Chitosan Microspheres and Sponges: Preparation and Characterization

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ABSTRACT: In this study, chitosan microspheres and sponges were prepared and characterized for diverse biomedical applications successfully. The chitosan microspheres were obtained with a "suspension crosslinking technique" in the size range of 30–700 μm. The stirring rate of the suspension medium and the chitosan/acetic acid ratio, emulsifier, and crosslinker, that is, the glutaraldehyde concentration in the suspension medium, were evaluated as the effective parameters on the size/size distributions of the microspheres. The microsphere size/size distributions were increased with the decreasing of all effective parameters except the chitosan/acetic acid ratio. In the second part of the study, chitosan sponges were prepared with a solvent-evaporation technique and sponges were cross-linked either during the formation or after the formation of sponges by using a cross-linker, that is, glutaraldehyde. When the sponges were crosslinked during the formation, fibrillar structures were obtained, while the leaflet structures were obtained in the case of crosslinking after the formation of sponges. In the last part of the study, the swelling behavior of both the chitosan microspheres and sponges were evaluated using different amounts of the crosslinker. The swelling ratio was increased in both types of structures, that is, microspheres and sponges, by decreasing the amount of the crosslinker. © 2000 John Wiley & Sons, Inc. J Appl Polym Sci 76: 1637–1643, 2000

Key words: chitosan microspheres; chitosan sponges; swelling; biomedical applications

INTRODUCTION

Chitosan [(1 → 4)-2-amino-2-deoxy-β-D-glucan] is a polyaminosaccharide, normally obtained by alkaline deacetylation of chitin, which is a polysaccharide that is widely spread among marine and terestrial invertebrates and lower forms of a plant kingdom.[1,2] Chitosan's availability in a variety of useful forms and its unique chemical and biological properties make it a very attractive biomaterial and it is extensively used in many types of applications, that is, treatment of wastewater,[3,4] chromatographic support,[5,6] enzyme immobilization,[7,8] wound-healing dressing,[9,10] dental applications,[11] adhesion bandages for surgery,[12–15] and drug-delivery systems.[16–18] In these applications, chitosan's key properties are biocompatibility, nontoxicity (its degradation products are known natural metabolites), it is ability to improve wound healing and/or clot blood, its ability to absorb liquids and to form protective films and coatings, and its selective binding of acidic lipids, thereby lowering serum cholesterol levels. Furthermore, chitosan's ability to be made into films, fibers, and beads as well as powders and solutions lead to many commercial applications.[19]

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Although there are a number of excellent publications, that is, books and reviews on chitosan and numerous articles and patents concerning chitosan microspheres and membranes, there is lack of investigation on the determination of effective parameters on microsphere size/size distributions and sponge-type chitosan structures. The first aim of this study was to investigate those effects and, second, to prepare chitosan sponges by novel techniques, such as direct precipitation of chitosan in the form of fibrillar or leaflet structures for diverse biomedical applications, that is, controlled-release systems and wound-healing studies. Furthermore, the swelling behavior of the obtained structures, that is, microspheres and sponges, was also investigated using different amounts of a cross-linker.

EXPERIMENTAL

Preparation of Chitosan Microspheres and Sponges

Chitosan was supplied commercially with the molecular weight of 650,000 (Fluka, Steinheim, Germany). The aqueous acetic acid solution was used as the solvent in both microsphere and sponge preparation. Glutaraldehyde, 50% (Fluka) was used as the cross-linker. The following chemicals were all obtained and used as reagent grade from Fluka: petroleum ether, mineral oil, ethyl alcohol, acetic acid, sodium bisulfide, and acetone.

