Polymer Journal (2011) 43, 654–661

www.nature.com/pj

ORIGINAL ARTICLE

Development of non-woven nanofibers of egg albumen-poly (vinyl alcohol) blends: influence of solution properties on morphology of nanofibers

Gundloori Venkata Naga Rathna[1], Jyoti Prakash Jog[1] and Anil Bhikaji Gaikwad[2]

Egg albumen (EA), a highly functional globular protein with desirable properties, is the least-explored material for biomaterial applications, although it is available in abundance. In our studies, we explored the viability of EA and various blends with biocompatible and non-toxic poly (vinyl alcohol) (PVA) to produce nanofibers for biomedical applications. EA and PVA blends were prepared in various compositions. Electrospinning was used to fabricate non-woven nanofibers. Solution properties, such as viscosity and electrical conductivity, were evaluated for various prepared solutions. Solution viscosity increased with increasing polymer concentration. Solutions with higher contents of EA recorded increased conductivity, which decreased with increasing PVA content. The influence of solution properties on the morphological appearance of as-spun products was studied using scanning electron microscopy. Instead of nanofibers, nanoparticles and microparticles of EA were produced at even higher contents. In contrast, a gradual increase in the addition of PVA content to 8% EA solution resulted in the transformation of particles from large agglomerates to very fine fibers ($\approx$100 nm in diameter) because of the influence of polymer content, viscosity and conductivity. The polymer–polymer interactions in the prepared materials have been validated by Fourier transform infrared spectroscopy, differential scanning calorimetry, X-ray diffraction and gel electrophoresis.
Polymer Journal (2011) **43,** 654–661; doi:10.1038/pj.2011.34; published online 18 May 2011

Keywords: biocompatibility; biodegradable; blends; nanotechnology; proteins

INTRODUCTION

Nanofibers[1–6] derived from natural or synthetic polymers are gaining importance in areas such as biomedicine, food science, cosmetics and pharmaceuticals for novel applications. These polymeric nanofibers are gaining value because of their intrinsic properties, including high surface area, flexibility, enhanced hydrophilicity, biocompatibility and biorecognition.

From the literature,[1–6] it is understood that very fine polymer-based nanofibers can be produced using electrospinning, which is a relatively simple and versatile technique to achieve smooth, fine nanofibers with large surface areas. Nanofibers obtained using the electrospinning technique are considered more oriented and therefore exhibit better mechanical and thermal properties than do those obtained using other methods.[7–10] Hence, the development of nanofibers based on biopolymers is gaining increasing attention for applications in food, cosmetics and biomedicine.[1,4–6] At present, natural and synthetic polymer-based electrospun nanofibers[1,4–6] are used to fabricate filters, cosmetic skin masks, nanosensors, military protective clothing, control drug delivery matrices and wound dressings.

Earlier investigations[2,4] have specified that highly functional proteins are the best candidates for developing electrospun nanofibers for biomedical applications because of their outstanding properties, such as water solubility, biocompatibility and biodegradability: moreover, proteins can be tailored as desired by modifying their functional groups. In addition, proteins are often readily available in abundance as byproducts of food processing and are economically viable. Gelatin, fibrin, collagen and elastin are fibrous proteins that, at higher concentrations, have the ability to form nanofibers.[2,5,11–13] However, protein alone may not always produce nanofibers with desired diameters ($<$100 nm) owing to either a mild or a complete lack of viscoelastic properties. Conversely, these nanofibers lack sufficiently strong mechanical, thermal and barrier properties, thereby limiting the scope of their applications. To some extent, investigations were performed to develop nanofibers using synthetic and fibrous proteins so as to improve their properties.[14,15] In addition, nanofibers based on globular proteins such as soy,[16] wheat,[17,18] zein[19] and bovine serum albumin[20] were also studied. Wongsasulak *et al.*[21] have studied the electrospinning of egg albumen (EA)-polyethylene oxide and clearly explained the influence of polyethylene oxide in the development of nanofibers and their physical and thermal properties.

Egg albumen is a water-soluble and highly functional globular protein.[22] It is available in abundance as a byproduct of food

[1]Polymer Science and Engineering, National Chemical Laboratory, Pune, Maharashtra, India and [2]Centre for Materials Characterization, National Chemical Laboratory, Pune, Maharashtra, India
Correspondence: Dr GVN Rathna, Polymer Science and Engineering, National Chemical Laboratory, Homi Bhabha Road, Pashan, Pune 411008, Maharashtra, India.
E-mail: rv.gundloori@ncl.res.in
Received 7 December 2010; revised 8 March 2011; accepted 11 March 2011; published online 18 May 2011

processing and, hence, is economically viable. The various functional groups that are present in EA are –SH, –OH, –COOH and $–NH_2$. The sulfhydryl groups in EA form covalent disulfide (S–S) bonds at 70–80 °C, leading to irreversible gel formation. EA is used in food as a food matrix[23] and in the cosmetics industry as an emulsifier and thickener.[24] However, the use of EA in 'value-added' non-food applications such as drug-delivery matrices, scaffolds for tissue engineering and as matrices for wound dressing have not been extensively investigated.

Electrospinning is a complex process wherein the formation of nanofibers is governed by a number of parameters and solution properties. Accordingly, it has been interesting to explore the electrospinning of globular proteins with poly (vinyl alcohol) (PVA), which is a thermally and mechanically strong, hydrophilic, flexible, biocompatible and biodegradable, as well as Food and Drug Administration-approved synthetic polymer. Thus, PVA is being used in food, biomedical, cosmetic and pharmaceutical applications.[25–27] In addition to these properties, PVA has the ability to form nanofibers.[28,29] In this communication, we report the electrospinning of PVA-EA blends and their characterization.

EXPERIMENTAL PROCEDURE

Materials

Egg albumen (14–43 kDa) and formic acid 85% were procured from Merck (Mumbai, Maharashtra, India), and PVA of molecular weight ~1 25 000 Da was obtained from S.D. Fine Chemicals Ltd (Mumbai, Maharashtra, India). CBB G-250 (Coomassie brilliant blue), sodium dodecyl sulfate and acrylamide were procured from Sigma-Aldrich (St Louis, MO, USA).

Methods

Various compositions of EA-PVA blends were prepared by dissolving EA and PVA polymers in 85 wt% formic acid. The respective polymers and their blends were dissolved in a water bath at 60–70 °C. The various compositional ratios of EA:PVA (1:0, 1:0.2, 1:0.5, 1:0.8. 1:1 and 0:1) with the total polymer concentration varying from 8 to 16 wt% were prepared as listed in Table 1. Dispersions of PVA, a high-molecular-weight polymer, were left overnight in a water bath at 60–70 °C for dissolution. For rapid and homogeneous dissolution, dispersions were frequently vortexed. Before electrospinning, solutions were cooled to room temperature.

