Biomimetic nanofiber scaffolds of Silver NanoParticles (SNPs) incorporated PVA matrices as novel drug eluting reservoirs for healthcare applications

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Abstract

Controlled release of drugs from uniform porous membrane matrices attracts great interest as emerging drug reservoirs in the healthcare industries. Here, we report a novel process for the rapid synthesis of Silver Nanoparticles (SNPs) using the aqueous extract of marine macroalgae *Padina boergesenii* to be a biomimetic drug eluting reservoir for the release of SNPs as well as bioactive drugs of our interest towards the targeted disease. The potentials of the algae extract and its medicinal compounds were found responsible for SNP's synthesis. The as-synthesized SNPs were mixed with the biopolymer Poly (vinyl alcohol) (PVA) to obtain a uniform sol solution and further fabricated as a nano-fibrous (NF) membrane using the electrospinning technique. The fabricated electrospun NF scaffolds were analyzed with FESEM to find the morphological characters; HRTEM was performed to obtain the microstructure features; and the XRD pattern to investigate the crystalline nature of the NFs. The characteristic peak of Fucoidan (sulfated polysaccharides) and nanoparticles were confirmed by ATR-FTIR analysis. The contact angle measurements of PVA alone and NFs were found to be 35±1º and 37±0.5º with de-ionised water, respectively. The feasibility and potential of PVA nanofibrous scaffold as a drug delivery vehicle for SNPs release was investigated. In initial stage, burst release for NFs was observed as 20 % at 0.5 h and later, the release was slow and sustains to give an end point release at 80.5±1.5%. This sustained release mechanism could help us investigate more on these polymer matrix based metal oxide nano-scaffolds to promise us efficient drug reservoirs in the near future.

Keywords: Biomimetic scaffolds, *Padina boergesenii,* controlled release, drug reservoirs, nanofibers.

1 Introduction

The Phaeophyceae or brown algae are intertidal a large group of marine multicellular algae commonly grow along sea rocky. Most brown algae containing fucoxanthin pigment which has responsible for "greenish-brown" colour it gives them their name brown algae are unique it's called Fucoidan, is a sulphated fucan; is composed of fucose, galactose, uronic acids, xylose and sulphated fucose, variations are observed between the species of which have an impact on the determination of the polysaccharides structure. They have cellulose walls with alginic acid and also containing polysaccharides, fucoidan is the amorphous sections of their cell walls brown marine algae Allender & Kraft 1983, Womersley 1987. Fucoidan is the bioreducing agent of silver nitrate to silver nanoparticles (SNPs).

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Nanofibrous scaffolds has originated from naturally synthesised nanoparticles encapsulated into the polymers fibers have increasing attention for their application of the biomedical sector, e.g. anticancer activity, antioxidant , antibacterial ,tissue engineering scaffolds, wound dressing and drug delivery [1-4]. Natural polymers and synthetic biopolymer has possessed proven tissue biocompatibility. Poly (vinyl alcohol) is a highly hydrophilic synthetic polymer with low toxicity and good biocompatibility, biodegradablity and easily spinning ability largely used in the alimentary, pharmaceutical, cosmetic industry and drug releasing [5]. However, the advantages associated of the PVA hydrophilicity and cause difficult associated with its low mechanical properties. PVA has revealed good potential for use in drug-eluding polymeric devices, most notably with gels. Biocompatible and biodegradable nanofibrous scaffolds were prepared by electrospinning an artificial 3-D scaffold which is used for biomedical applications such as bioactivity, controlled drug delivery and wound healing with wound dressing process. [6-13]. The advantage of using electrospinning for synthesizing nanofibrous scaffolds for drug delivery applications is that it does not demolish any molecular structure and bioactivity of the incorporated nanoparticles drug. The ultra-thin electrospun nanofibrous structures are similar like native human tissue Extracellular Matrix (ECM) due to their high surface area and porosity [14-15]. The efficiency of the nanofibrous scaffold in wound healing and wound dressing has several advantages in retaining certain properties to allow good oxygen permeability, flexibility, high surface to volume ratio and biocompatibility with sequential reformation in the cell growth process (16). In the present study, we devise a strategy for avoid toxic reagents in the chemical crosslinking of PVA at the same time, incorporated silver nanoparticles with crosslinked PVA is a releasing moiety for allowing drug delivery for topical applications. This strategy has based on the hydroxyl groups of PVA with nanoparticles (AgNPs) capable of establishing intra, intermolecular ester linkages with the PVA polymer chains. Seaweeds, a mediated synthezied silver nanoparticle have low toxicity and are already used for medical applications for treating cancer cell lines [17]. Thus opening a new perspective for using SNPs/ PVA nanofiber scaffold a releasing biomaterials in topical applications for increasing accelerating wound healing and drug delivery system.

