



DESIGN, OPTIMIZATION AND IN-VITRO EVALUATION OF *URARIA PICTA* EXTRACT LOADED SILVER NANOPARTICLES FOR CONTROLLED DRUG RELEASE

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Abstract

The Plant based nanoparticles synthesis is a new technique where biological molecules and their combinations in plant aqueous extract decrease metal salts, which have a dual role in the fabrication and stabilization of metallic NPs. **Aim:** The present study aimed to develop and optimize green synthesized silver nanoparticles (AgNPs) using *Uraria picta* (UPAgNps) plant extract through an eco-friendly and cost-effective method. **Materials and Methods:** Silver nanoparticles were synthesized via plant-mediated reduction using aqueous silver nitrate (1–3 mM) and varying concentrations of *Uraria picta* extract (50–250 mg/mL). Optimization was performed using a Box–Behnken Design (BBD) considering AgNO₃ concentration, extract concentration, and stirring time as independent variables. Particle size and zeta potential were selected as response parameters. The optimized formulation was characterized by UV–Visible spectroscopy, particle size analysis, zeta potential measurement, scanning electron microscopy (SEM), entrapment efficiency, and in vitro drug release studies using the dialysis membrane method. Drug release kinetics were evaluated using Zero-order, First-order, Higuchi, and Korsmeyer–Peppas models. **Results:** The optimized formulation showed significant model validity with a Model F-value of 85.01 ($p < 0.05$). Extract concentration and AgNO₃ concentration significantly influenced particle size and zeta potential. The optimized nanoparticles exhibited desirable particle size with good stability and a zeta potential indicating sufficient electrostatic stabilization. The entrapment efficiency was found to be 88.45%. SEM analysis confirmed nanoscale morphology. In vitro drug release demonstrated sustained release up to 12 hours, and kinetic modeling suggested diffusion-controlled release behavior following Higuchi and Korsmeyer–Peppas models. **Conclusion:** The study successfully formulated optimized *Uraria picta* extract-mediated silver nanoparticles using a green synthesis approach. The developed nanoparticles showed high entrapment efficiency, controlled drug release, and good stability, indicating their potential as an effective and eco-friendly nanocarrier system for improved drug delivery applications.

Keywords : AgNO, Nanoparticles, Extract, *Uraria picta*, Particle size, UV–Visible spectroscopy

1. Introduction

Recently, nanotechnology has presented advanced preferences for the treatment of various diseases. The antibacterial, antioxidant, and anticancer potentiality of nanoparticles has been extensively evaluated. Nanoparticles have the widespread range of surface area and pore volume, causing major advantages for absorption and loading of drugs inside pores. The pharmaceutical application of nanoparticles affords the significant contribution to the modern medical treatment (Mosleh et al., 2021; Aurore et al., 2018).

The neat silver nanoparticles (Ag NPs) significantly is influenced by aggregation problems, which could restrict their applications (Dehnoee et al., 2023). Covered Ag NPs as a core particles with a shell material is the strategy to overcome this problem. The material of shell can improve the stability and functionality of core, and also affect the charge of core surface (Mosleh et al., 2022). Plant-mediated synthesis of nanoparticles offers a sustainable alternative to conventional chemical methods by eliminating toxic reagents, reducing energy input, and utilizing renewable biomolecules as natural reducing and stabilizing agents (Salem et al., 2023). This eco-friendly route not only minimizes environmental burden but also enhances biocompatibility, aligning with principles of green chemistry and circular economy (Dubadi et al., 2023). *Uraria picta* Desv., commonly known as Prishnaparni or Pithvan, is a perennial herb from the Leguminosae family, growing 90-180 cm tall. It features rough-haired stems and imparipinnate leaves with 5-9 leaflets that are lanceolate, variegated, and shiny above (McNeill et al., 2007). The inflorescence is a long, many-flowered raceme, with pink to reddish flowers and distinctive fruit. This plant is widely found in India, Bangladesh, Sri Lanka, and many parts of Asia and Africa (Yusuf et al., 1994). It is a key component of “Dashmula,” an Ayurvedic formulation used for various ailments, including fatigue and gynecological disorders. Additionally, the leaves exhibit antianxiety effects (Yadav et al., 2009).

