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RESEARCH INTO SORPTION PROPERTIES AND STRUCTURES OF POLYMER HYDROGEL IMMOBILIZED BY DOXORUBICIN**S.M. Mammadova, Sh.Z. Tapdigov, S.F. Humbatova, N.A. Zeynalov,
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The sorption of doxorubicin by hydrogeles obtained through cross-linking of polyacrylic acid with varied quantity (% mass) of N, N-methylene-bis-acrylamide within pH = 1÷10 interval for 24 hours was performed. It revealed that protonation of active functional groups of hydrogel in acidic media (pH ≤ 4) impedes electrostatic interaction with identical charges in hydrogel and doxorubicin. A small degree of swelling at low pH of medium also impedes the penetration of molecule of antibiotic into internal pores of hydrogele. Note that pH changes toward alkaline medium, deprotonation and thereby negative charging of hydrogele surface leads to the sorption of positively charged molecule of doxorubicin. As the pH changes to the alkaline, the deprotonation of the hydrogel surface and, on the contrary, the negative loading that causes the easy sorption of positively charged doxorubicin molecule. On the other hand, the high swelling of hydrogel in alkaline medium also contributes to the increase of sorption level. Also, the nature of chemical interaction between hydrogel and antibiotic was examined by infrared spectroscopy methods, as well as by scanning electron microscopy. It found that the relation between the polymer and the doxorubicin occurs mainly due to hydrogen bond, electrostatic and hydrophobic interaction.

Keywords: *doxorubicin, immobilization, cross-linking, polyacrylic acid, methylene-bis-acrylamide, hydrogel.*

1. INTRODUCTION

Hydrogels are water swollen polymer matrices, with a huge tendency to absorb water. Their ability to swell under physiological conditions makes them an ideal material for biomedical applications[1]. The hydrophilicity of the network is due to the presence of chemical residues such as hydroxyl, carboxyl, amidic, primary amidic, sulphonic, etc. that can be found within the polymer backbone or as lateral chains [2,3]. Moreover, some “intelligent” hydrogels could undergo reversible and significant changes in the structure, shape, and/or property after being exposed to external physical/chemical/mechanical stimuli, such as pH value, temperature, ionic strength, enzymatic activity, glucose concentration, light, electric and magnetic fields, pressure and solvent composition or combination thereof [4-6]. Therefore, these stimuli-sensitives are

expected to contribute significantly to the exploration and development of the next generation of biomaterials for biological and biomedical applications, such as self-regulated and site-specific drug delivery systems, specialized separation systems or bioreactors. The polymeric hydrogels from synthetic sources are advantageous, because they usually possess controllable chemical structure and architecture, degradation rates, and mechanical strengths. However, most of them are lacking biological cues for biological applications, especially for proliferation of cells and tissue regeneration [7–9].

With the development of understanding in drug delivery mechanism and cancer pathology, a set of criteria have gradually been established for guiding the preparation of drug delivery system for cancer treatment. Drug delivery system should not cause severe long-

term side effect, so biocompatible and biodegradable materials have predominantly been used for the development of drug delivery system. In order to effectively kill cancer cells and suppress tumor proliferation, significant drug loadings are required for achieving remarkable therapeutic effect. Stimuli-responsive drug release from drug delivery system in tumor tissue is highly preferred to minimize their systemic toxicity and improve drug delivery efficiency [10-12].

As is known, functionalized polymers [13] and polymer composites with silver nanoparticles are widely used in medicine as carrier to immobilize bio-nanocomposites, bio-nanocatalysts, antibacterial drugs and biologically active substances depending on their particle size [14,15].

Polymersomes were prepared using poly(ethylene glycol)-*b*-poly(lysine)-*b*-poly(caprolactone), biocompatible tri-block

copolymer. To chemically cross-link the polymersomes, the primary amine of the tri-block copolymer came into reaction with a disulfide-containing cross-linker. Doxorubicin was chosen as a model anti-cancer drug, and was effectively encapsulated into the cross-linked bio-reducible polymersomes. The drug-loaded polymersomes were greatly retarded in releasing doxorubicin under physiological conditions (pH 7.4) whereas the release rate of doxorubicin rose remarkably in the presence of 10 mM glutathione mimicking an intracellular environment [16].

