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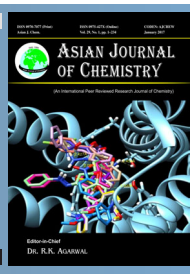

Physical Interaction Between Water-Soluble Poly(Acrylamide/ Itaconic Acid) with Acriflavine and Procainamide Hydrochloride

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In this study, the water-soluble polymers, poly(itaconic acid acrylamide) (PAI) and poly(acrylamide) (PAAm), were synthesized through radical addition reactions. Structural characterization of poly(itaconic acids acrylamide) and poly(acrylamide) was performed by Fourier transform infrared and scanning electron microscope. Release and anti-release profile of the pharmaceutical active agents [acriflavine (AF) and procainamide hydrochloride (PH)] from the polymers (PAI and PAAm) were monitored by ultraviolet-visible spectrophotometry. It has been observed that the entire drug (1 mg procainamide hydrochloride), which was added to the solution with 1 g of polymer, was absorbed in around 3 h by poly(itaconic acids acrylamide). Also, 0.6 mg of acriflavine drug was absorbed in around 3 h. Thus, poly(itaconic acids acrylamide) and poly(acrylamide) polymers were tested for acriflavine and procainamide hydrochloride drugs. In the light of these results, the polymers that are suitable for the release of acriflavine and procainamide hydrochloride drugs were identified.

Keywords: Acrylamide, Itaconic acid, Procainamide hydrochloride, Acriflavine, Water-soluble polymers.

INTRODUCTION

Polymers possess many useful properties that are quite important for the design of controlled drug release. Synthesized polymer should convey the active substance to the target area in optimal conditions. In addition, the synthesized polymer must have the following characteristics as well:

- not interacting with the active substance,
- must be biocompatible and biodegradable,
- should be produced easily and economically [1-3]

Controlled drug release systems designed to meet these requirements are poly(itaconic acids acrylamide) and poly-(acrylamide). Water-soluble polymers are the materials that are useful in many applications and industrial areas such as drugs, cosmetics, foods and paint [4]. Particularly in pharmaceutical industry, drugs with controlled release are generally preferred for the treatment cancer patients [5]. Water-soluble polymers have high water adsorption in dry forms. Such polymers are soluble in mild condition (temperature), however the dissolved polymer precipitates when they are cooled or heated to a specific temperature (when they held both at the same time). Since these polymers are particularly used in medical field, they should be synthesized in accordance with the purpose of main treatment. Furthermore they should be suitable for the targeted tissue and approved by the preclinical studies performed on animals. Water-soluble polymers are used for the treatment of many diseases because of their drug release properties. The molecular weight of anticancer drugs is low, thus they can pass through the cell membrane and then kill both healthy and cancer cell lines [6]. Even though a lot of studies have been conducted in this area, Helmut Ringsdorf synthesis was the first one. Polymer-drug systems were first designed by German polymer chemist, Helmut Rinsdorf, in 1975. His model was mainly concerned with anticancer drugs to obtain both controlled drug release profile and also minimum side effects [7].

This study involves physical interaction between acriflavine (anticancer active compound) and procainamide hydrochloride (PH) with poly(acrylamide) and poly(acrylamide-itaconic acid).

EXPERIMENTAL

For the preparation of poly(acrylamide) (PAAm) and poly(itaconic acids acrylamide) (PAI) polymers, acrylamide (AAm) was procured from Merck (Darmstadt, Germany), whereas itaconic acid (It), N,N,N′,N′-tetramethylethylenediamine (TEMED) and ammonium persulfate (APS) were procured from Sigma (Sigma Chemical Co., ABD). Active substances

of the drugs, namely acriflavine (AF) and procainamide hydrochloride (PH) were obtained from the company Sigma (Sigma Chemical Co., USA).

Synthesis of PAI and PAAm polymers: Water soluble acrylamide polymers with itaconic acid (AI) were prepared through radical addition polymerization. First 12.67 mmol acrylamide and 0.8 mmol itaconic acid monomers were dissolved into 1 mL water; then 0.1 mmol ammonium persulfate was added as starter and 0,5 mmol N,N,N',N'-tetramethylethylenediamine was added as accelerator; they were filled into pipettes and kept at 22 °C for 24 h. To prepare poly(acrylamide); 14 mmol acrylamide was dissolved into 1 mL water; then 0.1 mmol ammonium persulfate was added as starter and 0.07 mmol N,N,N',N'-tetramethylethylenediamine was added as accelerator; they were filled into pipettes and kept at 22 °C for 24 h.

