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Research Article

Keywords: Gold nanoparticles, LSPR, Biosensor, Creatinine, Morin, Catalytic Activity

Posted Date: October 21st, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11083241/v1>

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Additional Declarations: No competing interests reported.

Multifunctional Citrate-Stabilized Gold Nanoparticles for LSPR-Based Creatinine Sensing, Antioxidant Activity, and Catalytic Oxidation of Morin

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Abstract

Herein, we report the synthesis of gold nanoparticles (Au NPs) via a citrate-mediated chemical reduction method and investigation for its structural, sensing, antioxidant, and catalytic properties. Au NPs were characterized using transmission electron microscopy (TEM), X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), and UV–visible spectroscopy.

TEM analysis revealed well-dispersed spherical morphology of the nanoparticles with an average particle size of 15.61 ± 2.24 nm, whereas the XRD and XPS confirmed the formation of highly crystalline metallic Au NPs. UV–visible spectroscopy demonstrates a characteristic surface plasmon resonance band at 524 nm. A tapered single-mode optical fiber coated with Au NPs was fabricated for localized surface plasmon resonance (LSPR)-based creatinine sensing. The sensor shows a concentration-dependent shift in resonance wavelength over the creatinine range of 70–140 μM with a sensitivity of $0.0129 \text{ nm } \mu\text{M}^{-1}$, linearity ($R^2 = 0.9812$) and LoD = 65.11 μM . Reproducibility studies using three independently fabricated probes suggests good reproducibility. Au NPs also shows a notable antioxidant activity with IC_{50} values of 48.63 ± 0.11 and $42.03 \pm 0.20 \text{ } \mu\text{g mL}^{-1}$ via DPPH and phosphomolybdate assays, respectively. Additionally, Au NPs showed efficient catalytic activity toward morin oxidation, achieving 68.59% degradation within 20 min and following pseudo-second-order kinetics. These outcomes demonstrate the multifunctional potential of Au NPs for optical sensing, catalysis, and biomedical applications.

Keywords: Gold nanoparticles; LSPR; Biosensor; Creatinine; Morin; Catalytic Activity.

1. Introduction

Nanotechnology has appeared as one of the most emerging grounds because of its wide applications in biomedical science, catalysis, sensing, environmental remediation, and pharmaceutical research [1–3]. Among the various nanomaterials, Au NPs have drawn substantial attention due to its unique optical, electronic, chemical, and biological characteristics [4,5]. Due to its outstanding biocompatibility, high surface-to-volume ratio, and strong surface plasmon resonance (SPR) properties of Au NPs make them highly suitable for applications in biosensing, imaging, drug delivery, and antioxidant systems [6–8]. Specifically, the optical characteristics of Au NPs have empowered their wide-ranging use in optical and plasmonic sensing technologies [9,10].

Several chemical, physical, and biological approaches have been used for the synthesis of Au NPs [11]. Among all of the methods, the chemical reduction method using trisodium citrate is one of the most widely used approach because of its simplicity, cheap, reproducibility, and ability to produce stabilized nanoparticles with controlled size and excellent morphology [12,13]. In this approach, trisodium citrate acts as both reducing as well as the stabilizing agent, which aids in the reduction of Au^{3+} ions into metallic Au^0 nanoparticles simultaneously preventing the possible aggregation. The synthesized Au NPs shows significant stability and

nanorange dimensions, which are highly useful for the sensing as well as biological applications.

The physicochemical characteristics of the synthesised Au NPs, like particle size, morphology, crystallinity, and surface chemistry, strongly effect the efficiency of sensing and biological potential. Consequently, thorough characterization is crucial to confirm nanoparticle formation and examine their structural characteristics. Advanced analytical methods like transmission electron microscopy (TEM), X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), and UV–visible spectroscopy are extensively used to examine the morphological structure, crystalline nature, elemental constituent, and optical behaviour of Au NPs.

Recently, optical fiber-based sensors have drawn significant attention for biochemical sensing applications because of its high sensitivity, rapid response, compact size, flexibility, and immunity to electromagnetic interference [14–16]. In comparison to conventional sensing methods, optical fiber sensors provide advantages such as real-time monitoring, high stability, and very small amount of sample [17,18]. Among various configurations, the tapered single-mode optical fibers (SMFs) are specifically attractive due to the tapering which improves the evanescent field interaction between light and the surrounding medium, thereby improving analyte detection sensitivity [19–21]. Furthermore, the incorporation of gold nanoparticles (Au NPs) onto the sensing region further enhances sensor performance through localized surface plasmon resonance (LSPR) effects which results in the improvement of optical response and signal amplification.

Creatinine is a vital biomarker that is used for the evaluation of kidney function and diagnosing renal disorders [22,23]. Abnormal creatinine levels may confirm kidney dysfunction and other metabolic abnormalities, which make its accurate detection clinically important [24,25]. Conventional creatinine detection methods usually include expensive instrumentation, complex sample preparation, and time-consuming steps, which creates the need for more simple, rapid, and sensitive sensing platforms [26–28]. Moreover, to sensing applications, the Au NPs also shows remarkable antioxidant potential because of their large surface area and electron transfer capability, which enables the effective scavenging of free radicals and reactive oxygen species (ROS) [29–31]. These unique characteristics make the Au NPs promising materials for sensing and biomedical applications [32,33].

Au NPs can efficiently activate oxidizing agents like hydrogen peroxide, producing reactive oxygen species that promote degradation and transformation of organic compounds [34–36].