The goal of the current research was to develop the novel *Uria picta* Silver nanoparticles (UPAgNPs) formulation via the cost-effective route to improve drug delivery performance to decrease the dose of the drug and reduce adverse effects. Materials and methods

1.1 Chemical and instruments used

All reagents utilized were of analytical grade and high purity levels. Silver nitrate (AgNO₃) by Merck, Plant was collected from the Amar Shaheed Baba Ajit Singh Jujhar Singh Memorial (ASBASJS) College, located in Bela, Ropar (Punjab), (Authentication no:-COP-BELA/Plant/2025/1016).

1.2 Biogenic synthesis of silver nanoparticles

Aqueous solution of silver nitrate (1-3mM) was prepared in a conical flask and stirred continuously for 20 minutes. Different dilutions of the *Uraria picta* plant extract (50 mg/mL, 150 mg/mL, and 250 mg/mL) were prepared in distilled water and subsequently filtered.

From each filtrate, 1 mL was transferred into a beaker, followed by the addition of 9 mL of the AgNO₃ solution. The mixture was stirred continuously for 15-45 minutes. The reaction mixtures were then kept in a dark chamber until the color changed from yellow to dark yellow or brown, indicating the formation of silver nanoparticles. After approximately 30 to 180 minutes, the characteristic brown coloration appeared (Hasnain et al., 2019).

1.3 Optimization of the silver nanoparticles

The systematic optimization of silver nanoparticles was performed using a Box–Behnken Design (BBD) with the help of Design of Experiment (Version 12.0.1.0) software. Three influential factors—AgNO₃ concentration, stirring time, and concentration of *Uraria picta* extract—were selected as independent variables (factors) for optimization at three levels: low (−1), medium (0), and high (+1). The selected design suggested a total of 12 experimental trials, as presented in Table

1. Particle size (nm) and zeta potential (mv) of the synthesized silver nanoparticles were measured as the response variables (Sangolkar et al., 2012).

1.4 Evaluation parameter

1.4.1 Colour change

The color change of *Cassia fistula* extract and silver nitrate solution with respect to time was observed

1.4.2 UV spectroscopy

The green synthesized AgNPs were characterized using various spectroscopic and imaging techniques. Bio-reduction of silver ions was monitored by recording of UV–Vis spectrum between 200 and 800 nm on UV spectrophotometer (Shimadzu 1700) (Patel et al., 2022).

1.4.3 Particle Size Measurement

The diluted sample was analyzed in a clean cuvette at room temperature, yielding values for average particle size and polydispersity index (PDI), which reflect particle distribution uniformity (Jilsha & Viswanad et al., 2019).

1.4.4 Zeta Potential

The diluted sample was analyzed in a zeta potential cell at room temperature to determine the electrophoretic mobility of the nanoparticle and calculate the corresponding zeta potential, revealing insights into the electrostatic interactions between particles (Clogston and Patri et al., 2010).

1.4.5 Entrapment efficiency

The entrapment efficiency of *Uraria picta* plant extract silver nanoparticles was determined by adding 10 ml of phosphate buffer of pH 7.4 and sonicated in a bath sonicator and filtering. 1 ml of filtrate is made up to 10 ml with phosphate buffer and was assayed spectrophotometrically at 365 nm (UV spectrophotometer (Shimadzu 1700) (Condurache et al., 2022). The amount of entrapped drug was calculated from the equation:

Entrapment efficiency EE (%) = [(Total drug added – amount of free drug)/total drug added] x 100

1.4.6 SEM

The sample was dried under a lamp and subjected to SEM analysis using a Jeol JSM-6490A Analytical Scanning Electron Microscope at King Saud University, Riyadh.(Singhal et al., 2011).

1.4.7 In vitro release study by dialysis method

The drug release kinetics of *Uraria picta* plant extract silver nanoparticles were determined by plotting the following kinetic models, using the data collected from *in vitro* release studies (zero order, first order, and Higuchi equations). The mechanism of drug release was determined by using the Korsmeyer-Peppas equation (Chime et al., 2013)

1. Zero-order kinetics: The zero-order rate (Eq. 1) describes the systems where the drug release rate is independent of its concentration.

$$C = K_0 t \quad (1)$$

Where K_0 is the zero-order rate constant expressed in units of concentration/time and t is the time.

2. First-order kinetics: First-order kinetics was first applied for drug dissolution studies. The first-order equation (Eq 2 & 3) describes the release of a drug from a system where the release rate is concentration-dependent.

$$\log C_t = \log C_0 + K_1 t / 2.303 \quad (2)$$

$$\log C_0 - \log C_t = K_1 / 2.303 \quad (3)$$

Where C_t is the amount of drug released in time t , C_0 is the initial concentration of the drug, k_1 is the first order constant, and t is the time.

