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Commentary

Green synthesis of silver nanoparticles and their effect on the activity of alkaline phosphates

Vijaya Giramkar[1,*], Girish Phatak[1], Sushama Sabharwal[2]

[1] Centre for Materials for Electronics Technology, Panchwati, Off Pashan road, Pune 411008, Maharashtra, India
[2] Biochemistry Division, Department of Chemistry, Savitribai Phule Pune University, Pune 411007, Maharashra, India
*** Corresponding author:** Vijaya Giramkar, vijaya@cmet.gov.in

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Abstract: Nanoparticle-based assays offer rapid, sensitive, and real-time detection of biomolecules and are increasingly applied in bioanalytical sensing. However, due to the widespread applications of alkaline phosphatases in medicine, research and industry have a preference for this rapid, nanobased sensitive assay for detecting alkaline phosphatase activity. In this study, a simple and cost-effective nanobioassay was developed for the detection of alkaline phosphatase activity using green-synthesized silver nanoparticles. The assay is based on the ability of the enzyme substrate adenosine triphosphate (ATP) to inhibit salt-induced aggregation of silver nanoparticles. Synthesised silver nanoparticles were used for the bioassay, which served as both reducing and capping agents, avoiding the use of hazardous chemicals. Nanoparticle formation was confirmed by a characteristic surface plasmon resonance peak at ~420 nm in UV–Vis spectra, and dynamic light scattering analysis revealed an average particle size of ~80 nm.

Keywords: green synthesis; silver nanoparticles; *Arachis hypogaea*; alkaline phosphatase; selective detection

1. Introduction

Metal nanoparticles are widely explored in sensing and biomedical applications due to their size-dependent optical properties governed by localized surface plasmon resonance (LSPR) [1–6]. Variations in particle size, aggregation state, and surface chemistry produce distinct plasmonic shifts, enabling simple optical detection methods [7–10]. Silver nanoparticles (Ag NPs), in particular, exhibit strong plasmonic responses and are well suited for colorimetric assays [11–14].

Green synthesis using plant-derived biomolecules has emerged as an environmentally benign alternative to conventional chemical methods, offering improved biocompatibility while avoiding toxic reagents [15–18]. Phytochemicals such as flavonoids and phenolics act as both reducing and stabilizing agents during nanoparticle formation [19–21].

In this study, we present a rapid colorimetric assay for alkaline phosphatase activity based on ATP-mediated stabilization of green-synthesized Ag NPs against salt-induced aggregation. Enzymatic hydrolysis of ATP alters nanoparticle aggregation behavior, producing measurable optical changes for sensitive enzyme detection.

2. Experimental

2.1. Materials

2.1.1. Preparation of *Arachis hypogaea* crude protein Extract

Arachis hypogaea seed meal (100 g) was extracted with 500 mL of physiological saline solution (0.145 M NaCl) for 4 h at 4 ℃. The extract was then filtered through cheesecloth and clarified by centrifugation (12, 080 g) [22].

2.1.2. Synthesis of silver nanoparticles

Silver nanoparticles (AgNPs) were synthesized using crude plant protein extract as a reducing and stabilizing agent. Before synthesis, all glassware was thoroughly cleaned and rinsed with double-distilled water.

2.1.3. Preparation of tris buffer

Tris buffer (10 mM, pH 8.0) was prepared by dissolving the required amount of Tris(hydroxymethyl)aminomethane in double-distilled water. The pH was adjusted to 8.0 using 1 N HCl, and the final volume was made up with double-distilled water. The buffer was filtered and stored at 4 ℃ until use. The crude plant protein extract was prepared and maintained in Tris buffer to ensure protein stability and optimal reaction conditions during nanoparticle synthesis.

2.1.4. Synthesis procedure

For the synthesis of silver nanoparticles, 50 mL of crude *Arachis hypogaea* protein extract prepared in Tris buffer was mixed with 50 mL of aqueous silver nitrate ($AgNO_3$) solution (0.1 mM) in a 1:1 ratio. The reaction mixture was stirred continuously using a magnetic stirrer at room temperature. A visible color change from pale yellow to yellowish-brown indicated the formation of silver nanoparticles due to surface plasmon resonance. The reaction mixture was then kept undisturbed overnight to allow complete reduction of Ag^+ ions. To study the effect of reaction time on the synthesis of AgNPs, a reaction mixture containing crude protein extract and 0.5 mM $AgNO_3$ (1:1 ratio) was stirred continuously under identical conditions. Aliquots were withdrawn at regular intervals (4, 6, 8, 12, 24, and 48 h), and the formation of silver nanoparticles was monitored using UV–visible spectroscopy in the range of 200–800 nm. The effect of $AgNO_3$ concentration on AgNP synthesis was evaluated by conducting the reaction with varying concentrations of $AgNO_3$ (0.1–2.0 mM), while keeping the volume and concentration of the crude protein extract constant. The synthesis process and monitoring conditions remained the same as described above. Similarly, the influence of crude protein extract concentration on nanoparticle synthesis was investigated by varying the volume of the protein extract while maintaining a constant $AgNO_3$ concentration. The formation and stability of AgNPs were assessed by observing color change and UV–visible spectral analysis.

