

Fe_3O_4 Nanoparticle-Enabled Glucose Biosensor and Its Application in the Development of a Joint Pain Relief Formulation

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Abstract

The primary aim of this study is to synthesize and characterize Fe_3O_4 nanoparticles and silver nanoparticles (AgNP's) for their potential application in biomedical sensing and drug delivery systems. Specifically, Fe_3O_4 nanoparticles are chosen for their known magnetic properties, biocompatibility, and enzyme-mimetic activity, making them suitable for non-enzymatic glucose bio sensing and targeted biomedical applications. Silver nanoparticles are selected due to their high electrical conductivity, catalytic activity, and well-documented antimicrobial and drug delivery capabilities, making them ideal for loading and releasing therapeutic agents such as acetaminophen for joint pain management. AgNP's were made by a simple chemical reduction process, whereas Fe_3O_4 nanoparticles were created by co-precipitation. The Precursor material for the synthesis of silver nanoparticles (AgNP's) is silver nitrate (AgNO_3), which serves as the metal salt precursor in the process. Aniline acts as the reducing agent in the synthesis process. CTAB is used as a stabilizer for the nanoparticles. SEM and X-ray diffraction (XRD) were used to investigate the structural and morphological characteristics of the nanoparticles. The Fe_3O_4 nanoparticles have an uneven, non-uniform grain size and a cubic spinel shape. Additionally, the rotational and vibrational modes of Fe_3O_4 nanoparticles were investigated using Fourier-transform infrared spectroscopy (FTIR). The effectiveness of the nanoparticles in glucose sensing was confirmed by electrochemical measurement, which showed a linear response to rising glucose concentrations. The drug release efficiency of acetaminophen-loaded AgNP's was evaluated using an open-ended tube method, achieving 76.23% in vitro drug release efficiency after 24 hours. These findings suggest that Fe_3O_4 and AgNP's hold significant potential for applications in glucose biosensing and joint pain management.

Keywords: Biomaterials; Bioelectrocatalysis; Sol-gel preparation; Drug Delivery Systems; Antimicrobial Activity; Joint pain formulation; electrochemical sensors & Glucose detection

Introduction

Many researchers have shown that nanoparticles/nanomaterials like silver nanoparticles (AgNP's) [1, 2] magnetic nanoparticles [3] gold nanoparticles [4] and carbon nanotubes [5] have significant potential to hold biological molecules and opens new possibilities for scientists and researchers in the different fields. Gold nanoparticles have been used to carry biological molecules. However, silver nanoparticles were chosen in this study due to their strong catalytic activity, high electrical and thermal conductivity, and proven antimicrobial effects as compared to the other metals. These materials also offer a proper controlled environment to maintain the different biological activities [10] and enable the transfer of electrons among the trapped biomolecules and the electrode surface. In the context of scientific experiments, particularly those involving nanoparticles and drug formulations, the terms "controlled environment" and "reaction temperature" are crucial for ensuring consistent and reliable results. A controlled environment refers to a setting where specific conditions such as temperature, humidity, and pressure are maintained at constant levels throughout an experiment. This is essential to minimize external variables that could affect the outcome of the experiment. Reaction temperature is the specific temperature at which a chemical reaction occurs. It can significantly influence the rate of reaction and the properties of the products formed. Gold typically exists in oxidation states of +1 and +3, while silver primarily exists in the +1 oxidation state. Magnetic nanoparticles like Fe₃O₄ can exist in multiple oxidation states, including +2 and +3, depending on their composition and synthesis method. Silver nanoparticles are gaining more interest due to their multifunctional properties, including strong antimicrobial effects, efficient drug delivery capabilities, and their role in enhancing biological activities. Their ability to facilitate electron transfer between biomolecules and electrodes further enhances their applicability in bio sensing and therapeutic contexts. In cooperating detection with drug delivery such Nano particles can release antimicrobial drug to kill any invading pathogens [6-7]. This makes them particularly suitable for therapeutic applications such as drug delivery and antimicrobial coatings. Loading of drug with nanostructures of silver metal is another advantage in biomedical field especially drug delivery systems In this present research work, acetaminophen loaded silver nano particles are successfully synthesized and selected to explore their potential role in joint pain Formulation application. Open ended tube method is used to determine the drug release efficiency of acetaminophen loaded silver nano particles. Aqueous suspensions of silver nano particles were prepared by mixing different amounts (10 mg, 20mg, 30mg, 40mg, 50mg, and 60mg) of acetaminophen drug. After that these mixtures of drug and nanoparticles was incubated for 24 hours under constant stirring. Then resulting mixtures are centrifuged for 60 minutes by adjusting rotation speed 10000 rpm/ minutes. As prepared samples were utilized for further characterization.

In the last two decades, magnetic nanoparticles have gained the attention of researchers due to their amended physio-chemical properties in many universally emerging applications [8]. Magnetic nanoparticles have promising applications as hyperthermia treatment, drug delivery; biosensors cell separation, etc. [9]. Magnetic nanoparticles in the range of 2-20 nm are significantly essential due to its applications in magnetic storage devices. In this research, magnetic nanoparticles specifically Fe₃O₄ were selected because of their excellent magnetic properties, biocompatibility, and ease of surface functionalization, which are critical for biosensor applications. The unique properties of magnetic nanoparticles rise especially because of its condensed size and inter-particle interactions are almost negligible [10]. There are various metal oxide nanoparticles, among them, Fe₃O₄ nanoparticles have antique chemical and biological properties and could enhance the functionality of the biosensing devices by coating with enzymes, antibodies and catalyst. Furthermore, iron oxide provides a biocompatible and catalytically active surface, making it ideal for enzyme immobilization and promoting electron transfer on electrodes [11-12].

