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Synthesis of a New Drug Delivery System Based on Alginate via Graft Copolymerization

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The present work focused on the design of drug delivery system (DDS) based on a pH-sensitive hydrogel. The hydrogels were prepared via graft copolymerization of acrylonitrile (AN) monomer was directly grafted onto alginate using ammonium persulfate (APS) as an initiator and sodium hydroxide (NaOH) as a crosslinking agent under an inert atmosphere. Porous structure of hydrogel was essential in this system to yield a large surface area so that Metronidazole release could be facilitated. Due to the reversible swelling behavior of the hydrogels, the synthesized networks can sense the environmental pH change and achieve an oscillatory release pattern. The concentration of released Metronidazole loaded was monitored at 278 nm on the UV spectrophotometer.

Key words: Alginate, Hydrogel, Metronidazole, Releasing drug.

Hydrogels have been of interest to biomaterial scientists for many years because of their hydrophilic character and potential to be biocompatible[1-8]. These networks are special soft and pliable polymeric materials that can be absorb large quantities of water, saline or physiological solutions while the absorbed solutions are not removable even under pressure[2].

To date, many types of hydrogels as drug carriers have been widely investigated. Some interesting drug delivery systems based on hydrogels have thus been proposed[9-12]. Among hydrogels, however, considerable research attention has been focused on so-called "smart" hydrogels which can transfer their volume in response to environmental stimuli, and this result in environmental stimuli modifying drug release. Among these, pH-sensitive hydrogels have been extensively investigated for potential use in site-specific delivery of drugs to specific regions of the gastrointestinal tract and have been prepared for delivery of low molecular weight drugs. Therefore, these hydrogels have important applications in the field of medicine, pharmacy, and biotechnology.

The principal requirement of any controlled release system is that the release profile and rate are controlled. Controlled or sustained release drugs provide many advantages in comparison with conventional forms including reduced side effects, drug concentration kept at effective levels in plasma, improved utilization of drug and decrease the dosing times[13].

In the current study we investigated the synthesis and utility of an anionic hydrogel from graft copolymerization of acrylonitrile onto alginate backbones, for the controlled release drug, metronidazole. Drug absorption and release capacities of hydrogel systems and influence of pH of the medium on the release properties were also examined.

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MATERIALS AND METHODS

Sodium alginate (chemical grade, MW 50000) was purchased from Merck Chemical Co. (Germany). The monomer, acrylonitrile (AN, Merck), was used after vacuum distillation. Sodium hydroxide and ethanol as reagent grade were used without further purification. The drug, metronidazole, was obtained from Jaberebne Hayan Pharmaceutical Co. (Tehran, Iran). the chemical structure of metronidazole is shown in figure 1. Ammonium persulfate (APS) was used without purification. All other chemicals were of analytical grade. double distilled water was used for the hydrogel preparation and swelling measurements.

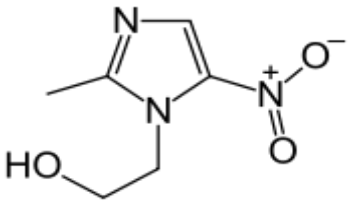


Fig. 1. Chemical structure of drug metronidazole

Preparation of hydrogel

Graft copolymerization of acrylonitrile onto alginate was carried out with APS radical initiator under argon atmosphere[11]. In a 100 mL flask, alginate (0.2-0.80 g) was dissolved in 50 mL of degassed distilled water. The flask was placed in a water bath with desired temperature (35-70 °C). A given amount of monomer, AN (1.62-4.05 g), was added to the flask and the mixture was stirred for 10 min. Then the initiator solution (7.0 mL) was added to the mixture and continuously stirred for 2 h. The product was then worked up with methanol (200 mL) and dried in oven at 50 °C for 5 h.

Determination of drug loading

Loading model drug into a hydrogel was performed using a contact adsorption technique. The vacuum dried powdered samples (1±0.0001 g), with average particle sizes between 40 and 60 mesh (250–350 μm), were accurately weighted and immersed in the aqueous solution of drug (0.6 g dissolved in 50 mL distilled water) at 0°C for 25h to reach the equilibrated state. The swollen hydrogels loaded with drug were placed in a vacuum oven and dried under vacuum at 37°C.

