

# LONG-TERM RADIATION-INDUCED EFFECTS ON SOLID STATE CHITOSAN

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## Abstract

*Treatment of chitosan with ionising radiation is one of the methods by which it is modified for various applications. However, in the case of chitosan irradiated in the solid state, the radicals formed during irradiation can be stabilised in its crystalline phase and subsequently cause post-irradiation changes. It has been observed that further degradation of the polymer occurs during storage of the irradiated polymer, resulting in an increase in oxidation products and a decrease in deacetylation degree. This effect results from the transformation of radicals leading to the formation of stable nitroxyl radicals, a process observed years after irradiation.*

**Keywords:** *chitosan, ionising irradiation, electron beam irradiation, post-irradiation effect*

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## 1. Introduction

The use of ionising radiation for polymer modification has been investigated since the middle of the XX century [1]. The current growing interest in the application of ionising radiation for polysaccharide treatment is a result of the development of reliable non-isotopic radiation sources, increasing awareness of pro-ecological technologies as well as increasing usage of natural compounds in many industries.

Irradiation with gamma rays, X-rays or electron beams can be a useful tool to modify the properties of chitosan for various applications [2]. The main effect of chitosan irradiation, either in solution or the solid state, is degradation, which influences its properties such as solubility [3], antioxidant activity [4], antimicrobial activity [5], fat-binding capacity [6] or plant-growth-promoting properties [7]. Irradiation of chitosan has also been reported as a method to obtain its nanostructures [8], to graft monomers [9] or to synthesise chitosan hydrogels [10]. Irradiation can be also used for sterilisation of chitosan-based end products for medicine such as wound dressings [11, 12].

To modify chitosan with ionising radiation, several approaches can be used: treatment of dissolved polymer or solids in dry conditions or suspended in solution [13]. Most often chitosan is irradiated in solution. In such conditions, the degradation accrues as a result of reactions of polymer molecules with products of water radiolysis. The reported yield of chain scission ( $G_s$ ) is  $4 \times 10^{-7}$  mol/J in nitrous oxide ( $N_2O$ )-saturated solution [14]. A much higher degradation yield has been observed for chitosan irradiated in a solution containing hydrogen peroxide. The  $G_s$  value for chitosan increased from  $2 \times 10^{-7}$  mol/J for a solution not containing hydrogen peroxide ( $H_2O_2$ ) to  $22 \times 10^{-7}$  mol/J for a solution containing 1%  $H_2O_2$ . This effect was observed due to an increase in the hydroxyl radical ( $\bullet OH$ ) concentration in solution generated in the process of  $H_2O_2$  radiolysis [15]. Finally, chitosan can be degraded by irradiation in the solid state as a result of direct interaction of ionising radiation with polymer molecules. The reported value of  $G_s$  varied from  $0.93 \times 10^{-7}$  mol/J [16] to  $6 \times 10^{-7}$  mol/J [6], depending on the properties of the initial polymer and the irradiation conditions.

When irradiating chitosan in solution, radical reactions only occur during irradiation. However, in the case of chitosan irradiated in a solid state, unpaired electrons formed as a result of ionising radiation interaction can also be transformed. Because chitosan is a polymer with a partly crystalline structure, radicals can be stabilised in its crystalline phase and cause post-irradiation changes. The reported post-irradiation effects include a decrease in the molecular weight of chitosan and an increase in oxidation products observed 10 and 55 days after irradiation [16, 17]. Such changes result from radical transformation, which can be observed using electron paramagnetic resonance (EPR) spectroscopy. In the case of chitosan irradiated in a solid state, observation of irradiated chitosan indicated post-irradiation transformation of radicals, which within a few months gave rise to the signal attributed to nitroxyl radicals [18].

The aim of the present work was to analyse the long-term effects of irradiation, which have to be considered parameters influencing the properties of chitosan treated in the solid state. Observations were made using samples immediately after irradiation and after storage for 5 years in an air atmosphere.

