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Influence of macromolecules conformation of chitosan on its graft polymerization with vinyl monomers and the copolymer properties



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ARTICLE INFO	A B S T R A C T		
Keywords: Chitosan The conformation of macromolecules Graft polymerization The efficiency of the process	The conformational states of chitosan macromolecules in solutions and their influence on the "grafting from" polymerization of 2-hydroxyethyl methacrylate and N-vinylpyrrolidone on the polysaccharide and the properties of their copolymers are studied. The conformational transition of macromolecules coil-rigid rod and rigid rod-globule in aqueous acidic solution was established by spectrophotometric method. The coil-rigid rod conformation transition depends on pH, it is sensitive to temperature and doesn't depend on the molecular weight of chitosan. The effective size of chitosan macromolecules for the rigid rod conformation was larger than for the coil conformation. The yield of the graft copolymer was significantly higher in case of where chitosan macromolecules were in the coil conformation (96 %) than in the rigid rod conformation (81 %). In the first case, the product of synthesis is a homogeneous solution, in the second case it is a colloidal solution. The copolymers were		

characterized by FTIR spectroscopy and gel-permeation chromatography.

1. Introduction

Chitosan is a semi-synthetic linear copolymer composed of variable units of β -(1–4) linked units of 2-acetamide-2-deoxy- β -D-glucopyranose and 2-amino-2-deoxy- β -D-glucopyranose, the distribution of the units depending on the chitin source and the processing method to derive the biopolymer (Desai, Kit, Li, & Zivanovic, 2008). Presently chitosan is widely used in different areas of bioengineering, medicine, pharmacology, and industry, primarily, due to unique properties of this polysaccharide, availability, and renewability of its crude material.

Due to hydrogen bonding between the functional groups of chitosan macromolecules, the polymer is poorly soluble in water, because this bonding is stronger than between the molecules of chitosan and water. Never the less high content of polar groups provides high hygroscopic properties of chitosan (Rosa, Moraes, & Pinto, 2010).

Chitosan is soluble in dilute solutions of organic and some inorganic acids (HCl). In medium pH < 6 protonation of its amino groups occurs and their positive charge causes electrostatic repulsion between the polymer's chains. It can strongly hold a solvent in its structure, as well as suspended particles. The diversity of functional groups and the specifics of chitosan structure determine the number of properties of this polymer, like adsorption, chelating and complexing properties (Chauhan, 2015).

Such properties as high biological activity, biocompatibility,

biodegradability, and mucoadhesiveness make chitosan an attractive object for the application in different areas.

There are a number of studies going on the possible application of chitosan as a polymer matrix for drugs delivery systems, because the majority of substances are characterized by low permeability through biological membranes (Cánepa et al., 2017; Huang, Liu, & Chen, 2017; Quiñones, Peniche, & Peniche, 2018; Wang et al., 2017). Chitosanbased biological systems can be used for transport of proteins/peptides, growth factors, anti-inflammatory drugs, antibiotics, antitumor, and other drugs to the diseased cells. Chitosan is used as a filler for pills and capsules that provide the controlled release of the active substance due to the combination of diffusion and slow decomposition of the macromolecule polymer matrix (Chavana, Bala, Pal, & Kale, 2017; Lin & Yeh, 2010). Chitosan is believed to be a highly potential component in tissue engineering (skin, bone, cartilage, nerve, liver tissues) due to its biocompatibility. It provides mechanical strength, steadily degrades facilitating the growth of new tissue, and acts as a cellular and molecular scaffold for the migration and cultivation of the required cells (Lian et al., 2009; Ilbasmis-Tamer, Çiftçi, Tu, Degim, & Tamer, 2017; Ribeiro, Vieira, Melo, Araújo, & Lima, 2017). There are studies on the application of polysaccharides for the production of new biodegradable suture material (Cruz, Diniz, Lisboa, & Fook, 2016; Li & Tang, 2016). Chitosan is used as a substance that contributes to weight loss and improves cholesterol metabolism and intestinal motility (Čopíková et al., 2015).

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Polymer and its derivatives are widely used in cosmetics as a moisturizer, gelling, film-forming and anti-inflammatory agent (Aranaz et al., 2018). During the past decade, the interest in the use of chitosan in the composition of hemostatic agents, like «Celox[™]» and «HemCon ChitoGauze PRO», increased. Chitosan-based hydrogels with different structures are synthesized via the formation of ions complexes with negatively charged molecules (sulfates, citrates, phosphates) and metal ions Pt²⁺, Pd²⁺, and Mo⁶⁺, polyelectrolyte complexes (with proteins, heparin, chondroitin sulfate, carboxymethylcellulose, etc.) or through the formation of polymer bonds between chitosan and other water-soluble nonionic polymers (polyvinyl alcohol, etc.) (Chauhan, 2015; Liu et al., 2018; Wu, Li, & Lee, 2017). The obtained hydrogels can be used as the basis for the development of wound healing bandages due to its compatibility with living tissues, viscoelastic nature.

In the process of studying chitosan and the prospects its application various methods for its modification were developed which was reflected in reviews and monographs (Argüelles-Monal, Lizardi-Mendoza, Fernández-Quiroz, Recillas-Mota, & Montiel-Herrera, 2018; Harish Prashanth & Tharanathan, 2003; Jayakumar, Prabaharan, Reis, & Mano, 2005; Mochalova & Smirnova, 2018; Ravi Kumar, 2000; Yu, Kecen, & Xiaosai, 2018). In these reviews, the authors examined in detail the chemical modification of chitosan by polymer-analogous reactions, graft and block copolymerization with vinyl monomers in liquid media and solid phase. A lot of attention in the reviews is given to factors that affect the efficiency of copolymerization processes such as the nature of the monomer and initiator, pH and temperature, molecular weight of polysaccharide and its degree of deacetylation.

