Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(6): 1428-1432
Received: 20-08-2020
Accepted: 09-10-2020

Helen Rani
Department of Nano Science and Technology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Raja K
Department of Nano Science and Technology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Syndhiya R
Department of Nano Science and Technology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Lakshmanan A
Department of Nano Science and Technology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Senthilkumar M
Department of Agricultural Microbiology, AC&RI, Eachankottai, Tamil Nadu, India

Corresponding Author:
Raja K
Department of Nano Science and Technology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Development and characterization of chitosan based polymer composite nanofibre as a smart carrier system for microbial immobilization

Helen Rani, Raja K, Syndhiya R, Lakshmanan A and Senthilkumar M

DOI: https://doi.org/10.22271/phyto.2020.v9.i6u.13147

Abstract
Electrospinning is a versatile and efficient technique which uses high electric field to produce ultrafine fibres from polymers. Nanofibers are widely used in biomedical and tissue engineering as scaffolds, sensors, filters, membranes, batteries, protective clothing, Wound dressing and as a catalyst. In agriculture, it is applied for controlled and targeted release of bioactive compounds. The main aim of this study was to develop E-spun nanofibre using biopolymer that act as a smart carrier system for beneficial bacteria to use it in seed coating. Polymer composite nanofibre was developed using chitosan and polyvinyl alcohol (PVA) by experimenting different ratio of PVA/Chitosan. The e-spin solution containing PVA and chitosan in the ratio of 8:2 was found to be optimum in producing fibre with desired diameter. The developed fibre was further characterized By Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) and Fourier Transform Infra-Red (FTIR) spectroscopy. The electron micrograph images provide the morphological structure of the PVA/Chitosan polymer composite nanofibre (100-150nm). Fourier Transform Infrared Spectroscopic (FTIR) measurement exhibited the interaction and existence of relevant functional groups of both PVA and chitosan in the nanocomposite fibres. This study provides a simple approach for the development of non-wovens that acts as a smart carrier to immobilize microbes which can be used for seed coating.

Keywords: Chitosan, nanocomposite nanofibres, electrospinning, PVA

1. Introduction
Electrospinning is widely used nanotechnology process wherein ultrathin fibres with nano scale dimension are produced from polymer solution. The process includes the application of high voltage electric field to the polymer solution, which forms a droplet. With increase in the electric field, the surface of droplet accumulates the electric charge which leads to increase in electrostatic repulsion accumulation against surface tension of polymer solution at the tip, thereby producing an elongated droplet, known as "Taylor cone". The excess electrical charges cause the polymer solution to stretch, bend and elongate which gets deposited on the ground collector as polymer fibre [10]. The stretching together with evaporation of the solvent renders fibres in nano size [11]. Electrospun fibres show high surface reactivity due to its increased surface area to volume ratio and because of its structural and size variations, the biologically active compounds like volatile organic compounds or microbes can be preserved in these fibres to protect it from the unfavourable environment [4]. The use of electrospun nanofibers in agriculture is comparatively novel and is still in its infancy. It includes encapsulation of plant growth hormones for seed germination [7], smart carrier of agricultural inputs like fertilizer [1], plant protection using pheromone-loaded nanofibers [3], sustained release of bioactive compounds [8] and effective encapsulation of beneficial microbes [2].
Microbial encapsulation for seed coating is widely accepted technique for enhancing the seed vigour and to increase microbial colonies in soil. The drawback in commercial inoculation technique is the exposure of microbes to harsh environment. Protecting the microbes in electrospun fibre is an efficient and reliable technique to increase the survivability of microbes and to maintain its viability. This study aims to develop nanofibre that can be used as a smart carrier to immobilize microbes in nanofibre using biopolymer.
Chitosan is a natural biopolymer obtained from the partial deacetylation of chitin and is used in various fields including agriculture, food science, biotechnology, pharmaceutics, because of its advantageous biological properties. The chitosan is widely used in agriculture for seed treatment due to its nontoxic, biocompatible, and biodegradable properties and it results in increased germination and seedling vigour of crop plants. Chitosan-based molecules are largely used as safe and environmental-friendly tools to ameliorate crop productivity [5] However, high viscosity and rigid chemical structure of chitosan limits its spinning properties

to form pure chitosan nanofibre [6]. Chitosan has better compatibility with PVA as a co-polymer. Also, PVA has excellent fibre forming ability and is highly hydrophilic in nature. The blending of PVA/Chitosan is a possible physical modification to develop chitosan-based nanofiber. The purpose of the study was to optimize the solution and spinning parameters of PVA/Chitosan to develop biopolymer composite nanofibers that can be used for the microbial encapsulation.

