RESEARCH ARTICLE

DOI: 10.34256/bsr1913

BULLETIN OF SCIENTIFIC RESEARCH

Ardil Protein Based Electro spun Mat for Medical Applications Investigation

M. Renuka Devi [a,*], D. Vijay Kirubakar Raj [a]

[a] Assistant professor, Department of Textile Technology, Anna University, Chennai-25, Tamil Nadu, India

* Corresponding Author: mrenuauc@gmail.com DOI: https://doi.org/10.34256/bsr1913

Received: 14-03-2019
Accepted: 12-05-2019

Abstract: The main objective is to produce an electro spun mat from ardil fibers in combination with polyvinyl alcohol which can be used for medical applications. Ardil fibers are the fibers extracted from the protein obtained from the defatted oil cake of groundnut. The inherent anti-microbial activity of ground nut allows the use of ardil fibers for medical applications. Since ardil fibbers are not capable of forming fibers of its own by electro spinning, they are used in blend with polyvinyl alcohol. Polyvinyl alcohol is chosen mainly because of its biocompatibility, biodegradability and non-toxicity. The electro spun mat produced from ardil and polyvinyl alcohol was checked for anti-microbial activity so that it can be used for medical applications and X-ray diffraction was seen to check the presence of proteins which was absolute. Other test such as moisture vapor transmission rate (MVTR) and thermo gravimetric analysis (TGA) were also conducted to check whether the produced electro spun mat can be used for the required end use. MVTR is checked to check the breathability of the mat and control of moisture. The MVTR is between the acceptance levels which can be used for medical applications. TGA is to check absorption and adsorption. From the results obtained from different results, it is obvious that the produced mat can be used for medical applications.

Keywords: Ardil, Electro spun, MVTR, Poly vinyl alcohol, TGA

1. Introduction

There is an increasing need to develop new biodegradable materials to be used in tissue engineering, wound cover or as dressings and barrier membranes, since there is a high demand for skin replacements and skin repair treatments. For instance, an ideal material to be used for medical application should associate availability with minimal storage requirements, long shelf life, versatility and biocompatible behavior. Many polymeric membranes have been investigated for the purpose of wound coverings and dressings on account of its importance in the treatment of burns, prevention of post-surgical adhesions and cosmetic surgery. These materials include synthetic polymers like polyurethane, polyethylene, polyglycolides and polyacrylonitrile. However some of these polymers have disadvantages in such application, i.e. poor biocompatibility and release of acidic degradation products.

One alternative approach involves the use of biodegradable polymers from renewable resources which include starch, gelatin, collagen, chitosan and other natural and regenerated protein based polymers (soy protein, ardil protein, casein, silk fibroin and

wheat). Conformed from the finding of Aditi Shankar *et al.,* (2018) All these polymers are widely available in nature and are biodegradable and non toxic [1]. Among these renewable polymers, the ardil protein, the major component of the groundnuts, has the advantage of being economically competitive and present good water resistance as well as storage stability [2-4]. The combination of these properties with a similarity to tissue constituent and a reduced susceptibility to thermal degradation makes an ideal template to be used as a biomaterial.

The ardil proteins rich in flavonoids, resorcinol, amino acids and phenylpropanoid compounds. This protein has fibre forming characteristics and woven and knitted into fabrics and it is also blended with wool for commercial use. It has compounds which promote health and tissue regeneration, and so like all other protein fibers it can be used for medical applications. This work will explore the extraction of ardil protein its blending with PVA and the production of nano fibers by electro spinning followed by the anlysis of characteristics and functional properties of the material [5].

2. Materials and Method

The materials used for the production of electro spun mat are ardil the protein obtained freom the defatted oil cake and poly vinyl alcohol of molecular weight 1,00,000 dalton.

2.1 Electro Spinning

The electro spining of pure ardil is not possible so it is blended with polyvinyl alcohol and spun into mat. The ardil protein isolate is mixed with ethanol. Polyvinyl alcohol solution is prepared separately by dissolving in water. The ardil and PVA is mixed in two different proportions such as 20:80 and 40:60 respectively. Multi syringe electro spinning machine is used for mat production. The prepared solution is loaded into 5 ml syringe fitted with 0.70 * 32 mm sized needle The needle tip was connected to the high voltage of constant 20 kV electric field, feed rate was 1 ml per hour and the distance between the tip and the collector is 15 cm. During this process, the solvent was evaporated from polymer and the fiber is deposited on the aluminum foil in the form of non woven fibrous mat [5, 6].

