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Optimization of concentration and applied voltage of electrospun zein nanofibers

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Abstract: Electrospinning is amongst the flexible techniques used to obtain ultrafine polymer fibres with a range of 10 μm to 100 nm. In this study, applied voltage and concentration were optimizedto fabricate zein nanofibers using the electrospinning technique. The electrospun nanofibers morphology, diameter and structure, were investigated by scanning electron microscopy (SEM), X-ray diffraction, Fourier transform infrared (FT-IR) and thermogravimetric analysis (TGA). The SEM images showed morphologies of the nanofibers affected by the concentration of the prepared zein solutions from 20 wt%, 25 wt% and 30 wt% and voltage variation from 15 kV, 20 kV and 25kV. The results showed that the concentration, average fibre diameter, and voltage increment influencedfibre size distribution and morphology. The XRD showed a broad, amorphous peak at $2\theta = 19.5^{o}$ for the different concentrations of zein nanofibresused, illustrating the retained structural conformation. FT-IR spectra confirmed the functional groups present in zein nanofibers, and the TGA thermograms showed no significant change on the zein nanofibres.

Keywords: Electrospinning, nanofibers, applied voltage, zein, weight per cent

1. Introduction

Electrospinning is an assuringtechnique that is commonly used for fabricating polymer fibres. The polymer nanofibers with a high surface area to volume ratio and tiny poresare the most critical nanostructured materials currently exploited in various fields. The fields include chemistry, biology, material science and engineering. Several applications such as adsorption, energy, catalysis, biomedical and environmental applications have been reported[1,2].In recent years, there has been progressive

growth in publications in electrospinning polymer fibres in many application fields. There has been an improvement in imaging techniques of polymer fibres and working parameters that fabricate uniform polymer fibres [3]. Electrospinning is a process that involves the application of an electrical field to continuously draw a polymer solution from a syringe needle towards a grounded collector. Electrospinning depends on several important factors such as solution, process and ambient parameters that influence fibre formation and morphology.Electrospinning has been investigated on various proteins such as collagen, wheat gluten, fibrinogen and zein [4,5, 6,7]. Zein is an amorphous natural polymer that is amphiphilic, and it is a dried "yellowish" powder obtained from ground maize or corn. It is a protein mixture of four classes: $\alpha, \beta, \gamma \; and \; \delta$, which are expressed sequentially in maize and are found to interact with each other to reach stabilization [8]. Zein has a large fraction of nonpolar amine and carboxyl functional groups.Its solubility in aqueous alcohol mediums like ethanol and acetic acid is attributed to its amino acid composition due to the high content of nonpolar amino acid residues. Zein nanofibers have some limitations, such as poor mechanical strength and morphological stability in wet conditions and possess distinctive properties such as nontoxicity, biodegradability and biocompatibility [9,10].

The successful preparation of ultrafine zein membranes was reportedby Miyoshi et al. [11]with 80 wt% aqueousethanolic solutions using the electrospinning technique. Themorphology of the formed beads and fibres was affected by polymer concentration and electric field parameters.An increase inthe concentration of the solution resulted in fewer wrinkled beads, and fibres became thicker. Zein nanofibers with diameters near 700nm were successfully produced by electrospinning.Yao et al.[7] studied the variation of electrospinning parameters to generate zein mats.Optimization conditions for zein produced nanofibers with a diameter of about 500 nm with an increase in the concentration of zein greater than 30% (w/v),fewer beads or ribbon-like nanofibers with a diameter of approximately 1–6 μm were obtained. The electrospun zein nanofiber mats were flexible and lustrous but showed poor mechanical properties.

