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OBTAINING A HYDROGEL BASED ON CHITOSAN, STUDY ITS STRUCTURE AND SORBTION ABILITY WITH LEVOFLOXACIN ANTIBIOTICS

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***Abstract**: Network polymer hydrogels with high swelling effect were synthesized by using of ultraviolet crosslinking, with crosslinking agent N,N'-methylene-bis-acrylamide with chitosan an average molecular weight of 100-300 kDa t. The amount of crosslinking agent made up 10% of chitosan weight. The gel structure was studied using FTIR and the mechanism of the cross-linking process identified. The sorption of levofloxacin on the obtained hydrogel was studied at different pH and the degree of sorption and the dependence of the sorption capacity of the hydrogel on the pH of the medium was studied. It found that the degree of sorption of hydrogel by levofloxacin is 90.4 % while the sorption capacity of gel increases as pH of the medium rises and at pH=8 it has the highest value equal to 8.9 mg/g.*

***Keywords:** chitosan, methylen-bis-acrylamide, crosslinking, hydrogel, swelling degree, levofloxacin, sorption capacity, sorption rate, infrared spectroscopy*
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Introduction

Many of them have a good therapeutic effect, but their biocompatibility and pharmacokinetics are weak, they remain in the body for a short time and show systemic toxicity after ingestion. Therefore, to prevent these problems, the drug delivery system has become an important tool to enhance the therapeutic efficacy by using delivery matrices such as nanoparticles, polymer micelles or hydrogels to transport drug molecules [1].

In this regard, hydrogels based on natural polymers are one of the topics in the focus of drug immobilization. Hydrogels are crosslink systems that can absorb and store large amounts of water. Natural hydrogels include collagen, silk fibroin, hyaluronic acid, chitosan, alginate, and hydrogels derived from desellulose tissues. They have unique properties such as biocompatibility, biological decomposition, low cytotoxicity, the ability to adapt the gel for hydrogel injection, and their unical to the physiological environment [2-6].

Among natural polymers, such as drug delivery systems, chitosan-based hydrogels are particular importance for their use in medicine. Chitosan is a derivative of chitin, the second most common polymer in nature, which is a protective material for the micells of crustaceans, insects and fungi. The point is about the family of cationic polysaccharides, the main chemical structure of (1,4)-2-amine-2-deoxy-D-glucans, produced commercially by partial deacetylation of chitin

obtained from the recycling of seafood waste. As a natural polymer, it has a great potential in wound healing due to its stimulating effect against leukocytes and antibacterial properties [7]. Chitosan is biodegradable, breaking down into harmless products (amine sugars) that can be easily absorbed by the human body [8]. Currently, chitosan and its derivatives have been studied in terms of many different medical applications, such as wound dressings, contact lenses, cell encapsulation, and drug delivery [9-12].

In recent years, the immobilization of antibiotics into hydrogels has been one of the main topics of various research studies. There are numerous classes of antibiotics, one of them is the fluoroquinolone series of antibiotics. One of the antibiotics of this class is levofloxacin. Levofloxacin is widely used in the treatment of various infection diseases, corneal and conjunctivitis, urinary and respiratory tract, skin and soft tissue infections [13, 14]. As a result of immobilization of levofloxacin in polymer hydrogels, its toxicity and side effects are reduced, and long-term effects are increased.

In the presented work hydrogel was synthesized on the basis of chitosan and the swelling degree was studied. The levofloxacin antibiotic was immobilized on the obtained hydrogel and its sorption capacity and structure studied.

2. Experimental

2.1. Hydrogels obtaining

The chitosan (CS)-based hydrogels was obtained through cross-linking CS with crosslinking agent N,N′-methylene-bis-acrylamide (MBAA). 1 g of CS with an average molecular weight of 100-300 kDa is dissolved in 30 ml of 1% acetic acid. Crosslinking agent-MBAA was added as 10% quantity of polymer mass - 0.1 g to the solution. 20 ml of 1% acetic acid was added to the solution and mixed for 4-5 hours. Then the solution was poured into a Petri dish, evaporated at room temperature and released from the solvent. Then polymer was continuously influenced with UV radiation for 5-6 hours for cross-linking. The sample was washed several times with deionized water and ethyl alcohol to remove both the polymer and the cross-linked sanple that were not involved in the crosslinking process.

