



ORIGINAL RESEARCH PAPER

Chemistry

INFLUENCING FACTORS ON PHYTOCHEMICAL ASSISTED BIOSYNTHESIS OF Au-Ag BIMETALLIC NANOPARTICLES: A SHORT REVIEW

KEY WORDS: Bimetallic Nanoparticles, Phyto-chemicals, Plant Extract, Bio-reducing, and Stabilizing agents.

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ABSTRACT

The paradigm of green fabrication of metal nanoparticles using plant extracts is gaining interest in research of nanotechnology because it does not require hazardous chemicals, solvents, and high energy, and not the production of toxic chemicals as byproducts which can harm living beings as well as the environment along with the fascinating properties which can be inculcated in various fields. This present work elaborately discussed the efficacy of photochemical like phenolic acids, Flavones, Alkaloids, terpenes, carbohydrates as a reducing and stabilizing agents for the biosynthesis of bmnps and influencing factors such as Ph of plant extract, the molar ratio of metal ions, concentration of plant extract, reaction temperature, and reaction time on the biosynthesis of bimetallic (Ag-Au) nanoparticles using the plant extract. This study will help to predict optimal conditions for the biosynthesis of bimetallic nanoparticles.

1) INTRODUCTION

The noble metals nanoparticles (NPS) have great interest, enthralling and diverse properties including size-related electronic, optical, thermal, and catalytic properties as a result of their exceptional size and surface area to volume ratio compared with their bulk counterparts. With the advancements in the field of nanotechnology [1]. Bimetallic nanoparticles exhibit more captivating properties than metal nanoparticles as they show better optical, electrical, and medical applications due to their peculiar mixing patterns and synergistic effects of two metal nanoparticles that form bimetallic Nanoparticles[2].

Nowadays, silver (Ag) and gold (Au) NPS are used in a wide range of biomedical, drug delivery, catalytic, agriculture, antioxidant, anticancer, antibacterial, anti-fungal, antibiotics, entomological, and parasitological applications [3]. Au–Ag BMNPs have attracted increasing attention due to their interesting properties like catalytic, antimicrobial agents, diagnosis, therapy, biosensing, drug delivery, and industrial catalysis. It is vital to prepare non-toxic and safe Nps to use in these applications. [4].

Different methods have been employed by researchers in preparing nanoparticles, these include chemical, physical and biological procedures. Unfortunately, there are disadvantages like maintaining extreme conditions in a physical method which renders the procedure very expensive. Also, the use of toxic chemicals as a reducing agent in the chemical method restricts application in clinical fields. Moreover, pose a threat to human health and the environment. Thus, green nanotechnology is considered a tool to overcome the aforementioned challenges. Green synthesis of metal nanoparticles involves the use of organisms, plant extract via phytochemicals like alkaloids, saponins, a flavonoid present in them as reducing agents. Plant-based synthesis of nanoparticles has many advantages as the method is considered to be environmentally friendly, cheap, and rapid.[5]

Using plant extract for nanoparticles synthesis can be beneficial over other biological processes because it eliminates the elaborate process of maintaining cell cultures and can also be suitably scaled up for large scale nanoparticles synthesis[6]. Bimetallic Au–Ag NPS have been prepared with Azadirachta indica leaf broth[7], spherical Au–Ag NPS(average size 20 nm) using Gloriosa superba leaf extracts [3]. Au–Ag bimetallic NPS were synthesized by using

leaf extracts of fenugreek, coriander, and soybean as reducing agents in a single-pot reduction process [8]. Similarly, Sharma et al. used an aqueous extract of clove buds to prepare Au–Ag bimetallic NPS[9]. Apart from this, other types of plant materials have also been used for a similar purpose [6].

II) PLANT EXTRACT AS A REDUCING AND STABILIZING AGENT.

KasiGopinath et al [3] proved with FT-IR spectroscopy analysis that, the possible biomolecules for reduction of Au+3 and Ag+ ions into silver, gold, and silver/gold bimetallic nanoparticles using the Gloriosa superba leaf extract. After the synthesis of the nanoparticles, the band in the 1259 cm-1 spectrum vanished, indicating that the glycosides and the water-soluble tannins that were present in the leaf extract facilitate the reduction of the metal ions to metal nanoparticles. S.Yallappa et al confirmed by FT IR spectroscopy that, the formation of Au, Ag, and Au–Ag NPS using aqueous J. sambac leaves extract, contains some proteins and metabolites such as polyphenols, flavonoids, and terpenoids are involved in the reduction and stabilizing agents of these NPS, After the reduction of metal ions into metal atoms, these biomolecules coveron the surface of NPS and serve as surfactants, which can prevent the aggregation of NPS.

