CONDIÇÕES CONFORMACIONAIS DE MACROMOLECULAS QUITOSANAS E SUA INFLUÊNCIA NA EFICIÊNCIA DO PROCESSO DE POLIMERIZAÇÃO DA QUITOSANA COM 2-HIDROXIETIL METACRILATO E N-VINILPIRROLIDONA

CONFORMATIONAL CONDITIONS OF CHITOSAN MACROMOLECULES AND THEIR INFLUENCE ON THE EFFICIENCY OF CHITOSAN POLYMERIZATION PROCESS WITH 2-HYDROXYETHYL METHACRYLATE AND N-VINYLPYRROLIDONE

КОНФОРМАЦИОННЫЕ СОСТОЯНИЯ МАКРОМОЛЕКУЛ ХИТОЗАНА И ИХ ВЛИЯНИЕ НА ЭФФЕКТИВНОСТЬ ПРОЦЕССА ПОЛИМЕРИЗАЦИИ ХИТОЗАНА С 2-ГИДРОКСИЭТИЛМЕТАКРИЛАТОМ И N-ВИНИЛПИРРОЛИДОНОМ

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RESUMO

A modificação da quitosana com polímeros sintéticos biocompatíveis é importante para o desenvolvimento de materiais médicos biodegradáveis com um conjunto de propriedades sob medida. A questão da influência da conformação de macromoléculas de polissacarídeos na síntese e nas propriedades dos produtos híbridos permanece aguda. As transições conformacionais de quitosana e sua influência no processo de polimerização enxertada de 2-hidroxietyl metacrilato e N-vinilpirrolidona sobre o polímero e as propriedades de seus copolímeros foram estudadas. Para interpretar as peculiaridades das propriedades das soluções de quitosana, sua viscosidade foi determinada pelo método de viscometria rotacional; as transições conformacionais dos polissacarídeos poliméricos foram investigadas pelo método da espectrofotometria; as características dimensionais das partículas de polímero foram avaliadas pelo método de espalhamento dinâmico da luz. Os copolímeros foram caracterizados pelos métodos de espectroscopia de IV e cromatografia de penetração em gel. A transição conformacional da hélice-espiral da macromolécula de quitosana foi sensível ao pH e à temperatura. O tamanho efetivo das macromoléculas de quitosana para a conformação da hélice foi 14-35% maior do que para a conformação espiral. O rendimento de copolímero enxertado (98%) foi significativamente maior nas soluções de quitosana, onde suas macromoléculas estavam na conformação da espiral. Os resultados obtidos permitem que os pesquisadores criem condições ideais durante o desenvolvimento de materiais biomédicos à base de copolímero, com características aprimoradas.

Palavras-chave: quitosana, conformação, modificação, a eficiência da polimerização.

ABSTRACT

Modification of chitosan with biocompatible synthetic polymers is important for the development of biodegradable medical materials with a tailor-made complex of properties. The issue of the influence of a polysaccharide macromolecule conformation on the synthesis and properties of the hybrid products remains acute. Conformational transitions of chitosan and their influence on the process of grafted polymerization of 2-hydroxyethyl methacrylate and N-vinylpyrrolidone onto the polymer and the properties of their copolymers were studied. To interpret the peculiarities of chitosan solutions properties, their viscosity was identified by the method of rotational viscometry; conformational transitions of the polymer polysaccharides were investigated by the method of spectrophotometry; dimensional characteristics of the polymer particles were assessed by the method of dynamic light scattering. Copolymers were characterized by the methods of IR-spectroscopy and gel-penetrating chromatography. Helix-coil conformational transition of the chitosan macromolecule was pH and temperature-sensitive. The effective size of the chitosan macromolecules for helix conformation was 14-35% larger than for the coil conformation. The grafted copolymer yield (98%) was significantly higher in the solutions of chitosan, where its macromolecules were in the coil conformation. The obtained results allow the researchers

to create optimal conditions during the development of the copolymer-based biomedical materials with improved characteristics.

