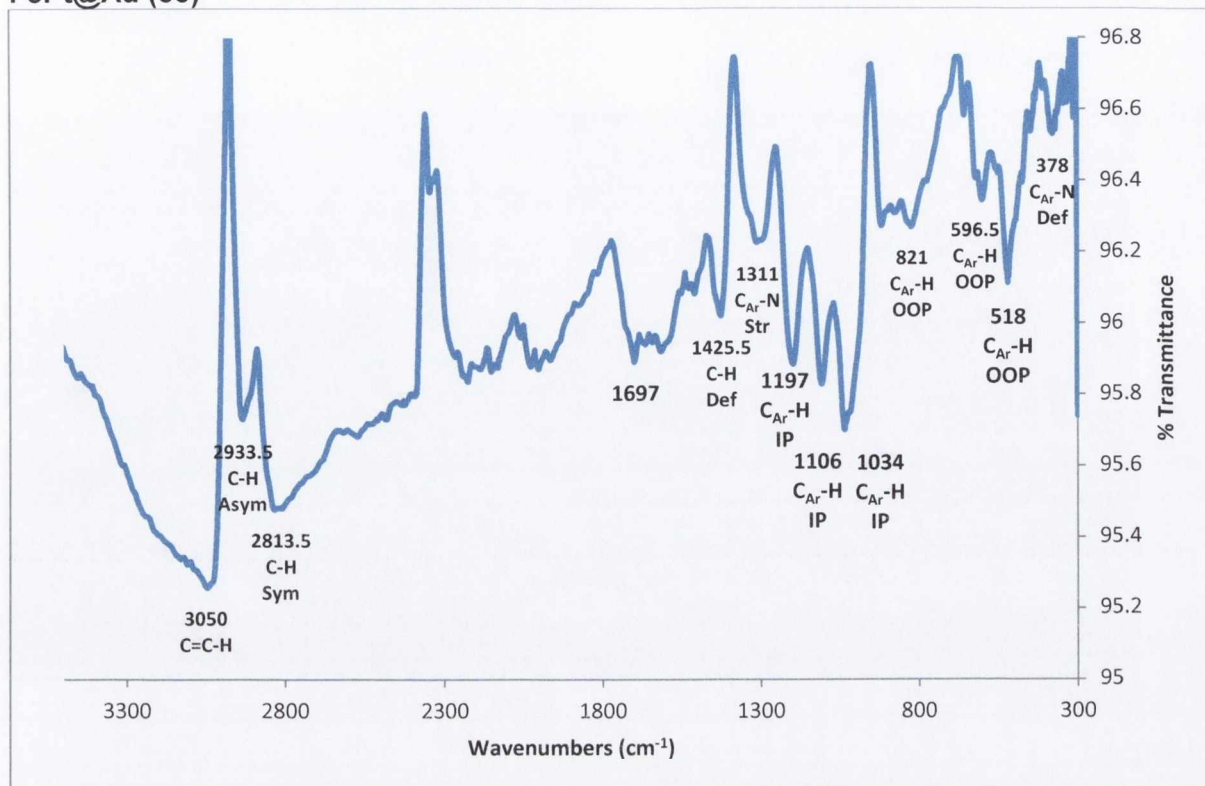
FePt₃ cubic | Au fcc (86)



A.4.9.3 FTIR FePt@Au (85)

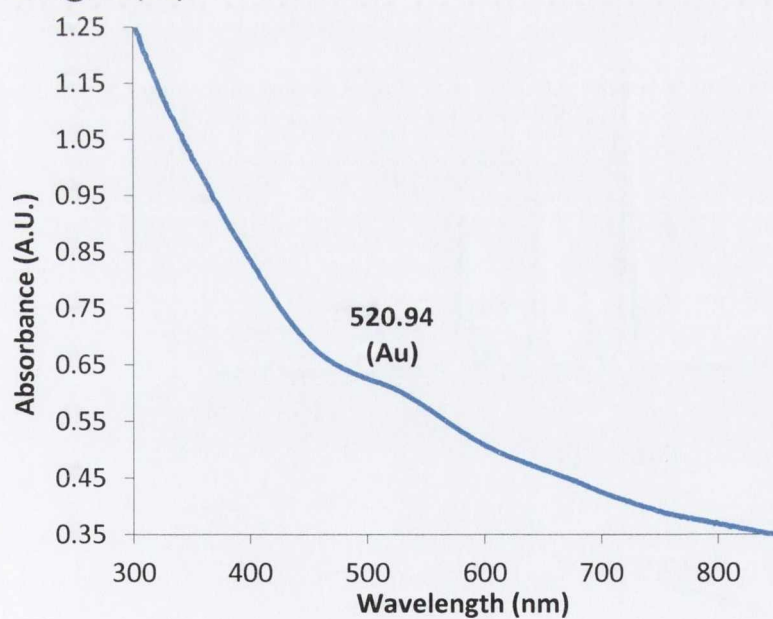


FePt@Au (86)

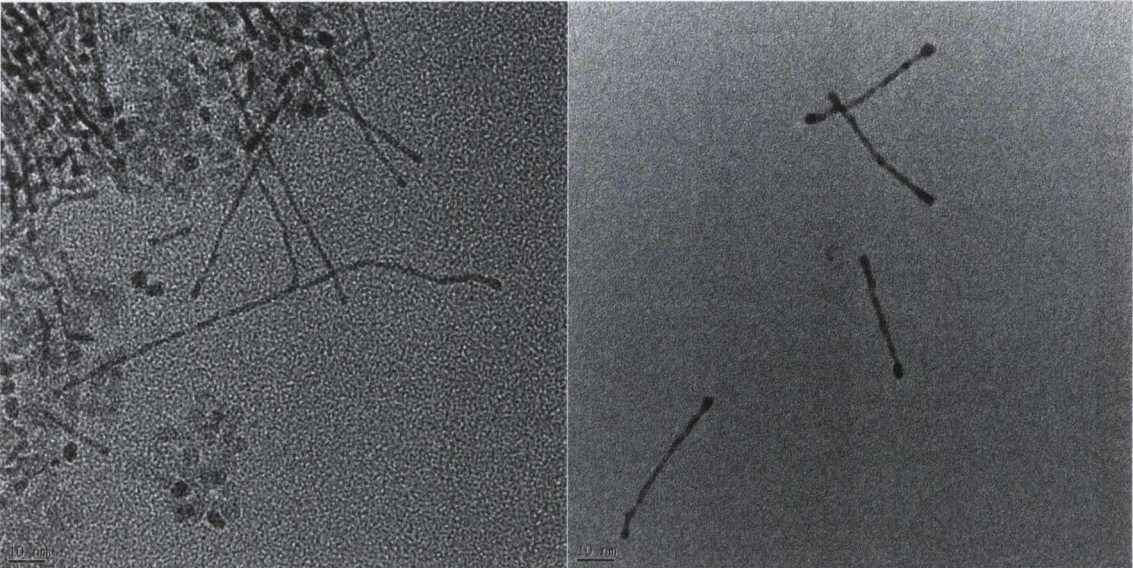


A.4.9.4 – UV-vis hexane

FePt@Au (85)



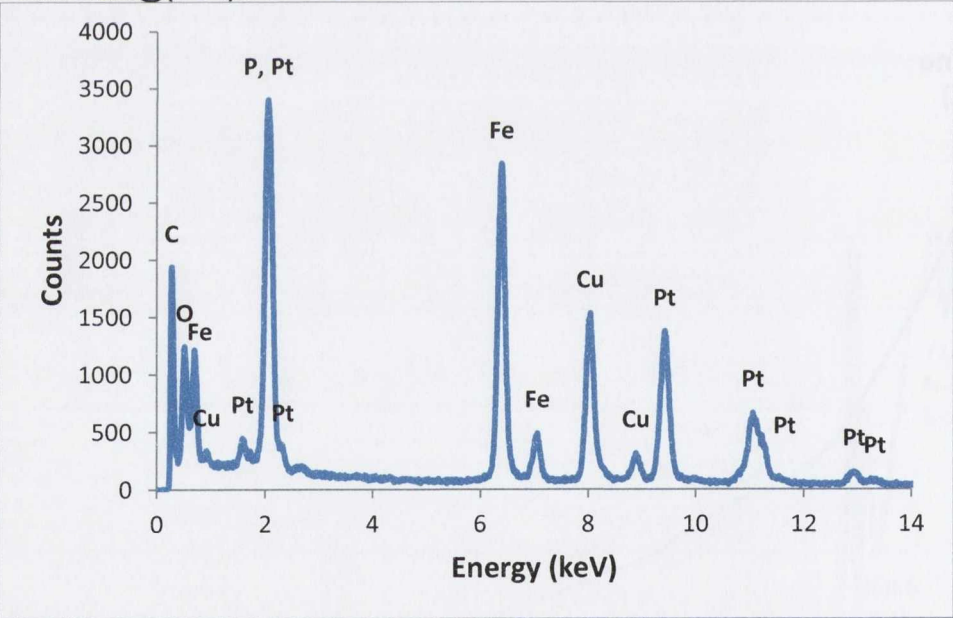
FePt@PEG-NH₂ (87)
TEM



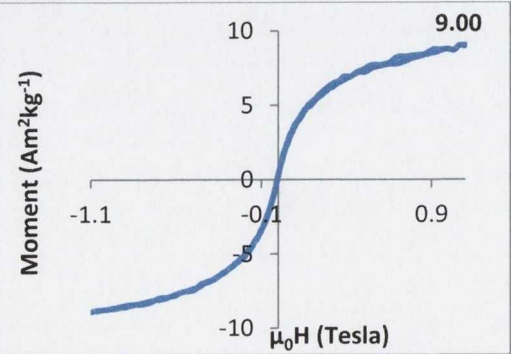
Scale Bar: 10 nm

Scale Bar: 10 nm

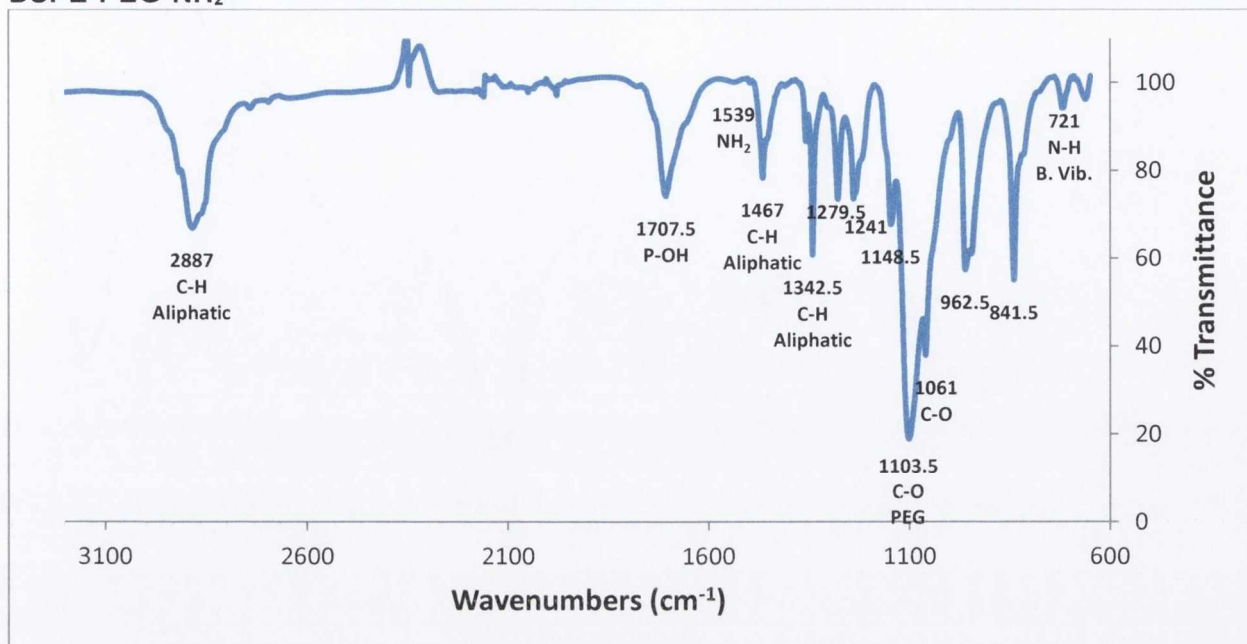
EDX
Fe_{67.2}Pt_{32.8}@(PO₃)_{53.8}-PEG-NH₂



