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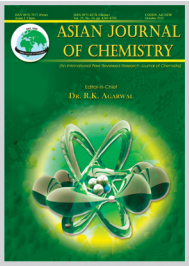

Chitosan-*g*-poly(acrylic acid-*co*-acrylonitrile) Hydrogels with Potential Biomedical Applications as Drug Delivery Systems

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In this work, acrylonitrile and acrylic acid monomers were directly grafted onto chitosan using ammonium persulfate as an initiator and methylenebisacrylamide as a crosslinking agent under an inert atmosphere. A mechanism for hydrogel formation was proposed and the structure of the product was established using FTIR spectroscopy. The chitosan-*g*-poly(acrylic acid-*co*-acrylonitrile) hydrogel exhibited a pH-responsive swelling-deswelling behaviour at pH's 2 and 8. This on-off switching behaviour provides the hydrogel with the potential to control delivery of bioactive agents. Release profiles of metronidozule drug, from the hydrogels were studied under both simulated gastric and intestinal pH conditions.

Key Words: Chitosan, Polyacrylic acid, Polyacrylonitrile, Metronidozule, Drug delivery.

INTRODUCTION

Highly swelling polymers, *i.e.* super absorbent hydrogels, are hydrophilic, three dimensional networks that can absorb water in the amount from 10 % up to thousands of times their dry weight[1]. They are widely used in various applications such as hygienics, foods, cosmetics and agriculture[2-4]. This accounts for increase in the worldwide production of super absorbent polymers (SAPs) from 6000 tons in 1983 to 450000 tons in 1996[1]. Nowadays, the worldwide production of super absorbent polymers is more than one million tons in year. Hence, synthesis and characterization of super absorbent hydrogels is the main goal of the several research groups in the world[5-8].

Because of their exceptional properties, *i.e.* biocompatibility, biodegradability, renewability and non-toxicity, polysaccharides are the main part of the natural-based super absorbent hydrogels. Graft copolymerization of vinyl monomers onto polysaccharides is an efficient route to preparation of hydrogels. The hydrogel forming ability through graft copolymerization of vinyl monomers onto polysaccharides such as starch, chitosan, sodium alginate, carrageenan and cellulose has been well documented[7-11]. Because of the presence of certain functional groups along the polymer chains, hydrogels are often sensitive to the conditions of the surrounding environment, which are referred to as "intelligent materials" or "smart materials". For example, the water uptake of these materials may be sensitive to temperature, pH, or ionic strength of the swelling solutions, or even to the presence of a magnetic field or ultraviolet light[12]. These smart hydrogels are of general interest for biomedical applications, such as artificial muscles or switches, biomedical separation systems and controlled release systems.

Indeed, chitosan is a linear natural polysaccharide composed of a partially deacetylated material of chitin. It is a basic polymer, having amine side groups. Due to its excellent biocompatibility and biodegradability, chitosan and its derivatives were widely applied to fabrication of biomedical materials, enzyme and cell immobilization, especially for drug delivery. Since chitosan is easily soluble in acidic solutions, crosslinking of chitosan to form a network is the only way to prepare chitosan hydrogels. When anionic monomer such as acrylic acid in present acrylonitrle monomer is grafted onto chitosan (in the presence of a divinyl crosslinking agent monomer), an ampholytic hydrogel containing both cationic and anionic charges is prepared. So, by introducing anionic charges (-COO-) onto chitosan, a hydrogel with swelling ability at various pHs is prepared. In the present work, we study the synthesis and characterization of chitosan-*g*-poly(acrylic acid-*co*-acrylonitrile) hydrogels with applications as drug delivery systems.

