

A Comparative Study of Iron Oxide Nanoparticles Surface Modified Using Carboxylic Acids

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ABSTRACT

In the last decade nanotechnology has greatly developed in many research fields such as engineering, electronic, biological and many others. They can offer several possibilities to design tools, to create new techniques or improve the already existing ones, to discover innovative applications. Nano-science is one of the most important research and development frontiers in modern science. Nanotechnology is now widely used throughout the pharmaceutical industry, medicine, electronics, robotics, and tissue engineering. For biological and biomedical applications, magnetic iron oxide nanoparticles are the primary choice because of their biocompatibility, super-paramagnetic behavior and chemical stability.

The purpose of this work is the design, development and surface modification of magnetic nanoparticles. Naked iron oxide nanoparticles have high chemical activity, toxicity and aggregate in the body fluid therefore providing surface coating for the stability of the magnetic nanoparticles. These protective shells not only stabilize the magnetic iron nanoparticles but also can be used for further functionalization. Here the iron oxide nanoparticles were prepared by co-precipitation method, then this nanoparticle is modified using acids- oleic acid and succinic acid and a comparative study is carried out. The TEM, FTIR and DSC characterization techniques were used to confirm the surface modification. After which, it was found the iron oxide nanoparticle with succinic acid gives a uniform coating of the three and can be used for further functionalization for various applications.

Keywords- Co-precipitation, Iron oxide nanoparticle, Oleic Acid, Citric Acid, Succinic Acid.

Nanotechnology covers fields from biology to material science, physics to chemistry, and can include development in a variety of specialities. Nanostructured materials have attracted considerable attention in recent years because they exhibit useful and unusual properties compared to conventional materials. They are of great interest in the creation of controlled micro and nanoarchitectures in an attempt to mimic the natural physical and biological environment that encourages tissue regeneration and growth. The hypothesis is that the nanoarchitectures can promote cell differentiation and functionality. By understanding how physical surface parameters influence cellular adhesion and differentiation we can more effectively design biomaterial surfaces for variety of tissue engineering applications. Further, nanostructured materials can be used as drug eluting interfaces for implantable devices, such as vascular stents, orthopedic implants, dental implants, etc. By precisely controlling the size of nanostructure, we can manipulate the release rates; thus releasing the drug at physiological levels.

1.1 Magnetic Nanoparticles

Magnetic nanoparticles are a class of nanoparticle, which can be manipulated using magnetic field. These particles commonly consist of magnetic elements such as iron, nickel and cobalt and their chemical compounds. The magnetic nanoparticles have been the focus of much research recently because they possess attractive properties which could see potential use in catalysis including nanomaterial-based catalysts,(1) biomedicine,(2) magnetic resonance imaging,(3) magnetic particle imaging,(4) data storage,(5) environmental remediation,(6) nanofluids,(7) and optical filters,(8) defect sensor(9) and cation sensors.(10)

1.2 Iron Oxide Nanoparticles

Researchers are constantly working on new applications for nanoparticles in biological, chemical, and industrial areas. Iron oxides have a useful set of properties for biomedical imaging and catalytic applications. The two main forms are magnetite (Fe_3O_4) and its oxidized form maghemite ($\gamma\text{-Fe}_2\text{O}_3$). They have attracted extensive interest due to their superparamagnetic properties and their potential applications in many fields.

I. INTRODUCTION

Nanotechnology is defined as the creation and exploitation of materials with structural features in between those of atoms and bulk materials, of dimension in the nanometer range ($1 \text{ nm} = 10^{-9} \text{ m}$). A property of materials having nanometric dimensions significantly varies from those of atoms as well as those of bulk materials.

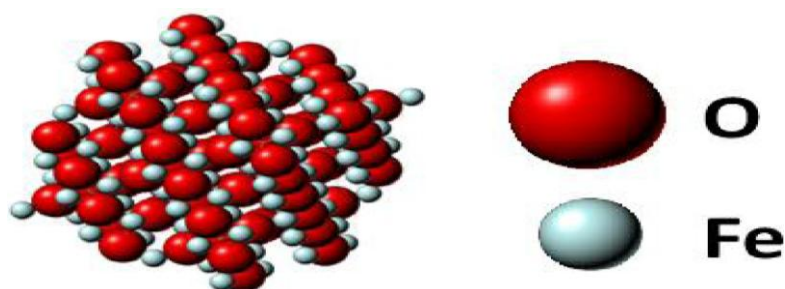


Figure 1.1: The crystal structure of iron oxide is a close-packed planes of oxygen anions (red) with iron cations (white) taking up some of the resulting octahedral or tetrahedral interstitial sites

Applications of iron oxide nanoparticles include terabit magnetic storage devices, catalysis, sensors, and high-sensitivity biomolecular magnetic resonance imaging (MRI) for medical diagnosis and therapeutics. These applications require coating of the nanoparticles by agents such as long-chain fatty acids, alkyl-substituted amines and diols.

