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Original Article

Effect of Co-solvent Ratios and Solution Concentrations on Morphologies of Electrospun Zein Nanomaterials

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Abstract

To investigate the effects of co-solvents on the morphology of nano-scale zein materials, zein solutions were electrospun with different co-solvent ratios of EtOH/H_2O. Different zein solution concentrations were used to study the effects of the zein content on the electrospun materials. The resulting electrospun materials were all characterized using field-emission scanning electron microscopy (FE-SEM) and transmission electron microscopy (TEM). The diameters of the electrospun nanoparticles and nanofibers were found to increase when increasing the EtOH ratio at certain zein concentrations. Furthermore, increasing the zein content changed the morphology of the electrospun materials from nanoparticles to nanofibers.

Keywords : Electrospinning, Zein, Nanofibers, Nanoparticles, Morphology

Introduction

Biomaterials are attracting wide spread interest due to their potential applications, especially in tissue engineering and drug delivery (Reddy et al. 2011; Chakraborty et al. 2009; Li et al. 2006). Examples of biomaterials include collagen, poly (lactic acid) (PLA), and zein, plus electrospun ultrafine fibers of protein-based biomaterials are preferred for their medical applications. The structures of these electrospun biomaterials resemble the three-dimensional (3D) fibrous networks found in a natural extracellular matrix (ECM) (Li et al. 2006; Lee et al. 2008; Marrhews et al. 2002; Sill 2008). Plant proteins, such as sorghum, corn, wheat, millet, and soybeans, are widely available, biodegradable, and have attracted numerous medical applications when compared with animal proteins, like bovine collagen. Yet, the limitation of solvents has made it difficult to develop biomaterials from these proteins (Reddy et al. 2011).
Zein is the major storage protein in corn, accounting for 40 – 50% of the total protein. Soluble in aqueous alcohol solutions (60 – 95%), the main constituents of zein are polar amino acids, proline, and glutamine, representing a good cell compatibility and more hydrophobic characteristics than other proteins. Zein is also one of the most hydrophobic, low toxic, renewable, and biodegradable proteins, which has already led to its widespread use in the packaging, food, pharmaceutical, cosmetic, and biomedical industries (Reddy et al. 2011; Lawton 2002; Shukla et al. 2001; Reddy et al. 2011). In addition, a three-dimensional (3D) zein scaffold for tissue engineering has also been reported (Tu et al. 2009).
As a rapid and efficient process, electrospinning is important for the production of nano-structured polymer materials with excellent properties (Reneker et al. 1996; Li et al. 2004; Cui et al. 2007; Han et al. 2006; Huang et al. 2003; Wang et al. 2002). The resulting nanomaterials canbe used for a wide range of applications, including separation filters, sensors, protective clothing, catalysis reactions, wound dressing materials, tissue scaffolds, and drug delivery (Sill et al. 2008; Wu et al. 2005; Choi et al. 2004; Zeng et al. 2003; Verreck et al. 2003). However, while the principle of electrospinning is simple, the process itself is difficult to control due to the influence of variables on the properties of the end product (Cui et al. 2007; Zong et al. 2002; Jiang et al. 2007; Lee et al. 2009). These variables can be broadly classified as the polymer solution parameters and processing conditions, such as the applied voltage and tip-collector distance. Thus, when changing the variables, the

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morphologies of the resultant electrospun materials can range from nanoparticles to nanofibers with pores on their surface via beaded nanofibers.

Accordingly, this report studied the influence of the co-solvent ratio and zein solution concentration on the morphology of nano-structured zein materials prepared by electrospinning from an ethanol/water solution. The morphologies and morphological changes were observed in detail using field-emission scanning electron microscopy (FE-SEM) and transmission electron microscopy (TEM).

Materials and Methods

Materials

Zeinfrom corn (molecular weight = 35,000) was purchased from Tokyo Chemical Industry Co. Ltd., Japan, and used as received without further purification. Ethanol (EtOH) of 96% (v/v) purity (Daejung Chemical & Materials Co. Ltd., Korea) and doubly distilled water (H_2O) were used as co-solvents to prepare all the solutions.