EXPERIMENTAL

Hydrogel preparation: A general procedure for chemically crosslinking graft copolymerization of acrylic acid and acrylonitrile onto chitosan backbones was conducted as follows. Chitosan was dissolved in degassed, distilled water containing

2 wt % of acetic acid. In general, 0.15-1.2 g of chitosan was dissolved in 35 mL of acetic acid. The reactor was placed in a water bath preset at 60 ºC. Then 0.03-0.13 g in 5 mL H_2O ammonium persulfate was added to the chitosan solution and stirred for 10 min at 60 ºC. Following this, acrylic acid (2.0-4.5 mL) and acrylonitrile (0.5 -3.0 mL) were added to the chitosan solution. Methylenebisacrylamide (0.01-0.13 g in 5mL H_2O) as a crosslinker was added to the reaction mixture after the addition of monomer and the mixture was continuously stirred for 1 h under argon atmosphere. After 1 h, the reaction product was allowed to be cooled to ambient temperature. The resulting hydrogel was neutralized to pH 8 by addition of 1 N NaOH solution. Then methanol (500 mL) was added to the gel product while stirring. After complete dewatering for 24 h, the product was filtered, washed with fresh methanol (2 × 50 mL) and dried at 50 ºC.

Drug loading efficiency and *in vitro* drug release: Powdered samples (1 g ± 0.0001), with average particle sizes between 40-60 mesh (250-420 µm), were accurately weighted and immersed in an alkaline solution of metronidozule (0.54 g dissolved in 50 mL distilled water) at 0 ºC for 25 h. The swollen hydrogels loaded with drug were placed in a vacuum oven and dried under vacuum at 37 ºC. The loading amount of drug in the hydrogels was calculated from the decrease in the concentration of the metronidozule solution which was determined using a UV spectrophotometer (UV-1201, Shimadzu, Kyoto, Japan). The loading efficiency of the chitosan-based hydrogels was calculated as the ratio of the final to the initial metronidozule concentration.

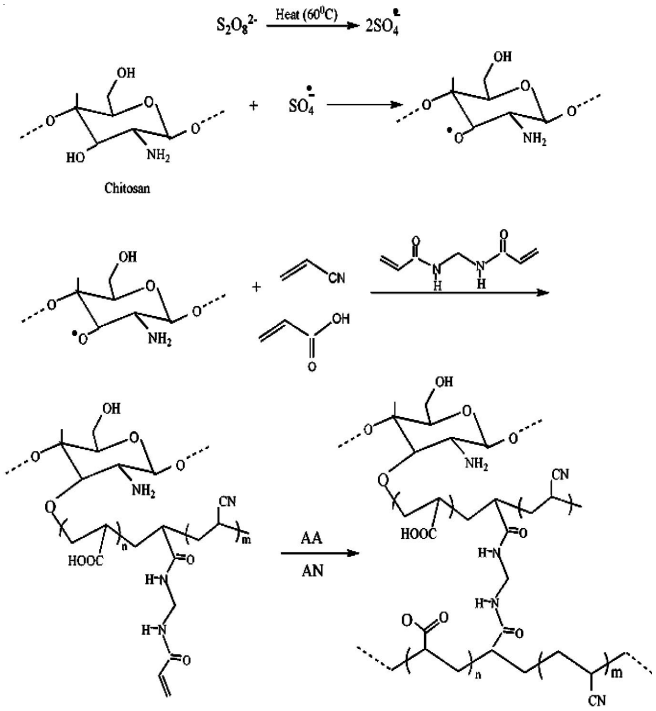
In vitro release was carried out in duplicate by incubating 0.01 ± 0.0001 g of the metronidozule-loaded hydrogels into a cellophane membrane dialysis bag (D9402, Sigma-Aldrich) in 50 mL of buffer solution (either pH 1.2 or 7.4) at 37 ºC. At specific time intervals, 1 mL aliquots of sample was withdrawn and after suitable dilution the concentration of drug released was measured by UV spectrophotometer. The drug release percent was calculated twice using the following equation:

$$\text{Released drug (\%)} = R_t/L \times 100 \quad (1)$$

where, L and R_t represent the initial amount of drug loaded and the final amount of drug released at time t.

