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Simple Biosynthesis of Silver/PVA Nanocomposites For Effective Antimicrobial Studies

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***Abstract*:** Silver nanoparticles (Ag NPs) are widely studied and extensively used in various applications because of their superior properties. Suitable structural modification with the right synthetic method is the key to obtain superior properties of Ag NPs. In the field of nanotechnology, the development of reliable and eco-friendly methods for the synthesis of nanoparticles is crucial. The conventional methods for the synthesis of NPs are costly, toxic, and not ecofriendly. To overcome these issues, natural sources such as plant, bacteria, fungi, and biopolymers have been used to synthesize AgNPs. These natural sources act as reducing and capping agents. With the aim of promoting a green approach for the synthesis of NPs, this study describes eco-friendly method for the preparation of biogenic NPs and the known mechanisms for their biosynthesis. Silver nanoparticles (AgNPs) have been reported to form composites with polymers such as polyvinyl alcohol, polypyrrole, polyvinylidene fluoride, chitosan, and cellulose. The formation of silver/polymer nanocomposite requires that the size of nanoparticles in the polymer matrix be controllable and that their distribution within the polymer matrix be uniform. In this study, extract from "orange peels" were utilized for the synthesis of silver/PVA nanocomposite (Ag/PVA Nps) from silver nitrate solution. The bioactive components of extracts and the morphology of Ag/PVA Nps were studied using FTIR, UV-Vis spectroscopy, SEM, XRD and AFM The antimicrobial and antifungal activities were also performed.

Key words: Polyvinyl alcohol, orange peels, FTIR, SEM, AFM, XRD.

I.INTRODUCTION

. Nanotechnology is the synthesis of particles with at least one dimension in the range of 1–100 nm, resulting in high surface to volume ratios. As the particle size decreases, not only the ratio of surface area to volume increases but also the physical, chemical and biological properties of the particles differ compared to their bulk counterparts . Noble-metal nanoparticles exhibit incredible physicochemical, optoelectronic and biochemical characteristics. They are being used for various purposes in industrial and pharmaceutical applications. Only a few of them such as gold, silver, palladium and platinum are synthesized extensively in nanostructured form. Among them silver nanoparticles have attracted much attention due to their unique characteristics for utilizing in various applications including pharmaceuticals, agriculture, water detoxification, air filtration, textile industries and as a catalyst in oxidization reactions. Furthermore, their predominant property is their high antimicrobial activity against a broad range of microbes. Bactericidal activity of AgNPs without toxicity to human cells can make them a proper substitute for antibiotics. AgNPs are being employed for eliminating microorganisms in medical devices, implants and hospital masks. They are also extensively being used in hospitals either supplemented with antibiotics or alone for preventing infections. The past decade has witnessed the vast development and involvement of nanomaterials in many different areas of research due to their unique optoelectronic and physicochemical properties.[1,2]

Polymer nanocomposites are materials of which composition includes atleast one constituent with dimensions less than 100 nm. Polymer nanocomposites consist of a polymer or copolymer having nanoparticles or nanofillers dispersed in the polymer matrix Polymer nanocomposites possess outstanding optical, catalytic, electronic, medicinal, and magnetic properties. With its unique properties polymer nanocomposites are favorable for many applications[3].Silver nanoparticles (AgNPs) have been reported to form composites with polymers such as polyvinyl alcohol, polypyrrole, polyvinylidene fluoride, chitosan, and cellulose. The formation of silver/polymer nanocomposites requires that the size of nanoparticles in the polymer matrix be controllable and that their distribution within the polymer matrix be uniform [4]. These silver/polymer nanocomposites can be used in a wide range of biomedical products, such as surgical gloves, antibacterial cloths and towels, and anti-infectious urinary catheters [5] also they can be incorporated into aseptic coverings for plastic surgery, traumatic wounds, leg ulcers, skin grafts, incisions, and abrasions. Further, they can be used in numerous household applications such as textiles disinfection in water treatment, food storage containers, and home appliances and in medical devices [6]. Many previous attempts to form silver/polymer nanocomposites have involved mixing of a nanoparticle solution into the polymerization mixture. However, the main methods currently utilized in their production are not environmentally friendly. Silver/PVA nanocomposite has now been established as an effective biomedical agent and acts on a wide range of both gram-negative and gram-positive bacteria[7]. PVA is a biodegradable polymer hence, it has a wide range of anti-bacterial properties. It has also been successful in demonstrating its antibacterial property on drug-resistant bacterial strains, qualifying it to be a potential candidate for pharmaceutical use. Several studies have been carried out on the mode of antibacterial action of AgNPs on bacteria. AgNPs were able to restrict bacterial growth . The cell wall damage was inspected and has been inferred to be caused due to the high level of interaction of AgNPs with compounds containing phosphorus and sulfur[8]. Moreover, the results suggest that an oxygen-rich environment induced the antibacterial activity of AgNPs in comparison with an anaerobic environment. These interactions can be assumed to be the reason for prevention of DNA replications, which eventually lead to bacterial death. Every year, thousands of patients, all over the world, die succumbing to infections from the contracted post-injury of surgery. AgNPs have shown fantastic antimicrobial properties and rapid healing properties.