VSM

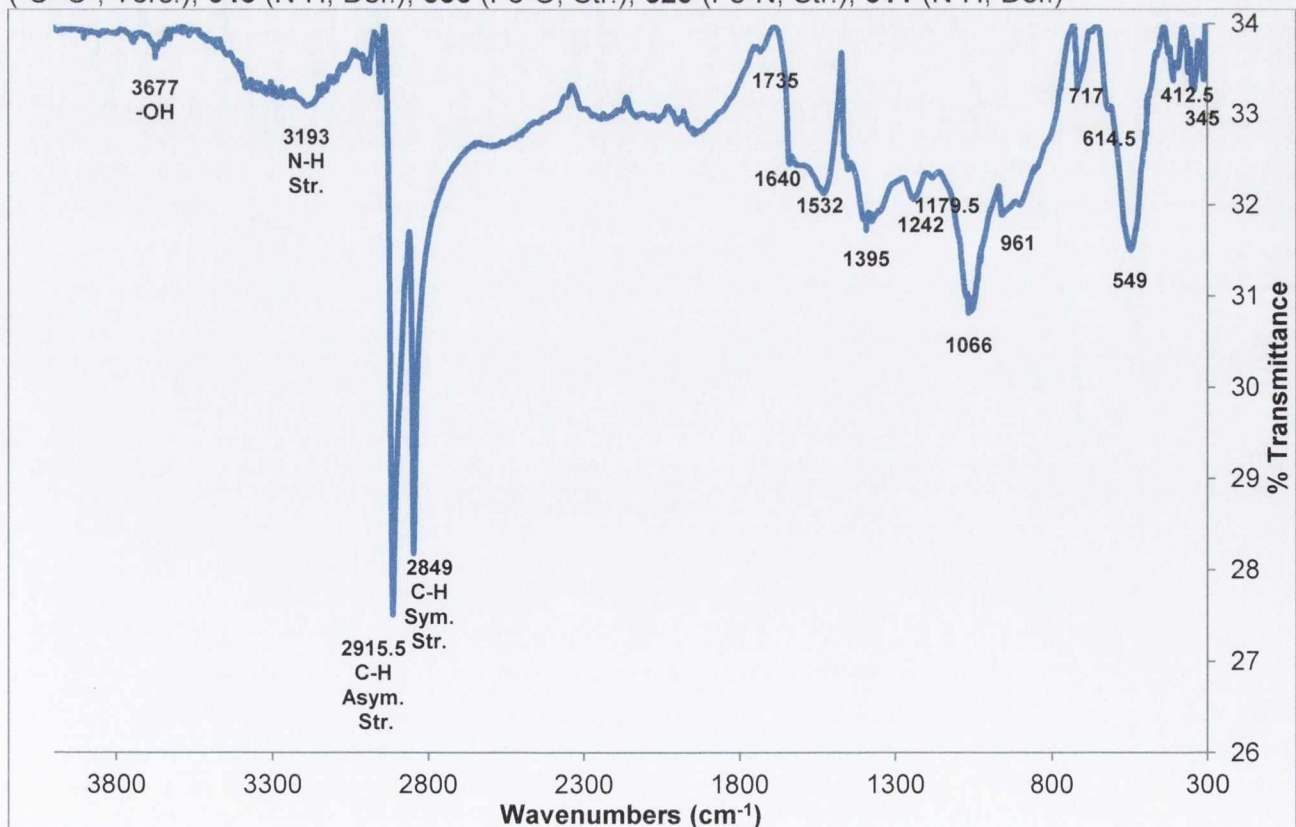


FTIR
DSPE-PEG-NH₂

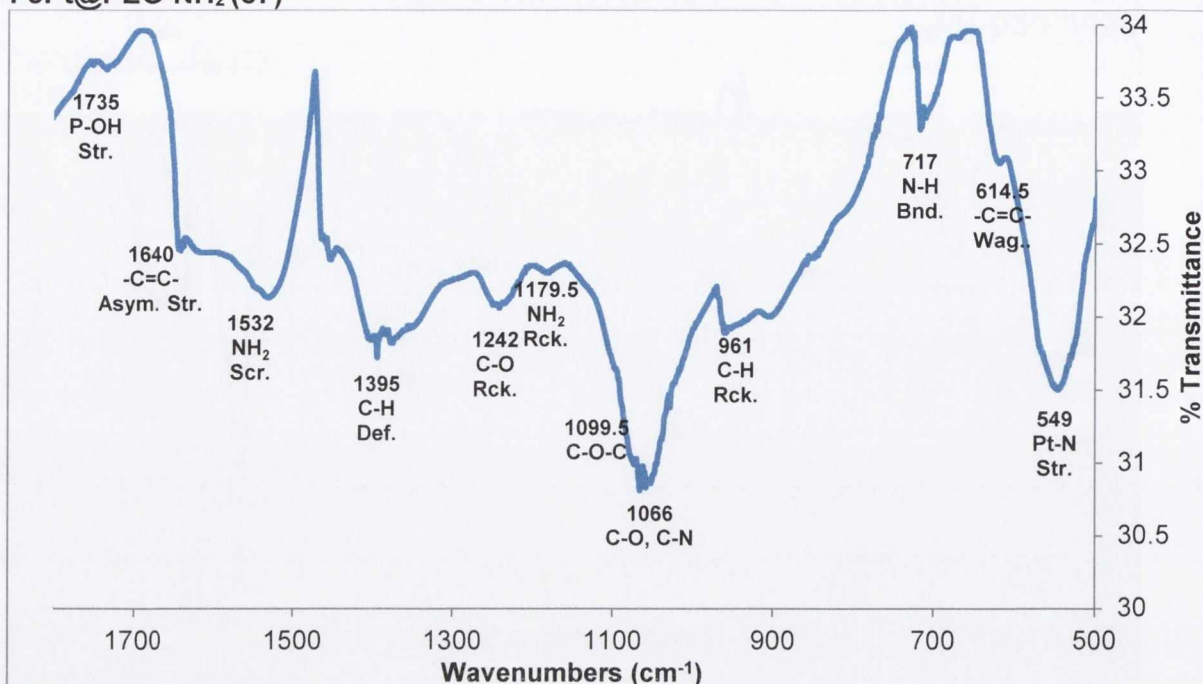


FePt@PEG-NH₂ (87)

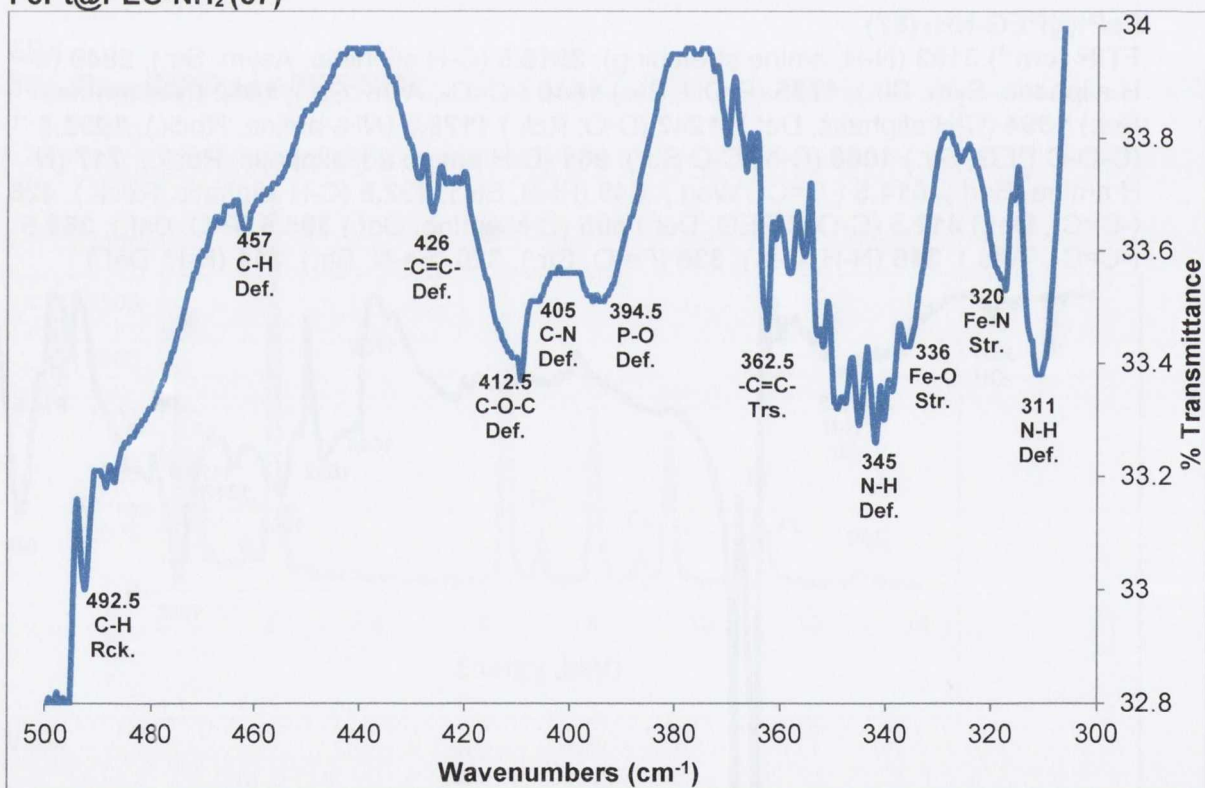
FTIR (cm⁻¹) 3193 (N-H, amine stretching), 2915.5 (C-H aliphatic, Asym. Str.), 2849 (C-H aliphatic, Sym. Str.), 1735 (P-OH, Str.) 1640 (-C=C-, Aym. Str.), 1532 (N-H amine, Scr.) 1394 (C-H aliphatic, Def.), 1242 (C-O, Rck.) 1179.5 (NH₂ amine, Rock.), 1099.5 (C-O-C PEG, Str.) 1066 (C-N, C-O Str.), 961 (C-H amino adj.aliphatic, Rock.), 717 (N-H amine, Bnd.), 614.5 (-C=C-, Wag.), 549 (Pt-N, Str.), 492.5 (C-H aliphatic, Rock.), 426 (-C=C-, Def.) 412.5 (C-O-C PEG, Def.) 405 (C-N amine, Def.) 394.5 (P-O, Def.), 362.5 (-C=C-, Tors.), 345 (N-H, Def.), 336 (Fe-O, Str.), 320 (Fe-N, Str.), 311 (N-H, Def.)



