# “SYNTHESIS & CHARACTERIZATION OF STABLE IRON OXIDE NANOPARTICLES: POTENTIAL FOR NUCLEIC ACID RECOVERY”

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IN PARTIAL FULFILLMENT OF THE REQUIREMENT

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**SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES & TECHNOLOGY**

**THIRUVANANTHAPURAM – 695011, INDIA**

# DECLARATION

I, **Sreelekshmi S R**, hereby declare that I had personally carried out the work depicted in the thesis entitled**, "SYNTHESIS & CHARACTERIZATION OF STABLE IRON OXIDE NANOPARTICLES: POTENTIAL FOR NUCLEIC ACID RECOVERY"** under the guidance of **Dr Francis Boniface Fernandez , Scientist C, Division Of Bioceramics ,** Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram, Kerala, India.

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# CERTIFICATE

This is to certify that the dissertation entitled "**Synthesis & Characterization of stable iron oxide nanoparticles: Potential for nucleic acid recovery"** is a bonafide work done by Ms. Sreelekshmi S R in partial fulfilment for the degree of Master of Philosophy in Biomedical Technology under my supervision and guidance at Division of Bioceramics, Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram, Kerala ,India.

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The Dissertation Entitled

**"Synthesis & Characterization of stable iron oxide nanoparticles: Potential for nucleic acid recovery**"

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For the degree of

**Master of Philosophy in Biomedical Technology**

Of

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## ABBREVIATIONS

| | |
|---|---|
| PEI | Polyethyleneimine |
| CTAB | Cetyltrimethylammoniumbromide |
| TEOS | Tetraethylorthosilicate |
| IONPS | Iron oxide nanoparticles |
| Si(1)-IONPS | One time silica coated IONPS |
| Si(2)-IONPS | Two time silica coated IONPS |
| P-IONPS | PEI coated IONPS |
| RB | Round Bottom Flask |

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# SYNOPSIS

Extensive use of nucleic acid for molecular diagnostics have increased the demand for their easy retrieval. As the DNA contains all the necessary information for the development and function of living things, retrieval must be rapid and effective. Existing Nucleic acid testing (NAT) method, involves the steps of extraction, amplification and detection, is currently labour intensive, expensive, time-consuming and equipment-dependent.

This led to the discovery of a non-contact and a rapid method for the retrieval of nucleic acid using magnetic nanoparticles. Along with an appropriate buffer system they possess larger binding capacity which can be modified with different coatings.

In view of that, we have synthesized iron oxide nanoparticles (IONPS) by coprecipitation method. These were further coated with silica and polyethyleneimine (PEI) to modify their DNA binding capacity. Results showed that PEI coated IONPS have maximum efficiency when compared with the uncoated and silica coated IONPS. The electrostatic attraction between negatively charged DNA and positively charged PEI coated IONPS resulted in their enhanced binding efficiency.

Commercially available IONPS for nucleic acid extraction are sold as suspension in appropriate buffer. This will reduce its shelf life as the buffer will lose its property within 3-6 months. IONPS synthesized here are stored in dried powder form which allows storage and ease of use at user end.

# CHAPTER 1

# INTRODUCTION

## 1.1 NUCLEIC ACID

Nucleic acids have been extensively used as molecular biomarkers in various applications such as medical diagnostics, food safety control and environmental monitoring.

In 1866, Gregor Mendel suggested the possibility of transfer of characteristics from one generation to next. This was modified by Friedrich Miescher and substantiated by Watson and Crick who provided a definitive proof.

Friedrich Miescher set a mile stone in 1869 with the discovery of Deoxyribonucleic Acid(DNA) while he was trying to learn the composition of lymphoid cells. A new molecule was accidently isolated , DNA with proteins which he called nuclein paved the way for a revolution in medicine and biology.(RalfDahm 2005) It was Albrecht Kossel, Nobel Prize winner and German biochemist who coined the name DNA. Between 1885 and 1901, Albrecht Kossel could isolate all the five nitrogen bases: adenine (A), cytosine (C), guanine (G) and thymine (T) in DNA replaced with uracil(U) in RNA. (Eugenio Frixione and Lourdes Ruiz-Zamarripa 2019) . Nuclein was renamed to nucleic acid by Richard Altmann in 1889. (RalfDahm 2005)

By 1940, DNA's role in genetic inheritance was explored and understood. Research was further carried out by many researchers; among them the role of Watson and Crick is critical. DNA's double helix, inter wined structure which is the fundamental to our existing knowledge was published by them.(J. D. Watson and F. H. C. Crick 1953)

Most of the living organisms have DNA within the cells. The distinctive profile of double helix DNA molecule as seen in most of the living organisms is composed of nucleotides. Nucleotides are long simple unit held together by a backbone made of sugars and phosphate groups.

DNA is considered as a long-term storage of cellular information necessary for the development and function of living things. They play a vital role in the storage of genetic instructions where the genes are the basic hereditable unit that carries them. Genome is an organism's complete set of nuclear DNA.

Nucleic acid testing (NAT) method, which generally involves the steps of extraction, amplification and detection, is currently labour intensive, expensive, time-consuming and equipment-dependent.

## REVIEW OF LITERATURE

### 1.2 ROLE OF DNA

DNA holds the instructions for an organism's or each cell's development, reproduction, replication, survival and ultimately death. It is often compared to a blueprint, since it contains the instructions to construct other components of the cell, such as proteins and RNA molecules.

The major function of DNA is to encode the sequence of amino acid residues in proteins, using the genetic code. Transcription and Translation are the two sequential process for protein synthesis. The size of genes varies from thousand bases to one million bases in humans. Apart from the nucleus, mitochondria also have DNA in them.

## 1.3 DNA ISOLATION – IMPORTANCE

With the discovery of DNA, the experiments for the isolation, retrieval were carried out.

Sir Archibald Edward Garrod in 1902 published the first finding from a study that deals with the inheritance of recessive genes. This opened the door for better understanding of genetic disorders that arise from the errors in chemical pathways in the body. Erwin Chargaff in 1905 discovered that DNA is responsible for heredity which varies between species. DNA also enables the treatment of genetic diseases.

DNA isolated from various disease-causing bacteria, virus, fungus etc can guide them to develop precise and personalised medicine against them.

Since the discovery of DNA, methods were implemented to isolate them. The extraction of biomolecules, DNA, RNA, and protein, are crucial methods used regularly in molecular biology. This is the starting point for downstream processes and product development including diagnostic kits.

### 1.3.1 Diagnostics

Detection of small number of base mismatches or single DNA is very complex due to the nature of DNA. This requires high sensitivity which is not provided by the conventional detection techniques. DNA alterations can cause a wide range of diseases which is extremely difficult to detect. The diagnosis of genetic-related diseases and conditions, for early stage treatment and monitoring is hard. (Fang Wei 2010)

Magnetic nanoparticles can also be tagged with specific ligands. These ligands can specifically bind with different types of DNA. Thus, magnetic nanoparticles can be used for specific DNA isolations.

Each pathogens have a unique complement of DNA which is the ideal molecular finger print aiding identification. Magnetic nanoparticles can also be tagged with certain ligands that can bind with those specific bacteria. This functionalization can be used to remove bacteria from the blood stream, fluids etc.(Jung-Jae Lee 2013)

This is utilised for RT-PCR, PCR, analysis of microbial DNA in clinical samples. Magnetic nanoparticles can isolate sufficient quantity of DNA even from the traces of samples of the order of nanogram per microlitre.(Gabrielle Heilek 2016)

### 1.3.2 Role in Genetics

DNA of an organism can be changed so as to bring benefits in them but this being a tedious and laborious process decreased its usage. The isolation needs to be simple, robust and automated. In vitro genetic modification within the cells allows biomanufacturing, disease modeling & enables cutting edge research. Isolation of quality nucleic acid sample is a key starting point. Each patient has unique biology and unique response to various diseases which can be studied. Various misleading conclusions regarding the individual response of patients which varies from the universal response can also be studied. This may open the lead to enable personalized medicine and its application in the future.

### 1.3.3 Scope of DNA in legal system

DNA samples aid in the forensic investigation of various criminal acts. DNA collected from the samples of unknown origins is used to carry out the investigations further. DNA typing is the primary method which directly compares the DNA profiles samples with known culprits whose DNA profiles are stored in computer data banks.

DNA profiles of a missing person can be reconstructed from parents/immediate relatives which can later be compared with the DNA obtained from unidentified body parts or bone fragments. (*DNA Technology in Forensic Science*. 1992)The DNA obtained is replicated through PCR.

An advantage of DNA profiling is how resilient the molecule is, it can survive even after exposure to chemicals and bacteria.

### 1.3.4 Nanotechnology

Three dimensional structures can be build using DNA strands, DNA origami. This involves nanostructures based on computer templates. Scientist can accurately control the dimensions, shape, size and the folding of these structures. These can further be configured into molecular nanomachines which will one day allow the development of DNA quantum computers.(“DNA The Source Code for Life,” 2019)

### 1.3.5 Predicting Ancestral Origin

DNA collected from the saliva sample is used to find a complete genetic picture about your ancestry. mtDNA can give information about matrilineal heritage or the female ancestors while Y-DNA gives information about patrilineal heritage or male ancestors.

An autosomal DNA test gives information about both matrilineal and patrilineal heritage.

DNA variations can pass down different generations which helps to trace your ancestry even thousands of years back. The DNA isolated from the sample is compared with millions of test in the database. This provides you a list of DNA matches which helps to extend your knowledge about ancestry. People with deep ancestral roots can have similar DNA which helps to identify the group that a person belongs, migration patterns of your ancestors.

### 1.3.6 Molecular Genetics

Except for twins, no two persons are genetically identical. DNA provides enormous information regarding the eyes color, shapes of earlobes. DNA helps to diagnose a wide variety of inherited traits ( cystic fibrosis, Huntington's disease ) and medical conditions . A stage will be reached when more common medical conditions like heart disease, diabetes, hypertension and Alzheimer's disease could be detected from DNA. Other behavioral traits like learning disabilities can also be detected.

### 1.3.7 Surgery

Nanobots can be used to treat cancer cells. Nanobots can be programmed in such way that they target it only to cancer cells without affecting the healthy cells.

Aptamer encoded logic gate will control the nanobots where nanoparticle of any type can be transformed into autonomous biocomputing structures which are capable of executing the Boolean logic. Logic gating functionality (NAND, NOT, AND and OR) can be incorporated into DNA that can be used to develop a diverse set logic circuit.

Nanobots can be developed using DNA origami which enables nanoscale folding to create two and three dimensional shapes in nano regime. These can interact with each other once they are introduced into the body which produce logical outputs which can enable and disable the logical circuit.(Devasena Umai R 2018)

## 1.4 DIFFERENT METHODS OF RETRIEVING

DNA was initially isolated by Friedrich Miescher in 1869 while he was trying to learn the chemical composition of the cells. Finding it difficult to isolate cells from lymph nodes, Miescher switched his isolation from lymph nodes to lymphocytes where he succeeded. During the test, Miescher noticed a substance that precipitates in acidic medium and dissolves in a basic one which was named as DNA afterwards. His initial focus was on the different types of proteins present in the leucocytes. (RalfDahm 2005) Different isolation methods were then discovered to isolate the DNA, among them centrifugation method was the most common one. This technique was used to demonstrate the semi conservative replication of DNA by Meselson and Stahl in 1958. Since then, different methods to isolate DNA were identified over- time.(Siun Chee Tan and Beow Chin Yiap 2012) The choice of method depends on the downstream use of the DNA. Different techniques developed can be classified as solid based techniques, fluid based techniques and extraction using magnetic nanoparticles.

