Reactive & Functional Polymers 73 (2013) 97–107

Contents lists available at SciVerse ScienceDirect

Reactive & Functional Polymers

journal homepage: www.elsevier.com/locate/react

The preparation, swelling characteristics, and albumin adsorption and release behaviors of a novel chitosan-based polyampholyte hydrogel

Hiroyuki Kono *, Ikuo Oeda, Taichi Nakamura

Department of Science and Engineering for Materials, Tomakomai National College of Technology, Nishikioka 443, Tomakomai, Hokkaido 059 1275, Japan

ARTICLE INFO

Article history:
Received 19 June 2012
Received in revised form 20 August 2012
Accepted 21 August 2012
Available online 27 August 2012

Keywords:
Chitosan
Polyampholyte hydrogel
Protein adsorption and release
Butanetetracarboxylic dianhydride
Solid state NMR

ABSTRACT

A novel chitosan (Ch)-based polyampholyte hydrogel was prepared from Ch dissolved in a 1:1 (v/v) mixture of 10% aqueous acetic acid and *N*-methyl-2-pyrrolidinone (NMP) by simple crosslinking using 1,2,3,4-butanetetracarboxylic dianhydride (BTCA). The detailed structure of the hydrogel was determined via FTIR and solid-state ^{13}C NMR spectroscopic analyses. The swelling behavior of the hydrogels was strongly dependent on the BTCA feed ratio, and the hydrogels exhibited a pH-responsive swelling ratio that was influenced by the presence of both cationic $—NH_3^+$ and anionic $—COO^-$ groups within their molecular structures. The Ch hydrogels also exhibited bovine serum albumin (BSA) adsorption capacity, which was maximal at pH 4.5, consistent with the isoelectric point of BSA (4.7). In addition, the BSA adsorption capacity of the hydrogel decreased with the increasing ionic strength of the adsorption medium, indicating that the capacity of the hydrogel to adsorb BSA is facilitated by hydrophobic as well as electrostatic interactions between the hydrogels and the BSA molecules. In addition, a high desorption ratio (89%) of BSA was achieved in aqueous solutions at pH 2.0.

1. Introduction

Hydrogels, which are high molecular-weight, complex, three-dimensional networks composed of a polymer backbone, water, and a crosslinking agent, continue to gain significance in a wide variety of pharmaceutical and related medical applications, such as artificial organs [1,2], contact lenses [3,4], and drug delivery systems [5–8]. Recently, particular interest has focused on the development of hydrogels that exhibit phase transitions in response to external stimuli, such as pH [9–12], ionic strength [10], and temperature [11,13]. Accordingly, the term "intelligent gel" has been coined to describe such gels. A number of so-called intelligent gels prepared by the copolymerization of a monomer containing an anionic functional group with a monomer containing a cationic functional group in the presence of a crosslinking agent have been studied, and the phase transitions of these hydrogels have been found to be strongly dependent on the charge density present within their molecular chains [9–11]. However, with the exception of succinyl chitosan (Ch) [14,15] and carboxymethyl Ch studies [16,17], investigations focusing on the phase transition of naturally occurring polyampholyte hydrogels remain limited, in contrast to the abundance of studies on synthetic polymer-based polyampholyte hydrogels.

Chitosan (Ch) is the deacetylated form of one of the most abundant naturally occurring polymers, chitin, which is extracted from the exoskeletons of arthropods, crustaceans, and insects. Ch is a cationic polymer that is composed of β-(1→4)-linked D-glucosamine residues (GlcN), where specific GlcN residues within the polymer chain can be replaced by *N*-acetyl-D-glucosamine residues (GlcNAc). Ch is well recognized as a promising biomaterial due to its biodegradability, non-toxicity, antimicrobial property, and biocompatibility [18]. The functionalization of Ch to *O*-succinyl Ch or *O*-carboxymethyl Ch via the introduction of $—COCH_2CH_2COOH$ or $—CH_2COOH$, respectively, onto the pre-existing —OH groups existing along the chitosan molecular chain produces amphoteric polyelectrolytes that contain both cationic and anionic fixed charges [14–17]. By varying the degree of deacetylation (DD) and the degree of substitution of the anionic groups, natural Ch-based polyampholyte hydrogels have been explored in an effort to produce hydrogels that exhibit varying phase transitions in response to changes in external pH conditions [16]. These Ch-based polyampholyte hydrogels might act as potential matrices for the loading of proteins or other hydrophilic bioactive drugs in drug delivery systems.

