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RESEARCH ARTICLE

NOVEL DRUG DELIVERY

CONTROLLED RELEASE OF A POORLY WATER-SOLUBLE DRUG FROM AN AMPHIPHILIC AND pH-SENSITIVE CHITOSAN-BASED HYDROGEL

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ABSTRACT

The present work focused on the design of drug delivery system based on an amphiphilic and pH-sensitive hydrogel. The hydrogels were prepared via graft copolymerization of acrylic acid (AA) onto chitosan backbones by a free radical polymerization technique. The structure and surface morphology of the hydrogels were characterized by FTIR and SEM, respectively. The swelling and pH-sensitive properties of the hydrogels were also investigated. Due to the reversible swelling behavior of the hydrogels, the synthesized networks can sense the environmental pH change and achieve an oscillatory release pattern. Using drug theophylline as a model molecule, loading and the *in vitro* controlled drug-release behaviors of these hydrogels were investigated. The loading yield was found to mainly depend on the crosslinker amount. The results also indicate that the main parameter affecting the drug-release behavior of hydrogels is the pH of the solution. The release rate of theophylline from hydrogel at pH 7.4 was faster than that at pH 1.2 due to the shrinkage of the hydrogel at pH 1.2.

KEYWORDS

Chitosan, Hydrogel, Acrylic Acid, Theophylline, Controlled Release.

INTRODUCTION

Hydrogels 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. In the swollen state, these become soft and rubbery, resembling a living tissue and some possess excellent biocompatibility [1]. Thus, polymeric hydrogels are of considerable interest as biomaterials in drug delivery research [2–6].
Drug release from solid matrices systems, made of polymer(s) and drug(s), is a basic concept for studies on controlled drug delivery. Recently, drug delivery systems based on natural hydrogels have been extensively explored to achieve the higher concentration of drugs in the specific region or tissue and the controlled release profile for extended time periods [7-10].

Hydrogels are formed from hydrophilic synthetic polymers and many natural polymers such as proteins and polysaccharides. Natural polymer gels are useful for pharmaceutical fields such as controlled delivery devices because of their non-toxic, low cost, free availability, biocompatibility and biodegradability.

Chitosan, a natural poly(aminosaccharide), is non-toxic and easily bioadsorbable. This biopolymer is a weak base with an intrinsic pK_a of 6.5 and with gel forming ability at low pH. In acidic solutions, the amine groups of a crosslinked chitosan are protonated and form a cationic hydrogel and result in swelling of the hydrogel network. For several years, chitosan has been largely evaluated as a potential vehicle for drugs administered orally. The development of hydrogel matrices incorporated with chitosan for oral drug delivery is still a virgin area of study.

Hence, in the current study we investigated the synthesis and utility of a hydrogel from graft copolymerization of acrylic acid onto chitosan backbones, for the controlled release of a poorly water-soluble model drug (theophylline). Drug absorption and release capacities of hydrogel systems were examined in detail.

MATERIALS AND METHODS

Chitosan was obtained from Aldrich, Milwaukee, WI, USA and used without further purification. N,N'-methylene bisacrylamide (MBA, from Merck), and ammonium persulfate (APS, from Fluka) were of analytical grade and used without further purification. Acrylic acid (AA, Merck) was used after vacuum distillation. The drug, theophylline, was received from Aldrich Chemical Co.

METHODS

Preparation of Hydrogel

Chitosan solution was prepared in a 1-l reactor equipped with mechanical stirrer and gas inlet. Chitosan was dissolved in degassed distillated water containing 1 wt% of acetic acid. In general, 0.50 g of chitosan was dissolved in 30 mL of distillated degassed 1 wt% acetic acid solution. The reactor was placed in a water bath preset at 60 ^{o}C. Then 0.10 g of APS as an initiator was added to chitosan solution and was allowed to stir for 10 min at 60^{o}C. After adding APS, the monomer (1.50 g) was added to the chitosan solution. Then, MBA solution (0.05 g in 5 ml H_2O) was added to the reaction mixture and the mixture

was continuously stirred for 1 h under argon. After 60 min, the reaction product was allowed to cool to ambient temperature and neutralized to pH 8 by addition of 1 N NaOH solution. Ethanol (500 ml) was added to the gelled product while stirring. After complete dewatering for 24 h, the hardened gel particles product were filtered, washed with fresh methanol and dried at 50 °C.

