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Synthesis and Antioxidant ability of Nanofiber mat prepared using Polyvinyl alcohol and Montmorillonite clay

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ABSTRACT: *Scaffold of Polyvinyl alcohol/Montmorillonite clay have been developed using electrospinning. SEM images of electrospun nanofiber mat showed that they had similar and smooth morphology without beads, and spindle shape. The addition of clay will results in decreasing the average fiber diameter and thereby increasing the surface area. The benefit of high surface area is obvious in drug delivery systems for poorly water-soluble drugs. Their FTIR spectra indicate that the PVA and MMT clay interacted intermolecularly via hydrogen bonds in the nanofiber mats. Their XRD patterns confirmed that they were amorphous, because of amorphization during electrospinning. The XRD analyses also strengthened the FTIR studies; namely, PVA and MMT formed intermolecular interactions in the electrospun nanofiber mats. From the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, the nanofiber mats exhibited very high antioxidant activities despite having been exposed to high voltage during electrospinning. Therefore, they are potential antioxidant products for food and pharmaceutics .*

Keywords: *Antioxidant, DPPH, Montmorillonite clay, Polyvinyl alcohol, Scaffold.*

I. INTRODUCTION

Electrospinning was one of the simple technique widely employed and have gained immense research interest among the research community to prepare scaffolds under laboratory conditions [1]. The advantage of the prepared nanofiber mat was it possesses high surface area to volume ratio and aspect ratio [2]. Electrospun nanofiber mats have been employed in numerous applications such as water filtration or heavy metal removal, textile industries, food and pharmaceutical fields [3-6]. The materials chosen for the current work was Polyvinyl alcohol and Montmorillonite clay. Polyvinyl alcohol was a promising material widely used by many researchers to perform electrospinning along with other polymer combinations. PVA is a semicrystalline hydrophilic polymer with good biocompatibility and non-toxicity that can be easily processed as nanofiber mats [7]. PVA with functional groups is useful in practical investigations of functional polymers because of its easy preparation as a bulk material, as films, and as fibers. Montmorillonite (MMT) clay is a useful inorganic material capable to show remarkable improvements in the polymeric properties when small amounts (1-10%) of MMT fillers are added [8]. Clay consists of nano layers with thickness around 1 nm and platelet aspect ratio of approximately 1000. The incorporation of clay to polyvinyl alcohol may improve its strength with decreasing fiber diameter [9]. DPPH assay was a simple and effective method commonly employed to measure the scavenging activity of the prepared sample [10]. This method can be used for solid or liquid samples and it applies to the overall antioxidant capacity of the sample [11]. The IC50 values can be determined to evaluate its scavenging ability in terms of high, moderate or weak anitoxidants. In the present work, polyvinyl alcohol and montmorillonite clay was electrospun and we report the optimum conditions such as applied voltage, flow rate and tip to collector distance. The prepared nanofiber was characterized using analytical techniques such as FT-IR, XRD and SEM. Also, we hereby evaluated the antioxidant ability of the nanofiber mat.

II. MATERIALS AND METHODS

2.1. Materials

Polyvinyl alcohol and Montmorillonite clay was supplied by HiMedia Laboratories Pvt. Ltd. Mumbai. 2,2-diphenyl-1-picrylhydrazyl (DPPH) was procured from Sisco Research Laboratories Pvt Ltd, Chennai. The entire chemicals used in the study were of analytical grade. Deionized water was employed for the preparation of PVA/MMT polymeric solution.

2.2. Preparation of spinning solution and Fabrication of Nanofiber mat using Electrospinning setup

First, the aqueous solution of 5% polyvinyl alcohol was prepared by dissolving 5 g of PVA in 100 ml of deionized water at 60° C for a period of 6 h. 1g of Montmorillonite clay was dispersed in minimum amount of deionized water and was ultrasonicated for a period of 5 hours. To the prepared homogeneous aqueous solution of PVA, the dispersed MMT clay solution was added and the stirring was continued for 48 h in a magnetic stirrer. The prepared solution was loaded into 2ml syringe and positioned vertically on the syringe pump in the ESPIN NANO (Model). Electrospinning of the solution was performed at the following operating parameters: applied voltage of 20 kV, solution flow rate of 0.55ml/h, the tip to collector distance of 12 cm. The positive end was connected to the metallic syringe tip while the negative terminal was connected to an aluminum foil. The electrospun nanofibers were deposited on a grounded stationary rotating drum collector wrapped with aluminium foil.