Keywords: *chitosan, conformation, modification, the efficiency of polymerization.*

АННОТАЦИЯ

Модификация хитозана биосовместимыми синтетическими полимерами актуальна для разработки биоразлагаемых медицинских материалов с индивидуальным комплексом свойств. Остается открытым вопрос о влиянии конформации макромолекулы полисахарида на синтез и свойства гибридных продуктов. Исследованы конформационные переходы макромолекул хитозана и их влияние на процесс привитой полимеризации 2-гидроксиэтилметакрилата и N-винилпирролидона на полимер, а также свойства их сополимеров. Для интерпретации особенностей свойств растворов хитозана изучили их вязкость методом ротационной вискозиметрии; конформационные переходы макромолекул полимера исследовали методом спектрофотометрии; размерные характеристики макромолекул полимера оценивали методом динамического рассеяния света. Соплимеры были охарактеризованы методами ИК-спектроскопии и гель-проникающей хроматографии. Конформационный переход клубок-спираль макромолекул хитозана чувствителен к pH среды и температуре. Эффективный размер макромолекул хитозана для спиральной конформации был на 14-35% больше, чем для конформации клубка. Выход привитого сополимера (98%) был значительно выше в растворах хитозана, где его макромолекулы находились в конформации рыхлого клубка. Полученные результаты позволяют исследователям подбирать оптимальные условия при разработке биомедицинских материалов с улучшенными характеристиками на основе сополимеров хитозана.

Ключевые слова: *хитозан, конформация, модификация, эффективность полимеризации.*

1. INTRODUCTION

Presently, chitosan, a natural-based polymer obtained by deacetylation of chitin, 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, chitosan is poorly soluble in water, because this bonding is stronger than between the molecules of chitosan and water. Never the less, the high content of polar groups provides high hygroscopic properties of chitosan (Smotrina *et al.*, 2015).

Amino groups in macromolecules provide good solubility of the polysaccharide in water solutions of organic and some inorganic acids (HCl). It can strongly hold a solvent in its structure, as well as diluted and suspended particles (Skryabin, 2013). The diversity of functional groups and the specifics of the chitosan structure determine the number of properties of this polymer, like absorptive, chelate, and complex formatting.

Such properties as high biological activity, biocompatibility, biodegradability, and muco-adhesiveness make chitosan an attractive object for the application in different areas.

Chitosan-based hydrogels with different structures are synthesized due to the formation of ions complexes with negatively charged molecules (sulfates, citrates, phosphates) and metal ions Pt^{2+} , Pd^{2+} , and Mo^{6+} , polyelectrolyte complexes (with proteins, heparin, chondroitin sulfate, carboxymethylcellulose, etc.) or due to the formation of polymer bonds between chitosan and other water-soluble nonionic polymers (polyvinyl alcohol) (Skryabin, 2013; Wu *et al.*, 2017; Liu *et al.*, 2018). 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, and capacity for the controlled release of the diluted substances. There are a number of studies going on the possible application of chitosan as a polymer matrix for drug delivery systems because the majority of substances are characterized by low permeability through biological membranes (Quiñones *et al.*, 2018; Wang *et al.*, 2017; Cánepa *et al.*, 2017; Huang *et al.*, 2017). Chitosan-based 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 (Skryabin, 2013; Chavana *et al.*, 2017; Lin

and 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; Ilbasmi-Tamer *et al.*, 2017; Ribeiro *et al.*, 2017). There are studies on the application of polysaccharides for the production of new biodegradable suture material (Cruz *et al.*, 2016; Li and Tang, 2016). Chitosan is used as a substance that contributes to weight loss and improves cholesterol metabolism and intestinal motility (Tuma *et al.*, 2011). Polymer and its derivatives are widely used in cosmetics as a moisturizer, gelling, film-forming, and anti-inflammatory agents (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.

Despite numerous studies on the properties of chitosan and chitosan derivatives and the success of the implementation of the obtained results, there are still some tasks that require improvement and further development. Among them, there are chitosan-based hemostatic and wound healing biodegradable materials with the tailor-made complex of properties and good physicochemical parameters. One of the solutions is seen as graft copolymerization of chitosan with biocompatible synthetic polymers. In this aspect, the issue remains relevant to the influence of the conformational condition of chitosan macromolecules on the synthesis of hybrid products and copolymer-based hemostatic and wound healing agents.