Magnetic iron oxide (IO) nanoparticles with a long blood retention time, biodegradability and low toxicity have emerged as one of the primary nanomaterials for biomedical applications *in vitro* and *in vivo*. IO nanoparticles have a large surface area and can be surface modified to provide a large number of functional groups for cross-linking to tumor-targeting ligands such as monoclonal antibodies, peptides, or small molecules for diagnostic imaging or delivery of therapeutic agents. IO nanoparticles possess unique paramagnetic properties, which generate significant susceptibility effects resulting in strong T_2 and T_2^* contrast, as well as T_1 effects at very low concentrations for magnetic resonance imaging (MRI), which is widely used for clinical oncology imaging. There are various methods currently used for the synthesis of nanomaterials. The following are a few common methods:

- Precipitation /wet chemical method
- Reduction of metal salt/ solution method
- Hydrothermal/solvothermal
- Hot thermolysis/colloidal synthesis

Here in my work I have used co-precipitation method for the synthesis of the nanoparticles.

This preparation method has a large effect on shape, size distribution, and surface chemistry of the particles. It also determines to a great extent the distribution and type of structural defects or impurities in the particles. All these factors affect magnetic behavior.

Co-precipitation

So far this is the most simple, cost effective and commonly employed method. As one convenient cheap method, chemical co-precipitation has the potential to meet the increasing demand for the direct preparation of well dispersed nanoparticles. Chemical co-precipitation can produce fine, high purity, stoichiometric particles of single and multicomponent metal oxides.

1.3 Surface Functionalization and It's Advantages

IO nanoparticles without any surface modification are not stable in aqueous media and readily aggregate and precipitate. For *in vivo* applications, the particles often form aggregates in blood and are sequestered by macrophages (Lee et al 2006). Therefore, the surface of IO nanoparticles should be coated with a variety of different moieties that can eliminate or minimize their aggregation under physiological conditions. Usually, two main approaches are used for coating magnetic IO nanoparticles, including *in situ* coatings with which the magnetic nanoparticles are coated during the synthesis process and post-synthesis coatings.(7).

The amphiphilic polymeric surfactants such as poloxamers, poloxamines and poly(ethylene glycol) (PEG) derivatives are usually used for coating the surface of IO nanoparticles, since they can minimize or eliminate opsonization of IO nanoparticles. Among them, PEG is the most used chemical material, which confers on IO nanoparticles several important properties such as high solubility and stability in aqueous solutions, biocompatibility, and prolonged blood circulation time.

Recently, a new class of superparamagnetic iron particles that have uniform sizes ranging from 5–30 nm were further functionalized through surface coating with amphiphilic polymers, which provide functional groups for conjugating tumor-targeting biomolecules such as peptides or antibodies(8). Hence surface modified magnetic nanoparticles are useful in biomedical applications. Here in my work I am coating the iron oxide nanoparticles using oleic acid and succinic acid.

II. EXPERIMENTAL PROCEDURE

2.1 General Description of the Work

First of all the iron oxide nanoparticles are synthesized using the co-precipitation method. As pure iron oxide nanoparticles are not stable in the aqueous media. IO nanoparticles without any surface modification does not have stability in aqueous media and readily aggregate and precipitate. For *in vivo* applications, the particles often form aggregates in blood and are sequestered by macrophages. Therefore, the surface of IO nanoparticles should be coated with a variety of different

moieties that can eliminate or minimize their aggregation under physiological conditions. Usually, two main approaches are used for coating magnetic IO nanoparticles, including *in situ* coatings with which the magnetic nanoparticles are coated during the synthesis process and post-synthesis coatings. Here the first one is done and the procedure is as explained below.

2.1.1 Synthesis of Pure Iron Oxide Nanoparticles

Materials and Reagents

Ferric Chloride Hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), (Nice Laboratory, Mumbai), Iron (II) sulphate Heptahydrate GR (Ferrous sulphate) (Merck, Mumbai), Liquor ammonia (Qualigens Fine Chemicals, Mumbai)

Methodology

Fe_3O_4 nanoparticles were synthesized by coprecipitation method consisting of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (2.36g) and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (0.86g) at the molar ratio 2:1 and they were dissolved in deionized water. After stirring for 10 minutes, NH_4OH (3.2 M) was then added to the solution which was kept for three hours of continuous stirring. The Fe_3O_4 nanoparticles were separated centrifugally at 15000rpm for 10 minutes and washed three times with water.

The above synthesized nanoparticle was considered for the further surface modifications.

III. SURFACE MODIFIED IRON OXIDE NANOPARTICLES

3.1 Synthesis of Iron Oxide Nanoparticles Coated with Oleic Acid

Materials and Reagents

Ferric Chloride Hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), (Nice Laboratory, Mumbai), Iron (II) sulphate Heptahydrate GR (Ferrous sulphate) (Merck, Mumbai), Liquor ammonia (Qualigens Fine Chemicals, Mumbai), Oleic Acid (Merck, Germany).