The amount of drug content entrapped in the hydrogels was determined by an indirect method. After the gel preparation, the washings were collected, filtered with a 0.45 Millipore filter and tested at λ_{max} 278 nm using UV/VIS spectrophotometer (UV-1201, Shimadzu, Kyoto, Japan).

The drug entrapped exhibited the same λ_{max} as the free drug. This clearly indicates that the drugs entrapped have not undergone any possible chemical reaction during the matrix formation. The difference between the amount of drug initially employed and the drug content in the washings is taken as an indication of the amount of drug entrapped.

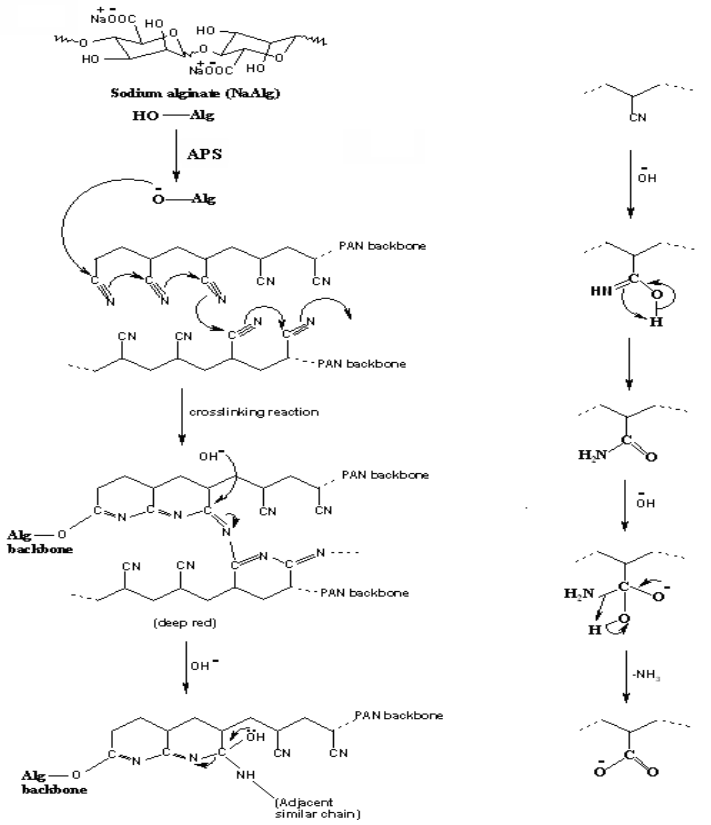
$$Drug\ entrapment\ (\%) = \frac{Mass\ of\ drug\ present\ in\ hydrogel}{Theoretical\ mass\ of\ drug} \times 100 \quad (1)$$

RESULTS AND DISCUSSION

Mechanism of hydrogel formation and Spectral Characterization

A general reaction mechanism for AN grafting onto alginate backbones and alkaline hydrolysis of the graft copolymer is shown in Scheme 1. At the first step, ammonium persulfate is decomposed under heating and produced sulfate anion-radicals that remove hydrogen from –OH groups of alginate backbones. So, this persulfate-saccharide redox system results in active centers capable to radically initiate polymerization of AN leading to graft copolymer. The graft copolymer, alginate-g-PAN, was then saponofied using sodium hydroxide aqueous solution to produce hydrophilic carboxamide and carboxylate groups. During the alkaline hydrolysis, ammonia was evolved and

an orange-red color developed due to conjugated imine formation. It has been reported, in the case of hydrolyzed starch-g-PAN (H-SPAN), a maximum conversion of 83% of nitrile to carboxyl groups and the remaining 17% are amide groups[14]. In fact, details of the chemical processes and mechanism involved in H-SPAN synthesis are not yet well understood. For instance, the incomplete hydrolysis is interpreted as being related to steric and polar factors .Therefore, in the case of our hydrogel, H-alginate -g-PAN, we realized that precise control of the ratio is practically impossible.