The hydrogel with high swelling capacity was synthesized in the present work. The sorption capacity of obtained hydrogel to doxorubicin was studied and the structure of complexes analyzed with the help of IR spectroscopy and scanning electron microscopy.

2. EXPERIMENTAL

2.1. Materials:

Average molecular mass of polyacrylic acid (PAA) with purity 90 % is 230 kDa to have been purchased from Fluca. Methylene-*bis*-acrylamide (MBAA) which is used as a crosslink agent was supplied by Sigma Aldrich and both reactives are used without further purification. Doxorubicin hydrochloride (Dox)

code ATX L01DB01 came from TEVA Pharmaceutical Industries (Israel). Deionized water which is used for preparation of solutions, diethyl ether to precipitate polyacrylic acid and NH_4OH , $\text{CH}_3\text{COONH}_4$, HCl , KOH for buffer solutions are chemically pure to come from Aldrich.

2.2. Methods

2.2.1. Preparation of Hydrogel:

100 mg PAA is dissolved in 50 mL of deionized water. Crosslink agent MBAA in 5, 10, 15, 20 % of polymer mass is added to the solution and mixed for 11 hours. The homogeneous solution is poured into Petri bottle and the solvent is evaporated under normal atmospheric pressure. Thin film is continuously influenced by UV radiation 4 h. After irradiation, the samples are washed two-three times consistently with deionized water, 0.01 N HCl and ethanol. Samples are dried up at 313-323 K and brought to a stable weight.

2.2.2. Absorbion practices:

Absorbion practices: Sorption of Dox was performed with PAA hydrogel by

compatible method: 0.5 mg gel obtained from cross-linking of 5, 10, 15 and 20 % ratio (mass) of MBAA which is stored for 24 hours in 10 mL deionized water. Then, by adding appropriate 5 mL 1÷10 pH it is kept for 30 min, and Dox solution with 1 mL $\times 10^{-3}$ mol/L concentration is added and kept for 24 hours in dark place. Then the solution is filtered, after the sorption the concentration is determined by comparing the concentration of antibiotic remaining in filtrate and optical density (UV-VIS 1800, SHIMADZU) in 510 nm area with pre-arranged graphic. According to the difference concentrations, sorption degree (SD, %) of Dox and sorption capacity (SC, mg/g) of hydrogel for antibiotic are calculated.

2.2.3. Structural analysis:

Functional group confirmations were assessed through the use of FTIR-spectroscopy. A film of the nano-fluid was dried up on the glass plate. The decomposition

of cross-linking PAA was studied with the help of scanning electron microscopy (SEM Sigma VP (Carl Zeiss Jena)).

3. RESULTS AND DISCUSSION

It is known that for sorption of organic and inorganic ions of absorbent from aqueous solutions the main affecting factor is pH of the environment. In considering that the H^+ and OH^- ions in the solution leads to the charging of surface, the volume of absorbent, or in other words, hydrogel is characterized by ionization and thus directly affects the sorption degree of sorbet and sorption capacity of hydrogel. The sorption (in the closed bottle, dark) of hydrogels obtained by cross-linking with MBAA in varied % (by weight) quantity of PAA with Dox has been carried out in terms of static conditions in the $pH = 1\div 10$ interval within 24 hours. As pH of environment rises, the sorption degree in the all samples goes up to 60-70 %. In acidic environments ($pH \leq 4$), the protonation of active functional groups ($>C=O$, $-OH$ and $-NH-$) occurs in the composition of hydrogels. Both hydrogel and Dox being of identical charge can't provide electrostatic encounter. Also, at low pH a swelling degree of hydrogel impedes the antibiotic molecule to let into pores of the hydrogel. Sorption of positively charged Dox molecule easily causes deprotonation of hydrogel surface and inversely affects negative charging as pH environment gets to alkali.

This time, on another side of alkali environment the hydrogels transition into high swelling shape also helps to increase a sorption degree [17].

Also, it found that when the amount of cross-linking agent changes, a sorption degree changes in all pH as well. It was established that hydrogel obtained by cross-linking PAA with 10 % MBAA maximally absorbs Dox in the $pH = 8$. It is related to a high swelling degree (600 %) of hydrogel and the rate of starting concentration of the given Dox in the same pH. Owing to $pH = 8$ sorption equilibrium, the concentration rate is directly related to more optimal swelling degree with the same concentration rate as with hydrogel surface.

