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ANTIBACTERIAL ACTIVITY OF ECO FRIENDLY SYNTHESISED ZERO VALENT IRON OXIDE NANOPARTICLES

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ABSTRACT

The emergence of nanoscience and nanotechnology in the last decade presents opportunities for exploring the bactericidal effect of metal nanoparticles. The bactericidal effect of metal nanoparticles has been attributed to their small size and high surface to volume ratio, which allows them to interact closely with microbial membranes and is not merely due to the release of metal ions in solution. In contrast, gram negative bacteria have a relatively thin cell wall consisting of a few layers of peptidoglycan. Surfaces of iron nanoparticles affect or interact directly with the bacterial outer membrane, causing the membrane to rupture and killing bacteria. Antibacterial activity of Zero valent iron oxide nanoparticles (ZVIO) synthesized by Eco friendly synthesis was evaluated by using standard Zone of Inhibition (ZOI) microbiology assay. Zero valent iron oxide nanoparticles (ZVIO) were synthesized using Iron (II) Precursor by Eco friendly method. Zero valent iron oxide nanoparticles (ZVIO) so obtained were characterized by UV- Visible spectroscopy, Fourier Transform Infrared Spectroscopy, and X-Ray diffraction analysis. X-Ray diffraction analysis proved the formation of Zero valent iron oxide nanoparticles (ZVIO) Zero valent iron oxide nanoparticles (ZVIO). SEM analyses showed the presence of nanocrystals. The Zero valent iron oxide nanoparticles (ZVIO) thus formed was subjected to Anti Microbial activity.

KEYWORDS: Fe (II) p-aminobenzoate, UV –Visible, XRD, FTIR, SEM and Antimicrobial activity.

1. INTRODUCTION

Nanotechnology is the art and science of manipulating matter at the atomic or molecular scale and holds the promise of providing significant improvements in technologies for protecting the environment. While many definitions for nanotechnology exist, the U.S. Environmental Protection Agency (EPA) uses the definition developed by the National Nanotechnology Initiative (NNI), a U.S. Government research and development (R&D) program established to coordinate multi-agency efforts in nanoscale science, engineering, and technology. Iron oxide nanoparticles are a promising material in biomedical application due to the material's low cytotoxicity, high biocompatibility, and outstanding magnetic properties. As a result, these particles have been widely investigated from the synthesis, to colloidal surface modification, and further to in vitro and in vivo biomedical tests. The most pivotal factor that affects iron oxide nanoparticles biomedical performance is the magnetic properties of the nanoparticles, which is significantly dependent upon the particles size, shape, oxidation state, and crystalline. Therefore, exploration of the particle formation mechanisms is critical for the successful synthesis of finely controlled nanoparticles to be used in theranostic applications. Despite the intensive motivation for the investigation of the mechanisms and the kinetics in the synthesis of these materials, there is still a considerable knowledge gap in relationship between the obtained particles size, shape, and crystallinity, and the reagent amount and reaction conditions, not to mention, the ability to precisely predict the particles morphology before reaction. This Paper is focused on the morphology control in the synthesis of iron oxide nanoparticles, the nucleation and crystal growth process during the fabrication process, in order to control and predict particles size and shape. These materials were further applied to a study on the magnetic hyperthermia properties to investigation how various morphology properties affect the iron oxide nanoparticles performance biomedical applications. The aim of the study was to investigate the treatment efficiency of the wastewater from rinsing after copper plating by zero valent iron oxide nanoparticles (ZVIO). Wastewater from metal finishing industry contains high concentrations of contaminants. Typical ones are cyanides and heavy metals.[1-9] Removal of copper and nickel was observed in this study. Tests were carried out in lab scale and performed on real wastewater from metal finishing. Metals were bound in complex compounds and difficult to remove from wastewater (ZVIO) showed possibility to solve this problem via environmentally friendly way.

1.1 Zero Valent Iron oxide Nanoparticles and its Antibacterial Activities

The emergence of nanoscience and nanotechnology in the last decade presents opportunities for exploring the bactericidal effect of metal nanoparticles. The bactericidal effect of metal nanoparticles has been attributed to their small size and high surface to volume ratio, which allows them to interact closely with microbial membranes and is not merely due to the release of metal ions in solution. A cell wall is present around the outside of the bacterial cell membrane and it is essential to the survival of bacteria. It is made from polysaccharides and peptides named peptidoglycon. There are broadly speaking two different types of cell wall in bacteria, called gram positive and gram negative. The names originate from the reaction of cells to the gram stain, a test long employed for the classification of bacterial species. Gram positive bacteria possess a thick cell wall containing many layers of peptidoglycan. In contrast, gram negative bacteria have a relatively thin cell wall consisting of a few layers of peptidoglycan. Surfaces of iron nanoparticles affect or interact directly with the bacterial outer membrane, causing the membrane to rupture and killing bacteria. Antibacterial activity of iron nanoparticles synthesized by electrolysis was evaluated by using standard Zone of Inhibition (ZOI) microbiology assay.[10-13]

