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Green chemistry approach for the synthesis of gold nanoparticles: Role of soybean extract as a bioreductant

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HIGHLIGHTS

- Soybean extract enables green AuNP synthesis at pH 3.0–8.5.
- Smallest SPR peak at 537 nm achieved at pH 7.4.
- Crystallite size reduced to 23–28 nm vs 37–42 nm (citrate).
- Hydrodynamic size reduced to ~108 nm using soybean extract.
- Neutral pH yields most uniform AuNPs.

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ABSTRACT

Gold nanoparticles (AuNPs) have gained considerable attention for biomedical applications, including biosensing, imaging, drug delivery, and cancer therapy; however, conventional chemical synthesis methods often employ toxic reagents and require complex purification, limiting environmental sustainability and clinical translation. Green chemistry provides an eco-friendly alternative through the use of biogenic reducing agents and benign solvents. In this study, AuNPs were synthesized by reducing chloroauric acid (HAuCl_4) using sodium citrate and soybean (*Glycine max*) extract under acidic (pH 3.0), physiological (pH 7.4), and alkaline (pH 8.5) conditions. The soybean extract, prepared via ethanol extraction and rotary evaporation, served as a reducing agent due to its high content of polyphenols, flavonoids, and carbohydrates. The synthesized AuNPs were characterized by UV–Vis spectroscopy, Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM), and dynamic light scattering (DLS). Surface plasmon resonance bands appeared at 537–589 nm, with soybean-mediated AuNPs exhibiting blue-shifted peaks and higher absorbance compared to citrate-derived nanoparticles, indicating smaller particle sizes and higher yields. SEM confirmed predominantly spherical nanoparticles below 50 nm, while XRD revealed a crystalline face-centered cubic structure. FTIR analysis demonstrated the involvement of soybean phytochemical functional groups in nanoparticle reduction. DLS results showed smaller hydrodynamic diameters (108–149 nm) for soybean-derived AuNPs relative to citrate-based counterparts, with optimal stability at pH 7.4. These findings highlight soybean extract as a sustainable, low-cost bioreductant for green AuNP synthesis with potential biomedical and catalytic applications.

1. Introduction

Nanotechnology represents an interdisciplinary field that integrates physics, chemistry, biology, and engineering to manipulate materials

at the nanoscale. Among the various nanomaterials, gold nanoparticles (AuNPs) have received considerable attention because of their unique optical, electronic, and physicochemical properties, including chemical

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stability, biocompatibility, and low toxicity [1,2]. These characteristics have enabled their use in catalysis, biosensing, environmental remediation, and a wide range of biomedical applications such as targeted drug delivery, imaging, and photothermal or photodynamic cancer therapies [3–5]. Their distinct red coloration, arising from surface plasmon resonance (SPR), also underpins their role in rapid diagnostic tools such as lateral flow immunoassays (e.g., pregnancy and COVID-19 kits) [3,4].

The phenomenon of SPR originates from the collective oscillation of conduction electrons at the nanoparticle surface when excited by light. This resonance can be precisely tuned by altering the particle size, shape, and inter-particle spacing, making AuNPs exceptionally versatile for biosensing and imaging applications [6–8]. For instance, their tunable optical response enhances sensitivity in surface-enhanced Raman spectroscopy (SERS), surface-enhanced infrared absorption (SEIRA), and fluorescence-based detection methods, as well as imaging modalities such as photoacoustic and computed tomography.

Despite their immense potential, the synthetic route used to produce AuNPs profoundly affects their size, morphology, stability, and biocompatibility, which in turn determine their functionality. Conventional synthesis approaches can be broadly classified as physical, chemical, and biological (green) methods, corresponding to top-down and bottom-up strategies [9]. Physical methods (e.g., laser ablation, milling) generally yield highly pure and crystalline nanoparticles but are energy-intensive and offer limited shape control, making them impractical for large-scale or biomedical applications [10]. Chemical reduction methods, such as the Turkevich and Brust–Schiffrin processes, allow better control over nanoparticle size and monodispersity but involve toxic reagents (e.g., NaBH_4 , thiols) and require post-synthesis purification, reducing biocompatibility [11,12].

In response to these limitations, green chemistry has emerged as a sustainable alternative. It relies on biological reducing agents-derived from microorganisms or plant extracts, to synthesize nanoparticles in non-toxic, aqueous environments [9,13,14]. While microbial synthesis (using fungi, bacteria, or algae) is effective, it often requires complex culturing, aseptic conditions, and long incubation times [15–17]. In contrast, plant extracts provide a faster, simpler, and more scalable method. They contain a wide range of phytochemicals, including polyphenols, flavonoids, reducing sugars, proteins, and alkaloids, that act both as reducing and stabilizing agents, enabling the formation of biocompatible AuNPs [15–20].