Though increasing a swelling degree of hydrogel after $pH = 9$, a sorption degree of Dox falls. In alkali environment, it is resultant in discharging the antibiotic surface and decreasing hydrophilicity.

The above-mentioned results can be seen also in dependence curves of pH environment (Fig. 1) of sorption capacity for Dox of hydrogels which is obtained by cross-linking of PAA with 5÷20 % MBAA.

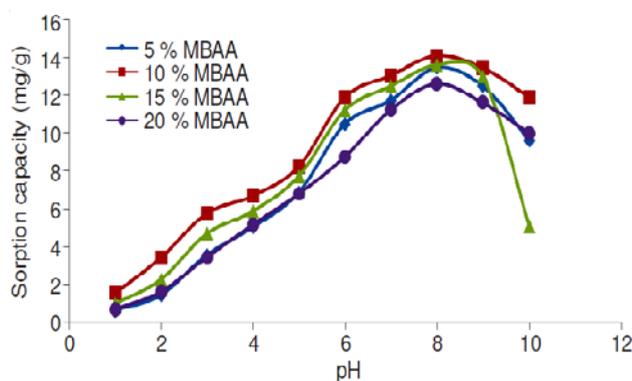


Figure 1. Dependence curves of pH environment of sorption capacity for doxorubicin hydrochloride of polyacrylic acid based on gel-cross-linking in different % ratio with methylene-bis-acrylamide (MBAA)

As follows from Fig. 1, the variation characterization of hydrogel sorption capacity has nearly the same shape that with sorption degree. Sorption capacity decreases accordingly to the increase in the quantity of crosslink reagent in hydrogel. It found that in case of sorption capacity for Dox of hydrogel based on PAA cross-linked with 10 % MBAA in pH = 8 is 14.12 mg/g, next comes the sorption capacity of polymer cross-linked with 20 % MBAA that makes up 12.64 mg/g. This is explained as being due to the decrease in the size of pores in hydrogel even despite the concentration of functional groups through the rise in the quantity of crosslink reagents. But comparing hydrogels obtained with the presence of 5 and 10 % MBAA, high sorption capacity of hydrogel obtained from 10 % crosslink reagent is related to its optimal value. From spatial point of view, it is more acceptable for Dox molecule in internal nets. In this respect, antibiotic molecule remains to be more stable and resistant in gel pores. But the polymer is cross-linked with 5 % MBAA because the gel-forming property is not full, sizes of pores are too big and the quantity of active functional groups in crosslink reagents is not enough for Dox, so the concentration of gel has little sorption capacity. Owing to the fact that the antibiotic molecule is not surrounded with enough quantity of functional groups, it is not resistant in the gel.

To determine a type of chemical interaction between Dox molecule and PAA-based gel, structures of starting substances and complexes both are identified with IR spectroscopy methods. We can see enough quantity of active functional groups, if we look at structures of Dox molecule, MBAA and polymer macromolecule.

It revealed that absorption strips having frequency of 1430, 1230, 1638 and 3345 cm^{-1} are observed according to $\text{CH}_2=\text{CH}-$, $>\text{C}=\text{O}$, -

OH functional groups in FTIR spectrum of PAA. But there are absorption strips 1645, 1445, 1650 cm^{-1} specific to $\text{CH}_2=\text{CH}-$, $>\text{CH}_2$, $>\text{C}=\text{O}$ and $-\text{NH}$ groups in crosslink reagent. There are observed decreasing intensity in absorption strip belonging to $>\text{CH}_2$ cross-linked polymer spectrum and intensity specific to $-\text{CH}_3$ group in spectrum. In 290 nm and 450 nm, two main maximums are observed in UV spectrum of Dox. In 485 and 525 nm, it is assigned to two smaller absorptions. Chemical shifts occur in absorption strips of functional groups belonging to both crosslink reagent and polymer in FTIR spectrum of PAA-Dox complex. In FTIR spectrum, it is impossible to track functional groups belonging to Dox antibiotic quantity which is less than 2 % in hydrogel. But one can specify which functional group is actively involved in the absorption strips by PAA and MBAA.

