

Research Article

Berberine Loaded Poly(vinylpyrrolidone) – Capped Silver Nanoparticles for Antioxidant and Antimicrobial Applications**B Dinesh¹, Lokesh Koodlur Sannegowda², Chandrashekhar G. Joshi^{1*}**¹Department of Biochemistry, Mangalore University, Mangalore - 574199, Karnataka, India.²Department of Studies in Chemistry, Vijayanagara Sri Krishnadevaraya University, Ballari - 583 105, Karnataka, India.**(Received: 15-08-2025****Revised: 17-12-2025****Accepted: 24-12-2025)**Corresponding Author: **Chandrashekhar G. Joshi** Email: josheejoshee@gmail.com**ABSTRACT**

Silver nanoparticles (AgNPs) are widely used in diverse fields, but have the limitation of stability and bioactivity which needs regular optimization. This study reports an ecofriendly approach in which the poly(vinylpyrrolidone) AgNPs was capped with glucose and loaded with Berberine(BBR) to form BBR loaded nanoformulation (PBR). The PBR was characterized using UV-visible spectroscopy, Scanning Electron Microscopy (SEM) with Energy Dispersive X-ray (EDX) analysis, X-ray diffraction, Dynamic Light Scattering (DLS), Zeta Potential analysis, and Fourier Transforms Infrared Spectroscopy (FTIR). The PBR showed a uniform spherical morphology with an average size of 78 nm and the zeta potential of -23.7 mV. FTIR study confirmed the loading of BBR through the shift in the functional groups in the spectra. PBR exhibited dose dependent antioxidant activity (18.5-45.9%), though lesser than the ascorbic acid. The antimicrobial activity revealed that PBR has moderate antifungal activity (40% inhibition against *Aspergillus* sp.). The moderate antioxidant and antifungal activity of PBR warrants further optimization to enhance its bioactivity.

Keywords: Silver nanoparticles; berberine; poly(vinylpyrrolidone); anti-oxidant activity; anti-microbial activity.

1. INTRODUCTION

The unique properties of the nanomaterials have revolutionized the advances in nanoscience and nanotechnology in the last decade [1]. Their size is 1-100 nm and these nanoparticles exhibit unique mechanical [2], electrical, magnetic, photochemical [3], and catalytic behaviors relative to those of their bulk counterparts [4]. The special features have helped them to have their applications in packaging, agriculture, energy, information technology, and health sectors [5].

AgNPs are notable metallic nanomaterials that have unique physical as well as chemical features [6]. They are extensively used in surface disinfectants, toys, textiles, air and water purification systems [7], gels, paints, food packaging, medical attire, and food product preservation [8]. Biological procedures of

AgNPs synthesis have become popular because of the high cost and hazard posed by the conventional physical and chemical techniques [9, 10].

The AgNPs biological activity is controlled by various parameters, such as their shape, surface chemistry, size, coating, morphology, composition, ion release efficiency, agglomeration, dissolution rate, cell type and specific reducing agents [11]. Poly (vinylpyrrolidone) (PVP) is a widely applied as stabilizer and reducing agent in the AgNPs production because it establishes strong interaction with metals via its carboxyl group and the nitrogen atoms on its pyrrolidine ring [12]. Recent studies have highlighted the improved stability of AgNPs capped with PVP compared with other agents [13].

Berberine (BBR), is a plant derived isoquinoline alkaloid, traditionally used in Chinese medicine for treating range of conditions including microbial infections, diabetes mellitus, diarrhea, ulcers, hypercholesterolemia, cardiovascular issues, polycystic ovary syndrome, fatty liver, and cancer [14-16]. Even though BBR and P-AgNPs (PVP capped AgNPs) have been studied independently, the knowledge about BBR-loaded P-AgNPs remains limited.

This work was intended to prepare green P-AgNPs by use of PVP and glucose as capping and reducing agent respectively. We explored the improved stability of BBR due to PVP capping on AgNPs and evaluated the antioxidant and antimicrobial activity of BBR loaded P-AgNPs.