Among various plants explored, soybean (*Glycine max*) is particularly promising due to its abundance of bioactive compounds. Soybean extract contains phenolic acids, isoflavones (daidzein, genistein, glycitein), and other antioxidants capable of donating electrons to reduce metal ions while stabilizing the resulting nanoparticles [21–23]. Its rich composition of proteins, lipids, and carbohydrates also provides capping functionality that prevents nanoparticle aggregation. These multifunctional phytochemicals not only enhance reduction efficiency but also impart biological activity to the resulting nanomaterials. Many studies reported that soybean is a rich source of isoflavones including aglycons (daidzein, genistein, and glycitein), their conjugated glycosides (daidzin, genistin, and glycitin), malonyl glucosides (6''-O-malonyl daidzin, 6''-O-malonyl genistin, and 6''-O-malonyl glycitin), and acetyl glucosides (6''-O-acetyl daidzin, 6''-O-acetyl genistin, and 6''-O-acetyl glycitin), as well as phenolic acids namely syringic, vanillic, caffeic, ferulic, p-coumaric and p-hydroxybenzoic acids, possessing antioxidative activity, have also been found in soybeans [24–27]. The capability of donating electrons and acting as a reducing agent to reduce HAuCl_4 to gold nanoparticles has been examined in this study.

Although several studies have examined plant-mediated gold nanoparticle synthesis, systematic comparative analyses between plant-based and conventional chemical reduction methods under controlled conditions remain limited, particularly for soybean extract. In addition, the influence of pH on nucleation behavior, and particle growth has

rarely been evaluated in a side-by-side framework across different reducing systems. To the best of our knowledge, no previous research has reported the use of soybean extract as a bioreductant and directly compared its performance with that of sodium citrate, a conventional reducing agent, under different pH conditions. Therefore, the present study aims to: synthesize gold nanoparticles using soybean extract via a green chemistry approach; synthesize AuNPs using a conventional citrate-mediated method for direct comparison; investigate the effect of pH (3.0, 7.4, and 8.5) on nanoparticle formation, and size control; and characterize the resulting nanoparticles using UV–Vis spectroscopy, SEM, FTIR, XRD, and DLS. By comparing the optical, structural, and morphological properties of AuNPs produced via both methods, this work introduces soybean extract as a novel, cost-effective, and environmentally friendly bioreductant capable of generating size-controlled gold nanoparticles, while providing new insights into pH-dependent synthesis mechanisms and their potential for biomedical and catalytic applications.

2. Materials and methods

2.1. Materials

All reagents used in this study were of analytical grade and used without further purification. Chloroauric acid (HAuCl_4) was obtained from Sigma-Aldrich (USA). Trisodium citrate ($\text{C}_6\text{H}_5\text{Na}_3\text{O}_7 \cdot 2\text{H}_2\text{O}$) and disodium hydrogen phosphate ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$) were supplied by Merck (Germany). Milli-Q water with a resistivity of 18.2 M Ω cm was used throughout all experiments.

2.2. Preparation of soybean extract

Soybean seeds were first washed, dried, and ground into fine powder using a laboratory mill. The resulting powder was extracted with 96% ethanol at room temperature for 72 h to isolate the bioactive phytochemicals. The mixture was then filtered through standard Whatman filter paper, and the filtrate was concentrated under vacuum at 40 °C using a rotary evaporator (EV311, LabTech) operating at 90 rpm. The ethanol was removed and condensed via a water-cooled system, and the concentrated soybean extract was stored until further use. This extract was later employed as a bioreductant agent in the green synthesis of AuNPs.

2.3. Green synthesis of gold nanoparticles

A 2 mM solution of HAuCl_4 was prepared by dissolving 0.1 g of the gold precursor in 250 mL of deionized water. For each reaction, 10 mL of this solution was mixed with 10 mL of phosphate buffer, prepared by dissolving 1.4196 g of $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ in 100 mL of deionized water. The pH of the buffer was adjusted to 3.0, 7.4, or 8.5 using HCl or NaOH. The reaction mixture was heated to 95 °C on a magnetic stirrer with heating capability (MS-20D, Wisd). Once the temperature stabilized, 0.5 mL of soybean extract was added dropwise to the solution and stirred continuously for 20 min. The gradual color change from pale yellow to ruby red or purple indicated the reduction of Au^{3+} ions and formation of gold nanoparticles due to SPR.

2.4. Wet chemical synthesis of gold nanoparticles

For comparison, AuNPs were also synthesized using a wet-chemical method based on citrate reduction. A 1% (w/v) sodium citrate solution was prepared by dissolving 0.5 g of trisodium citrate in 50 mL of deionized water. The same procedure as in Section 2.3 was followed: 10 mL of the HAuCl_4 solution was mixed with 10 mL of phosphate buffer at the desired pH, heated to 95 °C, and then 2 mL of citrate solution was added dropwise while stirring for 20 min. The resulting color transition from yellow to deep red confirmed nanoparticle formation.

2.5. Characterization

2.5.1. UV-Vis spectroscopy

Optical properties of the synthesized AuNPs were examined using UV-Vis spectrophotometry in the wavelength range of 300–800 nm to monitor SPR peaks and confirm nanoparticle formation.

2.5.2. FTIR spectroscopy

The functional groups involved in nanoparticle reduction and stabilization were identified using FTIR spectroscopy in the spectral range of 4000–400 cm^{-1} . Dried samples of both soybean extract and AuNPs were analyzed to detect chemical changes in functional groups before and after nanoparticle synthesis.

2.5.3. XRD and Energy-Dispersive X-ray (EDX) spectroscopy

The crystalline structure of the AuNPs was analyzed using XRD with Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$). The average crystallite size was calculated using the Debye-Scherrer [28]:

$$D = \frac{0.9\lambda}{\beta \cos \theta} \quad (1)$$

where D is the crystallite size, λ the X-ray wavelength, β the full width at half maximum (FWHM), and θ the Bragg angle. Elemental composition and sample purity were verified using EDX spectroscopy.

