RESEARCH ARTICLE

Biosynthesis of Platinum Nanoparticles PlNPs by Bacterial Strain *Rhodococcus erythropolis*

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ABSTRACT

The biosynthesis of platinum nanoparticles (PlNPs) using the bacterial strain *Rhodococcus erythropolis* offers a greener alternative to conventional chemical and physical synthesis methods. This study explores the potential of *R. erythropolis* to produce PlNPs by leveraging its biological machinery to reduce platinum ions and stabilize the resulting nanoparticles. The biosynthesis was conducted under varying conditions of pH, temperature, and sodium platinite concentrations to optimize yield and particle characteristics. The nanoparticles were characterized. The outcome reflects that PlNPs exhibit distinct size-dependent optical properties and crystallinity, with extracellular proteins from *R. erythropolis* playing a crucial role in nanoparticle stabilization. The optimized biosynthesis process produced PlNPs with high colloidal stability and significant potential for applications in catalysis and environmental remediation. This study advances the understanding of microbial nanoparticle production, highlighting a sustainable pathway for the synthesis of biocompatible and catalytically active platinum nanoparticles.

Keywords: Platinum nanoparticles, *Rhodococcus erythropolis*, Biosynthesis, Nanoparticle characterization, Green synthesis.

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INTRODUCTION

The synthesis of nanoparticles (NPs) environmental remediation. Among these, platinum nanoparticles (PtNPs) stand out due to their remarkable catalytic properties, stability, and biocompatibility. Traditional chemical and physical methods for PtNP synthesis, however, The manufacturing methods commonly employed often entail the utilization of risky chemicals and energy-intensive procedures, which give rise to apprehensions regarding both safety and environmental sustainability. As a greener alternative, biosynthesis methods utilizing microorganisms have emerged as a promising approach for NP production.[1]

One such microorganism, *Rhodococcus erythropolis*, has been identified as a capable bio-factory for the synthesis of PtNPs. This bacterium, known for its diverse metabolic capabilities and resilience in harsh environments, provides a unique platform for the environmentally friendly production of PtNPs. The biosynthesis process leverages the biological machinery of *R. erythropolis* to reduce platinum ions and stabilize the resulting nanoparticles, thus eliminating the need for toxic reducing agents and stabilizers typically used in conventional methods.

In this study, we explore the potential of *R. erythropolis* in the biosynthesis of platinum nanoparticles. We aim to elucidate the mechanisms by which this bacterium mediates, we examine the physicochemical properties of the biosynthesized PtNPs and evaluate their potential applications. By understanding and optimizing this biosynthetic pathway, we can pave the way for scalable, eco-friendly production of PtNPs, thereby advancing their application in various technological and industrial domains.[2]

MATERIALS AND METHODS[3-5]

Strain Acquisition and Storage

R. erythropolis strains were obtained from the National Chemical Laboratory (NCL) in Pune. The stock culture was preserved at 4°C on a nutrient agar slant, following the supplier's guidelines.

Biosynthesis Medium

For the biosynthesis medium, nutrient broth was prepared with peptone (1%), beef extract (1%), and NaCl (0.5%). The pH was adjusted to 7.4 after sterilization. Agar was used as a solidifying agent at a concentration of 2%. All research substances were procured from Sigma-Aldrich, AR grade.

Synthesis, Optimization, and Purification of Platinum Nanoparticles

Activation of stock culture

The stock culture of *R. erythropolis* was stored at 4°C on nutrient agar slants. For reactivation, a loopful of cells was transferred into 5 mL of nutrient broth in a test tube and incubated at 100 rpm and 37°C until the optical density at 600 nm reached the equivalent of one McFarland standard. This

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reactivation step was crucial to prevent a lag phase in bacterial growth during nanoparticle synthesis, ensuring consistent experimental conditions.[6]

