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Preparation of electrosprayed Chitosan/Gum Tragacanth Nanoparticles and their Antibacterial Properties

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Abstract

Electrospraying is similar to electrospinning. However, when the viscosity of the polymeric solution is not high enough to form nanofibers, electrospraying is used to make nanoparticles. In this study, chitosan/gum tragacanth blend nanoparticles were produced using an electrospinning / spraying device, and the chitosan and gum tragacanth concretions on the properties of the resulting nanoparticles were investigated. The morphology of electro - sprayed chitosan/gum tragacanth blend was characterized using a scanning electron microscope (SEM). The results showed that the electrospraying technique can form gum tragacanth - chitosan blend nanoparticles with an average size of 200 to 300 nm. The miscibility of the blend was determined using Fourier transform infrared spectrometer (FTIR). The antibacterial property of samples was also investigated. Wettability results of gum tragacanth chitosan nanoparticles (contact - angle measurements) indicated that the hydrophilic property of nanoparticles is necessary for biocompatible applications.

Keywords: Chitosan, Gum Tragacanth, Blend Nanoparticle, Antibacterial.

1. Introduction

Gum tragacanth, also known as tragacanth, is a natural biopolymer, that is a mixture of two polysaccharides. Tragacanthin, the galacturonic acid part of tragacanth, is water soluble and a neutral branched with high molecular weight, which gives highly viscous solutions, and bassorin, the other part of tragacanth, is a complex of methoxylated acids that is insoluble in water and swells to form a gel or viscous solution. Gum tragacanth has been used as wound dressing, adhesive, absorbent pad during surgery, drug release, and pharmaceuticals. Gum tragacanth is also known to have no antigenicity, and it is more economical to make it an attractive

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component for fabrication and incorporation into drug delivery systems and wound healing materials [1-5]. Another natural polymer similar in properties to tragacanth is chitosan which exhibits biodegradability, biocompatibility, and biological, antibacterial, and anti-inflammatory activity. It has gained increasing attention in the pharmaceutical field due to its favorable biological properties. It is widely accepted as a material for the basis of drug delivery carrier systems. Such CS-based delivery systems, especially in micro/nanospheres, have been shown to reduce drug side effects associated with systemic drug delivery and prolong drug release [1,2,6-9].

Due to the large aspect ratio of nanoparticles, they show different behavior compared to their bulk form. Electrospinning is a simple and physical process to fabricate submicron to nanometer nanofibers by applying electrostatic forces to a polymer solution. [9-11,1]. Electrospraying is similar to electrospinning, but when the viscosity of the polymeric solution is not high enough to form nanofibers, electrospraying can be used to make nanoparticles. So, when the viscosity of the polymer solution is sufficiently low, the electric charges draw the polymeric jet from the capillary nozzle in the form of a fine jet, which eventually disperses into droplets. Charge and droplet size can be finely controlled to some extent by the applied voltage, polymer solution concentration, nozzle-collector gap, flow rate, and needle diameter. After evaporation of the solvent, the size of the droplets decreases, and initial droplets break up into smaller ones as they travel from the tip of nuzzle to the surface of the collector. Droplet breakup, known as the Coulomb explosion, occurs through the repulsion of electronic forces that build up on the droplets [12-13].

In the present study, chitosan was blended with gum tragacanth and electrospray to nanoparticles and some properties have been investigated.

2. Experimental

2.1. Materials

Medium molecular weight chitosan powder with DD=75-85% obtained from Aldrich/USA, Gum tragacanth used in this study was a high-quality ribbon type, collected from the stems of flocculus species of astragalus bushes, grown in the central areas of Iran (Figure 1). Solvents that have been used are glacial acetic acid (AcOH) from chem-lab/ Belgium and distilled water.

Two bacterial strains: Gram-negative (Escherichia coli: ATCC 11303), Gram-positive (Staphylococcus:

ATCC 6538)



Figure 1. The schematic of gum tragacanth preparation from plant to powder

2.2. Nanoparticles fabrication

Chitosan and gum tragacanth were used to prepare solutions as shown in Table 1. Chitosan 3% by weight and gum tragacanth 0.5% by weight in acetic acid 20% were dissolved separately. Mixture solutions of chitosan gum tragacanth were prepared at a weight ratio of 100/0.50/50.0/100 (CH/GT) at 60°C. Polymers were fed into a 20 mL syringe, and needle gaged 21 was used as a nozzle in the electrospinning unit. The applied voltage was 30kV, and tip-to-collector distances and flow rates were fixed at 150 mm and 0.008 mm/min, respectively. Figure.2 shows the steps of preparing polymer solutions for electrospraying nanoparticles.



Figure 2. Schematic diagram of solution preparing and electrospraying process

Concentration(%w/v)	Weight Batio	Flow rate	Voltage(kv)	Distance between needle to collector(mm)
	Nauv			conector (mm)
Chitosan 3%	100	0.008	30	15
Gum Tragacanth0.5%	100	0.008	30	15
Gum Tragacanth() 5%	50/50	0.008	30	15

Tabel1. Electrospraying and solution parameters of nanoparticles samples

2.3. Fourier transform-infrared spectroscopy (FTIR)

The bonding configurations of the samples were characterized using FTIR/ATR (Tensor27, BRUCKER). All data were recorded employing a ZnSe Internal Reflective Element in the range of 500-4000 cm-1.