2.5.4. Field Emission SEM (FESEM), and DLS

The morphology and particle size distribution of AuNPs were examined using FESEM imaging. Prior to analysis, samples were gold-coated to enhance surface conductivity and imaging clarity. The hydrodynamic diameter and polydispersity index (PDI) of the colloidal AuNPs in aqueous suspension were measured by DLS, providing quantitative information on particle size uniformity and dispersion.

2.6. Experimental repeatability

All experiments were performed in triplicate to ensure reproducibility and minimize experimental uncertainty. Measurements are reported as mean values across repeated trials.

3. Results and discussion

This section presents the characterization and comparative analysis of AuNPs synthesized using two different reducing agents, sodium citrate (chemical method) and soybean extract (green method), under three pH conditions: 3.0, 7.4, and 8.5.

3.1. UV-Vis spectroscopy

Gold nanoparticles typically exhibit a strong absorption band between 500–600 nm, corresponding to SPR, which is highly sensitive to particle size, shape, and the surrounding medium. Experiments are executed in 3 different pH values for each reducing agent (Fig. 1). The UV-Vis spectroscopy results reveal distinct optical behaviors for nanoparticles synthesized using citrate and soybean seed extract as reducing agents, with clear correlations to pH conditions. Citrate-based nanoparticles consistently exhibit redshifted SPR peaks, progressing from $561 \pm 2 \text{ nm}$ to $572 \pm 1 \text{ nm}$ and stabilizing around $570 \pm 2 \text{ nm}$. This redshift is indicative of an increase in particle size, likely driven by slower nucleation followed by particle growth or agglomeration. The redshift becomes more pronounced at higher pH values (e.g., pH 8.5), where enhanced reduction kinetics and elevated reaction temperatures promote particle enlargement and aggregation. This is supported by DLS data, which show increased particle size at alkaline pH.

In contrast, nanoparticles synthesized using soybean seed extract initially display a blueshift in SPR peaks, shifting from $550 \pm 2 \text{ nm}$ to $537 \pm 1 \text{ nm}$, which suggests the formation of smaller, well-dispersed

nanoparticles. This blueshift is attributed to the size-dependent optical properties of metal nanoparticles, particularly the shift in SPR as particle size decreases. This behavior is most evident at neutral pH (around 7.4), where the enzymatic activity and bioactive compounds in the extract are preserved, facilitating efficient reduction and stabilization. At this pH, the nanoparticles exhibit higher absorbance intensity and shorter SPR wavelengths, indicating both higher concentration and smaller size. However, as the pH increases to 8.5, a redshift to $559.5 \pm 0.75 \text{ nm}$ is observed, reflecting particle growth or agglomeration, possibly due to enzyme degradation or changes in surface chemistry. Similarly, at acidic pH (e.g., pH 3.0), the SPR peaks become broader and less intense, suggesting poor nanoparticle formation, larger particle size, and reduced stability, likely due to disrupted reduction equilibrium and loss of enzymatic function.

Overall, the pH plays a critical role in modulating nanoparticle size, concentration, and optical properties. Neutral pH conditions (around 7.4) appear optimal for producing small, stable, and concentrated nanoparticles, particularly when using soybean extract. In contrast, both acidic and highly alkaline conditions tend to promote particle growth and agglomeration, leading to redshifted SPR peaks and reduced colloidal stability. These findings underscore the importance of pH control in green nanoparticle synthesis and highlight the superior performance of biogenic reducing agents under carefully optimized conditions.

These observations are consistent with previously reported plant-mediated AuNP synthesis systems, such as those using black tea granules, neem, and onion extracts, where blue-shifted SPR peaks are associated with smaller nanoparticle sizes and improved dispersion [29–31]. However, compared to these systems, the magnitude of the blue shift observed for soybean-mediated synthesis (down to 537 nm) indicates more effective size control under optimized conditions. This enhanced performance can be attributed to the combined action of phenolic compounds and isoflavones in soybean extract, which provide both strong reducing capability and efficient surface stabilization.

3.2. FTIR analysis

To identify the functional groups responsible for reduction and stabilization, FTIR spectra of soybean extract and synthesized AuNPs were recorded in the range of 4000–400 cm^{-1} (Fig. 2). The FTIR spectrum reveals that soybean contains polyphenolic compounds, various B vitamins, vitamin E, and the free radical DPPH [32,33]. A broad absorption peak at 3330 cm^{-1} corresponds to hydroxyl (–OH) groups, which are commonly found in polyphenols and sugars [34]. Peaks at 2926 cm^{-1} and 2857 cm^{-1} are consistent with methylene (–CH₂) and C–H stretching vibrations, associated with vitamin E and DPPH. The peak at 1743 cm^{-1} is consistent with the presence of carbonyl (C=O) groups, which may originate from lactones, acids, phenols, or B vitamins [32,33]. Additionally, a peak at 1100 cm^{-1} is consistent with C–O stretching, likely from C–O–H groups in sugars or C–O–C linkages in vitamin E [33,35]. These functional groups suggest the extract's potential role in reducing and stabilizing gold nanoparticles during synthesis.

Phytochemicals such as flavones, alkaloids, phenols, and anthracenes contain functional groups like C–O–C, C–O, and C=O, which play a key role in the synthesis of AuNPs. During this process, these naturally occurring compounds serve both as reducing agents and as stabilizing/capping agents, eliminating the need for external chemical additives [24,26]. This approach enables the extracellular formation of AuNPs while avoiding the use of toxic substances like sodium borohydride (NaBH_4).

