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Label free and high specific detection of mercury ions based on silver nano-liposome

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ABSTRACT

Herein, we report an eco-friendly, mild and one-pot approach for synthesis of silver nanoparticles via a lipopeptide biosurfactant - CHBS. The biosurfactant forms liposome vesicles when dispersed in an aqueous medium. The amino acid groups of the biosurfactant assists in the reduction of Ag^{+} ions leading to the production of homogeneous silver nanoparticles, encapsulated within the liposome vesicle, as confirmed from TEM analysis. Rate of synthesis and size of particle were greatly dependent on pH and reaction temperature. Kinetic analysis suggests the involvement of an autocatalytic reaction and the observed rate constant (k_{obs}) was found to decrease with temperature, suggesting faster reaction with increasing temperature. Furthermore, the silver nanoparticles served as excellent probes for highly selective and sensitive recognition of Hg^{2+} ions. Interaction with Hg^{2+} ions results in an immediate change in colour of nanoparticle solution form brownish red to milky white. With increasing Hg^{2+} ions concentration, a gradual disappearance of SPR peak was observed. A linear relationship ($A_{420}/_{660}$) with an R^2 value of 0.97 was observed in the range of 20 to 100 ppm Hg^{2+} concentration. Hg^{2+} ions are reduced to their elemental forms which thereby interact with the vesicles, leading to aggregation and precipitation of particles. The detection method avoids the need of functionalizing ligands and favours Hg^{2+} detection in aqueous samples by visible range spectrophotometry and hence can be used for simple and rapid analysis.

1. Introduction

Mercury in its ionic form (Hg^{2+}) is considered to be toxic and is one of the most hazardous environmental pollutants. Hg^{2+} state of mercury is highly stable and water soluble, hence is one of the most widely and easily available contaminant in water bodies [25]. It is highly carcinogenic and easily penetrates the skin and respiratory tissues affecting brain, central nervous system, endocrine and gastrointestinal system in humans [14]. These health issues and ecological risk associated with Hg^{2+}, demands a simple and sensitive method for its detection. A number of traditionally techniques as atomic absorption spectroscopy, inductively coupled mass spectrophotometry, atomic fluorescence spectrometry and high-performance liquid chromatography are used for detection of Hg^{2+} ions [21]. However, these are associated with the drawbacks of high equipment cost, complex instrumentation and protracted protocols. Metal nanoparticles (NPs) because of their exceptional optical properties have been used as potent agents in detection of Hg^{2+} ion. NPs, specifically silver nanoparticles (SNPs) tagged with mercury specific oligonucleotides, DNAzymes, fluorescent probes, etc. have been used for Hg^{2+} ion detection [26,28,29]. Though these methods provide a limit of detection (LOD) in the range of 2.2×10^{-6} mol/L to 10^{-10} mol/L [16,25], however suffer from the drawbacks of being lengthy and cumbersome as they involve tagging the NP surface with Hg-specific ligands. Therefore there is the need of a simple, single step protocol favouring onsite applicability. Biologically synthesized NPs are a promising solution in this regard as biological agents serve as both reducing and stabilizing agents and hence are naturally capped by functional groups of biomolecules. This thus precludes the need of conjugating target specific ligand molecules to NP surface. Surfactants (sodium dodecyl sulphate (SDS), Tween 80, triethyltetraamine, cetyltrimethylammoniumbromide (CTAB), polyvinylpyrrolidone (PVP), amine and carbooxylate surfactant) are generally used as stabilizing agents in NP synthesis [4,17,18,24,27,30]. Surfactants possess both hydrophilic and hydrophobic moieties and have been widely reported to assist NP synthesis by reverse micelle method [5,15]. Reverse micelle favours controlled synthesis of NPs within its core, thereby aiding homogenous synthesis and low polydispersity. Ganesh et al. [15] synthesized SNPs using a biosurfactant (BS) produced by the bacteria *Pseudomonas aeruginosa*, in an organic medium based on reverse micelle method. However, reverse micelle makes it difficult to utilize the synthesized NPs in an aqueous medium and necessitates transfer from the organic

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phase. Liposomes present a promising alternative to reverse micelle by providing a controlled environment, easy biofunctionalization and encapsulation of biomolecules within the hydrophobic core [19,22]. Liposomes for NP synthesis are generally synthesized using phospholipids by a pH jump method [22,23] and require stabilization in order to prevent fusion, which otherwise can lead to inclusion leakage and inefficient reactions [19].

In the present study we report an eco-friendly method for synthesizing SNPs using a lipopeptide BS. The BS produced by the bacteria *Bacillus tequilensis* CH served the twofold role of reducing and stabilizing agent and aided SNP synthesis by formation of liposome vesicles. The functional group of BS facilitates reduction of Ag^{+} ions with subsequent production of homogenous SNPs within the liposome vesicle. Effect of different parameters (pH and temperature) and kinetics behind SNP synthesis were studied. Further, the synthesized silver nano-liposome were found to be selectively specific towards Hg^{2+} ions compared to other heavy metal ions and thus were thoroughly investigated for their effectiveness as colorimetric sensor for detecting Hg^{2+} ions. Moreover, the method avoided the requirement of conjugating Hg-specific ligands prior to detection and favoured rapid detection in aqueous solution.

2. Materials and methods

2.1. Chemicals and instruments

Metal salts (analytical grade) were purchased from SRL Pvt. Ltd. All the metal solutions were prepared using Milli-Q water. Absorption spectra were recorded on an UV–Vis Spectroscope (CECIL) in the range of 200–800 nm. Dynamic Light Scattering (DLS) analysis was performed with a Dawn Heleos II (Wyatt) system operating at a wavelength of 658 nm, at regulated temperature of 25 ± 1 °C. FTIR analysis was carried using a FTIR-Perkin-Elmer - Model Spectrum 1 in the range of 4000–400 cm^{-1}. Transmission Electron Microscope (TEM) analysis was carried out on a FEI, TECNAI-G2, 20-TWIN microscope operating at 200 kV.

