

# On the possibility of obtaining the elastic and biocompatible film materials based on chitosan and N-succinyl chitosan

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## Abstract

For the first time, the studies of polyhydric alcohols (glycerol, ethylene glycol and propylene glycol) impact on the structure, deformation–strength characteristics and hemocompatibility of the film materials based on chitosan and N-succinyl chitosan were carried out. It was shown that the introduction of polyhydric alcohols into molding solutions during the creation of the films based on chitosan and succinyl chitosan in the amount up to 0.05 mol/l is accompanied by the tensile elongation value increase by 2–2.5 times. The value of the breaking stress in this case decreases by 3–4 times. There is also a decrease in the overall surface roughness and a sharp drop in the elastic modulus of the films. The stabilizing impact of the systems based on chitosan and succinyl chitosan in the presence of glycerol and propylene glycol upon the cell membranes in physiological conditions allows inferring their high hemocompatibility.

## Keywords

chitosan  
sodium salt of N-succinyl  
chitosan  
polyhydric alcohols  
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## 1. Introduction

The problem of wound healing and wound infection remains actual nowadays as the number of patients with various kinds of wounds does not decrease and makes up more than 50% of the total number of patients [1]. The use of traditional dressing materials is not efficient enough. That is why the development of new modern wound healing coatings possessing a complex of properties meeting the necessary demands is underway. These coatings should effectively remove the excess of the wound exudate and its toxic components, provide an adequate gas exchange between the wound and the atmosphere, prevent the secondary wound infection, promote the creation of the optimal humidity of the wound surface, possess anti-adhesive properties and have sufficient mechanical strength [2].

Proceeding from these demands, a proper base for the creation of effective wound coatings is the film materials based on bio- and hemocompatible biopolymers, e.g. polysaccharides. Thus, a promising one is polysaccharide chitosan (ChT) and its derivatives, e.g. N-succinyl chitosan (SChT). The advantages of chitosan and its derivatives in

the creation of the medical purpose materials are the biocompatibility with living tissues, the proximity to the derma components in vivo by their functional qualities, bacteriostaticity, the ability to bio-degradation and others [3–6].

However, ChT and the part of its described derivatives are polymers with a high degree of crystallinity and the materials based on them are characterized by the brittleness and the absence of elasticity [7, 8]. This limits the use of the film materials based on the unmodified ChT and its derivatives as the wound healing coatings. At the same time, it is known that the structure and, consequently, both physico-chemical and physico-mechanical properties (the plasticity being among them) of ChT films are defined by the way of their forming and modification [8–10].

The most widespread way of obtaining ChT and SChT-based films is the method of polymer solution irrigation on the solid base. That is why the most simple and the most accessible way of regulating plastic properties of the films is the introduction of a modifying additive into the forming solutions, which may behave as the polymers' plasticizers.

In the present work, polyhydric alcohols, such as glycerol, ethylene glycol and propylene glycol, were chosen as the plasticizing additives based on ChT and SChT. The

choice of polyhydric alcohols was due to the following reasons. On the one hand, they mix with water in any ratio and may be introduced into the system at the stage of polymer dissolution. On the other hand, glycerol, ethylene glycol and propylene glycol have a high boiling temperature and are not removed from the film being dried.

In the creation of medical purpose materials it is necessary to take into account that the substances contacting with the organism may enter the blood, the lymph and the tissues in a diluted form. Therefore, it is reasonable to study the blood indices' changes under the impact of ChT (SChT) – polyhydric alcohol systems being studied, which may prove their hemocompatibility.

Thus, the present paper aims to study the polyhydric alcohols' impact (glycerol, ethylene glycol and propylene glycol) on the deformation-strength characteristics and hemocompatibility of ChT and SChT-based film materials.

## 2. Experimental

### 2.1. Materials

Chitosan with molecular weight 113 kDa (deacetylation degree 82%) and sodium salt of N-succinyl chitosan with molecular weight 207 kDa (substitution degree of ChT by amino groups 75%) [11] were obtained from "Bioprogress" company (Russia). Propylene glycol, ethylene glycol and glycerol were used as film plasticizers and were supplied by Sigma-Aldrich (USA). Glacial acetic acid and sodium chloride were supplied by Sigma-Aldrich (USA).