Based on previous studies soybean extract have flavonoids, isoflavonoids, tannins, phenolic acids and proanthocyanidins [36]. The antioxidant-rich profile of soybean is well-established, with approximately 99% of its isoflavones existing as glycosides, primarily genistein (64%), daidzein (23%), and glycitein (13%), alongside phenolic acids

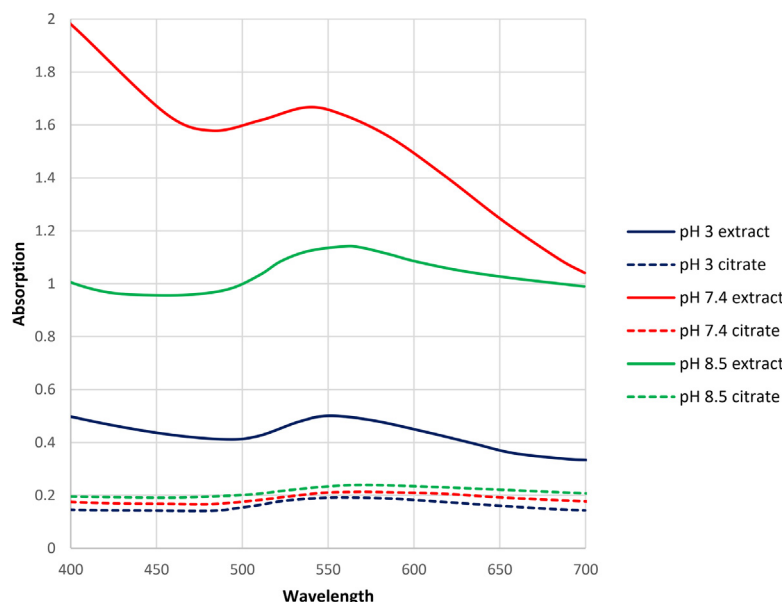


Fig. 1. UV-Vis absorption spectra of gold nanoparticles synthesized using soybean extract and trisodium citrate at different pH conditions (3.0, 7.4, 8.5).

such as syringic, vanillic, p-coumaric, and p-hydroxybenzoic acids [36]. These compounds contain hydroxyl (–OH) groups capable of donating hydrogen atoms and electrons [37], making them effective natural reducing agents in the green synthesis of AuNPs. Upon interaction with Au^{3+} ions, the phenolic –OH groups donate electrons and protons, reducing the gold ions to elemental gold (Au^0) and undergoing oxidation themselves, often forming quinones or other oxidized derivatives. This transformation is reflected in the FTIR spectrum, where the broad –OH stretching band typically observed around $3200\text{--}3600\text{ cm}^{-1}$ diminishes in intensity, while a sharper and more defined C=O stretching peak emerges around $1650\text{--}1750\text{ cm}^{-1}$. The presence of both –OH and C=O bands suggest partial oxidation of polyphenols, some hydroxyls remain, while others are converted to carbonyl groups. Such spectral evidence supports the proposed mechanism of Au^{3+} reduction and highlights the dual functionality of soybean extract as both a reducing agent and a stabilizer in nanoparticle formation [36].

A study by A. K. Chaurasia et al. reported no direct correlation between precursor concentration and nanoparticle yield, indicating that nanoparticle formation is primarily governed by the reducing capacity of the plant extract, provided sufficient Au^{3+} ions are available in the reaction medium [38]. In agreement with these findings, our results demonstrate that pH significantly influences the reducing ability of the extract, thereby playing a critical role in determining nanoparticle formation efficiency. pH plays a critical role in gold nanoparticle formation by simultaneously influencing the ionization state of phenolic reducing agents, the speciation of Au(III) , and the balance between nucleation and growth kinetics. Under acidic conditions, phenolic groups remain predominantly protonated (Ar-OH), resulting in weak electron-donating ability and limited reducing power. In addition, high proton and chloride ion concentrations stabilize AuCl_4^- and suppress its hydrolysis to more reactive hydroxo species, thereby reducing the overall reduction rate; increasing chloride concentration has been reported to decrease Au(III) reactivity by shifting the equilibrium toward AuCl_4^- [39]. As the pH increases, phenolic groups progressively deprotonate to form phenolate ions (Ar-O^-), which possess enhanced electron-donating capability and accelerate the reduction of Au(III) to Au^0 . Concurrently, AuCl_4^- undergoes pH-dependent hydrolysis to form mixed chloro-hydroxo complexes ($[\text{AuCl}_x(\text{OH})_{4-x}]^-$), which exhibit higher reactivity toward reduction than AuCl_4^- . The combined increase in reducing strength and precursor reactivity leads to faster supersaturation and nucleation. Under strongly alkaline conditions, this

rapid reduction results in burst nucleation and the formation of a high concentration of small nuclei in a short time; when stabilizing phytochemicals are insufficient to cap these newly formed surfaces, particle–particle collisions increase, promoting agglomeration rather than controlled growth. In contrast, near-neutral pH provides a balance between reduction rate, nucleation, and stabilization, enabling controlled growth and improved nanoparticle dispersion [40,41].

