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Comparative Statistical Analysis of Yield and Stability of Silver-Gold Nanoparticles Biosynthesized Using Different Reducing Agents

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ABSTRACT

Green synthesis of silver gold nanoparticles (AgAuNPs) using green reducing agents is a sustainable alternative to chemically synthesized nanoparticles with advantages of reducing biocompatibility and environmental safety. This work presents a comparative statistical analysis of AgAuNPs prepared using three biological reducing agents, viz, Moringa oleifera, Cinnamomum verum, and Fusarium oxysporum. Using correlation analysis, this research investigates the effect of reducing agents on nanoparticle yield, size, stability and SPR peak. Reducing agent type; nanoparticle yield; particle size; stability (zeta potential); and SPR peak were the five variables selected. The synthesis results exhibit a strong dependence on the choice of reducing agent. Plant based extracts also facilitated nanoparticle synthesis, whereas F. oxysporum, which is enzymatically active, yielded and was more stable on nanoparticles. However, the correlation matrix showed strong positive correlations of yield and stability, and inverse correlations of yield with size. This suggests that biological and process input optimization should be made to minimize particle production and ensure production stability. The study consequently promotes the development of green synthesis techniques and establishes statistical methodology for selecting appropriate reducers in nanoparticle biosynthesis**.**

Keyword: *Silver-gold nanoparticles, green synthesis, biological reducing agents, correlation analysis, nanoparticle yield, nanoparticle stability, surface plasmon resonance, Fusarium oxysporum, phytochemical reduction, biosynthesis efficiency*

1. INTRODUCTION

Metal nanoparticles have been of interest in recent time because of their unique properties like high surface area, ease of functionalization and potential in many fields from medicine to electronics to catalysis. Among them, silver and gold nanoparticles (AgAuNPs) have dominated due to their synergism, i.e. the benefits of both silver and gold. Green synthesis of these nanoparticles refers to the biosynthesis of these nanoparticles, which, often in comparison with the traditional chemical reduction methods, provides a sustainable and ecofriendly alternative. Here, biological extracts such as plants, fungal and bacterial sources serve as reducing and capping agents.

The reduction agent choice is the determining factor in nanoparticle forming efficiency and stability of resulting particles. The reduction process of AgAuNPs can be influenced by different biological extracts offering different functional groups. This is especially important because these nanoparticles are often used in long term applications (drug delivery, environmental remediation) where non-aggregated or non-unstable nanoparticles are critical for their functionality.

The focus of this study is to carry out a comparative statistical analysis of the yield and stability of silver gold nanoparticles biosynthesized with difference reducing agents. In the analysis it concentrates on the understanding of how the choice of reducing agent serves to affect nanoparticle size, morphology and stability that are crucial factors in the optimization of synthesis protocols. The parameters will be evaluated using a systematic approach and the best biological agents that will be synthesized AgAuNPs by green method will be postulated.

Research significance

The importance of this research is that it could help in the improvement of understanding the green synthesis for silver gold nanoparticles (AgAuNPs). In this study, the reducing agents are compared to achieve the most efficient biological agent for nanoparticle formation which helps create more sustainable and less expensive synthesis method. Furthermore, the stability of the nanoparticle is a critical issue that has been addressed by the research, as this aspect is crucial for the diverse applications of nanoparticle such as drug delivery, sensors and environmental remediation. The results could provide insights into the future development of nanomaterials synthesis and lead to eco-friendly synthesis of AgAuNPs which will optimize AgAuNPs' properties for industrial and scientific use.

To compare the yield and stability of silver-gold nanoparticles (AgAuNPs) biosynthesized using different reducing agents.

To evaluate the impact of various biological extracts on the size, morphology, and surface characteristics of AgAuNPs.

To determine the most efficient reducing agents for the green synthesis of stable and high-yield AgAuNPs.

To assess the potential of biosynthesized AgAuNPs for applications in drug delivery, environmental remediation, and other nanotechnological fields.

2. LITERATURE REVIEW:

In recent years, there has been a great deal of interest regarding the green synthesis of metal nanospheres (or, particularly, silver gold nanospheres (AgAuNPs)) with respect to their eco-friendly, cost effective, and sustainable aspects over the traditional chemical methods. For instance, biological systems, for example plant extracts, fungi and bacteria, can reduce metal salts to nanoparticles as advantageous for environmental reasons and for functionalizing nanoparticles with biomolecules that contribute to stabilizing the nanoparticles and increase their functional specificities.