### 2.2. Film preparation

The ChT and SChT based films were prepared by conventional solution-casting technique. Propylene glycol, ethylene glycol and glycerol at 0.03–0.3 mol/l were used as plasticizers to investigate the effect of each individual plasticizer on the developed films. As solvents in the preparation of the film forming solutions were used 1% acetic acid for ChT and Milli-Q quality ultrapure water for SChT.

The film preparation procedures are described as follows. Initially, 1 g/dl dispersions of chitosan and succinyl chitosan in appropriate solvents with different plasticizers were dissolved using a magnetic stirring plate at room temperature for 24 h. After dissolving, the film forming solutions were casted on a preliminary degreased surface of the glass Petri dishes. After 144 h of drying, the films were peeled from the Petri dish surfaces and stored in desiccators. In the preparation of ChT solutions, a 1% aqueous solution of acetic acid was used as a solvent. Therefore, chitosan in films is in the form of an acetate salt.

### 2.3. Thickness

Thickness of the films was determined using a Micrometer MK 0.25 to the nearest 0.1 mm. The selected values are the averages of at least ten random locations of the film sheets. The means were calculated and used in the determination of mechanical and physical properties.

### 2.4. Dimensions of the aggregates

The size of the aggregates was determined on a Zeta Sizer Nano photon correlation dynamic light scattering spectrometer (Malvern, UK). The light scattering angle is  $-1730^\circ$ . The source of laser radiation is a He-Ne gas laser. The wavelength is 633 nm. Power – 10 MW. The analysis of the signals was carried out by a single-board multi-channel correlator coupled with an IBM PC compatible computer. The pulse accumulation time is 5–7 min. The effective radius of the structures  $r$  was calculated by the Stokes-Einstein equation for spherical particles:

$$r = kT / 6\pi\eta D \quad (1)$$

where  $k$  – the Boltzmann constant,  $T$  – the temperature,  $\eta$  – the viscosity of the solvent.

### 2.5. Morphology and topography

The morphology and topography of the polymer films were studied using the Agilent 5500 AFM atomic force microscope (USA). The samples were scanned in air in semi-contact mode using silicon probes PPP-NCL (Nanosensors) with a hardness of 34 Nm and a resonant frequency of 172 kHz. Image processing and statistical processing of the results were carried out in the Gwyddion program. To visualize the scanned objects the PicoView 1.20 program was used.

### 2.6. Temperatures of relaxation transitions

The temperatures of relaxation transitions of polymer films based on ChT and SChT were determined by differential scanning calorimetry (DSC) on the DSC-1 device (NETZSCH) under the following conditions: temperature range 40–200 °C; dynamic mode – heating/cooling speed of 10°/min; medium – nitrogen.

### 2.7. Elastic properties

The elastic properties of the surface of the films were studied in the mode of force spectroscopy. To do this, when the probe was brought to the surface, the dependences of force on distance, the so-called "force curves", were recorded. The elastic properties (determination of the effective Young's modulus) of the films were evaluated based on the analysis of the obtained dependence within the framework of the accepted Hertz contact mechanics model by the expression for the dependence of the indentation depth ( $h$ ) on the force ( $F$ ):

$$h = \left( \frac{F^2}{RE_{eff}^2} \right)^{1/3}, \quad (2)$$

where  $R$  – probe tip radius,  $E_{eff}$  – effective Young's module.

### 2.8. Tensile properties

The mechanical properties of the films were tested using a tensile testing machine Autograph AGS-10 kNG equipped with the software "Trapezium" (Shimadzu, Japan). The length of the sample base was 20 mm, the width was 20

mm and the thickness was 0.1 mm. The films were pulled using a crosshead speed of 2 mm/min. The tensile stress ( $\sigma$ ) was determined considering the cross-sectional area of the analyzed sample and was expressed in MPa. The relative elongation at the rupture ( $\epsilon$ ) was calculated taking into account the initial film sample length taken for the testing and was expressed in percents. The values of the relative elongation at the rupture and the tensile stress were calculated as arithmetic averages of five parallel measurements.

