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Fabrication and physicochemical characterization of iron oxide nanoparticles for biomedical applications

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Abstract

This study was carried out for synthesis of iron oxide nanoparticle by conventional alkaline co-precipitation technique and determination of their physicochemical properties. For synthesis, ferrous and ferric salt were employed as iron precursor and ammonium hydroxide was incorporated as precipitating agent. A fine dark black coloured powder was obtained, which exhibits strong magnetism. On characterization, iron oxide nanoparticle displayed uniform nanoscale particle size, good colloidal stability and minimum aggregation tendency. The FT-IR and XRD analysis confirms the presence of specific functional group and highly crystalline nature of core magnetite. All the physicochemical evaluation ensures the successful fabrication of IONP for further biomedical application in wide range.

Keywords: Iron oxide nanoparticle, co-precipitation, DLS, FT-IR, XRD

Introduction

Nanotechnology has evolved as a transformative discipline in present-day science, offering applications pertaining to innovative solutions across medicine, biotechnology, and materials science (Pathakoti *et al.*, 2018; Malik *et al.*, 2023) [28, 21]. Iron oxide nanoparticles (IONPs) are gaining significant interest among other nanomaterials because of their distinct physicochemical and magnetic characteristics, biocompatibility (Xie *et al.*, 2018) [38], and ease of surface functionalization (Shah *et al.*, 2022) [33]. These qualities enable them to ideal for a variety of biological uses, such as biosensing (Magro *et al.*, 2019; Lee *et al.*, 2020) [20, 19], tissue engineering (Hasan *et al.*, 2018; Friedrich *et al.*, 2021; Truong *et al.*, 2024) [7, 5, 36], drug administration (Hussein-Al-Ali *et al.*, 2021) [9], magnetic resonance imaging (Khmara *et al.*, 2019), and magnetic hyperthermia (Martins *et al.*, 2025) [23].

Superparamagnetic property at nanoscale dimensions allows IONPs, especially magnetite to be modulated under an external magnetic field. This characteristic is particularly useful in diagnostic imaging and targeted drug delivery, as minimum aggregation and precise spatial localization are needed (Jeon *et al.*, 2021) [11]. Additionally, their biodegradability and comparatively low toxicity increase their potential for *in-vivo* applications. The process of IONP synthesis has remarkable impact on size, surface charge, size distribution, shape and stability of nanoparticles (Janik-Olchawa *et al.*, 2021) [10]. A variety of techniques have been developed including such as co-precipitation, thermal decomposition, hydrothermal synthesis and sol-gel methods (Nikitin *et al.*, 2018; Saqib *et al.*, 2019; Jesus *et al.*, 2024) [27, 31, 12]. Among all, chemical co-precipitation method is widely accepted considering its convenient nature, affordability, high yield and large scalability. Although, managing particle size distribution and minimizing the nanoparticle aggregation seem to be key challenges for co-precipitation technique (Guardia *et al.*, 2011; Ajinkya *et al.*, 2020) [6, 1].

In order to validate the structural, morphological, and functional characteristics of fabricated nanoparticles, characterization is paramount. The dynamic light scattering (DLS), offer insight on particle size, poly dispersity index, zeta potential of nanomaterial and indicates colloidal stability of nanomaterial (Mishra *et al.*, 2014) [25]. Fourier transform infrared spectroscopy (FTIR) is employed to identify functional groups and confirm surface modifications (Stoia *et al.*, 2016) [35], while scanning electron microscopy (SEM) visualises particle morphology and surface topography.

X-ray diffraction (XRD) analysis ascertained the crystalline structure and phase purity of the nanoparticles (Ali *et al.*, 2016) [2]. Customizing IONPs for enhanced therapeutic efficacy and biological compatibility has garnered more emphasis in recent years. Their stability, circulation time, and targeting ability can be dramatically improved by surface modification using polymers, surfactants, or bioactive compounds (Natarajan *et al.*, 2019) [26].

