INFLUENCE OF UV-IRRADIATION ON MOLECULAR WEIGHT OF CHITOSAN

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Abstract
In the present paper the results regarding the influence of UV-irradiation with 254 nm wavelength on the molecular weight of chitosan are presented. The concentration of chitosan solution was 0.5%(w/w) in 0.1 M acetic acid. The degree of deacetylation, determined by potentiometric titration in an acidic solution by alkaline, was 81%. Viscosity-average molecular weight of chitosan was determined using an Ubbelohde capillary viscometer. Mark–Houwink equation for the calculation of molecular weight was applied. The results showed that the viscosity-average molecular weight of chitosan was decreasing with increasing time of exposure to UV-irradiation. Spectroscopic analysis of UV-Vis and FTIR confirmed the photodegradation processes of chitosan. The increasing absorbance in UV-Vis spectra indicated the formation of new chromophoric groups after UV-irradiation of chitosan. Microscopic studies showed changes on the surface of the irradiated films of chitosan and decrease of the surface roughness.

Key words: *chitosan, UV-irradiation, photodegradation, photooxidation.*

1. Introduction

Chitosan is modified natural polymer, which is obtained from the polysaccharide chitin (poly-[β-(1-4)-2-acetamido-2-deoxy-D-glucopyranose]). The degree of deacetylation (DD) is the main factor in the solubility of this polymer, which determines its potential application [1 - 4]. The higher degree of deacetylation of polymer, the better soluble and useful it is. This degree influences the biocompatibility, which increases with increasing DD. Chitosan is known as nontoxic and biodegradable biopolymer. UV-irradiation can modify the surface properties of biopolymer film. Usually structural modifications of polymer as well as surface modifications of polymeric films appear as a results of photooxidation processes caused by the exposure to ultraviolet radiation [5 - 10]. This phenomenon is accompanied by formation of hydroxyl and carbonyl groups in UV-irradiated polymers and biopolymers. It was previously stated that chitosan is sensitive to various types of degradation such as oxidative, hydrolytic, thermo-, photo- and ultrasonic degradation [9, 11 - 15]. However, the influence of UV-irradiation with the wavelength of 254 nm on the molecular weight of chitosan in solution was rather poorly studied.

The aim of this study was to investigate the effect of UV- irradiation (254 nm) on the molecular weight of chitosan and on the surface properties of chitosan films. The changes in chemical structure were studied using UV-Vis and FTIR spectroscopy. The sample morphology was observed by an AFM technique and optical microscopy.

2. Materials and methods

Chitosan solution in 0.1 M acetic acid was prepared with final concentration of 0.5% (w/w). The degree of deacetylation was determined by potentiometric titration with a digital pH-meter equipped with a glass electrode in an acidic solution by alkaline. Chitosan films were obtained by solvent evaporation from chitosan solution poured onto glass plate. After drying the chitosan films were irradiated during 2, 8, 12 and 24 h by UV lamp (high pressure mercury lamp from Philips, the intensity of radiation I = 0.256 J/cm^2 min) with 254 nm wavelength (in room temperature).

Viscosity-average molecular weight of chitosan was determined using Ubbelohde's viscometer for viscosity measuring and a Mark–Houwink equation for the average molecular weight calculation:

$$[\eta] = K\overline{M_v}^{a} \quad [\frac{cm^3}{g}] \tag{1}$$

where:

$[\eta]$ – intrinsic viscosity [cm^3/g]

$\overline{M_v}$ – viscosity-average molecular weight [g/mol],

K, a – viscometric constants dependent on the type of solvent, the polymer and temperature. In this study the values of K and a were as follows: $K = 1.28\times10^{-2}$ cm^3·mol/g^2, $a = 0.85$.

Temperature was 25 °C. System of solvents was: 0.33M HOAc/0.2M NaOAc/0.67M Cl_2CH_2COOH [16].

Viscosity-average molecular weight ($\overline{M_v}$) was calculated based on the solvent flow time (t_0) and flow time (t) of the five chitosan solutions with defined concentrations.

Chitosan films, after exposition to different time of UV-irradiation were analyzed by UV-Vis spectrophotometer (UV-1601 PC Shimadzu Corporation, Japan) and FTIR spectrophotometer (Genesis II, Mattson, USA). Moreover, images by optical microscope were made in ambient conditions using Biological Microscope XSP-44 ENLARGEMENT with a magnification 40× - 1024×. Films surface were analyzed by computer program Ulead Video Studio 7 SE VCD with 4 and 40-times of magnification.

Microimages of samples were obtained using an atomic force microscopy (Atomic Force Microscopy - AFM) NanoScope III in ambient conditions. The surface of the specimens was analyzed by the bracket with a tip of pyramid-shape Si_3N_4 using force 10^{-8} N and frequency 39 Hz. Plotted road of the tip was registered using a laser radiation and the resulting signal was processed electronically on the image of the surface. Irradiated specimens (8 and 24 h) were studied in the same way. Base on the AFM microscopy the root mean square roughness (Rms) was calculated. The root mean square roughness is a statistical measure of roughness used in different fields.