Scheme 1. General mechanism for APS-initiated graft copolymerization of acrylonitrile onto alginate in the presence of sodium hydroxide

Infrared spectroscopy was carried out to confirm the chemical structure of the hydrogel. Figure 2 shows the FTIR spectra of Alg-PAN physical mixture and the resulted hydrogel, Alg-poly(NaAA-co-AAm). The band observed at 2244 cm^{-1} can be attributed to stretching of –CN group of polyacrylonitrile (Fig. 2c). The hydrogel comprise an Alg backbone with side chains that carry carboxamide and carboxylate functional groups that are evidenced by three new peaks at 1408, 1557, and 1676 cm^{-1} (Fig. 2b). These peaks attributed to C=O stretching in carboxamide functional groups and symmetric and asymmetric stretching modes of carboxylate groups, respectively. The stretching band of –NH overlapped with the -OH stretching band of the Alg portion of the copolymer. As shown in Fig. 2b and Scheme 1, after alkaline hydrolysis, most of the nitrile groups are converted to carboxamide and carboxylate groups.

To obtain an additional evidence of in situ crosslinking during alkaline hydrolysis, a similar reaction was conducted in absence of the polysaccharide. Since the resulted product became soluble, the crosslinks really formed between the alkoxide ions of Alg and the nitrile groups of PAN. This fact practically proves that the Alg hydroxyls are involved in the crosslinking[8].

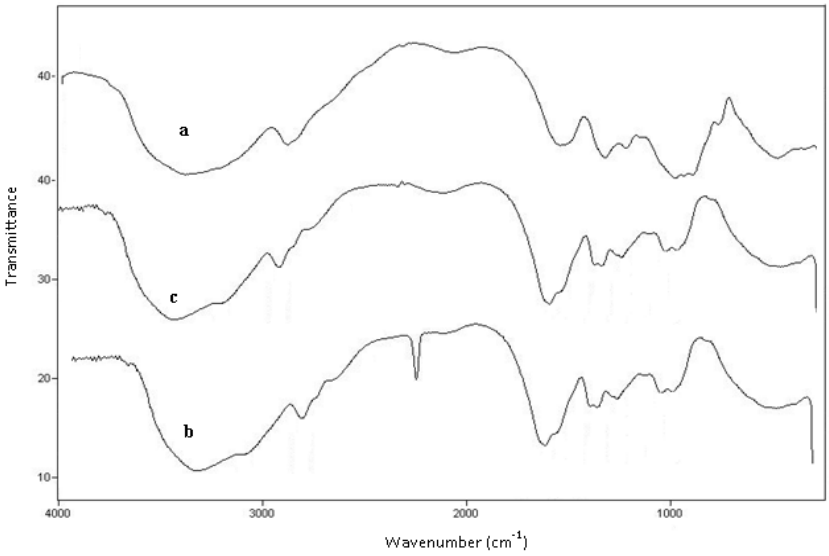


Fig. 2. FTIR spectra of (a) alginate , (b) alginate-g-poly(AN) and (c) H-Alg-poly(NaAA-co-AAm) hydrogels

Effect of pH on equilibrium swelling

In this series of experiments, swelling ratio for the synthesized hydrogels was measured in different pH solutions ranged from 1.0 to 13.0 (Figure 3). Since the swelling capacity of all "anionic" hydrogels is appreciably decreased by the addition of counter ions (cations) to the swelling medium, no buffer solutions were used. Therefore, stock NaOH (pH 10.0) and HCl (pH 1.0) solutions were diluted with distilled water to reach desired basic and acidic pHs, respectively. Maximum swelling (272g/g) was obtained at pH 8. In acidic media, most carboxylate groups are protonated, so decreased repulsion of anionic groups leads to a decreased swelling ratio. At higher pHs (3–8), some carboxylate groups are ionized and the electrostatic repulsion between carboxylate groups causes an enhancement of the swelling capacity. The reason of the swelling loss for the highly basic solutions is the charge screening effect of excess Na^+ in the swelling media, which shield the carboxylate anions and prevent effective anion–anion repulsion.