Morin is a naturally occurring flavonoid that is often used as a model substrate to test the catalytic oxidation activity of nanomaterials because of its well-defined optical properties and oxidation behaviour [37,38]. The catalytic oxidation of morin by Au NPs not only shows their catalytic efficiency but also indicates their potential applications in environmental remediation, wastewater treatment, and advanced oxidation processes. Thus, studying both the catalytic activity and the sensing performance of Au NPs can give a complete picture of their multifunctional capabilities.

Herein, we report Au NPs synthesized via chemical reduction method using trisodium citrate as both reducing and stabilizing agent. The synthesized AuNPs were characterized using TEM, XRD, and XPS analyses to investigate their structural and surface properties. Furthermore, an etched single-mode optical fiber sensor probe was fabricated for potential creatinine sensing applications, and the antioxidant activity of the synthesized Au NPs was examined via DPPH and phosphomolybdate assays as well as catalytic activity using morin. The obtained results show the multifunctional potential of Au NPs for optical sensing and biomedical applications.

2. Materials and methods

2.1 Materials

Chloroauric acid and trisodium citrate was used for the synthesis of Au NPs. Sodium phosphate dibasic and monobasic, ascorbic acid, sodium carbonate, methanol, ammonium molybdate (tetrahydrate), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) were used for antioxidant activity. Sulfuric acid, hydrochloric acid, nitric acid, ethanol and acetone were procured with analytical grade. morin hydrate and hydrogen peroxide was used for the catalytic activity. Creatinine was used for the sensing application.

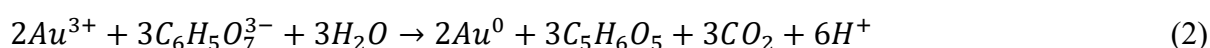
2.2 Synthesis of Au NPs by Chemical Reduction Method

Au NPs were synthesized using the chemical reduction method with trisodium citrate as both reducing and stabilizing agent [39]. For the initiation of the reaction, 100 mL of 1 mM chloroauric acid ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$) solution was prepared in deionized water and heated to boiling under continuous magnetic stirring. After a boil, 10 mL of 38.8 mM trisodium citrate solution was added dropwise to the precursor solution. The solution was then continuously stirred and maintained at boiling temperature for approximately 20 mins. During this time, the colour of the solution gets changed from pale yellow to greyish to finally wine red, which

confirms the formation of gold nanoparticles because of the reduction of Au^{3+} ions to Au^0 nanoparticles.

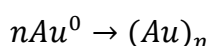
The synthesized Au NPs was then allowed to cool down to room temperature and was then stored in refrigerator at 4 °C for further characterization, sensing and antioxidant studies.

The reduction and formation of Au NPs using trisodium citrate can be explained by the following reactions:



Here:

- Au^{3+} = Au ions from chloroauric acid
- $C_6H_5O_7^{3-}$ = citrate ions (acted as reducing and stabilising agent)
- Au^0 = metallic Au atoms forming NPs
- Citrate is oxidized during the reaction and also acts as a stabilizing/capping agent.



here $(Au)_n$ represents the formed Au NPs.

The characteristic wine-red colour appears because of the surface plasmon resonance (SPR) of the synthesized Au NPs.

2.3 Characterization of Au NPs Nanoparticles

The phase and the structural characteristics of the Au NPs were carried out using Rigaku SmartLab 3 kW powder X-ray diffractometer by employing Cu $K\alpha$ radiation ($\lambda = 1.5403 \text{ \AA}$). The recorded diffraction patterns were used to assess the phase purity and the crystallinity. Morphological features and the particle size distribution were analyzed through the Transmission Electron Microscopy (TEM, Thermo Fisher Scientific TALOS L120C. The surface chemical composition and the oxidation states were examined by using X-ray Photoelectron Spectroscopy (XPS) with a PHI 5000 VersaProbe II instrument. The formation of the nanoparticles was further confirmed through UV-visible spectroscopy by using a Shimadzu UV-1900i spectrophotometer at the room temperature. All the analyses were performed under the controlled laboratory conditions to ensure the data reliability and reproducibility.

2.4 Antioxidant Activity Assays

Antioxidant potential of the Au NPs was examined using 2 different assays i.e., DPPH radical

scavenging and phosphomolybdate assays as per the previously reported methods [40–42]. For the DPPH assay, a stock solution was made by dissolving 3.9 mg of DPPH (2,2-diphenyl-1-picrylhydrazyl) in 100 mL of methanol. Different concentrations of synthesised Au NPs (20–100 μL) were then added to 3 mL of methanolic DPPH solution. This reaction mixture was thoroughly mixed and covered with aluminium foil and kept in the dark and incubated at room temperature for 40 min. After incubation, the absorbance was measured at 517 nm against a control (ascorbic acid) prepared without nanoparticles.

For the phosphomolybdate assay, the reagent solution was made by using 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. Various concentrations of Au NPs (20–100 μL) were properly mixed with this prepared reagent solution, and the reaction mixtures were incubated in a water bath at 95 °C for 90 min. It was later cooled down to room temperature, the absorbance was recorded at 765 nm wavelength. Ascorbic acid was used as the standard antioxidant agent. The antioxidant activity was calculated as % inhibition using the following equation:

$$\% \text{ Inhibition} = \frac{A_0 - A_1}{A_0} \times 100 \quad (3)$$

where A_0 is the absorbance of the control and A_1 is absorbance of the Au NPs/ascorbic acid.