2 Materials and mathods

2.1 Materials and methods and Collection of sample

Poly (vinyl alcohol) MW 1, 50000 Silver nitrate ($AgNO_3$, SRL) Nutrient agar, (Himedia Mumbai, India) Seaweeds belonging to the Phaeophyta (Brown) *Padina boergesenii* Allender & Kraft, were collected from the coastal region at intertidal gulf of mannar Mandapam, Rameswaram, Tamil Nadu, South India. The seaweed material has taxonomically identified as well as authenticated by the University of

Madras, CAS in Botany, Guindy Campus, and Tamil Nadu.

2.2 Source of microorganisms

Pure cultures of Bacteria *Escherichia coli* (ATCC 8739) *Streptococcus pyogenes* (ATCC 19615) were obtained from American Type Culture Collection.

2.3 Preparation of Algal Extract

Initially, seaweeds species viz., *Padina boergesenii,* Allender & Kraft, was washed thoroughly using freshwater to remove the salts & epiphytes and dried under shade. These dried seaweeds were powdered in an electric grinder and stored in polyethylene bags at room temperature. 5g of the dried powder was extracted in 100 mL of distilled water using water bath for 20 minutes and then it was centrifuged for 15 minutes at 10000 rpm (mode Centrifuge - eppendorf centrifuge 5810R). Then the supernatant was used for the further preparation of nanoparticles.

2.4 Synthesis of silver nanoparticles

In two different 100mL conical flasks, 95 mL aqueous solution of 1 x 10^{-3} M silver nitrate ($AgNO_3$) was taken individually. To one of the conical flask containing silver nitrate solution, 1 mL of the algal extract was poured to silver nitrate solution at room temperature under static condition. Both the setup was incubated in
The dark to minimize the photo activation of silver nitrate. It was noticed that, the algal extract reduces the silver nitrate solution resulting in the formation of brown-yellow solution. This indicates the formation of silver nanoparticles (SNPs).

3 Electrospinning Process

Poly (vinyl alcohol) MW-1, 50000 Polymer powder was dissolved in de-ionized water (DW) at 10 wt % and heated at 80^{o}C and gently stirred continuously 4-6 hours. SNPs was added to PVA solution and continuously stirred for 2 hours. The polymer and nanoparticles solution was taken in 2mL dispo van single use syringe which a needle tip of diameter 0.56 mm inner diameter was attached in the positive electric circuit of high voltage power supply has connected with the needle and the negative terminal to the drum collector which was covered with aluminium foil. Electric voltage was optimized at 20 kV. PVA and SNPs / PVA solutions were electrospun at a flow rate of 0.5 mL / hour and the tip to collector distance was kept at 15 cm, schematic diagram as shown in Figure 1.