3. Higuchi's model: Higuchi equation (Eq 4) describes drug release as a diffusion process based on Fick's law, square root time dependent.

$$Q = K_H t^{1/2} \quad (4)$$

4. Korsmeyer- peppas equations: Korsmeyer- Peppas equation (Eq 5) to analyze both Fickian and non-Fickian release of drugs.

$$M_t / M_\infty = K t^n \quad (5)$$

The logarithm form of equation (Eq 6) could be written as:

$$\text{Log} (M_t / M_\infty) = \text{Log} k + n \text{Log} t \quad (6)$$

2. Results

2.1 Optimized formulation

Table 1: Formulation trials as per Box–Behnken design

Formulation	Factor 1	Factor 2	Factor 3	Response 1	Response 2
	A:AgNo3 mM	B:Extract mg	C:Stirring Time min.	Particle Size nm	Zeta potential mV
UPNP1	1	150	45	598.7	-7.9
UPNP2	3	50	30	197.6	-14.1
UPNP3	2	250	15	171.1	-1.2
UPNP4	1	150	15	964.5	-5.8
UPNP5	3	250	30	183.2	-13.8
UPNP6	1	250	30	464.5	-1.4
UPNP7	3	150	15	203.3	-14.4
UPNP8	2	50	15	971.8	-21.4
UPNP9	2	50	45	215.6	-6.6
UPNP10	2	250	45	653.4	-12.8
UPNP11	3	150	45	194.1	-12.6
UPNP12	1	50	30	953.4	-13.9

Table 2: Response 1: Particle Size

Source	Sequential p-value	Adjusted R ²	Predicted R ²	
Linear	0.0477	0.4623	0.1202	
2FI	0.0002	0.9786	0.9441	Suggested
Quadratic	0.3369	0.9828	0.9248	Aliased

Factor coding is **Coded**.

Sum of squares is **Type III - Partial**

The **Model F-value** of 85.01 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, B, C, AB, AC, BC are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Final Equation in Terms of Coded Factors

Particle size (R1) = 480.93 Intercept -275.36AX1 -108.28BX2 +81.11CX3 OR 480.93 Intercept - 275.36 A-Extract concentration- 108.28 B- AgNo3 concentration-81.11 C-Stirring time.

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

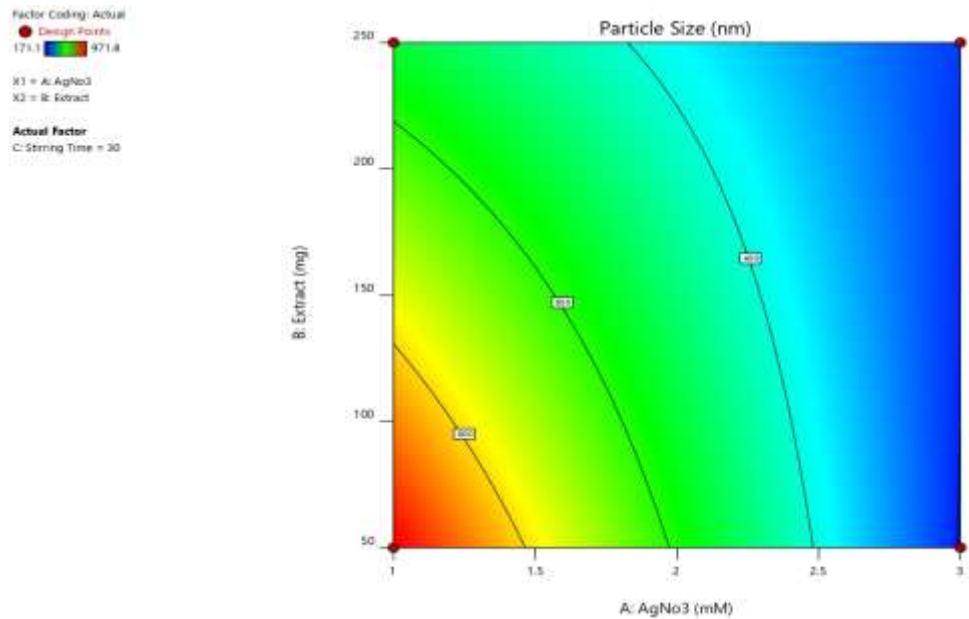


Figure 1 Two-dimensional (2D) contour plots for the effect of AgNo3 and extract concentration on particle size