2.4. Scanning electron microscopy (SEM)

chitosan3%

Scanning Electron Microscopy (SEM, AIS2100, Serontechnologies) was used to observe the morphology of the samples. All the samples were sputter coated with a gold layer beforehand. The diameters of the particles were determined by analyzing SEM images with snapshot software.

2.5. Water-contact Angle measurements (WCA)

Contact-angle measurements were performed according to international standard method no. ASTM D724-99. Deionized water was dropped onto the sample, and a photo of the drop was captured using a video camera (SSC-DC318P, Sony Co.). The contact angles could be calculated by the software by analyzing the shape of the drop (Image J).

2.6. Antibacterial Property of nanoparticles

To investigate the antibacterial effect of samples, the number of colonies was counted for a population of the test organisms on tryptic soy agar followed by incubation at 35°C for 24 hours. Two bacterial strains: Gramnegative (Escherichia coli: ATCC 11303), Gram-positive (Staphylococcus: ATCC 6538).

Antibacterial activity was calculated according to the following equation:

Antibacterial activity $(\%) = A-BA \times 100\%$

A and B are the bacterial colonies before and after shaking, respectively.

3. RESULTS AND DISCUSSION

3.1. Fourier transform-infrared spectroscopy (FTIR)

Figure 3 shows the FTIR spectra of a) chitosan, b) gum tragacanth, c) chitosan/ gum tragacanth blend. The amine band at 1552.52 cm-1 was observed in the FTIR spectrum of chitosan (Fig. 3a). Figure 3b represents the FTIR/ATR spectra of GT. The peaks at 1442 cm-1, 1637, and 1746 cm-1 are related to asymmetric and symmetric carboxylate and carbonyl stretching, respectively.

The spectrum of tragacanth-chitosan (Fig. 3c) reveals the main changes in 1415.12 cm-1, which relates to carboxylic salt and could not be observed in the tragacanth or chitosan spectrum. [14-17]



Figure 3. FTIR spectra of a) chitosan, b) gum tragacanth, c) chitosan/gum tragacanth nanoparticles

3.2. Scanning electron microscopy (SEM)

Figures 3a and 3b show SEMs of pure chitosan and tragacanth. The corresponding particle size distribution with an average diameter is 255.40±48.74nm and 264.94±136.1 nm, respectively. Figure 3c shows the average particle size of 50/50 chitosan/gum tragacanth, which is 294.28±149.48 nm. It can be concluded that almost uniform spherical nanoparticles can be produced by electrospray. The increase in nanoparticle diameter is due to higher entanglements of macromolecules in the polymers blend and more difficulty breaking up the droplets during Coulomb explosions in the electric field between the nozzle and collector [13].







3.3. Water-contact Angle measurements (WCA)

The surface hydrophilic property plays an important role in biomedical applications. To investigate the influence of blending polymers on the surface hydrophilic property of electrospray gum tragacanth/chitosan nanoparticles, the water contact angle measurement was done and shown in Figure 5. As can be seen, gum tragacanth/chitosan nanoparticles showed a contact angle of about 41° which is less than 90°, indicating that the surface is hydrophilic [14].



Figure 5. Water-contact Angle measurements (WCA) of gum tragacanth/chitosan

3.4. Antibacterial

The antibacterial property of biopolymeric nanoparticles plays a crucial role in their application as a wound dressing. However, for pure chitosan and chitosan-gum tragacanth nanoparticles, there is a higher grade of suppression of bacterial colonies (Table 2). It is due to the interaction between a negatively charged bacterial cell surface and positively charged chitosan. On the other hand, the antibacterial effect of chitosan is more effective against Staphylococcus aureus compared to Escherichia coli due to stronger binding and lysis on Grampositive bacterial cell walls. It is assumed that the L-sugars found in tragacanth (L-arabinose and L-fucose) are responsible for the resistance to microbial attack since most organisms would be unable to metabolize these foreign sugars [15].

Sample	E. coli	S. aureus
	Bacterial Activity	Bacterial Activity
Chitosan	83.79%	93.5%
Gum Tragacanth	78.89%	63.87%
Gum tracaganth/chitosan	80.5%	89.65%

Table 2. Anti-bacterial activity of nanoparticles with Gram-positive S. aureus and Gram Negative E. coli

4. Conclusion

Nanoparticles of chitosan/gum tragacanth blend with an average particle size of 200 to 300 nm were electrospray. Regarding FTIR results, gum tragacanth, and chitosan were found to be miscible. Wettability results of tragacanth-chitosan nanoparticles indicated the hydrophilic property of nanoparticles, which is essential for biocompatible applications. The hybrid nanoparticles showed antibacterial properties due to the presence of chitosan and gum tragacanth, which are antibacterial biopolymers

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