Therefore, the present study primarily targeted synthesis of iron oxide nanoparticles using an appropriate methodology and their thorough characterisation incorporating DLS, FTIR, SEM, and XRD techniques. The results are intended for the development of efficient and biocompatible nanomaterials for biological and clinical applications.

Materials & Methods

Materials

All chemical reagents utilized in the current study were of analytical grade, which ensures high purity. Anhydrous ferric chloride (FeCl_3) and ferrous sulphate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) were procured from Molychem and HiMedia Laboratories Pvt. Ltd., respectively. Additionally, ammonium hydroxide solution (18-20%) was employed as precipitating reagent and absolute ethanol was used as solvent.

Fabrication of iron oxide nanoparticles

The synthesis of iron oxide nanoparticles was conducted using the chemical coprecipitation method, following the methods outlined by Dehghanpour (2020) [4] together with a couple of changes. Anhydrous ferric chloride and ferrous sulphate heptahydrate were utilized as iron precursors. For this 1.62 gm of ferric chloride was added in 5 mL distilled water to prepare 2M solution and 1.39 gm of ferrous sulphate was added into another 5 mL of distilled water to obtain 1M solution. Then each solution were transferred into a conical flask and mixed with the help of magnetic stirrer at the speed of 1000 rpm, 80 °C for one hour. After that, 2M solution of ammonium hydroxide was incorporated drop by drop until the solution has turned completely black in colour. The resultant mixture was stirred vigorously for next 60 minutes.

The mixture was subsequently allowed to settle at ambient room temperature, following that the supernatant was removed. The black sediment was subsequently retrieved and washed thrice with absolute ethanol and thrice with double distilled water, then dried in a hot air oven at 60 °C for 4-6 hours.

Characterization of iron oxide nanoparticle

Estimation of particle size, zeta potential and poly dispersity index (PDI)

Determination of particle size, PDI and zeta potential was carried out on the basis of dynamic light scattering technique. In our study SZ-100, Zetasizer (HORIBA) instrument was used (Perissé Moreira and Grasseschi, 2022) [29].

Surface morphology of iron oxide nanoparticles

Scanning electron microscopy (SEM) is a sophisticated imaging technique extensively employed for the morphological characterization of nanoscale materials. The analysis was carried out using a Hitachi UHR FE-SEM SU8010 instrument. The dried powder samples were evenly

dispersed onto a carbon-coated aluminium stub and then coated with a thin layer of gold using a sputter coater. The specimens were subsequently examined under high-vacuum conditions at an accelerating voltage of 10 kV and micrographs were obtained at different magnifications (Selvam, *et al.*, 2024) [32].

Fourier-transform infrared spectroscopy (FT-IR) of iron oxide nanoparticles

Fourier-transform infrared spectroscopy (FT-IR) is a widely applied analytical method for recognizing and characterizing functional groups and chemical bonds present in given sample. The FT-IR spectra of iron oxide nanoparticle were obtained within the spectral range of 4000-400 cm^{-1} employing a PerkinElmer Spectrum equipment. A pellet of iron oxide nanoparticle powder sample was prepared with potassium bromide (KBr) prior to analysis (Zakariya, *et al.*, 2022) [40].

X-Ray Diffraction (XRD) analysis of iron oxide nanoparticles

X-ray diffraction (XRD) examination was conducted to assess the crystalline structure, phase composition, and crystallite size of the produced iron nanoformulation and iron-zinc nanocomposite. The diffraction patterns have been obtained through a PANalytical X-ray diffractometer employing Cu K- α_1 radiation ($\lambda = 1.5406 \text{ \AA}$), functioning at 45 kV and 40 mA. The finely prepared powdered specimens were meticulously affixed to the sample holder to guarantee a smooth and uniform surface before examination. The acquisition of data was conducted within a 2θ scanning range of 10° to 80° . The obtained diffraction peaks were analyzed against standard reference patterns from the Joint Committee on Powder Diffraction Standards (JCPDS) database to identify the crystalline phases present (Saqib, *et al.*, 2019) [31].

