

The Release of Doxorubicin's Active Ingredient from the Hydrogels with Poly (HEMA/Acrylamide/ Itaconic acid) and Their Biological Function

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ABSTRACT

In this study, Poly (HEMA/Acrylamide/ Itaconic acid) (HAI) polymer was synthesized for using in the release of Doxorubicin drug. Poly (HEMA/acrylamide/Itaconic acid) (HAI) hydrogel was prepared by a radical addition reaction in aqueous media formed by HEMA, acrylamide, Itaconic acid, in the presence of TEMED (N,N,N',N'-tetramethylenediamine). The characterization of the polymer was performed by FTIR analysis. At the same time, swelling and drug absorption capabilities of the polymers were analyzed. It has been observed that the entire drug, which was added to the solution with 0.1 mg of polymer, was absorbed in around 25 hours. This measurement showed that the absorption of this polymer is quite high compared to many polymers in the literature. Moreover, this polymer showed 80% swelling at 1000 minutes. In the light of these results, it has been concluded that this new polymer is very suitable for the release of Doxorubicin drug.

Key words: Doxorubicin, Poly (HEMA/acrylamide/Itaconic acid), Drug release, FTIR, kinetic

INTRODUCTION

Drug-polymer interactions are accepted as the first step of the controlled release, where the polymeric structure, in which active ingredient is adsorbed, is as important as the active ingredient of the drug. Synthesized polymers should not interact with the active ingredient, should be biocompatible and biodegradable, should be produced easily and cost effectively. Network structures that are swellable in water are called as hydrogels. Hydrogels are quite useful on controlled drug release systems because they respond the changes on pH, temperature, ionic concentration; they have swelling capability; and they control the diffusion of drug molecules. With the macro pores that they have, they show fast swelling kinetics.

Doxorubicin is an anti-cancer agent that is frequently used in the research and development of drug carrying systems. Doxorubi-

cin, which is a derivative of anthracycline, makes intercalation into DNA as all other anthracycline. The full action mechanism of doxorubicin is complex and not fully understood yet, however it is guessed that it interacts with DNA through intercalation.¹ It is known that doxorubicin interacts by intercalation and prevents its biosynthesis.² If doxorubicin is present in the media, the advancement of Topoisomerase II enzyme, which allows the opening of DNA, is prevented. During the replication of DNA, doxorubicin stabilizes the topoisomerase-DNA complex that is formed after the cut of DNA by topoisomerase and prevents the reunion of DNA double helix, thus it stops the replication of the DNA.^{3,4} Combined treatment trials performed using Sirolimus (rapamycin) and doxorubicin was promising in healing Akt-positive lymphomas of mice.⁵ Doxorubicin is also used in HIV treatment. The immuno-composite that

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was obtained by binding doxorubicin to a monoclonal mouse antibody could eliminate HIV infection in mice. Antiretroviral treatment (ART) still leaves some amount of HIV in the host body. The immuno-composite containing doxorubicin has the potential of being a complementary treatment for destroying cells that continue to express antigen.⁶ But, doxorubicin was seen to be more effective in the soft tissues and in breast cancer.

Acrylamide can usually join radical reactions with its carbon-carbon double bonds, thus it is a chemical that is widely seen in the structure of the polymers that were synthesized for drug absorption and release. In fact, it has been found that it is formed as a result of heat treatment from foods rich in starch, after which the studies on its residues remaining in the body are increasing. The formation of acrylamide is observed on many foods rich in protein. There are many studies aiming to remove / decrease if from the product because it is considered as a potential carcinogen. Therefore, even though the synthesized polymer was carefully purified, cytotoxicity tests were performed for acrylamide.

On the other hand, the risks of HEMA, which is easily polymerized as acrylamide, to human health are not known, thus cytotoxicity tests gained more importance. HEMA has the $H_2C=CCH_3COOCH_2CH_2OH$ formula and similar to acrylamide it can easily join polymerization through double bonds. Itaconic acid is a white, crystal chemical, with formula $C_5H_6O_4$. In addition, it is a chemical whose *in vitro* effect against bacteria was proven.

This study aims to perform the interaction of Poly (HEMA/Acrylamide/ Itaconic acid) (HAI) polymer with doxorubicin's active ingredients, which is used as an anti-cancer agent, and examine its absorption capability and cytotoxic impact of the polymers.

