Physicochemical Properties of Intravitreal Implant Based on Chitosan/Polyvinyl Alcohol Saturated with 5-Fluorouracil

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The aim of the investigation was to evaluate the possibility of using intravitreal introduction of the implant based on chitosan-polymer film saturated with 5-fluorouracil.

Materials and Methods. There was performed a study of physicochemical properties of chitosan/polyvinyl alcohol-based implant saturated with 5-fluorouracil (5-FU) in concentrations of 0.05 and 0.1 ml. The implant size was 8.0×1.0×0.35 mm. The process of drug release was studied by UV spectroscopy based on the characteristic absorption maximum. To assess the sterilization ability of chitosan films, their thermal stability was studied. The possibilities of interaction between chemical groups of chitosan, polyvinyl alcohol and 5-FU were studied by analyzing the infrared spectra of these substances.

Results. It was established that the process of drug release from the system occurs in three stages: 1) sorption of water by the film and swelling; 2) the drug diffusing in the film to the interphase boundary between the polymer system and the environment; 3) the drug diffusing into the solvent volume. Release of 5-FU from the implant to Ringer–Locke solution occurs almost completely within 7–8 h without undergoing any change. Thermal degradation of the implant begins at the temperature of 200°C. Infrared spectroscopy data evidence that 5-FU immobilized on chitosan film with polyvinyl alcohol undergoes no chemical changes and, consequently, does not lose its pharmacological properties.

Conclusion. The study of physicochemical properties of chitosan/polyvinyl alcohol-based implant saturated with 5-FU cytostatic proves the feasibility of its use in ophthalmology to reduce the initial peak concentration of 5-FU and, consequently, eliminate its toxic effects and prolong the drug action.

Key words: proliferative vitreoretinopathy; 5-fluorouracil; 5-FU; chitosan; intravitreal implant; chitosan-based implant.

Proliferative vitreoretinopathy (PVR) is one of the most severe eye diseases and a serious medical and social problem. PVR is considered to be a typical pathological process within the eye characterized by local scarring as means of eliminating tissue alteration occurring during such an ophthalmic diseases as retinal detachment, hemophthalmia, trauma, diabetes [1].

In vision disability, PVR accounts for 2–9% and 84–89% of those suffering from the disease are persons of working age [2–4]. Proliferative vitreoretinopathy requires complex surgical treatment which is often performed in several stages [5]. If the disease remains untreated, blindness occurs in 100% of cases [6–8].

In recent years, progress has been made in retinal detachment surgery, which allowed reducing the number of intra- and postoperative complications, significantly improving anatomical and functional results of operations for this pathology. However, despite the new level of modern diagnostic possibilities and a significant step forward in the field in vitreoretinal surgery, the number of successful operations, according to many authors, is only 61.5 to 97.5% [9–13].

PVR progression in the postoperative period is one of the main causes of unsuccessful surgical treatment for retinal detachment and it is observed in 2.2–29.4% of cases [9, 11, 13, 14]. Retinal detachment recurrence due to PVR progression occurs in 2.20 to 20.0% of cases [11,
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13, 15–17). Therefore, the search for and development of new treatments reducing surgery invasiveness and the risk of operative and postoperative complications is under way.

At present, there have been new trends in PVR treatment such as application of antiproliferative agents on various carrier implants used during intravitreal interventions. In recent years there has been a growing interest of specialists for applying as a carrier chitosan polymer material which is completely destroyed and absorbed by the body, has anti-inflammatory and antiproliferative effect, high biocompatibility, improves regeneration of cells and tissues [18–20]. Kazakh Scientific Research Institute of Eye Diseases in cooperation with the laboratory of polymer synthesis, A.B. Bekturov Institute of Chemical Sciences (Kazakhstan), developed the vitreosynthetic Vitrenal which is an aqueous solution of chitosan polymer [6]. Clinical studies have confirmed the efficacy of intravitreal introduction of Vitrenta in surgical treatment for PVR with retinal detachment and traumatic eye injuries.

Currently, one of the trends in vitreoretinal surgery is the use of medicines whose action is aimed at inhibiting scar tissue formation, in particular, cytostatics. The most famous representative of this group is 5-fluorouracil (5-FU) [21, 22]. This therapeutic agent is an antimetabolite inhibiting DNA synthesis and proliferation of fibroblasts. However, different authors show ambiguous results of its application, which determines the necessity of further investigations to explore the drug potential, in particular, as chitosan implant coating.

