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Optimization of process parameters for nozzle - free electrospinning of poly (vinyl alcohol) and alginate blend nano-fibrous scaffolds

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Abstract: The study describes the optimization of the process parameters for the fabrication of nano- fibrous scaffolds of poly (vinyl alcohol) (PVA) and sodium alginate (SA) blends using nozzle free electrospinning machine. The solutions prepared by varying the proportions of PVA and SA solutions were characterized by surface tensiometer and conductivity meter which showed reduction in the surface tension and conductivity of the solutions with increase in PVA concentration. The process optimization was tried by tailoring the applied voltage and roller – collector distance to obtain maximum production with least diameter fibers. The scaffolds were characterized by SEM, FT-IR, XRD and TG studies. The applied voltage, roller-collector distance of the optimized scaffold was 75 kV, 12cm respectively. A decrease in fiber diameter was observed with the increase in the proportion of SA. Inter – molecular hydrogen bonding between PVA and SA molecules and the amorphous nature of the scaffolds was suggested through FTIR and XRD studies respectively.

Keywords: Tissue Engineering, Nozzle free Electrospinning, Scaffold fabrication, Alginate.

INTRODUCTION

The field of tissue engineering is an inter-disciplinary subject which deals with the regeneration of tissues either under in vitro or in vivo conditions. For the successful regeneration of tissues, there is a need to simulate the extracellular matrix artificially. The artificial extracellular matrix, which provides support to the growth of the cells, is regarded as scaffolds. The scaffolds are porous in nature and allows proliferation of cells in 3-dimension. Many methods (e.g. salt leaching, freeze-drying and electrospinning) have been proposed for the development of the scaffolds. Electrospinning is a relatively modern method which is a simple, versatile and an expedient process for the fabrication of polymer and polymer composite based nanofiberous scaffolds. The nanofibers formed by electrospinning may be converted into nanofiber mats by randomly depositing the fibers. The mats, so formed has better surface area, aspect ratio and porosity as compared to the scaffolds prepared from the other techniques [1-2]. Due to this reason, the efficiency of the scaffolds prepared by electrospinning method has been reported to have improved cell attachments and viability within the scaffold matrix.

Alginate is a natural biopolymer obtained from brown seaweeds. It has been extensively used in various biomedical applications (e.g. wound dressings, drug delivery and sutures) due to its inherent biocompatibility, biodegradability and gel forming ability. There are many alginate wound dressings already available in the market, especially for absorbing the exudative wounds [3]. Alginate has also been electrospunned to form scaffold which regulated cell adhesivity by covalent coupling of adhesive ligands to the biopolymer backbone [4-5]. Even though alginate has been used extensively, electrospinning of pure alginate still remains a challenge. This may be attributed to its polyelectrolyteic nature and insufficient chain entanglement amongst the alginate chains. The electrospinnability of the alginate may be improved by preparing alginate blends with biocompatible synthetic polymer (e.g. polyethylene oxide and polyvinyl alcohol) [6-7]. Electrospinning of high molecular weight alginate has been prepared using blends of alginate and polyethylene oxide. The supporting polymers induced charge repulsion amongst the alginate chains. This, in turn, improved the flexibility of the polymer chains thereby resulting in the formation of scaffolds with improved properties [6, 8]. Electrospinning of alginate using polyvinyl alcohol blends have also been extensively studied.

The use of poly vinyl alcohol improves the properties of alginate (mechanical properties, chemical resistance and biological properties) [9-10]. Electrospinning of polyvinyl alcohol – alginate blends have been reported to produce nanofibers having diameter in the range of 100 nm to 1000 nm [11]. Electrospun nanofibers and the mats for wound dressings have been reported

in many literatures using polyvinyl alcohol/alginate blends. The studies have focused mainly over the polymer blend nanofiber formation using needle based electrospinning technique. However, no study has been coincided citing the preparation of nanofiberous mats of polyvinyl alcohol – alginate blends using needle free electrospinning methodology. The main advantage of nozzle free electrospinning is the ability to produce nanofiberous mats on a mass scale. This would allow a better economic value in the market [12].

Taking the inspiration from the above, in the current study attempts were made to optimize the process parameters for the fabrication of nano – fibrous mats using blends of sodium alginate and polyvinyl alcohol by nozzle free electrospinning technology.