Biosynthesis of platinum nanoparticles

About 2 mL of the reactivated culture were added to 100 mL of nutrient broth in a 250 mL Erlenmeyer flask. Different concentrations of sodium platinite (2–10 mM) were introduced in separate experiments. The solution was agitated on a rotary shaker at 100 rpm at temperatures between 20 and 50°C. The formation of PlNPs was visually confirmed by a color change from yellow to faint pink, then dark pink, and finally brown.[7]

Optimization of PlNPs synthesis

Several parameters were adjusted during the optimization process, including the pH of the media (ranging from 6–8), the temperature (ranging from 20–50°C), and the concentration of sodium platinite (from 2–10 mM). The reaction mixture was incubated for up to 72 hours, with samples collected at 24, 48, and 72 hours for purification.[8]

Purification of biosynthesized PlNPs

The culture media were sonicated at 20 kHz for 2 minutes to release nanoparticles from the surface of *R. erythropolis* cells.[9]

Centrifugation Process

The centrifugation process began with an initial centrifugation at 3000 rpm for 5 minutes to pellet the bacterial cells, leaving the platinum nanoparticles (PlNPs) in the supernatant. The supernatant was then transferred to a new tube and centrifuged at 12000 rpm for 10 minutes to pellet the PlNPs. Following this, the supernatant was discarded, and the PlNPs were resuspended in phosphate buffer (pH 7.4, 0.1 mM). This high-speed centrifugation at 12000 rpm for 10 minutes was repeated three times to remove loosely bound extracellular proteins and other biological macromolecules from the PlNPs. The purified PlNPs were then stored at 4°C for various characterizations. The stages involved in activating the bacterial culture, preparing for biosynthesis, optimizing conditions, and purifying the PlNPs are illustrated in Figure 1.[10]

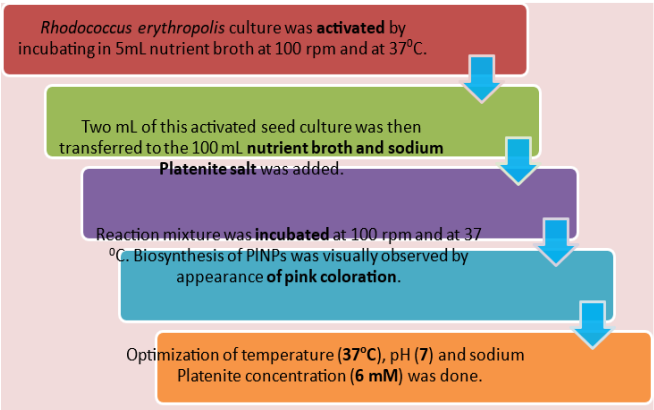

Figure 1: Step-by-step process, highlighting the key stages involved in activating the bacterial culture, preparing for biosynthesis, optimizing conditions, and purifying the resultant PlNPs

Characterization of Platinum Nanoparticles (PlNPs)[11]

UV-visible spectrophotometry

The optical properties of biosynthesized platinum nanoparticles (PlNPs) were assessed using a UV-visible double beam spectrophotometer (model UV-2700, Shimadzu, Japan). The wavelength range for the measurements was from 200 to 800 nm with a resolution of 2 nm.

X-ray diffraction

The crystallographic structure of the PlNPs was analyzed using a Rigaku Miniflex 600 system with Cu-Kα radiation (λ = 1.5418 Å). The 2θ range for the analysis was set from 10° to 90°. The crystallite size was determined using the Scherrer equation, based on the full width at half maximum (FWHM) of the most intense diffraction peak, with analysis performed using X-pert high score software and data referenced from the Joint Committee on Powder Diffraction Standards (JCPDS).

Dynamic light scattering

The PSS/NICOMP 380 ZLS nanoparticle sizing system was employed to measure the zeta potential and hydrodynamic diameter (HDD) of the biosynthesized PlNPs. This system used a red diode laser at 632.8 Å and measurements were conducted at a static angle of 90° in a plastic cell with water.