### 2.9. Hemocompatibility

Hemocompatibility of the ChT (SChT)-polyhydric alcohol systems was evaluated by determining the osmotic resistance of red blood cells by the degree of their hemolysis in hypotonic sodium chloride solutions. The expediency of using solutions for determining the osmotic resistance of erythrocytes is due to the fact that the components of film materials based on ChT and SChT, upon contact with body tissues, can enter the blood only in a dissolved form.

Blood was produced by decapitation of rats and collected in test tubes with an anticoagulant – heparin at a concentration of 150 u/ml of blood. The decapitation of animals was carried out in accordance with the "Rules for the work using experimental animals", under chloral hydrate anesthesia intraperitoneally (2.5% solution, 1 ml per 100 g of animal body weight).

After pouring 5 ml of the working solutions' mixture of NaCl with the concentration of 0.1 to 0.9% into several centrifugal tubes, 0.02 ml of the heparinized blood was added into the sodium chloride solutions. The samples were incubated for an hour at a room temperature and centrifuged at 1500 rpm for 10 min. The degree of erythrocytes' lysis was evaluated photometrically for the absorbance of released hemoglobin at the wavelength of  $\lambda=530$  nm against the distilled water with the layer thickness of 1 cm. In the experiments *in vitro* we added 0.2 ml of SChT or ChT solutions with a concentration of 1 mmol/l and their mixtures with polyhydric alcohols to the sodium chloride solutions. Then 0.02 ml of the intact rats' blood was added into the hypotonic solutions. With the values obtained, we plotted the lysis kinetics depending on the type of the system under study.

The percentage of hemolysis was calculated using the following equation [12]:

$$\text{Hemolysis (\%)} = \frac{D_{\text{sample}} - D_{\text{negative control}}}{D_{\text{max}} - D_{\text{negative control}}} \cdot 100 \quad (3)$$

where  $D_{\text{sample}}$  – the value of the optical density of the experimental sample;  $D_{\text{max}}$  – the value of optical density at complete (100%) hemolysis in a test tube with 0.1% sodium chloride solution;  $D_{\text{negative control}}$  – the value of the optical density of the control sample (a characteristic of spontaneous hemolysis of erythrocytes under experimental conditions).

## 3. Results and Discussion

The process of obtaining materials from polymer solutions is carried out by processing semi-diluted solutions in which macromolecules aggregate with each other. Thus, even in the solutions of ChT and SChT with a concentration of about 0.1 g/dl already contain aggregates of macromolecules [12, 13] as determined by the method of dynamic light scattering (Table 1). Naturally, the higher the concentration of the polymer in the solution, the larger the size of the aggregates formed.

