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Research Article

Phytochemical Synthesis and Preliminary Characterization of Silver Nanoparticles Using Hesperidin

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This paper is the first of its kind for development of rapid and ecofriendly method for synthesis of silver nanoparticles from aqueous solution of silver nitrate using the flavonoid "hesperidin" and optimization of the methodology. There is formation of stable spherical silver nanoparticles in the size range of 20–40 nm. Optimization of methodology in terms of concentration of reactants and pH of the reaction mixture reduced the reaction time for silver nanoparticle formation to 2 mins. Silver nanoparticles (AgNPs) were characterized by UV-Vis spectroscopy and transmission electron microscopy (TEM). UV-vis spectroscopy derived spectrum demonstrated a peak of 430 nm which corresponds to the plasmon absorbance of silver nanoparticles. Transmission electron microscopy revealed spherical shaped silver nanoparticles in the size range of 20–40 nm.

1. Introduction

Nanoparticles have become a subject of intense interest among the research community. These interests have been aroused by the lacunae in knowledge associated with silver nanoparticles and also the gaining momentum with which researches are uncovering the obscurities in knowledge surrounding the nanosciences. There is a rapid growth in the field of nanotechnology over the past few years and it has successfully ventured into the field of clinical medicine. Silver nanoparticles (Ag NPs) have occupied a central position in terms of research among all other metal nanoparticles [1]. There are several methods of synthesis of silver nanoparticles most of which fall under categories like physical methods, chemical methods, and the ecofriendly biomimetic methods [2]. Biomimetic methods of synthesis are the result of a growing need to develop cheap and ecofriendly methods for synthesis [3].

Physical approach for synthesis has several methods like evaporation/condensation and laser ablation, while in the chemical approach, the metal ions in solution are reduced in conditions favoring the subsequent formation of small metal clusters or aggregates [4]. From the utility perspective, along with nanodimensions the particles should be dispersed without agglomeration as well [2]. The properties exhibited by the metallic nanoparticles are size- and shape-dependent [5–7]. Biomimetic synthesis of silver nanoparticles is cost effective and environmental friendly and is also safe for applications in therapeutics [8]. Biological methods have emerged as an alternative to the conventional methods for synthesis of silver nanoparticles. Nanoparticle synthesis by biological methods results in a safer and biocompatible end product which is also cost effective and suitable for large scale supply since the production process can be scaled up [9, 10]. Apart from plants, microbial sources like bacteria and fungus are also used in the silver nanoparticle synthesis [11]. For the preparation of silver nanoparticles (AgNPs) as colloid dispersion in water or organic solvents, chemical reduction is the most frequently applied method. Colloidal silver with particle diameters of several nanometers is the general yield of reduction of silver ions ($Ag+$) in aqueous solution [12]. Many research papers have reported the use of plant extracts for synthesis of silver nanoparticles, with a few of them being *Sorghum bicolor*, *Helianthus annuus*, *Oryza sativa*, *Basella alba*, *Saccharum officinarum*, and *Zea mays* [11]; pine, *platanus* leaves, *ginkgo*, *magnolia*, and persimmon [13]; *Jatropha curcas* seeds [14]; *Acalypha indica* leaf [15]; banana peel [16]; mulberry leaves [17]; *Arbutus unedo* leaf [18];

Chenopodium album leaf [19]; *Rosa rugosa* [20]; *Trianthema decandra* roots [21]; *Ocimum sanctum* (*Tulsi*) leaf [22]; *Murraya koenigii* (curry) leaf [23]; *mangostana* (mangosteen) leaf [24]; *Ocimum sanctum* stems and roots [25]. Many studies have highlighted the fact that phytochemical constituents present in the plant extracts play a major role in the reduction of silver ions into metallic silver and subsequent capping to prevent agglomeration. Phytochemicals are found in edible fruits and vegetables and help in the prevention of chronic and degenerative diseases by human metabolism modulation [26]. An one of the important groups of phytochemicals, flavonoids have proven pharmacological properties like anti-inflammatory, antiallergic, antibacterial, and antiviral properties [27–29] and have also been found to have cytotoxic antitumor properties and to be effective in neurodegenerative diseases [30, 31]. Flavonoids are free radical scavengers acting as antioxidants against free radicals [32]. Flavonoids are COX inhibitors [32]. Flavonoids prevent synthesis of PGs that suppress T-cells [33]; there are a huge number of research studies done in which silver nanoparticles are synthesized using plant extracts rich in flavonoids. Literature search reveals lacunae in synthesis of silver nanoparticles using flavonoids directly rather than using flavonoid rich plant extract.