Similar reduction mechanisms involving phenolic compounds have been reported in other plant-mediated systems such as *Artemisia vulgaris* (Mugwort); however, the presence of isoflavones such as genistein, daidzein, and glycitein in soybean extract provides an additional functional advantage [24,42]. These compounds not only enhance electron transfer during Au^{3+} reduction but also contribute to stronger capping interactions, leading to improved nanoparticle stability. Compared to many plant extracts such as *Musa paradisiaca* (Banana), *Terminalia arjuna* (Arjun tree), *Theobroma cacao* (Cocoa) that rely primarily on polyphenols, soybean extract offers a more complex phytochemical matrix [43–45], which may explain the improved control over nanoparticle size and dispersion observed in this study.

3.3. XRD analysis

The XRD test was performed to determine the crystalline structure of AuNPs synthesized through bio- and chemo-synthesized methods under different pH conditions (3.0, 7.4, and 8.5). The 2θ scan range was selected between 35° and 80° , and the resulting diffraction patterns were analyzed using X-Pert software (Fig. 3). Regarding the databank of the Joint Committee on Powder Diffraction Standard (JCPDS No. 00-004-0784 and No. 00-004-0783) all significant peaks depict in figure is corresponded to crystallographic planes of face-centered cubic (fcc) gold nanoparticles.

The four common peaks at 2θ angle, namely 38.31° , 44.55° , 64.73° , and 77.71° , corresponding to the (111), (200), (220), and (311) planes diffraction, respectively. Clearly, the corresponding peak to the plane (111) is narrower, sharper and more intense than other Bragg reflection peaks at (200), (220), (111) denoting that synthesized Au nanoparticles are more tended to dominantly oriented along this plane. The observed peak broadening can be attributed to variations in diffraction domain size, distortions in the crystal lattice, and defects including stacking and twinning [46].

In general, peaks in AuNPs synthesized by extract are broader than peaks in those of synthesized by citrate which suggest smaller size

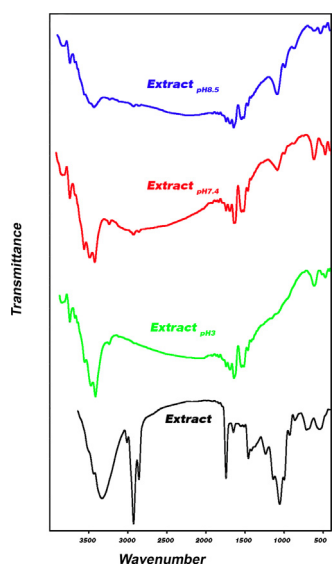


Fig. 2. FTIR spectra of soybean extract and corresponding gold nanoparticles synthesized using soybean extract.

and more amorphous characteristic due to biomolecules interfering the crystalline growth. Sharper peaks in pH 7.4 in citrate comparing to pH 3 concludes that neutral pH favors crystal growth in citrate-mediated synthesis. At pH 8.5 in extract peaks are slightly sharper than pH 3, this shows a modest improvement in crystallinity but not to the level achieved with citrate reduction. Average crystallite size estimations corroborate the observed peak shapes and intensities, showing that nanoparticles synthesized via the green method (soybean extract) possess smaller crystallite sizes ($23\text{--}28 \pm 1.50$ nm) compared to those produced through the chemical citrate route ($37\text{--}42 \pm 3.25$ nm). These findings are consistent with the SEM and UV-Vis results, confirming that green synthesis yields finer nanocrystals. These findings are also in agreement with previous reports on plant-mediated AuNP synthesis, where broader diffraction peaks are typically associated with smaller crystallite sizes due to biomolecular interference during crystal growth [14]. However, the crystallite sizes obtained in this study ($23\text{--}28 \pm 1.50$ nm) are smaller than or comparable to many reported plant-based systems [24], further confirming the effectiveness of soybean extract in controlling nanoparticle nucleation and growth.

3.4. Morphological and particle size distribution assessments

FESEM imaging revealed clear morphological differences between the nanoparticles synthesized by citrate and soybean extract under different pH conditions (Figs. 4 and 5). In all cases, particles appeared spherical with varying degrees of uniformity using 1% citrate and soybean seed extract as reducing agents.

SEM imaging across six synthesis conditions reveals clear distinctions in particle size and morphology between citrate- and extract-based AuNPs. Citrate-mediated synthesis consistently produced larger and more crystalline nanoparticles, with sizes ranging from 35–43 nm (39.37 ± 3.04 nm) at pH 3.0, 27–55 nm (37.18 ± 10.47 nm) at pH 7.4, and 19–39 nm (30.94 ± 8.27 nm) at pH 8.5. These particles exhibited well-defined boundaries and relatively uniform shapes, consistent with sharper and more intense XRD peaks. In contrast, soybean extract-based synthesis yielded smaller particles: 23–48 nm (34.86 ± 9.02 nm) at pH 3.0, 13–17 nm (15.31 ± 1.68 nm) at pH 7.4, and 20–53 nm (32.50 ± 12.60 nm) at pH 8.5. The extract samples showed broader size distributions and more irregular morphologies, especially at lower pH, reflecting broader XRD peaks. Notably, increasing pH improved particle definition and size in extract-based synthesis, with neutral

