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Characterization of ultrahigh-molecular weight cationic polyacrylamide using frit-inlet asymmetrical flow field-flow fractionation and multi-angle light scattering

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ABSTRACT

In this study, frit inlet asymmetrical flow field-flow fractionation (FlFFF) with multi-angle light scattering (MALS) and differential refractive index (DRI) detection is utilized for size separation, determination of molecular weight (MW), and conformation of ultrahigh-MW (10^7–10^9 g/mol) cationic polyacrylamides (C-PAMs), a class of water-soluble copolymers based on acrylamide and vinyl-type comonomers with quaternary ammonium cations that are widely used in wastewater treatment and in paper industries. Linear and branched C-PAM copolymers prepared in two different polymerization methods (solution and emulsion) from varying amounts of crosslinking agent and initiator were size fractionated by FlFFF with field-programming. It was found experimentally that the linear copolymers from both polymerization methods were less than 10^8 g/mol in MW with compact, nearly spherical structures, while the branched C-PAM copolymers from the emulsion polymerization showed a significant increase in average MW up to ~10^9 g/mol, which was about 20-fold greater than those from the solution method, and the branched copolymers had more compact or shrunken conformations. While both linear and branched copolymers less than 10^8 g/mol MW were well resolved in an increasing order of MW (normal mode), it was noted that branched copolymers prepared through emulsion polymerization exhibited significantly larger MWs of 10^{8}–10^9 g/mol and eluted in the steric/hyperlayer mode, in which the elution order is reversed in an extreme run condition (strong initial field strength followed by a fast field decay during programming).

1. Introduction

Cationic polyacrylamides (C-PAMs) are a class of water-soluble acrylamide-based polymers copolymerized with a vinyl-type comonomer having quaternary ammonium cations [1–3]. Acrylamide-based homopolymers (PAMs) are water soluble and are important in a number of industrial applications such as flocculants in paper manufacturing [4,5], enrichment/recovery of minerals from mining [6], wastewater treatment [7], and as rheology control agents [8]. However, due to the nonionic nature of PAMs, the incorporation of a charged group into an acrylamide chain provides a polyelectrolyte-like property, which results in a markedly improved performance in flocculation compared to that of nonionic PAMs. Specifically, C-PAM copolymers have been widely used as flocculants in paper manufacturing [2,9] and waste water treatment [7,10].

C-PAM copolymers are synthesized with acrylamide (AM) and cationic comonomers of quaternary nitrogen-containing structures such as acryloxyethyltrimethyl ammonium chloride (DAC) [10,11], methacryloxyethyl ammonium chloride (DMC) [12], acryloxyethyl dimethylbenzyl ammonium chloride (AODBAC) [13,14], etc. [1]. Since a branched polymer has a relatively lower viscosity than a linear polymer of the same molecular weight (MW), along with improved solubility in water, branched copolymers exhibit better performance [15,16]. The efficiencies of C-PAM copolymers as flocculants are known to depend on a number of parameters, including MW, molecular conformation, and degree of charge density [2,14]. Specifically, the determination of accurate MW and molecular weight distribution (MWD) of C-PAM is important in controlling the material properties in accordance with the

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intended applications. Size characterization of typical branched C-PAM molecules (<10^7 g/mol) has often been carried out using intrinsic viscosity measurement [11], size exclusion chromatography (SEC) based on calibration using PAM standards [13,14], or on multi-angle light scattering (MALS) analysis [17]. However, for ultrahigh-MW C-PAM molecules (>10^7), the determination of average MW has relied on intrinsic viscosity measurements [2] or on batch analysis using MALS [3] due to the lack of relevant SEC columns having packing materials of sufficient pore sizes to accommodate ultrahigh-MW molecules. In the case of the viscosity measurements, while branched polymers exhibit viscosity values lower than linear polymers of the same MW, it is complicated to determine accurate MWs and MWDs of ultrahigh-MW C-PAM materials. Even with SEC, ultrahigh-MW C-PAM molecules often shrink, resulting in difficulties in determining accurate MWs due to the addition of a considerable amount of salts in the mobile phase for successful elution through the SEC column.

Flow field-flow fractionation (FlFFF) is an elution-based method that is capable of separating macromolecules according to hydrodynamic size and has been widely utilized for characterization of polymers (both aqueous and organic) and proteins as well as nano- and micro-sized particles including those having biological importance such as exosomes, cells, and subcellular organelles [18–22]. Separation in FlFFF is carried out in an unobstructed rectangular channel space without a stationary phase by utilizing two flow streams (a migration flow to drive sample components down the channel and a cross flow moving across the channel to retard the migration of sample species) [23]; therefore, it is rather well suited to handle ultrahigh-MW species without danger of sample loss. Since the separation of components in the typical normal mode of FlFFF is achieved by an increasing order of diffusion coefficients of the sample materials and thus their hydrodynamic diameters [18], the online use of FlFFF with MALS (FlFFF-MALS) offers an outstanding feature for separation and MW characterization of various water-soluble polymers such as polysaccharides [24], polyacrylamides [25], amylopectin [26,27], and hyaluronic acid [28–32].

