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Fabrication and Characterization of PVA/Chitosan Composite Nanofiber for Encapsulation of Active Ingredients

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ABSTRACT: Electrospinning is a versatile technique used to produce nanofibers from polymer solution to encapsulate the active ingredients. It is hard to fabricate chitosan nanofiber, because high viscosity of chitosan solution hinders the fabrication process. To overcome this difficulty, poly vinyl alcohol (PVA) was blended with chitosan (CS) to lower the solution viscosity and produce composite nanofiber by electrospinning method. Different concentrations of PVA and CS were tested for optimum nanofiber formation. Fabricated nanofiber was characterized by using FTIR, Scanning electron microscope (SEM), Transmission electron microscope (TEM) and Energy Dispersive X-Ray Analysis (EDAX). The results confirmed that the fine nanofiber formation was perfect without beads from PVA/CS composite at a ratio of 9:1. FTIR analysis of nanofiber confirmed the presence of characteristics peaks of PVA and CS at 2934 cm^{-1} (C-H stretching) and 1550 cm^{-1} (N-H bending). SEM and TEM observation showed the smooth surface and smaller diameter of nanofiber which are important parameters for nano carriers. The concentration of chitosan plays a significant role in minimizing the beads in the fiber. The successfully fabricated PVA/CS composite nanofiber has wide applications in biomedical and agricultural field for smart and targeted delivery of active ingredients.

Keywords: Electrospinning, PVA, Chitosan, nanofiber, encapsulation.

INTRODUCTION

Electrospinning is an unique technique used to produce nanofibers of various diameter ranging from few nanometers (nm) to 500nm by stretching the polymer solution under electrostatic force. During the electrospinning, the applied voltage reduces the surface tension of polymer droplet at tip of the needle and stretches the droplet into nanofiber, which was dried and collected on the collector (Haider *et al.*, 2018). The excess charge applied to the polymer solution, stretches together with solvent evaporation and grounded to nanofiber (Xue *et al.*, 2019). The physical properties of nanofiber are high surface to volume ratio, nanometer in diameter, high porosity, high aspect ratio, fine surface morphology, high alignment and thermal stability (Khan *et al.*, 2013). Those properties of nanofiber lead to its wide application in agriculture field, batteries, biomedical applications, tissue engineering and protective clothing (Farias *et al.*, 2019). In agriculture, nanofiber used for the smart delivery of pesticides, fertilizers (Azarian *et al.*, 2018), growth enhancing hormones (Raja *et al.*, 2020), pest tricking pheromones (Kannan and Kolanthasamy, 2020), beneficial microbes (Gregorio *et al.*, 2017) and effective encapsulation of bioactive molecules (Rajan *et al.*, 2020). The nanofiber encapsulation technology is an alternate for conventional agrochemicals often causing fast evaporation, runoff, leaching and frequent reapplication (Khandelwal *et al.*, 2016).

There are a wide range of polymers used in the electrospinning process for fabrication of nanofibers. Natural, synthetic or composite of both can be used for producing nanofibers (Bhardwaj and Kundu, 2010). The natural polymers are widely used for its biocompatibility and biodegradability than synthetic

polymers in biomedical and agricultural field. Chitin is second most plentiful natural polysaccharide made by crustaceans. Chitin is insoluble in common solvents, so it is deacetylated to chitosan and readily soluble in acidic, neutral and alkaline solution. Chitosan is used commonly in various fields such as agriculture, biomedical, biotechnology, food science and pharmaceuticals for its beneficial biological properties. The properties of chitosan are nontoxicity, biodegradability and biocompatibility, which brands it as safe ecofriendly tool in seed invigoration to enhance crop productivity. Pure chitosan does not form nanofiber due to its high viscosity even in very low concentration (Abdelgawad *et al.,* 2014. and Khan *et al.,* 2013). To avoid this problem, chitosan is blended with other polymers to enhance its nanofiber forming characteristics. PVA is one among that polymer for blending with chitosan, which is nontoxic, biodegradable and biocompatible. PVA is an anionic polymer which mixed with chitosan cationic polymer to improve the fiber forming character by reducing crystallinity of chitosan (Li and Hsieh, 2006). Several optimizing experiments should be established to fine tune the smooth nanofiber fabrication by using PVA/CS composite polymers.

