



Synthesis and Characterization of Silver Nanoparticles (AgNPs) using Chemico-physical Methods

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ABSTRACT

Silver nanoparticles (AgNPs) have several applications in nanoscience and nanomedicine such as antibacterial, antifungal, anti-inflammatory and anti-angiogenic properties. In the present study, the AgNPs were synthesized by the chemical method and characterised using a combination of UV-Visible (UV-Vis) spectrophotometry, Fourier Transform Infra-Red (FTIR) spectroscopy, transmission electron microscopy (TEM) and X-ray diffraction (XRD). The AgNPs were made by chemically reducing AgNO₃ with NaBH₄ leading to the formation of a characteristic yellowish-brown colour of AgNPs. To investigate the formation, crystalline behavior, and quality of AgNPs powder, XRD measurements were performed. The produced AgNPs were found to be spherical, dispersed, and scattered in form and the particle size of the AgNPs formed was about 15 nm and they were crystalline in nature. The interaction of AgNO₃ with biomolecules may have numerous applications in nanoscience and nanomedicine.

1. INTRODUCTION

Nanotechnology, a rapidly advancing field of science and technology, is devoted to designing, producing, and using structures, devices, and systems by manipulating atoms and molecules at the nanoscale i.e., having one or more dimensions of the order of 100 nanometres [1]. Using nanotechnology, materials can effectively be made stronger, lighter, durable, reactive, sieve-like, or better electrical conductors, among many other traits [2]. The nanoparticles have become an integral component in a wide range of applications such as medicine, semiconductors, catalysis and energy [3]. A nanoparticle is usually defined as a particle of matter with a size ranging from 1-1000 nm [2]. The synthesis of nanoparticles has also generated a keen interest in biomedical sciences for their potential applications in drug transport, molecular imaging and therapeutics [4].

Nanoparticles can be synthesised by physical, biological and/or chemical methods. The physical methods of synthesis are evaporation-condensation, arc discharge method, laser ablation, and hydrothermal method. The chemical method includes chemical reduction, irradiation, electrochemical, microemulsion, photoreduction whereas the biological methods use the reduction property of plant, fungus and bacteria [5]. Due to their small-size and large surface-to-mass ratio, the nanoparticles may bind to proteins, nucleic acids and lipids [6]. The interaction of nanoparticles



with proteins is the basis of nanoparticle bio-reactivity. This interaction gives rise to the formation of a dynamic nanoparticle-protein corona. The protein corona may influence cellular uptake, inflammation, accumulation, degradation and clearance of nanoparticles. Furthermore, the nanoparticle surface can induce conformational changes in adsorbed protein molecules which may affect the overall bio-reactivity of nanoparticles [7]. Therefore, understanding the interaction of nanoparticles with proteins and drugs [8-11] may be beneficial for human health and environment.

Silver is the most common nano-material used in products, followed by carbon-based nano-materials and metal oxides such as TiO_2 . The silver nanoparticles (AgNPs) are the most significant nanoparticles due to their antibacterial, antifungal, anti-inflammatory and anti-angiogenic properties [12]. They are very significant amongst other metallic nanoparticles, because silver possess biomedical applications in cancer diagnosis and therapeutics. AgNPs are mainly used for antimicrobial and anticancer therapy, and also applied in the promotion of wound repair and bone healing, or as vaccine adjuvant, anti-diabetic agent and biosensors [13]. They have been used extensively as anti-bacterial agents in health industry, food storage, textile coatings and environmental applications [14]. The AgNPs have great potential in a wide range of applications in biomedical device coatings, drug-delivery carriers, imaging probes, and diagnostic and optoelectronic platforms due to their discrete physical and optical properties [15]. The AgNPs usually have a silver content of 20-15000 atoms [12]. The size of AgNPs can be manipulated according to their specific applications e.g., AgNPs prepared for drug delivery are generally larger than 100 nm to accommodate the drugs delivered. This adjustment permits a precise control of their size, shape, monodispersity and surface [16].