This study attempts to synthesize silver nanoparticles using flavonoid directly rather than flavonoid rich plant extracts. Hesperidin was selected from the flavonoid inventory because of its easy availability and since it has been an integral phytochemical present in most of the fruits that we consume.

2. Materials and Methods

Hesperidin (HPLC 90%) was purchased from Xian Xiaocao Botanical Development Co., Ltd., China. Silver nitrate (99.9999% trace metals basis) was purchased from Sigma Aldrich. 99.8% methanol was used. Milli-Q system was used for deionized water.

3. Synthesis of Silver Nanoparticles and Optimization of Methodology

Silver nanoparticles were prepared by chemical reduction of salt solution of silver nitrate using the flavonoid hesperidin. Aqueous silver nitrate solution was prepared with the strength of 1 mM and 3 mM. Hesperidin solution was prepared by using methanol (99.8%) as a solvent. In two glass flasks, 10 mg and 30 mg of hesperidin were taken, respectively. Into the two flasks 100 mL of methanol was added and the mixture was subjected to sonication for 5 mins in order to ensure that there is no particle matter left in the solution. The resultant strengths of the two hesperidin solutions were 100 μgm/mL and 300 μgm/ml. The pH of the hesperidin solution which is acidic is raised to a basic level of around 12 by adding few drops of freshly prepared NaOH.

90 ml of aqueous solution of silver nitrate was taken in a flask and kept on a magnetic stirrer into which 10 ml of hesperidin solution was added drop by drop using a micropipette. In total there were 5 flasks in the combinations as mentioned in Table 1.

Table 1: Combinations in the flasks.

10 mL hesperidin (100 μgm/mL) in 90 mL silver nitrate solution (1 mM) (1 : 10 ratio)
10 mL hesperidin (300 μgm/mL) in 90 mL silver nitrate solution (1 mM) (1 : 10 ratio)
10 mL hesperidin (100 μgm/mL) in 90 mL silver nitrate solution (3 mM) (1 : 10 ratio)
10 mL hesperidin (300 μgm/mL) in 90 ml silver nitrate solution (3 mM) (1 : 10 ratio)
10 mL methanol in 1 mM silver nitrate solution (90 mL) (1 : 10 ratio): control

4. Characterization Studies

Color of the reaction mixture was observed. UV-vis absorption spectra were measured using Synergy H4 Hybrid Multimode Microplate Reader (BioTek). Through UV-vis absorption spectra, formation process of silver nanoparticles, confirmation of formation of silver nanoparticles, and the stability of the synthesized silver nanoparticles were studied. Sample preparation was done by diluting a small aliquot of the reaction mixture solution in Milli-Q water. The sample was taken as soon as half of the hesperidin solution was added, which corresponds to the start of change in color of the reaction mixture, after the entire 10ml of hesperidin solution was added and soon after the color of the reaction mixture changed completely to brownish yellow, that is, around 2 mins after the last drop of hesperidin solution was added and also at regular time intervals after that.