MATERIALS AND METHODS

Synthesis of HAI Polymer

Poly (HEMA/acrylamide/ Itaconic acid) (HAI) polymer was prepared by a radical addition reaction. 5 mmol HEMA, 3 mmol acrylamide, and 1, 5 mmol Itaconic acid were dissolved in water. 0,1 mmol EGDMA (Ethylene glycol dimetacrilat) was added as cross binder, 0,02 mmol APS (ammoniumpersulphate) was added for starting polymerization and 0,06 mmol TEMED (N,N,N',N'-tetramethylethylenediamine) was added as accelerator, mixed for 1 minute and filled into pipets; they were held at 22° C for 24

hours. Then polymers were removed from pipets, cut in pieces of 3-4 mm, washed with double distilled water and dried first in air, then in the vacuum incubator.

Characterization of HAI Polymer

Structural characterization of HAI polymer was performed through swelling experiments and Fourier Transform Infrared (FTIR).

Cytotoxicity

Cultivation of the Cells

Cell cultivation studies should provide appropriate conditions and required medium for the existence and multiplication of the cells *in vitro* environment. Inactive serum should be definitely added to the medium for fulfilling the needs of the cells and acting as a buffer. The medium environment that we have used in our study was DMEM medium consisting of 10% Fetal bovine serum (FBS), 1% L-glutamine, 100 IU/mL penicillin and 10 mg/mL streptomycin. Cancer cell lines that were produced in DMEM medium were multiplied in the incubator at 37°C, with 95% humidity and 5% CO₂. Cytotoxicity tests were performed on Mouse Fibroblast Cell Lines (L929) through XTT Test, where L929 have been procured from ATTC.

Cytotoxicity Tests

Determination of Composites' IC₅₀ dosage for Cancer Cell Lines

IC₅₀ dosage indicates the inhibitor's concentration required to kill 50% of the target. IC₅₀ dosage of the composites have been determined by XTT (2, 3-bis (2-methoxy-4-nitro-5-sulphophenyl)-5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide) test, using "Cell Proliferation Kit". Even though XTT is a tetrazolium salt, it is decomposed by the dehydrogenase enzyme, which is present in the mitochondria of the metabolically active cells, and it is transformed to formazan, which is soluble in water. The density of the orange color arising from formazan is proportional to the number of living cells. Cell viability is determined by reading the density of the orange color, which has been formed at the end of the incubation period, in a micro plate reader, at 450-475 nm wavelengths.

At the end of the operation, the concentration that kills half of the healthy cells is set as IC₅₀ value. Sterile 96-wells plates are planted in a way that there is 10x10⁴ cells in each plate. Meanwhile, for sterilization purposes polymers were held under UV light for 4 hours; steril-

ization was established by holding them in 70% alcohol for 1 hour and washing with PBS 3 times; afterwards polymers were placed into wells. Following 24 hours of incubation, polymers were removed from wells and XTT kit was applied.

Cytotoxic Impact of HAI Polymer

Cytotoxic impact of HAI polymer was evaluated using mouse fibroblast cell line (L929). For sterilization purposes HAI polymer was hold under UV light for 4 hours. Sterilized polymers were placed into 10^4 cell planted plates. Cytotoxicity analysis were performed after 24 and 48 hours of incubation, through XTT (2, 3-bis (2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenyl amino) carbonyl]-2H-tetrazolium hydroxide). Results were expressed as percent viability.

Polymer (HAI) Doxorubicin Couple

HAI polymers were hold with the active ingredient of doxorubicin drug in phosphate buffer (PBS) for 5 days, in an incubator at 37°C . Absorption amounts were calculated through the samples taken from the solution every day. The samples were read in UV-Vis at 485 nm wavelengths and it was found that 98% was absorbed at the end of 5th day. Dried polymers were placed into PBS solution for release, in an incubator at 37°C . The release was monitored for 20 days by reading the samples that were taken in pre-determined time intervals in UV-Vis at 485 nm. The drug absorption, which has occurred at physiological pH and body temperature (37°C), has provided the basic approach for the polymer that contains the active pharmaceutical ingredient to be a bio-material.