Fourier-transform infrared spectroscopy

The interaction between PlNPs and biomolecular functional groups secreted by the bacterium was investigated using a Perkin-Elmer spectrometer, which operated in the range of 450 to 4000 cm^{-1}. The samples were prepared as KBr pellets.

Scanning electron microscopy

The morphological characterization of the PlNPs was conducted using a JEOL-JSM-6510 model scanning electron microscope, with a substrate of glass and an operating voltage of 20 kV.

Sample preparation for SEM

The sample preparation for scanning electron microscopy (SEM) involved several steps. The samples were first fixed with glutaraldehyde, then air-dried, and heat-fixed onto a glass slide. Subsequently, they underwent dehydration through a series of alcohol treatments, starting from 10% and progressing to pure alcohol. This preparation allowed for a detailed examination of the size and morphology of the synthesized platinum nanoparticles (PlNPs).[12]

Energy-dispersive X-ray spectroscopy

The elemental composition of the PlNP samples was analyzed using an Oxford IE150 instrument. This technique provided insights into the elemental makeup of the nanoparticles.

Steps for Quantification of PlNPs[13]

Preparation of Se^0 standards

Aqueous solutions of sodium platinite (ranging from 1–20 mM) were prepared in test tubes. To each tube, 25 mM hydroxylamine hydrochloride (HN2OH·HCl) was added to reduce the sodium platinite to elemental selenium (Se^0). The

reaction mixtures were then dried by passing nitrogen gas over them.

Formation of platinum sulfide

One milliliter of 1 M sodium sulfide (Na2S) was added to each dried tube. The mixtures were gently stirred and incubated at room temperature for 1-hour, resulting in the formation of red-brown platinum sulfide.

Measurement of absorbance

The absorbance of the solutions was measured at 500 nm using a spectrophotometer. Each experiment was performed in triplicate, and the average absorbance values were used to establish standard Se^0 concentrations.

Calibration curve and calculation

A calibration curve was plotted using the standard Se^0 concentrations. The concentration of PlNPs in the bacterial media was then calculated based on this calibration curve.

This spectrophotometric method provided a reliable means to quantify the amount of platinum nanoparticles synthesized by *R. erythropolis*, ensuring a precise assessment of the biosynthesis process.

RESULTS AND DISCUSSION

Biosynthesis of Platinum Nanoparticles

The biosynthesis of PlNPs was investigated with respect to time and the concentration of sodium platinite. Activated *R. erythropolis* cultures, along with the sodium platinite precursor. Solution exhibited a distinct color changes, which is depicted in Figure 2.

Initially, the reaction mixture was a clear, light-yellow solution. After 24 hours of incubation, the solution developed a light pink hue. Over the next 48 hours, the color intensified, turning a more pronounced pink. At 72 hours, the solution had transitioned to a brownish-pink color. This final hue did not change with further incubation, suggesting that the biosynthesis reaction had reached completion.

The process of biosynthesizing PlNPs by reducing sodium platinite using the bacterium *R. erythropolis* was visually monitored through distinct color changes in the suspension culture. These changes occurred at specific time intervals during the incubation of the reaction mixture, as detailed below:

Time zero (0 hours)

The initial reaction mixture exhibited a clear, light-yellow color (Figure 2a).

24 hours

After 24 hours of incubation, the color of the reaction mixture turned light pink (Figure 2b).