1.2 ANTIOXIDANTS

Antioxidant compounds in food play an important role as a healthprotecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties.[14-16] Some compounds, such as gallates, have strong antioxidant activity, while others, such as the mono-phenols are weak antioxidants.[50] Magnetic ferrite nanocrystals have gained much popularity during recent years and are increasingly being studied. This is because of their properties which are largely different compared to large compounds natural magnetic properties and especially due to their magnetic characteristics. In the present work, monodisperse Zerovalent iron oxide nanoparticles (ZVIO) with diameter of 8 and 12 nm were synthesized by the Fe(II) precursors from various aromatic acids like p-aminobenzoic acid, m-aminobenzoic acid, athranilic acid, formic acid.

2. EXPERIMENTAL

2.1 Materials and Reagents

All chemicals were of analytical grade and purchased from Merck and Sigma Aldrich. Ferrous Sulphate, Sodium Hydroxide, P-Aminobenzoic Acid, M-Aminobenzoic Acid, Anthranilic Acid, Formic Acid, Oleylamine, L-Valine, Ethanol.

2.2 Mechanochemical synthesis / Green chemical reduction method

In this process, chemical reaction is induced by mechanical energy. The chemical precursors such as Fe(II) p-aminobenzoate, Fe(II) m-aminobenzoate, Fe(II) anthranilate, Fe(II) formate were produce as a complex in which they act as an ultra fine particle. The present investigation reports, the novel synthesis of ZVI nanoparticles using mechanochemical reduction method using morter and pestle. The green chemistry approach used in the present work for the synthesis of nanoparticles is simple, cost effective and the resultant nanoparticles are highly stable and reproducible. This method was developed to synthesize ZVI nanoparticles by Fe (II) precursor with oleylamine.[17 -19]

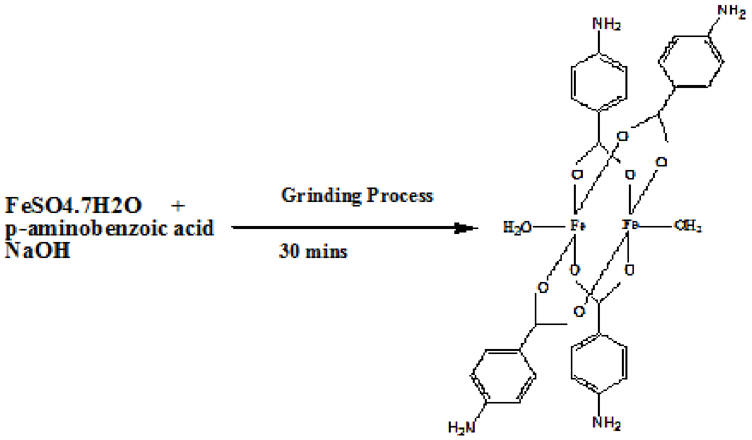
2.3 Preparation of Fe (II) p-aminobenzoate precursor

2.74 gms of p-aminobenzoic acid (20mmol) and 1.6 gms of Sodium Hydroxide (20mmol) is mixed in a mortar pestal and grinded it for fifteen minutes and the mixture is colorless. Then add Ferrous Sulphate Hepta Hydrate $FeSO_{4}.7H_2O$ (2.78, 10mmol) which is light green colour into the mixture and grind it for 45 minutes. The light brown colour mixture was changed into dark deep brown colour, this is washed with the help of ethanol and filtered and dried. This procedure by the other organic acids.

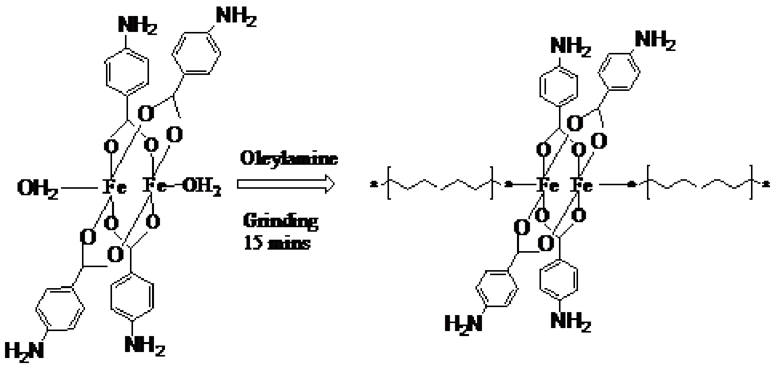
2.4 Preparation of ZVIO Nanoparticles from Fe(II) p-aminobenzoate precursor

The dark deep brown colour of Fe(II) p-aminobenzoate (0.691gm) is taken and it is reduced with the help of L-valine (0.351gm) in a mortal pestle for an hour in ambient temperature. The dark deep brown colours was gradually changed in to a dark brown colour powder and add sodium hydroxide (0.12gm) further grinding process continued for half an hour. Finally add an oleylamine (5ml) which act as surfactant, stabilizing agent or capping agent grinded the mixture was grinded on the constant speed for half an hour in the ambient temperature. The colour changes to black. The resultant mixture obtained was oleylamine encapsulated ZVIO nanoparticles. This procedure followed by the other four Precursors.