So, chemical shifts of absorption strips specific to $>\text{C}=\text{O}$, $-\text{OH}$, $-\text{NH}-$, $-\text{CO}-\text{NH}$ groups in PAA and MBAA occur to 1670, 1428, 1635, 3340 cm^{-1} area. It has been mentioned that a little difference between chemical shifts results in creating not covalent bond with Dox and PAA, so it is mainly hydrogen bond and electrostatic interaction that forms a complex between Dox with PAA.

The changes in the morphology and structure of the polymer after cross-linking of PAA with MBAA were analyzed with the help of SEM method. It found that the structural granulation occurs in PAA, thus, the polymer breaks into pieces and the heterogeneous structure becomes available. The cross-linked polymer has a number of layers that point to the cross-linking.

SEM images obtained from different parts of the PAA are given below. The structure of the hydrogel based on the cross-linked PAA with MBAA at a 10% rate of polymer is given in Fig 2.

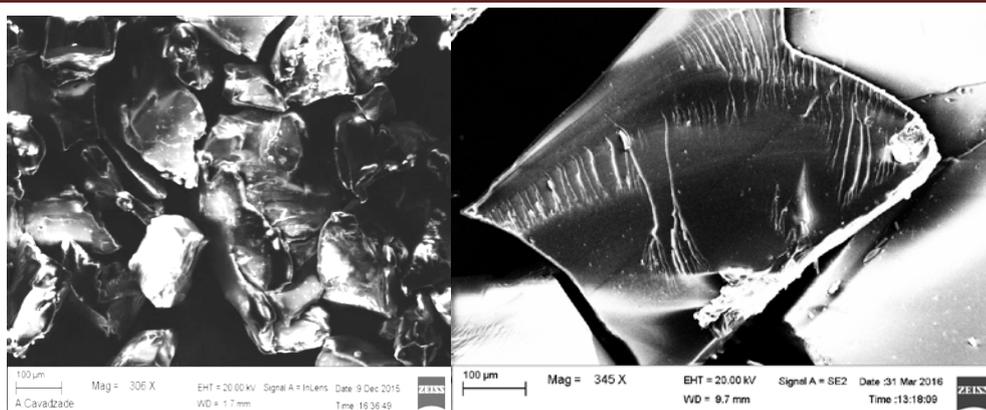


Figure 2. Scanning electron microscopy of 10 % polyacrylic acid crosslinked with MBAA

As it could be seen, fibrous structures are observed in the surface morphology that characterizes the cross-linking. Such relatively regular structures give a clearer representation of 10% mass of the MBAA. But at the 5; 15; 20% mass of MBAA, the morphology of the

formed gels are irregular, scattered structures that does not characterize heterogeneous or stable cross-linking.

Besides, there is a different morphology in SEM images of PAA with Dox which is cross-linked by 10% mass of MBAA (Fig. 3).

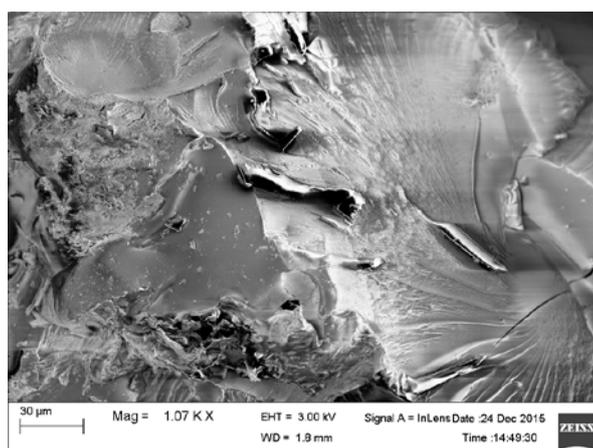


Figure 3. Scanning electron microscopy of crosslinked polyacrylic acid/doxorubicin composites.

Thus, the adsorption gel pores of the Dox molecule breaks the surface morphology of the polymer even despite its significance. As is seen from Figure 3, the smooth surface

of the cross-linked polymer is replaced by nonsmooth. That proves the concentration of Dox on the surface of molecule than on inner pores of the gel.