Preparation of zein solutions

A certain amount of zein was dissolved in a mixture solvent of EtOH/H_2O under magnetic stirring for 30 min at room temperature. EtOH/H_2O mixture solvents with 7/3, 8/2, and 9/1 (v/v) mixing ratios were used to prepare the zein solutions for electrospinning. The concentration of zein was maintained at 10, 15, 20, 25,and 30 % based on the weight of the solution.

Electrospinning of zein solutions

During the electrospinning, a high voltage of 10 kV(CHUNGPA EMT Co., Korea) was applied to the zein solution contained in a syringe via an alligator clip attached to the syringe needle. The solution was delivered to the blunt needle tip using a syringe pump to maintain the flow rate of the solution at 0.04 ml/h. The electrospun materials were then collected on electrically grounded aluminum foil placed 15 cm from the needle tip (Lee et al. 2009; Lee et al. 2009; Karim et al. 2009, Ji et al. 2009). The electrospinning process is shown schematically in Figure 1.

Characterizations

After gold coating, the morphologies of the electrospun zein materials were studied using a field-emission scanning electron microscope (FE-SEM) (JEOL, model JSM-6380). Based on the FE-SEM images, the average diameters of the electrospun zein nanoparticles and nanofibers were then measured using Adobe Photoshop 5.0 software. The transmission electron microscopy (TEM) analysis was conducted on an H-7600 model machine (Hitachi, Ltd., Japan) with an accelerating voltage of 100 kV.

Results and Discussion

Three series of experiments were performed using a fixed co-solvent ratio and various zein solution concentrations. Figure 2 shows FE-SEM images of the electrospun nanostructured materials obtained from the different zein solution concentrations. The co-solvent EtOH/H_2O, ratio was fixed at 7/3 (v/v), while

