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RESEARCH ARTICLE

UV irradiation-H_2O_2 system as an effective combined depolymerization technique to produce oligosaccharides from chitosan

Seyed Ahmad Ayati Najafabadi[1] · Hengameh Honarkar[2] · Majid Moghadam[3] · Valiollah Mirkhani[3] · Mohammadreza Tahriri[4] · Lobat Tayebi[4,5]

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Abstract
UV irradiation hydrogen peroxide (H_2O_2) system is used as an effective, easy and low-cost combined depolymerization technique to produce oligosaccharides from chitosan. UV–Vis spectroscopic studies explained that with increasing treatment time, the absorption of the depolymerized chitosan solution has increased, indicating the increase in the carbonyl and amino groups in their structure. Fourier transform infrared spectroscopy and nuclear magnetic resonance (1H NMR) analysis showed that the 1,4-β-D-glucoside linkages of chitosan are degraded without important changes in chemical structure of decomposed samples. X-ray diffraction patterns verified the polymerization of chitosan to produce oligomers, changing in structure from crystalline to amorphous. Viscosity-average molecular weight measurements of fragmented chitosan samples and Mark–Houwink equation are used to demonstrate the efficiency of this depolymerization method. Finally, the obtained results ascertained that this combined method could produce water soluble chitosan with significant efficiency and no essential change in its chemical structure.

Keywords Chitosan · Depolymerization · Ultraviolet irradiation · Hydrogen peroxide · Oligosaccharides

Introduction

In nature, there are many useful materials that could be extracted from various resources, such as plants and animals. One of those natural materials is polysaccharides such as starch, chitin, chitosan and cellulose, which have been considered by scientists as biopolymers in many different fields like medicine, industry and engineering [1]. Polysaccharides have a wide range of molecular structures and excellent properties, differentiating them from synthetic polymers [2]. Chitin is the second most abundant biopolymer, produced by crustaceans, fungi and shrimps [3,4]. Chitosan is a cationic polysaccharide composed of 1,4-D-glucosamine and *N*-acetyl glucosamine and is synthesized by the partial deacetylation of chitin [5,6]. The existence of free amino ($-NH_2$) groups in its structure makes chitosan a highly functional polysaccharide that can interact with many anionic polymers [7]. Some of the excellent features of chitosan are non-toxicity, homeostasis, antibacterial, biocompatibility and biodegradability [8,9]. These advantageous qualities make chitosan suitable for a variety of biomedical purposes such as wound healing [10–13], tissue engineering scaffolds [14–17], drug delivery systems [18] and biosensors [19]. Despite its exceptional properties, chitosan has been of limited biomaterial use in biomedical engineering due to its high molecular weight making it insoluble in water at a neutral or basic pH and in other organic solvents. In fact, chitosan can only dissolve in acidic water and specific solvents like hexafluoroacetone and, therefore, a chemical modification is required to overcome this problem. Scientists have treated chitosan utilizing different methods in order to improve its

✉ Mohammadreza Tahriri
mohammadreza.tahriri@marquette.edu

1 Biomaterials Group, Faculty of Biomedical Engineering, Amirkabir University of Technology, Tehran, Iran

2 Polyurethane and Advanced polymers, Faculty of Science, Iran Polymer and Petrochemical Institute, Tehran, Iran

3 Catalysis Divisions, Department of Chemistry, University of Isfahan, Isfahan, Iran

4 Marquette University School of Dentistry, Milwaukee, WI 53233, USA

5 Department of Engineering Science, University of Oxford, Oxford OX1 3PJ, UK

solubility and enhance its effectiveness [20]. One potential approach to overcome this problem is the depolymerization of chitosan using acid [21], hydrogen peroxide [22,23], enzyme [24], or ultraviolet light (UV) irradiation [25] in order to obtain oligochitosan. To lower the molecular weight of chitosan, the glucosidal linkages in its skeleton must be broken, which can be accomplished through ultraviolet irradiation (UV irradiation). This is an effective hydrolysis method that provides the sufficient energy level to break glucosidal bonds. The ultraviolet irradiation mechanism is based on free radicals such as hydroxyl or oxygen radicals, which make the formation of chitosan oligomers more rapid and efficient during the photooxidation process [26]. Hydrogen peroxide (H_2O_2), the simplest peroxide, is unstable and constantly decomposing into water and oxygen. This allows H_2O_2 to be utilized as an oxidizing agent in depolymerization of polysaccharides. Hydrolyzing chitosan with hydrogen peroxide is a common method to generate free radicals that can attack and break the glycosidic linkages of chitosan. Recently, research studies on the depolymerization of polysaccharides show an interest in applying two hydrolysis techniques simultaneously, so that each technique can intensify the hydrolyzing efficiency of the other [27,28].