Viscosity

Viscosity measurements for all polymer solutions were performed using a Brookfield DV-II+ Prov, programmable viscometer (Middleboro, MA, USA). The solution sample (1 ml) was taken in a sample cup, and the cone spindle, CPE 42, was used for measurements at a shear rate of $5.75\,s^{-1}$. Viscosity measurements for all of the compositions were conducted in duplicate at 25 °C.

Table 1 Compositions of EA and PVA polymer dispersions, denoted as ratios of EA and PVA

Serial no.	*Ratio of polymer composition EA:PVA*	*Sample code*	*Viscosity (cP)*	*Conductivity ($mS\,cm^{-1}$)*
1	1:0	EA-1	6	7.46
2	1:0.2	EA-2	13.2	7.2
3	1:0.5	EA-3	40.8	6.63
4	1:0.8	EA-4	181	6.15
5	1:1	EA-5	394	5.9
6	0:1	PVA-6	183	2.4
7	2:0	EA-7	13.0	8.0
8	2.5:0	EA-8	55.8	8.56

Abbreviations: EA, egg albumen; PVA, poly (vinyl alcohol).
Viscosities and conductivities of polymers and their blends are shown herein.

Conductivity

A Mettler Toledo SevenMulti (Mettler Toledo, Columbus, OH, USA) device, a digital and dual instrument with a provision to measure both pH and conductivity, was used to measure conductivity of the solutions. Conductivity measurements for all samples were conducted twice at 25 °C, and the data are expressed as the mean of the two measurements.

Electrospinning

A 10-ml syringe equipped with a blunt-ended stainless steel hypodermic needle with a pore diameter of 0.8 mm was used as a nozzle. A syringe filled with 8 ml polymer blend solution was mounted on a syringe pump using a controller (Model 351, SAGE Instruments, Division of Orion Research Development, Boston, MA, USA) to control the flow rate of the solution. The syringe needle was connected to a high-voltage generator (GAMMA High RR40-3.75/DDPM, voltage-regulated DC power supply; Ormond Beach, FL, USA) operated in a positive DC mode. An aluminum plate set in a closed chamber was used as a grounded collector for nanofibers. The experiments were performed under the following conditions: the distance between the tip of the needle and the collector was 15 cm; the flow rate and the applied voltage were 0.075 ml min and 15 kV, respectively. All experiments were performed three times under identical conditions and at room temperature and the samples analyzed.

Scanning electron microscopy

The morphological appearance of the electrospun nanofibers was evaluated using scanning electron microscopy (SEM) (SEM-EDAX, Micro Analysis System, and Model Phoenix, Cambridge, England, UK). The sample used for SEM observations was prepared by mounting a small portion of grounded non-woven nanofibers on the SEM stub. Later, the mounted stub was sputtered with gold using a Coating Unit E5000 (Polaron Equipment Ltd., Watford, Hertfordshire, England, UK). The micrographic images of various nanofibers were scanned at 10 K. The average diameters of the particles and fibers were determined from SEM images.

Fourier transform infrared spectroscopy analysis

The functional characteristics of the electrospun nanofibers of the polymers were recorded using a Fourier transform infrared spectrometer (Perkin-Elmer, spectrometer I, Fourier transform infrared spectroscopy (FTIR) diffused reflectance (DRIFT) mode, Boesch, Huenenberg, Switzerland). Spectra recording were performed in the wavelength range from 4000 to $400\,cm^{-1}$ with a resolution of $4\,cm^{-1}$. Each spectrum was composed of an average of eight scans.

Differential scanning calorimeter

Thermal analysis was performed using a differential scanning calorimeter (Model Q10 DSC, TA Instrument, New Castle, DE, USA). Overall, 5–6 mg of the sample (polymer/nanofiber mat) was loaded in a differential scanning calorimetry (DSC) pan, and the pan was sealed by applying pressure. The sample was equilibrated to −90 °C for 2 min and in the first cycle, the sample was heated to 200 °C at 10 °C per min. In the second cycle, the sample was quenched to −80 °C at 100 °C per min. In the third cycle, the sample was heated to 200 °C to 5 °C per min. The same method was followed for all of the samples. Experiments were repeated twice for reproducibility.

X-ray diffraction

The powder samples of polymers and their nanofibers were characterized using a Philips 1830 X-ray diffractometer (Philips, Amelo, The Netherlands). X-rays were generated by a Cu Kα source at a wavelength of 1.54 Å. The samples were scanned in the 2θ range of 3–50 °C to investigate the change in crystal structure before and after the formation of nanofibers.

Gel electrophoresis

In addition to FTIR, DSC and X-ray diffraction (XRD) analyses, gel-electrophoresis studies were also performed to provide additional support to confirm the interactions between EA and PVA. Tetracell Bio-Rad gel electrophoresis (Bio-Rad, Hercules, CA, USA) was used for the studies. Samples prepared in 85% formic acid were further diluted with water to attain a concentration of $10\,\mu g\,\mu l^{-1}$. All samples were stained with CBB G-250 (Coomassie brilliant blue)

and loaded into the respective wells of 12% SDS-PAGE, and the gel was run at a constant voltage of 150 V for 1.5 h.

RESULTS AND DISCUSSION

Preparation of EA-PVA blend solutions

In our investigations, we aimed to produce non-woven nanofibers using solutions of EA, PVA and their blends. In addition, we evaluated the effect of solution properties on the morphology of nanofiber formation. To begin with, we tried to dissolve various blend compositions of EA and PVA in an aqueous medium as both polymers are water soluble. PVA is hot water soluble, whereas EA soluble at room temperature but, gels at high temperature; hence, PVA and EA were dissolved separately, and solutions were mixed in different ratios at room temperature. However, upon mixing the solutions, EA coagulated and phase separated from the solution of PVA (linear polymer) because of its globular structure.

From the literature, it was understood that a volatile solvent was better than water in achieving nanofibers.[2,5] Accordingly, formic acid was identified as a good organic solvent for various protein polymers to produce nanofibers because formic acid is more volatile than water.[21,30–35] Furthermore, formic acid is also considered non-toxic and is used in edible chitosan films.[36] The solution viscosity for globular proteins is reported to be higher in formic acid than in water[35] because of the unfolding and swelling of globular proteins. As a result of unfolding and swelling of EA in formic acid,[31] the interactions between EA and PVA in the blends would be enhanced, leading to a homogenous solution for electrospinning. The existence of these interactions was further analyzed using FTIR, DSC, XRD and gel-electrophoresis techniques.