2.3. Characterization of nanofibrous mat

2.3.1. FT-IR Spectroscopy

A thermo nicolet AVATAR 330 IR spectrophotometer was used to record the IR spectra within the range of 4000–400 cm^{-1}. The IR spectra were recorded in a solid state using a KBr pellet method.

2.3.2. X-Ray Diffraction

The molecular packing of the nanofiber mat was analyzed with X-ray powder diffractometer (XRD-SHIMADZU XD-D1) using a Ni-filtered Cu Kα X-ray radiation source. The crystallite size was calculated using Scherrer equation and it can be written as:

$$\tau = \frac{K\lambda}{\beta \cos\theta} \quad \text{----- (1)}$$

τ is the mean size of the ordered (crystalline) domains, K is a dimensionless shape factor, with a value close to unity. The shape factor has a typical value of about 0.94, but varies with the actual shape of the crystallite; λ is the X-ray wavelength; β is the line broadening at half the maximum intensity (FWHM) and θ is the Bragg angle.

b

The surface morphology of the prepared nanofiber mat was viewed and photographed using Scanning Electron Microscopy (SEM, JEOL Model JSM - 6390LV) in STIC, Kerala.

2.4. Antioxidant Activity

2.4.1. DPPH Assay

The antioxidant activity of the nanofiber mat was evaluated by measuring DPPH radical scavenging activity, which was determined by the method as proposed by Pereira et al. [12] with some modifications. Stock solution is prepared with different concentration of 100 µg, 200 µg, 300 µg, 400 µg, 500 µg and 600 µg in 50ml standard flask. From the solutions of different concentrations 3ml was pipetted out into a test tube and 1ml of 2,2- diphenyl picryl hydrazyl (DPPH) of normality 0.001N was added. Then, it was kept in a dark room for about 30 minutes and the the antiradical scavenging ability of nanofiber mat was determined by measuring the decrease in the absorbance of DPPH at 517 nm. The percentage of antioxidant activity was calculated using the formula.

$$\% \text{ of Antioxidant activity} = \frac{A_{control} - A_{sample}}{A_{control}} * 100 \quad \text{------ (2)}$$

where $A_{control}$ is the absorbance of the DPPH solution and A_{sample} is the absorbance of the sample (nanofiber mat). Further, a curve that represents the relationship between the concentration of PVA/MMT nanofiber mat and % AA is plotted with the concentration of nanofiber mat as the x-axis and % AA as the y-axis. A regression line is then fitted to obtain an equation ($y = mx+c$). The PVA/MMT nanofiber mat concentration that is expected to exhibit 50% inhibition (IC_{50} value) is determined by finding the x-value resulting in the y-value of 50% [13].

III. RESULTS AND DISCUSSION

3.1. FT- IR spectroscopy

The characteristic functional groups of the scaffold was monitored by FT-IR and was shown in Fig. 1. A broad absorption band at 3351.37 cm^{-1} was due to the –OH stretching i.e. intermolecular and intramolecular hydrogen bonding and the broad absorption indicates the sign of strong hydrogen bonding. A peak at 2939.57 cm^{-1} indicates the presence of CH stretching for CH_2 and CH_3 group and the peaks at 946.10 cm^{-1}, 850.62 cm^{-1} were due to the presence of Al –Al- OH, Al- Fe- OH, and Al- Mg- OH bending vibrations [14].