Methodology

$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (3g) and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (1.5g) were dissolved in almost 5ml of distilled water. Then this iron salt solution was kept for stirring for 5 minutes at 70°C in a nitrogen purged atmosphere. Then after 5 minutes, 5ml of liquid ammonia solution was added into the flask which gives a black precipitate which is followed by the addition of 450 μl of oleic acid. This oleic acid dispersed solution was kept for continuous stirring for three hours in nitrogen atmosphere. After, three hours of continuous stirring we observe a black solution with precipitates. This solution was then allowed to cool at room temperature for almost 20 minutes which was followed by the centrifugation in order to remove the excess salts. The resultant was then centrifuged at 8000rpm at 4°C for 20 minutes and later centrifuged at 15000rpm at 4 degree for 50 minutes. The resultant sample after centrifugation was kept for lyophilization. The nanoparticles thus obtained were characterized using TEM and FTIR in order to know the particle size and to confirm the chemical adsorption of $-\text{COOH}$ groups onto nanoparticle surface.

3.2 Synthesis of Iron Oxide Nanoparticles Coated with Citric Acid

Materials and Reagents

Ferric Chloride Hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), (Nice Laboratory, Mumbai), Iron (II) sulphate Heptahydrate GR (Ferrous sulphate) (Merck, Mumbai), Liquor ammonia (Qualigens Fine Chemicals, Mumbai), Citric Acid (Sigma Aldrich, USA).

Methodology

$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (3g) and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (1.5g) were dissolved in almost 5ml of distilled water. Then this iron solution was kept for stirring for 5 minutes at 70°C in a nitrogen purged atmosphere. Then after 5 minutes, 5ml of liquor ammonia solution was added into the flask which gives a black precipitate which is followed by the addition of 5g of citric acid dissolved in 10 ml of distilled water. This citric acid dispersed solution was kept for continuous stirring for three hours in nitrogen atmosphere. After, three hours of continuous stirring, this solution was then allowed to cool at room temperature for almost 20 minutes which was followed by the dialysis procedure in order to remove the excess salts. After the dialysis procedure the resultant solution was kept for lyophilization where the solution will be changed into powder form by extracting the water in order to remain stable and to make it easier to store at room temperature. The obtained nanoparticles are characterized using TEM and FTIR to know the particle size and to confirm the chemical adsorption of $-\text{COOH}$ groups onto the nanoparticle surface.

3.3 Synthesis of Iron Oxide Nanoparticles Coated with Succinic Acid

Materials and Reagents

Ferric Chloride Hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), (Nice Laboratory, Mumbai), Iron (II) sulphate Heptahydrate GR (Ferrous sulphate) (Merck, Mumbai), Liquor ammonia (Qualigens Fine Chemicals, Mumbai), Succinic Acid (Sigma Aldrich, USA).

Methodology

$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (3g) and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (1.5g) were dissolved in almost 5ml of distilled water. Then this iron solution was kept for stirring for 5 minutes at 70°C in a nitrogen purged atmosphere. Then after 5 minutes, 5ml of liquor ammonia solution was added into the flask which gives a black precipitate which is followed by the addition of 5g of succinic acid dissolved in 10 ml of distilled water. This succinic acid dispersed solution was kept for continuous stirring for three hours in nitrogen atmosphere. After, three hours of continuous stirring, this solution was then allowed to cool at room temperature for almost 20 minutes which was followed by the dialysis procedure in order to remove the excess salts. After the dialysis procedure the resultant solution was kept for lyophilization where the solution will be changed into powder form by extracting the water in order to remain stable and to make it easier to store at room temperature. The obtained nanoparticles are characterized using TEM and FTIR to know the particle size and to confirm the chemical adsorption of $-\text{COOH}$ groups onto the nanoparticle surface.

IV. RESULTS AND DISCUSSIONS

4.1 Tem Analysis

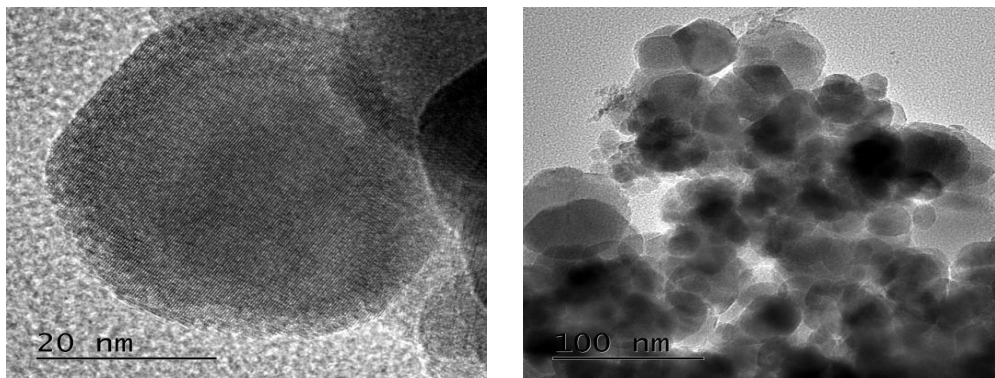


Figure 4.1.1: Pure Iron oxide nanoparticles

Inferences:

The TEM image of the iron oxide nanoparticle confirms the morphology and size of the nanoparticle.