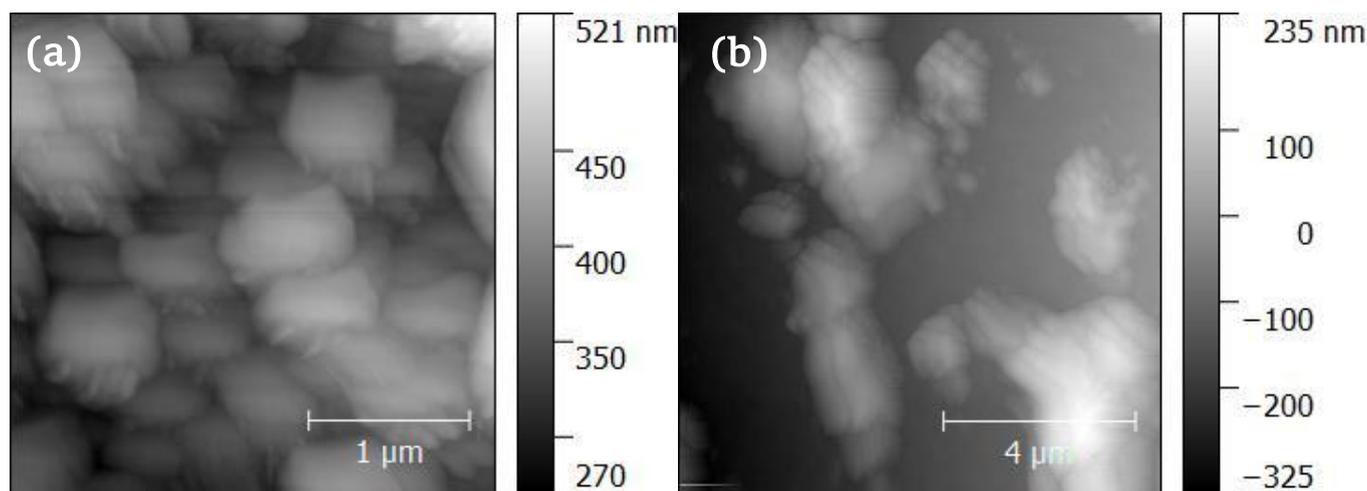
**Table 1** Diameter ( $D$ , nm) of aggregates in solutions of ChT and SChT in the presence of glycerol

Polymer	Polymer concentration, g/dl	Glycerol concentration, mol/l	$D$ , nm	
ChT	0.1	-	142±7	
		1.0	250±13	
		3.0	327±16	
	0.5	-	163±8	
		1.0	271±14	
		3.0	358±18	
	1.0	-	178±9	
		1.0	328±16	
		3.0	445±22	
	SChT	0.1	-	164±8
			1.0	197±10
		0.5	3.0	266±13
-			194±10	
1.0		1.0	250±13	
		3.0	342±17	
		-	221±11	
		1.0	387±19	
			528±26	

The use of polyatomic alcohols, for example, glycerol, leads to changes in the size of aggregates: so, as can be seen from the data in Table 1, in the presence of glycerol, the size of the resulting aggregates is significantly larger than in its absence. It can be assumed that the composition of polysaccharide aggregates when using polyatomic alcohols includes solvent molecules, i.e. the aggregates are intermolecular in nature due to the ability of polyatomic alcohols to "cross-link" macro chains.

The features of the supramolecular state of polymers in the solution in the presence of polyatomic alcohols are preserved during the transition to films. For example, as can be seen from atomic force microscopy data (Fig. 1), in film samples (as well as in solution) the size of supramolecular formations in the presence of polyatomic alcohols is larger than in their absence.

At the same time, the addition of polyhydric alcohols to films based on ChT and SChT leads to a sharp drop in the elasticity modulus of the films (Table 2), which may indicate their plasticizing effect.



**Fig. 1** Phase contrast AFS images of the surface of ChT films obtained from initial solutions in the absence of glycerol (a) and in the presence of glycerol with a concentration of 2 mol/l (b)

**Table 2** The modulus of elasticity of films of SChT films with a thickness of 0.1 mm with the addition of glycerol

Glycerol content in the initial solution, mol/l	$E_{eff}$ , MPa
0	2300
0.03	1.51
0.05	0.57

The physico-mechanical properties of films in the presence of polyatomic alcohols undergo the most serious changes.

The general character of the physico-mechanical behavior of the polymer body is determined by its phase state: amorphous and crystalline. ChT and many of its derivatives are the typical polymers being in the crystalline state and undergoing brittle damage [14]. However, as it follows from Fig. 2 data, SChT in contrast with ChT can reveal forced-elastic deformation. Such changes of the properties may be connected with some change in the molecule package density of SChT film stipulated by the presence of the comparatively voluminous salt groups in its structure; they also may cause a plasticizing effect as a result of intermolecular interaction weakening and the segmental macromolecules mobility increase. But, in spite of this, the level of the forced-elastic deformation in SChT films is not enough for the use of unmodified films based on it as the wound healing coatings. For example, the value of the tensile stress of the human skin is in the range of 20–30 MPa and the value of the tensile elongation makes up 40–80% (depending on the age, sex and the section from which the skin for the study was taken).

