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A Novel Ag/CS-PVC Nanomaterial with High Antimicrobial Properties: A Potential Self-Sterilizing Biomaterial

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***Abstract*-** The field of manufacturing technology has totally been renovated with the emergence of nanotechnology; and the mergence of nanoscience with material science, biology, biotechnology and medicine has completely novelized various arrays of biomedical engineering. For the nanomedicine landscape we have designed and developed Ag/CS-PVC nanocomposite material having superb antimicrobial properties which can effectively be used as self-sterilizing catheter biomaterial especially for the cardiovascular system. Arjuna bark, one of the best cardio-protector agents, has been involved for the production of silver nanoparticles. Several techniques have been used for the characterization of nanoparticles and the nanomaterial developed. This biomaterial is cost effective and extremely biocompatible.

***Index Terms*-** antimicrobial, biomaterial, nanocomposite, nanoparticles, self-sterilizing

I. INTRODUCTION

Advances in the field of nanomaterial manufacturing for biomedical engineering largely depend on the ability to synthesize nanoparticles in an eco-friendly manner without using any toxic chemicals in the protocol. In our present work, bioreduction and formation of silver nanoparticles has been achieved, by using the bark of the plant *Terminalia arjuna* which is commenly known as arjuna bark or arjun. Since time immemorial arjuna bark is considered to be an excellent astringent, hypolipidemic, anticoagulant, hypocholsteremic, antihypertensive, antiviral, antithrombotic, cardiac stimulant, hemostatic, rejuvenative tonic, lithontriptic, antifungal and antibacterial mean [1].

In our present investigation the silver nanoparticles produced involving the arjuna bark, has been incorporated into a chitosan (CS) – poly vinyl chloride (PVC) blended matrix, to develop a novel silver/chitosan-poly vinyl chloride (Ag/CS-PVC) antimicrobial self-sterilizing nanocomposite biomaterial, for the first time. The blending of PVC with CS was enabled through simultaneous casting of their separate solutions in 2:1 proportion of CS and PVC in suitable solvents, glutaraldehyde (GA) was used to ensure cross-linking between the two polymers. Chitosan, a natural biopolymer composed of poly (β-(1-4)-2-amino-2-deoxy-D-glucose), is one of the structural polysaccharide which is abundantly available in nature [2]. PVC is one of the most commonly used synthetic polymers in biomedical applications, especially as a catheter material for the circulatory system [3]. Earlier studies shed an important light on the underappreciated and significant risks of biomaterials associated infection (BAI) through peripheral IVs as well as other cardiovascular catheters composed of PVC [4]. It indicates that it is immensely important to initiate interventions to combat the risk of BAI.

So in our study we have approached to design blending of PVC with CS, using GA as a cross-linker to enhance the CS/PVC compatibility and endow desired properties to the hybrid material for biomedical use. In addition, we have also dispersed the formed silver nanoparticles to this crosslinked copolymer matrix which renders the developed nanomaterial self-sterilizing [5], with superb antimicrobial response for effective use as a catheter material to splendidly subside biomaterials associated infections (BAI).

II. EXPERIMENTAL

A. Materials

CS (degree of deacetylation: 79 %, molecular mass: 500,000 g/mol) purchased from Sea Foods (Cochin), India; PVC (molecular weight 10,000) purchased from Sigma Aldrich; GA, tetrahydrofuran, acetic acid glacial and silver nitrate of analytical grade purchased from Thomas Baker (Chemical) Pvt. Ltd. India. Arjuna bark was collected freshly from the garden.

B. Preparation of Arjuna Bark Extract

Arjuna bark was properly washed and air dried. 21 g was cut into fine pieces, boiled in 100 ml of distilled water for 15 min at 100 $^{\circ}$C. The crude bark extract was filtered using Whatman No. 41 filter paper and stored at 4 $^{\circ}$C for further use.

