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Hydroxyl functionalized thermosensitive microgels with quadratic crosslinking density distribution

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Abstract

N-isopropylacrylamide (NIPA) based uniform thermosensitive microgels were synthesized by dispersion polymerization by using relatively hydrophilic crosslinking agents with hydroxyl functionality. Glycerol dimethacrylate (GDMA), pentaerythritol triacrylate (PETA) and pentaerythritol propoxylate triacrylate (PEPTA) were used as crosslinking agents with different hydrophilicities. A protocol was first proposed to determine the crosslinking density distribution in the thermosensitive microgel particles by confocal laser scanning microscopy (CLSM). The microgels were fluorescently labeled by using hydroxyl group of the crosslinking agent. The CLSM observations performed with the microgels synthesized by three different crosslinking agents showed that the crosslinking density exhibited a quadratic decrease with the increasing radial distance in the spherical microgel particles. This structure led to the formation of more loose gel structure on the particle surface with respect to the center. Then the use of hydrophilic crosslinking agents in the dispersion polymerization of NIPA made possible the synthesis of thermosensitive microgels carrying long, flexible and chemically derivatizable (i.e., hydroxyl functionalized) fringes on the surface by a single-stage dispersion polymerization. The microgels with all crosslinking agents exhibited volume phase transition with the increasing temperature. The microgel obtained by the most hydrophilic crosslinking agent, GDMA exhibited higher hydrodynamic diameters in the fully swollen form at low temperatures than those obtained by PETA and PEPTA. Higher hydrodynamic size decrease from fully swollen form to the fully shrunken form was also observed with the same microgel.

Keywords: Thermosensitive polymer; *N*-isopropylacrylamide; Dispersion polymerization; Latex; Microgels; Pentaerythritol; Glycerol dimethacrylate; Confocal laser scanning microscopy

1. Introduction

Dispersion copolymerization is most widely used technique for the production of *N*-isopropylacrylamide (NIPA) based thermosensitive microgels [1–3]. Thermally reversible and pH sensitive microgels carrying anionic groups have been produced by the copolymerization of NIPA with acrylic acid or boronic acid functionalized comonomers [4–12]. The cationic thermosensitive microgels were synthesized by the dispersion copolymerization of NIPA with the amine carrying monomers like 2-aminoethyl methacrylate, 2-dimethylaminoethyl methacrylate, or *N*-(3-dimethylaminopropyl) methacrylamide [13–22]. These microgels were used as support materials in the development of diagnostic tests and the purification of proteins, nucleotides and nucleic acids [9–11,20,21]. 2-Vinylimidazole and 4-vinylpyridine are the other comonomers commonly used in the synthesis of NIPA based microgels with cationic structure [23–31].

In almost all studies involving the preparation of poly(NIPA) based microgels, *N*,*N*-methylenebisacrylamide (MBA) was commonly used as the crosslinking agent. Although the research on the preparation of NIPA based microgels have been continued for approximately 20 years, limited num-

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ber of studies were found on the influence of crosslinker type on the structure and swelling behavior of thermosensitive microgels [32–37]. Duracher et al. [32] investigated the effects of MBA concentration on the average size and thermosensitivity of N-isopropylmethacrylamide based latex particles. Monodisperse nanoparticles of NIPA were synthesized for biomedical applications by using polyethylene glycol dimethacrylate as the crosslinking agent in the dispersion polymerization [33]. The volume transitions of NIPA based latex particles prepared by different crosslinking agents (i.e., ethylene dimethacrylate (EDM), triethyleneglycol dimethacrylate (TEGDM) and MBA were investigated by small angle neutron scattering (SANS) and dynamic light scattering (DLS) [34]. The crosslinkers, EGDM and TEGDM were found to form particles with larger swelling/deswelling capacities (i.e., higher thermosensitivity) compared to MBA [34]. Poly(NIPA) latex particles crosslinked with polyethylene glycol dimethacrylates with higher number of ethylene oxide units (i.e., 9 and 14) also exhibited higher thermosensitivity [35]. Recently, poly(NIPA)-based microspheres were prepared by precipitation and dispersion polymerization. The effects of type and concentration of the crosslinker (MBA or EDM) were investigated [37].