Polymers were removed from the pipettes, they were cut in 3-4 mm size, they were dried first in the air, then in the vacuum oven [1].

SEM images of PAI and PAAm polymers: Surface morphology of PAI and PAAm polymers was investigated by scanning electron microscope.

FTIR studies: FTIR spectra of PAI and PAAm polymers were obtained by using a FTIR spectrophotometer (FTIR 8000 Series, Shimadzu, Japan).

Polymer acriflavine pair: The ability of absorbing drug substances is the first step of polymer usage as a drug carrier system. In his study, PAAm was kept in 10 mL phosphate buffer with 7.4 pH, containing 1 mg acriflavine drug substance, in an oven at 37 °C, for 5 days for incubation. Sample was taken from the media at certain time intervals and the drive force was kept constant by adding fresh buffer solution to the media. Then, the samples were kept at -20 °C for 2 days. The polymer was purified by washing with 98 % cold ethyl alcohol and dried in the oven at 37 °C, for 24 h. The amount of adsorbed acriflavine was read by UV-visible spectrophotometer at 452 nm. 0.1 mg of procainamide hydrochloride was dissolved into 10 mL PBS. This mixture was left in the shaking incubator for 5 days, at 37 °C, until it became turbid, viscous. Then, the samples were kept at -20 °C for 2 days. It was purified by washing with 98 % cold ethyl alcohol and dried in the oven at 37 °C, for 24 h. The amount of adsorbed procainamide hydrochloride was read by UV-visible spectrophotometer at 280 nm.

Measurement by UV-visible spectrophotometer: The amounts of released acriflavine and procainamide hydrochloride were monitored by UV-visible spectrophotometer at 280 nm wavelengths. 400 µL buffer samples, taken from the release media were diluted by adding 100 µL methanol. Acriflavine and procainamide hydrochloride solutions were prepared in different concentrations (buffer solution + ethanol mixtures) to draw a calibration curve. With the help of the developed graph, captured amounts of acriflavine and procainamide hydrochloride were calculated.

RESULTS AND DISCUSSION

Surface morphology: SEM images of PAI and PAAm polymers are displayed in Fig. 1a and 1b.

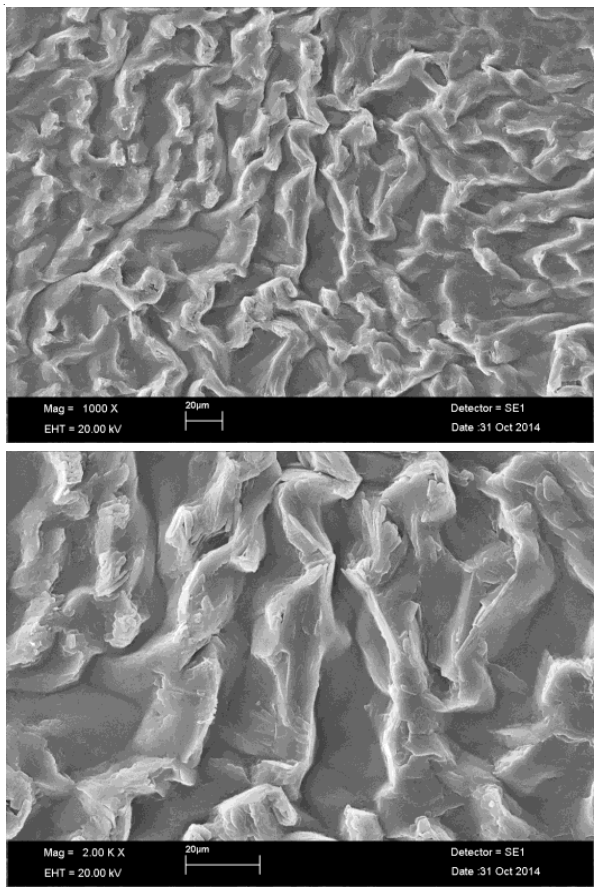

Fig. 1a. SEM images of poly(itaconic acid acrylamide)

