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PREPARATION, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY ON EVALUATION OF NOVEL POLYVINYL ALCOHOL/NANOCHITOSAN MAT

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ABSTRACT

Chitosan is a versatile polysaccharide derived from chitin that combines a unique set of physicochemical and biological characteristics which allows for a wide range of applications. This study was attempted to develop novel polyvinyl alcohol/nanochitosan mat through electrospinning technique with increased thermal stability and antibacterial activity. Chitosan nanoparticles were prepared by adopting ionic gelation method through crosslinking of chitosan with sodium tripolyphosphate and the particle size of nanochitosan was measured using Dynamic light scattering (DLS) technique. The prepared nanomat was characterized with analytical techniques of Fourier-Transform Infrared spectroscopy (FT-IR), Differential Scanning Calorimeter (DSC), Thermogravimetric analysis (TGA) and Scanning Electron Microscopy (SEM) to study the blending, thermal properties and morphology of nanomat. The antibacterial activity of nanomat was tested with the pathogens Shigella and Staphylococcus aureus.

KEY WORDS
Thermal stability, Antibacterial, Nanochitosan, Morphology

INTRODUCTION:

Electrospinning is a simple and quick technique for producing fibers with nanoscale diameters from a wide range of materials. [1] In this process a high electric field is applied to polymeric solution which is held in a plastic syringe with a capillary needle. Under this applied electrostatic force, the polymer is ejected from the nozzle, whose diameter is reduced significantly as it is transported to and deposited on a collector, which also serves as the ground for the electrical charges.[2] The fiber diameter of the prepared nanomat was influence by the factors such as molecular weight of the polymer, applied electric potential, flow rate, distance between the capillary and collection screen, and needle gauge. [3] As this formed nanofiber mats are exciting new class of material that have been extensively applied in various sectors such as wound dressing, adsorbent, energy storage, protein separation, immobilization, drug delivery, composites due to its high aspect ratio, high porosity and interconnected porous structure. [4]

Chitosan is a natural biodegradable and biocompatible polysaccharide derived from chitin, a substance that develops in the hard outer shells of crustaceans such as crab, crayfish, shrimp.[5] The glucosamine backbone of chitosan contains a high density of amino groups that can be protonated, and thus offer the opportunity to interact with anionic groups on the cell surface causing antibacterial activity against Gram-positive and Gram-negative bacteria which has been exploited in a number of studies.[6] The antimicrobial activity of chitosan is strongly influenced by factors such as the species of bacteria, concentration, pH, solvent, molecular weight and so on.[7,8]

In the current study, chitosan was converted to nanoform to offer unique properties like high surface area-to-volume ratio and small pore sizes. Another interesting polymer used along with nanochitosan was polyvinyl alcohol an excellent fiber forming material possessing high thermal stability with good mechanical properties. [9,10] Since electrospinning of chitosan is a difficult task due to its high viscosity between the ionic groups and generally resulting in beads instead of continuous fibers.

Hence in the present work we aimed to fabricate nanofibrous mat with the novel combination of polyvinyl alcohol, nanochitosan and have been evaluated for its antibacterial activity against the pathogens *Shigella* and *Staphylococcus.aureus.* We have also reported the optimum conditions for mat preparation with operating parameters of applied voltage of 20 kV, solution flow rate of 0.55 mL/h, and the tip to collector distance of 12 cm.

MATERIALS AND METHODS:

Preparation of nanochitosan:

Chitosan was crosslinked with sodium tripolyphosphate to prepare nanoparticles of chitosan. In this ionotropic gelation method, 400 mg of chitosan was dissolved in 35 mL of 2% (v/v) acetic acid prepared using deionized water and the solution was stirred well for 45 min. Then to the prepared chitosan solution, 0.5g of sodium tripolyphosphate dissolved in 10 mL of deionized water was added dropwise with rapid stirring to obtain milky emulsion and the nanochitosan formed was washed several times in deionized water and the particle size of nanochitosan was calculated by Dynamic Light Scattering technique using Malvern Zetasizer (Malvern Instruments, UK) and the average particle size of nanochitosan was found to be 100 nm.