Chitosan Microspheres

A suspension crosslinking technique was used for the preparation of chitosan microspheres.[20] In a typical procedure, a 4% chitosan solution was prepared using a 5% aqueous acetic acid solution, then, it was poured into the dispersion medium, dropwise, which was composed of mineral oil and petroleum ether (25/35, v/v) and an emulsifier (i.e., Span-85). During these processes, the dispersion medium was stirred with a mechanical stirrer in the range of 1000–2000 rpm at room temperature. Ten minutes later, 1 mL of glutaraldehyde was added into the dispersion medium. Similarly, 1 h later, 1 mL more of glutaraldehyde was added into the medium and then stirred for 2 h more. At the end of this period, the chitosan microspheres were collected by centrifugation and washed with petroleum ether, sodium bisulfide, and acetone consecutively. Then, the microspheres were dried in an oven at 40°C for 2 days and kept in a vacuum desicator for further analysis and uses. In this part of the study, the stirring rate of the dispersion medium, the chitosan/acetic acid solution ratio, the emulsifier concentration, and the amount of glutaraldehyde were changed for investigation of the effects of these parameters on the size and size distribution of chitosan microspheres in the ranges of 1000–2000 rpm, 5–20 mg/mL, 0.005–0.02 mL/mL suspension medium, and 0.0083–0.0333 mL/mL suspension medium, respectively.

Chitosan Sponges

Chitosan sponges were prepared with a solvent-evaporation technique.[21] Here, a 4% chitosan solution (which was prepared by using the 5% aqueous acetic acid solution) was poured into absolute ethyl alcohol containing different amounts of glutaraldehyde (i.e., 0.25–1.00%, v/v) in a vertical tube (inside diameter of the tube, 2 cm; height, 80 cm) via an injector equipped a dosage pump. The chitosan solution was formed as a fibrillar form during the precipitation and the chitosan sponges were kept in ethyl alcohol overnight. Afterward, the chitosan sponges were dried in an oven at 40°C for 2 days and kept in a vacuum desicator for further analysis and uses. The form of the sponges were of a fibrillar structure. On the other hand, chitosan sponges were also prepared with the same procedure given above without glutaraldehyde as non-crosslinked sponges. Crosslinking was achieved after formation of the sponges by addition of glutaraldehyde. In this case, the obtained sponges were of a leaflet structure. The obtained chitosan sponges were washed with sodium bisulfide to remove unreacted glutaraldehyde in the sponges.

Size Determination of Chitosan Microspheres

The size and size distributions of the chitosan microspheres were determined from micrographs taken with an optical microscope (Nikon, Alphaphot, Japan). Average size and standard deviations of the microspheres on the micrographs (each containing approximately 25–50 microspheres) were evaluated.

The morphological characterization of the chitosan microspheres and sponges were made with a scanning electron microscope (SEM, JEOL, Japan). A 100-μL aqueous suspension of chitosan microspheres was dropped (or a piece of chitosan sponge was fixed) on a sample holder (a stap) and

placed in a vacuum oven at room temperature for 24 h to dry. The samples were coated with gold, and then SEM micrographs were obtained.

Swelling Behavior of Chitosan Structures

Dynamic swelling properties of the chitosan structures were determined by the volumetric method for chitosan microspheres and the gravimetric method for chitosan sponges. In the volumetric method, the chitosan microspheres of a known amount (50 mg) were placed in a tube (internal diameter, 5 mm; height, 100 mm) and the top point of the microspheres was signed. Afterward, the tube was filled with distilled water and the height of the microspheres were signed periodically (i.e., for each 30 min). The percentage of swelling of the microspheres in the tube was calculated from the following formula:

$$S_{CM} = \frac{h_t - h_0}{h_0} \times 100$$

where S_{CM} is the percentage of swelling of the microspheres, h_t denotes the height of the microspheres at time t, and h_0 is the initial height of the microspheres in the tube.

To determine the swelling ratio of the chitosan sponges, the gravimetric method was applied as mentioned before. Here, the sponges of a known amount (around 50 mg) were placed in distilled water for a required period of time. The swollen sponges were collected and the wet weight of the swollen sponges was determined by first blotting the sponges with filter paper to remove absorbed water on the surface and then weighing immediately on an electronic balance. The weight of the swollen sponges was recorded at a predetermined time period. The percentage of swelling of the sponges in the media was then calculated from the following formula:

$$S_{CS} = \frac{w_t - w_0}{w_0} \times 100$$

where S_{CS} is the percentage of swelling of the sponges, w_t denotes the weight of the sponges at time t, and w_0 is the initial weight of the sponges.

RESULTS AND DISCUSSION

Characterization of Chitosan Structures

Chitosan is a multifunctional polysaccharide and has been the focus of much research in biomedical and pharmaceutical applications because of its biocompatibility, biodegradability, and nontoxicity and it is a very abundant naturally occurring raw material, that is, chitin.[1] In this study, we prepared and characterized chitosan structures in the form of microspheres and sponges for diverse biomedical applications.