2. Materials and method

2.1 Materials used

Partially hydrolysed cold water-soluble poly (vinyl alcohol) (PVA) with molecular weight 1,60,000 kDa was supplied by (HiMedia). Medium molecular weight with 15% degree of acetylation chitosan (CS) and Glacial acetic acid was purchased from Sigma Aldrich Pvt, Ltd.

2.2 Methodology

2.2.1 Optimization of chitosan concentration for nanofibre development

Chitosan solution with varying concentration of 1.0, 1.5, 2.0 and 2.5% w/v were prepared by dissolving medium molecular weight chitosan in 2% acetic acid. The solution was taken in a syringe and subjected to the electrospinning process by applying high voltage current. Spinning parameters like tip to collector distance, voltage, and flow rate were optimized and is shown in Table 1.

Table 1: Chitosan solution and spinning parameters for the development of chitosan nanofibre using acetic acid as the solvent

Chitosan concentration (%)	Solvent concentration	Voltage(kV)	Tip to collector distance(cm)	Flow rate (mL/hr)
1.0,1.5,2.0,2.5	1% Acetic acid	25,27,29,30	14.5 - 16	0.3-0.7
1.0,1.5,2.0,2.5	2% Acetic acid	25,27,29,30	14.5 - 16	0.3
1.0,1.5,2.0,2.5	2% Acetic acid	25,27,29,30	14.5 - 16	0.5
1.0,1.5,2.0,2.5	2% Acetic acid	25,27,29,30	14.5 - 16	0.7

2.2.2 Development of polymer composite nanofibre using PVA and Chitosan

The spin dope solution for the fabrication of polymer composite nanofibre was prepared using PVA and chitosan. 10% (w/v) PVA solution was prepared by dissolving 10 g PVA in 100 mL distilled water at 500 rpm for 2 h at room temperature and 2.5% (w/v) chitosan was prepared by slowly dissolving 0.25 g chitosan in 10 mL 2% acetic acid with a constant stirring at 250 rpm for 6 hrs. The electrospinning solution was prepared by blending different ratios of PVA to chitosan (herein mentioned as CS) in magnetic stirrer. Each ratio was subjected to spinning by applying high voltage. Table 2 shows the ratio of PVA to chitosan with the spinning parameters.

Table 2: Solution and spinning parameters for the development of PVA/Chitosan nanofibre

10% PVA (mL)	2.5% Chitosan (mL)	PVA/CS Ratio	Flow Rate (mL/hr)	Distance (cm)	Voltage (kV)
1	9	1:9	0.5	15	25-28
2	8	2:8	0.5	15	25-28
3	7	3:7	0.3	15	25
4	6	4:6	0.3	15	25
5	5	5:5	0.3	15	25
6	4	6:4	0.3	15	25
7	3	7:3	0.3	15	25
8	2	8:2	0.3	15	25

2.2.3 Scanning Electron Microscope

The morphology and diameter of electrospun nanofibre was characterized in Scanning Electron Microscope (Quanta 250, FEI, and Netherlands). The fibre was placed on the stub using double adhesive carbon tape and coated with conductive gold layer using sputter coater (EDWARDS). The nanofibre were then kept over the aluminium stub and operated at 10-15 kV the topography was observed at different magnifications.

2.2.4 Energy dispersive X-ray spectroscopy (EDAX)

Energy dispersive X-ray spectroscopy is an analytical technique used for the elemental analysis and chemical characterization. EDAX of PVA/Chitosan nanofibres was analysed in FEI QUANTA 250 EDAX while characterizing the fibre for surface morphology.