In 2015, research revealed that about 415 million people suffered from diabetes and 318 million people were at risk of diabetes in the future [13]. The glucose level in diabetic patients' blood increases up to a significant concentration due to an increase in the level of glucose in the blood compared to the normal range, causing serious health problems. The International Diabetes Federation has revealed that every six seconds, a person dies due to health problems related to diabetes. Although these problems could be prevented by examining the glucose level in the blood [14].

One of the most widely used detection methods for the determination of glucose in blood and food is glucose biosensors, which utilize immobilized oxides for the conversion of the target analytes into electrochemically detectable products [15]. In biosensor prepara-

tion, one of the crucial factors is the immobilization of enzymes [16]. To improve the enzymatic activity and immobilize enzymes, many methods have been used. Nowadays, there are a bundle of advantages of using nanoparticles, such as high surface reaction activity, a large surface-to-volume ratio, and strong adsorption ability to immobilize desired biomolecules [17].

To immobilize enzymes, as well as to exploit electrically contacted enzyme electrodes or protein-functionalized electrodes, there is growing interest in the application of nanoparticles. Moreover, due to their suitable size and physical properties, nanoparticles like Fe₃O₄ are ideal because they promote electron transfer between electrodes and the active site of the enzyme, enhancing sensor sensitivity [18]. This limitation motivated the use of cost-effective alternatives like Fe₃O₄, CuO, TiO₂, and Fe₂O₃, which are abundant and show good electrochemical performance. Extensive research has been found out about the several nanomaterials to be used for the electrode material for the fabrication of non-enzymatic glucose sensors. Numerous glucose biosensors have been developed earlier based on noble metals such as Pt, Au, but these metals have high cost and very rare. Nowadays, several glucose sensors have designed by using different metals oxides such as Cu, TiO₂ and Fe₂O₃ etc. The crystal structure of materials used in biosensors can influence their electrochemical properties, which are crucial for the detection of glucose level.

A well-defined crystal structure can enhance the stability and activity of the enzyme immobilized on the sensor, leading to improved sensitivity and specificity in glucose level detection. Scientist has proved that Fe₃O₄ NP's in fact possessed an intrinsic enzyme mimetic activity similar to that natural peroxidase. It showed that Fe₃O₄ NP's not only offered a good micro environment for retaining the bioactivity of enzymes, but also acted as natural peroxidase to oxidize organic substrates. It has further confirmed that the Fe₃O₄ NP's possessed intrinsic peroxidase-like activity and developed this new biosensor in the application of detection of H₂O₂ and glucose by colorimetric method [10]. The results that Fe₃O₄ NP's were used to enhance electro catalytic and bioelectric catalytic transformation have been emphasized with the aim to highlight a new concept. These nanomaterials can be used for electrochemical measurements of glucose because of their easy synthesis, long term stability and low cost [11, 12].

In this work, Fe₃O₄ was synthesized by the hydrothermal method because it allows better control over particle size and crystallinity, which are essential for consistent sensor performance. After that, these nanoparticles were used for the fabrication of a working electrode using glassy carbon. Glassy carbon was selected due to its wide potential window, chemical inertness, and excellent electrical conductivity, all of which are desirable for reliable electrochemical measurements. Moreover, the electrochemical measurements were carried out using the three-electrode method.

Materials and Method

The selection of Fe₃O₄ and AgNP's is motivated by their proven effectiveness in bio sensing and drug delivery applications. The enzyme-mimetic activity and magnetic properties of Fe₃O₄ make it ideal for non-enzymatic glucose detection, while AgNP's' high electrical conductivity and drug-loading capacity support their use in delivering therapeutic agents such as acetaminophen for joint pain management.

AgNO₃ (Sigma-Aldrich 99.9%), CTAB (Sigma-Aldrich 99.9%), and Aniline (Sigma-Aldrich 99.9%) are used for the synthesis of Ag nanoparticles. Doubly distilled and deionized water is used as a solvent for preparing stock solutions of all reagents. Aniline solution is stored in an amber-colored bottle to prevent aerial oxidation. For the preparation of Fe₃O₄, the materials used in this research work are FeCl₃·6H₂O, FeCl₂·4H₂O, NaOH, N₂H₄, Nafion, and Al₂O₃ powder. All the materials are of analytical grade and were used without further purification.

Preparation of Ag NP's

Initially, a 0.01 M AgNO₃ solution (Solution A) was prepared by dissolving 0.975 g of AgNO₃ in 500 mL of water. On the other hand, a 0.01 M CTAB solution (Solution B) was prepared by dissolving 1.75 g of CTAB in 500 mL of water. Then, both solutions were mixed and named as Solution C. A transparent, colorless mixture was obtained upon mixing the silver nitrate solution and the CTAB solution. This mixture contains silver nitrate as the metal salt precursor and CTAB as the stabilizing agent.