## 2. Materials and Methods

### 2.1. Irradiation

Two different forms of chitosan – one with a medium deacetylation degree (DD; 70%, Marine Chemicals, India) and one with a high DD (95%, Nicechem Co. Ltd., China) – were used in the experiments. All samples were irradiated in the solid state at room

temperature in an air atmosphere with an electron beam at the energy level 10 MeV with use of a Elektronika10-10 electron beam accelerator. The doses of irradiation were 25-500 kGy. Doses above 25 kGy were delivered as a cumulative dose controlled using Riso calorimeters. After irradiation, the samples were stored at room temperature in air for 5 years, subjected to seasonal temperature and humidity change of the Central European climate.

## 2.2. Analysis

The analysis of untreated and irradiated chitosan aimed to characterised long-lived radical products and changes in chemical structure resulting from irradiation and post-irradiation process.

EPR analyses of chitosan samples were carried out with a Bruker EMX/12 spectrometer equipped with a data-acquisition system. The spectra were analysed by computer simulation with the Bruker package Simfonia. Ultraviolet-visible (UV-Vis) light spectroscopy was used to examine 0.5% chitosan solutions prepared in 1% acetic acid. The analyses were performed using the Beckman DU-64 UV-Vis DIODE ARRAY spectrometer. A Perkin Elmer 1600 Series Fourier-transform infrared (FT-IR) spectrometer was used. The measurements of solid samples were performed using the attenuated total reflection (ATR) method. The data were collected in the range of 450-4400  $\text{cm}^{-1}$  with a resolution of 2  $\text{cm}^{-1}$  and scan accumulation 64.

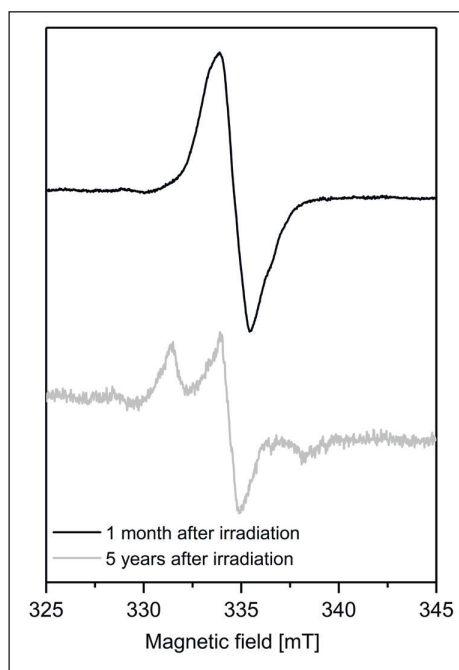
## 3. Results and Discussion

The main effect of polysaccharide irradiation in the solid state is polymer degradation as a result of the direct interaction of ionising radiation with molecules. The primary products of ionising radiation interaction with chitosan are ionised or excited molecules that undergo further transformation, leading to the scission of glycosidic bonds as well as scission of bonds between carbon atoms in aminoglucose units [19]. Unlike the case of irradiating a solution, where radical reactions are fast and occur mainly during irradiation, in the solid state, radicals can be trapped in the crystalline structure [17]. As reported by Chmielewski *et al.* [20], the crystalline phase of chitosan does not change as a result of irradiating solid-state chitosan. Thus, the post-irradiation effects have to be considered for a long time.

