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Synthesis, Characterization of Magnetic Iron Oxide Nanoparticles and its Toxicity Assessment in Tilapia fingerlings

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Abstract: *Nowadays, the most recent advances in nanoscience and nanotechnology are widely used in a variety of industries, including pharmaceuticals, medicine, electronics, robotics, and tissue engineering. The current study focuses on the chemical synthesis of Magnetic Iron Oxide Nanoparticles, Followed by characterization using UV-visible spectrometry, FTIR, SEM, and EDS. Toxicity tests were performed on fresh water fish Tilapia (Oreochromis mosambicus) fingerlings exposed to as synthesized Magnetic* Fe_3O_4 *NPs* LC_{50} *concentration of* Fe_3O_4 *NPs were 12.45 mg/L for 96 hrs and sub-lethal* LC_{10} *concentrations (10 ppm and 20 ppm) for 96 hours. When compared to controls, treated groups showed significant changes in biochemical indicators such as protein and catalase activity after 96 hours, which may be related to* Fe_3O_4 *NPs in fish muscles. The cause of nanoparticle toxicity is attributed to their own specific characteristics of greater surface-to-volume ratio, chemical composition, size, and dosage, body retention, and organ specific toxicity. The comprehensive data can provide the important empirical parameters as well as recent field developments. The extensive data is able to provide both the crucial empirical aspects and the most recent advancements in this field.*

Keywords*:* Fe_3 O_4 NPs, Characterization, toxicity, Fish and Biochemical test

I. INTRODUCTION

Iron Oxide Nanoparticles (NPs) have earned a significant attention at present, in relation to their unique properties which has basic scientific value but also for a wide range of biomedical and technological applications [1] [2]. The synthesis of Iron Oxide Nanoparticles (NPs) involves a range of intricate, costly, physical, and chemical procedures that result in dangerous, toxic waste that is detrimental to human health and the environment [3]. The synthesis of Iron Oxide Nanoparticles (NPs) involves a range of intricate, costly, physical, and chemical procedures that result in dangerous, toxic waste that is detrimental to human health and the environment. Nanoparticle research is expanding due to their unique physical and chemical properties [4] [5] [6]. The high surface-area-to-volume ratio of nanoparticles opens up numerous opportunities for producing antibacterial medicines to treat bacterial illnesses [7]. The possible health concerns posed to non-target creatures in the environment make it critical to set adequate rules for safe IONP management and disposal in order to protect the aquatic environment [8] [9]. However, despite the fact that it has been shown that Iron Oxide Nanoparticles can enter the human body through a number of routes, such as ingestion, injection, dermal penetration, and inhalation, little is known about the impact of these particles on human health, their potential side effects, and how their systematic circulation can change them in different tissues [10] and control over the production, use, and discharge of these nanoparticles in aquatic environments. When compared to conventional formulations, the growing exploration of NPs revealed many properties such as enhanced magnetic, catalytic, optical, and mechanical properties. Many commercial applications, such as fillers, catalysts, filters, semiconductors, microelectronics, and so forth, use these materials extensively, exposing people both directly and indirectly to environmental and medical risks. According to investigator [11], there are certain nanoparticles that are used in biomedical applications like protein immobilization, magnetic resonance imaging (MRI), gene and medication delivery, cell separation, and so forth. The persistence of nanoparticles after long-term exposure to Fe_3O_4NPs through treatment

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withdrawal to confirm the persistent impact of nanoparticles on the exposed organism were investigated [12]. Iron nanoparticles (FeNPs), showing, compete favourably with dangerous chemical pollutants in water and the environment [13].

Although magnetic nanoparticles must have specific properties, such as a persistent, switchable magnetic state in data storage applications to indicate bits of information that are influenced by thermal effects [14] and other investigator evaluated the altered the quantity of Iron Oxide Nanoparticles and noted the changes on growth, haematological and biochemical parameters of Koi Carp [15].

