Selective and sensitive colorimetric detection of platinum using Pseudomonas stutzeri mediated optimally synthesized antibacterial silver nanoparticles

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In this work, Pseudomonas stutzeri was used for the optimum biogenic synthesis of antibacterial silver nanoparticles (AgNPs) which were applied for colorimetric detection of platinum ions (Pt\(^{+2}\)). The optimum synthesis conditions were 2 mM AgNO\(_3\), pH 9 and incubation at 60 °C for 24 h. The FTIR spectra indicated that biomolecules such as amino acids, proteins or enzymes from P. stutzeri were involved in the synthesis of AgNPs in the size range of 10–50 nm. Among the various metal ions tested and screened initially, the colloidal AgNPs probe-based colorimetric assay selectively detected Pt\(^{+2}\) with 50 ppm as the limit of detection (LOD). The assay demonstrated in the present study quantitatively recovered Pt\(^{+2}\) in the range of 70–150 ppm with good accuracy and precision. Further, the test of antibacterial activity of AgNPs alone, and in combination with ampicillin showed excellent activity against four of the six tested bacteria.

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1. Introduction

Nanomaterials (NMs) and nanoparticles (NPs) are considered as the bridge between atomic structure and bulk materials. Due to their unique properties such as small size, large surface-to-mass/volume ratio, surface-enhanced Raman scattering (SERS), quantum size effect, and super-magnetism, NPs are gaining importance in nanotechnology. Metal NPs (MNPs) are purely aggregates of the metals atoms in the size range of 10–100 nm that exhibit unique physical, chemical, electronic, magnetic, thermal and optical properties different from that of bulk equivalent. In the nano-biotechnology, which is a currently emerging sub-branch of nanotechnology, MNPs are used for medical imaging, separation of biomolecules, disease diagnosis, pharmaceutical products, wound dressings, cosmetics, drug delivery, cancer therapy, hyperthermia of tumors, etc. [1–5]. Among the various MNPs, AgNPs are the most promising as they can be used in a wide range of biomedical applications. Besides showing excellent antimicrobial and anti-biofilm activities, AgNPs have also been demonstrated for their use in biomedical applications like wound dressings, antiseptic sprays and topical creams, drug targeting, drug delivery, cell imaging, colorimetric detection of metal ions, biosensors and colorimetric sensing of various drugs and biological samples [6–8].

Koduru et al. (2018) have reviewed phytochemical mediated synthesis of AgNPs and its application for antimicrobial activity along with its brief mechanism [9].

For the range of applications, AgNPs can be synthesized by various physical and chemical methods. However, these conventional methods are capital, materials, and energy-intensive. Also, these methods depend on the use of toxic or environmentally harmful chemicals and therefore, generate hazardous by-products [10]. As an alternative to physical and chemical methods, MNPs such as AgNPs synthesis by biological systems (biogenic) is preferred as an eco-friendly approach as it does not require any external stabilizing and capping agents [11]. Among all biological sources, bacteria mediated synthesis methods are explored due to their advantages over other biological sources. Easy handling, manipulation of genetic material, adaptability in a different type of environmental conditions make bacteria more useful for biogenic synthesis [7,12].

In the present study, the bacterial strain Pseudomonas stutzeri KDP_M2 was employed for the optimized biogenic synthesis of AgNPs for the development of a colorimetric probe for the detection of metal platinum. To optimize the biogenic synthesis, the effects of different parameters such as the concentration of precursor, pH, temperature, and reaction incubation time on AgNPs synthesis were studied and used for optimum synthesis. The optimally synthesized biogenic AgNPs were then characterized by various spectroscopic and imaging techniques. Further, the biogenic AgNPs were then demonstrated for their application in selective and specific colorimetric detection of platinum (Pt\(^{+2}\)).

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Also, the antibacterial activities of these biogenic AgNPs were tested against six bacterial strains and minimum inhibitory concentrations (MICs) for each bacterial strain were also estimated.