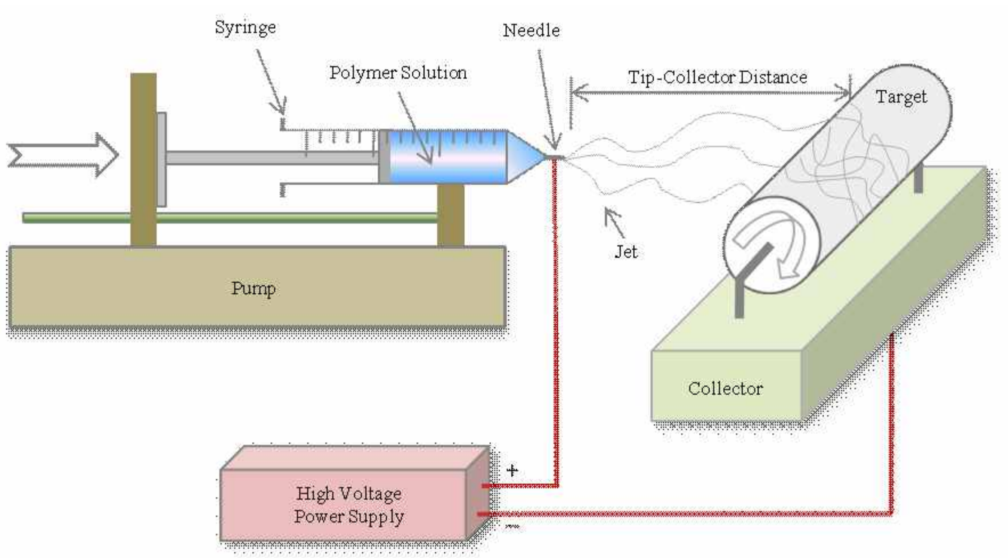

Figure 1. Schematic representation of electrospinning process

beads were also

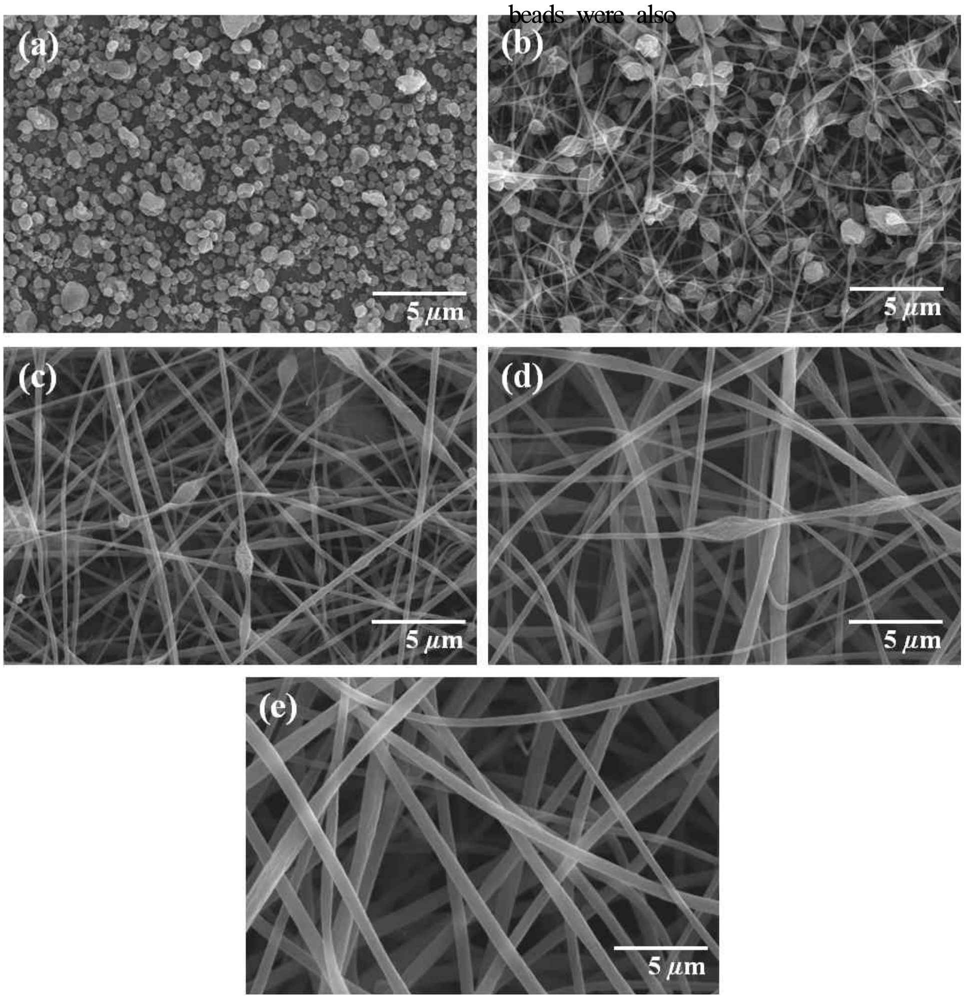

Figure 2. FE-SEM images of electrospun zein materials. Zein solution concentrations are (a) 10 wt.%, (b) 15 wt.%, (c) 20 wt.%, (d) 25 wt.%, and (e) 30 wt.%. EtOH/H_2O ratio is 7/3 (v/v). (TCD = 15 cm and applied voltage = 10 kV).

the zein contents were varied from 10 to 30 wt. % on the basis of the solution's weight. The applied voltage (10 kV) and tip to collector distance (TCD) (15 cm) remained fixed throughout the experiments. Thus, the effects of a variable zein content on the morphologies of the electrospun materials were observed with a fixed co-solvent ratio and fixed electrospinning process parameters. As shown in Figure 2(a), when using the lowest zein content (10 wt. %), the electrospinning produced only spherical-shaped nanoparticles with different sizes. When increasing the zein content to 15 wt. %, tiny nanofibers connected by many beads were obtained, as shown in Figure 2(b). Moreover, the shapes of the beads were crinkled up and remained spherical-like nanoparticles. In the case of the 20 wt. % zein content, the bead density was decreased and the nanofibers were more uniform and thicker(Figure 2 (c)). The stretched, creating an elliptical shape. When using the 25 wt. % zein content, there was almost no bead density, as the beads were merged with the fibers. The nanofibers were also more uniform and thicker than the fibers obtained from the solutions with lower zein contents (Figure 2 (d)). When the zein content reached 30 wt. % (Figure 2(e)), uniform and thick zein nanofibers were obtained without any bead defects. Thus, each zein concentration hada distinct effect on the morphology of the electrospun zein materials, where the low zein concentration caused nanoparticle formation, the middle zein concentrations generated nanofibers connected with beads, and the high zein concentration produced nanofibers with uniform sizes and shapes.