The focus of our study is to investigate the application of two depolymerization techniques (UV irradiation and hydrogen peroxide hydrolysis) simultaneously as a promising approach to promote the hydrolysis efficiency of chitosan and produce oligochitosan to make it soluble in water. We obtained different chitosan samples to investigate the effect of this combined method on the depolymerization of chitosan, which was evaluated using viscosity-average molecular weight, UV–Vis spectroscopy, Fourier transform infrared spectroscopy, XRD diffraction and ^{1}H NMR spectroscopy.

Experimental procedures

Materials

Chitosan with medium molecular weight (M_υ = 190−310 kDa, DD = 75−85%) was purchased from Aldrich Co. 35% hydrogen peroxide (H_2O_2), acetic acid (CH_3COOH), acetone and NaOH were obtained from Merck Co. All solutions were prepared using distilled water.

Depolymerization of chitosan using UV irradiation and H_2O_2

The first step requires preparation of a homogenous purified chitosan. Chitosan is dissolved in 1% (v/v) aqueous acetic acid solution with a constant stirring rate for 24 hours at room temperature. A solution containing suspended particles is obtained [20]. The homogenous solution involving chitosan fragments is separated by centrifugation (rpm = 4000, t = 6 min). Then, chitosan fragments are collected by evaporation of acidic water, washed with distilled water four times and dried by evaporation.

The second step consists of producing oligochitosan. Purified chitosan is dissolved in an aqueous media with a concentration of 1% (w/v), and then 2 mL H_2O_2 is added to the chitosan solution, stirring constantly. Immediately, the final solutions are exposed to UV irradiation by UV lamp, at a temperature of 60 °C and pH = 5.0 for different periods of time (i.e., 1, 3, 6, 10, 15 and 20 min).

The source of UV irradiation was a UV lamp (ECO/ T5-SLIM) purchased from Shenzhen ECOMAX Lightening Co. Ltd. (Shenzhen, China). The lamp produces short-wave ultraviolet light in the range of 254 nm with the intensity of radiation I = 0.256 [(J/(cm^2/min)]. Its technical data: 180–240 V, 50–60 Hz. The process was completed in a chamber to prevent harmful effects from exposure to ultraviolet (UV) radiation.

When the depolymerization process is completed, decomposed chitosan solutions are produced at mentioned times. The obtained solutions are cooled down to 0 °C and neutralized with 2M NaOH. Last, the precipitated oligosaccharide particles are recovered by filtering, washed with acetone several times and dried under vacuum overnight at room temperature.

Characterization

All experiments were conducted at room temperature and in the air atmosphere. UV–Vis spectra are obtained using a UV/Vis spectrophotometer model PG Instruments T80 Double Beam, Eppendorf. Fourier transform infrared spectroscopy (FTIR) spectrum of the samples is recorded using a FT Infrared Spectrometer, JASCO, FT/IR-6300 (400−4000 cm^{-1}), Japan. X-ray diffraction patterns of the original chitosan and treated chitosan samples are carried out using a Bruker, D8 Advance diffractometer (Germany) (Co Kα target (λ = 1.7890 Å), voltage 40 kV, current 40 mA). Untreated chitosan and low molecular weight chitosan samples are analyzed by nuclear magnetic resonance (^{1}H NMR) spectroscopy. ^{1}H NMR spectra are obtained on NMR spectrometer 400 MHz, Avance III 400 Bruker, (Germany). The viscosity of samples is measured using NDJ-1 Rotational viscometer (Shanghai Changji Geological Instrument Co., Ltd., China), after replicating each chitosan sample three times. The result of viscosity measurements is used to calculate the average molecular weight of samples using the Mark–Houwink equation (Eq. 1), shown as follows [29,30].

$$[\eta] = 1.40 \times 10^{-3}\, M_v^{0.83}. \quad (1)$$

Table 1 Values of absorbance after the different time of UV irradiation of the chitosan in the presence of 2% H_2O_2 ($\lambda_{irr} = 300$ nm)

