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# Small intestine- and colon-specific smart oral drug delivery system with controlled release characteristic

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ABSTRACT

In recent years, there has been a significant increase in strategies for the development of small intestine (and colon)-specific oral drug-delivery systems to maximize the efficiency of therapeutic agents and reduce side effects. However, only a few strategies are capable of working in the complicated environment of the human intestinal tract. In this study, the preparation of a basic pH/temperature-responsive *co*-polymer (p-NIVIm) and its *in-vitro*-drug delivery function in the pH range of 1–8 and temperature range of 25–42 °C are reported. The basic copolymer was prepared by radical copolymerization of *N*-isopropyl acryl amide (NIPAAm) and *N*-vinylimidazole (VIm). The lower critical solution temperature (LCST) of p-NIVIm was higher in stomach pH (~1.0) conditions (36.5–42 °C) and lower in small intestine and/or colon pH (~8.0) conditions (35.8–38.2 °C). The ability to uptake a model protein (BSA) at body temperature and to release it in conditions of 37 °C and pH 1–8 was determined. The drug loading capacity (0.231 mg per 1.0 mg copolymer) and efficiency (92.4%) were high at 37 °C/pH 7. The drug carrier showed a slow release pattern at pH 1 (~0.084 mg; ~35%) and then a sudden release pattern (~0.177 mg; ~73%) at pH 8. The cytotoxicity of p-NIVIm to MCF-7 cells *in vitro* was minimal at concentrations < 168.9 μg/mL after 72 h. The prepared copolymer with its pH-/temperature-responsive protein-entrapping and -releasing behavior at body temperature may potentially be applied as a novel small intestine (and colon)-specific oral drug delivery system.

## 1. Introduction

The oral administration of drugs is the preferred route for disease treatment in the gastrointestinal tract, because the method is simple, safe, equipment-free, and infection-free [1,2]. Therefore, many researchers have attempted to develop a strategy for the oral administration of pharmaceutical agents which are typically administrated through non-oral routes, for instance, most peptide- and protein-based pharmaceuticals including all types of insulin and most vaccines [3]. The oral administration of drugs is still limited by the poor availability of dosed drugs due to much loss of drugs caused by biochemical degradation at the highly acidic stomach (pH 2) and a speedy passage through the gastrointestinal tract, which can potentially lead to overdose. In order to solve this problem, a site-specific oral delivery method using functional polymer drug carriers has been proposed. This drug delivery system has three major prerequisites for effective delivery to a target site: first, protecting the drug during delivery to the target site [4], second, site specific drug-release by stimuli-responsive characteristics of drug carriers [5,6], and third, controlled drug-release to sustain adequate drug blood levels [7–9].

pH-responsive polymers have been widely employed for oral drug delivery in the gastro-intestinal tract [10]. It is well known that the pH values in the gastro-intestinal tract change from 1 to 2 in an empty stomach to 5–7 and 6–7.5 in the small intestine and colon, respectively [11,12]. pH-responsive drug-carrying materials also require the ability to protect drug molecules, to release them only at the target site, and to control their release. The many kinds of pH-responsive copolymers available generally contain not only weakly basic or acidic moieties, such as amine groups or carboxylic acid groups in the polymer chain [13,14], but also ester moieties that are degradable in acidic or basic conditions [15,16]. These functional moieties can change the polarity, solubility, and/or the degradability of the polymer backbone in different pH conditions, leading to drug-leakage from the polymer carrier. For this reason, pH-sensitive polymers have received considerable

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attention for application in oral drug delivery systems [9,17–19]. For instance, carrier polymers with basic moieties have been applied in acidic gastric conditions due to their increased solubility (or degradability) in that condition [17,20–23]. On the other hand, drug-carrying polymers with weakly acidic moieties have been used in neutral or weakly basic small-intestinal and colonic conditions because of their increased solubility (or degradability) in those conditions [9,16,18,19,24,25].

However, these types of pH-responsive polymers have limits in applications for drug delivery in the gastrointestinal tract because pH-dependent solubility (or degradability) change of the polymers in the pH-range of 1–8 is too slow and tedious to achieve controlled release [17,21–27]. The irreversible drug release process controlled by chemical (or physical) degradation of the polymer carrier wall has shown several problems, compared with that controlled by a reversible on-off system. First, the relatively slow degradation mechanism for drug-carrying polymers in the pH range of 1–8 compared with the speed of the drug carrier passing through the gastrointestinal tract [9,17–19,21–30], and second, uncontrollable drug release once the polymer carrier wall is decomposed [21–27]. These limitations could lead to problems such as the reduction of drug delivery efficiency for the exact delivery of the orally administered amount of drug to the target site, and an overdose to maximize the delivered drug amount. For these reasons, there is a strong need for the development of drug nanocarriers equipped with a reversible on-off system and additional smart functions such as drug-protection, site-targeting, and controlled release [31–35]. In that sense, development of a smart drug delivery vehicle that can respond to local pH and temperature conditions in the human body has been most widely attempted, because these two factors are crucial to the human body and are more effective for controlling the drug release time (or site) than any other lone factor [36–39].

Recently, temperature-responsive polymers have been developed for smart drug delivery systems. Poly N-isopropylacrylamide (p-NIPAAm) is one of the most famous thermo-responsive polymers [37]. The lower critical solution temperature (LCST) of p-NIPAAm is around 32 °C in aqueous solution, but this is too low to apply in the human body as a drug delivery system. To increase the LCST, many types of temperature-responsive copolymers have been synthesized by copolymerization with hydrophilic monomers [40–42].