There are different methods for synthesis of AgNPs but the chemical method provides a simple and easy way for their synthesis in solution [17]. It is based on the reduction of a metal salt via a reducing agent in the presence of a protective material. The synthesis of metal nanoparticles in solution is carried out using the following components: i) metal precursor, ii) reducing agent, and iii) stabilizing agent [18]. The mechanism of formation of colloidal solutions from the reduction of silver (I) ions consists of two stages: nucleation and growth steps. The nucleation step requires high activation energy while the growth step requires low activation energy. The size and shape of the nanoparticles will depend on the relative rate of these processes which can be controlled through the adjustment of reaction parameters [17, 18]. Chemical reduction has become an accessible and useful alternative to obtain AgNPs, which is also low cost with a large-scale production capacity.

The present study attempts to synthesise very stable and effective AgNPs by chemical reduction method and to characterise them by using multi-spectroscopic techniques. The characterization was performed using UV visible absorption spectroscopy, fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM) and X-ray diffractometry (XRD). These techniques were used for the determination of different parameters such as particle size, shape, crystalline behaviour, fractal dimensions, pore size and surface area [19]. Moreover, orientation, intercalation and dispersion of nanoparticles and nanotubes in nanocomposite materials could be determined by these techniques. For example, the morphology and particle size could be determined by using TEM [20]. Moreover, XRD is used for the determination of crystallinity, while UV-Vis spectroscopy is used to analyse the sample formation through surface plasmon resonance (SPR).

2. EXPERIMENTAL METHOD

2.1 Materials

Silver nitrate, sodium borohydride and sodium citrate were obtained from Sisco Research Laboratories (SRL), India. All other chemicals and reagents were analytical grade commercially available.

2.2 Synthesis of silver nanoparticles

The AgNPs were synthesized by the chemical method of nanoparticles synthesis using a metal precursor (silver nitrate, AgNO_3), a reducing agent (sodium borohydride, NaBH_4), and a stabilizing

agent (sodium citrate, $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$) [21]. 10 ml of 1 mM sodium citrate and 100 ml of 0.1M NaBH_4 were added to a 100 ml solution of 1 mM AgNO_3 in milli-Q water followed by vigorous stirring. This resulted in the formation of a characteristic yellowish-brown coloured solution of AgNPs.

2.3 Characterization of silver nanoparticles

The absorbance of AgNPs suspensions was measured at room temperature at a resolution of 1 nm using a Perkin-Elmer Lambda 25 double beam UV-visible spectrophotometer. The reduction mechanism of Ag^+ into AgNPs in solution was investigated using UV-visible spectra. The FTIR measurement of dried AgNPs was conducted using potassium bromide (KBr) pellet in the ratio of 1:100 in the wavelength range of $500\text{-}4000\text{ cm}^{-1}$ [22]. The transmittance mode of FTIR spectrum was acquired using a JASCO FT/IR-6300 with a resolution of 4 cm^{-1} [23]. The TEM was used to examine the surface morphology of the synthesized AgNPs. TEM uses an electron beam to form an image of nanoparticles formed, providing much higher resolution. It is the most preferred method to directly measure nanoparticles size, distribution and morphology. Then, to investigate the formation, crystalline behavior and quality of AgNPs powder, XRD were performed. XRD is based on the scattering of X-rays due to the revolution of electrons in the atom's nucleus when the rays strike the nanoparticles. It is an ideal method for the crystallographic characterisation of bulk, nano and thin film materials.