2.5 Catalytic oxidation of morin

For the catalytic oxidation of morin, a 300 μL volume of Au NPs (0.49 M, 1.47×10^{-6} μL) was used. H_2O_2 (180 μL , 24 mM, 4.32×10^{-3} mmol) was used as the oxidizing agent, and 300 μL of carbonate buffer solution (50 mM, 0.15 mmol) was used to maintain the pH at 10, for stability of H_2O_2 . A 1 mM stock solution of morin was freshly prepared and subsequently diluted to a concentration of 0.15 mM for the catalytic activity. The reaction was monitored using a UV–vis spectrophotometer at 410 nm [43].

2.6 Fabrication of Etched SMF Sensor Probe

For the sensor probe fabrication, a single-mode optical fiber (SMF) was used. The SMF optical fiber consisted of a silica core having a diameter of 9 μm and a core–cladding diameter of 125 μm . A 40% HF acid solution was used to etch the sensing region of about 3 cm in length at the centre and then washed with a three-step cleaning process, using acetone, piranha solution, and DI water [44]. Before applying the Au NP coating, an adhesive layer of MPTMS to create SH groups, followed by EDC/NHS, was deposited on the sensor probe. A 48-hour dip-coating

method was used to deposit Au NPs on the sensor probe; a schematic representation is also given in **Figure 1**. The coated sensor probes were washed with ethanol to remove the unbound Au NPs.

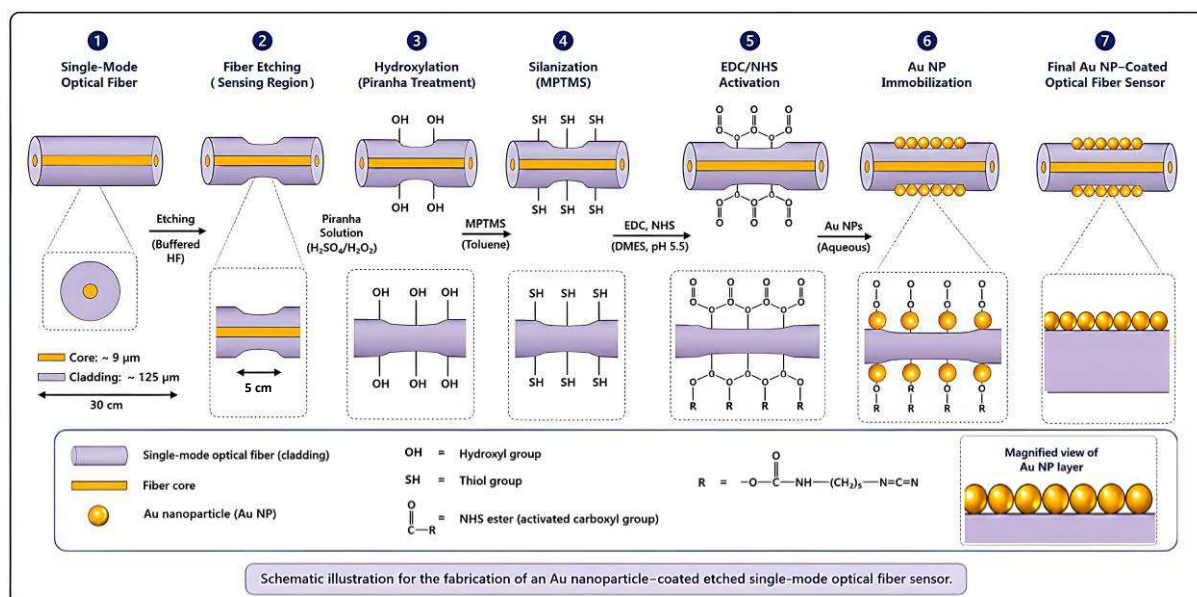
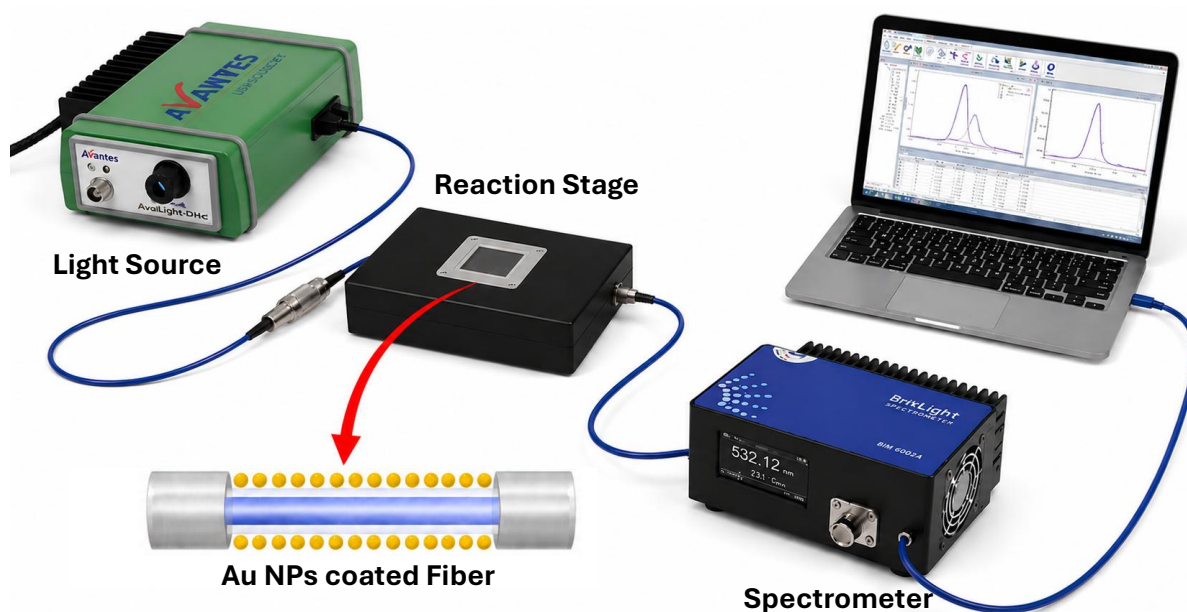