2.2.5 Transmission Electron Microscope

The TEM FEI Technai Spirit was used to analyze structure of the nanofibre. Copper grid was placed over the horizontal collector and the fibre was projected over the copper grid until the fibre deposition and viewed with a W-source at an ultra-high resolution pole piece at different magnifications.

2.2.6 Fourier Transmission Infrared Spectroscopy (FTIR)

Interaction between the two molecules, PVA and chitosan, was studied using FT/IR-6800 type A equipped with Attenuated Total Reflectant Unit (ATR Pro One) sensor. The spectra of the samples were recorded over a wavenumber range of 400-4000 cm^{-1} at a resolution of 4 cm^{-1} and a total of 64 scans were accumulated for the signal-averaging of each IR spectral measurement.

3. Results and Discussion

3.1 Development of electrospun nanofibre

3.1.1 Electrospinning of chitosan solution

Chitosan solution at various concentrations (1, 1.5, 2 & 2.5%) was prepared using acetic acid as a solvent. The solubility of chitosan at 1% acetic acid was less and the viscosity of the chitosan solution at 2 and 2.5 % was high resulting in the solution to held back in the syringe, while 1 and 1.5% solution resulted in electrospraying. Further, 1, 1.5, 2 & 2.5% chitosan solution prepared by increasing the concentration of solvent to 2 % acetic acid produced fibre with round beads with the spinning parameter set to 0.3mL/hr flowrate, 16.5 cm collector distance and applied voltage of 24 kV. Further increasing the flow rate to 0.5 mL/hr and voltage to 25kV and decreasing the collector distance to 14.5 cm produced fibre

with numerous spindle shaped beads at 2.5% chitosan concentration. The other parameters resulted in tip drying, electrospraying and leakage depending upon the viscosity and conductivity of the solution. The repulsive forces between ionic groups within the polymer backbone during electrospinning due to the application of a high electrical field restrict the formation of continuous fibres and often produce beads.

In electrospinning, solution viscosity is one of the most challenging parameters affecting the formation of fibre. The chitosan solution viscosity was influenced tremendously by molecular weight, concentration, and solvent characteristics. Solution viscosity in turn effects on fibre diameter and morphological structure. On the other hand, solvents play a key role in the electrospinning process by influencing the surface tension of the solution. Nozzle tip to collector distance affects jet flight time and electric field strength. Blending natural polymer with synthetic polymers like poly (ethylene oxide) (PEO), poly (vinyl alcohol) (PVA), poly (lactic acid) (PLA) and poly (caprolactone) (PCL) can enhance its mechanical properties [9, 12]. Therefore, the formation of nanofibers is facilitated by blending chitosan with co-polymers poly (vinyl alcohol) (PVA).

3.1.2 Electrospinning of PVA and chitosan composite nanofibre

PVA was used as an additive polymer due to its nontoxicity, biodegradability, biocompatibility, and possibility of making hydrogen bonds with chitosan. Electrospinning of PVA/ Chitosan nanofibre was performed at different ratios and different spinning parameters as mentioned in Table 2. Among the different ratio attempted, PVA to chitosan at 8:2 produced smooth and homogenous fibre with diameter ranges from 120nm to 150nm. Increase in the chitosan concentration, larger fibre diameters were found. The fibre development at 8:2 ratio could be due to the perfect bonding between PVA and chitosan functional groups at that concentration. A large number of beads were found in remaining ratios of (PVA/Chitosan) samples because of increased surface tension of solution owing to higher concentration of chitosan resulting in strong polyelectrolyte effect that causes an impulsion between chains and leads to breakage of physical bonds between the two polymers.

3.2 Characterization of developed nanofibre

3.2.1 SEM analysis of Chitosan e-spun fibre

The SEM image of electrospun chitosan fibre at different concentration from 1%-2.5% dissolved in 2% acetic acid is given in figure 1. The image (Fig 1.A&B) confirms the spraying of polymer solution at 1% and 1.5% concentration of chitosan solution while at 2% elongation of sprayed particles occurred (Fig 1.C). Further increasing the concentration to 2.5%, fibres were produced with large number of beads (Fig 1.D).