Many approaches have been developed to prepare the chitosan-based polyampholyte hydrogels from *O*-succinyl Ch or *O*-carboxymethyl Ch, including chemical- [19–22] and physical-based [23,24] crosslinking methods. Although the physically crosslinked hydrogels have the advantage of formation without the use of crosslinking entities, they exhibit disadvantages in the precise control of

* Corresponding author. Tel./fax: +81 144 67 8036.
E-mail address: kono@sem.tomakomai-ct.ac.jp (H. Kono).

1381-5148/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.reactfunctpolym.2012.08.016

qualities of chemical functionalization, including, for instance, gel pore size, degradation or dissolution. Chemical crosslinking methods use small bifunctional molecules, such as glutaraldehyde or diglycidyl ether, to improve the mechanical properties of the hydrogel compared to physical hydrogels. Therefore, many chemical crosslinking agents have been used to prepare Ch-based hydrogels.

In this study, crosslinked hydrogels were prepared from cellulose that was dissolved in either LiCl/NMP [25], LiCl/*N*,*N*-dimethylacetamide [26], or tetrabutylammonium fluoride/dimethylsulfoxide [26] via esterification-crosslinking using 1,2,3,4-butanetetracarboxylic dianhydride (BTCA). BTCA contains two acid anhydrides within its structure, each of which reacts readily with specific functional groups, including isocyanate, amine, and hydroxyl groups, to undergo crosslinking. In the reaction of the hydroxyl groups of cellulose with BTCA, two free carboxyl groups are formed, simultaneously esterification-crosslinking the cellulose chains. Following the conversion of the carboxyl groups to sodium carboxylates by neutralization with NaOH solution, the resulting product exhibits an absorbency of 720 times its dry weight. The hydrogels also exhibit favorable biodegradability, with a maximal degradation rate of 95% within 7 days using cellulase. The advantage of this crosslinking method that uses BTCA is the simultaneous introduction of anionic charges and crosslinks into the polysaccharides. On this basis, the preparation of Ch-based polyampholyte hydrogels from chitosan by one-step reaction might be possible.

In this study, novel crosslinked polyampholyte hydrogels were prepared from Ch by a crosslinking reaction using BTCA, and the pH-responsive swelling of the Ch hydrogels was assessed. In addition, the protein adsorption and desorption capacities of the Ch hydrogels were evaluated using BSA as a model protein. The results are discussed herein.

2. Materials and methods

2.1. Materials

Ch (viscosity average molecular weight = 4.6×10^4 and DD = 0.86) was purchased from Tokyo Kasei Kogyo, Japan. The DD value of the Ch was determined via solid-state ^{13}C NMR analysis; the details are presented below. The BTCA and BSA were purchased from Sigma–Aldrich. All other chemicals (purchased from Kanto Chemicals, Japan and Wako Pure Chemicals, Japan) were of reagent grade and were used as received.