3. RESULTS AND DISCUSSIONS

3.1. Material Characterization

The AgNPs were successfully synthesized by chemical reduction method of nanoparticles synthesis. AgNO_3 (colorless) turns to yellow to dark brown, which indicated the formation of AgNPs. AgNPs were made by chemically reducing AgNO_3 with NaBH_4 in the presence of sodium citrate. Ag^+ was reduced to Ag^0 by NaBH_4 and the synthesized AgNPs were capped and stabilized by sodium citrate. The synthesis of AgNPs was demonstrated by the transformation of AgNO_3 (colorless) to dark yellow/brown. The reaction kinetics was monitored in the visible range of 400-500 nm by UV-Visible absorption spectroscopy. The absorption spectrum of AgNPs is dominated by the surface plasmon resonance (SPR), observed at 450 nm (**Figure 1**). UV-Vis spectroscopy reveals the reaction kinetics in the visible range (400-500 nm), where AgNO_3 show a SPR band due to free electron excitation at 450 nm (**Figure 1**). The SPR of AgNPs can be tuned throughout the visible and near-infrared region by their shape and size. SPR is the manifestation of a resonance effect due to the interaction of conduction electrons of metal nanoparticles with incident photons [24]. AgNPs are known to exhibit a SPR band due to excitation of free electron. The absorption band in the visible region is a characteristic of AgNPs.

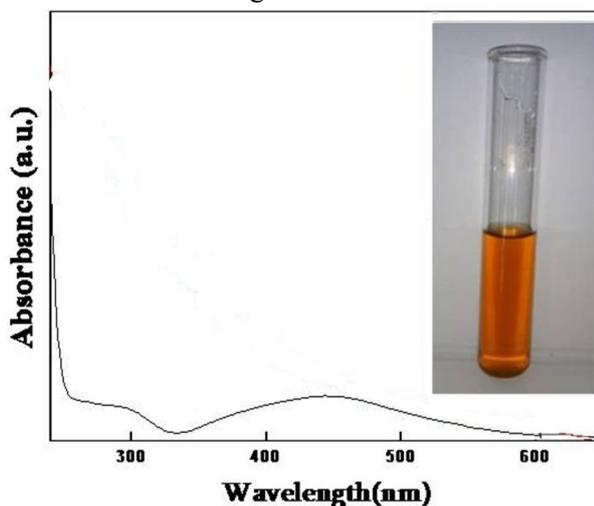


Figure 1. UV-Visible absorption spectrum of synthesized AgNPs.

The FTIR analysis was used to investigate the functional groups responsible for the reduction and stabilization of the synthesized AgNPs. The observed intense bands were compared with standard values to identify the functional groups. The FTIR spectrum shows absorption bands at 3472 cm^{-1} , 2912 cm^{-1} , 2867 cm^{-1} , 1768 cm^{-1} , 1642 cm^{-1} , 1450 cm^{-1} , 1377 cm^{-1} , 1043 cm^{-1} , 845 cm^{-1} and 621 cm^{-1} indicating the presence of capping agents in process of AgNPs synthesis (Figure 2). As a result, Ag^+ was reduced to Ag^0 by NaBH_4 and the synthesized AgNPs were capped and stabilized by sodium citrate.

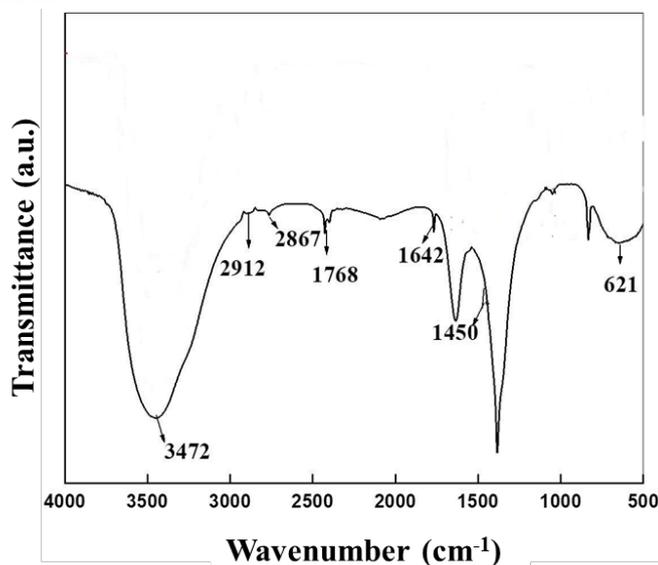


Figure 2. Fourier Transform Infra-Red (FTIR) spectrum of AgNPs.