Another 500 mL of a 0.01 M aniline solution was prepared and added to Solution C dropwise. This transparent, colorless solution gradually turned pale yellow upon the addition of the aniline solution, which acts as a reducing agent. The appearance of the pale-yellow color is an indication of silver nanoparticle formation. Silver ions (Ag^+) have an oxidation state of +1 in their ionic form. During the reduction process, silver ions gain electrons, transitioning to a metallic state (Ag^0) with an oxidation state of 0. This reduction is facilitated by a reducing agent, aniline, which donates electrons to the silver ions. The formation of metallic silver from silver ions is a key step in synthesizing silver nanoparticles, which exhibit unique properties due to their Nano scale size and structure. These pale-yellow precipitates of AgNPs were filtered and dried.

Proposed mechanism to the Joint pain formation with Ag-nanoparticles

The reaction starts when a silver ion (Ag^+) and an amine group (NH_2) quickly coordinate to produce an intermediate complex known as $\text{NH}_3\text{-Ag}$. After that, this complex proceeds through a long, rate-determining step called homolytic cleavage, which produces a radical species, a proton (H^+), and a silver atom (Ag^0 , a crucial nucleus). The overall rate of nanoparticle creation is controlled by the slow kinetics of this phase.

The silver trimer cation (Ag_3^+) is created when the produced silver atom (Ag^0) quickly combines with more Ag^+ ions. The hexasilver dication (Ag_6^{2+}) is then created by the dimerization of two Ag_3^+ species. The establishment of small, stable clusters is indicated by the aggregation process, which is linked to the development of the yellow color characteristic of the initial silver nanoparticle sol.

The produced radical species can quickly react with Ag^+ ions and hydroxide ions (OH^-). In addition to producing more metallic silver atoms (Ag^0) and water (H_2O), this reaction produces azobenzene ($\text{N}=\text{N}$) as a byproduct. These secondary Ag^0 atoms aid in the development of new nuclei or the expansion of already-existing nanoparticles.

Mechanism to the formation of Ag nanoparticles.

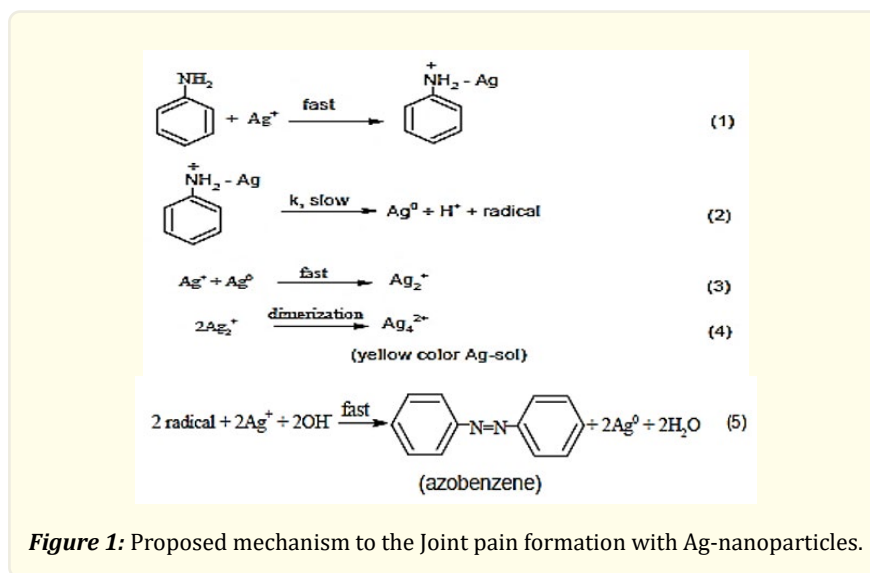


Figure 1: Proposed mechanism to the Joint pain formation with Ag-nanoparticles.

Loading of Acetaminophen (paracetamol) on Ag NP's

A suspension of AgNPs is prepared in DI (deionized) water. Then, 10 mg, 20 mg, 30 mg, 40 mg, 50 mg, and 60 mg of acetaminophen are accurately measured and mixed into 100 mL of the AgNPs suspension. The mixture of acetaminophen and silver nanoparticles is incubated for 24 hours under stirring. The resulting mixture is then centrifuged at 10,000 rpm for 60 minutes.

Preparation of Fe₃O₄ NP's

For the preparation of Fe₃O₄ nanoparticles, FeCl₃·6H₂O and FeCl₂·4H₂O were mixed in distilled water. A NaOH solution was prepared in distilled water. The ratio of FeCl₃·6H₂O, FeCl₂·4H₂O, and NaOH was expressed as 2:1:8. Then, NaOH solution and N₂H₄ were added dropwise into the solution of FeCl₃·6H₂O and FeCl₂·4H₂O under continuous stirring for 10 minutes. This solution was then added into a Teflon-lined stainless steel autoclave. The autoclave was thermally treated for 2.5 hours at a temperature of 200°C in the oven. The nanoparticles were collected using a permanent magnet and washed several times with distilled water, followed by ethanol. Finally, the nanoparticles were dried in an oven at 60°C for 1 hour. Dispersive nanoparticles refer to nanoparticles that are evenly distributed within a medium, preventing agglomeration and ensuring that they maintain their individual properties. The average size of nanoparticles is a critical parameter that influences their physical and chemical properties, including surface area, reactivity, and interaction with biological molecules. Smaller nanoparticles typically have a higher surface area-to-volume ratio, which can enhance their effectiveness in applications such as glucose bio sensing and drug delivery.

