Effect of Solution Properties and Operating Parameters on Needleless Electrospinning of Poly(Ethylene Oxide) Nanofibers Loaded with Bovine Serum Albumin

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Abstract: This paper presents the effect of solution properties and operating parameters of polyethylene oxide (PEO) based nanofiber using a wire electrode-based needleless electrospinning. The feed solution was prepared using a PEO dissolved in water or a water-ethanol mixture. The PEO solution is blended with bovine serum albumin protein (BSA) as a model drug to study the effect of the electrospinning process on the stability of the loaded protein. The polymer solution properties such as viscosity, surface tension, and conductivity were controlled by adjusting the solvent and salt content. The morphology and fiber size distribution of the nanofiber was analyzed using scanning electron microscopy. The results show that the issue of a beaded nanofiber can be eliminated either by increasing the solution viscosity or by the addition of salt and ethanol to the PEO-water system. The addition of salt and solvent produced a high frequency of smaller fiber diameter ranging from 100 to 150 nm. The encapsulation of BSA in PEO nanofiber was characterized by three different spectroscopy techniques (i.e. circular dichroism, Fourier transform infrared, and fluorescence) and the results showed the BSA is well encapsulated in the PEO matrix with no changes in the protein structure. This work may serve as a useful guide for a drug delivery industry to process a nanofiber at a large and continuous scale with a blend of drugs in nanofiber using a wire electrode electrospinning.

Keywords: Needleless electrospinning, polyethylene oxide, electrospinning parameters, drug embedding nanofibers.

1. INTRODUCTION

In the field of biomaterials, a three-dimensional porous material plays a crucial role in drug delivery application. In the past two decades, researchers in the field of targeted drug delivery focused on producing porous materials with the capabilities of loading and delivering drugs along with degradation properties. Obtaining a consistent and producing a bead free fiber in nanoscale is a constant challenge for the nanofiber manufacturing industry.

Electrospinning technology is often used to produce a nanofiber; however, product consistency is very sensitive to the operating conditions and feed material properties. Moreover, the operating condition and feed material properties set up for electrospinning are not always generic to all types of polymer. For instance, the organic solvent-soluble polymer such as PCL does not require strict control of humidity as that of PEO, which is water-soluble. In addition, polymer-like poly(vinyl alcohol) (PVA) readily produces nanofiber when electrospun. However, nanofiber based on PEO is of great interest due to its biocompatibility, since PEO is water-soluble negating the needs of organic solvent in its preparation, thus eliminating the trace solvent contamination in the final product.

Preparation of PEO based nanofiber is challenging owing to the needs of strict moisture control and physical properties i.e. viscosity and conductivity of the feed solution. In some cases, the electrospinning machine design itself plays an important role in obtaining a consistent nanofiber. For instance, the physical properties of the feed polymer changes with the polymer solution concentration during the electrospinning process, thus equipment design that utilizes a large
exposed solvent is prone to produce a discordant fiber. Usually, single needle-based electrospinning has a polymer stored in a syringe not prone to solvent evaporation, whereas the needleless electrospinning technique utilizes a large exposed solvent, hence prone to solvent evaporation. However, the productivity of needleless electrospinning is much greater than that of single needle electrospinning [1, 2], hence it is important to study the effect of solution parameter on the former.

The first-generation needle-free electrospinning was achieved by partially immersing the rotating drum spinneret into the polymer solution bath which in turn forms a multiple jet of fibers produced by Elmarco and commercially known as Nanospider [3]. The rotating drum spinneret design may fail to generate the nanofiber at low viscosity and low rotation speed due to insufficient layer of polymer on the spinneret. Meanwhile, a higher rotation speed increases the chance of Taylor cone formation [4]. Further development in needleless electrospinning paved the way for the development of wire electrode design using a fixed wire strung over the spinning area. The carriage containing the spinning solution move along the wire electrode, hence coating its surface with the solution enabling the nanofiber to spin from the wire. The latest design has a small surface area of the solvent which in turn reduces the solvent evaporation from the reservoir. However, limited study available on the effect of operating parameters and feed solution properties using the fixed wire needleless electrospinning and hence this is the aim of this work.