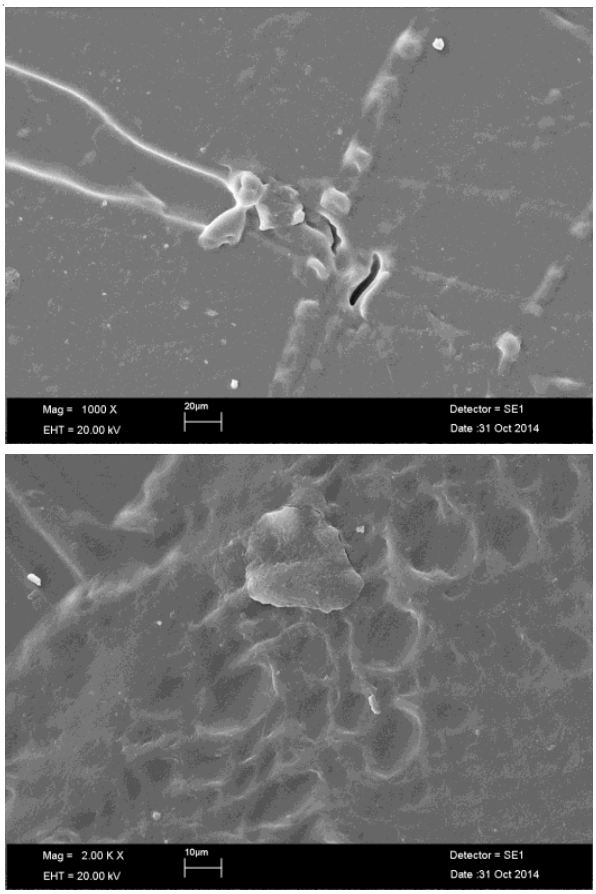

Fig. 1b. SEM images of poly(acrylamide)

From the SEM images of the polymers, it can be seen that the image of the polymer was changed after adding itaconic acid. It can be observed that some holes were obtained by adding acid to the polymer. In terms of absorption, the surface area of the polymers has a particular significance. The enlargement on the surface area of these polymers is noticeable. Especially, the wavy structure of PAI polymer's surface is an indicator that the surface area of this polymer is larger than PAAm polymer. When acid is added, surface area enlarges more and also some expansion may be observed on wavy pores.

FTIR: As can be seen from Fig. 2(a), $–NH_2$ vibration signals of the polymer were observed at about 3300 cm^{-1}, whereas this polymer's vibration signals belonging to carbonyl group were observed around 1648 cm^{-1} and –NH vibrations were seen around 1600 cm^{-1}. Fig. 2(b) shows the vibration signals after the absorption of the drug. It can be noticed that C-N vibrations were shifted to around 1311 cm^{-1} and they give a sharper peak. In addition, aromatic –CH vibrations, observed around 1070 cm^{-1}, were shifted to around 1106 cm^{-1} due to the aromatic group in the drug. Moreover, –C=C vibrations of the polymer observed around 2312 cm^{-1} could not be observed after the attachment of the drug [8-10].

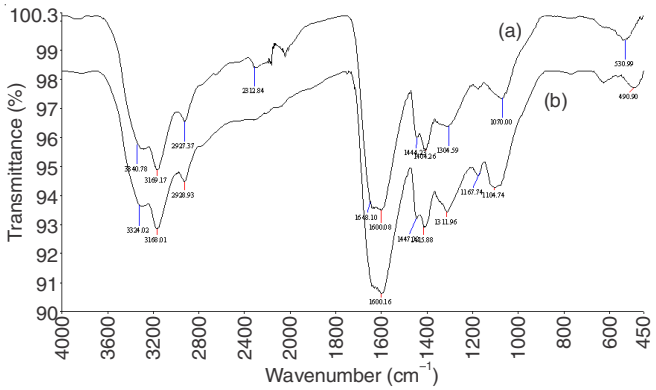

Fig. 2. FTIR spectrums of (a) P(AAm); (b) P(AAm)-AF

As can be seen from Fig. 3(a), $–NH_2$ vibration signals of the polymer were observed at in 3296-3160 cm^{-1} region, whereas this polymer's vibration signals belonging to carbonyl group were observed around 1645 cm^{-1} and –NH vibrations were seen around 1600 cm^{-1}. Fig. 3(b) shows the vibration signals after the absorption of the drug. It can be noticed that C-N vibrations were still around 1349 cm^{-1} and there is no change. Aromatic –CH vibrations were observed around 1170 cm^{-1}. In addition, aliphatic –CH vibrations of the polymer observed around 857 cm^{-1} were combined with aromatic –CH vibrations and became more apparent. It has been also noticed that aromatic –C=C vibrations became more apparent around 1414 cm^{-1} [10,11].