2.2. Production and extraction of biosurfactant

The biosurfactant producing bacteria, *Bacillus tequilensis* CH originally isolated by Pradhan et al. [2] was used as source of biosurfactant (CHBS) in this study. For the sake of easy readability CHBS is mentioned as BS hereafter. The procedure of bacterial growth, extraction and partial purification of BS was carried out as per the details of the work carried out by Pradhan et al. [2].

2.3. Synthesis and characterization of SNPs

The extracted BS was concentrated, dissolved in dimethyl sulphooxide (DMSO) and diluted to obtain varying BS concentration. Diluted BS solution was then used for SNP synthesis by adding silver nitrate ($AgNO_3$) such that the final concentration was 1 mM. Reaction mixture (BS solution + 1 mM $AgNO_3$) was incubated at room temperature (RT) and absorption spectra recorded at different time intervals. Different parameters as pH, BS concentration and temperature were studied to optimize efficient synthesis of SNPs. Effect of temperature on SNP synthesis was studied by incubating the reaction mixture at RT (37 ± 1 °C), 40 °C, 50 °C, 60 °C, 70 °C and 80°C. To understand the kinetics of SNP synthesis absorption spectra and DLS were monitored at regular intervals.

2.4. Colorimetric detection of mercury ions

Metal detection ability of as-synthesized silver nano-liposome was studied by individually treating 1 mL of colloidal SNP with 2 mL of different heavy metal ions (Hg^{2+}, Pb^{2+}, Cd^{2+}, Cu^{2+}, Ni^{2+}, Ba^{2+}, Zn^{2+}, Ca^{2+}), such that the final concentration of metal salt in the assay solution was 100 ppm. Absorption spectra of assay mixture were recorded at regular intervals. Experiments were carried out at RT and under similar conditions for all the tested metals. We further examined the ability of the system to detect Hg^{2+} ions, in presence of the tested metal ions. For the study, the metal ions with and without Hg^{2+} ions was treated with silver nano-liposome. The concentration of the metal ions in the assay solution was 100 ppm.

In subsequent studies we focused on analyzing the sensitivity of silver nano-liposome towards Hg^{2+} ions. For the study, gradually increasing concentration of Hg^{2+} ions in the range of 1–100 ppm were treated with silver nano-liposomes. All the analyses were conducted in triplicates and standard errors were calculated to ensure reproducibility.

3. Result and discussion

3.1. Silver nanoparticle synthesis

The biosurfactant producing bacteria, *Bacillus tequilensis* CH, was isolated by Pradhan et al. [2]. The biosurfactant molecule 'CHBS' was reported to be lipopeptide in nature, containing a hydrophobic chain of lipid and hydrophilic peptide group. Details regarding the extraction procedure and characterization of the BS can be obtained from the studies conducted by Pradhan et al. [2]. Subsequent to extraction and concentration of BS, dry weight of the BS was measured and thereafter dissolved in DMSO. The lipopeptide BS solution was treated with $AgNO_3$, to demonstrate NP synthesis. Different parameters (pH, temperature) were studied for optimizing and understanding the reaction kinetics.

3.1.1. Effect of pH

Briefly, 0.2 mg/mL of aqueous BS solution was taken and the initial pH was varied from 5 to 10. Solutions were then treated with 1 mM $AgNO_3$ and incubated at room temperature (RT). As depicted in Fig. 1a, after 24 h of incubation, evident SPR peak for SNPs was observed in reaction mixture having alkaline pH (8–10). The characteristic red brown colour of SNPs was observed in the alkaline reaction mixtures indicating the synthesis of colloidal SNPs (10,11). However, complete absence of SNP synthesis in reaction mixture of pH 5, 6 and 7 suggests the capability of BS to synthesize SNPs at an alkaline pH only. Reaction mixture at pH 9 showed maximum intensity of SNP with distinct narrow peak at 420 nm. Hence, subsequent studies were carried out using BS solution maintained at pH 9. Control experiments (BS only and $AgNO_3$ only) did not show any Surface Plasmon Resonance (SPR) peak for SNP nor was there any change in colour of the solutions. This confirmed that the lipopeptide BS was competent in synthesizing SNPs and functioned as an efficient reducing agent. As the lipopeptide BS molecule contains —OH groups, pH serves as a major determining factor in NP synthesis.

TEM analysis of the solution before and after SNP synthesis demonstrates that the lipopeptide BS forms liposome vesicle in an aqueous medium, wherein the synthesized SNPs are formed within the vesicle. The vesicles were of an average size of 0.5 to 1 μm (Fig. 1b-A). TEM-EDX analysis show peaks for Ag confirming the synthesis of SNPs. Presence of Cu peaks is due to the use of Cu-grids in TEM analysis (Fig. 1b). The SNPs synthesized within the vesicle were basically small monodispersed particles suggesting that the interior of liposomes provides an efficient reaction medium favouring controlled synthesis of NPs (Fig. 1b-B). The BS being amphiphilic in nature forms spherical liposome like vesicle in an aqueous solution. On addition of $AgNO_3$, the functional groups of BS facilitate the reduction of Ag^{+} ions presenting efficient nucleation sites for SNP synthesis. The membrane permeability of the vesicle favours encapsulation of Ag^{+} ions and their subsequent reduction resulting in formation of SNPs. Thus, the inefficiency of liposome BS to synthesize SNPs at an acidic pH is due to the fact that low pH neutralises the negative charge on the molecule responsible for