Results

Synthesis of iron oxide nanoparticles

The synthesized iron oxide nanoparticle appeared as black colour fine powder. From one batch we obtained approximately 800 mg iron oxide nanoparticles. These prepared iron oxide nanoparticle showed characteristic magnetism property as depicted in Figure 1

Characterization of iron oxide nanoparticle

Particle size, zeta potential and poly dispersity index (PDI)

The synthesized iron oxide nanoparticle showed average particle size around 99 nm. The poly dispersity index was around 0.416 and zeta potential found -45.9 mV (Figure 2 and Figure 3).

Surface morphology

Scanning electron microscopy (SEM) images of iron oxide nanoparticle exhibited almost spherical shape (Figure 4). However, few particles showed irregular shaped and aggregations. Surface of iron oxide particles displayed nearly smooth texture. The size and shape of prepared nanoparticles mostly uniform.

Fourier-transform infrared spectroscopy (FT-IR)

The FT-IR spectrum of iron oxide nanoparticle is depicted in Figure 5. The FT-IR spectrum of iron oxide nanoparticles

revealed presence of distinct prominent peaks at 3411.83 cm^{-1} , 1628.27 cm^{-1} and 586.92 cm^{-1} which are associated with the presence of hydroxyl group (O-H), water molecules (H-O-H) and metal oxide stretching (Fe-O). Few minor peaks were also recorded around 1402 cm^{-1} and 1109 cm^{-1} showed presence of organic solvent residue and peak and 797 cm^{-1} was indicated the presence of metal oxide related stretching.

X-Ray Diffraction (XRD)

The iron oxide nanoparticles displayed six distinct diffraction peaks on XRD analysis. These different diffraction peaks were located at $2\theta \approx 30.17^\circ$, 35.42° , 43.56° , 53.12° , 57.05° , and 62.99° , corresponding to the (220), (311), (400), (422), (511), and (440) planes of spinel iron oxide structure (Figure 6).

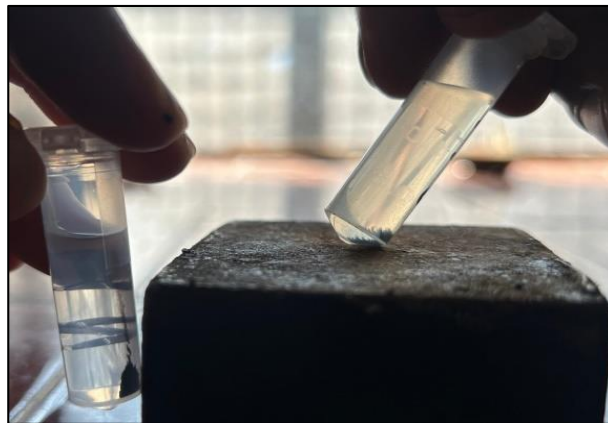


Fig 1: Photograph showing magnetism in iron oxide nanoparticles (IONPs)