The phytomolecules based stabilization of metallic NPS typically renders negative charge on the surface of NPS, and this can be verified with the zeta potential values of all the NPS, which were determined to be negative and were in the range of 9 to 19 mv at pH = 7. This gave additional evidence that the prepared metallic NPS were indeed capped by Phyto molecules of PE [9].

The shape and Morphology of synthesized BMNPs may affect by the reaction temperature, Reaction Time, concentrations of the extract, Concentration of precursor solution (metal ions), and pH(10).

1) Effect of the P^H of plant Extract (PE)

The PH of plant Extract (PE) is a critical factor influencing the size, stability shape, and composition of the nanoparticles (11). Mostafa et al [12] studied the efficacy of the Gmelina leaf extract in the synthesis of Ag, Au, and Ag-Au nanoparticles within the PH range of 3-10. The study revealed that the OD of the SPR band increased with PH, along with a blue shift. The results reported that at the low PH the Au, Ag NPS prefers to

aggregate to form large particles rather than nucleation. However, higher PH facilitates the nucleation and the ensuing formation of more number of smaller nanoparticles. This can be explained as increasing the PH would increase the electric charge of biomolecules attached on the surface of Nanoparticles, further it affect the stabilizing abilities of biomolecules and followed by the growth of the Nanoparticles (13). Moreover, the particles formed in the acidic medium were less stable and precipitated within 12 hrs, while the particles prepared at pH 9 were stable for one week. TEM analysis revealed that the prepared Au nanoparticle was spherical with an average size of 10 nm, while the AgNPs and Ag-Au BMNP were exhibited a range of different morphologies, the Ag NPD wherein the range of 70 nm and Ag-Au BMNP were smaller than Ag NPS.

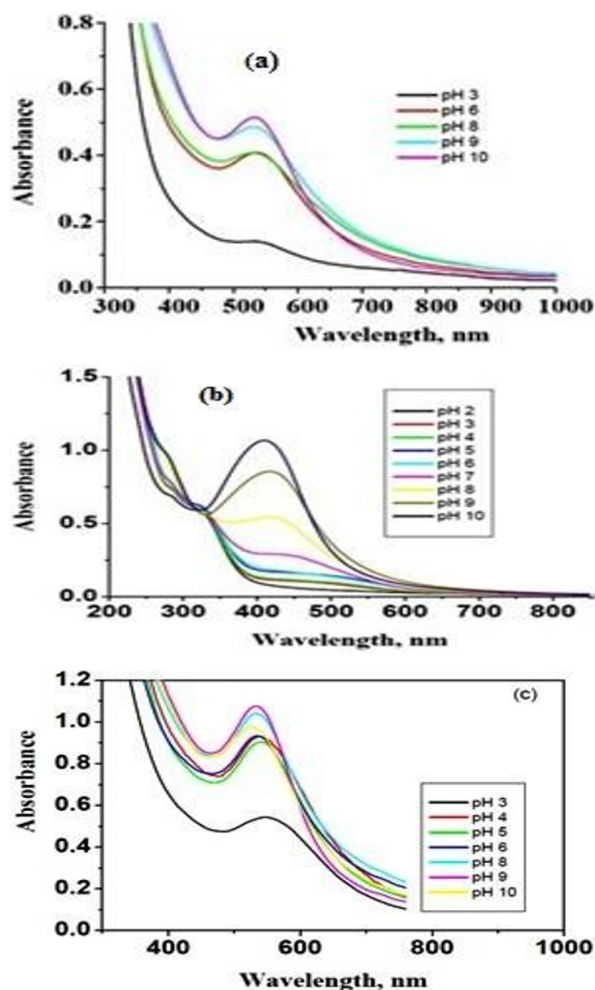


Figure 1: UV-Vis spectra of (a) AuNPs (b) AgNPs (c) AuAgNPs . formed using different pH of Gmelina extract.[12]