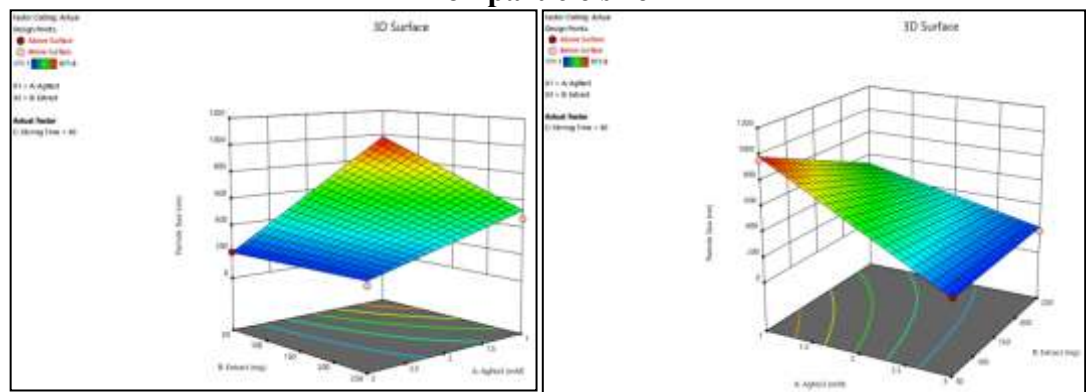


Figure 2: Three-dimensional (3D) Response surface plot showing combined effect of AgNo3 and extract on particle size of silver nanoparticle formulation Fit Summary

Table 3 Response 2: Zeta potential

Source	Sequential p-value	Adjusted R ²	Predicted R ²	
Linear	0.1735	0.2370	-0.2486	
2FI	< 0.0001	0.9857	0.9625	Suggested
Quadratic	0.5781	0.9834	0.9277	Aliased

Factor coding is Coded.

Sum of squares is Type III - Partial

The Model F-value of 127.22 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, B, AB, AC, BC are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Final Equation in Terms of Coded Factors

Zeta potential (R2) = -10.49 Intercept -3.24 AX1+3.35 BX2 +0.3625CX3 OR -10.49 Intercept - 3.24A-Lipid concentration+3.35B-Surfactant concentration +0.3625 C-Stirring time.

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are

coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

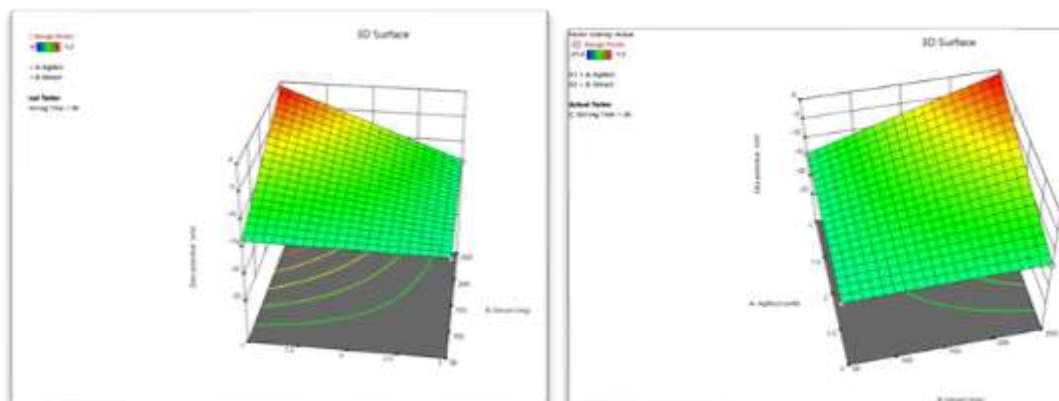


Figure 3: Three-dimensional (3D) Response surface plot showing combined effect of AgNO_3 and extract on zeta potential of silver nanoparticle formulation