Furthermore, the potential eco-toxicological consequences, bioavailability, and detrimental effects of nanoscale contaminants in the aquatic environment can be evaluated using appropriate biomarkers for aquatic species, like fish [16]. Therefore, it is necessary to synthesize a simple, cost-effective, reproducible technique to synthesize Fe_3O_4 nanoparticles. The toxicity of MNPs is thus determined by a variety of factors, including size, shape, structure, surface modification, concentration, dosage, bio-distribution, bioavailability, solubility, immunogenicity, and pharmacokinetics, making it critical to understand such variables [17] [18]. The precipitation technique is used to chemically method for nanoparticles and Tilapia was selected for this study since it is a traditional nutritious eating fish in India. Hence in this present work is link up for synthesis, characterization, and toxicological assessment of Iron Oxide nanoparticles (Fe_3O_4) in fish Tilapia fingerlings.

II. MATERIAL AND METHODS

The chemicals were all of analytical grade. The precipitation method for FeCl3 and FeSO4 was used for synthesis of Iron Oxide Nanoparticles and NaOH were used as precursors [19] [20]. Characterization of Iron Oxide NPs carried out by FTIR, SEM, EDS, and UV-visible spectrometry. Characterizations (SEM and EDS) of Iron Oxide NPs were carried out at Physic Department of SPP University, Pune and also FTIR at Chemical Technology department of KBC NM University Jalgaon. Healthy Tilapia fingerlings were procured from Fish Farm in Jalgaon, Maharashtra, India, and acclimatized for about 15 days before the experiment began. For assessment of Fe_3O_4 NP toxicity, fish with an average length of 12 ±0.5 cm and average weight of 10.2 ±4 g were selected. Toxicity tests were conducted by synthesized Fe_3O_4 NPs at exponential concentrations (10 ppm and 20 ppm) for 96 hrs. Significant differences in biochemical parameters protein and catalase activity were found after 96 hours in treated groups compared to controls, which could be attributed to Iron Oxide NPs in fish muscles. The estimation of protein concentration was done by Biuret method using kit. The technique was used to estimate Catalase enzyme activity [21].

III. RESULT AND DISCUSSION

Synthesized Iron oxide nanoparticles by chemical method as NaOH is added to $FeCl_3$ and $FeSO_4$, the colour of solution changes as of reddish brown to black, is presented in Fig.1 depicts the formation of Iron Oxide Nanoparticles (Fe_3O_4NPs). Precipitation was observed at pH to 11. Fig 1. Indicates the experimental design. Fig 2. Chemical Synthesis of Iron Oxide Nanoparticles (Fe_3O_4 NPs). The characterization of Iron Oxide nanostructures was further confirmed by the UV-Vis spectra, FTIR, SEM, EDS and (Figure 3, 4, 5 and 6) signifying high density chemically synthesized Fe_3O_4 NPs.

Characterization of Iron Oxide NPs

UV-Vis Spectrophotometry Analysis

Preliminary confirmation was obtained by measuring the absorbance of the produced iron oxide nanoparticles in UV/Vis spectra within the 200–800 nm range. As shown in the present study, the concentrations of the associated functional groups may be directly correlated with the band of absorption intensities. The synthesis of iron oxide nanostructures was further confirmed by the UV-Vis spectra (Figure 2), which displayed high density chemically generated Fe_3O_4. The findings are consistent with earlier research [22].

Fourier transform infrared (FTIR) spectra of Iron Oxide Nanoparticles

FTIR Functional Group bond of Fe_3O_4 NPs. Fourier Transform Infrared spectroscopy studies were performed to identify the probable functional groups responsible for the reduction of Fe ions by chemically synthesized iron oxide nanoparticles. FTIR characterization of Fe_3O_4 nanoparticles were carried out shown in Figure 4 and Table 1, the data plot indicates the wave number of infrared light as prominent absorption peaks in particular wavenumbers that result from the vibration of certain functional groups. FTIR is an analytical technique used to identify the functional groups in a sample as well as determine the microcrystalline structure of the particles. The 4000–500 cm-1 range was studied using FTIR spectroscopy, which is sensitive to molecule structure, conformation, and environment. In Figure 3, the FTIR spectra reveal the three characteristic peaks at 670 cm-1, and 855 cm-1 which are attributed to the Fe-O bond [23].