TEM is an electronic spectroscopic imaging technique capable of producing 2D images of greater resolution. TEM is a quantitative method employed to determine the particle size, shape, distribution and surface morphology of nanoparticles. The prepared AgNPs were found to be spherical, dispersed, and scattered in nature (Figure 3a). The particle-size distribution revealed that the synthesized AgNPs ranged in size from 10-20 nm, with an average size of 15 nm (Figure 3b).

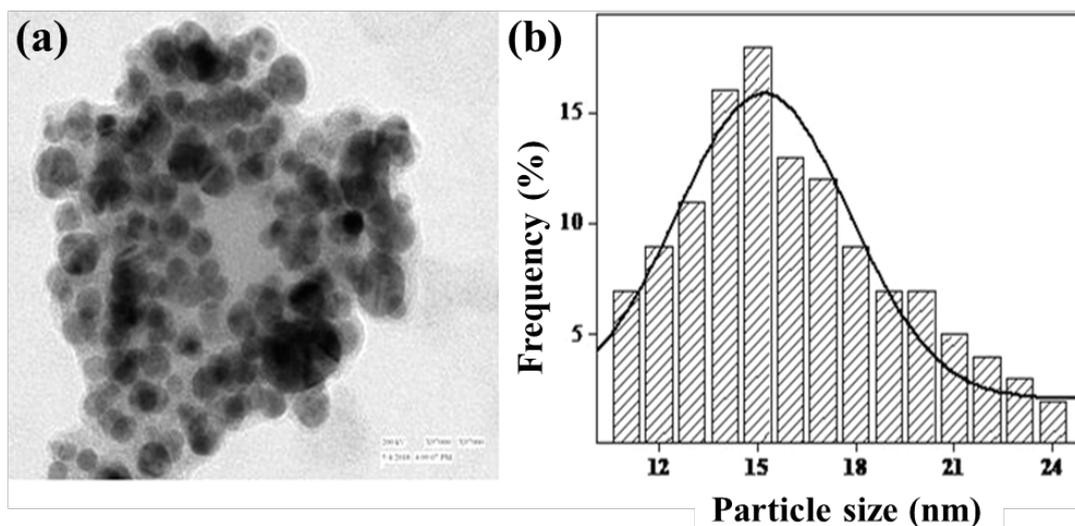


Figure 3. Transmission electron microscopy of silver nanoparticles (a) TEM images of spherical shape AgNPs and (b) particle size distribution curve.

The XRD study confirmed that the nanoparticles in the prepared sample are AgNPs having face centred cubic crystal structure. XRD determined the crystalline character of the chemically

synthesized AgNPs. The XRD pattern for AgNPs prepared via chemical reduction method shows the diffraction peaks having weak-medium intensities at 2θ values of 37.4° , 44.0° , 64.2° and 77.2° , which corresponds to the (111), (200), (220) and (311) planes of metallic silver having FCC crystal symmetry (**Figure 4**) [24-26]. The resulting XRD spectrum, when combined with TEM image, indicates that the prepared AgNPs were crystalline in nature.

