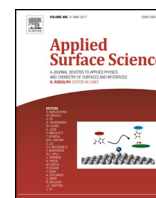




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Surfactant mediated interaction of vancomycin with silver nanoparticles

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ABSTRACT

In this present study, spherical AgNPs have been synthesized by chemical reduction method in the presence of different surfactants i.e. trisodium-citrate and polyvinylpyrrolidone. Further, these synthesised AgNPs have been functionalized with different concentrations of glycopeptide antibiotic (vancomycin). The interaction between AgNPs and antibiotic has been studied using various analytical techniques i.e. UV–vis absorption spectroscopy, Dynamic light scattering, and X-ray diffraction. Further, the nature of bonding between antibiotic and AgNPs has been probed by Fourier transform infrared spectroscopy, which shows the amine bonding between vancomycin and silver nanoparticles surface. The role of different nature of surfactants on attachment and interaction mechanism of drug with AgNPs has been studied.

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1. Introduction

In the recent years, there is an enhancement in the investigation of antibacterial activities of metal nanoparticles, due to increase in bacterial resistance to some of the classic antibiotics [1,2]. For example, *S. aureus* (Gram-positive) and *Enterococci* (Gram-negative) shows resistance to various antibiotics like vancomycin, ampicillin, amikacin, and so forth. Nanoparticles represent one of the most innovative non-invasive approaches for the delivery and targeting of drugs and pharmacologically active substances. Moreover, the drug-loaded nanoparticles provide several advantages with respect to the free drugs, such as protection against degradation and thus promote a suitable, selective and specific targeted therapy and the increase in the patient compliance [3]. Metallic nanoparticles due to some of their attractive properties like high stability and their ability to modify the surface characteristics easily, have been found useful to study drug targeting, drug delivery systems, and enhancement of drug bioavailability. Among all metallic nanoparticles, silver nanoparticles (AgNPs) are one of the most widely studied nanomaterials due to their excellent antimicrobial and antibacterial activities [4]. Silver nanoparticles are also incorporated in various medical supplements, like, catheters and wound dressings to inhibit the growth of pathogens.

A large number of other applications of silver nanoparticles include water purification, biosensors, cosmetics, bone prostheses, and gene delivery etc. [5–8].

Silver is very much popular since ancient times for its antibacterial properties [9,10]. But due to the solubility characteristics of silver salts and silver metal makes it impractical for various medical applications. Therefore, synthesis of colloidal silver nanoparticles have been a subject of great interest among researchers nowadays [11–13]. Previously many studies have demonstrated that nanoparticles such as solid lipid nanoparticles, dendrimers, liposomes, polymeric nanoparticles, and particularly metal nanoparticles were able to reduce the harmful effects of various antibiotics [14]. Therefore, the main advantages of using drug-coated metal nanoparticles for drug delivery systems are (1) increases the resistance time in the body, (2) its site of action (targeting drug to the specific location), and (3) improvement in the bioavailability by enhancing aqueous solubility.

These advantages of metal nanoparticles lead to the reduction in the quantity of a particular drug which is required and also reduces its dosage toxicity. So, the drug-loaded nanoparticles enable the safe drug delivery to a particular location and protection of non-target cells from the severe side effects [15]. In this present study, spherical silver nanoparticles have been synthesized by chemical reduction method by using different protecting agents such as sodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$), polypyrrolidone (PVP) and citrate + PVP (PVP/citrate) and sodium borohydride (NaBH_4)

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as a reducing agent. These capped silver nanoparticles have been modified with the glycopeptides antibiotic, named as, vancomycin.

Vancomycin is an antibiotic from glycopeptide family of antibiotics used to treat a number of bacterial infections caused by gram-positive bacteria [16]. Various methicillin- resistance bacteria such as *Staphylococcus aureus* (MRSA) and *Staphylococcus epidermidis* (MRSE) were treated with vancomycin. Vancomycin was recommended intravenously as a first-line treatment for complicated skin infections, bloodstream infections, endocarditis, bone and joint infections, and meningitis caused by methicillin-resistant *S. aureus* [17]. But in 1988, the first vancomycin-resistance gram positive bacterium was detected, named *Enterococci* (VRE). With time, it has been found that the resistance property of this drug may transfer to various other bacteria including *Staphylococcus aureus* (VRSA) [18]. Hence, numerous antibiotic conjugated nanoparticles such as ciprofloxacin, ampicillin, gentamycin, neomycin and various antibodies-capped particles appeared in the past few decades. The goal of this study was to develop and characterize the vancomycin-loaded silver nanoparticles. Hence, in this present work, we have studied the nature of interaction between vancomycin and silver nanoparticles coated with three different surfactants. Firstly, spherical silver nanoparticles can be obtained with different capping agents via two kinds of mechanism depending upon whether capping agent is ionic or non-ionic or polymer. Here, we have used one ionic (cationic) capping agent trisodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$) and the other, polymer surfactant PVP. The first mechanism involves by which, ionic surfactant ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$) interact with silver nanoparticles, is based on electrostatic stabilization mechanism. However, polymers or non-ionic surfactants (like PVP) interact with silver nanoparticles through steric repulsion mechanism [19–22]. Further, the nature of interaction between capped silver nanoparticles and drug was studied by using various characterization techniques, such as, UV–vis optical spectroscopy, FTIR, DLS and XRD.

