

# Anionic Polyelectrolyte-Stabilized Nanoparticles via RAFT Aqueous Dispersion Polymerization

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Supporting Information

**ABSTRACT:** We report the synthesis of anionic sterically stabilized diblock copolymer nanoparticles via polymerization-induced self-assembly using a RAFT aqueous dispersion polymerization formulation. The anionic steric stabilizer is a macromolecular chain-transfer agent (macro-CTA) based on poly(potassium 3-sulfopropyl methacrylate) (PKSPMA), and the hydrophobic core-forming block is based on poly(2-hydro-xypropyl methacrylate) (PHPMA). The effect of varying synthesis parameters such as the salt concentration, solids content, relative block composition, and anionic charge density has been studied. In the absence of salt, self-assembly is problematic



when using a PKSPMA stabilizer because of lateral repulsion between highly charged anionic chains. However, in the presence of added salt this problem can be overcome by reducing the charge density within the coronal stabilizer layer by either (i) statistically copolymerizing the KSPMA monomer with a nonionic comonomer (2-hydroxyethyl methacrylate, HEMA) or (ii) using a binary mixture of a PKSPMA macro-CTA and a poly(glycerol monomethacrylate) (PGMA) macro-CTA. These diblock copolymer nanoparticles were analyzed by <sup>1</sup>H NMR spectroscopy, gel permeation chromatography (GPC), dynamic light scattering (DLS), transmission electron microscopy (TEM), and aqueous electrophoresis. NMR studies suggest that the HPMA polymerization is complete within 2 h at 70 °C, and DMF GPC analysis confirms that the resulting diblock copolymers have relatively low polydispersities ( $M_w/M_n < 1.30$ ). NMR also suggests a significant degree of hydration for the core-forming PHPMA chains. Depending on the specific reaction conditions, a series of spherical nanoparticles with mean diameters ranging from 50 to 200 nm with tunable anionic surface charge can be prepared. If a binary mixture of anionic and nonionic macro-CTAs is utilized, then it is also possible to access a vesicular morphology.

# INTRODUCTION

Amphiphilic block copolymers can self-assemble into a wide range of complex morphologies in dilute aqueous solution, including micelles,<sup>1</sup> vesicles,<sup>1-5</sup> tubules,<sup>6-9</sup> toroids,<sup>10</sup> and large complex micelles.<sup>11</sup> The precise morphology obtained is dictated by various parameters, including the hydrophilic/hydrophobic balance, the copolymer molecular weight, the copolymer concentration, the choice of solvent, and the processing route.<sup>12</sup> In the latter case, various protocols have been developed to promote self-assembly, including dialysis, pH switching, and thin film rehydration. For example, Eisenberg and co-workers reported a range of morphologies for asymmetric amphiphilic diblocks by initial dissolution in a good solvent for both blocks (e.g., DMF), followed by slow dialysis against water. More specifically, reducing the hydrophilic block (typically either poly(acrylic acid) or poly(ethylene oxide)) while fixing the hydrophobic polystyrene block led to morphological transitions from micelles to rods to vesicles to large compound micelles.<sup>1,13</sup> Increasing the hydrophobic block led to morphological transitions from spheres to rodlike or vesicular structures.<sup>13,14</sup>

More recently, there has been increasing interest in exploiting polymerization-induced self-assembly for the production of spherical micelles, wormlike micelles, or vesicles.<sup>15–30</sup> For example, Hawker's group synthesized lightly cross-linked poly(N,N'-dimethylacrylamide)poly(N-isopropyl acrylamide) diblock copolymers via reversible addition-fragmentation transfer (RAFT) polymerization at 70 °C in aqueous solution.<sup>15</sup> On cooling, these spherical copolymer particles became nanogels due to the swelling of the poly-(N-isopropyl acrylamide) chains below their LCST of around 32 °C.<sup>15</sup> This concept has been recently extended to include other thermosensitive core-forming monomers by An et al.<sup>16,17</sup> Pan and co-workers have published a series of papers describing the synthesis of block copolymer spheres, wormlike particles, and vesicles via RAFT alcohol dispersion polymerization. However, monomer conversions were always substantially incomplete (typically only 30-70%), and the removal of large volumes of unreacted monomer makes such formulations unsuitable for industrial scale up.<sup>24–27</sup> Charleux et al. have recently developed rather more efficient aqueous emulsion polymerization formulations based on the living radical polymerization of water-immiscible