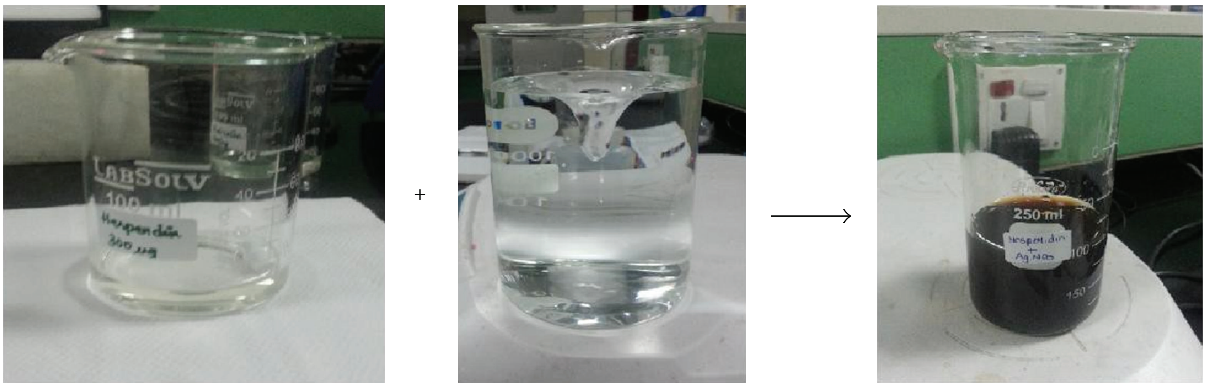
Transmission electron microscopy (TEM) analysis of synthesized silver nanoparticles was done using Tecnai G2 spirit Biotwin TEM system (FEI electron microscope). Sample preparation for TEM analysis was done by putting a small drop of the colloid solution on the TEM grid (formvar with carbon coating) and allowing it to dry at room temperature for two hours.

5. Results and Discussions

Physical observation revealed the change in color of the solution from colorless to brownish yellow within minutes of adding the hesperidin solution to the aquous silver nitrate solution (Figure 1). Similar change in color was seen in all the four combinations except control (Figure 2).

The color started to change when around 5 ml of hesperidin out of the 10 ml was added and completely changed to brownish yellow within 2 mins of adding the last drop.

The change in color was more or less the same in all the samples except the control sample. Formation of silver nanoparticles by reduction of the aqueous sol of silver nitrate on exposure to hesperidin solution was observed from the change in color of the reaction mixture. Silver nanoparticles exhibit a yellowish brown color in aqueous solution due to excitation of surface plasmon vibrations [34]. The change in



Hesperidin solution

Silver nitrate solution on magnetic stirrer

Silver nitrate solution (90 mL) + hesperidin solution (10 mL)

Figure 1: Formation of silver nanoparticles.

Figure 2: Different combinations.

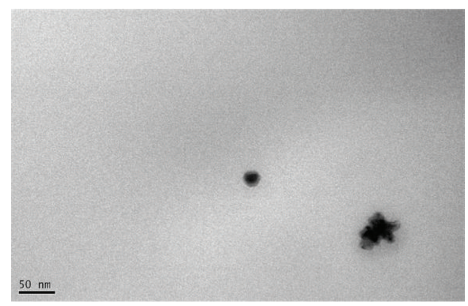


Figure 3: TEM image at 180,000 magnification.

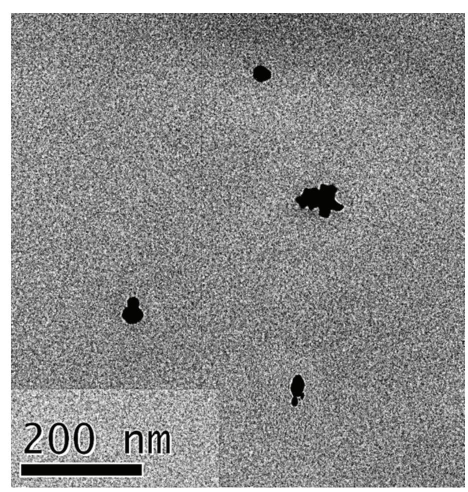


Figure 4: TEM image at 49,000 magnification.

color indicated the formation of silver nanoparticles. This suggests that there is a formation of elemental silver having nanodimensions from the silver ions in the silver nitrate solution. The basis of synthesis can be explained in two steps. In the first step silver atoms (Ag0) are formed initially due to the reduction of various complexes with Ag+ ions followed by the second step in which there is a formation of oligomeric clusters due to agglomeration and these clusters eventually lead to the formation of colloidal Ag particles [12].