The fiber quality is affected by the solution properties (viscosity, surface tension, and conductivity) that have to be first optimized for producing the micro or ultrafine fibers [5]. The viscosity of polymer solution is one of the parameters, which can affect the spinning of a polymer solution. According to Tiwari and Venkatraman [6], there are four different concentration regimes for polymer solutions viz dilute, semidilute unentangled, semidilute entangled, and concentrated. A uniform beaded nanofiber is generated when the polymer solution is at semidilute entangled regimes (concentration ≥ Ce). The present work focuses on a range of concentrations between the semidilute unentangled and semidilute entangled regimes, especially showing the effect of solvent type and electrolyte addition to the nanofiber quality.

The polymer solution with low viscosity can lead to the formation of beaded fiber due to unentanglements of the polymer chains. In the electrospinning process, increasing the coulombic forces plays a role, by increasing polymer concentration the polymer chain entanglement increases, which in turn leads to stabilizing the jet formation [7-12]. The quality of the fiber is also affected by the surface tension of the polymer solution even if the optimum viscosity was achieved. The surface tension affects the charge balance of the polymer solutions [13]. It was known that the solution with low surface tension and optimum viscosity can produce an ultrafine fiber [7]. Meanwhile, a solution with a higher surface tension leads to the formation of beaded fibers [9]. Surface tension plays a vital role when the electrical current is applied for the coulombic repulsion and elongation of fibers [13, 14]. The conductivity of the polymer solution also plays an important role in the elongation of the fiber. The fibers can be formed using a low conductive polymer, but the quality of the fiber may be compromised. This can be improved by the addition of polyelectrolyte salt [15, 16].

The effect of solution parameters has been extensively studied for the electrospinning with the needle tip, but only very few experiments were carried out on wire-based electrospinning. The effect of solution properties on needle-based electrospinning may not be similar to that of the wire electrode. Thus, this work focused on understanding the effect of solution parameters and the quality such as the fiber diameter of resulted in nanofiber produced using the wire electrode. This work also focused on the encapsulation of model protein bovine serum albumin (BSA) in fiber and its subsequent structural integrity in PEO nanofiber. BSA was chosen as a model drug because it is thermostable and hence it is important to study the effect of high voltage (35 kV) in wire electrospinning to the structural integrity of BSA. Therefore, this paper aims to evaluate the production of nanofiber for commercial application with drug encapsulation for the outlook of drug delivery application. Most of the previous work on electrosprun PEO is based on small-scale production using a single needle, which is sufficient for lab-scale testing but not suitable for large scale commercial uses.

The Nanospider needless electrospinning works under a similar principle like the conventional electrospinning equipment except the single needle was replaced by a wire. In this system, the nanofiber is collected in a substrate attached on top of the wire electrode. The working area depends on the length of the wire electrode attached, which is helpful in the production of a large-scale uniform fiber [17, 18]. Nanofiber can be produced continuously by setting the substrate cloth to revolve slowly depending upon the desired thickness of fiber required. The main advantages of Nanospider are high production rate and can run a continuous production of the nanofiber. To our knowledge, only a limited study concerning the effect of solution properties and operating parameters on the nanofiber quality made using needleless wire electrospinning. Moreover, the current work includes the evaluation of the effect of the electrospinning process to the stability of the loaded protein.