2.2. Swelling determination

In order to determine the swelling degree of the synthesized CS-based hydrogels in water and at different pH, the hydrogel samples are divided into disc-shaped fragments of about 30 mg, brought to a constant weight and accurately weighed. These synthesized fragments were placed in a closed glass beaker and placed on the swelling by adding 10 ml of 1% acetic acid, 5 ml of deionized water and 5 ml of buffer solution. In this case, after 24 hours of storage, the non-sorbed water was carefully separated at different times by filter paper, the swollen gel mass was determined, and the swelling degree was determined according to formula (1) below [15].

$$W = \frac{W_w - W_d}{W_d} \times 100\% \qquad (1)$$

Where W_d is the dry weight of the sample and W_w is the post-swelling weight.

2.3. Preparation of samples for the calculation of sorption capacity

To determine the sorption of CS-based hydrogels in buffer solutions, the cross-linked polymers were dried, crushed and pulverized. A sample of 10% cross-linked CS was weighed 0.05 g is added to the beaker, 10 ml of 1% acetic acid added and stored for 1 day. 5 ml bufer solution was added to each bucket, pH = 1 to the first, pH = 5 to the second, and pH = 10 to the third. 1 ml of LVF solution (C = 4.1 mg / l) was added to each bucket and stored for 1 day. After 1 day, each solution was filtered and the UV (UV-VIS1800, SHIMADZU) spectrum recorded. The UV spectrum was recorded in the range of 200-400 nm. The concentration was calculated, and the sorption degree (SD) and sorption capacity (ST) were calculated according to the following formula (2) by finding the post-sorption density from the pre-established degree graph [16].

$$SD = \frac{C_{init} - C_{end}}{C_{init}} \times 100\% \qquad ST = \frac{C_{init} - C_{end}}{g} \times V \qquad (2)$$

Here, C_{init} and C_{end}, respectively, are the concentrations of levofloxacin (LVF) before and after sorption, V – is the total volume of the solution to be sorbed, in ml, and g – is the expression in mg of hydrogel taken for sorption.

2.4. Structural analysis

Functional groups of CS-based hydrogels, as well as SHIMADZU IR Fourier infrared (FTIR) and UV (UV-VIS1800, SHIMADZU) spectroscopy methods were used to determine the interaction of functional groups between hydrogel and LVF antibiotic. FTIR spectra were obtained using KBr disks and were recorded in the spectral range of 4000-400 cm^{-1}.

3. Result and discussions

When hydrogels are used as a drug delivery, one of the main factors that will affect their structure, the degree of ionization of their functional groups and the environment in which the immobilized biologically active substances are exposed is the pH of the environment [17]. In this regard, the dependence of the degree of swelling of the synthesized CS-based gels on the pH of the medium was studied. Swelling rates of hydrogels obtained by crosslinking with MBAA at 10% (mass) ratios with an average molecular weight of 100-300 kDa in acidic, neutral and alkaline environment were analyzed (Fig.1).

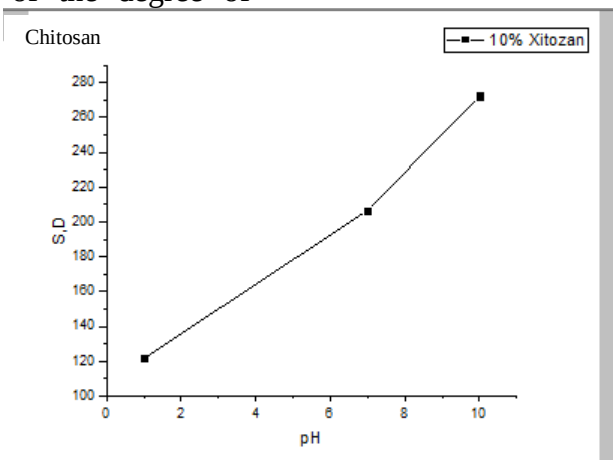


Fig. 1. Graph of the swelling degree of chitosan-based hydrogel depending on the pH of the medium.

As it seems in Fig. 1, the maximum swelling rate of gels obtained from the cross link of XZ with 10% MBAA was at pH = 10 and ~ 280%. It found that the polymer had a low swelling degree, due to the protonation of the functional groups in the gel at low pH. The rate of hydrogel swelling gradually increased due to occurence of deprotonization as it passes into the alkaline environment [18, 19].