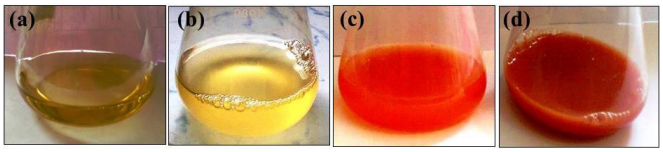

Figure 2: Change of color tith respect to time PlNPs

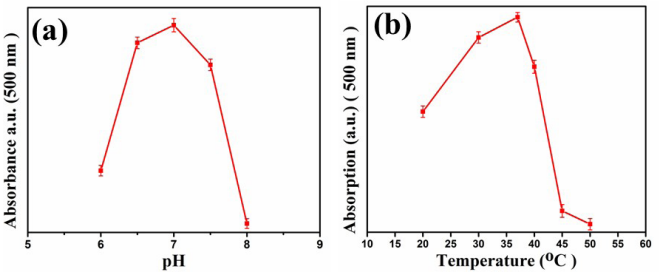

Figure 3: pH and temperature effect on PlNPs biosynthesis

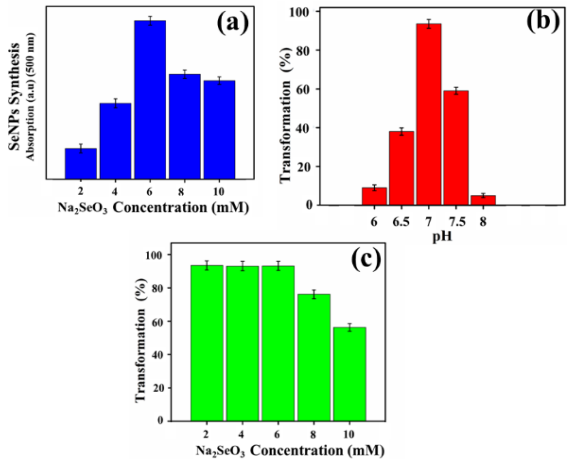

Figure 4: Precursor concentration optimization for biosynthesis of PlNPs

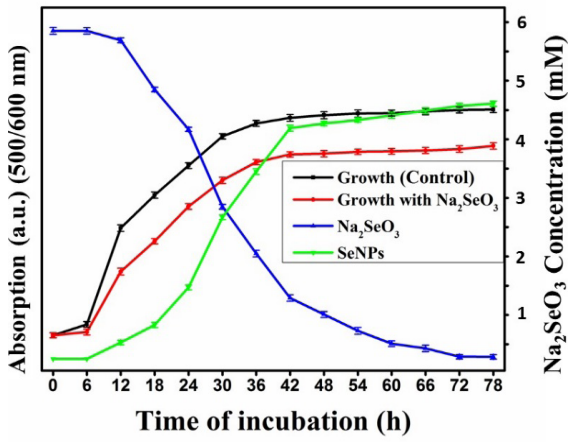

Figure 5: Study of growth Curve

48 hours

The solution gradually became pink after 48 hours of incubation (Figure 2c).

72 hours

By the 72-hour mark, the color transitioned to a brownish-pink, indicating the completion of the PlNPs formation (Figure 2d).

The effect of pH and temperature is shown in Figure 3, whereas Figure 4 depicts precursor concentration optimization and growth study in Figure 5.