2. Experimental details

2.1. Materials

Luria Bertani (LB) Broth, Muller Hinton (MH) broth, silver nitrate (AgNO₃), agar-agar, sodium hydroxide (NaOH), and metal salts such as aluminum chloride (AlCl₃), barium hydroxide (Ba(OH)₂), calcium sulfate (CaSO₄), cadmium chloride (CdCl₂), cobalt sulfate heptahydrate (CoSO₄·7H₂O), copper sulfate pentahydrate (CuSO₄·5H₂O), chromium chloride hexahydrate (CrCl₃·6H₂O), ferrous sulfate heptahydrate (FeSO₄·7H₂O), lithium perchlorate (LiClO₄), manganese sulfate monohydrate (MnSO₄·H₂O), magnesium sulfate heptahydrate (MgSO₄·7H₂O), mercuric chloride (HgCl₂), molybdenum trioxide (MoO₃), nickel chloride monohydrate (NiCl₂·H₂O), palladium chloride (PdCl₂), potassium chloride (KCl), potassium tetrachloroplattinate (K₂PtCl₆), selenium dioxide (SeO₂), titanium dioxide (TiO₂), zirconium chloride oxohydrate (ZrOCl₂·8H₂O), etc. were obtained from Sigma-Aldrich, India. All chemicals used were of analytical grade.

Sterile double distilled water (SDDW) was used for stocks and working solutions preparations.

2.2. Bacterial culture and preparation of bacterial biomass (BB)

Previously, iron tolerant bacterial strain was enriched, isolated and identified as P. stutzeri_KDP. This strain was found capable of synthesizing iron oxide magnetic NPs (IOMNPs) and gold nanoparticles (AuNPs) data communicated. For the present study, the same P. stutzeri_KDP was used for the synthesis of AgNPs. To this end, a loopful of 24 h old culture of this strain was inoculated in LB broth (1 L) and incubated at 37°C on shaking incubator agitating at 120 rpm for 72 h. After incubation, culture was centrifuged at 8000 rpm for 10 min., the supernatant was separated. Bacterial biomass (BB) was collected and subsequently washed 2–3 times with SDDW and then re-suspended in SDDW to the final concentration of (0.1 g/100 mL). Such re-suspended BB was used as a source of reducing and capping agents for the optimization of biogenic synthesis of AgNPs.

2.3. Optimization of AgNPs synthesis

For the study of effects of various synthesis parameters, the concentration of precursor (AgNO₃), pH, temperature, reaction time, etc. were varied in the ranges of 0.5–2 mM, 5–9, 30–70°C and 15 min–24 h and studied for their effects on AgNPs synthesis. During these optimization studies, AgNPs synthesis was monitored by recording UV–vis spectra in the range of 350–750 nm on BioSpectrometer (Eppendorf, Hamburg, Germany) as well as visually by observing the change in color of the reaction mixture from whitish to brown. AgNPs were synthesized on a large scale using optimized conditions and used for further study.

2.4. Characterization of AgNPs

A range of various spectroscopic and imaging techniques was used for the characterization of biogenic AgNPs. The UV–Vis. spectra were recorded in the range of 350–750 nm. The identifications of functional groups of biomolecules that could have acted as reducing and capping agents for biosynthesis were done by recording Fourier Transform Infra-red (FTIR) spectra on FTIR spectrometer (Shimadzu, Japan) whereas, the elemental compositions were detected using energy-dispersive X-ray spectroscopy) EDS with x-act with INCA® and Aztec® EDS analysis software (Oxford Instruments, UK). The X-ray powder diffraction (XRD) patterns were recorded on XRD (Bruker AXS Analytical Instruments Pvt. Ltd., Germany) and used to study the crystalline nature of AgNPs. The hydrodynamic particle size of AgNPs was determined by Zetasizer Ver. 7.11 (Malvern instruments Ltd, UK). Morphology, size, crystallinity and selected-area electron diffraction (SAED) patterns were studied by transmission electron microscopy (TEM) (Jeol/JEM 2100, USA) operating at an acceleration voltage of 300 kV.

