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A Green Approach to Synthesize Silver Nanoparticles from Natural Polymer for Biomedical Application

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ABSTRACT
Natural polymer (Pectin) was used to synthesize the silver nanoparticles (SNPs) in a spontaneous way without using any reducing agent. SNPs have been obtained by the mixing of various amounts of silver nitrate with 2% of aqueous pectin. The formed nano silver-pectin solutions were caused to get the nanocomposite thin films. The formation of SNPs have been characterized by ultraviolet-visible (UV-vis), Fourier transform infrared, and transmission electron microscopy (TEM) analyzes. The formation of SNPs confirmed by a characteristic absorption peak at 420 nm obtained from UV-vis spectrum. The average particle size SNPs was examined by TEM, i.e., 10 nm. The synthesized SNPs were evaluated for an antibacterial activity toward Bacillus and Escherichia coli, the result suggested that they are high potent in nature. The potentiality of this method is which it is possible to prepare SNPs without any synthetic reducing agents. Hence, it is environmentally safe and has a promising potential future in the medical or pharmaceutical area.

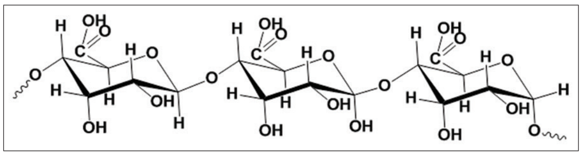
Key words: *Green synthesis, Pectin, Silver nanoparticles, Antimicrobial property, Nanocomposite.*

1. INTRODUCTION

In the recent year, green synthesis of silver nanoparticles (SNPs) is a growing need to develop sustainable, clean, and renewable materials. Hence, the focus turned into the synthesis of biocompatible SNPs by using various bio micro and macromolecules. The syntheses of silver as well as other metal nanoparticles using biological systems are recently reported, which include bacteria, yeast, fungi [1-6], and plants [7-10]. In general, the utilization of bio/plant extracts for fabrication of metal nanoparticles is advantageous when compared to the microorganisms, it is because of ease of scale up, less biohazard, and can reduce the lengthy process of maintaining cell culture.

Pectin is an anionic polymer, which is widely used in the present studies. It is a natural polymer functionalized with carboxylic groups (Scheme 1). It is a ubiquitous component of the cell walls of land plants and green algae [11]. Its main advantage of this polymer is to provide mechanical strength for the cell walls. In addition to this, it plays an important role in various processes in the cells, e.g., binding of water, morphological development, and fruit ripening [12]. Pectin has been used in the field of pharmaceutical and medical applications such as detoxicant, to control cholesterol levels, anticancer agents, chemosensitizing agents, controlled-release dosage forms, and carrier for drug delivery [13-16]. Naturally available polyelectrolytes have versatile applications in the field of biotechnology and medicine due to their biodegradability, biocompatibility, availability and ease of functionalization. The polyelectrolytes exhibit the better physicochemical, mechanical, topography, stability, etc.

The naturally abundantly available pectin polymer not only cost effective but also has potentiality in



Scheme 1: Schematic representation of pectin chemical structure.

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surface-active properties, which would be helpful to enhance size and shape of metal nanoparticles. So, authors explored a facial green approach to the synthesis of SNPs by reducing with sunlight, using pectin as a stabilizing agent. Considering the biologically inert, biocompatible and superior optic-electronic properties of SNPs, pectin-Ag nanocomposite (P-Ag-NC) film results the merits of pectin and as well as SNPs, so that it explores its potential application in biomedical and pharmaceutical field.

2. EXPERIMENTAL

2.1. Materials

Pectin and $AgNO_3$ were purchased from Merck, India. The double distilled water was used throughout the experiment.

2.2. Preparation of P-Ag-NC

The P-Ag-NCs were developed by mixing of 2% aqueous pectin solution and different $AgNO_3$ solution (26, 52, 78, and 126 mg) at 27°C (Table 1). This solution was kept under sunlight for 1 hour. The colorless of pectin and $AgNO_3$ mixture was started to brown violate, it clearly indicates reduction Ag^+ ions to Ag^0. The resulting P-Ag-NC solution was then casted onto glass plates and allowed to dry in a dust free environment.