2. MATERIALS AND METHOD

PEO (Mw-600,000) and BSA protein were obtained from Sigma-Aldrich and NaCl from R&M chemicals. Ethanol (99.8% absolute) was purchased from Merck. Deionized water was produced through MilliQ UV8 (Merck). The PEO solutions for the samples S1, S2, and S3 were prepared by dissolving a predetermined amount of PEO in deionized water. The samples S4 and S5 were prepared by dissolving 3 wt.% of PEO in (1:1) aqueous ethanol mixture. NaCl was added to improve the conductivity of the PEO polymer solution for formulation S3 and S5. The sample S6 is a PEO-water-NaCl system blended with BSA (0.5 wt.%). All the six samples were prepared using a magnetic stirrer over a period of 12 h at 35 °C. Table 1 shows the various solution concentrations used in this work. All solutions considered in this work are a weak base system with pH ranging from 8.43 to 9.11.

A wire electrode-based needleless electrospinning manufactured by Elmarco (NS Lab Nanospider) was used to pro-
duce the nanofibers. The collector is made of antistatic treated spun bound polypropylene cloth fitted on the top of the wire electrode. The collector substrate was set stationary during the experimental run. The height of the collector electrode was adjusted to 21 cm from the wire electrode throughout this work. The wire electrode was positioned in the center of the metal insert with inner orifice fitted to the polymer carriage reservoir, which moves along the fixed wire electrode surface immersed in the polymer solution. The metal insert with a size of 0.7 was used for all the experiments. The prepared polymer solutions were loaded into the carrier tank for electrospinning. The speed of the carriage movement on the surface of the wire electrode was set at 100 mm/s along and the voltage of 35 kV was used.

3. EXPERIMENTAL

The viscosity of the polymer solutions was measured using a plate and cone geometry (Kinexus Lab+, Malvern UK) which is monitored and controlled using rSpace software. All the experiments were carried out in a controlled environment of 25 °C. The viscosity experiment was carried out by ramping the shear rate from 100 to 1000 for a period of 5 min to obtain 50 measurement points. The conductivity of the polymer solution was measured using a multi-range conductivity meter (Hanna instruments HI-3388) fitted with HI-76301 probe.

The surface tension was measured using a tensiometer (DCat 9, Dataphysics) equipped with a DIN certified Wilhelmy plate method. The plate is oriented perpendicular to the interface, and the force exerted on it is measured. A 100 ml of the polymer solution is loaded into the sample cup, which is placed inside the sample holder, and the height of the sample holder is raised until it reaches the SFT plate and ensures the plate is not touching the sample initially. The experiment was carried out under a controlled environment of 25 °C.

All the samples were imaged using a Hitachi tabletop scanning electron microscope (TM3030plus) for initial screening of the fiber quality. The samples are imaged at 5-15 kV using a mix secondary electron (SE) and backscattered electron (BSE) option. The sample with a good fiber quality (S2 to S6) is further imaged using a field emission scanning electron microscopy (FESEM) (JEOL JSM-7800F). All samples were also imaged using the Phenom ProX desktop scanning electron microscope for the measurement of fiber size distribution using a Fibermetric software. Approximately 500 measurements were carried on five different regions on each sample for more accurate fiber size calculations.

Circular dichroism spectra (CD) were recorded on a Jasco J715 spectropolarimeter at room temperature. A stock solution of BSA-PEO nanofiber was prepared by dissolving approximately 14.5 × 11 cm of BSA-PEO nanofiber in 3 ml of double-distilled water. This solution was 100 times diluted and the resulting solution was transferred to 1 mm (diameter) rectangular quartz cell for CD measurements. The concentration of 100 times diluted BSA solution was estimated using Nanodrop OneC spectrophotometer (Thermo Scientific) and the concentration was found to be 0.32 mg/ml. For control, a stock solution (1 mg/ml) of BSA in double-distilled water was diluted to get 0.4 mg/ml. PEO solution was prepared by dissolving 1 mg of PEO in 1 ml of double-distilled water. CD spectra were recorded from 250 to 190 nm with a scan rate of 100 nm/min. The other parameters such as the bandwidth, response time, and data pitch were set to 1 nm, 1 s, and 0.5 nm, respectively. Each CD spectra depicts an average of 3 scans.