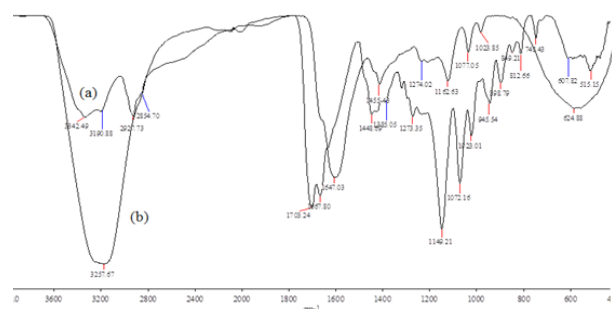


Figure 1: (a)-(b) FTIR analysis of HAI and Doxorubicin adsorbed HAI polymer

RESULTS

FTIR

IR spectrum of Poly (HEMA/acrylamide/Itaconic acid) (HAI) polymer is shown in Figure 1a and Figure 1b. In Figure 1 (a) the peak belonging to hydroxyl peak of the active ingredient is seen at 3257 cm^{-1} , whereas in Figure 1 (b) it was shifted to $3342\text{-}3090\text{ cm}^{-1}$ due to HEMA/acrylamide/Itaconic acid polymer. In addition a proton was added to the group because of the binding of the drug active ingredient's to the carbonyl group and alkyl peaks were observed at 2927 and 2854 cm^{-1} . Strong carbonyl peaks observed at $1708\text{-}1667\text{ cm}^{-1}$ in Figure 1 (a) were shifted to 1647 cm^{-1} at figure 1 (b). Following the binding of the drug, dense C-C peaks were identified around 1448 and 1385 cm^{-1} . Many splits and flourishing were observed on the peaks of the polymer observed around $1162\text{-}1023\text{ cm}^{-1}$ in Figure 1 (a).

Similarly, the peak of the drug observed at 624 cm^{-1} was seen as two separate peaks in figure 1 (a). According to FTIR analysis, Doxorubicin's active ingredient was bond to the synthesized polymer.⁷⁻⁹

Swelling

This polymer showed 170-175% swelling at 1000 minutes, in Figure 2. The swelling properties of polymer have been saturated $> 200\%$ at 1600 minutes. In the light of these results, it has been concluded that this new polymer is very suitable for the release of Doxorubicin drug.⁷

Adsorption of Doxorubicin's Active Ingredient on HAI Polymer

The adsorption of doxorubicin's active ingredient on HAI polymer is as given in Figure 3. The adsorp-