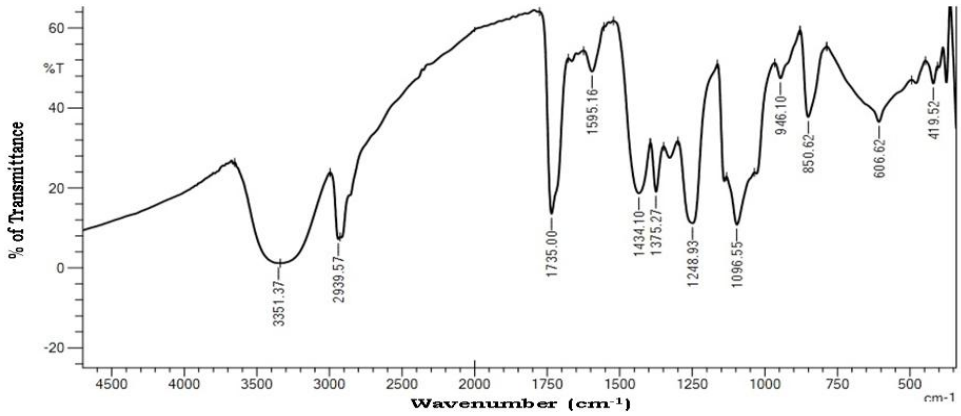

Figure 1: FT-IR spectrum of Polyvinyl alcohol/Montmorillonite clay nanofiber mat

The peak at 1735 cm^{-1} was assigned to the C=O and C-O stretching from the residual acetate groups in the PVA matrix [15]. The peaks at 606.62 cm^{-1} and 419.52 cm^{-1} are due to Si-O bending confirms the presence of MMT clay in the mat and the introduction of Si-O group in the PVA matrix will modify the semicrystalline nature of Polyvinyl alcohol to amorphous [16] which was confirmed by the decrease in crystallite size calculated using scherrer equation in the XRD. The FT-IR measurements exhibited indicates the existence of relevant functional groups of polyvinyl alcohol and montmorillonite clay.

3.2. X-Ray Diffraction studies (XRD)

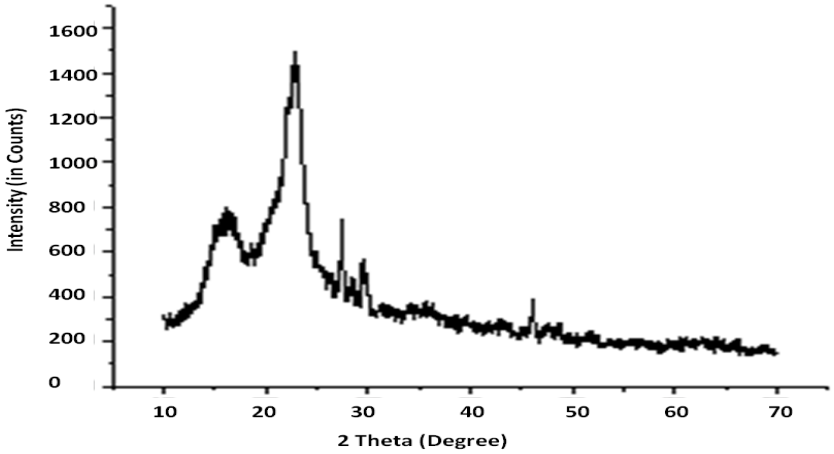

Figure 2: XRD pattern of PVA/MMT nanofiber mat

An XRD pattern of PVA/MMT nanofiber mat was shown in Fig. 2. The graph recorded depicts, intense crystalline band at 2□ =22.8 and shoulder peaks at 2□ values of 20.6, 27.6 and 29.6. The crystallite size values were measured using scherrer equation, the d-spacing values and full width at half maximum were tabulated in Table 1.

Table 1: Crystallite size values using Scherrer equation:

2□ □	d spacing value (nm)	FWHM Full width at half maximum (radian)	Crystallite size (nm)
20.6	0.4309	0.0075	18.72
22.8	0.3898	0.0068	20.77
27.6	0.3230	0.0056	25.31
29.6	0.3016	0.0053	27.22

By applying scherrer equation the crystallite size values for the nanofiber mat was calculated and it was in the range of 18.72 – 27.22 nm. This was due to the fact during electrospinning the time required to crystallize was lesser as compared to the required time for the evaporation of solvent and solidification of nanofiber takes place simultaneously [17]. Hence this fact facilitates the decrease in crystallite size and also decreases the semicrystalline nature of PVA to amorphous.