All Fourier transform infrared spectra (FTIR) were collected on a Jasco FTIR 4700 spectrometer (Japan). A small amount of PEO powder and a small piece of BSA-PEO nanofiber were placed on the crystal of ATR where the spectra were collected at a resolution of 8 cm⁻¹ with an average of 32 scans.

The samples utilized in CD measurements were used for fluorescence measurements. All fluorescence spectra were recorded on a Cary (eclipse) spectrometer. The emission spectra were measured between 300 and 550 nm with an excitation wavelength of 280 nm. The scan speed was kept at medium and both the emission and excitation slit width were set at 10 nm.

A transmission electron microscopy (TEM) system (FEI Tecnai G2 20 S-TWIN) that was equipped with a LaB₆ electron source operated at 200 kV was used to determine the internal structure of the nanofiber. The instrument has an embedded high-angle, annular dark-field (HAADF) STEM detector to generate (atomic resolution) dark-field STEM images with 2k × 2k Veleta Side Mount CCD camera. The nanofiber sample was electrospun directly on a copper grid and air-dried before measurement.

### Table 1. Polymer system and solution properties.

<table>
<thead>
<tr>
<th>Sample</th>
<th>PEO (%)</th>
<th>NaCl (%)</th>
<th>BSA (%)</th>
<th>pH</th>
<th>Conductivity (mS/cm)</th>
<th>Surface Tension (mN/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>2.5</td>
<td>-</td>
<td>-</td>
<td>9.11</td>
<td>0.09</td>
<td>62.18</td>
</tr>
<tr>
<td>S2</td>
<td>5.0</td>
<td>-</td>
<td>-</td>
<td>8.64</td>
<td>0.08</td>
<td>61.45</td>
</tr>
<tr>
<td>S3</td>
<td>5.0</td>
<td>0.5</td>
<td>-</td>
<td>8.72</td>
<td>7.60</td>
<td>61.45</td>
</tr>
<tr>
<td>S4</td>
<td>3.0</td>
<td>-</td>
<td>-</td>
<td>8.43</td>
<td>1.95</td>
<td>32.00</td>
</tr>
<tr>
<td>S5</td>
<td>3.0</td>
<td>0.3</td>
<td>-</td>
<td>8.53</td>
<td>4.50</td>
<td>32.00</td>
</tr>
<tr>
<td>S6</td>
<td>5.0</td>
<td>0.5</td>
<td>0.5</td>
<td>8.64</td>
<td>5.51</td>
<td>47.88</td>
</tr>
</tbody>
</table>
4. RESULTS AND DISCUSSION

4.1. Role of Humidity

The ambient parameter such as relative humidity and temperature greatly affects the quality of the fibers [19]. Therefore, in this work, the humidity was controlled at 40% RH using a dehumidifier attached to the needleless electrospinning, Nanospider equipment. All the PEO samples were electrospun under the controlled humidity of 40%, which produces a good quality fiber, but when the relative humidity increase above 40% splashes of polymer solution reaches the collected fiber, which affects the quality of nanofiber. It is understood that the PEO fiber dried almost immediately at relative humidity 40% to produce a very good nanofiber. It is clear that evaporation and solidification can affect fiber diameter and morphology.

4.2. Role of Carriage Speed and Voltage

In this experiment, the voltage is kept constant at 35 kV, which is optimum for the nanofiber formation. At a lower voltage, it was found that formation of Taylor cone jet is limited and hence difficult to obtain a nanofiber. Moreover, it was understood from a previous study that an increase in voltage leads to an increase in nanofiber diameter [20]. In addition, high voltage may cause a degradation of the loaded drug in nanofiber. Therefore, a constant voltage was used in this work.