Time of UV irradiation (min)	Absorption
1	0.909 ± 0.06
3	0.970 ± 0.07
6	1.330 ± 0.21
10	1.790 ± 0.12
15	1.960 ± 0.31
20	2.394 ± 0.01

Results and discussion

UV–Vis analysis

The maximum absorption of the solution of depolymerized chitosan samples in different treatment times (1, 3, 6, 10, 15 and 20 min) around 300 nm is observed using UV–Vis spectroscopy. During the UV irradiation process, the color of the solutions becomes brown gradually, and the maximum absorption of treated chitosan solutions increases throughout the time period. As the molecular weight of chitosan decreases, the color solution turns brown, increasing the absorption [31], as shown in Table 1. In other words, the carbonyl and amino groups in the chitosan solutions increase by increasing UV irradiation time [32]. Table 1 shows that the absorbance had increased with the increase in browning of chitosan solution with increasing time, and the absorbance reached its highest point after 20-min irradiation, which is two times more than depolymerized chitosan solution in 1 min.

It is worth mentioning that the values of absorbance depend on the browning of irradiated samples. It means the increase in absorbance, the increase in browning and the decreasing molecular weight. When the solution's color goes brown by increasing the irradiation time, the molecular weight of irradiated samples would be reduced. Although the sever browning (discoloration) or even black samples can affect the properties of depolymerized samples such as decreasing the solubility and we tried to prevent this phenomenon by using a combination depolymerization method which resulted irradiation's time would be decreased [31,33].

FTIR analysis

Figure 1 shows the FTIR spectra of the original (a) and the depolymerized chitosan samples (b) and (c) by UV irradiation with 2% H_2O_2 after 6 and 20 min, respectively. The difference in the FTIR spectra of initial chitosan and its degraded samples is almost the same. Hydroxyl groups in the initial chitosan have a broad absorption band at 3438 cm^{-1}, and this band is shifted to lower intensity in depolymerized

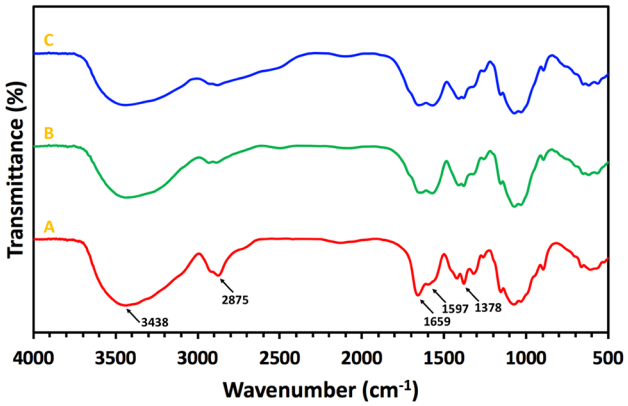


Fig. 1 FTIR of the samples: *a* original chitosan and the depolymerized chitosan samples by UV irradiation in the presence of 2% H_2O_2 after *b* 6 min and *c* 20 min

samples, due to formation of sugar rings with five functional groups after depolymerization. The broad absorbance band centered at 3438 cm^{-1} was the characteristic band of the stretching vibration of −OH and $-NH_2$ [34]. The band found at 2875cm^{-1} is attributed to C–H stretching of the alkyl substituent [35]. The absorption bands at 1659, 1597 and 1378 cm^{-1} in the original chitosan are assigned to the amide I, N–H bending NH_2 groups and amide III, respectively. Although there is no important difference between the spectrum of the original chitosan and its depolymerized samples in the amide III band, the amide I band in treated samples decreased to a lower value because of formation hydrogen bonds during hydrolysis [36]. The IR spectra of the original and treated chitosan samples confirm that the saccharide structure was similar between the two materials [37].

XRD analysis

XRD patterns of the original (a) and the depolymerized chitosan samples (b and c) were obtained by UV irradiation in the presence of 2% H_2O_2 for 6 and 20 min and are shown in Fig. 2, respectively. The XRD pattern of the original chitosan sample (a) had one sharp peak at $2\theta = 13°$ and a broad peak at $2\theta = 24°$ [37]. As the time of depolymerization process increases, the intensity of the characteristic peak at $2\theta = 24°$ in depolymerized sample (b) decreased sharply, and crystalline regions became broadened [38]. Hydrolysate sample (c) had a broad peak, which confirmed that as the degradation process progressed, the structure of chitosan transformed from a crystalline state to an amorphous one [39].

