

# Swelling Behavior and Cytotoxicity of Maleic Acid Grafted Chitosan

Sevil Yuçel, Zafer Omer Özdemir, Cem Kesgin, Pinar Terzioglu, Simten Unlu, Yagmur Erdogan, and Kubra Pusat

**Abstract**—Chitosan is an attractive polysaccharide obtained by deacetylation of an abundant natural biopolymer called chitin. Chitin and chitosan are excellent materials. To improve the potential of chitin and chitosan modification is needed. In the present study, grafting of maleic acid on to chitosan by cerium ammonium nitrate in acetic acid solution was investigated with use of a microwave and reflux system. The grafted chitosan was characterized by using a Fourier-transform infrared spectrometry. The solubility and swelling behavior of grafted chitosans were determined in acetate buffer (pH 3.6), citrophosphate buffer (pH 5.6 and pH 7.0), and boric buffer (pH 9.2) solutions. The sample obtained by microwave system with use of a chitosan/maleic anhydride/ceric ammonium nitrate 0.2/3.922/0.99 gram of raw material within 30 minute showed the maximum swelling ratio (13.6) in boric buffer solution.

**Keywords**—Chitosan, cytotoxicity, grafted, maleic acid, swell.

## I. INTRODUCTION

CHITIN is one of the most abundant natural, white, hard, hydrophobic, inelastic and nitrogenous polysachharide which is consist of 2-acetamido-2-deoxy-b-D-glucose through a  $\beta$  (1 $\rightarrow$ 4) linkage [1]. Exoskeleton of arthropods, cell walls of fungi and yeast are precursor of the chitin, whilst crab and shrimp shells are widely used for commercial production [2]. The utilization of chitin is limited due to its low water sorption ability [1], [3]. Chitosan is the deacetylated derivative of chitin, which is soluble in common solvents make it preferred over chitin for many applications ranged from biomedicine and pharmacy to agriculture [2], [4]. Chitosan has superior abilities such as being hydrophic, biocompatible, biodegradable, antibacterial, antioxidant, antitumor, antiulcer, immunostimulatory, ion-chelating agent, and a remarkable affinity for many proteins and fats [5], [6]. Therefore, chitosan

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is receiving greater attention as a biomaterial for wound-healing, gene delivery, cell culture, and tissue engineering applications [7].

The modification of these valuable biomacromolecules has a great significance to bring out and develop their potential functions. A variety of chemical modifications have been reported to improve properties of chitin and chitosan to diversify its usage area [3], [4], [8]. Graft copolymerization is one of the most preferred chemical modification methods. Different redox initiators as Fenton's reagent ( $\text{Fe}^{+2} - \text{H}_2\text{O}_2$ ), potassium per sulfate, and cerium (IV) ammonium nitrate have been used to graft monomers onto chitosan [9], [10]. Also, acrylonitrile, vinyl acetate, methyl acrylate, methyl methacrylate, acrylamide, acrylic acid and maleic acid have been used as vinyl monomer sources [9]. In this study, initially maleic acid was used to modify chitosan with use of a reflux system and a microwave system. Moreover, the water sorption ability of modified chitosans under various pH conditions was investigated.

## II. EXPERIMENTAL PROCEDURE

### A. Materials

Chitosan and ceric ammonium nitrate were purchased from Sigma-Aldrich. All other chemicals were of reagent grade.

### B. Grafting of Chitosan

Maleic acid grafted chitosan was prepared by a modified method to that described by Hasipoglu et al. [8]. Chitosan (0.4g) was dissolved in acetic acid solution (20mL, 1% v/v) with constant stirring using a magnetic stirring bar. The mixture was charged into a three-necked round bottom flask followed by adding maleic anhydride (3.922g) and ceric ammonium nitrate (0.99g). Nitrogen gas was bubbled during the reaction that continued for 3h at 70°C under continuous stirring. 20mL of distilled water was added to terminate the reaction. Then the products were precipitated in acetone (50mL), filtered and dried at 60°C.

The experiments were also conducted in a microwave system (Milestone, Microsynth). Batch microwave tests were performed on single-mode operating systems, running at 2.45GHz. Stirring was performed at 400rpm, with a magnetic nucleus.

Time (15, 30, 60 minute) and mass of chitosan (0.2, 0.4 gram) were studied as reaction variables for both of the methods.

### C. Characterization of Grafted Chitosan

The Fourier transform infrared spectra of the samples were obtained with use of a SHIMADZU, IR Prestige 21; USA in the range of 800 to 4000 $\text{cm}^{-1}$ .

The percentage of grafting was calculated according to the following equation:

$$\text{Grafting (\%)} = [(W_g - W_c) / W_c] \times 100 \quad (1)$$

where  $W_g$  and  $W_c$  are the weights of the grafted copolymer sample and the mass of chitosan, respectively.