The TEM image shows that the particles are aggregated because the metal oxide nanoparticles tend to react more with each other.

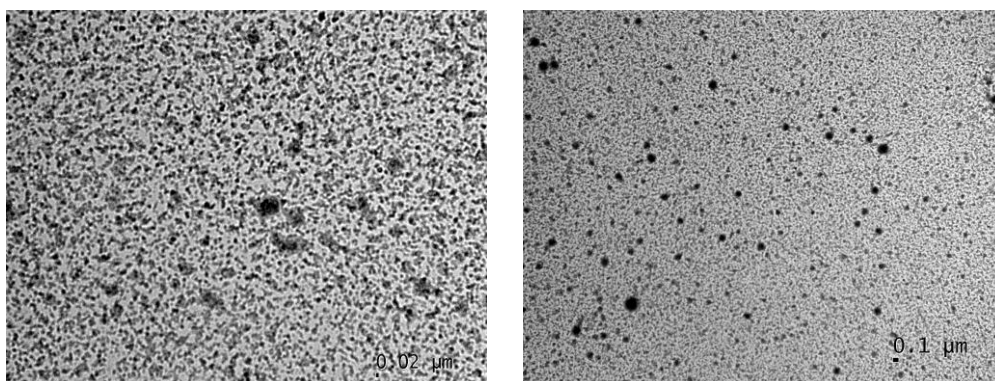


Figure 4.1.2: Iron oxide nanoparticles coated with oleic acid

Inferences:

Figures above shows TEM images and size distribution of iron oxide nanoparticles coated with oleic acid. It can be seen that plain iron oxide nanoparticles were polydisperse and seriously aggregated. After surface modification by oleic acid the particles maintained their

original spherical shape with a good monodispersity. The particle size is very uniform with the average size of about 100 nm- 200nm. From the magnified image due to the presence of excess oleic acid the aggregation of individual particles with an incomplete coating by oleic acid molecules can be observed.

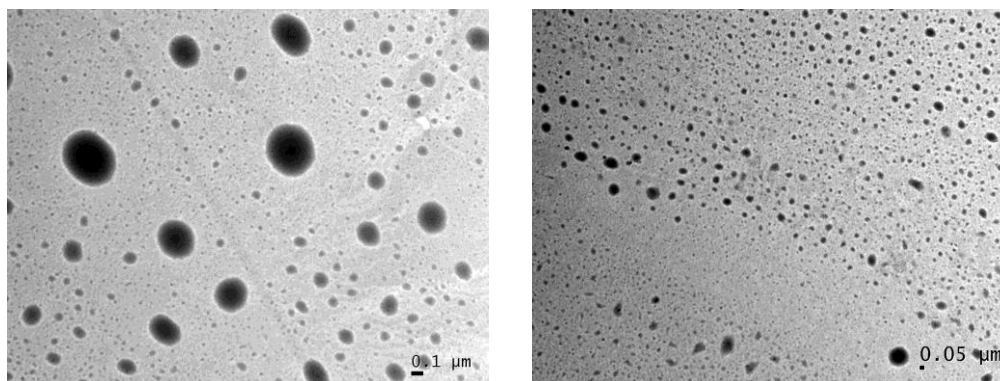


Figure 4.1.3: Iron oxide nanoparticles coated with succinic acid

Inferences:

After surface modification by citric acid the particles maintained their original spherical shape with a good monodispersity. The particle size is very uniform

with the average size of about 50nm - 200nm. It can be observed that the excess of citric acid is present hence this surface coating was also neglected.

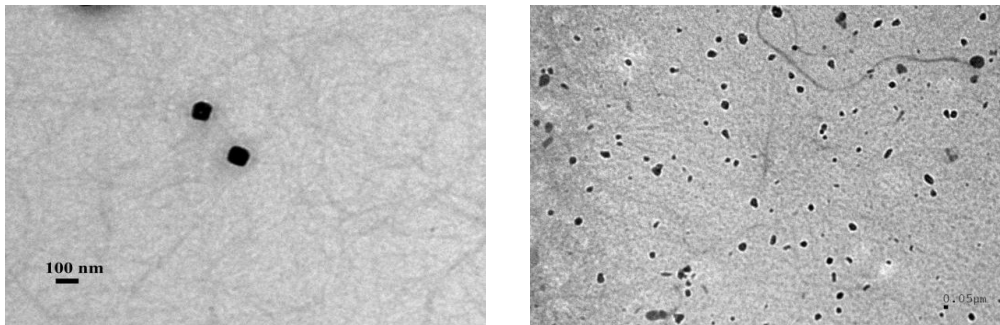


Figure 4.1.4: Iron oxide nanoparticles coated with succinic acid

Inferences:

Figures above shows the TEM images and size distribution of iron oxide nanoparticles coated with succinic acid. It can be seen that plain iron oxide nanoparticles were polydisperse and seriously aggregated. After surface modification by succinic acid the particles

maintained their original spherical shape with a good monodispersity. The particle size is very uniform with the average size of about 50nm- 100nm. It can be observed that the excess of succinic acid is present but that was washed off using dialysis procedure.