2.2 Isolation of protein from peanut

Extraction of oil from the peanut pods

↓

Washing of dried defatted oil meal with hexane

↓

Drying of the oil meal

↓

Stirring of the oil meal in solution of 1N NaoH and and 1N Hcl (pH 4 to 10) at 1200 rpm for an hour

↓

Centrifuging at 3000 rpm for 20 mins

↓

Supernatant is collected, pH is adjusted to 4.5 with 1N H_2SO_4, the protein is precipitated

2.3 Scanning Electron Microscope (SEM)

The morphology of the electro spun web was observed under a scanning electron microscope at an accelerating voltage of 20 kV. Based on the SEM photographs, the diameter of the fiber was analyzed using image visualizing software.

2.4 Fourier Transform Infrared Spectroscopy(FTIR)

Fourier transform infrared spectroscopy was used to identify the change in functional group of Ardil proteins and PVA. The

spectra were scanned between 400 and 4000 cm^{1}.

2.5 X-Ray Diffraction (XRD)

The XRD test is primarily used for phase identification of crystalline structure of the unit cell. The unit cell or the crystalline structure of the protein is to be determined by the diffraction or the deflection of the rays by the protein.

2.6 Thermal Gravimetric Analysis (TGA)

Thermal Gravimetric Analysis is the thermal analysis of mass a material which changes over the increasing temperature over the time. It provides information about physical and chemical phenomena such as phase transitions, absorption, adsorption, desorption and thermal decomposition, chemisorptions and solid gas reactions [7].

2.7 Moisture Vapor Transmission Rate (MVTR)

The Moisture Vapor Transmission Rate (MVTR) of the electro spun web was determined according to ASTM E 96. With the diameter of 50mm, the materials were mounted on the mouth of cylindrical beakers containing 10 ml of water with negligible water vapor transmittance. The material was fastened across the edge using a Teflon tape to prevent any water vapor loss across the boundary and kept at 37 °C and 65% relative humidity in an incubator. The assembly was weighed at regular intervals of every one hour and after 24 hours the weight loss of the sample was recorded and the slope was calculated

MVTR was calculated from the equation:

Slope * 24/ A $g/m^2/day$

where A is the test area of the sample in m^2

2.8 Anti Bacterial Activity

The electro spun matt produced is tested for antibacterial property of the protein is retained in the web. The activity is checked with E.coli and staphylococcus bacteria over the agar medium and zone of inhibition is calculated after 24 hours.

3. Results and Discussions

Ardil protein does not form nanofibres of its own, so the synthetic inert polymer polyvinyl alcohol is added to increase chain entanglements in the solution and enable the formation of continuous fiber [8]. Ardil and PVA is mixed in 20:80 and 40:60 respectively. The electro- spinning was carried with both these proportions. The 40:60 proportion gives continuous fibers with better quality compared to 20:80. Hence the above proportion was taken for this study and analyzed.

3.1 Scanning Electron Microscope (SEM)

The ardil/PVA electro spun web is characterized by SEM with accelerating voltage of 20-30 kV and the image is shown in Figure 1. From the figure, it was observed that the diameter of the fiber sample is found to be 124 nm.

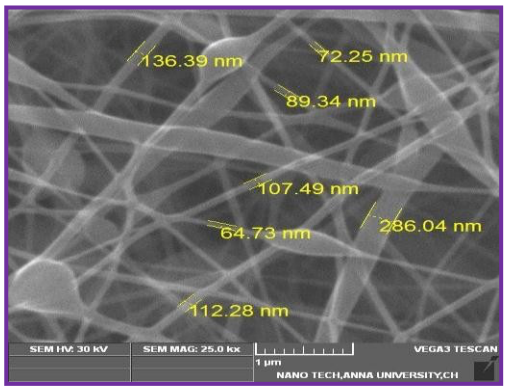

Figure 1 SEM image of electrospun mat (60: 40 PVA: Ardil)

3.2 Fourier Transform Infrared Spectroscopy(FTIR)

FTIR spectra of ardil /PVA blended electro spun web was obtained in the wave length ranged from 4000 – 400 cm^{-1}.It was evident from the Figure 2 and table 1, the characteristic band of amines functional group N-H bond stretch is at 1632 cm^{-1}. The functional group of alkenes (C –H) was observed in the spectra at 2980-2850cm^{-1}. The carboxylic group (C=0) bond was observed in the spectra at 1241-1472 cm^{-1}. This conforms the functional group amines, alkenes and carboxylic groups present in ardil protein. This is in agreement with the findings of [9].

