



Copolymer of chitosan with acrylamide: electron beam stimulated synthesis, structure and properties

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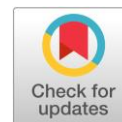
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Abstract

The aim of this research was to obtain the grafted copolymer of chitosan with acrylamide using the electron beam irradiation. Radiation dose was varied from 6 to 160 kGy. The highest yield of the product was observed at radiation dose of 12–40 kGy. Further increase in the dose caused a decrease in the product yield as well as its solubility in water. Using gel permeation chromatography, it was confirmed that unreacted chitosan remained in the product. NMR study of the water-soluble part of the product obtained under the doses of 6, 12, and 20 kGy showed that the length of the side chains of grafted acrylamide was about 2 elementary units. Investigation of chitosan solutions by means of dynamic light scattering revealed the presence of chitosan agglomerates in the solution. The possibility of obtaining dense films was demonstrated. Mechanical treatment of the copolymer in the ball mill caused an increase in the solubility of the samples obtained even at radiation doses of 80 and 160 kGy. It was determined by means of chromatographic methods that there were no products with low molecular weight in the ball-milled product, and unreacted chitosan did not undergo mechanocracking during the mechanical treatment.

Keywords

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Key findings

- The new method for modification of chitosan with acrylamide by using the electron beam irradiation.
- Confirmed that unreacted chitosan remained in the reaction product. Thus, modification of chitosan may proceed as a heterogeneous reaction.
- Mechanochemical cracking of the product was observed to be non-uniform.

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1. Introduction

Chitosan is a biopolymer formed through partial or complete deacetylation of chitin, one of the most widespread natural macromolecular polysaccharides. Chitosan and its derivatives have been attracting increasing attention due to their ecological safety, high biological activity, biocompatibility, and biodegradability [1, 2]. Chitosan finds application in medicine, pharmaceuticals, cosmetics, agriculture and food industry. Chitosan and its derivatives are widely used

to develop drug delivery systems [3, 4]. Among the disadvantages of chitosan, its low solubility in water and instability in acid solutions may be mentioned [5], as well as low mechanical strength and poor plasticity when used in films [6]. One of the most efficient methods that are used to improve the physicochemical properties of chitosan, such as its sensitivity to the pH of the solution, is obtaining chitosan copolymers with different structures [7, 8]. Using the copolymers opens up opportunities for the development of "smart" materials, which can change their properties under

the influence of temperature, pH of the medium, and other external factors.

These materials are particularly attractive for creating the systems with controlled release of biologically active substances into solution due to their sensitivity to the solution pH [9, 10].

Chitosan contains primary and secondary hydroxyl groups as well as amino groups, which may form bonds with the functional groups of acrylamide to form a copolymer. The interaction is possible through $-OH$ and $-NH_2$ groups of chitosan. In Figure 1, possible products of chitosan interaction with acrylamide are presented [11].

There are several methods of chitosan derivatives preparation, such as photochemical [12, 13], microwave synthesis [14], chemical [15, 16] and block copolymerization [17]. Moreover, polysaccharides like chitosan can be grafted with acrylamide in solution using the initiators of radical polymerization, in particular, heavy metal ions [18, 19]. Shortcomings of these methods include process inhomogeneity as a consequence of diffusion hindrance under the action of the initiating agent, substantial process duration, the necessity of heating and subsequent removal of the residues of polymerization initiators and the products of their transformations from the product. An alternative method is copolymerization under the action of ionizing radiation. This method is free from chemical initiators and allows uniform treatment of reaction mixtures without heating [20, 21].

Previously [22], we demonstrated the possibility of obtaining chitosan copolymer with acrylamide using graft polymerization stimulated by the electron irradiation. The grafted chitosan copolymer was used to prepare the betulin composites with the help of mechanical treatment. It was shown that the rate of betulin release into the aqueous solution during mechanocomposite dissolution depends on solution pH, which allows considering mechanocomposites based on chitosan copolymer with acrylamide as promising means for the controllable delivery of betulin and, probably, other pharmaceutical substances.

The goals of the present work were to determine the optimal conditions for obtaining grafted copolymer of chi-

tosan with acrylamide under the action of ionizing radiation and to study their structure and physicochemical properties. In addition to gel permeation chromatography, X-ray phase analysis, NMR spectroscopy and dynamic light-scattering spectroscopy were used to study chitosan and its copolymer in the solid state and in the solution. Bearing in mind the possibility of using chitosan copolymers as the means of drug delivery in the form of mechanocomposites, we carried out experiments to study the effect of mechanical treatment on the stability and solubility of the obtained copolymer.

2. Experimental

2.1. Reagents and objects

Chitosan (Bioprogress, the Moscow Region, Shchelkovo) obtained from crab crusts was used in the work. It had an average molecular mass $M_w = 1600$ Da (g/mol), polydispersity index $D = 1.05$, and deacetylation degree 80–82%. Anhydrous chitosan was prepared by precipitation from acetic acid solutions with the addition of acetone. Acetone (Reactive, Russia) of pure reagent grade was used.

Acrylamide for electrophoresis, after quadruple crystallization (Diaem, Russia) was used without preliminary purification and drying.

Acetic acid (Ekros, Russia) of chemically pure reagent grade was used to prepare acetic solutions.

Double distilled water was obtained from the BE-2 Laboratory distiller (LIAM, Russia).