### 1.4.1 Fluid based methods

Fluid phase based methods are based on series of washing and precipitation methods which may involve the use of highly toxic substances. Large number of steps involved will increase the degradation risk and will result in the loss and contamination of

samples.(Berensmeier 2006) This limits the retrieval from smaller sample for high-throughput screening and diagnostic tests(Lee et al 2018)

1.4.1.1 Guanidinium Thiocyanate-Phenol-Chloroform Extraction

Organic solvent - mediated phenol-chloroform extraction a widely used technique for the isolation of nucleic acid. Cell lysis , inactivation of cellular nucleases and separation of desired nucleic acid from cell debris are the general steps involved in DNA Isolation.(Siun Chee Tan et al. 2009) Even though organic extraction methods provide pure DNA it involves a number of centrifugation step and with lower yields of recovered DNA.(Saiyed et al. 2007)

1.4.1.2 Alkaline Extraction Method

This method works on the selective alkaline denaturation of high molecular weight chromosomal DNA while covalently closed circular DNA remains double stranded. Alkaline lysis is used to isolate plasmid DNA and *E. coli* . Plasmid DNA can be recovered from the supernatant after the denatured material has been removed by centrifugation.

1.4.1.3 CTAB Extraction Method

Cetyltrimethylammoniumbromide (CTAB) is a non-ionic detergent that has the ability precipitate nucleic acids and acidic polysaccharides from low ionic strength solutions. In solutions of higher ionic strength CTAB forms complexes with proteins. Purification of nucleic acid can be done from those organisms which produce large quantities of polysaccharides such as plants and certain Gram-negative bacteria. This is followed by the centrifugation step to remove the insoluble particles.

### 1.4.2 Solid-phase nucleic acid purification method

Incomplete separation causes downstream applications to fail in liquid-liquid extraction method. This can be avoided in solid-phase nucleic acid purification method where the nucleic acid will be adsorbed during extraction. The adsorption may vary based on the pH and salt content of the buffer. Cell lysis, nucleic acid adsorption, washing and elution are the processes involved which is normally performed using a spin column, operated under centrifugal force. Rapid nucleic acid purification is its advantage over the conventional methods. (Siun Chee Tan et al. 2009)

For the separation of DNA, mechanisms like anion exchanger, affinity and size exclusion mechanisms which forms a part of solid phase process are used. The process is based on the hydrogen-binding interaction between an underivatized hydrophilic matrix and ionic exchange under aqueous condition by means of an anion exchanger, affinity and size exclusion mechanisms. (Berensmeier 2006)

### 1.4.3 Nucleic Acid Purification using Magnetic nanoparticles

Magnetic nanoparticles along with an appropriate buffer system can be used in DNA isolation techniques which provide a platform for various DNA applications. Nanoparticles have larger surface area to volume ratio that increases its reactive sites. As the size reduce down to nanometre range, more is the probability for the nucleic acid to bind with magnetic nanoparticles. Isolation via this method eliminates the centrifugation steps, do not require toxic chemicals nor any sophisticated equipment. They do not require column separation or vacuum filtration and can be carried out in resource constrained environments.

Limiting aerosol generation is also key to the success of this method. Aerosol or airborne transmission is a major route for many human pathogens that include viruses ( Zoster virus ), Bacteria ( Mycobacterium Tuberculosis , Acinetobacter spp., Pseudomonas spp. and Legionella spp) and Fungi ( Aspergillus spp.) Extensive work has documented that short range, large droplet transmission is possible for the transmission of most respiratory agents which spread via aerosol route (J.W. Tang a, et al. 2006) e.g. tuberculosis, measles and chickenpox. (Raymond Tellier et al. 2019) This offers a large probability to infect the ones who diagnose and handle it. The droplets can escape during the analysis/ diagnosis which evaporates and form more smaller droplets which becomes airborne.(J.W. Tang et al. 2006)

Large scale methods to isolate DNA must be made available which meets the approval of regulatory agencies. (Kang et al. 2009)This method being a centrifugation free technique can limit the aerosol spread of diseases. The extreme sensitivity of these methods allows the detection of target DNA present at very low levels.

Magnetic nanoparticles have higher binding capacity which can further be modified based on the requirements. They can be coated with silica, polymers, ligands whose adsorption of DNA are driven by ligand binding interactions, electrostatic and hydrophobic interactions.

Paramagnetic nanoparticles have the ability to bind as well as release the DNA and can be used for both retrieval as well as delivery of DNA.

## 1.5 MAGNETIC NANOPARTICLES

Materials at the nano scale have properties altered irrespective of their physical or chemical nature. Similarly, their magnetic properties also alter from the established

laws governing magnetic phenomena in bulk. The basics of the nanoparticle research was established by Michael Faraday in the middle of 1800s, when he discovered the optical properties of gold colloids. Frenkel and Dorfman were the first to predict the single magnetic domain phenomenon of ferromagnetic materials. This phenomenon occurs below a critical size which was roughly estimated by Kittel. (S. Bedanta et al. 2013)

Particles having average diameter above 100nm is referred as bulk and those below 100nm falls in the category of nanoparticles. The magnetic properties of bulk material depend mainly on the chemical composition, crystal structure, presence of vacancy defects etc. But in the case of magnetic nanoparticles size and shape matters. Magnetic property possessed by the nanoparticles is an added advantage as these particles can be easily guided by an external magnetic field which helps in various biological applications.

Ferromagnetic and ferrimagnetic materials have domain structure within which spins are oriented. These materials can be influenced by an external magnetic field. Bulk is made up of multi magnetic domains with each of them corresponding to different spin between them while same spin within each domain. In the case of nanoparticles multi domain is reduced to single domain, their size will be comparable to that of single domain.

Magnetism in a material is a result of magnetic moment(μ) of electrons, that arise due to both spin and orbital motion of the unpaired electrons. Orbital angular moment(l) contributes to orbital magnetic momentum($m_l$), while spin(s) produces to spin angular momentum($m_s$).

$m_l = g_l l$

$m_s = -g_s \mu_B s$

$g_l$ and $g_s$ are the orbital and spin g-factors

$\mu_B$ is the Bohr magneton.

In addition to the magnetic property of the material , chemical reactivity , high surface area to volume ratio also increase the interest of researchers to use it for nucleic acid retrieval.(Justine Wallyn et al. 2019). Delivery of nucleic acid based agents is also plausible via this route.

For biological applications magnetic nanoparticles are classified as paramagnetic, ferromagnetic and superparamagnetic.

Paramagnetic particles have small net magnetic moment due to random orientation of spin. They orient themselves in the direction of external magnetic field.(Christine Rümenapp et al. 2012).In ferromagnetic particles, they already possess certain magnetic moment even in the absence of external magnetic field. Their spins are arranged in the same direction. When the temperature is raised above a critical temperature called curie temperature the ferromagnets loses its ferromagnetic property and becomes paramagnetic in nature. Ferrimagnetic particles have an unequal arrangement of spin in antiparallel and parallel direction creating a net magnetic moment. Superparamagnetism is a magnetic behavior exhibited by both ferromagnetic and ferrimagnetic nanoparticles. As the size of the particles reduce below 100 nanometers, they tend to form single domain particles. Their transition to paramagnetism occurs below curie temperature.

Among them we have focused on ferrimagnetic iron oxides. Iron oxide nanoparticles (IONPS) shows low toxicity, stable magnetic properties and ease of preparation which increases its relevance in biological applications.(A G Pershina et al. 2014) The small controllable size of IONPS and their similarity in size with respect to the biological entities allows an easily interaction between them. Certain fluorescent ligands can be also attached to those entities. This can provide more precise view of them upon illumination.

Metallic ferrous nanoparticles exists as three main iron oxides : hematite ($\alpha$-$Fe_2O_3$), maghemite ($\Upsilon$- $Fe_2O_3$) and magnetite ($FeO.Fe_2O_3$) which can be identified by their colour. Hematite and maghemite will show colour from red-brown while magnetite is black in colour. Ultrasmall iron oxide crystals below 10 nm are called Super Paramagnetic Iron Oxide Nanoparticles (SPIONS) whose super paramagnetism will vary with respect to the size of nanoparticles. In the single domain state superparamagnetic property increases with increase in size. (Justine Wallyn et al. 2019)

Magnetic nanoparticles along with an appropriate buffer system can be used in DNA isolation techniques which provides a platform for various DNA applications. Entire process can be automated and the nucleic acids can be isolated from comparatively larger sample volumes.

## 1.6 IRON OXIDE NANOPARTICLES(IONPS)

### 1.6.1 Crystal Structure

Metals like Fe, Cr, Mo, V are body centered cubic structure(bcc). For bcc structures, unit cell has one by eight of an atom at all the eight corners and one atom at the center of the cube. All together contributing to 2 atoms per unit cell with 8 as its coordination number.

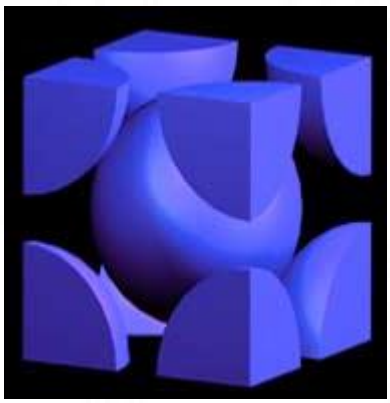

**Figure 1**: Schematic representation of BCC structure of unit cell (adapted from Anthony R West, 2014)

But in the case of iron oxide nanoparticles they have an inverse spinel structure.

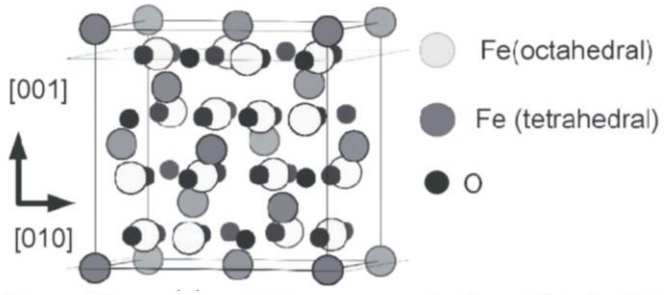


**Figure 2** : Schematic representation of magnetite having inverse spinel structure (adapted from Diego Tozini et al., 2015)

Represented as,

$$[B]^{tet}[A,B]^{oct}O_4 ; Fe^{3+}(Fe^{2+}Fe^{3+})O_4$$

Where A and B are the cations occupying interstitial sites. Half of the B ions occupy tetrahedral sites, and the remining B ions along with A ions occupy octahedral sites. i.e $Fe^{3+}$ occupy tetrahedral sites while $Fe^{2+}$ along with the remaining $Fe^{3+}$ occupy the octahedral sites.