Protonation of chitosan amino groups of different degrees is observed at chitosan dilution in media with different pH. This property is determined by different conformational models of its macromolecules in the solution (from the coil to helix), which was confirmed by many studies (Fedoseeva *et al.*, 2008; Franca *et al.*, 2008; Morris *et al.*, 2009; Slivkin *et al.*, 2014; Costa *et al.*, 2015; Desbrieres *et al.*, 2001). It is known that the conformational model of the molecules in different pH media determines the size of macromolecules in the solution. It should be considered that chitosan macromolecule conformation can significantly influence the process of polysaccharide modification by monomers, as well as on the properties of final

products.

The aim of the present study was to establish the conformational transitions of chitosan macromolecules and their influence on the process of chitosan modification by graft polymerization with 2-hydroxyethyl methacrylate (HEMA) and N-vinylpyrrolidone (VP) and on the properties of their final products.

2. MATERIALS AND METHODS

The following substances were used in the study. Chitosan – poly ((1,4)-2-amino-2-deoxy)- β -D-glucose, as such, 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»).

Tetrahydrate cerium sulfate (IV) was used as an initiating system for graft copolymerization. The initiator was soluble in water.

Homopolymer extraction was performed in a Soxhlet apparatus by isopropyl alcohol. Isopropyl alcohol had the purity of GOST 9805-84 (CJSC Khimreaktiv).

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

The authors 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 to 5.0. Before the measurement, the solutions were kept for 48 hours.

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

$$\eta = A \cdot \exp \cdot \left(\frac{\Delta E_a}{RT} \right) \quad (\text{Eq. 1})$$

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

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

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). The particle 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 2.

$$D = \frac{k_B T}{6\pi\eta R} \quad (\text{Eq. 2})$$

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

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 to 1.2, 2.4, 4.8 mol/(molar ratio), respectively. The initiator was introduced into the medium. The system was kept for 15 minutes 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 5×10^{-3} mol/L. The synthesis was going on for 2 hours. After that, the temperature in the thermostat was raised to 80°C, and the system was kept for 30 minutes until the completion of the 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 performed by the gas chromatograph GCMS-QP2010, Shimadzu equipped with a thermal conductivity detector and the system of computer readings registration. The column: Equity-1 (length - 30 m, diameter – 0.25 mm, sorbent particles size – 0.25 μm), gas vehicle: helium (He). The rate of the gas vehicle in the column was 1 ml/min; the temperature of the column for HEMA (VP) identification was 230°C.

The synthesis of grafted copolymers HEMA (VP) with chitosan was established by IR-spectroscopy (spectrophotometer "Perkin-Elmer"). The samples of the reaction products were used for the analysis. The authors got a homopolymer poly-HEMA (PVP) extracted in the Soxhlet apparatus. The extraction was performed by isopropyl alcohol for 36 hours. The time was established by the blank test of poly-HEMA (PVP) extraction from the precipitated physical mixture of the respective homopolymers. Further, the samples were dried out by vacuuming to the fixed mass.

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

The degree of grafting (DG) was calculated by the Equation 3. The effectiveness of the grafting (EG) can be calculated by the Equation 4.

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 TSKgelG3000SWxl 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 1×10^3 to 4.1×10^5 Da (Fluca).

Chitosan-based and chitosan copolymer-based films were obtained by casting their solution on a lavsan substrate in the conditions of even evaporation of the solvent to the fixed-mass at room temperature (lavsan substrate is another name for a smooth polyethylene terephthalate (PET) surface. In our work, the polymer films were obtained by casting a chitosan and copolymer solutions onto smooth lavsan substrates). Further, the films were exposed to vacuum for 4-6 hours at $t=30^\circ\text{C}$ in the vacuum chamber.

Mechanical properties of the film (break strength σ and tension strain ϵ) 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.

The authors (Morris *et al.*, 2009) reviewed the experimental data obtained by different researchers, and theoretical calculations showed that conformational conditions of chitosan macromolecules were different depending on the medium pH: coil – to the pH values up to ~ 3.6 and helix – up to pH values ~ 4.8 -5.5. The analyses of the reactions of chitosan copolymerization with HEMA and VP in the solution were performed in different conformational conditions of polysaccharide macromolecules – helix and coil. Since grafted copolymerization involved different MW of chitosan, it was relevant to develop the method of efficient identification of certain conditions (MW, pH) of conformational conditions of chitosan macromolecules.