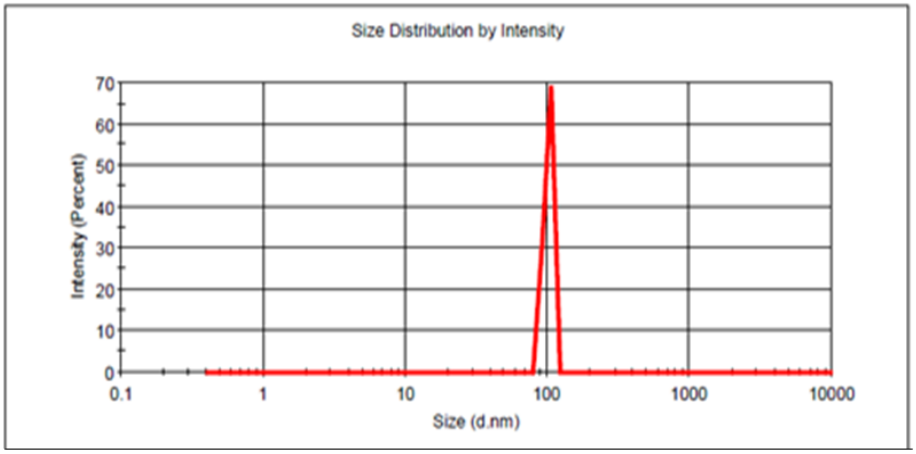

Figure 1: Particle size analysis of Nanochitosan

Fabrication of nanofiber mat using electrospinning setup:

First, the aqueous solution of 5% polyvinyl alcohol was prepared by stirring 5 g of polyvinyl alcohol in 100 mL of deionized water at 60° C for a period of 6 h. To this aqueous PVA solution, the prepared nanochitosan solution was added and stirred continuously for 48 h in magnetic stirrer to get homogeneous polymeric solution. The solution was loaded into 2ml syringe and spinned in ESPIN NANO.

CHARACTERIZATION OF NANOFIBER MAT:

FT-IR spectroscopy:

Shimadzu spectrophotometer was used to record the IR spectra within the range of 4000–400 cm^{-1}. The IR spectra were recorded in solid state using a KBr pellet method.

Differential scanning calorimeter (DSC):

The thermal transition behavior of the prepared mat was examined with Differential scanning calorimeter model Shimadzu DSC-50 (Kyoto, Japan) at a heating rate of 10^0C/min under nitrogen atmosphere at a flow rate of 30 mL/min.

Thermogravimetric Analysis (TGA):

Thermogravimetric analysis was conducted on a SDT Q600 V8.0 Build 95 instrument in the temperature range of 50° to 800°C and the heating rate of 20° K/min in nitrogen atmosphere.

Scanning electron microscopy (SEM):

The surface morphology of novel PVA/NC mat was viewed and photographed by Scanning Electron Microscopy (SEM, JEOL Model JSM-6390LV) in STIC, Kerala.

Antibacterial Activity:

For antibacterial test, disk diffusion method was followed using Mueller–Hinton Agar (MHA) medium for bacterial activity. The prepared bacterial suspension was inoculated on the medium with the sterile cotton tipped swap to form a uniform layer. After solidification of Mueller–Hinton Agar medium, small circular cavities are punctured in the culture agar plates and the sample was placed. The plates were incubated on individual racks for 24 h and after the corresponding time given the inhibition zone was measured in millimeter using a ruler. The samples were analyzed for their antibacterial activity against *Shigella* and *Staphylococcus aureus.*

RESULTS AND DISCUSSIONS:

FT-IR spectroscopy

Fourier transform infrared spectroscopy is an efficient analytical technique that is being used to characterize the functional groups participated in the formation of nanomats. The FT-IR spectral details of polyvinyl alcohol and polyvinyl alcohol/nanochitosan nanofiber mats have been shown in **Figure 2 (a) and (b)** respectively. A broad peak at 3273.20 cm^{-1} is assigned to inter and intramolecular -OH stretching vibration. The bands observed at 2929.87 cm^{-1}, 1726.29 cm^{-1} corresponds to alkyl -CH stretching vibration, carbonyl stretching vibration of remaining acetate groups respectively. [11]

