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Conference Paper

Formation of Polyelectrolyte Complexes from Chitosan and Alkaline Gelatin

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Abstract

The interaction of chitosan and alkaline gelatin biopolymers in formation of polyelectrolyte complexes has been studied by the Fourier IR spectroscopy and scanning electron microscopy. Also the influence of chitosan on the morphology of gelatin hydrogels has been observed. It has been shown that in the range of chitosan (C)/gelatin (G) (w/w) ratio $Z = g_C/g_G$ from 0.1 to 1.5, chitosan--gelatin complexes are formed due to electrostatic interactions of charges amino-groups of chitosan and carboxylic groups of gelatin as well as due to intermolecular hydrogen bonds. In this case, the share of collagen-like triple helices in the spatial network of hydrogel decreases. The formation of chitosan--gelatin complexes leads to the qualitative changes in the hydrogel morphology even at low ratios of biopolymer (at $Z = 0.025$ g_C/g_G). The fiber-like structure of gelatin gels transforms and less structured but denser zones appear in the gel network.

Keywords: chitosan, gelatin, polyelectrolyte complexes, Fourier IR spectroscopy, scanning electron microscopy

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1. Introduction

There is a modern tendency to increasing demand for new materials based on polyelectrolyte complexes (PECs) from natural polyelectrolytes in biotechnology, medicine, pharmaceuticals, food industry and the other innovation-based industries connected with health and nutrition of humans. Such complexes are formed from oppositely charged (complimentary) biopolymers -- proteins, polysaccharides and nucleic acids [1--4]. PECs are called stoichiometric if the ratio of the number of charged groups of one polyelectrolyte to the number of opposite charged groups of the other polyelectrolyte is close to 1:1. Otherwise the complexes are called non-stoichiometric [5--8].

It is worth mentioning that one of the most promising biopolymers (in terms ofsustainable use of biological resources) is cationic polysaccharide chitosan, which can be a peculiar matrix for the formation of PECs. The biological precursor of chitosan is chitin,

which is a cellulose similar biopolymer. Chitin is the base of the skeletal system of *Arthropoda*. This polymer is second after cellulose in terms of natural polysaccharides. Linear chains of chitin consist of chitobiose units (residues of 2-deoxy-2-acetamido-β-D-glucan) connected by (1-4) glikozid bonds (Figure 1a). The substantial part of chitobiose units in chitosan, unlike chitin, is deacetylated (Figure 1b) [9, 10]. The formation and stability of chitosan-based PECs depend on numerous factors: the degree of ionization, density of charges and their distribution along a chain, the nature and location of the ionic group at polymeric chains, molecular weight and ratio of masses of polyelectrolytes, flexibility of polymeric chains, order if mixing, and time of component interaction, temperature, ionic power and pH of a medium [11--14].

Polyampholyte gelatin is a product of the brake-up of collagen which is a protein in the tissue of *Chordata*. The negative charge in gelatin is due to residues of glutamic (*Glu*) and aspartic (*Asp*) acids. Their content per 1000 amino-acid residues of alkaline gelatin is, 72 and 46--48, respectively [15--17]. According to some data [18], the stable chitosan--alkaline gelatin complexes can exist in the pH range higher than 4.7 (above pH of alkaline gelatin) and below 6.7 (until the precipitation of chitosan from a solution).

The authors in their previous studies considered colloidal properties of chitosan--alkaline gelatin complexes in acid solutions [19], as well as the rheological properties of hydrogels obtained at cooling of these solutions [20]. The goal of this work was to study the influence of intermolecular interactions between chitosan and gelatin in the formation of their complexes and the morphology of the PECs chitosan--alkaline gelatin hydrogels. Fourier IR analysis was used for investigation of biopolymers intermolecular interactions and the scanning electron microscopy was applied for hydrogel morphology examination. According to the classification of the techniques of structural investigation of biopolymer gels presented by [21], these methods are classified as molecular and macromolecular probes, respectively.

2. Materials and Methods

2.1. Materials

A sample of chitosan obtained from shrimp shells and supplied by *Sigma--Aldrich* (Iceland) was used without additional purification. The degree of chitosan deacetylation was 86%.