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commodity monomers such as styrene and *n*-butyl acrylate to prepare diblock copolymer spheres, worms, or vesicles. In particular, it was shown that the hydrophilic/hydrophobic balance dictates the final copolymer morphology and that self-assembly is much more problematic if polyelectrolytic stabilizer chains are utilized because their mutual repulsion hinders in situ selfassembly.<sup>18,22,23</sup> This difficulty could be circumvented by either reducing the stabilizer charge density via statistical copolymerization with nonionic comonomers and/or by the addition of sufficient salt to screen the strong electrostatic repulsive forces.<sup>1</sup> We recently reported a robust RAFT aqueous dispersion polymerization formulation for the production of well-defined nanoobjects.<sup>30</sup> This protocol is based on the use of a poly(glycerol monomethacrylate) (PGMA) stabilizer block and 2-hydroxypropyl methacrylate (HPMA) as a core-forming monomer. HPMA is one of only a few vinyl monomers that are water-miscible (up to 13% at 25 °C) but produce a water-insoluble polymer. This is an essential prerequisite for a successful aqueous dispersion polymerization formulation. Our initial report focused on the production of either low-polydispersity spherical nanoparticles ranging from 26 to 105 nm diameter or relatively polydisperse vesicles of around 500 nm. GPC analyses of such block copolymer nanoparticles revealed relatively high polydispersities, which were due to a dimethacrylate impurity in the HPMA monomer. More recently, we have shown that if this impurity is removed polydispersities of less than 1.20 can be obtained, even when targeting mean degrees of polymerization of up to 1000 for the core-forming block.<sup>28</sup> Moreover, very high HPMA conversions (>99% as judged by <sup>1</sup>H NMR) are routinely achieved within 2 h at 70 °C, with an enhanced rate of polymerization being observed just after the onset of nucleation. Careful sampling of the reaction solution confirmed that spheres were first converted to worms and then vesicles, with useful insights into the precise nature of the worm-to-vesicle transition being obtained.<sup>28</sup> In a separate study, the first detailed phase diagram for any polymerization-induced self-assembly formulation was elucidated for a PMPC-PHPMA formulation (where PMPC is poly(2-(methacryloyloxy)ethyl phosphorylcholine)). This is important because it enables the predictable and reproducible synthesis of pure phases of spheres, worms, or vesicles.<sup>29</sup> Finally, the effect of cross-linking the core-forming PHPMA chains with ethylene glycol dimethacrylate was studied, and it was shown that a novel "lumpy rod" phase could be accessed, albeit over a relatively narrow range of conditions.<sup>29</sup>

In the present work, we have replaced the nonionic poly-(glycerol monomethacrylate) stabilizer block with an anionic polyelectrolytic block based on poly(potassium 3-sulfopropyl methacrylate) (PKSPMA). A systematic study of the effect of varying the diblock copolymer composition, the overall solids content, and the effect of added salt is presented for this RAFT aqueous dispersion polymerization formulation. In addition, we have explored the effect of diluting the coronal charge density by either (i) the statistical copolymerization of KSPMA with HPMA or (ii) using binary mixtures of PKSPMA and PGMA macromolecular chain-transfer agents (macro-CTA).

