Grafting of Chitosan: Structural, Thermal and Antimicrobial Properties

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Summary: In this study, some new chitosan materials were synthesized by the grafting of chitosan with the monomers such as 1-vinylimidazole (VIM), methacrylamide (MAm) and 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS). First of all, chitosan methacrylate was prepared by esterification of primary -OH group with methacryloyl chloride a 25.13% yield by mole. The monomers were grafted into chitosan methacrylate via free radical polymerization using 2,2'-Azobisisobutyronitrile as an initiator in N,N-dimethylformamide. The graft copolymers were characterized by FT-IR spectra and elemental analysis. Thermal stabilities of the graft copolymers were determined by TGA (thermo gravimetric analysis) method. The synthesized chitosan methacrylate and its graft copolymers were tested for their antimicrobial activity against bacteria and yeast.

Keywords: Chitosan, Chitosan methacrylate, Biodegradable polymers, Antimicrobial activity, Graft copolymer.

Introduction

Chitosan is one of the most common biopolymers in nature, and it is obtained by alkaline deacetylation of chitin. It can easily be obtained by processing the residues from a large portion of marine organisms. Due to its superior properties such as biocompatibility, biodegradability and low toxicity, it is used in many areas such as medical, cosmetics, agriculture, and recycling [1]. Recently, the use of chitosan as an adsorbent has been increasing in the removal of heavy metals and dyes [2]. Biologically compatible chitosan is used as an appropriate matrix in various drug formulations. Various medicines are placed in the chitosan matrix (film, microcapsules, coated tablets, etc.) [1,3]. In the acidic medium the NH_2 group is in the form of $-NH_3^+$ and it electrostatically interacts with the anionic groups in the environment. In the protonated state, it exhibits cationic polyelectrolyte behavior, forms viscous solutions, and interacts with oppositely charged molecules and surfaces. Chitosan interacts with negatively charged drugs, polymers, bioactive molecules and organoclay because of its free amine group $-NH_3^+$ cationic property [4]. In addition, chitosan could be a good choice to be applied in many areas including pharmaceuticals, foods, cosmetics, chemicals, agricultural crops, etc. [5]

In the literature, antimicrobial activity studies against different microorganism species have been carried out with different chitosan species (molecular weight, deacetylation degree, derivative form, etc.) [5,6]. The author team has previous researches on cellulose and starch grafting [7-9], while this study is about grafting on chitosan and its thermal and antimicrobial activity properties. There are many reports upon the synthesis, characterization, and properties of chitosan; but less attention has been paid to the chitosan graft copolymers. Thus, present study describes the esterification of a part of OH chitosan groups, and graft copolymerization of chitosan with VIM, MAm and AMPS monomers. In addition to characterizing graft copolymers, thermal stabilizers and antimicrobial activities were investigated.

Experimental

Materials and Instrumental Measurements

Chitosan was purchased from Sigma-Aldrich with degree of deacetylation: ≥75% and average molecular weight: 310.000-375.000 g/mol. Potassium tert-butoxide ($(CH_3)_3CO^-K^+$), methacryloyl chloride, 2,2'-Azobisisobutyronitrile (AIBN) as initiator, 1-vinylimidazole (VIM), methacrylamide (MAm) and 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) as commercial monomer were purchased from Sigma-Aldrich. N,N-dimethylformamide, acetonitrile, ethanol, diethyl ether were used as solvent. The IR spectra of all samples were performed with a PerkinElmer Spectrum Two (UATR) IR spectrometer in the range of 4000-450 cm^{-1} Leco CHNS-932 for elemental analysis and Hitachi 7000 thermo instruments for thermal analysis measurements were used.

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Preparation of Chitosan Graft Copolymers

In order to obtain chitosan graft copolymers, chitosan methacrylate was synthesized. Chitosan methacrylate (Cts.met) and its graft copolymers were synthesized according to the literature [7-11]. Raw chitosan was allowed to swell in the acetonitrile solvent at room temperature, and then $(CH_3)_3CO^-K^+$ dissolved in acetonitrile was added, and stirred for 24 h. Methacryloyl chloride dissolved in acetonitrile was added into the obtained mixture dropwise. The reaction mixture was refluxed for 32 hours in a controlled manner and then filtered by cooling, and Cts.met was synthesized. Synthesized Cts.met was washed with acetonitrile, water, ethanol, and diethyl ether in order to remove salt and impurities. The synthesis reaction of the chitosan methacrylate is shown in Fig. 1.