### D. Evaluation of Solubility and Swelling of Grafted Chitosans

Different buffer solutions were used to determine the pH-sensitivity of the samples. Acetate buffer (pH 3.6), citrophosphate buffer (pH 5.6 and pH 7.0), and boric buffer (pH 9.2) were used as buffer solutions.

A completely dried grafted sample was put into a weighed tea bag and immersed in 100ml buffer solution and allowed to soak at room temperature. The weight of the swollen samples was measured after the sample was allowed to drain by removing the tea bag from water and hanging for 1 minute. Also, the weight of bag was measured to determine the weight of the swollen sample [4]. The experiments were performed at different time intervals of 10, 20, 30, 60, 90, 120, and 1440 (24h) min.

The solubility and swelling behavior of the samples obtained by microwave system with use of 0.2 gram of chitosan, 20mL of 1% acetic acid for 15 (M1), 30 (M2), 60 (M3) min and by reflux system 0.2 gram of chitosan, 20mL of 1% acetic acid for 3h (R1) were determined.

The swelling ratio of the samples was determined according to the following equation:

$$Qt = Wt/Wi \quad (2)$$

where  $Wt$  and  $Wi$  are the weights of the swollen sample at equilibrium and the dry sample, respectively.

### E. MTT Assay

In this study, the L929 mouse fibroblast cells were treated with various concentrations of maleic acid grafted chitosan particles ranging from 3 to 100 $\mu\text{L}$  for 24 hour, and MTT assay was used to measure the cell viability. The number of viable fibroblast cells is proportional to the amount of the purple formazan salt crystals formed. L929 mouse fibroblast cells were seeded in 96 well tissue culture plates for 24 hour. After 24 hour, 3, 10, 20, 40, 60 and 100 $\mu\text{L}$  of maleic acid grafted chitosan 5 wt. % in PBS was added and incubated for 24 hour. Then, 10 $\mu\text{L}$  of MTT solution (10mg/mL) was added to each well and incubated at 37 $^{\circ}\text{C}$  for 4 hour. The purple formazan salt crystals occurred. Finally, 100 $\mu\text{L}$  of solubilization buffer was added on to the each well and waited for 30 minute to dissolve the formazan dye. The absorbances were measured at 570nm by use of ELISA microplate reader [11].

## III. RESULTS AND DISCUSSION

### A. Effect of Time and Acetic Acid Percentage on Product

The percentages of grafting results were given in Table I. The optimum grafting condition was obtained by microwave system with use of a chitosan/maleic anhydride/ceric ammonium nitrate 0.2/3.922/0.99 gram of raw material within 15 minute. Therefore, for the grafting of chitosan, with use of microwave system (15 minute) instead of reflux system (3 hour) will shorten the reaction time. Microwave system will provide advantages for industrial scale up grafted chitosan production.

TABLE I  
 THE EFFECT OF REACTION CONDITIONS ON PERCENTAGE OF GRAFTING

System	Time (minute)	Chitosan (g)	Percentage of Acetic Acid (%v/v,20 mL)	Percentage of Grafting (%)
Reflux	180	0.2	1	25
Microwave	15	0.2	1	160
Microwave	30	0.2	1	35
Microwave	60	0.2	1	30
Microwave	15	0.4	1	20
Microwave	30	0.4	1	22.5

### B. FT-IR Analysis

The FT-IR spectra of maleic anhydride, chitosan, ceric ammonium nitrate and grafted chitosan is given in Fig. 1. The board peak at about 3500 $\text{cm}^{-1}$  was related to the alcohol and amine groups. The region of 1680 to 1300 $\text{cm}^{-1}$  represented the ring stretching bands and amide bands [9]. Ether linkage in pyranose units was observed t at 1060 $\text{cm}^{-1}$ . As can be seen from the spectra of grafted chitosan (blue line) new amide bands were observed at 1640 $\text{cm}^{-1}$ . An O-H and C-H bending vibration was observed at 1400 $\text{cm}^{-1}$  and 1200 $\text{cm}^{-1}$ , respectively. The peak at around 1380 $\text{cm}^{-1}$  was related to C-N stretching. The small peak at 1715 $\text{cm}^{-1}$  was related to the C=O vibrations of maleic acid introduced onto the chitosan backbone. Therefore, there are many evidence supporting the incorporation of the maleic acid onto the chitosan [8].