Fabrication of Working Electrode

Hydrothermal co-precipitation was used to create magnetite (Fe₃O₄) nanoparticles. Aqueous solutions of Fe₃Cl₂·6H₂O and Fe₃Cl₃·4H₂O were combined with NaOH while being stirred, and then hydrazine hydrate was added as a reducing agent. The mixture was autoclaved at 200°C for two hours, and the separated nanoparticles were then cleaned and dried at 60°C. In order to create a stable composite film for electrochemical applications, a glassy carbon electrode (GCE) was dropped-cast with an ethanol suspension of Fe₃O₄ nanoparticles and Nafion binder after being polished with Al₂O₃, ultrasonically cleaned in water/ethanol, and dried at 35°C.

The working electrode was fabricated by the incorporation of glassy carbon. The glassy carbon was cleaned with aluminum oxide powder. For further purification, the glassy carbon was ultrasonicated in distilled water and ethanol for 20 minutes each. A suspension of Fe₃O₄ NPs was prepared in ethanol and Nafion. Then, the suspension of Fe₃O₄ NPs was dropped onto the glassy carbon using a dropper. Finally, the electrode was dried in an oven at 35°C for 10 minutes. The figure is given below:

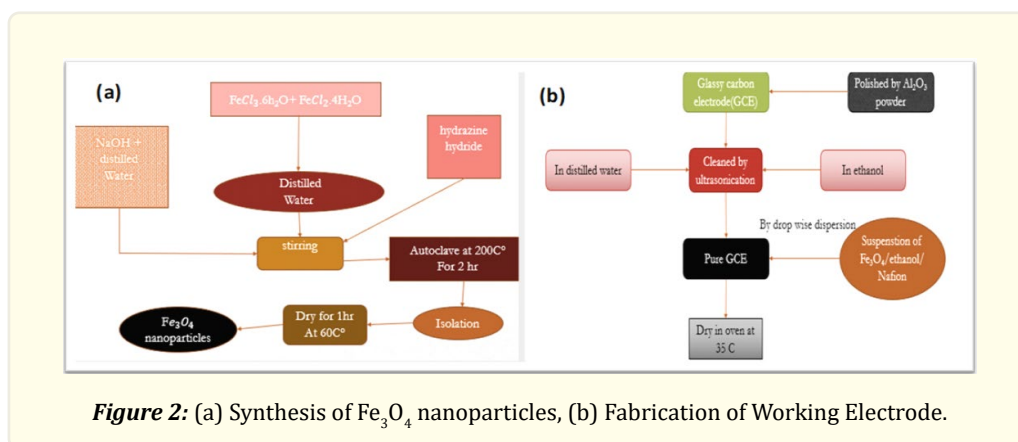


Figure 2: (a) Synthesis of Fe₃O₄ nanoparticles, (b) Fabrication of Working Electrode.

Results and Discussion

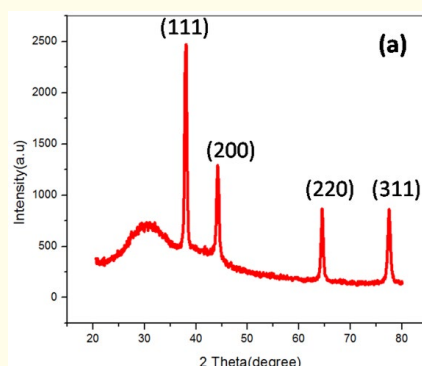


Figure 3: (a-b) displays the XRD pattern and SEM image of AgNP's.

XRD and SEM Analysis of AgNP's

X-ray diffraction pattern and SEM image of synthesized Ag NPs are shown in Figure 1(a-b). From Figure 1(b), it can be observed that the morphology of Ag NPs is not uniform, and some agglomeration is present between the nanoparticles. From Figure 1(a), it is evident that Miller indices (h, k, l) are assigned to each peak in the XRD pattern. The strong Bragg reflections corresponding to (111), (200), (220), and (311) are observed, and no spurious peaks are seen, which indicates that no crystallographic impurities are present in the given sample. The most intense peak, corresponding to (111), is observed in the given sample, which is characteristic of an FCC lattice. A high degree of crystallinity is present in the silver nanoparticles, as indicated by the intensity of the peaks. The crystallite size is very small due to the broadening of the peaks. The XRD pattern shows that the silver nanoparticles are crystalline. The Debye-Scherrer formula is used to calculate the size of the silver nanoparticles. The crystallite size is 34 nm, as calculated by the Debye-Scherrer formula. Five diffraction planes of silver nanoparticles are observed, corresponding to Bragg angles 2θ at 38.3571°, 44.5191°, 64.6431°, and 77.5651°, and compared with JCPDS silver file no. 04-0783. The XRD confirms that the silver nanoparticles are FCC silver nanoparticles.

$2\theta^\circ$	D	$1000/d_2$	$(1000/d^2)/6062$	Hkl
38.3571°	2.35	181.10	3	111
44.5191°	2.03	241.72	4	200
64.6431°	1.44	482.30	8	220
77.5651°	1.23	661.00	11	311

Table 1: Peak indexing from d-spacing.