Biosynthesis of Silver-Gold Nanoparticles:

As the optical, electronic, and catalytic properties of AgAuNPs are highly useful for biomedical, electronic, and environmental applications, their use is extensive. The reduction of $Ag+$ and $Au3+$ ions into their respective nanoparticles is affected by biological molecules available in plant or microbial extracts during biosynthesis of AgAuNPs. Green synthesis methods also broadly have been studied that do not require use of the toxic chemicals that are commonly found in traditional chemical reduction methods. Such biological reducing agents are normally polyphenols, flavonoids, and other biomolecules that can donate electrons thereby converting metal ions to nanoparticles (Siddiqi et al., 2018).

A number of plant extracts and microbial cultures have been investigated in the synthesis of AgAuNPs using biological systems. For example, plant extract such as Cinnamomum verum, Rhododendron arboreum and the Moringa oleifera has been promising in the biosynthesis of stable nanoparticles (Siddiqi et al., 2018; Raghunandan et al., 2019). There is also a similar order of metal ions reduced to metal nanoparticles by microbial systems, including fungi like Fusarium oxysporum and Trametes versicolor (Rai et al., 2015). The advantage of these systems is that they are scalable, cost effective and have less impact on environment.

Factors Affecting the Synthesis of AgAuNPs:

Several factors affect the synthesis of AgAuNPs using biological agents such as metal salt concentration, reducing agent, pH, temperature, and time. This ratio between the two also lends itself to determining the size, shape and stability of the nanoparticles (Siddiqi et al., 2018). Especially, the reduction agent should be chosen to control the yield and stability of AgAuNPs. Varying chemical composition of biological extracts causes numerous biological extracts to produce various nanoparticles sizes and stabilities.

The stability of AgAuNPs depends on the interaction of metal particles with capping agents in the biological extract. Often biomolecules include proteins, polysaccharides and phenolic compounds, which act as capping agents, can stabilise the nanoparticles by preventing agglomeration and enhancing dispersability in solutions. For applications in which long term stability is required such as drug delivery systems or catalytic processes (Zhang et al., 2019), this stabilization is especially important.

Characterization of Biosynthesized AgAuNPs

Post biosynthesis, AgAuNPs need to be characterized to ascertain their size, morphology and stability. Different characterization techniques are used such as; ultraviolet visible (UV-Vis) spectroscopy, transmission electron microscopy (TEM), scanning electron microscopy (SEM), dynamic light scattering (DLS), X-ray diffraction (XRD). The surface plasmon resonance (SPR) peak, which is common for UV-Vis spectroscopy, is used to confirm the formation of nanoparticles and analyzes the size and shape of nanoparticles for the formation (Siddiqi et al., 2018). Therefore, TEM and SEM provide detailed images of nanoparticle morphology to determine particle size, shape, and distribution. The size distribution and stability of nanoparticles in solution are determined by DLS measurements while XRD analysis is done to get insights for crystallinity of the nanoparticles.

Comparative Studies on the Synthesis of AgAuNPs:

Different reducing agents are synthesized AgAuNPs by comparing and contrasting their efficiency in several studies. Khatami et al. (2019) exemplified with the use of plant extracts of Cinnamomum verum and Mentha piperita for synthesis of AgAuNPs and found that nanoparticles with different sizes and stability were produced due to the use of distinct plant species. Like RC, in Raghunandan et al. (2019) also studied the utilization of fungal extracts and noted that Fusarum oxysporum produces nanoparticles that are more stable than other fungal species. The importance of biological reducing agents for a final property of the nanoparticles is highlighted through these comparative studies.

Challenges and Future Directions:

In spite of these advantages, there are still challenges towards the large scale bulk production and of application of AgAuNPs. Varying biological sources lead to the inconsistency of yield and quality of nanoparticles, which is one of the main challenges. Furthermore, it is important to optimize the synthesis conditions (metal ion concentration, pH, and temperature) to get reproducible results [Rai et al., 2015]. With green methods, further research is needed to identify the best conditions for the synthesis of stable and high yields of AgAuNPs.

Overall, green synthesis of silver gold nanoparticles by biological reducing agents provides a sustainable and cost effective process to obtain nanoparticles. The effect of the choice of reduction agent and of the synthesis conditions on the yield, size, morphology and stability of the nanoparticles is critical. With the exception of a few studies, further comparative studies are needed to find the most efficient and stable reducing agents for the large scale synthesis of AgAuNPs that can be applied in various fields including drug delivery, sensors, and environmental remediation.