2.3. Characterization of P-Ag-NCs

Ultraviolet-visible (UV-vis) absorption spectra of P-Ag-NC was recorded on an LAB India, (UV-3092 model), in the range of 250-800 nm. Fourier transform infrared (FTIR) spectra were recorded for the pure and P-Ag-NCs shown in Figure 3. The P-Ag-NCs were dried in an electronically controlled oven at 40°C. The FTIR spectra of P-Ag-NCs were recorded between 500 and 4000 cm^{-1} by KBr method on a Perkin Elmer Spectrum Two model. The size of the SNPs in the P-Ag-NC was determined using a dynamic light scattering (DLS) method using Brookhaven BI-9000 AT instrument, USA. TEM study of P-Ag-NC was performed by placing a drop of aqueous P-Ag-NC solution on a copper grid and subsequently drying at room temperature, before transferring them to the microscope operated at an accelerated voltage of 200 kV.

Table 1: Feed composition of silver-pectin nanocomposites.

Code	2% pectin (ml)	$AgNO_3$ (mg)
P0	4	13
P1	4	26
P2	4	52
P3	4	78
P4	4	126

2.4. Antibacterial Activity

The antibacterial activity of P-Ag-NC (1 $mg.mL^{-1}$ and 2 $mg.mL^{-1}$) was performed by disc method of Bauer-Kirby [17]. Furthermore, we have followed the procedure for this study is as per published earlier work of our group [18].

3. RESULTS AND DISCUSSION

Pectin is a naturally available miracle macromolecule due to their unique physicochemical properties. Pectin based materials has been used for different potential applications [18,19]. In the present contribution, SNPs were fabricated by reducing Ag^+ with naturally available biodegradable pectin. Here, authors prepared P-Ag-NC films by mixing of different amounts of aqueous solutions of polymer and $AgNO_3$. In general, pectin was dissolved in double distilled water, after forming homogeneous viscous solution, $AgNO_3$ solution with different portions was injected into this solution rapidly and allowed 1 hour under natural sunlight, a violet blue solution was formed in the solution (Figure 1). The resulting silver nanocomposite solution is poured in Petri dishes to form thin films of size around 25 µm. These SNPs in the film functionalizes as physical cross-link agents to strengthen the polymeric film. This type of nanocomposite structure provides functional channels for water molecules to facilitate them in and out from the film.

3.1. UV-vis Spectroscopy

In a series of P-Ag-NC, the SNPs formation depends on the pectin content as well as the amount of the silver ions. UV-vis spectrophotometer confirmed that increase of pectin content resulted increase in absorbance of the characteristic band at 420 nm, which is responsible for the surface plasmon of SNPs. As pectin content is increases, the reduction capability will be increased it is due to the presence of –COOH

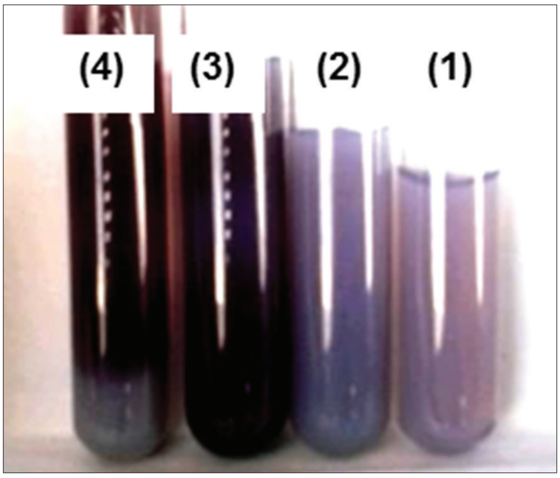

Figure 1: Similar pectin solutions (5 ml) contain different $AgNO_3$ concentrations (26, 52, 78, and 126).

and –OH functional groups. The UV-vis spectrum of the P-Ag-NC is presented in Figure 2.