To determine the mechanism of crosslinking process of CS crosslinking with MBAA, it was investigated the structure of the initial substances and hydrogels were studied by FTIR spectroscopy. Based on the change in the value of the absorption strips belonging to the functional groups in the CS macromolecule, the probable mechanism of the crosslinking process was determined.

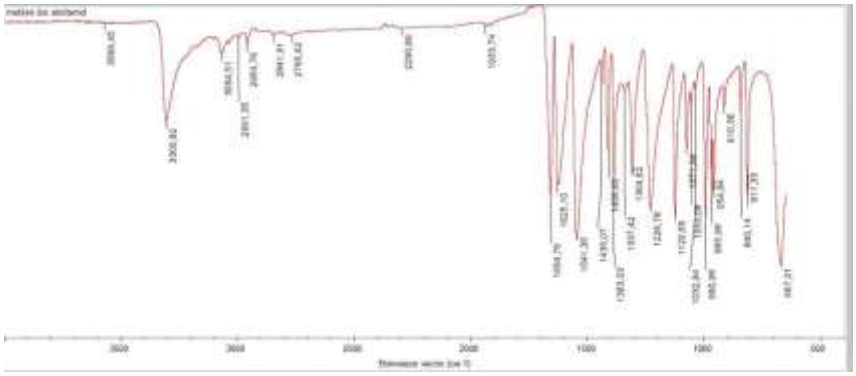


Fig. 2. IR spectrum of N, N`-methylene-bis-acrylamide.

The IR spectrum of the crosslink agent MBAA used in the cross-linking of the CS was also studied and compared (Fig.2). In other words, it was determined which functional groups of the polymer chain and the cross-link agent was involved in the cross-link process. Thus, 3500-3300 cm^{-1} and 1390-1000 cm^{-1} absorption bands belonging to -NH_2 groups are observed in the IR spectrum of CS. Also, a wide absorption band of medium intensity was observed in the region 1320-1387 cm^{-1}, which shows the valence vibration of -OH bonds (figure 3).

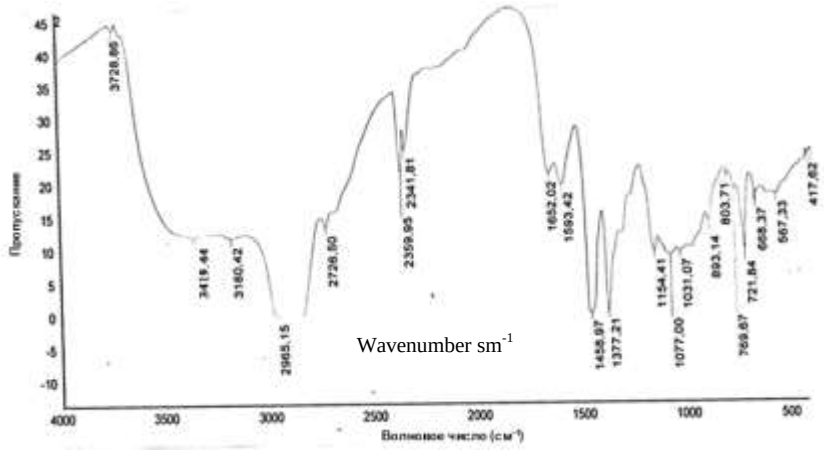


Fig. 3. IR spectrum chitosan.

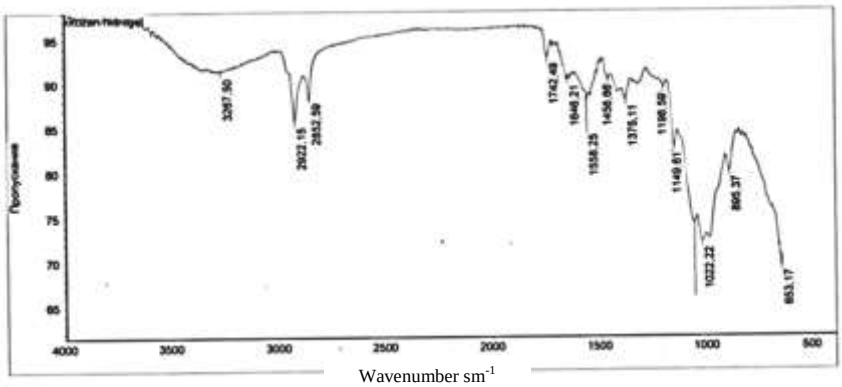


Fig. 4. IR spectrum of cross-linked chitosan.