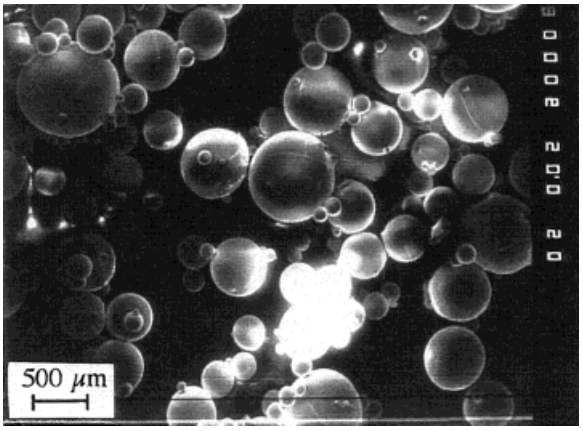


Figure 1 Representative SEM micrograph of chitosan microspheres.

Chitosan Microspheres

Chitosan microspheres were prepared using the suspension crosslinking technique in the size range of 30–700 μm. The obtained chitosan microspheres were evaluated by SEM micrographs to investigate the morphology of the microspheres. A sample of an SEM micrograph is given in Figure 1. The produced chitosan microspheres have a spherical shape and rather smooth surfaces, as seen in this figure. Furthermore, effective parameters (i.e., stirring rate of dispersion medium, chitosan/acetic acid solution ratio, emulsifier concentration, and amount of glutaraldehyde) on microsphere size and the size distribution were evaluated using optical micrographs and related results are given in the following subsections with details.

Effects of Stirring Rate. The stirring rate of the suspension medium was varied in the range of 1000–2000 rpm in order to investigate the effect of the stirring rate on microsphere size and the size distribution. During these studies, the emulsifier concentration, chitosan/acetic acid solution ratio, and glutaraldehyde concentration were kept constant at 0.02 mL/mL of the suspension medium, with a 20 mg chitosan/mL acetic acid

solution, and at 0.0333 mL/mL of the suspension medium, respectively. Figure 2 shows the average size and size distribution (as standard deviations) of the chitosan microspheres obtained under these conditions.

As seen in Figure 2, the average size of the chitosan microspheres decreased and the width of the size distribution was reduced by increasing the stirring rate, and the smallest microspheres in size were obtained with a 2000 rpm stirring rate as 144.86 ± 44.90 μm. Here, the stirring rate provides the required energy to the chitosan solution to be dispersed as fine droplets in the suspension medium (or oil phase) and, therefore, higher stirring rates create microspheres smaller in size and with a narrower size distribution, as reported in the related literature.[22–25]

Effects of Chitosan/Acetic Acid Solution Ratio. The chitosan/acetic acid solution ratio was varied in the range of 5–20 mg/mL in order to investigate the effect of the chitosan/acetic acid solution ratio on the microsphere size and the size distribution. During these studies, the stirring rate, emulsifier concentration, and glutaraldehyde concentration were kept constant at 1500 rpm, 0.02 mL/mL of the suspension medium, and 0.0333 mL/mL of the suspension medium, respectively. Figure 3 shows the average size and size distribution (as standard deviations) of the chitosan microspheres obtained under these conditions.

As seen in Figure 3, the average size and size distribution of the chitosan microspheres increased by increasing the chitosan/acetic acid solution ratio and the largest microspheres in size were obtained with a chitosan/acetic acid solution of 20 mg/mL. This can be explained by considering the viscosity of the initial chitosan–acetic acid solution. The more viscous chitosan solutions were obtained when higher amounts of chitosan were used. It was more difficult to disperse the solutions with higher viscosities; therefore, larger microspheres were obtained. Similar tendencies were obtained in our earlier studies, in which polylactide and poly(ethylene glycol) polylactide microspheres[23,26] and polyhydroxybutyrate microspheres[22] were prepared, and in those studies by other researchers.[27]