3. Results and discussion

The degree of deacetylation of chitosan used in this study was 81%. Molecular weight of chitosan can be determined by several methods. In our study we used viscosity measurements to estimate the molecular mass of chitosan. The results of viscosity measurements were used for calculation of viscosity-average molecular weight of chitosan. The results one can find in ***Table 1***. By analyzing measurements of viscosity-average molecular weight one can see that with increasing time of UV-irradiation, the viscosity-average molecular weight of chitosan decreases. The decrease of viscosity-average molecular weight of chitosan is due to the breaking of biopolymer chain by ultraviolet radiation. The decrease of viscosity-average molecular weight of chitosan can be a result of free radical reactions that may occur after the absorption of UV radiation. However, free radicals formed during UV exposure can not only lead to chain scission of chitosan. Free radicals formed during UV exposure of chitosan may also interact with each other and form additional crosslinks between the chains. In particular, the very active ·OH radicals derived from irradiated chitosan can migrate within the chain and produce new radicals and macroradicals. As the cleavage of the main chain and the crosslinking process may occur simultaneously, it is not possible to calculate the rate constant of chitosan photolysis.

Table 1. *Viscosity-average molecular weight of chitosan before and after different time of UV-irradiation.*

Time of UV-irradiation [h]	0	2	8	12	24
$\overline{M_v}$ [g/mol]	2.12×10^5	1.19×10^5	2.07×10^4	1.21×10^4	7.88×10^3

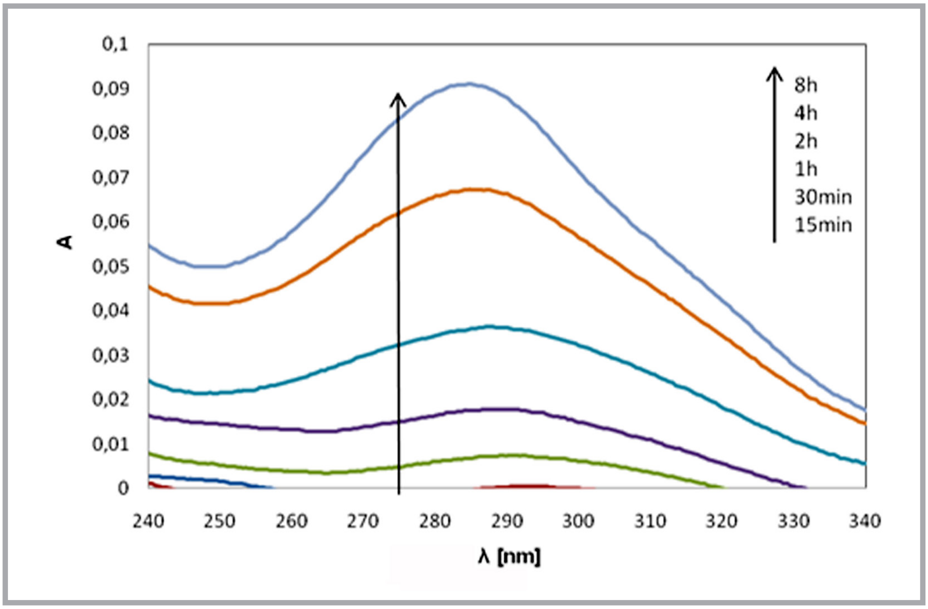

Figure 1. *The absorption spectra of chitosan samples with a concentration 0.043% (w/v) before and after UV – irradiation.*

Figure 1 presents the changes in absorption spectra of chitosan films after exposure to UV-irradiation. One can see that absorbance in the region 260 – 330 nm increases after UV-irradiation. The spectral sensitivity of chitosan photodegradation was studied previously [17]. It was stated that chitosan is sensitive to UV- irradiation. After UV irradiation the absorption spectrum was altered with the development of a distinct absorption band at about 290 nm being formed, and the loss of a shoulder at 250 nm. The absorption of irradiated samples was consistently lower than that of the control samples only after short time of UV-irradiation. The chromophores responsible for the absorption band at 250 nm are apparently destroyed by UV irradiation and new chromophores are generated that absorb at approximately 290 nm wavelength. A study by Andrady et al [17] suggested that the increase of absorbance is due to an increase in the carbonyl and amino groups in chitosan after irradiation. In this study UV-Vis spectroscopy analysis confirmed that during the processes of photodegradation of chitosan new chromophoric groups were formed, probably as a result of photooxidation [18]. The values of absorbance after the different time of UV exposure of chitosan one can see in ***Table 2***.