Among the various methods of chitosan modification, the graft radical polymerization method opens up the possibility of producing hybrid copolymers that combine the useful properties of a synthetic polymer (high physical-mechanical characteristics) and polysaccharide. Graft copolymers are prepared by a) a "grafting through", b) a "grafting from", c) "grafting onto" polymerization process. "Grafting from" mechanism begins by creating free radical sites on the trunk polymer chain whereby vinyl monomer can react with the radical to propagate into a new polymer chain that is covalently bonded to the trunk polymer. This type of copolymerization offers the possibility of creating novel polymer systems that permanently combine the properties of both polymer chains (Jenkins & Hudson, 2001).

Particular interest is the preparation of chitosan derivatives for biomedical applications. From this point of view, graft copolymers of chitosan with 2-hydroxyethyl methacrylate (HEMA) and N-vinylpyrrolidone (VP) are interesting.

HEMA belongs to vinyl monomers containing mobile hydrogen atoms. Based on it, water-soluble, water-swellable materials and hydrogels are obtained (Faccial, Pardini, & Amalvy, 2015; Ma & Lee, 2009). HEMA polymers are biocompatible due to their hydrophilic properties and low toxicity. They are applied to obtain embolizing materials that are used in the treatment of certain neoplasms (ischemia of tumors of the liver, kidney, myomatous nodes, etc.). The high porosity of materials based on poly-HEMA positively affects the compatibility of the material with body tissues and promotes the growth of connective fibrous tissue in the pores of the hydrogel (Horak, Gumargalieva, & Zaikov, 2003). HEMA cross-linked polymers are also used in the preparation of materials for the manufacture of prostheses of various organs, in ophthalmology as part of contact lenses and in the development of anti-burn dressings as a carrier for drug delivery (Dong, 2008; Singh, Sharma, Sharma, & Dhiman, 2017).

The authors describe methods for producing graft chitosan-HEMA copolymers in an aqueous solution using ammonium persulfate (Mun et al., 2008), potassium thiocarbonate bromate ([El-Tahlawy & Hudson, 2001), cerium (IV) ions (Joshi & Kumar Sinha, 2006), dinitrilazoisobutyric acid, UV- and γ -radiation (Casimiroa, Botelho, Leal, & Gil, 2005) as initiators. It should be noted that a significant disadvantage of using peroxide compounds and redox systems based on them is the destruction of the main chain of the polysaccharide

(Zohuriaan-Mehr, 2005).

Therefore, it is important to use initiators that provide high efficiency of the graft polymerization of HEMA to chitosan without breaking the polysaccharide chain. It is known that Ce⁴⁺ compounds are used for this purpose (Jenkins & Hudson, 2001). Initiator compounds containing Ce⁴⁺ ions are known in the literature (Pourjavadi, Mahdavinia, Zohuriaan-Mehr, & Omidian, 2003; Yilmaz, Adali, & Yilmaz, 2007; Metzler, Chylińska, & Kaczmarek, 2015; Doba, Rodehed, & Ranby, 1984). However, it should be noted that the researchers describe the initiation process in different ways. Yilmaz et al. (2007) proposed the mechanism of grafting via oxidation and chitosan chain breakdown. The authors (Metzler et al., 2015) do not exclude the involvement of chitosan amino groups, as well as hydroxyl groups, in the process of vinyl monomers polymerization onto chitosan. Celli, Sabaa, Jyothi, and Kalia (2015) are discussed the mechanism of initiation by complex formation between the Ce(IV) ion with the - NH₂ and - OH groups at C-2 and C-3 positions of chitosan, respectively. The dissociation of the formed complex produced the macroradical which is responsible for the initiation of the graft polymerization in the presence of the vinyl or acrylate monomers. The disadvantage of using Ce-ions as initiators is the low yield of the target product, which does not exceed 71 % as shown in the article (Metzler et al., 2015).

VP is also of great interest in the modification of chitosan since polymers based on it have a unique combination of physicochemical properties (biocompatibility, non-toxicity, chemical stability, good solubility in water and many organic solvents) (Teodorescu & Bercea, 2015). Due to its structure, high polarity and the ability to accept protons, poly-VP is cross-linkable and it forms chemical complexes. Poly-VP is physiologically inert and hemocompatible. The authors (Yazdani-Pedram & Retuert, 1997) investigated the effect of chitosan concentration, monomer and initiator, solvent volume, reaction time and temperature on the degree of grafting. Modification of chitosan by grafting of VP was carried out inhomogeneous phase using potassium persulfate as redox initiator. Values for grafting percentages up to 290 % were reached.

An analysis of numerous studies on chitosan modification revealed the ambiguity of the synthesis results - the efficiency of the process is often very low. From our point of view, this may be due to the influence of the conformational state of chitosan macromolecules in solution on the synthesis process and properties of copolymers. This question in scientific literature remains open.

Chitosan macromolecules have in solution a semi-flexible rod conformation or a rigid coil, depending on several parameters: protonation degree, value and molecular weight distribution, N-acetylglucosamine groups content, pH value. Chitosan macromolecules may form various shape configurations in solutions specified with parameter α in the Mark-Houwink-Kuh-Sakurada equation. It is known that parameter $\boldsymbol{\alpha}$ depends on the macromolecules conformation of polyelectrolytes in solution and varies from zero for very globular structures to 2 for rodshaped ones. In a solution of a strong polyelectrolyte with a sufficiently high ionic strength of the solution $\alpha = 0.5$, i.e., the chain has the coil conformation. For a weak polyelectrolyte, as well as for polypeptides in the α -helix conformation 1,5 < α < 2 (Harding & Tombs, 1997; Masuelli, 2014). The shapes of chitosan macromolecules assume the form of stiff, impenetrable spheres, flexible balls enabling the flow of solvent and disentangled, stiff rods (Franca, Lins, Freitas, & Straatsma, 2008; Morris, Castile, Smith, Adams, & Harding, 2009; Slivkin et al., 2014; Christensen, Vold, & Varum, 2008; Costa, Teixeira, Delpech, Souza, & Costa, 2015; Desbrieres, Brugnerotto, Heux, & Rinaudo, 2001; Dimonie, Dima, & Petrache, 2013; Fedoseeva, Smirnova, & Fedoseev, 2008; Kasaai, 2007; Chen & Tsaih, 1998).