## EXPERIMENTAL SECTION

**Materials.** All reagents were purchased from Sigma-Aldrich and were used as received, unless otherwise noted. 4,4'-Azobis-4-cyanopentanoic acid (ACVA, >98%) was used as an initiator. 2-Hydroxyethyl methacrylate (HEMA) and 2-hydroxypropyl methacrylate (HPMA, 97%)

were kindly donated by Cognis UK Ltd. (Hythe). HPMA comprises approximately 75% 2-hydroxypropyl methacrylate and 25 mol % 2-hydroxyisopropyl methacrylate.<sup>31</sup>

Synthesis of 4-Cyano-4-(2-phenylethane sulfanylthiocarbonyl) Sulfanylpentanoic Acid (PETTC). 2-Phenylethanethiol (10.5 g, 76.0 mmol) was added over 10 min to a stirred suspension of sodium hydride (60% in oil, 3.15 g, 79.0 mmol) in diethyl ether (150 mL) at a temperature of between 5 and 10 °C. The vigorous evolution of hydrogen was observed, and the grayish suspension became a viscous white slurry of sodium phenylethanethiolate over 30 min. The reaction mixture was cooled to 0 °C, and carbon disulfide (6.00 g, 79.0 mmol) was gradually added to provide a thick yellow precipitate of sodium 2-phenylethanetrithiocarbonate, which was collected by filtration after 30 min and used in the next step without purification. A suspension of sodium 2-phenylethanetrithiocarbonate (11.6 g, 0.049 mol) in diethyl ether (100 mL) was treated by the gradual addition of solid iodine (6.30 g, 0.025 mol). The reaction mixture was stirred at room temperature for 1 h, and the insoluble white sodium iodide was removed by filtration. The yellow-brown filtrate was washed with an aqueous solution of sodium thiosulfate to remove excess iodine and dried over sodium sulfate, and the solvent was removed under reduced pressure to leave a residue of bis-(2-phenylethane sulfanylthiocarbonyl) disulfide (~100% yield). A solution of 4,4'-azobis(4-cyanopentanoic acid) (ACVA, 2.10 g, 7.50 mmol) and bis-(2-phenylethane sulfanylthiocarbonyl) disulfide (2.13 g, 5.00 mmol) in ethyl acetate (50 mL) was degassed by nitrogen bubbling and held at reflux under a dry nitrogen atmosphere for 18 h. After the volatiles were removed under vacuum, the crude product was washed with water (five 100 mL portions). The organic phase was concentrated and purified by silica column chromatography (7:3 petroleum ether/ethyl acetate, gradually increasing to 4:6) to afford 4-cyano-4-(2-phenylethanesulfanylthiocarbonyl)sulfanylpentanoic acid as a yellow oil (yield 78%).

<sup>1</sup>H NMR (400.13 MHz, CD<sub>2</sub>Cl<sub>2</sub>, 298 K):  $\delta$  1.89 (3H, -CH<sub>3</sub>), 2.34–2.62 (m, 2H, -CH<sub>2</sub>), 2.7 (t, 2H, -CH<sub>2</sub>), 3.0 (t, 2H, -CH<sub>2</sub>), 3.6 (t, 2H, -CH<sub>2</sub>), 7.2–7.4 (m, 5H, aromatic).

<sup>13</sup>C NMR (400.13 MHz, CD<sub>2</sub>Cl<sub>2</sub>, 298 K): δ 24.2 (CH<sub>3</sub>), 29.6 (CH<sub>2</sub>CH<sub>2</sub>COOH), 30.1(CH<sub>2</sub>Ph), 33.1 (CH<sub>2</sub>CH<sub>2</sub>COOH), 39.9 (SCH<sub>2</sub>-CH<sub>2</sub>Ph), 45.7 (SCCH<sub>2</sub>), 118.6 (CN), 127.4, 128.8, 129.2, 144.3 (Ph), 177.4 (C=O), 222.2 (C=S).