3.3. Scanning Electron Microscopy (SEM)

SEM micrograph of the nanofibrous mat was shown in Fig. 3. The operating parameters such as applied voltage, tip to collector distance and flow rate have direct influence on the morphology of the prepared electrospun nanofiber [18]. From the SEM micrograph it was evident that the prepared PVA/MMT nanofiber mat was more uniform and thinner indicating the homogeneous favorable solution viscosity with optimized operating parameters was achieved during electrospinning. The average fiber diameter was measured using Image J software and it was plotted in the fiber diameter distribution graph. The average fiber diameter of lesser than 250 nm was achieved and the maximum number of fibers should be in the range of 150-200 nm. This uniform and thinner fiber diameter indicates eminent dispersion and exfoliation of MMT layers was achieved within the nanofiber matrix [19].

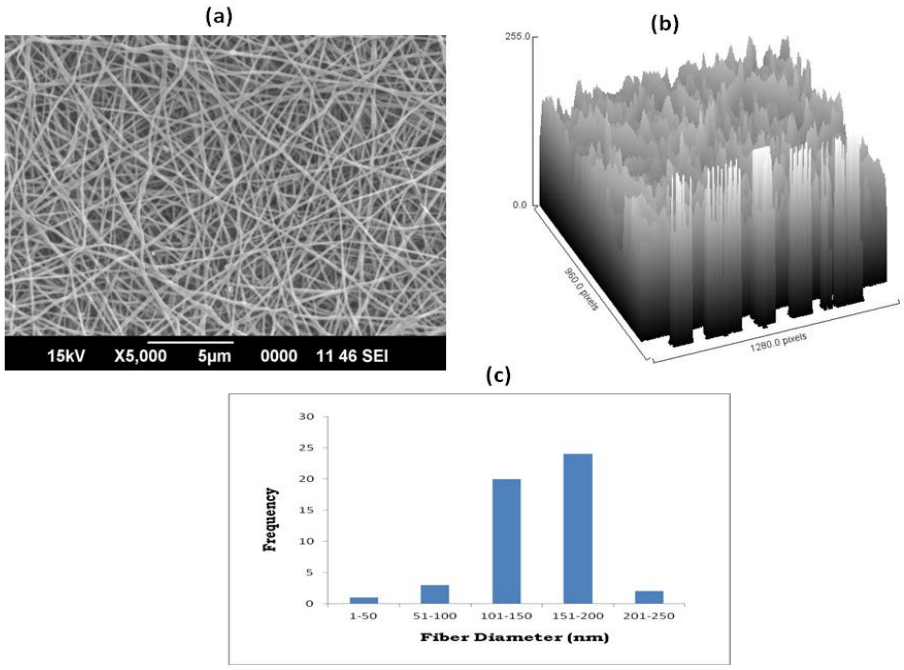

Figure 3: SEM micrograph (b) Three dimensional surface plot and (c) Fiber diameter distribution graph of PVA/MMT nanofiber mat

3.4. Antioxidant activity

DPPH assay is a rapid technique for screening the radical scavenging activity of the specific compounds [20]. The scavenging activity of the nanofiber mat was plotted and was respresented in Fig. 4. The antioxidant activity of the nanofibrous mat increases with increase in concentration and this result indicates nanofiber mat reduces the radicals to the corresponding hydrazine when it reacts with DPPH.

Antioxidant activity was described by IC_{50} values, in which antioxidant activity was inversely proportional to IC_{50} values i.e lower the IC_{50} value stronger the antioxidant activity [21, 22]. In general a compound is stated to be "very high" if the IC_{50} value is within the range of 1 to 50 μg/mL, "high" if the value is within 50 to 100 μg/mL, "moderate" if the value is within 101 to 150 μg/mL, and "weak" if the value is larger than 150 μg/mL [23].

The IC_{50} value of the nanofiber mat was calculated and was found to be 116 μg/mL. From this observation it was concluded the nanofiber mat has moderate scavenging activity which could be a potential antioxidant product to be applied in food and pharmaceutical products.