2. MATERIALS AND METHODS

Poly(vinylpyrrolidone) capped AgNPs Synthesis

Production of PVP-capped AgNPs followed the protocol of Chen *et al.*, [17]. To summarize, 15 mM AgNO₃ and PVP (1.5 mM in comparison with AgNO₃) dissolved in 50 mL of distilled water followed by stirring. Then, dropwise 10 mM glucose in 25 mL water and 10 mM KOH were added. The mixture was allowed to form the nanoparticles on a magnetic stirrer with heating plate at 60 degree temperature after this. The P-silver nanoparticles were then centrifuged at 11500 rpm (20 min) and collected after drying in stores.

P-AgNPs (PBR) loading of Berberine

The fusion of berberine (BBR) into P-AgNPs followed the protocol of Bhanumathi *et al.*, [18] with minor changes. In a short time, 10 mg P-AgNPs and 10 mg BBR were suspended in 5 mL dimethyl sulfoxide (DMSO) and stirred in each other overnight to allow binding. The resulting mixture was dialyzed against distilled water for 24 h to eliminate unbound components. Subsequently, centrifugation at 11,500 rpm was carried out to obtain the BBR-loaded P-AgNPs (PBR), separating them from free molecules and residual impurities.

UV– visible absorbance spectroscopy

PBR solution (0.5 ml) UV-1800 (Shimadzu, Japan) was investigated with precisions of ± 1 nm at range of 200 to 700 nm wavelength and scan

rate of 200 nm/min to confirm the formation of PBR through BBR loading on P-AgNPs. AgNO₃ solution was used as a control at A (15 mM) [19].

Energy-dispersive X-ray spectroscopy and SEM

The morphology and dispersion of thin layers of PBR were studied using SEM applied on a copper grid plus carbon (ZEISS EVO 15, Germany). Elemental composition of the PBR was also determined by EDX (Oxford Instruments, AztecLive, UK) [20].

X-ray diffraction

X-ray diffractometer (Empyrean, 3 rd generation, Malvern PANalytical) 40 kV and 40 mA and Co K alpha radiation were utilized to find out the structural features of PBR. Peak broadening was the evidence of the crystalline structure of the nanoparticles. The DebyeScherrer equation was used to estimate the mean particle size [21] as follows.

$$D = \frac{K\lambda}{\beta \cos \theta} \quad (1)$$

D = depth of the nanocrystal,

K= constant,

λ = X Rays wavelength,

β = half maxima width at the (111) reflection at the angle of Bragg,

θ = Bragg's angle.

Zeta potential and dynamic light scattering

The laser diffraction with multiple scattering (3 LitesizerTM 500, Anton-Paar, Austria) was used to determine the hydrodynamic size of the PBR together with DLS. Zeta potential was found using a 3 LitesizerTM500(Anton-Paar, Austria) at 60 s and the mean zeta potential was found.

Fourier transform infrared spectroscopy

A Nicolet 6700 spectrometer operating with a resolution of 0.5 cm⁻¹ were used to perform FTIR spectroscopy on both BBR and PBR using the KBr pellet technique. To do an analysis, the sample was finely ground with KBr, pressed into a transparent pellet, and loaded in the spectrometer holder. The spectra were measured within the 400 4000 cm⁻¹ range, where the data on characteristic molecular vibrations and functional groups were obtained, and the structure of BBR and PBR [22] was characterized.

DPPH radical scavenging assay

The evaluation of this activity was done according to Hulikere *et al.*, [23] with slight modifications. PBR and ascorbic acid with different concentrations were added to 150 μL of DPPH solution (2 mM) and left to incubate after 15 min in the dark. A reading at 517 nm was then taken with an iMark microplate reader (Bio-Rad). The share of percentage of scavenging of free radical was calculated as follows:

$$\text{Percentage of Radical Scavenging Activity} = \left[\frac{A_c - A_s}{A_c} \right] \times 100 \quad (2)$$

“ A_c is the control absorbance of the DPPH radical + methanol;

As is the sample absorbance of the DPPH radical + sample AgNPs/Ascorbic acid”

Antimicrobial activity

PBR was tested against the pathogenic strains *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and fungus *Aspergillus sp.* through the well diffusion assay [24]. Agar plates were prepared by using 5 mm cork borer, and 100 μL of PBR at 100, 250, and 500 μg was loaded in the respective wells of 5 mm diameter of agar plates. Amikacin (25 $\mu\text{g/mL}$) and fluconazole (25 $\mu\text{g/mL}$) served to act as positive controls with bacterial and fungal strains, respectively.