3.2.2 SEM analysis of polymer composite nanofibre

The SEM images of composite nanofibre with various ratio of PVA/chitosan as mentioned in Table 2 is provided in Fig 2. The images show that the increase in chitosan concentration to PVA ratio increases the number of beads that are round in shape, while increasing the PVA concentration with respect to chitosan concentration, elongated spindle fibre were produced indicating the stretching of polymer to form fiber. Fig 2H confirms the development of uniform bead free smooth fibre at PVA/Chitosan ratio of 8:2 with the average diameter ranging from 150-200 nm.

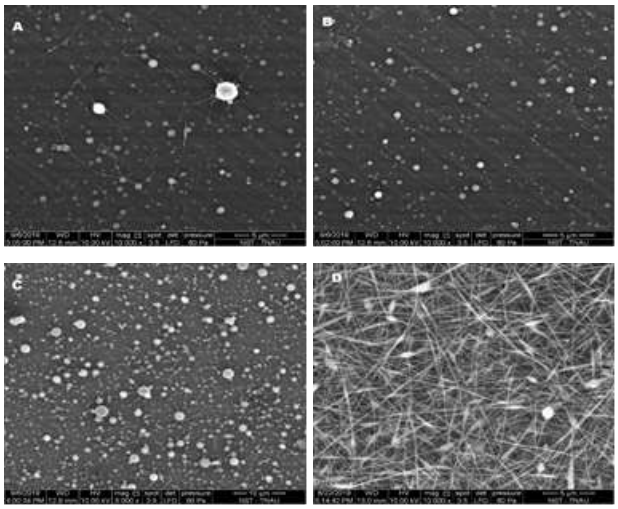

Fig 1: SEM image of electrospun chitosan fibre prepared at different concentrations (A) 1% (B) 1.5% (C) 2% (D) 2.5% using 2% acetic acid as solvent. Number of beads (Fig 1.D).

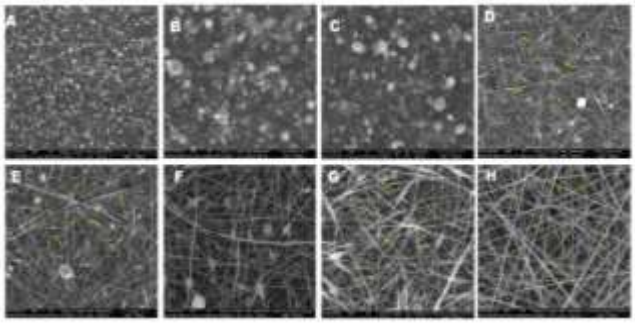

Fig 2: SEM image of PVA/Chitosan composite nanofibre prepared at different ratios of PVA to chitosan such as 1:9 (A) 2:8 (B) 3:7 (C) 4:6 (D) 5:5 (E) 6:4 (F)7:3 (G) 8:2 (H).

3.2.3 EDAX analysis of polymer composite nanofibre

The result of SEM-EDAX analysis is given in the form of graph below (Fig.3). The elemental composition of C, O indicates the presence of PVA, and N indicates the presence of chitosan in the PVA/Chitosan composite nanofibre.

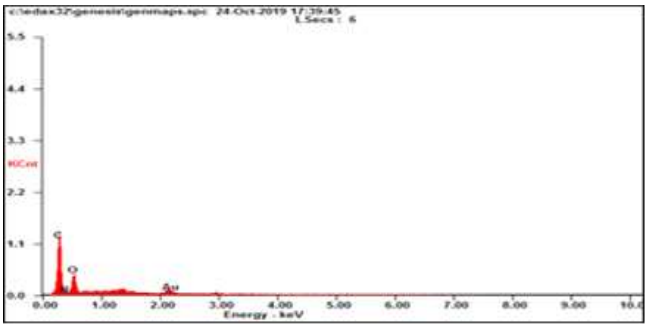

Fig 3: EDAX spectra of PVA/Chitosan blended composite nanofibre

3.2.4 TEM analysis of polymer composite nanofibre

The spinning parameters and SEM image revealed the development of smooth fibre at the PVA/Chitosan ratio 8:2. Hence, the developed fibre at this optimum ratio was further analysed in TEM for the confirmation of size and morphology. The TEM image exhibited the fibre diameter 195 to 295nm (Fig. 4A&B)