Many studies have been carried out on nanofiber fabrication, based on PVA/CS composite polymers (Sajeev *et al.,* 2008 and Desai *et al.,* 2008). The purpose of this study is to optimize the different concentration ratios of PVA and CS for fabricating composite nanofiber and investigated for its morphology, diameter, elemental composition and presence of functional group through SEM, TEM, EDAX and FTIR. The composite nanofiber can be used in the encapsulation of various agricultural inputs.

MATERIAL AND METHODS

A. Materials

Partially hydrolyzed PVA (Molecular weight 1,60,000 kDa) purchased from HiMedia, medium molecular weight Chitosan and glacial acetic acid obtained from Sigma-Aldrich.

B. Method

(i) Optimization of chitosan nanofiber. The different percentage (2.5 and 3) of chitosan solution was prepared by dissolving low molecular weight chitosan (Mol.wgt- 50,000-190,000 Da) in 1% acetic acid. The solution was stirred for 48 hours, to the complete dissolution of chitosan in the 1% of acetic acid. The solution was filled in the syringe for nanofiber formation using electrospinning unit. The parameters were set as voltage-17kV, tip to collector distance-15cm and flow rate-0.5ml/hr.

(ii) Development of PVA/CS composite nanofiber. PVA 10% solution was prepared by dissolving in distilled water and stirred at 300rpm @ 70^{o}C until complete dissolution. Chitosan 2% prepared by dissolving chitosan in 2% acetic acid and stirred continuously at 200rpm until fine dissolution. The different ratios of PVA and CS (5:5, 6:4, 7:3, 8:2 and 9:1) were blended to acquire homogenous polymer solution and filled in syringe equipped with metal needle. The metal needle was supplied with high voltage and collector was connected with cathode. The spinning parameters were set as follows: flow rate,0.5 mL hr^{-1}; tip to collector distance, 15cm and Voltage, 17kV. After electrospinning, the fabricated nanofiber was stored and characterized with FTIR, SEM and TEM.

(iii) Surface morphology of nanofiber. The surface morphology and diameter of composite nanofiber was analyzed by Scanning Electron Microscope (Quanta 250, FEI, Netherland). The nanofiber was placed on carbon tape sticked on aluminium stub and sputter coated with gold to make sample surface conductive. Then the sample was analyzed under different kV at different magnification.

(iv) Transmission Electron Microscope & EDAX. The structure of nanofiber was analyzed by using TEM FEI Technai Spirit. The copper grid was sticked over the collector and the nanofiber was deposited on grid. Then the fabricated nanofiber was viewed under W source at different magnifications. The elemental composition of nanofiber was analyzed by TEM-EDAX, for the confirmation of PVA and chitosan groups.

(v) Functional group analysis of composite nanofiber. Fourier Transform Infrared (FTIR) spectroscopy analysis was used to examine functional groups of PVA and chitosan present in the nanofiber. This study was done by using FT/IR-6800 type A (M/s. Jasco, Japan) equipped with the sensor Attenuated Total Reflectant Unit (ATR Pro One). The spectral scan of sample was recorded from 400-4000 cm^{-1} at 4 cm^{-1} resolutions by using TGS detector.

RESULT AND DISCUSSION

A. Development of nanofiber

The different concentrations (2.5 and 3%) of chitosan solution were prepared by dissolving in acetic acid. The parameters were set as i.e., voltage – 17kV, tip to collector distance – 15cm and flow rate – 0.5ml/hr. The electrospinning of different concentrations of chitosan creates no fiber formations. The flow rate was also adjusted by modifying voltage and related parameters, but resulted in round bead formation due to the high viscosity of the chitosan solution. The conductance and viscosity of the solution decide the morphology of nanofiber and thereby reducing the bead formation. The higher concentration of polymer requires higher voltage for the reduction of surface tension and stretching of polymer into nanofiber (Kusumastuti *et al.,* 2016). To reduce the viscosity and surface tension of natural polymer, another polymer (PVA) was blended with chitosan at different ratios (5:5, 6:4, 7:3, 8:2 and 9:1) and the spinning was done with electrospinning. While evaluating varying concentrations of PVA and CS, 9:1 ratio produced beadless smooth nanofiber due to perfect binding between the functional molecules of the polymers. In other ratios of PVA/CS, beaded nanofiber structures were produced due to lack of physical bonding between them.