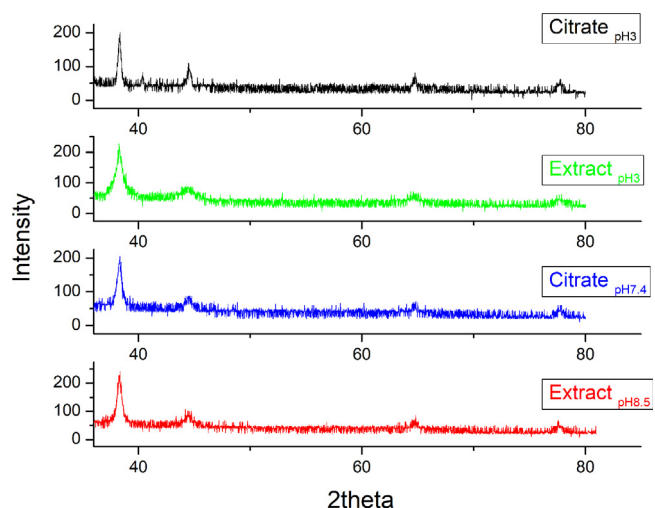


Fig. 3. XRD diffraction patterns of AuNPs synthesized using trisodium citrate and soybean extract under different pH conditions, showing characteristic fcc peaks at (111), (200), (220), and (311) planes.

conditions (pH 7.4) emerging as the optimal point, yielding the smallest and most uniform particles. However, citrate remained superior in producing consistently larger and more crystalline AuNPs across all conditions.

DLS revealed clear trends in particle size and dispersion behavior across citrate- and extract-mediated AuNPs. It worth mentioning that DLS measures hydrodynamic diameter, influenced by corona/aggregation [47], so it is expected to exceed SEM sizes. While both methods produced multimodal distributions, soybean-extract-mediated synthesis consistently yielded smaller average hydrodynamic diameters and lower polydispersity indices (PDI), indicating superior colloidal stability.

At pH 3.0, citrate-reduced AuNPs exhibited a mean size of 140.90 ± 13.05 nm with moderate dispersion (PDI $\sim 0.3 \pm 0.02$), consistent with SEM core sizes ($\sim 35\text{--}43$ nm) and XRD crystallite sizes ($\sim 37\text{--}42$ nm). In contrast, extract-synthesized AuNPs showed a smaller mean size of 109.6 ± 29.98 nm but a higher PDI (PDI $\sim 0.8 \pm 0.07$), reflecting broader size distribution and reduced stability under acidic conditions, despite finer nucleation.

At pH 7.4, citrate-based particles increased in size to 180.50 ± 35.12 nm with broader distribution and signs of aggregation. Extract-mediated AuNPs maintained a significantly smaller mean size of 108.10 ± 9.02 nm and the lowest PDI among all conditions, confirming optimal dispersion. These findings align with SEM-defined nanodots, smaller crystallite sizes (XRD), and the highest UV-Vis absorption intensity with SPR near 520 nm, indicating well-dispersed, optically active nanoparticles.

At pH 8.5, citrate-synthesized AuNPs reached their largest mean size (183.9 ± 30.14 nm) and exhibited the widest distribution, with visible aggregation. Extract-based AuNPs remained smaller (149.1 ± 18.21 nm) and showed improved dispersion relative to pH 3.0, though not as stable as at pH 7.4. SEM confirmed smaller cores ($\sim 19\text{--}39$ nm for citrate; $\sim 20\text{--}53$ nm for extract), while XRD maintained sharp peaks for citrate and broader ones for extract, reflecting crystallinity differences.

When compared with other green synthesis approaches reported in the literature, including Velgosova et al. demonstrated that precursor pH plays a decisive role in controlling nanoparticle morphology and size in green tea-mediated synthesis, with spherical nanoparticles of ~ 18 nm formed under alkaline conditions (pH 8–10), flower-like structures of ~ 60 nm at intermediate pH (4–6), and highly heterogeneous particles ranging from 20–250 nm under strongly acidic conditions (pH 1.3–2). In addition, reduced colloidal stability was observed at very low

