ELECTROSPUN CHITOSAN/PVA NANOFIBERS FOR DRUG DELIVERY

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ABSTRACT

Electrospinning technique is a versatile method to fabricate continuous fibers with diameters ranging from a few micrometers to a few nanometers. In this study, chitosan/ poly(vinyl alcohol) (PVA) nanofibers were fabricated by an electrospinning method. The effects of chitosan molecular weights and ratio of chitosan/PVA were studied. The characteristics and surface morphologies of nanofibers were observed by the Scanning Electron Microscope (SEM). The diameters of nanofibers were in the range of 100 nm to 250 nm. The chitosan/PVA nanofibers with different molecular weights were applied for curcumin delivery. Curcumin was loaded in the chitosan/PVA nanofibers. Then, release profile of curcumin was investigated. In the results, the encapsulation efficiency and the release rate demonstrate that chitosan/PVA nanofibers would be potential carriers for curcumin and promise to create a prospective drug delivery in the field of medicine.

Keywords: chitosan, curcumin, electrospinning, nanofibers.

1. INTRODUCTION

Electrospinning is a simple and versatile method for creating nanofibers [1]. The electrospinning has many advantages such as low cost, high production rate [2]. Electrospun nanofibers have a high surface-to-volume area. Thus, electrospun nanofibers have been applied for filtration, reinforcement in composite material, tissue engineering, energy-related applications, environmental applications, and especially in drug delivery [2 - 6].

Chitosan, poly-β-(1→4)-D-glucosamine, is a partially deacetylated chitin by concentrated alkaline [7 - 12]. The number of N-acetyl-glucosamine units is higher than 50 % [13, 14]. The amino groups of chitosan can be positively charge in dilute acid medium (pKa 6.3, pH < 6) to become fully soluble [7, 13]. Chitosan is an attractive material for use in drug delivery due to
controlled biodegradability and biocompatibility [7, 8, 15 – 17]. In previous studies, several solvents used in the electrospinning of chitosan are diluted hydrochloric acid, acetic acid, and neat formic acid [6, 7]. However, only TFA can successfully produce chitosan nanofibers with the best homogeneity [7]. TFA can destroy the rigid electrostatic interactions between the chitosan molecular chains and the high volatility of TFA can enhance the rapid solidification of chitosan fibers. With other acids, the formation of protonation with amino groups causes serious repulsion forces under a high applied voltage in electrospinning, resulting in the instability of electrospun fibers. So, conditions of chitosan electrospinning are often controlled very precisely due to the positive groups of chitosan, which repulse each other during electrospinning process. Moreover, the viscosity of chitosan solutions is very high. Thus, chitosan blended with electrospunable polymers, such as PVA enable the fabrication of homogeneous CS nanofibers [18 – 20].

Curcumin, a natural polyphenolic phytochemical extracted from the rhizomes of turmeric (Curcuma longa L.), has attracted considerable attention in recent years due to its great variety of beneficial biological and pharmacological activities including anti-inflammatory, anti-cancer, anti-oxidant, wound healing and anti-microbial effects [21 – 23]. However, widespread clinical application of this relatively efficacious agent in cancer and other diseases has been limited due to poor aqueous solubility, and consequently, minimal systemic bioavailability [24]. Attempts to improve water solubility, stability, and bioavailability of curcumin by complex formation or interaction with macromolecules including gelatins, polysaccharides and phospholipids were previously reported [25]. However, toxic organic solvents were still used in these procedures. Recently, nanoparticle technology has emerged as a potential area of targeted drug delivery systems and made biologically availability of therapeutic agent. Nanoparticles are easier to pass through cell membranes in organisms and get interacted rapidly with biological systems. The development of effective approaches to improve the aqueous solubility and bioavailability of curcumin is therefore necessary. Therefore curcumin compound is incorporated into chitosan-PVA nanofibers to improve significantly the therapeutic efficacy of the fiber film [24, 26 – 28].

In this study, chitosan/PVA nanofibers were fabricated by electrospinning method. The effects of chitosan molecular weights as well as ratio of chitosan/PVA were investigated. The nanofibers were also applied for curcumin release. The encapsulation efficiency and release profiles were established.

2. MATERIALS AND METHODS

2.1. Materials

Chitosan, acetic acid (glacial, 99 - 100 %), ethanol, PVA (146-186 kDa of molecular weight with the degree of acetylation, 87 - 89 %), hydrogen peroxide, hydrochloric acid, sodium hydroxide, and phosphate buffered saline (PBS) were purchased from Sigma-Aldrich. Curcumin (CUR) was obtained from Viet Nam Institute of Dietary Supplements (Viet Nam).