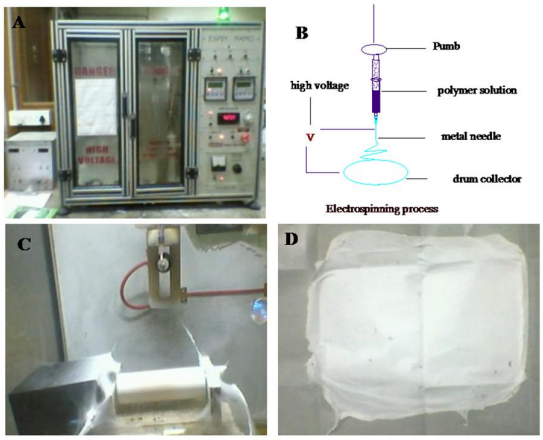

Figure: 1. Schematic diagram of electrospinning process

4 Characterisation

synthesis process was completed by reducing metal ion solution with *Padina boergesenii* extract, Surface Plasmon Resonance (SPR) of silver nanoparticles (SNPs) was easily confirmed by diffuse reflectance UV–Vis spectroscopy, the reaction mixture was sampled at regular intervals and the absorption maximum was scanned at the wavelength of 400–800nm using (Model UV-HITACHI U-2900 Spectrophotometer). The biosynthesized SNPs gave sharp peak in the visible region of the electromagnetic spectrum. After the SNPs incorporated into PVA nanofibrous membrane was analysis Surface Morphological Studies (SMS) of SNPs / PVA nanofibers were observed by FESEM (HITACH; S-3400N - FESEM) 5-20 kV and HRTEM (FEI, 30S TECHNAI G2) analysis with an accelerating voltage of 200 kV , with an elements were analyzed using energy dispersive analysis of X-ray (EDAX) attachment of HRTEM. The nanofibers diameters of about 100-300 different fibers were measured by help of the Image tool to obtained their average diameter. X-ray Diffraction analysis crystallinity of the prepared SNPs and SNPs / PVA nanofibers was examined using PANalytical X'pert pro Instrument using Cu Kα radiation (1.5406 A; 45 kV, 30 mA) in the range of 20°–80° peaks were matched with the tool of (JCPDS No. 01-1174) . The obtained pattern has cubic crystal structure. Attenuated Total Reflection - Fourier Transform Infrared Spectroscopy ATR-FTIR spectra the functional groups of SNPs and nanofibrous scaffolds were obtained on Perkin-Elmer spectrophotometer RX 100 in the range of 400 cm^{-1} to 4000 cm^{-1}. Thermal Analysis The thermo gravimetric analysis (TGA) of the nanofibers scaffold was performed by TGA/DTA model SDT 2600. Hydrophilicity of the electrospun SNPs / PVA nanofibrous scaffold was evaluated by the contact angle measurements by placing the sample on the instrument of holder Euromex Optical Microscope equipped with a CCD camera. A drop of de-ionized water (0.5 µL) was deposited on the sample surface. The contact angle of the drop of the sample surface was measured at room temperature (25°C), measured contact angle were calculated with the help of "ImageJ tool" software.

4.1 *In Vitro* Drug Release Studies

Experimental method:

In vitro studies of nanoparticles loaded SNPs / PVA nanofibrous scaffolds release were studied in 10 ml phosphate buffer solution (PBS) (Ph 7.4) medium in a rotating incubator at room temperature 36°C at 120 rpm. At regular time intervals, aliquots of 5 ml were replaced with same quantity of PBS. The amount of SNPs / PVA nanofibers drug in releasing media was determined by observing the absorbance at λmax at 428 nm using UV-Visible spectrophotometer. The experiment was carried out at different time intervals and the media was replaced with the 5 mL of absolute buffer solution periodically.

4.2 Antimicrobial efficacy studies

Bacterial activity studies were carried out to ascertain the biological activity of the nanoparticles loaded nanofibrous scaffold(SNPs / PVA) discs in comparison with control only PVA

nanofibrous scaffold without nanoparticles discs (5 mm diameter disc) against pathogenic organisms *Streptococcus pyogenes* strain (ATCC 19615) *E. coli* strain (ATCC 8739). The discs were placed on the nutrient agar media surface using sterilized forceps. The plates were kept at room temperature for 30 mins for solidification and then incubated at 37^{o}C for 24 h. The bacterial inhibition diameter of the Zone of Inhibition (ZOI) was obtained and measured by an "image tool".