Figure 1: Schematic representation of etching to coating of SMF

The developed sensor probe was tested by connected its one end to a visible optical light source which is operated in the wavelength ranging from 360 to 2500 nm (AvaLight-Hal-S-MINI2), whereas the other end was connected to a spectrometer with a detection range from 300 to 1100



nm (BIM 6002A) for real-time monitoring of the spectrum of light transmission as shown in **Figure 2**. It enables precise control over the etching procedure and further ensures the reproducible fabrication of a sensing region with a length of 5 cm (sensing region) as shown in **Figure 1**. The etched structure pointedly increases the penetration depth of the evanescent field, which increases the light–matter interaction and increases the overall sensitivity of the optical fiber sensor.

2.7 Statistical analysis

All the experiments were done in three replicates. Statistical analysis and the graphs are plotted using Origin Pro 2026 and Microsoft Excel. The IC_{50} values were calculated using a linear regression curve, where an x-y plot was made, and the data were fitted with a straight line.

3. Results and discussion

3.1 Transmission Electron Microscopy (TEM)

TEM analysis of the synthesized Au NPs is given in **Figure 3 (a–b)**. It reveals the successful formation of well-dispersed nanoparticles without any visible agglomeration, which may be attributed to the citrate ions for their stabilising effect. The nanoparticles majorly exhibit spherical to quasi-spherical morphology with uniform distribution.

The particles appear dark and distinctly separated, which shows good structural integrity and particle stability. The observed morphology confirms the successful reduction of gold ions (Au^{3+}) into metallic nanoparticles (Au^0).

The particle size distribution histogram is shown in **Figure 3 (c)**, which shows that the synthesized Au NPs have an average particle size of 15.61 ± 2.24 nm. Most of the NPs were distributed within the range of approximately 12–19 nm, which confirms a narrow size distribution and relatively uniform synthesised NPs. The smaller particle size and homogeneous distribution are majorly beneficial for increasing the physicochemical and biological properties of the synthesized Au NPs.

3.2 X-ray Diffraction (XRD)

XRD pattern of the Au NPs is given in **Figure 3 (d)**. The diffraction peaks observed at 2θ values of 38.01° , 44.12° , 64.34° , and 77.46° correspond to the (111), (200), (220), and (311) crystallographic planes, respectively which ultimately confirms the formation of face-centred cubic (fcc) crystalline Au NPs. The sharp and intense diffraction peak at 38.01° associated with

the (111) plane further confirms the preferential crystalline orientation and high crystallinity of the synthesized Au NPs. All the peaks are in good agreement with the standard JCPDS card no. 04-0784 for metallic Au. No additional peaks further confirm that there is no impurity present, suggesting the high purity of the Au NPs. The sharp diffraction peaks further confirm the nanoscale crystalline nature and successful synthesis of stable Au NPs.

3.3 X-ray Photoelectron Spectroscopy (XPS)

XPS analysis was carried out to examine the elemental composition and surface chemical states of the Au NPs, and the obtained spectra are presented in **Figure 3 (e–h)**. The survey spectrum shown in **Figure 3 (e)** confirms the presence of Au, C, and O elements in the synthesized nanoparticles. The Au 4f signal confirms the successful formation of metallic Au nanoparticles, whereas C 1s and O 1s peaks may originate from residual organic species of citrate ions which comes from the reducing agent and also acts as stabilising agent for the nanoparticles during the chemical reduction process.

The high-resolution spectra of O 1s is given in the **Figure 3 (f)** which shows a characteristic peak centered at 531.74 eV, this peak is attributed to oxygen-containing functional groups such as hydroxyl and carbonyl species adsorbed on the surface of the Au NPs. These surface oxygen species may arise from the citrate which is reducing and stabilizing reagents used during synthesis and contribute to nanoparticle stabilization.

The deconvoluted Au 4f spectra is given in the **Figure 3 (g)**, it shows 2 distinct peaks at the binding energies of 82.88 eV and 86.53 eV, which correspond to the Au 4f_{7/2} and Au 4f_{5/2}, respectively. The characteristic spin–orbit splitting confirms the metallic Au⁰ state, which confirms the successful reduction of Au³⁺ ions into elemental Au NPs. The sharp and characteristic peaks further confirm the high purity and crystalline nature of the synthesized Au NPs.

The high-resolution spectra of C 1s is given in the **Figure 3 (h)** which illustrates the predominant major peaks at 284.53 eV and 287.68 eV, these peaks are assigned to C–C/C=C and C=O functional groups, respectively. These carbon-related species are linked with surface-adsorbed carbonaceous constituents or residual stabilizing compounds present after the chemical reduction synthesis of Au NPs.