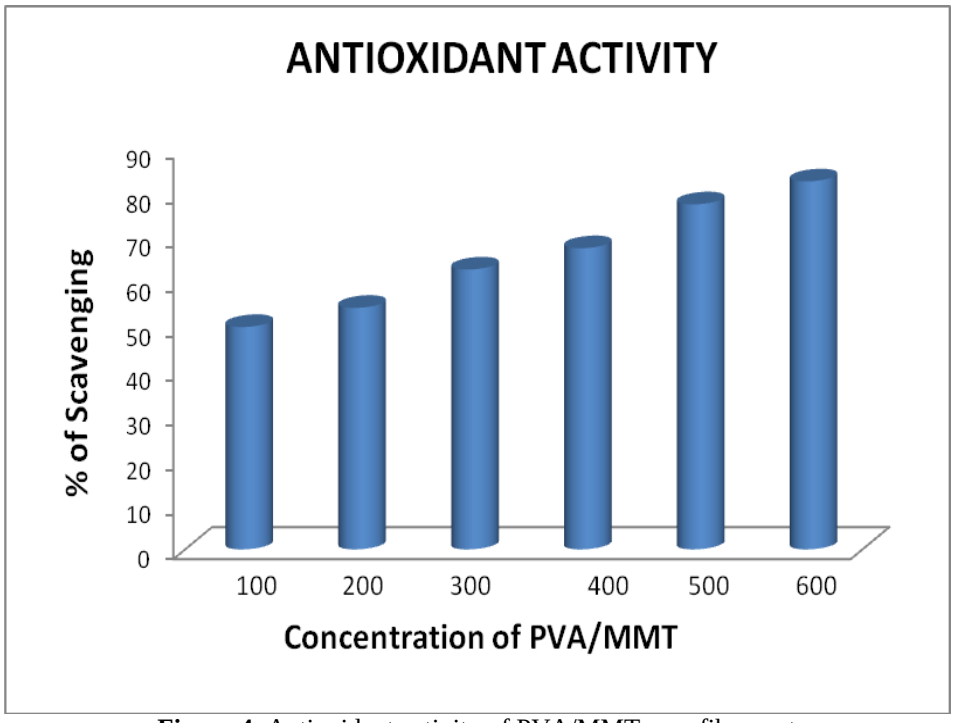

Figure 4: Antioxidant activity of PVA/MMT nanofiber mat

IV. CONCLUSION

In the present study, nanofiber mat of PVA/MMT was prepared using electrospinning method. FT-IR results confirms effective blending has taken place between the individual polymers of Polyvinyl alcohol and Montmorillonite clay. The XRD datas confirms the reduction in crystallite size was successfully achieved by electrospinning. The uniform morphology and the nanosized fiber diameter as given by their SEM images indicates good spinnability and high surface area was achieved successfully. The antioxidant abiliity of the nanofiber mat was evaluated using DPPH assay and the results revealed that the mat possess moderate scavenging activity which could find application in food and pharmaceutical industries as a potential antioxidant product.

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REFERENCES

[1]. M.-X. Wang, Z.-H. Huang, F. Kang, and K. Liang, Porous carbon nanofibers with narrow pore size distribution from electrospun phenolic resins, Materials Letters, 65, 2011, 1875-1877.
[2]. M. Sadri, A. Maleki, F. Agend, and H. Hosseini, Fast and Efficient Electrospinning of Chitosan-Poly(ethylene oxide) nanofibers as potential wound dressing agents for tissue engineering, Journal of Applied Polymer Science, 126(6), 20112, 2077-2084.
[3]. X.J. Han, Coaxial Electrospinning of PC (Shell)/PU (Core) composite nanofibers for textile application, Polymer composites, 27, 2006, 381-387.
[4]. C.-I. Su, Pan-based carbon nanofiber absorbents prepared using Electrospinning, Fibers and Polymers, 13(4), 2012
[5]. X.-H.Qin, and S.-Y. Wang, Filtration properties of Electrospinning nanofibers, Journal of Applied Polymer Science, 102(2), 2006, 1285-1290.
[6]. M.S. Islam, Effect of pullulan/poly(vinyl alcohol) blend system on the montmorillonite structure with property characterization of electrospun pullulan/ poly(vinylalcohol)/ montmorillonite nanofibers, J. Colloid and Interface Science, 368, 2012, 273-281.
[7]. A. Idris, E. Misran, N. Hassan, A.A. Jalil, and C.E. Seng, Modified PVA-alginate encap-sulated photocatalyst ferro photo gels for Cr (VI) reduction, Journal of Hazardous Material, 227, 2012, 309–316.
[8]. J.H. Park, Effect of montmorillonite on wettability and microstructure properties of zein/ montmorillonite nanocomposite nanofiber mats. Journal of Composite Materials, 0(0), 2012, 1-7.