^{1}H NMR analysis

The chemical structure of the original chitosan and depolymerized chitosan samples was analyzed by ^{1}H NMR spectroscopy (Fig. 3). The ^{1}H NMR spectra of the original

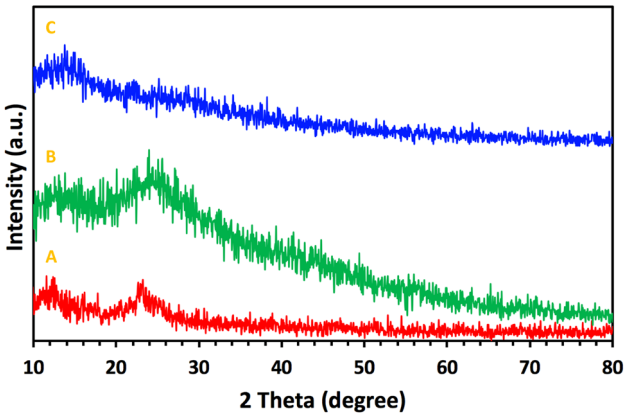


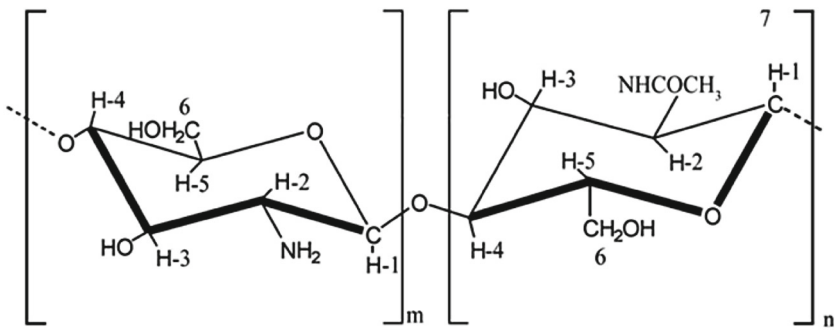
Fig. 2 XRD patterns of the original chitosan *a* and the depolymerized chitosan by UV irradiation in the presence of 2% H_2O_2 after *b* 6 min and *c* 20 min

chitosan (Fig. 4a) and its fragmented samples (Fig. 4b, c) after 6- and 20-min depolymerization are represented, respectively. Chitin is not completely deacetylated to chitosan (DD: 75–85%) and contains the N-acetyl glucosamine units (acetylated units); therefore, a small peak at 4.8 (H1) and a sharp signal at 1.9 ppm (H7) appear [40]. A resonance H2 peak occurs at about 3.3 ppm which corresponds to –CH2–OH [41]. There are peaks present between 3.34–4 ppm, which are attributed to H3, H4, H5 and H6 caused by the ring methane protons [40]. The ^{1}H NMR spectra show that the degraded chitosan samples and the original chitosan have almost the same chemical structure [42].

M_v viscometry measurements

During the decomposition of the macromolecules to oligomers, the molecular weight of products is calculated by measuring their viscosities. This provides a way to investigate the efficiency of UV irradiation H_2O_2 system on depolymerization of chitosan [26]. Figure 5 shows that fragmented chitosan sample, treated using a constant concentration of H_2O_2 (2% v/v) alone, has a slight decrease in molecular weight throughout the time period. The molecular weight of depolymerized products, by only UV irradiation, is reduced with a higher slope than the irradiated sample. However, when a combination of UV irradiation and 2% H_2O_2 is used to decompose the original chitosan, a sharp fall in the molecular weight of treated samples occurs in the first 10 min and then drops gradually in the remaining time period. This indicates that chitosan with high molecular weight depoly-