2.2. Chitosan degradation and determination of molecular weight (Mv)

Depolymerization of chitosan using hydrogen peroxide has been described in the previous research. Chitosan (3 g, 300 kDa) was dissolved in 200 ml of a 0.5 % HCl acid solution. The solution was added 2 ml of 30 % H2O2, and reacted at 40 °C for 2, 4, and 8 h before it was filtrated and adjusted to pH 9-10 by using NaOH. The chitosan became immiscible after
increasing pH and was suspended in the aqueous solution. Then, the deposited chitosan was rinsed with deionized water.

The viscosity- average molecular weight of chitosan (Mₐ) was determined by the Mark-Houwink equation:

\[ [\eta] = 1.81 \times 10^{-3} \left( \text{cm}^3 \cdot \text{g}^{-1} \right) M_v^{0.93} \]  

(1)

where the intrinsic viscosity \([\eta]\) of a chitosan solution was measured by the Ubbelohde capillary viscometer at 25 °C in triplicate. After that, the viscosity- average molecular weight of chitosan was determined by the regression of Mark-Houwink equation. The results indicate that different molecular weights (100 kDa, 150 kDa, and 200 kDa) of chitosan were obtained.

2.3. Electropsinning of chitosan/PVA

PVA were dissolved in distilled water to prepare a PVA solution of 8 g.L⁻¹. After stirring for 6 hours at 60 °C, the PVA solution was obtained. Chitosan with various molecular weights was dissolved in 2 % (v/v) of acetic acid to form a chitosan solution of 2 g.L⁻¹ by stirring for 8 hours, at room temperature. Both the polymer solutions were mixed with various weight ratios of chitosan/PVA at room temperature, yielding the homogeneous chitosan/PVA solutions.

The polymer solutions were placed into a syringe. The feeding rate of solution was controlled by a syringe pump (Cole Palmer, Model 74900, USA) at 0.5 mL.h⁻¹. The tip-to-collector distance was 10 cm and the applied voltage was 15 kV. The experiments were performed in air at room temperature.

2.4. Characterization of nanofibers

The morphology of the electrospinning fibers was observed on a Scanning Electron Microscope (SEM, JSM 5500, JEOL, Japan). Samples for SEM were dried at 37 °C. A small section of the fiber mat was placed on the SEM sample holder and sputter-coated with gold for 60 second with an accelerating voltage of 10 kV. The average diameter and the distribution of electrospun fiber were obtained by analyzing the SEM images with an ImageJ software.

2.5. Determination of encapsulation efficiency and loading capacity of curcumin

Curcumin was dissolve in ethanol to form the solution of 5 % wt. 30 mg chitosan/PVA nanofibers was immerged in 30 mL curcumin solution. After 24 hours, the solution was analyzed for total curcumin content by a UV spectrophotometer at 425 nm. Curcumin loading capacity (LC) and encapsulation efficiency (EE) were calculated by the following equations.

\[ \text{LC} = \frac{\text{Amount of curcumin in the nanofibers}}{\text{Amount of the nanofibers}} \times 100 \]  

(1)

\[ \text{EE} = \frac{\text{Amount of curcumin in the nanofibers}}{\text{Amount of initial curcumin}} \times 100 \]  

(2)

2.6. In vitro release of curcumin

Chitosan-curcumin nanofibers (20 mg) were incubated in 50 mL PBS at pH 7.4 and 37 °C with shaking (120 rpm). At a given time, the sample was centrifuged. Then, 8 mL of the solution
was taken out for analysis and 8 mL of fresh PBS was added into the releasing bath. Curcumin amount was measured by a UV spectrophotometer at 425 nm. Each experiment was performed in triplicate and the results report mean value ± standard deviation.

3. RESULTS AND DISCUSSION

3.1. Effect of chitosan molecular weight

Figure 1. SEM images of chitosan/PVA nanofibers with various chitosan molecular weights: (A) 100 kDa; (B) 150 kDa; (C) 200 kDa; (D) 300 KDa.

The morphology and diameter of electrospun nanofibers depend on the various parameters. Among these parameters, the chitosan molecular weight of the electrospinning solution was one of the most effective variables to control the fiber morphology and diameter. To study the effect of chitosan molecular weight, the other main electrospinning parameters were fixed as 2% of acetic acid concentration, 15 kV of applied voltage and 0.5 mL.h⁻¹ of flow rate.