Sellinget al.[12] studied the impact of solvent on electrospinning of zein and analysis of resulting fibres. Zeinfibres were produced by electrospinning from acetic acid, aqueous methanol, ethanol and isopropyl alcohol. It was reported that ethanol (60-90 %) produced ribbon-like zein nanofibers, isopropyl alcohol (80 %), and methanol (60-90 %) did not produce any nanofibers; instead, it electrosprayed, and glacial acetic acid gave round fibres with a narrower distribution of diameters under suitable spinning conditions. Therefore quality nanofibers of zein could be produced from acetic acid and ethanol solutions. Li et al.[13] studied electrospun zein fibres as carriers to stabilize (−) -epigallocatechin gallate.Fiber-forming solutions with various zein concentrations (10% to 30%, w/w) and aqueous ethanol concentrations (60% to 90%, w/w) were electrospun at 15 and 20 kV. SEM results showed that the morphology of zein fibres was affected by aqueous ethanol concentration, zein concentration, and the applied voltage. The optimal condition for forming bead-less fibres was 20 % protein and 70 w/w% alcohol, and 15 kV. Zein nanofibers with diameters ranging from 150 to 600 nm were successfully electrospun. The effective immobilization of EGCG was achieved by ageing the fibre for at least one day under dry conditions at ambient temperature.Therefore, the major limitation of electrospinning zein is its poor mechanical strength that influences the morphological stability of zein nanofibers.

In this report, the electrospinning method was used to prepare zein nanofibers. The effect of concentration and voltage on the morphology and diameter of the electrospun fibres were investigated.

2. Experimental

2.1. Materials

All chemicals were purchased from Sigma Aldrich. Zein powder and ethanol (99.8%).

2.2. Instrumentation

The FE-SEM (Leo, Zeiss) scanning electron microscopy operated at 1.00 keV electron potential was used to study the surface morphologies of the zein polymer nanofibers; the samples were placed on the sample holders coated with carbon tape and dried at room temperature.The X-ray diffraction patterns were recorded by a Bruker D2 diffractometer at 40 kV and 50 mA. Secondary graphite monochromated Co K alpha radiation (l = 1.7902 A°) was used to confirm the crystallinity of electrospun zein polymernanofibers.The measurements were taken at high angle 2θ in a range of 5°–90° with a scan speed of 0.01° 2θ s^{-1}. The chemical structure and composition of zein nanofibres were studied using a Thermo Scientific Nicolet iS50-FTIR spectrometer, universal ATR with the diamond detector, with a wavelength range of 4000 cm^{-1} to 400 cm^{-1} to determine the interaction between the polymers with each other and the nanoparticles.The thermal stability of electrospun zein nanofibers was studied using a PerkinElmer STA 600 Simultaneous Thermal Analyzer (Waltham, USA). The analysis was accomplished using nitrogen atmosphere, 3.2 bar pressure, 20 mL/min flow rate, and a heating rate of 10°C/min starting from 30 °C to 900 °C.

2.3. Experimental procedure

The preparation of zein polymer fibres followed work by Selling et al. [12] and Lin [14]. Zein (20, 25, 30 wt %) were prepared and dissolved in 70 wt % ethanol solution. The solutions were stirred at 70°C for two hours. The solutions were electrospun by varying applied voltage (15, 20, 25 kV) and the spinneret to collector distance (10 cm), respectively.

3. Results and Discussion

3.1. Effect of concentration on zein nanofibres.

Solution concentration plays a vital role in nanofibre diameter. Hence surface tension influences the fibre morphology at lower viscosities resulting in beads or fibres with beads. At higher concentrations, producing fibres is hindered by the inability to control and maintain the flow of a polymer solution to the tip of the needle[15]. Distance to the collector also affects fibre production; therefore, minimum distance should be kept to allow the fibres sufficient time to dry before reaching the collector to prevent beads from occurring [16]. **Figure** 1 shows SEM images and average fibre distribution of the zein nanofibres electrospun at 20, 25 and 30 wt% with an applied voltage of 15 kV and a distance of 10 cm. The results obtained illustrated no bead formation on the morphology of the fibres, and during electrospinning, the yellow zein solution changed from yellow colour to white zein nanofibre-based structures. At 20 wt%, uniform ribbon-like fibres were obtained with an average diameter of 478 ± 0.75 nm, and as the concentration was increased to 25 wt%, the morphology of the fibres became thicker and had an amorphous ribbon shape with an increase in fibre diameter to 819 ± 0.95 nm.