2.2. Obtaining grafted chitosan copolymer with acrylamide using electron-beam irradiation

To make the grafted copolymer of chitosan with acrylamide, 4 g of chitosan was dissolved in 200 mL of distilled water or a 2 vol.% solution of acetic acid under mixing at a speed of 600 rpm to obtain a homogeneous transparent solution. Then 3.5 g of acrylamide was added in portions (the ratio of the number of elementary units of chitosan to the number of acrylamide moles was 1:2). After that, mixing at a speed of 600 rpm was continued for 30 min.

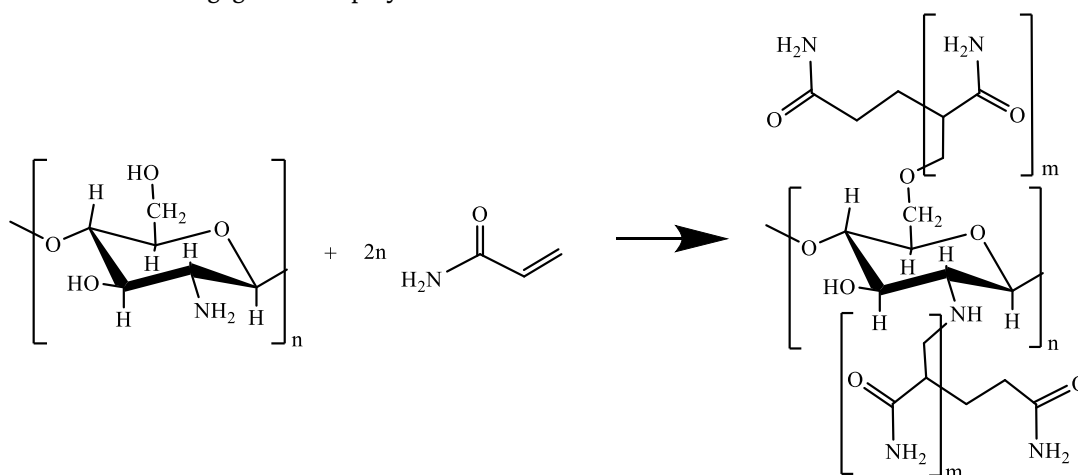


Figure 1 Addition of acrylamide to chitosan.

The electron beam treatment was performed using a pulsed electron accelerator ILU-6 (INP SB RAS, Russia). The electron energy was 2.4 MeV, the pulsed beam current was 328 mA and the pulse recurrence frequency was 2–2.5 Hz. The solution was treated with ionizing radiation with the solution layer thickness not more than 1 cm by moving it at the velocity of 2 cm/s under the outlet port of the accelerator. Treatment time and, consequently, the corresponding dose of ionizing radiation were varied by changing the number of passes under the outlet port of the accelerator. The dose was either 6, 12, 20, 40, 80, or 160 kGy (J/g).

The grafted copolymer was isolated by salting-out with acetone. The precipitate was separated by centrifuging at 8000 rpm at room temperature for 15 min and dried at room temperature and reduced pressure for 24 h. The dried products were extracted with a mixture of acetone with water (60:40 vol.%) for 24 h to remove the homopolymers [23]. Then the precipitate was washed with the same mixture of acetone with water and centrifuged 3 times. Final drying to a constant weight was carried out at 60 °C at reduced pressure (drying furnace SHS-11/2,5-C, MIUS, Russia). The degree of polymer grafting was determined from the sample mass increment with respect to the amount of initial acrylamide. The yield of the product was determined as a ratio of the mass of the isolated product to the sum of the masses of initial reagents.

Around 1 g of the obtained copolymer was placed in 100 mL of water and stirred at a speed of 600 rpm at room temperature for 1 h. The resulting dispersion was filtered through preliminary weighed paper filters. The filters were dried at reduced pressure at a temperature of 60 °C to the constant weight, and weighed to determine the weight of unreacted chitosan.

The filtrates were evaporated using an IR-1M rotary evaporator (Labtex, China) to a constant weight at the temperature up to 80 °C. As a result of evaporation, copolymer samples were obtained as films. The as-obtained films were weighed to determine the amount of dissolved copolymer. The films, representing the water-soluble part of the product, were studied by means of gel permeation chromatography, NMR spectroscopy, and electron microscopy.

Mechanical treatment was carried out using a SPEX 8000 mill (CertiPrep Inc., USA). A steel cylinder, 60 mL in volume, and steel balls, 6 mm in diameter, were used. The ratio of sample mass to ball mass was 1:40, and the acceleration of the balls was 8–10 g. The treatment time was 5, 15, and 30 min.

2.3. Methods of samples investigation

The FTIR spectra were recorded using a FT-801 Fourier spectrometer (Simex, Russia), KBr (1:300), 4 cm⁻¹.

X-ray powder diffraction analysis was carried out using a D8 Advance diffractometer (Bruker, Germany) with a single-coordinate detector Lynx-Eye, Cu K α radiation, Bragg-Brentano geometry, $2\theta = 5\text{--}70^\circ$.

The molecular mass distribution (MMD) in the samples was determined by means of gel permeation chromatography (GPC) using an Agilent 1200 chromatograph (Agilent Technologies, USA) with a refractive-index detector (30 °C, PLaquagel-OH 40 300 \times 7.5 mm, 0.1 M LiNO₃, 1 mL/min). The concentrations of solutions were 0.1–0.2 wt.%.