Mukti Sharma et al [14] noticed the effect that PH of seed extract of Madhuca longifolia on the morphology of Au–Ag bimetallic nanoparticles in simultaneous co-reduction. At PH 4 and 6, initially, a single peak of Au has observed at 510 and 500 nm, respectively. At both PH values over time, one more peak of Ag appeared at 430 and 420 nm. The delay in the second peak due to the relatively slow formation of Ag NP compared to Au NPS and formed Ag NPs assembled on the surface of the Au NPs to results in the core-shell structure of BMNP which was confirmed by the formation of two SPR peaks. At PH 8 and 10, a single peak at 470 and 460 nm appeared respectively. However, no change was discerned at a further higher PH. The Blueshift from 470 to 460 nm at PH 10 is attributed to the formation of small-sized and more stable bimetallic nanoparticles. The TEM analysis confirmed the

spherical shape of bio fabricated bimetallic Au–Ag nanoparticles having a diameter range of 34–66 nm.

Sabyasachi Ghosh et al [15] revealed that as increasing the PH of leaf extract of *P. Mongolia*, the O.D increased to different extents for Ag, Au, and Au-Ag (1:1)

NPS at 415 nm, 535nm and 470nm, respectively, the optimum phof leaf extract for the synthesis of Au, Ag, Au-Ag alloy predicted as 9, 11, and 12 respectively. Further increase in PH (beyond optimum PH values) led to a decrease in O.D at their respective λ_{max} values.

2) Effect of molar ratio

Ganaie et al [16] studied the effect of molar ratio on Size, Shape, and morphology in co reduction of Au-Ag BMNPS at PH 4 @ 10 using aqueous extracts of *Antigonon leptopus* plant.

a) Effect of concentration of Au(III) At PH 4:

As the proportion of Au (III) added to the reaction mixture of Ag(I) and the extract was increased from 0.5-fold to 1-fold, two spectral peaks were seen at 400 nm and 520 nm respectively, of which the OD increased with the increase in the proportion of Au (III). At Ag (I) @ Au (III) ratios were 1:2, and still higher proportions of Au (III), the intensity of the peak at 522 nm increased while the peak at 400 nm disappeared [16].

b) At PH 10:

When Au(III) and Ag(I) of 0.5:1 ratio were mixed with extract, there was an intense sharp band at 417 nm. Further, an increase in the proportion of Au(III) from 2-fold to 4-fold relative to the Ag(I), led to the continuous redshift in λ_{max} from 417-510 nm along with the broadening of peaks, which signifies a shift from isotropic to anisotropy [16].

c) Effect of concentration of Ag(I) At PH 4:

In the reaction mixture, as the proportion of Ag(I) was increased from 0.5-fold to 1-fold relative to Au(III), a spectrum with two peaks appeared, a sharp peak at about 510 nm and a broader peak at about 417 nm associated with an increase in OD. This pattern was reversed with a further increase in Ag(I) as to Au(III) proportions (2:1 and above), which led to a progressive loss in OD [16].

d) At PH 10:

A single spectral peak was seen at 575-420 nm. An increase in the proportions of Ag(I) from 0.5-fold to 4-fold as to Au (III), resulting in a blue shift of the λ_{max} from 575 to 420 nm along with the sharpening and simultaneous increase in OD. The increase in OD reflects an increase in BNP formation whereas the sharpening of bands indicates the shift from anisotropy to isotropic, with an increase in the proportion of the Ag (I) [16].

Sabyasachi Ghosh et al[15] notice that, in his research work of Biosynthesis of Ag, Au, and Au-Ag NPs with aqueous leaf extract of *P. longifolia* The SPR peaks of different Au–Ag compositions (1:0), (0.75:0.25), (0.50:0.50), (0.25:0.75) and (0:1) were recorded at λ_{max} 535, 505, 470, 440 and 415 nm, respectively. The SPR peak (λ_{max}) of Au–Ag NPS shifted from 415 to 535 nm (Red-shift) and broadening results with increasing Au+3 mole fraction, which indicates the formation of the larger and anisotropic structure of Alloy BMNPS. However, the SPR peak intensity decreases as the Au+3 mole fraction increased from 0 to 1.0 suggesting that the strong alkaline medium is not appropriate for the reduction of Au³⁺. They observed that maximum O.D of the SPR band achieved with 1mM of an equimolar ration of Au³⁺ and Ag⁺, as increased the concentration of precursor solution to 2mM the O.D of SPR band decreased rapidly, consequently low concentration of precursor metal ion/ions solution is suitable for the biosynthesis of Mono and metallic NPS.

