STRUCTURE AND PROPERTIES OF CHITOSAN-BASED FILMS FOR BIOMEDICAL PURPOSES

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The sorption, elastic–plastic, surface structural, bactericidal, and medicinal properties of films made of different chemical forms of chitosan were studied. Conversion of the polymer from polycationic to polybasic form resulted in changes in the sorption ability, strength and elastic characteristics, and surface structure. Films made of polybasic chitosan, as distinct from polycationic ones, did not inhibit *Escherichia coli* and *Staphylococus aureus* growth. Fairly high adhesion and proliferation activity of MA-104 epitheliocytes were found on the film substrates examined. The effectiveness of our chitosan film matrices in the treatment of second- and third-degree burns was assessed clinically. The use of chitosan films significantly promotes the process of wound healing (in comparison with traditional therapy), creates an optimal medium for regeneration, and protects wounds from infection and traumas.

Keywords: chitosan of various chemical forms, film, sorption kinetics, elastic–plastic properties, surface structure, bactericidal properties, cultivation of epithelium-like cells, burn treatment

In the past few years, a large number of new pharmacological preparations and cosmetics using the amino polysaccharide chitosan have appeared [1–4]. Such properties of chitosan as biocompatibility with living tissues, functional similarity to dermal components *in vivo*, biodegradability, and ability to act as a biocide determine the further development of novel medical–biological materials on its basis, namely film wound dressings, matrices as carriers of epithelium-like and epithelial cells, matrices for controllable liquation of medicines, and other materials [5–11].

The macromolecule of chitosan has a heterochain structure and is built up from *D*-glucosamine units (mainly) and *N*-acetyl-*D*-glucosamine units linked by β-1,4-glycoside bonds. The presence of an amino group in the elementary unit leads to two possible chemical forms of chitosan in ready films, namely the polycationic and the polybasic form, depending on the preparation technique used. Polycationic chitosan is well soluble in water, whereas the polybasic form is hydrophobic. Films made of these two forms of chitosan differ in other physicochemical characteristics as well.

The aim of this work was to study and comparatively analyze the structure and properties of biomedical-purpose films made of different chemical forms of chitosan.

Materials and methods of research

Powdered chitosan (Bioprogress Corp., Russian Federation) was used. The physicochemical characteristics of the samples are given in Table 1.

Table 1

Properties of powdered chitosan samples

Sample	Molecular mass (kDa)	Deacetylation degree DD (mol%)	$[\eta]^{25°C}$ in acetate buffer (dl/g)	Powder density $\rho_н$ (g/cm^3)	Moisture content W (%)
CTS-87	87	83,6	2,0	0,34	6,5
CTS-200	200	82,0	3,7	0,32	9,7
CTS-280	280	80,8	5,4	0,24	10,8
CTS-550	550	81,0	8,2	0,25	9,9
CTS-640	640	82,6	11,1	0,16	10,9

Films made of polycationic and polybasic chitosan served as the objects of study. These were formed by the dry technique by casting a polymer solution onto a polyethylene support. The chitosan concentration in the working solution was 2 g/dl. A 2% aqueous solution of acetic acid was used as a solvent. The films were formed at room temperature and normal atmosphere pressure for 3–4 days. The readiness of the films was determined visually by the separation of the film sample from the support. In fresh films, the polymer was polycationic. To make it polybasic, we soaked the film samples in a 1 N NaOH solution or in a 50% aqueous solution of triethanolamine (TEA) for 1 h. Then, the samples were washed with distilled water to achieve a pH value of 7 and were dried at 22 ± 2°C. The moisture content of the initial film samples did not exceed 20–22%. The formed films are characterized in Table 2.