High-performance liquid chromatography (HPLC) analysis was performed using a Prominence 20A chromatograph (Shimadzu, Germany) with the refractive-index and diode matrix detectors (45 °C, Shim-pack GIS 5mm C18, 150 \times 4.6 mm, H₂O(A) – AcN(B), 5-100-100% B, 1 mL/min). For calibration we used standard ReadyCal set poly(methyl methacrylate) (Sigma-Aldrich, 81506 – 1EA). Concentration of solutions was much lower than 1 wt.%

Investigation by means of nuclear magnetic resonance (NMR) spectroscopy was carried out using an Avance III 500 spectrometer (Bruker, Germany). The ¹H and ¹³C spectra were recorded in D₂O solutions. Water-soluble components of initial chitosan and the samples obtained under the doses of 6, 12 and 20 kGy were studied.

Electron microscopic studies were carried out using scanning microscopes S-3400N and TM-1000 (Hitachi, Japan). Before investigation, a layer of gold was deposited onto the films by means of magnetron sputtering.

Investigation of supramolecular structures in the solutions was carried out by means of dynamic light scattering (DLS) using a multi-angle particle size analyzer Photocor Complex (Photocor, Russia). The configuration for DLS was as follows: thermally stabilized at 25 °C semiconductor laser (450 nm, 25 mW), cuvette Macro (2.5 mL minimum volume). The solutions containing 2–4 g of chitosan in 200 mL of water or in a 2 vol.% solution of acetic acid were prepared. The solutions were subjected to centrifuging before the measurements.

3. Results and Discussion

3.1. Obtaining grafted copolymer of chitosan with acrylamide under irradiation with high-energy electrons

The data on grafting degree and product yield as well as the data on the solubility of resulting samples depending on the dose of irradiation of acetic acid solutions are presented in Figure 2.

The grafting degree and the yield of the product increase with the increase in the radiation dose and drop when the dose reaches 160 kGy. The highest product yield is observed for the radiation doses of 20–80 kGy. The solubility of the obtained product in acetic acid solutions is almost perfect for the radiation dose up to 20 kGy. The application of higher radiation doses causes at first a decrease in the fraction of water-dissolved substance as a consequence of cross-linking and gel formation, and then copolymer destruction resulting in a decrease in the grafting degree and product yield.

It should be noted that the FTIR spectra of copolymers obtained at radiation doses of 20 and 160 kGy almost coincide with each other, suggesting that the radiation-induced grafting may be performed even at low radiation dose.

At the same time, if the synthesis is carried out in aqueous solutions, lower product yields are obtained: for the doses of 6, 12, 20, and 40 kGy, the product yield is 46, 49, 79, and 80%, respectively. In this case, the quality of the product worsens; its solubility does not exceed 90%. This effect is probably due to the existence of associates in the aqueous solutions of chitosan [24], which hinders the access of acrylamide to reaction centers. In the case of acetic acid solutions, the number of associates is evidently smaller than in aqueous solutions, and the resulting conversion is higher.

It has been already shown [22] that the FTIR spectra of the obtained copolymer do not exhibit the C=C vibrations appearing in acrylamide in the region of 990–900 cm^{-1} as a doublet. This is the evidence of copolymerization proceeding during irradiation. A broad band of chitosan at 3700–3300 cm^{-1} shifted towards low frequencies suggests that OH- and NH-groups of chitosan participate in the formation of bonds with acrylamide. The same changes in the FTIR spectra were observed in [14].

The results of the investigation of molecular mass characteristics of chitosan and its copolymer with acrylamide obtained at different radiation doses are shown in Figure 3. The values of M_w , M_n and M_p for the observed peaks are shown in Table S1.

The data presented in Figures 4, S1 and S2 show that in water and acetic acid solutions the number of chitosan particles with the size of 100–1000 nm increases slightly with time. This phenomenon may be explained by the formation of chitosan agglomerates due to charged groups in polymer chains and their mobile counterions, which leads to the formation of both intra- and intermolecular reversible junctions [25].

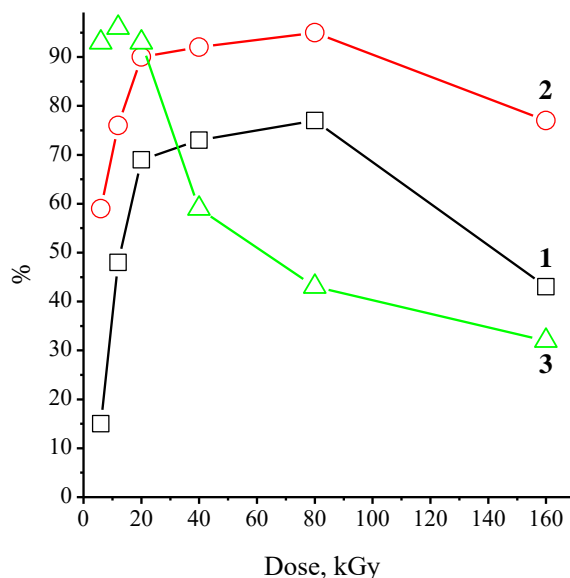


Figure 2 Grafting degree (1), product yield (2), and the fraction of water-soluble product (3) depending on the radiation dose.

In addition, the combined effect of the hydrophobic interaction and the hydrogen bonding can too act as the driving force of aggregate formation [26]. A similar phenomenon is observed in the solution of chitosan in acetic acid solution. Due to the presence of chitosan agglomerates in the solution, it may be assumed that the interaction of acrylamide with chitosan may proceed as a heterogeneous reaction.