4.2 Ftir Analysis

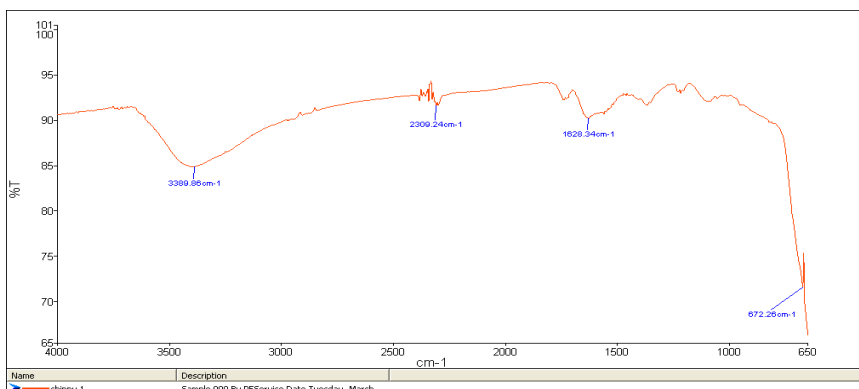
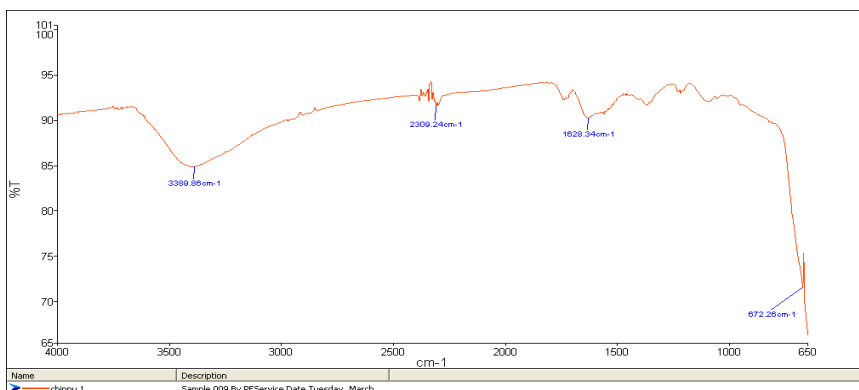


Figure 4.2.1: Pure iron oxide nanoparticles

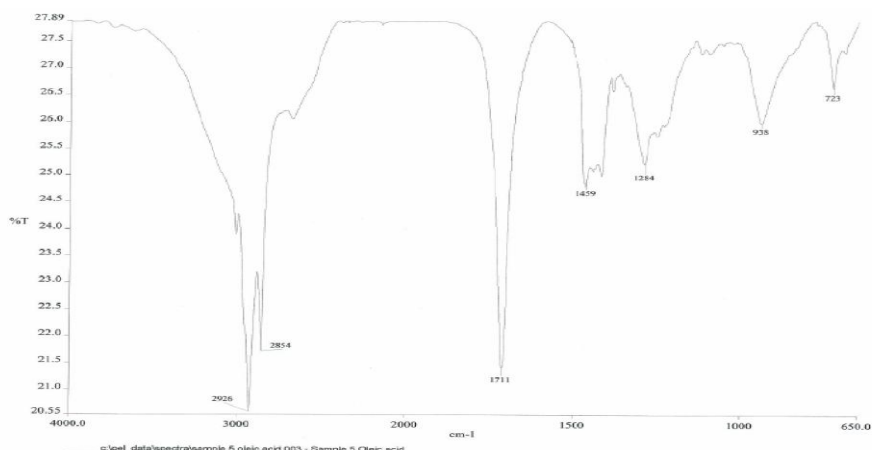
Inferences:

Figure above shows the FTIR result of pure iron oxide nanoparticles. Here the analysis indicated

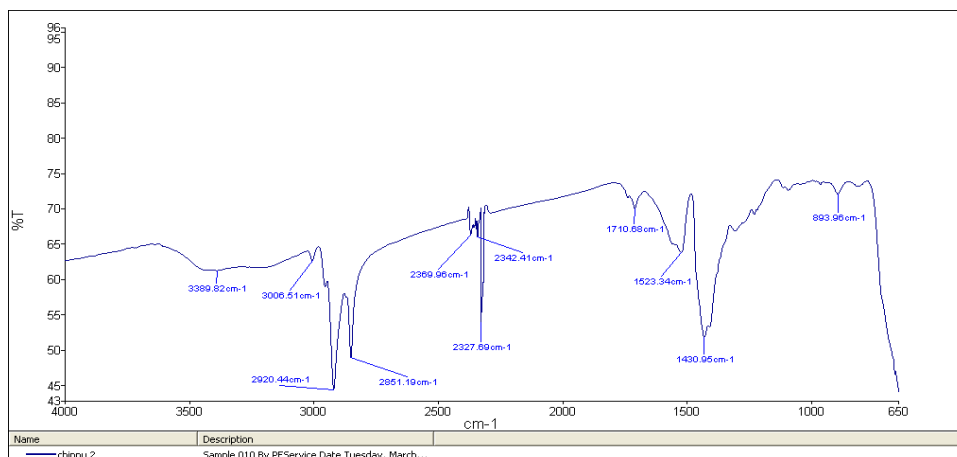
absorption peaks at 672.26 cm⁻¹, 1629.34 cm⁻¹, 2309.24 cm⁻¹ and 3389.86 cm⁻¹ corresponding to the Fe–O vibration related to the magnetite phase.