Electrospinning of EA-PVA blends

Figures 1a–h show SEM images for the electrospun polymers and blends of various compositions of EA and PVA. The micrographs of the various compositions listed in Table 1 were scanned at 10 K. Figures 1a–c show electrosprayed micrograph images for 8, 16 and 20% solutions of pure EA-1, EA-7 and EA-8, respectively. From these figures, we recognized that EA solutions were electrosprayed instead of being electrospun. As a result of electrospraying, separate aggregates of nanoparticles or microparticles were produced. The shape and size of the particles were irregular and non-uniform, with an average particle size in the range of 400 nm to 2 μm. To determine the feasibility of pure EA to produce nanofibers, we further increased the EA concentration to 25%, but the solution gelled. The failure of EA to produce nanofibers is because of its deficient spinnability (viscoelastic property). These results were similar to those reported by Wongsasulak *et al.*[21] However, their EA solutions gelled beyond 5%, unlike the EA solutions we used, which were stable up to 20%. The observed difference may be attributed to the difference in EA molecular weights and the concentration of formic acid.

According to earlier reports, PVA demonstrates the ability to produce nanofibers because of its viscoeleastic properties.[28,29] Thus, blends of EA with PVA were prepared to impart electrospinning ability to EA. As can be seen from SEM images (Figures 1a–g), blending of PVA with EA enabled the electrospinning of EA at various compositions as listed in Table 1. Figures 1a–c represent the electrospinning of EA solutions at various concentrations (8, 16 and 20% by weight). No fibers could be seen in SEM images, confirming the inability of EA to be electrospun. Figures 1d–g are micrographic images of the blends containing increasing amounts of PVA. In Figures 1d–g, it is observed that nanofiber formation is achieved, with the gradual transformation of particles to agglomerates to beaded fibers and finally to smooth fibers, which is attributed to the increasing addition of PVA content in the blend solution. Thus, the gradual transformation of particles to nanofibers was noted as the content of PVA increased. The evolution

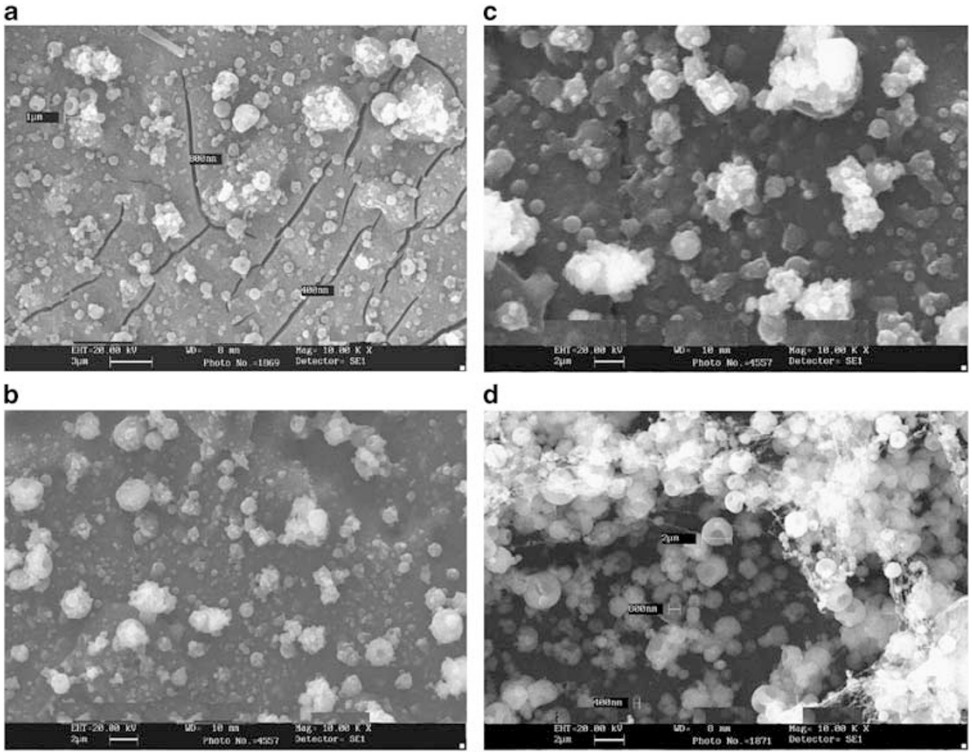

Figure 1 SEM images of electrospun structures of polymer compositions. (**a**) EA-1 (8%, 1:0), (**b**) EA-7 (16%), (**c**) EA-8 (20%) (**d**) EA-2 (1:0.2), (**e**) EA-3 (1:0.5), (**f**) EA-4 (1:0.8), (**g**) EA-5 (1:1), (**h**) pure PVA (8%, 0:1). EA, egg albumen; PVA, poly (vinyl alcohol); SEM, scanning electron microscopy.

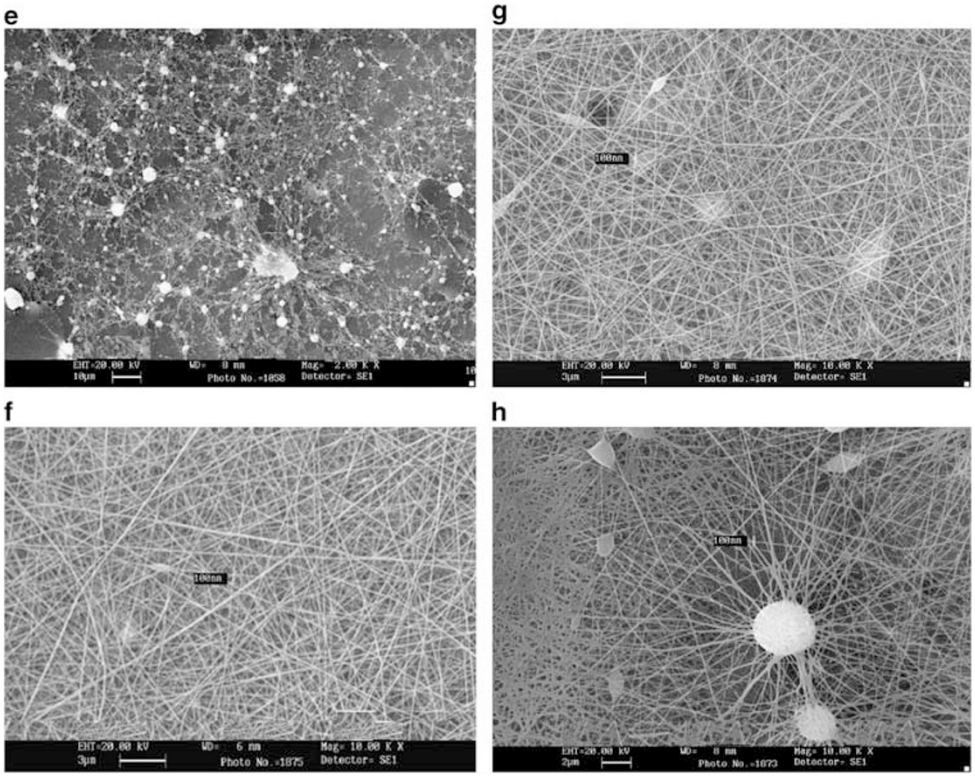

Figure 1 Continued.

of EA-PVA nanofibers can be explained on the basis of blend compositions, viscosity and conductivity, which have significant roles in electrospinning.