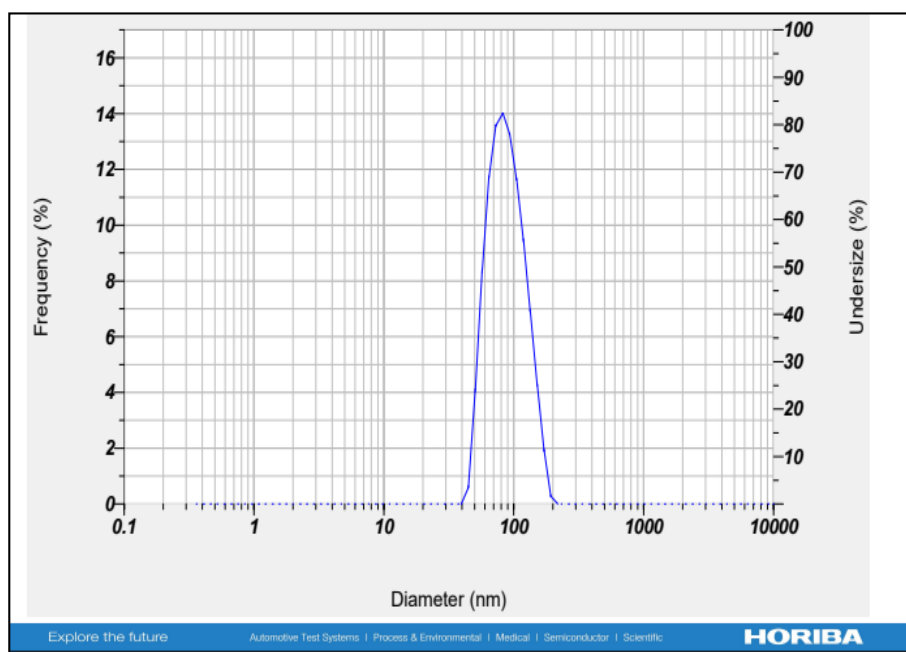


Fig 2: Particle size of iron oxide nanoparticle (IONPs)

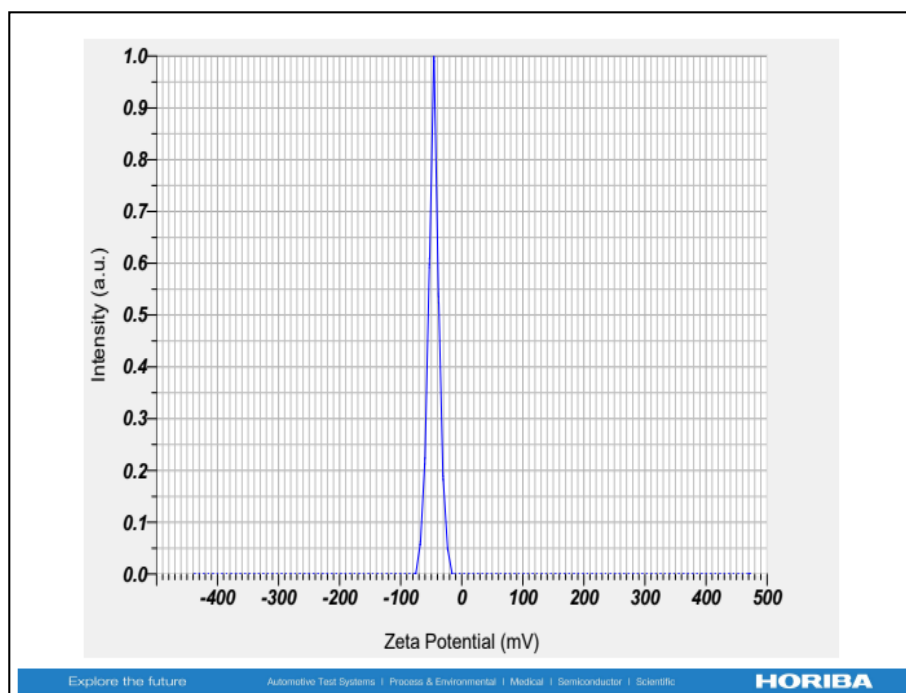


Fig 3: Zeta potential of iron oxide nanoparticle (IONPs)