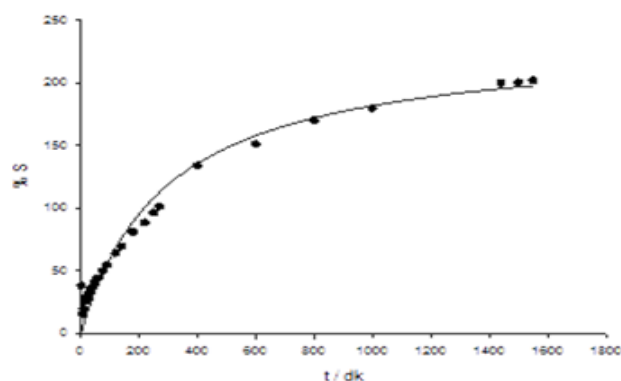


Figure 2: Swelling graph of HAI polymer

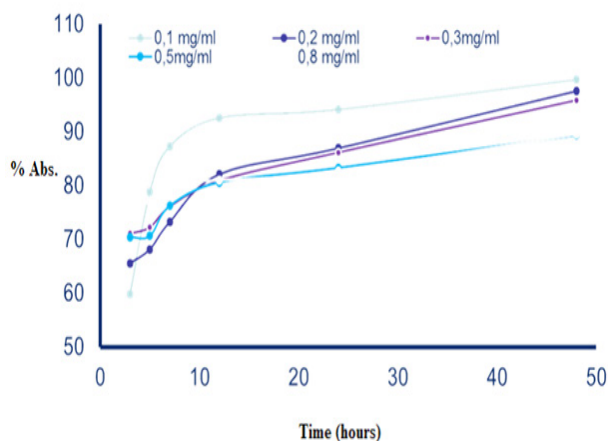


Figure 3: Adsorption Amount of the Polymer over Time in Various Concentrations

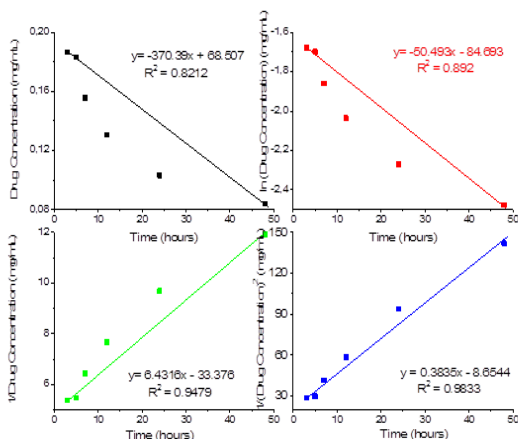


Figure 4: The speed mechanism of the polymer in drug adsorption

tion of the active pharmaceutical ingredient by HAI polymer was examined between 0.1-0.5 mg/L concentration levels. It has been observed that the adsorption of the polymer decreases as the concentration of the active ingredient increases. It has been found that adsorption amount doesn't significantly increases when the concentration of the active ingredient exceeds 0.2 mg/L. However, it was seen that the increase of the adsorption over time was very high when 0.1 mg/L active ingredient, which is the lowest value, is added.

At the end of the study, HA-D polymer released 60% of the active pharmaceutical ingredient.

Adsorption Kinetics

Many methods can be used to explain the kinetic of a reaction; graphic method is the most practical one.

Graphic method can also be used for adsorption speed equations, in Figure 4.

Therefore, we tried to explain the kinetic by drawing graphs of 0° (concentration-time), 1° (ln(concentration)-time), 2° (1/ concentration - time) and 3° (1/(concentration)²- time). These graphs are given in Figure 3. In 0° mechanism the slope of concentration-time graph is negative; in 1° mechanism the slope of ln (concentration)-time graph is also negative; in 2° mechanism the slope of 1/ concentration – time is positive; and in 3° mechanism the slope of 1/(concentration)²-time is also positive. In this case, the graph with the most linear line determines the degree of the mechanism. It can be seen that it has 3rd degree mechanism.⁷

$$-\frac{d[\text{drug}]}{dt} = k[\text{drug}]^3 \tag{1}$$

$$\int_{[\text{drug}]_0}^{[\text{drug}]_t} d[\text{drug}]^3 = -k \int_0^t dt \tag{2}$$

$$\frac{1}{[\text{drug}]^2} = \frac{1}{[\text{drug}]_0^2} + kt \tag{3}$$

Equities (3), (4) and (5) are the generic expressions derived for a 3rd degree speed mechanism. Here the notations show the followings: [drug]: final concentration of the drug, [drug]₀: initial concentration of the drug, t: time, d [...] is a mathematical expression meaning that the term is derivable. As can be seen, equation (5) is similar to a linear equation such as y=a + bx and the equations of the curves that we have shown in Figure 4. Thus, adsorption coefficients of the drug on the polymer may easily be derived from these equations. Accordingly the initial speed is 8.654 when adsorption

$$\Delta G_a^0 = -RT \ln k \tag{4}$$

coefficient is around 38.35x10⁻², half time is 6,651 hours. According to these speed kinetics results, adsorption speed of the drug may be controlled quite regularly through this polymer.^{5,9} According to Eyring's transition state theory, the explicit speed of the mechanism is proportional to the concentration of the activated complex and the frequency of the vibration; thus based on Eyring's equations the free enthalpy of adsorption ΔG_a⁰;

Accordingly, ΔG_a⁰ = 2.375 kJ/mol.