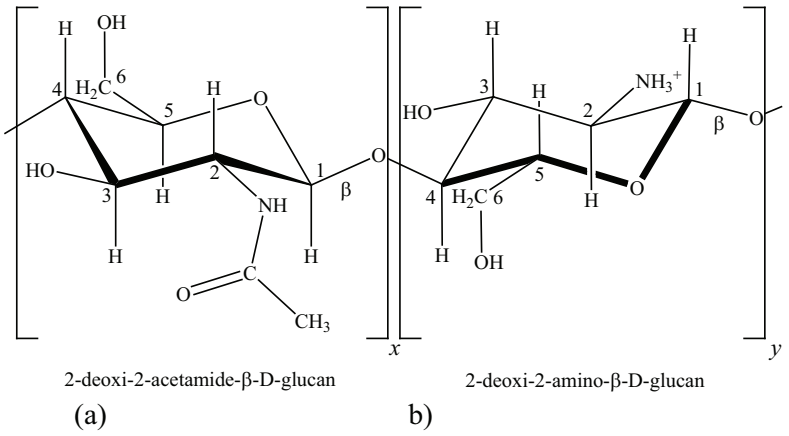

Figure 1: Structure of chitosan.

A sample of alkaline grade gelatin Type B from bovine skin 225 Bloom produced by *Sigma-Aldrich* (USA) was used. Its isoelectric point (pI_G) of 4.7 was defined by viscometric and turbidimetric methods.

The viscosity-average molecular weights of biopolymers $\bar{M}_\eta$ were defined by Mark--Kuhn--Houwink equation. The apparent radius of macromolecular biopolymer coil at the infinite dilution R^η_{app} was calculated from the $\bar{M}_\eta$ value. For chitosan sample $\bar{M}_\eta$= 260kDa and R^η_{app}= 29 nm; for gelatin sample, $\bar{M}_\eta$= 96 kDa and R^η_{app}= 9 nm [19].

The molecular weight distributions (MWD) of biopolymers were found by the high-performance liquid chromatography method [22]. Chromatograph LC-10A (*Shimadzu*, Japan) with column TSKgel Alpha-4000300×7.8 mm, 13 μm (*Tosoh*, Japan) was used. The eluation rate was 1.0 cm^3/min with the column temperature was 20 °C. The refractive index detector RID-10A was used. MWD of the chitosan and gelatin samples are shown in Figure 2. The samples are characterized by a rather quite narrow MWD. The weight-average molecular weight for chitosan $\bar{M}_w$= 250 kDa, for gelatin $\bar{M}_w$= 110 kDa, these data agree with the $\bar{M}_\eta$ values.

2.2. Methods

2.2.1. Preparation of solutions and gels

Solutions of chitosan and gelatin have been prepared separately. Initially, samples of chitosan and gelatin of the given weight were swelled in 0.1 M acetic acid (*HAc*) at 20 °C for 1 day and 1 hour, respectively. The acetic acid solution was used as solvent because the acidic medium is required to dissolve chitosan [10]. Then samples were dissolved

at elevated temperatures, 70 °C and 50 °C, respectively. This protocol allowed us to obtain homogeneous colloidal solutions (sols) of both biopolymers.

After that, chitosan solutions were added to gelatin solutions at a ratio corresponding to required biopolymer concentrations.The pH values of mixtures were in the range of 3.2--4.0 (below pI_G). In this pH range, neither phase separation nor coacervation of the mixtures was observed in the studied concentration range. Hydrogels were obtained from native chitosan and native gelatin solutions or from mixtures of chitosan with gelatin after cooling to 12 °C. The chitosan/gelatin (w/w) ratio, $Z = g_C/g_G$, varied from 0.025 to 1.5.

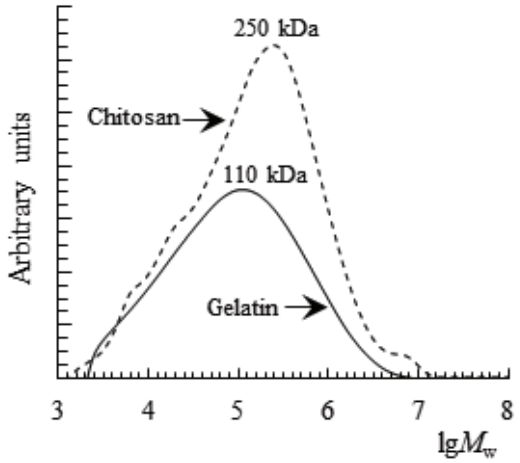

Figure 2: Molecular weight distributions for chitosan and gelatin samples.