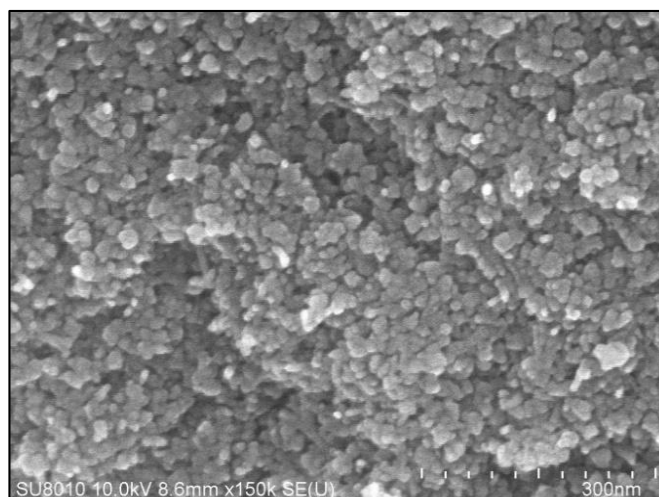


Fig 4: SEM image of iron oxide nanoparticle (IONPs) at 150K magnification

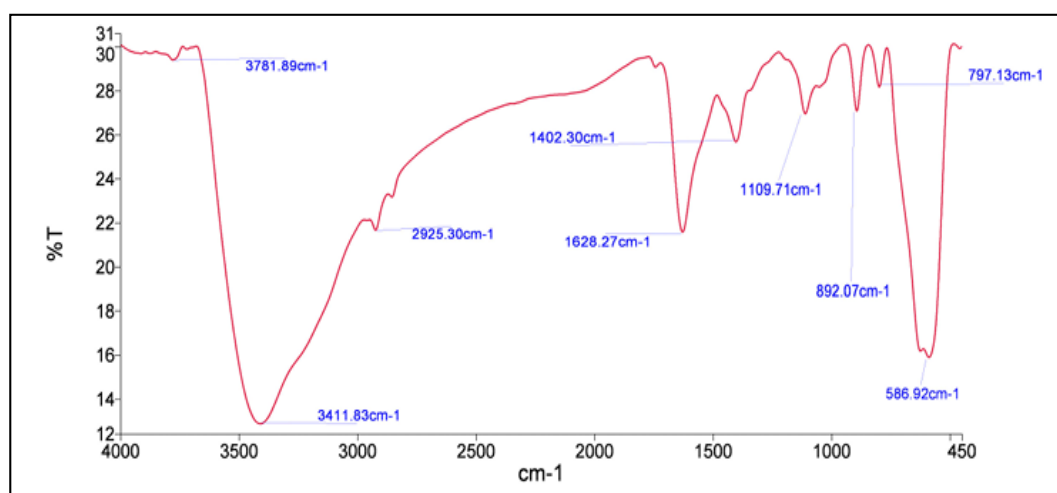


Fig 5: FT-IR spectrum of iron oxide nanoparticle (IONPs)