C. Synthesis of Ag Nanoparticles

10 ml of the bark extract was added to 200 ml of 2 mM silver nitrate ($AgNO_3$) solution. This reaction mixture (RM) was incubated at RT for stabilization.

D. Dispersion of Ag Nanoparticles in the CS Matrix

After 48 h RM was centrifuged separately at 9,000 rpm for 15 min and the residue (AgNP pellet) was dispersed in 20 ml of

chitosan solution [2% (w/v) in 1% (v/v) acetic acid] and sonicated for 10 min. Finally nanocomposite solution (NC) was prepared and the composite solution was properly kept for further use.

E. Development of Ag/CS-PVC Nanocomposite Biomaterial

NC solution and PVC solution [1% (w/v) in tetrahydrofuran] were taken in the ratio 2:1, poured simultaneously in a beaker portion-wise while stirring and 1 ml of 25% GA for proper cross-linking was added to the mixture. The obtained product, Ag/CS-PVC nanocomposite was collected, dried in vacuum at 37 °C for 24 h.

F. Characterization of the Formed Ag Nanopartcles

The reduction of Ag^+ to Ag^0 was monitored by measuring the UV-Vis. spectrum of reaction mixture RM after 48 h. The spectra of bark extract alone and $AgNO_3$ solution were also taken. The UV-visible spectra were recorded using UV- visible spectrophotometer (Shimadzu UV – 2450) from 200 to 800 nm. Transmission electron microscopy (TEM) of silver nanoparticles synthesized were performed on (HR TEM TECNAI 20 G^2) instrument operated at an accelerating voltage of 200 kV.

G. Characterization of the Ag/CS-PVC Nancomposite Material

The structure and morphological properties of Ag/CS-PVC nanomaterial was examined in a scanning electron microscope (SEM) (JEOL JXA 8100). For studying the chemical properties FTIR spectrum was recorded over the range of (500-4000) cm^{-1} with (FTLA 2000 ABB). The structure and physical properties were studied using X-ray diffraction (XRD, Philips, Xpert, Cu Kα) at a scanning speed of 2% min.

H. Swelling Properties Evaluation of Ag^+ Release

The pre-weighed and dried Ag/CS-PVC nanocomposite biomaterial was equilibrated in 250 ml of phosphate buffer saline solution (PBS) pH ~ 7.0 and 250 ml of deionized water separately, at room temperature. The solvent uptake by the nanomaterial, at time intervals, was measured using analytical balance. The equilibrium swelling ratio (ESR) was calculated using the equation: $ESR = (W_s-W_d) / W_d$, where W_s is the weight of swollen material and W_d is the weight of dry material. Therefore ESR is the ratio of, increase in swollen weight (W_s-W_d) to the weight of dried nanomaterial(W_d).

Study using atomic absorption spectroscopy (AAS) was carried out for the quantitative determination of the silver ion concentration in the analyte. The analyte was prepared by taking 250 ml of phosphate buffer saline solution (PBS) pH ~ 7.0, which resembles human extracellular fluid; in this Ag/CS-PVC nanomaterial weighing 16 mg was dipped for 48 h. 100 ml of this analyte was analysed through atomic absorption spectrophotometer (ELICO Ltd. SL 194) for the estimation of Ag^+ released by the nanocomposite biomaterial.

I. Antimicrobial Assay

The Ag/CS-PVC composite nanomaterial was assayed for antimicrobial activity against *Escherichia coli* (gram negative), *Pseudomonas aeruginosa* (gram negative), and *Staphylococcus aureus* (gram positive). *Staphylococcus aureus* is the pathogen most often associated with serious and costly catheter-related bloodstream infection [4]. Disc diffusion method was used to find out the standard zone of inhibition (ZOI). The nanomaterial was cut into disc shape having ~ 5 mm diameter, sterilized by UV radiation and placed on different cultured agar plates. These plates were incubated at 37°C for 2 days and then examined for evidence of zones of inhibition.