RESULTS AND DISCUSSION

Mechanism of hydrogel formation: Super absorbent hydrogels were prepared by graft copolymerization of acrylic acid and acrylonitrile onto chitosan in the presence of methylenebisacrylamide as a crosslinking agent. Ammonium persulfate was used as an initiator. The persulfate is decomposed under heating and produced sulfate anion-radicals that remove hydrogen from -OH groups of chitosan backbones[8]. So, this persulfate-saccharide redox system results in active centers capable to radically initiate polymerization of acrylic acid and acrylonitrile ledding to graft copolymer. Since the crosslinking agent, methylenebisacrylamide, is presented in the system, the copolymer comprises a crosslink structure. A possible mechanism of the polymerization of acrylic acid and acrylonitrile onto chitosan in the presence of methylenebisacrylamide was shown in **Scheme-I**.



Scheme-I: General mechanism for ammonium persulfate-initiated graft copolymerization of acrylic acid and acrylonitrile onto chitosan in the presence of methylene-bisacrylamide

For identification of the hydrogel, infrared spectroscopy and SEM were used. The FTIR spectra of pure chitosan and super absorbent hydrogel based on chitosan are shown in Fig. 1. In Fig. 1(a) a broad band at 3418 cm^{-1} corresponds to the associated -OH stretching vibrations of the hydroxyl groups and the peak at 1611 cm^{-1} corresponds to the N-H deformation bending of chitosan. The super absorbent hydrogel product comprises a chitosan backbone with side chains that carry sodium carboxylate and cyanide functional groups that are evidenced by new peaks at 1563 and 2246 cm^{-1} respectively. The very intense characteristic band at 1576 cm^{-1} is due to C=O asymmetric stretching in carboxylate anion that is reconfirmed by another sharp peak at 1448 cm^{-1}, which is related to the symmetric stretching mode of the carboxylate anion. To obtain an additional evidence of grafting, a similar polymerization was conducted in absence of the crosslinker. After extracting the homopolyacrylic acid and homopolyacrylonitrile (3.5 %), appreciable amount of grafted chitosan was concluded. The graft copolymer spectrum was very similar to Fig. 1(a). For more confirming structure of hydrogels, we applied scanning electron microscopy. One of the most important properties that must be considered is hydrogel microstructure morphologies. The surface morphology of the samples was investigated by scanning electron microscopy. Fig. 2 shows SEM micrograph of the polymeric hydrogels obtained from the fracture surface[2,3]. The hydrogel has a porous structure. It is supposed that these pores are the regions of water permeation and interaction sites of external stimuli with the hydrophilic groups of the graft copolymers.

pH-Reversibility for Chitosan-*g*-poly(acrylic acid-*co*-acrylonitrile) hydrogel: Since the present hydrogels show different swelling behaviours in various pH solutions, we

investigated the pH reversibility of these hydrogels in 0.01 M solutions with pH 2 and pH 8 (Fig. 3). At pH 8, the hydrogel swells up to 295 g/g due to anion-anion repulsive electrostatic forces, while at pH 2, it shrinks within a few minutes due to protonation of carboxylate groups. This sharp swelling-deswelling behaviour of the hydrogels makes them as suitable candidate for controlled drug delivery systems. Such on-off switching behaviour as reversible swelling and deswelling has been reported for other ionic hydrogels[5].

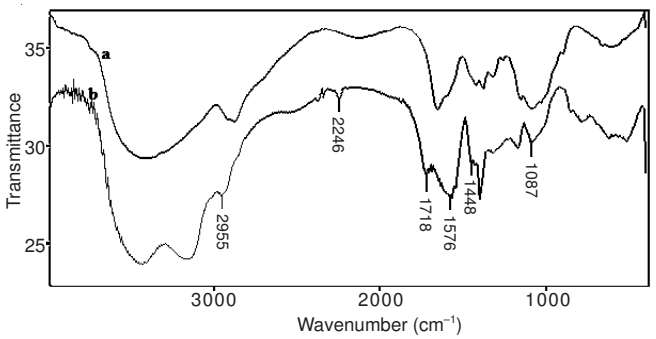


Fig.1. FTIR spectra of (a) Chitosan and (b) Chitosan-*g*-poly(NaAA-*co*-AN)