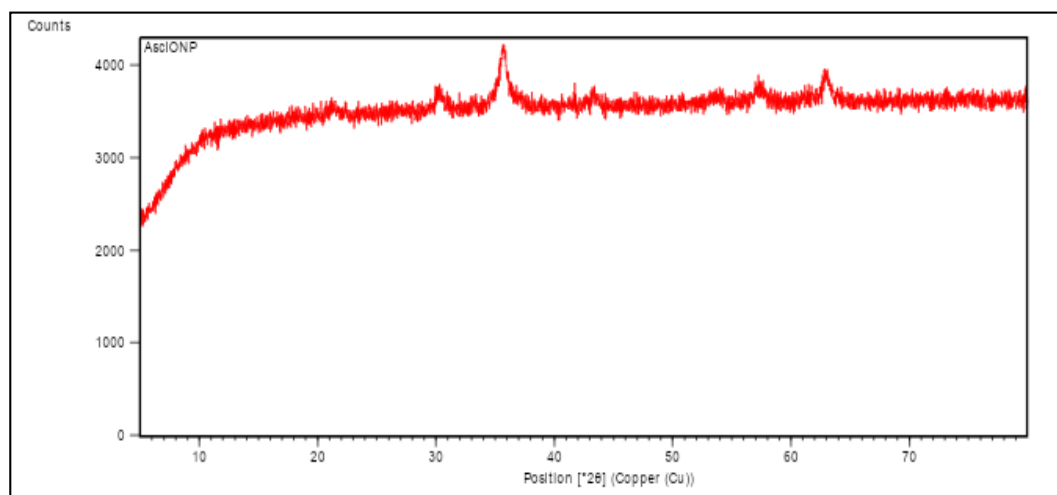


Fig 6: X-ray diffraction (XRD) pattern of iron oxide nanoparticle (IONPs)

Discussion

Iron oxide nanoparticles were successfully produced via chemical co-precipitation technique including ferrous sulphate and ferric chloride as precursors in alkaline conditions. Formation of nanoparticles was signified by a sudden alteration in colour of solution, indicative of the nucleation and development of IONP. Similarly, comparable synthesis methods utilizing ferric and ferrous salts along with ammonium hydroxide as a precipitating agent have

been extensively documented (Mandel *et al.*, 2011; Kim *et al.*, 2014; Khanam *et al.*, 2022) ^[22, 16, 14]. However, some studies (Khalil *et al.*, 2015; Mazrouaa *et al.*, 2019) ^[13, 24] reported the use of sodium hydroxide as alkaline reagent instead of ammonium hydroxide.

Zeta potential measurement and dynamic light scattering (DLS) were used to determine the surface charge and particle size of the synthesized IONPs. The successful synthesis of nanoscale particles having a relatively narrow

size range was recorded hydrodynamic diameter of 99 nm. Sheel *et al.* (2020) [34], also reported the similar particle size. A wide range of the particle size ranging from 5 nm to 150 nm was also reported in various studies (Kovrigina *et al.*, 2022) [18], which might be attributed to difference in protocol employed for synthesis of IONP. The nanoscale dimension is crucial for potential application in biomedical, as it facilitates the higher bioavailability, longer circulation time and enhanced cellular uptake (Salehrozveh *et al.*, 2024) [30].

The formed IONP showed around -45 mV zeta potential, suggesting a very stable colloidal dispersion. Zeta potential is key indicator of stability of nanoparticle and higher than ± 25 mV often reflects strong electrostatic repulsion between particles and prevents aggregation. These findings are supported by previous study (Constantino *et al.*, 2024) [3], who also found high negative zeta potential (-63 mV). The presence of strong negative ZP may be associated with occurrence of hydroxyl (-OH) groups on surface of IONP. The IONP revealed typical absorption peaks at 3400 cm^{-1} , 1600 cm^{-1} and 580 cm^{-1} indicating the presence of hydroxyl groups (O-H) stretching, adsorption of water molecules (H-O-H) and metal oxide bond (Fe-O), respectively. These findings are closely align with earlier investigations (Winiarczyk *et al.*, 2021; Hussain *et al.*, 2023) [37, 18], who also reported similar characteristic peak of Fe-O bond. Although, this peak generally recorded between $450\text{--}600\text{ cm}^{-1}$ regions.

The XRD pattern of synthesized IONP showed six characteristic diffraction peaks, which ensure the presence crystalline iron oxide core and high phase purity. Similarly, six distinct diffraction peaks were also recorded (Yoonus *et al.*, 2021; Kiwumulo *et al.*, 2022) [39, 17] at $2\theta \approx 30^\circ$, 35° , 43° , 53° , 57° and 63° .

Conclusion

Iron oxide nanoparticles (IONP) were effectively fabricated using simple and economic chemical co-precipitation method. Physicochemical characterisation confirmed their nanoscale size, uniform size distribution, good stability and crystalline nature along with distinctive Fe-O bond. These attributes suggest that the produced IONPs are suitable for biomedical uses including drug delivery and imaging. However, further studies on biocompatibility and *in vivo* efficacy are required to validate their clinical use.

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