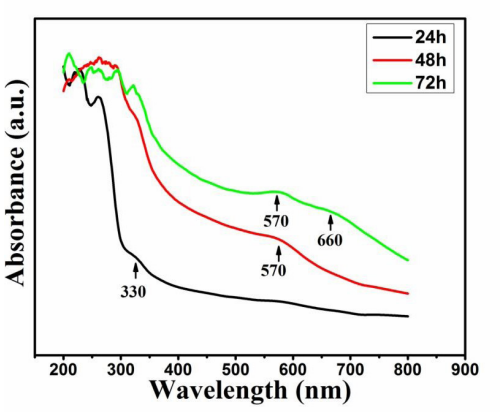

Figure 6: UV-vis. spectrum of PlNPs at different time intervals

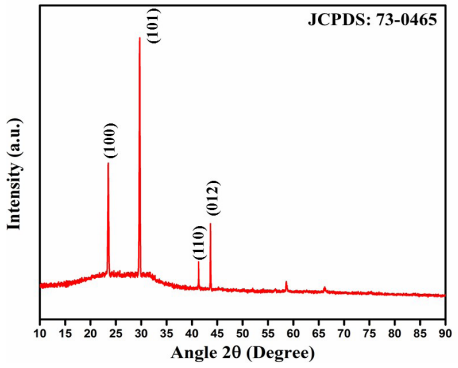

Figure 7: XRD Pattern

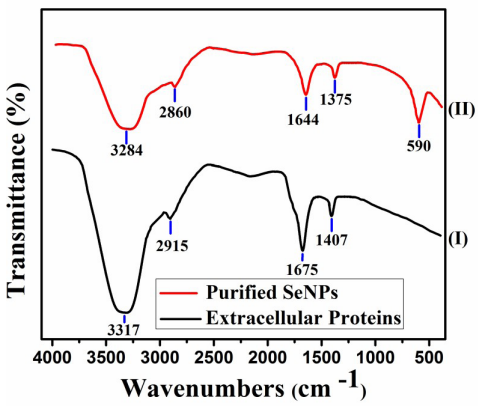

Figure 8: FTIR spectra

UV-visible Spectroscopy Study

Figure 6 illustrates the UV spectra of platinum nanoparticles (PlNPs) synthesized by *R. erythropolis* at different time intervals. The UV spectrometry data reveals distinct absorption behaviors of the PlNPs corresponding to their sizes.

24 hours

The absorption spectrum of PlNPs purified after 24 hours shows an absorption maximum (λ_{max}) at 330 nm. These NPs, with a dia. of 103 nm (Avg.), exhibit absorption primarily in the ultraviolet region.

48 hours

PlNPs synthesized and purified after 48 hours exhibit an absorption maximum at 570 nm. Having dia. of 140 nm (Avg.), these nanoparticles demonstrate increased absorption in the visible region compared to the 24-hour PlNPs.

72 hours

PlNPs synthesized and purified after 72 hours display absorption maxima at both 570 and 660 nm. These nanoparticles, exhibit absorption in the visible region, indicating further growth and size increase compared to the 48-hour PlNPs.

The observed size-dependent absorption behavior is consistent with that observed in chemically synthesized PlNPs. The shift in absorption maxima towards longer wavelengths as the nanoparticle size increases is indicative of plasmon resonance phenomena characteristic of metallic nanoparticles.

Additionally, the UV-visible spectra of all three types of PlNPs show significant absorption between 260 and 280 nm. Which is due to the presence of proteins secreted by *R. erythropolis* during the biosynthesis process. These proteins act as capping agents, contributing to the stability and dispersibility of the PlNPs[14]

Crystallography Study

X-ray diffraction (XRD) analysis (Figure 7)

- *Diffraction peaks and crystallographic analysis*

Major diffraction peaks were observed at 23.5°, 29.6°, 41.3°, and 43.6° 2θ values. These diffraction peaks matched well with the JCPDS card no. 73-0465, confirming the crystalline nature of the PlNPs. The absence of precursor peaks indicated the complete reduction of the precursor material to form crystalline nanoparticles. The high intensity of these diffraction peaks and the lack of amorphous peaks confirmed a high degree of crystallinity in the PlNPs. The crystallite size was determined using the Scherrer formula, resulting in a calculated size of 77.9 nm after 24 hours of incubation.[15]

FTIR analysis (Figure 8)

The FTIR analysis revealed that extracellular proteins from *R. erythropolis*, collected during the log phase, showed peaks at 3317, 2915, 1675, and 1407 cm^{-1}. In the spectra of purified PlNPs, these peaks shifted to lower values: 3284, 2860, 1644, and 1375 cm^{-1}, indicating an interaction with the nanoparticles. Additionally, a new peak appeared at 580 cm^{-1}, corresponding to platinum-oxygen (Pt-O) vibration. This suggested that the extracellular proteins acted as capping agents for the biosynthesized PlNPs. These proteins likely served as nucleation centers, facilitating the reduction of platinite ions and the growth of nanoparticles. Positively charged amino acid sites on the proteins attracted negatively charged platinite ions, promoting nucleation and subsequent growth of the nanoparticles.[16]

Observations

Spherical morphology

The images (Figure 9) revealed the biosynthesized PlNPs constituted spherical type shape with a smooth surface.