Synthesis of Poly(potassium 3-sulfopropyl methacrylate) [PKSPMA] Macro-CTA. In a typical experiment, a round-bottomed flask was charged with KSPMA (5.00 g, 20.0 mmol), PETTC (0.197 g, 0.580 mmol, dissolved in 1.0 mL dioxane), ACVA (33.0 mg, 0.220 mmol), and an aqueous 1:7 acetic acid/sodium acetate buffer (4.00 g, 100 mM, pH 5.5). The sealed reaction vessel was purged with nitrogen and placed in a preheated oil bath at 70 °C for 2 h. The resulting PKSPMA macro-CTA (96% conversion;  $M_n = 9800$  g mol<sup>-1</sup>,  $M_w = 11500$  g mol<sup>-1</sup>,  $M_w/M_n = 1.18$ ) was purified by dialysis against 9:1 water/methanol and isolated by freeze-drying overnight. The mean degree of polymerization (DP) for this macro-CTA was calculated by <sup>1</sup>H NMR spectroscopy by comparing the integrated aromatic proton signals due to the PETTC chain end at 7.2–7.4 ppm to those due to the methacrylic polymer backbone at 0.4–2.5 ppm.

Synthesis of Poly(potassium 3-sulfopropyl methacrylate-stat-2-hydroxyethyl methacrylate) P(KSPMA-stat-HEMA) Macro-CTA. In a typical experiment, a round-bottomed flask was charged with KSPMA (1.00 g, 4.10 mmol), 2-hydroxyethyl methacrylate (HEMA, 2.64 g, 20.3 mmol), PETTC (0.270 g, 0.800 mmol, dissolved in 1.0 mL dioxane), ACVA (45.5 mg, 0.160 mmol) and 1:7 acetic acid/sodium acetate buffer (3.0 g, 100 mM, pH 5.5). The sealed reaction vessel was purged with nitrogen and placed in a preheated oil bath at 70 °C for 2 h. The resulting polymer (97% conversion,  $M_n = 13300 \text{ g mol}^{-1}$ ,  $M_w =$ 15 600 g mol<sup>-1</sup>,  $M_w/M_n = 1.17$ ) was purified by dialysis against 9:1 water/methanol and isolated by freeze-drying overnight. The mean DP of the purified macro-CTA was calculated as described previously. Scheme 1. RAFT Aqueous Dispersion Polymerization of 2-Hydroxypropyl Methacrylate to Produce Sterically-Stabilized Diblock Copolymer Nanoparticles at 70 °C Using (a) Anionic Poly(potassium 3-sulfopropyl methacrylate) Macro-CTA and (b) Anionic Poly(potassium 3-sulfopropyl methacrylate-*stat*-2-hydroxyethyl methacrylate) Macro-CTA



Block Copolymer Nanoparticle Synthesis via RAFT Aqueous Dispersion Polymerization. For a typical aqueous dispersion polymerization synthesis conducted at 10 wt % solids, the protocol was as follows. HPMA (1.00 g, 6.90 mmol), ACVA (0.780 mg, 0.003 mmol), and P(KSPMA<sub>6</sub>-stat-HEMA<sub>29</sub>) macro-CTA (73.0 mg, 0.014 mmol) were codissolved in deionized water (9.66 g). The reaction mixture was sealed in a round-bottomed flask and purged with nitrogen for 20 min before being placed in a preheated oil bath at 70 °C for 24 h. The higher solids content (15, 20, and 25 wt %) formulations were treated similarly by reducing the amount of solvent, as required.

**Copolymer Characterization.** Block copolymer molecular weight distributions were assessed by DMF gel permeation chromatography (GPC) using two Polymer Laboratories PL gel 5  $\mu$ m mixed C columns and one PL polar gel 5  $\mu$ m guard column arranged in series and maintained at 60 °C, followed by a Varian 390 LC refractive index detector. The eluent contained 10 mM LiBr, and the flow rate was 1.0 mL min<sup>-1</sup>. Ten near monodisperse poly(methyl methacrylate) standards (ranging from 625 g mol<sup>-1</sup> to 618 000 g mol<sup>-1</sup>) were used for calibration ( $K = 2.094 \times 10^{-3}$ ,  $\alpha = 0.642$ ).

Molecular weight distributions for the PKSPMA macro-CTA precursors were determined using aqueous GPC. The standard GPC protocol involved using a Pharmacia Biotech Superose 6 column connected to a Polymer Laboratories ERC-7517A refractive index detector. The eluent was an aqueous solution of 0.20 M NaNO<sub>3</sub> containing 50 mM Trizma buffer at pH 7. Calibration was achieved using a series of near monodisperse poly(ethylene oxide) standards ranging from 440 to 288 000 g mol<sup>-1</sup>.