5 Results and discussion

The UV-vis spectra show a well-defined surface plasmon band centered at around 428 nm Figure 2, which is the characteristic of SNPs and clearly indicates the formation of SNPs in solution. It may be due to the excitation of surface Plasmon resonance (SPR) effect and reduction of $AgNO_3$ – ions. The synthesised SNPs / PVA electospun fibrous scaffolds were analysed Surface morphological studies observed by field emission scanning electron microscope (FESEM) with accelerating voltage of 15.0 kV to 20.90 kV. The morphology of the electrospun fibers depends on several parameters such as viscosity, distance between the needle tip and collector drum conductivity, applied voltage, diameter of the needle and the flow rate of the polymer solution. Bead free and random nanofibers were obtained from PVA and SNPs / PVA nanofibers, fine nanofibers were obtained for SNPs / PVA nanofibers. The diameter range of PVA nanofibers and SNPs / PVA nanofibers were found to be in the range of 100-285 nm respectively as shown in Figure 3. The dispersed SNPs in PVA was visible as black color spots in the HRTEM image of a single nanofiber of SNPs / PVA as confirmed presenting nanoparticles with an elements were found in energy dispersive of X-ray (EDAX) very low concentration of SNPs were presenting SNPs as shown in Figure 4. The crystallinity of SNPs and SNPs / PVA nanofibers was scanned in the range of 2θ between 10^{o} to 90^{o} by XRD. The SNPs and electrospun nanofibrous scaffold were pressed inside the sample holder and XRD data were collected in the step scan mode. The XRD profiles are shown in Figure 5. five XRD diffraction peaks were observed at 38°, 45°, 67°, 78° and 84° in the 2θ range can be indexed to the (111), (200), (220), (311), (222) reflection planes of cubic structure of metallic SNPs nano powders and the diffraction pattern of SNPs / PVA showed a characteristic peak at 2θ= 19^{o}. The diffraction peaks for SNPs mild peaks were reported at 38°, SNPs / PVA showed only a broad peak stretching between 19^{o}-84^{o} characteristic of crystalline phase after incorporation of SNPs into PVA were reported by many authors [18-21]. The UATR-FTIR spectra of PVA and SNPs / PVA nanofibrous scaffold are shown in Figure 6. The ATR-FTIR revealed strong broad band peak absorbance appearing at 3297cm^{-1} is assigned for O–H stretching vibration indicating the presence of hydroxyl groups, fucoxanthin is a kind of the pigment have reductive properties and released to solution by diffusion rich in hydroxyl groups it could be responsible for the reduction of silver nitrate to SNPs as confirmed from ATR-FTIR

analysis. 2920 cm^{-1} can be assingned as carboxyl group and secondary amines respectively. Peak appearing at 596 cm^{-1} assigned very weak band this indicates that presenting silver nanoparticles from synthesised *Padina boergesenii* extract are secondary metabolites. The TGA graph of PVA and SNPs / PVA nanofiber scaffold are shown in Figure 7 The weight loss below 100^{0}C was due to evaporation of water. The decomposition temperature of PVA was 266^{0}C whereas SNPs / PVA were 240^{0}C. The higher decomposition temperature of PVA shifted to lower temperature in SNPs / PVA nanofibers thus confirming nanoparticles conversion to amorphous form. The contact angle measurements of PVA alone and SNPs/PVA NFs were found to be 35±1^o and 37±0.5^o with de-ionised water, respectively as shown in Figure 8.

The amorphous form of the drug dispersed in the polymer matrix has the benefit of increasing the aqueous solubility and thus the bioavailability of low water soluble drugs. Hence, antibacterial activity result of loaded NPs NF showed significant inhibitory activity of SNPs incorporated nanofibers scaffolds against the tested microorganisms *(E .coli , Streptococcus pyogenes*), as shown in Figure 9. While the PVA nanofibers without SNPs (control) did not show any inhibition of antibacterial activity. The diameter of the zone of inhibition *E .coli* 14 mm and *Streptococcus pyogenes* 22mm diameter, silver ions are released when the SNPs loaded nanofibers were brought in contact with the test bacterial cultures in the petridish plate, which resulted in the formation of zone of inhibition in the SNPs NF steps bacterial growth by inhibiting protein synthesis, specifically it binds in to the 16S rRNA of the bacterial ribosome [22].

In vitro Drug Release Studies, the rate of release of SNPs from the PVA nanofiber scaffold is dependent on two main factors, viz, the thickness of the SNPs / PVA nanofiber, and the rate at which PVA degrades and allows for the optimization of SNPs release within the body. In general drugs can be released in a controlled manner with first order kinetics. In the present study, the feasibility and potential of PVA nanofibrous scaffold as a drug delivery vehicle for SNPs release was investigated. In initial stage, burst release of SNPs / PVA was observed as 20 % at 1/2h. It could be due to the release of loosely bound SNPs / PVA on the surface of the nanofibers. Later, the release was slow and sustained. The maximum cumulative release percentage of SNPs / PVA over into the PBS period of 24 h is 80.5% as shown in Figure 10. These loosely bound SNPs might be released by a mechanism of diffusion through the aqueous pores on the surface created by the water uptake by nanoscaffold immediately after being exposed. At the later stage, the SNPs release was slow, whose rate was determined by the diffusion of PBS into the PVA scaffold, because the release of nanoparticles from SNPs / PVA nanofibers depending upon the diffusion path filled up by PBS. The percentage cumulative SNPs release at the end of day period was 80.5±1.5%. There is no significant difference in the drug release was observed between nanoscaffold containing nanoparticles in the conducted release period.