(a) Iron oxide nanoparticles



(b) Oleic Acid



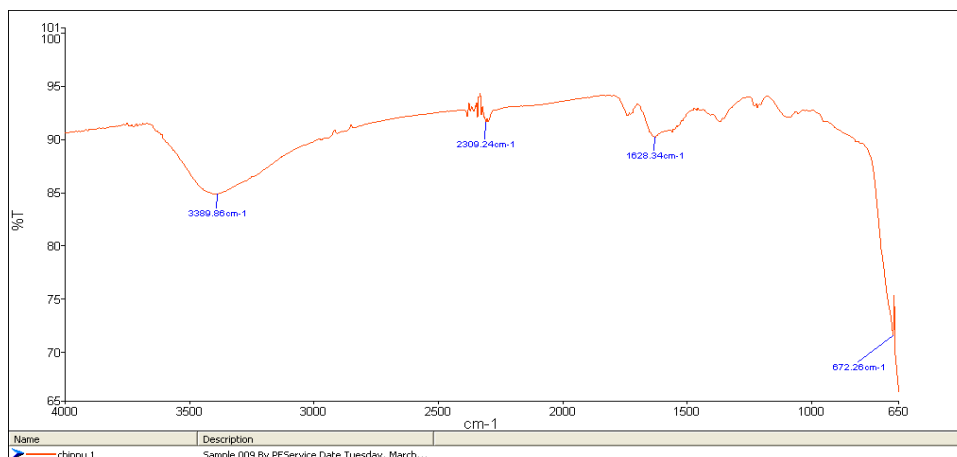
(c) Iron oxide nanoparticles coated with oleic acid

Figure 4.2.2: Comparison of FTIR Analysis of (a) Iron oxide nanoparticle, (b) Oleic Acid and (c) Iron oxide nanoparticle coated with Oleic Acid

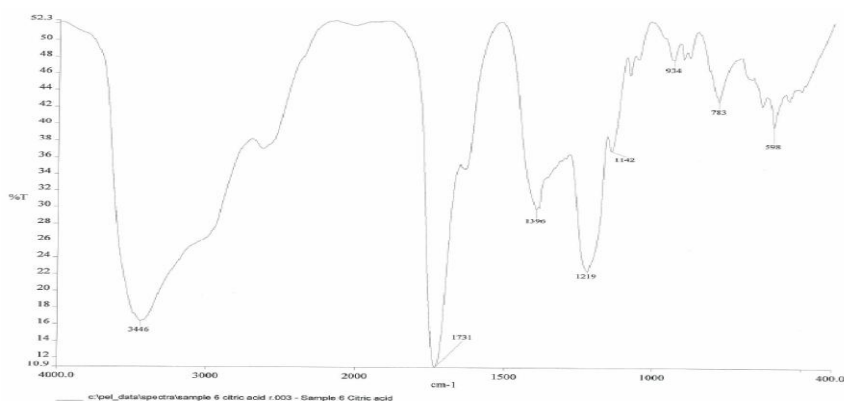
Inferences:

The two absorption peaks at 2920.44cm⁻¹ and 2,851.19 cm⁻¹ were attributed to the asymmetric CH₂ stretching and the symmetric CH₂ stretching,

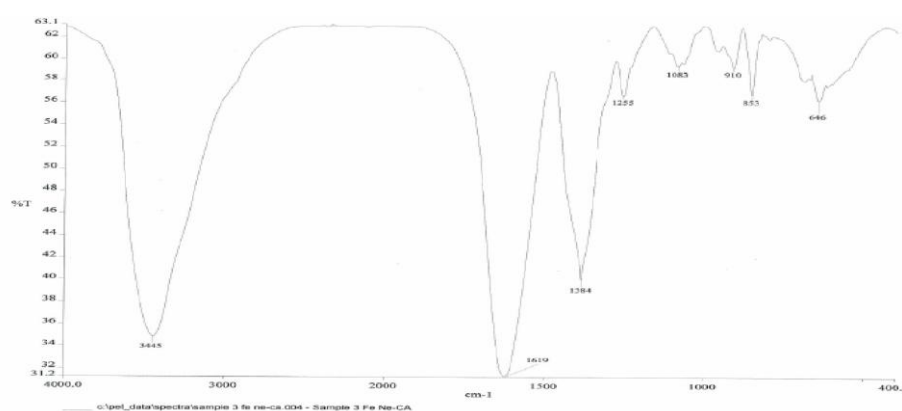
respectively. The intense peaks at 2327.69 cm⁻¹ and 1430.95 cm⁻¹ was due to the overlapping of the absorption bands of the carboxyl groups and the double bonds of OA.