FE-SEM Images and EDS

SEM image of Fe_3O_4, the image depicts Fe_3O_4 particles formed of small particles. The FE-SEM examination reveals information about the surface shape and particle size of the produced samples. Figure 4 displays the iron oxide nanoparticle FE-SEM analysis. Spherical particles of different sizes and dispersion can be seen in microscopic pictures. Furthermore, it was shown that the ratio of particle agglomeration increased due to the electrostatic contact between the layers of nanoparticles. Similar results noted from SEM analysis of Iron Oxide Nanoparticles [24] [25].

Toxicity and Biochemical analysis

Toxicity tests were performed on fresh water fish Tilapia (*Oreochromis mosambicus*) fingerlings exposed to as synthesized Magnetic Fe_3O_4 NPs at LC_{50} concentration of Fe_3O_4 NPs were 12.45 mg/L for 96 hrs, and sub-lethal LC_{10} concentrations (10 ppm and 20 ppm) for 96 hours. Preliminary toxicity analyses have been carried out on Tilapia fingerlings to assess the 96 hrs median lethal concentration of Fe_3O_4. Significant variations in biochemical parameters, including protein and catalase activity, were seen after 96 hours between the treatment and control groups. Effect of Iron Oxide Nanoparticles on the Catalase (CAT) in Gills and Muscles of the fish Tilapia (µmol H_2O_2/ mg protein/ min) noted in Table 3. Whereas Effect of Iron Oxide Nanoparticles on the Catalase (CAT) in Gills and Muscles of the fish Tilapia (µmol H_2O_2/ mg protein/ min) showed in Graph 1. Similar results are noted by [26] [27]. The non-uniform scattered nanoparticles morphology was noted, as seen in Fig 5. Iron oxide nanoparticles synthesized chemically are hexagonal and spherical in structure. In earlier studies on the acute toxicity of nanometals to aquatic life, concentrations in the mg–lg/L range can be fatal for fish. These results indicate that iron oxide nanoparticles prefer to combine due to intrinsic magnetic attraction [28]. Fish tissues all showed a marked reduction in protein levels following exposure to iron oxide nanoparticles. Acute toxic stress and generalized increased proteolytic activity lead protein breakdown to outweigh synthesis, suggesting a tissue state that favours anaerobic respiration to meet energy demands when aerobic oxidation is diminished [4] [29] [30] [31]. Earlier investigation was identified fish mortality in higher amounts of Iron Oxide NP treated groups, which could have resulted from an excessive accumulation of Iron Oxide NPs in the fish's body [10] [16]. The findings are the same as those of former research conducted [32]. Several publications have investigated Magnetic Iron Oxide Nanoparticles [33] [34] [35] [36] based on their biological activity, which includes antibacterial, toxicity, and drug delivery, they have been employed as model organisms in bio-system multifunctional nanomaterials. The biological system adjusts the activities of antioxidants like catalase (CAT) and related enzymes as ROS levels rise in order to build a first line defence mechanism [37]. Thus, the increase in catalase activity implies that iron oxide nanoparticles (NPs) have the ability to cause oxidative stress in fish [38] [39] [40] [41].

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TABLE I

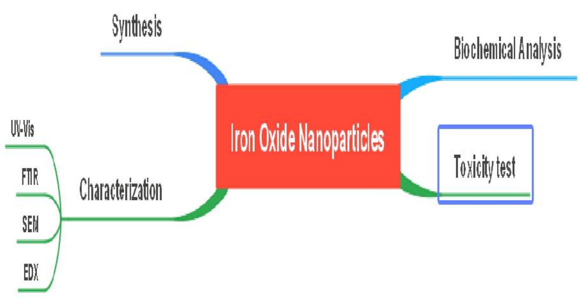

Fig 1. Experimental design

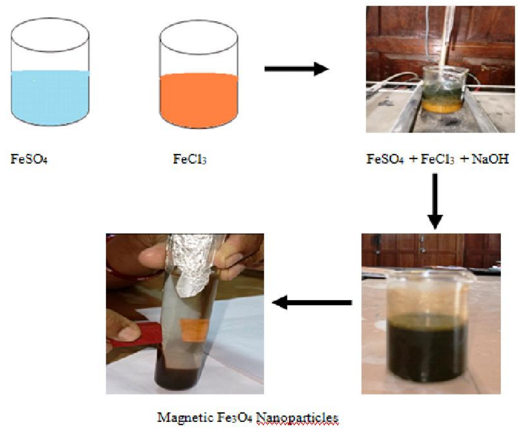

Fig 2. Chemical Synthesis of Iron Oxide Nanoparticles (Fe_3O_4 NPs)