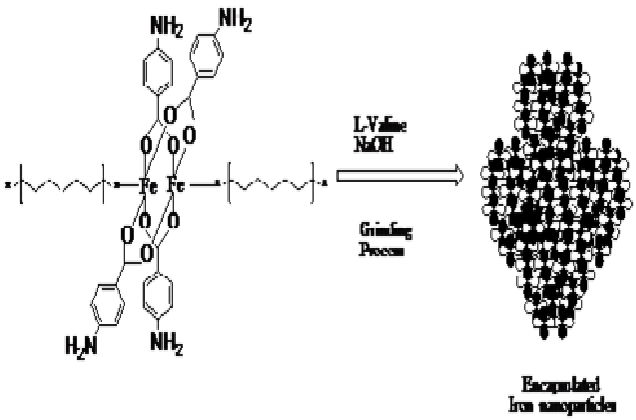
Mechanochemical synthesis / Green chemical reduction method



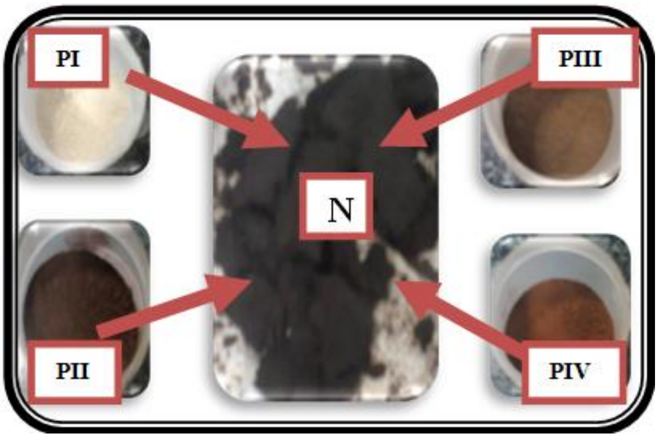
Scheme 1. The preparation of Fe (II) p- amino benzote.



Scheme 2. The preparation of Fe (II) p- amino benzoate encapsulated oleylamine.



Scheme 3. The preparation of Zero valent Iron oxide Nanoparticles encapsulated oleylamine.



Photographic Picture Shows Fe (II) Precursors and Zero valent iron oxide nanoparticles (ZVIO).

[PI] Fe(II) formate Precursor,
[PII] Fe(II) anthranilate Precursor,
[PIII] Fe(II) p amino benzoate,
[PIV] Fe(II) m amino benzoate,
[N] Zero valent Iron oxide nanoparticles (ZVIO)

3. RESULTS AND DISCUSSION

The Zero valet iron oxide nanoparticles (ZVIO) are divided into two parts. In the first part, the Fe(II) precursor as prepared and UV, FT- IR analysis. The stability of Zero valet iron oxide suspended (ZVIO) nanoparticles in oleylamine for more than two weeks. In the second part, the magnetite (ZVIO) nanoparticles after purification were dried overnight at 80° for XRD, SEM, FT-IR and UV analysis.

3.1. Electronic Spectra

Extinction spectra of synthesized Zero valet iron oxide nanoparticles (ZVIO) are shown in Figure 1 & Table1. Characteristic surface Plasmon absorption band is observed at 312 nm for the brownish block colour Zero valet iron oxide nanoparticles (ZVIO) synthesized from Fe(II) precursor.[20]

Table. 1: UV-Visible spectral data of Fe(II) precursors and zero valent iron oxide nanoparticles.

Fe(II) Precursor	π-π*	n- π*	(ZVIO) Nanoparticles	Λ MAX (nm)
P I	-	380	**N I**	312
P II	292	-	**N II**	236
P III	216	306	**N III**	306
P IV	278	354	**N IV**	300