Extracellular synthesis

PlNPs were synthesized extracellularly outside the bacterial cell, indicating an external deposition of the nanoparticles.

Size variation over time

The analysis revealed that PlNPs with an average diameter of 103 (24 hours), 140 (48 hours), and 288 nm (72 hours) were synthesized.

Statistical analysis

Detailed statistical analysis provided measurements such as minimum size, maximum size, and standard deviation for each time point.

Size increase over time

Showed an enhancement in the size and diameter of biosynthesized PlNPs over the incubation period, while shape remained same.

Dynamic light scattering (DLS) and zeta potential analysis: DLS: In order to gauge the distribution of hydrodynamic diameter (HDD) of the PlNPs synthesized in a colloidal solution, DLS was employed.

Observations

24-hour reduction reaction

PlNPs produced after a 24-hour reduction reaction showed a hydrodynamic diameter (HDD) distribution ranging from 100 to 200 nm.

72-hour reduction reaction

After a 72-hour reduction reaction by the bacteria, the PlNPs exhibited a broader HDD distribution, ranging from 260 to 450 nm.

Comparison with SEM graphs

The average HDD distribution values, as illustrated in Figure 10, exceeded the corresponding diameters observed in SEM graphs. This discrepancy was attributed to the formation of a hydration shell around the nanoparticles, which is detected by DLS but not visible in SEM images.

Hydration layer formation

The observed variation between the HDD measurements from DLS and the sizes depicted in SEM images was ascribed to the development of a hydration shell surrounding the nanoparticles. This layer increases the apparent size of the nanoparticles when measured in a liquid suspension.

Colloidal stability

The HDD data confirmed the colloidal stability of the biosynthesized PlNPs in suspension, suggesting their potential for diverse applications due to their stable dispersion in colloidal form.

Zeta Potential Analysis of Biosynthesized PlNPs

Surface charge analysis

The zeta potential analysis was conducted to assess the surface charge of the biosynthesized platinum nanoparticles (PlNPs) produced by *R. erythropolis* at different stages of the reduction reaction. The attachment of biomolecules, primarily proteins secreted by the bacteria, to the surface of the nanoparticles imparts a characteristic charge to them.[17,18]

Zeta Potential Observations

24-hour reduction reaction

PlNPs with an approximate size of 103 nm exhibited a zeta potential of -28 mV.

48-hour reduction reaction

PlNPs with an approximate size of 140 nm exhibited a zeta potential of -39 mV.

72-hour reduction reaction

PlNPs with an approximate size of 288 nm exhibited a zeta potential of -49 mV.

Interpretation of Results

Negative surface charge

All three sizes of PlNPs exhibited a negative surface charge. The magnitude of the negative charge increased with the nanoparticle size.

Size-dependent charge variation

Smaller PlNPs (103 nm) had a zeta potential of -28 mV, medium-sized PlNPs (140 nm) had a zeta potential of -39 mV, and larger PlNPs (288 nm) had a zeta potential of -49 mV.

Surface biomolecules

The increase in negative charge with larger nanoparticle sizes is likely due to the greater surface area available for protein attachment. More protein molecules attaching to the surface result in a higher negative charge.