UV-Vis spectroscopy analysis was done in the wavelength range of 300 nm to 700 nm. The absorption peak (wavelength at mean maximum optical density) was obtained to be 420 nm (for 100 μgm/ml hesperidin solution combinations) and 430 nm (300 μgm/mL hesperidin solution combinations) for the samples taken after the complete change of color except control. Typical absorption band for silver nanoparticles was observed in visible light region for silver nanoparticles [35]. The plasmon peak and the full-width of half-maximum (FWHM) depend on the extent of colloid aggregation [35].

The process of synthesis was complete in few mins of reaction time and the time dependent change in the absorption peak is presented (Figures 7, 8, and 9).

It can be seen how the absorption peak which corresponds to nanoparticle synthesis has evolved over a period of time and corresponds to the change in the color of the reaction mixture

To monitor stability of the colloid solution, absorption of the colloid was measured at the time of complete color change and various intervals thereafter. There was no obvious change in peak position (wavelength at mean maximum optical density) and maximum optical density when studied at various time intervals which suggests a highly stable colloid and aggregation after formation not to be of significance [36]; as the particles increase in size, the absorption peak

Well	Mean min OD (spectrum)	Wavelength at mean min OD (spectrum)	Mean max OD (spectrum)	Wavelength at mean max OD (spectrum)
1 mM/300 (E7)	0.094	700	3.78	430

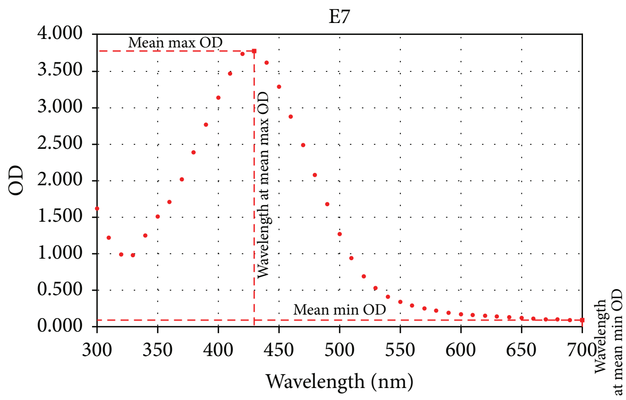


Figure 5: UV-Vis data of 300 μl/ml hesperidin + 1 mM of silver nitrate solution.

Well	Mean min OD (spectrum)	Wavelength at mean min OD (spectrum)	Mean max OD (spectrum)	Wavelength at mean max OD (spectrum)
3 mM/300 (E9)	0.117	700	2.355	430

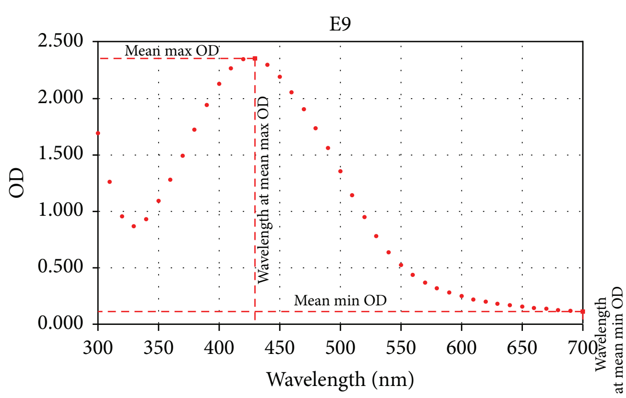


Figure 6: UV-Vis data of 300 μl/ml hesperidin + 3 mM of silver nitrate solution.

usually shifts toward the red wavelengths [37] which further reinforces the fact that there was no change in the particle size over a period of time.

UV-Vis spectroscopy data for the following combinations "1 mm silver nitrate –300 μgm/mL hesperidin" and "3 mm silver nitrate –300 μgm/mL hesperidin" is presented (Figures 5 and 6).