Further increase of the concentration to 30 wt%, the fibres became thicker and was tubular shaped with an average fibre diameter of 992 ± 1.04 nm. It was observed that as concentration was increased, there was a gradual increase in the fibre diameter, which influenced the morphology of the fibres. There was less clogging observed during electrospinning of 25 wt% and continuous uniform fibre formation compared to 20 wt% and 30 wt% zein solutions. Miyoshi et al. [11]studied the preparation of ultrafine

fibrous zein membranes via electrospinning. It was previously reported that ultrafine fibrous zein membranes were successfully produced by electrospinning zein/ethanol solutions above 21 wt% with an electric field of 15 kV. Therefore, the results obtained in this study with correlation to concentration effect on fibre morphology were similar to the study conducted.

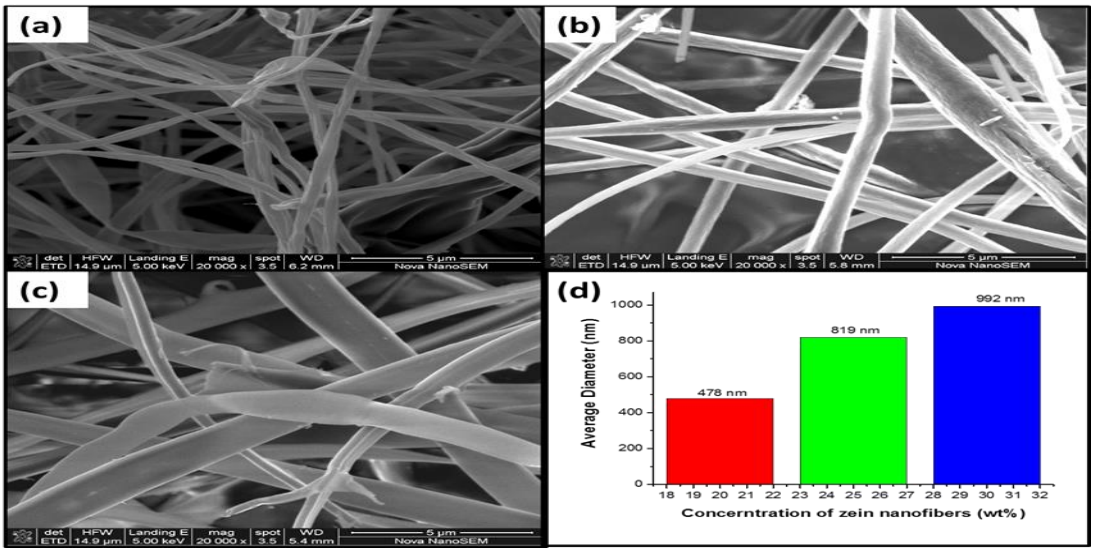

Figure 1. SEM images and average fibre distribution of zein nanofibres at different zein concentration solutions (a) 20 wt%, (b) 25 wt%, (c) 30 wt% and (d) fibre size distribution.

3.2. Effect of applied voltage on zein nanofibres

Electrospinning occurs when the applied voltage is higher than the surface energy of a droplet of a polymer solution. Generally, higher voltage causes more significant stretching of the jet due to the increase in Columbic force exerted by the charges. Increasing the applied voltage can reduce fibre diameters due to increased stretching of the jet. Jacobs et al.[17] and some researchers reported that applied voltage is one of the most critical parameters in the electrospinning process due to its direct influence on the dynamics of the polymer solution flow. The changes made in the applied voltage are reflected in the droplet's shape suspended at the tip of the needle. Its surface charge, the velocity of the flowing polymer solution, and hence on the structural morphology of electrospun fibres. The SEM images of **Fig. 2** show 25 wt% zein fibre size distribution at different voltages and illustrate that the increment in applied voltage directly influences fibre morphology. At 15 kV, the tubular fibres with an average diameter of 819 ± 0.95 nm and as the voltage were increased to 20 kV, the average diameter of the fibres decreased to 699 ± 0.77 nm, and the fibres became more interconnected, rougher and amorphous still maintaining the ribbon-like shape. It was further observed that as the voltage increased to 25 kV, the fibre morphology became more interconnected and had an average diameter of 625 ± 0.26 nm. Therefore the increment in voltage results in a reduction of fibre diameter, and also the rapid evaporation of ethanol solvent from the fibres enhanced uniform fibre production. Therefore 25 kV was the optimum voltage for polymer fibre fabrication because smooth interconnected fibres were produced at this voltage.

