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Aqueous chromatography system using pH- and temperature-responsive stationary phase with ion-exchange groups

Eri Ayano [a], Kyoko Nambu [a], Chikako Sakamoto [a], Hideko Kanazawa [a,*], Akihiko Kikuchi [b], Teruo Okano [b]

[a] *Department of Physical Pharmaceutical Chemistry, Kyoritsu University of Pharmacy, 1-5-30 Shibakoen, Minato-ku, Tokyo 105-8512, Japan*
[b] *Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, 8-1 Kawadacho, Shinjyuku-ku, Tokyo 162-8666, Japan*

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Abstract

We report on the development of a novel analytical HPLC technique of nonsteroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen, ketoprofen and naproxen, with an isocratic aqueous mobile phase. In this study, we designed a new pH- and temperature-responsive copolymer of *N*-isopropylacrylamide (NIPAAm), butyl methacrylate (BMA) and *N*,*N*-dimethylaminopropylacrylamide (DMAPAAm). The copolymer was modified with cross-linked poly(NIPAAm-*co*-BMA-*co*-DMAPAAm) (IBD) hydrogel on to aminopropyl silica beads, and the products were evaluated as HPLC packing materials for an ion-exchange- and temperature-responsive chromatography. The property of the surface of the stationary phase was altered from hydrophilic to hydrophobic, and from charged to non-charged by changes in the temperature and pH. In addition, it is possible that ion-exchange groups can appear or be hidden on the polymer chain surface by temperature changes. The interactions of NSAIDs with this stationary phase were controlled by the temperature and the pH with a constant aqueous mobile phase. PH- and temperature-responsive chromatography is expected to be useful for the separation of pharmaceuticals and biomolecules.
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Keywords: Poly(*N*-isopropylacrylamide); HPLC; pH- and temperature-responsive chromatography; NSAIDs; Ibuprofen; Ketoprofen; Naproxen

1. Introduction

Recently, various polymers that respond to stimuli have been developed, and are widely utilized in drug-delivery systems [1], cell-culture dishes [2], cell sheets [3] and bioconjugates [4,5]. Some of these polymers change their structure and functions in response to the surrounding conditions, such as the pH [6], electric field [7], light [8,9] and temperature [1]. These stimuli-responsive materials, termed "intelligent materials", respond to the intensity of one or more external stimuli, and change their structure and functions. Poly(*N*-isopropylacrylamide) (PNIPAAm) is one of the extensively studied thermosensitive polymers. PNIPAAm exhibits a thermally reversible phase transition in aqueous solution at 32 °C. This transition temperature is called the lower critical solution temperature (LCST) [10]. In water, the polymer chains of PNIPAAm hydrate and expand below the LCST, while they dehydrate to form a compact, insoluble conformation above it. We previously reported a reversible alteration in the surface hydrophilic/hydrophobic properties of PNIPAAm grafted surfaces in response to changes in temperature [11–13]. Taking advantage of these characteristics, we developed a high-performance liquid chromatography (HPLC) for the separation of analytes by controlling the external column temperature. This HPLC system is very simple because elution can be controlled merely by adjusting the temperature, and only aqueous solutions are used as the mobile phase [11–15]. Using this system, we previously achieved the separations of steroids, peptides and proteins [11,12,14]. Temperature-responsive chromatography is an analytical method with little load on environmental pollution because no organic solvent is used in the mobile phase. We have designed and synthesized a novel temperature-responsive copolymer, which responds to the temperature and other environmental stimuli, such as changes in the pH [16,17]. They showed hydrophilic–hydrophobic phase transitions in response to temperature changes, and the transition temperatures were affected by the pH [18].

Nonsteroidal anti-inflammatory drugs (NSAIDs) are frequently used to treat inflammatory conditions, such as rheumatic disease, arthritis, bursitis and tendinitis [19]. Though these drugs

* Corresponding author. Tel.: +81 3 5400 2657; fax: +81 3 5400 1378.
E-mail address: kanazawa-hd@kyoritsu-ph.ac.jp (H. Kanazawa).

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have a similar anti-inflammatory action of steroid drugs, serious side effects do not appear, since they are not powerful, like steroid drugs. Also, NSAIDs are relatively inexpensive, and are frequently the first line of medication used to relieve pain and to reduce inflammation. Very low-dose NSAIDs may be prescribed for people with cardiac disease. It seems to be necessary to construct in the future a convenient analytical method for producing drugs with a high use frequency.

The purpose of the present contribution is to describe the design of new stationary phases for the effective separation of bioactive compounds and pharmaceuticals. In this study, we synthesized pH- and temperature-sensitive polymers that involve a copolymer of NIPAAm and anion-exchange groups, DMAPAAm. The stationary phase modified with the copolymers was expected to exhibit a hydrophilic, positively charged property at temperatures below the LCST. We prepared a pH- and temperature-responsive terminally-modified surface on the surface of packing materials and achieved the successful separation of various acidic drugs such as salicylic acid classes. Moreover, we prepared a pH- and temperature-responsive hydrogel-modified surface in order to enhance the density of PNIPAAm chains on the surface of packing materials. Using the pH- and temperature-responsive hydrogel-modified column, we have now achieved the successful separation of NSAIDs using only an aqueous solution as a mobile phase without an organic solvent.

