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# EVALUATION OF CROSSLINKED CHITOSAN FILM LOADED FLUOROURACIL

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**Abstract.** The purpose of this study was to develop a crosslinked chitosan film with glutaraldehyde (GA) of controlled drug delivery. This study examines the performance of novel dosage form as chitosan (Ch) film loaded fluorouracil (FU), which were prepared by the solution casting method using 2% chitosan polymer in 1% v/v hydrochloric acid solution. The drug loading efficiency, film size and chemical compositions of the film loaded drug were confirmed by UV–vis spectrophotometer and Fourier transform infrared spectroscopy. In vitro release kinetics of drug from the polymeric films was investigated to determine the drug release properties. In vivo study was showed the efficacy and no toxicity of this formulation. Further uses of the film loaded FU may provide an efficiency deliverable for ophthalmic administration.

**Key words:** fluorouracil, glutaraldehyde, crosslinked chitosan, *in vitro* release, *in vivo* study.

**Introduction.** FU is an anti-cancer drug with a broad spectrum. However, due to toxicity its clinical use is limited [1]. The chemotherapy agent FU has been in use against cancer for about 40 years [2]. The ability of FU to reduce fibroblast proliferation and subsequent scarring has made it one of the most often used antimetabolites in ophthalmology [3].

Natural polymers, such as polysaccharides, are widely used in pharmaceutical applications due to their good biocompatibility and biodegradability. Chitosan is a widely used natural polymer in pharmaceutical formulations due to its biocompatibility [4-6].

The preparation method of chitosan film in aqueous phase by crosslinking agent GA has been studied [7-9].

Development of film dosage forms received from biocompatible polymer is actual area in pharmaceutic technology. The medical film containing various drugs widely applied in dentistry and ophthalmology. The prolonged effect in such films is reached by the immobilization of drugs on various polymeric carriers. The main advantage of medicinal films is possibility of the programmed delivery of drug by regulation of the nature of polymeric matrix. Using various methods it is possible to change the physical and chemical properties of polymer matrix and respectively the release of drug [10].

The purpose of present work is development of polymeric medicinal forms with the controlled action by immobilization of the anticancer drug FU on polymeric films. The drug release characteristics of such systems are discussed.

## Experimental

Chitosan with molecular weight of 250 kDa was purchased from Sigma-Aldrich (USA) and was used without further purification. Pharmaceutical grade fluorouracil - *5-fluoro*-2,4-(*1H,3H*)-*pyrimidinedione* - was produced by "Grindeks" (Latvia). Sodium chloride, potassium chloride and calcium chloride was purchased from Biopharma (Ukraine). Glutaraldehyde, hydrochloric acid and distilled water were used in this experiment. Glutaraldehyde was used as cross-linked agent.

10 chinchilla rabbits (range of weight from 2,5 to 3,5 Kg) at the age of 4-6 months were used. Implantation into the intravitreal implantation of polymer film loaded 5-FU was produced in amounts of 125 mg/g and 250 mg/g, with deducing them from the experience with subsequent enucleation in different periods (7th, 14th and 28th days). Distribution eyes of experimental animals in groups and period enucleation was presented in Table 1.

Table 1 – Distribution eyes of experimental animals

| Type of surgery | Group | Periods of enucleation | | |
|---|---|---|---|---|
| | | $7^{th}$ day | $14^{th}$ day | $28^{th}$ day |
| OD – partial vitrectomy + polymer film loaded 125 mg/g of 5-FU | 1 main | 3 eyes | 3 eyes | 3 eyes |
| OS – partial vitrectomy + polymer film loaded 250 mg/g of 5-FU | 2 main | 3 eyes | 3 eyes | 3 eyes |
| Intact animals | control | – | – | – |

Film was prepared by the solution casting method using FU (125 mg/g and 250 mg/g), 2 wt.% Ch solutions were prepared by dissolving Ch in 1% hydrochloric acid solution at ambient temperature with stirring for 4-5 h. The solution was filtered by filter before use. This solution was further cross-linked with 0.05 wt% of GA. The mixture was poured on the horizontal glass surface and dried for 2-3 days at 20-40°C until residual moisture was of 5-7%. Polymer composite film loaded 5-FU was carefully removed from the cup and placed in a sterile plastic bag. Samples of size 8 × 1 × 1 $mm^3$ were formed from the obtained chitosan film loaded 5-FU. Prepared film was characterized for swelling coefficient.