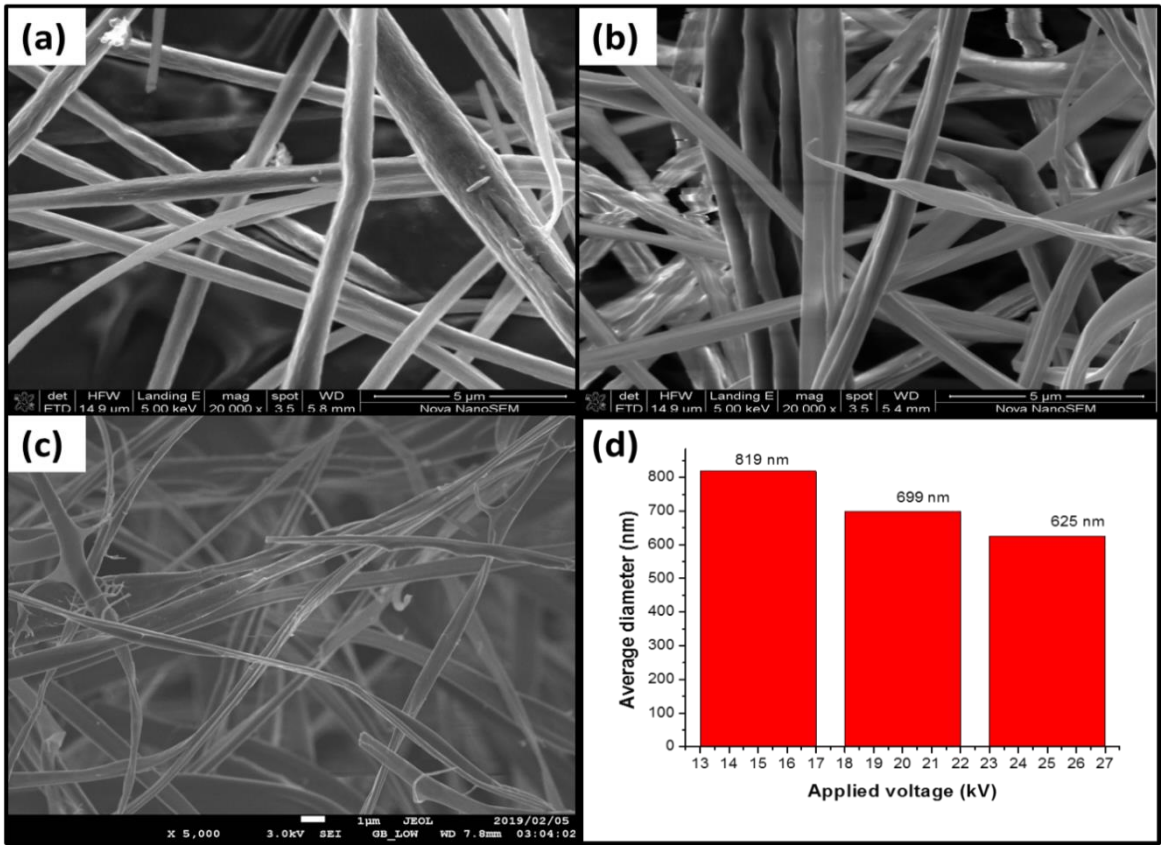

Figure 2. SEM images and size distribution of 25 wt% zein nanofibres at different voltages (a) 15kV, (b) 20kV and (c) 25kV.

3.3. XRD analysis of the zein polymer

XRD was used for phase identification of zein crystallinity. The XRD patterns in **Fig. 3** showed low-intensity peaks at 2θ = 12.6 and 27.2° with broad, amorphous peaks for the different concentrations of zein nanofibres at 2θ = 19.5°. The diffraction peaks at 12.6°, 19.5° and 27.2 ° are related to the average backbone distance within the α-helix structure of zein. The result indicates that the electrospun zein is nanofibres retained their structural conformation. Oliveira et al.[18] reported similar diffraction patterns with three broad peaks having maxima at 2θ = 14 °, 17 ° and 25 ° for electrospun zein/tannin bio-nanofibres. Miao & Yang [19] studied poly(L-lysine) modified zein nanofibrous membranes as an efficient scaffold for neural stem cell adhesion, proliferation, and differentiation. They reported the XRD of two diffraction peaks of 2θ =9.2° and 19.4°, which are related to the α-helix and β-sheet structures of protein. Yao et al. [7] reported that the difference between literature and experimental XRD parameters could be attributed to secondary and tertiary zein structure variations. The parameters also include the different solvents used during the electrospinning process, ethanol, and zein powder's medium molecular weight. These parameters used in this study might have influenced the results obtained, corresponding to other reported results. The polymer's molecular weight, tacticity and glass transition temperature strongly influenced the fibre's crystallinity and orientation.