J. In vitro Biocompatibility/Cytotoxicity Test

The *in vitro* biocompatibility/cytotoxicity test using direct contact method was performed using the sample Ag/CS-PVC nanomaterial. The culture of mouse fibroblasts were established on Petri-dish in contact with the sample. The cell culture was recorded after 120 hr and the viability was calculated.

III. RESULTS AND FINDINGS

A. UV-Visible Spectral Studies

The UV–visible absorption spectrum recorded from the nanoparticle suspension of the reaction mixture, (RM) is shown in Figure 1. Surface plasmon resonance (SPR) band absorption peak appears between 420-480 nm, which is characteristic of silver nanoparticles. Peak position of RM occurs at 422 nm. Absorption spectrum of aqueous $AgNO_3$ solution was at ~220 nm and of bark extract at ~310 nm without any maxima or minima.

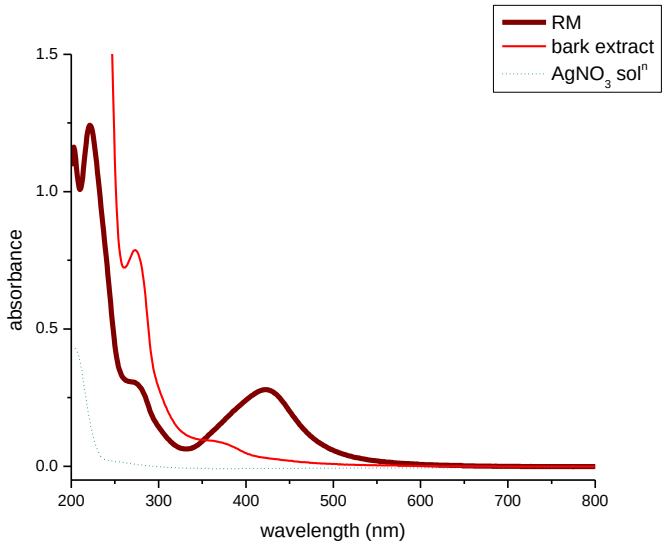
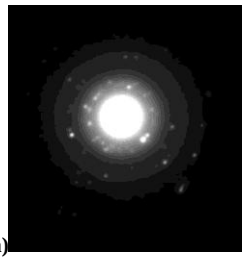


Figure 1: UV-Vis. spectra of, Ag nanoparticle suspension in (RM); bark extract; $AgNO_3$ solution.

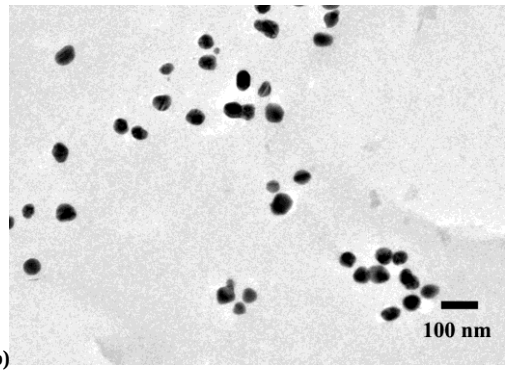
B. TEM Observations

TEM images shown in Figure 2 (b), revealed that silver nanoparticles formed are chiefly spherical but are also irregular in shape and non uniform in size. The ring-like diffraction

pattern and the approximately circular nature of the selected area electron diffraction (SAED) spots indicated in Figure 2 (a) reflects that the particles are crystalline in nature.



