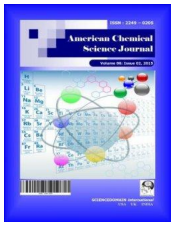

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Investigation Chemical Interaction Type of Polyacrylic Acid Based Hydrogel with Doxorubicin Hydrochloride

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Authors' contributions

This work was carried out in collaboration between all authors. Author SZT designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors DBT and NAZ managed the analyses of the study. Author SMM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

In alkali environment hydrogels with high swelling ability have been synthesized as a result of cross-linking well water soluble and non-toxic polymer - polyacrylic acid (M_η=230 kDa) with 5, 10, 15 and 20% ratio (by weight) of N,N`-methylene-bis-acrylamide. Absorption processes of obtained hydrogels with doxorubicin hydrochloride from water environment have been researched. Dependence of sorption degree and sorption capacity of antibiotic on environment pH and starting concentration of doxorubicin has been researched and nature of chemical interaction between hydrogel and antibiotic has been found out via infrared, ultraviolet, nuclear-magnet resonance spectroscopy methods. It was determined that in pH=8 hydrogel taking 10% cross-linking reagent

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inside has 570% swelling degree and with increasing environment pH sorption capacity increases up to 13.4 mg/g as per doxorubicin of gel. It was investigated with UV, FTIR and NMR spectroscopy methods that interaction hydrogel with antibiotic occurs due to hydrogen bonds and electrostatic forces.

Keywords: Doxorubicin; immobilization; cross-linking; polyacrylic acid; methylene-bis-acrylamide; hydrogel.

1. INTRODUCTION

Antibiotics are one of the drugs, which are of particular importance in medicine and biotechnology. Antibiotics unusual due to the fact that, unlike other drugs, their target receptor is not located in human tissue, but located in the cells of microorganisms. In addition, the activity of antibiotics is not forever, it gradually decreases [1-5].

Antibacterial polymers are also recorded as polymer biocides. It was determined that the length of alkyl fragments and spatial structure in the polymer chain affects on its antibacterial activity. In general, the results indicate that with increasing alkyl groups these polymers show higher activity. This effect can be explained in two ways. First, the long chains have more active pores of the adsorption capacity with bacteria cell wall and cytoplasm membrane. Secondly, aggregation form of long chains is more different than short chains, which also provides better adsorption [6-8]. From this point fixing antibiotics to polymer composites especially against to the cancer and synthesis of composites which has long lasting effect in their little concentration currently is in focus of the world biochemistries [9-11].

The research shows that inclusion of novocaine, insulin, penicillium, tetracycline, ofloxacin and other antibiotics with polymers such as poly-N-vinylpyrrolidon, polyvinyl alcohol, dextran into to the body in mixture form even without chemical bond increases the impact time and decreases toxicity of them [12,13].

Two directions should be noted in the synthesis of polymer derivatives of antibiotics: - the synthesis of polymer salts and obtaining of *"poly-antibiotics"* by combination of a covalent bond with polymer chain of antibiotics. For example, 50 nm polymer micelles were obtained by fixing doxorubicin to the grafted copolymers of polyuronic acids of o-succinyl with chitosan. At this time 73-74% efficiency of encapsulation has been identified [5,14].

In other work adsorption of doxorubicin hydrochloride from aqueous solution by graphite oxide has been conducted, kinetic and thermodynamic parameters were determined. It was determined that the adsorption capacity of the carrier is 1428 mg/g and the adsorption isotherm results conform to Langmuir model. The adsorption kinetics is a pseudo-second model and adsorption is spontaneous and endothermic [15].

Saman and colleagues [16] prepared nano-zeolite composites with hydrogels based on polyethylene glycol, polyacrylic acid and polyacrylamide and immobilizied amoxicilin antibiotic. Separation of antibiotics to solution at pH=7.8 and 6.8 depending on time and amount of nano-zeolite in the composition. It was determined that at pH=7.8, 37°C 4% nano-zeolite containing composite amoxicillin antibiotic which contains 4% nano zeolite amoxicillin separation to the solution is 27% during 500 minutes.

Bonding type of carboxyl derivatives of hyperbranched polyether polyols having different fictionalization degree with doxorubicin drug was investigated. The sizes of aggregates obtained based on doxorubicin- polyether polyacrylic acid is changing between 30-200 nm and they are stable depending on time. Aggregates can be effectively used for carrying doxorubicin [17].