The present study focuses on the preparation of a small intestine-(and/or colon-) specific polymer drug delivery system and its *in vitro*-application in a neutral pH environment at body temperature. To minimize the dosed amount of therapeutic agent and to maximize site-specific delivery, the development of a polymer with multi-stimuli responsive characteristics is strongly required. The preparation and characterization of a pH- and temperature-responsive *co*-polymer, and its *in vitro* application as a site-specific drug delivery system in small intestinal (and/or colonic) environments (pH 6–8 and body temperature) are reported herein. A basic copolymer (p-NIVIm) was prepared by radical copolymerization of N-isopropyl acryl amide (NIPAAm; 85 mol%) and N-vinylimidazole (VIm; 15 mol%). The prepared copolymer with a surface charge of +9.1 mV at pH 7 showed a minimum release amount (~0.084 mg per 1.0 mg polymer) of the model protein (BSA) in the stomach pH condition (1.0) due to the high LCST of 36.5–42 °C, and a maximum release amount (~0.18 mg per 1.0 mg polymer) of BSA in the small intestinal (and/or colonic) pH condition (6–8) due to the low LCST of 35.8–38.2 °C. The potential combined application of pH- and temperature-responsive characteristics will provide a new possibility for effective oral delivery of pharmaceutical protein agents to the small intestine (and/or colon) in a site-specific manner.

## 2. Experimental

### 2.1. Materials

N-isopropylacrylamide (NIPAAm) was purchased from ACROS (USA). N-hexane (HPLC grade), 1-vinylimidazole (VIm), ammonium persulfate (APS), and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) were purchased from Sigma-Aldrich (USA) and used as received. Bovine serum albumin (BSA) was used as a model drug and purchased from Sigma-Aldrich (USA). The Bio-Rad DC Protein Assay II kit was supplied by BMS (Korea). All other reagents and solvents used were analytical grade and used as received.

### 2.2. Synthesis and characterization of a basic copolymer, poly(NIPAAm-co-VIm) or p-NIVIm

A basic copolymer with amide and amine moieties, poly(NIPAAm-*co*-VIm) or p-NIVIm, was synthesized. NIPAAm (2.304 g; 20 mmol) and VIm (0.336 g; 3.6 mmol) in the molar ratio of 1.0:0.18 were dissolved in 40 mL of deionized water (D.W), then 10 μL (0.5 mmol) of APS solution (10 w/v%) as an initiator and 15 μL (0.1 mmol) of TEMED as an activator were added to the solution. Prior to polymerization, the reaction solution was purged with nitrogen for at least 30 min to remove oxygen. Polymerization was performed for 24 h at 60 °C. After completion of the reaction, all possible impurities were removed by dialysis (membrane tubing, molecular weight cutoff 3500 Da, Spectrum Laboratories, Savannah, GA, USA) for 3 days in D.W. The purified aqueous solution was freeze-dried to obtain p-NIVIm powder for further use. The yield was 2.471 g.

The physicochemical identification of the synthesized copolymer product was performed by FT-IR spectroscopy (Nicolet 380, Thermo Fisher, Wisconsin, USA) and $^1$H NMR (Bruker, Ultrashield 500 PLUS, Billerica, USA). FT-IR spectra of NIPAAm, VIm, and p-NIVIm were obtained using a KBr pellet. The $^1$H NMR spectrum of the copolymer product was obtained at 500 MHz using $d_6$-dimethyl sulfoxide as the solvent. The pH- and temperature-responsive behaviors (pH-dependent LCST value change) of the copolymer were determined using a UV–vis spectrophotometer (Evolution 300, Thermo Scientific, Wisconsin, USA) fitted with a temperature control system. For this, aqueous solutions (0.1 wt%) of the copolymer were prepared in phosphate-buffered saline (PBS) at pH 1–9 and about 1 mL of the prepared solution was added to a polystyrene UV cell located on the cell holder. The percentage transmittance of light at 500 nm was scanned for each sample during the heating process at a rate of 1 °C per 10 min between 34 and 43 °C. The desired pH was adjusted using HCl or NaOH solutions, and pH values were measured using a pH-meter (Orion 3 star, Thermo Scientific, Singapore, Singapore). The particle morphologies and mean sizes of the copolymer samples prepared in different dry conditions were measured using SEM (S-4300, Hitachi, Tokyo, Japan). For this, the polymer solutions (0.1 mg/mL) at 25 °C/pH 7, 37 °C/pH 7, 42 °C/pH 7, and 37 °C/pH 1were dropped onto cover glasses and then dried at each temperature in an oven fitted with a temperature control system. The Zeta (ξ) potential values were recorded using a Zetasizer (ZEN 3600, Malvern, Malvern, UK) at room temperature as a function of pH (1 − 10) using sodium acetate buffer solution (0.1 M acetic acid and 0.2 M sodium acetate in water). Matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectroscopy (Voyager-DE STR, Negative Polarity, Foster City, USA) was successfully employed to determine the average molar mass of the basic copolymer. The copolymer sample (10 mg) was dissolved in 1 mL of D.W and mixed with α-cyano-4-hydroxycinnamic acid (HCCA) matrix solution at a ratio of 1:9 (*v*/v, polymer:matrix). The average molecular weights ($M_n$ and $M_w$) were calculated according to the following formula:

$$Mn = \frac{\sum_i NiMi}{\sum_i Ni}, \quad Mw = \frac{\sum_i NiMi2}{\sum_i NiMi}$$