In Fig. 4(a), $–NH_2$ vibration signals that belongs to the polymer were observed at 3343-3190 cm^{-1} region, whereas this polymer's vibration signals belonging to carbonyl group were observed around 1645 cm^{-1} and –NH vibrations were seen around 1600 cm^{-1}. Fig. 4(b) shows the vibration signals after the absorption of the drug. Regarding them, the intensity of NH vibration signals is very apparent after the adsorption of the drug. It can be noticed that, in the polymer C-N vibrations

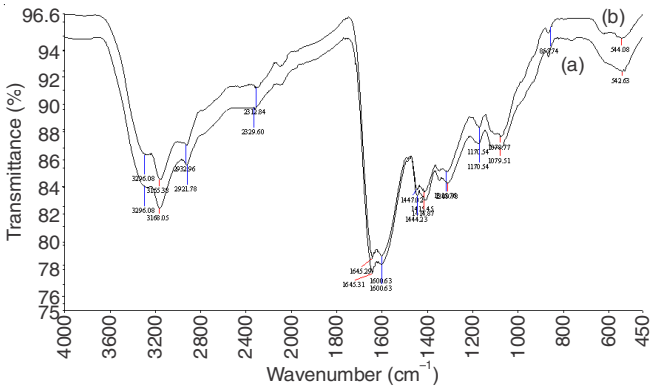

Fig. 3. FTIR spectrums of (a) P(AI); (b) P(AI)-AF

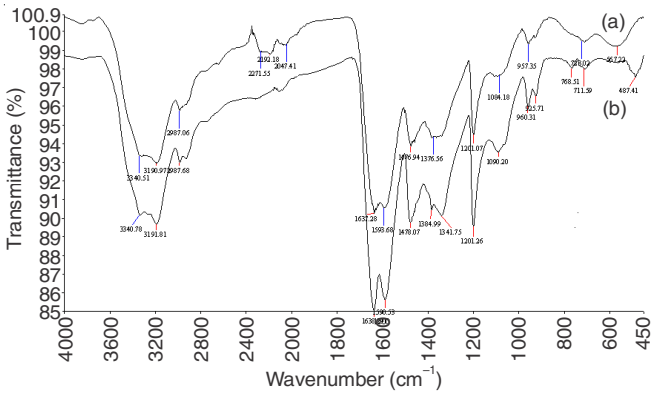

Fig. 4. FTIR spectrums of (a) P(AAm); (b) P(AAm)-PH

were around 1376 cm^{-1}, however they appeared more clearly around 1384 and 1341 cm^{-1} after the adsorption of the drug. Aromatic –CH vibrations which were observed around 957 cm^{-1}, were combined with aromatic –CH vibrations of the drug and they can be observed clearly at 960-925 cm^{-1}. The same effect was also identified around 761 and 711 cm^{-1}. As can be seen, the changes on the structure of the polymer that occurred after the adsorption of the drug, even the small ones, can be easily identified through infrared signals. This is one of the most obvious proofs showing that our drug was adsorbed by the polymer [10-12].

Adsorption graph: The slope of adsorption *versus* time graphs give information about the adsorption speed. poly-(itaconic acid acrylamide) polymer has regularly absorbed 1 mg procainamide hydrochloride drug substance (Fig. 5) in 200 min, whereas the absorption speed of PAAm has been decreased before reaching 200 min. Afterward, a very small increase can be observed on the PAAm polymer's absorption of procainamide hydrochloride drug substance. On the other hand, procainamide hydrochloride absorption of PAI was constant after 200 min. This is because acrylamide contains more hydroxyl functional groups after the bonding of itaconic acid. Thus, the probability of inter-molecular hydrogen bonds was increased and made contribution to absorption. In addition, as can be seen from SEM images of PAI polymer, its surface area is larger than PAAm's polymer. Large surface area also increases absorption amount.