Many biological routes of AgNPs synthesis have been reported using plant extracts, such as oranges, [9] lemons, [10] pepper,[11] red cabbage, [12,13] Aloe vera,[14] Nigella sativa, [15] Pulicaria glutinosa, , [16] Justicia glauca, , [17] Mimusops elengi L, [18] and coffee. [19] Very few reports have been performed on AgNPs stabilized by nanocellulose. [20] This material can be extracted from various types of biomasses, including oranges, [21] and presents a great potential as a renewable nanomaterial owing to its sustainability, high versatility (in the form of nanofibrils, nanowhiskers, or nanocrystals), and ease of extraction. With the aim of promoting a green approach for the synthesis of AgNPs, this work describes eco-friendly method for the preparation of biogenic NPs. In this study, extract from "orange peels" were utilized for the synthesis of silver/PVA nanocomposite from silver nitrate solution. The bioactive components of extracts and the morphology of AgNps were studied using FTIR, UV-Vis spectroscopy, SEM, XRD and AFM. The antimicrobial activity was performed.

II. MATERIALS AND METHOD

2.1 Materials Required

Shade dried orange peel, Silver nitrate, polyvinyl alcohol, and toluene .

2.2 Green synthesis of Ag nanoparticles

1 g of shade dried orange peel was crushed to which 60 ml of toluene was added with vigorous stirring for 30 minutes at 60^{o}C to prepare the extract of orange peel. The extract then was centrifuged for 5 minutes for 6000 rpm at room temperature. Then, 2 g of $AgNO_3$ was dissolved in 40 ml of toluene with vigorous stirring at 70^{0}C for 5 minutes. Thereafter 5 ml of orange peel extract was added to the solution of $AgNO_3$ the colour changed to brown which indicated reduction of Ag ions and the formation of silver nanoparticles. Then the precipitate was allowed to dry it turns into black colour.

2.3 Synthesis of Ag/PVA nanocomposites

Various methods are employed to prepare antimicrobial AgNPs/PVA nanocomposite [22]. In our study, solution method was used to prepare antimicrobial AgNPs/PVA nanocomposites. 2 g of Polyvinyl alcohol (PVA) was added to the silver nanoparticles that were dispersed in toluene and synthesized as described in the previous section. The solution was stirred under vigorous stirring at 60∘C until PVA completely dissolved and the solution turns into brown colour indicating the formation of Ag/PVA nanocomposites. Then, the solution was cast in a watch glass and the toluene was allowed to evaporate at room temperature, to produce the nanocomposite. The precipitated nanocomposite was then removed from the watch glass after 24 hours.


Fig1: AgNPs Fig2:Ag/PVA nanocomposite

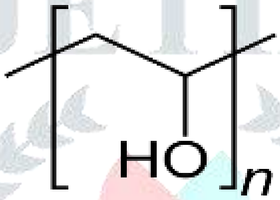

Fig3: Molecular structure of PVA

III. RESULTS AND DISCUSTUTION

3.1.Characterization of Ag/PVA nanocomposite

3.1.1 FTIR;

The electrostatic interactions between PVA and silver nanoparticles were studied by FTIR spectroscopy. In case of PVA there is a strong broad band at 3278 cm-1 which is assigned to O-H stretching frequency indicating the presence of hydroxyl groups. The bands observed at 2933 cm-1 and 2851 cm-1 correspond to C–H stretching vibrations and that at 1723 cm -1 correspond to C=O stretching vibrations, resulting from the conversion of an alcohol group (-C-OH) during the reduction of Ag^{+} ions and the absorption band at 1683 cm -1 arises due to C=C stretching. The absorption bands at 1405 cm -1 is assigned to CH2 bending and that at 1321 cm-1 is due to CH2 wagging. The band at 1080 cm-1 is due C-O stretching. The absorption band appearing at 840 cm -1 is due to the out-of-plane vibration of C–H group[22]. An important band centered at 913 cm-1 symbolizes the presence of the syndiotactic structure of PVA suggesting the regular inter-chain bridging in a layered structure via sequential distribution of OH groups, conferring the H-bonding functionality to planarize the polymer backbone. Finally the continuous decrease in intensities of bands in the region 1440-1080 cm -1 indicates the decoupling between OH and CH vibrations due to electrostatic interaction between OH and embedded silver nanoparticles.