Solution properties of polymer compositions and their influence on morphology of nanofibers

It was well documented[36–40] that the process of electrospinning is affected or controlled by process parameters such as spinning voltage, solution flow rate, solvent volatility and the distance between the needle tip and the ground electrode. The structure and morphology of electrospun fibers are also influenced or regulated by solution properties, such as polymer content, polymer molecular weight, viscosity, conductivity, dielectric constant and surface tension.[36–40] In electrospinning, one of the conditions required for the formation of nanofibers is that the polymer solution should have an optimum viscosity. Below the optimal viscosity, electrospraying of a solution occurs; above the threshold, the polymer is dried. The optimum viscosity varies from polymer to polymer and can be determined by trial experiments. The desired viscosity can be obtained either by increasing the molecular weight of the polymer or by increasing the polymer content in the blend. During electrospinning, a jet of polymer solution leaves the tip of the needle, wherein the polymer is stretched and entangled as it travels toward the collector. This entanglement of the polymer chains prevents the electrically driven jet from breaking the continuity of fiber formation. With an increase in viscosity, the entanglements of polymer chains are increased, which is required to maintain the continuity of the jet during electrospinning.[36–40] Surface tension also has an important role in nanofiber formation. If surface tension is dominant over viscosity, fibers containing beads are formed because of the presence of more solvent molecules and fewer chain entanglements. The formation of nanofibers is improved if viscosity dominates surface tension, provided the polymer satisfies the viscoelastic requirement. At higher viscosity, the amount of polymer chain entanglements is increased, and the charges on the electrospinning jet allow the polymer solution to stretch with an even distribution of solvent molecules; hence, smooth and continuous nanofibers are formed.[36–40]

In our studies, the processing parameters were fixed to study the influences of solution properties on the morphology of formed nanofibers.

The viscosity measurements of all polymers and blend solutions were recorded using a spindle CPE 42 at a shear rate of 5.75 s^{-1} at 25 °C. The solution conductivity of various polymers and blends was recorded using a conductivity electrode at 25 °C. According to earlier findings,[21] and as explained above, our studies also revealed increases in viscosity and conductivity (viscosity is inversely proportional to surface tension) with increased pure EA content from 8 to 25%; thus, we expected nanofiber formation (Table 1). However, our experiments proved that pure EA protein was unable to produce nanofibers; even though the protein had sufficient polymer concentration, viscosity, conductivity and surface tension, it lacked viscoelastic properties. The viscosities of various blend compositions prepared by blending 8% of EA with an increased ratio of PVA in the blend composition were investigated. The results are expressed in a tabular form (Table 1) and in a graphical form (Figure 2). The contour in Figure 2 represents changes in viscosity as a function of the EA-to-PVA ratio. There was a significant increase in viscosity from 6 to 13.2, 40.8, 181 and 394 cP with an increase in PVA content from 1.6 to 8%, which indicated ~2-, 6-, 30- and 65-fold increases in viscosity over 8% EA (EA-1). The difference in viscosity with the addition of PVA could be attributed to the increased interactions and entanglements between polymer chains as a result of increased concentration of PVA.[17,21,40]

The influence of viscosity on the gradual changes in the morphology of nanofibers in EA with various concentrations of PVA can be observed in Figures 1d–g. The results clearly indicate that an increase

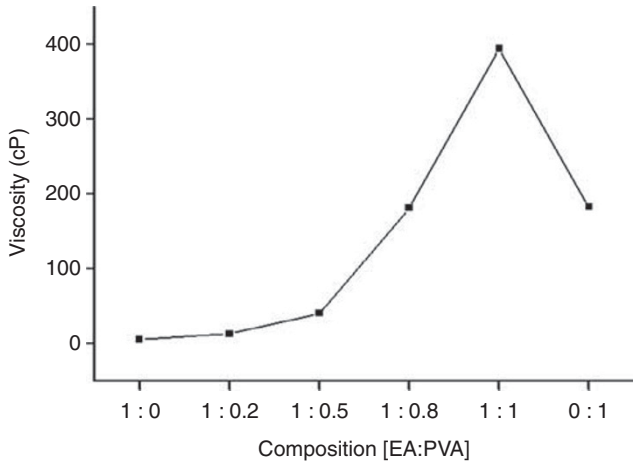

Figure 2 Viscosity measurements for dispersions of polymeric blends (EA-PVA) as functions of composition ratios. EA, egg albumen; PVA, poly (vinyl alcohol).

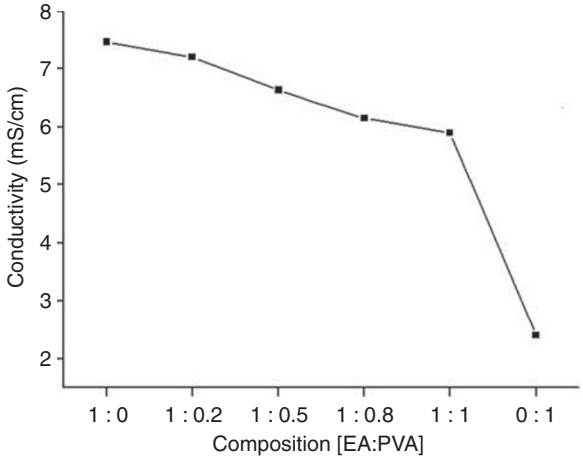

Figure 3 Conductivity measurements for dispersions of polymeric blends (EA-PVA) as functions of composition ratios. EA, egg albumen; PVA, poly (vinyl alcohol).

in viscosity due to the addition of 1.6% of PVA to an 8% EA solution in the blend composition enabled a transformation from nanoparticles and microparticles of EA (Figure 1a) to large agglomerates with connecting fibers (Figure 1d). Moreover, a further increase in viscosity with the addition of 4, 6.4 or 8% PVA to an 8% EA solution causes the agglomerates to form fibers containing beads (Figure 1e) and to form very fine nanofibers that were $\sim$100 nm in diameter (Figures 1f and g). The above findings indicate that an increased addition of PVA, a viscoelastic polymer, to the blend composition caused an increase in viscosity with an apparent reduction in surface tension, owing to the strong molecular interactions (polymer solvent) and chain entanglements (polymer–polymer) that occurred between PVA and EA. Consequently, during electrospinning, the electrically driven jet was prevented from breaking the continuous formation of fibers and thus produced fine nanofibers. Pure 8% PVA-6 recorded a viscosity of 183 cP, which was 30 times higher than for EA-1 (6 cP) at the same content. At a viscosity of 183 cP, pure PVA-6 produced nanofibers with large bulbs instead of smooth fibers, whereas a blend of EA-PVA (EA-4), with a viscosity of 181 cP, produced smooth nanofibers that were 100 nm in diameter. The reason for this ambiguity is explained in the subsequent paragraph.