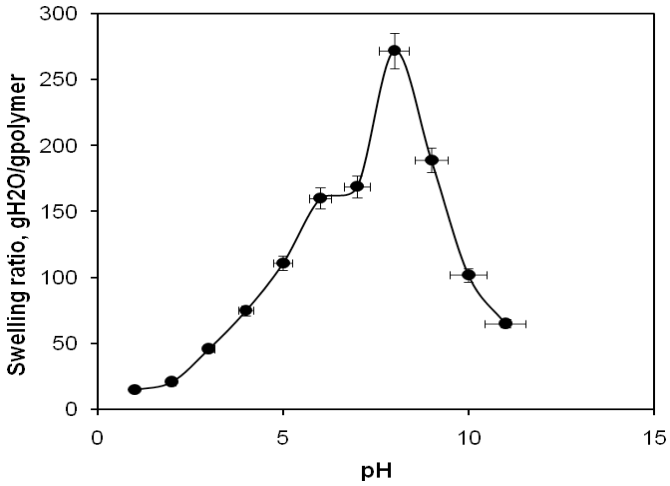


Fig. 3. pH-dependent swelling of the H-Alg-poly(NaAA-co-AAm) superabsorbent hydrogel

pH-responsiveness behavior of the hydrogel

Since the hydrogels show different swelling behaviors at various pHs, we investigated their pH-reversibility in the solutions buffered at pHs 2.0 and 8.0 (Figure 4). The figure shows a stepwise reproducible swelling change of the hydrogel at 25°C with alternating pH between 2.0 and 8.0. At pH 8.0, the hydrogel swells up to 272 g/g due to anion–anion repulsive electrostatic forces, while, at pH 2.0, it shrinks within a few minutes due to protonation of carboxylate groups. This sharp swelling-deswelling behavior of the hydrogels makes them suitable candidates for controlled drug delivery systems.

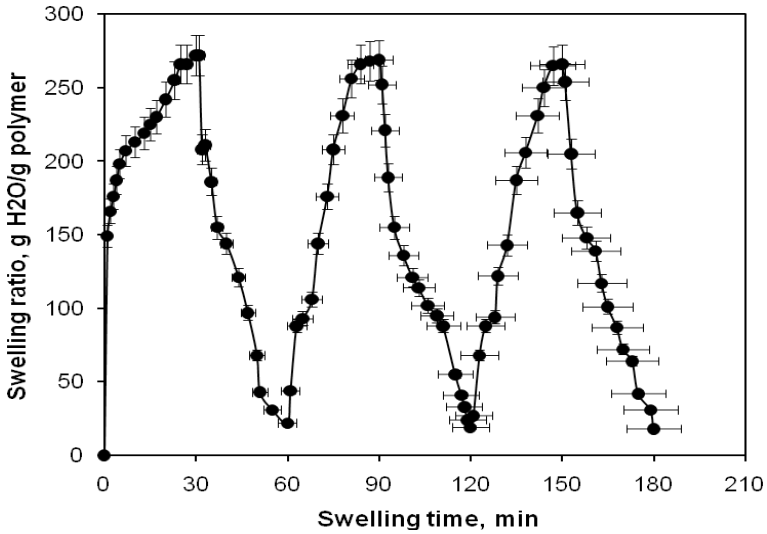
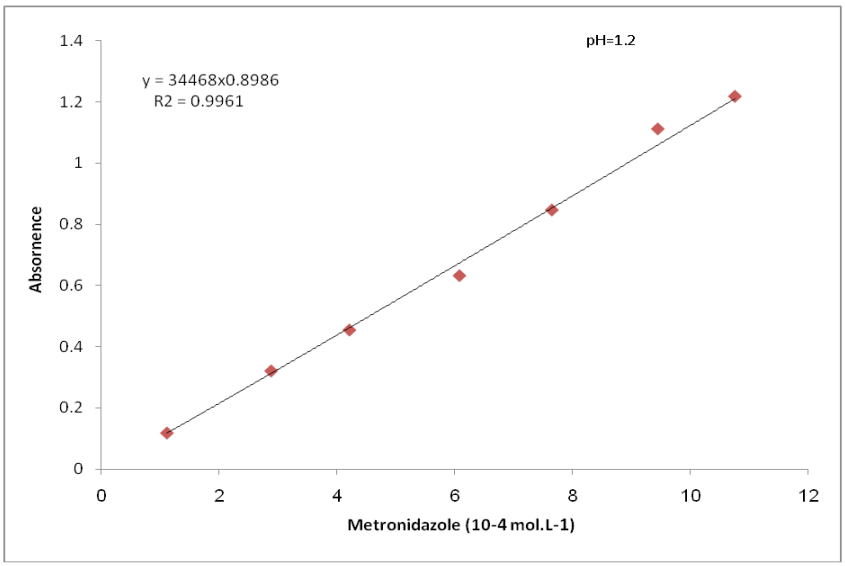