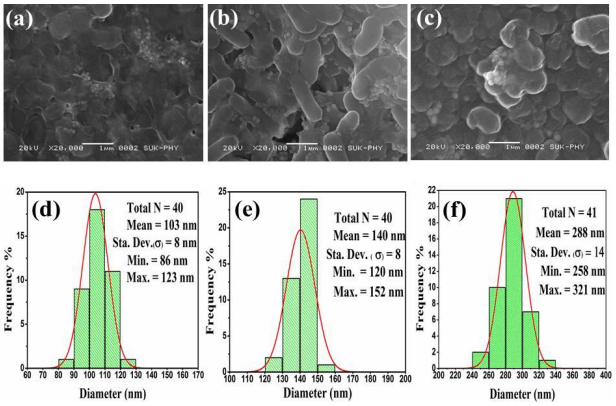

Figure 9: SEM images for each time point are displayed in (d), (e), and (f) to illustrate the distribution of PlNP diameters

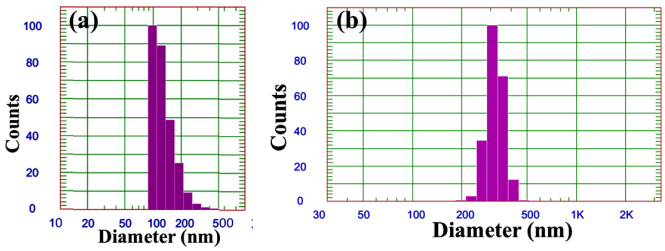

Figure 10: Illustrates the HDD distribution for PlNPs after 24 hours and 72 hours of reduction reaction by the bacterium *R. erythropolis*

Colloidal stability

The negative charge on PlNPs is crucial for preventing aggregation in suspension. Electrostatic repulsion between negatively charged nanoparticles enhances their colloidal stability.

Significance for Biomedical Applications[19]

Surface charge and biological interactions (Figure 11)

The surface charge of the biosynthesized PlNPs significantly affects their interactions with biological systems. The negative charge on PlNPs can influence various factors such as cellular uptake, biodistribution, and overall biocompatibility. Ensuring colloidal stability is crucial for maintaining the effectiveness of nanoparticles in biomedical applications, as it prevents aggregation and enhances their interaction with biological environments. Various evaluation parameters are presented in Table 1.

- (a) Zeta potential of PlNPs after 24 hours of reduction reaction (-28 mV)
- (b) Zeta potential of PlNPs after 48 hours of reduction reaction (-39 mV)
- (c) Zeta potential of PlNPs after 72 hours of reduction reaction (-49 mV)

Elemental Analysis[20]

The elemental analysis conducted through energy-dispersive X-ray spectroscopy (EDS), as illustrated in Figure 12, provides crucial insights into the composition of the PlNPs synthesized by *R. erythropolis*. This quantitative examination confirms the purity of the PlNPs and reveals the presence of specific elements integral to the biosynthesis process. One of the prominent observations from the EDS spectra is the characteristic peak for platinum (Pl) at approximately 1.3 keV, unequivocally confirming the existence of crystalline PlNPs within the sample. Alongside platinum, the spectra also exhibit peaks corresponding to oxygen (O), carbon (C), phosphorus (P), sodium (Na), and sulfur (S). These elemental signatures hint at the intricate interplay between the nanoparticles and biological entities, such as oxidized states interacting with biological molecules, organic molecules likely from bacterial biomass and extracellular proteins, and contributions from nucleotides