Figure 1 shows the SEM images with CS molecular weights range from 300 to 100 kDa. An increase in the chitosan molecular weight from 100 kDa to 300 kDa, the morphology of electrospun nanofibers was changed. At 100 kDa and 300 kDa, chitosan/PVA nanofibers and large beads were obtained (Fig. 1a and 1d). In contrast, only chitosan/PVA nanofibers were obtained with the chitosan molecular weight of 200, and 150 kDa as shown in Fig. 1b and 1c. The results revealed that chitosan with medium molecular weight were advantageous to form continuous nanofibers due to the low viscosity of solution. It was due to the strong entanglement force in the polymer chains which is indicated by the high viscosity of the solution. The high molecular weight, which indicates the longer polymer chains, results in a higher degree of entanglement between polymer chains. With low molecular weight, the entanglement forces were not enough to keep the continuous fibers. Thus, chitosan with 150 kDa and 200 kDa were proper for electrospinning.
3.2. Effect of ratio of CS/PVA

Figure 2. Effect of ratio of PVA/chitosan with chitosan molecular weight of 150 kDa: (A) 1/3.6; (B) 1/3.2; (C) 1/3; (D) 1/2.8.

Conditions of chitosan electrospinning are often controlled very precisely due to the positive groups of chitosan, which repulse each other during electrospinning process. Moreover, the viscosity of chitosan solutions is very high. Thus, chitosan blended with PVA enable the fabrication of homogeneous chitosan nanofibers.

Figure 2 shows the SEM images of chitosan/PVA nanofibers with various ratio of PVA/chitosan with chitosan molecular weight of 150 kDa. An increase in ration of PVA/chitosan, the morphology of electrospun nanofibers was changed. With the ratio of PVA/chitosan higher than 1/3 (Figure 2c and 2d), the uniform nanofibers were obtained, where the average diameter was about 200 nm. It can be explain because the PVA adding could decrease the viscosity of polymer solutions as well as destroyed the rigid structure of chitosan. However, chitosan has several outstanding properties that can apply for drug delivery in compare with PVA. Thus, the higher ratio of chitosan/PVA was proper for fabrication of electrospun nanofibers. Thus, the ratio of PVA/chitosan was chosen for further experiment was 1/3.

3.3. Encapsulation efficiencies and loading capacities of curcumin

Encapsulation efficiencies (EE) and loading capacities (LC) of curcumin, determined by equations (1) and (2), are shown in Table 1. EE and LC decreased slightly increased increasing CS molecular weight. Compared with previous studies of curcumin adsorption from chitosan nanoparticle, EE prepared by an electrospun technique of our study was higher [29-31]. Thus, chitosan/PVA nanofibers were efficiently adsorption curcumin.

Table 1. EE and LC of curcumin on chitosan/PVA nanofibers with different molecular weights.

| Mv (kDa) | EE (%)       | LC (%)    |
|---------|--------------|-----------|
| 150     | 79.67 ± 1.105| 4.76 ± 0.041|
| 200     | 76.45 ± 1.234| 4.63 ± 0.035|
3.4. Curcumin release in vitro experiment

![Figure 3](image-url)  
*Figure 3. In vitro release profiles of curcumin from different molecular weights of chitosan: (a) 150 kDa, (b) 200 kDa.*

The drug release profiles are shown in Figure 1. After 148 hours, 79.5 % and 65.4 % of loaded curcumin was released from the nanofibers of 150-kDa chitosan and 200-kDa chitosan, respectively. The overall release can be divided into two stages: a rapid release in the first 20 hours and a slow release after 20 hours. In the initial release within the first 20 hours, the releasing percentage of curcumin reached 56.4 % for 150-kDa chitosan and 51.2 % for 200-kDa chitosan. In this stage, drug presented on the nanofibers surfaces was released, and thus release rate mainly depended on curcumin desorption. After 20 hours, releasing rate became slow and was almost constant up to 148 hours (zero-order kinetics of drug release). The release rate of this step mainly depended on the interaction between curcumin and chitosan nanofibers. The release rate of curcumin from 150-kDa-CS nanofibers was somewhat higher than that from 200-kDa-CS nanofibers. The higher viscosity of the high molecular weight CS will form a gel layer and it covers the curcumin. This layer hinders the curcumin release.

4. CONCLUSION

Chitosan/PVA nanofibers were successful prepared by an electrospinning method. The chitosan molecular weights and ratio of chitosan/PVA were strong effects on the morphology of electrospun nanofibers. 150-kDa-chitosan and 200-kDa-chitosan molecular weights were proper for electrospinning. Ratio of chitosan/PVA, smaller than 1/3, could create uniform nanofibers. The average diameter of nanofibers was 200 nm. The adsorption efficiency and release of curcumin from chitosan with 150-kDa chitosan were better than that of 200-kDa chitosan. The release time of curcumin was prolonging until 148 hours.

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