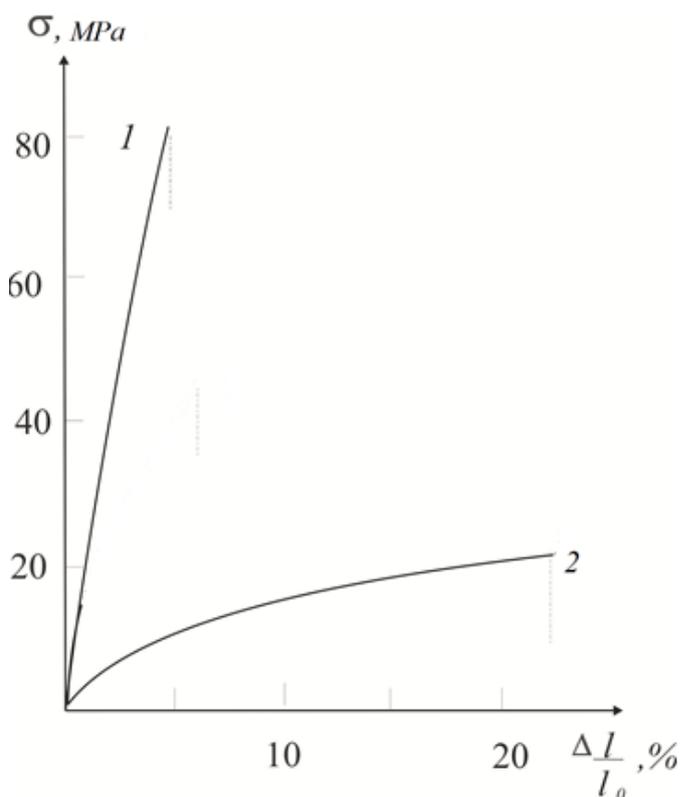
Thus, the problem of obtaining elastic film materials based on ChT and SChT has not been solved, which limits its use in the medical practice. One of the ways of regulating the elastic properties of the films based on ChT is the plasticizer use.

It is well known that the polymer plasticizers are the substances able to shift the glass transition temperature to the area of lower temperatures [10]. The fact that polyatomic alcohols play the role of plasticizers is evidenced by

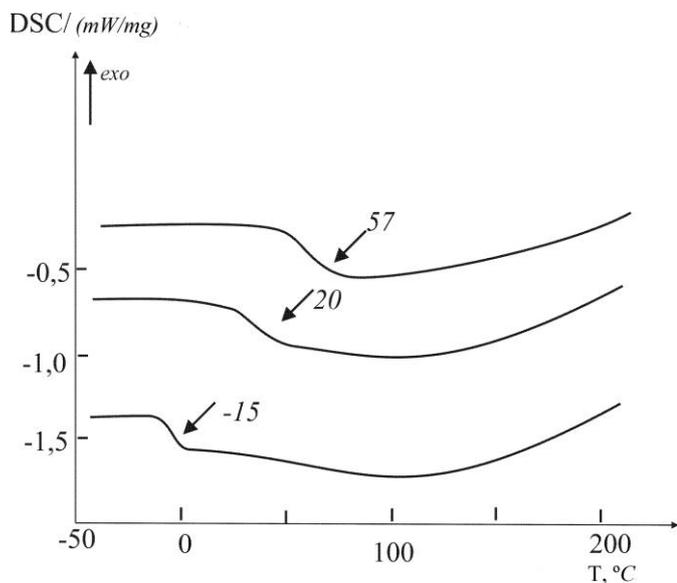
the DSC data presented in Fig. 3. It can be seen that the values of relaxation transition temperatures in films formed in the presence of polyatomic alcohols decrease.

Very often plasticizers are introduced not only for the purpose of glass transition temperature reduction, but also for the reduction of the elasticity module, the elasticity increase, namely, the material deformation at the mechanical force impact in all the three physical states.