In this study, ultrahigh MW (10^7–10^9 Da) C-PAM copolymers synthesized from AM monomers with a cationic comonomer, DAC, were examined using FlFFF-MALS. The FlFFF system employed in this work was a frit inlet asymmetrical FlFFF (FIAF4) channel [33], which is a modified form of asymmetrical FlFFF utilizing the hydrodynamic relaxation of sample components without the focusing/relaxation method that has been commonly applied for conventional asymmetrical FlFFF. With hydrodynamic relaxation, sample components injected into the channel are expected to achieve equilibrium between the two counter-directed forces (the external field force generated by the crossflow and the diffusion of components against the channel wall) while they continuously migrate through the channel. The C-PAM copolymers examined in this study were synthesized using two different polymerization methods, solution polymerization and emulsion polymerization, by varying the amount of an initiator with or without addition of a cross-linking agent. Thus, the effects of polymerization method and branching on MW, MWD, and molecular confirmation of C-PAM could be examined using FIAF4 with MALS. While most of the C-PAM species examined in this work were analyzed in normal mode of FlFFF separation, we observed that a few extremely high-MW branched C-PAM species were separated by steric/hyperlayer elution mode. Steric/hyperlayer elution is typical for the separation of micronsized particles since diffusion of larger particles becomes negligible and particles are lifted from the channel wall by hydrodynamic lift forces, therefore larger species elute more rapidly than smaller ones [34]. While the steric/hyperlayer elution is common with miconsized particles, it has not been found for polymeric species in FlFFF to the best of our knowledge.

2. Experimental

2.1. Materials and reagents

The C-PAM copolymer samples examined in this study were poly(acrylamide-co-N,N,N-trimethyl aminoethyl chloride acrylate) synthesized from AM monomers copolymerized with DAC. Eight C-PAM copolymer samples were obtained in powdered form from Kolon Life Science Inc. (Gwacheon, Korea) and were synthesized using two different polymerization methods (solution polymerization and emulsion polymerization) by varying the amounts of cross-linking agent, initiator, and chain transfer agent in order to form linear and branched polymers of different MWs, as listed in Table 1. The identification of each sample shown in Table 1 is designated with L for linear polymers, B for branched polymers, S for solution polymerization, and E for emulsion polymerization. To prepare aqueous solutions, copolymer samples were dissolved in deionized water (>18 MΩ) at a concentration of 0.5 mg/mL by stirring at 300 rpm for 2 h using a glass-blade impeller installed in a WiseStir® HS-30E model stirrer from Daihan Scientific Co. (Wonju, Korea) and reducing the stirring rate to 150 rpm for 22 h. Next, $NaNO_3$ from Sigma–Aldrich Co. (St. Louis, MO, USA) was added to the dissolved C-PAM samples to produce a total 0.1 M $NaNO_3$ solution (300 rpm for 30 min) for FIAF4-MALS analysis.

2.2. FIAF4-MALS

The FIAF4 channel utilized in this study was modified from an Eclipse® channel model LC (long channel) from Wyatt Technology Europe GmbH (Dernbach, Germany) by replacing the inlay (a plastic block used as a depletion wall) with a frit-inlet type of inlay equipped with a ceramic inlet frit (35 mm × 18 mm × 7 mm) at the inlet end of the polycarbonate inlay, as described in a previous study [32]. Through the inlet frit, a tubing port was created by drilling a hole to insert a Teflon tube (0.03 inch i.d. and 1/16 inch o.d.) for the channel inlet so that the sample materials could be delivered to the FIAF4 channel. The channel spacer was a 195-μm-thick Mylar sheet cut into a trapezoidal channel design having an initial breadth of 1.6 cm, which decreased to 0.4 cm at the outlet end. The length of the channel was 26.5 cm, with triangular sections at both ends. The actual length of the inlet frit exposed to the channel space was 3.5 cm. At the accumulation wall, a regenerated cellulose membrane from Microdyn-Nadir GmbH (Wiesbaden, Germany) with a pore size of 10 k Da was placed above the stainless steel frit. A model 7725i loop injector from Rheodyne (Cotati, CA, USA) was installed between the channel inlet and a model SP930HPLC pump from Young-Lin Co. (Seoul, Korea) for sample loading. Frit inlet flow was delivered from a model 1260 Infinity HPLC pump from Agilent Technologies (Palo Alto, CA, USA), and the flow rates were controlled (frit flow, cross flow, and outflow) with the Eclipse system from Wyatt Technology Europe GmbH. The carrier solution of FIAF4 (for both sample injection flow and frit flow) was prepared with deionized water (>18 MΩ) containing 0.1 M $NaNO_3$ and 0.02% NaN_3 from Sigma-Aldrich as bactericide, and the prepared solution was filtered through a Durapore® Membrane filter (0.1 μm pore, polyvinylidene fluoride) from Merck Millipore (Darmstadt, Germany) prior to use. To remove any particulate species contained in the carrier solution, a PEEK inline filter unit with a VVLP membrane with 0.1-μm pores from Wyatt Technology Europe GmbH was placed right after the outlet of each pump.

FIAF4 operation was achieved through hydrodynamic relaxation, in which samples were injected through the inlet of the channel while a high-speed frit flow (more than 20 times faster than the sample stream) was introduced through the inlet frit so that the sample components could be pushed toward the accumulation wall of the channel to achieve equilibrium during continuous