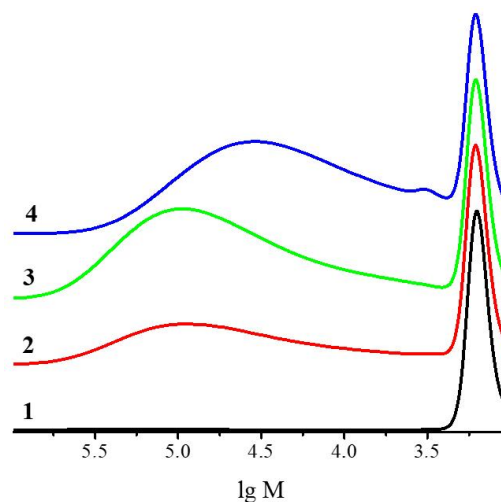


Figure 3 Gel chromatograms: chitosan (1) and copolymers of chitosan and acrylamide, obtained at the radiation doses 6 (2), 12 (3), and 20 (4) kGy.

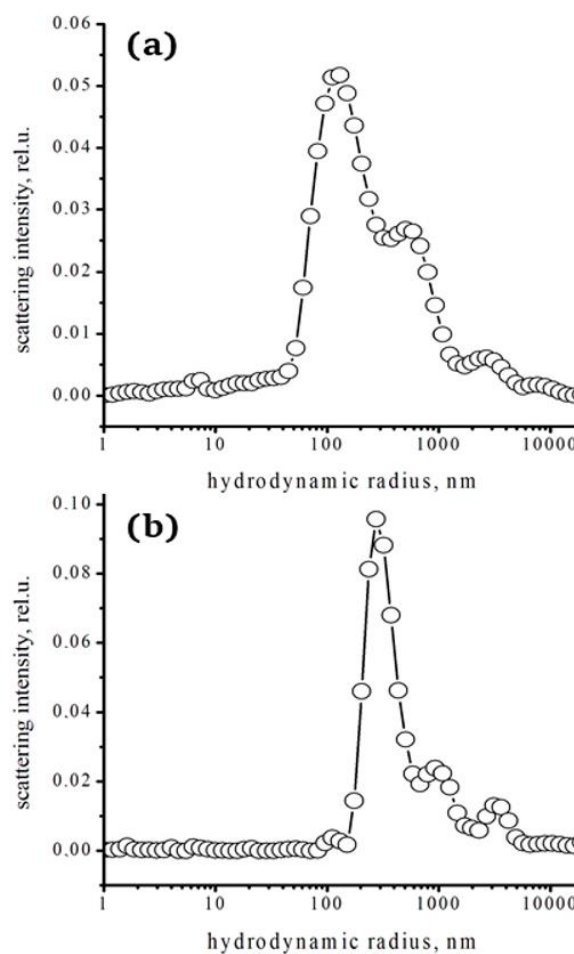


Figure 4 Distribution of particle sizes in chitosan 2 wt.% – solutions in water after 2 days (a); in 2 vol.% acetic acid after 2 days (b).

Investigation of the copolymers of chitosan with acrylamide obtained at the radiation dose of 20 kGy was carried out by means of ^1H and ^{13}C NMR-spectroscopy. Substitution degree at different groups of chitosan was determined from the ratio of intensities of changed and unchanged signals. An example of ^{13}C NMR spectrum for the calculation of substitution degree is presented in Figure 5a.

The signals from C2 (50 ppm) and C6 (54 ppm) are shown in the inset along with atom numbering. Chitosan modification with acrylamide leads to the appearance of additional signals. In the case of C6, a shift to weaker field is observed, while in the case of C2, the signal is observed to shift to stronger field. Gaussian signal expansion gives the substitution degree $\sim 40\%$ at C2 atom and $\sim 50\%$ at C6 atom. Taking into account the presence of unreacted chitosan in the samples, we may state that the conversion in the reacted part is much higher.

The selectivity of chitosan interaction with acrylamide was determined with ^1H NMR (Figure 5b). The signals of acrylamide added directly to chitosan (7.0 ppm) and acrylamide forming oligoacrylamide (7.8 ppm) are shown in the inset. Comparing the obtained values with the grafting degree (Figure 2), we may assume that the average length of the side chains of acrylamide is slightly more than 2 elementary units.

The X-ray powder diffraction patterns of chitosan and its copolymer with acrylamide obtained at different radiation doses are presented in Figure 6. Along with the diffraction pattern of initial chitosan (Figure 6, curve 1), the diffraction pattern of the sample obtained by chitosan precipitation from acetic acid solution is shown (Figure 6, curve 2). Thus, recrystallized chitosan is anhydrous [27, 28], and its crystallinity is higher than that of initial chitosan. The formation of chitosan with higher crystallinity may be a consequence of its low molecular mass, promoting a decrease in the energy barriers of chain mobility and diffusion, thus simplifying crystallization, and a narrow molecular mass distribution, promoting the formation of regular structures. In addition to the basic reflections of chitosan, broad maxima at $2\theta \sim 30, 34$ and $39\text{--}40^\circ$ may be distinguished, providing evidence of additional arrangement of the short-range order during recrystallization. Grafting polymerization leads to the appearance of halos with the maximum at $2\theta \sim 22^\circ$ (Figure 6, curves 3–8). Broad maxima at $2\theta \sim 30, 34$ and $39\text{--}40^\circ$ are observed to disappear. The diffraction patterns of the samples obtained at the doses of 6–40 kGy clearly exhibit reflections that may be related to chitosan at $2\theta \sim 12\text{--}15^\circ$, which agrees with the data of gel permeation chromatography.