2. Experimental materials and methods

2.1. Materials

Silver nitrate (AgNO_3), sodium borohydride (NaBH_4), trisodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$), and polyvinylpyrrolidone (PVP40) ($\text{C}_6\text{H}_9\text{NO}$)_n were used for the synthesis of colloidal silver nanoparticles (AgNPs). Antibiotic named as: vancomycin ($\text{C}_{66}\text{H}_{75}\text{Cl}_2\text{N}_9\text{O}_{24}$) was used for the functionalization of silver nanoparticles. All chemicals were purchased from Sigma Aldrich and were used without any further purification. Double-distilled water was used to prepare all the samples.

2.2. Methods

2.2.1. Synthesis of spherical silver nanoparticles

Metal nanoparticles synthesised by chemical reduction methods usually experience nucleation and growth stages. So, various stabilizing agents have been used for the synthesis of spherical nanoparticles. In present study, colloidal silver nanoparticles were synthesised by following the chemical reduction method described in the previous literature, [23] using trisodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$) and PVP as capping agents (stabilizing agents) and sodium borohydride (NaBH_4) as a reducing agent (Fig. 1). The solution of silver nanoparticles (AgNPs) was synthesised by using 1.0 mM AgNO_3 as a precursor and 2.0 mM of NaBH_4 as a reducing agent, in the presence of capping agents, trisodium citrate (0.5 mM), PVP 0.3% and combination of both $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$ and PVP, by continuous stirring until the colour of solution changes to deep brown or brownish

yellow. The solutions were kept at low temperature for further characterizations and experiments.

2.2.2. Characterization of synthesised spherical silver nanoparticles

The appearance of brownish yellow colour indicates the formation of AgNPs. Furthermore, the synthesis of capped nanoparticles was assured by monitoring the surface plasmon resonance (SPR) of colloidal solution by using UV–vis spectrometer. The optical properties of spherical AgNPs are highly dependent on the nanoparticle size and uniformity. Smaller nanoparticles primarily absorb light with peaks near 400 nm, while larger ones exhibit increased scattering, broader spectral peaks and peak intensities at longer wavelengths [24]. Dynamic light scattering (DLS) was carried out to determine particle size distribution and polydispersity in aqueous solution. DLS is a valuable technique to evaluate particle size and size distribution of nanoparticles in aqueous solution. It measures the fluctuation of the intensity of the scattered light which is caused by particle movement. DLS (Dynamic light scattering) has been carried out by using Zeta analyzer. The FTIR (Fourier-transform infra-red) spectra in the range of $4000\text{--}400\text{ cm}^{-1}$ were recorded by using FTIR spectrophotometer which reveals the different functional groups corresponds to different vibrational frequencies. XRD (X-Ray diffraction) pattern was recorded on an X'Pert PRO X-ray diffractometer using $\text{Cu K}\alpha_1$ radiation in the 2θ range of $30^\circ\text{--}80^\circ$ at a scanning rate of $2^\circ/\text{min}$. To record XRD pattern, the films were deposited by dropping colloidal solution of AgNPs on clean glass slides.

2.2.3. Vancomycin loading on citrate and PVP stabilized silver nanoparticles

The colloidal solution of silver nanoparticles with all three different capping agents was modified with vancomycin by adding different concentrations of vancomycin (0.1 mM, 0.2 mM, 0.3 mM, 0.4 mM and 0.5 mM). Take 5 ml colloidal solution of citrate-capped silver nanoparticles and add 0.1 mM vancomycin to it and stir it for 15–20 minutes. Similarly, with other concentrations five different samples of vancomycin capped AgNPs (Van@AgNPs) were prepared by adding 0.2 mM, 0.3 mM, 0.4 mM and 0.5 mM vancomycin to each 5.0 ml solution of silver nanoparticles, by stirring for the same duration as for the first sample. All the samples kept at room temperature for 24 h. After 24 h incubation, solution was stored in refrigerator for further characterizations (Fig. 2).

3. Results and discussion

3.1. SPR (surface plasmon resonance) studies of AgNPs and Van@AgNPs

Fig. 3 shows the UV–vis absorption spectra of vancomycin, bare spherical AgNPs, Van-citrate@AgNPs (vancomycin coated citrate-AgNPs), Van-PVP@AgNPs (vancomycin coated PVP-AgNPs) and Van-PVP/citrate@AgNPs (vancomycin coated PVP/citrate-AgNPs). The surface plasmon band for the spherical citrate-capped AgNPs, PVP-capped AgNPs and PVP/citrate-capped AgNPs were observed at 403, 431 and 410 nm respectively, in visible region of UV–vis absorption spectra and vancomycin absorbed in UV region around 290 nm. After the addition of different concentrations (0.1 mM, 0.2 mM, 0.3 mM, 0.4 mM, and 0.5 mM) of vancomycin to the solution of silver nanoparticles, the absorption peak shows significant red shift for Van-citrate@AgNPs and Van-PVP/citrate@AgNPs around 25–43 and 16–25 nm respectively. However, for Van-PVP@AgNPs there was a blue shift of about 2 nm. It could be explained on the basis of electronic transitions which take place when UV–vis light is passed through sample.

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