Sorption properties were examined at 22 ± 2°C and at 37°C. Distilled water, its vapors, and the vapor medium above a 0,5 N HCl aqueous solution (chosen to imitate wound exudates) were used as sorbates. The sorbate vapor experiment has been described elsewhere [12]. The degree of polymer sorption of vapors (C_s, wt%) was estimated gravimetrically on an OHAUS Discovery DV215CD (USA) and an E. Mettler Zurich (Germany) analytical balance, with an accuracy of ± 0,0001 g. C_s per absolutely dry film was calculated with account taken of the conditional moisture content of the film sample. There were not less than three replicates.

Table 2

Properties of chitosan films

Sample	Chemical form	Base	Thickness d (μm)	Moisture content W (%)
CTS-87	C	-	45	19
	O	NaOH	80	16
		TEA	70	17
CTS-200	C	-	50	20
	O	NaOH	85	15
		TEA	70	17
CTS-280	C	-	50	20
	O	NaOH	85	16
CTS-550	C	-	50	21
	O	NaOH	90	17
		TEA	70	18
CTS-640	C	-	55	22
	O	NaOH	95	17

Physicomechanical properties were examined on a TiraTest 28005 uniaxial tensile testing machine (Germany) with a loading cell of 100 N. The ultimate tensile stress (σ, MPa) and relative elongation (ε, %) were calculated with account taken of the cross-section area and the initial length of the film sample, respectively. The values of σ and ε were obtained by averaging the results (not less than five samples). The Young modulus (E, MPa) was estimated as the σ/ε ratio, and the tension modulus (E_0, MPa) was calculated from the slope of the initial straight segment of the $\sigma = f(\varepsilon)$ tension curve. When calculating E and E_0, we expressed ε in unit fractions.

The film surface structure was examined on a Solver P47-PRO scanning probe microscope (NT MDT, Russia). A 50 μm × 50 μm × 3 μm scanner and cantilevers were used for contact and noncontact microscopy.

The bactericidal properties of the chitosan films were tested on a gram-negative (*Escherichia coli*) and a gram-positive (*Staphylococus aureus*) microorganism grown on AGV solid nutrition medium. The exposure time was 18 h. Bactericidal activity was estimated by the diameter of the inhibition zone for the microbial culture at the places to which film samples were applied.

For estimating the biocompatibility of the film matrices, a transformed cell line of the fetal epithelium of the rhesus monkey kidney, MA-104 (collection of the Virology Institute of the RAMS, Moscow), was used as a test culture. A cell culture grown in Costar plates was used as a reference. Cell adhesion and proliferation were observed with a Biolam P inverted-stage microscope (Russia).

Clinical tests were done at Urban Clinical Hospital No. 7 (The Thermal Wound Center), Saratov, in accordance with GOST (State Standard) P 52379-2005, «Good Clinical Practice». For these tests, films were prepared under aseptic conditions and were additionally sterilized by UV radiation for 1 h.

Results of research and their discussion

Both polycationic and polybasic chitosan films are known to possess high moisture absorption [13, 14]. As polycationic chitosan is water soluble, sorption was performed in a vapor phase for a comparative analysis of the sorption properties of films made of the different chemical forms of chitosan. Fig. 1 shows the kinetics of sorption of H_2O vapors and the vapors above an aqueous solution of 0.5 N HCl by both chitosan forms at 22 ± 2 °C. It can be seen that polycationic chitosan films, in contrast to polybasic ones, were characterized by unlimited sorption kinetics and did not reach an equilibrium swelling degree. After about 72 h of exposure to the vapor medium, the acetate chitosan films started to dissolve (the dashed line). The total sorption degree of water vapors is always higher than that of the vapors above a hydrochloric acid solution. It also should be noted that the sorption degree of polycationic films is about four to six times higher than that of polybasic films. However, after losing their hydrophilicity, modified polybasic chitosan films acquire specific sites of binding to the receptors of cell cultures (see below).

For polycationic chitosan, the sorption rate and the maximum value of Cs were higher for the films made of high-molecular weight polymer samples (Fig. 2).