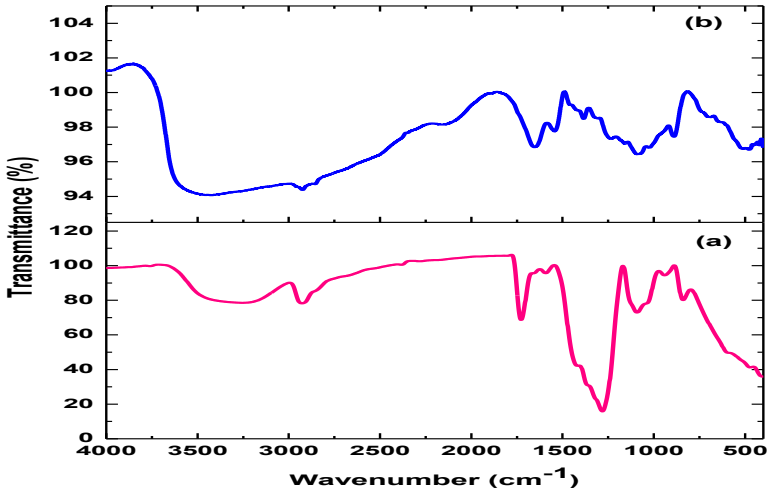

Figure 2: FT-IR spectrum of (a) Polyvinyl alcohol (b) Polyvinyl alcohol/Nanochitosan mat

For polyvinyl alcohol/nanochitosan nanofiber mat the -OH and -NH stretching vibrations is observed at 3456.50 cm^{-1} which is wider attributing to the enhanced intermolecular hydrogen bonding formed between the polymers. The presence of CH stretching attributing to CH_2 and CH_3 group was obtained at 2925.10 cm^{-1}, peak lie at 1384.91 cm^{-1} corresponds to OH stretching (O=P–OH) confirms the presence of nano-chitosan and the band obtained at 1090.76 cm^{-1} is assigned to P-O stretching vibration of nanochitosan.[12] These noticeable peaks for P=O, P-O stretching vibrations indicate the conversion of chitosan in nano range by chemical crosslinking with sodium tripolyphosphate (TPP) was successfully achieved. On compared to pure polyvinyl alcohol the presence of new peaks for P=O, P-O stretching vibration in figure 1(b) confirms the presence of nanochitosan in the binary mat of polyvinyl alcohol/nanochitosan.

Differential scanning calorimeter (DSC)

DSC is widely used thermal analytical tool which helps to understand the thermal behavior of polymers. It provides information such as glass transition temperature (Tg), melting temperature (Tm), crystallization temperature (Tc) values for each process. [13]

Figure 3 show the DSC thermogram of PVA/NC nanomat. From the figure 3 it is evident that the PVA/NC

nanofiber mat shows a broad endothermic event at 106.1°C with a weak peak at 230.4°C indicating the crystallization temperature of binary mat and an exothermic peak was obtained at 205.7°C attributing to the melting of semi-crystalline PVA and nanchitosan phases in the mat. A single glass transition temperature was detected at 160°C indicating a good miscibility between nanochitosan and polyvinyl alcohol. The broadening of the endothermic peak indicates the declination in the –OH content of PVA as these hydroxyl groups are highly interconnected by hydrogen bonding with the nanochitosan network, leading to high glass transition temperature value of 160°C as compared to PVA. A similar trend was observed by Guirguis and his coworkers [14] in which the transition in endothermic and exothermic peaks was obtained with addition of hydroxypropyl cellulose (HPC) in polyvinyl alcohol matrix (PVA).