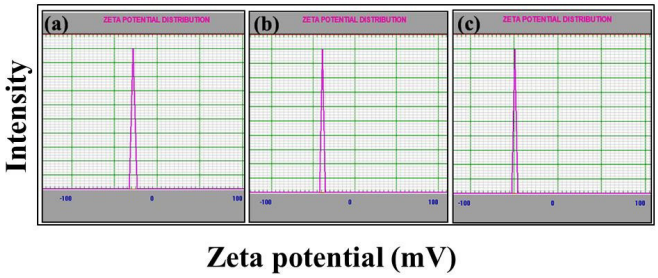

Figure 11: Zeta potential analysis of PlNPs biosynthesized by *R. erythropolis*

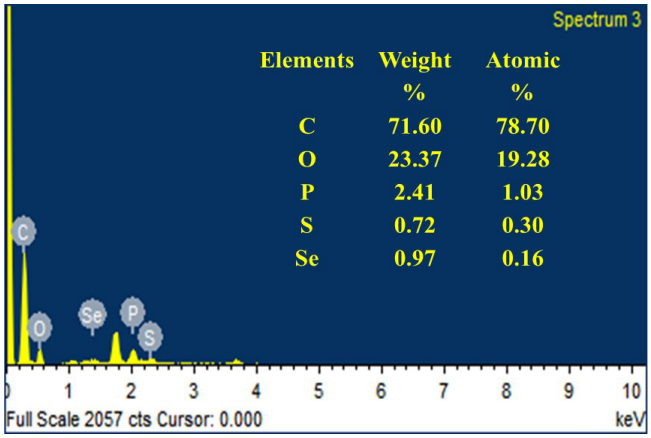

Figure 12: Elemental analysis for the PlNPs

or phospholipids present in bacterial secretions. Moreover, the presence of sodium peaks suggests possible interactions with the culture media or other biological components, while sulfur peaks hint at the involvement of amino acids from proteins attached to the PlNPs. Such detailed elemental analysis not only validates the formation of the PlNPs but also underscores the significant role played by bacterial biomolecules in their synthesis and stabilization.

The elemental stoichiometry observed in the EDS spectra aligns with the expected composition for biosynthesized PlNPs, providing further confirmation of their formation and highlighting the crucial influence of bacterial biomolecules in the synthesis process. This confirmation is particularly significant as it solidifies the understanding of the nanoparticles'

Table 1: Evaluation parameters of nanoparticles

Parameter	*R. erythropolis (24 hours)*	*R. erythropolis (48 hours)*	*R. erythropolis (72 hours)*	*Lactobacillus paracasei (24 hours)*
Average diameter (SEM) (nm)	103	140	288	90.3
**Hydrodynamic diameter (DLS) (nm)	145	-	320	-
Zeta potential (mV)	-28	-39	-49	-
XRD peaks (2θ)	23.5°, 29.6°, 41.3°, 43.6°	-	-	-
FTIR peaks	3284, 2860, 1644, 1375, 590 cm^{-1}	-	-	-
EDS composition	Pt, C, O, P, Na, S	-	-	-
Shape and surface	Spherical, smooth	Spherical, smooth	Spherical, smooth	Spherical, smooth
Crystallite size (XRD)	77.9 nm	-	-	-

elemental makeup and elucidates the mechanisms underlying their production. Furthermore, the identification of specific elements and their corresponding peaks in the EDS spectra offers valuable insights into the interactions between the PlNPs and biological entities. These interactions, including the attachment of biomolecules like proteins secreted by *R. erythropolis*, play a pivotal role in the production, stability, and functionality of the nanoparticles. The comprehensive elemental analysis provided by EDS not only serves as concrete evidence of the elemental composition of the biosynthesized PlNPs but also sheds light on the intricate interplay between biological components and nanoparticles, furthering our understanding of their potential applications in various fields, including biomedicine and nanotechnology.[21]

CONCLUSION

In conclusion, the study successfully demonstrated the biosynthesis of platinum nanoparticles (PlNPs) through environmentally friendly methods facilitated by bacterial biomolecules. Optimization of synthesis parameters such as temperature, pH, and sodium platinite concentration resulted in the production of PlNPs with desirable characteristics. The physicochemical properties of the nanoparticles varied depending on the bacterial strain used, with different sizes observed over various time intervals. Characterization techniques including SEM, DLS, zeta potential, XRD, FTIR, and EDS confirmed the spherical shape, stability, crystalline nature, biomolecule interaction, and elemental composition of the PlNPs. Importantly, the eco-friendly and cost-effective synthesis methods hold promise for scalable production of PlNPs with potential environmental and economic benefits. Overall, this study underscores the potential of efficient and sustainable PlNPs synthesis for various applications, with the ability to tailor nanoparticle properties through synthesis parameter adjustments.

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