Overall, the XPS outcomes suggests the successful synthesis of metallic Au NPs with high purity and shows the presence of surface functionality linked with the chemical reduction and stabilization process.

3.4 UV-Visible spectroscopy

The synthesis of AuNPs by the chemical reduction method was further confirmed using UV–Visible spectroscopy the spectra for the same is given in the **Figure 3 (i)**. A characteristic surface plasmon resonance (SPR) absorption peak was recorded at 524 nm, which ultimately confirms the successful synthesis of Au NPs. As per literature, the SPR band in this region is basically for the spherical shaped Au NPs and which arises from collective oscillation of surface electrons upon the irradiation of light. The recorded sharp absorption peak confirms the formation of well-dispersed Au NPs with excellent colloidal stability. Furthermore, the absence of any additional peaks at higher wavelengths again confirms purity and minimal aggregation of the synthesized Au NPs.

The obtained TEM, XRD, XPS and UV-visible outcomes further confirms the successful synthesis of highly crystalline, stable, and uniform distribution of the synthesised Au NPs with nanoscale dimensions. Due to the small particle size, high surface area, and metallic Au⁰ characteristics of the Au NPs makes it highly suitable for optical fiber-based creatinine biosensing by increasing the surface interaction, signal sensitivity, and sensing performance.

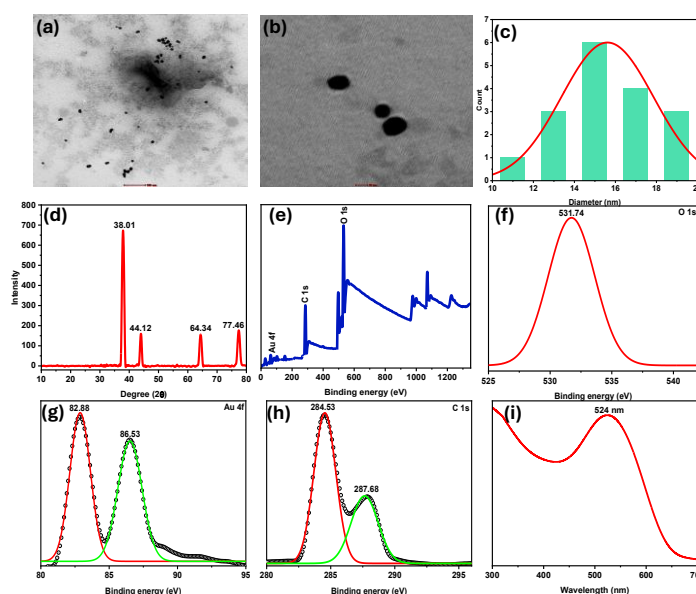


Figure 3: (a,b) TEM micrographs showing the morphology and dispersion of the synthesized Au NPs, revealing spherical to quasi-spherical nanoparticles. (c) Particle size distribution

histogram with Gaussian fitting, showing an average particle size of 15.61 ± 2.24 nm. (d) XRD pattern of the Au NPs exhibiting characteristic diffraction peaks of face-centered cubic (fcc) gold. (e) XPS survey spectrum confirming the presence of Au, C, and O elements. (f) High-resolution O 1s XPS spectrum showing a peak centered. (g) Deconvoluted Au 4f XPS spectrum, confirming the metallic Au⁰ state. (h) High-resolution C 1s XPS spectrum. (i) UV–visible absorption spectrum of the synthesized Au NPs, confirming the successful formation of stable Au nanoparticles.

3.5 Antioxidant activity

Antioxidant potential of the synthesized Au NPs was examined using DPPH radical scavenging and phosphomolybdate assays, and the outcomes are IC₅₀ values (μg/mL) in the form of mean ± standard deviation. Stronger antioxidant potential is indicative of lower IC₅₀ values. In the DPPH assay, Au NPs show an IC₅₀ value of 48.63 ± 0.11 μg/mL, when compared to the standard ascorbic acid, which demonstrated a lower IC₅₀ value of 19.51 ± 0.12 μg/mL, confirming the comparatively stronger radical scavenging potential of the marketed standard. Likewise, in the phosphomolybdate assay, the Au NPs show an IC₅₀ value of 42.03 ± 0.20 μg/mL, whereas the standard ascorbic acid demonstrated an IC₅₀ value of 15.79 ± 0.46 μg/mL as shown in **Figure 4**. The antioxidant potential of the synthesised Au NPs may be because of