FePt@PEG-NH₂ (87)



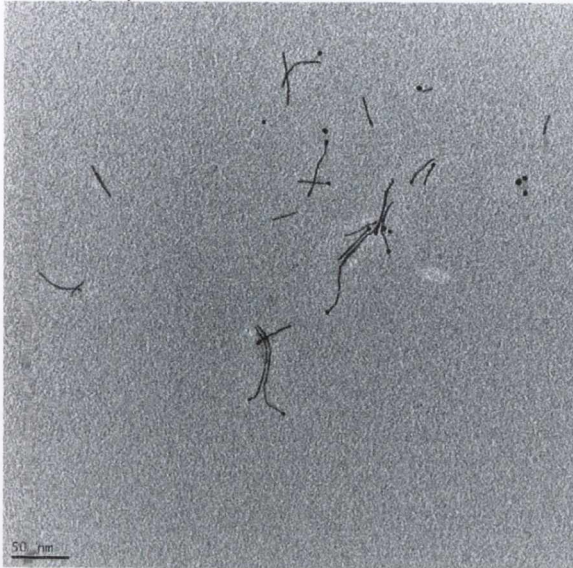
FePt@PEG-NH₂ (87)



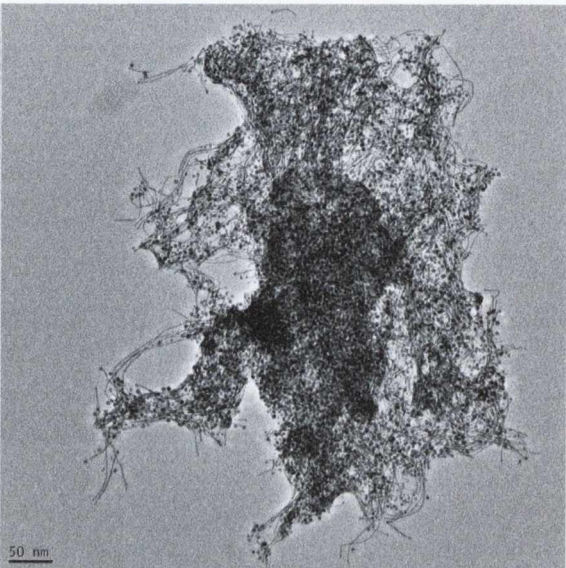
A.4.11.1 – Cobalt-Platinum Nanostructures

TEM

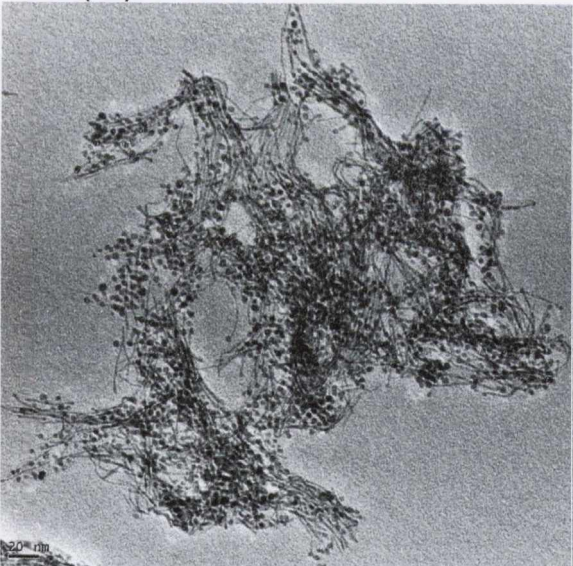
CoPt (69)



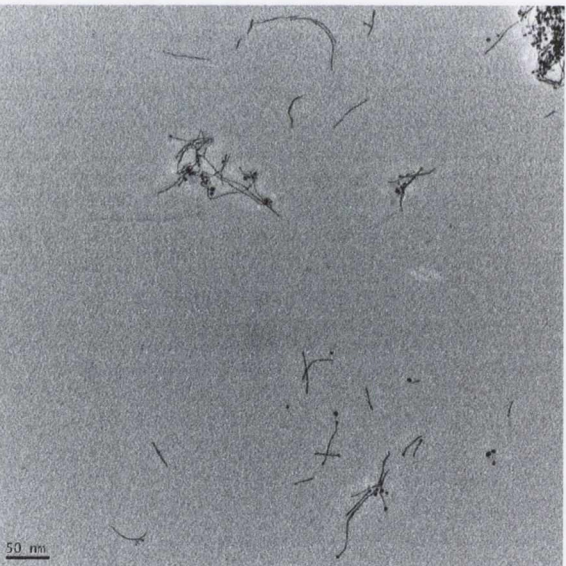
Scale Bar: 50 nm
CoPt (70)



Scale Bar: 50 nm

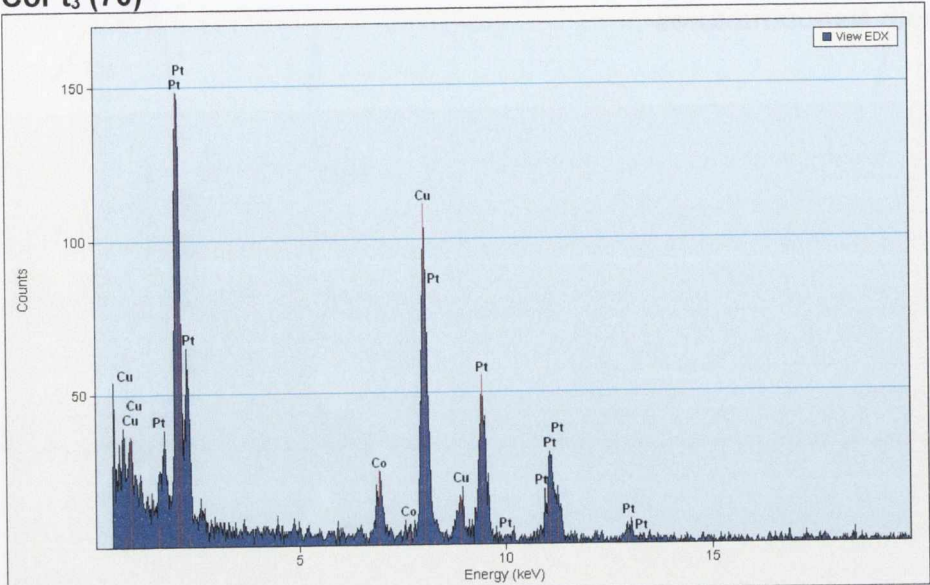


Scale Bar: 20 nm

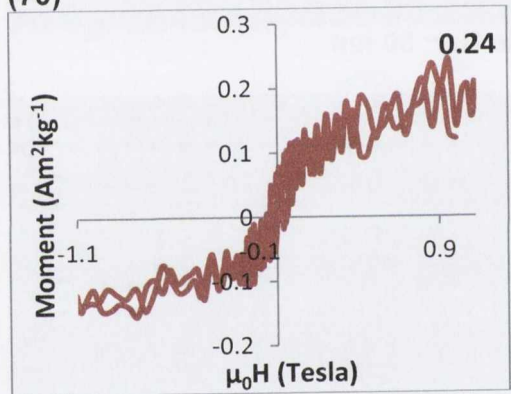


Scale Bar: 50 nm

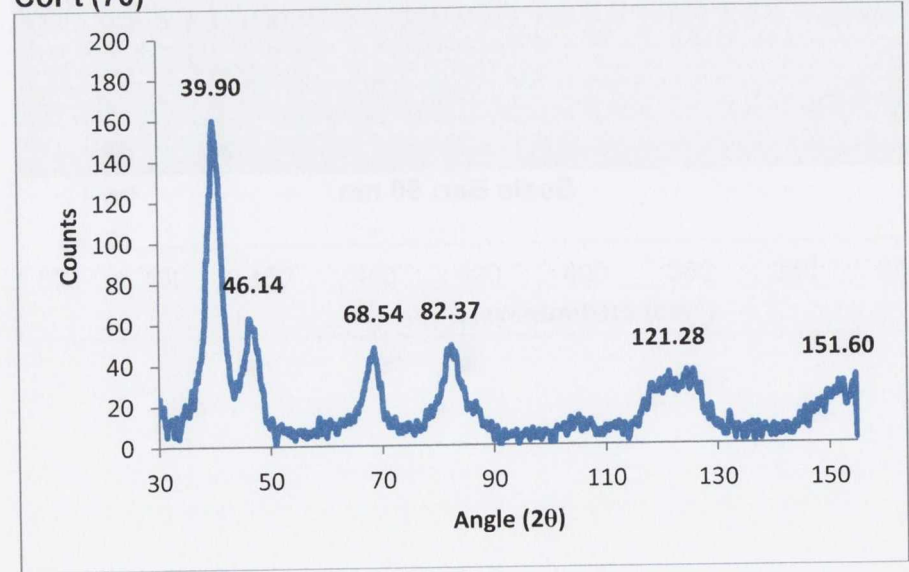
EDX
CoPt₃ (70)



VSM
(70)