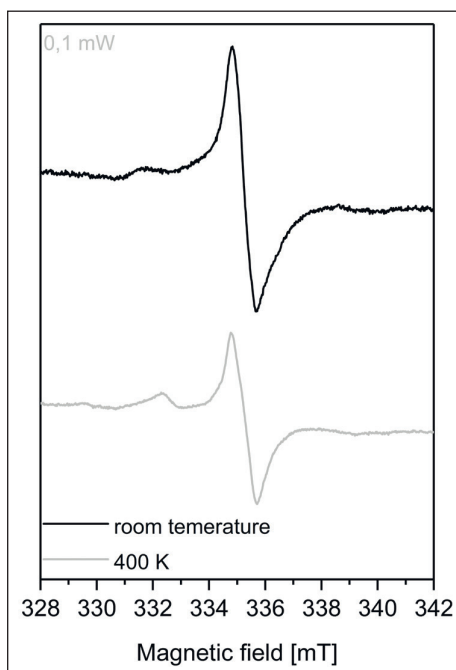
In the present study, the post-irradiation transformation of radicals during storage was observed using EPR spectroscopy. For chitosan irradiated in the solid state, the signal recorded at room temperature after irradiation is a broad singlet, assigned to radicals formed as a result of hydrogen abstraction from carbon C2. Increasing the irradiation dose above 200 kGy produced an overlapping signal consisting of an asymmetric triplet with an overall splitting of 6.8 mT. This signal belongs to stable nitroxyl-type radicals [18]. In such radicals, an unpaired electron is localised on the N-O bond. Importantly, there is a lack of hydrogen atoms in the alpha position of the nitrogen atom. Radicals of this structure can be stored for months under ordinary conditions [21].

As shown in Figure 1, the EPR signal of irradiated chitosan underwent a transformation during storage in air. This transformation, leading to the disappearance of a singlet signal and the formation of a triplet signal, was observed for years after irradiation. Indeed, spectra recorded 1 month after irradiation were still composed mainly of a singlet with a poorly formed additional broad signal. We hypothesised that, due to its high stability, nitroxyl radicals are observed in irradiated chitosan for a long time. Five years after irradiation, the nitroxyl radicals were still easily detectable. The recorded signal was also thermally stable as tested during heating up to 400 K (Figure 2).

Because of their stability, nitroxyl radicals are used as spin markers that can be studied in a system in which there are normally no paramagnetic centres. Nitroxyl radicals



**Figure 1.** Electron paramagnetic resonance spectra of chitosan (95% deacetylation degree) irradiated with 250 kGy and recorded after storage in air for 1 month and 5 years



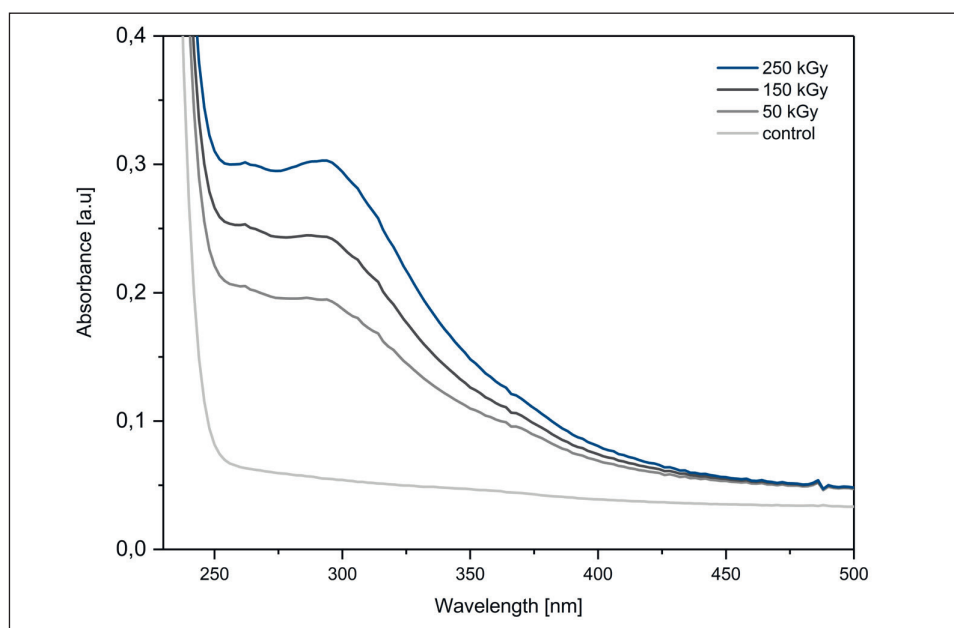
**Figure 2.** Electron paramagnetic resonance spectra of chitosan (70% deacetylation degree) irradiated with 250 kGy and after storage in air for 1 month at room temperature and after heating at 400 K

