In Situ Surface-Selective Modification of Uniform Size Macroporous Polymer Particles with Temperature-Responsive Poly-*N*-isopropylacrylamide

Ken Hosoya,* Etsuko Sawada, Kazuhiro Kimata, Takeo Araki, and Nobuo Tanaka

Department of Polymer Science & Engineering, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606, Japan

Jean M. J. Fréchet

Department of Chemistry, Baker Laboratory, Cornell University, Ithaca, New York 14853-1301

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ABSTRACT: A new *in situ* surface-selective modification procedure for the incorporation of temperature-responsive poly-*N*-isopropylacrylamide (poly-NIPAM) into porous polymer beads has been developed. This procedure allows the incorporation of the poly-NIPAM either on the internal surface of the macroporous beads or on their external surface selectively. The process involves the addition of NIPAM monomer and a water-soluble radical initiator to a polymerizing mixture consisting of uniformly sized monomer and porogen particles prepared by a two-step swelling and polymerization method. NIPAM polymerizes in the aqueous phase but soon precipitates out because the upper critical solution temperature of poly-NIPAM is exceeded. If cyclohexanol is used as the porogen for the monodispersed beads, poly-NIPAM dissolves in the cyclohexanol and is able to penetrate all pores of the beads where it becomes grafted at their surface. With toluene as the porogen, poly-NIPAM being insoluble in the porogen cannot penetrate the pores but only becomes grafted onto the external surface of the beads. The characteristics of the poly-NIPAM-modified particles were confirmed by a simple chromatographic process.

Introduction

The preparation and chromatographic use of macroporous glass beads modified with temperature-responsive polymer poly-*N*-isopropylacrylamide (poly-NIPAM) has been reported[1] recently. In this report, the temperature responsiveness of poly-NIPAM[2,3] was used for the control of pore size in high performance liquid chromatography (HPLC) through changes in column temperature. This application is rather interesting because separation selectivity in HPLC had previously been controlled mostly by changes in the mobile phase and/or the stationary phase, while this early report[1] suggested that temperature could be also used to control separation selectivity in HPLC. However, the preparation method was too complicated to make this method widely applicable to the preparation of stationary phases for HPLC.

The surface modification of ordinary support materials with a variety of reactive substances is a very useful technique to prepare effectively functionalized materials, because the reactive substances can be incorporated in such a way that they are located only at the surface of the support materials. For these modifications, the reactive functional groups located at the surface of the support materials are treated with the reactive substances to afford the surface-modified support materials. However these modification reactions are difficult and incomplete due to the relatively slow mass transfer and steric hindrance within the support materials. This problem is especially acute with highly cross-linked macroporous polymeric support materials,[4] for which accessibility to the reactive groups is severely limited.

Therefore, a copolymerization technique that directly incorporates functional monomers within the polymeric support materials has been used extensively. However, this method wastes a significant part of the functional monomers because of their incorporation at sites such as the inner framework of the beads[4] that are inaccessible to the substances that diffuse through the pores of the medium during the separation process.

In this report, we describe a useful novel method that achieves the *in situ* surface-selective modification of uniform size macroporous polymeric materials by a graft-type copolymerization of the temperature-responsive polymer poly-NIPAM. This new widely applicable modification method improves on the current modification and preparation techniques of surface-functionalized beads.

Experimental Section

Materials. *N*-Isopropylacrylamide was a gift from Kohjin Co., Ltd. (Tokyo, Japan), while ethylene dimethacrylate was purchased from Wako Pure Chemical (Osaka, Japan). These monomers were purified to remove radical inhibitors by standard techniques. The reagents for the preparation of uniform size seed particles, such as styrene and potassium peroxodisulfate, were purchased from Nacalai Tesque (Mukoh, Japan), while those utilized in the two-step swelling and polymerization method were purchased from Tokyo Kasei (Tokyo, Japan).

Procedures. Uniform size polystyrene seed particles were prepared through an emulsifier-free emulsion polymerization method and purified by the previously reported method.[5] The size of the seed particle was ca. 1 μm in diameter.