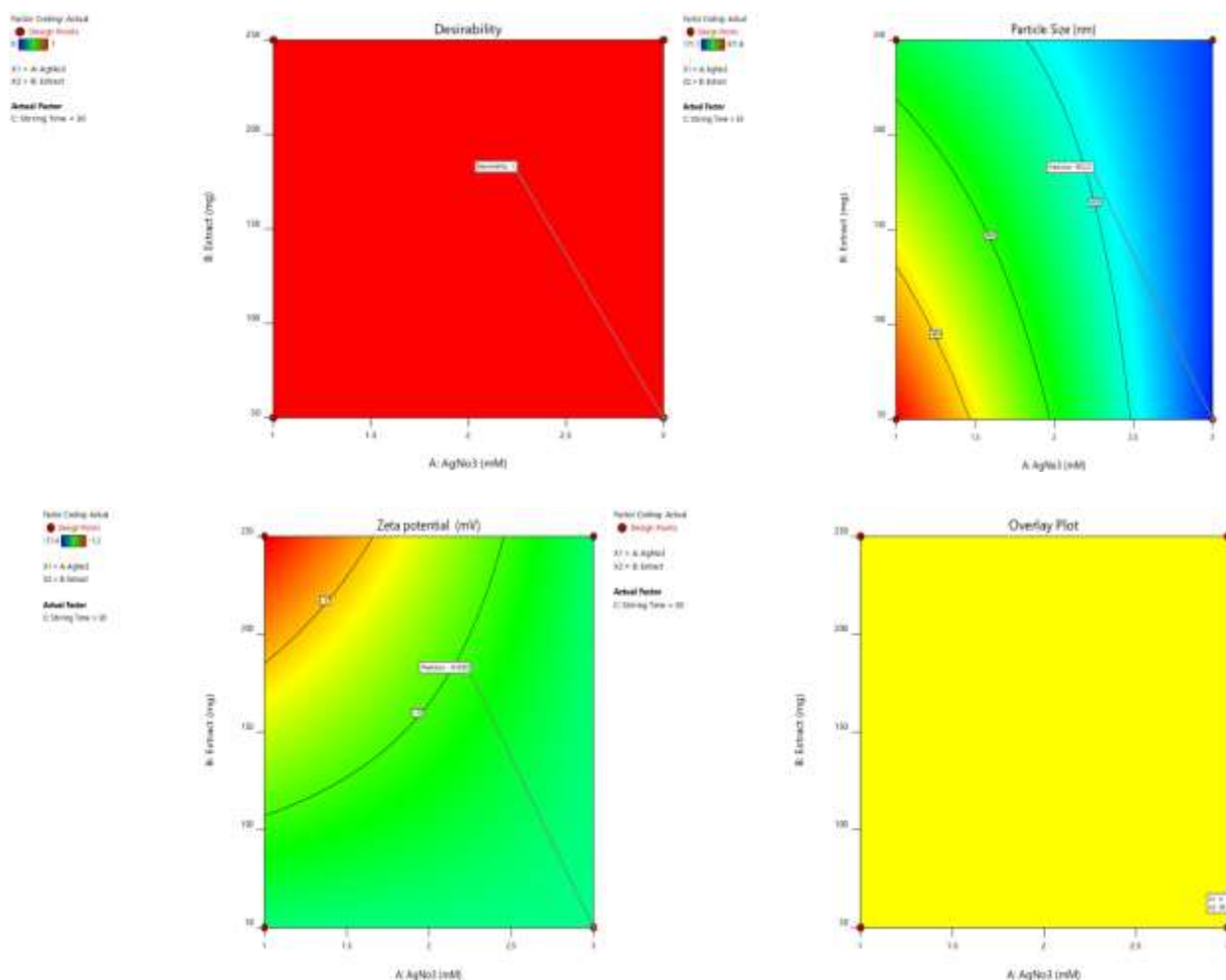


Figure 4: Response surface plot showing prediction data for optimization and Overlay plots of optimization formulation