Some shifts of reflection positions may be attributed to the effects of chitosan recrystallization from the solutions of acetic acid with the formation of an anhydrous form.

Figure 7 and 8 shows the results of electron microscopic studies of the films obtained from the copolymer (radiation dose 20 kGy) and initial chitosan along with the light scat-

tering curves for their 1 wt.% solutions in water. The copolymers of chitosan with acrylamide obtained under irradiation with high-energy electrons are amorphous to a high extent, unlike those obtained in solution, where crystallinity degree decreased only from 44 to 19% [15]. Moreover, the crystallinity of the copolymer decreases with an increase of the radiation dose.

The film obtained from the copolymer solution is composed of rounded particles up to $5\ \mu\text{m}$ in size. On the contrary, the film obtained from initial chitosan consists of submicron particles with clearly pronounced faces and has porous structure.

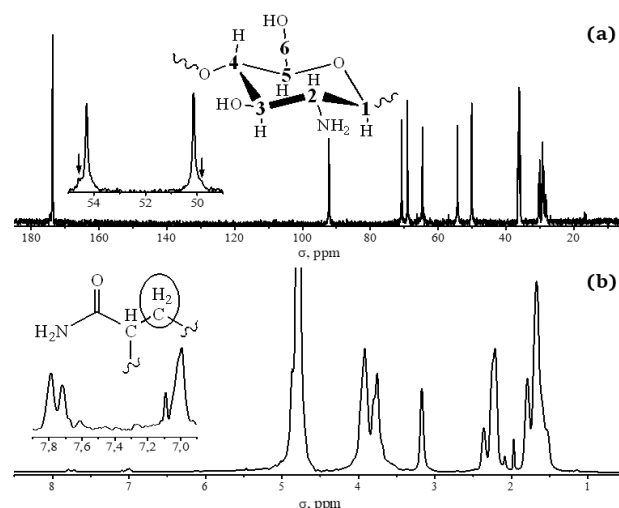


Figure 5 NMR spectra of the sample obtained at the radiation dose of 20 kGy for ^{13}C (a) and ^1H (b).

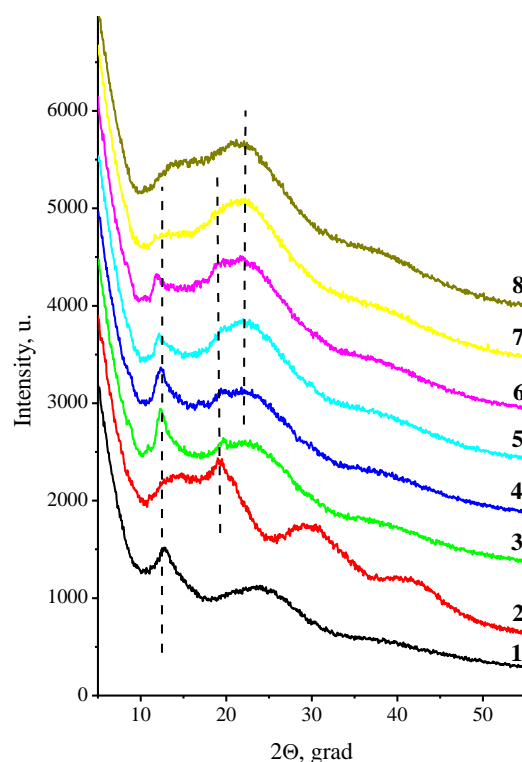


Figure 6 X-ray powder diffraction patterns of initial (1), recrystallized (2) chitosan, copolymers of chitosan with acrylamide, obtained at the radiation dose 6 (3), 12 (4), 20 (5), 40 (6), 80 (7), and 160 (8) kGy.

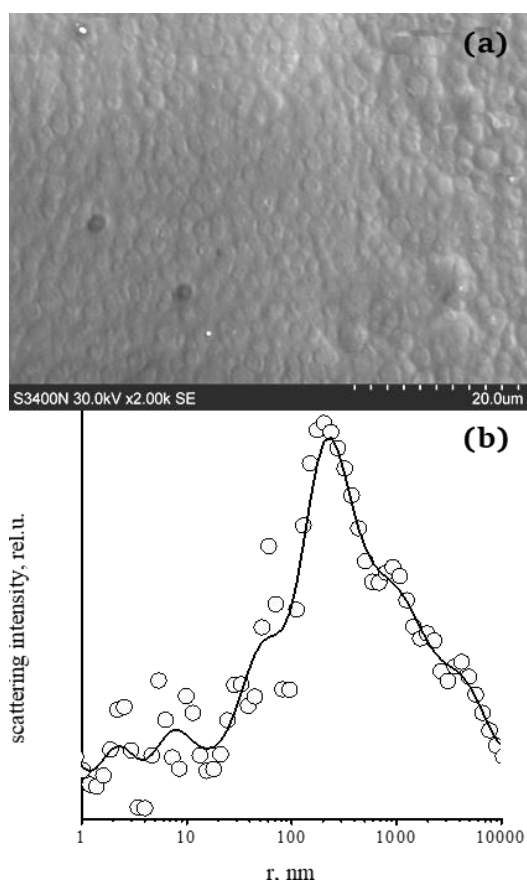


Figure 7 Microphotographs of the films (a) and light scattering curves (b) 2 h after dissolution for the solutions of chitosan copolymer obtained at radiation dose of 20 kGy.