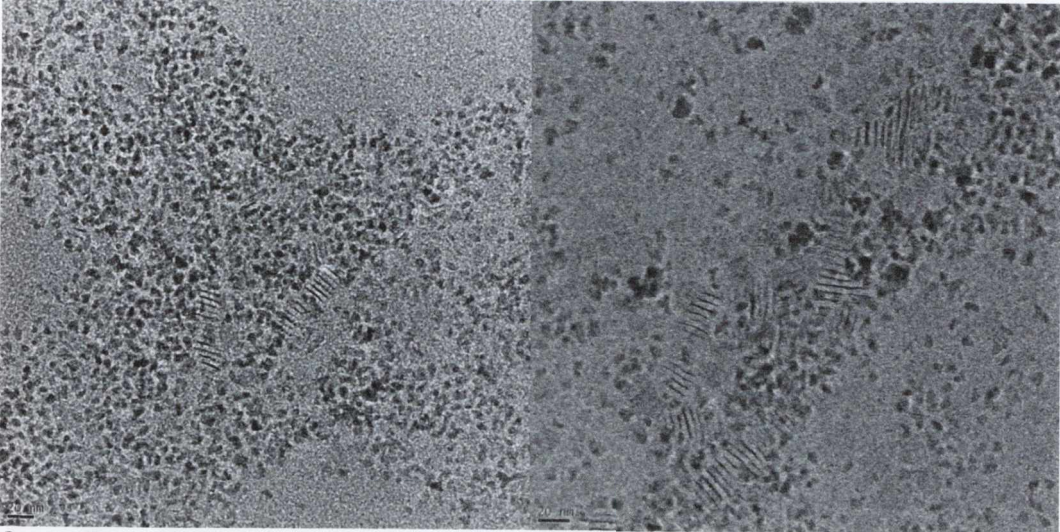
XRD
CoPt (70)



A.4.11.2 –Iron-Cobalt Alloy Nanostuctures

TEM

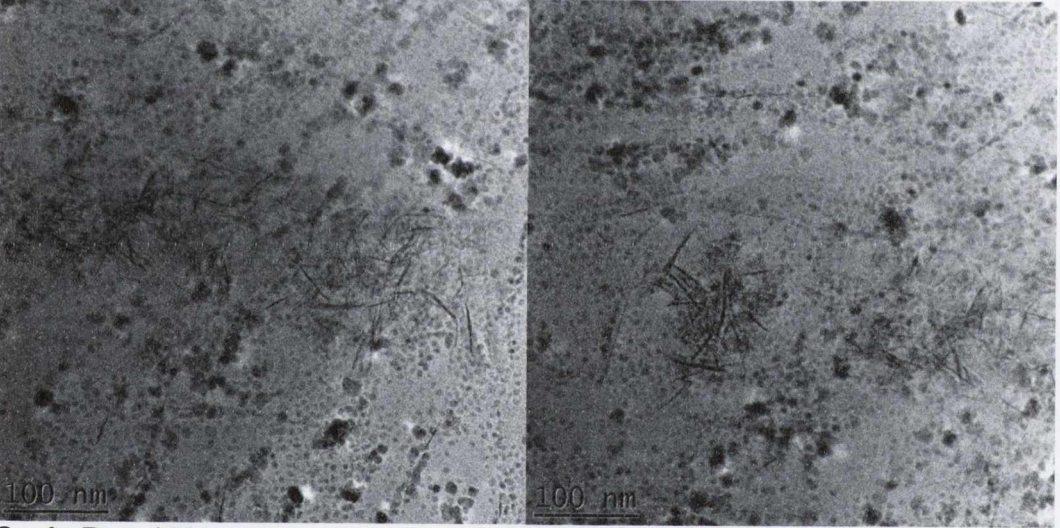
Fe₈₉Co₁₁ (71)



Scale Bar: 20 nm

Scale Bar: 20 nm

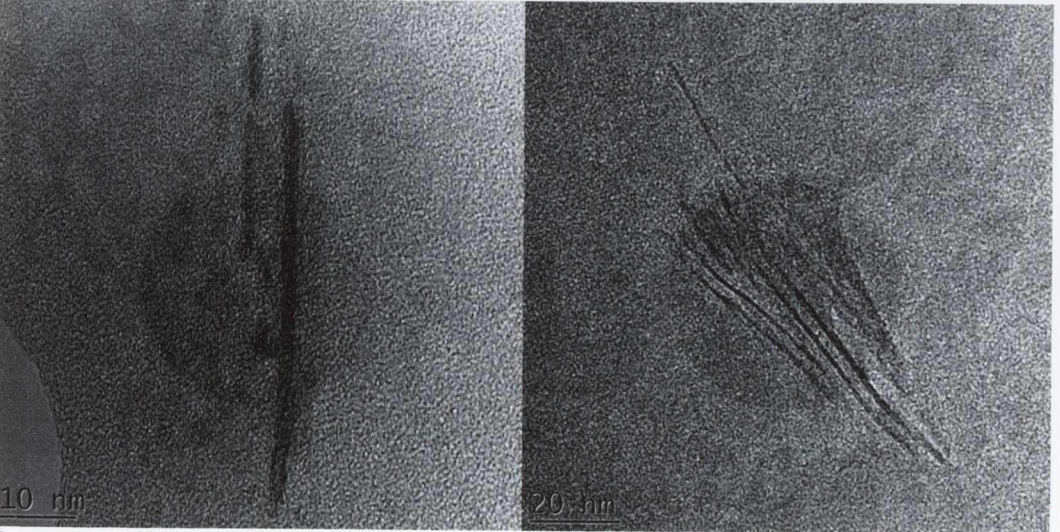
Fe_{81.5}Co_{18.5} (72)



Scale Bar: 100 nm

Scale Bar: 100 nm

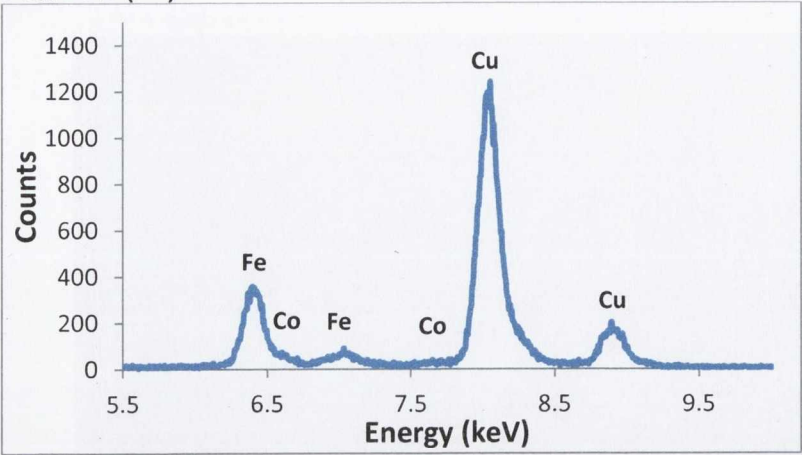
Fe₈₀Co₂₀ (73)



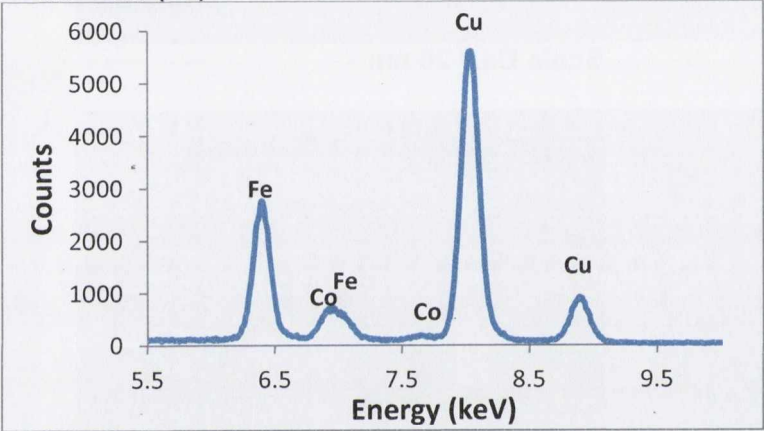
Scale Bar: 10 nm

Scale Bar: 20 nm

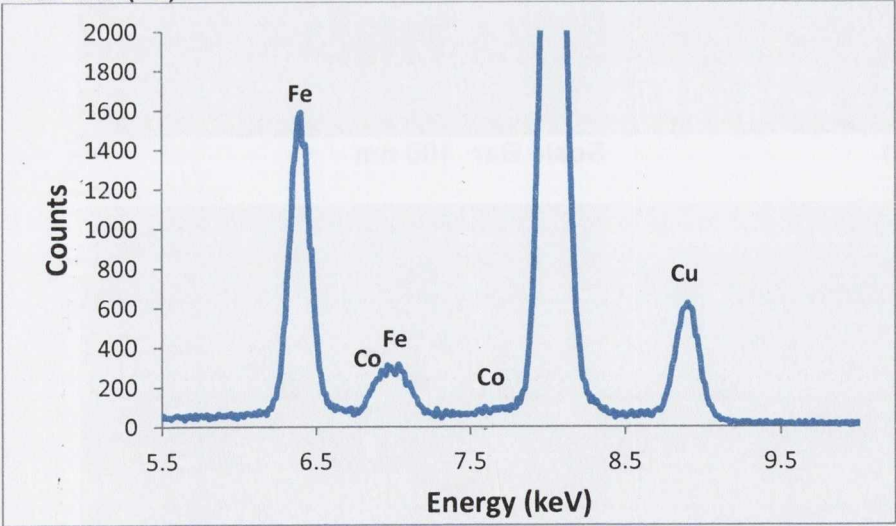
EDX
Fe₈₉Co₁₁ (71)



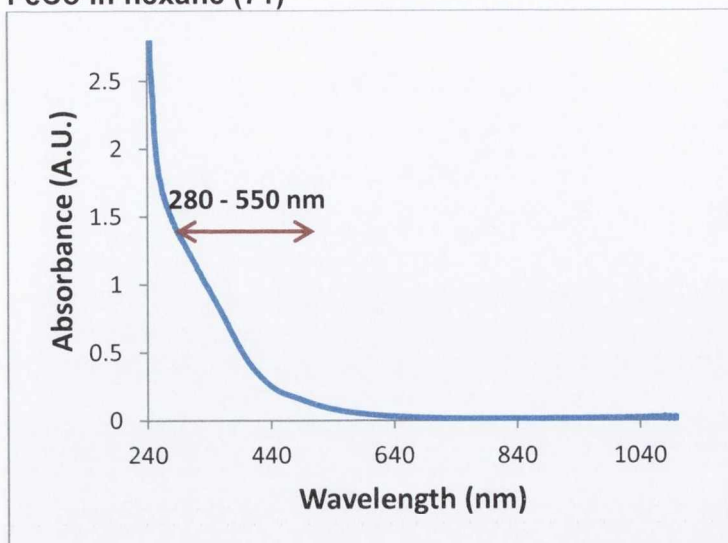
Fe_{81.5}Co_{18.5} (72)



