PREPARATION AND CHARACTERIZATION OF CRYOGELS BASED ON PECTIN AND CHITOSAN

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Abstract

Novel cryogels based on pectin and chitosan were obtained by a cryotropic gelation method. Percentage of chitosan in the cryogels was estimated by elemental analysis. The obtained values were from 3.50 to 15.03 % for Apple pectin/Chitosan cryogels and from 9.44 to 17.64 % for Heracleum pectin/Chitosan cryogels. Internal structure and porosity of the cryogels were measured by scanning electron microscopy. According to the scanning electron microscopy, cryogels have a macroporous sheet-like structure. In the future, cryogels can be used in biotechnology and medicine as biocompatible, biodegradable materials.

Key words: *pectin, chitosan, cryogels, SEM, elemental analysis.*

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1. Introduction

Currently, development of new materials based on polysaccharides of natural origin is attracting increasing interest among researchers. The main advantages of these polymers are availability, biocompatibility, biodegradability and low cost. It makes them extremely attractive for using in biomedicine. The major importance of biopolymers is their application in development of targeted drugs and antigens delivery systems, wound healing, tissue engineering, and in many other fields of biomedicine. Among many natural, biocompatible polysaccharides, it is important to highlight the potential use of pectin and chitosan in creation of new biomaterials. Chitosan is a cationic deacetylated derivative of natural polysaccharide chitin, which is the main component of the integument of arthropods. Chitosan consists of D-glucosamine residues and N-acetyl-D-glucosamine units linked by β-1.4-glycosidic linkages [1, 2]. Pectin is a natural anionic polysaccharide, a major component of plant cell walls. Primary carbohydrate chain of pectin consists of 1.4-α-D-galacturonic acid residues [3]. When dissolved, chitosan is in cationic form and polyelectrolyte pectin is in anionic form, which allows to construct different biomaterials, such as gels, films, multilayer micro- and nanoparticles by complexation between oppositely charged polyionic polysaccharides or lower molecular weight molecules and ions [4 - 7]. Materials on the polysaccharides basis have a broad spectrum of biological properties, including good biocompatibility, biodegradability, low toxicity and high mucoadhesiveness [8]. Thus, pectin and chitosan polysaccharides are promised to be good materials for cryogels based on them. The objective of this study was to prepare and to characterize of cryogels based on pectin and chitosan with different physical and chemical properties.

2. Materials and methods

2.1. Materials

Apple pectin (AP) Classik AU 701 with molecular mass (MM) 401 kDa and degree of methylesterification (DM) 36% (Herbstreith & Fox Corporate Group, Germany), *Heracleum L.* pectin (HP) with MM 419 kDa and DM 9% (Vyatka State University, Russian Federation), Chitosan from crab shells with MM 200 kDa and deacetylation degree (DD) 95% (ZAO «Bioprogress», Russian Federation).

2.2. Preparation of chitosan samples

2.2.1. Reprecipitation of chitosan

Purification of initial chitosan by reprecipitation was done from a 1% chitosan solution in 1% acetic acid. The solution was centrifuged at 5000 rpm for 10 min. Then, pH was adjusted to the value of 8.5 using a 12% solution of NH_4OH. The solution was centrifuged at 6000 rpm for 15 min. The precipitate was washed twice with distilled water and was dialyzed for 3 days and then was freeze-dried.

2.2.2. Acid hydrolysis of chitosan

Low molecular weight chitosan samples were prepared by chemical hydrolysis with 6M hydrochloric acid. The ratio of chitosan and hydrochloric acid was 1:3 (w/v) [9]. The mixture was heated at 95 °C in a water bath with stirring for 3 hours. Then, the mixture was held for 12 hours at room temperature. The precipitate was separated by vacuum filtration, suspended in distilled water, heated until complete dissolution (95 °C) and filtered over a sintered glass filter to remove mechanical impurities. Then, the solution was chilled to 5 °C, the resulting precipitate was washed with ethanol (60 °C) and dried on the filter; it was further reprecipitated from an aqueous solution by 12% NH_4OH, dialyzed and freeze-dried.

2.2.3. Deacetylation of chitosan

Deacetylation of chitosan was carried out according to the method of Lim S-H et al. [10]. Commercial chitosan (20 g) was dispersed in 200 mL of 10% (w/w) NaOH solution containing $NaBH_4$ (2 g) as a reducing agent. After 5 h of stirring at 110°C, the mixture was filtered over a glass filter and washed with distilled water to neutral pH. The chitosan was further washed with MeOH and acetone and dried at 70 °C under vacuum overnight.