Statistical analysis

Data from the experiments is presented in mean plus standard deviation (SD) of three independent repetitions. We conducted a statistical analysis with one-way ANOVA software Origin 8.0.

3. Results and Discussion

Evaluation of UV–visible spectroscopy

PBR UV-visible spectral analysis was done to ensure that the BBR has been loaded successfully on the surface of the P-AgNPs (Figure 1). Characteristic absorption peaks of BBR were observed at 232, 266, 346, and 428 nm, consistent with findings of Daiz *et al.*, [25]. Upon interaction with P-AgNPs, peaks were shifted toward 220, 260, 348, and 425 nm indicating the formation of PBR [18].

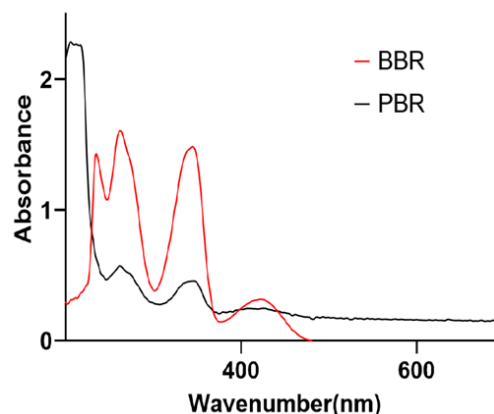


Figure 1: Ultraviolet-visible absorption spectra of Berberine (BBR) and P-AgNPs loaded with BBR (PBR)

Energy dispersive X-ray spectroscopy and SEM

SEM imaging was employed to examine the surface of PBR (Figure 2a). The images revealed the uniformly distributed, spherical, non-aggregated particles ranging from 60–100 nm in diameter with a mean particle size of 78 nm. In comparison, Bhanumathi et al have reported the size range of 35–50 nm for BBR-loaded AgNPs based on transmission electron microscopic studies [18]. The differences in the results are due to the use of different electron microscopes which work on dissimilar principles yielding slight variation in morphological data.

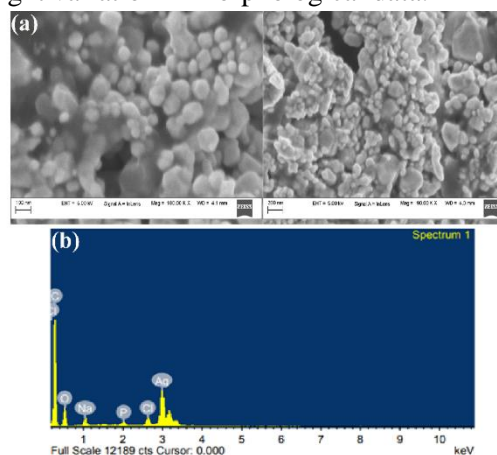


Figure 2: The morphology and elemental composition of P-AgNPs loaded with Berberine (PBR) in (a) SEM and (b) EDX

The energy dispersive X-ray (EDX) analysis with at 3 KeV absorption peak, revealed the purity and complete chemical composition of the PBR (Figure 2b). A significant peak was observed for silver (12.45%) along with other

notable components such as oxygen (52%), sodium (4.4%), chlorine (2.36%), and carbon (26.9%). Our results establish the role of the other organic molecules in the capping and stabilization of PBR [26].

X-ray diffraction evaluation

XRD pattern revealed clear diffraction peaks at 38.07° , 44.32° , 64.42° , 77.35° , and 81.45° as the crystal structure of the fcc metallic silver crystallographic plane (111), (200), (220), (311), and (222) (Figure 3). The lattice constant (4.047), interplanar spacing values (dhl) of 2.363, 2.043, 1.446, 1.232, and 1.180 were also matching with the standard Ag values JCPDS-PDF -card 04-0783. Weak signals at 10.0° and 20.0° are explained by BBR [27].