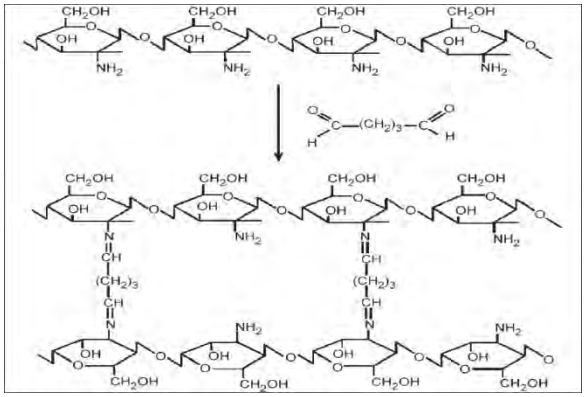

Figure 1 – Crosslinking process of chitosan treated with glutaraldehyde

The Ch film was cut into 3 × 3 $cm^2$ to determine their dry weight (*W*d). The Ch film was dropped in distilled water at 37 °C for 2 h. The wet weight (*W*t) was determined by removing adsorbed water on the surface with filter paper. The percentage swelling of the film was calculated from the formula:

$$W\,(\%) = [(W\mathrm{t} - W\mathrm{d})/W\mathrm{t}] \times 100.$$

Film thickness was measured using Micrometer (MK 102, Russia), with the unit measured count of 0.01 mm.

Spectra of samples of polymer films were analyzed by Nicolet model 5700 FT-IR spectroscopy (USA). The peak of adsorption between 4000 and 400 $cm^{-1}$ were detected to monitor interaction of chemical groups of Ch and GA. Samples were made in form of tablets KBr.

Biomedical tests of obtained films were carried out jointly with the staff of the Centralized Research Laboratory of KazNMU.

Implants of size 8 × 1 × 1 $mm^3$ were formed from polymer film.

In order to determine the prolonged properties *in vitro* the release of FU from cross-linked Ch films was investigated. The drug release was determined using UV/VIS spectroscopy (Evolution 300, USA) at 266 nm in quartz cuvettes with thick of 10 mm at 37 °C in Ringer-Locke solution. Ringer-Locke solution - a standard isotonic solution 6.5 g NaCl, 0.42 g KCl, 0.25 g $CaCl_2$ and 1 mole of sodium bicarbonate is dissolved in one Liter of distilled water. Visual acuity was assessed using a projector ACP 7EM" firm «Topcon» (Japan).

## Results and discussion

In figure 2 a,b, FTIR spectra of Ch and ChGA were displayed, respectively. The main peaks for Ch can be assigned as follows: 3439 $cm^{-1}$ (N–H and O–H stretching vibration), 2925 $cm^{-1}$ ($CH_3$ symmetric stretch), 1666 $cm^{-1}$ (C=O stretching vibration), 1438 $cm^{-1}$ (C–N stretching vibration), 1363 $cm^{-1}$ ($CH_3$ bending vibration), 1155 $cm^{-1}$ (C–O–C bending vibration), and 1073 $cm^{-1}$ (C–OH stretching vibration). However, some major changes have been observed in the spectrum of ChGA by comparing the spectral differences in the 4000–500 $cm^{-1}$ region of FTIR spectra between Ch and GA.