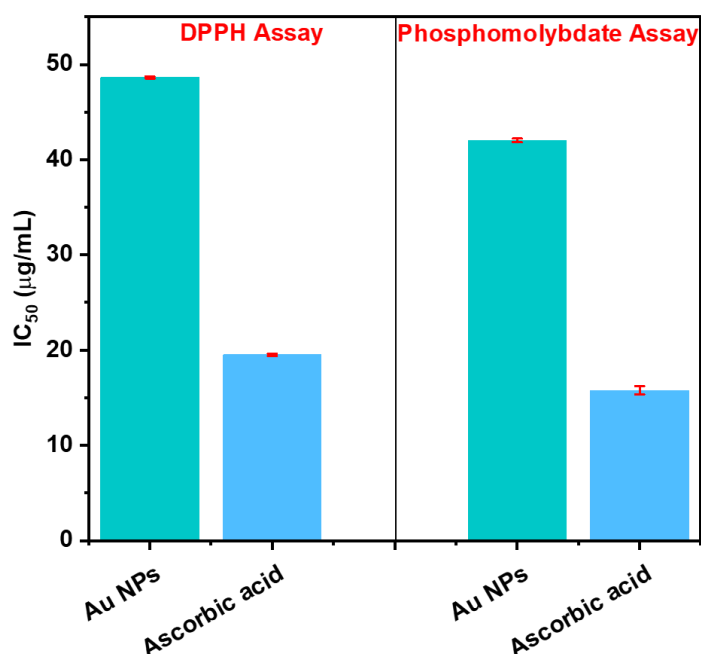


Figure 4: Antioxidant activity of Au NPs using 2 different assays

their high surface area and electron transfer capability, which enables the neutralization of the free radicals and reduction of oxidizing species. Moreover, the presence of citrate ions on the surface of the synthesised Au NPs which originates from the sodium citrate reduction process during synthesis, may contribute to the stabilization and antioxidant activity of the synthesised Au NPs.

3.6 Catalytic Oxidation of Morin Using Biosynthesized Gold Nanoparticles

In the presence of Au NPs as a catalyst and H_2O_2 the catalytic oxidation of morin was carried out, and the progress of the reaction was monitored by UV-visible spectroscopy. Morin is a naturally occurring flavonoid with several hydroxyl groups and an extensive conjugated aromatic system, which leads to the characteristic absorption bands in the UV-visible region. AuNPs during the oxidation process help to activate H_2O_2 to generate reactive oxygen species, which oxidize morin to its corresponding quinone derivatives. The oxidation reaction was accompanied by a gradual decrease in absorbance intensity as a function of reaction time, indicating continuous degradation of the morin molecules.

Figure 5 (a) shows the time-dependent UV-visible spectra measured during the catalytic oxidation process. Initially (0 min) morin showed a strong absorption band with an absorbance intensity close to 1.0 a.u. The intensity of absorbance was decreased continuously during the reaction time of 20 min after the addition of AuNPs and H_2O_2 . Absorbance spectra registered every minute showed gradual oxidation of morin. The reaction showed a spectacular decrease in the absorption band characteristic for morin, which is evidence of the progressive destruction of the chromophoric structure of morin.

The oxidation process was also visually confirmed by the colour change of the reaction mixture. The reaction solution was pale yellow in colour, characteristic of morin at the initiation. As oxidation progressed, the intensity of yellow colour gradually decreased and after 20 min of reaction the solution was found to be almost colourless. This colour change offers a straightforward visual confirmation of the degradation of morin molecules and correlates well with the observations from UV-visible spectroscopic studies. **Figure 5 (a)** schematically depicts the proposed oxidation pathway where morin is oxidized to the quinone form via AuNP activation of hydrogen peroxide.

The catalytic oxidation efficiency was determined using the absorbance values obtained from UV-visible spectroscopy according to the following equation:

$$\text{Degradation Efficiency (\%)} = \frac{A_0 - A_t}{A_0} \times 100 \quad (4)$$

where A_0 represents the initial absorbance of morin and A_t represents the absorbance after a reaction time t .

The degradation efficiency of morin was found to be 68.59% after 20 min, based on the absorbance reduction during the reaction, which means that the oxidation of the flavonoid molecule was considerable. The high catalytic activity of AuNPs can be ascribed to the large specific surface area, abundant active surface sites, efficient electron-transfer capability, and strong interaction with hydrogen peroxide and morin molecules, etc. The phytochemicals adsorbed on the surface of the biosynthesized nanoparticles may enhance the catalytic activity further by promoting the adsorption of substrate and charge-transfer processes.

The oxidation mechanism probably involves the adsorption of morin molecules on the surface of AuNPs, followed by the decomposition of H_2O_2 into highly reactive hydroxyl radicals ($\bullet\text{OH}$) and other reactive oxygen species. These radicals attack the hydroxylated aromatic rings of morin, leading to cleavage of the conjugated π -electron system and formation of oxidized quinone intermediates. The continuous oxidation results in the further deterioration of the molecule, which causes a significant reduction in the absorbance intensity and the disappearance of colour. The high catalytic efficiency observed demonstrates the excellent ability of Au NPs to mediate oxidation reactions under mild reaction conditions.

The experimental data were fitted to pseudo-zero-order, pseudo-first-order and pseudo-second-order kinetic models in order to gain insight into the reaction kinetics.

3.7 Pseudo-Zero-Order Kinetic Analysis

The pseudo-zero-order kinetic model assumes that the reaction rate remains independent of the concentration of morin and can be expressed as:

$$A_t - A_0 = -kt \quad (5)$$

where k is the apparent zero-order rate constant.

The corresponding linear fitted plot of $(A_t - A_0)$ versus reaction time is illustrated in **Figure 5 (b)**. A reasonably linear trend was observed throughout the reaction period; however, noticeable deviations from linearity occurred at longer reaction times. The correlation coefficient obtained from linear fitting was around $R^2 = 0.9432$, which confirms that the pseudo-zero-order model

does not adequately describe the oxidation process. This confirms that the reaction rate is influenced by the concentration of morin and cannot be considered concentration-independent.

3.8 Pseudo-First-Order Kinetic Analysis

The pseudo-first-order kinetic model is determined by [45]:

$$\ln\left(\frac{A_t}{A_0}\right) = -kt \quad (6)$$

A plot of $\ln(A_t/A_0)$ versus reaction time is illustrated in **Figure 5 (c)**. The experimental data exhibited excellent linearity over the entire reaction period, yielding a correlation coefficient of $R^2 = 0.9802$. The high R^2 value indicates that the oxidation rate is significantly dependent on the concentration of morin present in the reaction medium.