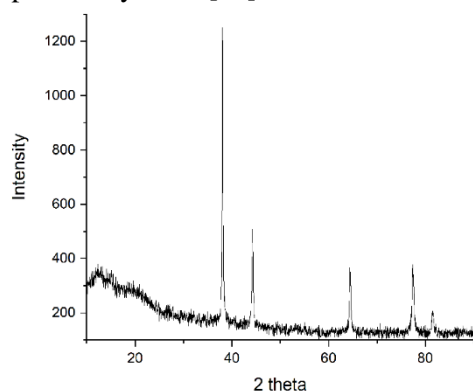


Figure 3: X-ray diffraction pattern of P-AgNP loaded with Berberine (PBR)

Evaluation of Zeta Potential and Dynamic light scattering

DLS evaluation using light interaction of PBR revealed the average hydrodynamic diameter of 284 nm (96% intensity) as shown in Figure 4a. The bigger size of the PBR observed in DLS compared to XRD, and SEM is attributed to the impact of the coating, stabilizing materials that build up on the surface, and the metallic core [28]. In addition, non-uniform dispersion of particles in the colloidal solution used in the DLS is also responsible for the observed differences in the results.

The surface charge of PBR and its stability were assessed by Zeta potential analysis (Figure 4b). PBR exhibited a modest stability with 23.7 mV zeta potential. Even though the PBR did not displayed the strong positive or negative zeta potential (greater than ± 30 mV) required for particle separation and disaggregation [29],

negative charge resist the aggregation and promote the stability due to electrostatic repulsion.

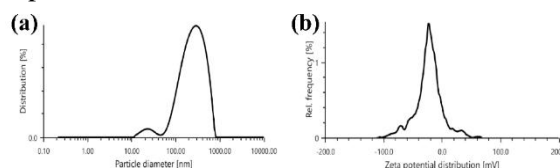


Figure 4: a) Hydrodynamic size measured by DLS, b) Surface charge determined by zeta potential analysis of P-AgNP loaded with BBR (PBR)

Fourier transform infrared Spectroscopic evaluation

The FTIR of PBR (Figure 5) spectrum was characterized by clear absorption bands at 1000.44, 1634.52, 2847.53, 2964.84 and 3548.85 cm^{-1} . The band of 1634.52 cm^{-1} OH is associated with the carbonyl (C=O) of BBR, which proves that it becomes conjugated with P-AgNPs. The peaks at 2847.53 cm^{-1} and 2964.84 cm^{-1} of the aliphatic C-H stretching vibrations are due to either PVP or BBR attached to the nanoparticles. The strong band at 3548.85 cm^{-1} showed the presence -OH or -NH group in the compound. All in all, the FTIR results show that BBR is conjugated to P-AgNPs

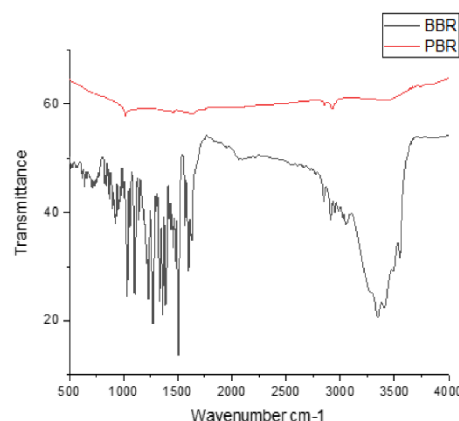


Figure 5: the FTIR spectra of berberine (BBR) and of P-AgNP loaded with BBR (PBR).

DPPH radical scavenging assay

This assay was employed to determine PBR antioxidant activity at 0.4-2mg/mL (Figure 6). PBR increased its radical scavenging activity in dose-dependent manners, reaching a maximum DPPH scavenging activity of 45.9% at the highest concentration tested. Even though its activity was relatively lower than the standard

ascorbic acid, an apparent improvement in the activity with higher concentration was noted. It is possible that the antioxidant activity of PBR remains observed due to the functional groups of BBR conjugated with P-AgNPs.