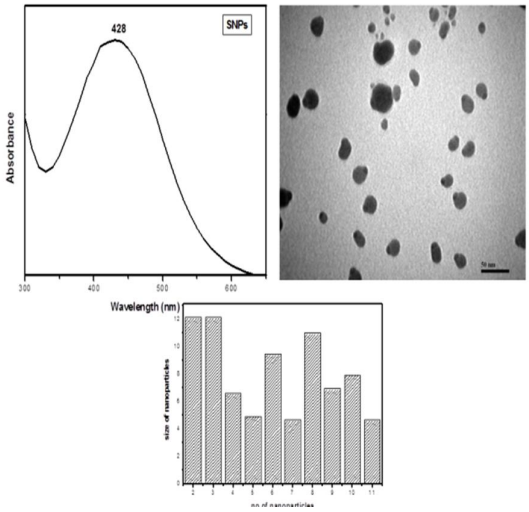

Figure: 2. UV spectrum of SNPs b) Bio-tem images of SNPs c) Bar diagram of SNPs Particle size

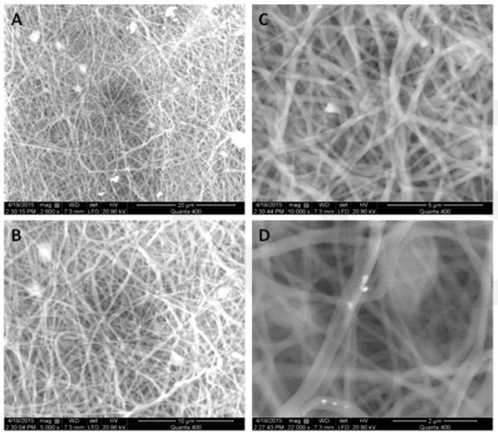

Figure: 3. FE-SEM images of electrospun SNPs/PVA nanofibrous scaffolds (1 Wt %), SNPs loaded (10 Wt %) (PVA) Poly (vinyl alcohol)

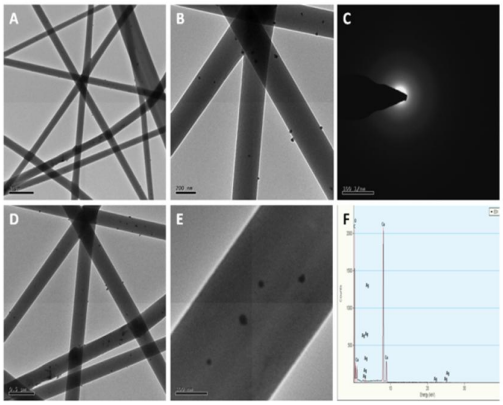

Figure: 4. HRTEM images of electrospun SNPs loaded PVA single nanofiber different scale with EDAX

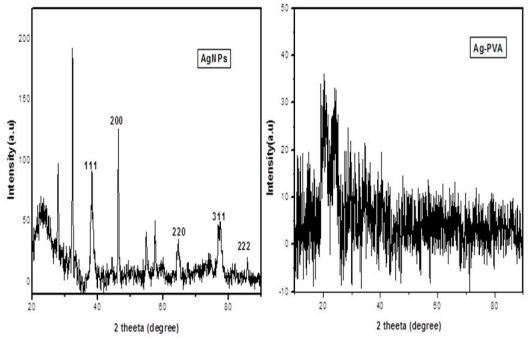

Figure: 5. XRD graph of SNPs and electrospun SNPs/PVA nanofibrous scaffolds

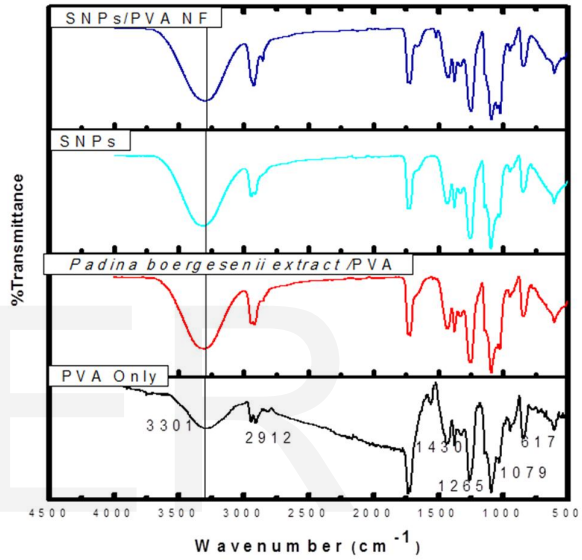

Figure: 6. UATR-FTIR spectroscopy a) PVA b) Padina boergesenii extract with PVA c) SNPs d) SNPs/PVA