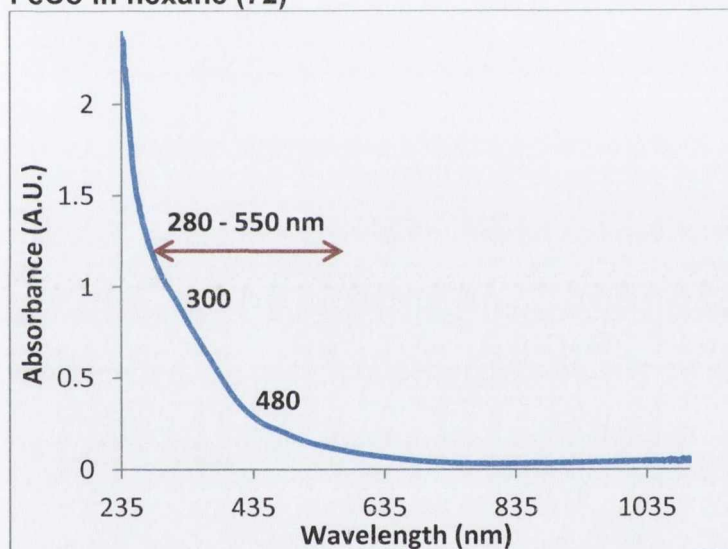
Fe₈₀Co₂₀ (73)



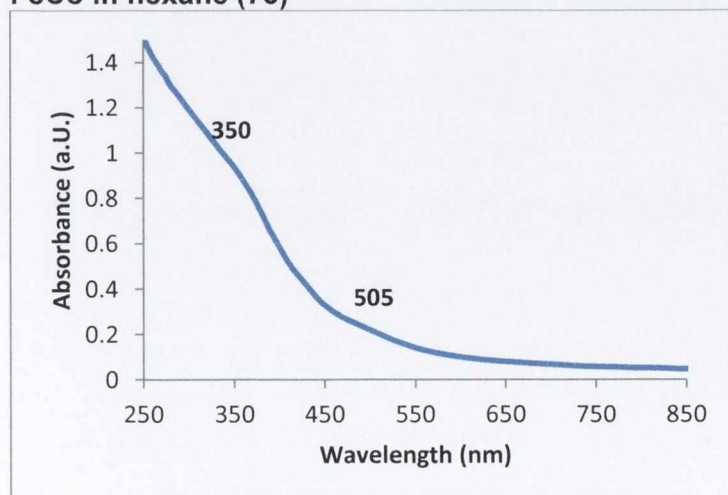
UV-vis
FeCo in hexane (71)



FeCo in hexane (72)

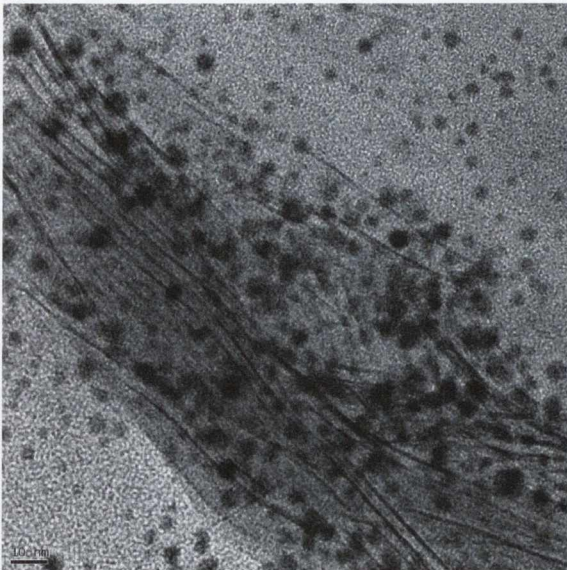


FeCo in hexane (73)

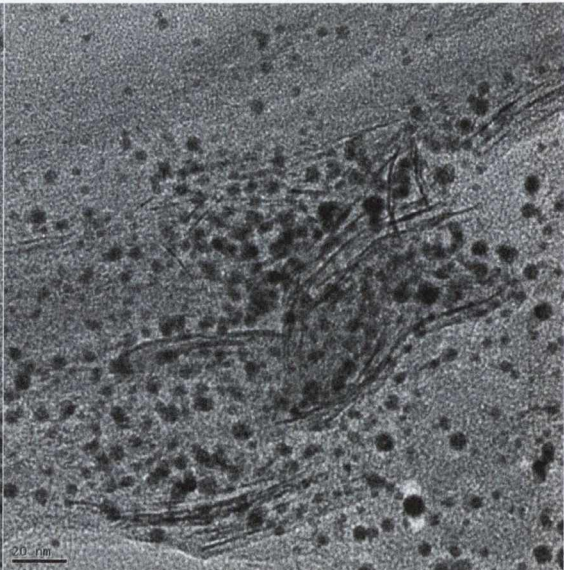


A.4.11.3 – Iron-Gold Alloy Nanostructures (74)