As the performed studies demonstrated, the introduction of polyhydric alcohols in the film forming process leads to a significant improvement of their physical and mechanical characteristics.



**Fig. 2** Stress-strain relationship for ChT (1) and SChT (2) films



**Fig.3** DSC curves of ChT films obtained from initial solutions in the presence of glycerol with a concentration of 0, 0.05 and 0.15 mol/l (from top to bottom)

Thus, it is clearly seen from Fig. 4 that an increase in the content of glycerin and propylene glycol in the initial solutions during the production of films based on ChT and SChT to 0.05 mol/l is accompanied by a significant increase in discontinuous elongation. In this case, the values of the breaking voltage naturally decrease. But, since the values of the breaking stress in any case remain at the required level, the observed drop in strength indicators is not of a fundamental nature. Similar results were obtained for films in the presence of ethylene glycol. An increase in the content of polyatomic alcohol in the initial solutions on the basis of which the films were obtained, more than 0.05 mol/l, is accompanied by a sharp loss of strength and it is not possible to remove deformation and strength indicators for them.

The fact of the elasticity increase of the films based on ChT and SChT in the presence of polyhydric alcohols may have the following explanation. The films were prepared

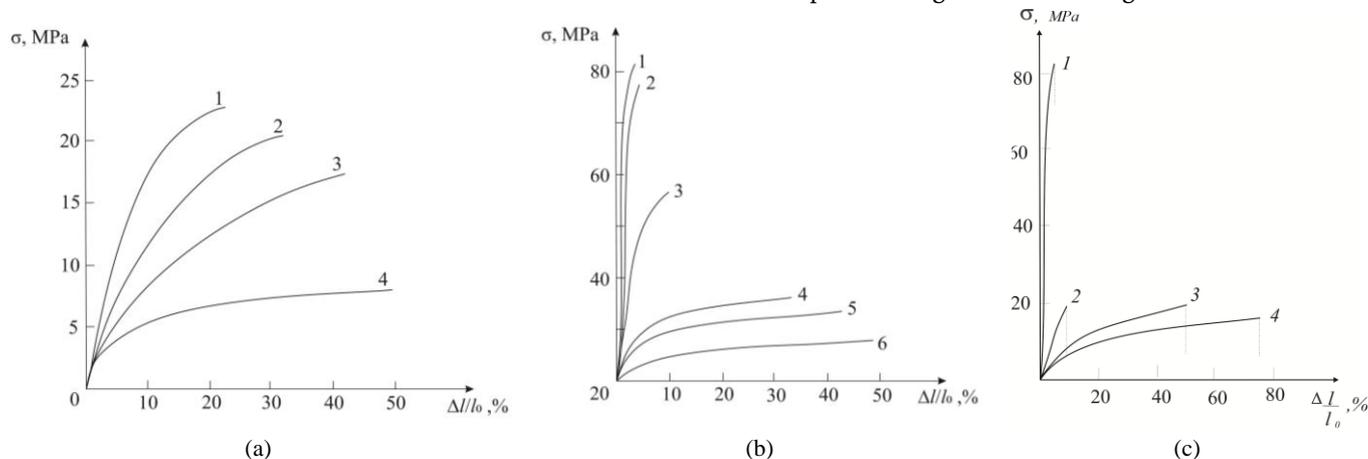
from solutions of polyelectrolytes–chitosan N-succinyl sodium salt (polyanion) and chitosan acetate (polycation). The dissolution of polyelectrolytes is accompanied by dissociation. Thus, the charged macromolecules become solvated molecules of the solvent that leads to the decrease of the interactions among the macromolecules. But in the film formation, the solvent evaporates and the intermolecular interaction among the chains increases. If in the process of dissolution polyhydric alcohols are added to SChT (ChT)-water system, the situation changes. Taking into account a high boiling temperature of the alcohol, it remains in the film even after removing the solvent. As a result, it is the decrease of polymer-polymer intermolecular interaction in comparison with the initial unmodified film that leads to the increase of the molecular mobility of the whole system. This causes the change of the whole complex of physical, chemical and physico-mechanical properties of ChT and SChT-based films.