## 1.7 SYNTHESIS TECHNIQUES OF MAGNETIC NANOPARTICLES

### 1.7.1 Chemical Methods

1.7.1.1 Co-precipitation Method

$FeCl_2$ and $FeCl_3$ are coprecipitated for the synthesis of iron oxide nanoparticles. They are precipitated in the ratio 1:2 by the addition of a base (NaOH) purged with Nitrogen. Ferrous chloride and ferric chloride will supply the iron ions for the reaction,

$$Fe^{2+}\,(aq) + 2Fe^{3+}\,(aq) + 8OH^{-}\,(aq) \rightarrow Fe_3O_4\,(s) + 4H_2O$$

The physical and chemical properties of the synthesised nanoparticles are affected by the pH and temperature. Iron ions are usually supplied as ferrous and ferric chlorides or sulfates. Magnetite or maghemite IONPS with an inverse spinel structure is obtained in this technique. (Kamyar Khoshnevisan et al. 2012 ; Morteza Mahmoundi et al. 2009)

1.7.1.2 Microemulsions

Two immiscible liquids are mixed together to form a thermodynamically stable transparent isotropic solution. Anionic (AOT, sodium dodecylsulphate), non-anionic(polyethoxylates, lutensol) and cationic surfactants (CTAB) are the most commonly used ones for synthesis for the stabilization of liquids. When iron salts and base medium emulsions are introduced, the microplates will collide with each other.

This will breakdown the microplates resulting in the formation of a precipitation of micelles.(Bagwe R P and Kanicky J R 2001)

In order to synthesize core shell structures microemulsions are preferred.

1.7.1.3 Thermal Decomposition

When iron precursors (Fe(acetylacetonate)$_3$) are decomposed in the presence of hot organic surfactants and solvents (steric acid, fatty acid) thermal decomposition occurs. Reaction time, temperature, concentration of reactants can greatly influence the physicochemical characters of the IONPS.(Gupta A K and Gupta M 2005)

For the synthesis of narrow size distribution, uniformity and highly crystalline iron oxide nanoparticles this technique is used. These IONPS also possess high saturation magnetization and high initial susceptibility.

1.7.1.4. Sol gel

Sol of nanoparticle is formed when the molecular precursors in the solution undergoes condensation and hydroxylation. It is a wet method that can synthesize monodispersed nanoparticles whose properties depends upon temperature, nature, concentration, pH etc. In order to obtain crystallinity these particles are heated in a required temperature.

This technique can yield maghemite, hematite goethite and iron hydroxide nanoparticles.(Ennas G et al. 1998)

1.7.2.5. Polyol

In this synthesis technique, polyols like ethylene glycol, diethylene glycol are used. This will reduce the ratio of metal salts to metal. Magnetic nanoparticles synthesized

will possess a hydrophilic polyol ligands on its surface which will reduce its dispersion in solvents.(Joseyphus R J et al. 2007)

The nanoparticles synthesized in this technique are isolated from the oxidizing atmosphere thus ensuring more stability and are highly crystalline in nature.(Morteza Mahmoundi et al. 2009)

### 1.7.2 Physical Methods

Synthesis of magnetic nanoparticles by chemical method uses toxic surfactants that can decrease the biocompatibility of the materials. This can be reduced with the use of physical methods.

#### 1.7.2.1 Pulsed Laser Deposition

A pulsed laser beam is focused at the tip an iron wire. This will decompose the iron and vaporizes it to form a plasma plume that gets deposited on the nearby substrate. The particle size can be decreased by increasing the laser power density. This technique reduces the possibility of contamination and increases the biocompatibility.(Wang Z et al. 2006)

#### 1.7.2.2 Powder Ball Milling

In this technique, nanoparticles can be produced by high energy ball milling of micrometer sized metal particles. The mixture is powdered and cold welded through a mechanical alloying process. Apart from controlling the crystalline size of nanoparticles , this method can induce the phase transition from $\alpha$-$Fe_2O_3$ to $\Upsilon$-$Fe_2O_3$ and vice-versa. (Jiang J Z et al. 1997)

This is preferred only for laboratory usage due to its limited yield.(Morteza Mahmoundi et al. 2009)

**Co-precipitation** technique is the synthesis route selected here for the nanoparticles.

## 1.8 SURFACE MODIFICATION

Strong magnetic dipole-dipole attractions between particles tend to aggregate the nanoparticles and reduces the homogenous distribution of particles in suspension. Modification of these nanoparticles can provide useful functionality & beneficial properties. This can be tuned based on the agents used. Modification inhibits the agglomeration of nanoparticles and give hydrophilic properties to them. Commonly used agents are silica based systems, polymers and peptides.

In addition to this, oxidation of magnetic nanoparticles from magnetite to maghemite occurs in long term storage of magnetite nanoparticles. This will change its magnetism from paramagnetic to ferromagnetic which will reduce saturation magnetism and thus reducing the magnetic property of the nanoparticle.(Lee et al. 2018) A corona around the IONPS can protect it from early oxidation and will increase the stable shelf life of IONPS. Surface modification can increase the stability of nanoparticle an can also protects them from acid and basic corrosion, release of metal ions.(A G Pershina et al. 2014)

### 1.8.1 Cetyltrimethylammonium bromide (CTAB)

Cetyltrimethylammoniumbromide (CTAB), $C_{19}H_{42}BrN$ is a common surfactant used. This when used as a coating agent can reduce the size of the particle and will increase the homogenous dispersion the particles in solution. (Kamyar Khoshnevisan et al.

2012) CTAB is a cationic reagent having positive charge and has the potential to reduce the surface charge of the particle thereby increasing the stability.

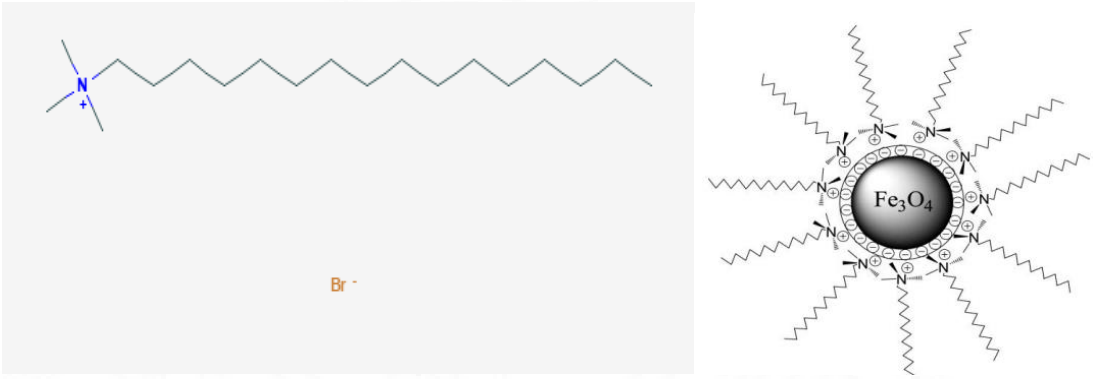


**Figure 3** : (a)Structure of CTAB (b)Surface modification of Iron oxide nanoparticle by CTAB(adapted from Kamyar Khoshnevisan et al. 2012)

The interaction between the head group of CTAB and the hydroxyl group on the surface of IONPS will results in the formation of micelles by making an organic chain of CTAB around it(Adheesha N. Danthanarayana1 et al. 2018) which will help the nanoparticle to remain more dispersed in the solution. This will reduce the aggregation of the nanoparticles and increase the loading area.

1.8.2 Silica

Silica coated magnetic nanoparticle binds non electrostatically with the nucleic acid thereby ensuring an easy release of DNA molecule from the surface. (J.R Sosa-Acosta 2018) A strong ionic bond exist between the particles and the phosphate backbone of DNA molecules thereby increasing the amount of DNA that binds to the IONPS(J.R Sosa-Acosta 2018)

Silica provides more resistance towards biodegradation when compared with organic coating materials like chitosan etc. They exhibits higher stability for a wider range of pH in aqueous conditions.(Dae-Won Lee et al. 2018)

The simplicity and robustness of the method described here makes it very valuable for large-scale, low cost use in DNA and RNA separations (Lee et al. 2018)The thickness of silica shell can be controlled by TEOS concentration. This change in thickness can subsequently change the charge possessed by the nanoparticle. (Kang et al., 2009)

1.8.3 Polyethyleneimine(PEI)

PEI is an organic polymer alkyl chain with primary, secondary and tertiary amines. (DanielleC.Arruda et al. 2017)They exist in three forms ; linear , partly branched or repetitively branched. Linear contains only primary amines while branched PEI has secondary and tertiary amines. In PEI, the one nitrogen atom present in every three atoms present in the backbone provides a positive charge upon protonation.

IUPAC Name : Poly(iminoethylene)

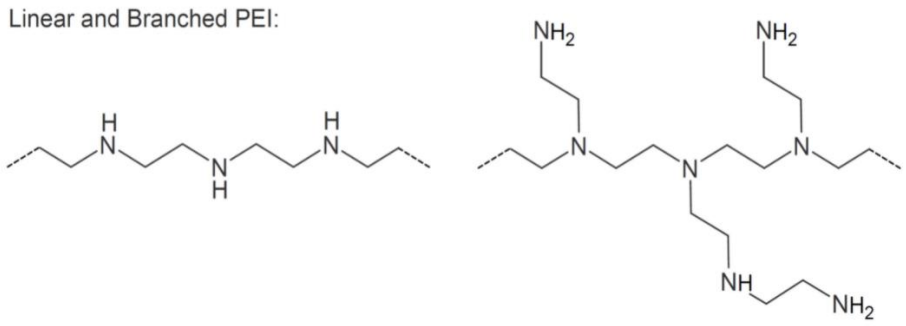


**Figure 4 :** Structure of Polyethyleneimine(PEI)
(adapted from Ubong Eduok et al. 2019)

Being a cationic polymer it interacts with negatively charge phosphate backbone of DNA via electrostatic attraction.(Adheesha N. Danthanarayana1 et al. 2018 ; Qiyi Feng et al. 2018 ; S. C. McBain et al. 2007)). The phosphate anion gets adsorbed on the IONPs at physiological pH due to their interaction with $Fe^{3+}$ in octahedral sites.

PEI possess an inherent property to condense genetic material which can be utilised to deliver DNA/siRNA Only those PEI coated IONPS having positive charge comparable to the negative charge DNA can significantly bind to each other. (S. C. McBain et al. 2007)

Apart from using for DNA isolation, PEI is used for the synthesis of various colloidal particle, gene- delivery vehicle, surface modified adsorbent. They are also utilizing for antimicrobial coatings for medical devices as they can rupture bacterial cell membrane.

## 1.9 COMMERCIALLY AVAILABLE SYSTEMS

Matrices based on silica, porous glass, cellulose , agarose , polystyrene and silane are the commercially available magnetic particles. Non-specific nucleic acid adsorption , sedimentation of magnetic particles with glass coating , time consuming , complex spray and additional mechanical mixing processes will pull back the applications of magnetic particles.(Berensmeier 2006) These products like Mag-bind, MagJET, Magmax are available in suspension form in appropriate buffer.

## 1.10 HYPOTHESIS

Magnetic nanoparticles can be modified to interact with DNA via electrostatic or non electrostatic bonding which can be used for the isolation of DNA.

## 1.11 OBJECTIVES

The work will involve

- Synthesis and physicochemical characterization of iron oxide nanoparticles (IONPS).
- Surface modification of iron oxide particles with silica and polyethyleneimine.
- Demonstration of DNA binding and Retrieval efficiency of coated and uncoated IONPS.