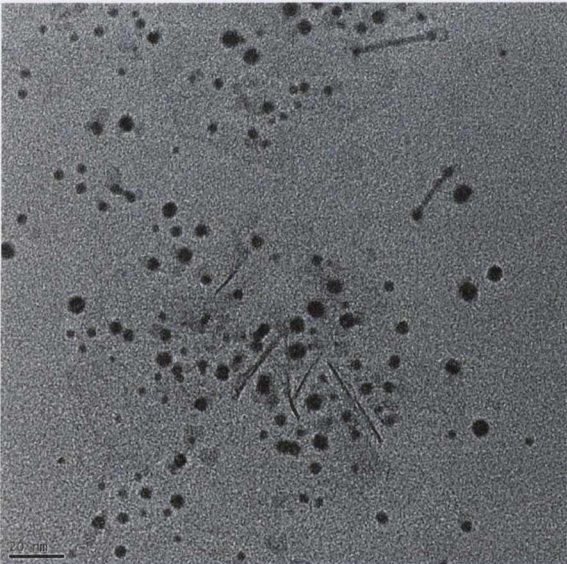
TEM



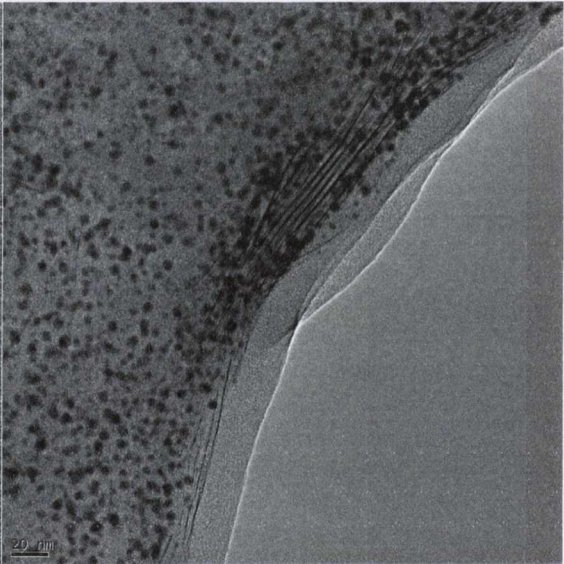
Scale Bar: 20 nm



Scale Bar: 20 nm

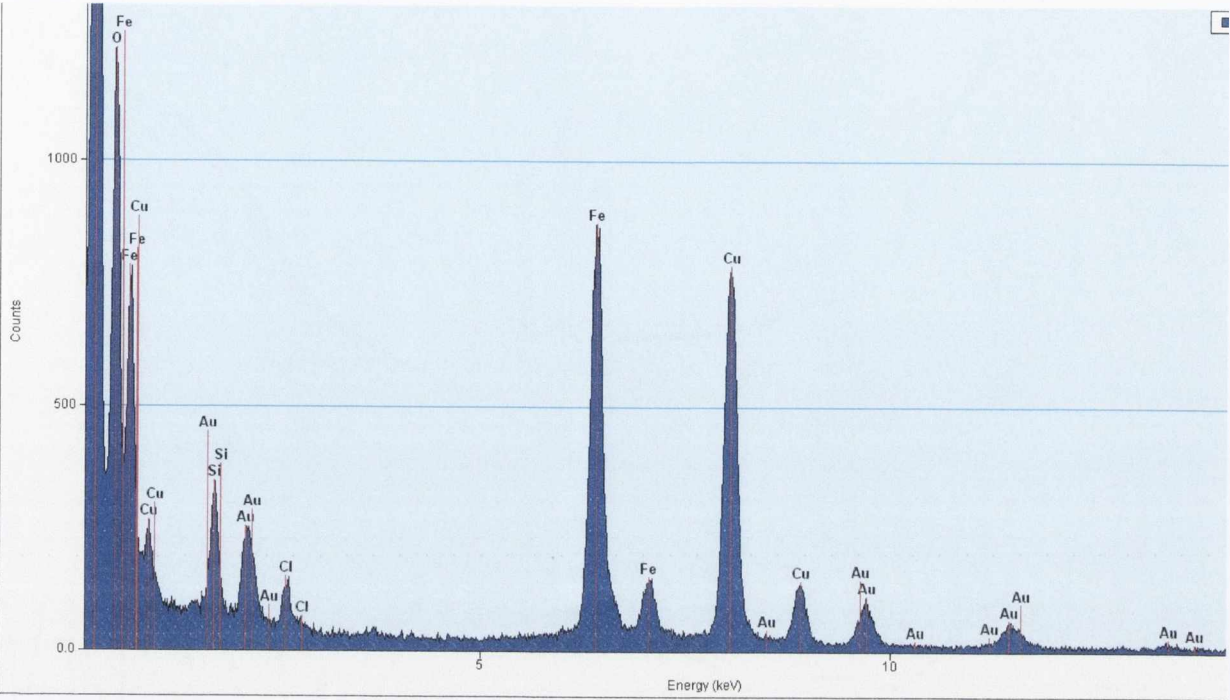


Scale Bar: 20 nm

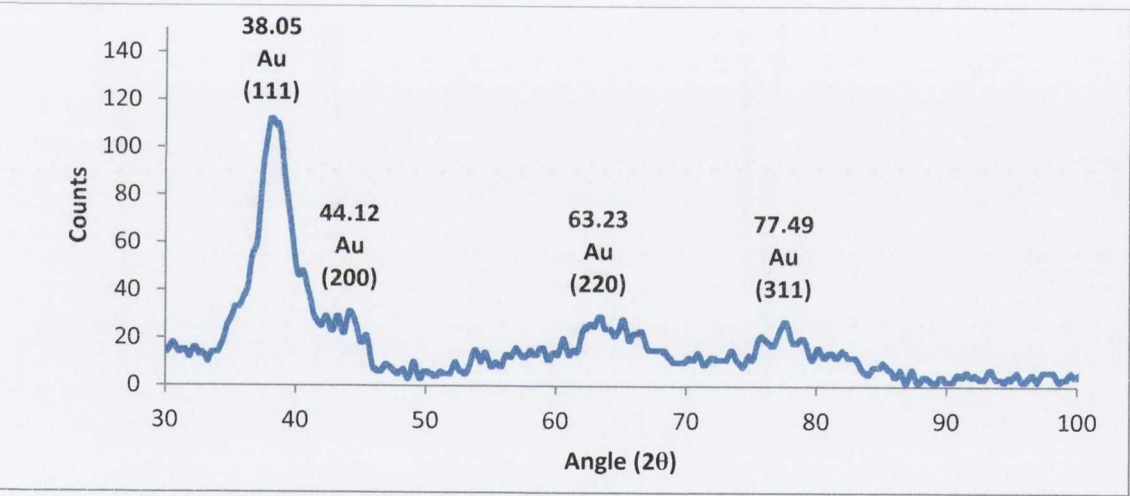


Scale Bar: 20 nm

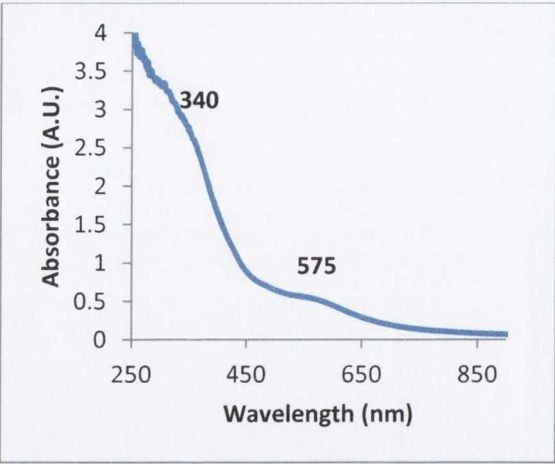
EDX



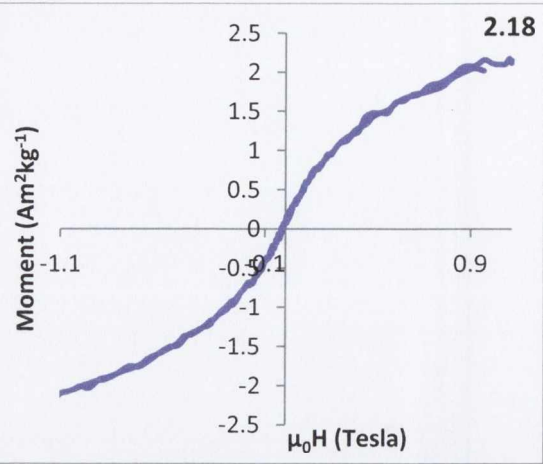
XRD Au fcc



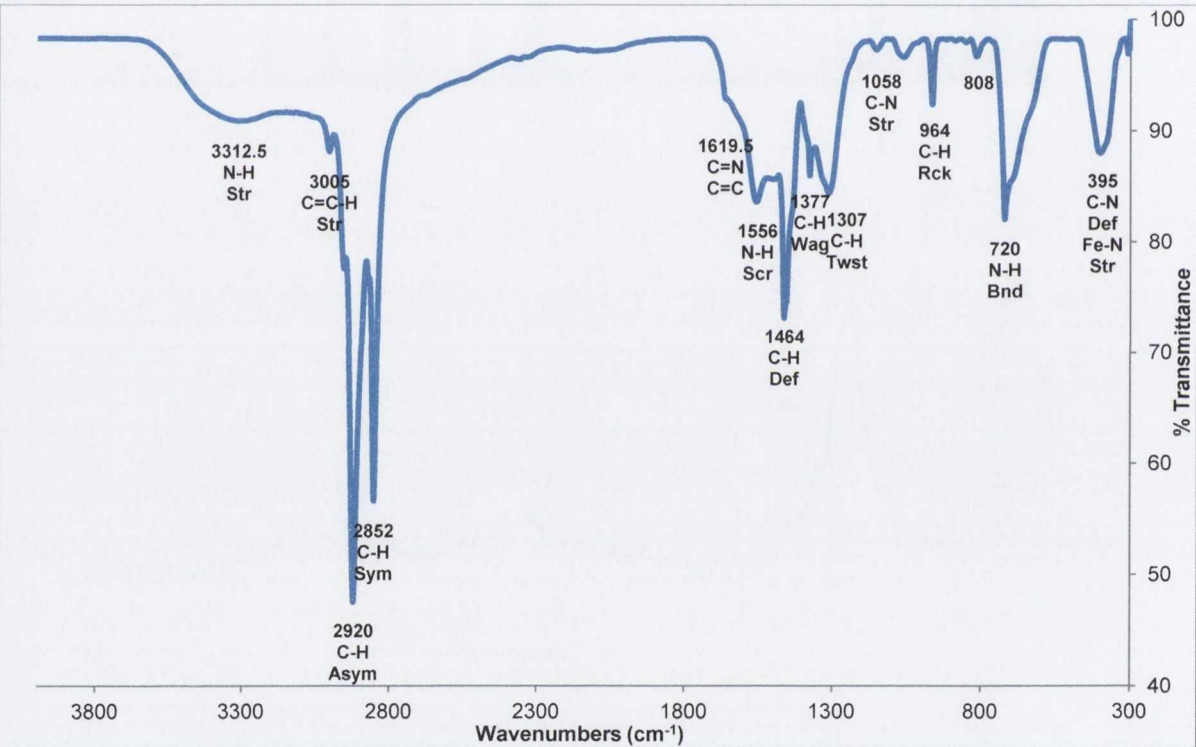
UV hexane



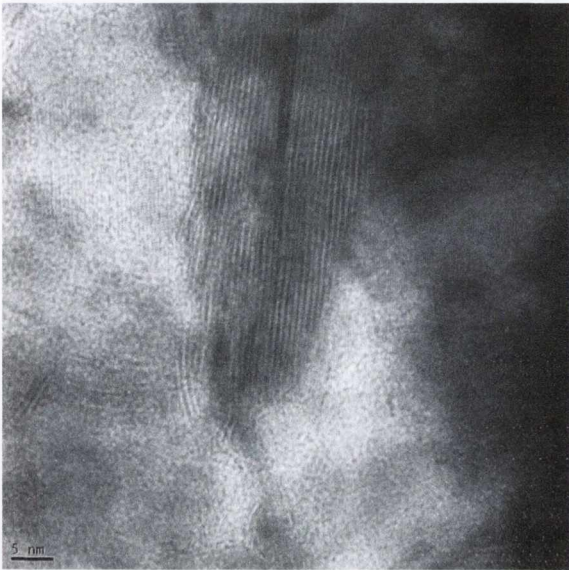
VSM



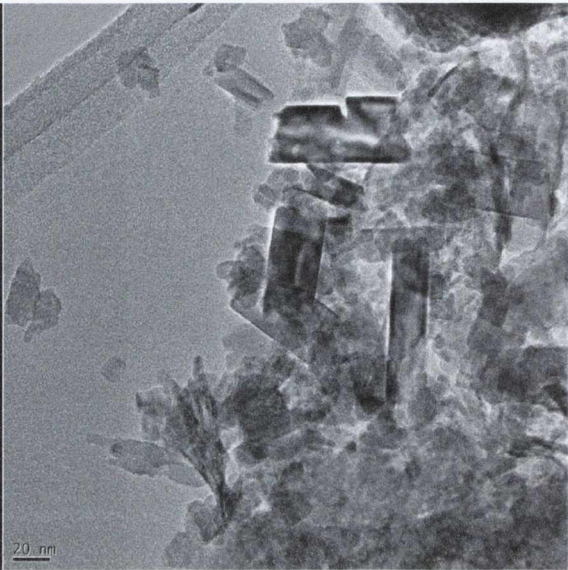
FTIR



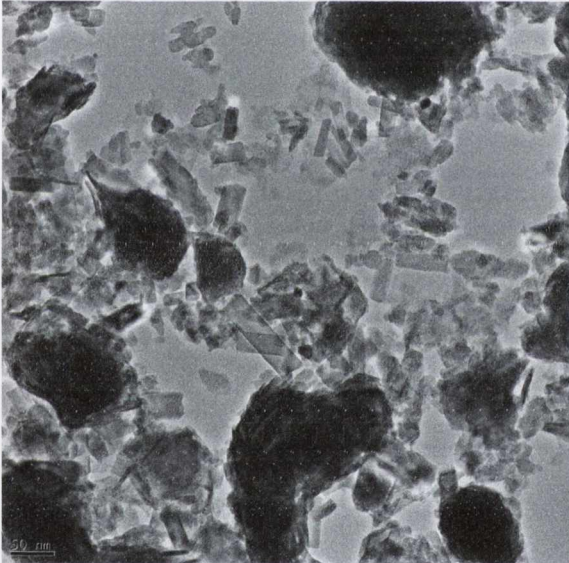
A.4.11.4 –Manganese-Bismuth Alloy Nanostructures (75) TEM