The authors consider the possibility of existence the forms of chitosan macromolecules depending on degree protonation its aminogroups: rigid rod-type structure (Chen & Tsaih, 1998; Kasaai, 2007;) or a semi-flexible coil (Dimonie et al., 2013; Velásquez, Albornoz, & Barrios, 2008). In case of pH by 5.57, the chitosan macromolecules are

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rigid because the amino groups which were weakly protonated form intermolecular bonds with the oxygen of adjacent chains generating certain structures with lower stability (Ravi Kumar, 2000). In a solution with pH near 4, the polymer macromolecules are more flexible and can take the coil conformation (Dimonie et al., 2013).

The authors (Morris et al., 2009) describe the conformation of chitosan macromolecules in the solution as a semi-flexible rod (or stiff coil) based on global analysis: multi_HYDFIT and use other method for conformation analysis, including Mark-Houwink-Kuhn-Sakurada power law relation linking intrinsic viscosities [n] and molecular weight Mw, translational frictional ratio, Wales-van Holde ratio and sedimentation conformation zoning.

The authors (Fedoseeva et al., 2008) suggest that in moderately concentrated solutions ($\nu_{acetic} acid/\nu_{chitosan} \leq 5$) chitosan macromolecules have a rigid-rod type conformation. They based their conclusions by determining the parameter α in the Mark – Houwink equation by measuring the intrinsic viscosity [η] of chitosan solutions. It is known that the parameter α of the Mark - Kuhn - Hauwink equation depends on the conformation of the polyelectrolyte macromolecules in solution and varies from 0 for globular structures to 2 for rod-shaped structures. (Harding & Tombs, 1997; Masuelli, 2014). Based on the measurement of intrinsic viscosity author (Fedoseeva et al., 2008) determined the coil-rigid rod conformational transition of chitosan macromolecules is in the pH range 3.2–4.3.

It should be noted that the conformation of chitosan macromolecules can significantly affect the process of modification of polysaccharides, as well as the properties of the final products. For example, we previously found that the polymerization of styrene in chitosan solutions revealed a significant effect of the pH of the reaction medium on the grafting parameters: as the pH of the reaction mixture changed from 4.2–5.5, the degree of polymerization increased from 13.4 wt. % to 27 wt. % (Mochalova et al., 2015). The molecular weight of the graft polystyrene chains on chitosan increases ~ 4 times as the pH of the reaction mixture decreases from 5.5 to 3.9.

A change in the conformation of polymer macromolecules is manifested in a change in the packing density of the chains and their sizes and, accordingly, in the intensity of the scattered light (Tanford, 1961). Based on this, we considered the possibility of using the spectrophotometric method to determine the conformational state of polymer macromolecules.

The aim of the present investigation was to establish the chitosan macromolecules conformational transitions using spectrophotometric method and their influence on process graft polymerization HEMA and VP on chitosan and the final product properties.

2. Experimental

2.1. Materials and reagents

The following substances were used in the study.

Chitosan obtained from crab shells (OJSC "Bioprogress", Moscow, Russia) with different MW - 1.10×10^5 , DD = 0.82 and 2.20×10^5 , DD = 0.82. Mass fraction of minerals in chitosan did not exceed 0.1 %, moisture content - 6 %, insoluble compounds - 0.1 %.

Chitosan solution was prepared with acetic acid of "chemical purity" GOST 61–75 (99.5 %, density 1.049 g/cm^3), hydrochloric acid (HCl) of "chemical purity" (36.8 wt% at 20 °C, density– 1.19 g/cm^3).

Modification of chitosan was performed by graft polymerization with HEMA (content of the main component 99.9 %, produced by «Sigma-Aldrich»; before the synthesis, the monomer underwent vacuum distillation with full condensation in the headspace at lower pressure) and VP (content of the main component 99.9 %, produced by «Sigma-Aldrich»).

Soluble in water tetrahydrate cerium sulfate (IV) was used as an initiating system for graft copolymerization.

Homopolymer extraction was performed in a Soxhlet apparatus by

2.2. Dynamic viscosity

Dynamic viscosity of chitosan solutions was measured by rotational viscometer Brookfield DV-II + Pro with spindle shafts № 18 and № 31. The precision of viscosity measurement was \pm 1.0 % of the upper range limit.

We studied the dependence of chitosan solution dynamic viscosity on the MW of polymer, concentration, temperature, and medium pH. Aqueous acetic acid solutions of chitosan with different concentrations of the polymer (from 1 to 4 wt %) were prepared in the media with pH ranging from 3.3–5.0. Before the measurement, the solutions were kept for 48 h. The temperature of the solutions increased from 0 °C to 50 °C.

The apparent activation energy of the solution viscous flow (Δ Ea, kJ/mol) was calculated by the equation of Arrhenius-Frencel-Eyring:

$$\eta = A \cdot exp(\frac{\Delta E_a}{RT})$$

Where *A* – constant, *R* – universal gas constant (*J*/mol×K), *T*– temperature (K), $\eta = \eta_{max}$.

2.3. Spectrophotometric method

Conformational transitions of chitosan were established by the spectrophotometric method. For the analysis, 0.03 wt % aqueous solution of chitosan in hydrochloric acid with MW of the polymer 2.2×10^5 and 1.0×10^5 were prepared. The reading of pH-meter MettlerToledo LE902 ranging from 3.3–6.0 were recorded and the transmittance spectra of the solutions were registered by the UV-vis spectrophotometer UV-1650 (Shimadzu) during the introduction of some amount of 2.5 % NaOH solution.