Determination of Drug Loading

Hydrogel (0.10 g) was immersed in 10 mL of the phosphate buffer solution (pH 7.4) in a 50 mL beaker for completely swelling. The swollen hydrogels were crushed in an agate mortar with a pestle and transferred into a conical flask, and then about 20 mL of the fresh phosphate buffer solution was added to the conical flask and the homogeneous mixture was sonicated for 20 min. The theophylline solution was separated from the mixture after being centrifuged for 20 min at 5000 rpm. The amount of theophylline was determined using UV spectrophotometer (UV-1201, Shimadzu, Kyoto, Japan). The drug loading (%) was calculated using the following equation:

$$Drug\ \ Loading\ \ (\%) = \frac{Weight\ \ of\ \ drug\ \ in\ \ hydrogel}{Weight\ \ of\ \ hydrogel} \times 100 \qquad (1)$$

In Vitro Drug Release

The *in vitro* release of theophylline was evaluated using dissolution methodology in simulated gastric and intestinal fluids (SGF and SIF). *In vitro* release was carried out in duplicate by incubating 0.1±0.0001 g of the theophylline-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°C. At specific time intervals, 1 mL aliquots of sample was withdrawn through a sampling syringe attached with a 0.45 μm Millipore filter and after suitable dilution, the concentration of released drug was measured by UV spectrophotometer at 272 nm. The drug release percent was calculated twice using the following equation:

$$\mathrm{Re}leased\ \ drug(\%) = \frac{R_t}{L} \times 100 \qquad (2)$$

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

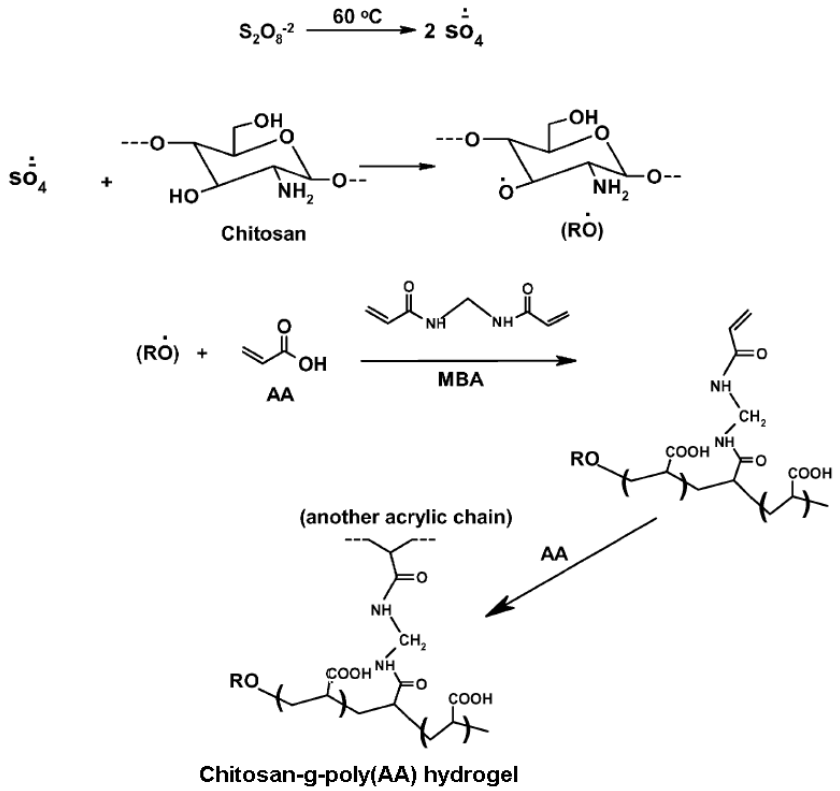
RESULTS AND DISCUSSION

Synthesis and Spectral Characterization

Poly(acrylic acid) was grafted onto chitosan in a homogeneous medium using APS as a radical initiator and MBA as a crosslinking agent under an inert atmosphere (Scheme 1). In the first step, the thermally dissociating initiator, i.e. APS, is decomposed under heating (60 °C) to produce sulfate anion-radicals. Then the anion-radicals abstract hydrogen from the chitosan backbones to form corresponding macroinitiators. These macroradicals initiate grafting of AA onto chitosan backbones leading to a graft copolymer. Crosslinking reaction also occurred in the presence of the crosslinker.

The grafting was confirmed by comparing the FTIR spectra of the chitosan substrate with that of the grafted products (Figure 1). In the spectra of the hydrogel the characteristic band at 1562 cm^{-1} was attributed to C=O asymmetric stretching in the carboxylate anion. This was confirmed by another peak at 1402 cm^{-1} which is related to the symmetric stretching mode of the carboxylate groups. The absorbed band at

1665 cm^{-1} is attributed to the carbonyl functional groups of carboxylic acid of acrylic acid grafted polymer.



Scheme 1

Proposed mechanistic pathway for synthesis of the chitosan-g-poly(AA) hydrogel.

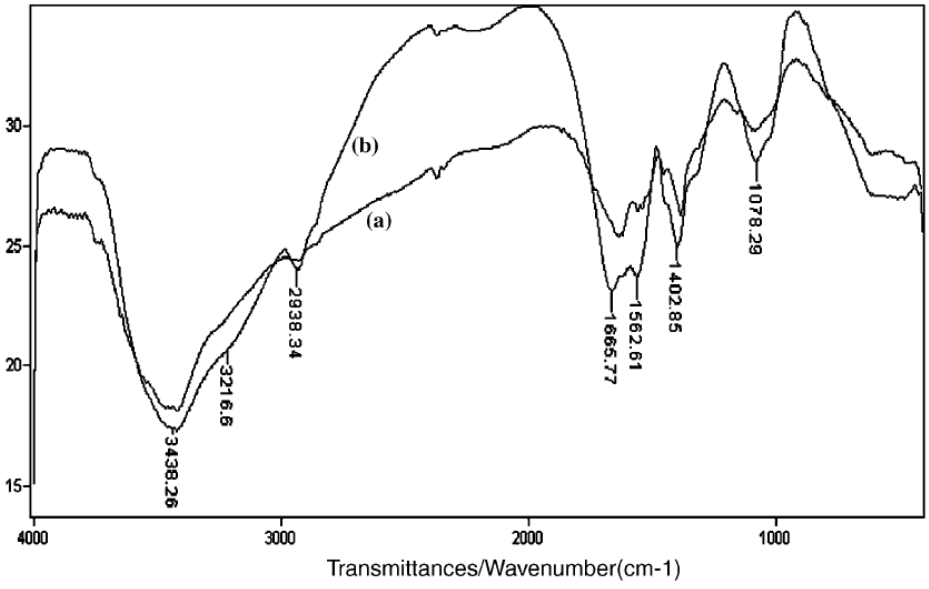


Figure 1

FTIR spectra of (a) chitosan and (b) chitosan-g-poly(AA) hydrogel.

One of the most important properties that must be considered is hydrogel microstructure morphologies. Figure 2 shows the scanning electron microscope images of the hydrogel. This picture verifies that the synthesized polymer in this work have 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.