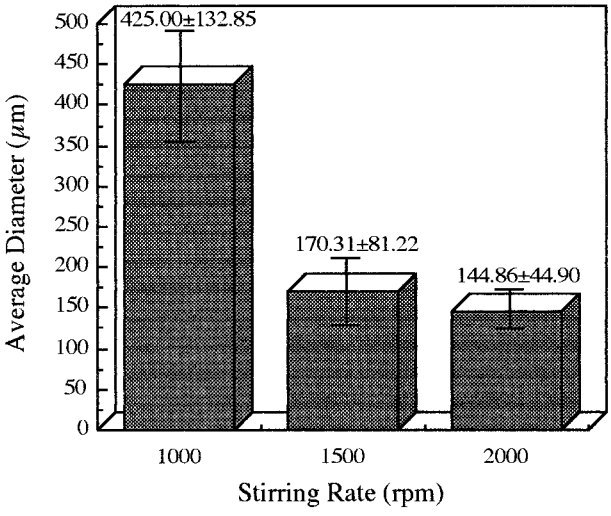


Figure 2 Effects of stirring rate on chitosan microsphere size and size distribution.

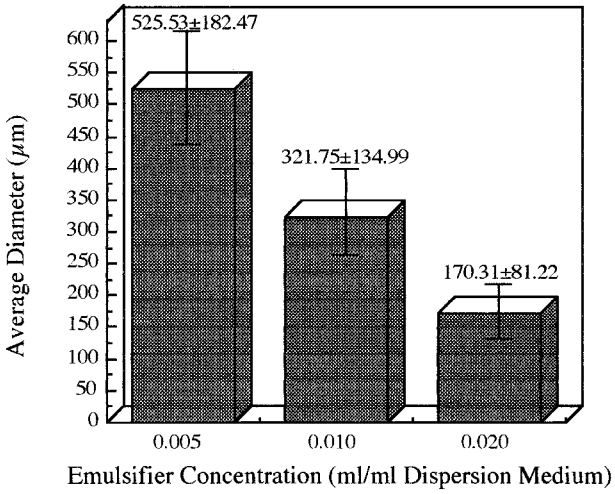


Figure 3 Effect of chitosan/acetic acid solution ratio on chitosan microsphere size and size distribution.

Effects of Emulsifier Concentration in Suspension Medium. In the chitosan microsphere preparation studies, different types of emulsifier were used, for example, dioctylsulfosuccinate[28] and Span 85,[29] to provide a stable oil phase while some of the studies were performed without any emulsifier.[30] In our preliminary studies, we used Tween-80 as an emulsifier to produce chitosan microspheres, but the microspheres were smaller than the desired size.[20] Therefore, we tried to use another type of emulsifier, that is, Span-85. On the other hand, the emulsifier concentration in the suspension medium was evaluated as the effective parameter on the size and size distribution of the chitosan microspheres similarly as in the other studies.[20] Here, the emulsifier concentration was varied as 0.005–0.02 mL/mL of the suspension medium. During these studies, the stir-

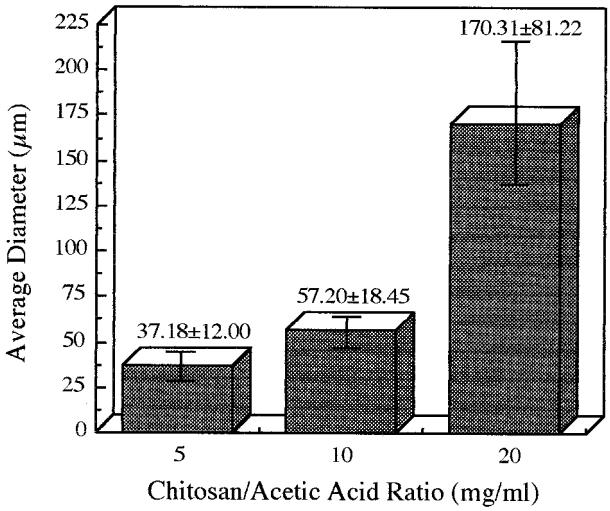


Figure 4 Effect of emulsifier concentration in suspension medium on chitosan microsphere size and size distribution.

ring rate, chitosan/acetic acid solution ratio, and glutaraldehyde concentration were kept constant at 1500 rpm, 20 mg of the chitosan/mL acetic acid solution and 0.0333 mL/mL of the suspension medium, respectively. Figure 4 shows the average size and standard deviations of the chitosan microspheres obtained under these conditions.