2.2.4. Acetylation of chitosan

The reaction was carried out in aqueous-alcoholic medium (1: 1.7 v/v). Chitosan (1 g) was dissolved in 1% acetic acid-methyl alcohol medium with stirring for 0.5 hours [11]. Acetylating agent was added in an amount of 6.4 mM to 1 g of chitosan. The reaction time was 5 minutes at 22 °C. Then the chitosan was dialyzed and freeze-dried.

2.3. Characterization of chitosan samples

2.3.1. Deacetylation degree

DD of chitosan samples was determined by ^{1}H-NMR. ^{1}H-NMR spectra were measured in deuterium chloride (DCl) using a 400 MHz spectrometer Bruker AMX (Vernon Hills, IL, USA).

2.3.2. Molecular mass

MM of chitosan samples was determined by viscometric method. The intrinsic viscosity [η] of chitosan samples was measured with an Ubbelohde viscometer in 0.2 M CH_3COOH/ 0.1 M CH_3COONa aqueous solution at 30±0.05 °C.

MM of chitosan samples was calculated based on the DD by the Mark-Houwink equation:

$$[\eta] = \kappa M^{\alpha}$$

where [η] – intrinsic viscosity; k, α were selected for each sample according to the Wang W. et al. method [12].

2.3.3. Dynamic viscosity

Chitosan was dissolved in 1% acetic acid under magnetic stirring at room temperature. The resulting solution was filtered. The dynamic viscosity of the solution was measured at 25°C by Brookfield DV-I + Viscometer (Brookfield, USA).

2.4. Preparation of pectin/chitosan cryogels

$CaCl_2$ was added to a 0.7% chitosan solution in acetic acid (0.25%) for preparation of cryogels. Concentration of $CaCl_2$ in the resulting solution was 0.12 M. The solution was frozen at -18 °C for 1 hour. Then 2 ml of 1% pectin solution in distilled water was poured onto frozen chitosan with calcium. Cryogels were made through diffusion of the solution pectin to the frozen chitosan-Ca solution; while yielding thawing. Gels were formed for 8 hours at room temperature. The formed gel was freeze - dried and sterilized by UV-irradiation for 1 hour.

2.5. Characterization of cryogels

2.5.1. Elemental analysis

N elemental composition of the chitosan and prepared pectin/chitosan cryogels was determined using an elemental CHNS-analyzer (Vario Microcube Elementar, Germany).

2.5.2. Morphology

The morphology, porosity and the microstructure of the samples were studied using scanning electron microscopy (TESCAN VEGA-II XMU, Czech Republic) at 30 kV without applying any coating procedure. Cryogels were cut with a razor scalpel.

3. Results and discussion

Samples of chitosan with different physical and chemical characteristics (MM, DD) were obtained from reprecipitated chitosan using various methods. Chitosan samples were characterized by ^{1}H-NMR and viscometric methods. Characteristics of chitosan samples are shown in table 1. In this work AP and HP were used. HP was characterized in Vyatka State University. Data on MM (HPLC) and DM are shown in table 2.

Table 1. Physical and chemical characteristics of chitosan samples

Method of preparation	Abbreviations	DD $_{1_{H\text{-}NMR}}$, %	MM, kDa	Dynamic viscosity, cP
Acetylation	Chi230/38	38	230[a]	155
Reprecipitation	Chi200/95	95	200	294
Deacetylation	Chi255/98	≥98	255	223
Hydrolysis	Chi31/98	≥98	31	32

[a] - calculated by the elementary units of chitosan according to the DD.

Table 2. Physical and chemical characteristics of pectin samples

Source of pectin	Abbreviations	MM, kDa	DM, %	Content of GalA[a], %
Heracleum L.	HP	419	9	88
Apple	AP	401	36	86,5

[a] – galacturonic acid

The polymeric cryogels are macroporous heterophase gels formed as a result of the freezing, storage in the frozen state, and subsequent thawing of the initial solutions in which preconditions for structure formation and transition into a gel are already present or are specially created [13]. In this work, low methylesterified pectin is the basis of gels, which is able to form a gel in the presence of divalent ions, in this case these are Ca^{2+} ions. The cryogels were made through diffusion of the solution pectin to the frozen chitosan solution; while yielding thawing. Formation of the pectin/chitosan polyelectrolyte complex occurs due to electrostatic attraction between ionized amino groups of chitosan (NH_3^+) and ionized carboxyl acid groups (COO^-) of pectin [4]. Ions of Ca^{2+} also diffuse from the layer of chitosan to pectin, and ionic interactions between dissociated carboxyl groups via Ca^{2+}-bridges are formed [14]. The pectin/chitosan-based cryogels formation scheme is presented in Fig. 1.