2.2.2. Fourier transform infrared (FTIR) analysis

FTIR spectra of the sample under study were registered with the Fourier IR-spectrometer Shimadzu IR Tracer-100 in the middle range of IR wave lengths (800--3600 cm^{-1}) [22, 23]. FTIR spectra were measured using dried samples obtained from chitosan, gelatin and their mixtures. The following protocol of sample preparation was used. Hydrogels were maintained at 12°C for 12 h. Then they were frozen at --6 °C, unfrozen in the dark, and centrifuged. The formed sediment dried in a drying cabinet at 50 °C for 5 h and finally at 25 °C for 20 h. Obtained dry films were ground in a ball mill until reaching the state of highly dispersed powder, and this powder was pressed with KBr forming a pellet, which was used for FTIR spectroscopy measurements.

2.2.3. Microstructure observation

Hydrogel microstructure was examined by the scanning electron microscopy (SEM) using the microscope S405-A (*Hitachi*, Japan) supplied with the SEM LEO-420 software. Hydrogel samples preliminary stored in a desiccator at 12 °C for 1 day. Before examination, samples of the 3×3×3 mm size were frosted (in vacuum) and then metalized with thin (app 20 Å) gold layer under vacuum.

3. Results and Discussion

Figure 3 shows the FTIR spectra for samples of native chitosan, native gelatin as and for chitosan--gelatin mixtures of different chitosan/gelatin w/w ratios *Z*. The interaction between chitosan and gelatin macromolecules has been detected by the changes in the FTIR spectra.

The attribution of lines in the FTIR spectrum of gelatin to the vibration of corresponding functional groups is based on data of the publications [16,23--27]. In the native gelatin spectrum, the main lines are wideband with a peak at 3400 cm^{-1} (Amide A, stretching vibration of NH and OH groups), characteristic absorption at 1654 cm^{-1} (Amide I, stretching vibration of the CO and CN groups), 1538 cm^{-1} (Amide II, vibration of NH and CN groups) and 1238 cm^{-1} (Amide III, stretching vibration of NH and CN groups). Bands at 1165 cm^{-1} are obliged to a stretching vibration of carboxyl (COOH) groups of *Glu* and *Asp* in gelatin [22, 23]. For chitosan, the band Amide A is also characteristic and this band is shifted relative the gelatin line in the direction of higher wave numbers till 3450 cm^{-1} [9]. The analysis of the IR spectrum of chitosan (Figure 3) shows the Amide A peak of the sample under study corresponds to 3439 cm^{-1}.

In the systems containing stoichiometric chitosan--gelatin PECs ($Z < 0.8$, according to [20]), the Amide A band should be at 3306--3309 cm^{-1}. So, the comparison of this band with the Amide A band for gelatin shows that a rather strong shift towards the short range (from 3401 to 3306--3309 cm^{-1}) is observed. If to compare this shift for chitosan, the observed shift is even more significant (from 3439 to 3306--3309 cm^{-1}). This can be explained by the formation of the hydrogen bonds between chitosan and gelatin molecules, as well as the electrostatic interaction between amino-groups in chitosan and carboxyl groups in *Glu* and *Asp* in gelatin at the formation of PECs. Similar effects were observed in the study by the FTIR spectroscopy of films prepared from the blends of gelatin added to sodium alginate [28], κ-carrageenan [29], and chitosan [27]. In the further increase in *Z* to 1.5 (to the range of the formation of non-stoichiometric PECs),

the Amide A peak appears at 3401 cm^{-1} (see Figure 3). This can be explained by the influence of the free (not connected with gelatin) NH and OH in chitosan that give the additional input into the resulting location of the Amide A peak in non- stoichiometric PECs.