<sup>1</sup>H NMR spectra were acquired using either a 250 or 400 MHz Bruker spectrometer in  $d_6$ -DMSO or D<sub>2</sub>O. Sodium 3-(trimethylsilyl)-1-propanesulfonate was used as an internal standard. TEM studies were conducted using a Philips CM 100 instrument operating at 100 kV. To prepare TEM samples, 5.0 μL of a dilute aqueous copolymer solution was placed onto a carbon-coated copper grid, stained using uranyl formate, and then dried under ambient conditions. DLS studies were conducted using a Malvern Instruments Zetasizer Nanoseries instrument equipped with a 4 mW He–Ne laser operating at 633 nm, an avalanche photodiode detector with high quantum efficiency, and an ALV/LSE-5003 multiple  $\tau$  digital correlator electronics system. Aqueous electrophoresis studies were performed on aqueous 0.01 wt % copolymer solutions using the same Malvern Instruments Zetasizer Nanoseries instrument. The solution pH was adjusted by the addition of 0.01 M HCl or 0.01 M KOH using an autotitrator, and the background electrolyte was  $10^{-3}$  M NaCl.

## RESULTS AND DISCUSSION

In this work, we examined the synthesis of sterically stabilized diblock copolymer nanoparticles via the RAFT aqueous dispersion polymerization of HPMA using an anionic polyelectrolyte stabilizer as a macro-CTA (Scheme 1). The RAFT synthesis of the block copolymer nanoparticles was performed in two steps. First, KSPMA was polymerized using 4-cyano-4-(2-phenylethanesulfanylthiocarbonyl)sulfanyl pentanoic acid (PETTC) as the CTA and 4,4'-azobis(4-cyanopentanoic acid) as the free radical initiator (CTA/initiator molar ratio = 5:1) in an aqueous buffer solution at pH 5.5 so as to minimize the hydrolysis of the RAFT chain ends<sup>32</sup> and hence ensure good block efficiencies. A small amount of 1,4-dioxane cosolvent was used to ensure complete dissolution of the CTA. The solution polymerization was terminated after 2 h, and the resulting PKSPMA macro-CTA was purified by dialysis against a 9:1 v/v water/methanol mixture. Polydispersities ranged between 1.18 and 1.20, as indicated by aqueous GPC. The second step was performed under RAFT aqueous dispersion polymerization conditions using this near monodisperse macro-CTA, which was dissolved in distilled water along with HPMA and 4,4'-azobis(4-cyanopentanoic acid) initiator.

The macro-CTA/initiator molar ratio was fixed at 5.0, and HPMA polymerization was conducted at 70 °C. Initially, the reaction mixture was transparent, but it became increasingly turbid as the polymerization proceeded, indicating in situ phase separation. This is because the HPMA monomer is water-soluble but the growing PHPMA chains are hydrophobic, which leads to polymerization-induced self-assembly after around 45 min and ultimately to colloidally stable sterically stabilized dispersions.

A series of PKSPMA<sub>34</sub>-PHPMA<sub>x</sub> diblock copolymers were synthesized in aqueous media with mean target degrees of polymerization (*x*) of 100, 200, or 300 simply by adjusting the [HPMA]/[macro-CTA] molar ratio. This systematic approach led to a series of spherical nanoparticles exhibiting a range of mean diameters. However, the mean nanoparticle diameter did not simply increase monotonically as longer PHPMA chains were targeted, as described previously by Li and Armes for diblock copolymer particles prepared via RAFT aqueous dispersion polymerization using a nonionic stabilizer.<sup>30</sup> This qualitative difference is most likely due to the strong polyelectrolyte nature of the PKSPMA block, which leads to lateral repulsive electrostatic forces between neighboring stabilizer chains, thus impeding their efficient self-assembly. This results in the formation of rather large, ill-defined micellar aggregates comprising relatively hydrated



**Figure 1.** <sup>1</sup>H NMR spectra obtained for (a) PHPMA<sub>50</sub> homopolymer in CD<sub>3</sub>OD, (b) PKSPMA<sub>34</sub> homopolymer in D<sub>2</sub>O, and (c) PKSPMA<sub>34</sub>-PHPMA<sub>300</sub> in D<sub>2</sub>O.