Table 1 and Figure 3 illustrate the electrical conductivity of EA, PVA and the blends of EA-PVA. In Figure 3, it can be observed that the conductivity of the 8% EA solution gradually decreased from 7.46 to 7.2, 6.63, 6.15 and 5.9 mS cm^{-1} with EA:PVA ratios of 1:0, 1:0.2, 1:0.5 and 1:0.8, respectively. The decrease in conductivity with increase in PVA content to EA in the solution could be attributed to the decrease in the number of charged species in the formic acid solution. In general, during electrospinning, the solution is stretched because of the repulsion between the charges located on the surface. The electrospinning jet carries more charges if the conductivity of the solution is high, which increases the stretching of the solution. As a result, smooth nanofibers are formed. By and large, solutions of natural polymers carry more ions than do synthetic polymers because of their high functionality. Hence, in our studies, the pure EA solution recorded the highest conductivity because of its high functionality, which increased with an increase in EA content. The 8, 16 and 20% pure EA solutions recorded conductivities of 7.46, 8.0 and 8.56 mS cm^{-1}, respectively (Table 1). Nevertheless, in Figures 1a–c, it can be observed that the presence of a large number of ions with an increase in the content of EA did not benefit nanofiber production as EA lacked viscoelasticity. On the other hand, as a synthetic and less functional polymer, the pure 8% PVA solution recorded the lowest conductivity (2.4 mS cm^{-1}). This polymeric solution, in spite of its low conductivity, still produced nanofibers, owing to its high viscoelasticity (Figure 1h). Nonetheless, the so-formed nanofibers contained a significant number of large beads because of deficient conductivity or viscosity at that concentration. These large beads disappeared and fine fibers were produced when blended with an 8% EA solution (EA-5, 1:1). As explained above, with the addition of EA, the conductivity of PVA increased from 30 to 80% (2.4–5.9 mS cm^{-1}). Owing to the 50% increase in the conductivity, the repulsion between charges increased, allowing for increased stretching in the polymer solution; thus, smooth fibers were formed. A similar effect was observed for the blend EA-4 (1:0.8). However, the blends EA-3 and EA-2 lacked the desired viscosity and conductivity for the formation of smooth fibers; hence, agglomerates and beaded fibers were obtained. Studies based on EA-PVA blends revealed that to form nanofibers, it is essential for the polymer solutions to have the desired viscosity and conductivity, both of which differ from polymer to polymer.

FTIR analysis

Figures 4a and b represent the FTIR spectra for the pure EA-1 and PVA-6 nanofibers and nanofibers of blends of different compositions of EA and PVA (EA-2, EA-3, EA-4 and EA-5). The spectra of pure EA-1 showed peaks at 3286 and 3070 cm^{-1} due to –NH stretching of the secondary amide, –C=O stretching at 1658 cm^{-1} (amide-I) and –NH bending at 1542 cm^{-1} (amide II), –CH stretching at 2962 cm^{-1} and a weak peak at 2869 cm^{-1} due to –SH stretching present in EA. A peak due to plane wagging was observed at 704 cm^{-1}. The FTIR (Figure 4a) spectra of PVA-6 nanofibers showed a broad peak at 3159 cm^{-1}, indicating –OH stretching and a prominent and significant peak at 2922 cm^{-1} due to the backbone of –CH stretching of PVA and a strong peak at 1717 due to –C=O. The spectra of the blends of the EA-PVA nanofibers (EA-2, EA-3, EA-4 and EA-5) showed all of the peaks because of the presence of EA and PVA, except for a few changes due to hydrogen interactions between the polymers. A single and broad peak appeared at 3286 cm^{-1} due to the merging of the characteristic peaks of –OH and –NH of PVA and EA, respectively, indicating interactions between the polymers. The broadness of the peaks increased with an increase in PVA content in the blend composition. As shown in Figure 4b, the broadness of the peak

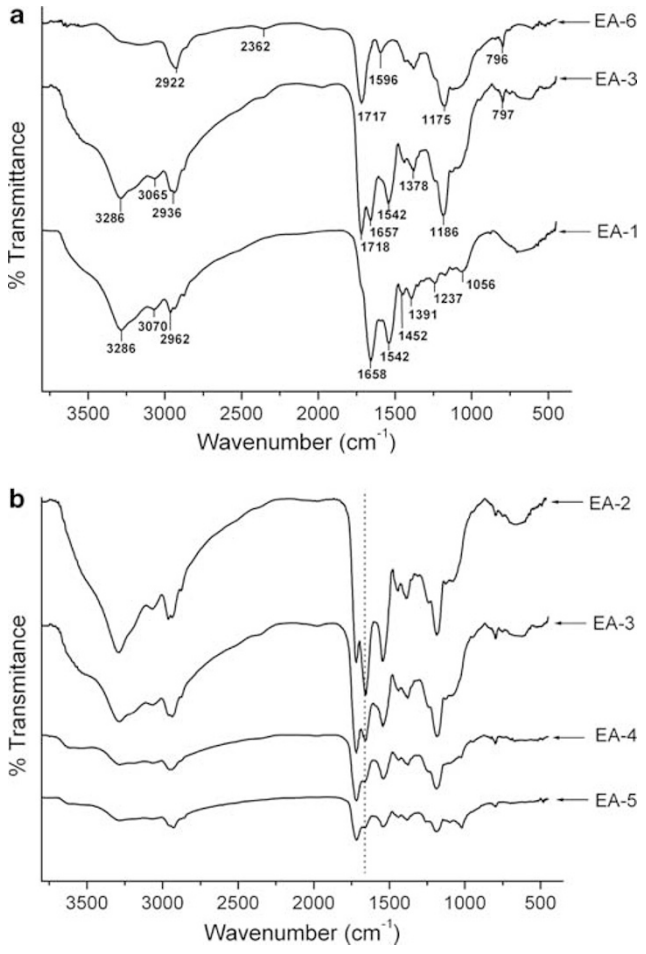

Figure 4 (**a**) FTIR spectra (4000–400 cm^{-1}) of electrospun nanofibers for EA-1, PVA-6 and blend of EA-PVA, that is EA-3. (**b**) The merging and broadness of the carbonyl peak and amide I peaks of PVA and EA, respectively, due to polymer–polymer interactions. EA, egg albumen; FTIR, Fourier transform infrared spectroscopy; PVA, poly (vinyl alcohol).