B. Characterization of nanofiber

(i) Scanning Electron Microscope. According to the SEM analysis, the bead less nanofiber was formed at

the 9:1 ratio of PVA/CS polymer. Fig. 1c-1g showed that the bead in the nanofiber was reduced gradually from PVA/CS ratio 5:5 to 9:1.

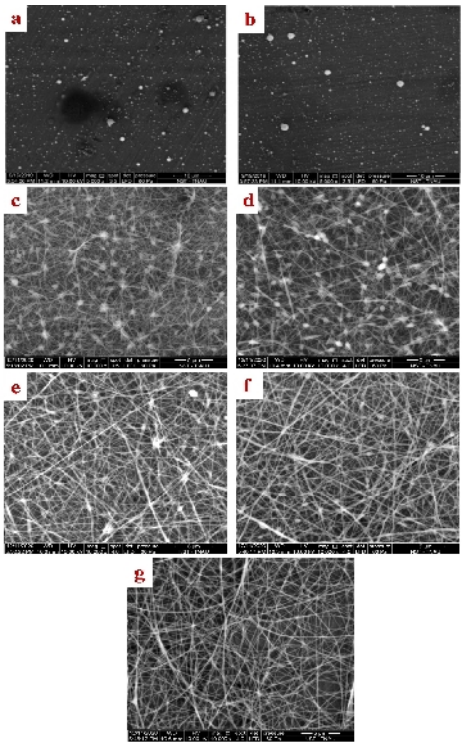

Fig. 1. SEM analysis of nanofiber (a & b) Chitosan concentration at 2.5 & 3%. PVA/CS different ratios (c) 5:5, (d) 6:4, (e) 7:3, (f) 8:2, (e) 9:1.

The reduction of bead in the nanofiber was due to the decreased surface tension and increased conductivity of polymer after blending of polymer as composite. Applying high voltage provides uniform charge distribution over the solution, which creates repulsion of ionic groups between polymers. This repulsion leads to the continuous nanofiber formation (Nokhasteh *et al.*, 2020). This repulsion was absent in the chitosan solutions at concentrations of 2.5 and 3%. The surface tension was higher in this concentration leading to the uneven distribution of charge among the chitosan solution and resulted in lagging of jet formation at the tip of needle. Fig. 1a-b showed the presence of only small beaded structures on the collector and no fiber was formed.

(ii) Transmission Electron Microscope & EDAX. The nanofiber formed at 9:1 ratio of PVA/CS was characterized with TEM and the image showed the smooth morphology of composite nanofiber. The result showed that the optimum concertation for the fine nanofiber formation was 9:1 ratio PVA/CS. The average diameter of the nanofiber was 120 nm (Fig. 2a & b). Similar TEM results were reported by Helen *et al.*, (2020), in which they found that PVA/CS composite nanofiber found to have 195 to 295 nm diameter. TEM-EDAX confirmed the presence of PVA/CS groups in the nanofiber (Fig. 2c). The presence of C and O group represents PVA and N group due to chitosan amide in the nanofiber.

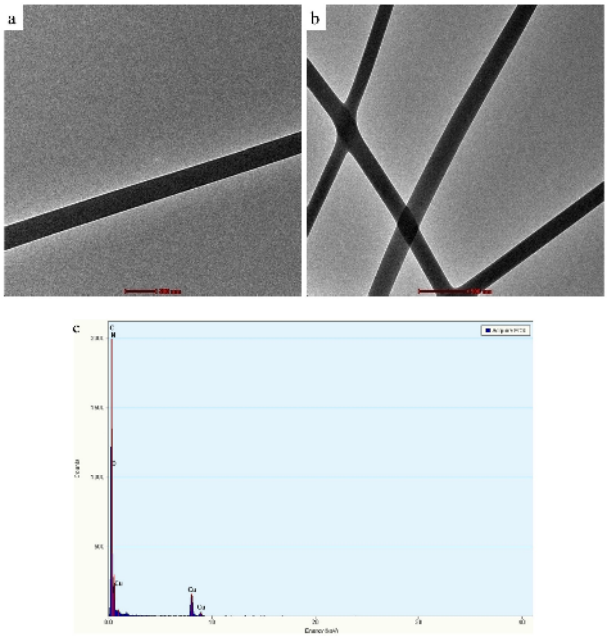

Fig. 2: (a, b) TEM analysis of 9:1 ratio PVA/CS nanofiber, (c) EDAX of composite nanofiber.