The good agreement with the pseudo-first-order model suggests that the oxidation process involves direct interaction between oxidizing species generated from H_2O_2 and morin molecules adsorbed on the nanoparticle surface. Similar first-order behaviour has been reported for the catalytic oxidation of phenolic compounds and flavonoids using noble metal nanoparticles.

3.9 Pseudo-Second-Order Kinetic Analysis

The pseudo-second-order kinetic model is expressed as:

$$\frac{1}{A_t} = kt + \frac{1}{A_0} \quad (7)$$

The corresponding plot of $1/A_t$ versus reaction time is shown in **Figure 5 (d)**. The data displayed excellent linearity throughout the reaction duration, producing a correlation coefficient of $R^2 = 0.9951$, which is substantially higher than those obtained for the pseudo-zero-order and pseudo-first-order models.

The results showed that the oxidation of morin was best described by the pseudo-second-order kinetic model with the highest correlation coefficient and the best linearity. This observation suggests that the overall reaction rate is limited by the surface-mediated interactions involving the adsorption of the reactants on the nanoparticle surface followed by electron-transfer processes. In such systems, the number of catalytic active sites is an important factor that determines the reaction kinetics.

The pseudo second order model has a higher correlation coefficient which indicates that it better describes the oxidation process. Therefore, the catalytic oxidation of morin by AuNPs

with the presence of H_2O_2 follows mainly the pseudo-second-order kinetics. The excellent catalytic activity observed could be attributed to the synergistic effect between the metallic Au core and the phytochemical capping agents, present on the surface of the nanoparticles. These phytochemicals can not only stabilize the nanoparticles but also promote substrate adsorption, leading to enhanced catalytic efficiency.

Overall, the biosynthesized Au NPs showed excellent catalytic performance, with 68.59% oxidation of morin in 20 min at ambient conditions. The notable degradation efficiency, rapid reaction rate, change in visible colour, and excellent kinetic fitting confirm the overall suitability of the synthesized Au NPs as efficient nanocatalysts for flavonoid oxidation reactions. These results suggest their promising applications in environmental remediation, wastewater treatment, advanced oxidation processes, and catalytic transformation of organic pollutants.

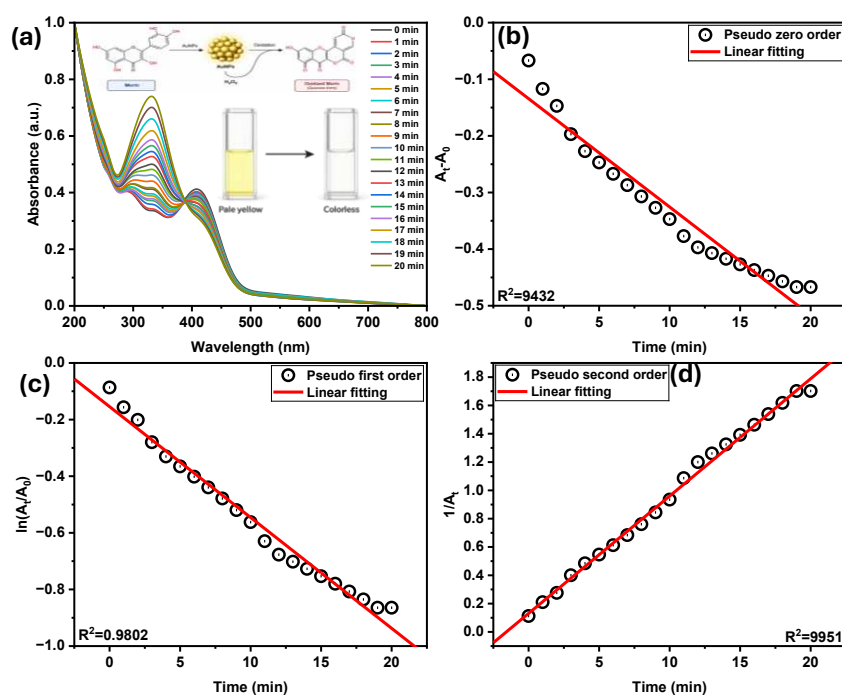


Figure 5: (a) Time dependent UV-visible graph showing oxidation of morin. (b) Pseudo zero order. (c) Pseudo first order. (d) Pseudo second order kinetics of the catalytic reaction.

3.10 LSPR Response of the Sensor

The LSPR response of the fabricated sensor probes were investigated for various concentrations of creatinine. The reduction of the fiber cladding enhanced the evanescent-field interaction with the surrounding medium, which enables the efficient excitation of localized

surface plasmons at the Au NPs surface. After the exposure to creatinine solutions of various concentrations, a distinct shift in the resonance wavelength was noticed, as illustrated in **Figure 6 (a)**. This spectral variation is arising due to the changes in the local refractive index near the Au NPs layer resulting from the interaction of creatinine molecules with the sensing surface. The dose-dependent resonance shift confirms the effective plasmonic response of the proposed sensor toward creatinine detection.

3.11 Creatinine Sensing Performance

The sensing performance of Au NP-coated etched SMF sensor was examined using creatinine concentrations ranging from 70 to 140 μM . Analysis were carried out sequentially from lower to higher concentrations for ensuring reproducibility and minimize experimental variability. Before each analysis, the sensor probe was properly rinsed with PBS buffer solution and later dried for the removal of any residual analyte from previous analysis.