As seen in Figure 4, the average size of the microspheres decreased with an increasing emulsifier concentration and the size distribution became narrower. This is because of the decreasing interfacial tension between the aqueous droplets and the organic suspension medium caused by the emulsifier increasing.

Effects of Glutaraldehyde Concentration in Suspension Medium. According to the suspension crosslinking technique to prepare chitosan microspheres, different types of bifunctional agents (i.e., glutaraldehyde, terephthaloyl chloride, hexamethylene diisocyanate, ethylene glycol diglycidyl ether) can be used as the cross-linker.[6,29] Glutaraldehyde is the most frequently used one for this purpose.[28–31] Furthermore, it is well known that chitosan has a hydrogel structure, and in the hydrogels, the reduction in water uptake by the accompanying microspheres increased the crosslinked density because of the crosslinking. Therefore, in this study, the amount of the crosslinker, that is, glutaraldehyde, was evaluated as the effective parameter on microsphere size and size distribution. Here, the glutaraldehyde concentrations were varied as 0.0083–0.0333 mL/mL of the suspension medium. During these studies, the stirring rate, chitosan/acetic acid solution ratio, and emulsifier concentration were kept constant at 1500 rpm, 20 mg of the chitosan/mL acetic acid solution, and 0.02 mL/mL of the suspension medium, respectively. Figure 5 shows the average size and standard deviations of the chitosan microspheres obtained under these conditions.

As seen in Figure 5, the average size of the microspheres decreased with increasing glutaraldehyde concentration and the size distribution became narrower. This was due to the increasing resistance to the water diffusing out from the microspheres during the microsphere formation caused by increasing glutaraldehyde. Hence, the microspheres reached a greater size.

Chitosan Sponges

The crosslinking procedure affected the morphology of the sponges. SEM micrographs of the chitosan sponges are given in Figure 6(A, B). As seen in these figures, when the crosslinker was applied during the formation of the chitosan sponges, the sponges have a fibrillar structure and the fibers are stuck at each contact point. In this case, the size (or diameter) of the chitosan fibers is dependent on the diameter of the needle which was used to prepare the chitosan sponges.[32] On the other hand, if the crosslinker was applied after the formation of chitosan sponges, the final form of the sponges is seem as the leaflet structure, as seen in Figure 6(B). The swelling ratios of both types of chitosan sponges are dependent on the

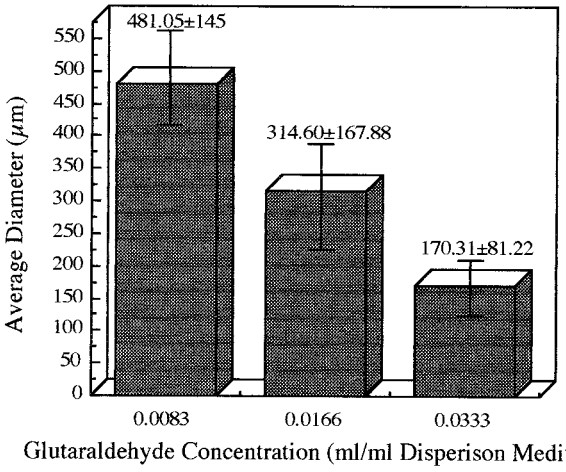
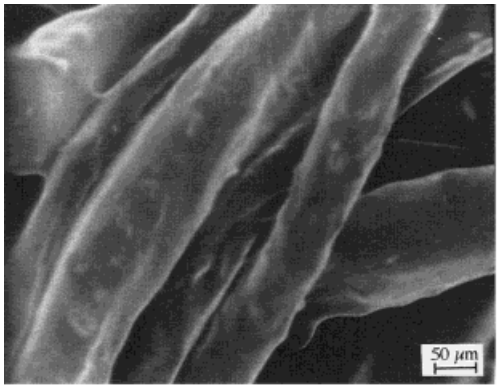
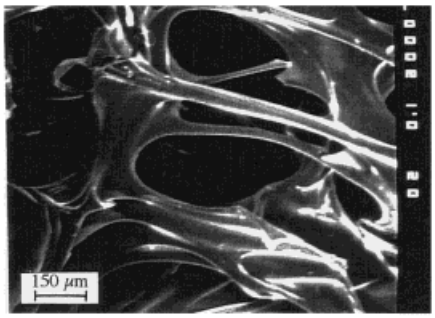


Figure 5 Effect of glutaraldehyde concentration on chitosan microsphere size and size distribution.