(iii) FTIR analysis of PVA/CS nanofiber. The FTIR analysis was carried out to characterize the functional groups of PVA and CS in the composite nanofiber. Fig. 3 shows the FTIR spectra of PVA, CS and 9:1 ratio PVA/CS composite nanofiber. The characteristics peaks of PVA were 3319 cm^{-1} (O-H stretch), 2918 cm^{-1} (C-H stretching) and 1075 cm^{-1} (C-O stretching). FTIR spectra of chitosan shows peaks at 1647 cm^{-1} and 1587 cm^{-1} due to C=O stretching and N-H bending. The PVA/CS composite nanofiber has PVA characteristic peaks at 3334 cm^{-1} (O-H stretch), 2934 cm^{-1} (C-H stretching) and 1090 cm^{-1} (C-O stretching), and also

chitosan characteristic peaks at 1649 cm^{-1} and 1550 cm^{-1} due to C=O stretching and N-H bending. The FTIR result revealed the presence of PVA and CS functional groups in the composite nanofiber. Similar, FTIR result was showed by Kuo *et al.*, (2017), in which they confirmed the existence of characteristic peaks of PVA at 1080 cm^{-1} (C–O stretching), 2925 cm^{-1} (C–H stretching) and 3273 cm^{-1} (O–H stretching), and also found chitosan characteristic peaks at 1661 cm^{-1} and 1584 cm^{-1} due to (C=O stretching) and (N–H bending) in PVA/CS composite nanofiber.

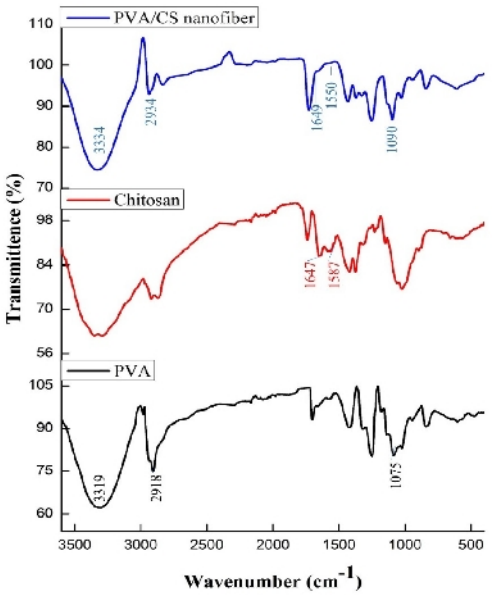

Fig. 3. FTIR spectra of PVA, CS and PVA/CS nanofiber.

CONCLUSION

The present study fabricated PVA/CS composite nanofiber by electrospinning technique. SEM image showed that increasing concentration of chitosan in polymer composite formed the beads in nanofiber and fine smooth nanofiber formed at lower concentration of CS with PVA. The result showed that 9:1 ratio of PVA/CS resulted in smooth and beadless nanofiber fabrication. EDAX and FTIR analysis confirmed the presence of principle elements and functional groups of PVA and CS in composite nanofiber. These results together demonstrate that the PVA/CS nanofiber has the potential and flexibility as an effective carrier for sustainable target delivery of active ingredients like drugs, nutrients, pesticides, fungicides, and nematicides. Further studies could bring out more insights on loading of multiple functional molecules and their release pattern from this ecofriendly biodegradable composite nanofiber.

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Conflict of interest. None.

REFERENCES

Abdelgawad, A. M., Hudson, S. M., & Rojas, O. J. (2014). Antimicrobial wound dressing nanofiber mats from multicomponent (chitosan/silver-NPs/polyvinyl alcohol) systems. *Carbohydrate polymers*, *100*: 166-178.

Azarian, M. H., Kamil Mahmood, W. A., Kwok, E., Bt Wan Fathilah, W. F., & Binti Ibrahim, N. F. (2018). Nanoencapsulation of intercalated montmorillonite-urea within PVA nanofibers: Hydrogel fertilizer nanocomposite. *Journal of Applied Polymer Science*, *135*(10): 45957.