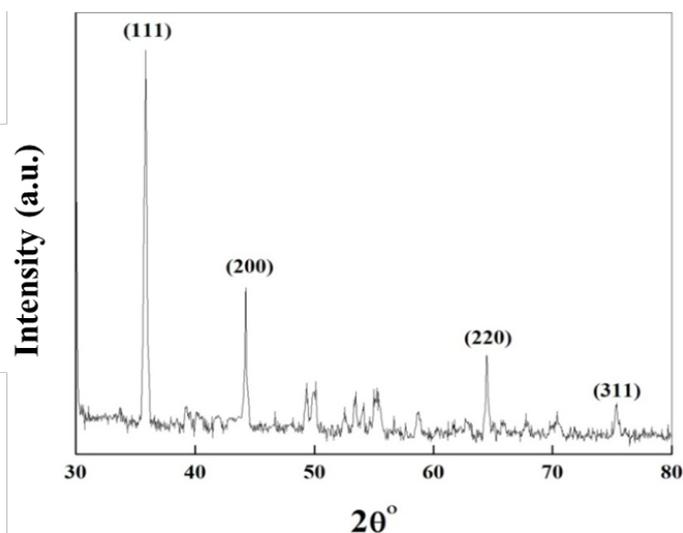


Figure 4. X-ray diffraction (XRD) pattern of AgNPs.

3.2. Discussion

The synthesis of nanoparticles for their potential applications in drug transport, molecular imaging, and therapeutics [2] has generated a keen interest in biomedical sciences. Due to their small-size and large surface-to-mass ratio, the nanoparticles may bind to proteins, nucleic acids, and lipids and the biomolecule-ligand interaction may have an important structural and functional role in biological systems [7]. The AgNPs are emerging as the next-generation application in nanomedicine, and their potential benefits as a prominent nanomaterial in biomedical and industrial sectors [15]. The AgNPs are significant and beneficial due to their antibacterial, antifungal, anti-inflammatory and anti-angiogenic properties [12]. The AgNPs have a diameter of less than 100 nm and a silver content of 20-15000 atoms [12]. The size of AgNPs can be adjusted according to the specific application e.g., AgNPs prepared for drug delivery are mostly greater than 100 nm to accommodate or quantify the drug to be delivered. With different surface properties, AgNPs can also be formed into various shapes, including rod, triangle, round, octahedral, polyhedral, etc. [27]. Moreover, AgNPs are used in antimicrobial applications with proven antimicrobial characteristics of Ag^+ ions [28]. The AgNPs can be utilized as highly sensitive nanoparticle probes for targeting and imaging of small molecules, DNA, proteins, cells, tissue, and tumours *in vivo* [29]. The AgNPs with stronger and sharper plasmon resonance have been widely used in imaging systems, particularly for cellular imaging with contrast agents via surface modification. For example, a AgNP-embedded nanoshell structure can be used in cancer imaging and photothermal therapy to explore the location of cancer cells by absorbing light and destroy them via photothermal effect [30]. The exceptional properties of AgNPs have enabled their use in nanomedicine, pharmacy, biosensing, and biomedical engineering.

The AgNPs are chemically synthesized mainly through the Brust-Schiffrin synthesis (BSS) or the Turkevich method [31]. The strength and type of reducing agents and stabilizers should be taken into consideration in synthesizing metal nanoparticles of a specific shape, size, with various optical properties. A stabilizing agent is typically used to avoid aggregation of nanoparticles. In the present study, the AgNPs were successfully synthesized by the chemical reduction method. AgNO_3

was chemically reduced with NaBH_4 in the presence of sodium citrate, which acts as a capping and stabilizing agent. AgNO_3 (colourless) turns to yellow and dark brown coloured solution (**Figure 1**), confirming the formation of AgNPs. Chemical reduction yields non-toxic, stable, colloidal dispersions of nanoparticles in water or organic solvents. The commonly used reductants are borohydride, citrate, ascorbate, and elemental hydrogen. The reduction of silver ions (Ag^+) in aqueous solution generally yields colloidal silver with a particle diameter of several nanometers [32]. Initially, the reduction of various complexes with Ag^+ ions lead to the formation of silver atoms (Ag^0), which is followed by agglomeration into oligomeric clusters. These clusters eventually lead to the formation of colloidal Ag particles [33]. It is important to use protective agents to stabilize the dispersive metal nanoparticles during their synthesis. The common protective agents can be absorbed on or bind onto the nanoparticle surface avoiding their agglomeration [34]. In this present study, we have used sodium citrate as a stabilizing agent. The significance of chemical reduction method for the synthesis of nanoparticles is its simplicity as well as its rapid synthesis. This technique also enables the variation in molar concentration of the reactant, dispersant and feed rate of reactant to produce AgNPs with controlled particle size, shape and distribution [34].