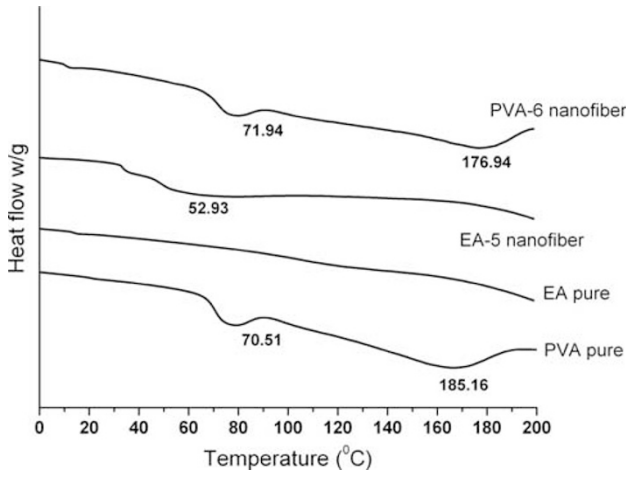

Figure 5 DSC thermographs of pure polymers and nanofibers of pure polymers (PVA and EA) and blend EA-5. DSC, differential scanning calorimetry; EA, egg albumen; PVA, poly (vinyl alcohol).

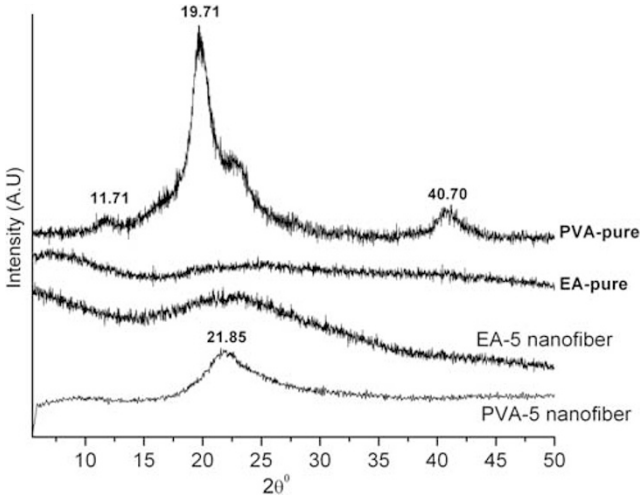

Figure 6 XRD patterns of pure polymers and nanofibers of pure polymers (PVA and EA) and blend EA-5. EA, egg albumen; PVA, poly (vinyl alcohol); XRD, X-ray diffraction.

increased with an increase in the PVA content in the blend composition due to enhanced intermolecular hydrogen interactions between the EA and PVA polymers. In addition, in the blend, because of polymeric hydrogen interactions, a single peak attributed to –CH stretching appeared at 2936 cm^{-1}. The –C=O peak in the EA-1 shifted from 1658 to 1657 cm^{-1} in EA-2 blends, and the –C=O peak of EA-2 shifted further (1700 cm^{-1}) and merged with the –C=O peak of PVA as the PVA content in the blend increased (Figure 4b) because of the hydrogen bond interactions between –OH and –C=O. As noted in Figures 4a and b, the –C=O peak, which is sharp and prominent in EA-1, merged with the –C=O peak of PVA; hence, a broad band is seen in EA-3 with a slight shift in the peak. With the increase in the PVA content of the blend, the two characteristic peaks merged and the result was one broad peak. As reported,[41] this is caused by the intermolecular interactions between the two polymers in the blend, and it resulted in an amorphous polymer. This interpretation is also supported by DSC and XRD studies.

Differential scanning calorimetric studies

Similar to the findings of others,[42] in our studies, the EA that possessed poor physical properties was blended with PVA at various ratios to improve physical properties. DSC is one of the common methods used to verify phase separation or polymer–polymer miscibility in any of the blends. Typically, changes in the glass transition temperature of blends versus individual homopolymers are determined. When one of the components is crystalline, the melting point depression of the polymer may also be used as evidence of the interactions between polymers. Figure 5 shows the changes that occurred before and after blending the polymers. Owing to its semi-crystallinity, pure PVA recorded a T_g and T_m of 70.5 and 185.1 °C, respectively, whereas nanofibers formed from PVA recorded a T_g and T_m of 71.9 and 176.9 °C, respectively. The depression in the melting temperature indicated a suppression of the degree of crystallinity of the PVA nanofibers, caused by the constrained orientation of molecular chains during electrospinning. This was also evident from the XRD results shown in Figure 6 as the intensity of the crystalline peak decreased. On the other hand, nanofibers of the EA-5 blend registered a new T_g (52.9 °C) without T_m, indicating miscibility due to enhanced interactions between PVA and EA and the loss of crystallinity. It is worth noting that the nanofibers of EA-polyethylene oxide showed an

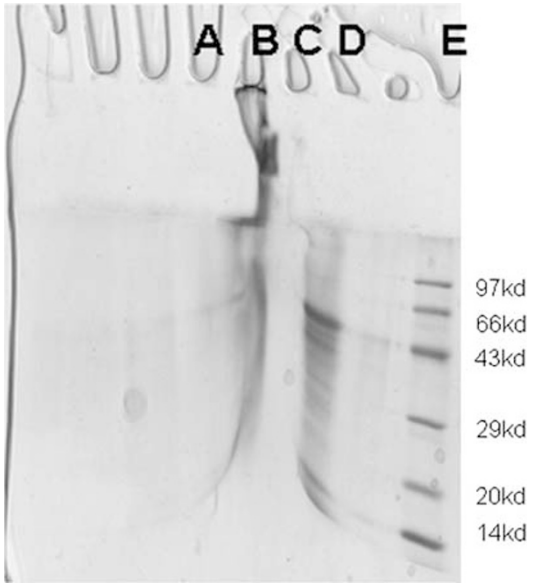

Figure 7 Gel electrophoresis of (A) gelatin, (B) EA-5 (blend), (C) PVA, (D) EA and (E) protein marker. EA, egg albumen; PVA, poly (vinyl alcohol).

increase in T_m with respect to those of polyethylene oxide, which is unlike EA-PVA systems.