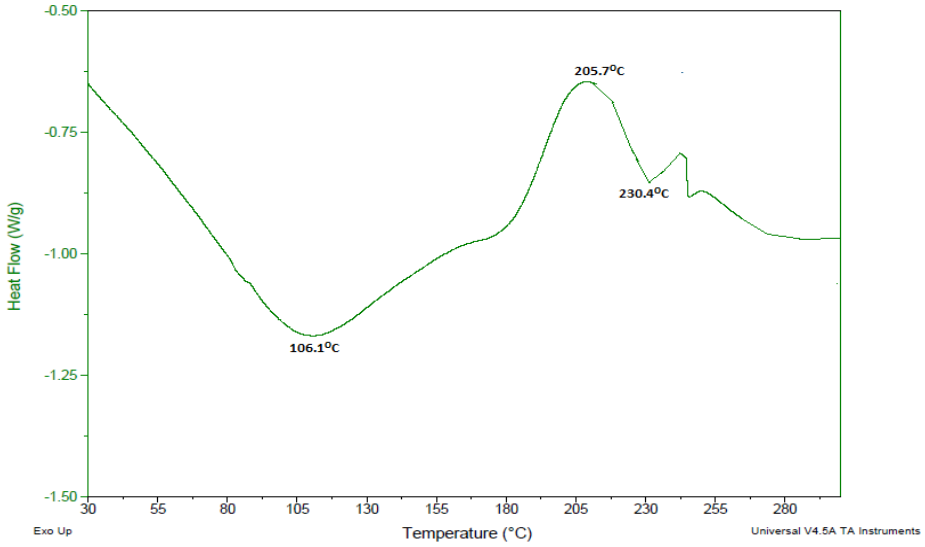

Figure 3: DSC thermogram of PVA/NC nanomat

Thermogravimetric Analysis (TGA)

Thermogravimetric analysis (TGA) is considered as one of the powerful tools generally used to access thermal stability of polymer and its blends. [15] This method was used to measure the change in physical and chemical properties of materials as a function of increasing temperature or time at a constant heating rate.