exhibit antioxidant properties. They can undergo reduction to the hydroxylamine radical  $-\text{RR}'\text{N}-\text{O}^\bullet \rightarrow \text{RR}'\text{N}-\text{OH}$  – or can be oxidised to a cation  $-\text{RR}'\text{N}-\text{O}^\bullet \rightarrow \text{RR}'\text{N} = \text{O}^+$  [22].

Post-irradiation transformation of radicals observed in irradiated chitosan, as a result of amine group decomposition, influence the chemical structure of the polymer [18]. As reported in the literature, a decrease in the chitosan molecular weight, which is the main effect of its irradiation, continues weeks after treatment [17]. Another effect observed is the oxidative degradation of chitosan, causing formation of carbonyl and carboxyl groups, which can be observed using UV-Vis spectroscopy as an increase in intensity of absorption bands at the wavelength 247 and 290 nm (Figure 3) [16].

The C=O groups are formed in the polymer structure under the influence of ionising radiation, and also as a result of the post-irradiation effects that occur during the storage of the irradiated solid state polymer. Rosiak *et al.* [16] noted that the increase in the intensity of the absorption band at the wavelength of 247 nm was about 11% during 10 days of chitosan storage in aerobic conditions.

In the present study, chitosan was analysed immediately after irradiation and after storage for 5 years in air. As shown in Figure 4, there was a linear increase in the absorption band at 290 nm. A greater increase in the intensity of the analysed absorption band was observed for chitosan with a higher degree; this finding indicates the presence of the  $-\text{NH}_2$  group has a prominent influence on the polymer degradation mechanism. As reported by Taskin [23], the higher the DD of chitosan, the more pronounced effects of irradiation on the molecular weight and rheological properties.



**Figure 3.** Ultraviolet-visible light spectra of non-irradiated and irradiated chitosan

The observations carried out for samples stored for 5 years after irradiation showed an increase in the intensity of the band at 290 nm exceeding 100% (Figure 4). This means that the oxidative degradation of irradiated polysaccharides is a process that takes place years after irradiation.

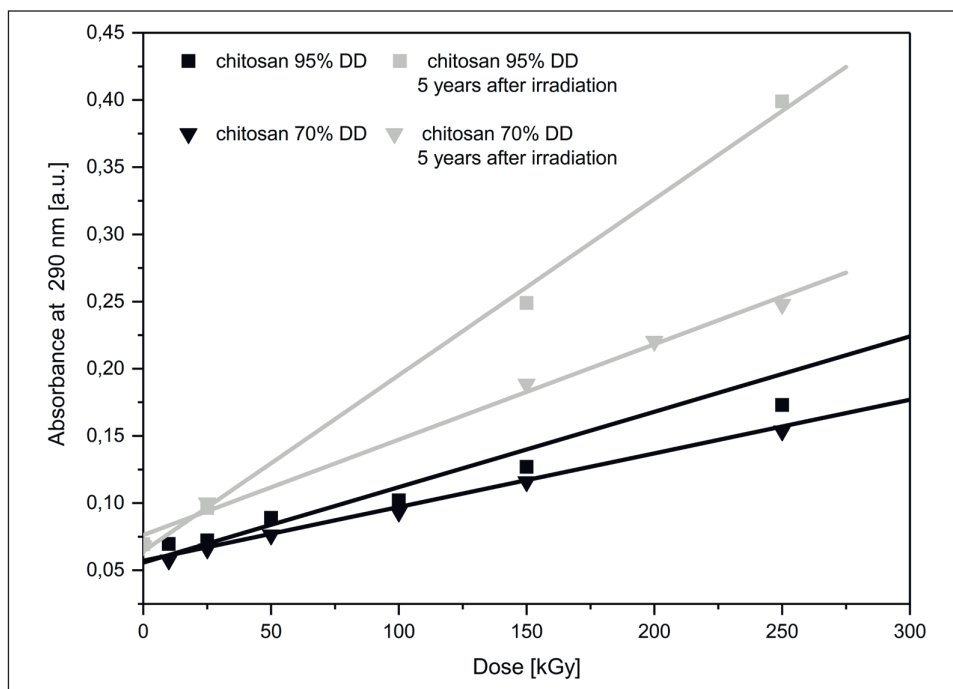
The chemical structure of chitosan was characterised using FTIR spectroscopy (Figure 5). The observed characteristic bands are related to the presence of the amine and amide groups in the polymer structure. For amines, the characteristic infrared absorption bands in the range of 1650-1500 and 1360-1250  $\text{cm}^{-1}$  are due to vibrations of the N-H and C-N bonds, respectively. On the other hand, amides have characteristic absorption bands of the carbonyl group in the range of 1690-1630 and 1570-1515  $\text{cm}^{-1}$ .