PHPMA cores. This interpretation is supported by inspecting a <sup>1</sup>H NMR spectrum (Figure 1) recorded for diblock copolymer PKSPMA<sub>34</sub>-PHPMA<sub>300</sub> dissolved in D<sub>2</sub>O because characteristic PHPMA signals are clearly visible at 3.8 ppm. Moreover, DLS indicates a relatively large hydrodynamic diameter of about 200 nm (and a relatively low scattered-light intensity), and TEM studies reveal rather ill-defined particles (Figure S1a). In principle, the covalent stabilization of the core-forming PHPMA block could minimize this problem. Thus, 1 mol % ethylene glycol dimethacrylate (EGDMA) cross-linker was added to a PKSPMA<sub>34</sub>-PHPMA<sub>300</sub> formulation, but unfortunately no improvement in particle morphology was observed (Figure S1b).

Alternatively, the addition of salt leads to charge screening, which should facilitate the close packing of the PKSPMA stabilizer chains within the corona. This approach has been recently explored by Charleux et al. in the context of RAFT aqueous emulsion polymerization.<sup>19</sup> In the present study, a series of PKSPMA<sub>34</sub>-PHPMA<sub>100-300</sub> diblock copolymer nanoparticles were prepared in the presence of 0.10, 0.20, or 0.30 M NaCl. Table S1 summarizes the dynamic light scattering results obtained for this series of dispersions. Relatively large particles are observed at each salt concentration for the PKSPMA<sub>34</sub>-PHPMA<sub>100</sub> diblock copolymer (244, 293, and 152 nm diameter, respectively). Increasing the DP of the core-forming chains reduces the mean diameter to 40-60 nm, presumably because of their more hydrophobic character and more efficient charge screening. Comparing TEM images obtained for diblock copolymer nanoparticles of the same chemical composition suggests that the addition of salt is an effective means of screening the anionic charge density and hence promoting self-assembly, as expected (Figure 2). Moreover, if the target DP of the core-forming PHPMA chains is increased up to 1000 or 5000 in the absence of any salt, then the hydrodynamic diameter increases monotonically from 130 to 175 nm (Table S2).

An alternative approach to salt screening is to reduce the charge density on the polyelectrolyte stabilizer chains. However, because the PKSPMA chains are strong polyelectrolytes, their anionic character cannot be varied by adjusting the solution pH. Instead, KSPMA was statistically copolymerized with a neutral comonomer, 2-hydroxyethyl methacrylate, to reduce the charge density within the coronal layer and hence promote more efficient chain packing. In practice, a series of P(KSPMA-*stat*-HEMA) statistical copolymers were synthesized, whereby the overall DP of the stabilizer chains was fixed at approximately 34 (for comparison with the performance of the PKSPMA<sub>34</sub> homopolymer stabilizer) and the number of KSPMA units per chain



Figure 2. Representative transmission electron micrographs obtained for PKSPMA<sub>34</sub>-PHPMA<sub>300</sub> spherical nanoparticles synthesized by RAFT aqueous dispersion polymerization at 70 °C in the presence of (a) no salt, (b) 0.10 M NaCl, (c) 0.20 M NaCl, and (d) 0.30 M NaCl (entries 3, 6, and 9 in Table S1).