Taking into account mentioned above, sorption regularities of doxorubicin antibiotic with hydrogel obtained on the basis of polyacrylic acid have been studied and explained the nature of chemical interaction between antibiotic with hydrogel in the presented paper.

It is known that doxorubicin hydrochloride which is used for chemical therapy of blood cancer is the highly effective anti-neoplastic drug having antracyclic ring.

Various routes of immobilization of anticancer substances on polymer carriers of synthetic,

natural or mixed natures can also be useful for developing water-soluble forms of water-insoluble drugs and providing them with prolonged action and addressed delivery to the target organ. Therefore, creating novel drug delivery systems that contain surface active polymeric carriers corresponding to these requirements is of great actuality.

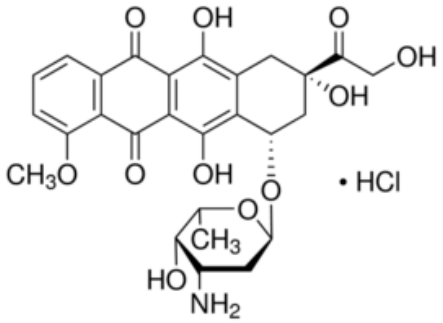

Fig. 1. Chemical structure of doxorubicin hydrochloride

2. EXPERIMENTAL

2.1 Materials

Average molecular mass of polyacrylic acid (PAA) with purity 90% is 230 kDa and was purchased from Fluka. Methylene-bis-acrylamide (MBAA) which is used as a crosslink agent was supplied by Sigma Aldrich and both of reagents are used without further purification. Doxorubicin hydrochloride (DOX) code ATX L01DB01 from TEVA Pharmaceutical Industries (Israel). Deionized water which is used for preparation of solutions, diethyl ether - to precipitate PAA and NH_4OH, CH_3COONH_4, HCl, KOH for buffer solutions are analytical chemically pure and from Aldrich Sigma.

2.2 Preparation of Hydrogels

100 mg PAA is dissolved in 50 ml deionized water. Cross-linking reagent - MBAA in 5, 10, 15, 20% of polymer mass is added into solution and mixed 1 hour. 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 hours. After irradiation, the samples are washed two-three times consistently with deionized water, 0,01N HCl and ethanol. Samples are dried at 313-323 K and brought to a stable weight [4].

2.3 Absorbtion 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 is stored 24 hours in 10 ml deionized water. Then, by adding appropriate 5 ml 1÷10 pH it is kept 30 minutes, and DOX solution with 1 ml×10^{-3} mol/l concentration is added and kept 24 hours in dark place.

Then the solution is filtered, after the sorption the concentration is determined by comparing concentration of antibiotic remained in filtrate and optical density (UV-VIS 1800, SHIMADZU) in 510 nm area with pre-arranged graphic. According to the difference concentrations of antibiotic are calculated sorption degree (SD, %) of DOX and sorption capacity (SC, mg/g) of hydrogel for antibiotic [18].

$$SD = \frac{C_{beg} - C_{end}}{C_{beg}} \times 100 \quad SC = \frac{C_{beg} - C_{end}}{g} \times V$$

Here, C_{beg}, C_{end} concentrations of DOX before and after sorption (mg/l), V-general volume of solution (ml), and - g is the hydrogel dose (mg).

Depending on concentrations of DOX for studying sorption isotherms [19] we used 0,05 g PAA which is cross-linked with 10% MBAA showing maximum swelling and sorption degree and sorption capacity at pH=8. Sorption processes were conducted at constant temperature under static condition by changing concentration of antibiotic at 0.01, 0.02, 0.03, 0.05, 0.1, 0.2, 0.3 and 0.5 mg/l interval.

2.4 Structural Analysis

To determine the interaction of functional groups between hydrogel based on PAA and DOX ultra-violet (UV) (SHIMADZU IR-affinity) and Fourier transformation infrared (FTIR) spectroscopy methods were used. FTIR spectra were taken at 4000 and 400 cm^{-1} spectral range.

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. Because the H^+ and OH^- ions being in solution are causes to the charging of surface and volume of absorbent in other words hydrogel that is ionization and this direct affects to the

sorption degree of sorbet and sorption capacity of hydrogel [20]. In this point at the static conditions in the pH= 1÷10 interval within 24 hours have carried out sorption (in the closed bottle, dark) of hydrogels which is obtained by cross-linking with MBAA in different % (by weight) quantity of PAA with DOX and the results are given in Table 1.