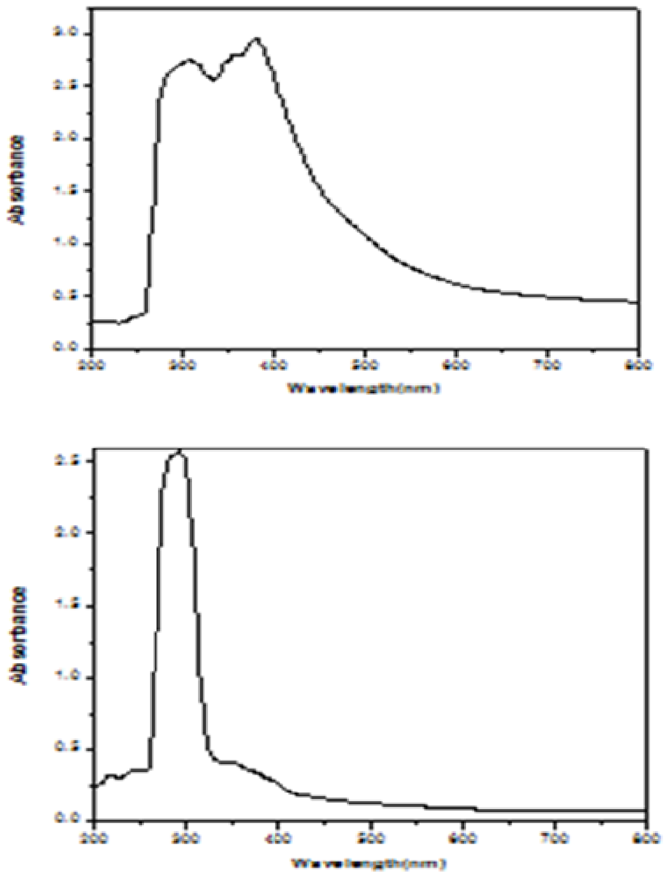


Fig 1: UV-Visible Spectra of for Fe(II) PI & ZVIO Nanoparticle of Nano NI.

3.2 IR – Spectra Analysis

In order to determine the role of the functional group in the reaction, the sample of oleylamine was characterized individually (a), Fe (II) Precursor (b) and ZVIO nanoparcicles (c). Before the reaction by FT-IR (Figure 2 (a) – (c)), we can see that the specific peaks of amine group are around 3350 cm-1 and in the fingerprint region (1650-1504 cm-1).[21] But the peaks in the fingerprint region (1650-1580) are also overlapped with C=O and C=C peaks of FeO. After the reaction, no N-H at 3300 cm-1 could be detected, which could be due to the relative intensity of the specific peaks. Overall the observation confirms the presence of oleylamine, which acts as a reducing agent and stabilizer for ZVIO nanoparticles.