CONCLUSION

Hydrogel obtained by cross-linking PAA with 10 % MBAA maximally absorbs Dox in 0.05-0.01 mg/L concentration at the pH = 8. At room temperature within 24 hour static condition the sorption rate of Dox is 88 % and

sorption capacity is 18-21 mg/L 2 % (1 g) Dox keeping compositions are used in medical biotechnology for transportation of mentioned antibiotic. It is also used as a long lasting and effective drug.

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REFERENCES

1. Gao D., Xu H., Philbert M.A., Kopelman R. Ultrafine hydrogel nanoparticles: synthetic approach and therapeutic application in living cells. *J. Angew Chem. Int. Ed.* 2007, vol. 46, pp. 2224-2227.
2. Lin C.C., Metters A.T. Hydrogels in controlled release formulations: network design and mathematical modeling. *J. Adv. Drug. Delivery Rev.* 2006, vol. 58, pp. 1379-1408.
3. Satish C.S., Satish K.P., Shivakumar H.G. Hydrogels as controlled drug delivery systems: Synthesis, crosslinking, water and drug transport mechanism. *Ind. J. Pharm. Sci.* 2006, vol. 68, pp. 133-40.
4. Qiu Y., Park K. Environment-sensitive hydrogels for drug delivery. *J. Adv. Drug Deliv. Rev.* 2012, vol. 64, pp. 49-60.
5. Hoffman A.S. Stimuli-responsive polymers: Biomedical applications and challenges for clinical translation. *J. Adv. Drug Deliv. Rev.* 2013, vol. 65, pp. 10-16.
6. Ward M.A., Georgiou T.K. Thermoresponsive polymers for biomedical applications. *J. Polymers.* 2011, vol. 3, pp. 1215-1242.
7. Li Y., Rodrigues J., Tomas H. Injectable and biodegradable hydrogels: Gelation, biodegradation and biomedical applications. *J. Chem. Soc. Rev.* 2012, vol. 41, pp. 2193-2221.
8. Annabi N., Tamayol A., Uquillas J.A., Akbari M., Bertassoni L.E., Cha C., Camci-Unal G., Dokmeci M.R., Peppas N.A., Khademhosseini A. 25th Anniversary article: Rational design and applications of hydrogels in regenerative medicine. *J. Adv. Mater.* 2014, vol. 26, pp. 85-124.
9. Caló E., Khutoryanskiy V.V. Biomedical applications of hydrogels: A review of patents and commercial products. *Eur. Polym. J.* 2015, vol. 65, pp. 252-267.
10. Fleige E., Quadir M. A., Haag R. Stimuli-responsive polymeric nanocarriers for the controlled transport of active compounds: concepts and applications. *J. Adv. Drug Delivery Rev.* 2012, vol. 64, pp. 866-884.
11. Du J. Z., Du X. J., Mao C. Q., Wang J. Tailor-Made Dual pH-Sensitive Polymer-Doxorubicin Nanoparticles for Efficient Anticancer Drug Delivery. *J. Am. Chem. Soc.* 2011, vol. 133, pp. 17560-17563.
12. Xiao N. Y., Liang H., Lu J. Degradable and biocompatible aldehyde-functionalized glycopolymer conjugated with doxorubicin via acid-labile Schiff base linkage for pH-triggered drug release. *J. Soft. Matter.* 2011, vol. 7, pp. 10834-10840.
13. Mammadova S.M., Tapdigov Sh.Z., Humbatova S.F., Safaraliyeva S.F., Hasanova M.Kh., Zeynalov N.A. Research into hydrogel swelling capacity on the basis of polyacrylic acid and immobilization of doxorubicin thereupon. *Kimya Problemləri - Chemical Problems.* 2016, no. 4, pp. 377-385. (In Azerbaijan).
14. Humbatova S.F., Mammadova S.M., Tapdıqov Sh.Z., Safarov N.A., Abbasov M.H., Zeynalov N.A. Research into structure of argentium nanoparticles obtained from varied polymer medium. *Kimya Problemləri - Chemical Problems.* 2018, no. 1, pp. 78-85. (In Azerbaijan).
15. Tapdigov Sh.Z., Zeynalov N.A., Taghiyev D.B., Akhmedova U.M., Mammadova A.I., Hasanova M.Kh., Amirov M.A. Research into properties and structure of basic polysaccharide in *prunus domestica* (cherry). *Kimya Problemləri - Chemical Problems.* 2018, no. 1, pp. 35-43. (In Azerbaijan).
16. Thavasyappan Thambi V. G., Deepagan H. K., Suh Y. D., Yi G. R., Lee J. Y., Leea D. S. and Park J. H. Biostable and bio-reducible polymersomes for intracellular delivery of doxorubicin. *J. Polym. Chem.* 2014, vol. 5, pp. 4627-4632.
17. Tapdigov Sh.Z., Mammadova S.M. and Zeynalov N.A. Spectroscopic-examined interaction between silver nanocomposites based of poly-N-vinylpyrrolidone and doxorubicin for drug delivery. *J. Chem. Chemical Eng.* 2014, vol. 8, pp. 800-804.

