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Synthesis of a Chitosan-Based Superabsorbing Hydrogel for Controlled Release of Gentamicin

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ABSTRACT: In the present article, attention is paid to synthesis and controlled release of a model drug, gentamicin from a superabsorbent hydrogel based chitosan and polyacrylonitrile (PAN). The physical mixture of chitosan and PAN was hydrolyzed by NaOH solution to yield chitosan-poly(sodium acrylate-*co*-acrylamide) superabsorbent hydrogel. The nitrile groups of PAN were completely converted to a mixture of hydrophilic carboxamide and carboxylate groups during alkaline hydrolysis followed by in situ crosslinking of the PAN chains by the alkoxide ions of chitosan. Swelling capacity was conducted in solutions with pH ranged from 1 to 13. The hydrogels exhibited a pH-responsiveness character so that a swelling-deswelling pulsatile behavior was recorded at pHs 2 and 8. This on-off switching behavior makes the hydrogel as a good candidate for controlled delivery of bioactive agents. Therefore, loading and the *in vitro* controlled drug-release behaviors of these hydrogels were investigated in detail.

Key Words: chitosan; hydrogel; polyacrylonitrile; superabsorbent; gentamicin.

INTRODUCTION

In recent years, much interest has been shown in the development of synthesis of natural-based superabsorbent hydrogels [1-4]. These biopolymer materials are crosslinked hydrophilic polymers, capable of absorbing large quantities of water, saline or physiological solutions [5].

Because of their excellent characteristics, superabsorbent hydrogels are widely used in many applications such as disposable diapers, feminine napkins, and soil for agriculture and horticulture, and have aroused considerable interest and been the subject of much research [6-8].

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 [9-12].

Free radical vinyl graft copolymerization onto polysaccharide backbones is a well-known method for synthesis of natural-based superabsorbent hydrogels [13-16]. Radical polymerization, however, has several disadvantages. Reproducibility of this method is poor, and there is little control over the grafting process, so that the molecular weight distribution is polydisperse. In addition, the necessity for inert gases, e.g. argon, for preparing of oxygen-free atmosphere and need to initiator, toxic and/or expensive monomer and crosslinker are another disadvantages of free radical polymerization reactions. For the first time, Fanta et al. with development a new method, tried to synthesis of hydrolyzed starch-graft-polyacrylonitrile (HSPAN) superabsorbent hydrogel [17]. They hydrolyzed the physical mixture of starch and polyacrylonitrile. The initially formed oxygen-carbon bonds between starch hydroxyls and nitrile groups of the PAN chains remain as crosslinking sites.

Chitosan is an amino polysaccharide produced from chitin, the most abundant biomass in the world [18]. It has potential applications ranged from biomedicine and pharmacy to water treatment. Chitosan has both reactive amino and hydroxyl groups that can be used to chemically alter its properties under mild reaction conditions. Chitosan is a weak base and easy bioadsorber, with gel forming ability at low pH. This article describes the synthesis and controlled release behavior of a superabsorbent hydrogel based on chitosan and polyacrylonitrile.

EXPERIMENTAL MATERIALS

Chitosan (chemical grade, MW 50000) was purchased from Merck Chemical Co. (Germany). Polyacrylonitrile (PAN) was synthesized through a method mentioned in the literature [15]. Double distilled water was used for the hydrogel preparation and swelling measurements.