DD is a parameter characterising the degree of conversion of chitin to chitosan; one of the methods that can be used for its determination is infrared spectroscopy [24]. In the present experiments, DD was determined based on FTIR spectra of chitosan using the equation and baselines given by Brugnerotto *et al.* [25]:

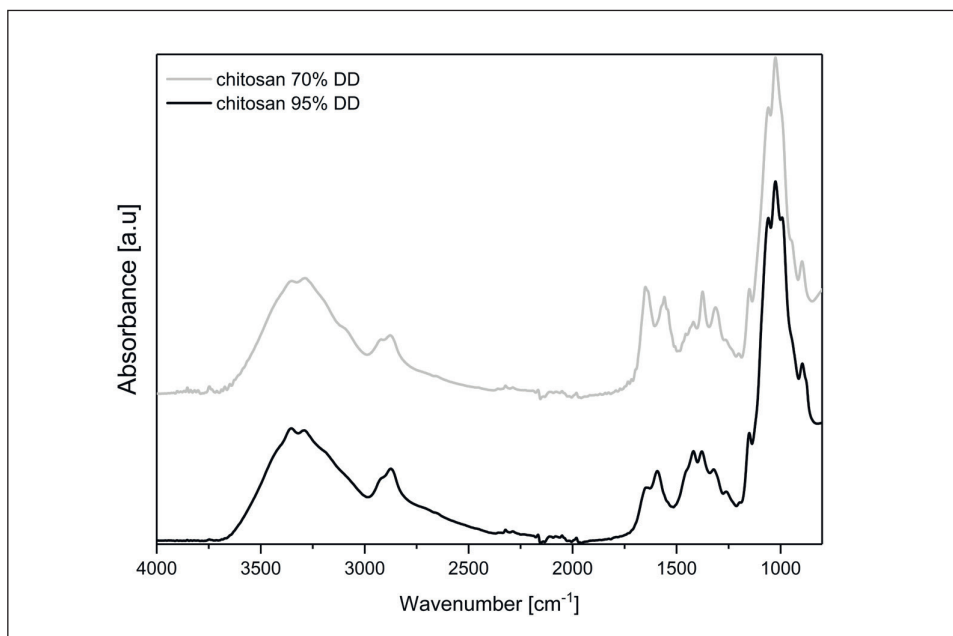
$$\frac{A_{1320}}{A_{1420}} = 0.3822 + 0.03133 \text{ DA and} \\ \text{DD} = 100\% - \text{DA},$$

where  $A_{1320}$  is the band intensity corresponding to the content of acetyl groups and  $A_{1420}$  is a reference band and DA is the acetylation degree.