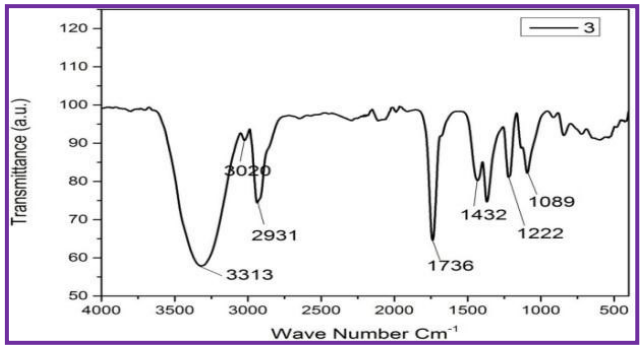

Figure 2 FTIR analysis of ardil/PVA electro-spun mat

Table 1 Wave number with peaks and corresponding groups

S.no	Wave Number (at peaks)	Group
1.	1632 cm^{-1}	Amines
2.	2980-280 cm^{-1}	Alkenes
3.	1241-1472 cm^{-1}	Carboxylic

3.3 X-ray Diffraction (XRD)

The XRD results have produced peaks scanning the sample at 2 Theta /◦ , axis 2:1. It was inferred from the Figure 3 and table 2 the peaks obtained conforms the presence of elements (calcium, potassium, magnesium, silicon, iron, cobalt and molybdenum.) in the protein fibre. This is in agreement with the findings of [9, 10].

Table 2 Percentage of various elements present

Elements	Percentage (%)
Calcium	47.24
Iron	11.92
Silicon	11.01
Potassium	15.04
Aluminium	9.33
Chromium	4.48

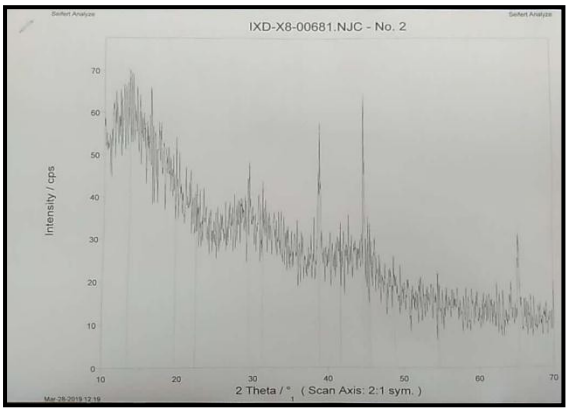

Figure 3 XRD graph of the electro-spun mat

3.4 Thermal Gravimetric Analysis (TGA)

The thermal gravimetric results for the sample show the response or the degradation of the electro spun web to the increasing temperature over time.

The Figure 4 indicates that as the temperature increases the weight decreases. It is inferred from the graph that between room temperature to 100°C, there is only slight variation in weight loss. Between 100°C and 200°C, the weight loss is not obvious. There is a sudden drop of weight from temperature 250°C to 300°C. After 300°C, there is a gradual decrease in the weight. This shows that the fiber can be used for medical applications since it can withstand autoclave and sterilization temperature [11].

3.5 Moisture Vapor Transmission Rate (MVTR)

MVTR of the electro spun mat of PVA and protein were calculated as the gradient of weight loss versus time for every one hour. The result shows that the water loss is lesser hence can promote rapid healing. The water permeability of the electro spun mat is about 2041 g/m^2/day. The water loss per hour is about 125 g/m^2/h. Hence this rate of moisture vapor transmission helps to keep on the moist environment which is essential to heal the wound.

Permeability is the transmission rate of a vapor normalized for both the thickness of the film and the difference in pressure. The rate at which a gas will permeate through a membrane is decided by the properties of membrane.

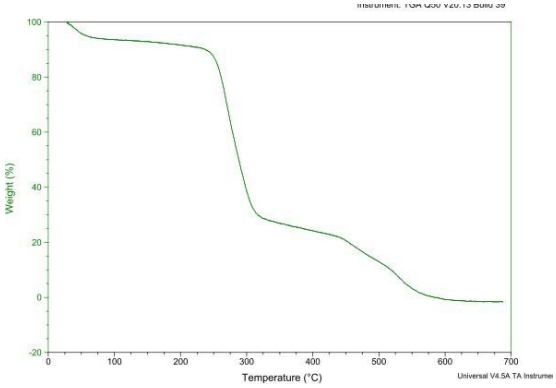

Figure 4 TGA analysis of ardil/PVA Electro-spun mat

The optimal rate of moisture vapor transmission which is suitable for materials used for medical application is 2028±237 g/m^2/day. Since the determined MVTR for ardil/PVA mat is between the acceptance leve. So, it can be used for medical applications [12].

3.6 Antibacterial Activity

The anti-microbial activity was qualitatively evaluated by agar well- diffusion method. It is found that the zone of inhibition varies with the concentration. Higher the concentration more is the zone of inhibition.