(a)

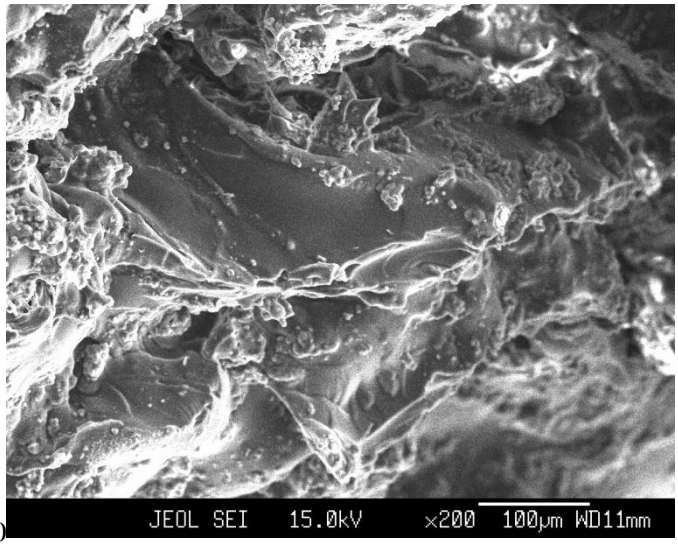


(b)

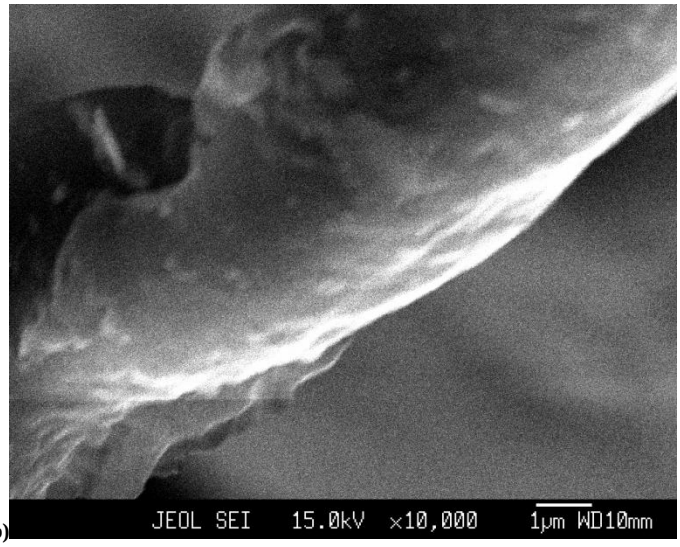
Figure 2: (a) SAED pattern of silver nanoparticles, (b) TEM image of silver nanoparticles

. C. Characterization of Ag/CS-PVC Nanocomposite

SEM micrographs in Figure 3 (a, b) elucidates homogenous mixing and optimized blending of the two polymer components in the modified biomaterial and show nanoparticles dispersed in the CS-PVC copolymer matrix with minimum aggregation. The interaction, between the lone pair of electrons present at the amine group of chitosan and the partial positive charge developed at the surface of the silver nanoparticles due to electron drift, effectively stabilizes the silver nanoparticles and prevents from agglomeration.



(a)



(b)

Figure 3: (a) and (b) SEM micrographs of Ag/CS-PVC nanocomposite at different magnifications.

The FTIR spectrum of the composite nanomaterial in Figure 4, illustrates intense bending vibrations between 1600-1000 cm^{-1} indicating possible interaction between silver nanoparticles and amino group of chitosan.

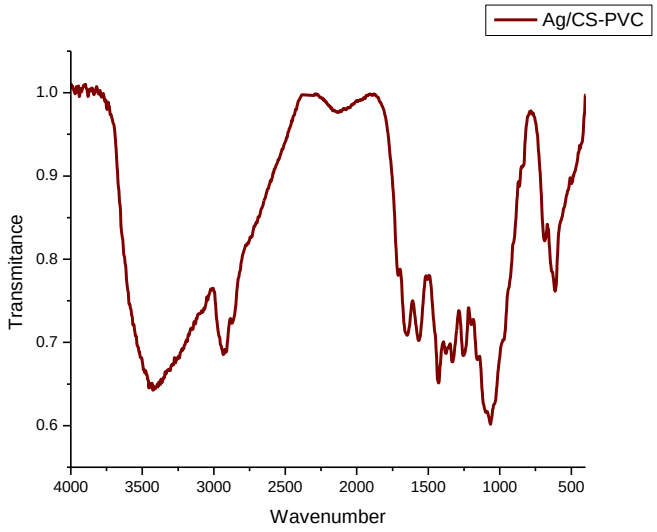


Figure 4: FTIR spectrum [Transmission/Wavenumber(cm^{-1})] of Ag/CS-PVC nanocomposite.