3.2. FTIR Spectral Analysis

FTIR spectroscopy is one of the best tools for detection of an interaction between silver and macromolecules. Figure 3 describes the FTIR spectra of P-Ag-NC and the FTIR spectra of the pure pectin shows the typical absorption band at 1588 cm^{-1} and 3340 cm^{-1} corresponds to the stretching frequency of the -COO^- and –OH groups, respectively. The band at 2920 cm^{-1} is due to -C-H stretching vibration. The bands at around 1416 and 1322 cm^{-1} are assigned to -CH_2 scissoring and -OH bending vibration, respectively. The band at 1019 cm^{-1} is due to [-CH-O-CH_2] stretching. Whereas, FTIR spectra of P-Ag-NC illustrates the similar characteristic bands with slight change in their vibration frequencies (1598 [-COO^-], 3256 [-OH], 2948 [-CH_2], and 1022 [-CH-O-CH_2]). Finally, results suggest that the presence of SNPs in P-Ag-NC which are having a strong interaction with pectin polymer chains.

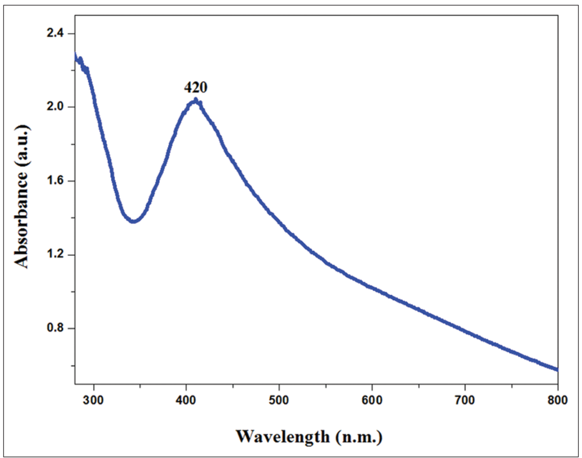

Figure 2: Ultraviolet-visible spectra pectin silver nanocomposite films.

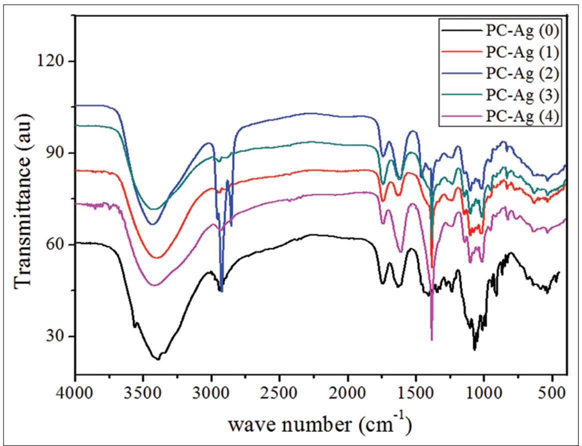

Figure 3: Fourier transform infrared spectra of pure pectin and P-Ag-NC films with different $AgNO_3$ concentrations (13, 26, 52, 78, and 126 mg).

3.3. TEM and DLS Analysis

To study the surface morphology and size of the metal nanoparticles, TEM and DLS are the promising techniques (Figures 4 and 5). The TEM picture of P-Ag-NC has clearly revealed that the formation of SNPs in the pectin films. The dark core of the nanoparticles should correspond to the metallic silver. A clear ring pattern for SNPs is appeared in electron diffraction pattern study (Figure 5). From the TEM image, SNPs have a size ~20-40 nm in diameter, and these results are supported by DLS, form the DLS histograms the average diameter of the 35.7 nm.

3.4. Antimicrobial Studies

The potential application of pectin SNPs was already well established on photodynamic therapy. Applications of the photodynamic effects are mainly explored on cancer treatment as an alternative to chemotherapy or radiotherapy procedure [1,2,10]. Moreover, photodynamic methods are already in use either routinely or in experimental studies on several medical fields, such as dermatology, ophthalmology, gastroenterology, cardiology, neonatology [1,2,20], fungal, and bacterial infections [1,2,21,22].