The same series of experiments, using the same solution concentrations and electrospinning parameters, were then repeated when changing the co-solvent ratio from 7/3 to 8/2 (v/v) (EtOH/H_2O).The FE-SEM images obtained from this series are listed in Figure 3. The effect of the co-solvent ratio can be easily understood when comparing Figure 3 with Figure 2. Figure 3(a) shows that electrospinning the lowest zein concentration (10 wt. %) with the increased amount of EtOH (80 vol. %) produced nanoparticles with uniform sizes and shapes. However, the 15 wt. % zein solution and increased amount of EtOH (80 vol. %) produced nanoparticles with only a minimal number of nanofibers (Figure 3(b)), whereas the 15 wt. % zein solution and the lower amount of EtOH (70 vol. %) produced nanofibers connected with many beads (Figure 2 (b)), indicating that the co-solvent ratio had a significant effect on the morphology of the electrospun product. Similarly, twisted and irregular nanofibers connected with lots of crinkled and large-sized beads were obtained when using the 20 wt. % zein solution and increased amount of EtOH (80 vol. %) (Figure 3 (c)), representing clear morphological changes from those in figure 2 (c). Meanwhile, figure 3(d)shows that ribbon-like fibers with very few beads were produced when using the 25 wt. % zein solution and increased amount of EtOH (80 vol. %). Plus, the shapes of the beads were changed from elliptical