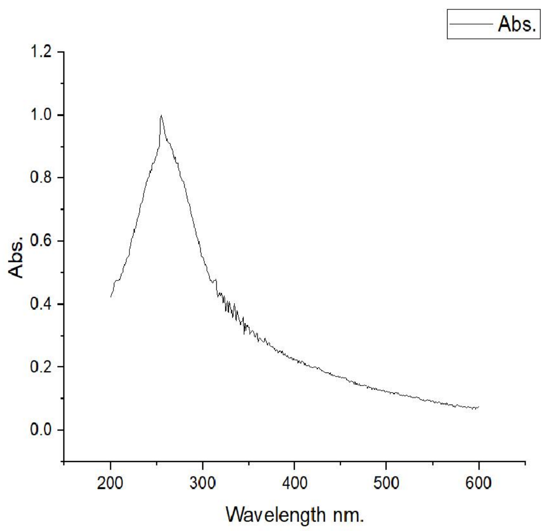

Fig.3 : UV of Fe_3O_4 NPs

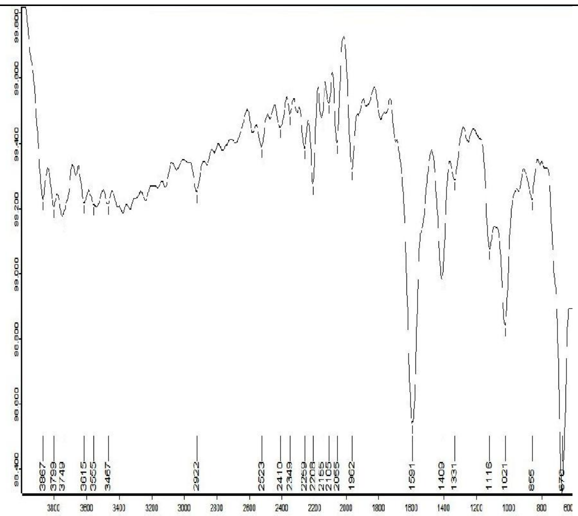

Fig. 4 FTIR of Fe_3O_4 NPs

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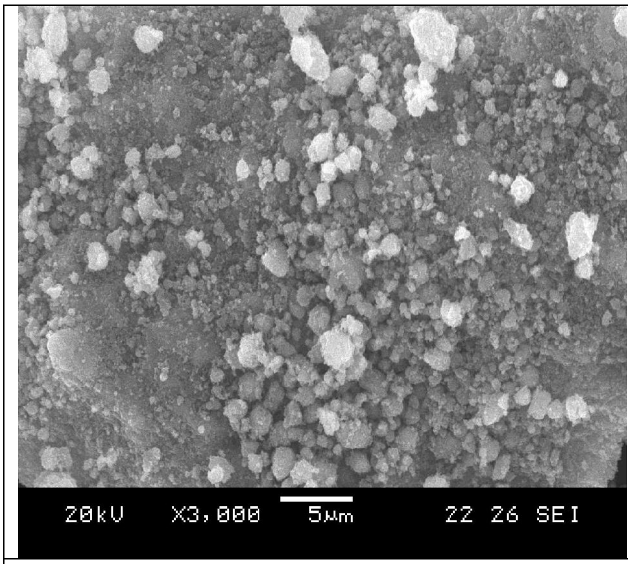


Fig 5. SEM Image of Iron oxide Nanoparticles

Sr. No.	Wave Number (cm-1) Fe3O4 NPs	Functional Group
1	658-506	Fe-O stretching
2	1672-1367	H-O-H bending
3	3028-3400	O-H stretching
4	2920-2855	C-H stretching