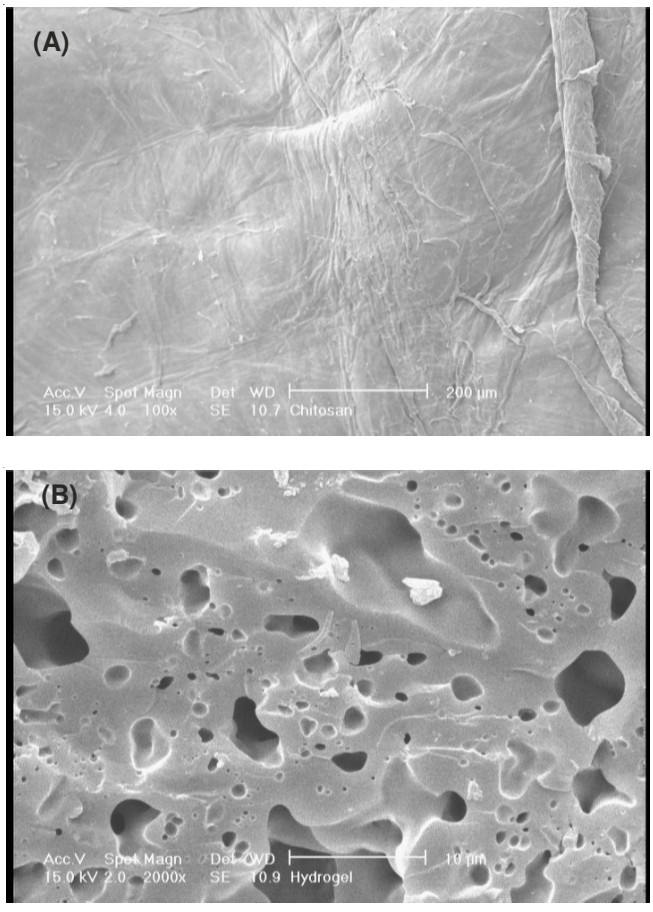


Fig. 2. SEM photograph of Chitosan (A) and the optimized super absorbent hydrogel (B)

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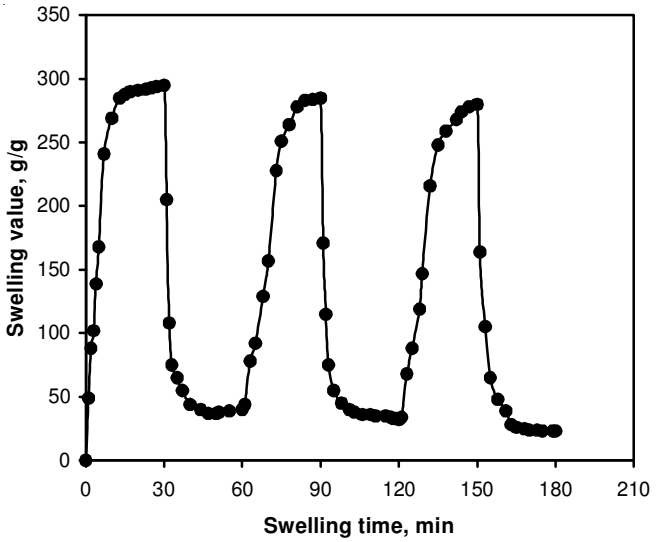

Fig. 3. On-off switching behaviour as reversible pulsatile swelling (pH 8) and deswelling (pH 2) of the hydrogel

***In vitro* metronidozule release in the simulated human gastrointestinal system:** To determine the potential application of chitosan-based super absorbent containing a pharmaceutically active compound, we have investigated the drug release behaviour metronidozule form this system under physiological conditions. The percent of released drug from the polymeric carriers as a function of time is shown in Fig. 4.