<i>Experimental diffraction angles of silver specimen</i>	<i>Standard diffraction angles silver specimen</i>
44.5191°	44.3°

Table 2: Experimental and standard diffraction angles of silver specimen.

<i>Diffraction peaks</i>	<i>Sample value</i>	<i>Conventional value</i>
(200) and (111)	0.54	0.40
(220) and (111)	0.32	0.25

Table 3: Ratio between the intensities of the diffraction peaks sample value and Conventional value.

Theoretically, the value of the unit cell is calculated using the formula $a = 4r / \sqrt{2}$, where r is the atomic radius of a silver atom, whose value is 144 pm. The theoretical value of the unit cell of silver nanoparticles is 4.086 Å. The experimental value is 4.07 Å, which is in agreement with the theoretical value given in the literature.

The ratio of the intensity of the (200) and (111) diffraction peaks is slightly higher than the conventional ratio of the (200) and (111) diffraction peaks. The ratio of the intensity of the (220) and (111) diffraction peaks is also slightly higher than the conventional (220) and (111) diffraction peak ratio. These ratios are summarized in Table 3.

XRD particle size calculations are shown in Table 4. The average particle size is calculated using the Debye-Scherrer formula:

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

Where:

D = average particle size.

λ = wavelength of X-rays.

β = FWHM (Full Width at Half Maximum).

θ = Bragg diffraction angle.

The interplanar distance (d) is calculated using Bragg's equation:

$$2d \sin\theta = n\lambda$$

These results are summarized in Table 4.

<i>Diffraction angles</i>	<i>hkl</i>	<i>FWHM</i>	<i>Crystallize size (nm)</i>	<i>d-spacing (nm)</i>	<i>Lattice parameter(a)</i>
38.3571°	111	0.0043	34	0.235	4.070
44.5191°	200	0.0052	29	0.203	4.068
64.6431°	220	0.0052	31	0.144	4.073
77.5651°	311	0.0072	24	0.123	4.079

Table 4: XRD Crystallize size Calculation data.

Calculation of Dislocation Density

The irregularities or crystallographic defects within the crystal structure are called dislocations. Many physical properties strongly depend upon dislocations. A mechanism for dislocation formation is essential to be activated in the material, because dislocation density increases with plastic deformation. The possible mechanisms for dislocation formation are homogeneous nucleation, dispersed phases, reinforcing fibers, grain boundary initiation, and precipitation. The movement of dislocations is impeded by the movement of all neighboring atoms. The hardness of the material increases with the increase in dislocation density. Dislocation density increases as grain size decreases. The strength of the material increases with the decrease in grain size, especially when the grain size approaches 20 nm.

The dislocation density of the given nanomaterial is calculated using the formula:

$$\delta = 15\beta \cos\theta / 4\alpha D$$

Where:

β = FWHM.

θ = Bragg diffraction angle.

α = lattice constant.

D = particle size.

The dislocation density of the given silver nanoparticles is found to be $9.4 \times 10^{14} \text{ m}^{-2}$.

Crystallinity index measurement

The peak breadth of a specific phase is directly proportional to the mean crystallite size of the material; larger crystallite materials show a sharper XRD peak. The crystallinity index is measured using the formula:

$$\text{ICRY} = D_p (\text{SEM}) / D_{\text{cry}} (\text{XRD})$$

Where:

ICRY = crystallinity index.

D_p (SEM) = particle size obtained from SEM morphological analysis.

D_{cry} (XRD) = particle size obtained from XRD analysis.

The crystallinity index has a value greater than 1. This data also shows that silver nanoparticles are highly crystalline, and the FCC phase structures are well indexed. The given sample of silver nanoparticles is polycrystalline because the crystallinity index is higher than 1.

Sample	D_p	D_{cry}	I_{cry}	Crystallite
Silver Nano particles	99	34	2.90	FCC

Table 5: The crystallinity index of silver nanoparticles.

Calculation of Specific Surface Area

Specific surface area (SSA) is a material property. The properties and types of a material are determined by this scientifically calculated SSA value. SSA values have great importance in adsorption, heterogeneous catalysis, and surface reactions.

SSA is the surface area per unit mass and is calculated by the formula:

$$\text{SSA} = \text{SA}_{\text{part}} / V_{\text{part}} \times \rho$$

or

$$\text{SSA} = 6 \times 10^3 / (D_{\text{p}} \times \rho)$$

Where:

- SSA is the specific surface area.
- SA_{part} is the surface area.
- V_{part} is the volume of the particle.
- ρ is the density.
- D_{p} is the particle size.

The SSA value is found to be $24 \text{ m}^2/\text{g}$.

Calculations of Unit Cell Parameters

Unit cell parameter values calculated from XRD are enumerated in Table 6.

Space group	Values	Space group	Values	Space group	Values
Space group	fm-3m	Symmetry of lattice cubic	close-packed	VOL.UNIT cell(V)	67.42A3
Space group number	225	Particle size	34nm	Radius of atom	144pm
Point group	m-3m	Bond angle	$\alpha=\beta=\gamma=90$	Density	10.5g/cm ³
Packing fraction	0.74	Lattice parameter	a=b=c=4.070 Å	Dislocation density	9.2*10 ¹⁴ m ⁻²
Mass	107.8682amu				

Table 6: XRD parameters of silver nanoparticle Structure FCC.