1 g Cts.met, 5 g monomer that VIM, MAm and AMPS in N,N-dimethylformamide and 0.05 g AIBN were added to the tube as initiator. Each monomer was provided to graft onto Cts.met in different tubes at ±70 °C by stirring in inert gas for 24 h. Grafted copolymers were filtered and thoroughly washed with N,N-dimethylformamide, acetonitrile, ethanol, and diethyl ether to eliminate oligomer and homopolymer that can form in the reaction. The synthesis reaction of the grafting of chitosan methacrylate with VIM, MAm and AMPS monomers is shown in Fig. 2.

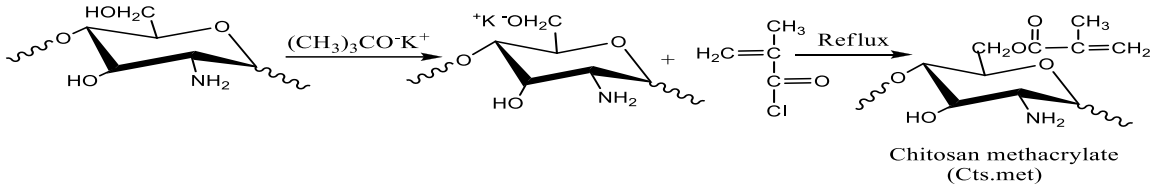

Fig. 1: Synthesis of chitosan methacrylate.

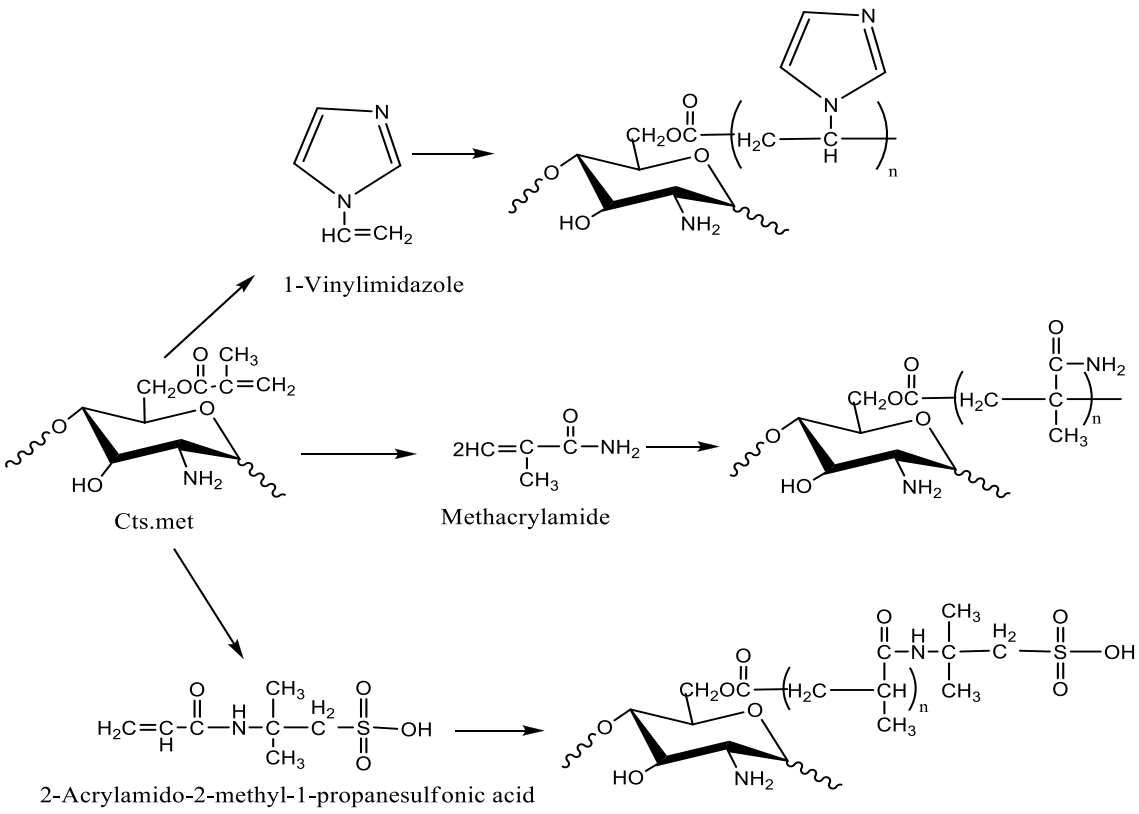

Fig. 2: The grafting of chitosan with some monomers.