For polybasic chitosan films, increasing the molecular mass of the polymer did not change the character of the swelling curve and affected the sorption degree only slightly (Fig. 3). The sorption rate was always maximal at the initial stage ($t < 30$ min). The reagent (an inorganic or organic base) in chitosan conversion to the basic form did not change the character of $C_s = f(t)$ either, but it influenced the maximum value of C_s. For instance, a polybasic chitosan film obtained by NaOH treatment sorbs, on the average, about 20 wt% more vapor than does a TEA-treated film. Unless otherwise specified, further work used basic films prepared by modification in an NaOH solution.

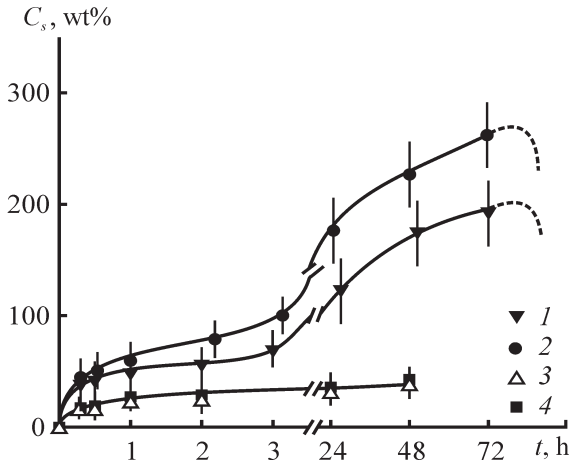


Fig. 1. Kinetic curves for the sorption of vapors of 0.5 N HCl (1, 3) and H_2O (2, 4) by polycationic (1, 2) and polybasic (3, 4) CTS-87 films at 22 ± 2°C. NaOH was used to convert CTS to the polybasic form. The dashed line shows the onset of film dissolution

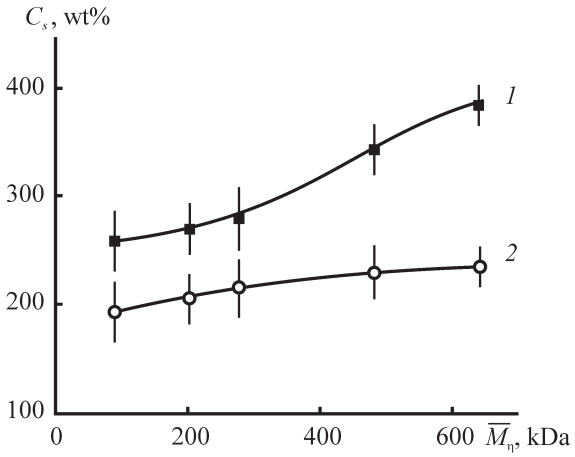


Fig. 2. Maximum degree of sorption of vapors of water (1) and 0,5 H HCl (2) by polycationic chitosan films as a function of the polymer's molecular mass; T = 22 ± 2°C