As it is shown from Table 1 by increasing of pH of environment the sorption degree are increases in the all samples up to 60-70%. In acidic environments (pH≤4) protonization of active functional groups (>C=O, -OH, and –NH-) occurs in the composition of hydrogels. Both hydrogel and DOX being same charge can't provide electrostatic encounter. Also at low pH, being less of swelling degree of hydrogel prevents to penetrate the antibiotic molecule into pores of the hydrogel. Sorption of positive charged DOX molecule easily causes deprotonization of hydrogel surface and inversely negative charging as environment pH getting to alkali. This time from other side in alkali environment hydrogels transition into high swelling shape also helps increasing of sorption degree.

Also it was determined that when amount of cross-linking agent changes, sorption degree changes in all pH too. It was studied that hydrogel obtained by cross-linking PAA with 10% MBAA maximally absorbs DOX at the pH=8. It is related with high swelling degree (600%) of hydrogel and rate of starting concentration of taken DOX at the same pH. Because in pH=8 occurring sorption equilibrium at the concentration rate is directly related to more optimal swelling degree with the same concentration rate of hydrogel surface. Though increasing swelling degree of hydrogel after pH=9, the sorption degree of DOX decreases. In alkali environment it much more causes discharging of the antibiotic surface and decreasing hydrophilicity as a result [21].

The above-mentioned results can be seen also in dependence curves of pH environment (Fig. 2) of sorption capacity for DOX of hydrogels which obtained by cross-linking of PAA with 5÷20% MBAA.

As it seems in Fig. 2 the variation characterization of hydrogel sorption capacity is almost the same shape with sorption degree. Sorption capacity decreases accordingly to increasing quantity of cross-linking reagent in hydrogel. It is determined that in case sorption capacity for DOX of hydrogel based on PAA cross-linking with 10% MBAA in pH=8 is 14.12 mg/g, then sorption capacity of polymer cross-linking with 20% MBAA is 12.64 mg/g. It can be explained by decreasing size of pores in hydrogel despite concentration of functional groups rises by increasing quantity of crosslink reagents. But comparing hydrogels obtained with presence of 5% and 10% MBAA, high sorption capacity of hydrogel obtained from with 10% crosslink reagent is related with being optimal or from spatial point of view and conformationally more acceptable for DOX molecule of internal nets. This case provides antibiotic molecule to stay more stable and resistant in gel pores. But in polymer cross-linking with 5% MBAA because gel forming property is not full, sizes of pores are too big and quantity of active functional groups in cross-linking reagents is not enough for taken DOX, so concentration gel has little sorption capacity [15,22]. Because antibiotic molecule is not surrounded with enough quantity functional groups, as a result it is not resistant in the gel.

During research it was studied sorption process of PAA cross-linking with 10% MBAA with 50 mg DOX in 0.01÷0.5 mg/l concentration interval in 293 K in pH=8 (Fig. 3).

As it seems if DOX concentration is increased from 0.01 mg/l to 0.5 mg, sorption degree decreases accordingly from 86% to 14%. It is related with increasing concentration of antibiotic in stable rate of hydrogels taken quantity and the emergence of a state of equilibrium. It is known that gel will absorb DOX according to equivalent amount of functional groups in it and whichever rate of antibiotic concentration forms sorption equilibrium the same cross-linking degree is considered optimal for hydrogel. Meantime, balance constant rate emerged between quantity of DOX absorbed into gel pores and quantity remaining in solution, influences to this process too. It is determined that sorption degree in 0.05 mg/l concentration rate of DOX being maximal is equal to 88%. As DOX concentration increases functional groups in gels are not enough for much more occurring of sorption and so, sorption capacity rate becomes stable in the next increases of DOX concentration [12,23]. After 0.02 mg/l rate of concentration both of sorption capacity and sorption degree become stable. In the 0.3-0.5 mg/l rate of concentration, despite 20-21 mg/g of sorption capacity, sorption degree takes 15-18%. Regarding this, gel based on 0.05 gr PAA cross-linking with 10% MBAA can be optimally and invariably accepted with DOX for sorption of starting solutions of 0.05-0.1 mg/l concentration in 293 K within 24 hours.