2.2 Evaluation parameter optimized formulation

2.2.1 Physical appearance



Figure 5: Optimized silver nanoparticle of plant extract

2.2.2 UV spectroscopy

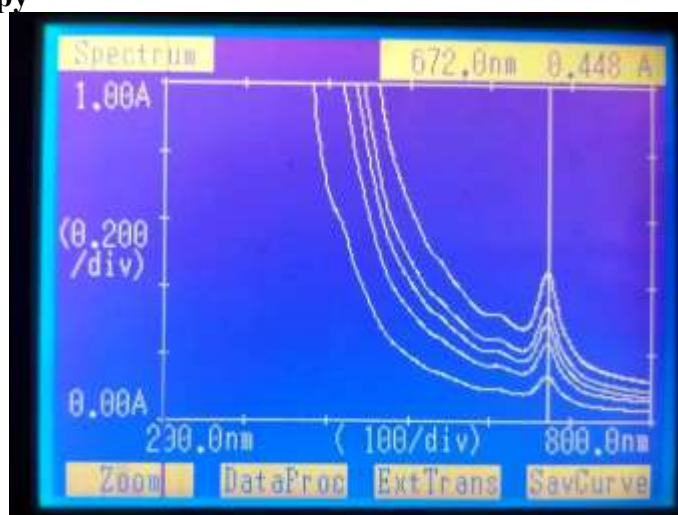


Figure 6: UV Graph of optimized formulation in different time interval

2.2.3 Particle size analysis

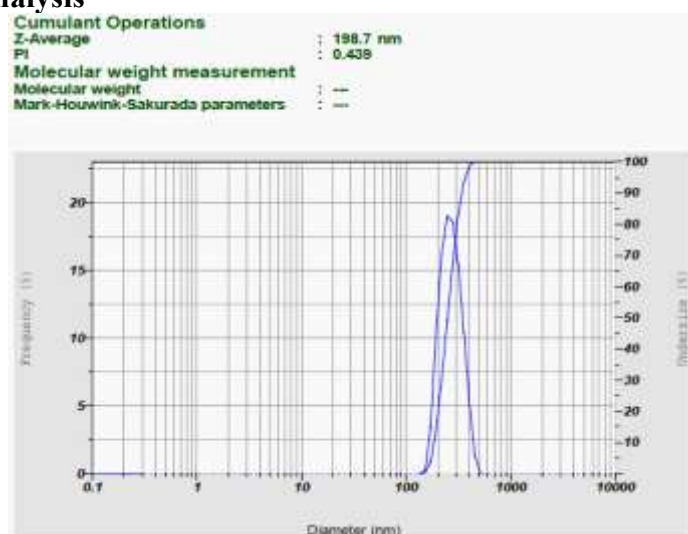


Figure 7: Particle size of optimized formulation

2.2.4 Zeta potential analysis

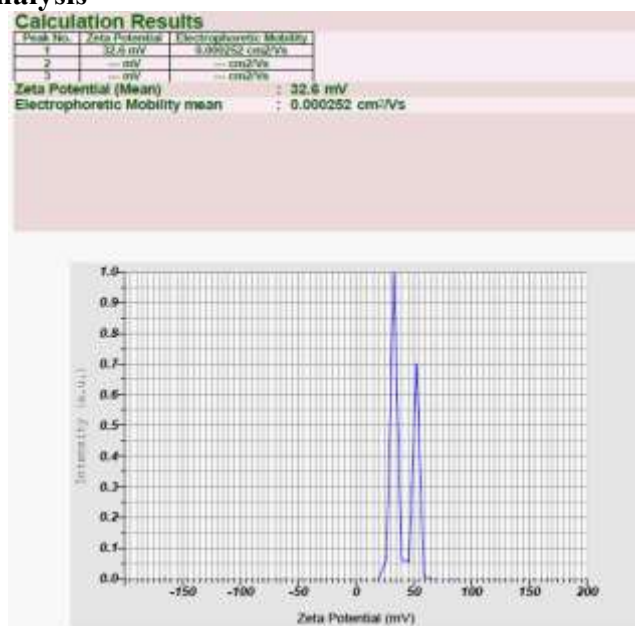


Figure 8: Zeta potential of particle size

2.2.5 SEM