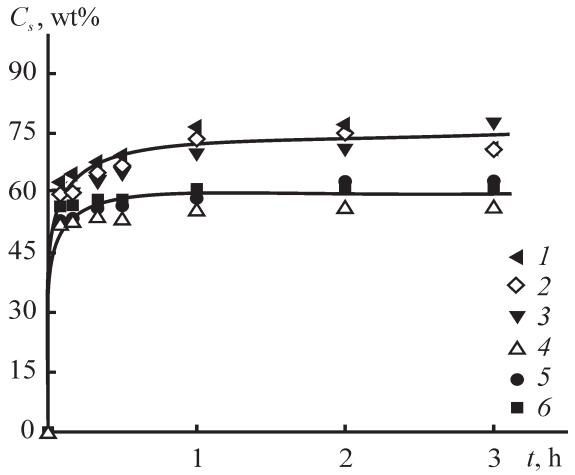
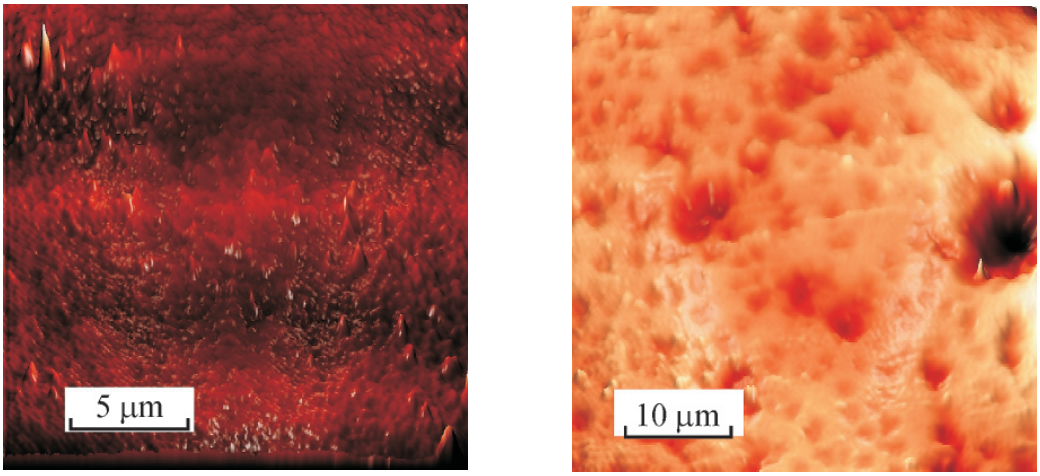


Fig. 3. Swelling kinetics of the films made of CTS-87 (1, 4), CTS-200 (2, 5), and CTS-550 (3, 6) and converted into the polybasic form with NaOH (1 - 3) and TEA (4 × 6) in water; T = 22 ± 2°C

As chitosan materials are promising for biomedical applications, the sorption and diffusion characteristics of the film samples under study were estimated at 37°C. The sorption curves for the differing chemical forms of chitosan were similar, as in our previous experiments. However, increasing the temperature somewhat reduced the rate and degree of sorption of sorbate vapors.

UV sterilization of the different chemical forms of chitosan films did not affect their sorption properties.

The differences in the chemical structure of chitosan were reflected in the surface structural characteristics of the film samples. The results of scanning probe microscopy showed that the surface structures of chitosan films in the different chemical modifications differed essentially in their morphological relief. For example, polycationic films had a densely packed structure with microspikes (Fig. 4 a), whereas in polybasic films, a porous structure with pore diameters of ~1–3 μm was observed (Fig. 4 b). There were bulges on the surface of the films of both types. With account taken of these differences in the surface relief, it was supposed that the morphological features of the film samples should have reflected on other physicochemical properties, e.g., the elastic–plastic characteristics.



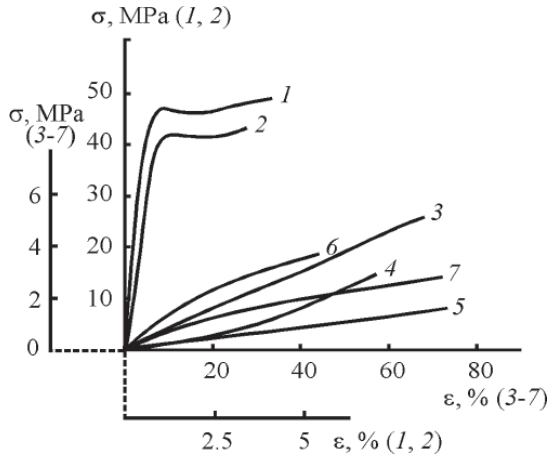
a b

Fig. 4. A topographic image of the morphological surface structure of films made of CTS-87 in the polycationic (a) and the polybasic (b) form. Scanning probe microscopy. NaOH was used to make CTS polybasic

Chitosan conversion from polycationic to polybasic form almost did not change the character of deformation of the film samples. The tension curves of both forms of chitosan films displayed portions with elastic and plastic deformation (Fig. 5 a, curves 1, 2). However, alkaline treatment deteriorated both strength and elasticity of the films. Polycationic films, unlike polybasic ones, were characterized by higher values of ultimate tensile stress and elongation at fracture and also by higher values of the Young and elasticity moduli (Table 3). A similar character of σ variations for chitosan films with = 86 and 31 kDa in the polycationic and the polybasic form was noted in Ref [15].