[9]. H.W. Lee, M.R. Karim, H.M. Ji, J.H. Choi, H.D. Ghim, S.M. Park, W. Oh, and J.H. Yeum, Electrospinning Fabrication and Characterization of Poly(vinyl alcohol)/Montmorillonite Nanofiber Mats, Journal of Applied Polymer Science,113, 2009, 1860-1867.
[10]. H.E. Miller, F. Rigelhof, L. Marquart, A. Prakash, and M. Kanter, Antioxidant content of whole grain breakfast cereals, fruits and vegetables, J. Am. Coll. Nutr., 19. 2000, 312-319.
[11]. P. Nithya, and C. Madhavi, Antioxidant activity of 3-arylidene-4-piperidones in the 1,1-diphenyl-2-picrylhydrazyl scavenging assay, Journal of Taibah University for Science, 11, 2017, 40-45.
[12]. J.A. Pereira, I. Oleiveira, A. Sousa, P. Valentão, P.B. Andrade, I.C. Ferreira, F. Ferreres, A. Bento, R. Seabra, and L. Estevinho, Walnut (Juglans regia L.) leaves: Phenolic compounds, antibacterial activity and antioxidant potential of different cultivars. Food and Chemical Toxicology, 45, 2007 2287-2295.
[13]. I. Sriyanti, D. Edikresnha, A. Rahma, M. Miftahul Munir, H. Rachmawati, and K. Khairurrijal, Correlation between structures and Antioxidant activities of Polyvinylpyrrlidine/Garcinia mangostana L. Extract composite nanofiber mats prepared using Electrospinning, Journal of Nanomaterials, Article ID 9687896, 2017, 1-10.
[14]. M. Baek, J.-H. Choy, and S.-J. Choi, Montmorillonite intercalated with glutathione for antioxidant delivery: Synthesis, characterization, and bioavailability evaluation, International Journal of Pharmaceutics, 425, 2012, 29-34.
[15]. M.E. Mahmoud, M.T.H.A. Kana, and A.A. Hendy, Synthesis and implementation of nanochitosan and its acetophenone derivative for enhanced removal of metals, International Journal of Biological Macromolecules, 81, 2015, 672-80.
[16]. E.F.D. Reis, F.S. Campos, A.P. Lage , R.C. Leite, L.G. Heneine, W.L. Vasconcelos, Z.I.P. Lobato, and H.S. Mansur, Synthesis and Characterization of Poly (Vinyl Alcohol) Hydrogels and Hybrids for rMPB70 Protein Adsorption, Materials Research, 9(2), 2006, 185-191.
[17]. N.S. Sambudi, M.G. Kim, S.B. Park, The formation of web-like connection among Electrospun chitosan/PVA fiber network by the reinforcement of ellipsoidal calcium carbonate, Materials Science and Engineering C, 60, 2016, 518-525.
[18].] Z.-M. Huang, Electrospinning and mechanical characterization of gelatin nanofibers, Polymer, 45, 2004, 5361-5368.
[19]. Md.S.R. Islam, Md. S. Rahaman, and J.H. Yeum, Electrospun novel super-absorbent based on polysaccharide-polyvinyl alcohol-montmorillonite clay nanocomposites, Carbohydrate Polymers, 115, 2015, 69-77.
[20]. N. Sreejayan, and M.N.A. Rao, Nitric oxide scavenging by curcuminoids, Journal of Pharmacology, 49, 1997, 105-108.
[21]. M.S. Blois, Antioxidant determinations by the use of a stable free radical, Nature, 181(4617), 1958, 1199- 1200.
[22]. P. Molyneux, The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity, Songklanakarin Journal of Science and Technology, 26(2), 2004, 211–219.
[23]. I. Fidrianny, S. Natalia, and M. Insanu, Antioxidant capacities of various fruit extracts from three varieties of tomato and correlation with total phenolic, flavonoid, carotenoid content, International Journal of Pharmaceutical and Clinical Research, 7(4), 2015, 283–289.