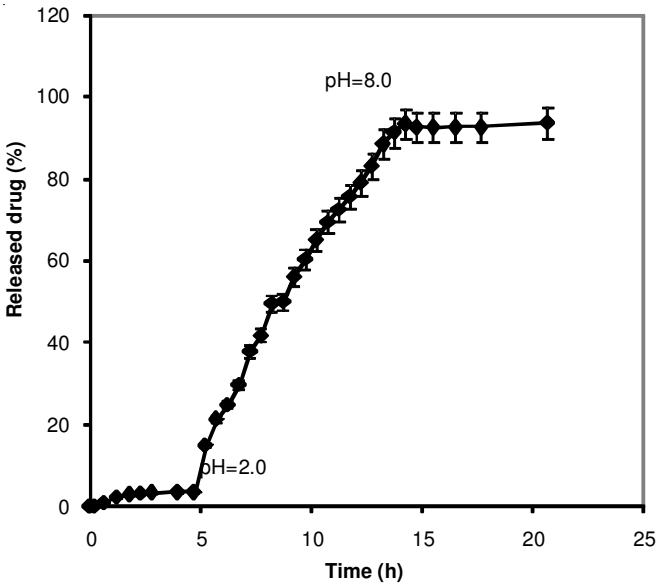

Fig. 4. Release of metronidozule from hydrogel carrier as a function of time and pH at 37 ºC

The concentration of metronidozule released at selected time intervals was determined by UV spectrophotometer. The metronidozule-loaded hydrogels with high degrees of drug loading (> 83 %) were prepared by the swelling-diffusion method. The amount of metronidozule released in a specified time from the Pectin-based hydrogel decreased as the pH of

the dissolution medium was lowered, indicating better release in a medium with a pH much higher than that of the stomach.

At low pH values, electrostatic repulsion between the carboxylic acid groups of backbone is low, thus decreases gel swelling and minimizes release of metronidozule *via* diffusion. However, in alkaline media the presence of OH^- increases the electrostatic repulsion between carboxylate groups, thus increases the gels swelling degree and so the release of metronidozule was increased[12]. The amounts of the loaded drug in super absorbent hydrogels was significantly affected by the loading time (Fig. 5). With increasing loading time, the amount of drug loaded is initially increased and then begins to level off.

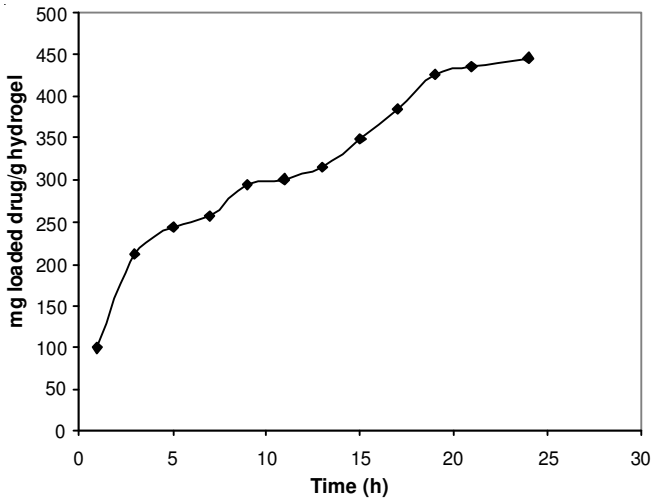

Fig. 5. Dependency of the drug loading amount to the loading time

Conclusion

Super absorbent hydrogels, Chitosan-*g*-poly(NaAA-*co*-AN) hydrogel, were synthesized through grafting of acrylic acid and acrylonitrile monomers onto chitosan using ammonium persulfate as an initiator and methylene bisacrylamide as a crosslinking agent under an inert atmosphere. The super absorbent hydrogels exhibited high sensitivity to pH, so that, the reversible swelling-deswelling behaviour in solutions with acidic and basic pH, contributes to the suitability of these hydrogels as candidates for controlled drug delivery systems. In vitro drug-release studies in different buffer solutions showed that the most important parameter affecting the drug-release behaviour of hydrogels is the pH of the solution. The release value of from hydrogels at pH 8 was higher than that at pH 2 due to the electrostatic repulsion between carboxylate groups.

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