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Controlled release of 5-aminosalicylicacid from chitosan based pH and temperature sensitive hydrogels

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ABSTRACT

A series of temperature and pH responsive hydrogels based on chitosan and poly(N-isopropyl acrylamide) (PNIPA) was prepared by redox polymerization. Effect of the composition on swelling behavior of the hydrogels and the release of 5-aminosaylcilic acid (5-ASA) at different temperatures and pHs have been investigated. Ammonium persulphate and TEMED were used as a redox pair at room temperature. As a cross linker, methacrylated chitosan was synthesized through the reaction of chitosan with glycidyl methacrylate (GMA). Introduction of the cross-linker provided the hydrogels with pH and temperature sensitivities. The phase transition temperatures of the hydrogels were determined by derivative differential scanning calorimeter (DDSC). Their phase transition temperatures were increased by chitosan content. Swelling behaviors and the release of 5-ASA varied significantly with pH, temperature and the gel composition. The release of 5-ASA from the hydrogels was followed by UV–Vis and fluorescence spectroscopy.

1. Introduction

Stimuli-responsive hydrogels are well known "smart materials" in terms of responding to environmental signals such as pH, temperature, ionic strength, charge density by changing their shape and volume. Chitosan, which is a high molecular weight polysaccharide, is a well known pH responsive natural polymer, derived by fully or partly N-deacetylation of natural chitin. Its free reactive amino and hydroxymethyl groups are convenient functional groups for further modifications. Amino groups can easily be protonated in aqueous medium to form positively charged chitosan soluble in aqueous acids and insoluble in alkaline and neutral solutions, as well as in common organic solvents. This property therefore makes chitosan pH sensitive. Additionally, protonation of free amino groups makes chitosan bind to some drugs and bio-macromolecules, such as proteins, DNAs, enzymes and antibodies, which makes chitosan suitable for transportation applications of bio-molecules and some drugs [1–3]. Moreover, due to its biodegradable, biocompatible and non-cytotoxic properties, and antimicrobial, antifungal and anticancer activities, chitosan and its copolymers and blends have been attracting increasing attention for various biomedical applications, such as wound healing [4–8], immunology, drug and gene delivery [9–15] and antibacterial food packaging [16]. On the other hand, relatively poor mechanical properties of chitosan cause some limitations in its applications as film materials. Thus, improvement in its physical properties through blending and copolymerization has been the subject of various research groups [17,18].

Thermo-sensitive hydrogels undergo volume phase transition with temperature, among which cross-linked poly(N-isopropyl acrylamide) (PNIPA) is a well known one, having a lower critical solution temperature (LCST). It has a volume transition at around 32 °C from the swollen to collapse state, which makes it suitable for controlled drug delivery in biomedical applications as the transition temperature is close to human body temperature [19–21]. pH and thermo sensitive hydrogels are hydrophilic network structures, carrying both thermo and pH sensitive segments. By considering the varying local pH values and the body temperature, preparation of thermo and pH sensitive hydrogels, leading to specific drug delivery processes, is particularly important. Thus, pH sensitivity of chitosan and the volume transition temperature of PNIPA have been the interest of various groups to develop materials for biomedical applications, such as drug carriers. Syntheses and

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characterizations of hydrogels based on PNIPA grafted chitosan, using gamma-irradiation, were previously studied by Cai et al. [22]. They reported the effect of NIPA concentration and irradiation dose on grafting efficiency and swelling behavior of the hydrogels. Han et al. prepared chitosan-PNIPA hydrogels, using photo polymerization technique, who studied acid orange (AO8) and 5-flurouracil (5FU) release [23]. Therapeutic effect of 5-ASA loaded chitosan capsules for ulcerative colit was investigated by Tozaki et al. [24]. They observed that chitosan capsules are useful 5-ASA carriers for colon-specific drug delivery. Separately, a pH sensitive methacrylated 5-ASA as a pro-drug was prepared and its swelling characteristics and release properties were studied [25]. On the other hand, to the best of our knowledge, there is no report available in the literature on controlled release of 5-ASA by thermal and pH sensitive chitosan-PNIPA hydrogels.

Although 5-ASA is an important anti-inflammatory drug used to treat inflammatory bowel diseases such as ulcerative colit and Crohn's disease, it causes gastric irritation when administrated through oral system. However, controlled release of 5-ASA by pH and temperature sensitive hydrogel in intestine system, rather than stomach, reduces such irritation. A report demonstrated the release of 5-ASA from chitosan capsules in a controlled way in bowel system for the treatment of Crohn's disease [24].

As independent and mutual influences of both pH and temperature on release kinetics of a drug, complete release of 5-ASA in small and/or large intestine is possible by tuning the chitosan/NIPA ratio. This study focused on the syntheses of pH and temperature sensitive chitosan-PNIPA based hydrogels, employing redox polymerization technique and determination of their potential use in controlled release of 5-ASA in intestinal system. Methacrylated chitosan was synthesized and applied as a cross-linker in redox polymerization of NIPA. Phase transition, swelling and collapsing behaviors of the hydrogels and their controlled release of 5-ASA as a function of temperature, pH and composition were examined.