# CHAPTER 2

# MATERIALS AND METHODS

## 2.1 MATERIALS

Materials were purchased from established sources and are specified as follows.;

| ***Chemical Name*** | ***Formula*** | ***Brand*** | ***Assay*** |
|---|---|---|---|
| *Ferrous chloride tetrahydrate* | $FeCl_2.4H_2O$ | Sigma Aldrich | 98% |
| *Ferric Chloride* | $FeCl_3$ | Sigma Aldrich | 98% |
| *λ-DNA* | | TaKaRa | |
| *Sodium Hydroxide Pellets* | NaOH | S.D.fine | |
| *Polyethyleneimine (branched, MW – 25000)* | PEI | Sigma Aldrich | |
| *Ammonium solution* | $NH_4OH$ | Avantor | 25% |
| *Tetraethylorthosilicate(TEOS)* | $SiC_8H_{20}O_4$ | Sigma Aldrich | 98% |
| *Cetyl trimethyl Ammonium Bromide(CTAB)* | $C_{19}H_{42}BrN$ | Sisco Research Laboratories | 99.5% |
| *Agarose Low EEO Superior Grade* | | Sisco Research Laboratories | |

**Table 1**:Materials used for synthesis of IONPS,Si(1)-IONPS,Si(2)-IONPS,P-IONPS

## 2.2 METHODS

### 2.2.1 Synthesis of Iron oxide nanoparticles

Iron oxide nanoparticles (IONPS) was synthesized by co-precipitation method. Ferrous chloride tetrahydrate, $FeCl_2.4H_2O$ (0.1M) and Ferric chloride , $FeCl_3$(0.1M) were dissolved in HCl in the stochiometric ratio of $Fe_3O_4$. The mixture is then dropped into 2M NaOH with a continuous stirring and heating for 20 minutes in the presence

of nitrogen. 25% ammonia is added as required, followed by 30 minutes stirring after complete addition. The black slurry formed is cooled, centrifuged and peptized. The suspension of IONPS in distilled water is preserved at room temperature and lyophilized.

Samples are characterized by SEM, TEM, FT-IR and EDS analysis. DLS and Zeta Potential measurements provided the size and charge of the IONPS.

**Retrieval of DNA using IONPS**

The synthesized IONPS (as per 2.2.2) were used for DNA retrieval. IONPS was prepared as per the BOMB Protocol.(Oberacker et al. 2019)

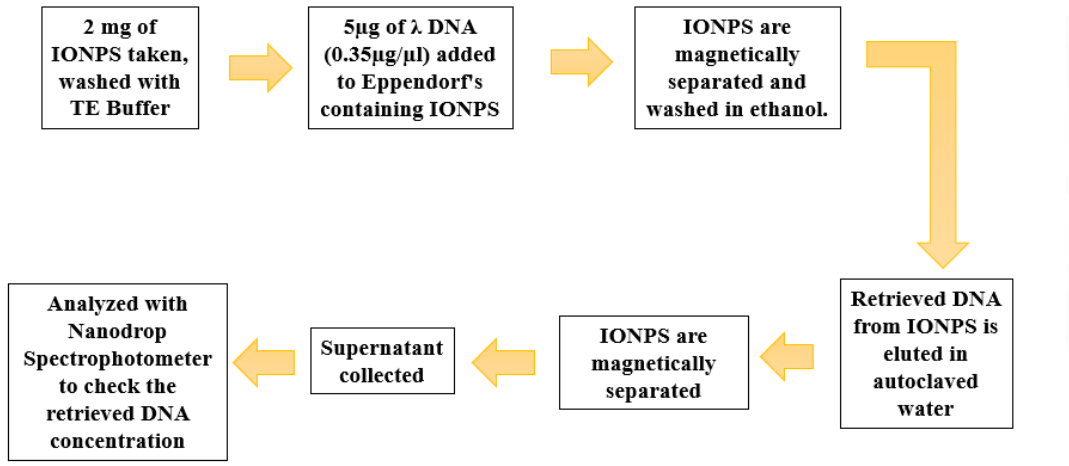


**Figure 5**: Schematic representation of DNA retrieval using magnetic nanoparticles.

Purity of DNA is analysed using Nanodrop Spectrophotometer. The quantity of retrieved DNA was also confirmed by gel run in a 1% Agarose gel. Retrieval Efficiency is calculated as,

$$\text{Retrieval Efficiency} = \frac{\text{Quantity of DNA Retrieved}}{\text{Initial Conectraation of DNA}} * 100$$

### 2.2.2 Synthesis of Iron oxide nanoparticles with Silica coating

**Synthesis of Iron oxide nanoparticles**

$FeCl_3$ and $FeCl_2.4H_20$ is mixed in required proportion in distilled water with nitrogen purging. 28-30% of Ammonia was added drop wise to the solution under continuous stirring. Solution is heated for 1 hour without boiling followed by cooling, centrifugation and peptization. IONPS synthesized was resuspended in distilled water.

**Surface Modification by Silica**

IONPS were magnetically separated and an aqueous sodium citrate solution was added to it. The suspension was stirred and again magnetically separated. The separated IONPS were added to 90% ethanol taken in Round Bottom flask(RB) under continuous stirring. The solution was heated at a temperature below boiling point. TEOS diluted in purified ethanol is added drop wise to RB followed by the addition of Ammonia. Stirring was continued for 4 hours and were lyophilized.

Samples are characterized by SEM, TEM, FT-IR and EDS analysis. DLS and Zeta Potential measurements provided the size and charge of the IONPS.

**Retrieval of DNA using Si-IONPS**

The IONPS synthesized as per 2.2.2 is used for DNA retrieval. Purity of DNA is analysed using Nanodrop Spectrophotometer and later on confirmed by gel run. Retrieval efficiency was calculated as mentioned in 2.2.1.

### 2.2.3 Synthesis of Iron oxide nanoparticles with PEI coating

**Synthesis of Iron oxide nanoparticles**

$FeCl_2.4H_20$ and $FeCl_3$ are dissolved separately in deionized water in the presence of nitrogen and are mixed together. 0.1M CTAB is added to it followed by the addition of 5M Ammonia with continuous stirring for 40 minutes. The suspension is magnetically separated, washed and stored in vacuum desiccator for drying.

**Surface modification using PEI**

5M $NH_4OH$ is added drop wisely to 20% PEI solution prepared in distilled ethanol. The solution is heated up to 50°C. Required quantity of dried IONPS is added and stirred continuously for 30 minutes. The suspension is magnetically separated, washed and stored in vacuum desiccator for drying. The samples were preserved at room temperature.

Samples are characterized by SEM, FT-IR and EDS analysis. DLS and Zeta Potential measurements provided the size and charge of the IONPS.

**Retrieval of DNA using P-IONPS**

The IONPS synthesized as per 2.2.3 is used for DNA retrieval. The result is quantified using Nanodrop Spectrophotometer later on confirmed by gel run. Retrieval efficiency calculated as mentioned in 2.2.1.

## 2.3 ANALYSIS TECHNIQUES

### 2.3.1 PHYSICAL STUDIES

2.3.1.1 DLS Measurement

The sample concentration is adjusted to 0.1% w/v particles in distilled water followed by sonication to minimize the diffusion of particles in the solution. The particle size is determined by the measuring the fluctuation in scattering intensity using DLS (Zetasizer Nanoseries , Nano ZS, Malvern Instruments).

2.3.1.2 Zeta Potential

The sample concentration is adjusted to 0.1% w/v particles in distilled water followed by sonication to minimize the diffusion of particles in the solution. Zeta Potential determines whether the particles have the tendency to flocculate. Large negative charges or positive charges will repel the particles thus reducing the tendency to flocculate. (Zetasizer Nanoseries , Nano ZS, Malvern Instruments).

### 2.3.2 MORPHOLOGICAL STUDIES

2.3.2.1 Scanning Electron Microscope(SEM)

Magnetic nanoparticles suspended in distilled water is dropped onto a glass plate after sonication. Further coated with Au/Pd material to ensure the conductivity to carry the charging electrons using sputter coater (E1010, Hitachi). SEM uses high energy electron beams to obtain the surface morphology of the sample( FEI, Quanta) .

2.3.2.2 Transmission Electron Microscope(TEM)

The IONPS dispersed in distilled water after sonication is dropped on a TEM grid made of copper and dried. TEM uses electron beam ejected out from the electron gun at a high voltage to obtain a magnified image even at nano scale.

### 2.3.3 CHEMICAL STUDIES

2.3.3.1 Fourier Transform Infrared Spectroscopy (FT-IR)

Different functional groups and components present in the sample is obtained using Thermo Nicolet 5700 spectrometer (USA). The spectra were obtained in DRIFT mode. The sample is mixed with KBr powder (IR Grade) and scanned under the electromagnetic spectrum between the range 4000$cm^{-1}$ - 400$cm^{-1}$(mid IR Region). Number of scans is 64 with a resolution of 4$cm^{-1}$. Spectrum was taken using KBr as background. Based on the light absorbed/transmitted/reflected different vibrational modes corresponding to the composition of the compound is determined. IR pattern is curve of Absorbance/Transmittance/Reflectance v/s wavenumber.

2.3.3.2 Energy Dispersive X Ray Spectroscopy (EDS)

The surface elemental compositions were analyzed using energy dispersive X-ray spectroscopy (FEI, Quanta). This microanalysis technique comes with SEM. X-Rays are emitted from the sample during SEM Analysis. These can give information regarding the elemental composition of the sample.

2.3.3.3 X Ray Diffraction (XRD)

Crystallinity and phase were analyzed by XRD (Bruker D8 Advance, Germany). Information regarding the size, crystal structure, composition etc. of sample can be determined. Sample was scanned with Cu-Kα radiation at a current 30mA and voltage of 40KV. X-ray beams gets diffracted as they pass through the samples depending on the arrangement of planes in them. XRD pattern is a 2Θ v/s Intensity curve where Θ is the angle of diffraction. 2Θ ranges from 20-80 at the rate of 2°/minute at a step size of 0.02 degree.

### 2.3.4 BIOLOGICAL STUDIES

2.3.4.1 Nanodrop Spectrophotometer

NanoDrop™ 2000/2000c Spectrophotometer are full spectrum, UV-Vis spectrophotometers used to quantify and to determine the purity of DNA retrieved. They are microvolume spectrophotometers which requires samples less than 0.5 µl. The sample directly dropped on the optical measurement surface of the instrument. Ratio of absorbance at 260nm, 280nm (A260/280) and 260nm, 230 nm (A260/230)

Ratio of absorbance at 260nm and 280nm gives the concentration and purity of the DNA sample. For pure DNA 1.8 and for pure nucleic acid 1.8-2.2 is the generally accepted ratio.

Detection range of nanodrop spectrophotometer is between 0.4ng/µl and 15000ng/µl.

2.3.4.2 Luminescent Image Analyzer

Luminescent Image Analyzer(LAS 4000 Fuji Film , Japan) gives the fluorescence image of the gel after run. Multipurpose CCD camera will provide images upon illumination by various filters. When illuminated with 312nm and 365 nm filter (UV region) EtBr intercalated with DNA shows enhanced fluorescence. Exposure time is adjusted to $1/30^{th}$ and $1/15^{th}$ of a second.