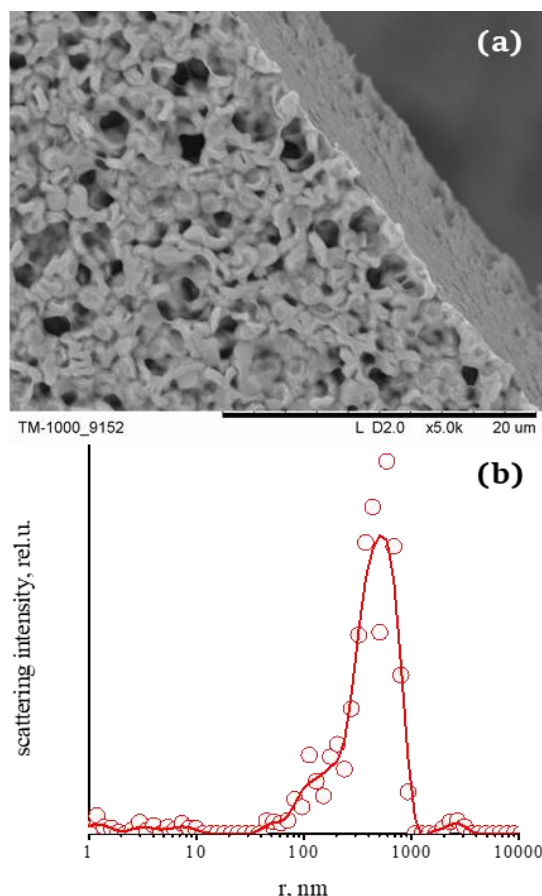


Figure 8 Microphotographs of the films (a) and light scattering curves (b) 2 h after dissolution for the solutions of initial chitosan.

The film structure is likely to be determined by the presence of supramolecular structures in the solution. The dependences of the intensity of dynamic light scattering on particle size are presented in Figures 7b and 8b. In the case of chitosan, the maximum light scattering relates to the region corresponding to particle size 400–600 nm. In the case of copolymer, the maximum light scattering corresponds to particle size 200–300 nm. Substantial light scattering is also observed for small particles, 1–10 nm in size, corresponding to the sole polymer molecules. It can be assumed that the presence of a wider size distribution of inhomogeneities in the solution makes it possible to obtain a more uniform film. The properties of such films are of interest for future research, for instance, for obtaining the complexes of triterpenoids similar to the complex of betulin diacetate with arabinogalactan [29].

3.2. The effect of mechanical treatment on the properties of copolymer of chitosan with acrylamide

GPC analysis of chitosan copolymer with acrylamide (radiation dose of 20 kGy) after mechanical treatment for different time intervals (Figure 9, Table S2) shows that unreacted chitosan is almost insensitive to mechanical action.

This is consistent with the results of other authors, who showed that low molecular mass chitosan does not significantly change its molecular mass during mechanical processing [30]. At the same time, the high-molecular component is subjected to noticeable mechanocracking, which is observed as a sequential shift of the maximum (M_p) from ~40000 to 15000 Da. (Table S3)

Moreover, mechanical treatment causes an increase in solubility for copolymer samples obtained at the doses of 80 and 160 kGy, up to 56% and 58%, respectively. This may also be due to the breaking of bonds between elementary units of chitosan in the copolymer and the formation of lower molecular mass products.

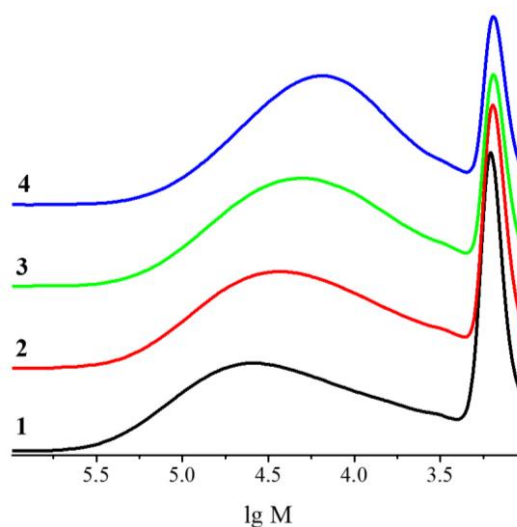


Figure 9 Gel chromatograms: copolymer obtained at the dose of 20 kGy: initial (1) and subjected to mechanical treatment for 5 (2), 15 (3), and 30 (4) min.

The possibility of formation of such products, including acrylamide itself, under mechanical milling was studied using HPLC method. For this, the extracts were obtained using acetone-water mixture 60:40 (vol.%) or pure acetone. The reference samples were extracts from copolymer samples that were not subjected to mechanochemical treatment. The analysis of the extracts of copolymers after mechanical milling revealed the absence of new components in the samples, compared to the reference samples. This is a convincing proof of the absence or a very insignificant formation of substances with low molecular weight during mechanochemical treatment of the samples of chitosan copolymer with acrylamide.

4. Limitations

Chitosan solutions were instigated. In the future, we plan to investigate the size of chitosan particles in solutions with different temperature, the solutions with different chitosan concentration, and the toxicity of the product in the future.