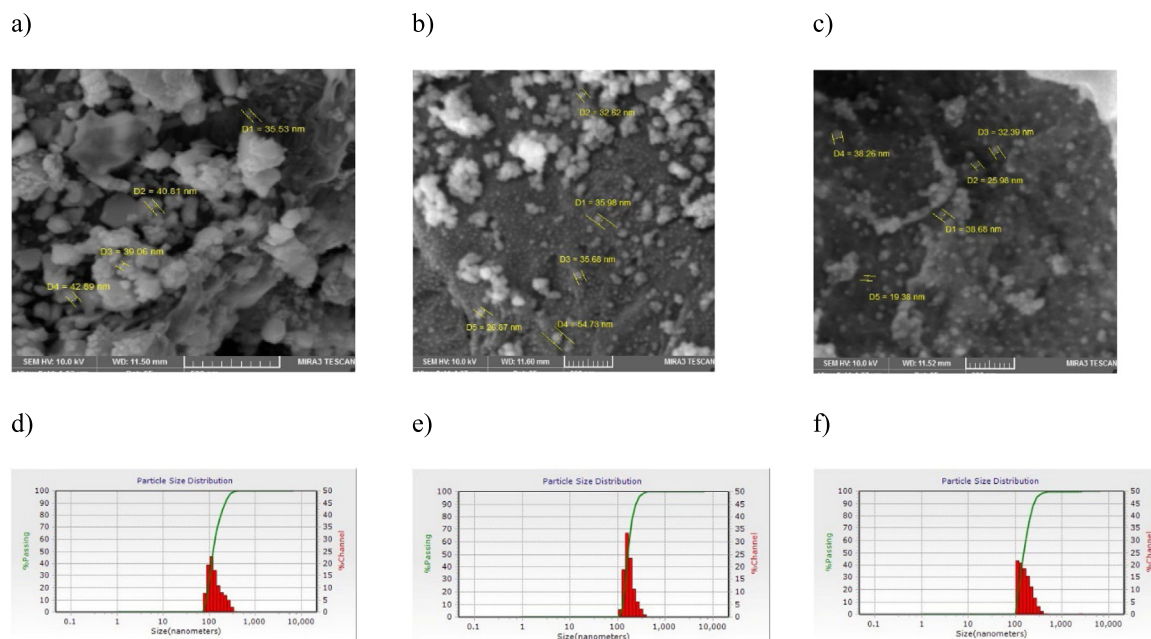


Fig. 4. FESEM micrographs and particle size distributions of gold nanoparticles synthesized using trisodium citrate at pH 3.0, 7.4, and 8.5 (a–c, respectively). (d–f) Corresponding DLS histograms indicating mean hydrodynamic diameters of 140.9 nm, 180.5 nm, and 183.9 nm, respectively.

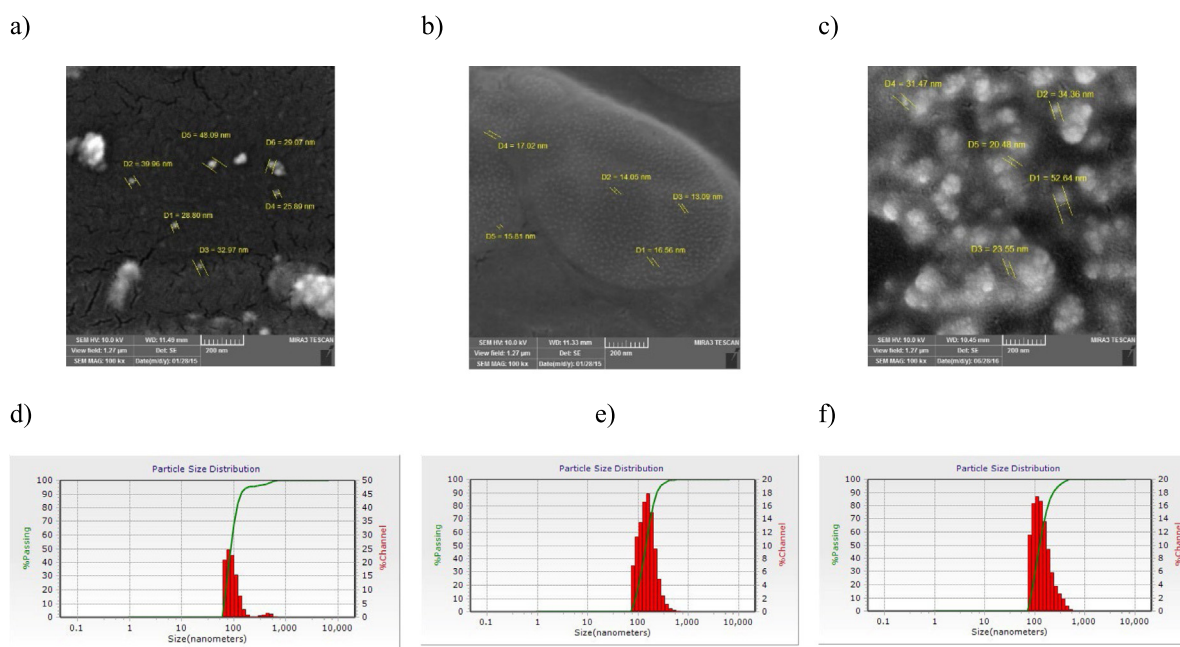


Fig. 5. FESEM micrographs and particle size distributions of gold nanoparticles synthesized using soybean extract at pH 3.0, 7.4, and 8.5 (a–c, respectively). (d–f) Corresponding DLS histograms displaying mean hydrodynamic diameters of 109.6 nm, 108.1 nm, and 149.1 nm, respectively.

pH, as evidenced by decreased absorbance intensity over time [48]. Consistent with these findings, the present study confirms the critical role of pH in governing nanoparticle formation and stability in plant-mediated systems. However, the soybean extract system exhibits more defined size control under optimized conditions, producing narrowly distributed nanoparticles (13–17 nm) at neutral pH (7.4). This indicates that, while pH-driven variability is an inherent feature of green synthesis, soybean extract enables improved control within a specific pH window. Furthermore, the inclusion of a parallel sodium citrate-mediated synthesis provides a direct comparative framework, allowing systematic evaluation of pH-dependent behavior across both biogenic and conventional reduction pathways.

Overall, the DLS results (summarized in Table 1) showed that soybean-extract-mediated AuNPs consistently yielded smaller average sizes at all pH values compared to citrate-reduced ones (109–149 nm vs. 140–184 nm, respectively). These findings are in strong agreement with the UV–Vis spectra, where soybean-synthesized nanoparticles exhibited shorter absorption wavelengths and higher intensities—indicative of smaller particle size and higher concentration.