Bhardwaj, N., & Kundu, S. C. (2010). Electrospinning: a fascinating fiber fabrication technique. *Biotechnology advances*, *28*(3): 325-347.

De Gregorio, P. R., Michavila, G., Ricciardi Muller, L., de Souza Borges, C., Pomares, M. F., Saccol de Sá, E. L., & Vincent, P. A. (2017). Beneficial rhizobacteria immobilized in nanofibers for potential application as soybean seed bioinoculants. *Plos one*, *12*(5): e0176930.

Desai, K., & Kit, K. (2008). Effect of spinning temperature and blend ratios on electrospun chitosan/poly (acrylamide) blends fibers. *Polymer*, *49*(19), 4046-4050.

Farias, B. V., Pirzada, T., Mathew, R., Sit, T. L., Opperman, C., & Khan, S. A. (2019). Electrospun polymer nanofibers as seed coatings for crop protection. *ACS Sustainable Chemistry & Engineering*, *7*(24): 19848-19856.

Haider, A., Haider, S., & Kang, I. K. (2018). A comprehensive review summarizing the effect of electrospinning parameters and potential applications of nanofibers in biomedical and biotechnology. *Arabian Journal of Chemistry*, *11*(8): 1165-1188.

Kannan, M., Elango, K., & Govindaraju, K. Nanotechnological Approaches in Plant Protection.

Khan, W. S., Asmatulu, R., Ceylan, M., & Jabbarnia, A. (2013). Recent progress on conventional and non-conventional electrospinning processes. *Fibers and Polymers*, *14*(8): 1235-1247.

Khandelwal, N., Barbole, R. S., Banerjee, S. S., Chate, G. P., Biradar, A. V., Khandare, J. J., & Giri, A. P. (2016). Budding trends in integrated pest management using advanced micro-and nano-materials: Challenges and perspectives. *Journal of environmental management*, *184*: 157-169.

Kuo, T. Y., Jhang, C. F., Lin, C. M., Hsien, T. Y., & Hsieh, H. J. (2017). Fabrication and application of coaxial polyvinyl alcohol/chitosan nanofiber membranes. *Open Physics*, *15*(1): 1004-1014.

Kusumastuti, Y., Putri, N. R. E., & Dary, A. R. (2016). Electrospinning optimization and characterization of chitosan/alginate/polyvinyl alcohol nanofibers. *In AIP Conference Proceedings* (Vol. 1755, No. 1, p. 150007). AIP Publishing LLC.

Li, L., & Hsieh, Y. L. (2006). Chitosan bicomponent nanofibers and nanoporous fibers. *Carbohydrate research*, *341*(3): 374-381.

Nokhasteh, S., Molavi, A. M., Khorsand-Ghayeni, M., & Sadeghi-Avalshahr, A. (2019). Preparation of PVA/Chitosan samples by electrospinning and film casting methods and evaluating the effect of surface morphology on their antibacterial behavior. *Materials Research Express*, *7*(1): 015401.

Raja, K., Prabhu, C., Subramanian, K. S., & Govindaraju, K. (2020). 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*, *78*(11): 6429-6440.

Rani, H., Raja, K., Syndhiya, R., Lakshmanan, A., & Senthilkumar, M. (2020). Development and characterization of chitosan-based polymer composite nanofibre as a smart carrier system for microbial immobilization. *Journal of Pharmacognosy and Phytochemistry*, *9*(6): 1428-1432.

Ranjan, S., Chandrasekaran, R., Paliyath, G., Lim, L. T., & Subramanian, J. (2020). Effect of hexanal loaded electrospun fiber in fruit packaging to enhance the post harvest quality of peach. *Food Packaging and Shelf Life*, *23*: 100447.

Sajeev, U. S., Anand, K. A., Menon, D., & Nair, S. (2008). Control of nanostructures in PVA, PVA/chitosan blends and PCL through electrospinning. *Bulletin of Materials Science*, *31*(3): 343-351.

Xue, J., Wu, T., Dai, Y., & Xia, Y. (2019). Electrospinning and electrospun nanofibers: Methods, materials, and applications. *Chemical reviews*, *119*(8): 5298-5415.

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