Hydrogel preparation

Chitosan solution was prepared in a 1-l reactor equipped with mechanical stirrer (Heidolph RZR 2021, three blade propeller type, 300 rpm), and gas inlet. Chitosan (0.50-2.50 g) was dissolved in 30.0 ml of distillated degassed water containing 1wt. % of acetic acid solution. After complete dissolution of chitosan, certain weight percent of sodium

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hydroxide (1.0-15.0 wt %) was added to the solution at 90 °C. The mixture was allowed to stir for 120 min. The various amount of polyacrylonitrile (0.50-1.50 g) was dispersed in the reaction mixture to saponify for certain times and temperatures. During the saponification, NH_3 gas was evolved and a color change from red to light yellow. This discoloration was an indication of the reaction completion. The pasty mixture was allowed to cool to room temperature and neutralized to pH 8.0 by addition of 10 wt % aqueous acetic acid solution. Then, the gelled product was scissored to small pieces and poured in ethanol (200 mL) to dewater for 5 h. The hardened particles were filtered and dried in oven (50 °C, 10 h). After grinding, the powdered superabsorbent hydrogel was stored away from moisture, heat and light.

Swelling measurements using tea bag method

The tea bag (i.e. a 100 mesh nylon screen) containing an accurately weighed powdered sample (0.5 ± 0.001 g) with average particle sizes between 40–60 mesh (250-350 μm) was immersed entirely in distilled water (200 mL) or desired salt solution (100 mL) and allowed to soak for 3 h at room temperature. The tea bag was hung up for 15 min in order to remove the excess fluid. The equilibrated swelling (ES) was measured twice using the following equation:

$$ES(g/g) = \frac{Weight\ of\ swollen\ gel - Weight\ of\ dried\ gel}{Weight\ of\ dried\ gel} \quad (1)$$

Swelling in buffer solutions

Two buffer solutions with pH 2 (citric acid/hydro-chloric acid) and pH 8 (boric acid/potassium chloride-sodium hydroxide) were used to study of pH-sensitivity of the hydrogel. The pH values were precisely checked by a pH-meter (Metrohm/820, accuracy±0.1).Then 0.10g of dried sample was used for the swelling measurements in both buffers according to the above mentioned method.

Drug loading on hydrogels

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} 266 *nm* using UV/VIS spectrophotometer (UV-1201, Shimadzu, Kyoto, Japan).

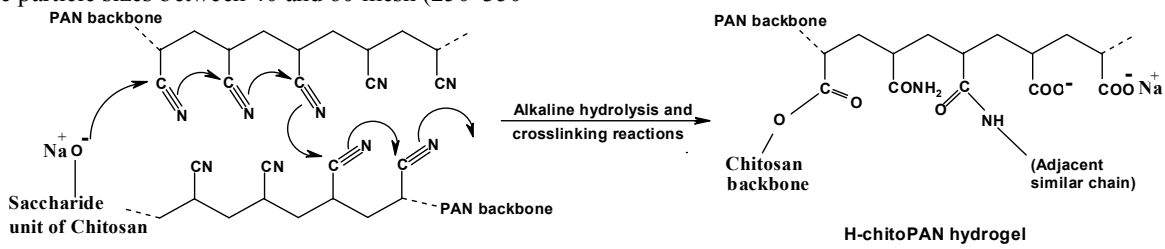
In vitro drug release of hydrogels

The release of gentamicin was followed as a function of time by measuring the light-absorbance of the outer aqueous phase at 266 nm using an UV/VIS spectrometer The samples (0.1±0.0001 g) were immersed into 50 mL of the release medium (simulated gastric and intestinal fluids, SGF and SIF) with different pH values (pH 1.2 or 7.4) at 37oC with agitation using a magnetic stirrer. Also, gentamicin release experiments were performed in deionized water at 20 oC and 40 oC, respectively. The same volume of fresh release medium was used to replace what was removed.

RESULTS AND DISCUSSION

Mechanism of hydrogel formation

A general reaction mechanism for H-chitoPAN synthesis is shown in Scheme 1. The hydroxyl groups of chitosan substrate was converted to corresponding alkoxide ions using sodium hydroxide solution. Then, these macroalkoxides initiate crosslinking reaction between some adjacent polyacrylonitrile pendant chains. This reaction leads to intermediate formation of naphthyridine cyclic structures (including imine, -C=N-, conjugated bonds) with deep red color. The intermediate was then hydrolyzed using residual sodium hydroxide aqueous solution to produce hydrophilic carboxamide and carboxylate groups with a resulting color change from red to light yellow. This sharp color change was used as an indication to halt the alkaline treatment.