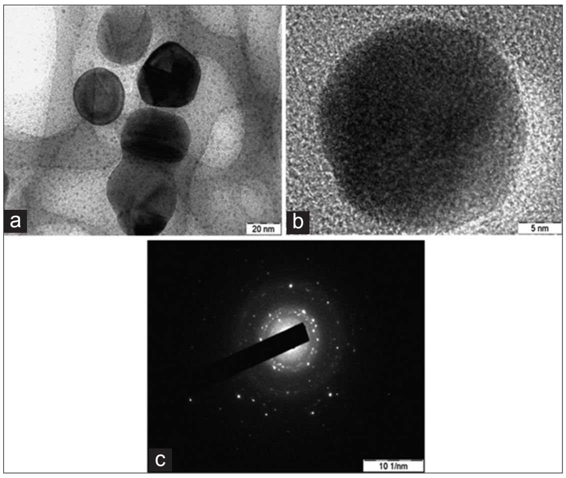

Figure 4: Representative transmission electron microscopy images of pectin-silver nanocomposites, with higher magnification in (b). The scale bars of (a) and (b) are 20 nm and 5 nm, respectively. (c) EDS pattern of SNPs

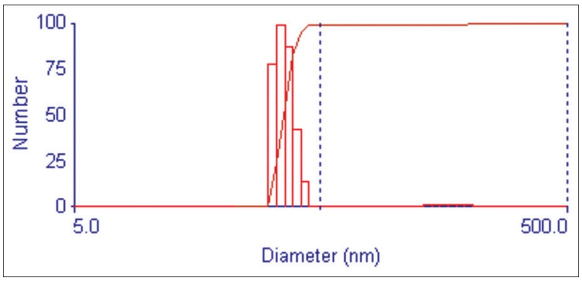

Figure 5: Dynamic light scattering size distribution histograms of silver nanoparticles from P-Ag-NC.

Furthermore, recently SNPs synthesized from natural products for antimicrobial applications [23-25]. Here pectin, a complex carbohydrate found in plants primary cell walls, isolates the silver nanospheres increasing the biocompatibility of the colloid and controlling chemical interactions among SNPs and other structures. The antibacterial activity of P-Ag-NC against the *Bacillus subtilis* and *Escherichia coli* was studied using Bauer-Kirby method by measuring the diameter of the zone of growth inhibition [23] (Figure 6). Results suggested that antibacterial activity of the P-Ag-NC had considerable differences in their activities, which depends on the microorganisms tested. The inhibition zones were observed, i.e., 10.3±0.7 mm for Gram-negative *E. coli,* and the maximum inhibition zones of 15.3±0.5 mm were shown by Gram-positive *B. subtilis*. The zone of inhibition was compared with streptomycin (*30 mcg units) where it shown 19.6±0.5 for *E. coli* and 20.3±0.5 for *B. subtilis*. Finally, the antibacterial activity of P-Ag-NC found to exhibit good results in Gram-positive *B. subtilis* and the moderate result was shown in Gram-negative *E. coli*. However, the results were summarized that the P-Ag-NC films were show efficient antimicrobial activity on both the strains.

4. CONCLUSION

In this study, we described environment-friendly biosynthesis of SNPs using pectin natural polymer. Results suggested that bio-reduction and stabilization of SNPs are maintained by pectin macromolecular chains which are comprised of hydroxyl and carboxyl functional groups. The P-Ag-NC films were shown efficient antimicrobial activity on two strains namely *Bacillus* and *E. coli.* This type of biosynthesis of SNPs does not contain any toxic reagents and thus has the potential for use in biotechnology and biomedical fields. These are the preliminary studies of P-Ag-NC, in continuation of this work we will develop the drug delivery systems for potential anticancer applications.

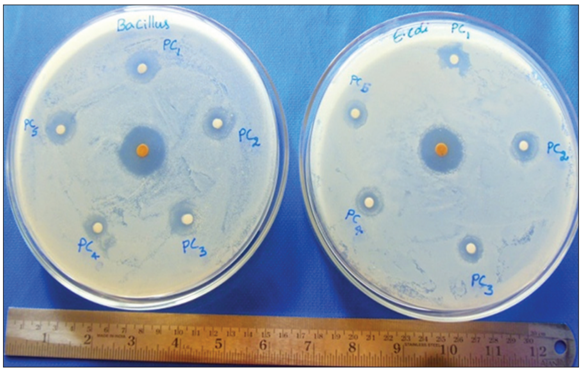

Figure 6: Antimicrobial activity of pectin-Ag-nanocomposite particles in the *Bacillus* and *Escherichia coli*.

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