XRD studies

The XRD technique has been extensively used to determine the crystallinity in polymer blends. XRD patterns were recorded, as shown in Figure 6, to understand changes in the crystallinity of pure polymers, nanofibers of pure polymers and blends. As a semi-crystalline polymer, PVA exhibited a typical peak[43] at $2\theta=19.71^{\circ}$, whereas no crystalline peak was recorded for pure EA, indicating that it is amorphous. As explained above, EA did not produce any nanofibers; however, the nanofibers of PVA recorded a low-intensity peak at $2\theta=21.85^{\circ}$ because during electrospinning, the flow-induced molecular orientation of molecular chains of the polymer is constrained, resulting in the loss of crystallinity. Meanwhile, the nanofiber produced from the blend EA-5 recorded a very broad peak, indicating the loss of crystallinity due to interactions between the polymer chains of PVA, with EA leading to further restriction of chain mobility.

Gel electrophoresis

In addition to FTIR, DSC and XRD studies, gel-electrophoresis studies were also conducted to support the existence of hydrogen ion interactions between polymers. Figure 7 shows an SDS-PAGE for various polymers and protein markers. Each well was loaded with gelatin, EA-PVA blend, EA, PVA or protein marker. It was observed that gelatin, EA and the protein marker produced multiple bands representing proteins of various molecular weights. In general, SDS-PAGE is used to determine the presence of various molecular weights in proteins (poly peptides); however, as a non-protein, PVA was not stained by CBB G-250; hence, a band representing the molecular weight of PVA was not seen. In contrast to pure EA, the blend of EA-PVA (EA-5, 1:1) recorded a smear, with some part of it being retained in the well instead of separating into bands; this was likely due to suspected hydrogen ion interactions between EA and PVA, which led to chain entanglements. As a result of chain entanglements, the blend became bulky, thereby inhibiting band separation.

Fourier transform infrared spectroscopy, DSC, XRD and gel-electrophoresis studies have enabled us to confirm the interactions between natural and synthetic polymers that enhance the compatibility between polymers. As a result, blends of EA will definitely show improved mechanical and thermal properties essential for biomedical applications.

CONCLUSIONS

In summary, we have shown that pure EA, up to a 20% concentration, could not be electrospun to produce nanofibers because of a lack of viscoelasticity. Blending EA with PVA resulted in enhanced solution properties, which were attributed to the interactions between EA and PVA. Increasing the PVA content in the blend enabled a continuous change in both viscosity and conductivity. As a result, the surface morphology of electrospun mats transformed from that of agglomerates to that of beaded fibers to that of smooth nanofibers. The studies also confirmed that specific polymer content was required to achieve the preferred viscosity and conductivity. The molecular interactions between EA and PVA in the blends were enhanced, leading to a homogenous solution for electrospinning. The existence of these interactions was confirmed using FTIR, DSC, XRD and gel-electrophoresis techniques.

Finally, the biocompatible nanofibers mats of EA-PVA polymer blends may have potential biomedical applications such as in tissue engineering, scaffolds, drug delivery and implants. Studies on these applications are in progress.

ACKNOWLEDGEMENTS

We thank Ms B Hemangi for conducting the gel electrophoresis and Dr PR Chatterji and Dr SD Prasad for their valuable suggestions during our investigations.

1 Zhang, Y., Lim, C. H., Ramakrishana, S. & Huang, Z.- M. Recent development of polymer nanofibers for biomedical and biotechnological applications. *J. Mater. Sci. Mater. Med.* **16,** 933 (2005).
2 Nauanchan, C., Kunawan, A., Duangdao, A.- O., Chidchanok, M. & Pitt, S. Electrospun gelatin fibers: effect of solvent system on morphology and fiber diameters. *Polym. J.* **39,** 622 (2007).
3 Hong, Y., Li, Y., Shang, L., Wang, C., Wang, X. & Jing, X. Synthesis using electrospinning and stabilization of single layer macroporous films and fibrous networks. *J. Memb. Sci.* **276,** 1 (2006).
4 Li, M., , Mondrinos, M. J., Gandhi, M. R., Ko, F. K., Weiss, A. S. & Lelkes, P. I. Electrospun protein fibers for tissue engineering. *Biomaterials* **26,** 5999 (2005).
5 Rho, S. K., Jeong, L., Lee, G., Seo, B.- M., Park, Y. J., Hong, S.- D., Roh, S., Cho, J. J. & Min, B.- Y. Electrospinning of collagen nanofibers: effects on the behavior of normal human keratinocytes and early-stage wound healing. *Biomaterials* **27,** 1452 (2006).
6 Hong, K. H., Park, J. L., Youk, H. J. & Kang, T. J. Preparation of antimicrobial poly (vinyl alcohol) nanofibers containing silver nanoparticles. *J. Polym. Sci. B Polym. Phys.* **44,** 2468 (2006).
7 Huang, Z.- M., Zhang, Y.- Z., Kotaki, M. & Ramakrishna, S. A review on polymer nanofibers by electrospinning and their applications in nanocomposites. *Compos. Sci.Technol.* **63,** 2223 (2003).
8 Bongnitzki, M., Czado, W., Frese, T., Schaper, A., Hellwig, M. & Steinhart, M. Nanostructured fibers via electrospinning. *Adv. Mater.* **13,** 70 (2001).
9 Shao, C., Kim, H. Y., Gong, J., Ding, B., Lee, D. R. & Park, S. J. Fiber mats of poly(vinyl alcohol)/silica composite via electrospinning. *Mater. Lett.* **57,** 1579 (2003).
10 Huang, L., McMillan, R. A., Apkarian, B., Pourdeyhimi, E. L., Conticello, V. P. & Chaikof, E. L. Generation of synthetic elastin-mimetic small diameter fibers and fiber networks. *Macromolecules* **13,** 70 (2000).
11 Matthews, J. A., Winek, G. E., Simpson, D. G. & Bowlin, G. L. Electrospinning of collagen nanofiber. *Biomacromolecules* **3,** 232 (2002).
12 Chen, H.- C., Jao, W.- C. & Yang, M.- C. Characterization of gelatin nanofibers elctrospun using ethanol/formic acid/water as a solvent. *Polym. Adv. Technol.* **20,** 98 (2009).
13 Zoccola, M., Aluigi, A., Vineis, C., Tonin, C., Ferrero, F. & Piacentino, M. G. Study on cast membranes and electrospun nanofibers made from keratin/fibroin blends. *Biomacromolecules* **9,** 2819 (2008).