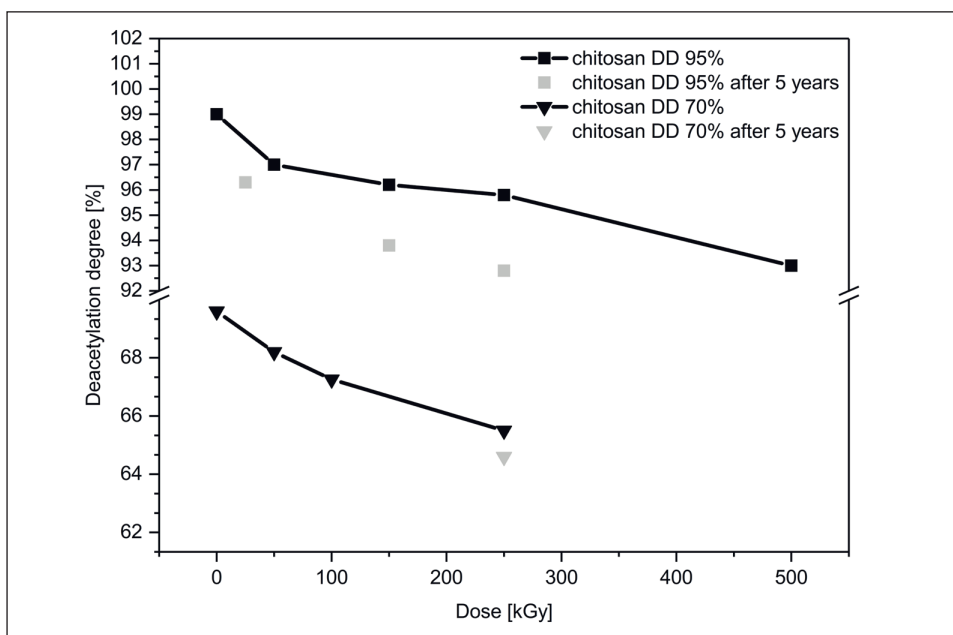
Changes in chitosan DD upon irradiation are caused by breaking bonds between carbon and nitrogen, which result in the emission of ammonia. However, the observed synergetic effect regarding the change in DD is also influenced by detachment of acetyl groups from non-deacetylated units [26].



**Figure 4.** The change in the intensity of the absorption bands of chitosan (95% and 70% deacetylation degree [DD]) at 290 nm occurs under the influence of ionising radiation and during storage in the air atmosphere



**Figure 5.** Fourier-transform infrared spectra of nonirradiated chitosan with different deacetylation degrees (DD)



**Figure 6.** Dependence of chitosan deacetylation degree (DD) on irradiation dose

In the present study, there were changes in the chitosan DD immediately after irradiation and after storage for 5 years in air. For irradiated samples, the DD decreased as the dose of irradiation increased (Figure 6). Irradiation with 250 kGy decreased the DD by 4% for both tested chitosan samples. After 5 years of storage, there was a further decrease in the DD; it was more prominent for the chitosan with the higher initial DD. Based on these results, the important parameter that must be considered is experimental error, which for the method of DD determination using FTIR spectroscopy is high [24]. The formula selected for DD calculation has been reported to give a smaller experimental error independent of the technique and state of the material [25]. As shown in Figure 6, independently of the measurement error, the results show a trend regarding a change in the DD after irradiation and storage.

#### 4. Conclusions

The use of ionising radiation to modify natural polymers is one of the directions of radiation technology development. Currently, the main area of research is biopolymer radiation degradation to increase their reactivity by modifying the chemical and morphological structure, as well as the sterilisation of biopolymer-based medical devices. However, changes that can continue after radiation treatment must be considered at the stage of a final product to estimate its significance on the product properties. Based on the radical transformation mechanism and the observed formation of stable nitroxyl radicals, it can be concluded that even 5 years after irradiation, this transformation is accompanied by a decrease in the DD and an increase in oxidative degradation products. For this reason, for polysaccharides intended for solid state irradiation – for example, sterilisation – the post-irradiation effects must be considered. Such processes are factors that can influence the properties of the end product during its lifetime.



## 5. Acknowledgements

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