(A)



(B)

Figure 6 Chitosan sponges; crosslinked (A) during formation of sponges and (B) after formation of sponges.

crosslinker concentration. This effect is discussed in the following subsections. On the other hand, it is possible to prepare chitosan sponges by using the chitosan gel in an acetic acid solution with freeze-drying or lyophilization,[33] but our method seems to be much more simpler than this one.

Swelling Behavior of Chitosan Structures

The swelling ratio (or crosslinking density) is significantly dependent on the amount of the crosslinker which was used for crosslinking.[34,35] Therefore, in this study, the amount of the crosslinker was evaluated as the most effective parameter on the swelling behavior of the chitosan structures. The obtained results related to the swelling ratios are given in the following subsections for both the chitosan microspheres and chitosan sponges.

Swelling Behavior of Chitosan Microspheres

The swelling ratio variations with time are given in Figure 7 depending on the amount of the crosslinker. As seen in this figure, in all cases, the microspheres reach the equilibrium value of swelling within 30–60 min. The maximum swelling ratio, that is, 75%, was achieved by using the lowest crosslinker concentration and the swelling ratio was decreased by increasing the crosslinker concentration.

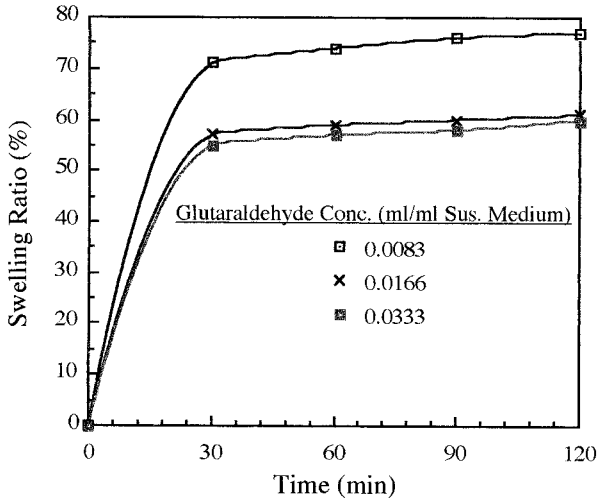


Figure 7 Swelling behavior of chitosan microspheres.

Swelling Behavior of Chitosan Sponges

The swelling ratio variations with time are given in Figure 8 depending on the amount of the crosslinker. As seen in this figure, in all cases, the sponges reached the equilibrium value of swelling at around 90 min. The maximum swelling ratio, that is, 130%, was achieved by using the lowest

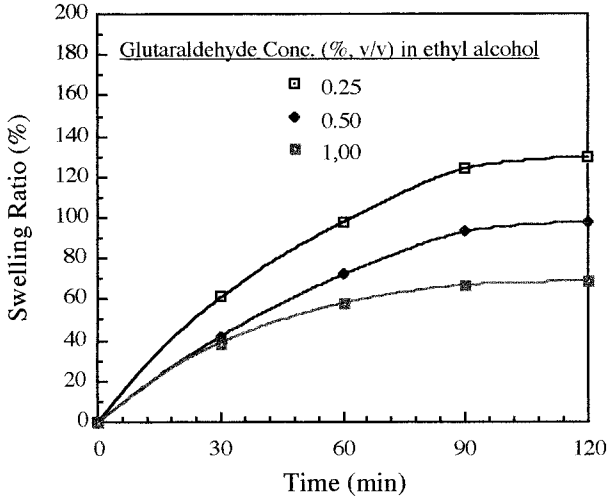


Figure 8 Swelling behavior of chitosan sponges.

crosslinker concentration and the swelling ratio was decreased by increasing the crosslinker concentration as in the case of chitosan microspheres.

This swelling behavior can be explained by the permeation mechanism in hydrogels, meaning that in the hydrogels the water permeation occurs via a "pore" mechanism, the reduction in water uptake by the microsphere (or sponge) accompanying increased cross-linking density being an important factor.[34]

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