The aim of the investigation was to evaluate the possibility of using intravitreal introduction of the implant based on chitosan-polymer film saturated with 5-fluorouracil.

Materials and Methods. There was performed a study of physicochemical properties of chitosan and polyvinyl alcohol based implant saturated with 5-fluorouracil (5-FU) in concentrations of 0.05 (250 mg/g) and 0.1 ml (500 mg/g), which corresponds to 2.5 and 5.0 mg of dry matter. Polyvinyl alcohol was used to improve physicochemical properties of the films. The dosage of 5-FU was chosen for a good reason. According to the literature, 0.1–0.15 ml of 5% 5-FU is considered the optimal therapeutic dose. In the study by D.N. Sharipova [21], marked toxic changes in the eye structures were observed when a single intravitreal injection of 0.15 ml of 5-FU was administered, whereas introduction of the same dose on polyurethane carrier had no such effect. We chose the therapeutic dose (0.1 ml) and the dose 2 times lower than the therapeutic one (0.05 ml) in recognition of the fact that the implant (chitosan) by itself provides antiproliferative and anti-inflammatory action.

Scanning electron microscopy images of polymeric medicinal forms were obtained using the Superprobe-733 electron probe microanalyzer (JEOL, Japan) equipped with energy dispersive spectrometer INCA Energy (Oxford Instruments, USA). The powder was applied to the conductive tape, then, to improve image contrast, it was covered with a thin layer of gold using the Fine Coat installation (JEOL, Japan). Imaging was carried out in the mode of secondary electrons.

To determine the kinetics of 5-FU release from the chitosan film, a special device consisting of a metal basket, a thermostatic glass and a mechanical stirrer was used. The drug release was studied in vitro. For this, a certain quantity of chitosan films was placed in a metal basket immersed in 70 ml of water at room temperature. Constant rate of release medium stirring (100 rpm) was provided with a magnetic stirrer, temperature control was maintained using a flow cell. At certain time intervals, 2 ml of solution was taken to determine the drug content by UV spectroscopy.

To assess the ability of the implants for sterilization, their thermal stability was studied by thermogravimetric analysis using the TGA/SDTA 851 device (Mettler Toledo, Switzerland).

To evaluate the possibility of chemical interaction between chitosan, polyvinyl alcohol, and 5-FU, infrared spectra of samples of these substances were recorded and analyzed. IR spectra were recorded on the Nicolet 5700 FT-IR spectrophotometer (Thermo Electron, USA) with Fourier transform in the region of 4,000–400 cm⁻¹. The samples were prepared in the form of tablets with KBr crystals.

Results and Discussion

Scanning electron microscopy. Scanning electron microscopy of chitosan and the implant was performed using the Superprobe 733 electron probe microanalyzer equipped with energy dispersive spectrometer INCA Energy (Figure 1). The smooth surface of chitosan was clearly visible, the implant had a random distribution of large structures with pores of the same size, coming up to 42.68 nm. Thus, the implant had mesopores.

The release of 5-FU from the implant. To assess the prolonged release properties, 5-FU release from chitosan-polyvinyl films cross-linked with glutaraldehyde was studied in vitro. The release process was studied by UV spectroscopy based on the characteristic absorption maximum of the drug at A=266 nm. The spectra were detected on the Jasco UV/VIS 7850 spectrophotometer (JASCO International, Japan) in quartz cuvettes of 10 mm thickness at 25°C. The amount of 5-FU was identified with the calibration graph of optical density versus concentration. Freshly prepared Ringer–Locke solution was used as the environment for release.

It was established that the process of drug release from the system consists of three stages: 1) sorption of water by the implant and its swelling; 2) the drug diffusing in the implant to the interphase boundary between the polymer system and the environment; 3) the drug diffusing into the solvent volume.

Chitosan films with 250 and 500 mg/g of 5-FU were submerged into the solution to determine the effect of the drug dose (concentration) on release kinetics. It was
noted that the change in the drug concentration in the polymer matrix had no significant effect on the diffusion process. 5-FU diffused almost completely in Ringer–Locke solution within 60–70 h (Figure 2). The obtained parameters of the release are presented in the Table.

Kinetic curve profile suggests that the drug release takes place according to first-order kinetics and is controlled by diffusion of the therapeutic agent in the matrix.

It is known that with the implant thickness increasing 3 times the drug diffusion coefficient decreases approximately 2 times. Diffusion of the therapeutic agent in the matrix plays the limiting role in the process of 5-FU release from the chitosan implant as evidenced by the inverse relationship between release rate and film thickness.