Table 1. According to DOX the dependence rate of pH of environment from sorption degree of gel based on PAA cross-linking 5 ÷ 20% ratio with MBAA. m = 0.5 g, T = 293 K, V = 15 ml, C_{DOX} = 1×10^{-2} mg/l

pH	Sorbtion degree, %			
	5% MBAA	10% MBAA	15% MBAA	20% MBAA
1	4.26	9.78	6.27	4.52
2	9.34	21.67	14.36	10.27
3	22.41	36.27	29.42	21.76
4	31.64	42.18	36.82	32.41
5	43.16	51.76	48.56	42.63
6	65.76	74.62	70.24	54.82
7	73.46	81.74	78.32	70.46
8	84.26	88.24	85.22	79.16
9	78.36	84.36	81.42	72.86
10	60.24	74.42	68.23	62.74

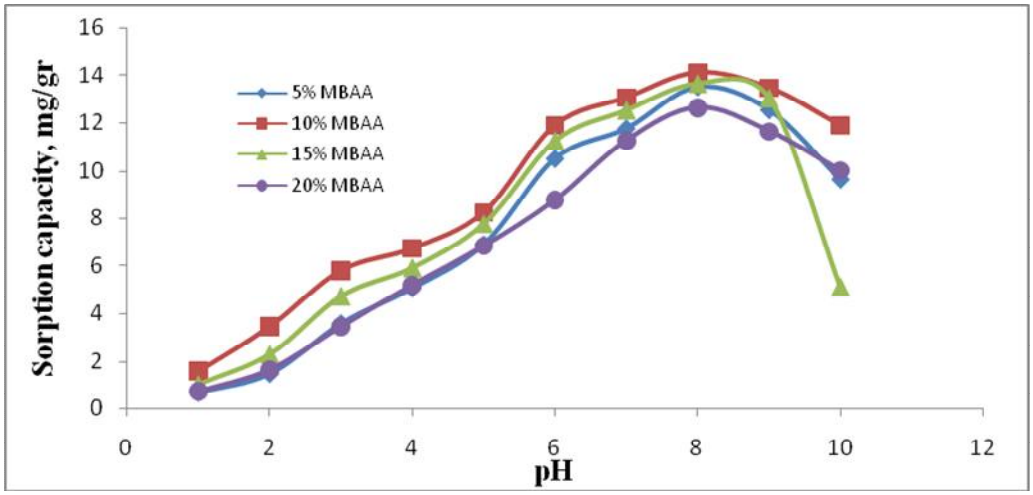

Fig. 2. Dependence curves of pH environment of sorption capacity for DOX of PAA based gel cross-linked in different % ratio with MBAA

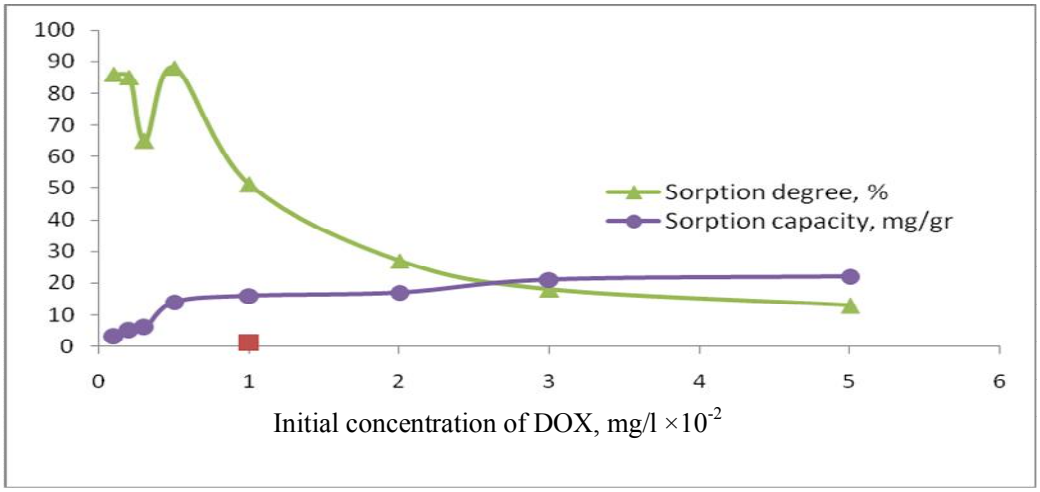

Fig. 3. Dependence curves of sorption capacity of 10% cross-linking PAA acid for DOX and dependence curves of sorption degree on starting concentration of antibiotic

To determine kind of chemical interaction between DOX molecule and PAA based gel, structures of starting substances and complexes both are identified with IR spectroscopy methods (Fig. 4). We can see enough quantity of active functional groups, if we look below structures of DOX molecule, MBAA and polymer macromolecule:

It is determined that, absorption strips having frequency of 1430, 1230, 1638 and 3345 cm^{-1} are observed according to CH_2=CH-, >C=O, -OH functional groups in FTIR spectrum of PAA (Fig. 3). But there are absorption strips 1645, 1445, 1650 cm^{-1} specific to CH_2=CH-, $>CH_2$, >C=O and –NH groups in crosslink reagent. There are observed decreasing intensity in absorption strip belonging to $>CH_2$ cross-linked polymer spectrum and intensity specific to $–CH_3$ 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 two smaller absorptions. Chemical shifts occur in absorption strips of functional groups belonging to both of crosslink reagent and polymer in FTİR spectrum of PAA-DOX complex. In FTIR spectrum it is impossible to track functional groups belonging to DOX as antibiotic quantity is less than 2% in hydrogel. But it can be determined which functional groups actively participates in absorption for chemical shifts of absorption strips by PAA and MBAA. So, chemical shifts of absorption strips specific to >C=O, -OH,-NH-, -CO-NH groups in PAA and MBAA occur to 1670, 1428, 1635, 3340 cm^{-1} area. It has been mentioned that little difference between chemical shifts cause to creating not covalent bond with DOX and PAA so mainly hydrogen bond and electrostatic interaction created complex between DOX with PAA.

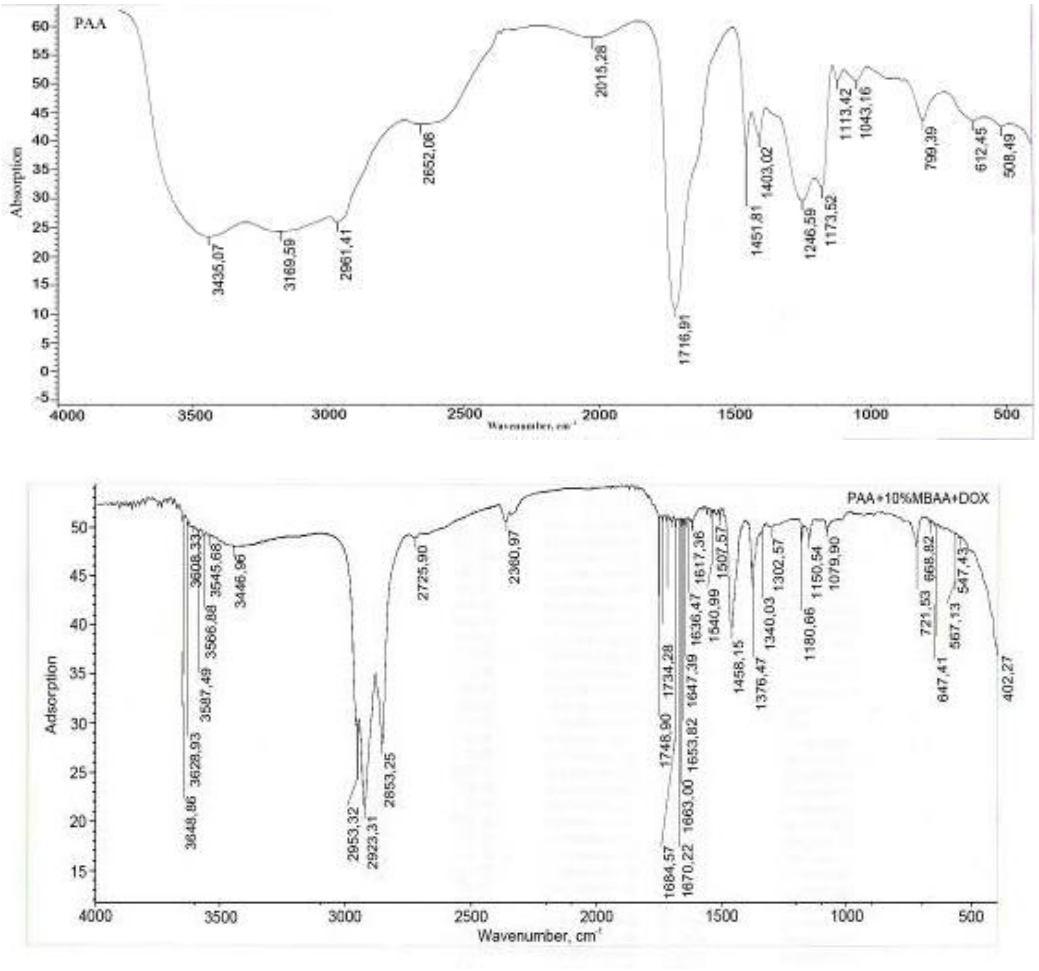

Fig. 4. FTIR spectroscopy of cross-linking PAA and PAA/DOX composite

By ^{1}H and ^{13}C NMR spectroscopy it was studied the chemical interaction between the macromolecule of DOX and the carrier. ^{1}H and ^{13}C NMR spectra of the studied systems are recorded at room temperature using a Bruker Fourier with operating frequency of 300 MHz. As a solvent used deuterated water D_2O and dimethyl sulfoxide DMSO-d_6 were used. This purpose is to use the PAA, PAA-DOX model systems with presence of (D_2O) solvent (Figs. 5-6).