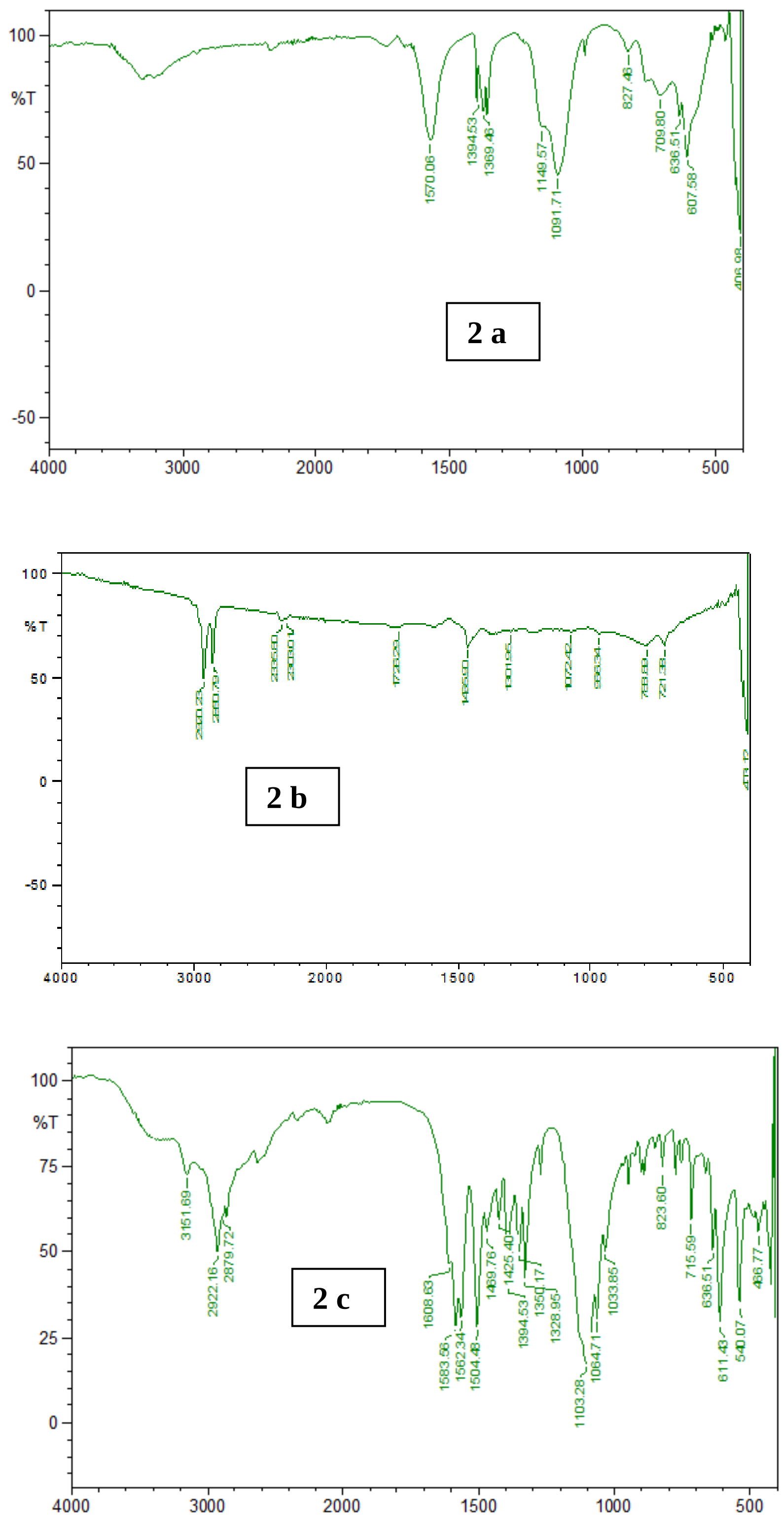


Fig 2: IR spectral graph for (a) free oleylamine (b) Fe(II) precursor & (c) ZVIO nanoparticles.

3.3 XRD – Analysis

XRD is an effective characterization to confirm the crystal structure of the synthesized magnetite nanoparticles. The XRD spectrum of magnetite nanoparticles is presented in Figure

3. The 2θ peaks at 23.55°, 32.81°, 38.48°, 48.52°, 54.59° and 62.34° are attributed to the crystal planes of magnetite at 204, 220, 311, 222 and 400, respectively. The magnetite nanoparticles are well- crystalline and the position and the relative intensity of the diffraction peaks match well with the standard XRD data for bulk magnetite (JCPDS file No. 19-0629). The average particle sizes of the synthesized magnetite nanonparticles were calculated using Debye-Scherrerformula.[22-24]

Table 2. Peak values for ZVIO Nanoparticle N1.

S. No.	2θ	Peak Position (hkl)	Nanoparticles
1.	23	204	FeO
2.	32	204	FeO
3.	38	220	Fe_2O
4.	48	200	FeO
5.	54	210, 400	Fe_3O_4
6.	65	211	Fe_3O_4

3.4 Particle Size Calculation

From this study, considering the peak at degrees, average particle size has been estimated by using Debye-Scherrer formula Particle Size prediction by the Debye-Scherrer formulae

$$D = 0.9\lambda / \beta\cos\theta$$

$\lambda = 1.5406 \times 10^{-10}$m

β = Full width at half maximum (radian)

Calculation of d-Spacing

The value of d (the interplanar spacing between the atoms) is calculated using

Bragg's Law: $2d\sin\theta = n\lambda$

$$d = \frac{\lambda}{2\sin\theta} \quad (n=1)$$

Wavelength λ = 1.5418 Å

Dislocation density (δ) is calculated with the crystalline size.

$$\delta = \frac{1}{D^2}$$

Table. 3: The grain size of Zero valent Iron Nanoparticles (ZVI).

S. No.	Compound	Size of the particle (D) nm	Dislocation density (δ)
1	N1	8.66	0.0133
2	N2	11.74	0.0072
3	N3	8.60	0.0135
4	N4	12.05	0.0068

The structure and chemical composition of all Zero valent Iron oxide Nanoparticles (ZVIO) samples synthesized in this work are confirmed with XRD. A typical XRD pattern of all Zero valent Iron oxide Nanoparticles (ZVIO) samples is shown in below figure and Table 2-3. The Zero valent Iron oxide Nanoparticles (ZVIO) synthesized got oxidized in due course. The XRD pattern is consistent with the spectrum of Zero valent Iron oxied Nanoparticles (ZVIO). The average particle size obtained from the XRD data of nanoparticles was around 8-12 nm.