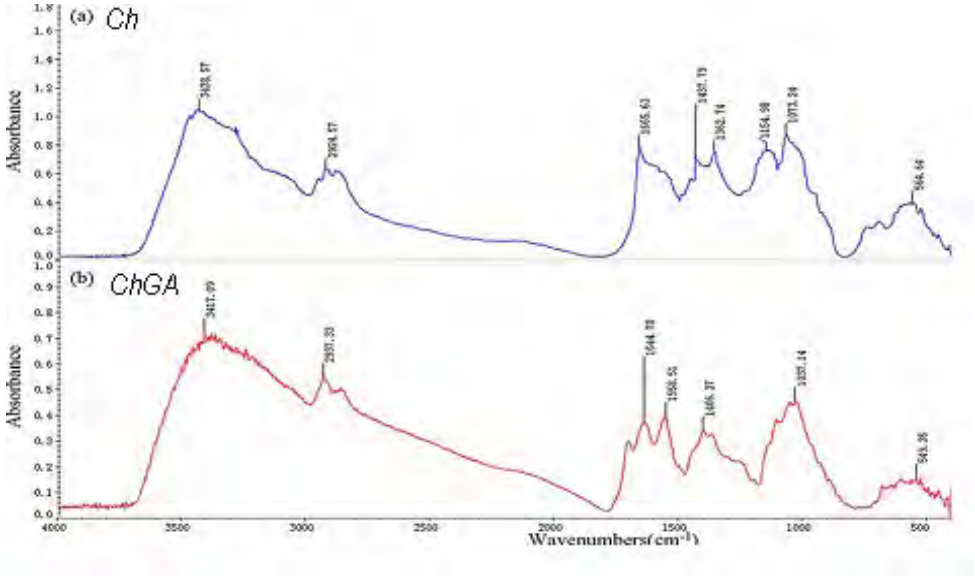


Figure 2 – FTIR spectra of (a) chitosan and (b) cross-linked chitosan-glutaraldehyde

*Partial operation "Vitrectomy"*- intravitreal injection of chitosan film loaded 5-FU was carried in Centralized Research Laboratory of KazNMU. Analgesic and antispasmodic effect is achieved by intramuscular injection of 0.1-0.4 ml of Xylavet, 3-multiple instillation of inokain. Polymeric implant introduction into the vitreous cavity was carried out as follows. Sclerectomy was hold applying blepharostat and undermining of c*onjunctiva* from the limbus *in* 5 mm. A partial vitrectomy was carried out and chitosan film with FU was implanted intravitreal in both eyes in a set dosage. Enucleation of both rabbits' eyes was on the 7 th, 14 th, 28 th days.

Thus, it was experimentally proved that the crosslinked chitosan film introduction into the vitreous cavity did not cause toxic effects on the eye. This method was shown high efficiency for the treatment of proliferative vitreoretinopathy.

*In vitro* release kinetics of 5-FU from the cross-linked chitosan films was investigated to determine the drug release properties. It was established that the

release consisted of three main stages: water sorption by a film and its swelling, the drug diffusion in a film at the phase interface “polymer system-environment” and the drug diffusion in the solvent volume. To determine the influence of drug loading on its release kinetics, polymer films were loaded with 125 and 250 mg/g of 5-FU. Increasing doses of the drug does not affect on the release of FU. Obtained results showed that the drug was diffused practically completely into Ringer-Locke solution within 15-20 h (Figure 3).

Diffusion coefficients for the cross-linked chitosan film, calculated at the initial stage of release was within $7.5 \cdot 10^{-6}$ and $8.3 \cdot 10^{-6}$ $cm^2$/sec. Increasing the film thickness by 3 times the diffusion coefficient decreased twice. Diffusion of the therapeutic agent in a matrix that was confirmed by inverse relationship between the release rate and film thickness played the limiting role in the release of 5-FU from polymeric film system