The construction of generalizing dependencies (Fig. 5) of the values of breaking stress and breaking elongation on the content of the modifying additive in the initial solution showed that for both studied polymers their introduction into the molding solution leads to a natural decrease in the values of breaking stress and an increase in the values of breaking elongation.

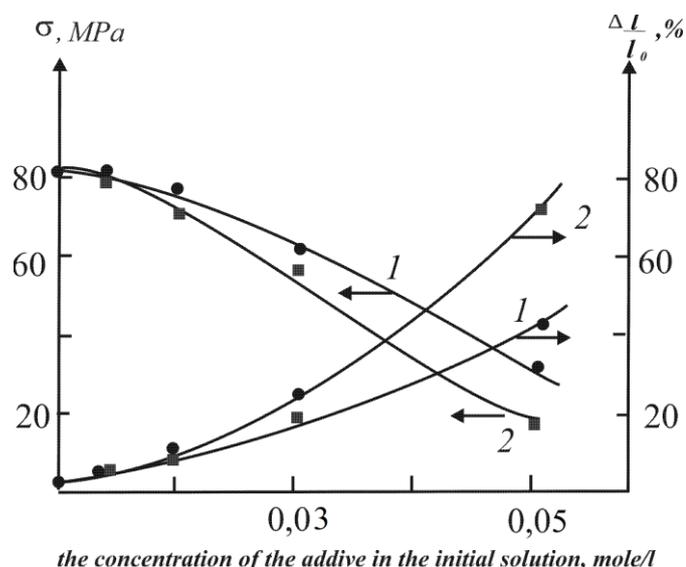
In fact, it is not possible to obtain a film with satisfactory strength from a solution containing polyatomic alcohol at a concentration above 0.05 mol/l.

Undoubtedly, the materials recommended for the medical purpose must undergo a complex study of the living organism's response to their use. Since the components of film materials based on ChT and SChT in contact with body tissues can enter into the bloodstream in dissolved form, it is advisable to study changes in blood parameters under their action.

The functional state of the erythrocytes' membranes is the most successful model for the analysis of the dynamics of many destructive changes taking place in the organism at the impact of exogenous and endogenous factors.



**Fig. 4** Tensile stress-strain curves for films obtained from initial solutions in the presence of polyhydric alcohols. The films based on SChT (1) and SChT in the presence of glycerol with a concentration of 0.03 (2), 0.04 (3), 0.05 (4) mol/l (a). The films based on ChT (1) and ChT in the presence of glycerol with a concentration of 0.03 (2), 0.04 (3), 0.05 (4), 0.075 (5) и 0.1 mol/l (6) (b). The films based on ChT (1) and ChT in the presence of propylene glycol with a concentration of 0.03 (2), 0.04 (3), 0.05 (4) mol/l (c).



**Fig. 5** Dependence of the breaking stress and breaking elongation of films based on the ChT- glycerol (1) and ChT-propylene glycol (2) systems on the concentration of the modifying additive in the initial solution

Physiological properties, such as deformity, osmotic resistance and the ability to aggregate, providing the movement of erythrocytes through the bloodstream and, as a consequence, the oxygen transport to organs and tissues, are determined by the lability of the erythrocyte membrane. Besides, metabolic processes, taking place in erythrocytes at the stress in the clinical pathology, are an integral reflection of the cells' reaction to the level of the whole organism. In the clinical practice, the most frequently used test for the qualitative characteristics of erythrocytes is the determination of the osmotic resistance of erythrocytes by the osmotic lysis model. The osmotic resistance of erythrocytes is an important integral physiological cell function, the changes of which are widely used as a marker reflecting the cell membrane state.