3) Concentration of Plant Extract

Merajuddin Khan et al[9] investigation proved the significant

role of concentration of plant extract of *Pulicaria undulate* on reaction time, in controlling the shape, size, stability, and morphology of nanoparticles in the biosynthesis of Au, Ag, and Au–Ag. At a low concentration of PE, the absorption bands of Ag, Au, and Au–Ag appeared after several minutes of adding the PE to the reaction mixture. At low concentrations of PE, the rate of reduction was slow in the formation of Au and Au–Ag alloy NPS and completed after 120 min, whereas the formation of Ag NPS was completed after 60 mints, the slow reduction rate and low concentration of PE resulting in slow nucleation and growth processes, the nuclei coalesced, resulting in the growth of anisotropic, irregular shaped NPS. At a higher concentration of PE, the characteristic absorption bands of Ag, Au, and Au–Ag appeared immediately after the addition of PE and the intensities of these bands increased rapidly with time, reaches a maximum within 15 and 30 mints respectively.

This shows a rapid reduction, and at the same time, enough concentration of PE available to stabilize the surface energy of the nuclei, resulting in a small size of quasi-isotropic NPS. The stability of NPS is attributed to the attachment of Phyto molecules on the surface of the NPS through electrostatic attractions, which stabilize metallic NPS by typically renders a negative charge on the surface of NPS [17]. The calculated zeta potential values of all the NPS were determined to be negative, and were in the range of 9 to 19 mv at pH = 7. Furthermore, the zeta potential data also revealed that with increasing concentration of PE, the stability of the NP suspension was also slightly increased [9]. Sabyasachi Ghoes et al [15] found a similar trend in the biosynthesis of Au–Ag Alloy NPS using aqueous leaf extract of *P. longifolia*.

Ganaie et al [16] reported that at pH 4 as the concentration of PE increased from 10 folds to 20 folds results in two SPR peak at 414 and 520 nm. On further increased the conc., to 40 folds, accompany the increase in O.D, which indicates the formation of more number of Core-Shell BMNPS but on the further increment of conc., of PE shown a reverse trend in O.D. As the proportion of extract was increased from 10-fold to 150-fold at pH 10, a single SPR band appeared between 420–495 nm, along with the progressive increase in its OD and change associated in the shape of the SPR band from broad and asymmetric to increasingly narrow and symmetric. This, in turn, indicates an increasing shift from polydispersed to monodispersed with an increase in the proportion of the extract, the blue shift signifies the decrease in the size of BNP's.

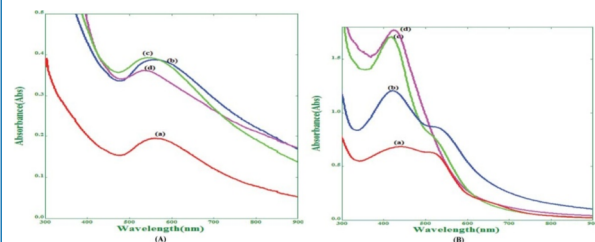


Figure 2. The UV visible absorption spectra of BNPs recorded at (A) at pH 4 by the mixing of Au(III) to AgNPs synthesised with leaf extract concentration: (a) 150 mg/L, (b) 300 mg/L, (c) 600 mg/L and (d) 1200 mg/L (B) at pH 10 by the addition of 1 mg/L Ag(I) to 15 mg/L of Au(III) and root extract concentration: (a) 150 mg/L, (b) 300 mg/L, (c) 600 mg/L, (d) 1200 mg/L and (e) 2250 mg/L. [16]—

Work by Mohamed et al [18] proved the significant role of plant extract in controlling the shape, size, and distribution of NPS. The PE acted as a surfactant by adhering to the particle surface, hence preventing aggregation of nanoparticles to form larger particles. Tamuly et al. [6] reported that interactions between biomolecules and nanoparticle surfaces led to isotropic growth and therefore results in

spherical nanoparticles. The presence of n-electrons possibly caused biomolecule-nanoparticle interactions. Mostafa et al [12] observed, At a low concentration of PE, the formation of Ag, Au NPS, and Au–Ag started after several minutes and completed by 2hr in the case of Au and alloy but in the case of Ag 60 mints. But at higher concentrations bio-reduction has started spontaneously and completed by 30 minutes.