5. Conclusions

Grafted copolymers of chitosan with acrylamide were obtained using the electron-beam processing. It was shown that synthesis efficiency and grafting degree depend on the dose of ionizing radiation, passing through a maximum with an increase in the dose. The solubility of the obtained products in water is almost perfect for the radiation doses up to 20 kGy. Above this dose, cross-linking occurs at first, leading to gel formation (40, 80 kGy), followed by product destruction (160 kGy). NMR investigation of the water-soluble part of the product obtained at the doses of 6, 12, and 20 kGy shows that the length of the side chains of grafted acrylamide is ~2 elementary units. Based on the obtained results, we assume that -NH₂(C2) and -OH(C6) groups both participate in the reaction. It is shown that the obtained product contains residual chitosan. Investigation of chitosan solutions by means of dynamic light scattering revealed the presence of agglomerates. These agglomerates and the presence of unreacted chitosan in the product may indicate a heterogeneous reaction route. The possibility for the product to form dense films was demonstrated.

Investigation of the transformation of product under ball-milling detected an increase of solubility, also occurring at high radiation doses (40, 80, 160 kGy). Mechanochemical cracking of the product was observed to be non-uniform: the residual chitosan did not undergo cracking. It was determined by means of HPLC analysis that there were no products with low molecular weight in the ball-milled copolymer.

• Supplementary materials

This manuscript contains supplementary materials, which are available on the corresponding online page.

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• Author contributions

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Formal analysis: M.M.

Investigation: I.A., M.M., S.K., A.B.

NMR investigation: I.A., I.E.

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• Conflict of interest

The authors declare no conflict of interest.

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References

1. Khajavian M, Vatanpour V, Castro-Munoz R, Boczkaj G. Chitin and derivative chitosan-based structures preparation strategies aided by deep eutectic solvents: A review. *Carbohydr Polym.* 2022;275:118702. doi:[10.1016/j.carbpol.2021.118702](#)
2. Bakshi PS, Selvakumar D, Kadirvelu K, Kumar NS. Chitosan as an environment friendly biomaterial – a review on recent modifications and applications. *Int J Biol Macromol.* 2020;150:1072–1083. doi:[10.1016/j.ijbiomac.2019.10.113](#)