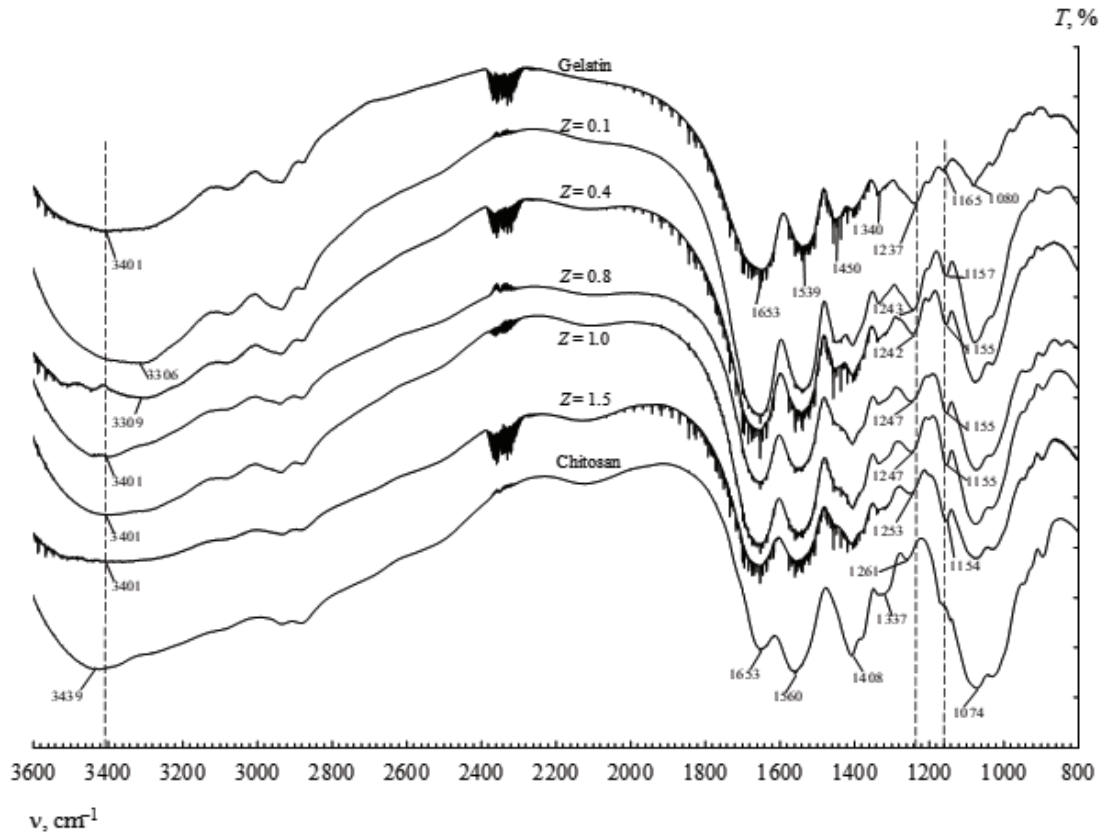

Figure 3: FTIR spectra for samples of native chitosan, native gelatin and mixtures of chitosan and gelatin of different chitosan/gelatin (w/w) ratios *Z*.

The introduction of chitosan also results in shift of the 1165 cm^{-1} band towards the low-frequency range until it reaches 1154--1157 cm^{-1}. The observed shift is the consequence of the interaction between negatively charged carboxyl groups COO^- in gelatin with positively charged amino-groups NH_3^+ of chitosan in the formation of PECs. Similar results have been demonstrated in the study of the films from fish gelatin, chitosan and their mixtures [27]. It must be emphasized that chitosan--gelatin films revealed the formation not only of the hydrogen bonds within and between polymer chains, but also of the electrostatic interactions between NH_3^+ of chitosan and COO^- groups of gelatin.

Chitosan has a much higher molecular weight than gelatin; consequently, molecular coils in chitosan sols are much larger than the gelatin coils (see 2.1). In an acidic media (pH 3.2--4.0, below pI_G, (see 2.2.1), free amino-groups of chitosan are almost completely ionized. So, polysaccharide macromolecules carry a high density of positive charge. The

carboxyl groups of *Glu* and *Asp* gelatin residues are ionized only slightly. It allows for presuming that several gelatin molecules are connected with a chitosan macromolecule via the electrostatic interactions and hydrogen bonds. As the result, chitosan--gelatin PECs appear where chitosan is a lyophilized component, and gelatin is a blocking polyelectrolyte [13, 27, 30]. The upper boundary in the formation of the stoichiometric PECs corresponds to the ratio Z = 0.78. Then, based on the molecular weights on biopolymers, it is reasonable to suppose that a single chitosan macromolecule is connected with 3--4 gelatin macromolecules [20].

The Amide III absorption band also is shifted from 1237 cm^{-1} to 1253 cm^{-1} at Z = 1.5 (see Figure 3). According to [26, 31, 32], these up-shifts could be attributed to a reduction of intermolecular interactions between gelatin chains within a collagen-like triple helical structure. In other words, this effect can be associated with the increasing ratio of bonds between the random coil of gelatin and secondary structure of collagen. A decrease in the degree of spiraling in gelatin in the formation of chitosan--gelatin PECs can be explained by the mutual electrostatic repulsion positively charged complexes in a acidic medium. This hinders intermolecular interaction between gelatin molecules entering different complexes. Positive charging of PECs is provided by the base groups of the following amino-acids of gelatin: arginine *Arg*, lysine *Lys*, hydroxylysine *Hyl*, and histidine *His*. Their content per 1000 amino-acid residues of alkaline gelatin is 48--49, 22--28, 4--9, and 4--5, respectively [15--17]. According to [33], the complex formation of gelatin with the other polysaccharide, such as anionic κ-carrageenan, in the pH range close to the pI_G leads to the opposite effect increasing the degree of polypeptide spiraling.