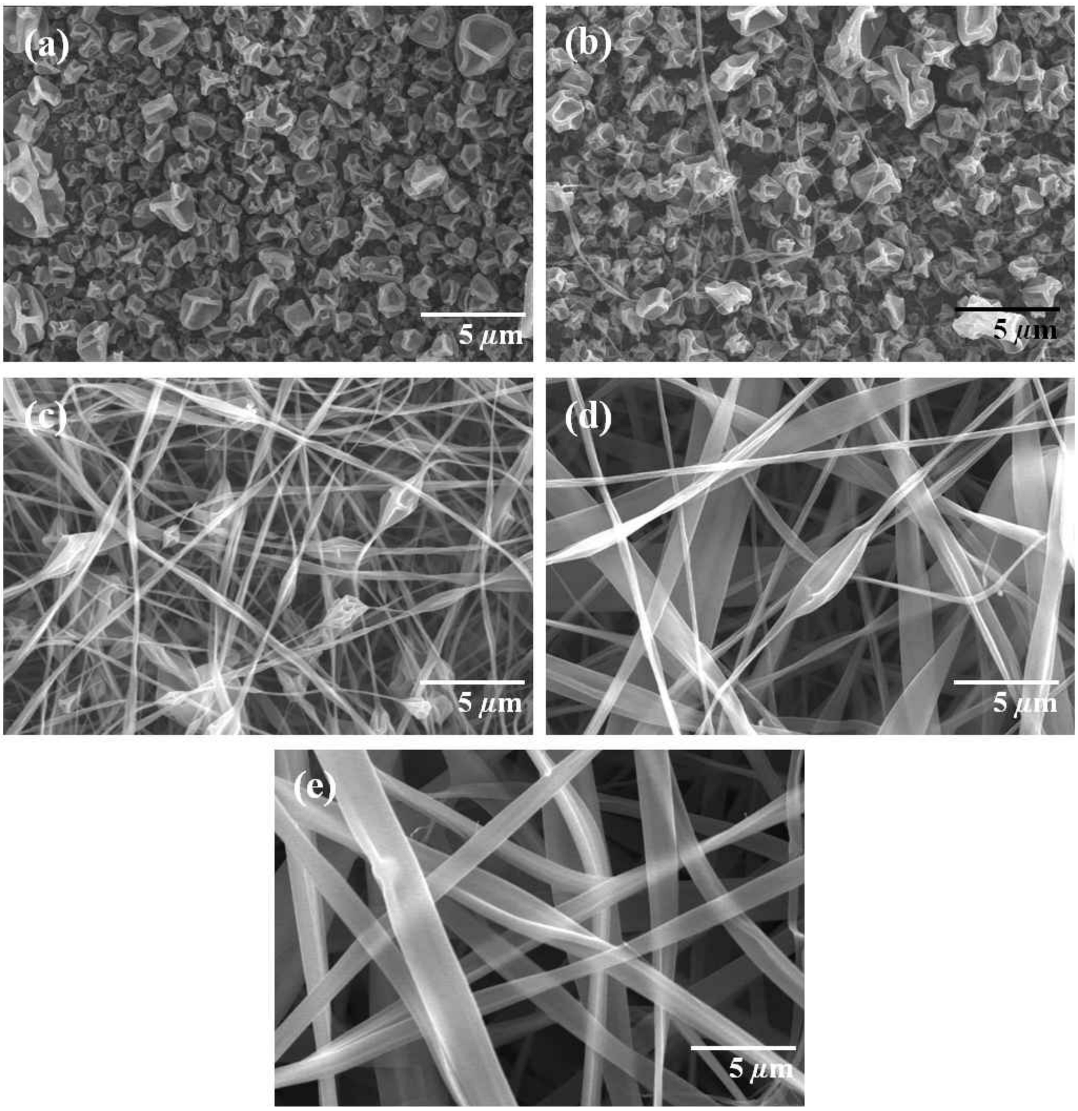

Figure 3. FE-SEM images of electrospun zein materials. Zein solution concentrations are (a) 10 wt.%, (b) 15 wt.%, (c) 20 wt.%, (d) 25 wt.%,and (e) 30 wt.%. EtOH/H_2O ratio is 8/2 (v/v). (TCD = 15 cm and applied voltage = 10 kV).

to flat and the sizes increased. Finally, the 30 wt. % zein solution and increased amount of EtOH (80 vol. %) produced bead-free nano ribbons with regular shapes and sizes (Figure 3e). Thus, when comparing Figures 3e and 2e, the higher EtOH ratio (80 vol. %) changed the morphology of the electrospun nanofibers from a tubular shape to a half-tube shape and also increased the sizes.

Figure 4 shows FE-SEM images of the nanostructured electrospun zein materials obtained when further increasing the amount of EtOH, the co-solvent, to an EtOH/H_2O, ratio of 9/1 (v/v). All the other electrospinning parameters remained the same as in the previous experiments. When comparing Figure 4 with Figures 2 and 3, the structures, shapes, and sizes of the electrospun zein nanomaterials were all altered when using the higher amount (90 vol. %) of EtOH. In the case of the 15 wt. % zein solution, no nanofibers were produced (Figure 4 (b)), whereas the 30 wt. % zein solution produced uniform nano-ribbons (see Figure 4e), reconfirming the influence of the co-solvent ratio and the zein content on the morphology of electrospun zein materials.

From the above results, it was found that all the co-solvent ratios with the lowest zein concentration (10 wt. %) produced only nanoparticles, regardless of size and shape. Meanwhile, all the co-solvent ratios with the highest zein concentration (30