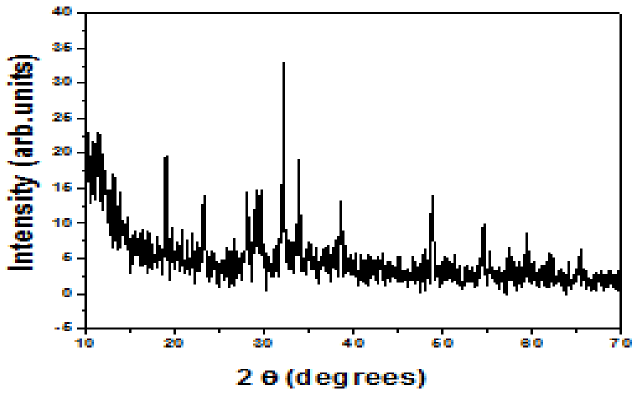
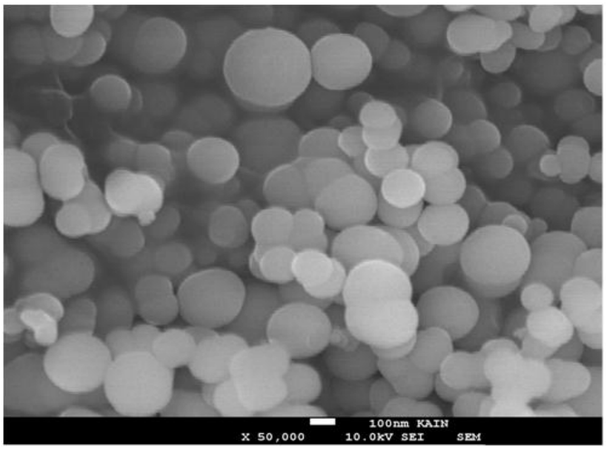


Fig 3: XRD graph for ZVIO nanoparticles coated with oleylamine compound N1.

3.5 Scanning Electron Microscope

The scanning electron microscopy (SEM) micrograph for Zero valent Iron oxide Nanoparticles (ZVIO) is shown in Figure 4. The particles had a rather narrow size distribution, where most of the Zero valent Iron oxide Nanoparticles (ZVIO) were within 8-12 nm. Therefore Zero valent Iron oxide Nanoparticles (ZVIO) were successfully synthesized by this green method using oleylamine as reducing agent and stabilizer for nanoparticles.



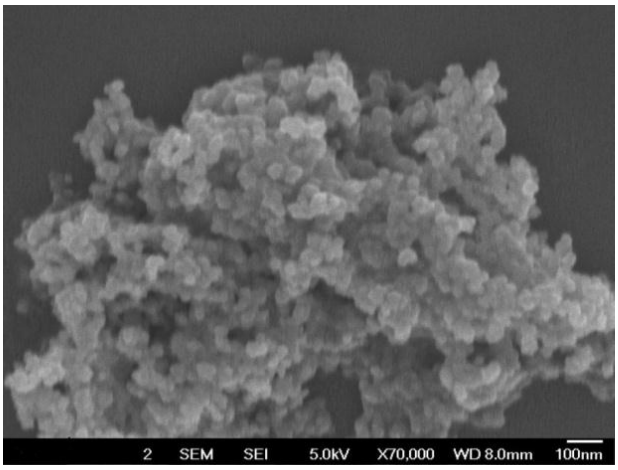


Fig 4: SEM image of oleylamine encapsulate ZVIO nanoparticles.

3.6 Antimicrobial Activity

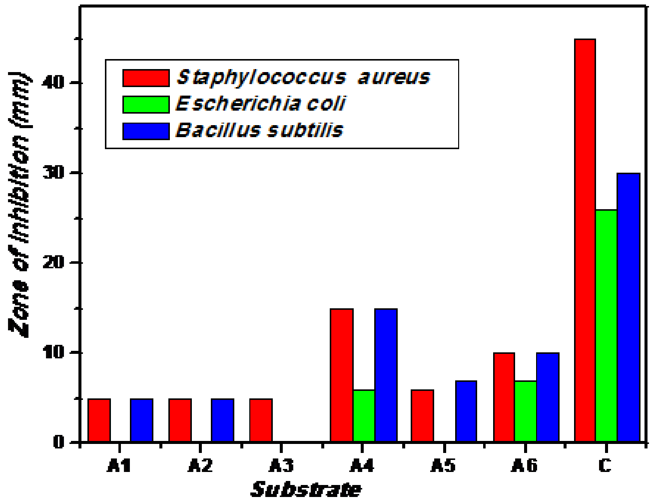
Antimicrobial analysis was followed using standard agar well diffusion method to study the antimicrobial activity of compounds[25] Each bacterial isolate was suspended in Brain Heart Infusion (BHI) broth and diluted to approximately 10^5 colony forming unit (CFU) per mL. They were flood-inoculated onto the surface of BHI agar and then dried. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer and 30 µL (5µg compound in 500 µL DMSO) of the sample solution were poured into the wells. The plates were incubated for 18 h at 37°C for bacteria. Antimicrobial activity was evaluated by measuring the diameter of the zone of inhibition in mm against the test microorganisms and the solvent. DMSO was used as solvent control. Ciprofloxacin was used as reference antibacterial agent. The tests were carried out in triplicates.