The small peaks of the absorption band 1415 cm^{-1} and 1707 cm^{-1} go back to C = O groups. Besides it, 1655 cm^{-1} absorption band belonging to amide groups is observed. In the composition of the cross-link agent, the absorption band, which is characteristic of the CH_2 = CH- group associated with the group > C = O, provides intensive absorption in the range of 1600 - 1680 cm^{-1}. When the polymer is cross-link, the CH_2 = CH- group is transformed into the -CH_2-CH- group due to the opening double bond contained in the crosslink agent. In this case, a decrease in the frequency of the CH_2 = CH- group and an increase in the frequency of the -CH_2-CH- group (-CH_2- 1465 cm^{-1}, > CH-1340 cm^{-1}) are observed in the spectrum. After crosslinking the polymer the absorption band 1655 cm^{-1} belonging to the amide groups also disappears in the IR spectrum (figure 4).

Analysis of the absorption bands belonging to the functional groups of CS, MBAA and obtained hydrogels by IR spectroscopy revealed that the cross-link of the polymer occurs due to the combination hydrogen atom of amine groups to the -CH group of cross-link agent.

One of the main factors influencing the sorption of organic and inorganic ions by the adsorbent from aqueous solutions is the pH of the environment. Because the H^+ and OH^- in the solution results in the ionization of the hydrogel which has a direct effect on the sorption rate of the sorbate and the sorption capacity of the hydrogel. From this point of view, the hydrogels obtained from the cross-link of CS with different 10% (mass) amount of MBAA were sorbed with LVF in the interval pH = 1 ÷ 10 for 24 hours, while sorption capacity and sorption rate were calculated and the results given in Table 1 [20].

Table 1. pH dependence of sorption rate and sorption capacity for levofloxacin of chitosan-based gel cross-linked at 10% mass \ ratio of N, N` - methylene-bis-acrylamide

pH	**pH=2**	**pH=5**	**pH=8**
SD%	31.21	61.3	90.4
ST mg/g	6.5	8.2	8.9

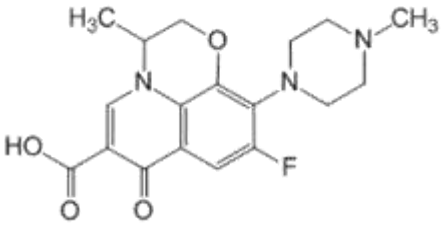
As follows from Table 1, the sorption degree is increased as the pH of the medium increases. Protonation of active functional groups (-NH_2) in hydrogel occurs in acidic environment (pH≤4). Also, the low swelling degree of hydrogel at low pH prevents the antibiotic molecule from penetrating into the internal pores of hydrogel. As the pH of the medium changes into alkali, the surface of the hydrogel becomes deprotonized, and, conversely, the negative charging causes the positively charged LVF molecule to be easily sorbed. On the other hand, the conversion of hydrogel to a highly swollen

form in an alkaline environment also helps to increase the sorption degree.

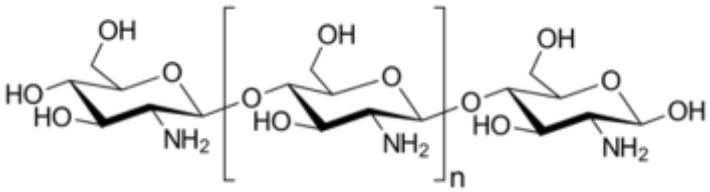
The sorption capacity of CS-based hydrogel in different environment was also calculated and the results shown in Table 1. It reveals that the nature of changes in the sorption capacity of hydrogel is almost the same as the rate of sorption, the sorption capacity of hydrogel for antibiotics increases as alkaline environment increases. CS-based hydrogel maximally sorbs LVF at pH = 8. This is related the high swelling degree of the hydrogel at same pH. After pH = 9, the sorption rate of LVF decreases, although the swelling degree of the hydrogel increases. This results in the decrease in the surface of the antibiotic in alkaline environment and consequently, in the decrease in its hydrophilicity.