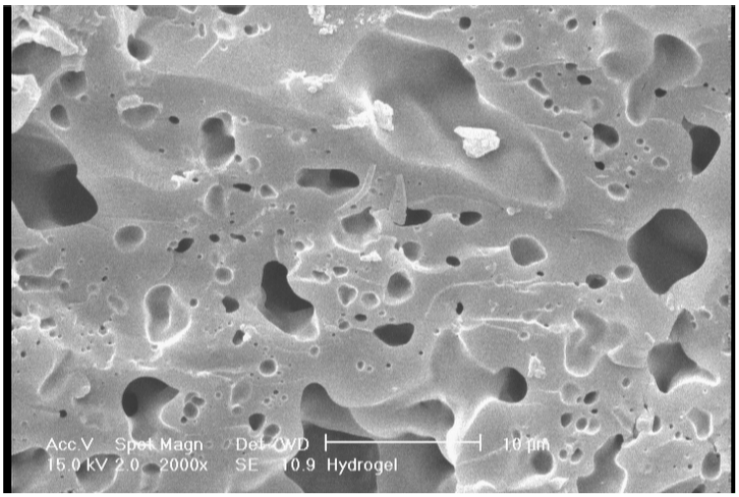


Figure 2

SEM photograph of the hydrogel. Surfaces were taken at a magnification of 2000, and the scale bar is 10 µm.

pH-Dependent Swelling of the Hydrolyzed Hydrogel

In this series of experiments, swelling ratio for the synthesized hydrogels was measured in different pH solutions ranged from 1.0 to 12.0 (Figure 3). According to Fig. 3, the two sharp swelling capacity changes can be attributed to high repulsion of $–NH_3^+$ groups in acidic media and $–COO^-$ groups in basic media. However, at very acidic conditions (pH ≤2), a screening effect of the counter ions, i.e. Cl^-, shields the charge of the ammonium cations and prevents an efficient repulsion. As a result, a remarkable decreasing in equilibrium swelling is observed. Around pH 5, the carboxylic acid component comes into action as well. Since the pK_a of the weak polyacid is about 6.4, its ionization occurring above this value, may favor enhanced absorbency. But under pH 6.4, at a certain pH range 4–6, the majority of the base and acid groups are as non-ionized forms, so hydrogen bonding between amine and carboxylic acid) may lead to a kind of crosslinking followed by a decreased swelling. At higher pHs, the carboxylic acid groups become ionized and the electrostatic repulsive force between the charged sites (COO^-) causes increasing in swelling. Again, a screening effect of the counter ions (Na^+) limits the swelling at pH 8–12 and opposed the swelling at pH>12, so that the hydrogel totally collapses at pH 13.

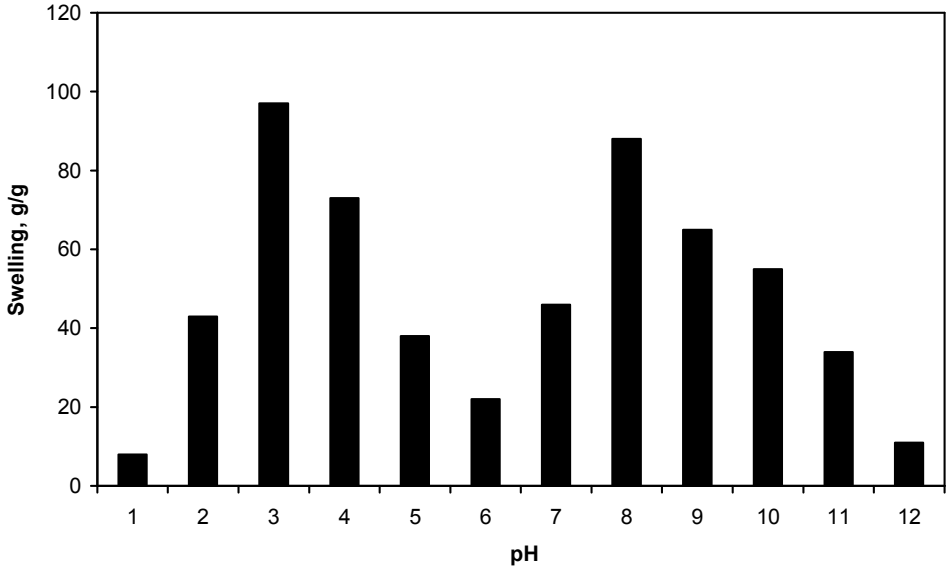


Figure 3
Swelling dependency of partially hydrolyzed chitosan-g-poly(AA) hydrogel on pH.