Table 3 presents results of elemental analysis of the obtained chitosan and of the formed pectin/chitosan cryogels. Chitosan mass percentage in the cryogels was estimated through nitrogen composition in a sample. The obtained values were from 3.50 to 15.03 % for the AP/Chi cryogels and from 9.44 to 17.64 % for the HP/Chi cryogels. This means that the composition of the cryogels is not identical. The final concentration of chitosan in cryogels based on HP was higher than in gels based on AP, despite the same initial ratio of polysaccharides (Pectin/Chitosan 3:1). These results can be explained by different densities of ionized groups in AP and HP. HP with DM 9% has a greater number of free carboxyl groups than AP with DM 36%, consequently, the percentage of chitosan in gels based on HP is more than in the gels based on AP. However, it does not reach 25%, which indicates that

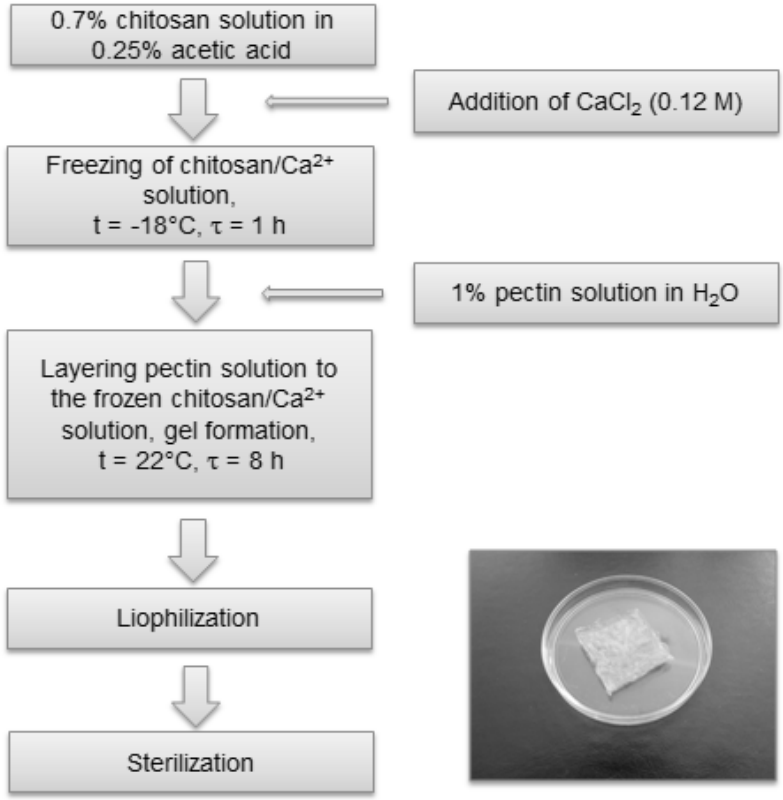


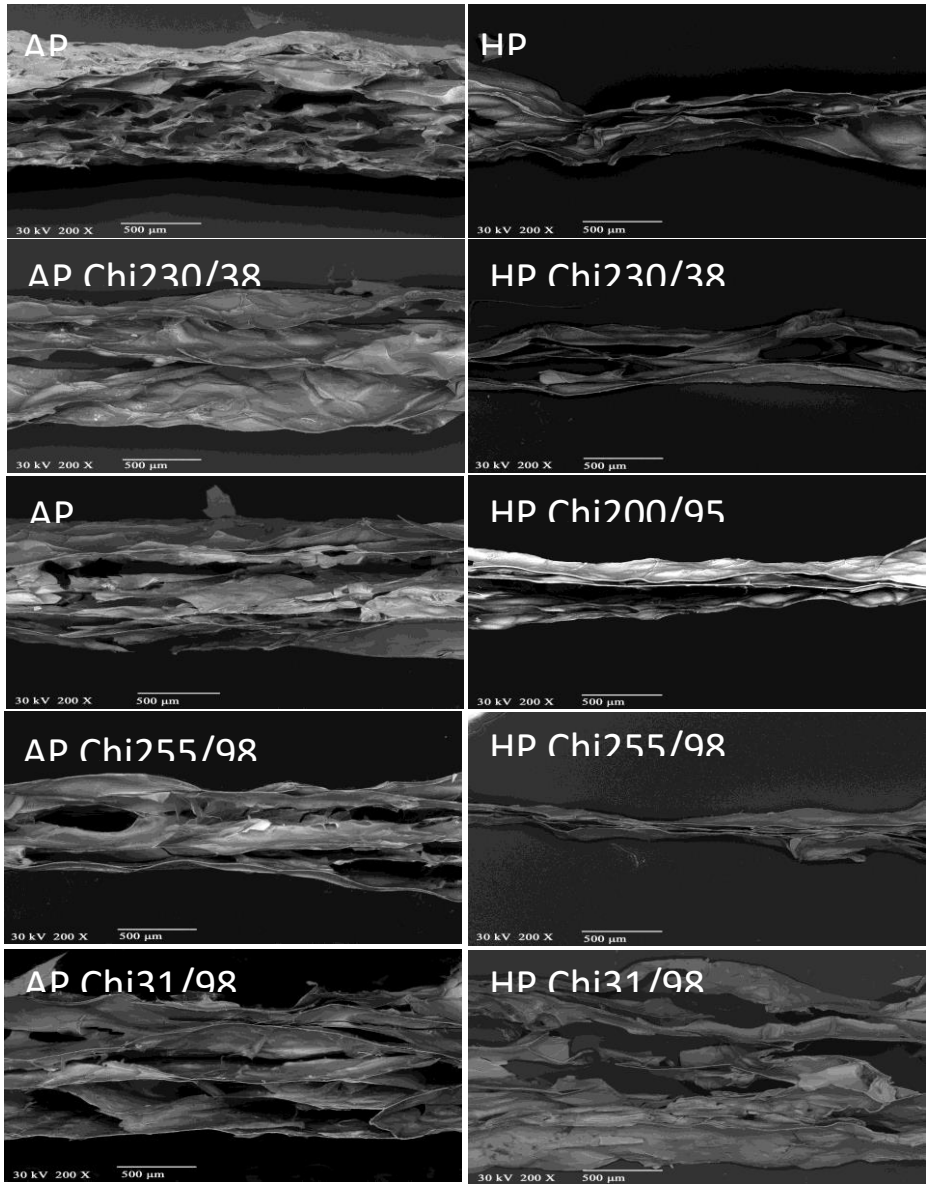
Figure 1. Formation scheme of pectin/chitosan cryogels.

Table 3. Elemental analysis results and estimated composition of the pectin/chitosan based cryogels.

Sample	Abbreviations	%N	% Chitosan in the cryogels (w/w)[a]
Chitosan	Chi200/95	8.58	-
	Chi230/38	7.50	-
	Chi255/98	8.65	-
	Chi31/98	8.68	-
Apple pectin gels	AP:Chi200/95	0.30	3.50
	AP:Chi230/38	0.47	6.27
	AP:Chi255/98	0.60	6.93
	AP: Chi31/98	1.31	15.03
Heracleum L. pectin gels	HP:Chi200/95	0.81	9.44
	HP:Chi230/38	1.17	15.60
	HP:Chi255/98	0.85	9.82
	HP: Chi31/98	1.53	17.64

[a] - Calculated based on the %N in the scaffolds and %N of chitosan.