Table 2. *The absorbance at the maximum of the peak in UV-Vis spectra for chitosan samples after different time of UV-irradiation.*

Time of UV-irradiation	λ [nm]	A_{max}
0 h	-	-
5 min	286.0	0.2965
10 min	287.0	0.2853
15 min	287.0	0.2780
30 min	285.5	0.2767
1 h	286.5	0.2747
2 h	288.0	0.3079
4 h	288.5	0.3646
8 h	288.5	0.4539

As one can see in ***Table 2*** after short time (from 5 minutes up to 1 hour) of UV-irradiation the absorption slightly decreased. Such decrease is probably due to the photochemical destruction of the chromophores responsible for the absorption in this region. However, after UV-irradiation time longer than 2 hours the absorption increased and after 8 hour of UV-irradiation was almost two time bigger than for specimens irradiated only 5 minutes. The increase of absorbance after prolonged irradiation time suggests that new chromophores are generated that absorb at approximately 290 nm wavelength. This new chromophores can be a result of photooxidation processes in chitosan, for example OH group in chitosan can undergo oxidation and turn to carbonyl groups.

FTIR spectroscopy for chitosan (***Figure 2***) shows the bands assigned to NH stretching vibrations (3366 cm^{-1}) and OH (3435 cm^{-1}) and also symmetric or asymmetric stretching CH_2, which belong to pyranose ring (2920 cm^{-1}, 2879 cm^{-1}, 1413 cm^{-1} and 1324 cm^{-1}). On the spectrum bands from amide group, stretching band C=O (1646 cm^{-1}) and bending CH_3 (1382 cm^{-1}), and also NH (from amino group) bending vibrations were recorded. There are bands of asymmetric vibrations C-O-C from the glucosidic bonds (1153 cm^{-1}) and skeletal stretching =CO (1079 cm^{-1}, 1035 cm^{-1}) which belong to characteristic bands of polysaccharides. UV-irradiation of samples caused systematic changes in the FTIR spectra of chitosan. The main changes are observed in hydroxyl and amino group regions (3200 - 3500 cm^{-1}) in spectra of irradiated samples.

The integral absorbance of the band at 3200 - 3600 cm^{-1} in chitosan decreases with UV- irradiation time (***Figure 2***). The integral absorbance for the bands at 1646 cm^{-1} and

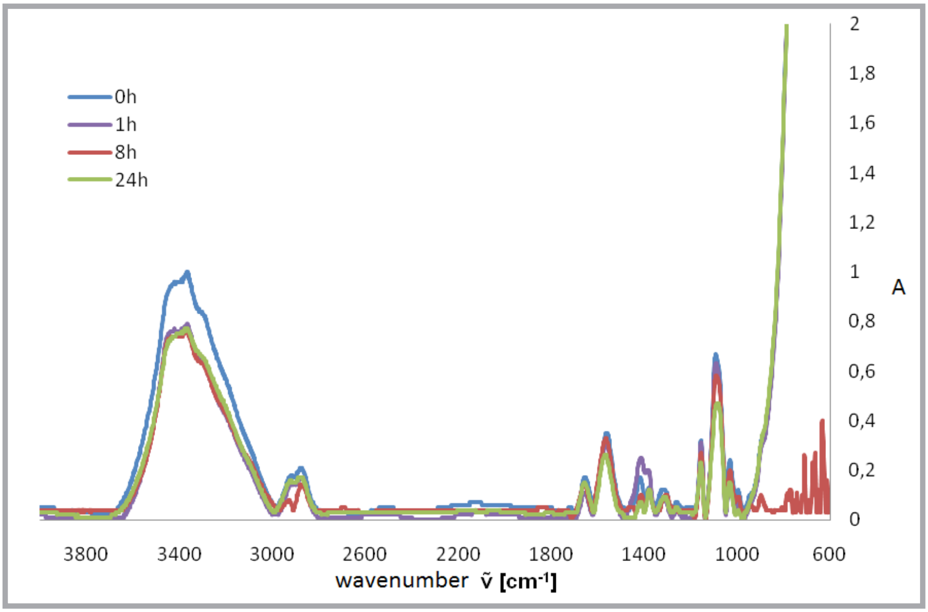

Figure 2. *FTIR spectrum for chitosan films before and after UV-irradiation.*

1413 cm^{-1} respectively decreases rapidly after UV-irradiation chitosan. The drop in the integral absorbance is more pronounced in samples irradiated during 1 hour. The prolongation of the irradiation time caused only small increase above the integral absorbance of 1 hour irradiated chitosan films. Using FTIR method we could observe the changes of integral absorbance of several bands after 1 hour of UV irradiation, whereas using viscosimetry methods and UV-VIS spectra for solution we could observe the changes after much shorter time of UV irradiation. One can conclude from this that the photodegradation of chitosan in solution occurs faster than the photodegradation of chitosan films.