**Figure 3.** Transmission electron micrographs obtained for (a) P(KSPMA<sub>11</sub>-*stat*-HEMA<sub>24</sub>)-PHPMA<sub>500</sub> with zero NaCl, (b) P(KSPMA<sub>11</sub>- *stat*-HEMA<sub>24</sub>)-PHPMA<sub>500</sub> with 0.30 M NaCl, (d) P(KSPMA<sub>6</sub>-*stat*-HEMA<sub>29</sub>)-PHPMA<sub>500</sub> with 2ero NaCl, (e) P(KSPMA<sub>6</sub>-*stat*-HEMA<sub>29</sub>)-PHPMA<sub>500</sub> with 0.20 M NaCl, (e) P(KSPMA<sub>6</sub>-*stat*-HEMA<sub>29</sub>)-PHPMA<sub>500</sub> with 0.20 M NaCl, and (f) P(KSPMA<sub>6</sub>-*stat*-HEMA<sub>29</sub>)-PHPMA<sub>500</sub> with 0.30 M NaCl at 10 wt % solids.



**Figure 4.** Conversion vs time data calculated from <sup>1</sup>H NMR spectra  $(D_2O)$  for the RAFT aqueous dispersion polymerization of HPMA using a P(KSPMA<sub>6</sub>-*stat*-HEMA<sub>29</sub>) macro-CTA at 70 °C, 10 wt % solids, and 0.10 M NaCl. The targeted diblock composition was P(KSPMA<sub>6</sub>-*stat*-HEMA<sub>29</sub>)-PHPMA<sub>500</sub>.

was 6, 11, or 25.<sup>33</sup> A series of PHPMA nanoparticles were synthesized via RAFT aqueous dispersion polymerization at 70 °C at 10 wt % solids using each of these copolymer macro-CTAs as the steric stabilizer (Table S3). Judging by the TEM images (Figure S2) and the DLS data (Table S3), it is clear that reducing the anionic charge density leads to the formation of smaller spherical nanoparticles with little or no change in polydispersity. Furthermore, conducting the HPMA polymerization in the presence of added salt using these P(KSPMA-*stat*-HEMA) macro-CTAs produces larger nanoparticles when targeting longer core-forming blocks (Table S4 and Figure S3). Typically, the hydrodynamic particle diameter increases from 40 to 80 nm as the target DP of the



**Figure 5.** Evolution of the number-average molecular weight  $M_n$  (vs poly(methyl methacrylate) calibration standards) and polydispersity  $(M_w/M_n)$  with monomer conversion as judged by DMF GPC when using a P(KSPMA<sub>6</sub>-stat-HEMA<sub>29</sub>) macro-CTA for the RAFT aqueous dispersion polymerization of HPMA at 70 °C, 10 wt % solids, and 0.10 M NaCl. The targeted diblock composition was P(KSPMA<sub>6</sub>-stat-HEMA<sub>29</sub>)-PHPMA<sub>500</sub>.

PHPMA chains increases from 100 to 500. Also, fused particles and short worms are observed when a macro-CTA containing the lowest proportion of KSPMA (six repeat units) is employed in the presence of either 0.20 or 0.30 M NaCl (Figure 3e,f).

The effect of the copolymer concentration (solids content) was also investigated to examine whether either worms or vesicles could be obtained, as recently reported by Sugihara and co-workers.<sup>29</sup> When copolymer stabilizer macro-CTA contained 11 KSPMA residues, increasing the solids content in the presence of salt still produced spherical particles (Figure S4) but higher-order



**Figure 6.** Evolution of GPC traces (refractive index detector) with time for the RAFT aqueous dispersion polymerization of HPMA using a  $P(KSPMA_6$ -stat-HEMA<sub>29</sub>) macro-CTA at 70 °C, 10 wt % solids, and 0.10 M NaCl. The targeted diblock composition was  $P(KSPMA_6$ -stat-HEMA<sub>29</sub>)-PHPMA<sub>500</sub>.