(a) Iron Oxide nanoparticle



(b) Citric Acid



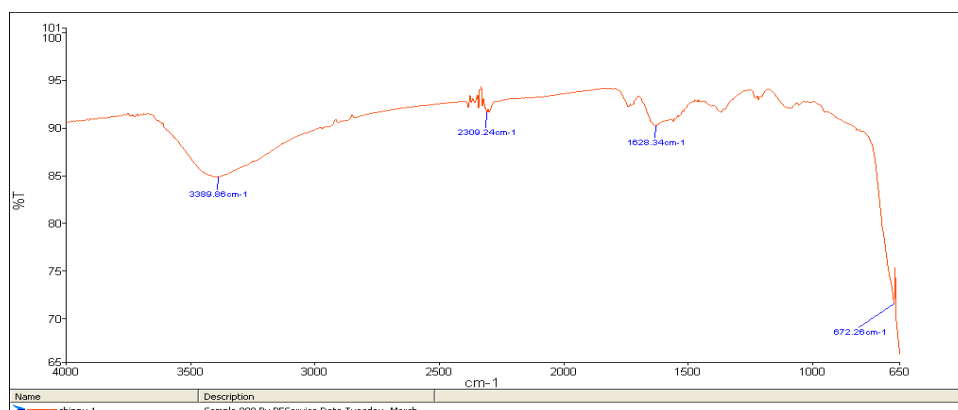
(c) Iron oxide nanoparticle coated with Citric Acid

Figure 4.2.3: Comparison of FTIR Analysis of (a) Iron oxide nanoparticle, (b) Citric Acid and (c) Iron oxide nanoparticle coated with Citric Acid

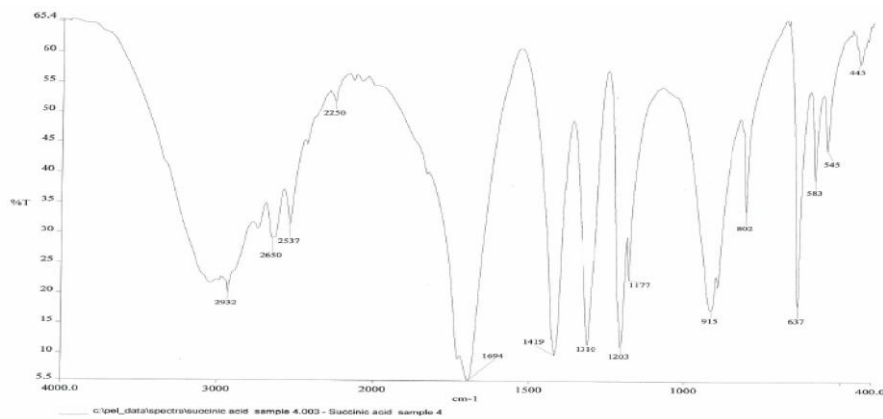
Inferences:

The absorption bands for pure citric acid are resolved but those of iron oxide nanoparticle coated with citric acid are broad and few. The intense band at 3446 cm^{-1} shows the presence of non dissociated OH groups of citric acid. The peak around 1731 cm^{-1} is due to the CH_2 stretching, peak at 1396 cm^{-1} may be due to the symmetric stretching of OH from $-\text{COOH}$ group. The peak at 1700 cm^{-1} of citric acid shows the $\text{C}=\text{O}$ vibration from the

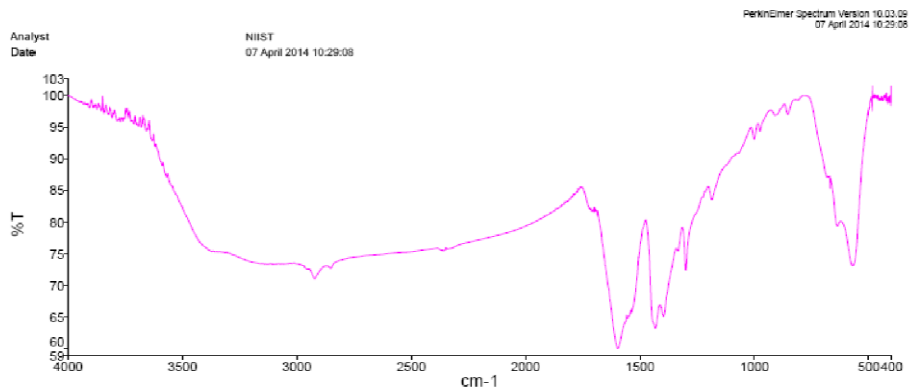
COOH group of citric acid. This peak shifts to an intense band at about 1255 cm^{-1} showing the binding of citric acid onto the magnetite nanoparticles. The next band at 1142 cm^{-1} can be due to the asymmetric stretching of CO from COOH group. The strong FTIR band observed at around 598 cm^{-1} can be due to the Fe-O stretching vibration of Fe_3O_4 . Therefore, we can say that the citric acid binds to the magnetite surface by carboxylate.