The characterization of prepared AgNPs was performed using various biochemical and biophysical methods. Multi-spectroscopic techniques such as UV visible absorption spectroscopy, fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM) and X-ray diffractometry (XRD) were used for the characterization of AgNPs. The reaction kinetics was monitored in the visible range of 400-500 nm by UV-Visible absorption spectroscopy [35-38]. The absorption spectrum of AgNPs is dominated by the surface plasmon resonance (SPR), observed at 450 nm (**Figure 1**). AgNPs are known to exhibit a SPR band due to excitation of free electron. The absorption band in the visible region is a characteristic of AgNPs. The FTIR spectroscopy determines the functional groups responsible for the reduction and stabilization of synthesized AgNPs. The observed intense bands were compared with standard values to identify the functional groups. The FTIR spectrum shows absorption bands at 3472 cm^{-1} , 2912 cm^{-1} , 2867 cm^{-1} , 1768 cm^{-1} , 1642 cm^{-1} , 1450 cm^{-1} , 1377 cm^{-1} , 1043 cm^{-1} , 845 cm^{-1} and 621 cm^{-1} indicating the presence of capping agents in process of AgNPs synthesis (**Figure 2**). As a result, Ag^+ was reduced to Ag^0 by NaBH_4 and the synthesised AgNPs were capped and stabilized by sodium citrate.

TEM reveals the particle size, shape, distribution and surface morphology. The prepared AgNPs were found to be spherical, dispersed, and scattered in nature. The particle-size distribution shows that the synthesized AgNPs ranged in size from 10-20 nm, with an average size of 15 nm. XRD determines the crystalline character [39] of the chemically synthesized AgNPs. The XRD study confirmed that the resultant particles in the prepared sample are AgNPs having face centered cubic crystal structure. The XRD pattern for AgNPs prepared via chemical reduction method shows the diffraction peaks having weak-medium intensities at 2θ values of 37.4° , 44.0° , 64.2° and 77.2° , which corresponds to the (111), (200), (220) and (311) planes of metallic silver having FCC crystal symmetry [32]. The resulting XRD spectrum, when combined with TEM image, indicates that the prepared AgNPs were crystalline in nature. Therefore, the AgNPs were successfully synthesized by the chemical reduction method and characterized by multi-spectroscopic techniques. The chemical method of synthesis yields metallic nanoparticles as a colloidal dispersion in aqueous solution or organic solvent by reducing their metal salts. A better understanding of the synthesis of AgNPs merits future research to broaden their nanomedical applications in diagnostics, therapeutics and pharmaceuticals.

4. CONCLUSION

AgNPs are emerging as a next-generation application in numerous subfields of nanomedicine due to their potential benefits as a prominent nanomaterial in biomedical and industrial sectors. In this study, AgNPs were successfully synthesized by chemical reduction

method and characterized by multi-spectroscopic studies. The characterization was performed using different techniques such as TEM, XRD, FTIR, and UV-Vis spectroscopy. These techniques are used for determination of different parameters such as particle size, shape, crystallinity, fractal dimensions, pore size and surface area. The morphology and particle size was investigated by using TEM. Moreover, XRD was used for the determination of crystallinity, while UV-Vis spectroscopy was used to determine sample formation through SPR. Hence, the interaction of AgNPs with biomolecules may have numerous applications in nanoscience and nanomedicine.

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