The change in optical transmission of the chitosan solutions (3 wt %) in a wide range of temperatures - 20-85 °C was studied. Precise systematic decrease of optical transmission in the range from 60 to 75 °C was observed.

2.4. Dynamic light scattering (DLS)

Dimensional features and polydispersity of chitosan particles in over-diluted chloride solutions were established by the method of DLS with the particle size and zeta potential analyzer Nano Brook Omni (Brookhaven Instruments Corporation, USA). Particles diffusion coefficient of the dispersed phase in the liquid was identified by the analysis of the correlation function of the diffused light intensity fluctuations. The most important parameter, established during the investigation of quasielastic light scattering, was the diffusion coefficient (D). The size of the spherical noninteracting particles was calculated by the Stokes-Einstein equation:

$$D = \frac{k_B T}{6_{\pi n R}}$$

Where k_B – Boltzmann's constant; T – absolute temperature; η – shear viscosity of the medium with the suspended particles with radius R.

2.5. Synthesis of graft copolymers

Graft copolymerization of HEMA (VP) onto chitosan was performed at constant stirring in a three-neck round-bottomed flask equipped with a backflow condenser and placed in a temperature-controlled thermostat. The concentration of chitosan in acetic acid solutions in all the tests was equal to 3 wt%, medium pH ~4.8 and 3.3, reaction temperature 60 °C. HEMA (VP) was introduced into the system after the temperature establishment in the reactor. The ratio [monomer]/[glucosamine] was equal to1.2, 2.4, 4.8 mol/(molar ratio), respectively. The initiator was introduced into the medium. The system was kept for 15 min for the establishment of the required temperature of the solution. Further, the weighted sample of the initiator was added. In all the cases, the concentration of the initiator was equal to $5 \cdot 10^{-3}$ mol/L. The synthesis lasted 2 h. After that, the temperature in the thermostat was raised up to 80 °C, and the system was kept for 30 min until the completion of HEMA (VP) polymerization process. During the synthesis, the probes were taken at certain time intervals, precipitated with isopropyl alcohol and centrifuged for the separation of the precipitation. The level of HEMA (VP) conversion was established by the analysis of the residual monomer by the method of gas chromatography. The concentration of the residual monomer was determined by the gas chromatograph GCMS-OP2010. Shimadzu equipped with a thermal conductivity detector. The column: Equity-1 (length - 30 m, diameter -0.25 mm, film thickness – 0.25μ m), carrier gas helium. The carrier gas flow was 1 ml/min, the temperature of the column for HEMA (VP) identification was 230 °C.

After the extraction, the efficiency (relation of the mass of the graft copolymer to the mass of the total polymerized monomer) and the degree of grafting (the relation of the mass of the graft polymer to the mass of chitosan) of HEMA (VP) onto chitosan were established.

The degree of grafting (DG) was calculated by the equation:

$$DG = \frac{m(graft \ polymer) - m \ (chitosan)}{m \ (chitosan)} \cdot 100 \%$$

The efficiency of the grafting (EG) can be calculated by the equation:

$$EG = \frac{m(graft \ polymer) - m(chitosan)}{m(graft \ polymer) - m(chitosan) + m(homopolymer)} \cdot 100\%$$

where m(graft polymer) – the mass of the copolymer in the sample, m (chitosan) – the mass of chitosan in the probe, m(homopolymer) – the mass of homopolymer extracted from the sample.

2.6. Gel-permeation chromatography (GPC)

MW of the copolymers was established by the method of gel permeation chromatography (GPC) with high-performance liquid chromatograph CTO20A/20AC (Shimadzu, Japan), software module LC-Solutions-GPC, column Tosoh Bioscience TSKgel G3000SWxl with the pore diameter 5 µm, and evaporative light-scattering detector ELSD-LT II. 0.5 M acetic acid solution was used as an eluent. The flow rate was 0.8 ml/min. MW was calculated based on narrow disperse samples of dextran in the range of MW from 1.0×10^3 to 4.1×10^5 Da (Fluca).

2.7. Fourier transform infrared spectroscopy (FTIR)

The synthesis of graft copolymers HEMA (VP) with chitosan was established using the FTIR spectrometer (spectrophotometer "Perkin-Elmer") at the frequency range of $500-4000 \text{ cm}^{-1}$ with resolution 2 cm^{-1} and 100 times scanning. The extraction homopolymer poly-HEMA (poly-VP) from the reaction products was performed by isopropyl alcohol for 36 h in the Soxhlet apparatus. The time was established by the blank test of poly-HEMA (poly-VP) extraction from the precipitated physical mixture of the respective homopolymers. Further, the samples were dried out by vacuuming to the fixed mass. Dry samples of copolymers and chitosan were ground with KBr at a sample/KBr ratio of 1:20. The sample powder and KBr were blended and triturated by agate mortar. The mixture was compacted using an IR-hydraulic press at a pressure of 400 kg/cm².

2.8. Physical-mechanical properties

Chitosan-based and chitosan copolymer-based films were obtained by casting their solutional lavsan substrate in the conditions of even evaporation of the solvent to the fixed-mass at room temperature. Further, the films were exposed to vacuum for 4-6 h at t = 30 °Cin the vacuum chamber. The process of acid neutralization in films was conducted by 0.5 wt % sodium hydroxide solutions and then they were washed with distilled water to neutral pH. Physical-mechanical properties of the film (break strength and tension strain) were established with a tensile testing machine ZWIC Z005 (Germany) at the rate of extension 50 mm/min.

3. Results and discussion

The universal technological step in the synthesis of chitosan-based and chitosan derivatives-based materials is the dilution of polysaccharides in aqueous acid solutions. The most available and widespread component in the preparation of chitosan solutions is acetic acid.