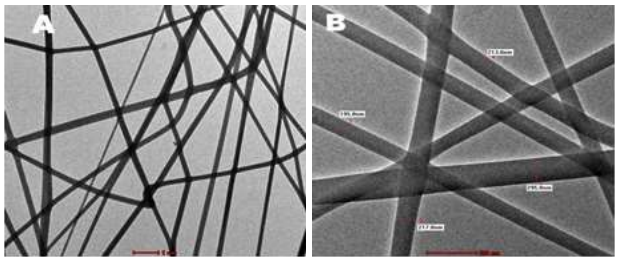

Fig 4: (A) & (B) TEM image of 8:2 ratio PVA/Chitosan composite nanofibre

3.2.5 FTIR analysis of nanofibre

The pure PVA registered the peaks at 1089 cm^{-1}, 841cm^{-1}, 1714 cm^{-1}, 2341cm^{-1}, 3292cm^{-1} due to C-O stretch, C-C stretch, C=O stretch, C-H stretch and O-H stretch, respectively. The spectrum of pure chitosan shows transmittance peak around 1021cm $^{-1}$ to 3291cm $^{-1}$ corresponding to the polysaccharide structures wherein the sharp peak at 2333 cm^{-1},1021cm^{-1}, 3291 cm^{-1}, 2854 cm^{-1} are due to C-H stretch, C-O stretch, N-H stretch, CH_3 symmetrical stretch, respectively. The FTIR spectra of the developed composite nanofibre confirmed the presence of both PVA and chitosan in the fibre by exhibiting the characteristics peaks of polymers at 1087cm^{-1}, 3309 cm^{-1} and 2379 cm^{-1} due to C-O stretch, N-H stretch, and C-H stretch, respectively.

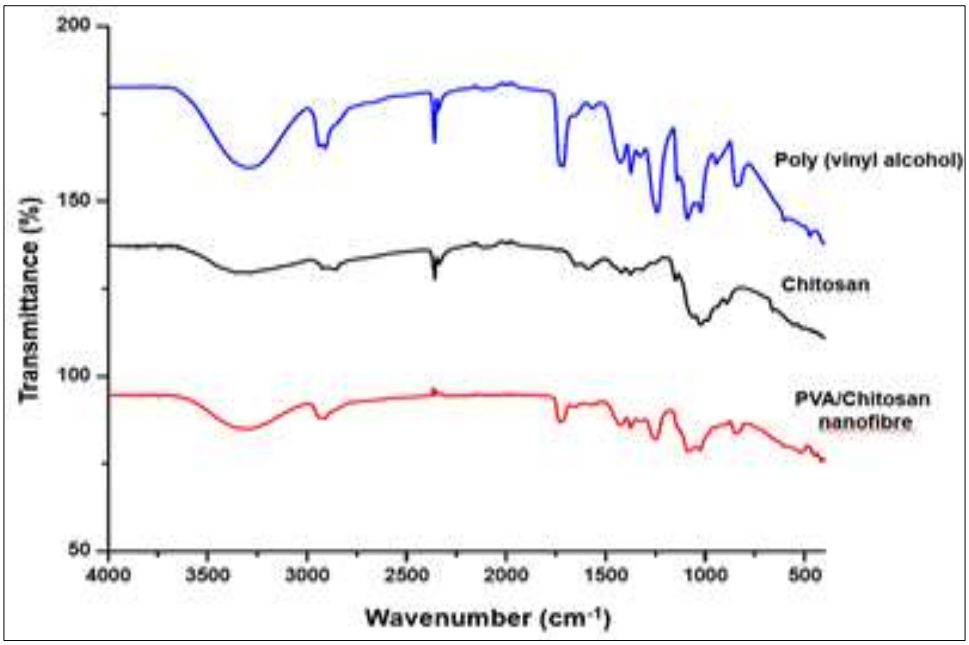

Fig 5: FT-IR spectra of pure PVA, chitosan and PVA/Chitosan composite nanofibres

4. Conclusion

Bead-free chitosan polyvinyl alcohol composite nanofibers at 8:2 ratio were obtained by electrospinning technique. The results demonstrated the successful production of the nanofibers. The composite nanofibres can be used as a carrier for the bioactive compounds and for the encapsulation of beneficial microorganism for sustained release.