Preparation of uniform size macroporous polymer particles by a two-step swelling and polymerization method was carried out as follows: As shown in Figure 1, the water dispersion of the uniform size polystyrene seed particles (9.5×10^{-2} g/mL) (1.4 mL) was admixed with a microemulsion prepared from 0.95 mL of dibutyl phthalate, activating solvent,[6] 0.085 g of benzoyl peroxide, 0.04 g of sodium dodecylsulfate, and 10 mL of distilled water by sonication. This first step swelling was carried out at room temperature while there was stirring at 125 rpm. Completion of the first step swelling was determined by the vanishing point of the oil droplets in the added microemulsion using an optical microscope. A dispersion of 9 mL of ethylene dimethacrylate and 10 mL of porogenic solvent such as toluene or

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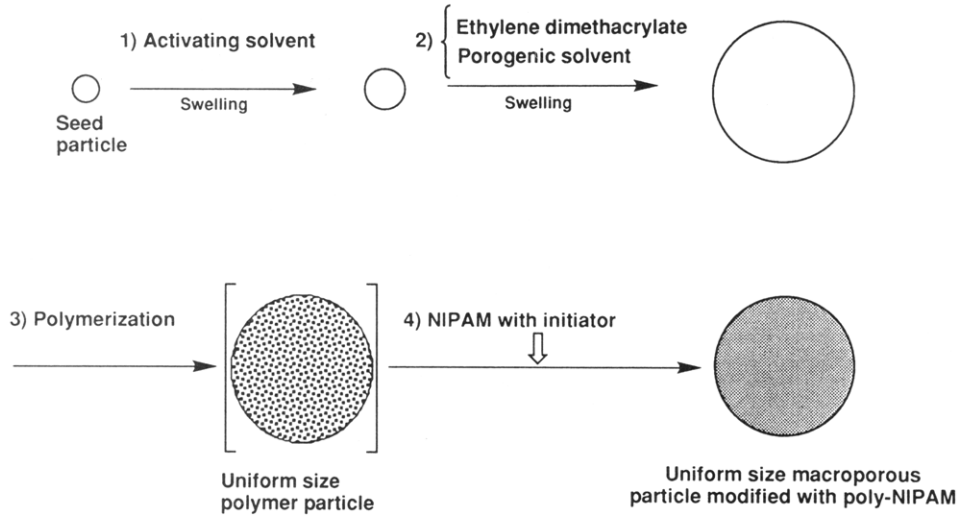

Figure 1. Preparation procedure of uniform size macroporous polymer particles modified with poly-NIPAM.

cyclohexanol into 90 mL of water containing 1.92 g of polyvinyl alcohol (dp = 500, saponification value = 86.5–89 mol %) as a dispersion stabilizer was added to the dispersion of swollen particles. The swelling was carried out at room temperature for 2 h while the dispersion was stirred at 125 rpm. After the second step swelling was completed, the polymerization procedure was started at 80 °C under argon atmosphere with slow stirring. After 4 h, 1 g of *N*-isopropylacrylamide (NIPAM) and potassium peroxodisulfate (0.02 g) were directly added to the aqueous polymerization medium. After an additional 20 h, the dispersion of polymerized particles was poured into 250 mL of cold water to remove the unbound poly-NIPAM and the supernatant was discarded after sedimentation of the particles. The polymer particles were redispersed into methanol, and the supernatant was discarded after sedimentation. This procedure was repeated three times in methanol and twice in tetrahydrofuran (THF), and then the polymer particles were filtered onto a membrane filter, washed with THF and acetone, and then dried at room temperature to determine yields.

Unmodified macroporous particles (base particles) were also prepared by utilizing the same procedure but without the addition of NIPAM.

The yield calculated from the amount of the monomers used was 94–100%. The prepared particles were packed into stainless steel column (4.6 mm i.d. × 150 mm) by a slurry technique to evaluate their characteristics.

Chromatography. High performance liquid chromatography was performed with a Jasco 880-PU intelligent pump or a Shimadzu LC-4A ternary gradient pump equipped with a Rheodyne 7125 valve loop injector. Peak monitoring was carried out with a Jasco UVIDEC-100-III or a Shimadzu SPD-2A UV detector set at 254 nm and an RI detector from a Waters R401 differential refractometer. Peak information was recorded on a Shimadzu C-R4A chromatopak.

Result and Discussion

Concept of *in Situ* Surface-Selective Modification.[7] Because NIPAM monomer is water soluble and a water-soluble radical initiator is present, the polymerization of NIPAM itself is initiated within the aqueous phase. While this polymerization proceeds, the polymerization of ethylene dimethacrylate also continues independently in the adjacent organic phase that makes up the uniformly sized oil droplets.

Once the growing NIPAM polymer chains reach a certain size, they become water insoluble due to their temperature responsiveness at the high polymerization temperature of 80 °C. This leads to precipitation of growing poly-NIPAM in the aqueous phase. At this moment, the partly polymerized droplets containing the porogen and the residual ethylene dimethacrylate monomer are still dispersed in the aqueous phase; consequently, the precipitating poly-NIPAM should distribute itself between aqueous and organic phases according to its partition coefficient.