MATERIALS & METHODS

Preparation and characterization of polymeric blend solutions

2% sodium alginate (viscosity: 2,000 cps, from SDFCL, Mumbai, India) was dissolved in double distilled water (DDW) at room temperature. 10% PVA (Mol Wt: 14,000, from Otto Chemie Pvt. Ltd., Mumbai, India) was prepared in DDW at 70°C along with continuous stirring for 4 hours. Apart from the above mentioned control samples, polyvinyl alcohol and sodium alginate were mixed in five different ratios, thus forming five different polymeric blends. The blend solutions of polyvinyl alcohol and sodium alginate were prepared in 80:20, 70:30, 60:40, 50:50 and 40:60 ratios. The solutions were continuously stirred for 1 hour before use. Surface tension analysis was done to evaluate the material properties through Whilmey plate method, using a surface tensiometer, Data Physics, Germany (DCAT-11EC). Viscosity of solutions was tested by Bohlin Visco 88 viscometer (Malvern Instruments, U.K.). Moore Model was applied for the analysis. Conductivity analysis was performed using Deluxe conductivity meter 601, EI Products, India.

Standardization for electrospinning of blend solutions

Process optimization of electrospinning was done in order to standardize the voltage, roller-collector distance and rotational speed for different blend solutions. Different voltages were applied at different roller – collector distances for the formation of cones and hence the nanofibers. Electrospinning of pure 2% alginate was also tried at different parameters.

Nanofiber fabrication

Nozzle free electrospinning (Elmarco, NS Lab 200) was used to fabricate nanofibers from the blended solutions of polyvinyl alcohol and sodium alginate. The machine has different types of electrode systems for the fabrication of nano-fibers. For the experiment roller electrode system was used. Fibers thus formed using the system was then collected as a mat on the collector which is located at the top. In the convention needle electrospinning machines the collector usually remains at the bottom whereas the needle lies at the top.

Scanning Electron Microscopy (SEM)

Fibers formed from blended solutions of polyvinyl alcohol and sodium alginate in different ratios were analyzed for their surface morphology using scanning electron microscope (JEOL-JSM 6480, Japan) after sputter coating with a thin layer of platinum (Pt). The diameters of the fibers were measured using ImageJ software. At least 20 fiber diameters at various locations were measured in micrograph to take the average diameter.

Fourier-transform infrared (FTIR) spectroscopy

All the blended nanofibers along with pure 10% PVA fibers were examined for spectroscopic analysis using FTIR spectroscopy. Nanofiber thin sheets were pressed under potassium bromide (KBr) layers. A pressure of 10 tons was applied using a pressing machine (KBr press Technosearch). These sample layers coated with KBr were analyzed using Shimadzu/IR prestige 21 with a scanning range of 4000 cm^{-1} to 500 cm^{-1} and resolution of 4 cm^{-1}.

X-Ray diffraction (XRD)

The polymeric blends were analyzed using X-ray diffractometer (PW3040, XRD-PANalytical, Philips, Holland) using monochromatized Cu – Kα radiation (λ = 0.154 nm) as a source, which was operated at 30 KV and 20 mA. Scanning of the samples was done at 5° - 30° 2θ with a rate of 2° 2θ /min. The analysis was performed at the room temperature.

Thermal Analysis:

Thermogravitometric apparatus (STA449C/4/MFC/G apparatus), Netzsch, Germany, was used to study the thermal properties of the prepared samples. Aluminium pans with the pierced lids were used for the study. The study was done under Nitrogen environment. The nitrogen gas was purged at 40 ml/ min flow rate. 5 mg of the nanofiber samples was analyzed within the temperature range of 25ºC-1000 ºC at a heating rate of 10ºC/min.

RESULTS AND DISCUSSION

Material Properties Analysis

Surface tension and conductivity of the prepared solutions were tabulated in Table (1). It was observed that there was a decrease in all the readings with increase in the amount of PVA in the blend solutions. This is due to the fact that increase in the concentration PVA tends to decrease the surface tension and the conductivity of an aqueous solution[13].

Table (1): Physical and chemical parameters for different polymeric blend solutions.