As mentioned above in the introduction it is known that the conformations of chitosan macromolecules were different depending on the pH of the medium: a coil — up to pH ~ 3.6 and a rigid rod — up to pH ~ 4-5.5. The research of the reactions of chitosan copolymerization with HEMA and VP in the solution were performed in different macromolecular conformations of chitosan – rigid rod and coil. Since graft copolymerization involved different MW of chitosan, it was relevant to develop the method of efficient identification of conformations of chitosan macromolecules.

3.1. Investigation macromolecular conformations of chitosan in solution

A complex of studies was performed for the interpretation of the peculiarities of chitosan solutions:

- Evaluation of the dependence of dynamic viscosity of the chitosan solutions on the pH of the medium;
- Identification of chitosan macromolecules effective sizes by the method of DLS;
- The investigation of the temperature-dependent viscosity variation of chitosan solutions with its macromolecules in different conformations and identification of energy of activation of polymer solution viscous flow;
- Evaluation of optical properties of the chitosan solutions.

3.1.1. Investigation dynamic viscosity of chitosan solutions

Chitosan samples with MW = 1.1×10^5 , DD 0.82 (Fig. 1, curve 1) and MW = 2.2×10^5 , DD 0.82 (Fig. 1, curve 2) were used for the evaluation of medium pH influence on the viscosity property of moderately concentrated solutions of the polysaccharide.

In Fig. 1 the curves are similar. The observed dependence of the chitosan solution viscosity at the medium pH was not linear, the curve



Fig. 1. Dependence of the dynamic viscosity of 3 wt % of chitosan solution on pH, $1 - MW = 1.1 \times 10^5$, DD = 0.82. $2 - MW = 2.20 \times 10^5$, DD = 0.82. T = 25 °C.

was divided into three areas: pH < 3.4 – the curves were steadily going upwards, pH = 3.8 – the curves plateaued, and then went on going steadily upwards. It can be suggested that such a curve structure is associated with the conformational condition of chitosan macromolecules: rigid rod – at pH > 4, coil – at pH = 3.3 – 3.5. Cooperative destruction of hydrogen bonds inside a molecule at some "critical" degree of protonation results in conformational transition coil-rigid rod (Dimonie et al., 2013; Fedoseeva et al., 2008). Thus, conformational condition of a macromolecule significantly influences the viscosity of polymer solutions.

3.1.2. The effective size of chitosan macromolecules determined by DLS

The conformational transition provides the polymer macromolecule size change, which was confirmed by the method of DLS. The first experiment was performed in over diluted solutions of chitosan with the polysaccharide concentration - 0.03 wt%. Chitosan macromolecule in rigid rod conformation was characterized larger effective diameter than in coil conformation regardless of MW. Thus, macromolecules of chitosan with MW = 1.1×10^5 in 4.8 medium pH (rigid rod conformation) have the size ~ 624 nm, and in 3.3 medium pH (coil conformation) they have the size ~ 537 nm. It should be noticed that the increase in WM of a polymer leads to a significant increase in the difference in size. Thus, macromolecules of chitosan with MW = 2.2×10^5 in 4.8 medium pH (rigid rod conformation) have the size ~ 868.9 nm, but at the same time in 3.3 medium pH (coil conformation) they have the size ~ 546.5 nm.

However, since these effective diameters of chitosan macromolecules were of the order of hundreds of nm apparently their associates were registered and macromolecules in the rigid rod conformation had a greater tendency to form associates. This was confirmed by repeated research of more dilute chitosan solutions (MW = 1.1×10^5).

The effective diameters of chitosan macromolecules in the conformation of the rigid rod were 34–39 nm, while in the conformation of the coil - 22–25 nm. In this way, the effective size of chitosan macromolecules determined by DLS for the rigid rod conformation was larger than for the coil conformation. In this particular case, the authors believe it is correct to fix the sizes that are determined by the diffusion rotation of the extended polysaccharide chains, whereas, in the coil conformation, the diffuse rotation of a macromolecule reflects its actual size that is determined by its radius.

3.1.3. The investigation of the temperature-dependent viscosity variation of chitosan solutions

Different conformational conditions of the polysaccharide macromolecules in various pH media were also observed in the studies of the temperature-dependent viscosity variation of moderately concentrated chitosan solutions (Fig. 2).

Within the range of temperatures from 0 to 30 °C, the viscosity of solutions of chitosan in the rigid rod conformation exceeds the viscosity of solutions of the polysaccharide in the coil conformation at the same concentration of the polysaccharide (3 wt%) and MW = 1.1×10^5 (Fig. 2). At T = 10 °C, the viscosity of the solutions is two times different. This can be explained by strong inter-chain interaction between the extended macromolecules in rigid rod conformation, and as a result, by the increased resistance of the flow. As the temperature rises, the viscosity of chitosan solutions in both systems decreases. We followed this trend for chitosan concentrations from 0.5 wt.% to 3.5 wt.%. At low chitosan concentrations, the viscosity at different pH values differs less critically; with increasing concentration, the difference becomes noticeably more significant.

Based on the temperature dependence data, the apparent activation energy of the chitosan solutions viscous flow for different conformational models of the macromolecules (ΔE_1 - rigid rod, ΔE_2 - coil) was calculated by the Arrhenius-Frenkel-Eyring equation using the semilog coordinates of the dependence of the viscosity on the temperature.



Fig. 2. Dependence of 3 wt % of chitosan solution dynamic viscosity on the temperature. MW(chitosan) = 1.1×10^5 : pH = 4.8-molecular rigid rod conformation; pH = 3.3- molecular coil conformation.