14 Kim, S. E., Heo, D. N., Lee, J. B., Kim, J. R., Park, S. H., Jeon, S. H. & Kwon, I. K. Electrospun gelatin/polyurethaneblended nanofibers for wound healing. *Biomed. Mater.* **4,** 11 (2009).
15 Huang, L., Nagapundi, K., Apkarian, R. P. & Chaikof, E. L. Engineered collagen- PEO nanofibers and fabrics. *J. Biomater. Sci. Polym.* **12,** 979 (2001).
16 Vega-Lugo, A.- C. & Lim, L.- T. Electrospinning of soy protein isolate nanofibers. *J. Biobased Mater. Bioenergy* **2,** 223 (2008).
17 Jing, D., Alexandru, D. A. & Richard, S. P. Aqueous electrospinning of wheat gluten fibers with thiolated additives. *Polymer* **51,** 3164 (2010).
18 Woerdeman, D. L., Ye, P., Shenoy, S., Parnas, R. S., Wnek, G. E. & Trofimova, O. Novel protein fibers from wheat gluten. *Biomacromolecules* **6,** 707 (2005).
19 Jiang, Q., Reddy, N. & Yang, Y. Cytocompatible cross-linking of electrospun zein fibers for the development of water-stable tissue engineering scaffolds. *Acta. Biomater.* **6,** 4042 (2010).
20 Chandra, M. V., Steven, D. & Xiaojun, Y. Polycaprolactone and bovine serum albumin based nanofibers for controlled release of nerve growth factor. *Biomacromolecules* **10,** 1084 (2009).
21 Wongsasulak, S., Kit, K. M., McClements, D. J., Yovidhya, T. & Weiss, J. The effect of solution properties on the morphology of ultrafine electrospun egg-albumen-PEO composite fibers. *Polymer* **48,** 448 (2007).
22 Rathna, G. V. N., Li, J. & Gunasekaran, S. Functionally-modified egg white albumen hydrogels. *Polym. Int.* **53,** 1994 (2004).
23 Lu, G. H. & Chen, T. C. Application of egg white and plasma powders as muscle food binding agents. *J. Food Eng.* **42,** 147 (1999).
24 Kinsella, J. E. in *In Relationship between Structure and Functional Properties of Food Protein, in Food Protein* (eds Fox, P. F., Condon, J. J.) 51–103 (Applied Science, London, 1985).
25 De-Merlis, C. C. & Schonekar, D. R. Review of the oral toxicity of polyvinyl alcohol (PVA). *Food Chem. Toxicol.* **41,** 319 (2003).
26 Sudhamani, S. R., Prasad, M. S. & Sankar, K. U. DSC and FTIR studies on gellan and polyvinyl alcohol (PVA) blend films. *Food Hydrocolloids* **17,** 245 (2003).
27 Pal, K., Banthia, A. K. & Majumdar, D. K. Preparation and characterization of polyvinyl alcohol - gelatin hydrogel membranes for biomedical applications. *AAPS Phar. Sci. Technol.* **8,** E1 (2007).
28 Safi, S., Morshed, M., Hosseini, S. A., Ravandi, H. & Ghiaci, M. J. Study of electrospinning of sodium alginate, blended solutions of sodium alginate/poly(vinyl alcohol) and sodium alginate/poly(ethylene oxide). *Appl. Polym. Sci.* **104,** 3245 (2007).
29 Naebe, M., Lin, T., Staiger, M. P., Dai, L. & Wang, X. Electrospun SWNT/PVA composite nanofibers; structure-property relationships. *Nanotechnology* **19,** 8 (2008).
30 Bucho, C. J., Chen, L. C., Shen, Y. & Martin, D. C. Processing and microstructural characterization of porous biocompatible protein polymer thin films. *Polymer* **40,** 7397 (1999).
31 Gupta, P., Elkins, C., Long, G. & Wilkes, L. Electrospinning of linear homopolymers of poly (methyl methacrylate): exploring relationships between fiber formation, viscosity, molecular weight and concentration in a good solvent. *Polymer* **46,** 4799 (2005).
32 Reneker, D. H., Yarin, A. L., Fong, H., Fong, H. & Koombhongse, S. Bending instability of electrically charged liquid jets of polymer solution in electrospinning. *J. Appl. Phys.* **87,** 4531 (2000).
33 Ki, C. H., Beak, D. H., Gang, K. D., Lee, K. H., Um, I. C. & Park, Y. H. Characterization of gelatin nanofiber prepared from gelatin–formic acid solution. *Polymer* **46,** 5094 (2005).
34 George, L. & Sander, W. Matrix isolation infrared and ab initio study of the hydrogen bonding between formic acid and water. *Spectrochim. Acta. A* **60,** 3225 (2004).
35 Martin, D. C., Jiang, T. & Buchko, J. in *Protein-Based Materials* (eds McGrath K., Kaplan, D.) (Birkhauser, Boston, 1997).
36 Caner, C., Vergano, P. J. & Wiles, J. L. Chitosan film mechanical and permeation properties as affected by acid, plasticizer, and storage. *J. Food Sci.* **63,** 1049 (1999).
37 Sonar, W. K., York, J. H., Leek, T. S. & Park, W. H. The effects of solution properties and polyelectrolyte on electrospinning of ultrafine poly (ethylene oxide) fibers. *Polymer* **45,** 2959 (2004).
38 Shenoy, S. L., Bates, W. D., Frisch, H. L. & Wnek, G. E. Role of chain entanglements on fiber formation during electrospinning of polymer solutions: good solvent, non-specific polymer–polymer interaction limit. *Polymer* **46,** 3372 (2005).
39 Zong, X., Kim, K., Fang, D., Ran, S., Hsiao, B. S. & Chu, B. Structure and process relationship of electrospun bioabsorbable nanofiber membranes. *Polymer* **43,** 4403 (2002).
40 Ramakrishna, S., Fujihare, K., Teo, W.- E., Lim, T.- K. & Ma, Z. in *An Introduction to Electrospinning and Nanofibers* (World Scientific Publishing Pvt Ltd, Singapore, 2005).
41 Aditya, M. K., Asit, K. C. & Arvind, K. B. FTIR studies on differential intermolecular association in crystalline and amorphous states of structurally releated non-steroidal anti-inflammatory drugs. *Mol. Pharm.* **5,** 937 (2008).
42 Young, J. L., Dong, S. S., Oh, W. K., Won, H. P., Han, G. C., Yong, R. L., Sung, S. H., Seok, K. N. & Won, S. L. Preparation of atactic poly (vinyl alcohol)/sodium alginate blend nanowebs by electrospinning. *J. Appl. Polym. Sci.* **106,** 1337 (2007).
43 Bajpai, A. K., Shefali, M., Bajpai, R. & Katare, R. On the mechanical strength of biocompatible semi-IPNs of polyvinyl alcohol and polyacrylamide. *Microsyst. Technol.* **14,** 193 (2008).