XRD and SEM Analysis of Fe₃O₄ NP's

The final black powder was attracted by a permanent magnet, confirming the formation of Fe₃O₄ NPs. Figure 2(c) shows the XRD spectrum of Fe₃O₄ NPs, having an FCC cubic spinel structure, and Miller indices (220), (311), (400), (511), and (440) matched with standard JCPDS card no. 96-900-5839. The average crystallite size was 60 nm, as calculated by using the Scherrer equation (I).

$$D = \frac{k\lambda}{\beta \cos \theta} \dots\dots\dots(I)$$

The SEM micrograph used to study the surface morphology of pure Fe₃O₄ NPs is shown in Figure 2(a). The visual observation of the SEM image indicates that the nanostructure is homogeneous and the material is dense. Furthermore, the grain morphology is irregular and non-uniform, consisting of both small and large grains [16].

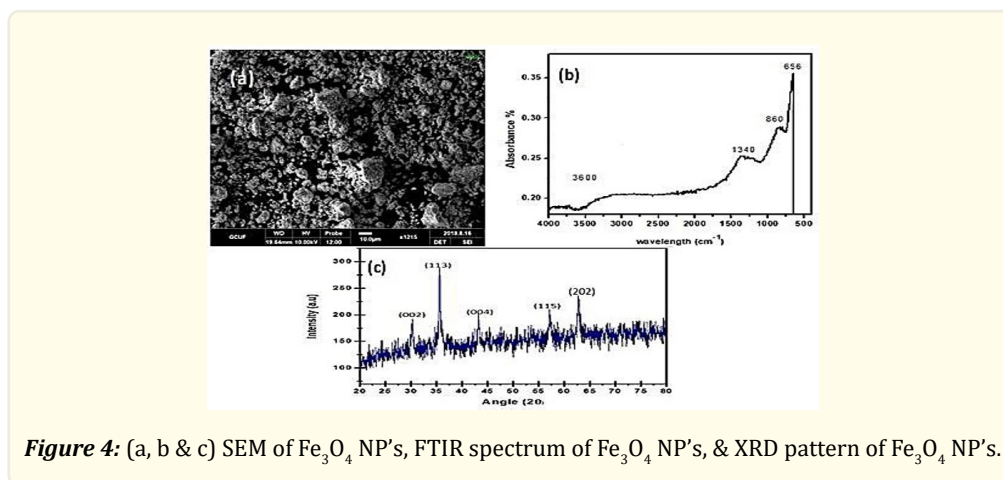


Figure 4: (a, b & c) SEM of Fe₃O₄ NP's, FTIR spectrum of Fe₃O₄ NP's, & XRD pattern of Fe₃O₄ NP's.

Fourier Transform Infrared Spectroscopy of Fe₃O₄ NP's

Elemental composition of the Fe₃O₄ nanoparticles using FTIR is shown in figure 4(b). The graph is plotted between wavelength and Absorbance and peaks at specific wavelengths show availability of certain Functional groups. Absorbance is often more sensitive to small changes in concentration or material properties. This sensitivity is crucial in applications like bio sensing, where detecting minute changes in glucose concentration is essential. Absorbance spectra are typically easier to interpret than transmittance spectra, especially when dealing with overlapping peaks. This clarity is beneficial for identifying specific functional groups in materials like Fe₃O₄ Nano particles. The broad band located on 1340cm⁻¹ is due to a hydroxyl group and the weak band located at 860cm⁻¹ is formed by N-H asymmetric stretching vibration. The sharp peak at 656cm⁻¹ is due to Fe-O. Small expansion throughout the material is due to hydrazine hydride. The weak band observed 3620cm⁻¹ is due to Na+ [17].

UV VIS Spectroscopic Analysis

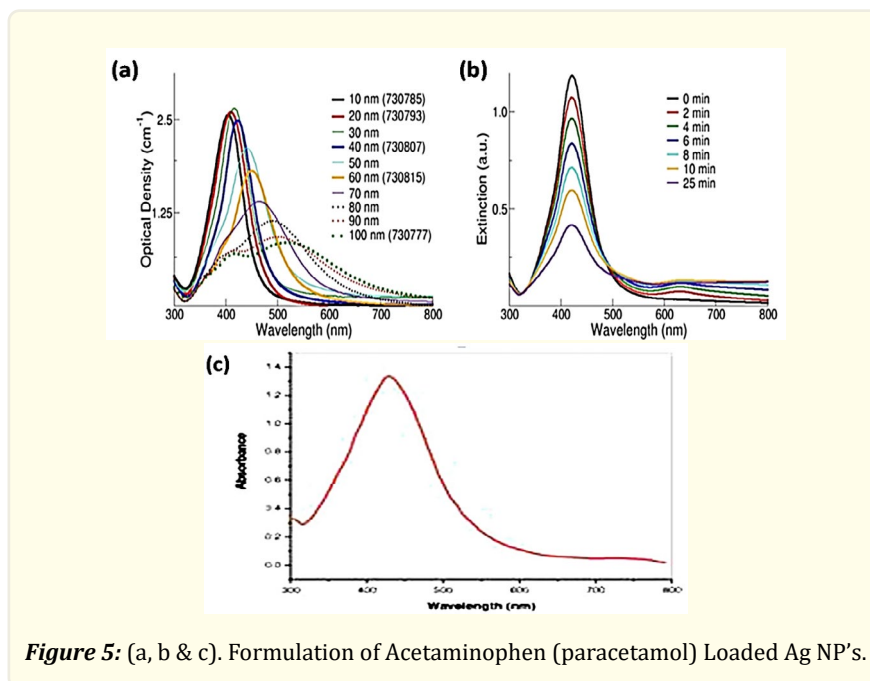


Figure 5: (a, b & c). Formulation of Acetaminophen (paracetamol) Loaded Ag NP's.