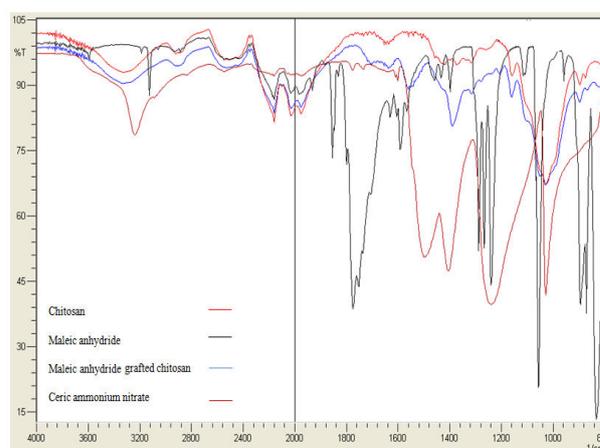


Fig. 1 FTIR spectra of maleic anhydride, chitosan, ceric ammonium nitrate and grafted chitosan

### C. Solubility and Swelling Behavior

The chemical structure and the medium are important characteristics effecting the swelling behavior of chitosan [4]. The solubility and swelling behavior of maleic acid grafted chitosans at various pH mediums were showed in Fig. 2. The grafted chitosans showed different swelling behaviors due to the pH of the buffer solutions. The M2 sample showed the maximum swelling ratio (13.6) at pH 9.2 when soaked for 24h. The M3 sample showed a minimum swelling ratio (0) was obtained at pH 5.6. The M1 sample is soluble at pH 3.6, 5.6 and 9.2 after 30 minute. The minimum swelling ratio of M1 is 0.82 at pH 3.6 when soaked for 30 min. The M3 sample is soluble at pH 3.6 when soaked for 20 minute and at pH 9.2 when soaked for the first time. It can be said that the M1, M2 and R1 samples are polyampholyte gel due to swelling in both acidic and basic buffer solutions.

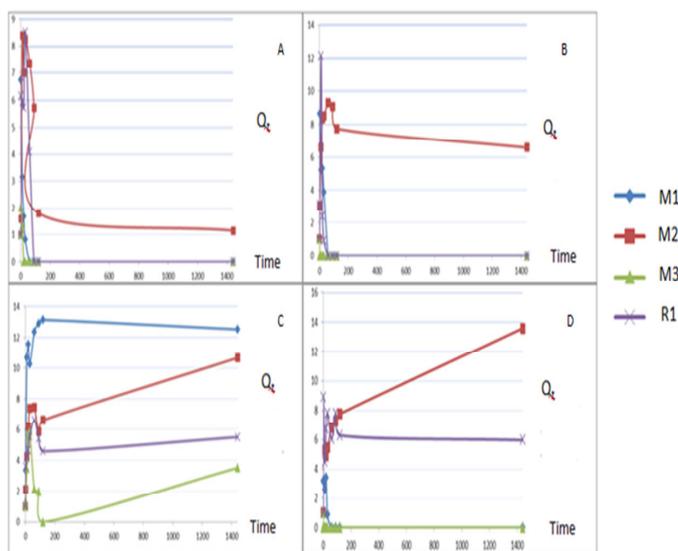


Fig. 2 The swelling behavior of maleic acid grafted chitosans at A) pH 3.6 B) pH 5.6 C) pH 7 D) pH 9.2

### D. MTT Assay

The effect of maleic acid grafted chitosans on the proliferation of L929 mouse fibroblast cells was assayed by MTT technique. Fig. 3 showed the effect of grafted chitosans on the viability of L929 mouse fibroblast cells. At lower concentrations (3, 10 and 20  $\mu\text{L}$  of grafted chitosan 5 wt. %) no significant difference occurred between the fibroblast cells. The viability decreased at 40, 60 and 100  $\mu\text{L}$  but especially differences between the values obtained at 60 and 100  $\mu\text{L}$ . According to these results, it can be said that maleic acid grafted chitosan has antibacterial properties.

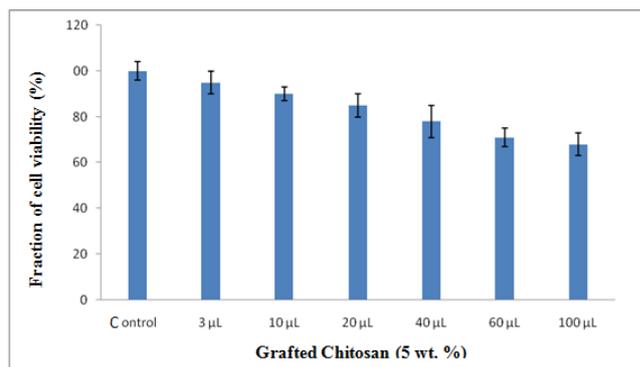


Fig. 3 The effect of maleic acid grafted chitosans on the viability of L929 mouse fibroblast cells

### IV. CONCLUSION

Chitosan is an attractive material that can be used in many applications in the form of hydrogels, membranes, nanofibers, beads, microparticles, nanoparticles and scaffolds with suitable modifications. Grafting of maleic acid on to chitosan was successfully achieved. The results indicated that the use of microwave system is an effective way to decrease the reaction time to produce soluble and insoluble grafted chitosans.

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