Interaction of DOX with PAA confirms the data by NMR spectroscopy. PAA proton signals are broadened and shifted toward to more weak fields (Fig. 5). At the same time, a ^{1}H NMR spectrum transformation occurs and some signals. Interaction between DOX with PAA can clearly be seen by the spectra recorded before and after the application of the DOX on the PAA. Observed signals at 42-40 ppm can be attributed to C_{α} carbon atoms, and the signals at 36.2-34.6 ppm- $C_{ß}$ to carbon atoms (Fig. 6). During interaction between DOX with PAA in the NMR spectra there is a noticeable change in the intensity and position signals of the DOX and PAA. Decrease of the intensity of the signals which is characteristic to carboxyl and amine groups is observed by this way. However, the obtained spectra do not allow detailing the nature of this interaction. It requires additional measurements, generally, obtaining of ^{13}C to obtain spectra for DOX/PAA systems [24].

Therefore, it can be assumed that displacement of DOX molecules and binding PAA macromolecules via hydrogen bonds with oxygen and nitrogen atoms of DOX and –OH, -NH- and >C=O groups of hydrogel leads to the formation of micelle-like structures with polymer hydrogel and free DOX molecules (Fig. 7).

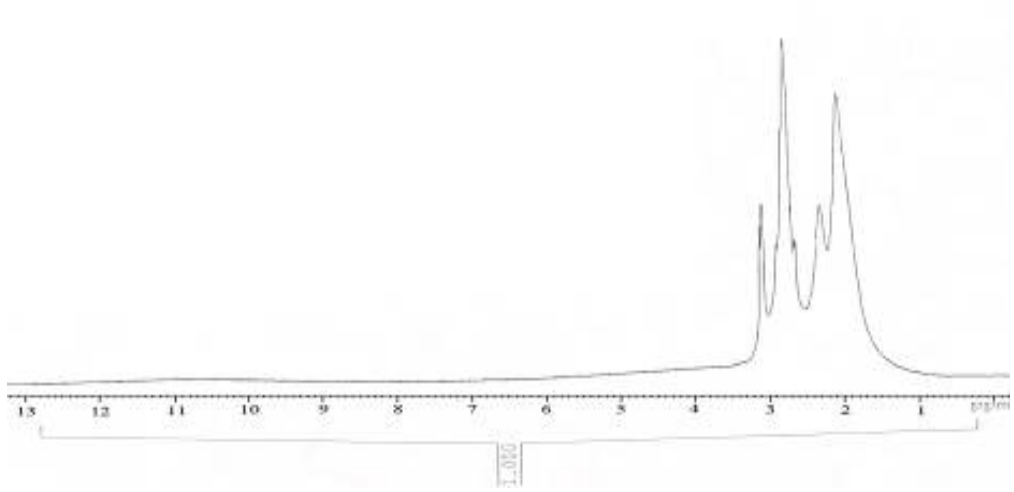

Fig. 5. ^{1}H NMR spectrum of PAA/DOX composite

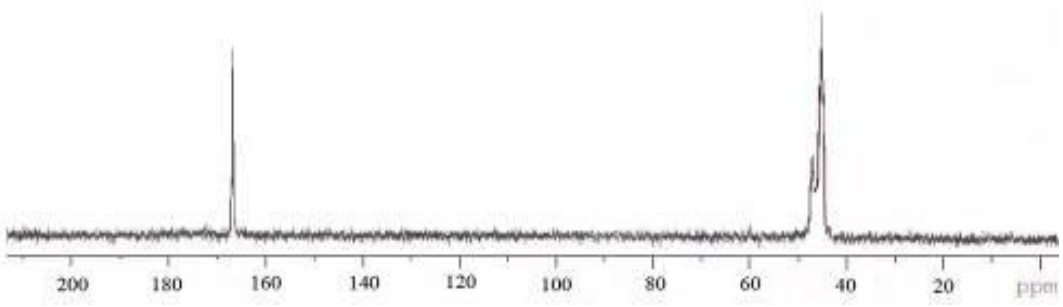

Fig. 6. ^{13}C NMR spectrum of PAA/DOX composite

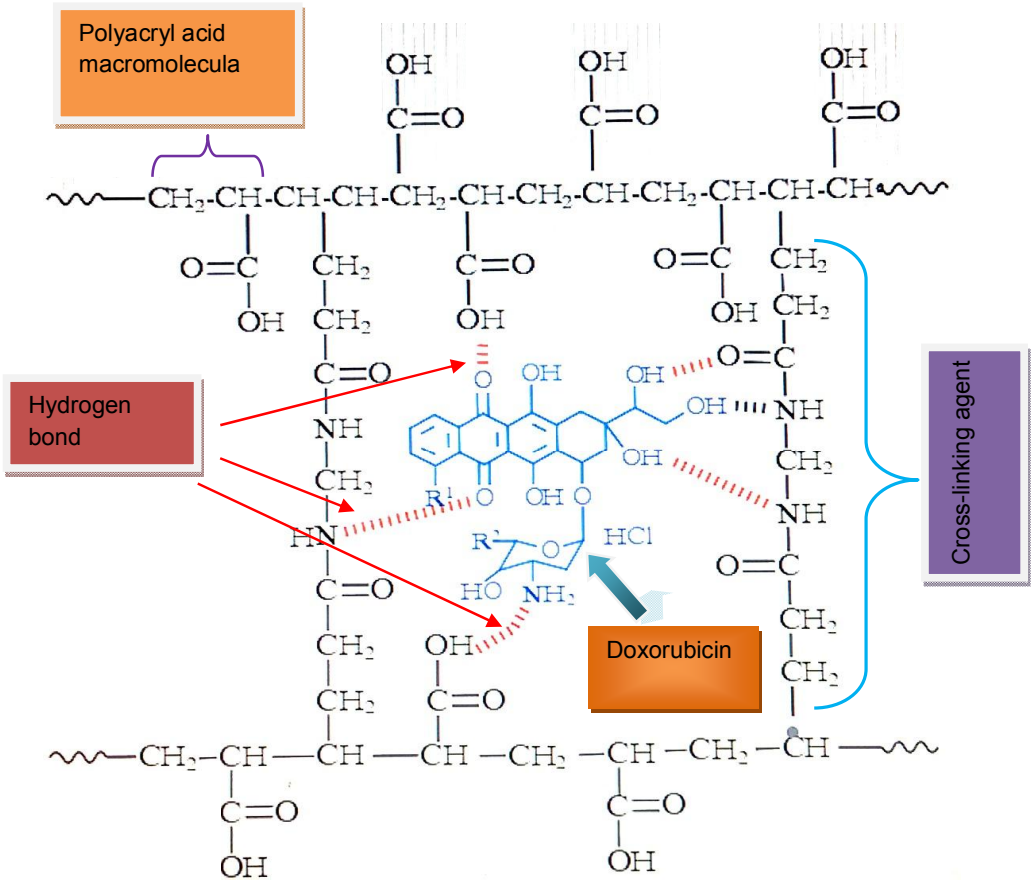