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;
- Investigation of temperature-related dependences between dynamic viscosity of chitosan solutions and macromolecules in different conformations and identification of energy of activation of polymer solution viscous flow;
- Evaluation of optical properties of the chitosan solutions.

Chitosan sample with $\text{MW}=1.1 \times 10^5$ and DD 0.82 was used for the evaluation of medium pH influence on the viscosity property of moderately concentrated solutions of the polysaccharide (Figure 1).

The observed dependence of the chitosan solution viscosity on the medium pH was not linear, and the curve was divided into three areas: pH < 3.4 – the curve was steadily going upwards, pH = 3.8 – the curve 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: helix – 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 helix-coil and provides the polymer macromolecule size change, which is confirmed by the method of DLS. Thus, the conformational condition of a macromolecule significantly influences the viscosity of polymer solutions.

The effective diameter of chitosan macromolecules in different pH media was established by the DLS method (Table 1).

It is shown in Table 1 that chitosan macromolecule in helix conformation, regardless of MW, is characterized larger effective diameter than in coil conformation. Thus, macromolecules of chitosan with $\text{MW} = 1.1 \times 10^5$ in 4.8 medium pH (helix 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 (Table 1). It must be mentioned that the MW of chitosan

diversely influence on the size of its macromolecules: the molecules in coil conformation with twofold different MW are similar in size, while the molecules in helix conformation, the molecule sizes are different by ~ 30%. In the last case, the authors cannot exclude the association of macromolecules that also provides increased values. However, the experiment was performed in over diluted solutions of chitosan with the polysaccharide concentration, not more than 0.03 wt%. In this case, the influence of the association can be ignored, and the conformation of the molecule remains unchanged. In this particular case, the authors believe that 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 the radius of its radius of gyration. The respective calculations on the radius of gyration by the equation of Flory-Fox based on the values of inherent viscosity confirmed the last statement.

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

Within the range of temperatures from 0 to 30°C, the viscosity of solutions of chitosan in the helix conformation exceeds the viscosity of solutions of the polysaccharide in the coil conformation at the same concentration of the polysaccharide (3 wt%) and MW=1.1×10⁵ (Figure 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 helix 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.

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 – helix, ΔE_2 – coil) was calculated by the Arrhenius-Frenkel-Eyring equation and the semilog coordinates of the dependence of the viscosity on the temperature (Figure 3).

The apparent activation energy (ΔE_a) of the 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 Equations 5 and 6.

$$\Delta E_1 = (36.5 \pm 0.4) \text{ kJ/mol} - \text{pH} = 4.8 \quad (\text{Eq. 5})$$

$$\Delta E_2 = (31.1 \pm 0.3) \text{ kJ/mol} - \text{pH} = 3.3. \quad (\text{Eq. 6})$$

The higher value of ΔE_a shows that inter-chain interaction between chitosan molecules that involve hydrogen bonds is stronger in the helix conformation.

This tendency agrees with the published data on other natural polysaccharides and their derivatives, in particular, on cellulose and its ethers. In the range of temperature ~50°C, the disruption of macromolecule associates in the helix 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 helix-coil 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.

Spectrophotometric analysis is known to be used for the evaluation of the dynamics of protein molecules in a solution. In the present study, for the first time, this method was used for the identification of chitosan macromolecules conformation. During the investigation of the solution viscosity (Figure 2), the authors suggested that its values were getting closer due to cooperative disruption of hydrogen bonds inside a macromolecule and, thus, by the conformational helix-coil transition. For this reason, the change in optical transmission of the solutions in a wide range of temperatures was studied (Figure 4). Precise, systematic decrease of optical transmission in the range from 60 to 75 °C was observed. Thus, the obtained data indicates the fact that conformational helix-coil transition is temperature-sensitive.

$$DD = 0.82; MW = 1.1 \times 10^5 \quad (\text{Eq. 7})$$

The changes in optical density of the diluted chitosan solutions in a wide range of MW dependence from the medium pH were evaluated at $T = \text{const.}$

In all the cases, the authors observed a sudden change in the optical transmission ratio at medium pH 3.4. The character of the curves was similar for all the chitosan samples with the same DD. Figure 5 illustrates the typical picture of optical density change in all the samples with MW 2.2×10^5 and 1.1×10^5 (DD = 0.82) depending on the pH of the medium.