S.No.	oncentration (PVA: Sodium Alginate)	urface Tension (m N/m) ± SD	onductivity (µS/cm) ± SD
1	100:0	42.214 ± 0.030	0.4527±0.005
2	80:20	42.424 ± 0.029	0.86±0.047
3	70:30	42.560 ± 0.029	0.896±0.003
4.	60:40	42.670 ± 0.030	1.0437±0.043
5.	50:50	42.810 ± 0.030	1.340±0.016
6.	40:60	42.980 ± 0.030	1.634±0.043
7.	0:100	69.693 ± 0.029	3.42±0.009

Nanofibers fabrication by electrospinning from blend solutions

To optimize the whole process of electrospinning, different concentrations of polymeric blends of PVA-Sodium alginate, were prepared and were electrospunned to obtain nanofibers. Each sample was tested for its electrospinnability at a voltage range of 20-80 kV. At this variable range of voltage, roller to collector distance was also varied from 10-19 cm and the effect was noted. The findings are tabulated in the Table (2). It was observed that the formation of cone usually started at around 35 kV for all the solutions when the roller-collector distance was 12 cm. But the fiber formation for all the solutions started at around 45 kV at the same distance. Mass fibers formation throughout the whole body of the roller was observed in all polymeric blend solutions at voltage range of 72-77 kV, with roller to collector distance keeping at 12 cm. At the roller-collector distance of 10cm and 11cm sparks was observed without the formation of fibers. To get the maximum output from the machine, it is necessary that the fibers should form from the whole body of the roller.

Table (2): Roller-collector distance and voltage standardization for electrospinning

S.No.	Polymer Solution	Distance (cm)	Voltage (V)	Comments
A.	**2% Alginate**	11-19	35-80	o fibers; Cone formation at 50 kV; Sparking at 80 kV
B.	**10%PVA+2% Alginate**			
1	80:20	11-19	35-80	Cone Formation at 35 kV; Fiber formation at 72 kV and 12 cm
2.	70:30	11-19	35-80	Cone Formation at 35 kV; Fiber formation at 76 kV and 12 cm
3.	60:40	11-19	35-80	Cone Formation at 35 kV; Fiber formation at 75 kV and 12 cm
4.	50:50	11-19	35-80	Cone Formation at 35 kV; Fiber formation at 72 kV and 12 cm
5.	40:60	11-19	35-80	Cone Formation at 35 kV; Fiber formation at 72 kV and 12 cm

It is importance to mention that above the 12 cm roller-collector distances and within the voltage capability of the machine, fibers were never seen to be coming out of the whole body of the roller. Thus the optimized roller-collector distance was found to be 12 cm. In all the cases the rotational speed of the roller was kept 16 rpm because that is the maximum achievable rotational speed of the roller. Normally, higher rotational speed of roller leads to lower diameter of the nanofibers. It was also found that the control, that is, 2% sodium alginate solution was not electrospinnable using the machine.

Scanning Electron Microscopy (SEM)

Surface morphology of electrospun fibers was examined through SEM. Different locations were selected to evaluate the diameter of different fibers and an average of all the recorded diameters was calculated. It was found that the fibers obtained are of diameter 100nm-400nm. Average diameters for all five samples can be seen in Figure (1).

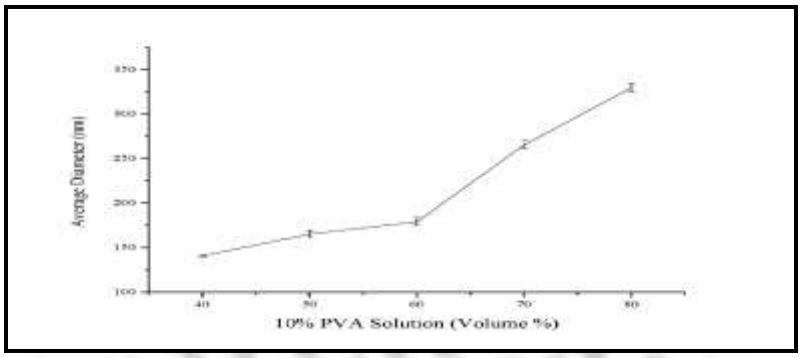

Figure (1): Average diameters of electrospun nanofibers with 10% PVA and 2% sodium alginate

It was observed that with the increase in the amount of 2% sodium alginate in the blended solutions, there was a decrease in the diameter of the fibers. Formation of small beads was also observed in the blended solutions having PVA and sodium alginate in the ratio of 40:60 (Figure (2(e))). The webs were observed in the solutions having PVA and sodium alginate in the ratio of 50:50 (Figure (2(d))).So, the 60:40 ratios of PVA and sodium alginate (2(c)) was found to have the minimum diameter without any imperfections.