2.2. The preparation of the Ch hydrogels

The Ch hydrogels were prepared from Ch according to the synthetic schematic presented in Fig. 1. The DD of the Ch used in this study was 0.86, indicating that 86% of the total monomer residues in the Ch were GlcN and that the remaining 14% of residues were GlcNAc. Thus, the average molecular mass of the Ch monomer was calculated to be 167 g/mol; this value was used to determine the molar mass of Ch. A typical run of the procedure for preparation of the hydrogel from Ch using a BTCA feed ratio of two is described in detail below. A solution of Ch (1.0 g, 6.0 mmol for the monomer unit) dissolved in aqueous 10% (w/w) acetic acid (50 mL) was diluted with NMP (50 mL) containing BTCA (2.4 g, 12 mmol). The crosslinking reaction of Ch with BTCA was allowed to proceed for 24 h at room temperature with stirring using a Teflon impeller (300 rpm), after which the reaction mixture was transferred into a mixture (500 mL) of methanol and water (1:1) with stirring to precipitate the product. The product was then neutralized to pH 7.0 with 10% (w/v) aqueous NaOH while monitoring with a pH meter. The resulting precipitate was filtered using a glass filter and was then purified twice by re-precipitation using methanol and water. The purified product was dried under reduced pressure, cut with a mixer, and screened through a 16 mesh (1.0 mm) sieve to obtain a granular product. The resulting powdered product was denoted as Ch_BTCA 2. The number following the abbreviation Ch_BTCA indicates the molar BTCA feed ratio to the monomer unit of Ch. A series of Ch_BTCA hydrogels were prepared using a procedure similar to that used to prepare Ch_BTCA 2 by varying the molar feed ratio of BTCA to Ch.

2.3. FTIR analysis

The FTIR spectra of Ch and of a series of Ch_BTCA hydrogels were obtained using a Perkin–Elmer Spectrum two spectrometer. All of the samples were analyzed as KBr pellets, which were prepared by blending 2 mg of the powdered polymer with 100 mg of KBr that was previously dried at 105 °C for 24 h. The FTIR spectra were obtained at a spectral resolution of 1 cm^{-1} by the accumulation of 16 scans within the frequency range of 4100–450 cm^{-1}.

2.4. Solid-state ^{13}C NMR analysis

Dipolar-decoupled solid-state ^{13}C NMR spectra were recorded on a Bruker BioSpin AVANCE II 500 spectrometer (^1H Larmor frequency of 500 MHz) with a 4 mm dual-tuned magic angle spinning (MAS) probe, set at an MAS frequency of 10 kHz. The ^{13}C-excitation pulse with a flip angle of 30°, the acquisition time, and the repetition time were set to 1.5 μs, 20 ms, and 20 s, respectively. During the acquisition period, the SPINAL64 (the small phase incremental alternation with 64 steps) ^1H decoupling sequence [27] was applied with a ^1H field strength of 100 kHz. Generally, the spectra were accumulated over 6000–10,000 scans to achieve an acceptable signal-to-noise ratio. The ^{13}C chemical shift was calibrated based upon the carbonyl carbon resonance of D-glycine at 176.03 ppm, which was used as an external reference. The lineshape analysis of the NMR spectra was performed using the solid-lineshape tool included in the Bruker BioSpin TopSpin 3.0 software, and the non-linear least squares methods was employed for the lineshape analysis using the Gauss–Lorentz functions, as previously described [25,28–32].

2.5. Swelling studies

The swelling ratio of each Ch_BTCA hydrogel was determined using the tea bag method, as previously described [25,26]. Briefly, a nylon tea bag (dimensions of 50 × 100 mm) was prepared from a nylon mesh sheet with a pore size of 255 mesh (57 μm) using a heat sealer. One hundred milligrams of the hydrogel was placed into the tea bag, and the tea bag was then immersed in water or in the indicated buffer solution at 25 °C. The tea bag was removed from the aqueous solution at regular intervals, and excess water was removed by draining for 10 min. The swelling ratio was calculated using the following equation:

$$\text{Swelling ratio} = (W_s - W_0 - W_b)/W_0,$$

where W_s is the weight of the hydrogel in the swollen state, W_0 the weight of the dry gel, and W_b is the weight of the empty tea bag after the water treatment. The swelling ratios were measured for five samples of each hydrogel, and the average of the five values was plotted against the swelling time. As an external solution for determining the swelling ratio, distilled water and 10 mM HCl–KCl (pH 2.0), 10 mM citric acid–sodium citrate (pH 3.0–6.0), 10 mM NaH_2PO_4–Na_2HPO_4 (pH 7.0), and 10 mM Tris–HCl (pH 9.0) buffers were used. In addition, the swelling ratios of the Ch_hydrogels were assessed in BSA-containing pH buffers, which were prepared by dissolving BSA in each buffer solution described above.