Table 2 – FU release from polymer film

| Total volume, ml | Time, h | Concentration, µg/ml | Amount of 5FU taken, µg | Amount of 5FU missing in the sample, µg | Amount found, µg | Total amount of FU, µg | Cumulative release of 5FU, % |
|---|---|---|---|---|---|---|---|
| 50 | 1 | 23,956 | 0 | 0,000 | 1197,8 | 1197,800 | 48,058 |
| 50 | 3 | 26,855 | 47,912 | 47,912 | 1342,75 | 1390,662 | 59,963 |
| 50 | 6 | 26,799 | 53,71 | 101,622 | 1339,95 | 1441,572 | 62,092 |
| 50 | 9 | 25,977 | 53,598 | 155,22 | 1298,85 | 1454,070 | 65,019 |
| 50 | 12 | 25,035 | 51,954 | 207,174 | 1251,75 | 1458,924 | 67,557 |
| 50 | 15 | 23,458 | 50,07 | 257,244 | 1172,9 | 1430,144 | 88,578 |
| 50 | 18 | 22,789 | 46,916 | 304,16 | 1139,45 | 1443,610 | 92,811 |
| 50 | 21 | 21,965 | 45,578 | 349,738 | 1098,25 | 1447,988 | 100,000 |

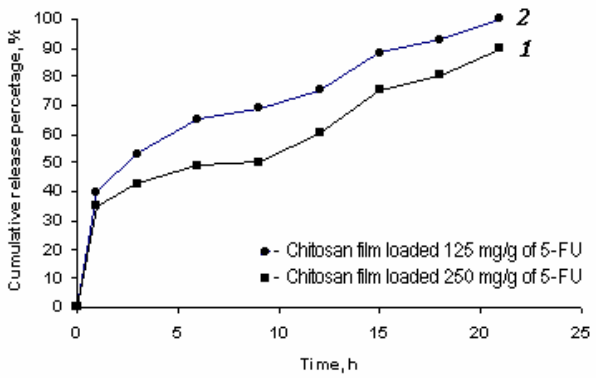


Figure 3 – FU release from cross-linked chitosan films

**Conclusion.** In this study using of chitosan film loaded fluorouracil and treated with glutaraldehyde in ophthalmology were generalized.

Immobilization of anticancer drug FU on chitosan film is carried out and drug release from polymeric forms is investigated. Film was prepared by the solution casting method. FTIR spectra of Ch and ChGA were analyzed. *In vitro* release kinetics of 5-FU from the cross-linked chitosan films was investigated. Obtained results showed that the drug was diffused practically completely into Ringer-Locke solution within 15-20 h. The determined consistent patterns allow to predict drug release and to create polymeric materials with necessary rate of delivery drug in organism.

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**Резюме**

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ОЦЕНКА СШИТЫХ ХИТОЗАНОВЫХ ПЛЕНОК, СОДЕРЖАЩИЕ ФТОРУРАЦИЛ

Синтезированы глазные лекарственные формы в виде пленок, содержащие различные дозы ФУ. В качестве основы для получения лечебных пленок использован сшитый полимер хитозан. Определены оптимальные условия получения пленочных лекарственных форм на основе хитозана. Определена скорость высвобождения препаратов офтальмологического назначения из макромолекулярных терапевтических систем.

**Ключевые слова:** фторурацил**,** глутаровый альдегид, сшитый хитозан, *in vitro* и *in vivo* тесты.

**Summary**

*B. A. Zhubanov*, *A. B. Ismailova*, *E. O. Batyrbekov, D. Loca, A. O. Baiyrkhanova*

EVALUATION OF CROSSLINKED CHITOSAN FILM LOADED FLUOROURACIL

Crosslinked chitosan film loaded various dose of FU was synthesized. The optimal conditions for obtaining the film formulations based on chitosan. The optimal conditions for obtaining the film formulations based on chitosan were determined. The rate of drug release from macromolecular therapeutic systems was defined.

**Key words:** fluorouracil, glutaraldehyde, crosslinked chitosan, *in vitro* release, *in vivo* study.