Abbreviation

PVA-poly vinyl alcohol
CS-Chitosan
SEM- Scanning Electron Microscope
TEM- Transmission Electron Microscope
EDAX- Energy- Dispersive X-ray Spectroscopy
FT-IR – Fourier Transform Infrared Spectroscopy

5. Acknowledgment

Authors are thankful to Tamil Nadu Agricultural University, Coimbatore-641003, Tamilnadu, India.

*Research Guide or Chairperson of Research: Dr Raja Kalimuthu

University: Tamil Nadu Agricultural University, Coimbatore-641003

Research project name: M. Sc Thesis.

Study area/sample collection: Department of Nanoscience and Technology, Tamil Nadu Agricultural University, Coimbatore-641003, Tamil Nadu

6. References

1. Azarian MH, Kamil Mahmood WA, Kwok E, Bt Wan Fathilah WF, Binti Ibrahim NF. Nanoencapsulation of intercalated montmorillonite-urea within PVA nanofibers: Hydrogel fertilizer nanocomposite. Journal of Applied Polymer Science 2018;135(10):45957.
2. De Gregorio PR, Michavila G, Ricciardi Muller L, de Souza Borges C, Pomares MF, Saccol de Sá EL *et al*. Beneficial rhizobacteria immobilized in nanofibers for potential application as soybean seed bioinoculants. Plos one 2017;12(5):0176930.
3. Kannan M, Elango Kolanthasamy. Nanotechnological approaches in plant protection 2020, 349-364.
4. Liu P, Wei YN, Cui S, Wei HY, Bu JL, Ni J *et al*. Fabrication of mesoporous TiVN powders and their electrochemical performance. Journal of the Ceramic Society of Japan 2019;127(10):728-735.
5. Malerba M, Cerana R. Recent applications of chitin-and chitosan-based polymers in plants. Polymers 2019;11(5):839.
6. Nasikhudin Diantoro M, Kusumaatmaja A, Triyana K. Preparation of PVA/Chitosan/TiO2 nanofibers using

electrospinning method. In AIP Conference Proceedings 2016;1755(1):150002. AIP Publishing LLC.
7. Raja K, Prabhu C, Subramanian KS, Govindaraju K. Electrospun polyvinyl alcohol (PVA) nanofibers as carriers for hormones (IAA and GA3) delivery in seed invigoration for enhancing germination and seedling vigor of agricultural crops (groundnut and black gram). Polymer Bulletin 2020, 1-12.
8. Ranjan S, Chandrasekaran R, Paliyath G, Lim LT, Subramanian J. Effect of hexanal loaded electrospun fibre in fruit packaging to enhance the post-harvest quality of peach. Food Packaging and Shelf Life 2020;23:100447.
9. Thien DVH. Electrospun chitosan/PVA nanofibers for drug delivery. Vietnam Journal of Science and Technology 2016;54(4B):185.
10. Wen P, Zong MH, Linhardt RJ, Feng K, Wu H. Electrospinning: A novel nano-encapsulation approach for bioactive compounds. Trends in Food Science & Technology 2017;70:56-68.
11. Xue J, Wu T, Dai Y, Xia Y. Electrospinning and electrospun nanofibers: methods, materials, and applications. Chemical reviews 2019;119(8):5298-5415.
12. Yousefi Abdolmaleki A, Zilouei H, Nouri Khorasani S, Abdolmaleki A. Optimization and characterization of electrospun chitosan/poly (vinyl alcohol) nanofibers as a phenol adsorbent via response surface methodology. Polymers for Advanced Technologies 2017;28(12):1872-1878.