X-ray diffraction patten denotes that the nanocomposite developed is having semi-crystalline nature in Figure 5. Broad XRD pattern of Ag/CS-PVC below 30^{o} indicates the semi-crystalline nature of the major bulk components i.e., CS and PVC. In addition, strong reflection exhibited around 38^{o} is characteristic of the silver nanoparticles.

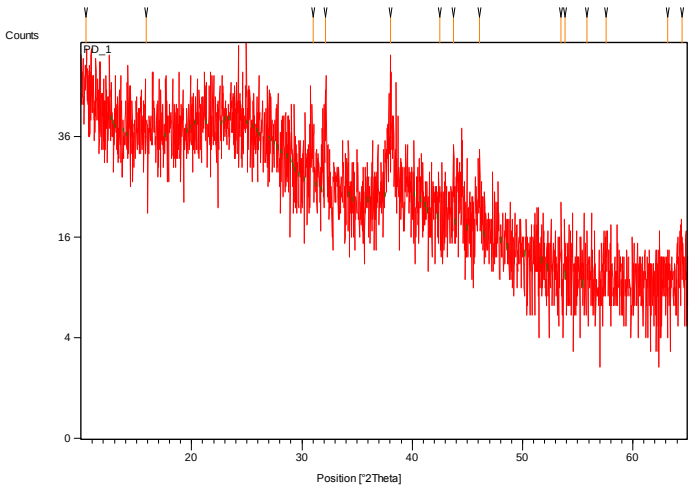


Figure 5: XRD spectrum of Ag/CS-PVC nanocomposite biomaterial.

D. Swelling properties Evaluation of Ag^+ release

As the swelling capacity of a nanocomposite material plays an important role in the antibacterial activity, wound healing capacity, and for various biomedical application due to their high water/solvent holding capacity. They can further absorb a slight to moderate amount of the wound exudates by swelling which helps in fast healing. The higher crosslink's within the nanomaterial restricts the penetration of solvent for swelling [6]. But the increase of chitosan content in the nanocomposite (2:1, chitosan: PVC) increases the swelling capacity significantly. Figure 6 shows swelling capacity and behaviour of Ag/CS-PVC nanocomposite with time.

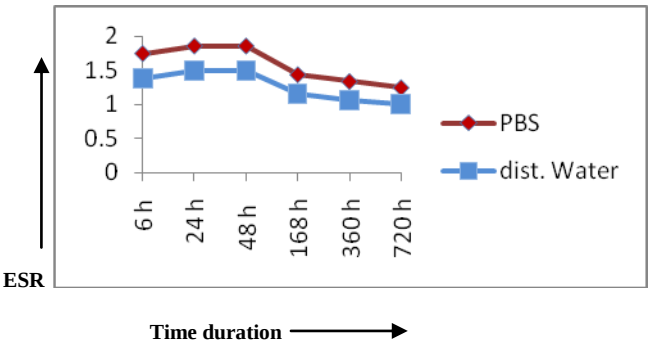


Figure 6: The swelling behaviour of the Ag/CS-PVC nanocomposite biomaterial in distilled water and phosphate buffer saline (PBS) solution shown with equilibrium swelling ratios (ESR) as a function of time duration (in hours).