In vitro Antimicrobial Activity Study

Four bacteria and a yeast were used as test organisms that these *Staphylococcus aureus (S.a)* ATCC 6538 (Gr+), *Bacillus subtilis (B.s)* ATCC 6633 (Gr+), *Echerichia coli (E.c)* ATTC 25922 (Gr-), *Enterobacter aeroginosa (E.a)* CCM 2531 (Gr-) as bacteria, and *Candida tropicalis (C.t)* ATCC 13802 as yeast. The polymers and standard antibiotic were homogenized in dimethyl sulfoxide at a sample concentration of 100 µg/ ml by using the antibiotic disk assay [12, 13]. The resulting inhibition zones on the plates were measured.

Results and Discussions

Grafting of Chitosan and Characterization

The primary OH groups on chitosan react with $(CH_3)_3CO^-K^+$, which is a basic compound, to form chitosan alkoxide. In another reaction, the chitosan alkoxide reacts with methacryloyl chloride to form chitosan methacrylate (Cts.met). Then, a series of vaccination studies were carried out on Cts.met by using AIBN as a free radical initiator with VIM, MAm and AMPS (Fig. 1 and 2). The FT-IR technique was the first spectroscopic method used to identify functional groups within a molecule. The spectrum of chitosan shows the characteristic bands at 3350 cm^{-1} (O-H strech), 2900 cm^{-1} (aliphatic C-H strech) 1380 cm^{-1} (C-H bending vibration), 3290 cm^{-1} (N-H strech) 1640 cm^{-1} (N-H bending vibration) 1025 and 1062 cm^{-1} (C-O symmetric and asymmetric vibration at C-O-C bonds) were observed [14-16].

A band 1735 (-C = O stretching), not seen in the chitosan itself, indicates that the methacrylate groups are attached to the chitosan. Moreover, the newly formed C = C peak in Cts.met overlaps with the N-H bending vibration peak from the chitosan (Fig. 3).

The elemental analysis of the polymers can be seen from Table 1. The degree of substitution in the glucose units of chitosan was 25.13% by mole (y) from and 32.29% by weight (Y) by percentage of carbon [7-11]. The percentage substitution of the glucose unit of chitosan was found by the following formula.

$$Y = \frac{A - B}{C - D} \times 100$$

where

A = % carbon found for modified chitosan
B = % carbon found for starting chitosan
C = % carbon calculated for 100% modification
D = % carbon calculated for starting chitosan.

When the FT-IR spectra of the grafting studies on Cts.met are examined, the following results are observed. FT-IR results of all Cts.met containing polymers show OH peaks at 3110 cm^{-1}, NH peaks at 3026 cm^{-1} and ester peaks at 1735 cm^{-1} region clearly. For Cts.met-g-MAm and Cts.met-g-AMPS, an increase in amide peaks due to polymerization of the monomer is observed. Furthermore, the weight increase in the synthesis of the graft copolymers as a result of the values calculated by gravimetric analysis is another evidence that graft copolymers are formed. FT-IR spectra of Cts.met, the graft copolymers of chitosan with poly(1-vinylimidazole) (Cts.met-g-VIM), poly(methacrylamide) (Cts.met-g-MAm), and poly(2-acrylamido-2-methyl-1-propanesulfonic acid) (Cts.met-g-AMPS) are shown in Fig. 3.

Thermogravimetric Analysis Results

Thermal analysis measurements were made at a heating rate of 10 ^oC/min at a N_2 gas flow of 10 ml/min. Referring to Figure 4 and Table 2, the thermal stability of the Cts.met and its graft copolymers are lower than chitosan. All the polymers show that thermal degradation was completed in one steps. Initial decomposition temperature, and temperatures of a weight loss have decreased compared to chitosan.

Antimicrobial Activity of Polymers Derived From Chitosan

The antimicrobial activities of the polymers were determined against four bacteria and a yeast as described in the experimental section. The zones of inhibition of the microorganism growth of the standard samples and synthesized polymers were measured by millimeter at the end of the incubation period. Fig. 4 shows the average data of microorganisms. The results show that the Cts.met graft copolymers have more antimicrobial properties than Cts.met itself. VIM, MAm and AMPS structures attached to the graft copolymers increased the zone. However, neither the graft copolymers of Cts.met nor Cts.met did not respond to *B.s.* bacteria. According to the literature relevant, VIM, MAm and AMPS monomers and polymers themselves have antimicrobial activity [12,13,17-20]. Therefore the test results concluded that synthesized polymers are effective on some of the microorganisms.

Table-1: Elemental analysis results of grafting on chitosan with some monomers.