# CHAPTER 3

## RESULTS AND DISCUSSION

### 3.1 IRON OXIDE NANOPARTICLES(IONPS)

#### 3.1.1 PHYSICAL STUDIES

3.1.1.1 Dynamic Light Scattering(DLS)

| | | | Size (d.nm): | % Intensity: | St Dev (d.n... |
|---|---|---|---|---|---|
| Z-Average (d.nm): | 143.1 | Peak 1: | 166.0 | 93.9 | 49.94 |
| PdI: | 0.311 | Peak 2: | 27.27 | 6.1 | 4.416 |
| Intercept: | 0.940 | Peak 3: | 0.000 | 0.0 | 0.000 |
| Result quality : | Good | | | | |

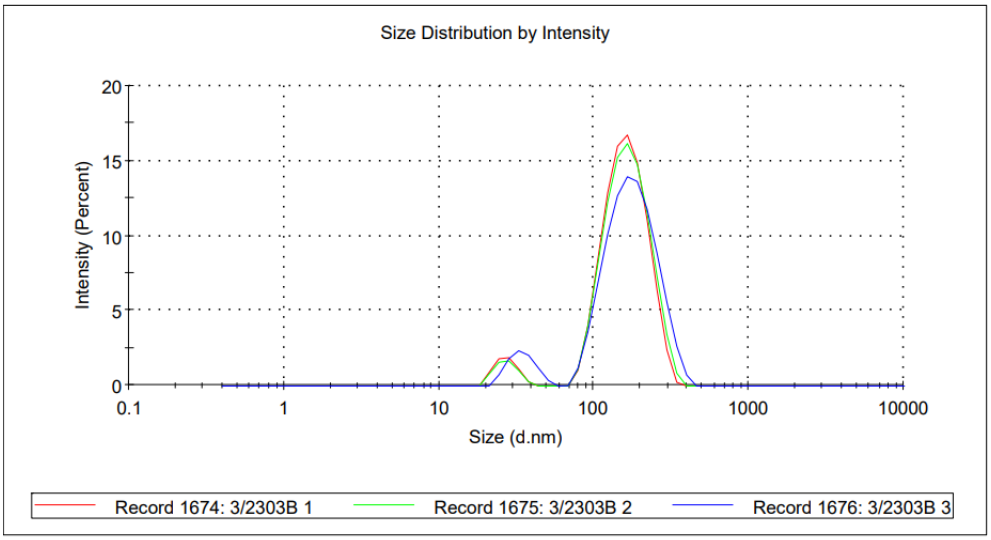


**Figure 6:** Dynamic Light Scattering(DLS) size measurements of IONPS

The DLS analysis indicated the presence of monodispersed nanosized particles in the range of 166nm. Size Distribution intensity peaks showed that 93.9% of the particles are of same size.

3.1.1.2 Zeta Potential

| Sample | Zeta Potential Dispersed in water |
|---|---|
| **IONPS** | **22.1mV** |

**Table 2** : Zeta potential measurements of IONPS.

Zeta potential of IONPS measured in aqueous medium (distilled water) is 22.7mV. This value is adequate to maintain the stability of nano particles. Large negative and positive values is due to higher electric repulsion between the particles. This prevents the aggregation of particles and ensures stability. Zeta Potential is found to decrease as pH decreases.

### 3.1.2 MORPHOLOGICAL STUDIES

3.1.2.1 Scanning Electron Microscope(SEM)

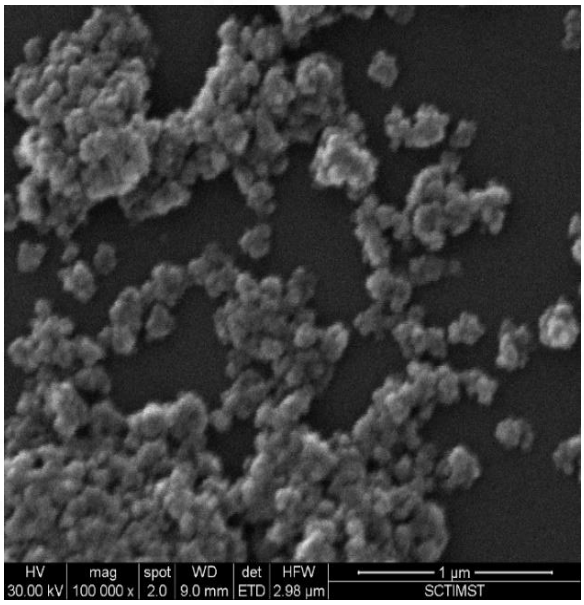


**Figure 7:** SEM micrographs of IONPS.

SEM images shows nearly spherical nanosized particles in aggregate form. A well defined spherial particles need not be the essential criteria to increase the binding. The increase in surface area to volume ratio(specific surface area)in the nanoparticles will increase the reactive site which consequently increases the binding capacity.

3.1.2.2 Transmission Electron Microscope(TEM)

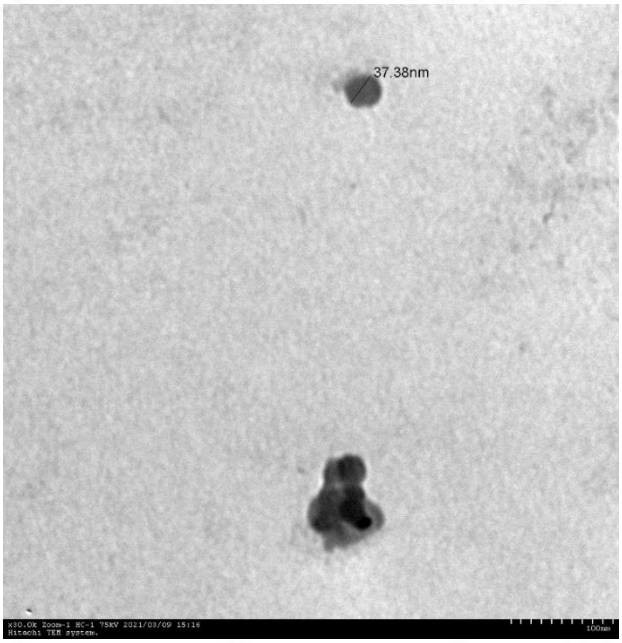


**Figure 8 :** TEM Micrograph of IONPS at 75kV

TEM micrographs confirms the near sphericity of nanosized particles having a particle size of 37.38nm.

### 3.1.3 CHEMICAL STUDIES

3.1.3.1 EDS Pattern

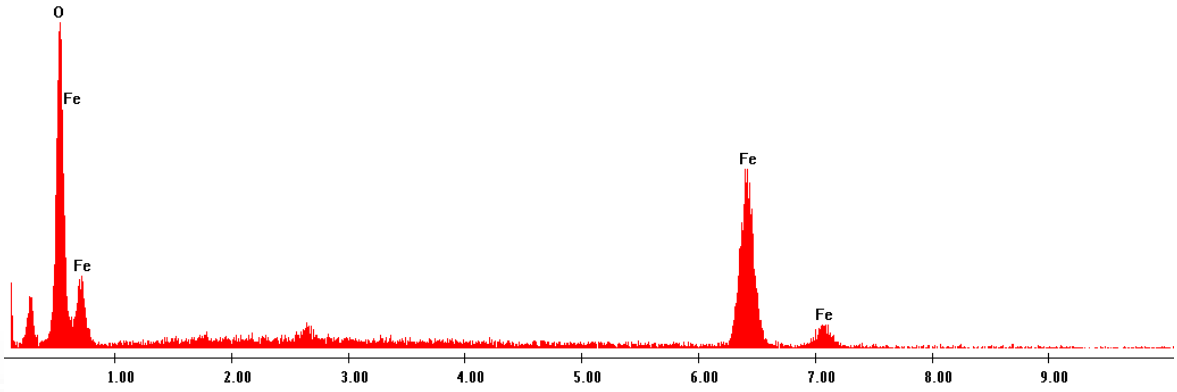


**Figure 9 :** EDS Pattern of IONPS

EDS Pattern confirms the presence of only Fe (Iron) and O(Oxygen) peaks thus confirming the chemical purity.

3.1.3.2 Infrared Spectroscopy

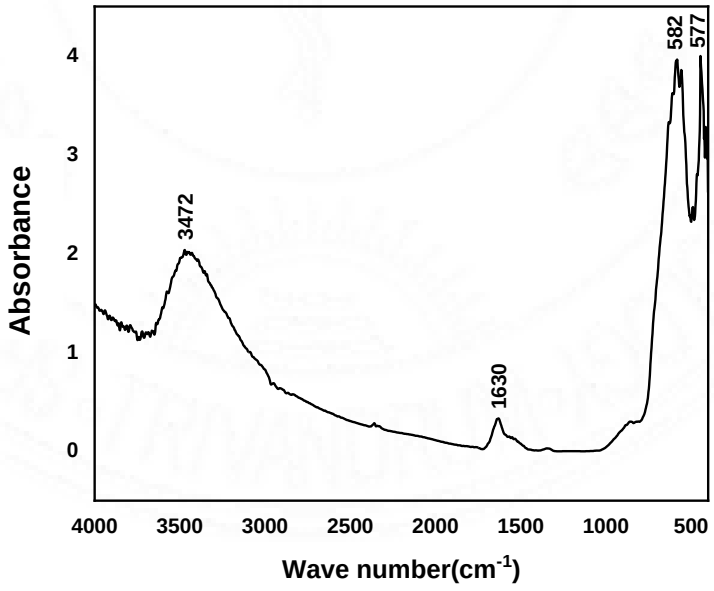


**Figure 10 :** IR spectrum of IONPS

IR bands at 582cm$^{-1}$, 557cm$^{-1}$indicated the presence of characteristic band of iron corresponding to the Fe-O stretching vibration.

3.1.3.3 X-Ray Diffraction (XRD)

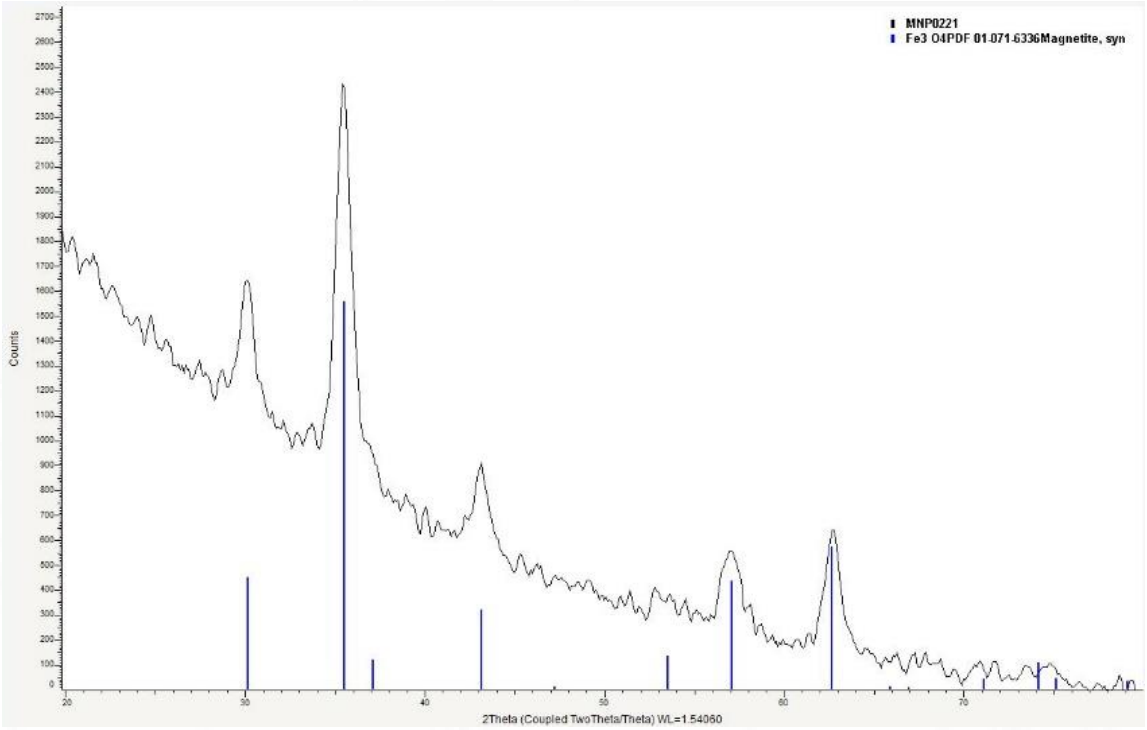


**Figure 11**: XRD Pattern of IONPS

XRD Spectrum of the IONPS exactly matches with the pattern of magnetite (pdf number – 01.071.6336 Magnetite). This confirms IONPS synthesized is magnetite and crystalline in nature. Phase purity is also achieved as no other peaks are recorded in the XRD Pattern.