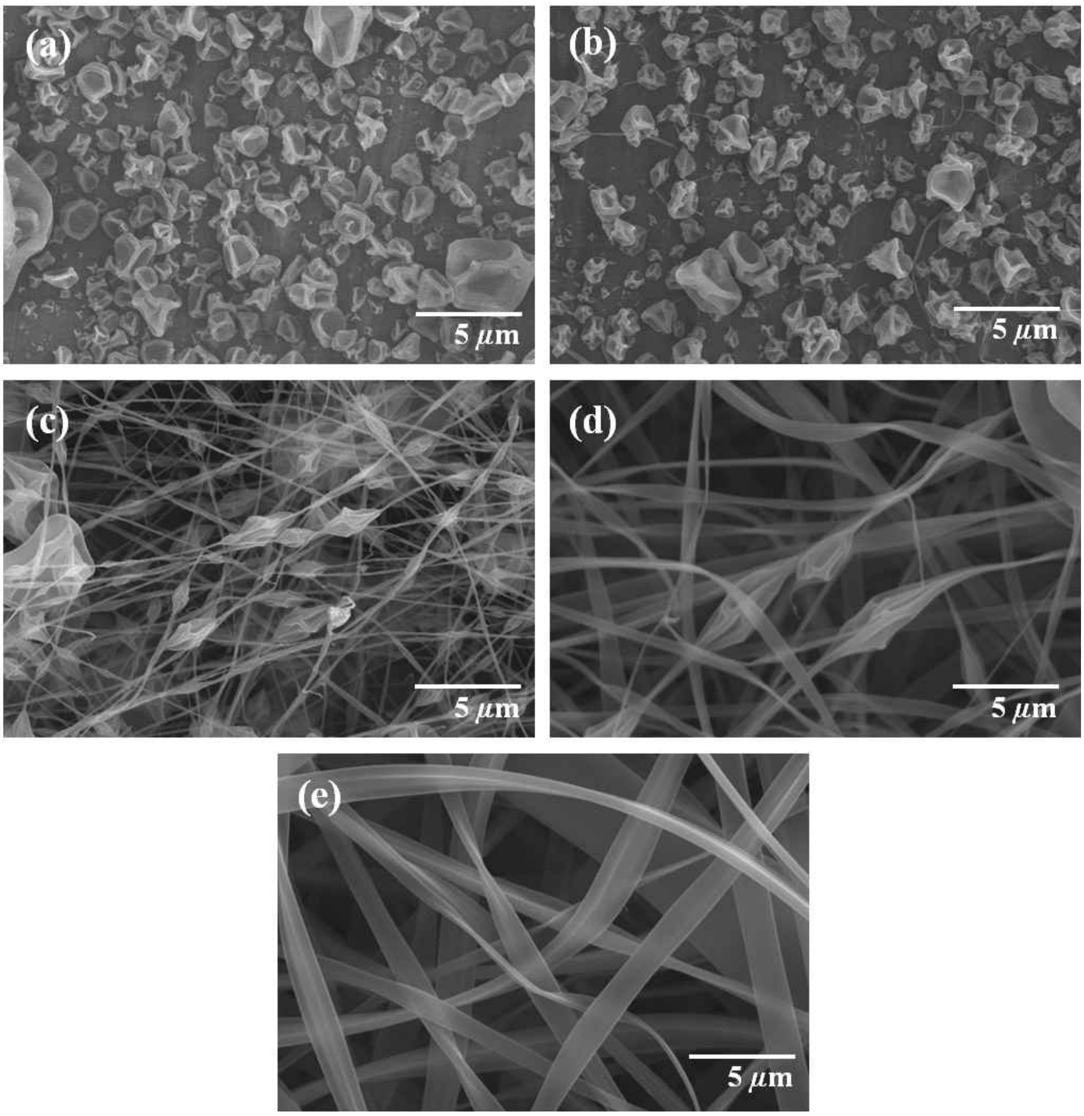

Figure 4. FE-SEM images of electrospun zein materials. Zein solution concentrations are (a) 10 wt.%, (b) 15 wt.%, (c) 20 wt.%, (d) 25 wt.%, and (e) 30 wt.%. EtOH/H_2O ratio is 9/1 (v/v). (TCD = 15 cm and applied voltage = 10 kV).

wt. %) produced only bead-free fibrous nanomaterials. However, figure 5 shows the effect of the co-solvent ratio on the average diameter of the nanoparticles and nanofibers, regardless of the individual morphologies, where the diameters of both the nanoparticles and nanofibers increased when increasing the EtOH ratio. Yet, the diameters of the nanofibers were larger than those of the nanoparticles at the same co-solvent ratio when using the highest zein concentration (30 wt%), indicating that the zein concentration had a greater effect on the diameters of the electrospun nanoparticles and nanofibers.