Thus, the influence of the systems ChT and SChT with polyhydric alcohols on the resistance of erythrocyte membranes to osmotic lysis was studied in vitro. Our study showed that in the control series of sodium chloride solutions, lysis of erythrocytes occurred in the range of 0.4–0.5% NaCl, which corresponds to the literature data.

A comparison of the studied samples based on SChT showed that, in combination with ethylene glycol and propylene glycol, SChT does not affect the stability of the erythrocyte membrane (it does not change the degree of hemolysis) over the entire range of NaCl concentrations. The minimum limit of osmotic resistance of erythrocytes, as in the control sample, is observed in physiological saline with a concentration of 0.45–0.50% (Table 3). In the range of low and high NaCl concentrations, the intensity of hemoglobin release from erythrocytes does not statistically significantly differ from the control values.

SChT in combination with glycerol has a pronounced membrane-stabilizing effect both in the zone of hypotonic and physiological concentrations of NaCl solutions. Moreover, the initial signs of hemolysis appear at higher concentrations of NaCl (about 0.5–0.6%) than in the control. At the same time, there is a two-fold decrease in the intensity of hemolysis relative to the control values at physiological NaCl concentrations of 0.7–0.8%.

Of particular note is the fact that unmodified SChT causes a slight destabilizing of erythrocyte membranes.

When studying the effect of ChT (Table 3), it was shown that both ChT itself and all ChT solutions in the presence of modifiers have a pronounced positive effect (especially when glycerol is used as a modifier). A decrease in the degree of erythrocyte hemolysis was observed both in hypotonic NaCl solutions and at physiological NaCl concentrations.

**Table 3** The values of the hemolysis degree ( $X$ ) under the action of a 1% polymer solution containing polyhydric alcohols with a concentration of 3 mol/l

Polymer	NaCl, %	$X$ (%)				
		Negative control	-	Ethylene glycol	Propylene glycol	Glycerol
SChT	0.1	99	98	98	98	97
	0.2	97	98	97	98	95
	0.3	96	98	96	97	90
	0.4	65	78	67	70	78
	0.5	21	31	25	28	75
	0.6	10	12	8	10	34
	0.7	7	8	6	7	3
	0.8	5	7	5	6	2
ChT	0.1	99	98	98	98	96
	0.2	97	96	96	96	95
	0.3	96	94	94	94	93
	0.4	65	64	65	65	58
	0.5	21	19	20	20	18
	0.6	10	9	10	10	8
	0.7	7	6	7	5	3
	0.8	5	5	5	3	2

According to the present opinion, the erythrocytes' stability to the osmotic lysis is determined to a high extent by the permeability of the membrane for water being regulated by the presence of aquaporins, the specific integral albumen of erythrocyte membranes. We may assume that polyhydric alcohols in combination with ChT and SChT block the action of aquaporins, reducing the water inlet into the cell and, at the same time, increasing the cell membrane resistance.

Thus, the stabilizing impact of SChT (ChT) – polyhydric alcohol systems upon the cell membranes in the physiological conditions allows inferring their high hemocompatibility.

#### 4. Conclusions

It was stated that succinyl chitosan, in contrast with chitosan, can reveal a slight forced – elastic deformation, but this is not enough for the use of unmodified film based on it as the wound healing coatings.

The presence of polyhydric alcohols (glycerol, ethylene glycol and propylene glycol) in molding solutions of N-succinyl chitosan and chitosan leads to an increase in the size of aggregates of macromolecules both in solution and in films based on them, as well as to a decrease in the overall surface roughness and to a sharp drop in the elastic modulus of films.

The use of polyhydric alcohols for molding solutions in the production of films based on chitosan and chitosan N-succinyl in an amount of up to 0.05 mol/l is accompanied by an increase in the value of tensile elongation by almost 2–2.5 times. The value of the breaking voltage is reduced by 3–4 times. The optimal content of polyatomic alcohols as plasticizers is 0.03–0.04 mol/l.

The stabilizing effect of systems based on chitosan and succinyl chitosan in the presence of polyhydric alcohols on the cell membranes in the physiological conditions indicates their high hemocompatibility.

#### Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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