DOKSORUBİSİN İMMOBİLİZƏ OLUNMUŞ POLİMER GELİN SORBSİYA XASSƏLƏRİ VƏ QURULUŞUNUN TƏDQIQI

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Poliakril turşusunun müxtəlif miqdar (% kütlə) N,N-metilen-bis-akrilamidlə tikilməsindən alınan hidrogellərin doksorubisin ilə $pH=1\div 10$ intervalında 24 saat ərzində sorbsiyası aparılmışdır. Müəyyən olunmuşdur ki, turş mühitlərdə ($pH\leq 4$) hidrogelin tərkibindəki aktiv funksional qrupların protonlaşması və bununla da həm hidrogelin, həm də doksorubisinin eyni yüklü olması onların elektrostatik qarşılışmasını təmin edə bilmir. Həmçinin aşağı pH-larda hidrogelin şişmə dərəcəsinin az olması antibiotik molekulunun hidrogelin daxili məsamələrinə nüfuz etməsinə maneçilik törədir. Mühitin pH-ı qələviyə doğru dəyişdikcə hidrogelin səthinin deprotonlaşması və əksinə olaraq mənfi yüklənməsi müsbət yüklü doksorubisin molekulunun asanlıqla sorbsiya olunmasına səbəb olur. Digər tərəfdən, sorbsiya dərəcəsinin artmasına qələvi mühitdə hidrogelin yüksək şişmə formasına keçməsi də köməklik göstərir. Eyni zamanda hidrogel ilə antibiotik arasındakı kimyəvi qarşılıqlı təsirin xarakteri infraqırmızı spektroskopiyaya, eləcə də skanediçi elektron mikroskopiyaya üsulları ilə öyrənilmişdir. Göstərilmişdir ki, polimerlə doksorubisin arasında əlaqə hidrogen rabitəsi, elektrostatik və hidrofob qarşılıqlı təsir hesabına baş verir.

Açar sözlər: doksorubisin, immobiizə, tikilmə, poliakril turşusu, metilen-bis-akrilamid, hidrogel.

ИССЛЕДОВАНИЕ СОРБЦИОННЫХ СВОЙСТВ И СТРУКТУРЫ ПОЛИМЕРНОГО ГЕЛЯ, ИММОБИЛИЗОВАННОГО ДОКСОРУБИЦИНОМ

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Проведена сорбция доксорубицина гидрогелем, полученным сшивкой полиакриловой кислоты с разным количеством (% масс) N,N' метилен-бис-акриламида в интервале $pH=1\div 10$ в течение 24 часов. Установлено, что протонизирование активных функциональных групп гидрогеля в кислой среде ($pH\leq 4$) препятствует электростатическому взаимодействию в связи с одинаковыми зарядами в доксорубицине и гидрогеле. Малая степень набухаемости при низких значениях pH среды также препятствует проникновению молекулы антибиотика во внутренние поры гидрогеля. Изменение pH в сторону щелочной среды, депротонизирование, и тем самым отрицательное заряджение поверхности гидрогеля приводит к сорбированию положительно заряженной молекулы доксорубицина. С другой стороны, высокая набухаемость гидрогеля в щелочной среде также способствует увеличению степени сорбции. Также изучен характер химической взаимосвязи между гидрогелем и антибиотиком методами ИК-спектроскопии, а также сканирующей электронной микроскопии. Показано, что связь между полимером и доксорубицином происходит, в основном за счет водородной связи, электростатического и гидрофобного взаимодействия.

Ключевые слова: доксорубицин, иммобилизация, сшивание, полиакриловая кислота, метилен-бис-акриламид, гидрогель.