(a) Iron oxide nanoparticle



(b) Succinic Acid



(c) Iron oxide nanoparticles coated with succinic acid

Figure 4.2.4 Comparison of FTIR Analysis of (a) Iron oxide nanoparticle, (b) Succinic Acid and (c) Iron oxide nanoparticle coated with Succinic Acid

Inferences:

Figure above shows the FTIR spectrum for the succinic acid coated nanoparticles. The spectrum confirms the presence of Fe-O stretches peaks around 580 and 620 cm^{-1} . The peaks at 3650 cm^{-1} shows the presence of non dissociated OH groups of succinic acid. Compared

to pure succinic acid, there is a shift in the peak at about 1646 cm^{-1} for iron oxide nanoparticles coated with succinic acid displaying the binding of the succinic acid onto the surface of Fe_3O_4 nanoparticles by chemisorption of carboxylate ions.

4.3 Differential Scanning Calorimetry

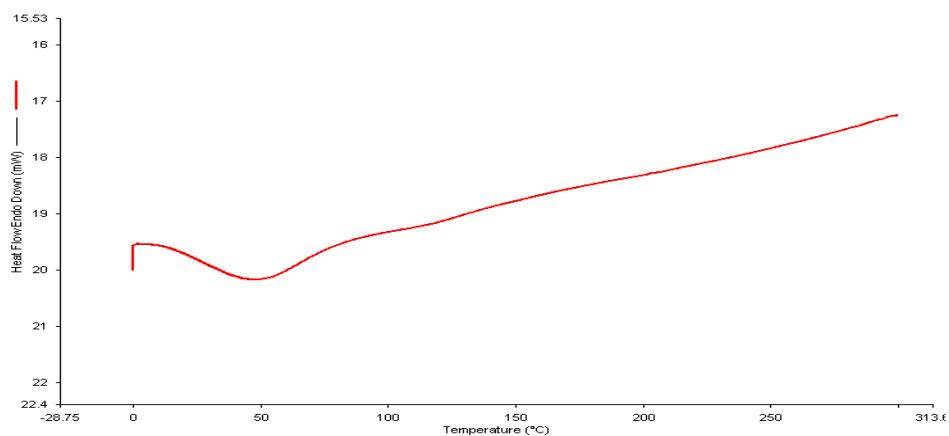


Figure 4.3.1: Pure iron oxide nanoparticles

Inferences:

The DSC curve of pure iron oxide nanoparticle is as shown above. It shows an exothermic curve. The

DSC curve gives us the temperature at the midpoint of the curve as 47.55°.

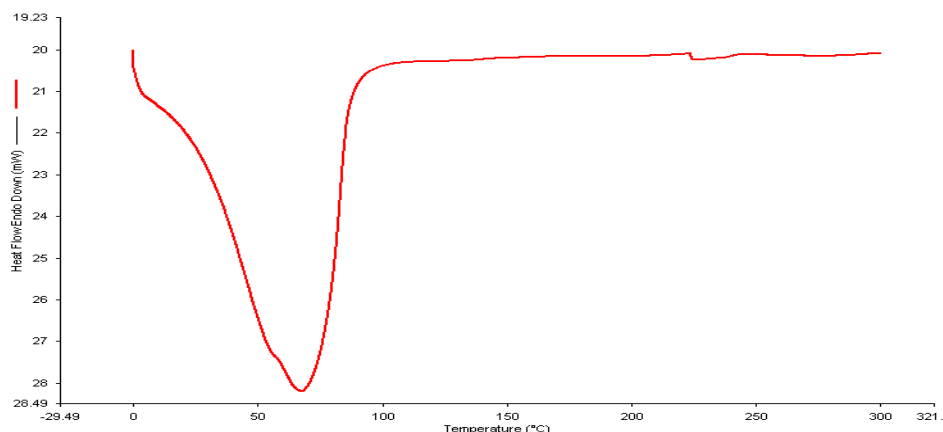


Figure 4.3.2: Iron oxide nanoparticles coated with succinic acid

Inferences:

Figure shows the DSC curve of iron oxide nanoparticle coated with succinic acid. The curve indicates an exothermic one. The temperature at the midpoint of the peak of iron oxide nanoparticle coated with succinic acid is 67.77°. So comparing with the DSC curve of pure iron oxide nanoparticle (figure 4.3.1), the curve gets shifted which shows that succinic acid binds to the magnetite surface by carboxylate.

V. CONCLUSION

The work investigated the synthesis of superparamagnetic iron oxide nanoparticles prepared by the simple and cost effective method, co-precipitation method. As the naked iron oxide nanoparticles cannot be used for the in vitro applications as they are toxic, agglomerates in the body fluid. To overcome this disadvantage the iron oxide nanoparticles were surface modified. The nanoparticles were surface modified using three types of carboxylic acids –oleic acid, citric acid and succinic acid. A comparative study was carried out among them using TEM, FTIR and DSC characterization techniques. Out of the three, iron oxide nanoparticle coated with succinic acid gives a uniform coating among them which was confirmed using TEM analysis and we came to a conclusion that we can use these nanoparticles for further functionalization which can be used for various applications.

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