The solution carriage speed is maintained at 100 mm/s, which is an optimum speed for nanofiber formation. It was observed that decreasing the carriage speed leads to the wastage of the solution from the wire electrode, which is due to the thick coating of a viscous polymer solution. This leads to insufficient charge balance on the coated polymer surface and failure in the formation of the Taylor cone from the wire electrode [11]. Whereas at high speed, a very thin coating was achieved. This thin coating leads to the nanofiber jet instability from the Taylor cone due to longer travel time from the wire electrode to the collector [14]. This similar effect can also be observed from the needle-based electrospinning based on the feed rate of the polymer solution. Thus, fixing the speed of the carriage movement plays an important role in controlling the feed rate of the polymer solution and the formation of bead free nanofiber.

4.3. Effect of Solution Properties

The rheological property of the PEO solution used in this work as shown in Fig. (1) indicates that all the prepared solutions behave as Non-Newtonian fluid except for sample S1, which behaves almost like a Newtonian fluid. Fig. (1) clearly shows the decrease in the viscosity when the PEO solution is subjected to shear from 100² to 1000² s⁻¹. The viscosity value of the polymer solution may not be constant throughout the experiment due to the shear-thinning effect of non-newtonian fluid. In addition, solvent evaporation may occur during the experiment thus exacerbating the shear-thinning effect further as the solution concentration changes.

The effect of the PEO solution viscosity on the morphology of the electrospun fibers is shown in Fig. (2). The SEM image clearly shows that the beaded fiber formation of sample S1 (Fig. 2A) can be resolved by increasing the percentage from 2.5 to 5 wt.% of PEO in water, which in turn increases the viscosity of polymer solutions. Even lower PEO concentrations (3 wt.%) can achieve a higher viscosity when a mixture of water and ethanol is used as the solvent. This is due to the change in the solution properties, which is altered when compared with pure water as shown in the samples S2 and S4 (Table 1). The addition of salt to the polymer solution does not show any significant changes in viscosity (see S2 and S3 in Table 1). Sample S6 is similar to S3 in terms of PEO and salt percentage with the only difference is the addition of BSA. Sample S6 shows similar effects of shear-thinning recorded for all the other samples.

The previous work on the PEO system at various concentrations suggested that polymer with low viscosity will not withstand the coulombic elongation force due to entanglement in electrospinning thus causing a beaded fiber formation. A lower solution viscosity is often associated with a lower polymer concentration and high wet ability of solvent used for mixing the polymer [16, 21]. A smooth fiber formation in electrospinning is produced when the solvent is dried as they travel between the electrodes [16, 22]. Previous work on poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) [11] shows that the formation of the beaded electrospun fiber is due to a lower concentration of the polymer solution. While the current work is based on needleless electrospinning, but the principle of electrospinning does not change even though the nanofiber processing equipment is different. The results obtained in the current work are in good agreement with reported work on single needle electrospinning regarding the effect of solution viscosity [23].

The PEO in ethanol solution S4 shows a conductivity of 1.9 mS/cm, while the addition of NaCl to PEO-ethanol solution improves the conductivity value to 4.5 mS/cm. Polar solvents like water are excellent for dissolving NaCl.
whereby it forms the Na\(^+\) and Cl\(^-\) ions surrounded by the polar water molecules, which in turn increase the solution conductivity. Ethanol has a polar and non-polar part, therefore water and ethanol are completely miscible.

The increase of conductivity of PEO in aqueous ethanol solution is due to the formation of hydrogen bonding between the hydrogen in -OH group of ethanol and oxygen in the water molecule. According to the interactions involved in ethanol-water mixtures such as the formation of hydrogen bonding and multi-hydration layers around the ethanol, molecules may alter the conductivity of the solution [24, 25]. For instance, the presence of 1% water in ethanol increases the conductivity from 0.219 \(\mu\)S/cm in pure ethanol to 0.516 \(\mu\)S/cm [26, 27]. The addition of salt to the PEO in aqueous ethanol increased the conductivity over twofold from 1.95 mS/cm for S4 and S5 to 4.5 mS/cm.