4) Reaction Time

Tamuley et al [6] studied the reduction of the Ag+ and Au3+ ions (1:1) mixture using *P. pedicellate* leaf extract and monitored as a function of time by UV–Visible spectrometer. It was interesting to note that the rate of increase in absorbance at max 582 nm was much faster in the case of the 1:1 Ag+: Au+3 mixture than in the case of the pure Au+3 and Ag+ ions. The absorbance of the SPR of Au NPS formed from the bimetallic solution reaches a maximum within 90 minutes while 3hrs the in case of pure Au+3 solution. This indicated that in a competitive reduction process involving both Ag+ and Au3+ ions. Concurrently absorbance of Ag NPS was not noticed due to the resulting smaller size of Ag NPS and does not form a uniform layer on the Au NPS surface hence Au–Ag Core-Shell BMNPS were not formed. TEM analysis confirmed the size range of 3 - 45nm of BMNPS.

Ganaie et al [16] investigated the trend of reduction of Au+3 and Ag+ ions with PE as a function of time at PH 4 @ 10. The OD of SPR bands at PH 4 increased gradually with time till it reached a maximum, evidently due to the increase in the concentration BNPs. At PH 10, the BNP formation was almost complete within a few minutes of the commencement of the synthesis. The OD of the reaction mixtures increased only marginally afterward and by the fourth hour had reached their maximum. The shape of the spectra remained unchanged which indicates that the pattern of mono dispersion of the BNPs and their shape isotropic.

Mostafa et al [12] observed vast variations in the reduction of Au+3 and Ag+. The reduction of Au+3 started within 5 mints, with time O D increases and reached to maximum after 35 mints, but in the case of formation of Ag NPS and Au–Ag BMNPS under similar condition, the reduction process started after 24hrs @ 60 mints respectively and completed after 4 days which implies that formed Ag

NPS are very stable without aggregations. Li sun et al [17] noticed a similar effect of reaction time on the rate of reduction of metal ions, as increasing reaction time the SPR band intensity increased along with the blue-shift in various proportions of Au+3 and Ag+. The Blue-shift attributes to an increase in the proportion of Ag+ ions to Au+3 ions and indicates the formation of small size on NPS.

5) Reaction Temperature

The no of particles requires enough energy to overcome the critical energy barrier, facilitated by increasing temperature. For this reason, the synthesis of Au NPS was carried out at boiling points of the mixture [19]. According to Mahrajuddin Khan et al [9] biomolecules (reductant) often require elevated temperature and longer reaction time for the biosynthesis of nanoparticles which may hamper broad applications. The formation of Ag nanoparticles with *P. glutinosa* PE required 24hrs at 90° C but in contrast, PE of *P. undulate* formed the metal nanoparticles at ambient temperature, which reflects strong reducing property of *P. undulates* due to the presence of more concentrated phytochemical.

Table: 1

BMNP	Temperature	Average Size	Structure	Ionic ration (Au: Ag)	Ref
Au–Ag	Room temp	10–20nm	Alloy	1:1	10
Au–Ag	Room temp	5–12	Alloy	9:1	4

Au-Ag	Room temp	10-20nm	Alloy	1:1	9
Au-Ag	30°C	3-45nm	Alloy	1:1	5
Au-Ag	70°C	5-15nm	Alloy	3:1, 1:1, 1:3	2
Au-Ag	Boiling temp	10.9nm	Alloy	1:5	6
Au-Ag	50°C	10-50nm	Core-Shell	0.5:1	11

Sabyasachi Ghosh et al[15] proved that with temperature formation of Ag, Au NPS and Au-Ag alloy NPS accelerated. But the maximum absorbance of Ag, Au NPS, and Au-Ag alloy was observed at 80°C, 50°C, and 70°C respectively. Further increase of temperature causes a decrease in absorbance thus the above-mentioned temperature is recorded as optimum temperatures for the biosynthesis of Ag, Au NPS, and Au-Ag alloy NPS.

III) CONCLUSION:

This work emphasis on the facile, one pat, and benevolent synthesis of Au-Ag Bimetallic Nanoparticles using green protocols for the reduction and stabilization of metal nanoparticles may promote green chemistry. Plant extracts contain secondary metabolites (such as phenolic acid, Flavonoids, Alkaloids, Carbohydrates, etc) acted as reducing and stabilizing agents. Utilization of plant extract has eliminated the requirement for Sophisticated equipment, high temperature, pressure, high energy, hazardous chemical and by products and stabilizing agents for the preparation of NPs, and also discussed the influencing factors such as the pH of Plant extract, reaction temperature, the concentration of plant extract, reaction time and the Molar ratio of the precursor solution on sizes, shape, and morphology of Nanoparticles.

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