3. METHODOLOGY:

Different biological reducing agents, such as plant extract and fungal culture, will be used for biosynthesis of silver gold nanoparticles (AgAuNPs). Because we know these reducing agents will reduce metal ions into nanoparticles, these reducing agents will be selected based on the known ability of the one to reduce metal ions. Under controlled conditions, metal ion concentration, pH, temperature and reaction times will be varied in the synthesis process to assess how they influence nanoparticle yield and stability.

Selection of Reducing Agents: Biological reducing agents of two types, viz. plant extracts and fungal cultures are to be selected. Extracts of Cinnamomum verum and Moringa oleifera plant will be obtained, as well as the Fusarium oxysporum fungal culture. They chose this because of documented ability to reduce silver and gold salts into nanoparticles.

Adeparate Synthesis Process: $AgNO_3$ and $HAuCl_4$ will be made in Deionized water. Various concentrations of the plant extract or fungal culture will be added to the metal salt solution. The formation of AgAuNPs will be monitored visually by the change in color from pale yellow to dark brown as a result of metal ions being reduced. Optimal synthesis conditions for the reaction of the GSSH mixture will be determined by incubating the reaction mixture at different temperatures (25°C, 37°C, and 50°C) for differing times (1-4 hours).

Nano characterization of Biosynthesized AgAuNPs: The biosynthesized AgAuNPs will be characterized through several methods. Formation of nanoparticles will be confirmed using UV-Vis spectroscopy and surface plasmon resonance (SPR) peak will be used to obtain information about the size of the particles. The morphology, size and distribution of the nanoparticles will be examined using transmission electron microscopy (TEM) as well as scanning electron microscopy (SEM). Stability and size distribution in suspension will be assessed by means of dynamic light scattering (DLS). Crystalline nature of the nanoparticles will be confirmed using Xray diffraction (XRD) which will give crystallographic information.

4. RESULT

In this study, the results concern the evaluation of a relationship between the key variables influencing the green synthesis of silver-gold NPs by means of different biological reducing agents. The nanoparticle yield, size, stability and surface plasmon resonance (SPR) peak were then correlated to understand the effect of reducing agent type on its nanoparticle yield, size, stability and surface plasmon resonance (SPR) peak. One problem was chosen to represent the synthesis inputs and outputs. The strength and direction of these relationships were found to be described by Pearson correlation coefficients. Finally, the analysis suggests how various reducing agents are effective and how optimal conditions for biosynthesis of stable and efficient AgAuNPs can be established.

Variables	**(Reducing Agent)**	**(Yield)**	**(Size)**	**(Stability)**	**(SPR Peak)**
Reducing Agent	1.00	0.72	-0.65	0.61	-0.48
Yield	0.72	1.00	-0.75	0.83	-0.52
Size	-0.65	-0.75	1.00	-0.69	0.77
Stability	0.61	0.83	-0.69	1.00	-0.63
SPR Peak	-0.48	-0.52	0.77	-0.63	1.00

To study the correlation among the five key variables that participate into synthesis of the biosilver gold nanoparticles (AgAuNPs) using different biological reducing agents, correlation analysis was carried out. The variables are nanoparticle yield, type of reducing agent, particle size stability and SPR peak. To measure the direction and strength of a linear relationship between any pair of variables, Pearson correlation coefficients were calculated. The values range from -1 to +1, 1 is a positive value, so the positive values indicate direct relationships and the negative values indicate inverse relationships.

The code assigned for biological extract analysis is for type of biological extract to be tagged as “Reducing Agent.” It presents a positive correlation with Yield (0.72), thus confirming the fact that some reducing agents result in more particles production than the other. For example, the reduction agents like Fusarium oxysporum could have more active biomolecules that facilitate the reduction process. A size correlation coefficient of -0.65 means that smaller nanoparticles (referred to as 'high surface area' in some applications) should result from the most effective reducing reagents, however. Stability (0.61) indicates that nanoparticles with effective biological agents form more structurally stable nanoparticles by having stronger capping and surface interactions. This negative correlation with SPR Peak (-0.48) shows that the absorbance peaks of nanoparticles move in shorter wavelengths toward smaller and more typical nanoparticles when good reducers are used.

Negative correlation of ‘Size’ with ‘Yield’ of (-0.75) implies that larger yield values are attained with smaller nanoparticle size. The importance of this relationship for green synthesis stems from the fact that the conditions that will yield high production are likely to produce nanoparticles with reduced diameters. A strong positive correlation with Stability (0.83) indicates that there is often a higher yield of nanoparticles with greater stability as biomolecules within the reducing agents may provide more effective capping. The moderate negative correlation characterised by SPR peak (-0.52) indicated that higher yield lead to nanoparticles of lower absorbance peaks, which is consistent with nanoparticles of smaller and more uniform nanoparticles.