Since hydrogel swells differently in media with different pHs, we have investigated its pH-dependent swelling reversibility. The hydrogels were also showed reproducible swelling-deswelling cycles at pH 3.0 and 10.0 as demonstrated in Figure 4. At pH 10.0, the hydrogel swells due to anion-anion repulsive electrostatic forces, while at pH 3.0, it shrinks within a few minutes due to protonation of carboxylate groups. This sharp swelling-deswelling behavior of the hydrogels makes them as suitable candidate for controlled drug delivery systems.

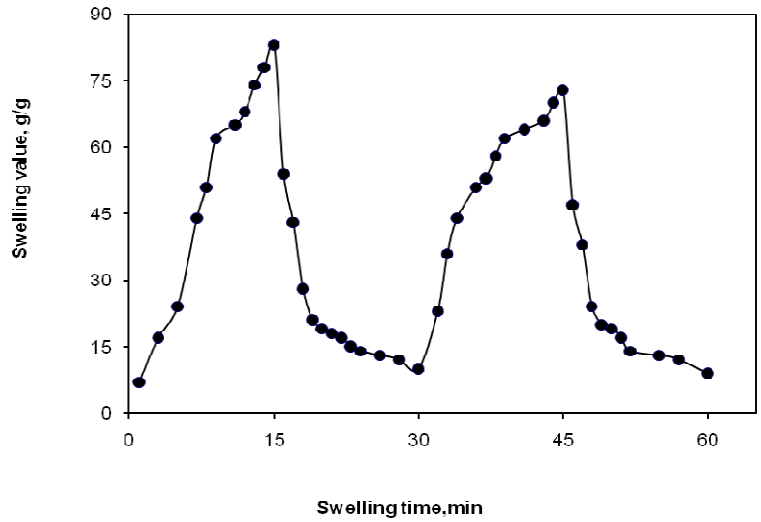


Figure 4
On–off switching behavior as reversible swelling (pH 3) and deswelling (pH 10) of the pH-responsive hydrogel, chitosan-g-poly(AA).

Drug Loading Efficiency

In this series of experiments, the drug loading of the hydrogels with different crosslinker content were shown in Figure 5. As can be seen, the amount of drug loaded in the hydrogel beads decrease with increasing the content of crosslinker, MBA. The greater the crosslinking density, the worse the elasticity of the polymer chains [11], which could restrict the penetration of theophylline into hydrogel, and then leads to the decrease of the loading amount for drug.

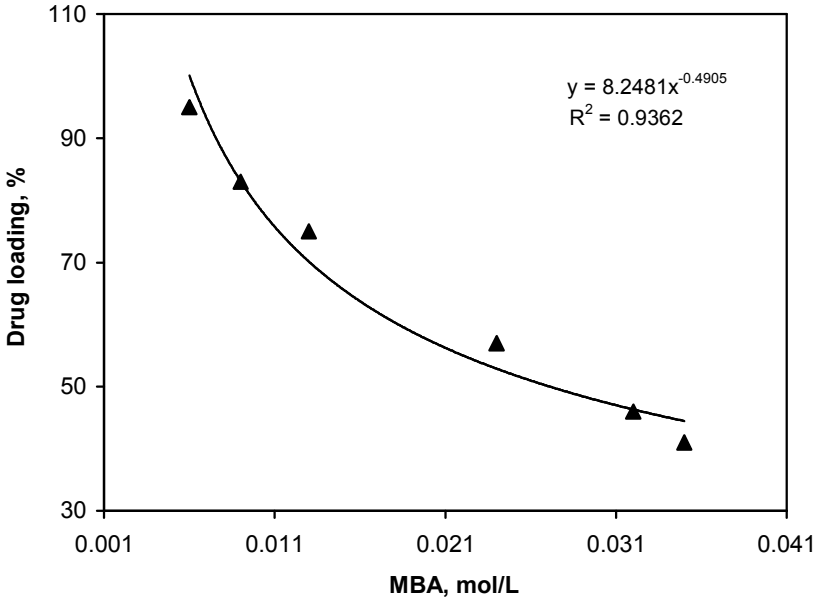


Figure 5

The dependency of the drug loading amount to the crosslinker concentration.