Cytotoxicity

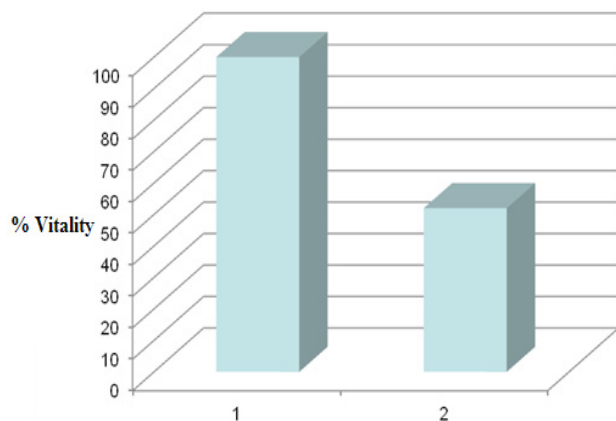


Figure 5: % Viability of the Polymer (1-Control, 2-HAI)

As can be seen from Figure 5, the viability ratio of the polymer was 30% lower than control group. This cytotoxicity impact of the polymer may be due to the lack of polymerization of methacrylate monomers and being imprisoned in the structure of the polymer. In spite of the purification, a certain amount of monomer may stay in the structure. Sorption-based monomers may stay in the holes of the polymers, which are similar to capillary vessels. The problems such as the imprisonment of the excess monomer by being surrounded by the big polymer mass, or the scattering of the monomer inside the polymer may be eliminated through various purification methods, but sorption may be a problem that cannot be solved with such physical methods. It is known in the literature that some monomers have cytotoxic impact.^{10,11} Therefore, cytotoxicity analysis should be performed in drug release studies. HAI polymer that we have synthesized exhibited low toxic impact.

CONCLUSION

Poly (HEMA/Acrylamide/ Itaconic acid) (HAI) polymers which are soluble in water were prepared by a radical addition reaction. The characterization of the polymer was performed by FTIR analysis. The drug active ingredients to the carbonyl group and alkyl peaks were observed at 2927 and 2854 cm^{-1} and strong carbonyl peaks observed at 1708-1667 cm^{-1} . We also studied adsorption and kinetics of this polymer. HA-D polymer released 60% of the active pharmaceutical ingredient. The kinetic study showed that the adsorption mechanism of drug-polymer was 3rd degree speed mechanism. Cytotoxicity test disclosed that this polymer is less toxic than the other polymers. Consequently, it has been concluded that these synthesized polymers are suitable for this active pharmaceutical ingredient.

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None

CONFLICT OF INTEREST

I can confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. I further confirm that the order of authors listed in the manuscript has been approved by all of us.

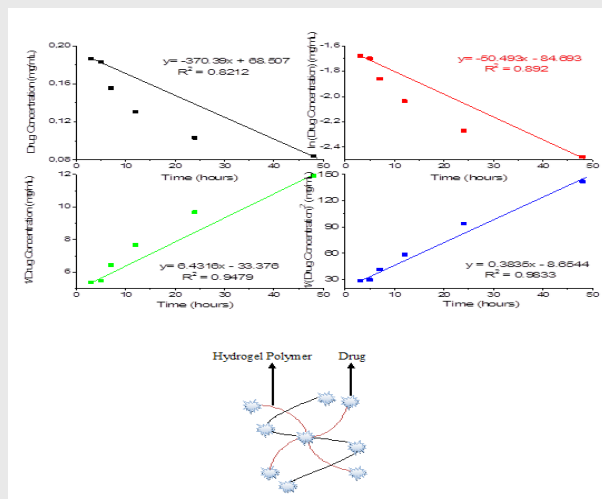
ABBREVIATIONS USED

FTIR: Far infrared spectroscopy; **HAI:** Poly (HEMA/Acrylamide/ Itaconic acid); **ΔG_0 a:** Free enthalpy of adsorption; **[drug]:** Final concentration of the drug; **[drug]₀:** Initial concentration of the drug; **EGDMA:** Ethylene glycol dimetacrilat; **APS:** Ammoniumpersulphate; **TEMED:** N,N,N',N'-tetramethylenediamine; **XTT:** 2, 3-bis (2-methoxy-4-nitro-5-sulphophenyl)-5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide).

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PICTORIAL ABSTRACT



SUMMARY

- Poly (HEMA/Acrylamide/ Itaconic acid) polymers are suitable for this active pharmaceutical ingredient.
- I hope that this polymer will be useful for drug release.

About Author



Afşin Kariper: He is a researcher at Erciyes University. He studied physical chemistry in his PhD. He has published thin films, material science, drugs, and science education articles. He is focused on the physical or physical chemistry properties of thin films, material science, drugs, and influential factors of their properties. He has been studying at Erciyes University.

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