2.2. Characterization of Ag NPs

The green synthesized silver nanoparticles were purified by repeated centrifugation and washing with distilled water, followed by drying under vacuum. The dried powder was used for FTIR and BET analysis, while freshly dispersed nanoparticles in aqueous medium were used for DLS measurements and surface morphology studies. The surface morphology of the nanoparticles was observed by FESEM and TEM.

2.3. Detection of alkaline phosphatase activity

These Ag NPs were used to detect alkaline phosphatase activity using ATP (*Adenosine Triphosphate*) as substrate. ATP serves as a substrate for alkaline phosphatase and also prevents the aggregation of the silver nanoparticles due to high salt concentration.

The assay was carried out by adding 5 μL of ATP (5 mM) to 200 μL of green synthesized silver nanoparticles (10 mg/mL) and 40 μL of Tris buffer (pH 8.0, 20 mM) and the mixture was kept at room temperature for 30 mins. After that, 100 μL alkaline phosphatase (2 units) in Tris buffer (pH 8.0) [23] was added and the mixture was incubated for 30 mins at room temperature, followed by the addition of 15 μL of 0.5 M NaCl. The final volume of the reaction mixture was adjusted to 1.0 ml using Tris buffer (pH 8.0, 20 mM) and its UV-vis absorption spectrum was recorded between 200–800 nm. Corresponding blanks without enzyme were run simultaneously.

2.3.1. Optimization of the bioassay

Optimization of the bioassay was carried out by studying the effect of reaction time as well as the effect of varying concentrations of salt. ATP and alkaline phosphatase by keeping all other parameters constant except the parameter under study as follows:

In order to optimize the salt concentration for alkaline phosphatase assay, different concentrations of NaCl (0.1 M, 0.2 M, 0.3 M, 0.4 M, and 0.5 M) were used in the assay and UV-visible spectra were recorded.

The effect of different ATP concentrations, for *Arachis hypogaea* alkaline phosphatase detection, varying concentrations of ATP were used *viz*. 2%, 4%, 6%, 8% and 10% (*v*/*v*) in the assay as described above and the UV spectra were recorded.

2.3.2. Selectivity for alkaline phosphatase

With the aim of to study the selectivity of the assay for alkaline phosphatase enzyme different enzymes used *viz*. *Fagopyrum esculentum* alkaline phosphatase, *Macrotyloma uniflorum* alkaline phosphatase *and Arachis hypogagea* alkaline phosphatase, which were partially purified in our laboratory; and commercial sources viz. *Aspergilus niger* alkaline phosphatase (ANL), bovine serum albumin (BSA), Trypsin and Papain.

3. Results and Discussion

3.1. Nano-based bioassay for alkaline phosphatases

Green synthesis of silver nanoparticles was carried out using a crude protein extract of *Arachis hypogaea* seeds. $AgNO_3$ solution (0.1mM) was mixed with the crude protein extract (1:1 *v*/*v*) at room temperature with constant stirring. The crude protein extracts act both as a reducing agent and as a capping agent. The conversion of silver ions into silver nanoparticles did not involve use of any hazardous chemicals. The UV- Vis spectra of the above yellow coloured solution of silver nanoparticles showed the characteristic absorbance at about 420 nm (**Figure 1**). The differential light scanning (DLS) results confirmed the effective size of the nanoparticles to be ~80 nm. (**Figure 2**). Similar observations have been reported by Mahdi et al. (2015) [24].

FESEM microscopic analysis (**Figure 3a**) and Transmission electron microscopic (TEM) (**Figure 3b**) analysis showed that the silver nanoparticles were well dispersed with a mainly spherical shape.

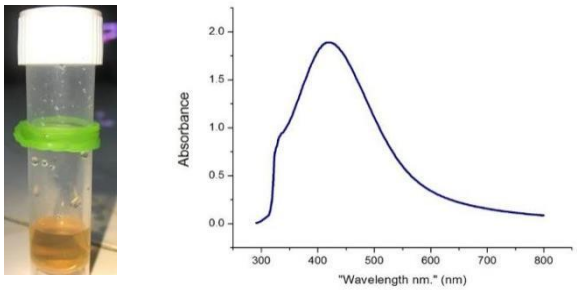

Figure 1. UV-vis absorption spectrum of green-synthesized silver nano particles.