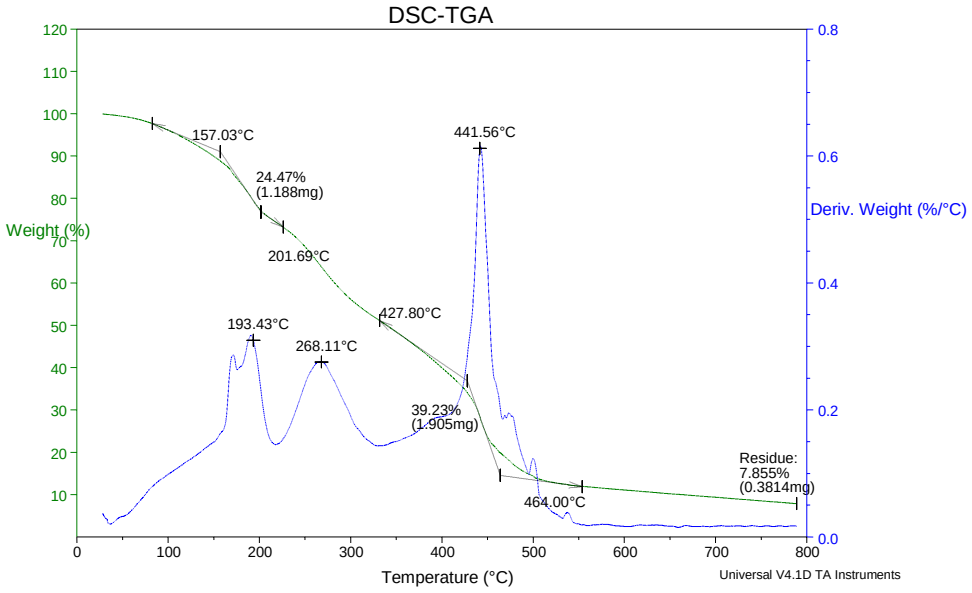

Figure 4:TGA thermogram of PVA/NC nanomat

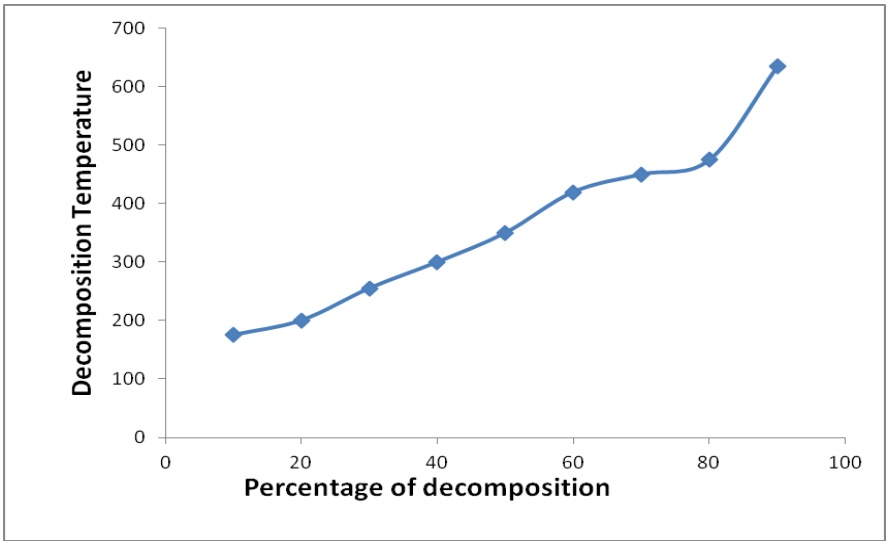

Figure 4a:TGA thermogram details of PVA/NC nanomat

TGA thermal details of PVA/NC were shown in **Figures 4** and **4a.** From the figures it was obvious that the nanofiber mat shows three major weight loses. First stage around 157 °C was due to desorption of volatile water molecule. The second stage corresponds between 201.69 ˚C to 427.80˚C was due to partial thermal decomposition of polymeric backbone of nanochitosan, semicrystalline polyvinyl alcohol and the third stage around 464 °C corresponds to degradation of higher stability crystalline parts of nanochitosan and PVA backbone.[16] At the end of the experiment nearly 7.855% of the sample remained as residue showing the higher thermal stability of the mat.

Scanning Electron Microscopy (SEM)

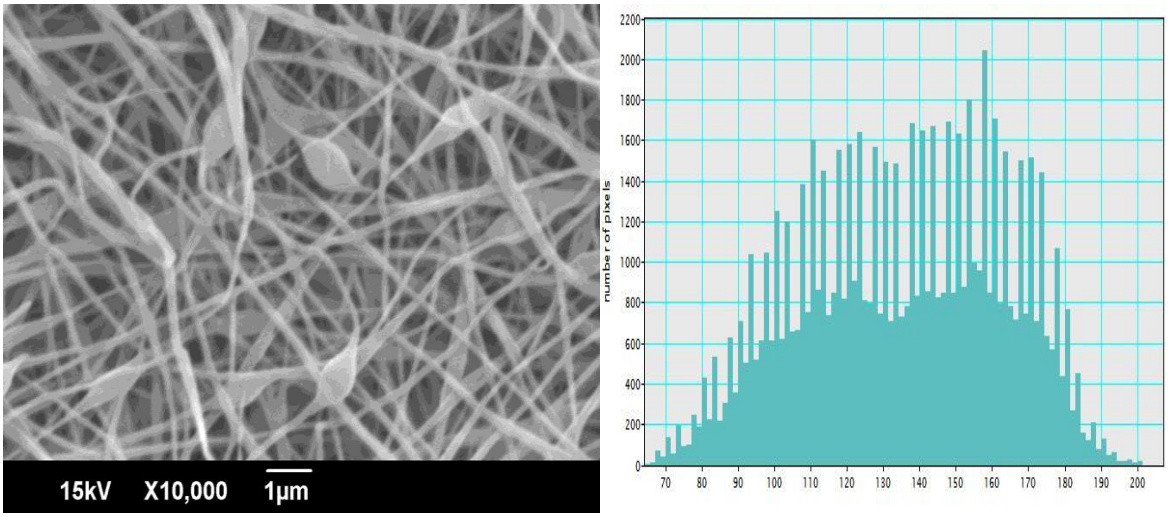

Figure 5: SEM micrograph and fiber diameter distribution graph of PVA/NC nanomat

The SEM micrograph of binary nanomat PVA/NC and its fiber diameter distribution graph were depicted in **Figure 5**. From the surface morphology it is obvious that rather uniformly aligned fibers with very few beads is achieved with the addition of chitosan nanoparticles to polyvinyl alcohol solution with the average fiber diameter of 160 ± 25 nm. Since no fibrous protrusions have been seen in the image indicating the size of chitosan nanoparticles were quite enough to get incorporated inside the fibers rather than trapping in between the fiber layers. As compared to the study conducted by Wu and his coworkers [17] in which the incorporation of intumescent non-halogenated flame-retardant additives in the polyamide 6/nanoclay mat

will show fibrous protrusions indicating the entrapment in between layers rather than inside them.