A solution of pale yellow colored AgNP's was prepared by dissolving DI water and stirred at 5000rpm for 15 minutes. This solution was transferred to a cuvette and exposed to UV-Vis radiation. UV-Vis (Ultraviolet-Visible) spectroscopy is a powerful analytical technique used to determine the absorbance of light by a sample at different wavelengths [18]. This method is particularly useful in characterizing nanoparticles and their interactions with biomolecules. Conjugation refers to the presence of alternating single and double bonds in a molecule, which allows for the delocalization of π (pi) electrons across the structure. This delocalization affects the electronic transitions that can occur when the molecule absorbs light. Here, the conjugation in nanoparticles like silver and iron oxide can influence their optical properties, which is analyzed using UV-Vis spectroscopy. UV-Vis spectroscopy is used to confirm the effectiveness of nanoparticles in glucose sensing [19-20]. The absorbance spectrum provides information about the concentration of glucose and the interaction between glucose and the nanoparticles.

The analysis can reveal the characteristic peaks corresponding to the electronic transitions of the nanoparticles, indicating their structural and functional properties. The absorbance of the solution was recorded and resonant peaks occur at different wavelengths for different nanoparticles due to surface Plasmon resonance phenomenon. According to the theory of resonance, maximum wavelength is absorbed in resonant wavelength typically silver nanoparticles have characteristic, surface Plasmon resonance at the wavelength in the range of 380nm to 440nm. Here from figure 4(a) we observed that spr for the given sample solution occurred at the wavelength of 410nm which confirms the presence of silver nanoparticles with the prepared solution [21].

The spr absorbance is sensitive to the nature, size, and shape of the particles present in the solution and depended upon their inner particles distant and surrounding media.

Electrochemical measurement of Fe₃O₄ based glucose biosensor

Electrochemical biosensors utilizing silver nanoparticles (AgNP's) for glucose detection are based on the principles of electrochemistry, where the interaction between glucose and the AgNP's [22] leads to measurable electrical signals. Here's a detailed explanation of the mechanism involved in the sensing process and the concentration description of glucose.

Mechanism of Sensing

Electrochemical Reaction

The glucose biosensor operates on the principle of oxidation-reduction (redox) reactions. When glucose is present, it undergoes oxidation at the surface of the AgNP's. In this reaction, glucose is oxidized to gluconolactone [23-24], while silver ions (Ag⁺) are reduced to metallic silver (Ag⁰), which can be detected electrochemically.

Role of Silver Nanoparticles

AgNP's enhance the electrochemical response due to their high surface area and excellent electrical conductivity. This allows for efficient electron transfer between the glucose molecules and the electrode surface [25]. The catalytic properties of AgNP's facilitate the oxidation of glucose, leading to a significant increase in the current response, which is proportional to the glucose concentration.

Detection Mechanism

The electrochemical measurement is typically performed using techniques such as cyclic voltammetry or amperometry [26]. In amperometry, a constant potential is applied, and the resulting current is measured. The current response increases linearly with the concentration of glucose, allowing for quantitative analysis. This linear relationship is crucial for determining glucose levels accurately [27].

Concentration Description of Glucose

Linear Response

The biosensor exhibits a linear response to varying glucose concentrations, which is essential for practical applications in monitoring glucose levels in biological samples. The sensitivity of the biosensor can be influenced by factors such as the size and distribution of the AgNP's, the electrode material, and the experimental conditions.

Concentration Range

The effective concentration range for glucose detection using AgNP's can vary, but typically, it is designed to cover physiological levels (around 0.1 mM to 20 mM) to ensure it meets the requirements for clinical diagnostics.

The ability to detect low concentrations of glucose is particularly important for diabetic patients who need to monitor their blood sugar levels regularly [28-30].

The response of the electrochemical potential difference of Fe₃O₄ NPs to changes in aqueous glucose solution was determined. It is shown in Figure 3(a) that the potential difference between the electrodes increases with increasing glucose concentration. Fe₃O₄ nanoparticles demonstrated high sensitivity at the working electrode. The biosensor setup is shown in Figure 3(b). Furthermore, these NPs were used to investigate the electrochemical measurements of glucose concentration.

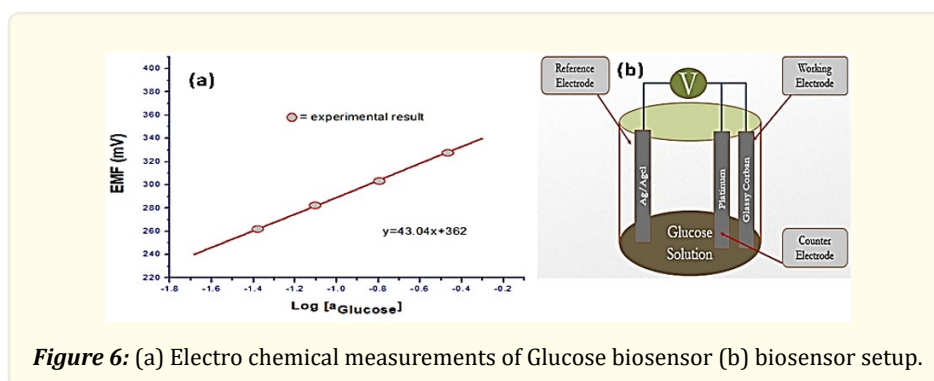


Figure 6: (a) Electro chemical measurements of Glucose biosensor (b) biosensor setup.