The apparent activation energy (ΔE_a) of viscous flow of the liquid is equal to the height of some potential of the energetic barrier that has to be exceeded by one mol of particles for a successive transition from one position of equilibrium to the other. ΔE_a parameter measures the intensity of intermolecular interaction in solutions, i.e. it is an indirect characteristic of polymer systems structure stability in solutions. The observed difference in the activation energy of the viscous flow of the chitosan solutions was the following:

 $\Delta E_1 = (36.5 \pm 0.4) \text{ kJ/mol- pH} = 4.8$

 $\Delta E_2 = (31.1 \pm 0.3) \text{ kJ/mol} - \text{pH} = 3.3.$

The higher value of ΔE_a shows that inter-chain interaction between chitosan molecules, that involve hydrogen bonds, is stronger in the rigid rod conformation. This tendency agrees with the published data on other natural polysaccharides and their derivatives, in particular for cellulose solutions (48 kJ/mol (N-methyl morpholine N-oxide / water solvent) and 22,6 kJ/mol (NaOH / water solvent) (Egal, 2006) and starch (3 wt%, 44.7 kJ/mol) (Gulnov, Nemtseva, & Kratasyuk, 2016). Near ~ 50 °C, the disruption of macromolecule associates in the rigid rod conformation begins, and the viscosity values in both systems are getting closer. This fact can be explained not only by the disruption of the associates but also by the disruption of hydrogen bonds between the links of polymer chains, which leads to the conformational coil-rigid rod transition. It should be mentioned that this effect can also be determined by the disruption of hydrogen bonds. To prove this suggestion, the authors performed a spectrophotometric analysis of chitosan solutions.

3.1.4. Spectrophotometric analysis of chitosan solution

Spectrophotometric analysis is known to be used for the evaluation of the dynamics of protein molecules in a solution. In the present study, this method was used for the identification of chitosan macromolecules conformation. During the investigation of the solution viscosity (Fig. 2), it was suggested that its values were getting closer due to cooperative disruption of hydrogen bonds inside a macromolecule and, thus, by the conformational coil-rigid rod transition. For this reason, the change in optical transmission of the solutions in a wide range of temperatures was studied. Precise systematic decrease of the optical transmission of the solution decreased and then increased up to the solvent values in the range from 60-75 °C. It can be assumed that in this temperature range, under conditions of the restructuring of the structure of macromolecules due to the violation of hydrogen bonds (the chaotic state of the system) the light transmission of the solution decreases. And as the isotropic structure of the coils, conformational macromolecules are formed, the optical density is restored. Thus, a conformational transition can occur in the temperature range 60–75 °C.

The changes in optical density of the diluted chitosan solutions in a



Fig. 3. Changes in optical transmission of 0.03 wt% chitosan solution depending on the pH of the medium. $1 - MW = 1.1 \times 10^5$, DD = 0.82. $2 - MW = 2.20 \times 10^5$, DD = 0.82. T = 25 °C.

wide range of MW dependent from the medium pH were mesured at T = const. In all cases, the authors observed a sudden change in the optical transmission coefficient at medium pH 3.4. The character of the curves was similar for all the chitosan samples with the same DD. Fig. 3 illustrates the typical picture of optical density change in all the samples with MW 2.2 \times 10⁵ and 1.1 \times 10⁵ (DD = 0.82) depending on the pH of the medium.

The two sharp lows were observed within the curves (Fig. 3) at pH values \sim 3.4 and 4.8-6. The results are reproduced in multiple experimentes. The first minimum at pH = 3.4 completely coincides with both MW of chitosan and lies within the same area of pH values that were registered by the authors: Fedoseeva, Smirnova,& Fedoseev (2008) based on the viscometric data. Unlike the mentioned study, the results of the present spectrophotometric analysis showed that chitosan macromolecule conformational transition rigid rod-coil was fixed very precisely. For the first time, it was shown that at similar DD, that transition did not depend on the MW. After the minimum, the curve did not reach the baseline optical transmission ratio, which is, probably, associated with a more intensive light scattering by the macromolecules in rigid rod conformation. The second lows on the curves were different for the samples with various MW: the bigger the MW of chitosan was, the earlier the change in optical transmission was observed. The second minimum was characterized by the chitosan macromolecule conformational rigid rod-globule transition. This is explained by the fact that at a slight increase in the medium pH above the minimum point of the curve, precipitation is observed, the solution clears and the ratio of optical transmission becomes close to the one of the solvent. Conformational rigid rod-globule transition at the pH value of polymer precipitation was different for chitosan with various MW, as well as the threshold value of the medium pH that corresponds to the precipitation of the polymer. For chitosan with MW = 1.0×10^5 , this transition was observed at pH = 5.7, while the macromolecules with twofold higher MW collapsed already at pH = 5.0. The test results explain the anomaly revealed during the study of chitosan solution viscosity. In the range of pH \sim 5.0-5.2 the heavier their MW is the lower the viscosity of chitosan solutions at similar DD. In the work (Fedoseeva et al., 2008) there is a certain pH range of the coil-rigid rod conformational transition determined by the viscometric method. Whereas in our study, in the same experiment, two conformational transitions of chitosan macromolecules can be observed simultaneously: a coil-rigid rod and a rigid rod-globule, which precedes precipitation.

The obtained results indicate the prospects of application of spectrophotometric analysis for the precise identification of conformational transition of chitosan macromolecules with the changing of the medium pH. It was shown that the interaction of chitosan macromolecules by means of hydrogen bonds in the rigid rod conformation was stronger than in the coil conformation. The fact that macromolecules of chitosan at the medium pH = 4.8 (rigid rod conformation) are bigger in size than at the medium pH = 3.3 (coil conformation) allows the preparation of solutions with reproducible viscous properties in different conditions.

3.2. Graft modification of chitosan using 2-hydroxyethyl methacrylate and N-vinylpyrrolidone

Despite numerous studies on the preparation of chitosan derivatives and the successful implementation of the results, it remains an open question of the influence of the conformational state of chitosan macromolecules on the synthesis process and properties of hybrid products.

The modification of chitosan was performed by graft polymerization with HEMA and VP. Due to hydrophilic properties and low toxicity, the polymers HEMA and VP have good biocompatibility and are used in medicine (Dong, 2008; Teodorescu & Bercea, 2015). The modification of chitosan via copolymerization can result in the synthesis of watersoluble (expanding) hybrid products or gels.