Antibacterial activity

Chitosan has been explored as an antimicrobial material against a wide range of target organisms like algae, bacteria, yeasts and fungi in tests including *invivo* and *in vitro* interactions with chitosan in different forms (solutions, films and composites).[18] Hence the fabricated nanofibrous polyvinyl alcohol/nanochitosan mat was tested for its bactericidal activity against the pathogens of *Shigella* and *S.aureus* using agar disk diffusion method and is shown in **Figure 6**. According to the standard antibacterial test "SNV 195920-1992", specimens showing more than 1 mm microbial zone inhibition can be considered as good antibacterial agents. Dimethyl sulphoxide (DMSO) was used as control as this nanomat was dissolved in the same solvent and was placed in the plate for further evaluation.

The zone of inhibition values revealed that the prepared mat shows strong antibacterial effect against both gram positive and gram-negative microorganism and the mechanism involved in both the cases were different. In case of gram negative bacteria there arise electrostatic interaction between the polycationic NH_3^+ ions with the outer layer of anionic components present in *shigella* causing cell death with higher value of circular inhibition zone as compared to gram positive bacteria *S.aureus* .[19] In case of gram positive bacteria the mat will adhere on the surface of bacteria and inhibit the nutrient adsorption on the microbial positive-charged cell wall causing the formation of circular zone of inhibition as shown in figure 6.

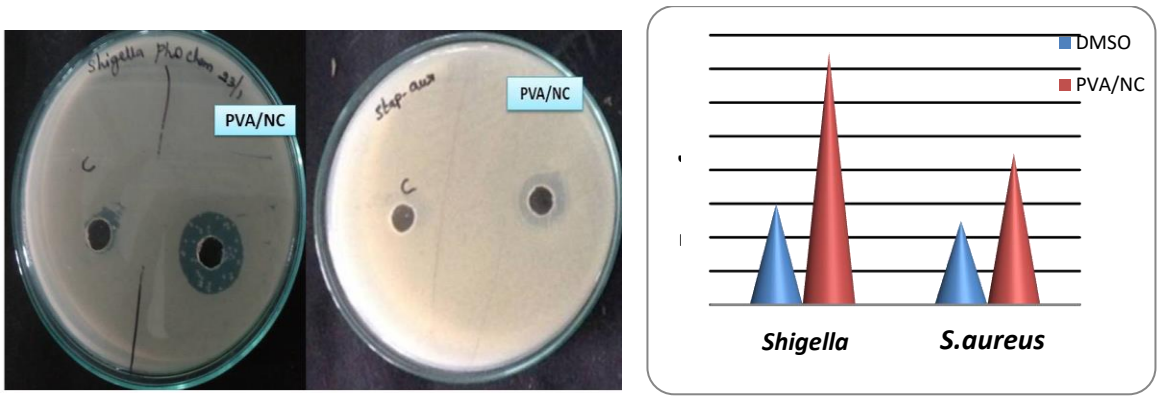

Figure 6: Zones of inhibition tests for PVA/NC nanofiber mat utilizing (a) Gram-negative *Shigella* and Gram-positive *S. aureus* bacteria (b) Graphical representation of inhibition zones

CONCLUSION:

In the present work, nanofiber mat of polyvinyl alcohol/nanochitosan was successfully fabricated by electrospinning technique. Investigation on the properties of electrospun nanomat displayed good miscibility between nanochitosan and polyvinyl alcohol as revealed by the results of FT-IR, excellent thermal stability of the mat was revealed by thermal studies of DSC and TGA which was due to the strong intermolecular hydrogen bonding existing between the amino groups of chitosan nanoparticles and the hydroxyl groups of polyvinyl alcohol. The continuous fiber morphology was evident with SEM results and the antibacterial activity of prepared nanomat was evident from the zone of inhibition values against the tested pathogens.

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