Evaluating the thermal stability of the chitosan implant saturated with 5-FU.

Thermal properties of the chitosan implant were studied using thermogravimetric analysis on the TGA/SDTA 851 device. Thermogravimetry is a method to determine mass variation when the substance undergoes controlled heat treatment. The analysis was carried out in the temperature range from 50 to 900°C with heating rate 5°C/min (Figure 3).

Thermogravimetric analysis curve for the chitosan implant with 5-FU shows that thermal degradation of the implant begins at the temperature of 250°C. In general, the degradation process occurs in the temperature range of 250–500°C.

The obtained data indicate that 5-FU-saturated implant based on chitosan and polyvinyl alcohol can be sterilized in an autoclave.

The study of interaction between chemical groups of chitosan, polyvinyl alcohol, and 5-FU.

To assess the level of interaction between chemical groups of glutaraldehyde cross-linked chitosan, polyvinyl alcohol, and 5-FU, infrared spectra of samples of these substances were recorded on the Nicolet 5700 FT-IR spectrophotometer with Fourier transform in the region of 4,000–400 cm⁻¹ and analyzed. The samples were prepared in the form of tablets with KBr crystals.

Figure 4 shows the infrared spectra of 5-FU, cross-linked chitosan films containing 5-FU, and cross-linked chitosan-polyvinyl film containing 5-FU, respectively. The main 5-FU peaks are observed in the spectrum regions.
Release of 5-fluorouracil from glutaraldehyde cross-linked chitosan-polyvinyl films in Ringer–Locke solution

| Release time (h) | 5-FU concentration (mg/ml) | 5-FU quantity (mg) | 5-FU quantity lost in the sample (mg) | 5-FU quantity found (mg) | Total 5-FU quantity (mg) | Total 5-FU release (%) |
|------------------|-----------------------------|-------------------|--------------------------------------|-------------------------|--------------------------|------------------------|
| 1                | 26.749                      | 0                 | 0.000                                | 1,872.43                | 1,872.4                  | 11.539                 |
| 5                | 48.975                      | 53.498            | 53.498                               | 3,428.25                | 3,481.7                  | 21.457                 |
| 10               | 108.963                     | 97.950            | 151.448                              | 7,627.41                | 7,778.8                  | 47.939                 |
| 15               | 156.622                     | 217.926           | 369.374                              | 9,248.50                | 9,617.8                  | 59.273                 |
| 20               | 166.232                     | 313.243           | 633.617                              | 10,516.24               | 11,149.8                 | 68.714                 |
| 25               | 183.812                     | 332.464           | 934.081                              | 10,626.84               | 11,560.9                 | 71.247                 |
| 30               | 144.038                     | 367.624           | 1,237.705                            | 12,042.52               | 13,260.2                 | 81.843                 |
| 35               | 173.336                     | 288.072           | 1,581.777                            | 12,133.52               | 13,175.2                 | 84.524                 |
| 40               | 180.184                     | 360.368           | 1,934.145                            | 12,322.88               | 14,267.0                 | 87.924                 |
| 45               | 176.416                     | 360.368           | 2,286.513                            | 12,069.12               | 14,355.6                 | 88.471                 |
| 50               | 162.932                     | 352.832           | 2,631.345                            | 12,245.24               | 14,876.5                 | 91.681                 |
| 55               | 139.156                     | 325.864           | 2,981.209                            | 12,260.92               | 15,242.1                 | 93.934                 |
| 60               | 157.848                     | 278.312           | 3,331.521                            | 12,449.36               | 15,780.8                 | 97.254                 |

Notes:
The drug (5-FU) concentration was determined using UV spectroscopy: \( y = mx + \text{intercept} \), where \( y \) is absorbance, \( m \) is slope, \( C \) is intercept, \( X \) is concentration. 2 ml of solution was taken with a pipette out of 70 ml volume bath (receiver) (where 5-FU films were placed) and tested for absorption with an interval of 5 h, the bath was filled with empty buffer.

The quantity of taken drug (5-FU) is the quantity of drug in the receiver at the time of sampling \( t \). To find the quantity of drug in the sample, each value was multiplied by the sample volume (2 ml) which was removed for testing the absorption, i.e. concentration \( 2 \text{ ml} \). For example, \( 26.749 \times 2 = 53.498 \).