Microimages from optical microscope show changes on the surface of chitosan films due to the action of ultraviolet radiation (***Figure 3***). Microscopic studies have shown that with increasing time of UV-irradiation the number of defects increased. The defect is understood as surface irregularity that disrupts the planar film surface. UV light can generate free radical reactions on the surface. These reactions may lead to polymer degradation and/or crosslinking. As a result on the surface one can see microcracks, wrinkles, bubbles and several other irregularities.

Using AFM technique the surface roughness was evaluated. Surface roughness is a measure of the texture of a surface. It is quantified by the vertical deviations of a real surface from its ideal form. Roughness plays an important role in determining how a real object will interact with its environment. Roughness may promote adhesion, what can be very important for the films for potential biomedical and cosmetic applications.

In this study the root-mean-square (Rms) surface roughness of chitosan films was measured by means of AFM microscopy. The values of Rms surface roughness of chitosan films are shown in ***Table 3***. As a result of UV exposure, the surface roughness of chitosan films is reduced. The reduction of surface roughness can be due to the photochemical oxidation process that can occur after UV-irradiation on chitosan surface. Moreover, the photodegradation process may lead to cleavage of several bonds and due to this process the architecture of the surface can be altered, for example photocrosslinikng reactions may lead to more ordered surface of polymeric films. The reduction of surface roughness can be also due to the water evaporation during UV-irradiation of chitosan films.

The extended application of chitosan in several fields requires several modification of this biopolymer [19]. It is very important to have several methods to produce chitosan with different molecular weights and/or modified surface properties of chitosan films. Our study showed that UV-irradiation can be used for molecular weight modification as well as for modification of the surface of chitosan films.

Table 3. *The root-mean-square (Rms) surface roughness of chitosan films before and after UV-irradiation.*

Time of UV-irradiation [h]	Rms [nm]
0	4.8
2	3.4
8	2.8

4. Conclusion

1) The determined degree of deacetylation of chitosan used in this study was 81%.

2) Ultraviolet radiation induced photodegradation of chitosan due to the cleavage

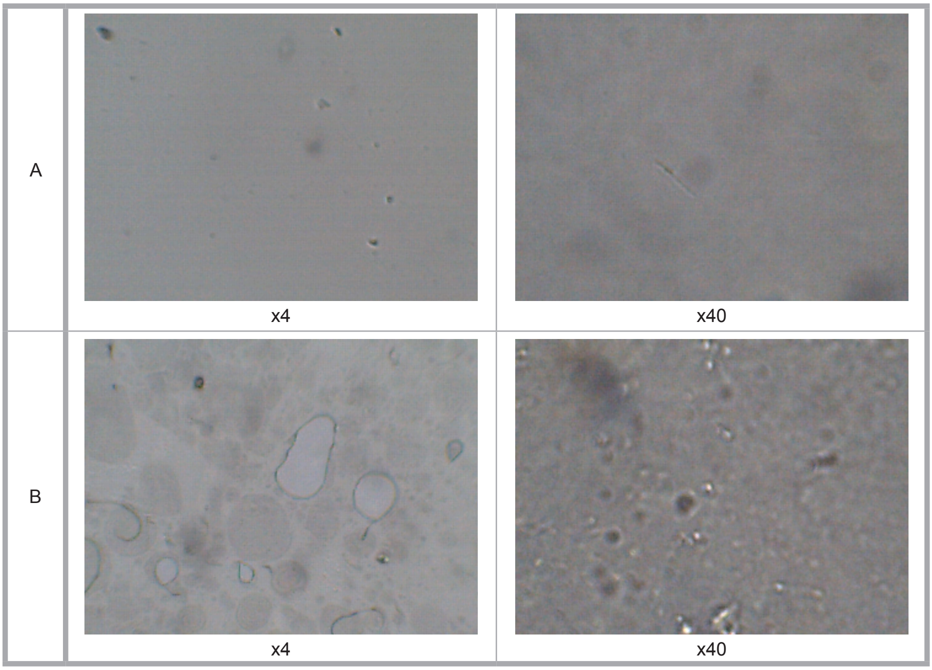

Figure 3. *Microimages of the surface of chitosan films before (A) and after 24 hours of UV-irradiation (B).*

of bonds in the main chain. As the results the reduction of average molecular weight of chitosan was observed.

3) UV-Vis and FTIR spectroscopy analysis confirmed the photodegradation processes of chitosan. The increase of absorbance in UV-Vis spectra indicates the formation of new chromophoric groups, probably as a result of photooxidation.

4) Defects on the surface of chitosan films are a result of the action of ultraviolet radiation. Porosity of the films increases with the time of exposure whereas the surface roughness decreases.

5) UV-irradiation can be considered as good method for molecular weight modification as well as for modification of the surface of chitosan films.

5. References

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