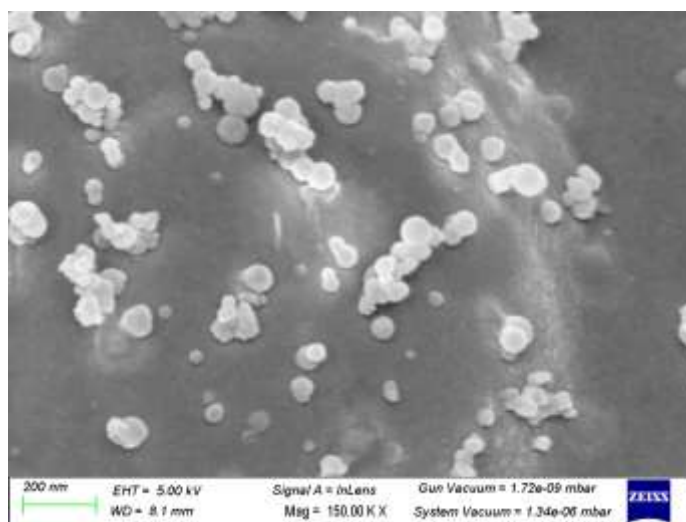


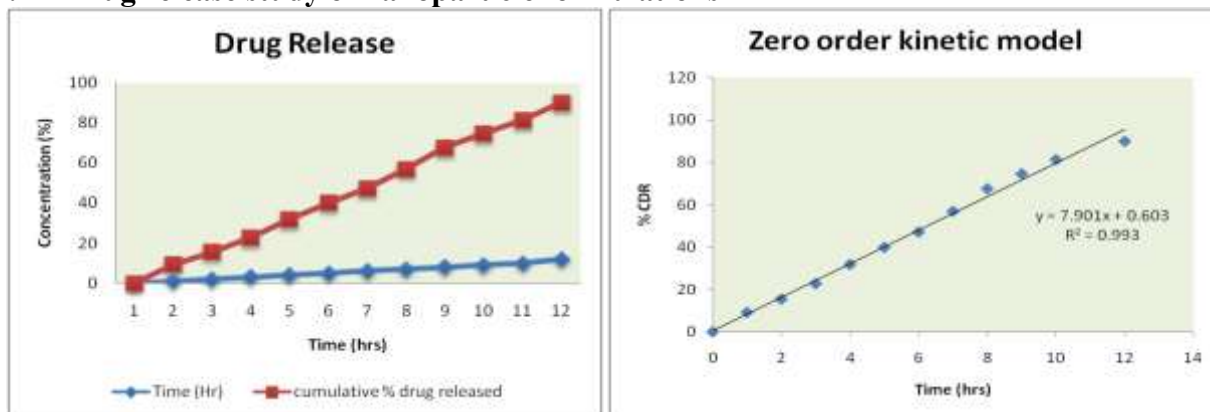
Figure 9: SEM image

The image illustrates the surface morphology of the optimized *Uraria picta*-mediated silver nanoparticles. The nanoparticles appear uniformly distributed with a nearly spherical shape, confirming successful green synthesis.

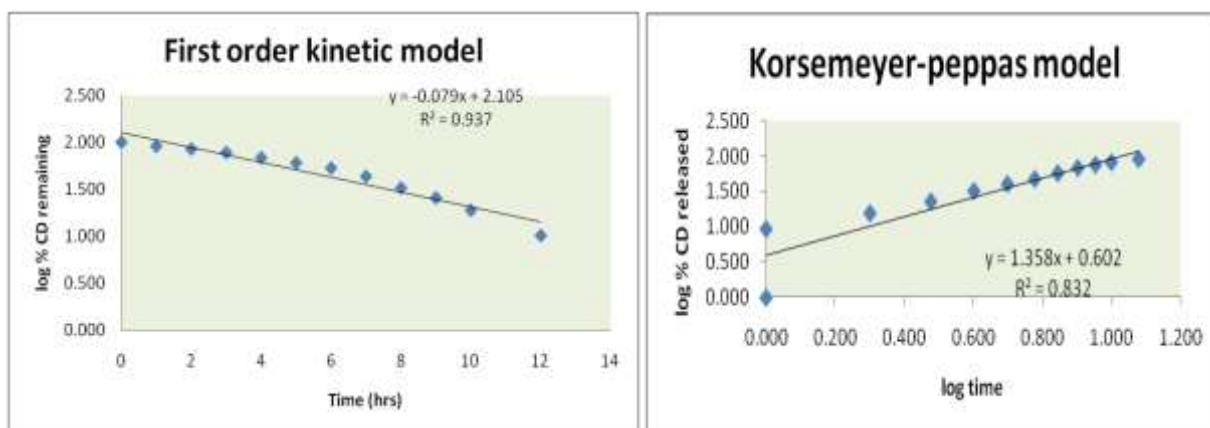
2.2.6 Entrapment efficiency

The entrapment efficiency of *Uraria picta* extract-mediated silver nanoparticles was found to be 88.45% for the optimized formulation.