2.5. Biogenic AgNPs probe-based colorimetric detection of metal ions

For biogenic AgNPs probe-based selective and sensitive colorimetric detection of metal ions, 20 different metal cations namely Al³⁺, Ba²⁺, Ca²⁺, Cd²⁺, Cr³⁺, Co²⁺, Cu²⁺, Fe³⁺, Hg²⁺, K⁺, Li⁺, Mg²⁺, Mn²⁺, Mo⁷⁺, Ni²⁺, Pd²⁺, Pt²⁺, Se⁴⁺, Te⁴⁺, Zn²⁺ and Zr²⁺ and were screened initially by exposing the colloidal AgNPs (100 μL) solution to 100 μL of each of these metal ions (500 ppm). The reactions were then monitored visually for change of color and by recording UV–Vis. spectra for the study of spectral shift in SPR peak. Further, for metal ions which were successfully detected as evident by a change of color and SPR shift, the limit of detection (LOD) and limit of quantification (LOQ) were studied as a test of sensitivity. To accomplish this, the selected metal ions in the range of 10–100 ppm were added to the colloidal AgNPs solutions and monitored for change of colors and shift in SPR peaks. The standard graph of concentration (ppm) of selected metal ion Vs absorption ratio (A₄20/A₄00) was plotted to study the linearity and obtain the equation of straight line and regression coefficient. The LOD was expressed as the lowest concentration of an analyte in a sample that could be detected but not necessarily quantified under the stated conditions whereas, the LOQ was estimated as the lowest concentration of an analyte that could be determined with acceptable precision and accuracy under the standard conditions. Further, the precision and accuracy of selected metal ion detection were determined percentage (%) recovery, intra-day repeatability, and inter-day reproducibility. To this end, water samples were spiked with known concentrations in the range of 50–90 ppm, then quantified at three different times in a single day (intra-day) and on three consecutive days (inter-day) for determination of % recovery and %RSDs.

2.6. Detection of Pt²⁺ ions in real water samples

Further, the potential efficacy of AgNPs probe based assay for the detection Pt²⁺ in real water sample (drinking water) was tested. To achieve this, the drinking water samples were spiked with 12.5, 13.0 and 13.5 ppm Pt²⁺ and used in a detection assay. The detection reaction was monitored by recording of UV–vis spectra for the spectral shift in SPR peaks. A standard plot of Pt²⁺ concentrations in the range of 4–14 ppm against ratio (A₄20/A₄00) was plotted, a trend line was added to obtain the equation of the straight line and regression value and used for determination of Pt²⁺ concentration and recovery from spiked real water test samples.

2.7. Antibacterial activity of AgNPs

The antibacterial activity of biogenic AgNPs was tested against two Gram-positive bacterial strains such as Bacillus subtilis, and Staphylococcus aureus and four Gram-negative strains namely Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, and Protease vulgaris by employing the well-diffusion assay with slight modifications as described previously [13]. These cultures were obtained from the National Centre for Industrial Microbes,
National Chemical Laboratory Pune, India. For antibacterial test, 50 μL of freshly prepared, 24 h old culture of each of bacterial strain under the study was spread evenly on an MH agar plate, wells were prepared and then 100 μL of each of test colloidal AgNPs (5 and 10 mg/mL) solutions, ampicillin (20 μg/mL) + AgNPs (10 mg/mL) and ampicillin (20 μg/mL) were loaded in the wells. Subsequently, the plates were incubated at 37 °C for 24 h and observed for zones of inhibition (ZOI) and measured to estimate the antibacterial activities. To determine the MIC, a broth dilution method as described previously by Saxena et al. (2016) was employed [14]. Briefly, the AgNPs solution (10 mg/mL) was prepared and diluted to get the solutions of different concentrations in the range of 1–5 mg/mL. The suspensions of each test bacterial strains were diluted in sterile MH broth to obtain final inoculums which were then inoculated in culture tube containing MH broth with AgNPs in the range of 1–5 mg/mL. These culture tubes were then incubated at 37 °C at 150 rpm for 24 h, then optical densities (OD) were determined at 600 nm. Culture medium containing test organisms without AgNPs was used as a negative control. MICs for each test organism were recorded as the minimum concentration at which no visible growth of test organisms was observed.

3. Results and discussion

It has been well established that whole cells biomass, crude cell extract and crude or purified enzymes from microorganisms play a major role as reducing and capping agents during the biogenic synthesis of NPs [15–17]. In our previous study, an iron tolerant bacterial strain P. stutzeri KDP_M2 (NCBI genebank accession No. MK312641) was successfully used for the biogenic synthesis of IOMNPs and AuNPs (data in the communication). Our initial experiments demonstrated that P. stutzeri was also capable of AgNPs synthesis and therefore, was used in the present study for biogenic synthesis of AgNPs.