The addition of NaCl to the PEO-water system reduces the fiber diameter as observed in Fig. (2B and 2C), due to an increase in conductivity of the polymer solution [28]. About 9 nm difference in average fiber size diameter between the samples S2 and S3 was observed. It has to be noted that the measurement is automated so the presence of salt particles is also counted in the fiber size measurement, which makes the measured fiber diameter bigger than the actual fiber diameter without the NaCl nodes. The data obtained from a benchtop SEM image, which is of lower resolution to that of the FE-SEM image in Fig. (2). Hence inevitably the salt on the surface of the fiber may have been measured as part of the fiber.

**Fig. (2).** FESEM image comparison of electrospun polymer sample obtained at different viscosity, conductivity, and humidity, A) S1 2.5% PEO in water, B) S2 5% PEO in water, C) S3 5% PEO in water with NaCl, D) S4 3% PEO in 50% aqueous ethanol mixture, E) S5 3% PEO in 50% aqueous ethanol mixture with NaCl, F) S6 5% PEO in water with BSA and NaCl. (A higher resolution / colour version of this figure is available in the electronic copy of the article).
Table 1 shows the measured surface tension of the prepared polymer solutions of the electrospun samples. As mentioned earlier, the quality of nanofibers is not inconsistent due to the presence of low viscosity, low conductivity, and high surface tension (62.18 mN/m). This observation is similar to the previous findings reported by Zhou et al. [29] and Fuhr et al. [30]. Surface tension prevails more than the force exerted by the applied electrical voltage resulting in non-elongation of the fiber formation from the Taylor cone causing a beaded nanofiber formation (e.g. Sample S1) instead of a smooth bead free nanofiber production [31]. Whereas when the surface tension decreases from 61.45 mN/m (Sample S2) to 32 mN/m (Sample S5) with the use of low surface tension solvent such as ethanol, which improves the quality of nanofibers with less or no bead formation [13]. This can be observed from the FESEM image in Fig. (2B and E).

The sample S6 is the same as S3 except that BSA was added in the solution as a model drug. The solution properties of the sample S6 have a surface tension of 47.88 mN/m and conductivity of 5.51 mS/cm. The presence of BSA reduces the overall surface tension of the PEO-BSA solution. The conductivity in sample S6 is higher than that of S2 and S4 due to NaCl addition. Essentially the viscosity of sample S6 is similar to that of S3 (see Fig. 1). Fig. (2F) shows an excellent fiber consisting of a nearly uniform nanofiber size with an average diameter of 114.89 nm. This is due to the higher conductivity of sample S6.

4.4. Structural Integrity of BSA in PEO-BSA Nanofiber

The nanofiber for drug delivery uses must be capable of preserving the structure and function of the loaded drug. In this work, the effect of coulombic repulsion and high current (35 kV) on the structure of the model drug, BSA, was evaluated. Three different spectroscopic techniques, namely circular dichroism (CD), Fourier-transform infrared (FTIR), and fluorescence, which are routinely used to characterize the structure of proteins [32-34] were employed to analyze the secondary and tertiary structure of BSA embedded in the nanofibers. CD spectra of BSA-PEO sheet dissolved in water show a negative double minimum at 208 and 222 nm followed by a positive maximum at 193 nm with a crossover around 200 nm (Fig. 3), which is a characteristic of proteins with predominantly α-helical structure [35]. The CD spectrum of a control sample made of pure BSA shows a similar feature to those embedded in nanofiber (PEO-BSA). The CD of pure BSA is consistent with a known BSA spectrum, which exhibits a similar sign and band positions [35]. In addition, the molar ellipticity of pure BSA (0.4 mg/ml) and BSA embedded with nanofiber (0.32 mg/ml) at 193, 208, and 222 nm exhibit similar values, indicating that the BSA structure is retained in the nanofiber. It should also be noted that pure PEO did not exhibit any characteristic bands in the region of 190 to 250 nm, where the characteristic bands for BSA are found.