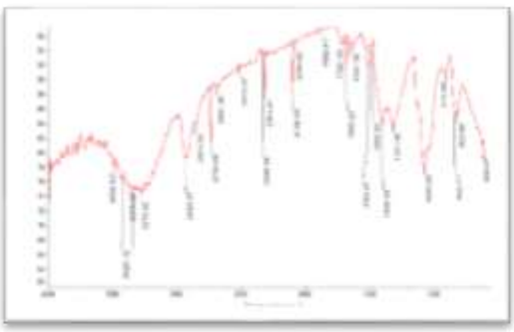

Fig 4 : FT-IR spectrum of Ag/PVA nanocomposite

3.1.2 UV;

In the present investigation, UV absorption spectra (400-600 nm) of Ag/PVA nanocomposite showed higher absorption in the region of 400-475nm Fig 5.indicates that increase in absorption in UV spectra may be due to the formation of Ag/PVA nanocomposite

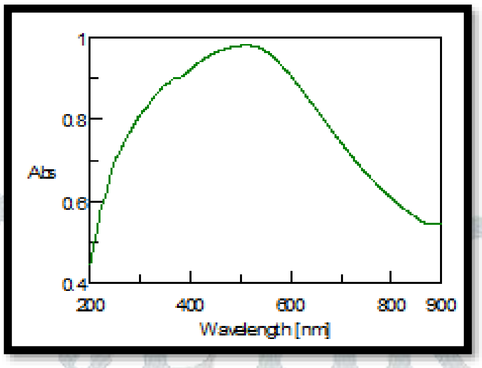

Fig 5 : UV spectrum of Ag/PVA nanocomposite

3.1.3 XRD;

Figure 6 shows the XRD pattern of the synthesized Ag/PVA nanocomposite and it is observed that the particles are crystalline in nature. Strong peaks from Braggs reflection were observed in the XRD pattern at $2\theta = 20°$, $23°$ and $40°$. It is well known that the peaks at 2θ nearby $20°$ may be due to the crystalline nature of the PVA polymer molecule while the other two peaks can be assigned to the cubic crystal structure of silver. The grain size of the synthesized Ag/PVA nanocomposite was calculated using Debye-Scherer's equation $D = K\lambda/\beta\cos\theta$, where the average grain size was found to be 6.63 nm.

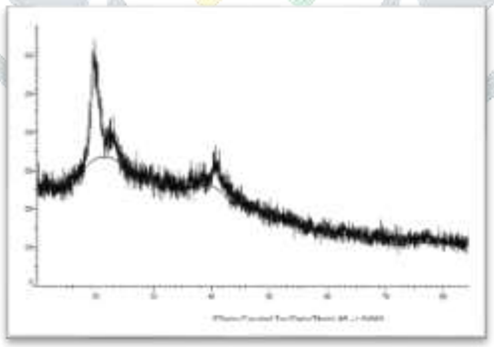

Fig 6 : XRD pattern of Ag/PVA nanocomposite

3.1.4 AFM;

Atomic force microscopy (AFM) is a powerful technique that enables the imaging of almost any type of surface, including polymers, ceramics, composites, glass and biological samples. AFM is able to build three –dimensional maps of surface properties in both air and liquid environments. AFM has several advantages over electron microscopy in the study of biological materials, including the ability to image in liquid with minimal sample preparation . AFM also allows the topographic characterization of surfaces at resolutions not achievable by optical microscopy. Here the AFM is used to determine how the Ag is dispersed on the surface of the polymer (PVA). This is confirmed by the graphical method(Fig 7).

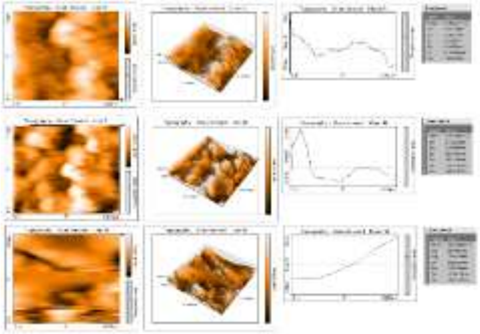

Fig 7 : AFM image of Ag/PVA nanocomposite

3.1.5 SEM;

SEM analysis is a powerful investigative tool which uses a focused beam of electrons to produce complex, high magnification images of a sample's surface topography. A scanning electron microscope (SEM) scans a focused electron beam over a surface to create an image. The electrons in the beam interact with the sample, producing various signals that can be used to obtain information about the surface topography and composition. Advances in scanning electron microscopy (SEM) enable the high-resolution imaging of single nanoparticles (NPs) with sizes well below 10 nm. SEM analysis shows that the synthesized nanoparticles were uniformly dispersed in the PVA matrix confirming the formation of Ag/PVA nanocomposite. SEM analyses of the synthesized silver/PVA nanoparticles were clearly distinguishable measured 60-70 nm in size[23] .