Visually, the elasticity of the film samples increased when they were held in the sorption media (Fig. 5 b). In this connection, the elastic–plastic characteristics of polybasic chitosan films having absorbed various quantities of liquid water were studied. The shape of the deformation curves obtained for swollen films attested to the development of plastic deformation (in the mode of forced deformation) in these samples (Fig. 5 a, curves 3–7). The value of elongation at fracture for the films having absorbed water exceeded the elongation value of the initial chitosan films in any form by 10–20 times (Table 3) [16]. The use (with other conditions being equal) of an organic base to change chitosan from the polycationic form to the polybasic one resulted in a higher value of ε for the swollen film samples (see, e.g., curves 4–7). This makes the modified film sample capable of modeling a complex-relief surface. In each case, the values of ultimate tensile stress and the Young and elasticity moduli decreased in comparison with those for both forms of the initial films (Table 3).

The bactericidal properties of both chemical forms of chitosan films were studied by using the microorganisms *E. coli* and *S. aureus* (major infecting agents found in wound exudates) as examples. Polycationic film samples were found to possess bactericidal activity and to inhibit the growth of cultures of the gram-negative and gram-positive microorganisms. Polybasic samples were not inhibitory to *E. coli* or *S. aureus* under the experimental conditions used.



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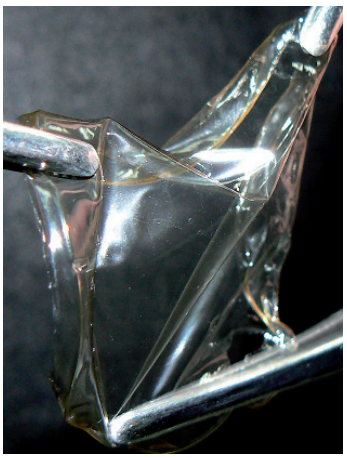

b

Fig. 5. (a) Elongation curves of the films made of CTS-87 (1, 3–5) and CTS-200 (2, 6, 7) in the polycationic (1, 2) and the polybasic (3–7) form, after the absorption of 60 (3), 90 (5, 6), and 95 wt% H_2O (4, 7). NaOH (3, 4, 6) and TEA (5, 7) were used to make CTS polybasic. (b) Photo of a film made of CTS-280 acetate after modification in a vapor medium formed by 0,5 N HCl at 20°C

Table 3

Deformation and strength properties of the chitosan films

Sample	Chemical form	Base	H_2O sorption degree C_s (wt%)	Ultimate tensile strength σ (MPa)	Elongation at fracture ε (%)	Young modulus E (MPa)	Elasticity modulus E_0 (MPa)
CTS-87	C	-	-	49 ± 4	4 ± 3	$1,2 \cdot 10^3$	$4,4 \cdot 10^3$
	O	NaOH	60	6,5 ± 0,5	69 ± 1	9,4	25
			95	3,6 ± 0,5	52 ± 5	6,9	4,0
		TEA	90	2,0 ± 0,5	78 ± 4	2,6	2,5
CTS-200	C	-	-	44 ± 3	3 ± 1	$1,5 \cdot 10^3$	$4,0 \cdot 10^3$
	O	NaOH	90	3,2 ± 1,2	45 ± 3	7,1	40
		TEA	95	2,8 ± 1	68 ± 5	4,1	10

Thus, it can be concluded that polycationic and polybasic chitosan films differ in the nature of sorption kinetics, strength and elastic properties, surface structure, and bactericidal activity. The totality of these properties satisfies the requirements placed upon wound dressings (film matrices).