3.1. Optimization of biogenic synthesis of AgNPs

In order to study if BB of P. stutzeri also synthesizes AgNPs, the initial reaction was set up between AgNO₃ (1 mM) and BB which resulted in the appearance of dark brown color and SPR absorption at 420 nm that clearly demonstrated and confirmed the AgNPs synthesis (Fig. 1a). The AgNO₃ in the range of 0.5–2 mM was noted to increase the intensities of SPR peaks and brown color (Fig. 1b). However, a single and sharp SPR peak with maximum intensity at 420 nm was observed at 2 mM AgNO₃ suggesting it as the optimum precursor concentration. In congruence with our observation, 2 mM AgNO₃ was also reported optimum for Penicillium aculeatum Su1 mediated biogenic synthesis of AgNPs [18].

Fig. 1c shows the effect of another synthesis parameter i.e. pH. In this case, characteristic SPR peaks and change of color were observed at alkaline conditions of pH 8 and 9. Between these two pH values, pH 9 was most suitable which was evident by the appearance of an intense peak at 420 nm. Thus, in the case of the synthesis of AgNPs, pH 9 was found most suitable (Fig. 1c). The literature survey suggests that alkaline conditions are most suitable for the biogenic synthesis of stable preparations of AgNPs. Our results are well in agreement with previous studies that also demonstrated the alkaline conditions as most suitable for the biosynthesis of MNPs [18,19].

Further, attempts were made to optimize the reaction parameters such as incubation temperature and time. Fig. 1d and e represent the effects of incubation temperature and time in the ranges of 30–70 °C and 15 min-24 h, respectively. The characteristic SPR peaks with the highest intensity and appearance of characteristic brown color were observed when reactions were incubated at 60 °C for 24 h (Fig. 1d). This study of the effects of temperature and time showed that P. stutzeri mediated optimum AgNPs synthesis required incubation at 60 °C for 24 h (Fig. 1e). Previously, Gurunathan and colleagues also reported optimum AgNPs biosynthesis at 60 °C [20]. When Baker and colleagues investigated the extracellular synthesis of

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**Fig. 1.** Optimization of P. stutzeri mediated synthesis of AgNPs. (a) UV–vis. absorption spectrum of initial AgNPs synthesis reaction (i) initial reaction (ii) reaction after synthesis of AgNPs; (b) effect of AgNO₃ (0.5–2 mM); (c) effect of pH (5–9); (d) effect of temperature (30–70 °C) and (e) effect of time (15 min–24 h) on AgNPs synthesis. (inset images show change in the colour of the reaction mixtures).
AgNPs by cell-free extract of *Pseudomonas veronii* AS 41 G, the synthesis was found rapid at increased temperature with 80°C being optimum [21]. The optimum and increased rate of synthesis can be attributed to increased kinetic energy due to increased temperature. This was well supported by the study of the effect of temperature on *Sclerotinia sclerotiorum* mediated AgNPs synthesis by Saxena and co-workers (2016) who noticed that an increase in temperature increases the kinetic energy of reaction which subsequently leads to faster synthesis rate [14].

After having optimized synthesis parameters, the large scale synthesis of AgNPs was carried out using optimized parameters such as 2 mM AgNO₃, pH 9 and incubation at 60°C for 24 h for AgNPs synthesis and characterized subsequently.