5-FU quantity lost in the sample is the volume in the receiver before sampling plus the volume taken from each previous sample; for cumulative calculation, all the previous concentration values were added to the current one before taking samples after 1, 2, 3 h etc. Next, to perform cumulative calculation, we started from the second sample, i.e., cumulative drug concentration for the 2nd sample was: the 1st sample concentration + the 2nd sample concentration. Similar calculation was made for the 3rd sample: the 1st sample concentration + the 2nd sample concentration + the 3rd sample concentration and so on until the last sample. For example, \( 0 + 53.498 = 53.498 \); \( 0 + 53.498 + 97.950 = 151.448 \).

5-FU quantity found (released drug quantity) is drug concentration \( \times 70 \text{ ml} \): \( 26.749 \times 70 = 1,872.43 \); \( 48.975 \times 70 = 3,428.25 \) etc.

Total 5-FU quantity is 5-FU quantity lost in the sample + 5-FU quantity found: \( 0 + 1,872.43 = 1,872.43 \); \( 53.498 + 3,428.25 = 3,481.7 \) etc.

Total 5-FU release is the total 5-FU quantity/quantity of encapsulated drug \( \times 100\% \): \( 1,872.4/16,226.7 \times 100 = 11.539 \); \( 3,481.7/16,226.7 \times 100 = 21.457 \) etc.

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Figure 3. Thermogravimetric analysis of chitosan-polyvinyl film containing 5-fluorouracil
of 3,084 cm\(^{-1}\) (N–H), 1,689 cm\(^{-1}\) (C=O), 1,250 cm\(^{-1}\) (C=C) and 1,180 cm\(^{-1}\) (C–F) (Figure 4 (a)).

Absorbance in 5-FU-containing chitosan film at 3,127 cm\(^{-1}\) demonstrated peaks of O–H chitosan groups and N–H stretching vibrations of 5-FU that overlapped each other. C=O-vibrations at 1,683 cm\(^{-1}\) were detected in chitosan film spectrum, which also indicates the presence of overlapping.

Absorption bands 1,247 cm\(^{-1}\) (C=C) and 1,172 cm\(^{-1}\) (C–F bond) showed 5-FU presence in the film spectrum (Figure 4 (b)). The presence of azomethine group in chitosan-glutaraldehyde interaction product was identified by the peak of C=N stretching vibrations, which was easily detected in the spectra at 1,634 cm\(^{-1}\) frequency due to high extinction coefficient. This peak intensity increased with the increasing number of protonated amino-groups in chitosan.

There was observed a transition of NH\(_2\) groups to −N=C− groups, disappearance of OH\(^{-}\) groups and formation of acetal and simple ether groups in the reaction of OH\(^{-}\) groups with CH\(_2\)O.

Infrared spectra of chitosan-polyvinyl film containing 5-FU are shown in Figure 4 (c). Absorption in the area of 3,134 cm\(^{-1}\) indicates the presence of O–H group of chitosan, O–N group of polyvinyl alcohol and N–H bond of 5-FU. In the area of 1,664 cm\(^{-1}\) there appear

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**Figure 4.** Infrared spectra of 5-FU (a), glutaraldehyde cross-linked chitosan film containing 5-FU (b) and cross-linked chitosan-polyvinyl film containing 5-FU (c)
absorption bands caused by C=O bond participation in the skeletal vibrations of polyvinyl alcohol molecules in chitosan-polyvinyl film.

Figure 4 also shows glutaraldehyde effect on the chemical structure of chitosan-polyvinyl film. Decrease of OH- and C–O bonds of polyvinyl alcohol was revealed in the region of 1,090 cm⁻¹, which indicates the appearance of C–O–C group at 1,090 cm⁻¹.

The obtained data show that 5-FU and polymers are compatible. 5-FU on chitosan with polyvinyl alcohol undergo no chemical changes and, consequently, loses no pharmacological properties.

Conclusion. The use of an intravitreal implant based on natural polymer chitosan saturated with 5-fluorouracil cytostatic provides gradual release of 5-fluorouracil during 60–70 h, which reduces primary peak concentration of the drug and prevents exceeding its therapeutic concentration in the environment. The established physicochemical properties of 5-FU-immobilized chitosan implant speak of the possibility to use it in ophthalmology with the aim of prolonging the drug action. Well-known antiproliferative and anti-inflammatory properties of natural polysaccharide chitosan confirm the feasibility of its use as a medicinal substance carrier.

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Conflicts of Interest. The authors have no conflicts of interest to disclose.

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