There are two sharp transitions observed within the curves (Figure 5) at pH values ~ 3.4 and $4.8 - 6$. The results are reproduced in multiple replications. 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 *et al.*, 2008) based on the viscometric data. Unlike the mentioned study, the results of the present spectrophotometric analysis showed that chitosan macromolecule conformational transition helix-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 a helix conformation. The second minimums 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 helix-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 one of the solvent. Conformational helix-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. These test results explain the anomaly revealed during the study of chitosan solution viscosity. In the range of pH $\sim 5.0 - 5.2$, the lower the viscosity of chitosan solutions is, the heavier their MW is at similar DD.

The obtained results indicate the

prospects of application of spectrophotometric analysis for the precise identification of conformational transition of chitosan macromolecules when the medium pH is changed. It was shown that the interaction of chitosan macromolecules by means of hydrogen bonds in the helix conformation was stronger than in the coil conformation. The fact that macromolecules of chitosan at the medium pH = 4.8 (helix 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.

The development of hemostatic and wound healing chitosan-based compositions is limited by the fact that chitosan is soluble only in aqueous acid solutions and is characterized by low physicomechanical properties. These issues can be solved by the modification of chitosan.

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 (Ma and Lee, 2009; Dong, 2008; Teodorescu and Bercea, 2015). The modification of chitosan via copolymerization can result in the synthesis of water-soluble (expanding) hybrid products or gels.

The influence of components ratio and chitosan conformation (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 concentration of the initiator in all the reaction mixtures was 5×10^{-3} mol/L.

This initiator was chosen because, in the mentioned concentration, it did not disrupt the chains of the polysaccharide. Ce^{4+} containing initiator compounds, used in grafted polymerization of monomers onto chitosan, are known (Pourjavadi *et al.*, 2003; Yilmaz *et al.*, 2007; Metzler *et al.*, 2015). However, it should be mentioned that the researchers describe the initiation process differently. The authors (Yilmaz *et al.*, 2007) proposed the mechanism of grafting via oxidation and chitosan chain breakdown (Scheme 1).

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 (Scheme 2). In this case, the degree of grafting at copolymerization does not exceed 71%.

The degree of monomer HEMA transformation during its graft polymerization onto chitosan, when the macromolecules of the polysaccharide have helix conformation (pH 4.8), is evaluated below. The complete reaction (Figure 6, a) was observed at the ratio of $[HEMA]/[chitosan] = 4.8 \text{ mol}/(\text{molar ratio})$: the degree of monomer transformation was ~81%, while at other ratios, the degree of transformation was less than 5%.

The significant influence of chitosan macromolecules conformation on the characteristics of the process was observed. The degree of HEMA transformation in helix conformation was 81% (Figure 6, a). It significantly increased to 96% during the process that involved chitosan in coil conformation (Figure 6, b) in analog 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 were in helix confirmation, the system had the properties of a homogenous solution. 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. Probably, intra and intermolecular interactions within a helix conformation limit the availability of the reactive centers of chitosan, which leads to the synthesis of a significant amount of the homopolymer. MW of the grafted copolymers was also different: in the synthesis with chitosan macromolecule helix confirmation, it was equal to $(2.0-2.5) \times 10^5$, for coil conformation - $(4.0-600) \times 10^5$. 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 grafted copolymer chains and, as a result, higher concentration and strong interaction.

Grafted polymerization is characterized by the degree and efficiency of polymerization (DP and EP). The highest polymer outcome was observed when chitosan macromolecules were used in coil conformation. In this synthesis, the EP was equal to 98% and DP – to 390% (Table 2).