To determine the type of chemical interaction between the LVF molecule and the CS-based gel, the structure of both the antibiotic (figure 5) and the antibiotic/hydrogel complex (figure 6) was studied by IR-Fourier spectroscopy.

If we look at the following structure of the LVF molecule and polymer macromolecule, we can see that the amount of active functional groups in its composition is sufficient:



Levofloxacin



Chitosan

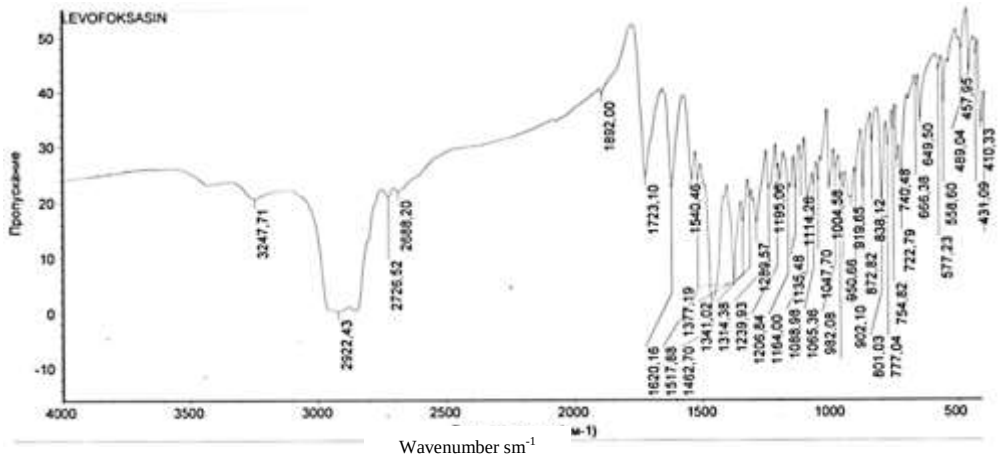


Figure 5. IR spectrum of levofloxacin.

In the IR spectrum of LVF, there is a 1723 cm^{-1} absorption band belonging to the C=O group. 3440 cm^{-1}, 3247cm^{-1}, 2500 cm^{-1} is a typical absorption band of the carboxyl group, and $\lambda_{C=O}$ = 1620 cm^{-1} is a characteristic absorption band of C=O bonds in the quinone

fragment. In the aromatic nucleus, characteristic absorption bands of wavelengths 1341 cm^{-1}, 1314 $^{cm-1}$, 1239 cm^{-1} corresponding to the C-F bond are visible. λ_{C-H} = 3050 cm^{-1} valence vibration of CH bonds in aromatic nucleus, $\lambda_{C=C}$ = 1517, 1540 cm^{-1} valence vibration of C = C bond in aromatic nucleus, λ_{C-H} = 838, 872, 740 cm^{-1} in aromatic nucleus is the absorption band of the deformation vibrations of C-H bonds. Also, an absorption band of 1461 cm^{-1} valence vibrations belonging to groups C-N 1206 cm^{-1} and C-O is observed. The occurrence of chemical shifts in the absorption bands of both polymer and antibiotic functional groups in the FTIR spectrum of the CS-LVF complex is indicative of the location of the antibiotic in the polymer structure (Fig. 6).