Table 1. Partition of NIPAM in Various Solvents (Poly-NIPAM δ = 11.18)[a]

initiator	cyclohexanol (δ = 11.4[b])	water	toluene (δ = 8.91[b])	water
none	0.0	100.0[c]	0.0	100.0[c]
PPS[d]	99.0[e]	1.0	1.0[e]	99.0[e]

[a] Phase ratios corresponding to those of the actual polymerization mixture. [b] Reference 8. [c] NIPAM monomer was recovered. [d] Potassium peroxodisulfate. [e] Poly-NIPAM was yielded.

Table 1 shows the solubility parameters[8] and partition properties of the poly-NIPAM determined separately by simulated experiments. As expected from their relatively similar solubility parameters, cyclohexanol completely dissolves poly-NIPAM. On the other hand, poly-NIPAM is completely insoluble in the toluene and, therefore, in experiments involving toluene as the porogen, a triphase system consisting of the aqueous phase, the organic phase, and the poly-NIPAM dispersed into the aqueous phase is observed during the experiments.

According to these results, if cyclohexanol is used as the porogenic solvent, the growing poly-NIPAM presumably distributes itself into the cyclohexanol phase filling the pores of the already polymerizing porous particles, modifying their internal surfaces through a graft-type copolymerization. In contrast, if toluene is used as the porogenic solvent, poly-NIPAM being insoluble in the porogen cannot penetrate the inner pores of the beads that are filled with toluene, and therefore, the internal surface of the beads remains unmodified. In this case, poly-NIPAM can only graft itself onto the external surface of the beads which is in contact with the aqueous phase.

Characteristics of the Prepared Particles. Scanning electron micrographs of these two kinds of modified particles are shown in Figure 2 in a comparison with the unmodified particles. The external appearance of the particles prepared with toluene as the porogen (d) is drastically different from that of the unmodified particles (c). In contrast, similar external appearances are observed for both the NIPAM-modified particles utilizing cyclohexanol as the porogen (b) and the unmodified particles (a). These differences in external appearances demonstrate that surface-selective modification[7] with poly-NIPAM is achieved through the selection of the porogenic solvents according to the concept described above.