Herein, we proposed relatively hydrophilic crosslinking agents carrying hydroxyl functionality for the synthesis of NIPA based thermosensitive microgels. For this purpose, glycerol dimethacrylate (GDMA), pentaerythritol triacrylate (PETA) and pentaerythritol propoxylate triacrylate (PEPTA) were selected. Thermosensitive microgels with a highly crosslinked core and loosely crosslinked surface layer including long flexible fringes were obtained by dispersion polymerization. The hydroxyl groups coming from selected crosslinking agents make easier the chemical derivatization of microgel particles. Here, we wish to report the synthesis and characterization of NIPA based thermosensitive microgels produced with new crosslinking agents.

2. Materials and methods

2.1. Materials

N-isopropylacrylamide (NIPA, Aldrich Chem. Co., Milwaukee, WI) was crystallized from hexane-acetone solution. Potassium perfulfate (KPS, Analar grade, BDH Chemicals Ltd., Poole, England) was used as the initiator. The crosslinking agents, glycerol dimethacrylate (GDMA), pentaerythritol triacrylate (PETA) and pentaerythritol propoxylate triacrylate (PEPTA, average M_n: 530) and N,N-methylenebisacrylamide (MBA) were supplied from Aldrich and used without further purification. N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide (EDC), and the fluorescent marker, 5-aminofluorescein (AF) were supplied from Sigma (St. Louis, USA).

2.2. Preparation of poly(NIPA) based microgels with different crosslinking agents

The dispersion polymerization was performed according to the following protocol by using different crosslinking agents. The monomer NIPA (1 g) and the selected crosslinking agent, GDMA (0.085 mL, 0.095 g) or PEPTA (0.105 mL, 0.123 g) or PEPTA (0.2 mL, 0.215 g) were added into distilled water (40 mL) in cylindrical pyrex reactor. The initiator, KPS (0.04 g) was dissolved in the resulting medium. The reactor was sealed by following the nitrogen purge for 10 min at room temperature. The polymerization was conducted at 85 ± 0.5 °C for 6 h in a shaken reactor placed in a temperature-controlled water bath. The latex was cooled down to room temperature and cleaned by a successive centrifugation-decantation procedure. In the polymerization runs, type and feed concentration of the crosslinking agent were changed for the synthesis of thermosensitive microgels with different properties.

2.3. Characterization of microgels

The final microgel yield was determined by a conventional gravimetric procedure [9,10]. Poly(NIPA) based microgels produced with different crosslinking agents were imaged in dry state by transmission electron microscope (TEM, JEOL, JEM 1200EX, Japan). For this purpose, poly(NIPA) based microgel dispersion was dropped onto a formvar coated copper grid and dried at room temperature. The dried particles were then imaged with 5000X magnification.

The distribution of hydroxyl groups in the particle structure was observed by Confocal Laser Scanning Microscope (CLSM, Leica SP2, Heidelberg, Germany) by an imaging protocol developed in this study. For this purpose, poly(NIPA) based microgel particles were fluorescently labeled with AF via their hydroxyl functionality. Typically, the fluorescent probe, AF (35 mg, 0.1 mmol) was linked to the particles bearing hydroxyl groups (0.05 g, theoretical GDMA content: 0.0425 mmol) by using EDC (0.02 g, 0.1 mmol) in an aqueous medium (5 mL, pH 7) containing acetone (10% v/v) for 6 h at room temperature. Approximately 2.5 fold excess amounts of EDC and AF with respect to the theoretical hydroxyl content of particles were used for complete consumption of all hydroxyl groups. AF-labeled particles were washed by applying a successive centrifugation-decantation protocol and extensively dialysed against water.

The aqueous dispersion of AF-labeled particles (solid content: 0.5% w/w) were then examined by CLSM equipped with He–Ne laser operated at an excitation wavelength of 489 nm. The serial optical sections of microgel particles were obtained by performing z-scan (i.e. sectioning in the vertical direction) in the aqueous dispersion. The thickness of each optical section was approximately 15 nm for all microgel samples.

The thermosensitive microgel particles obtained with hydroxyl functionalized crosslinking agents were also treated with AF under the same conditions, but by excluding EDC in the labeling protocol. These particles were also examined under CLSM by using identical conditions with those used for the hydroxyl functionalized thermosensitive microgels.

The hydrodynamic diameters of microgels were measured by Dynamic Light Scattering (DLS) (NanoS, Malvern Instruments, London, UK) in the aqueous medium at pH 7. The variation of hydrodynamic diameter by the temperature was also