### 3.2. Characterization of biogenic AgNPs

The FTIR spectrum of AgNPs showed bands at 3226.38, 2924.64, 1743.97, 1685.89, 1524.70, 949.46, 700.38 and 608.16 cm⁻¹ for biosynthesized AgNPs (Fig. 2a). A small peak at 3226 cm⁻¹ appeared due to hydroxyl group while, band at 2924 cm⁻¹ was found to correspond to C—H stretching, while another small peak at 1743.97 was found associated with stretching vibrations of carbonyl group (C=O), whereas peaks at 1685.89 and 1524 cm⁻¹ represented peaks representing amide I and amide II stretching of polypeptides/proteins. In addition, a peak observed in the range of 700 to 750 cm⁻¹ could have occurred due to amino groups or aromatic groups of biomolecules from BB in biogenic synthesis. It was observed that peaks at 2925, 1700–1743 and 1524–1525 could have occurred due to C—H vibrational stretching of the methylene groups of the protein, carbonyl group (C=O) and stretching of amide I and II groups of polypeptides/proteins, respectively. These peaks confirmed biomolecules such as amino acids, proteins or enzymes from *P. stutzeri* were involved in the synthesis of AgNPs. Further, XRD pattern of AgNPs (Fig. 2b) and its analysis revealed the Bragg’s reflections at 2θ values of 38.11°, 44.20°, 64.44° and 77.39° which represented [111] [200] [220] and [311] planes, respectively that were found to match with JCPDS card #03-065-2871. In addition, few low-intensity peaks were also observed which could have occurred due to the presence of biological molecules on the surface of AgNPs. Based on the XRD pattern and Scherer’s relation (D = kλ/βcosθ), the average crystalline size was calculated to be 32 nm. The EDS based elemental composition showed a strong absorption peak at 3.2 and 2.7 keV corresponding to the elemental signal of silver (Fig. 2c). The EDS of AgNPs also showed weak peaks corresponding to carbon, oxygen, phosphorous and iron confirming the biogenic synthesis. The estimation of hydrodynamic particle size (Fig. 2d) indicated AgNPs of an average size of 227 nm whereas, TEM imaging showed well-separated AgNPs of size ranging between 10–50 nm (Fig. 2e). The SAED pattern (Fig. 2f) showed well-defined circular rings of diffraction related to Bragg’s reflections at [111], [200], [220] and [311] that was well in congruence with XRD pattern. Both, SAED together with XRD demonstrated the nanocrystalline FCC structure of biogenic AgNPs.

### 3.3. Biogenic AgNPs probe-based colorimetric detection of metal ions

The initial screening of 20 metal cations for the study of metal ion detection indicated that only one metal ion namely Pt⁺² resulted in the change of color for detection reaction from brown to slight yellow along with spectral shifts after 1 h of incubation (Fig. 3a–f). Though few other tested ions showed slight spectral shifts, only Pt⁺² caused both, the prominent change in color and spectral shifts, therefore, was considered further for the test of sensitivity. The most prominent color change (brown to pale yellow) was observed upon addition of 200 ppm of Pt⁺² in 1 h whereas prominent SPR shift was observed upon addition of 50 ppm (Table 1). These initial screening reactions demonstrated that biogenic AgNPs of the present study could be employed for a selective, colorimetric probe for the detection of Pt⁺². The test of the sensitivity of Pt⁺² detection in the range of 50–90 ppm indicated that 50 ppm of Pt⁺² was the LOD as the reactions between

![Fig. 2. Characterization of AgNPs. (a) FTIR spectrum; (b) XRD pattern; (c) EDS spectrum; (d) particle size analysis by DLS; (e) TEM image at scale 20 nm and (f) SAED pattern of purified AgNPs.](image-url)
AgNPs and Pt\textsuperscript{2+} at < 50 ppm did not show the change in spectra. Thus, minimum concentrations of Pt\textsuperscript{2+} that could be colorimetrically detected using AgNPs was 50 ppm, indicating it to be LOD. The test of sensitivity in the range from 50–90 ppm required a minimum of 1 h for prominent SPR shift upon addition of Pt\textsuperscript{2+}. The recording of absorption spectra for the test of precision and accuracy of Pt\textsuperscript{2+} detection in the range of 50–90 ppm indicated that an increase in the concentrations of Pt\textsuperscript{2+} increases the SPR shift. Also, the absorption ratio \(A_{420}/A_{409}\) for Pt\textsuperscript{2+} was found to linearly decrease with increased concentration of analytes in the range from 50–90 ppm for Pt\textsuperscript{2+}. This demonstrated the direct and linear correlation between the concentration of analyte and absorption ratios (Fig. 3d and e) with the correlation coefficient \((R^2)\) of 0.9699. Table 2 represents the precision and accuracy of

![Figure 3](image)

**Fig. 3.** AgNPs probe-based colorimetric detection of Pt\textsuperscript{2+}. (a) Visual color change; (b and c) UV–Vis. absorption spectra showing SPR shifts for sensing reaction upon addition of different metal ions; (d) Spectral shift with all metal ions; (e) UV–Vis. absorption spectra of Pt\textsuperscript{2+} detection reaction in the range of 50–90 ppm; (f) plot of \(A_{420}/A_{409}\) ratio versus the concentration of Pt\textsuperscript{2+} in the range of 50–90 ppm.