Figure 4 demonstrates microphotographs obtained by SEM for a high-concentrated hydrogel of gelatin (28 wt%) without polysaccharide and the same sample with a small amount of chitosan (Z = 0.025). Both images are presented on the same scale with $\times 10^4$ magnification. Supramolecular structure in the samples of native gelatin is formed by transverse collagen-like fibrils (helices), creating a network (Figure 4a). The size of fibrils depends on the gelatin concentration. The gel structure also demonstrates the existence of discrete zones (cells) spread along fibrils inside the network. These elements may be formed by the non-helical sites of macromolecules.

The addition of chitosan (even at such a low concentrations) leads to significant changes in the supramolecular structure (Figure 4b). First, triple collagen-like gelatin helices become not so notable. Second, polysaccharide promotes the formation of a strengthened network in gel due to the formation of stoichiometric chitosan--gelatin PECs, which is accompanied by the formation of additional nodes in the network

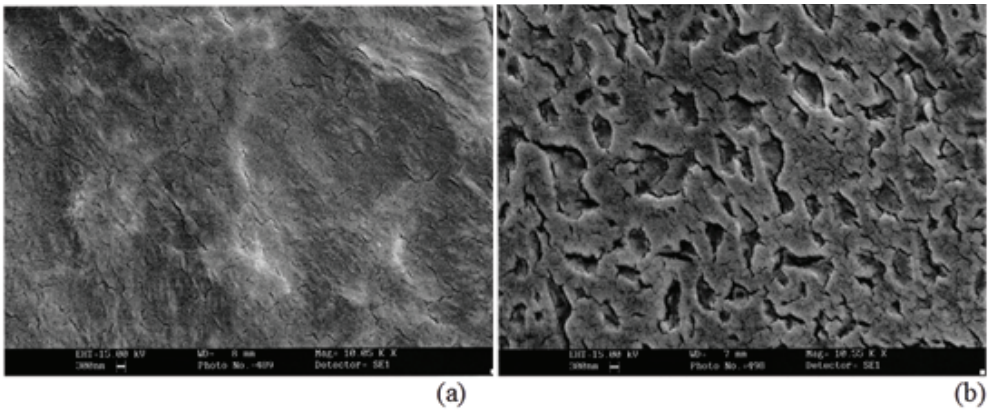

Figure 4: Microphotographs of gelatine hydrogel C_G = 28 wt% (a) and hydrogel formed by chitosan--gelatine PECs, C_G = 28 wt%, Z = 0.025 g_C/g_G (b).

(electrostatic interactions between NH_3^+ of chitosan and COO^- groups of gelatin and hydrogen bonds between both biopolymers). Similar changes in the morphology are also produced by other polysaccharides, for example, gellan [29], and κ-carrageenan [33]. Also it has been shown that the formation of the strengthened network the chitosan--gelatine PECs hydrogels leads to the significant increase in their viscoelastic parameters [20].

4. Conclusion

Intermolecular interactions between chitosan and gelatin during the formation of PECs have been studied using the FTIR spectroscopy method in varying the composition of these biopolymers in the range of Z (g_C/g_G) from 0.1 to 1.5. It has been shown that the electrostatic interactions between charged amino-groups in chitosan and carboxyl groups of gelatin contribute to the stabilization of chitosan--gelatin complexes. Besides, the formation intermolecular hydrogen bonds have been also found. The share of collagen-like triple spirals in the gelatin macromolecules at their entering the complex with chitosan decreases.

The PECs formation obliged to the interaction between chitosan and gelatin leads to the qualitative changes in the morphology of the gelatin gels which take place even at addition of small quantity of chitosan (Z = 0.025 g_C/g_G). Photomicrographs obtained by the SEM method allowed us to observe the disappearance of fiber-like structures and decrease of the gelatin spiraling as the main gel-forming agent. Besides, the appearance of some nodes (denser zones) in the spatial network of gel has been found.

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Conflict of Interest

The authors have no conflict of interest to declare.

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