Controlled Drug Release

To determine the potential application of starch-based superabsorbent containing a pharmaceutically active compound, we have investigated the drug release behavior form this system under physiological conditions. The release rate experiments were performed in SFG (pH 1.2) and SIF (pH 7.4) solutions at 37 ^{o}C (Figure 6). As can be seen from Figure 6, when pH of the medium is 1.2, the cumulative release ratio of theophylline from the test hydrogels is below 35% at the end of the experiment (24 h), whereas 90% of the loaded drug is released within 15 h in pH 7.4 medium. Again, these results indicate that the higher swelling ratios of the hydrogel create larger surface areas to diffuse the drug. In basic solutions (pH 7.4), the electrostatic repulsion between COO^{-} anions of grafted poly (sodium acrylate) on the hydrogel accelerates the release of theophylline from the hydrogel.

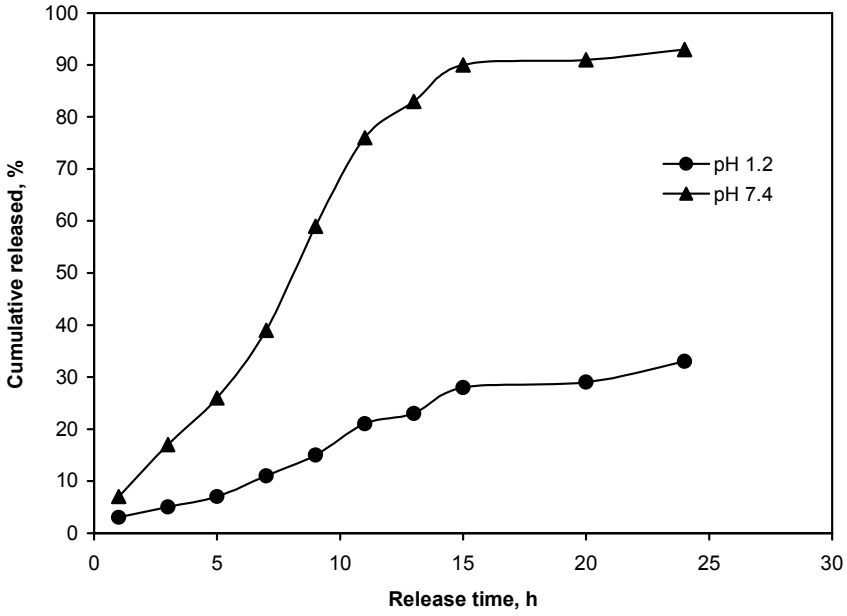


Figure 6
Release of theophylline from hydrogel carrier as a function of time and pH at 37°C.

CONCLUSION

Chitosan-g-poly(acrylic acid) hydrogel was synthesized through simultaneous crosslinking and graft polymerization of acrylic acid onto chitosan. The swelling of hydrogel exhibited high sensitivity to pH. This superabsorbent polyampholytic network intelligently responding to pH considered as an excellent candidate to design novel drug delivery systems.

It was observed that the release of theophylline was much higher in SIF compared to SGF, indicating that the release system is controllable and can be as a release system for intestine specific drug delivery. The drug loading efficiency was decreased with increasing crosslinker content.

Overall, the drug delivery systems based on hydrogels presented in this study may serve as a platform for a wide range of pharmaceutical uses to improve the bioavailability of non-steroidal anti-inflammatory drugs such as theophylline.

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