Atomic absorption spectroscopic (AAS) studies estimated the silver ion concentration in the analyte, *i.e.* in phosphate buffer saline solution (PBS) pH ~ 7.0, which resembles human extracellular body fluid. The characteristic nature of the Ag/CS-PVC nanocomposite material, is an Ag^+ emitter in biological and other aqueous environment. It was determined that Ag^+ present in 100 ml of the analyte solution taken for the test was < 0.1 ppm, which was the lower detection limit of the instrument. The Ag^+ release increases with the amount of silver nanoparticles present in the sample specimen and it is also rational to believe that Ag^+ release will be influenced by the equilibrium swelling characteristics of the nanocomposite specimen.

E. Antimicrobial Assay

Ag/CS-PVC has excellent antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, with remarkable ZOI, showing majestic self-sterilizing characteristic. Details of the result are in Figure 7, and listed in Table I.

Table I Zone of inhibition (ZOI) in [mm] against microbial strains.

Name of the micro-organism	**Ag/CS-PVC nanocomposite**
(a) *Escherichia coli*	43
(b) *Pseudomonas aeruginosa*	36
(c) *Staphylococcus aureus*	35

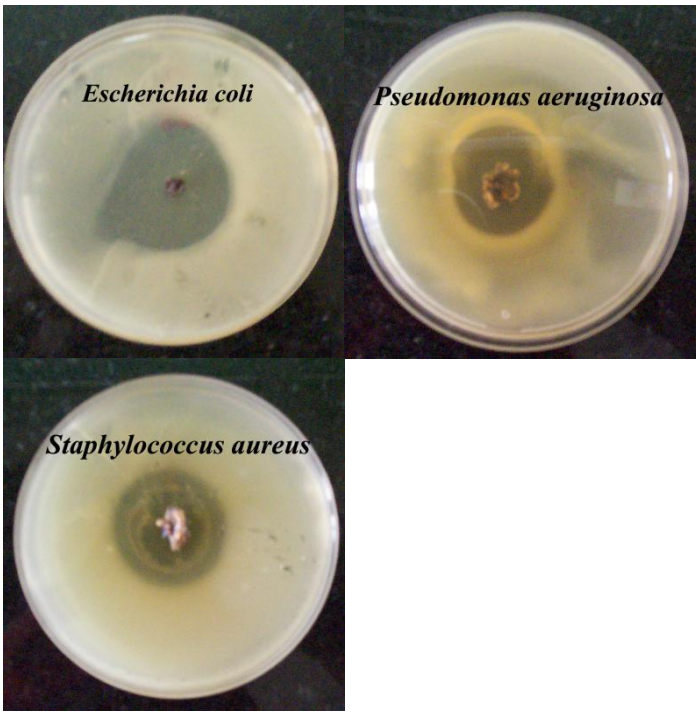


Figure 7: Photographs showing the antibacterial activity of Ag/CS-PVC nanocomposite biomaterial; through zone of inhibition (ZOI); against selected bacterial strains.

F. Biocompatibility Test

Ag/CS-PVC nanocomposite biomaterial showed non-cytotoxic response to fibroblast cells and a cell viability percent higher than 80%, which signified a good biocompatibility. Summarized data of the biocompatibility/cytotoxicity is elaborately given in Table II.

Table II. Cytotoxicity result of Ag/CS-PVC nanocomposite biomaterial (0.6×10^5 cells incubated for five days)

Sample	**Live cells** **X 10^5**	**Dead cells** **X 10^5**	**Total cells** **x 10^5**	**Viability** **%**
control	6.74	0.6	7.34	91.8
Ag/CS-PVC	5.29	0.9	6.19	85.4
chitosan	5.34	0.8	6.14	86.9
PVC	5.25	1.0	6.25	84.0

IV. CONCLUSION

This work presents a new approach for developing novel Ag/CS-PVC nanocomposite material, together with demonstrating a green method for generating silver nanoparticles. In this study, Ag/CS nanocomposite was first prepared and then transformed to Ag/CS-PVC nanocomposite blend. The developed Ag/CS-PVC nanocomposite material has exhibited good biocompatibility and superior antimicrobial properties with bright prospects to be used as catheter biomaterial to combat biomaterials associated infections (BAI).

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