The influence of components ratio and conformational state of chitosan macromolecules (pH of the medium) on the process of graft polymerization was studied. Cerium sulfate (IV) was used as an initiating agent during graft polymerization. The concentrations of initiator in all the reaction mixtures were 5×10^{-3} mol/L.

3.2.1. Graft polymerization of HEMA on chitosan

The degree of monomer HEMA transformation during its graft polymerization onto chitosan, when the macromolecules of the polysaccharide have rigid rod conformation (pH 4.8) is evaluated below. The most complete reaction (Fig. 4, **a**) was observed at the ratio of [HEMA]/[chitosan] = 4.8 mol/(molar ratio): the degree of monomer transformation was ~81 %, while at other ratios, the degree of transformation was less than 50 %.

A significant influence of chitosan macromolecules' conformation on the characteristics of the process was observed. The degree of HEMA transformation in rigid rod conformation was 81 % (Fig.4 **a**). It significantly increased to 96 % during the process that involved chitosan in coil conformation (Fig.4, **b**) in analogue conditions of synthesis. Besides, in this case, the change in the phase state of the reaction media was observed. During all the process when chitosan macromolecules



Fig. 4. Time-dependent changes in HEMA transformation during graft polymerization on to chitosan with $Ce(SO_4)_2$ as an initiator. T = 60 °C. **a** – pH 4.8; [HEMA]/ [chitosan] = 4.8 (1); 2.4 (2); 1.2 (3) (mol/(molar ratio). **b** – pH 3.3; [HEMA]/[chitosan] = 4.8 mol/(molar ratio).



Fig. 5. Chitosan-HEMA copolymer solutions, [HEMA]/[glucosa-mine] = 4.8 mol/(basic-mol), initiator-Ce(SO₄)₂. 1 - pH = 4.8; 2 - pH = 3,3.

were in rigid rod conformation, the system had the properties of a homogenous solution (Fig. 5, 1). On the contrary, when chitosan macromolecules were used in coil conformation, at the initial stages of the process, the system had the properties of a colloid solution (Fig. 5, 2). Probably, intra and intermolecular interactions within a rigid rod conformation limit the availability of the reactive centres of chitosan, which leads to the synthesis of a significant amount of the homopolymer. We suppose, that this result may be due to the following factors: the effect of acid concentration (PH = 3), provoking the release of the copolymer (deterioration of its solubility) or the conformational influence of chitosan with the following formation of a copolymer with high MW. The first assumption was not confirmed since acetic acid was added to the solution obtained in the first case at a concentration corresponding to the second synthesis. The copolymer does not precipitate when the concentration of acetic acid in the solution increases.

At the initial stages of the process, intramolecular and intermolecular interactions due to hydrogen bonds along the entire length of chitosan macromolecules in the rigid rod conformation (pH = 4.8) limit the availability of the reaction centers of the polysaccharide, which in this case (homogeneous solutions) leads to as will be shown below the formation of a large number of homopolymer. In contrast, in the case of a pH of \sim 3.3, the intra- and intermolecular interactions of chitosan macromolecules having the coil conformation are significantly lower. This has been proven by calculating the apparent activation energies of the viscous flow of chitosan solutions in various conformational states. Therefore, in more acidic solutions, the availability of the reaction centres of the polymer leads to the formation of a graft copolymer with a large MW. According to the results of GPC, the MW of the graft copolymer in a colloidal solution is 4.0–6.0 \times 10⁵ against 1.5–2.5 \times 10⁵ for homogeneous solution depending on the synthesis conditions. So, it can be suggested that the difference in the phase state of the synthesized products is explained by their limited solubility due to higher MW of the graft copolymer chains and, as a result, their higher concentration and strong interaction.

Graft polymerization is characterized by the degree and efficiency of grafting (DG and EG). The highest polymer outcome was observed when chitosan macromolecules in coil conformation were used. In this synthesis, EG was equal to 98% and DG – to 390% (Table 1).

Graft copolymer synthesis was confirmed by the results of FTIR spectroscopy. The spectrum of the original chitosan molecule (Fig. 6, 1) contained the peaks that corresponded to the valence vibration of amid

Table 1DG and EG in graft polymerization of HEMA onto chitosan, [chitosan] = 3 wt%,[HEMA]/[chitosan] = 4.8 mol/(molar ratio). Initiator – Ce^{4+} .

Conformation of macromolecules	q, %	DG, wt%	EG, wt%
Rigid rod (pH 4.8)	81	300	91
Coil (pH3.3)	96	390	98



Fig. 6. FTIR spectra of chitosan (1) and chitosan-HEMA copolymer (2).

functional groups at 1652 cm^{-1} , 1563 cm^{-1} . Besides the peaks typical for polysaccharides were observed at 1153 cm^{-1} , $1077 \text{ and } 1034 \text{ cm}^{-1}$ (functional group C–O–C). The peak observed at 1379 cm^{-1} belongs to the vibrations of the acetamide group. The results coincide with the values obtained by us earlier (Mochalova et al., 2015), as well as with the results of the other authors (Negrea, Caunii, Sarac, & Butnariu, 2015). The cleaned from the homopolymer synthesized product shows the typical for HEMA and chitosan peaks. A peak appears in the spectrum (Fig. 6, 2) at 1736 cm^{-1} , which corresponds to the vibrations of HEMA carbonyl (–C = O) groups (Islas, Burillo, & Ortega, 2018).

Chitosan modification was also obtained by the method of graft polymerization with VP. As a result, it was established that to provide water-soluble properties of the copolymer (pH = 7), it was sufficient to have the concentration of the components that did not exceed the value of the molar ratio [monomer]/[glucosamine] was 1.0. This ratio provided minimum content of the synthetic fragment in the structure of the copolymer and the required characteristics, like biocompatibility, water solubility and capacity for further modification of the copolymer for the creation of chitosan derivatives based wound healing and hemostatic materials.