Fig. 4. On-off switching behavior as reversible pulsatile swelling (pH 8.0) and deswelling (pH 3.0) of H-Alg-poly(NaAA-co-AAm) hydrogel

Standard calibration curve

The calibration curve of the absorbance as a function of the metronidazole concentration at 278 nm, shown in Figure 5, has a linear relationship with a correlation coefficient (r) of 0.996 and 0.998 at pHs 1.2 and 7.4, respectively.



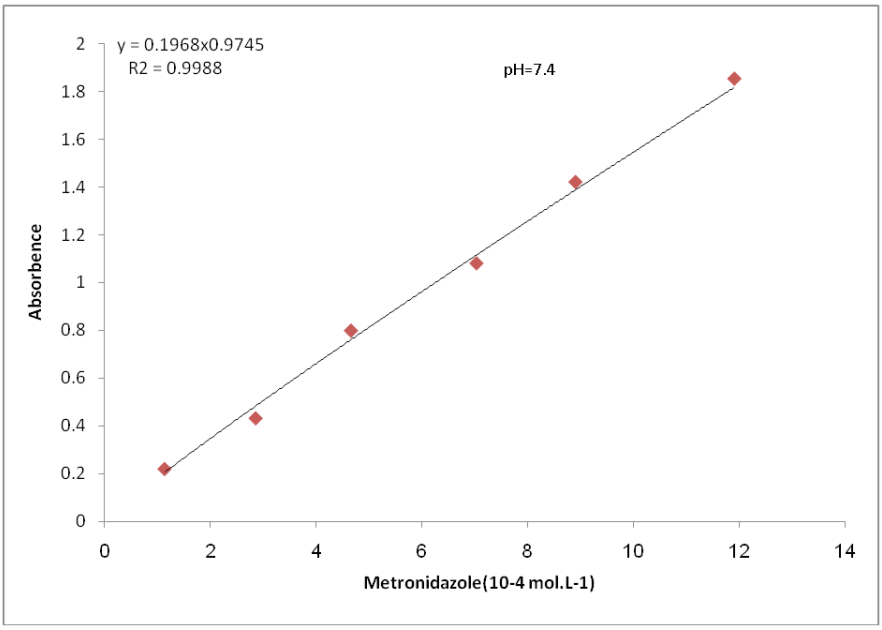


Fig. 5. The standard calibration curve of the absorbance as a function of metronidazole concentration at 278 nm on the UV spectrophotometer at pH 1.2 (a) and pH 7.4 (b)

CONCLUSION

Novel graft copolymer were synthesized by grafting of acrylonitrile onto alginate in the presence of ammonium persulfate as an efficient initiator in acidified aqueous medium, under inert atmosphere.

The based-alginate superabsorbent hydrogels exhibited high sensitivity to pH, so that, several swelling changes of the hydrogel were observed in pH variations of a wide range (1-13). Furthermore, the reversible swelling-deswelling behavior in solutions with acidic and basic pH makes the hydrogels a suitable candidate for controlled drug delivery systems.

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