Polymer	Elemental Analysis		
	% C	% H	% N
Chitosan	43.03	6.31	6.66
Chitosan methacrylate	45.51	5.23	6.45
Cts.met-g-VIM	46.72	6.70	8.86
Cts.met -g-MAm	47.99	6.47	7.28
Cts.met -g-AMPS	50.43	6.65	7.32

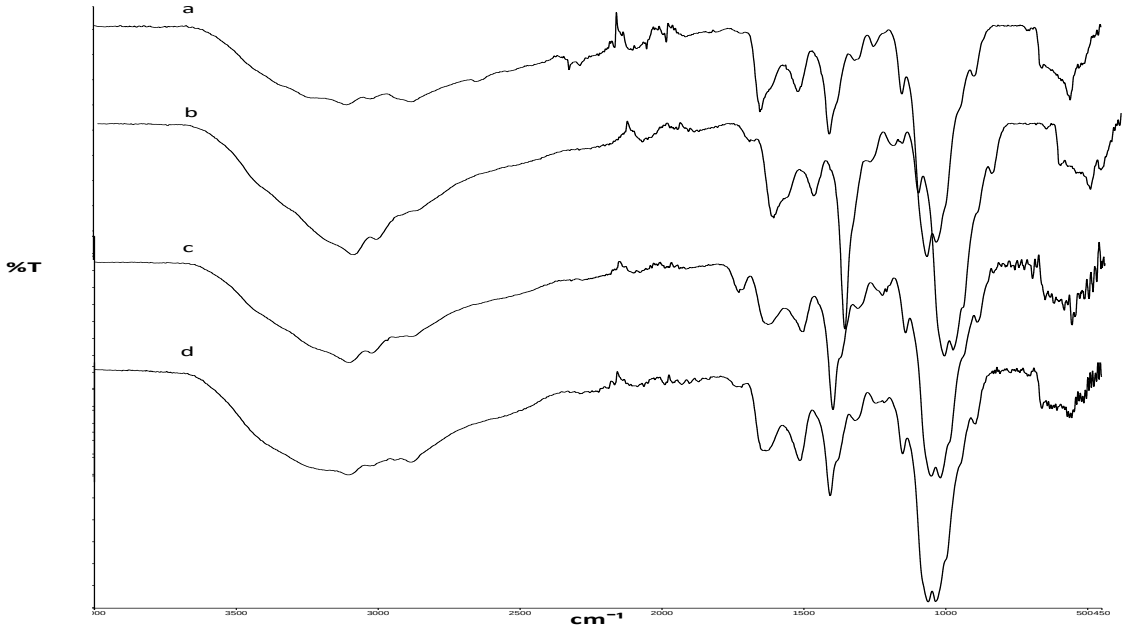

Fig. 3: FT-IR spectra of the a) Cts.met b) Cts.met-g-VIM c) Cts.met-g-Mam d) Cts.met-g-AMPS.