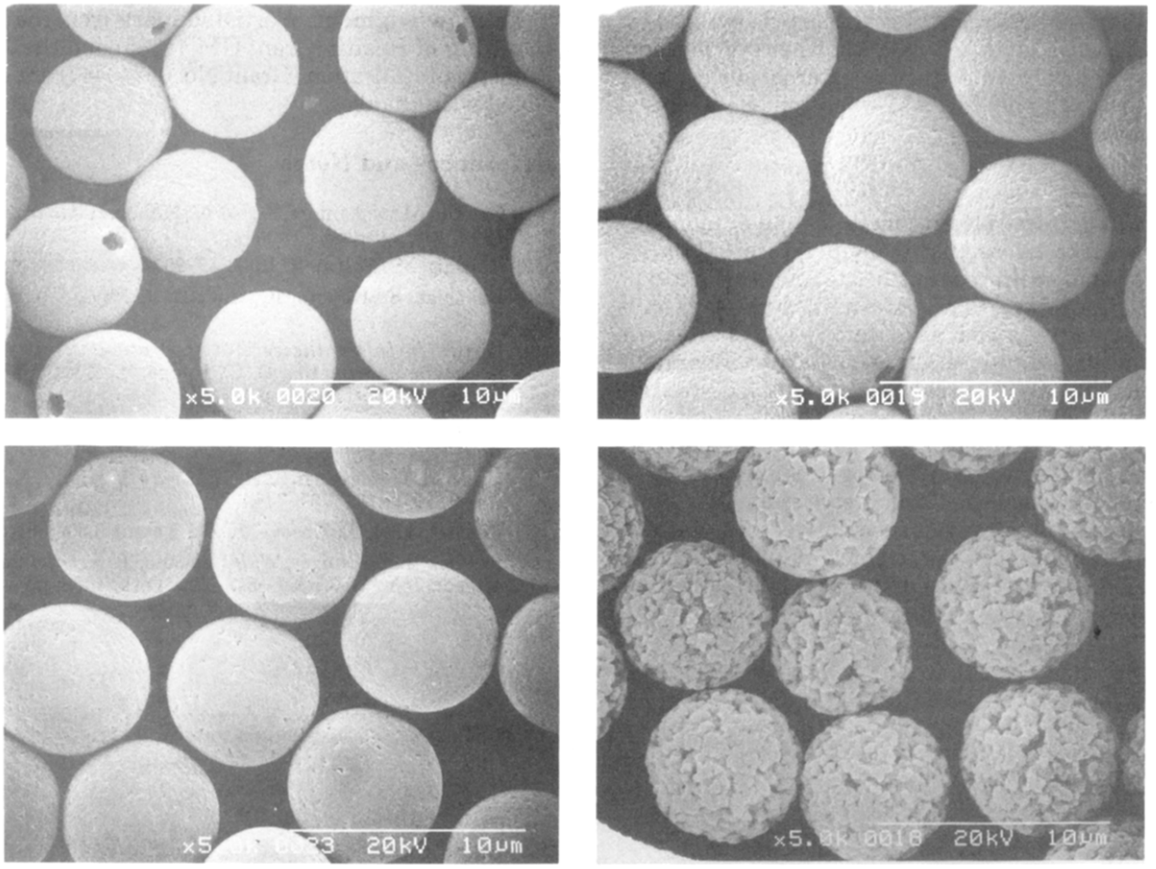

Figure 2. (a) Top left: Scanning electron micrograph of the unmodified particles using cyclohexanol as porogen. (b) Top right: Scanning electron micrograph of the modified particles using cyclohexanol as porogen. (c) Bottom left: Scanning electron micrograph of the unmodified particles using toluene as porogen. (d) Bottom right: Scanning electron micrograph of the modified particles using toluene as porogen.

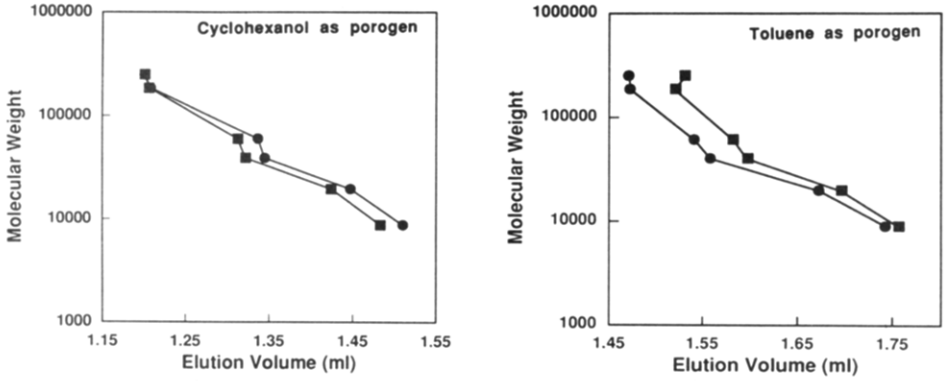

Figure 3. Temperature dependence of calibration curves observed in size exclusion chromatography. Chromatographic conditions: mobile phase, water; samples, dextran standard samples; ■, 30 °C; ●, 50 °C.

According to elemental analyses, 80% of the added NIPAM is incorporated into the base particles if cyclohexanol is used as the porogen, while only 60% of the added NIPAM is incorporated when toluene was used as the porogen. These data confirm that the poly-NIPAM is incorporated more extensively with cyclohexanol than with toluene because with toluene the added NIPAM, once polymerized, can only reach the external surface of the base particles. Therefore its effect is felt on a much smaller surface area than would be the case if it could also reach the much larger internal surface area of the beads.

Since one the most persuasive proofs of the temperature-responsive physical transformation of poly-NIPAM is the reversible transformation of the apparent volume of the polymer in the aqueous phase, a change of pore size and pore size distribution should be observed depending on the experimental temperature if poly-NIPAM is effectively incorporated on the internal surface of the pores. Due to the temperature responsiveness of poly-NIPAM between helix and random coil forms, the pore size was reported to be smaller below the critical temperature (32 °C) than observed above the critical temperature.[1] To verify that this transition occurs with our beads modified in surface-selective fashion, we used size exclusion chromatography (SEC) with water as the mobile phase to obtain information on the pore size and pore size distribution of the macroporous particles in HPLC.

As shown in Figure 3, the calibration curve observed with the poly-NIPAM particles prepared using cyclohexanol as the porogen affords the expected temperature-dependent pore size change. In contrast, the poly-NIPAM-modified particles prepared using toluene as the porogen clearly show the opposite behavior that is also observed with the unmodified particles. These findings strongly

suggest the internal surface of the base particles was indeed selectively modified with poly-NIPAM when cyclohexanol was used as the porogen, while the external surface only was modified with poly-NIPAM when toluene was used as the porogen.

Conclusion

The *in situ* surface-selective modification of monodispersed macroporous beads by temperature-responsive poly-NIPAM has been accomplished to afford a novel type of separation medium. The porosity of this medium is readily adjusted by a change in temperature, a feature that may prove useful in a number of chromatographic applications. The ability to control the site of modification within or at the surface of macroporous beads afforded by the combination of upper-critical solution temperature phenomenon and selection of porogen is unmatched in conventional techniques for bead preparation. While our conclusions are still somewhat speculative, this type of *in situ* modification method is extremely promising and its extension to other systems is under active investigation.

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