3. Sivanesan I, Gopal J, Muthu M, Shin J, Mari S, Oh J. Green synthesized chitosan/chitosan nanoforms/nanocomposites for drug delivery applications. *Polymers*. 2021;13-14:2256. doi:[10.3390/polym13142256](https://doi.org/10.3390/polym13142256)
4. Pari R. Drug delivery applications of chitin and chitosan: a review. *Environ Chem Lett*. 2020;18(3):577-594. doi:[10.1007/s10311-020-00963-5](https://doi.org/10.1007/s10311-020-00963-5)
5. Renbi B, Han W, authors. A chitosan solution and method of preparing the same. Patent WO2009035413A1. Publ. Date 19.03.2009 (Worldwide)
6. Thakhiew W, Champahom M, Devahastin S, Soponronnarit S. Improvement of mechanical properties of chitosan-based films via physical treatment of film-forming solution. *J Food Eng*. 2015;158:66-72. doi:[10.1016/j.jfoodeng.2015.02.027](https://doi.org/10.1016/j.jfoodeng.2015.02.027)
7. Kumar D, Gihar S, Shrivash MK, Kumar P, Kundu PP. A review on the synthesis of graft copolymers of chitosan and their potential applications. *Int J Biol Macromol*. 2020;163:2097-2112. doi:[10.1016/j.ijbiomac.2020.09.060](https://doi.org/10.1016/j.ijbiomac.2020.09.060)
8. Boominathan T, Sivaramakrishna A. Recent advances in the synthesis, properties, and applications of modified chitosan derivatives: Challenges and opportunities. *Top Curr Chem*. 2021;379(3):19. doi:[10.1007/s41061-021-00331-z](https://doi.org/10.1007/s41061-021-00331-z)
9. Concheiro A, Alvarez-Lorenzo C, Schneider HJ. *Smart Materials for Drug Delivery: Complete Set.*; Royal Society of Chemistry: Cambridge, 2013. 900 p.
10. Morrow B, Payne G, Shen J. pH-Responsive self-assembly of polysaccharide through a rugged energy landscape. *J Am Chem Soc*. 2015;137(40):13024-13030. doi:[10.1021/jacs.5b07761](https://doi.org/10.1021/jacs.5b07761)
11. Ibrahim AG, Sayed AZ, El-Wahab HA, Sayah MM. Synthesis of poly(acrylamide-graft-chitosan) hydrogel: optimization of the grafting parameters and swelling studies. *Am J Pol Sci Tech*. 2019;5(2):55-62. doi:[10.11648/j.ajpst.20190502.13](https://doi.org/10.11648/j.ajpst.20190502.13)
12. Saber-Samandari S, Yilmaz O, Yilmaz EJ. Photoinduced graft copolymerization onto chitosan under heterogeneous conditions. *J Macromol Sci A*. 2012;49:591-598. doi:[10.1080/10601325.2012.687970](https://doi.org/10.1080/10601325.2012.687970)
13. Sautrot-Ba P, Razza N, Breloy L, Abbad Andaloussi S, Chiappone A, Sangermano M, He lary C, Belbekhouche S, Coradin T, Versace D-L. Photoinduced chitosan-PEG hydrogels with long-term antibacterial properties. *J Mater Chem B*. 2019;7:6526-6538. doi:[10.1080/10601325.2012.687970](https://doi.org/10.1080/10601325.2012.687970)
14. Desnelli D, Eliza E, Mara A, Rachmat A. Synthesis of copolymer of chitosan with acrylamide as an adsorbent for heavy metal waste treatment. *IOP Conf Ser: Mater Sci Eng*. 2020;833:012064. doi:[10.1088/1757-899X/833/1/012064](https://doi.org/10.1088/1757-899X/833/1/012064)
15. Mochalova AE, Zaborshchikova NV, Knyazev AA, Smirnova LA, Izvozhikova VA, Medvedeva VV, Semchikov YuD. Graft polymerization of acrylamide on chitosan: Copolymer structure and properties. *Polym Sci A*. 2006;48(9):918-923. doi:[10.1134/S0965545X06090069](https://doi.org/10.1134/S0965545X06090069)
16. Mochalova AE, Smirnova LA. State of the art in the targeted modification of chitosan. *Polym Sci B*. 2018;60(2):131-161. doi:[10.1134/S1560090418020045](https://doi.org/10.1134/S1560090418020045)
17. Tatarinov PV, Mochalova AE, Belysheva IV, Smirnova LA, Bodikov IV. Induced degradation of chitosan, conjugated with block copolymerization with acrylamide. *Russ J Appl Chem*. 2010;83(7):1294-1298. doi:[10.1134/S1070427210070232](https://doi.org/10.1134/S1070427210070232)
18. Okieimen FE. Preparation, characterization, and properties of cellulose-polyacrylamide graft copolymers. *J Appl Polym Sci*. 2003;89(4):913-923. doi:[10.1002/app.12014](https://doi.org/10.1002/app.12014)
19. Shi A, Dai X, Jing Z. Tough and self-healing chitosan/poly(acrylamide-co-acrylic acid) double network hydrogels. *Polym Sci A*. 2020;62(3):228-239. doi:[10.1134/S0965545X20030128](https://doi.org/10.1134/S0965545X20030128)
20. Sun H, Wirsén A, Albertsson A. Electron beam-induced graft polymerization of acrylic acid and immobilization of arginine-glycine-aspartic acid-containing peptide onto nanopatterned polycaprolactone. *Biomacromolec*. 2004;5-6:2275-2280. doi:[10.1021/bm049703p](https://doi.org/10.1021/bm049703p)
21. Ponomarev AV. Radiolysis as a powerful tool for polymer waste recycling. *High Energy Chem*. 2020;54(3):194-204. doi:[10.1134/S0018143920030121](https://doi.org/10.1134/S0018143920030121)
22. Mikhailenko MA, Shakhtshneider TP, Antonov IM, Myz SA, Kuznetsova SA, Bryazgin AA. Radiation-thermal synthesis of copolymers of chitosan with acrylamide as a means of betulin delivering. *J Sib Fed Univ: Chem*. 2022;15(3):420-430. doi:[10.17516/1998-2836-0305](https://doi.org/10.17516/1998-2836-0305)
23. Al-Karawi AJM, Al-Qaisi ZHJ, Abdullah HI, Al-Mokaram AMA, Ajeel Al-Heetimi DT. Synthesis, characterization of acrylamide grafted chitosan and its use in removal of copper(II) ions from water. *Carbohydrate Polymers*. 2011;83:495-500. doi:[10.1016/j.carbpol.2010.08.017](https://doi.org/10.1016/j.carbpol.2010.08.017)
24. Vikhoreva GA, Pchelko OM, Gal'braikh LS, Rogovina SZ. The phase state and rheological properties of the chitosan-acetic acid-water system. *Polym Sci B*. 2001;43(6):1079-1084.
25. Blagodatskikh IV, Bezrodnykh EA, Abramchuk SS, Muranov AV, Sinitsyna OV, Khokhlov AR, Tikhonov VE. Short chain chitosan solutions: self-assembly and aggregates disruption effects. *J Polym Res*. 2013;20:73. doi:[10.1007/s10965-013-0073-0](https://doi.org/10.1007/s10965-013-0073-0)
26. Mandal P, Mukherjee M, Shunmugam R. Effect of the aqueous-organic solvent mixtures upon super-aggregation of chitosan. *J Polym Res*. 2023;30:26. doi:[10.1007/s10965-022-03404-9](https://doi.org/10.1007/s10965-022-03404-9)
27. Naito P, Ogawa Y, Kimura S, Iwata T, Wada M. Crystal transition from hydrated chitosan and chitosan/monocarboxylic acid complex to anhydrous chitosan investigated by X-ray diffraction. *J Polym Sci B: Polym Phys*. 2015;53:1065-1069. doi:[10.1002/polb.23748](https://doi.org/10.1002/polb.23748)
28. Mogilevskaya EL, Akopova TA, Zelenetskii AN, Ozerin AN. The Crystal Structure of Chitin and Chitosan. *Polym Sci Ser A*. 2006;48:116-123. doi:[10.1134/S0965545X06020039](https://doi.org/10.1134/S0965545X06020039)
29. Mikhailenko MA, Shakhtshneider TP, Eltsov IV, Kozlov AS, Kuznetsova SA, Karacharov AA, Boldyrev VV. Supramolecular architecture of betulin diacetate complexes with arabinogalactan from *Larix sibirica*. *Carbohydr Polym*. 2016;138:1-7. doi:[10.1016/j.carbpol.2015.11.047](https://doi.org/10.1016/j.carbpol.2015.11.047)
30. Pandit A, Indurkar A, Deshpande C, Jain R, Dandekar P. A systematic review of physical techniques for chitosan degradation. *Carbohydr Polym Technol Appl*. 2021;2:100033. doi:[10.1016/j.carpta.2021.100033](https://doi.org/10.1016/j.carpta.2021.100033)