The larger particle sizes obtained from DLS compared with SEM and XRD arise from the fundamental differences between the techniques. While SEM and XRD measure the dry-state core size and crystallite dimensions of nanoparticles, DLS determines the hydrodynamic diameter in solution, which includes contributions from the solvation layer,

Table 1

Mean hydrodynamic diameters of gold nanoparticles synthesized using trisodium citrate and soybean extract at different pH values.

pH	Mean size (nm) — Citrate	Mean size (nm) — Soybean extract
3.0	140.90 ± 13.05	109.6 ± 29.98
7.4	180.50 ± 35.12	108.10 ± 9.02
8.5	183.9 ± 30.14	149.1 ± 18.21

electrical double layer, and surface-bound molecules [49,50]. In the case of soybean-mediated AuNPs, the adsorption of phytochemicals such as polyphenols and proteins forms a biomolecular corona around the nanoparticle surface, significantly increasing the effective hydrodynamic size. In addition, DLS is highly sensitive to minor aggregation and is weighted toward larger particles, further contributing to the observed size discrepancy. These combined effects explain why DLS-derived sizes (108–184 nm) are substantially larger than core sizes observed by SEM (13–53 nm) and crystallite sizes calculated from XRD (23–42 nm).

The observed pH dependence aligns with the proposed mechanism of reduction:

- At low pH (3.0), the accumulation of HCl as a byproduct suppresses reduction efficiency, leading to fewer and slightly larger particles.
- At high pH (8.5), excess OH[−] ions reduce the electrostatic attraction between [AuCl₄][−] and the phenolic O–H groups in the extract, slowing reduction and promoting agglomeration.
- At neutral pH (7.4), optimal ion balance enhances reduction rate and nanoparticle stabilization, resulting in the smallest and most monodisperse particles.

These mechanistic insights highlight the importance of pH control and support the superior performance of soybean extract compared to traditional citrate reduction.

The comparative results demonstrate that soybean extract-mediated synthesis consistently produces smaller and more uniform AuNPs than the citrate-based chemical method. The neutral pH (7.4) condition was optimal for achieving the smallest particle size (15.31 ± 1.68 nm), and highest absorbance intensity. These outcomes confirm that soybean extract is a powerful, eco-friendly bioreductant capable of replacing synthetic agents in gold nanoparticle synthesis without compromising quality or functionality. Therefore, soybean extract not only enhances reduction efficiency compared to citrate but also provides natural stabilization through its phytochemical constituents, minimizing aggregation and yielding smaller, more uniform nanoparticles. In comparison with previously reported plant-mediated systems, the present results demonstrate improved control over nanoparticle size and dispersion, particularly under neutral pH conditions. These findings clearly highlight the effectiveness of soybean extract as a multifunctional bioreductant, offering a competitive and scalable alternative to conventional chemical methods.

4. Conclusion

This study demonstrates an eco-friendly and scalable strategy for the synthesis of gold nanoparticles (AuNPs) using soybean (Glycine max) extract as a natural bioreductant and systematically compares its performance with conventional citrate reduction across a range of pH conditions. The results confirm that soybean extract effectively mediates the reduction of Au³⁺ ions and may simultaneously help with stabilizing the resulting nanoparticles through phytochemical functional groups. Comprehensive characterization using UV–Vis spectroscopy, FTIR, XRD, FESEM, and DLS verified the successful formation of crystalline, predominantly spherical AuNPs. The green synthesis route produced smaller nanoparticles than the citrate-based method, with crystallite sizes of approximately 23–28 nm compared with 37–42 nm

for citrate-mediated particles. UV–Vis analysis revealed a blue-shifted SPR peak at 537 nm, corresponding to the smallest nanoparticles obtained at pH 7.4, while DLS measurements confirmed reduced hydrodynamic diameters (~108–149 nm) relative to citrate-derived nanoparticles (140–184 nm), along with improved size uniformity under these conditions. These results demonstrate that neutral pH provides the optimal balance between reduction kinetics, nucleation, and colloidal stabilization during soybean-mediated synthesis. The novelty of this work lies in its systematic, controlled comparison between soybean-mediated and citrate-mediated synthesis across multiple pH conditions, providing new mechanistic insight into pH-dependent nucleation and growth. The high phytochemical content of soybean extract, particularly phenolics and isoflavones, could enable simultaneous reduction and capping without the need for external stabilizers or chemical reagents. Consequently, soybean extract not only constitutes a viable green alternative but also affords enhanced control over nanoparticle size and dispersion, outperforming conventional citrate reduction under optimized conditions. Overall, this work establishes soybean extract as an effective, low-cost, and sustainable platform for AuNP synthesis and contributes to the advancement of green nanomaterial production. The resulting biogenic AuNPs have strong potential for catalytic, environmental, and biomedical applications. Future studies should address the mechanistic elucidation of phytochemical–metal interactions, long-term colloidal stability, and the functional performance of the synthesized nanoparticles.

CRediT authorship contribution statement

Ebrahim Izadi: Writing – original draft, Investigation, Formal analysis, Data curation. **Parisa Mirshahi:** Writing – original draft, Formal analysis. **Seyed Mahmoud Shahpar:** Writing – review & editing, Formal analysis. **Benyamin Ebrahimpour:** Writing – review & editing, Validation, Formal analysis. **Karim Osooli-Bostanabad:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization.

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Declaration of competing interest

On behalf of all authors, the author hereby declare that there are no known competing financial interests or personal relationships that could have appeared to influence the work reported in the manuscript.

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All authors have reviewed and approved the final manuscript and agree with its submission to Green Technologies and Sustainability.

Data availability

The authors confirm that all datasets generated and analyzed during this study are included within the manuscript.

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