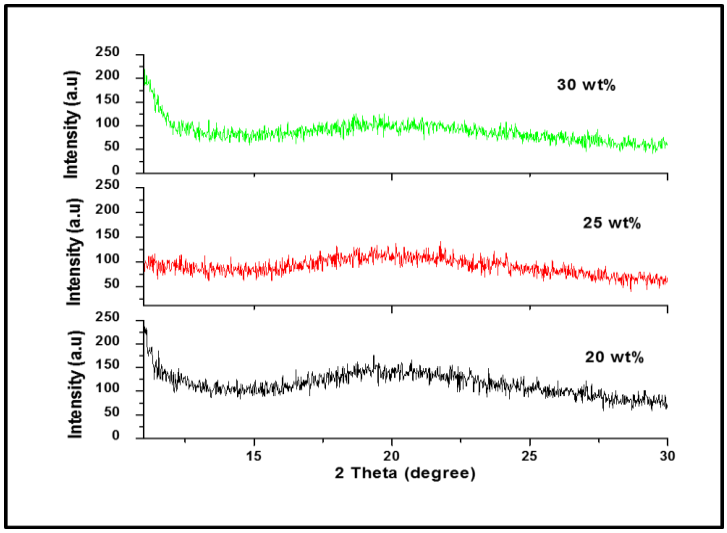

Figure 3.XRD patterns of different concentrations of zein solutions (a) 20 wt %, (b) 25 wt% and (c) 30 wt%.

3.4. FTIR spectral analysis of zein nanofibres

Zein proteins are classified according to their solubility and sequence homology into α, β, γ *and* δ zeins. It is highly hydrophobic due to apolar amino acids and rich in α *and* β sequence homology, which are soluble in an alcohol solution. The β sheet is generally visible with a display of a shoulder peak around the region of 1620 cm^{-1}.**Fig. 4** shows the FTIR spectra of electrospun zein nanofibres fabricated at different concentrations of 20 wt%, 25 wt% and 30 wt%. The zein nanofibres had a broad peak at 3294 cm^{-1} associated with amide I (NH_2 stretch vibrations). In addition, the peak observed at 1648 cm^{-1} was due to the carbonyl stretching vibration, which indicated a high content of α–helices was present due to the absence of a secondary peak around 1620 cm^{-1}. At 1530 cm^{-1}, a peak of amide II was observed related to the NH bending, and at 1448 cm^{-1}, a peak of C-H bending was observed.

Further, a peak at 1239 cm^{-1} was due to amide III (axial deformation vibration of the carbon-nitrogen stretching). Qureshi et al.[10]studied electrospun, zein nanofibre as green and recyclable adsorbents, and they reported similar peaks of zein functional groups illustrating that there was no β–sheets present (1662, 1614 and 1631 cm^{-1}). The results obtained indicate the predominate peaks of α-helices of zein nanofibres.