chitosan is not completely included in the composition of gels and pectin is the main component of the produced cryogels (it constitutes approximately more than 80% of the cryogel mass). The percentage of chitosan in gels also depends on physical and chemical characteristics of chitosan samples. The highest percentage of chitosan in a gel was observed when using low molecular weight and high DD (Chi31/98) chitosan. The strength of chitosan and pectin electrostatic binding increases with the number of free NH_3^+-groups. Due to the small size, a larger number of low molecular chitosan molecules penetrate the structure of the pectin gel.



***F*igure 2.** SEM micrographs (200X) of cryogels based on Apple and Heracleum L. pectin.

The morphology of the cryogels was analyzed by scanning electron microscopy (SEM). SEM images of the AP- and HP- based cryogels are shown in Fig. 2. The cryogels present an irregular, highly porous structural form (sheet-like structures). Moreover, pores were highly interconnected.

4. Conclusions

Novel cryogels based on pectin and chitosan were obtained by a cryotropic gelation method. Cryogels composition was evaluated by elemental analysis. The percentage of chitosan in a gel depends not only on the physical and chemical properties of chitosan, but also on the characteristics of the pectin. The internal structure and porosity of the cryogels were measured by scanning electron microscopy. According to SEM cryogels have a macroporous sheet-like structure. Cryogels combine advantages of both polysaccharides, such as biocompatibility, biodegradability and low toxicity, which allow further usage of these cryogels for medical purposes.

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