Full Width at Half Maximum is 0.909 from the XRD Pattern. From the Debye Scherrer's equation the size of the bare IONPS was found to be 102nm which agrees with the DLS measurements.

### 3.1.4 BIOLOGICAL STUDIES

3.1.4.1 Nanodrop Analysis

Spectroscopic analysis confirms the retrieval of DNA using Iron oxide nanoparticles. 131 ng/µl out of 341.7ng/µl of DNA was retrieved.

| Sample | Analysed time | Nucleic Acid concentration (ng/µl) |
|---|---|---|
| **Pure DNA** | Before retrieval | 341.7 |
| **IONPS (powder form)** | After retrieval | 131.0 |

**Table 3 :** Nanodrop results of λ-DNA retrieved using IONPS

**Retrieval Efficiency**

| Sample | Analysed time | Nucleic Acid concentration (ng/µl) | Retrieval Efficiency (%) |
|---|---|---|---|
| **Pure DNA** | Before retrieval | 341.7 | |
| **IONPS (powder form)** | After retrieval | 131.0 | 38.34 |

**Table 4 :** Retrieval Efficiency using IONPS

The Retrieval efficiency is calculated 38.34%. From the zeta potential measurements, the synthesized IONPS is positively charged. These particles will bind with DNA via electrostatic attraction.

DNA tends to coil around the IONPS randomly. This random orientation leads to the coagulation which results in the agglomeration of particles. Thus the precise

determination of the particle size is not plausible. The presence of DNA is confirmed using FT-IR Spectroscopy.

## 3.2 SILICA COATED IONPS

### 3.2.1 PHYSICAL STUDIES

3.2.1.1 DLS Measurements

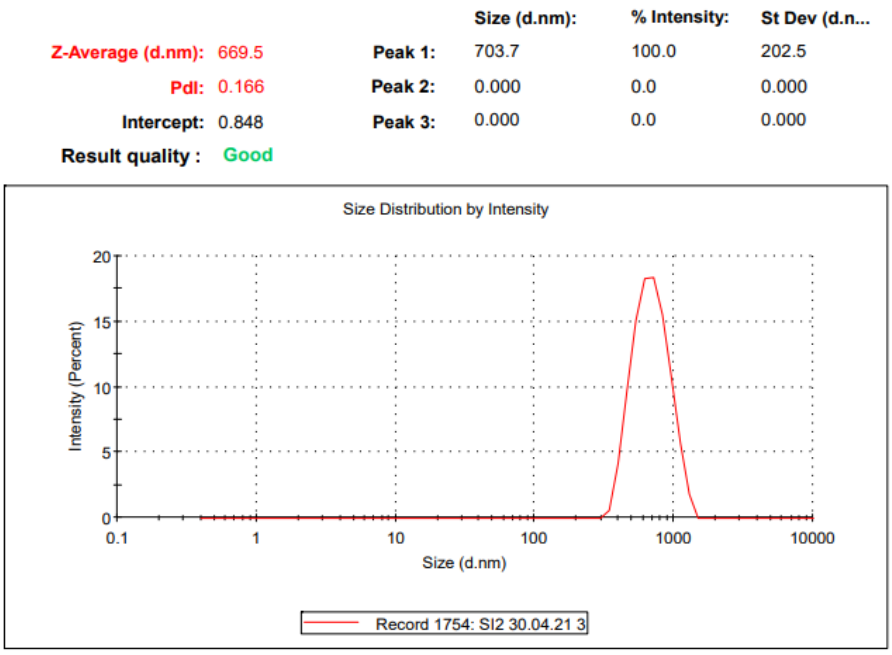


**Figure 12:** Dynamic Light Scattering(DLS) size measurements of Si(2)-IONPS

The particle size of the double time silica coated iron oxide nanoparticle is 669nm. This indicates an well established coating of Silica on the IONPS.

### 3.2.2 MORPHOLOGICAL STUDIES

3.2.2.1 Scanning Electron Microscope (SEM)

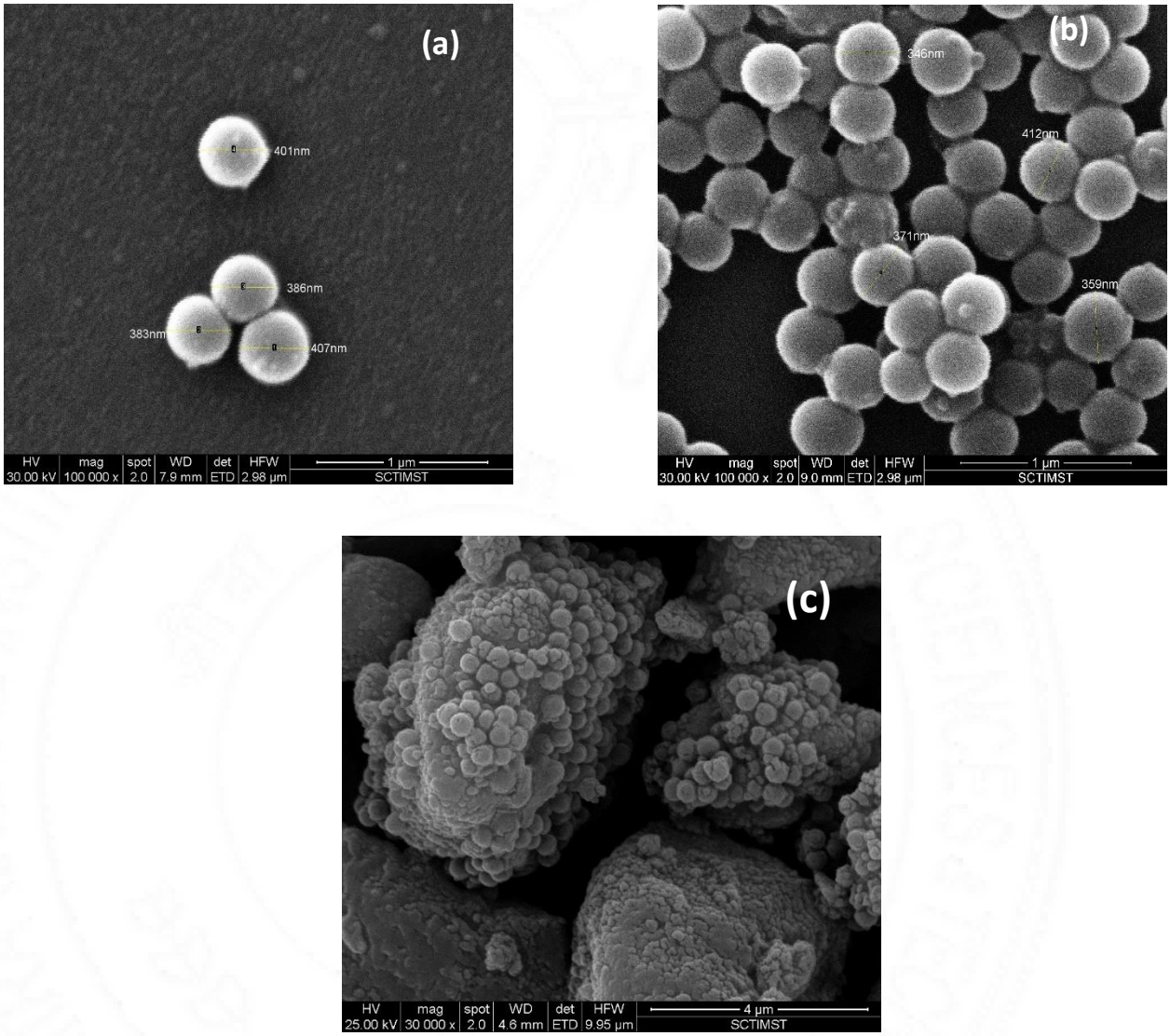


**Figure 13:** SEM micrographs of IONPS fig (a) IONPS fig (b) Si(1)-IONPS, fig (c) Si(2)-IONPS

SEM micrographs shows the circular shape of nanosized particles with nearly homogeneous matrices fig(a), fig(b). Average particle size for an uncoated nanoparticle is 372nm which increased to 394.5nm upon first time silica coating. This

slight change in the size confirms the formation of a thin layer of silica coating on the IONPS.

Small size and less aggregation of nanoparticles will increase the specific surface area for DNA to bind with.

After the second coating, nanoparticles fused together to form clusters fig (c) due to the gelation of silica. This agrees with the increase in size of particles as determined by DLS Analysis. Spherical nature of the particles is maintained but excess amount of silica resulted in the formation of clusters.

3.2.2.2 Transmission Electron Microscope (TEM)

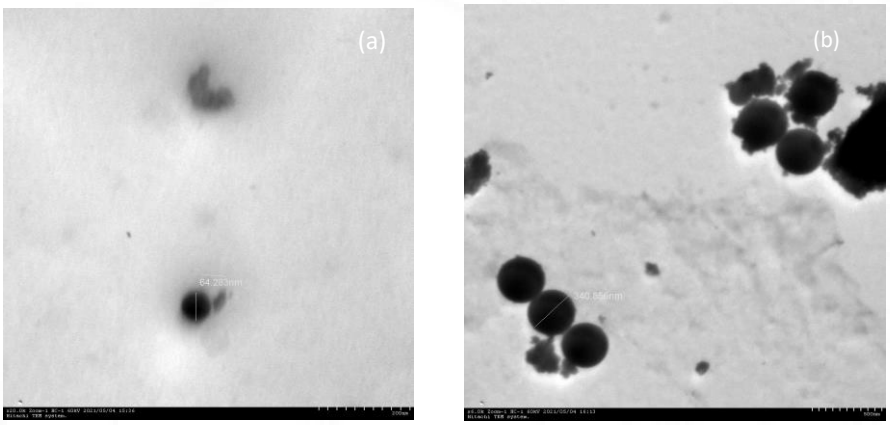


**Figure 14 :** TEM micrographs of fig (a) IONPS, fig (b) Si(1)-IONPS,fig (c) Si(2)-IONPS observed under 60kVwith 20k x and 6k x mag in 200nm and 500nm scale respectively.

Spherical morphology of silica coated nanoparticles is confirmed by TEM fig (a), fig (b). As TEM images are obtained by dropping the supernatant on grid after sonication, cluster formation is reduced. This enabled a clear view of spherical particles.

### 3.2.3 CHEMICAL STUDIES

3.2.3.1 Zeta Potential

| Sample | Zeta Potential (Dispersed in water) |
|---|---|
| **Single time Silica Coating** | **-50.3mV** |
| **Double time Silica coating** | **-44.7mV** |

**Table 5** : Zeta potential measurements of Si(1)-IONPS and Si(2)-IONPS

Zeta Potential is measured to be highly negative for single time coating and double time silica coated nanoparticle ensuring high stability. The charge decreased with coating. It is the deprotonation of silanol groups ($SiO^-$) that causes negative charge for the particles. The DNA binds with Si-IONPS through a non electrostatic bonding.