3.2.2. Graft polymerization of VP on chitosan

The study of the kinetics of VP graft polymerization on chitosan was performed at 60 °C and 70 °C and two medium pH (4.8 and 3.3), at a different molar ratio of the monomer to the links of glucosamine. Cerium sulfate (IV) was used as an initiating system. The degree of the VP conversion in the process of polymerization is presented in Fig. 7.

The difference in the kinetics of graft polymerization at 60 °C, when the macromolecules of chitosan were in different conformational states, were more evident in the case of VP polymerization than HEMA polymerization. The degree of monomer conversion (VP) in different of the polysaccharide conformations differed by more than 40 %. The graft polymerization of VP on to chitosan, performed at 70 °C, was characterized by close values of the ultimate depth in monomer transition of both chitosan rigid rod and coil conformation. It cannot be excluded that in this case, the process is cooperative due to simultaneous rigid rod-coil conformational transition of chitosan macromolecules. The



Fig. 7. Degree of VP conversion in the process of polymerization on to chitosan with $Ce(SO_4)_2$ as an initiator. Molar ratio [VP]/[chitosan] = 1.0.



Fig. 8. FTIR spectra of chitosan (1) and chitosan-VP copolymer (2).

Table 2

The degree and efficiency of graft polymerization VP on chitosan, ω (chitosan) = 3 wt %.

Initiator, temperature	Conformation of macromolecules	[VP]/[chitosan], mol/(molar ratio)	q, %	DG, wt %	EG, wt %
Ce(SO ₄) ₂ 60 °C	Rigid rod (pH = 4.8)	1.0	53	20,5	58
Ce(SO ₄) ₂ 60 °C	Coil (pH = 3.3)	1.0	95	44	70
Ce(SO ₄) ₂ 70 °C	Rigid rod (pH = 4.8)	1.0	85	45	80
Ce(SO ₄) ₂ 70 °C	Coil (pH = 3.3)	1.0	98	61	94

process, when macromolecules of chitosan were in coil conformation, was characterized by a higher rate. The depth of VP transition at 70 °C reaches 80 % 25 min after the beginning of the process and 98 % after the completion of the process.

It is similar to the process of polymerization with HEMA and is explained by the fact that chitosan macromolecules in the coil conformation form more available reactive sites as compared to chitosan macromolecules in rigid rod conformation (pH = 4.8) that forms stronger intermolecular interaction due to hydrogen bonds.

Fig. 8 shows the FTIR spectra of chitosan and chitosan-VP graft copolymers. The peaks typical of chitosan were observed on both curves and they correspond to the stretching vibrations of the polysaccharide described earlier for Fig. 6. The graft polymer cleaned from the homopolymer displays the peaks typical for VP and chitosan. The C=O groups of VP in the graft sample (2 curves) show a prominent peak at 1663 cm-1 in FTIR spectrum which is characteristic of C=O bond in VP (i.e., amide C=O bond) and the peak observed at 1290 cm-1 belong to the stretching vibrations of C-N of VP (Koczkur, Mourdikoudis, Polavarapu, & Skrabalak, 2015). These bands are taken as evidence of grafting of VP on to the chitosan chains.

Thus, the association of the molecules could be observed during the process of polymerization. The best graft polymerization parameters VP on chitosan were obtained when macromolecules of chitosan were in the coil conformation (as in the case with HEMA polymerization): the depth of VP conversion was 98 % and EG was 94 % (Table 2).

3.2.3. Investigation of mechanical properties of copolymers

Chitosan copolymers, modified by graft copolymerization, can be used as the components of new wound-healing materials in different forms: film and fibre products, gels, sponges, membranes, etc. Hence, it is necessary to investigate their mechanical strength and deformation properties.

Chitosan (tensile strength 25 MPa, deformation 1,9 %) barely expresses any deformation properties. HEMA film, obtained with the Ce $(SO_4)_2$ in reactive media with different pH (i.e. rigid rod or coil conformation of chitosan macromolecule), is characterized by the improved physical-mechanical properties in comparison with the original polysaccharide. This is especially noticeable in the case of films

obtained from solutions with the coil conformation of chitosan macromolecules: they have more tensile strength (up to 35 MPa) and elastic - 4.5 %. The film, cast from the solutions of chitosan in the coil conformation, has better physical-mechanical properties due to a higher degree of polymerization.

Chitosan-VPgraft copolymers film is two times more elastic and three times more tensile strength as compared to the original chitosan: burst stress values reach 60 MPa at 4 % deformation.

To increase the elasticity of the films glycerin was used as a plasticizer in the concentration range of 0.5; 0.7 and 1 wt. %. After glycerin was introduced into the copolymer solution increase in the elasticity of the films was observed with an increase in glycerol concentration – 17.6 %, 25.7 %, 33.9 % respectively, but the strength was decreasing to 20 MPa. It is known that the strength of polymeric materials decreases with increasing elasticity. It suffices for practical purposes. The introduction of 0.5 wt. % glycerol in the composition of the block copolymer makes the strength to reach 30.8 MPa at the deformation of 17.6 %. Such rates will allow the use of these films, for example, as a part of wound healing and hemostatic dressings in medicine and veterinary.

In general, it can be concluded that copolymer film cast from the solutions with chitosan coil conformation is slightly more tensile and elastic as compared to the one obtained from the solutions with chitosan rigid rod conformation.

4. Conclusion

For the first time, the authors on the example of the "grafting from" polymerization of 2-hydroxyethyl methacrylate and N-vinylpyrrolidone on chitosan showed a significant influence of the conformational state of chitosan macromolecules in solution on the degree of grafting, the efficiency of grafting, the phase state of the system and the properties of the final product. These results indicate the need to take into account the conformational state of polyelectrolyte macromolecules to increase the efficiency of chitosan modification processes during graft polymerization and its possible manifestation in other reactions.

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