Fig. 3 Structure of the chitosan



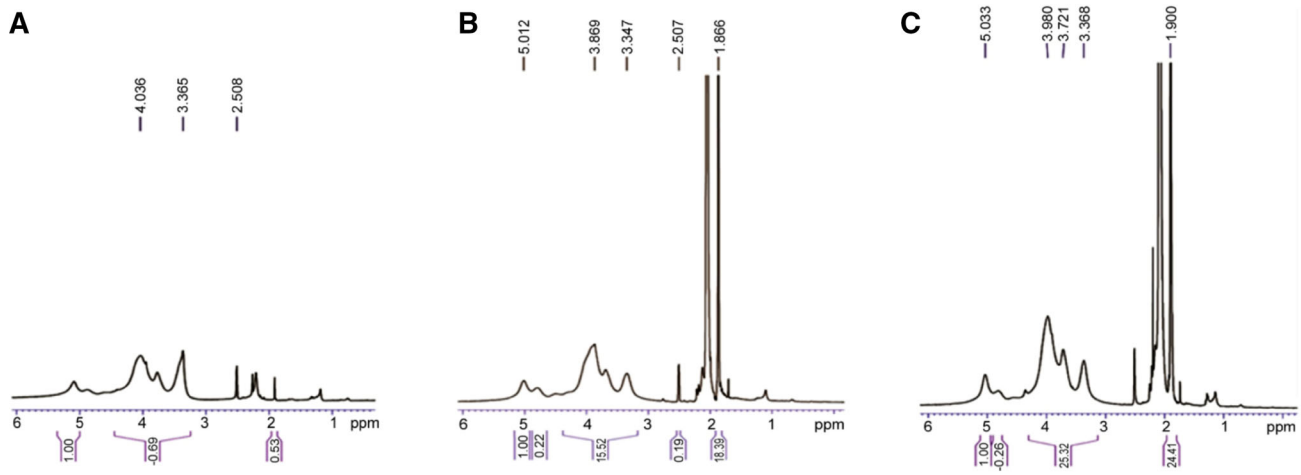


Fig. 4 ^{1}H NMR spectra: *a* original chitosan and irradiated chitosan samples in the presence of 2% H_2O_2 after *b* 6 min and *c* 20 min

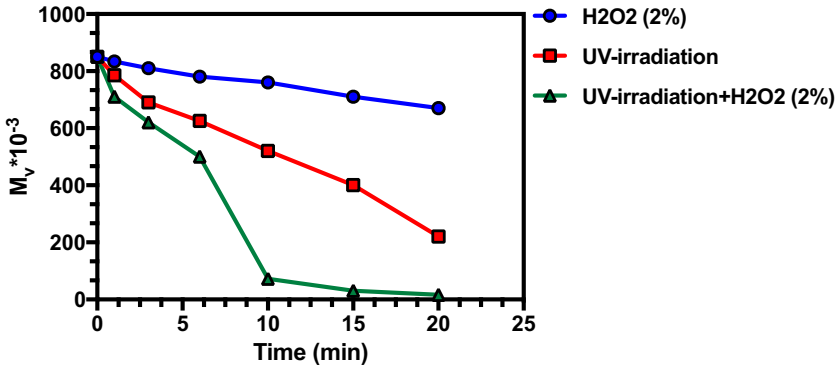
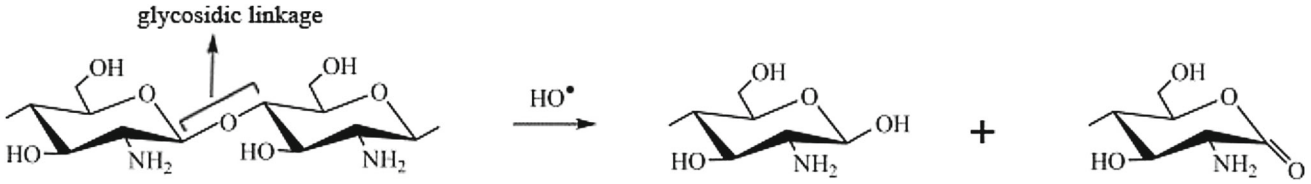


Fig. 5 Effect of UV irradiation and H_2O_2 on the molecular weight of chitosan



Scheme 1 The depolymerization mechanism

merizes more rapidly and efficiently than low molecular weight chitosan [40,41].

Depolymerization mechanism using UV irradiation and H_2O_2

Polysaccharides are a wide range of biopolymers with different structures that are constructed from repeating saccharide units bonded together by glycosidic linkages. The high molecular weight of these biomolecules has limited their performance as important biopolymers in various applications, especially biomedical ones [13,43]. Depolymerization techniques have been used to decrease the molecular weight of polysaccharides, such as chitin and chitosan, and make them water soluble. The depolymerization mechanism is mainly based on oxidative degradation process, which produces different free radicals, such as hydroxyl radicals (·OH) and oxygen radicals (·O), to attack glycosidic bonds in the backbone and shorten long chain polysaccharides (Scheme 1) [44]. Hydroxyl radicals (·OH) and oxygen radicals (·O) are highly reactive radical species, which can fragment many macromolecules [45]. This study proposes a free radical degradation mechanism of chitosan through simultaneous hydrogen peroxide and UV irradiation, which is utilized to form them effectively, while also promoting the efficiency of depolymerization process.