Scheme 1. Proposed mechanism for crosslinking during hydrolyzing nitrile groups of chitosan-PAN mixture to produce H-chitoPAN hydrogel.

Infrared spectroscopy was carried out to confirm the chemical structure of the hydrogel. Figure 1 shows the FTIR spectra of chitosan-PAN physical mixture and the resulted hydrogel, H-chitoPAN. The band observed at 2248 cm^{-1}

can be attributed to stretching of –C≡N group of polyacrylonitrile (Fig. 1a). The hydrogel comprise an chitosan backbone with side chains that carry carboxamide and carboxylate functional groups that are evidenced by three new peaks at 1410, 1561, and 1682 cm^{-1} (Fig. 1b). These peaks attributed to C=O stretching in carboxamide functional groups and symmetric and asymmetric stretching modes of carboxylate groups, respectively. As shown in Fig. 1b, after alkaline hydrolysis, most of the nitrile groups are converted to carboxamide and carboxylate groups.

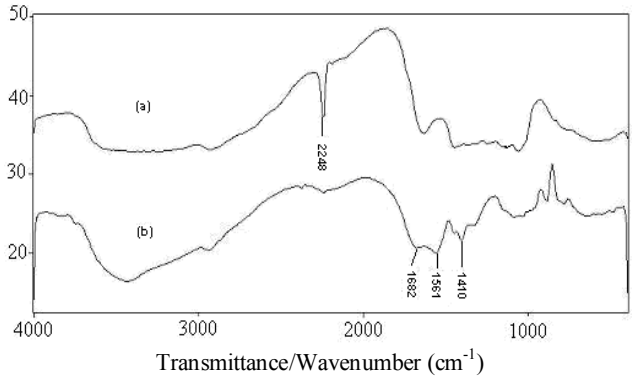


Figure 1. FTIR spectra of (a) the physical mixture of chitosan and PAN, and (b) the crosslinked H-chitoPAN hydrogel.

Equilibrium Swelling at Various pH Solutions

In this series of experiments, equilibrium swelling for the synthesized hydrogels was measured in different buffer solutions with pHs ranged from 1.0 to 13.0 (Fig. 2).

According to Fig. 2, 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 (gel collapsing). Around pH 5, the carboxylic acid component comes in to action as well. Since the pK 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 (and probable carboxamide groups) 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 8–11 and opposed the swelling at pH>12, so that the hydrogel totally collapses at pH 13. Such behavior has been reported for copolymeric gels from acrylic acid (the anionic constituent) and methacryl amidopropyl trimethyl ammonium chloride (the cationic constituent) [19]. In this system, a combination of attractive or repulsive electrostatic interactions and hydrogen bonding are the main reasons for existence of several phases observed in various environmental conditions

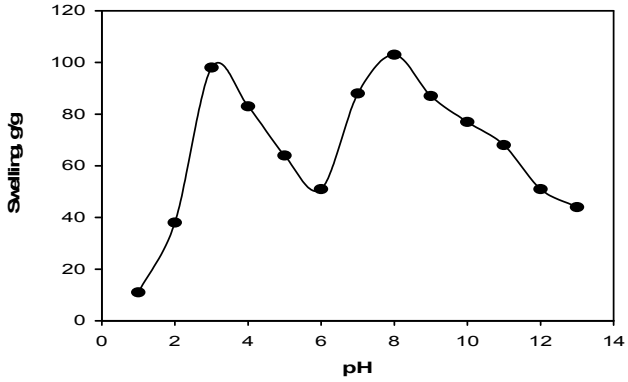


Figure 2. Effect of pH of buffer solutions on swelling capacity of H-chitoPAN hydrogel.

pH-responsiveness behavior of hydrogel

Since the present hydrogels show different swelling behaviors 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 8.0, the hydrogel swells up to 98 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 as suitable candidate for controlled drug delivery systems.