**Table 1**

| Metal ions | Spectral shift (nm) | Visible Colour change (Y/N) | Absorption maxima of AgNPs (nm) | Minimum concentration (ppm) required for color change | Time Required for color change |
|------------|---------------------|-----------------------------|-------------------------------|------------------------------------------------------|-------------------------------|
| Se\textsuperscript{2+} | 1                   | No                          | 419                           | –                                                    | –                            |
| Zr\textsuperscript{4+} | 1                   | No                          | 416                           | –                                                    | –                            |
| Pd\textsuperscript{2+} | 0                   | No                          | 415                           | –                                                    | –                            |
| Cd\textsuperscript{2+} | 0                   | No                          | 415                           | –                                                    | –                            |
| Mn\textsuperscript{2+} | 1                   | No                          | 416                           | –                                                    | –                            |
| Cu\textsuperscript{2+} | 2                   | No                          | 417                           | –                                                    | –                            |
| Mg\textsuperscript{2+} | 0                   | No                          | 415                           | –                                                    | –                            |
| Ti\textsuperscript{2+} | 0                   | No                          | 415                           | –                                                    | –                            |
| Ni\textsuperscript{2+} | 0                   | No                          | 415                           | –                                                    | –                            |
| Pt\textsuperscript{2+} | 13                  | Yes (brown to pale yellow)  | 409                           | 50                                                  | 1 h                          |
| Cr\textsuperscript{3+} | 0                   | No                          | 415                           | –                                                    | –                            |
| Co\textsuperscript{2+} | 0                   | No                          | 415                           | –                                                    | –                            |
| Ba\textsuperscript{2+} | 2                   | No                          | 417                           | –                                                    | –                            |
| Mo\textsuperscript{2+} | 0                   | No                          | 415                           | –                                                    | –                            |
| K\textsuperscript{+} | 0                   | No                          | 415                           | –                                                    | –                            |
| Ca\textsuperscript{2+} | 0                   | No                          | 415                           | –                                                    | –                            |
| Hg\textsuperscript{2+} | 1                   | No                          | 416                           | –                                                    | –                            |
| Fe\textsuperscript{2+} | 3                   | Yes                         | 423                           | –                                                    | –                            |
| Li\textsuperscript{1+} | 1                   | No                          | 416                           | –                                                    | –                            |
| Al\textsuperscript{3+} | 2                   | No                          | 417                           | –                                                    | –                            |

Precision of method was expressed as the % RSD of three replicate samples.
AgNPs based quantitative detection of Pt$^{2+}$ and shows % recovery and %RSD for intra-day repeatability and inter-day reproducibility of quantitative analyses performed on spiked water samples.

Using AgNPs probe-based assay employed in the present study, Pt$^{2+}$ was estimated to be recovered in the range of 81.27–115.44 % with % RSD in the range of 4.011–10.135 and inter-day in the range of 50.89–61.50 % with % RSD in the range of 2.56–14.44. Since, biogenic AgNPs based colorimetric assay employed in our study quantitatively recovered Pt$^{2+}$ in the range of 70–150 % with good precision (RSD < 20 %), it complies with the standards laid by United State Pharmacopeia (USP) [22,23] and therefore, is acceptable. As per our literature survey, this is the first report on colorimetric detection of platinum by AgNPs. There are no previous reports for Pt$^{2+}$ detection by AgNPs with colorimetric assay, thus making this study significant for probe based detection.

The possible mechanism of the colorimetric assay for sensing Pt$^{2+}$ is yet unknown but the methods of determination is reported to rely on their unique size and inter-particle distance-dependent absorption spectra and color change of the solution [24]. As per the previous studies that demonstrated the heavy metal detection by AgNPs, the bacterial proteins responsible for reduction of silver, its stabilization and the peptide linkage (—CONH—) may be responsible for formation of coordination complex with Pt$^{2+}$ [25]. By nature the transition elements like platinum metal is difficult to analyze. Though various traditional techniques such as acid test, fire assay, conductivity used for detection of Pt$^{2+}$ are cheap, but they are less reliable due to their approximate results. On the other hand, modern techniques like energy dispersion X-ray fluorescence (ED-XRF), optimal emission spectroscopy (OES), inductively coupled plasma optical emission spectroscopy (ICP-OES) that are used to improve the accuracy and sensitivity involve high costly, complex instrumentation and require expertise. In comparison to these methods, our study reports simple, cost effective and reliable colorimetric assay for sensing a transition metal like Pt$^{2+}$ by AgNPs.