3.6.1 Antibacterial activity

Zero valent Iron oxide Nanoparticles (ZVIO) showed antibacterial effect against gram-positive as well as gram-negative bacteria which clearly indicates that these nanoparticles are effective antibacterial agents. In Figure 5 and table 4 the control, low and high concentrations of Zero valent Iron oxide Nanoparticles (ZVIO) were shown, respectively. Many antibacterial studies were made using different nanoparticles. The reason for the bactericidal activity is due to the presence of reactive oxygen species (ROS) generated by different nanoparticles.

Table 4. Antibacterial activity of Fe(II) Precursors & ZVIO Nanoparticles.

S.NO	MICRO ORGANISMS	A1	B1	A2	B2	A3	B3	A4	B4	A5	B5	A6	B6	D	C
1.	Staphylococcus aureus	5	15	5	20	5	20	15	17	6	18	10	17	24	45
2.	Escherichia coli	-	15	-	20	-	23	6	17	-	19	7	16	22	26
3.	Bacillus subtilis	5	-	5	-	-	6	15	7	7	9	10	5	7	30



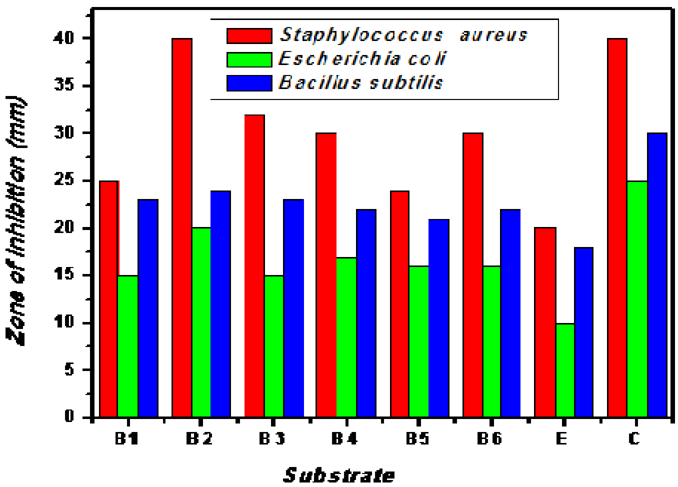


Fig. 5: Graphical diagram for antibacterial activity of Fe(II) Precursors & ZVIO Nanoparticles.

S.NO	MIRO ORGANISMS	B1	B2	B3	B4	B5	B6	E	D
1.	Aspergillus niger	15	20	20	17	18	17	7	24
2.	Aspergillus flavus	15	20	23	17	19	16	-	22
3.	Mucus indicus	-	-	6	7	9	5	5	7

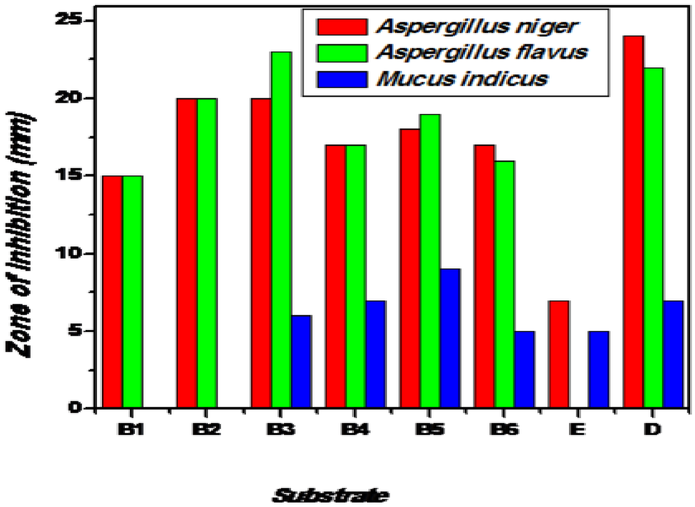


Fig. 6: Graphical diagram for antifungal activity of ZVIO nanoparticles.

3.7 Antioxidant

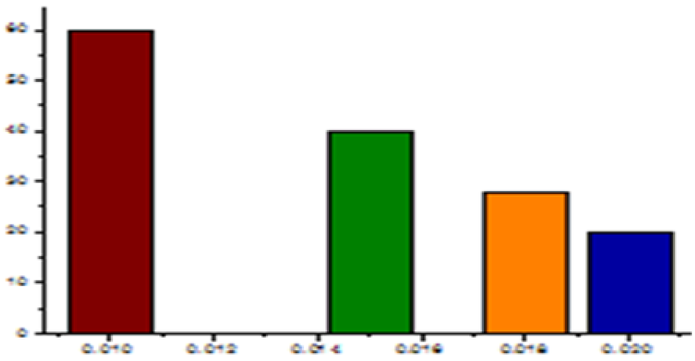
The ability to scavenge hydrogen peroxide was determined according to the method of Ruch et al (1989). Absorbance of hydrogen peroxide at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentages of hydrogen peroxide scavenging were calculated:

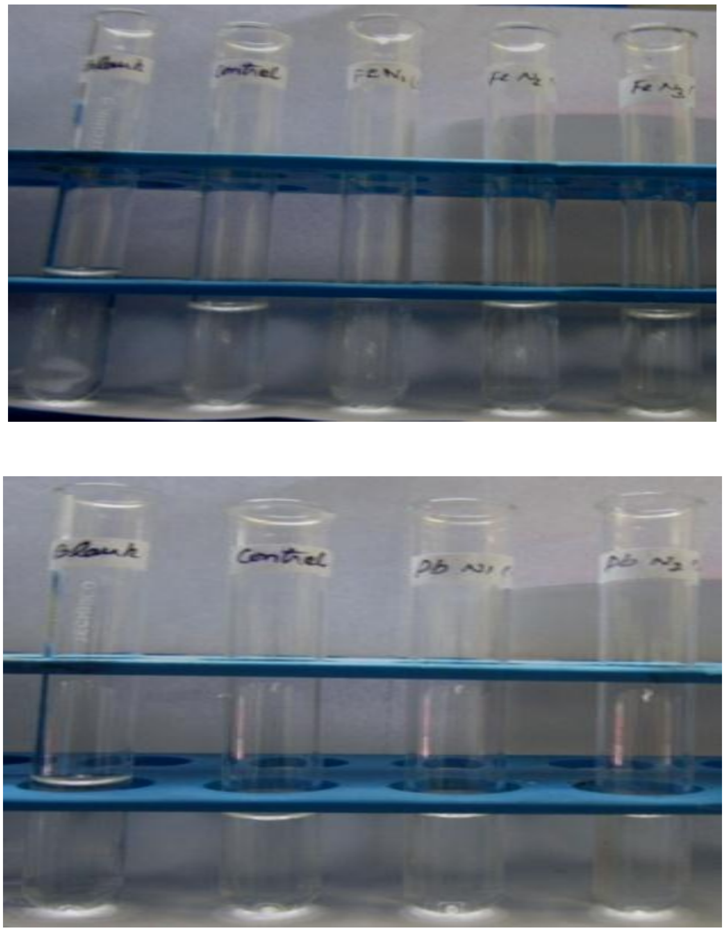
$$\% \text{ Scavenged } [H_2O_2] = [(AC - AS)/AC] \times 100$$

Where AC is the absorbance of the control and AS is the absorbance in the presence of the nanoparticles.

Table 6. Hydrogen peroxide Scavenging activity

Sample name	Sample Volume (µl)	Sample Abs.	% of Inhibition
N_1	25	0.010	60
N_2	25	0.015	40
N_3	25	0.020	20
N_4	25	0.020	20





Picture of Hydrogen Peroxide of ZVIO nanoparticles Antioxidant activity.

4. SUMMARY

In this research study, Zero valent Iron oxide Nanoparticles (ZVIO) with different sizes were synthesized from the Green method using oleylamine as reducing agent and stabilizer for nanoparticles. FTIR was used to identify the functional group present in the materials. The main groups are Fe-O and Fe-OH. SEM again gives the reproducible result for the synthesis of the nanoparticles. The novel facile Surfactant and capping agent oleylamine has been used to synthesis Zero Valent Iron oxide Nanoparticles (ZVIO) for the first time with aromatic acids. The XRD result and SEM results confirmed Zero Valent Iron oxide Nanoparticles (ZVIO) has the crystallite size less than 30 nm. The dislocation density has decreased with the increase in the crystallite size. The Zero valent Iron oxide Nanoparticles (ZVIO) showed their antibacterial properties on both gram positive and gram negative bacterial strains. As the diameter of the zone of inhibition is high, we can conclude that Zero Valent Iron oxide Nanoparticles (ZVIO) is a very effective antibacterial agent. Oleylamine coated Zero Valent Iron oxide Nanoparticles (ZVIO) exhibited good antibacterial activities against both Gram-Positive bacteria *staphylococcus auries*, Gram-negative bacteria *Escherichia coli* microorganism. The activities were more pronounced in the Gram-positive bacteria as compared to the Gram-negative bacteria. This could begin to unlock the unlimited possibilities of applications of oleylamine coated Zero Valent Iron oxide Nanoparticles (ZVIO) to the future of research in biomedicine and the production of pharmaceuticals. As the diameter of the zone of inhibition is high, we can conclude that Zero Valent Iron oxide Nanoparticles (ZVIO) is a very effective antifungal agent. Oleylamine coated Zero Valent Iron oxide Nanoparticles (ZVIO) exhibited good antifungal activities compare Fe(II) Precursor against both *Aspergillus niger, Aspergillus flavus and Mucus indicus* microorganism. The zero valent iron nanoparticles showed their antioxidant properties is more effective.

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