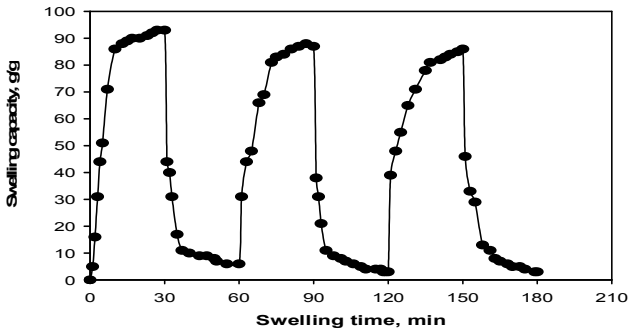


Figure 3. On-off switching behavior as reversible pulsatile swelling (pH 8.0) and deswelling (pH 2.0) of H-chitoPAN hydrogel. The time interval between the pH changes was 30 min.

Gentamicin Loading

The amounts of the loaded drug in superabsorbent hydrogels was also significantly affected by the impregnation times (Figure 4). It is obvious that with increasing the loading time, the amount of drug loaded is initially increased and then begins to level off. The initial increment in the amounts of the loaded drug with increasing the loading time can be ascribed to the increased drug diffusion into the swollen matrix. The most efficient time of

loading efficiency was 16 h, where a major amount of drug was encapsulated.

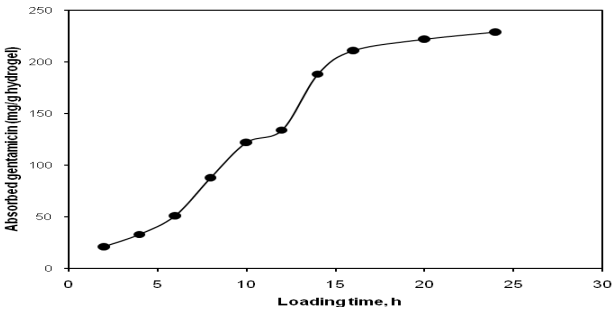


Figure 4. The dependency of the drug loading amount to the loading time.

Controlled Gentamicin Release

To determine the potential application of chitosan-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 5). As can be seen from Figure 5, when pH of the medium is 1.2, the cumulative release ratio of theophylline from the test hydrogels is below 40% at the end of the experiment (24 h), whereas 93% of the loaded drug is released within 16 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 gentamicin from the hydrogel.

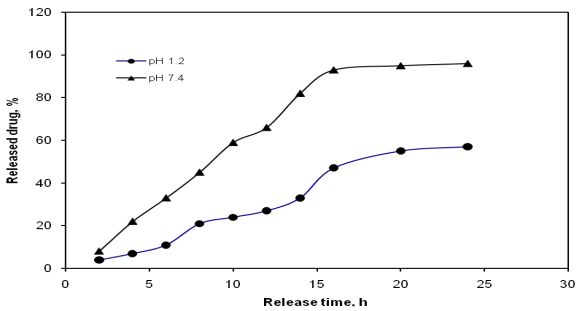


Figure 5. Release of gentamicin from hydrogel carrier as a function of time and pH at 37°C.

CONCLUSION

In the present study, we prepared a superabsorbent hydrogel, H-chitoPAN, by alkaline hydrolysis of chitosan/PAN physical mixture. The reaction of chitosan alkoxide anions with nitrile groups of polyacrylonitrile, forms crosslinking points and results in a three-dimensional network. Because a polymerization reaction is not involved, so there is no need to initiator, toxic and/or expensive monomer and crosslinker. In addition, this one-step preparative method conducted under normal atmospheric conditions in a short period of time. The dark red-yellow color change provides a visual indication for recognizing the reaction completion.

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

It was observed that the release of gentamicin 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.

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