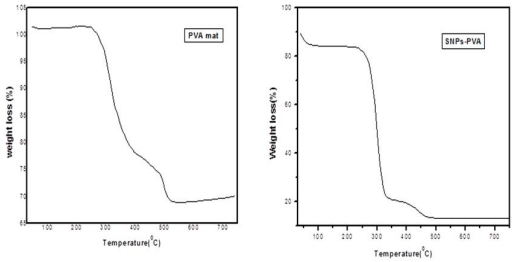

Figure: 7 TGA thermograms of the eletrospun SNPs /PVA nanofibrous scaffolds

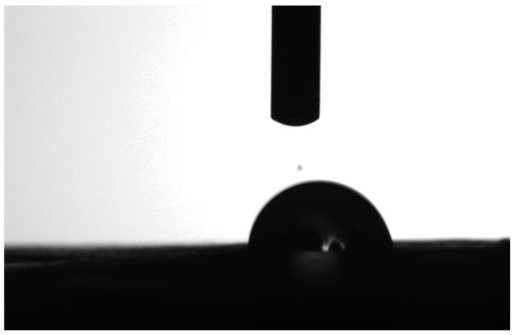

Figure: 8. Contact angle images of electrospun SNPs/PVA nanofibrous scaffolds

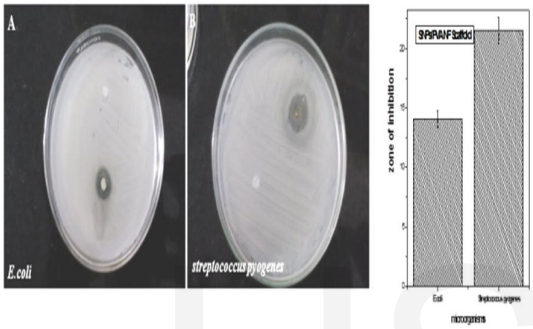

Figure: 9. Zone of inhibition of electrospun SNPs/PVA and without SNPs is control (PVA only)

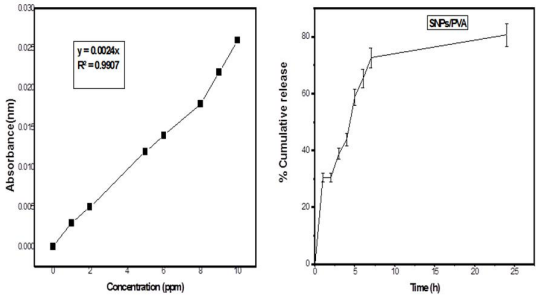

Figure: 10. *In vitro* SNPs release studies from SNPs loaded nanofibrous scaffolds

6 Conclusion

Silver nanoparticles (SNPs) were synthesized by a green route using *Padina boergessinii* brown seaweeds extract and characterized, SNPs incorporated into the PVA polymer and electrospun to developed SNPs / PVA nanofibrous scaffolds. Surface morphological analyses like FESEM, HRTEM revealed the presence of SNPs on the surface of the electrospun nanofibrous scaffolds. UATR-FTIR was analyzed presence of functional groups of fucoidan. PVA became more hydrophilic and biocompatible biodegradable upon very low loading of SNPs. This increase in hydrophilicity led to good for releasing drug delivery process and wound healing for reformation of cell growth and also exhibited promising antibacterial activities against test pathogenic bacterial strains, *E. coli, S. pyogenes.* The nanofibrous scaffold thus developed great potential for biomedical applications as well as the exploring of *In vitro* drug release studies has revealed incorporated SNPs loaded nanofibers released were analyzed UV-Vis spectrophotometer the peak was 428 nm the indicate that was released in a sustained mode over a 24 h period the percentage of cumulative SNPs release at the end of day period was 80.5±1.5%.

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