3.2.3.2 EDS Pattern

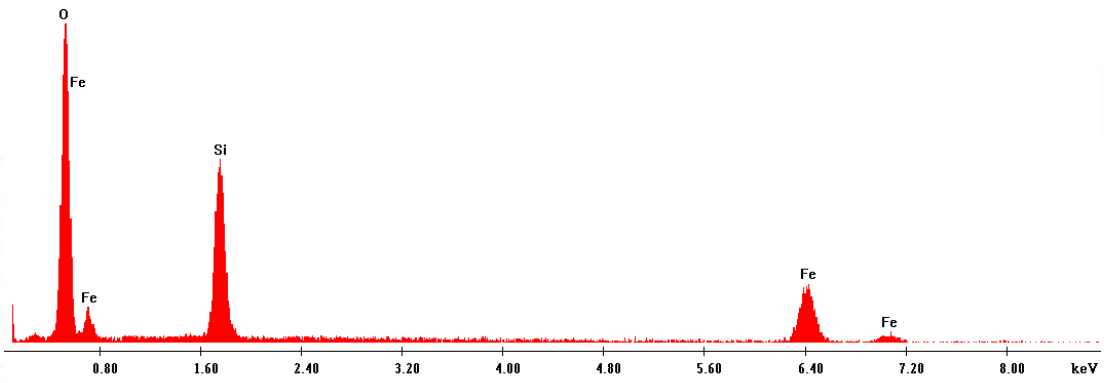


**Figure 15:** EDS Pattern of Si(1)-IONPS

Presence of Si (Silicon) peaks along with confirmed the coating on the IONPS along with Fe (Iron) and O(Oxygen) peaks. Purity is also ensured with the absence of other peaks.

3.2.3.3 Fourier Transform Infrared Spectroscopy (FT-IR)

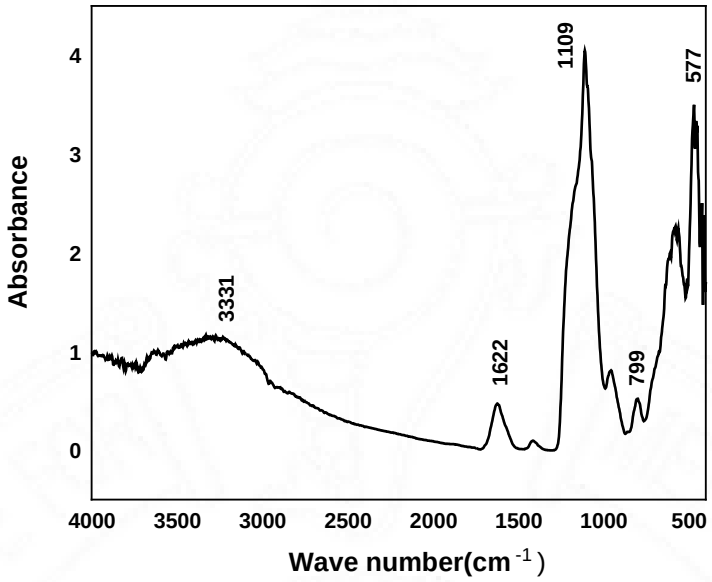


**Figure 16**:FT-IR pattern of Si(1)-IONPS

The band around 1109 $cm^{-1}$ is the characteristic one for asymmetrical stretching vibrations of Si-O-Si. The band at 799$cm^{-1}$ represents the Si-O stretching vibrations. The band around 577 is due to the characteristic absorption of Fe-O.

### 3.2.4 BIOLOGICAL STUDIES

3.2.4.1 Nanodrop Analysis

For nanodrop analysis, initial DNA concentration was recorded as 341.7 ng/µl. Using silica single time coated IONPS 116 ng/µl was retrieved while the double time silica coated IONPS could retrieve only 81.3 ng/µl from the initial concentration**.**

| Sample | Analysed time | Nucleic Acid concentration (ng/µl) |
|---|---|---|
| **Pure DNA** | Before retrieval | 341.7 |
| **Si(1)- IONPS** | After retrieval | 116.0 |
| **Si(2)- IONPS** | | 81.3 |

**Table 6 :** Nanodrop results of λ-DNA retrieved using Si(1)-IONPS, Si(2)IONPS

**Retrieval Efficiency**

| Sample | Analysed Time | Nucleic Acid concentration (ng/µl) | Retrieval Efficiency (%) |
|---|---|---|---|
| **Pure DNA** | Before retrieval | 341.7 | |
| **Si(1)-IONPS** | After retrieval | 116.0 | 33.95 |
| **Si(2)-IONPS** | | 81.3 | 23.79 |

**Table 7:** Retrieval Efficiency using Si(1)-IONPS, Si(2)IONPS

Quantification of the DNA retrieved using silica coated iron oxide nanoparticles is analysed. Single time coated IONPS showed a Retrieval efficiency of 33.95% which decreased to 23.79% upon double time coating. This showed that the multiple coatings did not enhance the efficiency of the nanoparticle in nucleic acid retrieval. Gelation of the Silica would have reduced the specific surface area for the DNA to bind with IONPS. Formation of clusters inhibited the retrieval efficiency.

## 3.3 PEI(Polyethyleneimine) COATED IONPS

### 3.3.1 PHYSICAL STUDIES

3.3.1.1 DLS Measurements

| | | | Size (d.nm): | % Intensity: | St Dev (d.n... |
|---|---|---|---|---|---|
| Z-Average (d.nm): | 743.7 | Peak 1: | 744.8 | 100.0 | 152.2 |
| PdI: | 0.233 | Peak 2: | 0.000 | 0.0 | 0.000 |
| Intercept: | 0.887 | Peak 3: | 0.000 | 0.0 | 0.000 |
| Result quality : | Good | | | | |

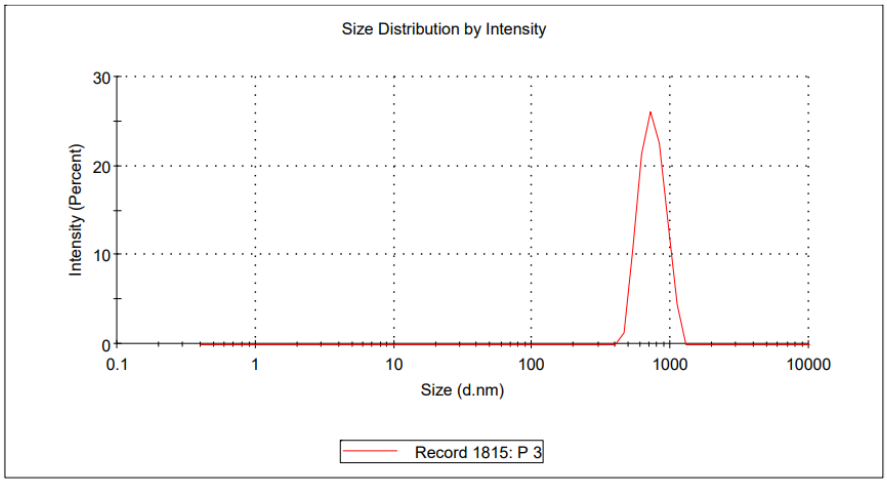


**Figure 17:** Dynamic Light Scattering (DLS) size measurements of P-IONPS

DLS measurement shows an increase in size of PEI coated IONPS when compared to uncoated IONPS. The size was measured to be 743.7nm while 166nm for uncoated IONPS. Agglomeration of particles after PEI coating may have resulted in the size increase.

3.3.1.2 Zeta Potential

| Sample | Zeta Potential Dispersed in water |
|---|---|
| **PEI Coating** | **43.4mV** |

**Table 8**: Zeta potential measurements of P-IONPS

High positive zeta potential ensures high stability due to greater repulsion between the particles. This positive charge of the PEI coated nanoparticles can electrostatically attract the DNA and bind them.

### 3.3.2 MORPHOLOGICAL STUDIES

3.3.2.1 Scanning Electron Microscope (SEM)

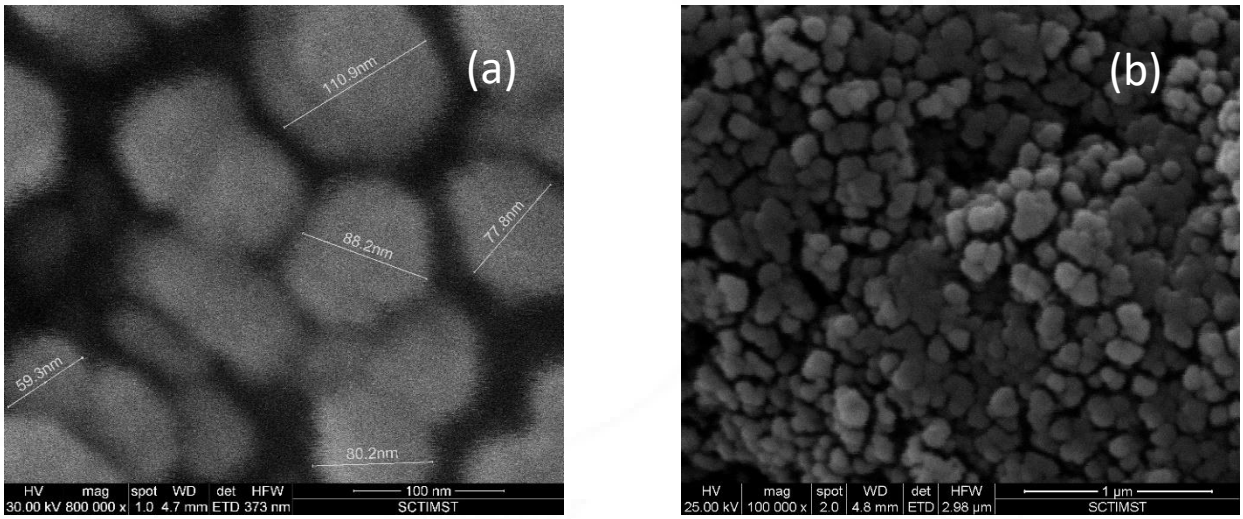


**Figure 18**: SEM micrographs of P- IONPS

SEM micrographs shows irregular arrangement of nanosized particles with decrease in sphericity.

### 3.3.3 CHEMICAL STUDIES

#### 3.3.3.1 EDS Pattern

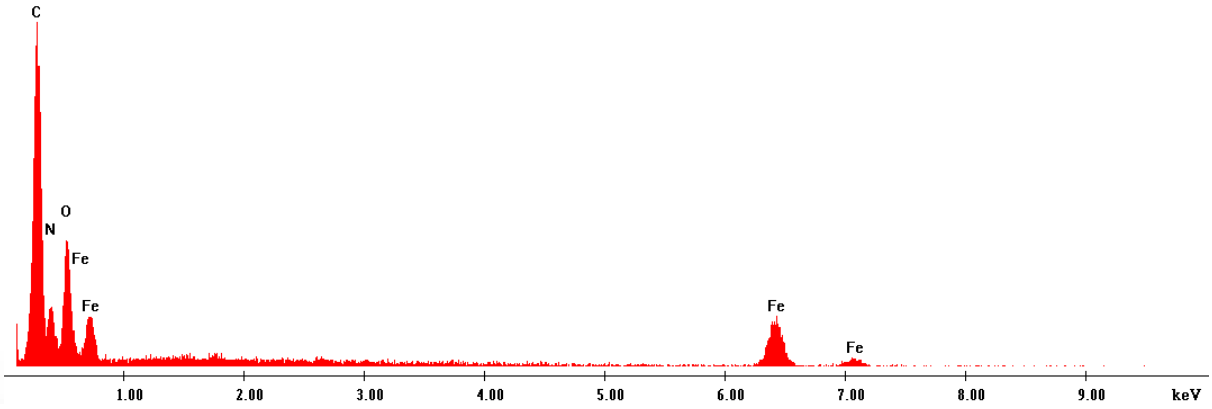


**Figure 19 :** EDS Pattern of P- IONPS.

Polymer coating is confirmed by the presence of Carbon, Oxygen and Nitrogen peaks in the EDS Pattern in addition to Fe peaks.