The recorded LSPR spectra shows a gradual red shift in the resonance wavelength along with the increase in the concentration of creatinine, as shown in **Figure 6 (a)**. This trend is attributed to the increase in the local refractive index which surrounds the Au NP-coated sensing region due to the adsorption and interaction of creatinine molecules at the surface of the sensor. The resonance wavelength increased systematically from approximately 713.6 nm to 714.5 nm as the concentration of the creatinine increased from 70 μM to 140 μM .

The relationship between the resonance wavelength and creatinine concentration was found to be highly linear, as illustrated in **Figure 6 (b)**, and can be expressed as:

$$\lambda = 0.0129C + 712.74 \quad (R^2 = 0.9812)$$

where λ is the resonance wavelength (nm) and C is the creatinine concentration (μM). The sensitivity obtained from the slope of the calibration curve was found to be 0.0129 nm μM^{-1} , which confirms a significant and reliable response toward creatinine concentration variations.

For the evaluation of the reproducibility of the sensor, analysis was done using three independently fabricated probes. The transmitted spectra of the three probes, illustrated in **Figure 6 (c)**, exhibited similar spectral profiles and resonance characteristics, which shows good fabrication consistency and reproducibility of the sensing platform. These outcomes confirm the suitability of the proposed LSPR-based fiber-optic sensor for sensitive and reliable

detection of creatinine. The sensor performance also relies on several analytic characteristics, limit of detection (LoD) is one of them which plays a significant role. The minimum concentration of analyte that can be detectable by a sensor is termed as LoD, which can be expressed as:

$$\text{LoD} = \frac{3 \times \text{SD}}{\text{Sensitivity}}$$

In the proposed work, standard deviation (SD) = 0.28 nm and Sensitivity = 0.0129 nm/ μM .

Therefore, LoD = 65.11 μM

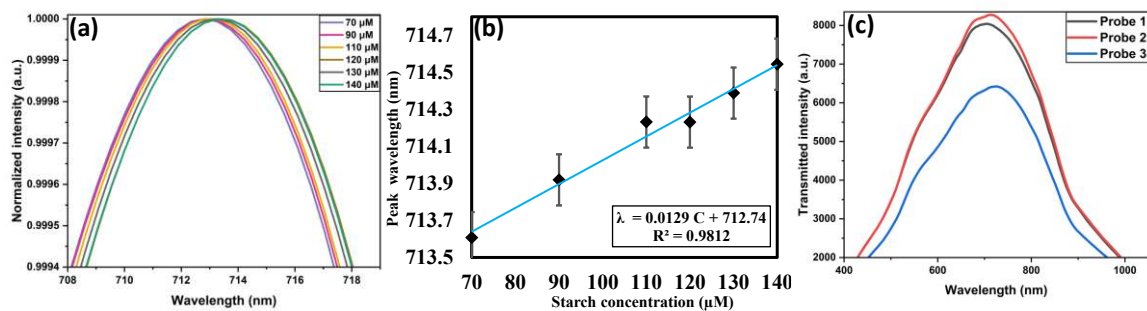


Figure 6: (a) Normalized LSPR spectra of the Au NP-coated etched SMF sensor for various concentrations of creatinine (70–140 μM), illustrating a concentration-dependent resonance wavelength shift. (b) Calibration curve showing the linear relationship between resonance wavelength and creatinine concentration. (c) Illustrates transmitted spectra recorded from three independently fabricated sensor probes, demonstrating good reproducibility and fabrication consistency of the proposed creatinine sensor.

4. Conclusion

In the current study, Au NPs were successfully synthesized using a simple and cost-effective citrate reduction method and were characterized using TEM, XRD, XPS, and UV–visible spectroscopy. Au NPs exhibited spherical morphology, high crystallinity, excellent colloidal stability, and an average particle size of 15.61 ± 2.24 nm. An etched Au NP-coated single-mode optical fiber sensor was developed and employed for LSPR-based detection of creatinine. The sensor demonstrated a clear concentration-dependent resonance wavelength shift within the creatinine concentration range of 70–140 μM , which provides a sensitivity of 0.0129 nm μM^{-1} , LoD of 65.11 μM and a strong linear response ($R^2 = 0.9812$). The reproducibility results obtained from three independent probes confirmed the reliability and reproducibility of the sensing platform. Moreover, Au NPs shown an appreciable antioxidant activity and excellent catalytic performance toward morin oxidation, achieving 68.59% degradation within 20 min,

with the best fitted model for the kinetics of the reaction as pseudo-second-order model. The combined sensing, antioxidant, and catalytic properties highlight the multifunctional nature of the synthesized Au NPs and shows their potential for applications in biomedical diagnostics, environmental remediation, and advanced nanotechnology-based sensing systems.

CRedit authorship contribution statement

Vishwajeet Bachhar: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Kuldeep Choudhary:** Conceptualization, Methodology, Investigation, Writing – original draft, Supervision, Project administration, Writing – review & editing. **Vibha Joshi:** Investigation, Writing – review & editing. **Manisha Duseja:** Project administration. **Chung-Hsien Hung:** Formal analysis. **Chien-Hsing Wu:** Formal analysis. **Guo-Ping Chang-Chien:** Project administration, Supervision. **Sakthivel Kogularasu:** Supervision, Writing – review & editing. **Santosh Kumar:** Supervision, Validation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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