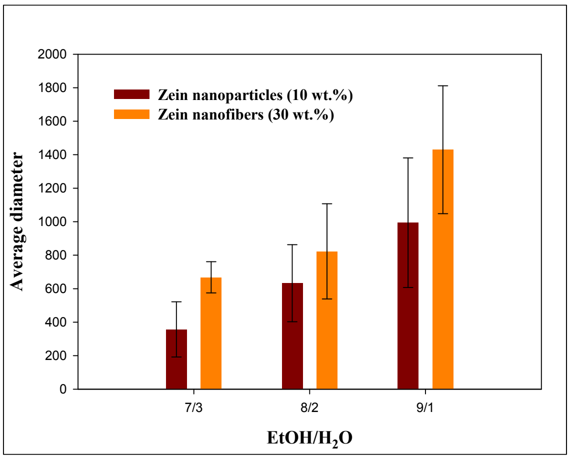

Figure 5. Average diameters of electrospun zein nanoparticles and nanofibers prepared using different $EtOH/H_2O$ ratios.

Electrospinning the lowest zein concentration (10 wt%) produced nanoparticles with different sizes and shapes according to the co-solvent ratio, where uniform nanoparticles were obtained when using the 8/2 (v/v) ratio of $EtOH/H_2O$. Meanwhile, nanofibers, half-tube nanofibers, and nano-ribbons were produced from the highest zein concentration (30 wt%) according to the co-solvent ratio, where uniform nanofibers were obtained when using the 7/3 (v/v) ratio of $EtOH/H_2O$. The appearance of zein nanofibers appeared to be related to the self-assembly of the protein during evaporation of the EtOH. The rapid evaporation of the solvent caused the circular fibers to become elliptical and then flat, forming a ribbon-like structure. Furthermore, when increasing the EtOH ratio, the diameters of the electrospun zein nanofibers and nanoparticles also increased (Figure 5), which was also supported by a TEM analysis. Figure 6 shows TEM images of a single isolated nanoparticle and nanofiber to clarify their exact morphology, where the nanoparticle and nanofiber were obtained from a solution containing 10 wt% zein in 8/2 (v/v) $EtOH/H_2O$ and 30 wt. % zein in 7/3 (v/v) $EtOH/H_2O$, respectively.

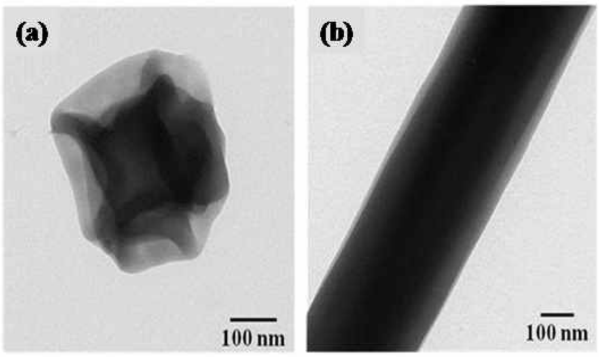

Figure 6. TEM images of electrospun zein (a) nanoparticles (zein concentration = 10 wt. % and $EtOH/H_2O$ ratio = 8/2 (v/v)) and (b) nanofibers (zein concentration = 30 wt.% and $EtOH/H_2O$ ratio = 7/3 (v/v)) (TCD = 15 cm and applied voltage = 10 kV).

Conclusions

The concentration of the zein solution and volume ratio (v/v) of the co-solvents, $EtOH/H_2O$, effectively altered the morphology of the electrospun zein materials, and these morphological changes were quite distinctive. At a fixed co-solvent ratio, the electrospun zein nanoparticles transformed to nanofibers when increasing the zein content. Meanwhile, increasing the amount of EtOH increased the diameters of the nanoparticles and nanofibers. Uniform nanoparticles were obtained from a 10 wt% zein solution in 8/2 (v/v) $EtOH/H_2O$. Bead-free zein nanofibers or nano-ribbons were obtained from a 30 wt% zein solution when changing theco-solvent ratio. Tubular nanofibers became ribbon-shaped when increasing the amount of EtOH. And nanofibers connected by beads were produced when electrospinning the medium zein concentrations (15, 20, and 25 wt%). Thus, the morphologies of the fibers and beads depended on the co-solvent ratios.

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