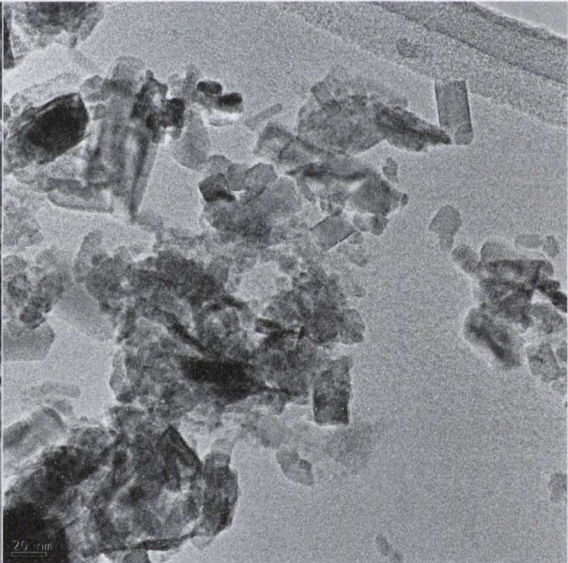
Scale Bar: 5 nm



Scale Bar: 20 nm

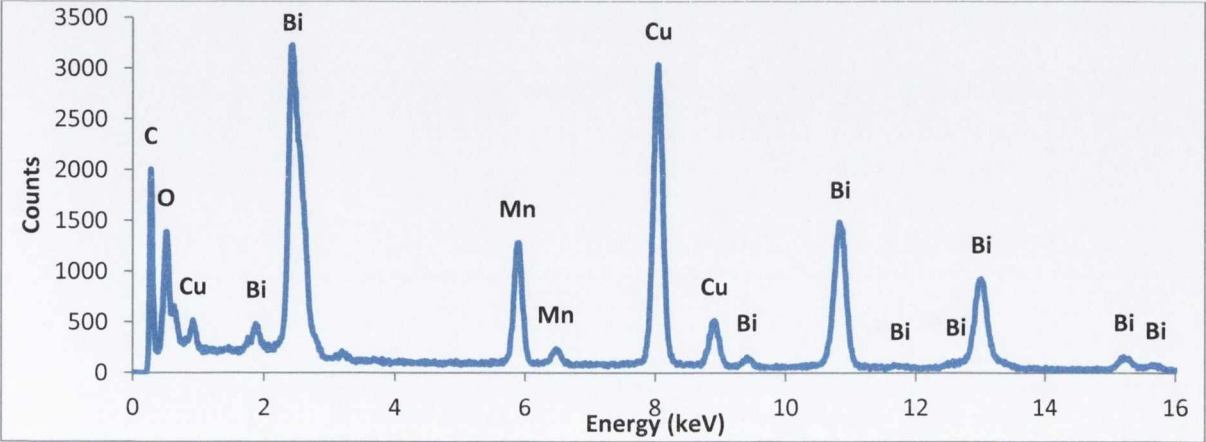


Scale Bar: 50 nm

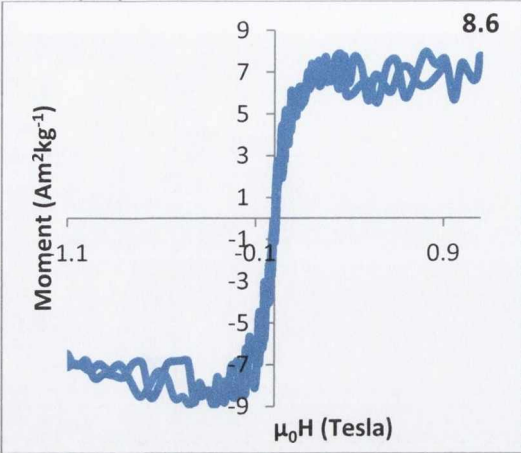


Scale Bar: 20 nm

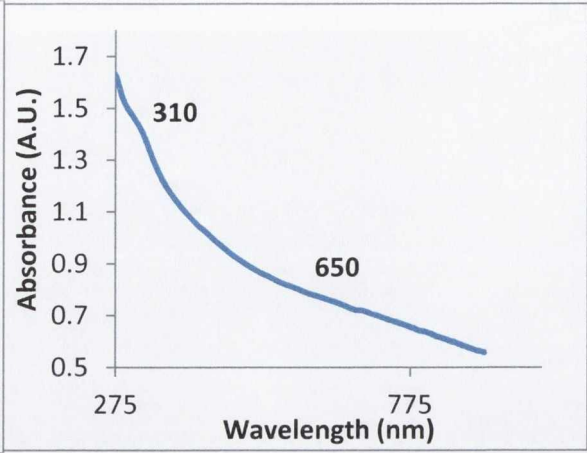
EDX $\text{Mn}_{50}\text{Bi}_{50}$



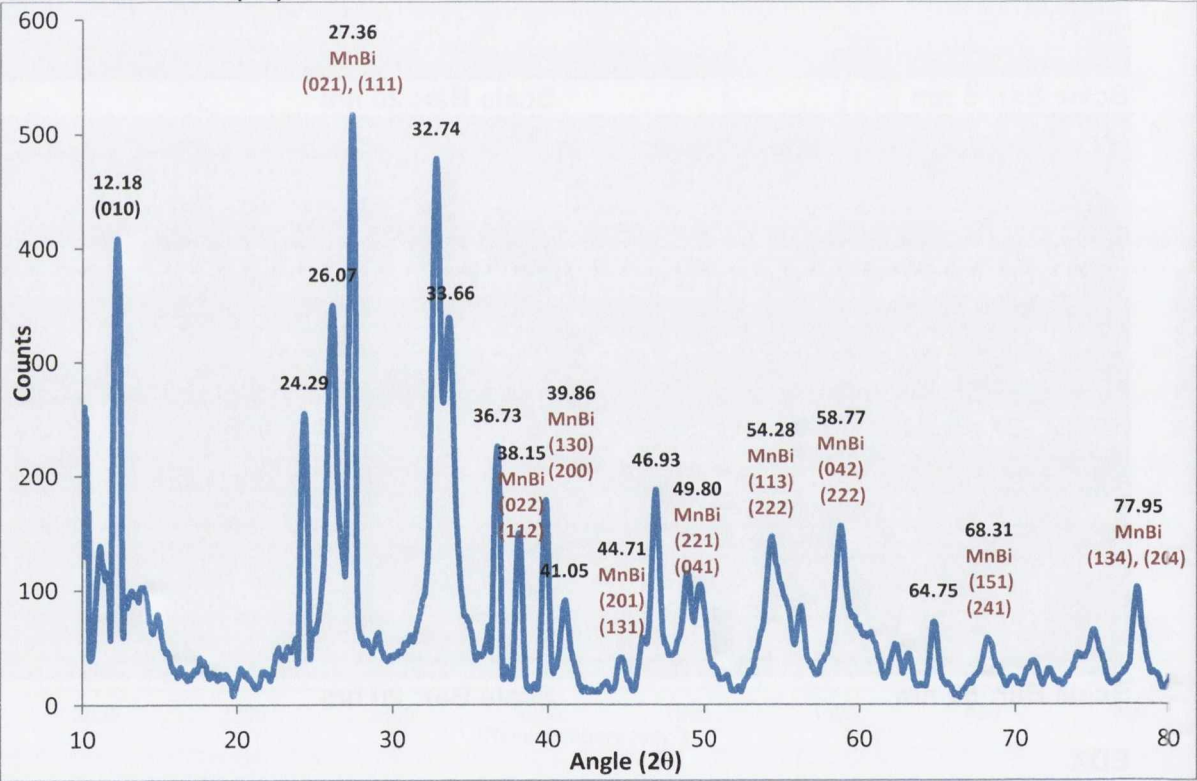
VSM (75)



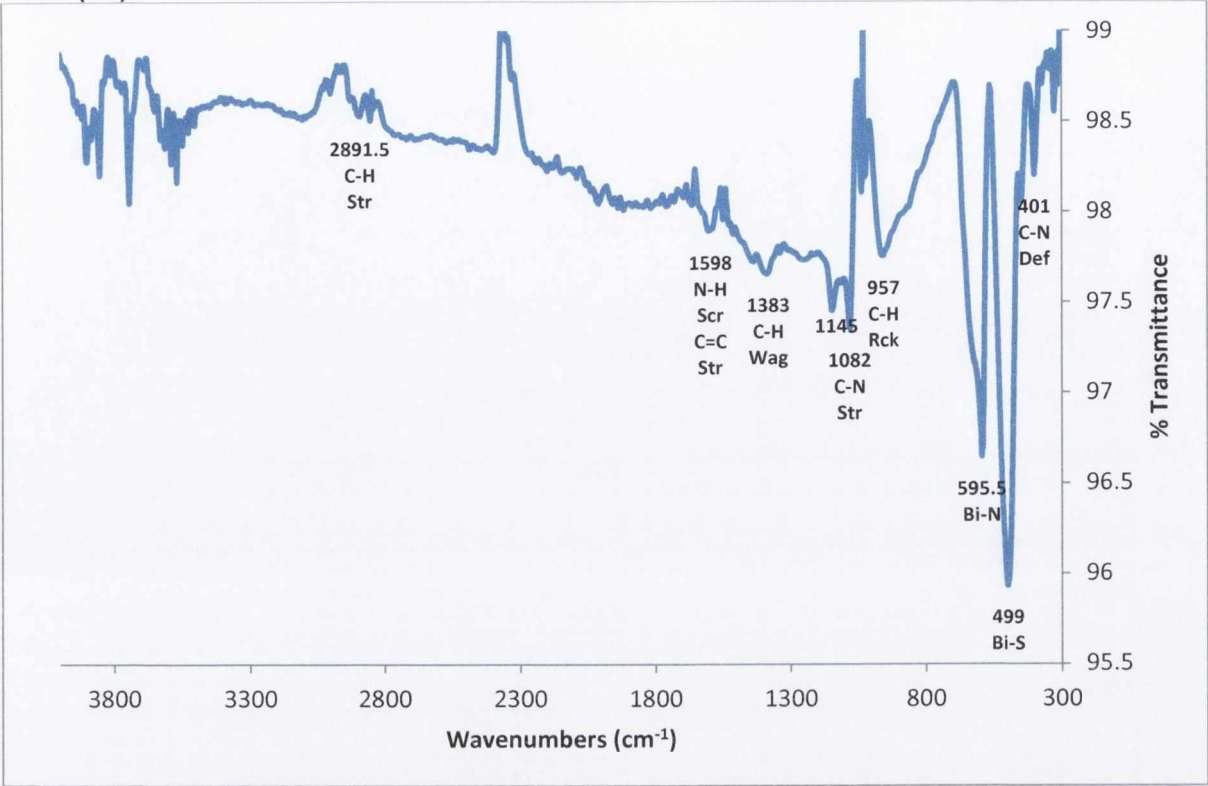
UV (75)



XRD MnBi ortho (75)

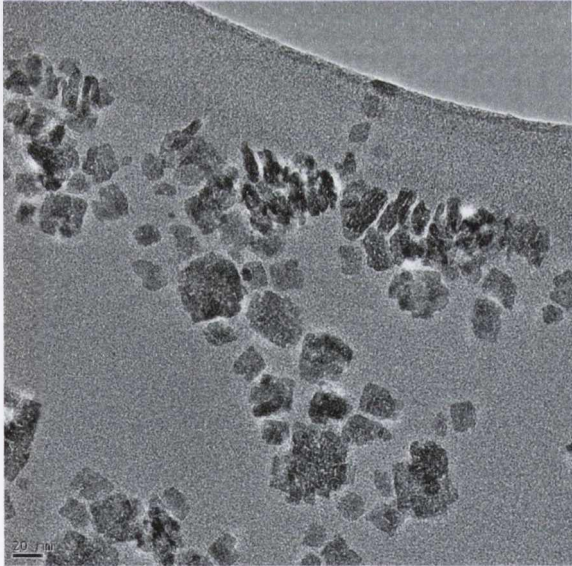


FTIR (75)

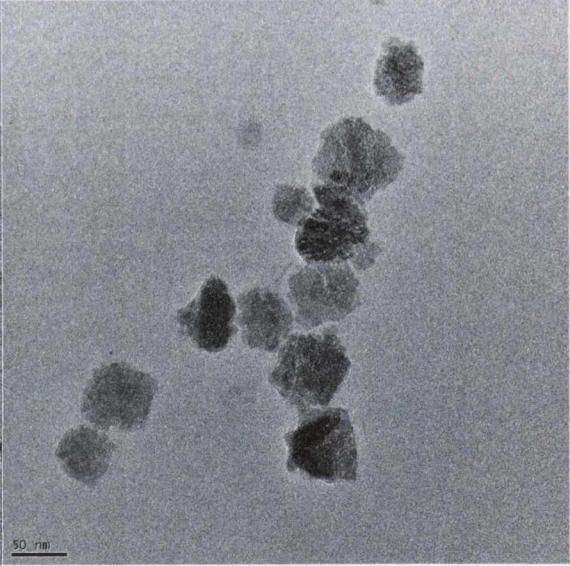


A.4.11.5 Manganese Sulfide Nanostructures (84)

TEM (84)