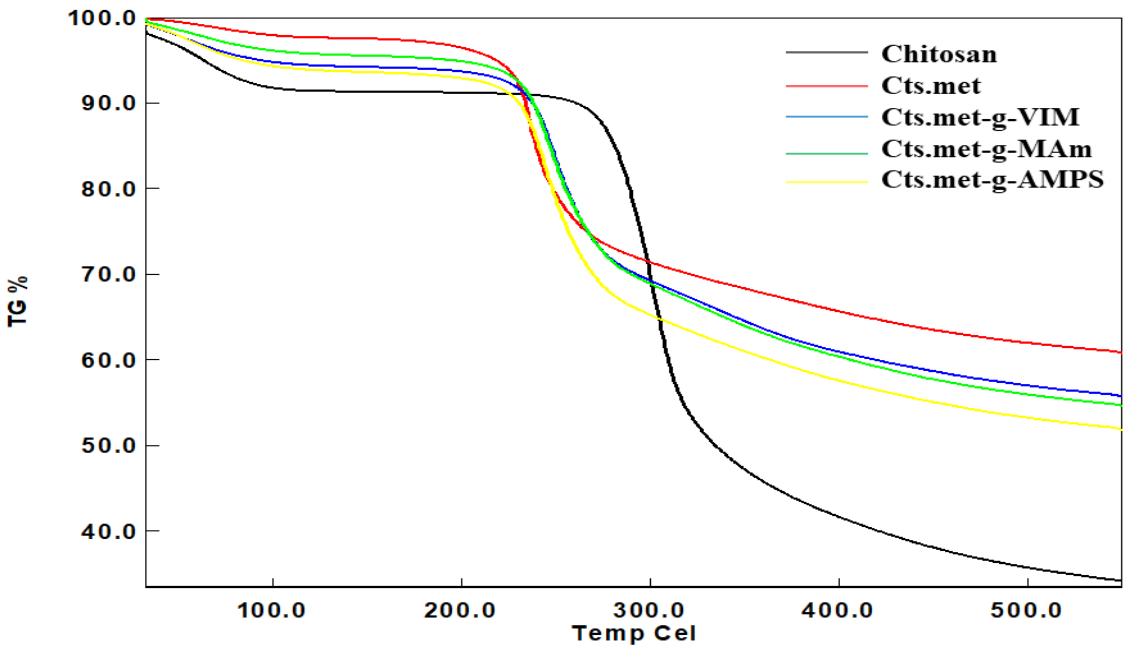

Fig. 4: TGA curves of chitosan, Cts.met and its graft copolymers.

Table-2: Thermal analyses results of grafting on chitosan with some monomers.

Polymer	Max. Decomposition Temperature (ºC)	Temperature of 30% weight loss (°C)	%Weight loss (300 ºC)	%Weight loss (400 ºC)	%Residue (550 ºC)
Chitosan	273	300	30	58	34
Chitosan methacrylate	236	317	29	34	61
Cts.met-g-VIM	249	293	31	39	56
Cts.met-g- MAm	247	290	31	40	55
Cts.met-g-AMPS	245	270	35	42	52

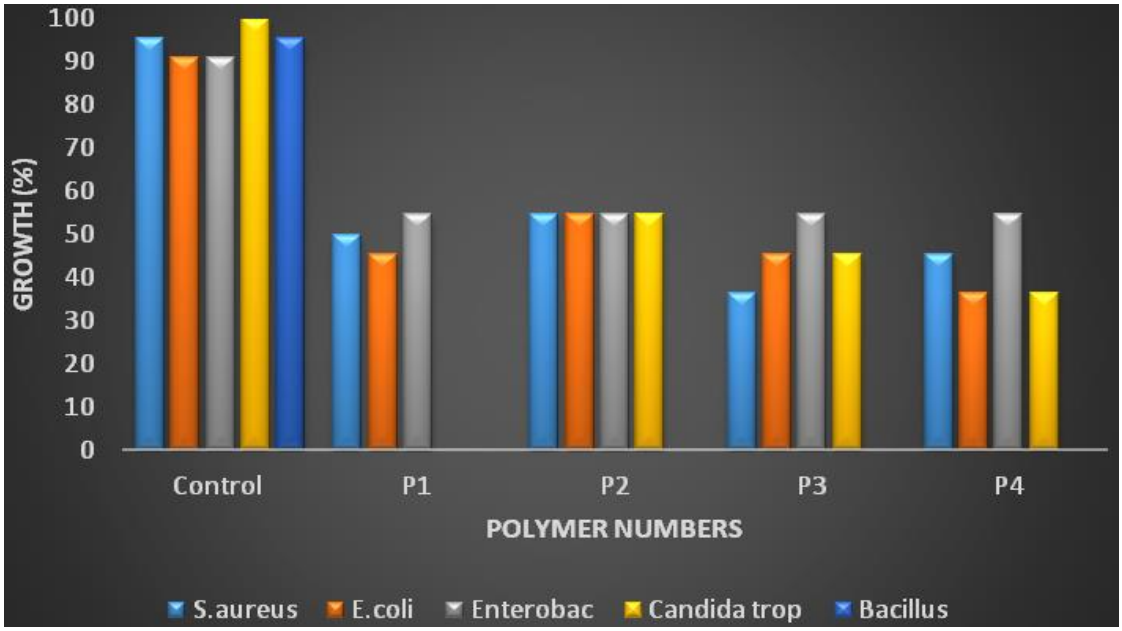

Fig. 5: Effect of polymers on percentage growth of microorganisms.

In this study, chitosan methacrylate was prepared by esterification 25.13% yield by mole. The monomers such as 1-vinylimidazole (VIM), methacrylamide (MAm) and 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) were grafted into the chitosan methacrylate via free radical polymerization. The graft copolymers were characterized by FT-IR spectra and elemental analysis. The synthesized chitosan methacrylate and its graft copolymers were tested for their antimicrobial activity against microorganisms. It was found out that graft copolymers containing chitosan are more resistant to bacteria compared to chitosan methacrylate. Thermal stability of the chitosan graft copolymers was determined by the TGA method and compared with each other. It was observed that the thermal stability of chitosan is higher than Cts.met and its graft copolymers. Since chitosan is low cost, biodegradable, and easily obtainable, the synthesized chitosan-containing polymers will also have the same properties. The newly synthesized biopolymer-containing graft polymers could increase the application area of the biomaterials.

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