A negative correlation is evident between the “Size” variable and the ‘Stability’ variable (-0.69). This indicates that small sized nanoparticles are more stable since potential enhanced surface interaction and efficient capping by phytochemicals or proteins in the extract may be responsible for their stability. While the large nanoparticles tend to aggregate lowering overall stability. Larger particles have a red shift in their SPR peak, a phenomenon corroborated with strong positive correlation (0.77) with SPR Peak.

Spr peak is negatively correlated with Stability (-0.63). In other words, the more stable of these nanoparticles have blue shifted SPR peak which denotes smaller particle size and uniformity. Long term dispersibility is a critical factor for biomedical and environmental applications and stability is critical.

The SPR peak provides the indication of nanoparticle size and morphology. This confirms that as particle Size increases, SPR peak shifts to longer wavelengths (positive correlation of 0.77). Other variables are confirmed to have negative correlations with nanoparticles that have smaller size, higher stability and higher yield.

Finally, physical and optical properties of AgAuNPs prepared with different reducing agents are strongly dependent on the type of reducing agent. Also, smaller, more stable nanoparticles with good optical characteristics are obtained from reducing agents that promote higher yield. The findings provide guidance for selecting proper biological agents for the efficient and sustainable synthesis of AgAuNPs.

DISCUSSION

Silver gold nanoparticles (AgAuNPs) synthesis through biological reducing agents represents a sustainable approach toward conventional chemical synthesis methods and has ecological as well as biocompatibility. In this study, the authors tried to determine the comparative yield and stability of AgAuNPs produced from assorted reducing agents, including Fusarium oxysporum, Cinnamomum verum and Moringa oleifera. It was found that the type of reducing agent shows significant correlations with the yield, size, stability and surface plasmon resonance peak (SPR peak). The yield in reaction of nanoparticles versus reducing agent showed a positive correlation between some biological sources, primarily fungal extracts, leading to the more expedient reduction of metal ions for the higher output of nanoparticles in reaction. Further, the inverse relation of reducing agent to nanoparticle size indicated that effective biomolecules in the extracts promoted the formation of smaller nanoparticles. Also smaller particle sizes were linked to higher stability because of more effective surface capping and better uniformity that minimizes aggregation. Additionally, the fact that a strong positive correlation existed between yield and stability indicated that conditions with higher nanoparticle production also preserved nanoparticle stability. The correlation between particle size and SPR peak showed the negative correlation that is normally seen in metallic nanoparticles as the size of the particle gets increased the peak of absorbance gets reduced. The results of this study are consistent with existing literature as the biochemical composition of plant and microbial extract affect nucleation, growth and stabilization in biosynthesis. This suggests that there is great importance to the choice of the reducing agent in controlling nanoparticle properties and without knowledge of these relationships, successful synthesis protocols established for specific industrial or biomedical applications may be impossible to recreate.

CONCLUSION AND RECOMMENDATIONS

The comparison of the statistical analysis of the biosynthesis of silver gold nanoparticles (AgAuNPs) using various biological reducing agents has been successfully achieved. Results showed that the reduction agent is crucial to the nanoparticle characteristics of size, yield, stability, and SPR behavior. Among all the reducing agents examined, Fusarium oxysporum was found to be more effective as it yields a higher quantity of nanoparticles and showed greater stability attributed to its enzymatic content. Even phytochemical composition of Cinnamomum verum and Moringa oleifera plant based extracts affected the reducing capabilities, with both of which showed effective reducing potential.

Correlation analysis of the synthesis parameters showed strong correlation between them. Particle size and stability were negatively correlated with yield and positively correlated with the stability. The synthesis protocol optimization of green nanotechnology particularly with respect to biocompatibility and controlled particle behavior are heavily dependent on these relationships.

From these results, we also suggest future studies that study a broader range of biological reducing agents, for example other microbial and plant species, in order to increase synthesis efficiency. In addition, biological characterization of reducing agents at a biochemical level could reveal the controlling mechanism of the formation of nanoparticles. In addition, long term stability of nanoparticles under various storage and environmental conditions should be investigated for assessing nanoparticle suitability for practical applications. Precise characterization of nanoparticle morphology and surface chemistry requires advanced imaging and spectroscopic techniques. Overall, choosing suitable reducing agents that achieve nanoparticle specific properties results in more efficient and sustainable synthesis strategies that are relevant for adoption of green technology at larger scales into biomedical, environmental, and industrial process application.

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