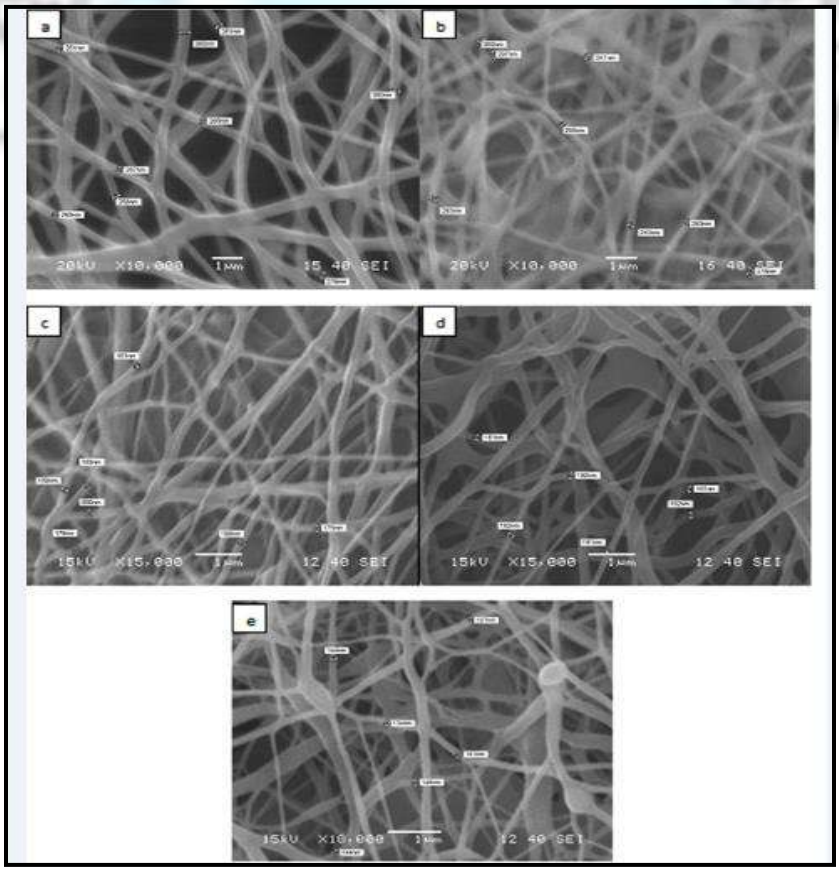

Figure (2): Scanning Electron Microscopy of the PVA – Alginate blend nanofibers in the ratios of (A) 80:20, (B) 70:30, (C) 60:40, (D) 50:50, and (E) 40:60

Fourier-transform infrared (FTIR) spectroscopy

In FTIR analysis, PVA show various peaks confirming the presence of different functional groups like CH_2 group at 2944 cm^{-1}, C-O group at 1096 cm^{-1} and hydroxyl group at 3435 cm^{-1} [14]. On the other hand, sodium alginate shows a characteristic peak at 3430 cm^{-1} for hydroxyl groups. Asymmetric and symmetric $–COO^-$ group peaks correspond to wave-number of 1615 cm^{-1} and 1417 cm^{-1} respectively. For the alginate sample, peaks were observed at 3430, 1615 and 1417 cm^{-1}.

Figure (3(A)) shows the FTIR spectra of different polymeric blend nanofibers with PVA. It was also observed, that hydroxyl peaks became broader as the quantity of alginate increased. It may be because of the formation of hydrogen bonding formed between hydroxyl groups of PVA and sodium alginate. According to Shahidul et al., the addition of PVA could improve the electrospinnability of alginate by stabilizing the bonds formed between the alginate molecules. Thus, it could be suggested that the incorporation of PVA improves the hydrogen bond formations as well as the electrospinnability of alginate.