Grafted copolymer synthesis was confirmed by the results of IR spectroscopy. The spectrum of the original chitosan molecule (Figure 7, 1) contained the stripes that corresponded to the valence vibration of amid functional groups at 1652 cm^{-1} , 1563 cm^{-1} . Besides, the stripes typical for polysaccharides were observed at 1153 cm^{-1} , 1077 , and 1034 cm^{-1} (functional group C-O-C). The stripes observed

at 1379 cm^{-1} belong to the vibrations of the acetamide group. The synthesized product, cleaned from the homopolymer, leaves the stripes typical for HEMA and chitosan. A stripe appears in the spectrum (Figure 7, 2) at 1736 cm^{-1} , which corresponds to the vibrations of HEMA carbonyl ($-C=O$) groups.

Chitosan modification was also performed 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 $[monomer]/[glucosamine] - 1.0 \text{ mol}/(\text{molar ratio})$. 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.

The study of the kinetics of VP graft polymerization onto chitosan was performed in two temperature modes (60°C and 70°C) and two medium pHs (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 VP conversion in the process of polymerization is presented in Figure 8.

The difference in the kinetics of grafted polymerization at 60°C , when the macromolecules of chitosan were in different conformational conditions, was more evident in the case of VP polymerization than HEMA polymerization. The degree of monomer conversion (VP) in different transitions of the polysaccharide conformations differed by more than 40%. The grafted polymerization of VP onto chitosan, performed at 70°C , was characterized by close values of the ultimate depth in monomer transition of both chitosan helix and coil conformation. It cannot be excluded that, in this case, the process is cooperative due to the simultaneous helix-coil conformational transition of chitosan macromolecules. The 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 minutes after the beginning of the process and 98% after the completion of the process.

It 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 helix conformation (pH = 4.8) that form stronger intermolecular interaction due to hydrogen bonds.

Thus, the association of the molecules could be observed during the process of polymerization. The best VP grafted polymerization parameters onto 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 EP was 94% (Table 3).

3.1 Investigation of physical-mechanical properties of copolymers.

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

Chitosan (tensile strength 27 MPa) barely expresses any deformation properties (Table 4). HEMA film, obtained with the $\text{Ce}(\text{SO}_4)_2$ in reactive media with different pH (i.e., helix or coil conformation of chitosan macromolecule), is characterized by the improved physicochemical properties in comparison with the original polysaccharide: they are more resistant to deformation (up to 33-35 MPa) 0 3.5 – 4%. The film, cast from the solutions of chitosan in the coil conformation, has better physicochemical properties due to a higher degree of polymerization.

Chitosan-VP graft copolymers film is two times more tensile as compared to the original chitosan (Table 5): burst stress values reach 60 MPa at 4% deformation.

To increase the elasticity of the film, glycerin was used as a flexibilizer. In practice, it is enough to introduce 0.5 wt% of glycerin into the polymer structure, which allows for the application of the obtained material in the composition of bandaging materials 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 helix conformation.

4. CONCLUSION

The obtained results showed that spectral analysis was feasible for the identification of the coil-helix and helix-globule conformational transition of chitosan macromolecules in aqueous acidic solutions. Conformational transition is pH-dependent, temperature-sensitive, and does not depend on the chitosan MW at the same degree of deacetylation. Dependence of the depth, degree, and efficiency of the HEMA and VP graft polymerization onto chitosan on the conformational model of its macromolecules allows the researchers to develop new chitosan-based and chitosan derivatives-based biomedical materials with a tailor-made complex of properties.

5. ACKNOWLEDGMENTS

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EQUATIONS

$$DG = \frac{m(\text{grafted polymer}) - m(\text{chitosan})}{m(\text{chitosan})} \times 100 \% \quad (\text{Eq. 3})$$

$$EG = \frac{m(\text{grafted polymer}) - m(\text{chitosan})}{m(\text{grafted polymer}) - m(\text{chitosan}) + m(\text{homopolymer})} \times 100\% \quad (\text{Eq. 4})$$

where $m(\text{grafted polymer})$ – the mass of the copolymer in the sample, $m(\text{chitosan})$ – the mass of chitosan in the probe, $m(\text{homopolymer})$ – the mass of homopolymer extracted from the sample

Figure 1. Dependence of the dynamic viscosity of 3 wt% chitosan solution on the medium pH, $MW = 1.1 \times 10^5$, $DD = 0.82$. $T = 25^\circ\text{C}$

Figure 2. Dependence of solution dynamic viscosity on the temperature in the chitosan samples with $MW = 1.1 \times 10^5$: curve 1 – molecular helix conformation; curve 2 – molecular coil conformation.