On the other hand, the curves showing the absorption of 1 mg acriflavine (Fig. 6) drug substance by these polymers are interesting. The absorption curve of PAAm is not much

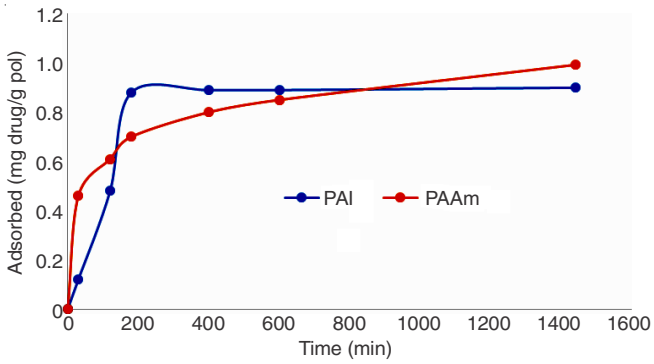

Fig. 5. Adsorption of 1 mg procainamide hydrochloride

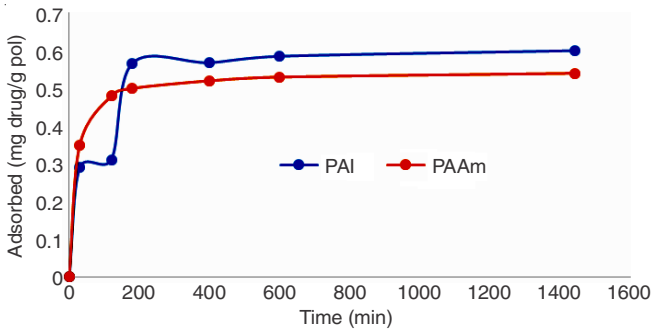

Fig. 6. Adsorption of 1 mg acriflavine

different than its procainamide hydrochloride absorption curve. However the absorbed amount is lower. The absorption curve of PAI seemed to stay constant at the end of the first 200 min; however it has continued to rise. The reason of the irregularity occurred in this absorption curve may be as follows; at the beginning acriflavine has been physically attached to the wavy pores located on the surface of PAI, but afterwards it might have performed chemical absorption through inter-molecular bonds. After filling these wavy pores for a while, drug substance might have been attached to the surface through inter-molecular bonds. Another important point is, acriflavine drug substance has not been attached to the surface of both PAI and PAAm by hydrogen bonds, but by van der Walls bonds, which is 10 % less strong than hydrogen bonds. This is because amine functional groups of acriflavine don't contain atoms with high electro-negativity due to the nitrogen atoms that they have. Thus, PAI did not show a regular absorption curve like PAAm, even though it contains more functional groups in its structure. At this point, PAI can be used for the high absorption of acriflavine drug. However it may be more reasonable to use acrylamide for a regular absorption and controlled drug release. On the other hand, the use of PAI is quite suitable for the controlled release of procainamide hydrochloride. Gavini *et al.* [13] have studied the release of acriflavine by bonding it to chitosan. Gavini *et al.* [13] stated that this drug with chitosan was released in 8 h. Another study about the release of acriflavine was conducted by Patel *et al.* [14]. They have absorbed acriflavine substance to poly(methyl methacrylate-*co*-maleic anhydride) polymer and they have observed that only 10 % of the loaded drug has been released in 1 day. Bolourtchian *et al.* have tested some surface active ingredients (sodium lauryl sulfate and sodium stearate as anionic surfactants, cetyl pyridinium chloride and cetyltrimethylammonium bromide as cationic and span 60 and Tween 80 as non-ionic surfactants) [15]. They have observed that only 60 % of the drug has been released in 2 h. But, they have shifted the pH of the environment to acidic area for the release, which may create complications to apply it to the livings. The literature contains many resources about the polymer release of these drugs. Since these drugs are frequently used for cancer treatment, researchers have conducted many studies in this area. When the results of our study were compared to the literature, it can be said that PAI is most suitable for drug release, except the interesting deviation observed in the absorption graph of acriflavine substance.

Conclusion

In this study, two substances have been absorbed, which are frequently used in cancer treatment, namely acriflavine (anticancer active compound) and procainamide hydrochloride on two different polymers, poly(acrylamide) and poly(acrylamide-itaconic acid) and tested their drug release. We have observed that bonding of itaconic acid to procainamide has increased the amount of drug absorption because of having more functional group in its structure and by enlarging its surface area. We confirm that 1 mg drug can be regularly released with this polymer in the first 200 min (except the fluctuation on the absorption curve mentioned before). In addition, as a result of the comparison of these polymers, it has been found that PAI has performed more regular release with procainamide hydrochloride drug substance, whereas PAAm has performed more regular release with acriflavine. In this study, the synthesis of new polymers with acrylamide proved that acrylamide's drug release can be controlled by changing its surface area or by functional groups.

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