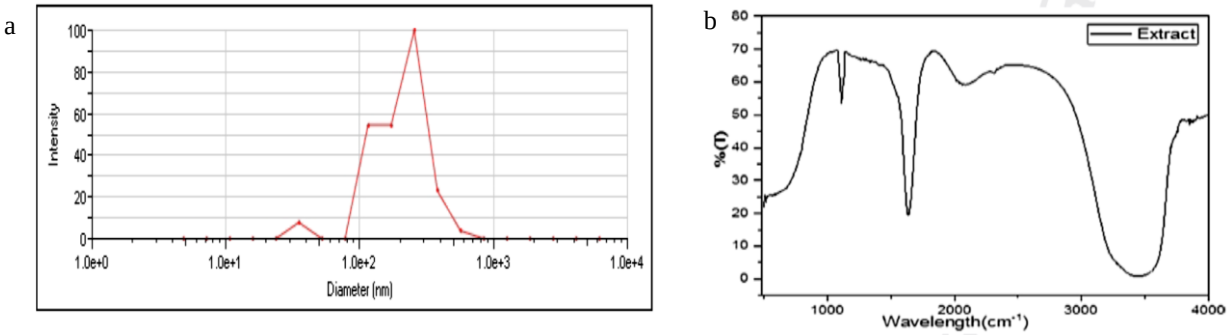

Figure 2. (**a**) DLS histogram and (**b**) FTIR image of synthesised silver nanoparticles.

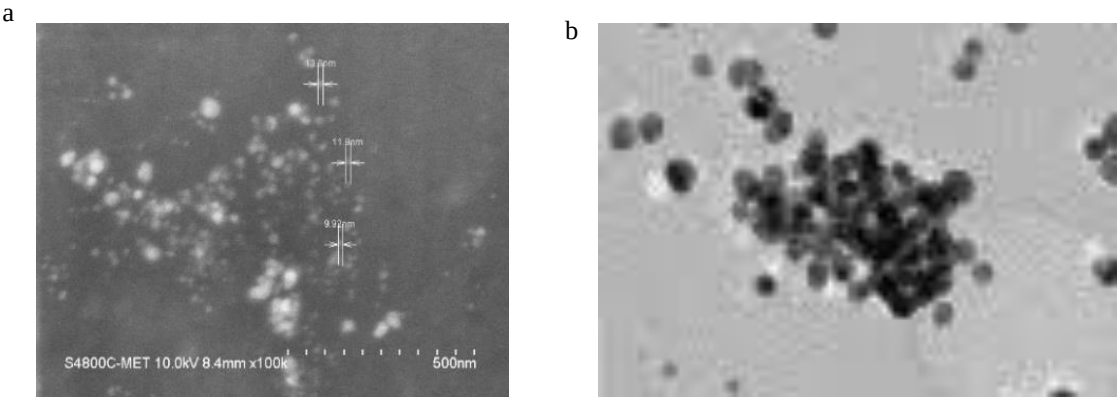

Figure 3. (**a**) FESEM and (**b**) TEM image of green synthesized silver nanoparticles.

3.2. Detection of *Arachis hypogaea* alkaline phosphatase activity

Above green synthesized silver nanoparticles were used to detect alkaline phosphatase activity in the presence of ATP and 0.5 M NaCl [25]. ATP has been used both as a substrate for alkaline phosphatase and for preventing silver nanoparticle aggregation at high salt concentration. Thus, ATP stabilized silver nanoparticle-based colorimetric bioassay for alkaline phosphatase has been developed.

For the bioassay, a solution containing ATP was pre-incubated with alkaline phosphatase, followed by the addition of silver nanoparticles and 0.5 M NaCl and the UV-visible spectra of the mixture were recorded. Corresponding control without

alkaline phosphatase was also run simultaneously. Since alkaline phosphatase hydrolyzed the ATP, and thus reduced the protection conferred by it against salt-induced aggregation of the silver nanoparticles the intensity of the absorption peak of the silver nanoparticles was lower in the presence of alkaline phosphatase (due to aggregation of the silver nanoparticles). This change is monitored with the UV-Vis spectrometer, as shown in the **Figure 4**.

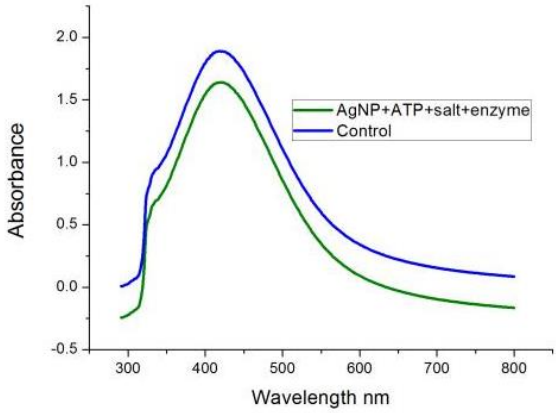

Figure 4. UV-vis absorption spectra of silver nano particles incubated with salt and ATP in absence and presence of alkaline phosphatase.