2. Experimental

2.1. Chemicals

N-isopropylacrylamide (NIPAAm) was kindly provided by KOHJIN, Tokyo, Japan, and was purified by recrystallization from *n*-hexane. *N*,*N*-dimetylaminopropylacrylamide (DMAPAAm) was purchased from KOHJIN. Butylmethacrylate (BMA), HPLC-grade methanol and ammonium acetate were purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan. 2,2′-Azobisisobutyronitrile (AIBN) (Wako Pure Chemical Industries) was purified by recrystallized from ethanol. 3-Mercaptopropionic acid (MPA), *N,N*-dimethylformamide (DMF), *N,N*′-dicyclohexylcarbodiimide (DCC) and *N,N*′-methylenebisacrylamide (MBAAm) were obtained from Kanto Chemicals, Tokyo, Japan. *N*-Hydroxysuccinimide was purchased from MERCK-Shuchardt. 4,4′-Azobis(4-cyanovaleric acid) (Wako Pure chemicals) was used after drying at 25 °C in vacuo. 1-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinolone (EEDQ) was purchased from Tokyo Chemical Industry, Tokyo, Japan. Aminopropyl silica beads (average diameter, 5 μm; pore size, 120 Å) were purchased from Nishio Industries, Tokyo, Japan.

Benzene (BEN), hydrocortisone (HC), hydrocortisone acetate (HCA), testosterone (TES), benzoic acid (BA), salicylic acid (SA), methyl salicylate (MS) and acethylsalicylic acid (Aspirin, As) were purchased from Wako Pure Chemical Industries. Cortisone (COR), prednisolone (PRE), dexamethasone (DEX), Ibuprofen, ketoprofen and naproxen, USP were purchased from Sigma Chemicals (St. Louis, MO, USA). Disodium hydrogenphosphate 12-water, sodium dihydrogen phosphate and citric acid monohydrate were purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan. Water used for sample preparation and the LC mobile phase was prepared using a Milli-Q water purification system (Millipore Corp., MA, USA).

2.2. Preparation of PNIPAAm terminally-modified silica

The synthesis of semitelechelic NIPAAm copolymer and a modification of aminopropyl silica with the NIPAAm copolymer were carried out by radical polymerization, as previously reported method [11,20]. AIBN and MPA were used as an initiator and chain transfer agent, respectively. Poly(NIPAAm-*co*-BMA) (IB) with a feed ration of NIPAAm/BMA molar ratio fixed to 95/5, poly(NIPAAm-*co*-BMA-*co*-DMAPAAm) (IBD) with a feed ration of DMAPAAm 5.0 mol%, and NIPAAm/BMA with a molar ratio fixed to 95/5 were synthesized in DMF with AIBN and MPA.

2.3. Preparation of PNIPAAm hydrogel-modified silica

4,4′-Azobis(4-cyanovaleric acid) and EEDQ, an initiator and a condensing agent, respectively, were dissolved in DMF in a separation flask. Aminopropyl silica beads were immersed in the solution, and the solution was bubbled with N_2 gas for 30 min. The mixture was degassed again for 30 min before the reaction was started. The reaction was carried out at 25 °C for 6 h under an N_2 gas atmosphere. Modified silica beads were washed with DMF and ethanol, consecutively, and dried in vacuo overnight. The polymerization of PNIPAAm hydrogel on silica beads was carried out by the following method: NIPAAm or NIPAAm:BMA:DMAPAAm (mol ratio; 90.25:4.75:5) and MBAAm were dissolved in 200 mL of ethanol in a glass ampule. Initiator-immobilized silica beads (4.0 g) were added to the solution of a monomer. The reaction mixture was then bubbled with N_2 gas for 1 h, and polymerization was carried out at 70 °C for 5 h under a N_2 gas atmosphere. PNIPAAm or P(NIPAAm-*co*-BMA-*co*-DMAPAAm) hydrogel-modified beads were filtered and washed three times with methanol by decantation to remove any un-immobilized hydrogels. These were rinsed in methanol at 25 °C, and then pure water at 5 °C, respectively, and dried in vacuo overnight. Polymer hydrogel-modified beads were obtained.

2.4. Transmittance measurements

The LCST of NIPAAm copolymers was determined by measuring the optical transmittance of copolymer aqueous solutions [11,12]. The copolymer solutions were prepared by water and phosphate/citrate buffer (0.5, w/v%). The optical transmittance changes at 500 nm of NIPAAm copolymer solutions were measured at various temperatures and pH using a spectrometer (Hitachi U-3000). The temperature of the observation cell was controlled with a deviation of ±0.02 °C with a LAUDA RC20 water bath. The LCST was defined as the temperature at 50% optical transmittance of NIPAAm copolymer solutions.

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