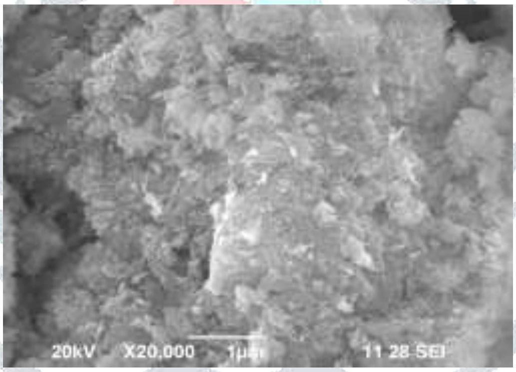

Fig 8: SEM image of Ag/PVA nanocomposite

3.2 Antimicrobial Activity:

Antimicrobial activity was performed on Escherichia coli, Staphylococcus and Candida albicans. using standard disk dispersal method. Fresh overnight cultures were taken and propagated for cultivation on agar and potato dextrose plates. Sterile paper discs 5 mm in diameter were saturated with orange peel extract. Silver /PVA nanocomposite and dual distilled water (as a control) were placed on each plate incubated at 37 ºC for 24 h and antimicrobial activity was measured on the basis of inhibition. The region around the disk was enriched with silver/PVA nanocomposite integrated into the orange peel extract. Various concentrations of Ag/PVA NPs derived from orange peel extract have been shown to be successful in inhibiting the growth of human pathogens, measuring 1 mg/ml concentration of orange peel inhibited bacteria and fungi Escherichia coli, Staphylococcus and Candida albicans. Table 1 explains the in vitro antimicrobial activity of orange peel extract and Ag/PVA Nanocomposite. The presence of Ag NPs in the cell membrane of bacteria has been proven in earlier studies. AgNPs disturb the permeability of the cell membrane by penetrating into it and causing intracellular ATP leakage and cell death. Silver ions' release[24] from AgNPs acting as reservoir causes antibacterial activity of Ag NPs. It appears that the positively charged ions such as Ag+ in this experiment have a high tendency to act with phosphorus and sulfur present in biomolecules such as DNA and RNA resulting in the disruption of DNA and RNA functions [25-30].

Table 1. The in vitro antimicrobial activity of orange peel extract and Ag/PVA Nanocomposite

Compound	E. coli	Staphylococci	C. albicans
Orange peel extract	7	10	11
Ag/PVA NPs	17	17	24
Standard**	5	5	7

Zone of inhibition in mm. Each value observed is within the error limits of ±2, R= Resistant. **Standard = (Ciprofloxacin and Cephalosporin)

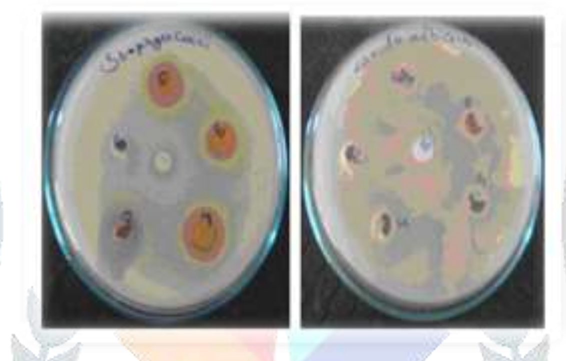

Fig 9: Zone of inhibition of AgNPs

IV. CONCLUSION

A simple and safe process is utilized for the synthesis of silver/PVA nanocomposite by using orange peel extract. No chemical reagent or surfactant template is required in the process, which consequently established the biosynthesis with the advantage of being environmentally friendly. In this study we successfully demonstrated that orange peel extract has the ability to synthesize the nanoparticles and maximum yield of nanoparticles was obtained. The electrostatic interactions between PVA and silver nanoparticles were studied by FTIR spectroscopy which confirmed the formation of Ag/PVA nanocomposite. In the present investigation, UV absorption spectra (400-600 nm) of Ag/PVA nanocomposite showed higher absorption in the region of 400-475nm indicating the formation of Ag/PVA nanocomposite. SEM analysis showed that the synthesized nanoparticles were uniformly dispersed in the PVA matrix confirming the formation of Ag/PVA nanocomposite Similarly XRD analyses of the synthesized silver/PVA nanoparticles were clearly distinguishable and measured 6.63 nm in size. AFM results confirm uniform particle distribution and surface topology. The Ag/PVA nanocomposite shows good antibacterial and antifungal activities when assayed by agar well diffusion method. This green synthesized nanocomposite could be used in the medical field against human diseases due to their high efficiency as antimicrobial agent.

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