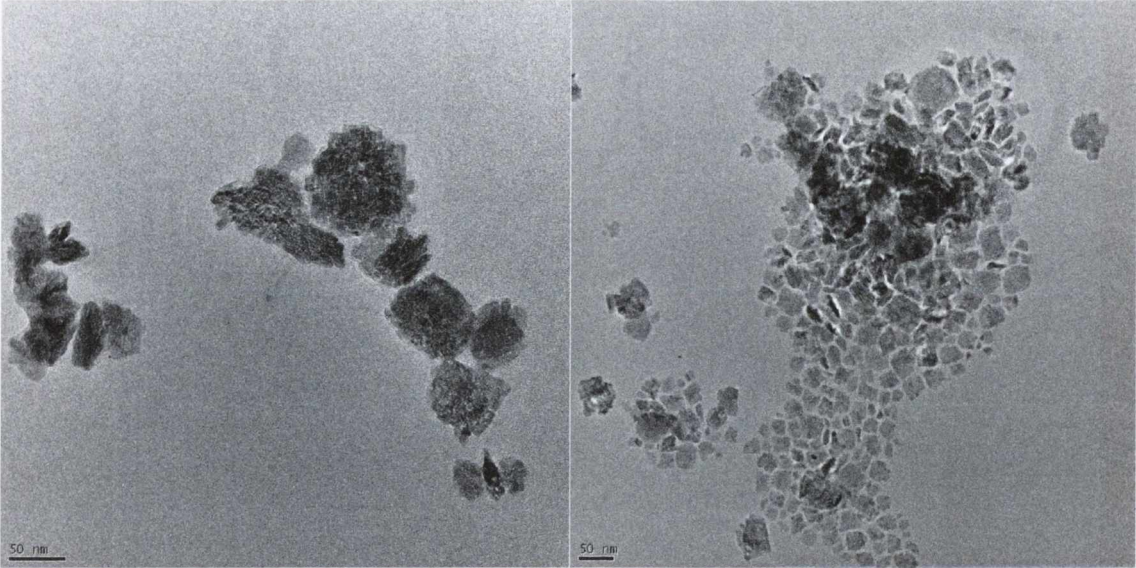


Scale Bar: 20 nm



Scale Bar: 50 nm

TEM (84)

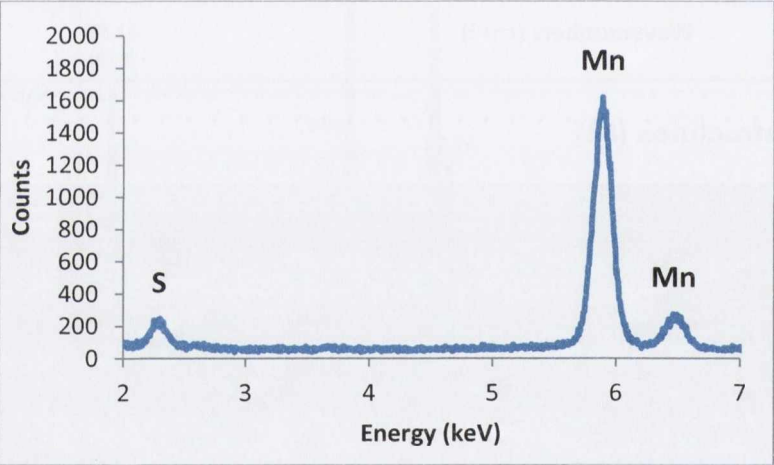


Scale Bar: 50 nm

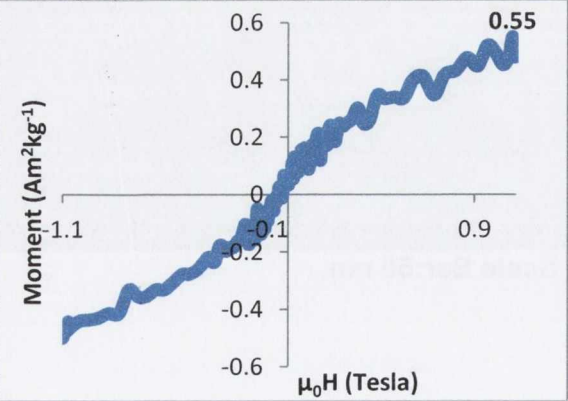
Scale Bar: 50 nm

EDX (84)

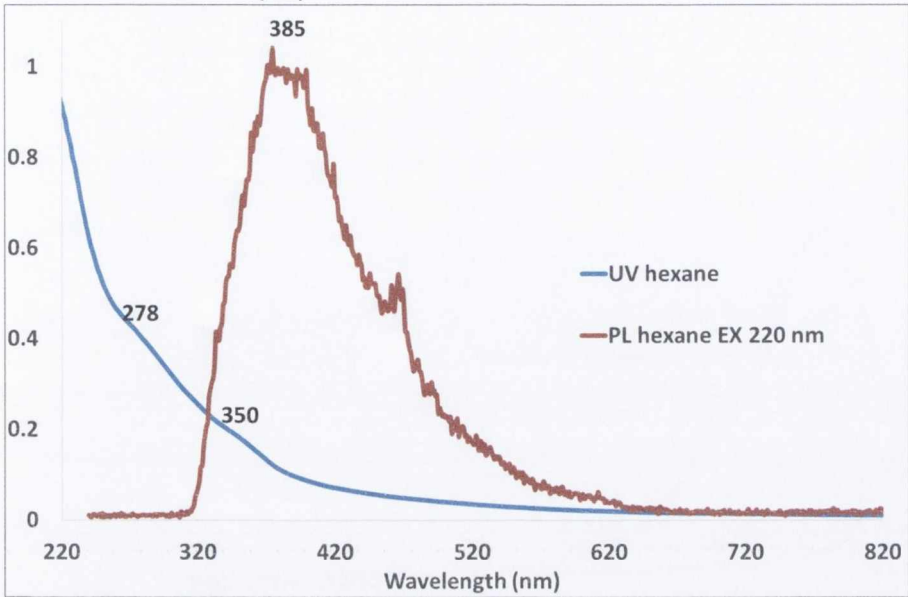
Mn₉₀S₁₀



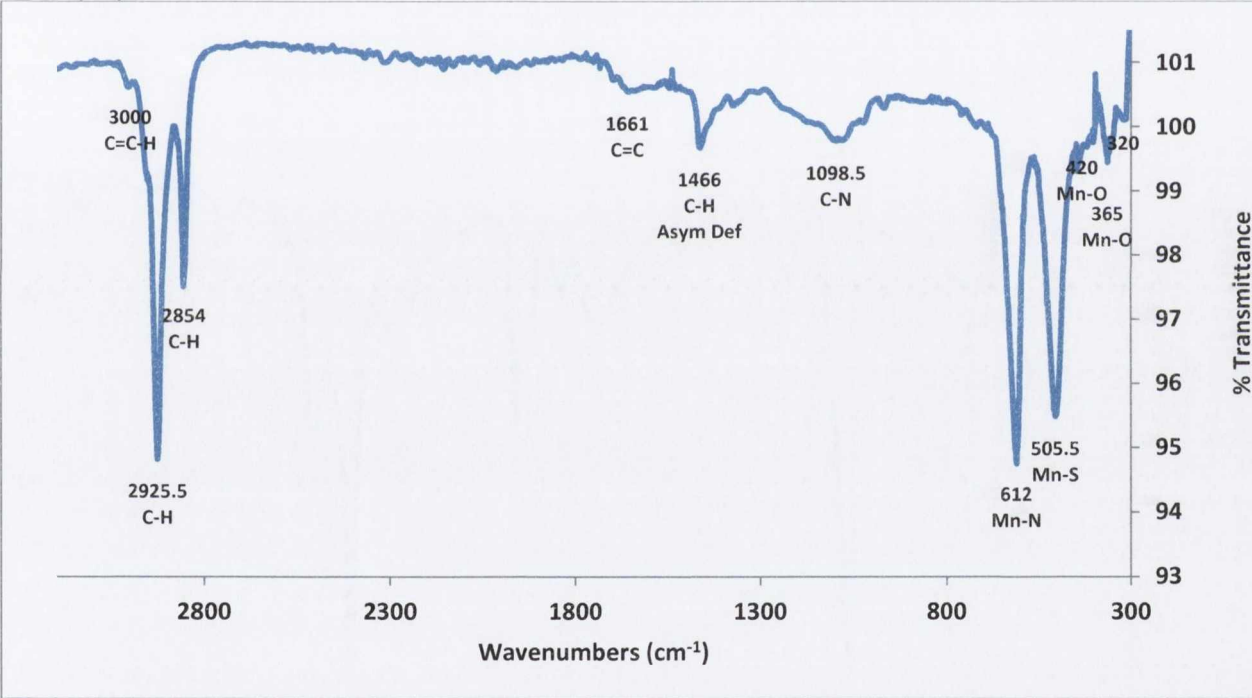
VSM (84)



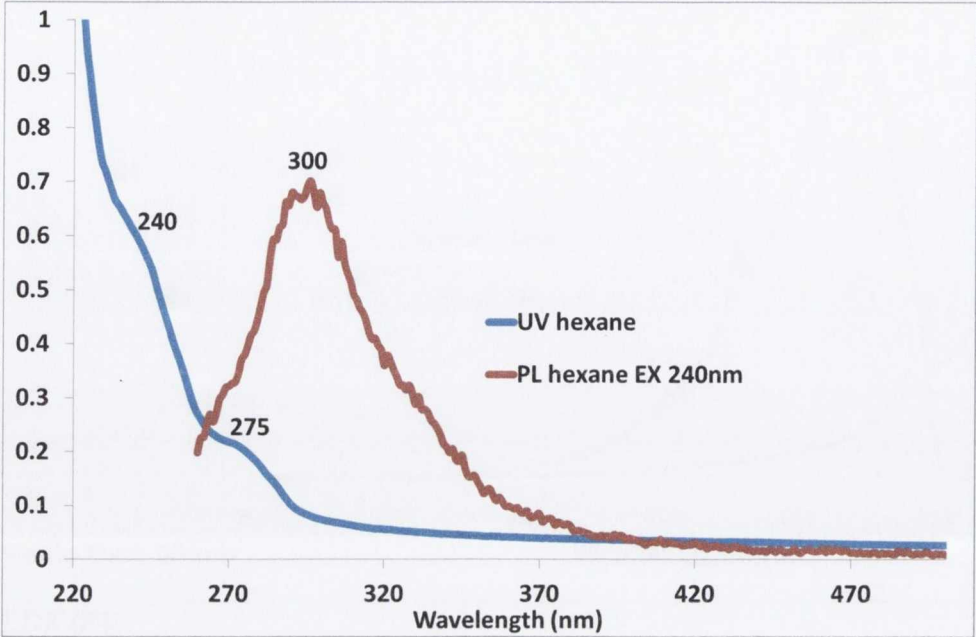
UV-vis/PL hexane (84)



FTIR (84)



A.4.11.6 Oleylamine
UV/PL in hexane



Sulfur-OAm
FTIR