#### 3.3.3.2 Fourier Transform Infrared Spectroscopy (FT-IR)

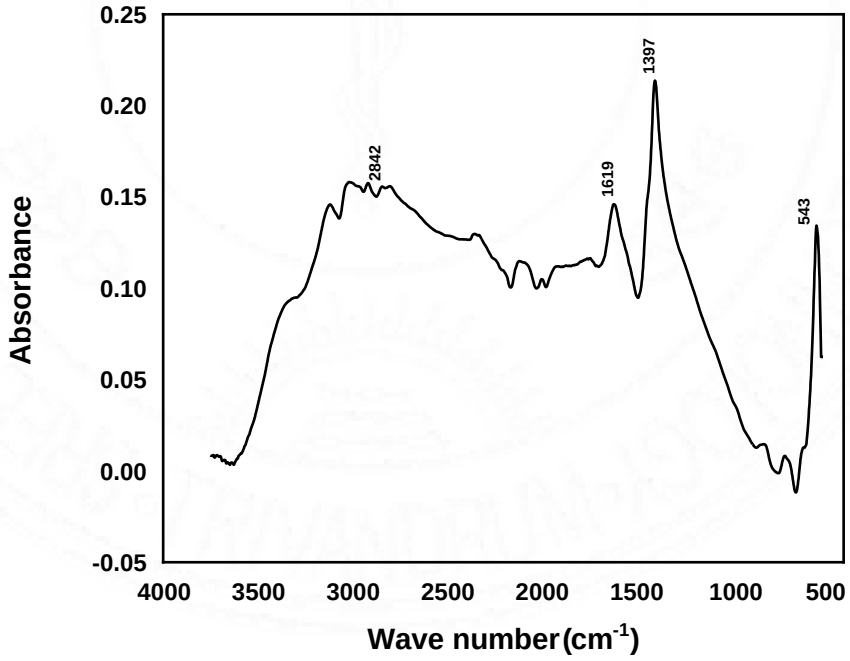


**Figure 20(a):** FT-IR Pattern of P- IONPS.

Band around 543 $cm^{-1}$ is due to the FeO vibration of present in $Fe_3O_4$. The bands around 1397 $cm^{-1}$ and 2848 $cm^{-1}$ are due to C-N and C-H bond stretching present in PEI. The bands between 1553 $cm^{-1}$ and 1646 $cm^{-1}$ are due to $NH_2$ bending present in PEI. This confirms the presence of PEI coating on the IONPS.

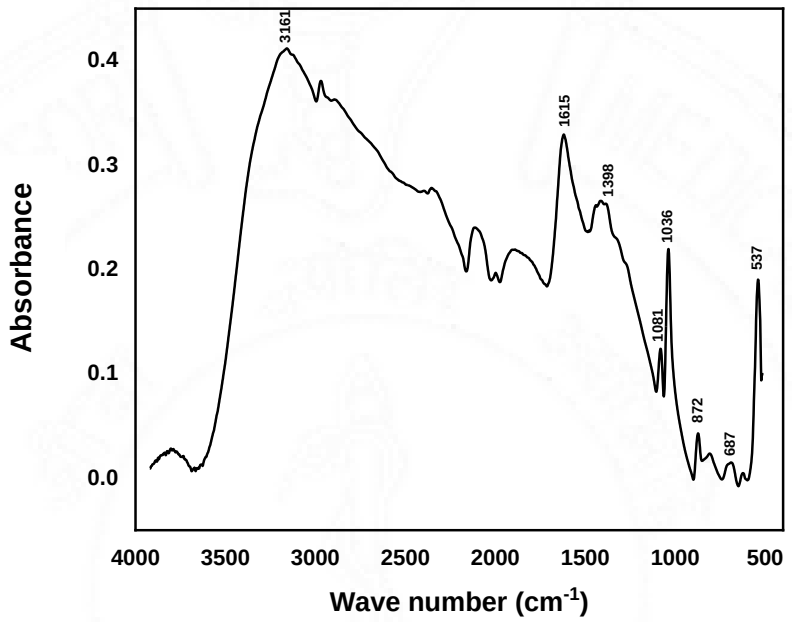


**Figure 20(b):** FT-IR Pattern of DNA bound P- IONPS.

Band around 537 $cm^{-1}$ is due to FeO vibration present in $Fe_3O_4$. The band around 1398 $cm^{-1}$ is due to C-N bond stretching present in PEI. The bands between 1553 $cm^{-1}$ and 1646 $cm^{-1}$ are due to $NH_2$ bending present in PEI. This confirms the presence of PEI coating on the IONPS.

Phosphate stretching vibrations, deoxyribose stretching of DNA backbone, ring vibrations of nitrogenous bases are found in the spectral region(400 $cm^{-1}$ to 750 $cm^{-1}$).

$NH_2$ band in PEI is observed at 1619 $cm^{-1}$ for P-IONPS (fig 20a). This band is shifted to 1615 $cm^{-1}$ and is more intense in DNA bound P-IONPS. This might be due to the cumulative effect of $NH_2$ bending present in DNA molecules and the $NH_2$ bending present in PEI. This confirmed the presence of DNA bound on P-IONPS

### 3.3.4 BIOLOGICAL STUDIES

3.3.4.1 Nanodrop Analysis

Initial concentration of DNA was 329.7 ng/µl . Using PEI coated nanoparticle 232.5 ng/µl could be retrieved.

| Sample | Analysed time | Nucleic Acid concentration (ng/µl) | Retrieval Efficiency (%) |
|---|---|---|---|
| **Pure DNA** | Before retrieval | 329.7 | |
| **P-IONPS** | After retrieval | 232.5 | 70.5% |

**Table 9:** Nanodrop results of λ-DNA retrieved using P-IONPS

**Retrieval Efficiency**

| Sample | Analysed time | Nucleic Acid concentration (ng/µl) |
|---|---|---|
| **Pure DNA** | Before retrieval | 329.7 |
| **P-IONPS** | After retrieval | 232.5 |

**Table 10:** Retrieval efficiency using P-IONPS

The spectroscopic analysis quantifies the amount of DNA retrieved using PEI coated nanoparticles and showed a Retrieval efficiency of 70.5. This increased efficiency is due the electrostatic attraction between the positively charged PEI coated IONPS and

negatively charged DNA. Modification with PEI has reduced the agglomeration of the molecules which increased the reactive site of IONPS.

3.3.4.2 Gel Doc

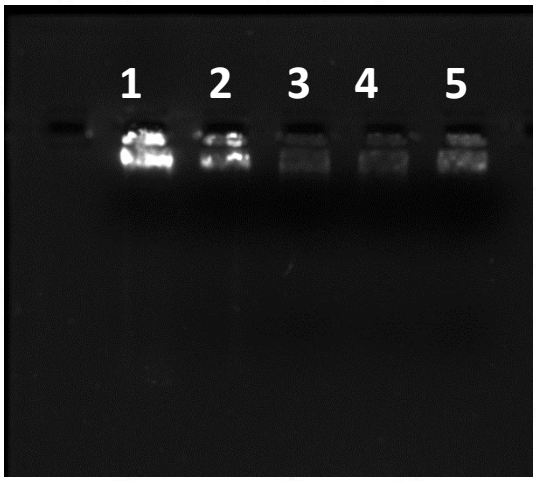


**Figure 21**: Agarose Gel Electrophoresis (1%) image of λ DNA retrieval using IONPS. Lane 1 for control [λ DNA], Lane 2 IONPS alone, Lane 3 Si(1)-ONPS, Lane 4 Si(2)- IONPS, Lane 5 P-IONPS

The Retrieval efficiency of nanoparticles as quantified by nanodrop spectrophotometer is confirmed by the agarose gel electrophoresis.

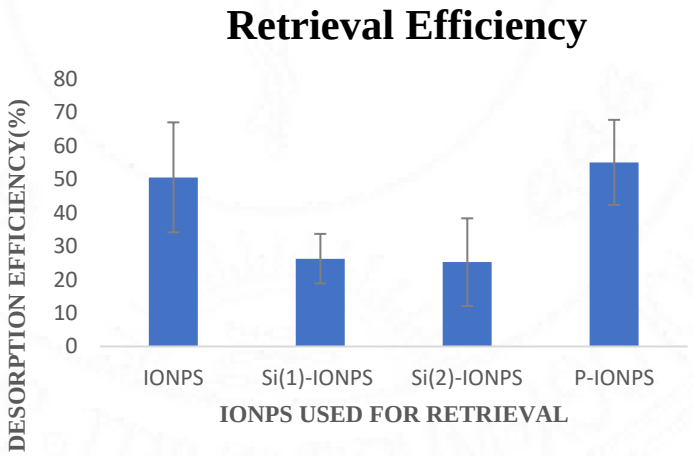


**Figure 22**: Retrieval Efficiency - Comparison of IONPS, Si(1)-IONPS, Si(2)-IONPS, P-IONPS(for 3 trials)

# CHAPTER 4

## 4.1 CONCLUSION

The Iron oxide nanoparticles (IONPS) were synthesized by co-precipitation method. Their surfaces were modified with silica and polyethyleneimine to study their binding efficiency. Physical, Chemical, Biological and Morphological studies of uncoated and coated IONPS were carried out. The results showed that all the particles synthesized are nano sized and exhibited DNA Retrieval Efficiency. SEM, TEM, DLS analysis confirmed the size of the particles. Zeta potential provided the charge while XRD, FT-IR and EDS analysis confirmed the purity of nanoparticles synthesized. Nanodrop Spectroscopy and Gel Doc quantified the nucleic acid retrieved.

Efficiency to retrieve nucleic acid was seen better for PEI coated nanoparticles than the rest.

The synthesized IONPS are stored in dried powder form while commercially available IONPS are in suspension form (Mag-bind, MagJET, Magmax) in proprietary buffer. Presence of any buffer can reduce saturation magnetization and magnetic property. This inconsistency can be solved by using nanoparticles stored in a stable powdered form. This allows for flexibility in storage, utilization and application based on end user needs. Parameters to increase the binding efficiency are being evaluated. This requires lots of optimization in which we are currently working on.

## 4.2 FUTURE SCOPE

With the advancement of technology and the increasing need for easy and fast retrieval of nucleic acid, magnetic nanoparticle assisted nucleic acid separation is a breakthrough. As these magnetic nanoparticles can be manipulated and targeted with an external magnetic field their application is extended in various fields like contrast agents, cell labelling, multimodal imaging, drug delivery vehicles, hyperthermia treatment etc.

Targeted drug delivery can be achieved by magnetic carriers. This can reduce the dosage of loaded drugs and can reduce side effects. In case of tumors, magnetic nanoparticles can be guided directly to the them and can induce hyperthermia artificially. This helps to destroy malignant cells without compromising any healthy cells.

Commercialization of magnetic field aided separation kits of nucleic acids and cells, protein labelling etc are in the initial phase. A nucleic acid extraction kit is to be developed from our findings to provide a better output in this field with minimal cost. Delivery of nucleic acid based agents via magnetic targeting is also plausible via this route.

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