Subsequently, a glassy carbon electrode was fabricated for biosensing applications. Potentiometric behavior was studied using a three-electrode configuration, consisting of a working electrode (a glassy carbon electrode coated with Fe₃O₄ at the nanoscale), a counter electrode (platinum), and a reference electrode (Ag/AgCl) [31]. A curve was plotted between potential difference and the logarithm of glucose concentration. The electrochemical potential showed a linear relationship with the logarithm of glucose concentration [18].

Drug Loading Efficiency of AgNP's

Drug loading efficiency is determined by the surface adsorption of the drug onto silver nanoparticles. The amount of drug is added to the silver nanoparticle suspension in an increasing manner [32]. The drug loading efficiency is 78% when 10 mg of acetaminophen is added. Drug loading efficiency increases to 82% when 20 mg of acetaminophen is added [33]. However, drug loading efficiency decreases with further increases in the amount of acetaminophen. Thus, saturation on the surface occurs at 20 mg. The percentage of drug loading efficiency is shown in Table 7.

<i>S. No</i>	<i>Formulation</i>	<i>Amount of drug</i>	<i>Percent drug loading</i>
1	S1	10	78.3
2	S2	20	81.95
3	S3	30	54.45
4	S4	40	47.73
5	S5	50	41.0

Table 7: Percentage drug loading efficiency.

In-vitro Drug Release Efficiency

In vitro drugs release efficiency of optimized formulation S3 carried out in buffer of Ph value 7.4. The percentage of drug release is shown in table 8.

<i>S. No</i>	<i>Times in hour</i>	<i>Cumulative percentage drug release</i>
1	0	0
2	1	12+-0.118
3	2	14.25+-0.537
4	3	16.40+-0.8621
5	4	18.95+-0.6035
6	5	20.44+-0.6415
7	6	25.90+-0.7840
8	7	27.92+-0.2281
9	8	30.42+-0.9821
10	24	76.23+-0.5411

Table 8: Percentage of drug release.

Conclusion

In summary, silver nanoparticles were synthesized by chemical reduction method using silver nitrate as metal precursor, aniline as reductant, and CTAB as stabilizer. The XRD characterization indicated that Ag NPs were FCC crystals with a particle size of 34 nm. SEM image shows the formation of crystals of spherical structure of silver nanoparticles with the non-uniform distribution. The drug loading efficiency was determined by centrifugation and analyzed by UV spectroscopy, and the optimized formulation was found to be formulation of Acetaminophen. In vitro drug release of optimized formulation was found to be 76.23 ± 0.54 at the end of 24 hours.

This formulation will produce a synergistic effect to treat the inflammation in rheumatoid arthritis efficiently and release the drug in a controlled manner to target the inflammatory site.

The crystallinity and surface morphology of co-precipitated synthesized Fe₃O₄-NPs were examined by XRD and SE analysis. Finally, the current development of Fe₃O₄-NPs based glucose biosensor provided a stable potentiometer response in a small interval of time. In an overall assessment, the Fe₃O₄-NPs based biosensor was stable and sensitive response up to the mark values even at room temperature. In future, Fe₃O₄ based sensor is also achievable for clinical diagnosis as well.

Author Declarations

Ethics approval

The study was carried out in compliance with the ethical standards set by COMSATS University Islamabad, Lahore Campus, as well as the 1964 Helsinki declaration and its subsequent amendments or similar ethical standards.

Consent to Participate

All individual participants engaged in the study provided informed consent. Before being included, participants were given comprehensive information regarding the research's objectives, procedures, potential benefits, and hazards. They were also notified of their entitlement to discontinue their participation in the study at any time without incurring any repercussions. Written consent documentation has been obtained and is accessible for examination by the Editorial Board if necessary.

Consent for Publication

All participants in this study have given their agreement for the publication of the data derived from this research. Participants provided written informed consent for publishing, with the assurance that their identities would remain anonymous and all data would be anonymised. The Editor of this publication has copies of the consent forms accessible for examination.

Availability of data and materials

The datasets produced and/or examined during the present investigation can be obtained from the corresponding author upon a reasonable request. The data supporting the findings of this investigation can be obtained from Dr. Akbar Ali, however there are restrictions on their availability. These data were utilized under license for the current study and are not publically accessible. The writers can provide the data following a reasonable request and with the authorization of the authority.

Declaration of No Competing Interests

The authors affirm that they do not have any conflicting interests in respect to this study. This statement confirms that there are no financial or personal connections with individuals or organizations that could improperly impact or prejudice the content of the paper.

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Contribution of the Authors

Dr. Akbar Ali, as the primary author, developed the research theme and supervised the entire research process, offering essential leadership and supervision to the research team.

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