Figure 3. Dependence of $\ln \eta$ on $1/T$ in the samples of chitosan with $MW = 1.1 \times 10^5$.
1 - pH = 4.8; 2 - pH = 3.3

Figure 4. Dependence of optical transmission of 0.03 wt% chitosan solution on the temperature.

Figure 5. Changes in optical transmission of 0.03 wt% chitosan solution depending on the pH of the medium. DD = 0.82. T = 25°C

Figure 6. Time-dependent changes in HEMA transformation during graft polymerization onto chitosan with $\text{Ce}(\text{SO}_4)_2$ as an initiator. $T = 60^\circ\text{C}$. a – pH 4.8; $[\text{HEMA}]/[\text{chitosan}] = 4.8$ (1); 2.4 (2); 1.2 (3) (mol/(molar ratio)). b – pH 3.3; $[\text{HEMA}]/[\text{chitosan}] = 4.8$ mol/(molar ratio)

Figure 7. IR spectra of chitosan (1) and chitosan-HEMA copolymer (2).

Figure 8. Degree of VP conversion in the process of polymerization onto chitosan with $\text{Ce}(\text{SO}_4)_2$ as an initiator. $[\text{VP}]/[\text{chitosan}] = 1.0$ mol/(molar ratio)

Scheme 1. The scheme of the mechanism of graft polymerization onto chitosan with cerium ions as an initiator

Scheme 2. The mechanism of chitosan initiation with cerium ions

Table 1. Effective diameter of chitosan macromolecules in different pH media. $\omega(\text{chitosan}) = 0.03\%$. Polydispersity ~ 1.25

MW of chitosan	Effective diameter, nm pH=4.8 (conformation – helix)	Effective diameter, nm pH=3.3 (conformation – coil)
1.1×10^5	623.9	536.9
2.2×10^5	868.9	546.5

Table 2. DP and EP in grafted polymerization of HEMA onto chitosan, $[\text{chitosan}] = 3 \text{ wt}\%$, $[\text{HEMA}]/[\text{chitosan}] = 4.8 \text{ mol}/(\text{molar ratio})$. Initiator – Ce^{4+}

Conformation of macromolecules	q, %	DP, wt%	EP, wt%
Helix (pH 4.8)	81	300	91
Coil (pH 3.3)	96	390	98

Table 3. The degree and efficiency of VP grafted polymerization onto chitosan, $\omega(\text{chitosan}) = 3 \text{ wt}\%$

Initiating system, temperature	Conformation of macromolecules	$[\text{VP}]/[\text{chitosan}]$, mol/(molar ratio)	q, %	DP, wt%	EP, wt%
$\text{Ce}(\text{SO}_4)_2$ 60°C	Helix (pH=4.8)	1.0	53	20,5	58
$\text{Ce}(\text{SO}_4)_2$ 60°C	Coil (pH=3.3)	1.0	95	44	70
$\text{Ce}(\text{SO}_4)_2$ 70°C	Helix (pH=4.8)	1.0	85	45	80
$\text{Ce}(\text{SO}_4)_2$ 70°C	Coil (pH=3.3)	1.0	98	61	94

Table 4. Physicomechanical properties of chitosan and chitosan-HEMA grafted copolymers

Initiator	pH of the reaction medium	DP	σ , MPa	ϵ , %
-	4.8	0	25.0 -27.0	1.9
$\text{Ce}(\text{SO}_4)_2$	4.8	300	32.5	3.5
$\text{Ce}(\text{SO}_4)_2$	3.3	390	35.0	4.2

Table 5. Physicomechanical properties of grafted chitosan-VP copolymers

$[\text{VP}]/[\text{chitosan}]$, mol/(molar ratio)	pH of the solution	σ , MPa	ϵ , %
1.00	4.8	53.4	2.6
1.00	3.3	60.0	4.0