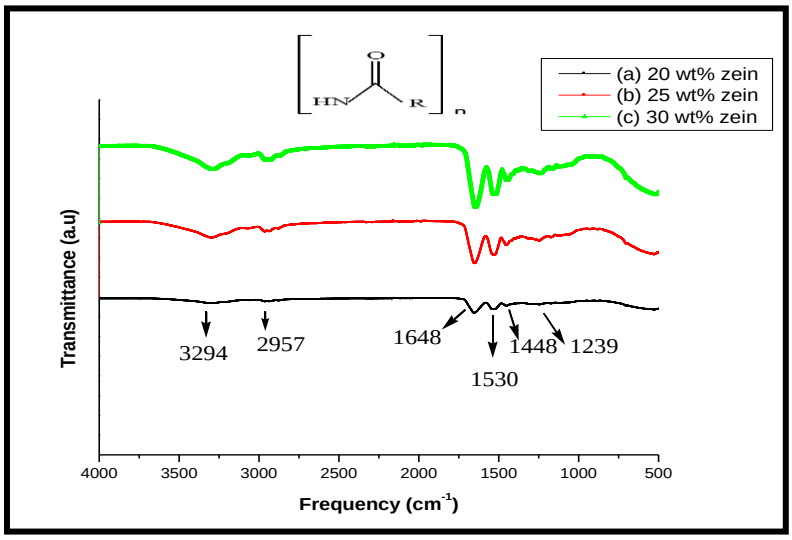

Figure 4. FTIR spectra of zein nanofibers prepared at concentrations (a) 20 wt%,(c) 25 wt% and (d) 30 wt%.

3.5. Thermogravimetric analysis (TGA)

Fig. 5 Show the TGA results of different electrospun zein nanofibre concentration 20 wt%, 25 wt% and 30 wt%. TGA results of 20 wt% and 25 wt% concentrations overlap, and the 30 wt% zein nanofibres are slightly above the 100 weight per cent loss that might have been affected by the loading capacity during TGA analysis. The results obtained illustrate a weight loss regime at 125 to 200 °C; this was due to the minor loss of the ethanol solvent. A significant weight loss regime was observed around 260 to 357 °C that was due to the presence of the remaining solvent present. The weight loss regime from 550 to 857 °C was due to the complete degradation temperature of zein nanofibres. The results reported illustrated less water present in the nanofibers, which agrees with the reported literature of zein nanofibres[8, 20,21].

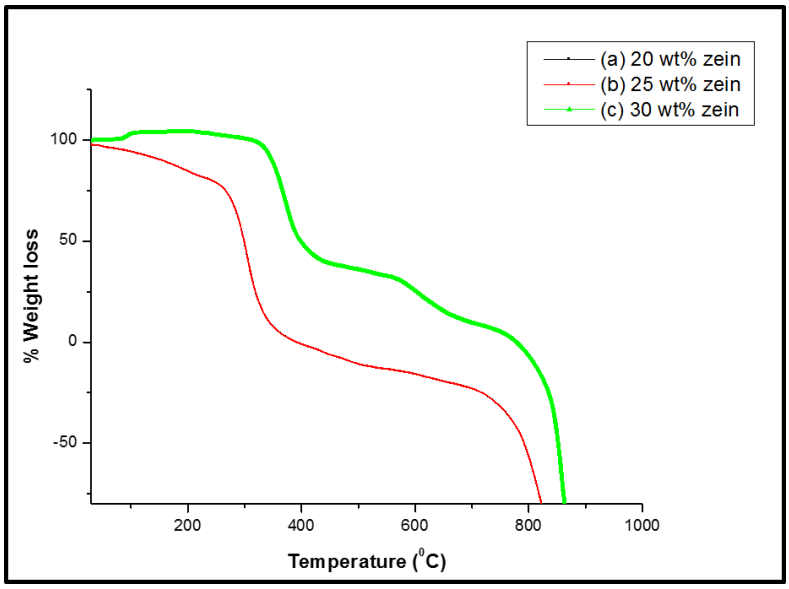

Figure 5. TGA curve of zein nanofibres electrospun at 25kV and distance: 10 cm

4. Conclusion

Zein nanofibers with diameters near 820 nm were produced by electrospinning of 70wt% ethanol aqueous solution. The morphology of the formed nanofibers was affected by parameters such as polymer concentration and applied voltage. SEM results of 25 wt% zein nanofibers electrospun were the suitable maximum condition due to continuous fibre formation, and25 kV was regarded as the optimum voltage for polymer fibre fabrication because smooth interconnected fibres were produced at this voltage.FT-IR confirmed the carboxyl and amino functional groups of zein nanofibers.The TGA curves showed no significant effect on the thermal properties of the zein nanofibers. The results from the electrospinning of zein nanofibres were obtained with improved stability with no further additives. Zein nanofibresalso possess potential usage fornumerous applications fields such as environmental and biomedical.

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