It is observed that both samples have a peak (wavelength at mean maximum optical density) of 430 nm. It is also observed that the mean maximum optical density is 3.78 cm^{-1} and 2.355 cm^{-1}, respectively. These values can be used to quantify the concentration of silver nanoparticles in various combinations. Using the Beer-Lambert law (which states that

Well	Mean min OD (spectrum)	Wavelength at mean min OD (spectrum)	Mean max OD (spectrum)	Wavelength at mean max OD (spectrum)
H0	0.113	700	1.938	300

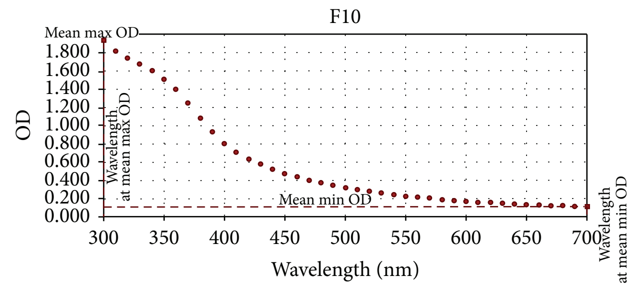


Figure 7: UV-Vis spectrometry data of reaction mixture at the start of change in color.

Well	Mean min OD (spectrum)	Wavelength at mean min OD (spectrum)	Mean max OD (spectrum)	Wavelength at mean max OD (spectrum)
H1	0.212	700	1.638	300

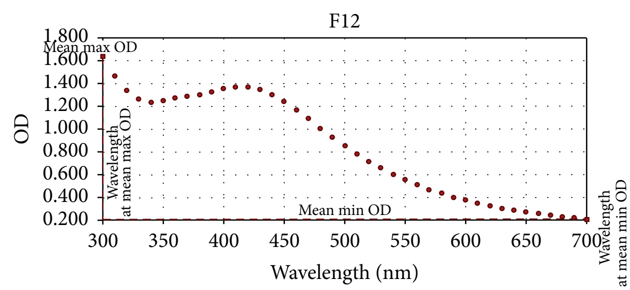


Figure 8: UV-Vis spectrometry data of reaction mixture taken after complete mixing of 10 ml hesperidin of 100 μgm per ml.

the optical density (OD, a measure of the amount of light transmitted through a solution) has a linear relationship with concentration) the concentrations of silver nanoparticle solutions were compared which pointed out that the combination "1 mm silver nitrate –300 μgm/mL hesperidin" has a better yield and was further studied using TEM.

Transmission electron microscopy helps analyzing the morphology and size of the particles. Observed images were of magnification of 180,000 (Figure 3) and 49,000 (Figure 4) and shows spherical silver nanoparticles in the size range of 20 to 40 nm with capping on it.Clumping of particles resulting in a larger particle is also seen.

6. Conclusion

We have developed a fast, ecofriendly, and convenient method for synthesis of silver nanoparticles from silver nitrate using hesperidin at room temperature. Color changes from colorless to yellowish brown due to surface plasmon resonance during the reaction with hesperidin solution

Well	Mean min OD (spectrum)	Wavelength at mean min OD (spectrum)	Mean max OD (spectrum)	Wavelength at mean max OD (spectrum)
H2	0.198	700	1.868	420

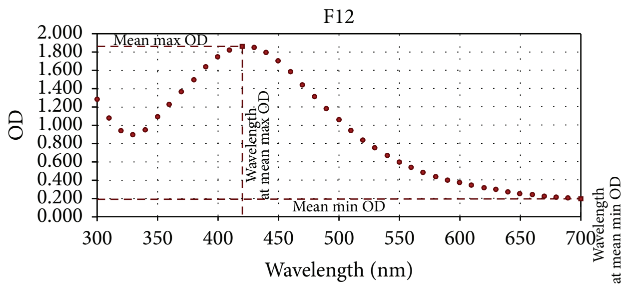


Figure 9: UV-Vis spectrometry data of reaction mixture after complete change in color (2 mins).

resulting in the formation of silver nanoparticles, which is confirmed by TEM and UV-vis spectroscopy. Hesperidin is found suitable for the synthesis of silver nanoparticles within two mins. Spherical, polydispersed AgNPs of particle sizes ranging from 20 to 40 nm with an average size of 30 nm are obtained. Further studies will be conducted to study the pharmacological properties of the synthesized silver nanoparticles from hesperidin.

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