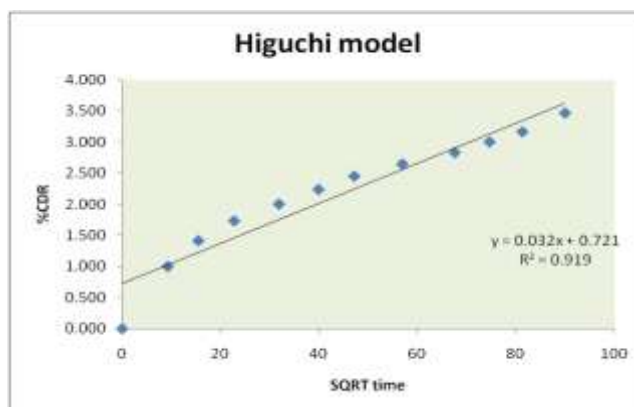
2.2.7 Drug release study of nanoparticle formulations



Graph 1: *In-vitro* drug release for nanoparticles prepared by optimization and Zero order plot



Graph 2 First order plot and Korsmeyer Peppas plot



Graph 3 Higuchi plot

Table 4 Correlation Value (R^2 value)

Formulation	Kinetic Model	Regression value
UPNP	Zero order	0.993
	First order	0.937
	Korsmeyer-peppas	0.837
	Higuchi	0.919

In-vitro drug diffusion studies were carried out using dialysis bag method. For kinetic study following plots were made: cumulative % drug release vs. time (zero order kinetic model); log

cumulative % drug remaining vs time (first order kinetic model); cumulative % drug release vs square root of time (Higuchi model); log cumulative % drug release vs log time (Korsmeyer–Peppas model). All Plots are shown in Graph 2 to 5 and results are summarized in Table 04. In the above table R^2 is correlation value. On the basis of best fit with the highest correlation (R^2) value it is concluded that in the optimized formulation of nanoparticles follow the zero order model

Discussion

The current study effectively used *Uraria picta* leaf extract to demonstrate the green synthesis and statistical optimization of silver nanoparticles. Indicating successful bioreduction facilitated by phytoconstituents found in the plant extract, the UV-visible spectrum analysis provided additional support for the creation of nanoparticles. Terpenoids, phenols, and flavonoids are examples of phytochemicals that probably served as stabilizing and reducing agents to stop excessive aggregation.

Particle size and zeta potential were shown to be considerably impacted by the concentration of extract and AgNO_3 , according to optimization utilizing Box–Behnken Design (BBD). The statistical model's robustness and dependability were validated by the high Model F-value (85.01) and significant p-values (<0.05).

Particle size analysis showed nanoscale distribution with improved uniformity, while zeta potential values indicated adequate electrostatic stabilization of the formulation. The optimized formulation demonstrated a zeta potential in the negative range, which suggests reduced aggregation tendency due to electrostatic repulsion among particles. SEM analysis further confirmed nearly spherical morphology and uniform dispersion, validating the effectiveness of the green synthesis approach.

Strong interaction between the components of the plant extract and the silver nanoparticles is indicated by the entrapment efficiency of 88.45%, which may be the result of surface binding or adsorption processes. Applications involving regulated drug administration are supported by such high entrapment, which also increases therapeutic loading capacity. Studies on drug release *in vitro* showed sustained release for up to 12 hours. Diffusion-controlled drug release behavior was indicated by the release kinetics, which were in accordance with the Higuchi and Korsmeyer–Peppas models. This implies that rather than straightforward concentration-dependent release, drug diffusion from the nanoparticle matrix is the primary release mechanism.

Overall, the findings confirm that plant-mediated synthesis offers a cost-effective, eco-friendly, and scalable alternative to conventional chemical methods. The optimized *Uraria picta* silver nanoparticles exhibited desirable physicochemical characteristics, high entrapment efficiency, and controlled drug release, highlighting their potential application in advanced nanotherapeutic drug delivery systems.

Conclusion

This study presents a systematic and sustainable approach for the development of silver nanoparticles via green synthesis using *Uraria picta* leaf extract. The green synthesis of silver nanoparticles using *Uraria picta* leaf extract is presented in this paper in a methodical and sustainable manner. The manufacture of AgNPs from *Uraria picta* leaf extract has a number of benefits, including being environmentally friendly and economically viable. Environmental and health outcomes improve through an economical and energy-efficient green synthesis method for large-scale nanoparticle production. This approach utilizes simple procedures, affordable raw materials, and is easily scalable while maximizing yield and stability of nanoparticles. Future research should clarify the mechanistic roles of specific phytochemicals, enhance scale-up strategies for industrial use, and tailor the physicochemical properties of UPAgNPs for biomedical applications including antimicrobial interventions, drug delivery, and imaging.

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