Fig. 7. The suggesting structural of PAA/DOX complexes

4. CONCLUSION

Hydrogel obtained by cross-linking polyacrilic acid with 10% N,N`-methylene-bis-acrylamide maximally absorbs doxorubicin antibiotic in 0.005-0.01 mg/l concentration at the pH=8. At room temperature during 24 hour static condition sorption rate of DOX is 88% and sorption capacity is 18-21 mg/l. 2% (1 g) DOX keeping compositions are used at medical, biotechnology for transportation of mentioned antibiotic, also is used as for a long time affecting composite material.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Wang YZ, He F, Zhuo RX. Synthesis and characterization of amphiphilic block copolymer containing PVP and Poly (5-benzyloxytrimethylene carbonate. Chinese Chemical Letters. 2006;17:239-242.
2. Korshak VV, Shitilman MI. Polymers in process immobilization and modification nature compounds. Book, Moscow. 1984; 260.
3. Aryal S, Prabaharan M, Pilla S, Gong S. Biodegradable and biocompatible multi-arm star amphilic block copolymer as a carrier for hydrophobic drug delivery. Int. J. Biol. Macromol. 2009;44:346-352.
4. Cuvier C, Roblot TL, Millot JM. Doxorubicin-loaded nanospheres bypass tumor cell multidrug resistance. Biochem Pharmacol. 1992;4:509-517.
5. Chen J, Ouyang J, Kong J, Zhong W, Xing MM. Photo cross-linked and pH-sensitive biodegradable micelles for doxorubicin delivery. ACS Appl Mater Interfaces. 2013;5:3108–3117.
6. Chung HJ, Go DH, Bae JW, Jung IK, Lee JW, Park KD. Synthesis and characterization of Pluronic grafted