2. Materials and methods

2.1. Materials

Chitosan was prepared by deacetylation of chitin from shrimp shell. N-isopropyl acrylamide (NIPA) (minimum 98% purity), glycidyl methacrylate (GMA, minimum 97% purity), N,N,N′,N′-tetramethylethylenediamine (TEMED, minimum 99% purity) and potassium peroxydisulphate (KPS, minimum 99% purity) were supplied from Aldrich, Fluka, Analyticals Carlo-Erba and J.T. Baker, respectively, and they were used as received. Acetic acid with 99–100% purity was supplied from Carlo Erba and used without further purification. Hydroquinone was supplied from Aldrich (minimum 99% purity) and used as inhibitor for undesirable polymerization of GMA in the course of methacrylated chitosan synthesis. Acetonitrile (AN) and tetrahydrofurane (THF) were supplied from Aldrich (99% purity) and used for isolation and purification of methacrylated chitosan. 5-Aminosalicilic acid (5-ASA) was 95% purity, obtained from Alfa Aeser and used without purification.

2.2. Instrumentation

Thermal gravimetric analyses (TGA) were recorded on Seiko-EXSTAR-TG/DTA7300 model thermal analyzer. Measurements (5–7 mg of the samples) were performed at heating rate of 10 °C/min under dynamic argon atmosphere at a flow rate of 20 ml/min. The phase transition temperatures were determined using Perkin Elmer Jade type differential scanning calorimeter (DSC) under argon atmosphere at 10 °C/min heating rate. Derivative of the DSC curves (DDSC) were plotted using Pyris TA software. Temperature calibration of DSC was conducted according to the indium melting point and melting enthalpy. The molar mass and molar mass distribution of chitosan were determined by gel permeation chromatography (GPC) system, equipped with Perkin Elmer-series 200 GPC high pressure pump, injector, serial connected four Water columns (Ultrahydrogel 120 + Ultrahydrogel 250 + Ultrahydrogel 500 + Ultrahydrogel 1000), Wyatt Optilab differential refractive index detector (RI) at 654 nm and Dawn Heleos multiangle light-scattering (LS) detector. The mobile phase was 0.1 M solution of sodium nitrate ($NaNO_3$) in 2% acetic acid water mixture, which had a flow rate of 1.0 ml/min. Measurements were carried out at 25 °C. Polymer concentrations were in the range of 0.5–2.0 mg/ml and all the samples were filtered through 0.2 μm filter prior to use. Drug release was followed in phosphate buffer, using Perkin Elmer Lambda-35 UV–Vis spectrophotometer and Scinco FS-2 fluorescence spectrophotometer, having Fluoro Master Plus Wave Scan software. The degree of deacetylation (DD) and methacrylation of glucosamine groups of chitosan were determined by ^1H NMR (Varian 600 MHz NMR) at 50 °C, applying a solvent system of 2% CF_3COOH in D_2O. Proton chemical shifts were recorded in ppm downfield from tetramethylsilane (TMS).

2.3. Preparation and characterization of chitosan

Chitosan was prepared from chitin of shrimp shell according to the literature procedure [26–28]. Briefly, chitin was isolated and purified by removing the minerals, proteins and pigments. Deacetylation of chitin was carried out in a 50% aqueous solution of NaOH at 80 °C for 8 h. The degree of deacetylation (DD) of chitosan was determined as 98% by ^1H NMR spectra. The specific refractive index increment (dn/dc) was found to be 0.128 ml/g in 0.1 M solution of sodium nitrate ($NaNO_3$) in a 2% acetic acid water mixture. The molar-mass and molar-mass distribution were determined by on-line GPC-LS technique. The polydispersity (Mw/Mn), absolute Mw and Mn values were found to be 1.6, 117.000 g/mol and 73.000 g/mol, respectively.

2.4. Synthesis of methacrylated chitosan [29]

5 g of chitosan (35 mmol glycosamine groups) and 250 ml of 3% (v/v) acetic acid/water solution were introduced into 500 ml three-necked round bottom flask (RB), equipped with a magnetic stirrer, gas inlet and a rubber septa. The solution was stirred under Ar atmosphere until a clear and yellowish solution was observed and 0.01 mg hydroquinone was added to prevent undesirable polymerization of GMA. pH was adjusted to 3.8 with KOH solution (0.1 M) and 5 ml GMA was slowly added with a vigorous stirring in an ice bath. After the reaction was allowed to stir at 60 °C for 4 h, the RB was placed into an ice bath to terminate the reaction. The product was precipitated in acetonitrile, washed twice with THF and kept under reduced pressure at room temperature. The conversion of chitosan was determined to be 12% by ^1H NMR spectroscopy.

2.5. Synthesis of chitosan-PNIPA hydrogels [30]

A solution of 0.2 g methacrylated chitosan (1.1 mmol of functional group based on 12% conversion) in 10 ml of 5% acetic acid was placed in a 20 ml reaction tube, to which 1.5 g of NIPA (13.2 mmol) and 0.2 ml of APS (0.05 M in water) were added. After the viscous solution was purged with argon for 10 min to remove oxygen, 0.1 ml of TEMED (0.05 M in water) was added and the solution was further purged with argon for 2 min. It was then transferred to a plastic straw of 5 mm diameter and 10 cm long, which was sealed and kept at room temperature until the cross-linking reaction was complete. The hydrogel was purified by consecutive swelling (pH 4 at 30 °C)