To assess the biocompatibility of the chitosan matrices, we examined the adhesion and proliferation of a model culture, MA-104, on films made of CTS-87 and CTS-200 in the polycationic form. As can be seen in Fig. 6, high adhesion and proliferation of the cell culture were observed as early as after 24 h of incubation. Depending on the molecular mass of the polymer, the formation of a cell monolayer was observed on day 3 or 4 of incubation. The best cell growth was recorded for the films made of CTS-200.

We investigated the effectiveness of using chitosan film matrices in the treatment of second- and third-degree burns. Various film modifications were applied, namely polycationic, polybasic, and polycationic after additional modification in water vapors (sorption degree, 90–100 wt%). Burns were treated by the closed method (films were fixed with bandages) in three groups, each consisting of 5 volunteer patients. Open wounds were inspected every 2–3 days. The effectiveness of the film action was evaluated by the character of the wound-process course, the degree of manifestation of the inflammation reaction, and the period of wound repair.

It was found that the films were convenient to use and could be easily and painlessly applied to a patient's wound. Their high moisture absorption ability and high air permeability enabled the modeling of the wound surface profile and a longer period of application to a wound, permitting the redressing frequency and the wound surface traumatization to be reduced.

Our studies showed that the use of chitosan film matrices to treat burn wounds was highly effective as compared with traditional treatment methods. The films sorbed wound exudates well. Polybasic and polycationic chi-

tosan films could be detached from the wound together with the bandage during redressing on day 2 or 3 and on day 5 or 6, respectively. When second-degree burns and intermediate third-degree ones were treated with the biodressing, complete epithelization occurred on day 5 or 6 and on day 15 or 16, respectively, no matter what chemical form of the matrix was used. In traditional therapy (a bandage impregnated with an antiseptic), second-degree burns and intermediate third-degree burns are fully healed on days 14 and 21, respectively, i.e., slower by 1,5–2 times. The treatment was accompanied by a 2–2,5-fold reduction in the bacterial infection level of the burn wound, as compared with what is observed in traditional therapy. No allergic reactions or irritating effects were observed.

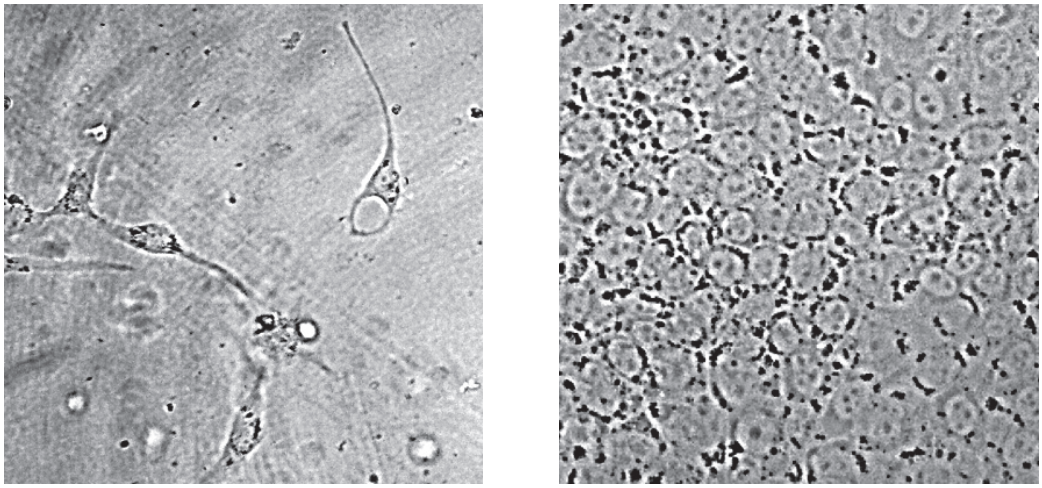

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Fig. 6. Epithelium-like cells of the rhesus monkey kidney, MA-104, after 1 (a) and 7 (b) days of cultivation on a CTS-87 film in the polybasic form. Light microscopy; magnification, ×400

Thus, chitosan films can be regarded an effective wound dressing for the treatment of burns and other surface wounds.

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