The CD result suggests that BSA maintains its secondary structure when embedded in the nanofibers of PEO and not affected by the needless electrospinning process. FTIR analysis, a complementary technique to Circular Dichroism, was also performed for the BSA-PEO sheet under the total attenuated reflectance (ATR) mode. The corresponding FTIR spectrum exhibits a characteristic amide I (associated with predominantly amide -C=O stretching) and II (the combination of N–H bending and C–N stretching) bands at 1653 and 1543 cm⁻¹ which are characteristic of BSA with predominantly α-helical structure (Fig. 4). Other researchers also observed a similar result for proteins with predominantly α-helical structure [36, 37]. The FTIR result confirmed that BSA exists in its native secondary structure within the BSA-PEO nanofiber.

The presence of PEO in the nanofiber was confirmed by the appearance of bands at 1465 and 1341 cm⁻¹, which were seen in the FTIR spectrum of pure PEO (Fig. 4). Fluorescence spectra (Fig. 5) is widely used to ascertain the tertiary structure of proteins [38]. PEO-BSA nanofiber and pure BSA (control) were dissolved in water prior to fluorescence spectra analysis to determine the tertiary structural changes of BSA after going through the electrospinning process. Fig. (5) shows that fluorescence spectra of pure BSA and BSA-PEO nanofiber have a similar wave length peak at 345 nm,
indicating that the tertiary structure of BSA was preserved during the needleless electrospinning process. Results from all the three spectroscopic methods performed in this work suggest that the BSA structure is preserved during the needleless electrospinning process. Thus, needleless wire electrospinning is suitable for producing a nanofiber embedded with the thermolabile drug.

The SDS-PAGE may also be used to determine the structural integrity of BSA [39]. However, SDS-PAGE is generally used to determine the molecular size, whereas FTIR, CD spectra, and fluorescence spectra can give information about the α-helical structure as well as the secondary and tertiary structure of BSA [38, 39]. Compared to SDS-PAGE, the Circular Dichroism is a better method, which can confirm the preservation of the secondary and tertiary structure of BSA [32]. In addition, the structural integrity of BSA was confirmed by the FTIR and fluorescence spectra, and hence SDS-PAGE analysis is not made in this work.

4.5. Fiber Size Distribution

Fibermetric software was used to calculate the fiber diameter distribution from the SEM images. Approximately 500 to 800 measurements on six SEM images were processed to automatically generate the frequencies of the fiber size. The sample S1 was not considered for measurement since fiber formation was not observed, instead, bead formation was observed for sample S1.

Fig. (6A) shows the frequencies of the nanofiber size distribution. The sample S2 consists of 5 wt.% of PEO in the water mixture. The calculated fiber diameter of this system shows that most of the fibers are below 300 nm with the presence of the maximum number of fibers are in the range of mean fiber diameter of 100 to 150 nm range. The average fiber diameter was found to be 142.2 nm. The sample S3 shows the average fiber diameter of 151.4 nm. As mentioned earlier, the NaCl nodes embedded in the fiber (see Fig. 2C) may be measured as part of the nanofiber, which makes the measured nanofiber size bigger than the actual fiber observed in the SEM image. The sample S3 consists of ionic salt in the polymer solution of PEO in water to improve the conductivity. Most of the fiber for sample S3 is below 300 nm, which is similar to the S2 system but has a higher number of fibers formed in the range of 150 to 200 nm compared to the S2. This can be due to the presence of ionic salt as shown in Fig. (2C), which is embedded within the nanofiber.