UV irradiation, in the presence of H_2O_2 as an effective photochemical oxidation system, can lead to two main processes that produce free radicals species. The first process is photooxidation, which is initiated with UV rays. UV radiation can produce free radicals from not only the atmosphere, but also from suspended oxygen molecules in solution with a constant stirring rate. Therefore, UV photons reduce molecular oxygen, leading to the production of oxidative free radical derivatives such as superoxide ($\cdot O_2^-$), H_2O_2 and hydroxyl radical (·OH), which contain exactly the right amount of energy to attack and break down the glycosidic linkages of chitosan without changing its chemical structure [33,39].

Superoxide formed during photooxidation (Eq. 2):

$$O_2 + e^- \rightarrow \cdot O_2^- \tag{2}$$

The dismutation of superoxide radicals produces H_2O_2 and O_2 (Eq. 3)

$$2H^+ + \cdot O_2^- + \cdot O_2^- \rightarrow H_2O_2 + O_2 \tag{3}$$

H_2O_2 is unstable and interacts with superoxide to produce ·OH or reduced to H_2O and O_2 completely (Eqs. 4, 5) [46]:

$$H_2O_2 + \cdot O_2^- \rightarrow \cdot OH + OH^- + O_2 \tag{4}$$

$$2H_2O_2 \rightarrow 2H_2O + O_2 \tag{5}$$

The second process that produces free radical species is the decomposition of H_2O_2 to OH by UV irradiation as a catalyst. H_2O_2 is naturally unstable and transforms gradually in the presence of UV irradiation as a catalyst. UV light catalyzes H_2O_2, which is added to the solution. It is worth noting that H_2O_2 is also produced from the dismutation of superoxide radicals as shown in Eq. (3). H_2O_2 generates ·OH as a very powerful oxidant as shown in Eq. (4) [44].

It is worth mentioning that the quality of producing oligomers had increasingly improved because of employing H_2O_2 and UV irradiation at the same time and had intensified

their efficiency to generate free radical species. It is mainly because when free radical species are produced constantly, rapidly and sufficiently and spread uniformly throughout the media, free radicals would have easy access to linkages and break them effectively. This is because the depolymerization process was done in an aqueous media, H2O2 was used to distribute properly in the liquid media to provide enough radicals. Besides, UV irradiation generated radicals by not only photooxidation process (Eq. 2) but also decomposing unstable H_2O_2 (Eq. 4) which means the liquid environment has enough powerful radical elements. Therefore, using this combination technique, depolymerization process's time reduces, and also, the process would be inexpensive. At the first stage of the hydrolyzing process, chitosan is only soluble in acidic media. The molecular weight of chitosan is gradually decreased and chitooligosaccharides are formed in the UV-H_2O_2 system, according to the following mechanism:

Oxidizing agents [(seen in Eq. (4)], especially hydroxyl radicals that are highly reactive, lead to an electrostatic reaction in the cationic amino groups ($-NH_3^+$) on the C-2 of chitosan. Consequently, 1,4-glycosidic linkages become unstable and fermented, followed by a chain scission occurring in the chitosan structure to produce short chain molecules [44,47]. The progressive breakdown of chain molecules over time increases their water solubility, and free radical species will therefore be able to easily attack amino groups on chitosan chains, making degradation occur easier and faster than the beginning of the process [46].

Conclusion

Free radical-based UV-H_2O_2 system was utilized as an effective depolymerizing method to prepare oligoglucosamine efficiently. UV–Vis spectroscopy showed that during the depolymerization process, an increase in absorption occurred, due to the increase in the carbonyl and amino groups in oligochitosan structure. Reducing sugars by fragmentation of 1,4-β-D-glycosidic bonds of the long chain chitosan during the depolymerization process was confirmed by FTIR and 1H NMR spectrum. XRD analysis showed that reduction in the molecular weight of chitosan caused a change in the crystallinity structure of its hydrolysates. FTIR, ^{1}H NMR, XRD and UV–Vis spectra confirmed that the chemical structure of chitosan remained unchanged during the depolymerization of chitosan using UV irradiation-H_2O_2 technique. Finally, measuring viscosity-average molecular weight was demonstrated by the time period, and macromolecules had broken down easily and rapidly.

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