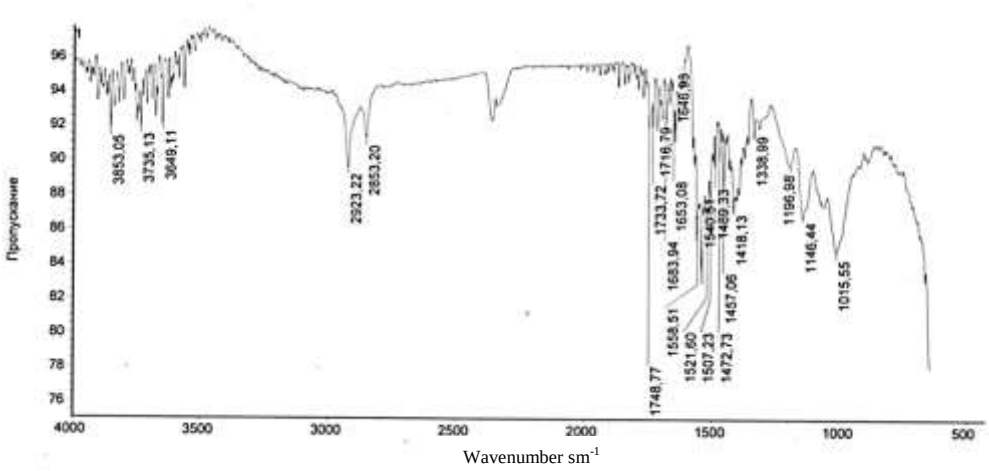


Fig. 6. IR spectrum of levofloxacin complex with chitosan-based hydrogel.

Conclusion

"Smart" hydrogels based on chitosan were obtained capable of swelling well in the water. The swelling degree of the obtained hydrogels were studied at different pH and it was found that the swelling degree of these hydrogels increases from acidic to alkaline environment. The sorption of the antibiotic levofloxacin on these hydrogels in various media was also studied and it was determined that the obtained hydrogel has a high sorption capacity. The structure of the obtained hydrogels by IR-Fourier was studied and the nature of the interaction between the antibiotic and the hydrogel was determined.

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XİTOZAN ƏSASLI HİDROGELLƏRİN ALINMASI VƏ ONUN LEVOFLOKSASİN ANTİBİOTİKİ İLƏ SORBSİYASININ ÖYRƏNİLMƏSİ

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Orta molekul kütləsi 100-300 kDa olan xitozanın 10% (kütlə) nisbətlərində tikici agent N,N'-metilen-bis-akrilamidlə ultrabənövşəyi şüa vasitəsilə tikilməsindən yüksək şişmə qabiliyyətinə malik torvari polimerlər sintez olunmuşdur. İQ FTİR spektroskopiya metodu ilə gelin quruluşu öyrənilmiş və tikilmə prosesinin mexanizmi açıqlanmışdır. Həmçinin, alınmış hidrogel ilə levofloksasin antibiotikinin sorbsiyası müxtəlif pH-larda öyrənilmiş və antibiotikin sorbsiya dərəcəsi və sorbsiya tutumlarının mühitin pH-dan, asılılığı tədqiq edilmişdir. Müəyyən olunmuşdur ki, tərkibində 10% tikici agent saxlayan hidrogelin levofloksasinə görə sorbsiya dərəcəsi 90.4 % təşkil edir, mühitin pH-nın artması ilə gelin levofloksasinə görə sorbsiya tutumu artır və pH=8-də ən yüksək olub, 8.9 mq/q təşkil edir.
Açar sözlər: *xitozan, metilen-bis-akrilamid, tikilmə, hidrogel, şişmə dərəcəsi, levofloksasin, sorbsiya tutumu, sorbsiya dərəcəsi, infraqırmızı spektroskopiya*

ПОЛУЧЕНИЕ ГИДРОГЕЛЯ НА ОСНОВЕ ХИТОЗАНА И ИЗУЧЕНИЕ ЕГО СТРУКТУРЫ И СОРБЦИОННОЙ СПОСОБНОСТИ ПО ОТНОШЕНИЮ К ЛЕВОФЛОКСАЦИНУ

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Ультрафиолетовым сшиванием, при помощи сшивающего агента N,N'-метилен-бис-акриламида с хитозаном со средней молекулярной массой 100-300 kDa синтезированы сетчатые полимеры с высоким набухающим эффектом. Количество сшивающего агента составляло 10% от массы хитозана. Изучена структура геля при помощи ИК-Фурье и показан механизм процесса сшивки. Также изучена сорбция левофлоксацина на полученном гидрогеле при различных pH и исследована степень сорбции и зависимость сорбционной емкости гидрогеля от pH среды. Установлено, что степень сорбции гидрогеля по левофлоксацину составляет 90.4 %, сорбционная емкость геля по левофлоксацину с увеличением pH среды увеличивается и при pH=8 имеет самое высокое значение, равное 16,68 мг/г.
Ключевые слова: *хитозан, метилен-бис-акриламид, сшивка, гидрогель, степень набухания, левофлоксацин, сорбционная емкость, степень сорбции, инфракрасная спектроскопия*