The fiber diameter distribution of S4 and S5 system with an average diameter of 378.9 and 144.4 nm, respectively. S4 and S5 consist of 3 wt.% PEO polymer with a mixture of ethanol and water. In the sample S5, ionic salt was added to improve the solution conductivity, in the same manner as S3. The fiber diameter of S4 shows that most of the fiber is in the range of 200 to 250 nm. Sample S4 has a lower PEO concentration resulting in lower surface tension, which engenders a significant increase in mean fiber size. The sample S5 shows the highest frequency of nanofiber was observed in the range of 100 to 150 nm. Compared to S4, which has the same PEO and solvent system, S5 has a better nanofiber formation with most of the fiber is smaller than 300 nm. This is due to the presence of ionic salt, which improves the solution conductivity. The result obtained in this work demonstrates that the size of the fiber produced through needleless electrospinning can be controlled either by improving the solution conductivity or viscosity.

Encapsulation of BSA in PEO causes a reduction in the fiber diameter whereby the highest frequency of fibers was formed in the range of 50 to 150 nm (sample S6). The average fiber diameter is 114.9 nm. This shows that the addition of protein in the PEO solution leads to a reduction in the size of the nanofiber. This reduction in size increases the density of nanofiber and surface area to volume ratio, thus allowing a high drug loading. The sample S6 shows that needleless wire electrospinning porous scaffold with drug encapsulation on the nanofiber itself, which may be useful for commercial drug delivery application.

The mean size of the nanofiber produced in this work is shown in Fig. (6B). It can be observed that the addition of BSA 0.5% to the PEO-water-NaCl system reduced the nanofiber diameter slightly, although the difference is not significant (p > 0.05). This can be attributed to the slight increase in surface tension and conductivity of sample S6 compared to S5, although the solution viscosity is comparable to that of S3. From the statistical point of view, the mean fiber size of S2, S3, S5, and S6 is not significantly different (p > 0.05). However, fiber size in the sample S4 is significantly larger (p < 0.05) than all other fiber samples, due to a lower surface tension by reduction of polymer concentration.

4.6. Fiber Size Distribution

The transmission electron microscope image of the BSA loaded nanofiber (S6) is shown in Fig. (7). The SAED pattern shows that the nanofiber is made of amorphous material. The square particle is likely a NaCl, which corroborates with the observation from the FESEM image (Figs. 2C, 2E, and 2F). The dark spot in the TEM image may be attributed to the presence of BSA.
Fig. (6). Comparison of fiber diameter distribution of electrospun polymer sample obtained at different viscosity, conductivity, and humidity. S2 5% PEO in water, S3 5% PEO in water with NaCl, S4 3% PEO in 50% aqueous ethanol, S5 3% PEO in 50% aqueous ethanol with NaCl, S6 5% PEO-BSA blend with NaCl. A) Fiber size distribution, B) Mean fiber size. (A higher resolution / colour version of this figure is available in the electronic copy of the article).

Fig. (7). TEM image of sample S6. (A higher resolution / colour version of this figure is available in the electronic copy of the article).
CONCLUSION

Wire-Based needleless electrospinning was used to evaluate the effect of polymeric solution properties and extrinsic parameters like relative humidity on the nanofiber diameter distribution and surface morphology. It was found that the polymeric solution properties play an important role to obtain a nanofiber with an average diameter of below 200 nm. The result shows that a lower PEO concentration (2.5 wt.%) with the surface tension of 62.18 mN/m and conductivity of 0.09 mS/cm failed to produce a good nanofiber (i.e. severe bead formation). An increase in the PEO concentration from 2.5 wt.% to 5 wt.% increases the solution viscosity and hence yielded a high number of nanofibers with a mean diameter of 100 to 150 nm. The addition of salt increases the solution conductivity, thus yielding a nanofiber with an average diameter below 150 nm. The voltage used in this work. Thus, needleless wire electrospinning (FiberMetric) performed in this work suggest that BSA structure is preserved during the needleless electrospinning process. BSA was not affected by the coulombic forces and high voltage used in this work. Thus, needleless wire electrospinning (NS Lab Nanospider™) is suitable for producing a nanofiber embedded with the thermolabile drug at a large scale.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

None.

CONFICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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