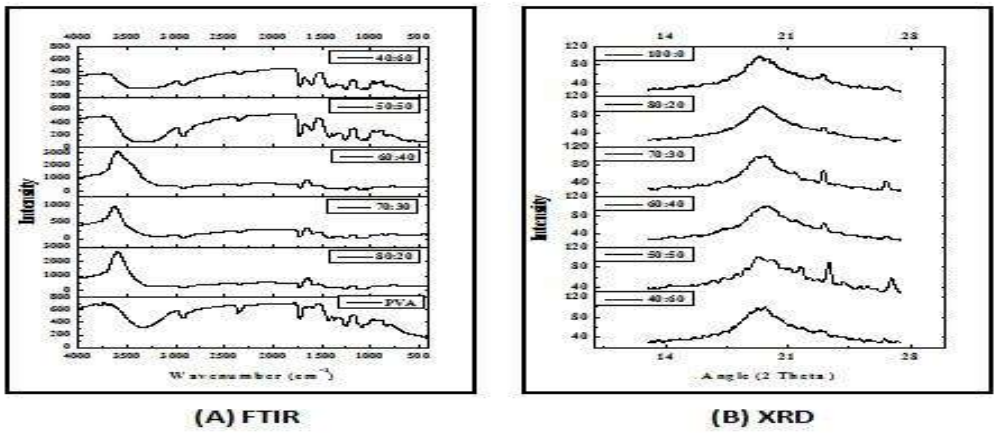

Figure (3): (A) FTIR and (B) XRD analysis data for electrospun nanofibers with 10% PVA and 2% sodium alginate in the different blended ratios

X-Ray diffraction (XRD)

Figure (3(B)) describes the XRD patterns of PVA and sodium alginate blend polymeric fibers. The diffractograms of the samples were analyzed using X'Pert Highscore (Version 1.0b, Philips analytical B.V). XRD pattern of PVA showed the sharp peak at 19.3° 2θ, corresponding to the Bragg's distance (d- spacing) of 4.583 Å, which was found to be in corroboration with the findings of Md. Shahidul et al. [14]. As the concentration of sodium alginate is increased in the corresponding blends, the peak of 19.3º became broader which may be due to reduction in crystallinity of the solutions. It was also confirmed from the increase in the Full Width Half Maximum (FWHM) (Table (3)). This reduction of crystallinity in turn may be due to the intermolecular hydrogen bonding between sodium alginate with PVA which was also evident from the FTIR studies.

Table (3): Peak Position (2θ) and FWHM values for different polymeric blend solutions.

S.No.	Concentration (PVA: Sodium Alginate)	Peak Position (2θ)	FWHM
1	100:0	19.34	1.102
2	80:20	19.60	1.152
3	70:30	19.84	1.259
4.	60:40	19.78	1.570
5.	50:50	19.55	1.5744
6.	40:60	19.63	1.7280

Thermal Analysis

According to the literature, PVA undergoes loss of water and breakdown into monomers when subjected to high temperatures more than 200ºC and 400ºC respectively [14]. Figure 4 shows the thermal profile of PVA and that of blended solutions of PVA with sodium alginate. The steep declination seen in the thermogram, up to temperature 100°C and then around temperature 230 - 260°C, mostly indicate the evaporation of moisture absorbed by the sample and thermal degradation of PVA in all the samples respectively. Literature also suggests that PVA forms a conjugated polyene structure around 245 ºC with elimination of water molecules [15]. It was also observed that with an increase in the amount of alginate, the degradation temperature was found to increase.

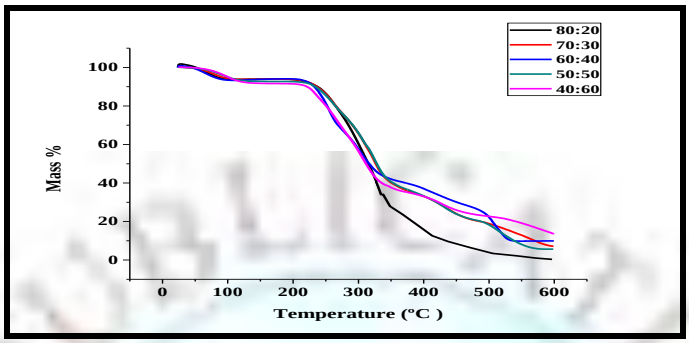

Figure (4): Thermogravitometric analysis data for electrospun PVA and alginate blend solutions.

CONCLUSION

The optimized process parameters of nozzle free electrospinning can be a useful in the industrial mass scale productions of alginate nanofibers. Hydrogen bonding between PVA and sodium alginate improves the electrospinning ability of alginate. Blends of 2% sodium alginate and 10% PVA in different volumetric ratios were found to be a suitable combination for the fabrication of uniform nanofibers. Incorporation of higher amounts of sodium alginate (10% PVA: 2% alginate = 60:40) was found to be helpful in reduction of fiber diameters without any formation of webs or beads. The optimized processes can be used as an applicative measure in tissue engineering, regenerative medicines and wound healing. Incorporation of certain potential drugs in the formulation would fetch a better wound dressing material.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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