### 3.4. Detection of Pt$^{2+}$ in real water samples

The possible efficacy of AgNPs probes based Pt$^{2+}$ detection assay was further extended and confirm by demonstrating the quantitative detection and recovery of Pt$^{2+}$ in spiked water samples. The colorimetric response in terms of blue shifts of the SPR peaks in the range of 4–14 ppm of Pt$^{2+}$ was found directly proportional and linear. The plot of ratio $A_{420}/A_{409}$ against Pt$^{2+}$ showed good linearity and regression ($R^2 = 0.9737$) (Fig. 4b). Pt$^{2+}$ was quantitatively estimated and recovered from spiked samples in the range of 91.76–104.96 % with % RSD in the range of 3.49–5.73 (Table S1). Our results were quite promising as the detection reaction was rapid, accurate and detected Pt$^{2+}$ at low concentrations with good recovery.

### 3.5. Antibacterial activity

The ever increasing antibiotic resistance in the bacterial kingdom is a major concern in the biomedical field [26]. AgNPs can be used as an alternative or in combination with antibiotics for enhancing efficiencies of antibiotics. Recently, AgNPs alone or in combination with antibiotics has been well demonstrated for their antimicrobial efficacy by various researchers [10,27,28]. In the present study, the test of antibacterial activities of AgNPs alone and in combination with ampicillin showed that AgNPs at 5 and 10 mg/mL clearly inhibited the growths of tested bacterial strains such as E. coli, B. subtilis, S. aureus and P. vulgaris which was clearly evident by clear ZOIs in the range 12–20 mm (Table S2 and Fig. 5). Noteworthy, AgNPs were not found to inhibit the growths of P. aeruginosa and S. typhi (Table S2 and Fig. 5). In addition, it was found that ZOIs of growths of E. coli and B. subtilis due to AgNPs in combination with Ampicillin was enhanced as compared to Ampicillin alone. Inhibitory effect of Ampicillin alone was not observed for S. aureus and P. vulgaris whereas Ampicillin in combination with AgNPs showed clear ZOIs indicating enhanced antibacterial effect. Further, experiments were carried out to

### Table 2

Accuracy and precision of quantitative detection of Pt$^{2+}$ spiked water samples.

| Spiked Conc. (ppm) | Estimated Conc. (ppm) | Recovery (%) | % RSD |
|-------------------|-----------------------|--------------|-------|
|                   | Intra day | Inter day | Intra day | Inter day | |
| Spike water 50    | 57.72     | 50.89     | 115.44    | 101.78    | 4.011 |
|                   | 63        | 61.44     | 105       | 102.4     | 9.195 |
|                   | 56.89     | 61.5      | 81.27     | 87.86     | 10.135 |

**Fig. 4.** (a) Plot of absorbance intensity difference of Pt$^{2+}$ concentration for drinking water sample; (b) Plot of ratio $A_{420}/A_{409}$ versus concentration for Pt$^{2+}$ detection.
estimate the MICs of AgNPs against four of the six most susceptible bacterial strains. Table S2 shows MIC values against E. coli, B. subtilis, S. aureus, and P. vulgaris. The MICs against E. coli and P. vulgaris were estimated to be 1 mg/mL whereas MICs against B. Subtilis and S. aureus were estimated to be 2 mg/mL. Thus, it can be said that P. stutzeri mediated biogenic AgNPs possess excellent antibacterial activity, therefore, they can be used either alone or in combination with Ampicillin for the formulation of antibacterial preparations.

4. Conclusion

In conclusion, iron tolerant bacterium P. stutzeri was successfully used in optimized biogenic synthesis of AgNPs. The biogenic AgNPs were demonstrated for possible application as colorimetric probes for sensing of Pt\(^{2+}\) with high precision and accuracy. In addition, biogenic AgNPs showed excellent antibacterial activity against four of the six tested bacteria namely B. subtilis, S. aureus, E. coli, and P. vulgaris. Thus P. stutzeri mediated biogenic AgNPs can be employed for the development of colorimetric probe for sensing of Pt\(^{2+}\) and AgNPs alone or in combination with ampicillin can be used as an antibacterial agent for biomedical applications.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.btre.2019.e00404.

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M.P. Desai et al. / Biotechnology Reports 25 (2020) e00404

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