The electro spun mat prepared from PVA and protein were used to determine the antibacterial activity in E.coli and Staphylococcus R bacteria. The microbial suspension was spread evenly over the face of the sterile agar plate. The electro spun matrix was applied to the centre ofthe agar plate and incubated. After 24 hours, zone of inhibition appeared around the samplesand was found to be over area 15 mm and 18 mm respectively [13].

It is found that the presence of certain groups assures inherent anti-bacterial activity. The groups such as tannins, flavanoids, alkaloids and terpenoids have been found to have invitro antimicrobial activity. The ardil fibres possess flavanoids and alkaloids which are responsible for anti-bacterial activity.

4. Summary and Conclusions

The electro spun web was produced by blending of ardil protein and PVA in different proportions. The proportion 40:60 is rich in ardil protein when compared to other proportions and hence it is take for further studies. The electro spun web was characterized by SEM and the average fiber diameter was found to be 124 nm.

FTIR results showed that the functional group amines, alkenes, carboxylic groups present in the ardil protein is conformed bond stretching in 1632cm^{-1}, 2980-2850cm^{-1} and 1241-1472cm-^{1} respectively.

The XRD peaks conforms that the minerals are present in the web. This indicates the presence of proteins in the web. MVTR results show that the electro spun mat has water permeability of 2041 g/m^2/day and hence provides a moist environment to promote rapid healing.

The TGA results give the degradation of the mat with increasing temperatures over time which is also acceptable. The antibacterial results are also positive. These are in

agreement with [14].

The results obtained from the various test conducted shows that the ardil/PVA blended electro-spun mat can be used for medical application due to its bio-compatibility, anti-bacterial activity, permeability and non-toxicity.

References

[1] A. Shankar, A. Seyam, S. Hudson, Electro spinning of soy protein fibres and their compatibility with synthetic polymers, Journal of Textile and Apparel, Technology and Management, 8 (2018) 1-14.

[2] N.N. Mahapatra, (2018) Groundnut protein fiber, its properties, and end use, *Textile today*, Accessed from: https://www.textiletoday.com.bd/groundnut-protein-fiber-properties-end-use/

[3] R.H.K. Thomson, The proteins of Arachis hypogeal and fibre formation, *Biochemical Journal*, 51 (1952) 118-123.

[4] R. Shafiquer, A. Islam, M.M. Rahman, M.B. Uddin, A.R. Mazumder, 2018, Isolation of protein from defatted peanut meal and characterize their nutritional profile, *Chemistry Research Journal,* 3 (2018) 187-196.

[5] H. Wang, Q. Jiang, S. Jiang, S. Jiang, Fabrication and characterization of PVA/SPI/SiO2 hybrid fibres via electro spinning technique, *polymer and polymer composites*, 20 (2012) 621-628.

[6] H. karakas, Electro spinning of nanofibers and their applications, *MDT electro spinning*, 1-35.

[7] J. Xue, J. Xie, W. Liu, Y. Xia, Electrospun nanofibers: new concept, materials and applications, Accounts of Chemical Research, 50 (2017) 1976-1987.

[8] T.S. Gaaz, A.B. Sulong, M.N. Akhtar, A.A.H. Kadham, A.B. Mohammed, A. Aminey, Properties and Applications of Polyvinyl Alcohol, Halloysite Nanotubes and Their Nanocomposites, *Molecules*, 20 (2015) 22833–22847.

[9] C. Li, W. Zhu, H. Xue, Z. Chen, Y. Chen, X. Wang, Physical and structural properties of peanut protein isolate-gum Arabic films prepared by various glycation time, *Food Hydrocolloids*, 43 (2015) 228-244.

[10] C. Yao, X. Li and T. sung, Electrospinning and crosslinking of zein nanofiber mats, *Journal of Applied Polymer Science*, 103 (2006) 380-385.

[11] R.M. Lopes, T.S. Agostini-Costa, M.A. Gimenes, D. Silveira, Chemical Composition and Biological Activities of *Arachis* Species, *Journal of Agriculture and Food Chemistry*, 59 (2011) 4321-4330.

[12] Li Yao, T.W. Haas, A. Guiseppi-Elie, G.L. Bowlin, D.G. Simpson, G.E. Wnek, Electrospinning and Stabilization of Fully Hydrolyzed Poly (Vinyl Alcohol) Fibers, *Chemistry of Materials*, 15(2003) 1860-1864.

[13] K.G. DeFrates, R. Moore, J. Borgesi, G. Lin, T. Mulderig, V. Beachley, X. Hu, Protein-Based Fiber Materials in Medicine: A Review, *Nanomaterials*, 8 (2018) 457.

[14] N. Bhardwaj, S.C. Kundu, Electrospinning: A Fascinating fibre fabrication technique, *Biotechnology advances*, 28 (2010) 325-347.

About the License