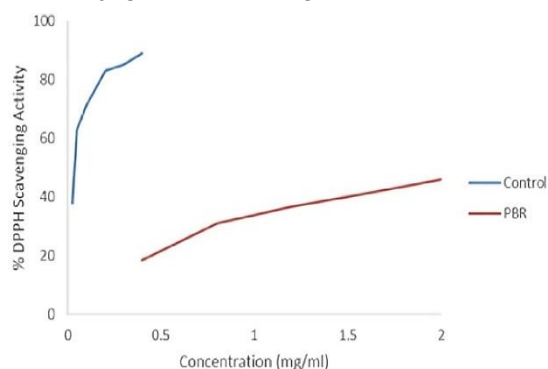


Figure 6: DPPH radical scavenging activity (%) of Control and PBR at different concentrations (mg/mL)

Antimicrobial activity

PBR and amikacin were tested against the microbe *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. typhi*. The tested bacteria did not show any antibacterial action in the PBR. The non-toxic PVP capping to AgNPs, the particle size, and the special ways of action explain the lack of the antibacterial action [30, 31]. In comparison, PBR displayed 40% of the standard drug inhibition of *Aspergillus sp.* growth. Although the fungal growth inhibition power of PBR was lesser than fluconazole (Table 1 & Figure 7), results indicate the potential antifungal activity of PBR, warranting further optimization.

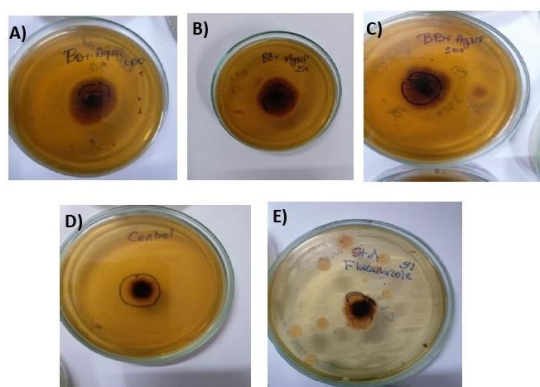


Figure 7: Antifungal activity of PBR against *Aspergillus sp.* showing zones of inhibition at different concentrations: (A) 100 µg, (B) 250 µg, (C) 500 µg of PBR. (D) Control, and (E) standard (fluconazole).

Table 1: Anti-microbial activity of the P-AgNP loaded with BBR (PBR), and standard (Amikacin and Fluconazole).

Test organism	PBR	Amikacin	Fluconazole	Control
Zone of inhibition (mm)				
<i>S. typhi</i>	-	12±0.6	-	-
<i>P. aeruginosa</i>	-	11±0.3	-	-
<i>E. coli</i>	-	10±0.7	-	-
<i>S. aureus</i>	-	9±0.4	-	-
<i>Aspergillus sp.</i>	3±0.4	-	2±0.7	-

4. Conclusion

In this study, AgNPs were synthesized, capped with PVP and loaded with BBR employing glucose as reducing agent at 60 °C under alkaline conditions without using any harsh chemicals. P-AgNPs and PBR formation was validated by using UV-visible spectroscopy, and their crystalline nature was established through XRD analysis. The SEM and EDX analyses revealed that their size was approximately 78 nm and was composed primarily of carbon, silver, and sodium. The zeta potential and the hydrodynamic size were -23 mV and 284 nm, respectively. The analysis of FTIR showed the successful loading of BBR onto P-silver nanoparticles. The PBR composite exhibited significant radical scavenging activity, moderately active against *Aspergillus sp.* and did not exhibit any antibacterial activity likely due to its size and morphology. These studies confirm the biomedical applications of the PBR warranting further studies to enhance the therapeutic efficacy.

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Author Contributions

Dinesh B was involved in the investigation, analysis of data, collection of data, writing of original draft, reviewing as well as editing, administration of the project and funding acquisition. Lokesh Koodlur Sannegowda helped to write the manuscripts, FTIR interpretation of data, and editing and reviewing. Chandrashekhar G. Joshi took part in the creation of the work, and designing methods of investigation, validation, formal analysis, data curation, provision of

resources, reviewing and editing of the manuscript, and overseeing the work.

Declaration

Ethical approval was not applicable.

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Conflicts of interest

The author's do not have any conflicts of interest.

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