3.3. Effect of different ATP concentration

ATP acts as the stabilizer against the aggregation of the silver nanoparticles, with an increasing concentration of ATP a decrease in aggregation of silver nanoparticles was observed using 0.5 M NaCl as shown in the **Figure 5**. Thus, 5 mM ATP was chosen as the optimum concentration for the assay.

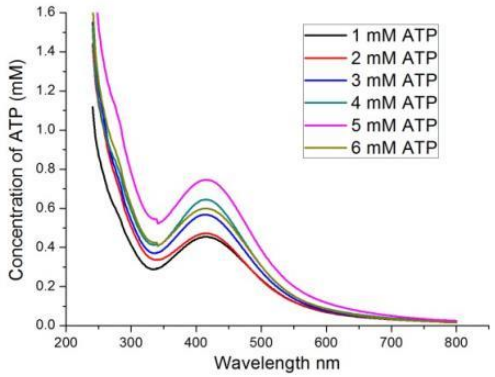

Figure 5. Effect of silver nanoparticles incubated with salt and different concentrations of ATP.

3.4. Optimization of alkaline phosphatase concentration for calibration curve

The minimum detection limit of the sensor was found to be 0.52 units per mL ATP acts as the stabilizer against the aggregation of the silver nanoparticles, with an increasing concentration of ATP a decrease in aggregation of silver nanoparticles was

observed using 0.5 M NaCl as shown in the **Figure 6**. Thus, 5 mM ATP was chosen as the optimum concentration for the assay.

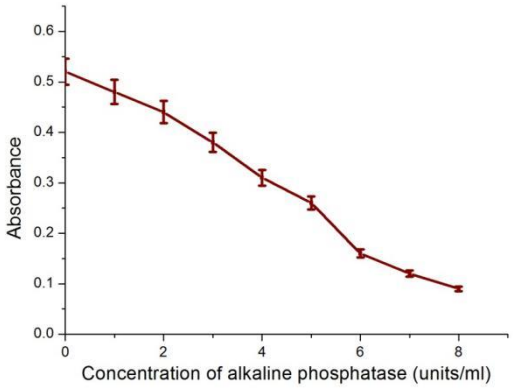

Figure 6. Effect of silver nanoparticles incubated with salt and ATP using different concentrations of alkaline phosphatase.

Thus, this sensor was found to be suitable for the detection of alkaline phosphatase in the range of 0.5 to 6 enzyme units/mL. For analysis of high concentrations of alkaline phosphatase, there is a need to dilute the sample before analysis.

3.5. Effect of pH on the assay

The effect of varying pH showed that the maximum aggregation occurs at pH 8 that can be explained as the optimum pH for the enzymatic catalysis (**Figure 7**).

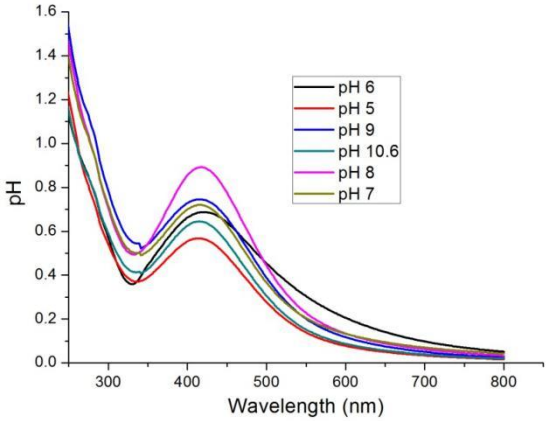

Figure 7. Effect of pH on nanoparticle based *Arachis hypogaea* alkaline phosphatase assay.

In order to investigate the selectivity of the developed sensing tool towards the detection of alkaline phosphatase, an assay was carried out by replacing alkaline phosphatase with other proteins viz BSA, papain, pepsin and trypsin (each of 10 µg per mL concentration). No significant change was observed in the UV-vis spectra of Ag NPs in the presence of these proteins, whereas a significant decrease in absorption

peak intensity was observed in the presence of alkaline phosphatase. This suggests that the developed sensing tool is specific for the detection of alkaline phosphatase.

4. Conclusion

This study reports a silver nanoparticle–based colorimetric assay for the detection of alkaline phosphatase, relying on enzyme-induced aggregation of AgNPs. Given the commercial and clinical importance of alkaline phosphatase, there is a clear need for a rapid, sensitive, and real-time detection method. The ATP-stabilized AgNPs system offers a simple and cost-effective sensing platform. Accordingly, green-synthesized silver nanoparticle-based nanobioassays have been developed to enable sensitive and economical detection of alkaline phosphatase.

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