chitosan copolymer as a novel injectable biomaterial. Current Applied Physics. 2005;5:485–488.
7. Keith WG, Jon C, James DK, Andrew RZ, Susan LB. Sorption of the antibiotic ofloxacin to mesoporous and nonporous alumina and silica. Journal of Colloid and Interface Science. 2005;283:160-170.
8. Chen S, Zhang XZ, Cheng SX, Zhuo RX, Gu ZW, et al. Functionalized amphiphilic hyber branched polymers for targeted drug delivery. Biomacromolecules. 2008;9: 2578- 2585.
9. Staroverov SM, Kuznetsov MA, Nesterenko PN, Vasiarov GG, Katrukha GS, Fedorova GB. New chiral stationary phase with macrocyclic glycopeptide antibiotic eremomycin chemically bonded to silica. J Chromatogr A. 2006;1108:263-267.
10. Andersen A. Quantitation of cell-associated doxorubicin by high-performance liquid chromatography after enzymatic desequestration. Cancer Chemother Pharmacol. 1994;34:197-202.
11. Parichart N, Kwanchanok VP. Development of doxorubicin - Core shell chitosan nanoparticles to treat cancer. International Conference on Biomedical Engineering and Technology. 2011;11:90-94.
12. Valuev LI, Valueva TA, Plate NA. Polymer system for controller separation biological active compounds. Uspexi Biologicheskaya Khimiya. 2003;43:307-328.
13. Missirlis D, Kawamura R, Tirell N, Hubbell JA. Doxorubicin encapsulation and diffusional release from stable, polymeric, hydrogel nanoparticles. European Journal of Pharmaceutical Sciences. 2006;29:120–129.
14. Pruitt J, Husseini G, Rapoport N, Pitt WG. Micelles with an interpenetrating network of N, N-diethylacrylamide. Macromolecules. 2000;33:105.
15. Shaoling Wu, Zhao Xindong, Li Yanhui. Adsorption of doxorubicin hydrochloride onto grapheme oxide: Equilibrium, kinetic and thermodynamic studies. Materials. 2013;6:2026-2042.
16. Saman S, Aboulfazl B, Reza D, Mohammad AF. Antibiotic release process from hydrogel nano zeolite composites. Middle-East Journal of Scientific Research. 2012;12:392-396.
17. Khannanov AA, Bondar OV, Surnova AV, Kutireva MP. Joint aggregates of doxorubicin with modified polyols. Student Scientific Journal. 2013;1:73-78.
18. Polyakova IV, Toshevikova AY. Sorption dynamics of antibacterial antibiotic eromomicina on carboxyl cationite. Structure and Dynamics of Molecular Systems. 2003;2:173-175.
19. Reem Al-Bayati A. Adsorption – Desorption of trimethoprim antibiotic drug from aqueous solution by two different natural occurring adsorbents. International Journal of Chemistry. 2011;3:21-30.
20. Beijnen JH. Aspects of the chemical stability of doxorubicin and seven other anthracyclines in acidic solution. Pharm Weekbl Sci. 1985;21:109-116.
21. Weenen H, Osterop AP, Poort SE, Lankelma J. Analysis of doxorubicin, 4'-epidoxorubicin, and their metabolites by liquid chromatography. J Pharm Sci. 1986; 75:1201-1204.
22. Lee CJ, Kang JS, Kim MS, Lee KP, Lee MS. The study of doxorubicin and its complex with DNA by SERS and UV-resonance raman spectroscopy. Bull. Of the Korean Chemical Society. 2004; 25:1211-1216.
23. Petrusevska K, Kuznetsov MA, Gedicke K, Meshko V, Staroverov SM. A Seidel-morgenstern chromatographic enantio-separation of amino acids using a new chiral stationary phase based on a macrocyclicglycopeptide antibiotic. J Sep Sei. 2006;29:1447-1457.
24. Tapdigov ShZ, Mammedova SM, Zeynalov NA. Spectroscopic investigated interaction between silver nanocomposites based of Poly-N-Vinylpyrrolidone and doxorubicin for drug delivering. J. Chemistry & Chemical Engineering. 2014;8:800-804.

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