Table 1. FTIR Functional Group bond of Fe_3O_4 NPs

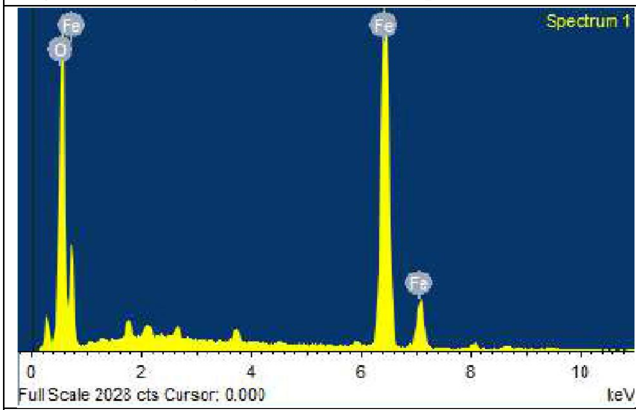


Fig 6. EDS Image of Iron oxide Nanoparticles

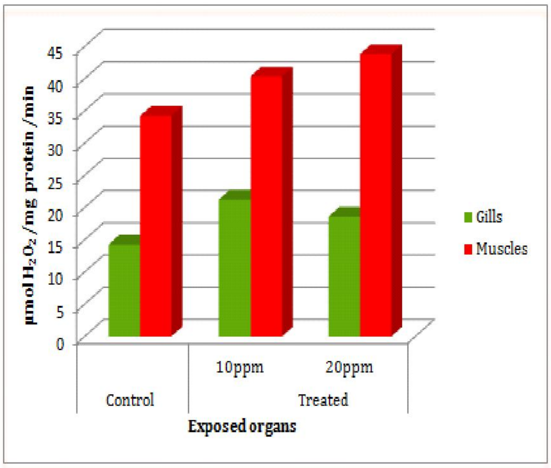
Element	Weight %	Atomic %
O K	44.03	73.30
Fe K	55.97	26.70
Total	100.00	

Table 2. EDS area analysis of Iron Oxide Nanoparticle

Organs	Control	Treated	
		10 ppm	20 ppm
Gills	14.24±0.2	21.24±0.2*	18.6±0.4*
Muscles	34.22±1.22	40.32±2.12***	43.8±2.45*

$P < 0.05$; ** = $P < 0.01$; ***= $P < 0.001$

Table 3. Effect of Iron Oxide Nanoparticles on the Catalase (CAT) in Gills and Muscles of the fish Tilapia (μmol H_2O_2/ mg protein/ min)



Graph 1. Effect of Iron Oxide Nanoparticles on the Catalase (CAT) in Gills and Muscles of the fish Tilapia (μmol H_2O_2/ mg protein/ min)

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IV. CONCLUSION

The safety assessment of synthetic Iron Oxide NPs included extensive characterisation, and fish toxicity testing, resulting in a better knowledge of their possible biological affects and environmental hazards. Using methods like UV-visible spectrometry, FT-IR, FESEM, EDS the physical and chemical characteristics of Iron Oxide NPs were fully examined. Present investigations showed that nanoparticles' size and form have a big impact on biological systems. Present data observed acute toxicity observed at LC50 concentration of Fe_3O_4 NPs were 12.45 mg/L for 96 hrs and toxicity at 10 ppm and 20 ppm for 96 hours freshwater fish Tilapia species exposed to as synthesized Fe_3O_4 NPs at LC10 concentration. After 96 hours, there were significant differences in biochemical measures such as protein and catalase activity in treated groups compared to controls, which could be due to iron oxide NPs in fish muscles. The concentration used in this study had a significant impact catalase activity on muscles of Tilapia fingerlings. These parameters are potentially useful as biomarkers or biological end points for determining the toxicity of manufactured Nanoparticles in aquatic creatures. The current study's findings emphasize the importance of safe metal oxide disposal and practices. However, further research is needed on the direct impact of metal ions and/or the release of metal ions from Nanoparticles.

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