**Figure 7.** Evolution of the intensity-average particle diameter and polydispersities (PDI) with time as judged by dynamic light scattering for the RAFT aqueous dispersion polymerization of HPMA using a  $P(KSPMA_6$ -stat-HEMA<sub>29</sub>) macro-CTA at 70 °C, 10 wt % solids, and 0.10 M NaCl. The targeted diblock composition was  $P(KSPMA_6$ -stat-HEMA<sub>29</sub>)-PHPMA<sub>500</sub>.

morphologies were observed as the solids content was increased on reducing the KSPMA content to an average of 6 residues per copolymer stabilizer chain in the presence of 0.20 M salt (Table S5 and Figure S5). Thus, spherical particles of around 30 nm were observed by TEM at 15 or 20 wt % for a target PHPMA DP of 100 or 200, but short worms were formed at a DP of 300 (Figure S5e) and micrometer-sized aggregates of worms were observed at a DP of 500 (Figure S4b). Block copolymer vesicles were obtained at a solids content of 25% when targeting a PHPMA DP of 300 (Figure S5f), but increasing the target DP further to 1000 produced only colloidally unstable aggregates (image not shown). The same conditions (i.e., using a PKSPMA<sub>6</sub>stat-HEMA<sub>29</sub> macro-CTA at 25 wt %) employed in the presence of 0.30 M NaCl resulted in spherical particles for PHPMA DPs of less than 300 but only unstable aggregates when the PHPMA DP exceeded 300 (image not shown).



**Figure 8.** Aqueous electrophoresis data obtained for PKSPMA<sub>34</sub>-PHPMA<sub>500</sub> (green ▼), P(KSPMA<sub>25</sub>-*stat*-HEMA<sub>11</sub>)-PHPMA<sub>500</sub> (blue ▲), P(KSPMA<sub>11</sub>-*stat*-HEMA<sub>24</sub>)-PHPMA<sub>500</sub> (red ●), and P(KSPMA<sub>6</sub>*stat*-HEMA<sub>29</sub>)-PHPMA<sub>500</sub> (black ■). In each case, these aqueous dispersions were prepared at 10 wt % solids in the presence of 0.20 M NaCl. Electrophoretic measurements were made on 0.01 wt % dispersions in the presence of  $10^{-3}$  M background NaCl.

Scheme 2. Schematic Preparation of Diblock Copolymer Nanoparticles with Mixed Coronas via RAFT Aqueous Dispersion Polymerization of HPMA at 70 °C Using a Binary Mixture of an Anionic PKSPMA Macro-CTA and a Nonionic PGMA Macro-CTA<sup>a</sup>



<sup>*a*</sup> The latter stabilizer reduces the overall coronal charge density and hence enables polymerization-induced self-assembly to occur in the presence of added salt.

In all cases, the polymerization of HPMA was monitored by <sup>1</sup>H NMR spectroscopy, and monomer conversions were calculated on the basis of the disappearance of the monomer vinyl signals relative to a convenient nonvolatile internal standard (sodium 3-(trimethylsilyl)propyl sulfonate). However, GPC analysis was limited to those diblock copolymers synthesized using the macro-CTA containing the lowest anionic charge density (i.e.,  $P(KSPMA_6-stat-HEMA_{29})-PHPMA_{\nu})$  because all other copolymers proved to be insufficiently soluble in the DMF eluent. A kinetic study was performed for this leastanionic formulation: it was found that approximately 100% conversion was attained within around 2 h (Figure 4). An enhanced rate of polymerization was observed after around 45 min in the semilog plot inset shown in Figure 4, which corresponds to the onset of turbidity in the reaction solution due to the nucleation of the growing PHPMA chains. The number-average molecular weight,  $M_{\rm p}$ , of the diblock copolymer increased linearly with conversion, with polydispersities remaining below 1.25 (Figure 5). This suggests good pseudoliving character for the HPMA polymerization, as expected for a RAFT formulation.



**Figure 9.** Electrophoretic mobilities obtained for 0.10% aqueous dispersions of the following diblock copolymer nanoparticles at pH 4.50: (a) PGMA<sub>60</sub>-PHPMA<sub>500</sub>, (b) PKSPMA<sub>34</sub>-PHPMA<sub>500</sub>, and (c) (1 PKSPMA<sub>34</sub> + 4 PGMA<sub>60</sub>)-PHPMA<sub>500</sub>. Originally (a) was prepared at 10 wt % solids in the absence of any salt and (b) and (c) were prepared at 10 wt % solids in the presence of 0.20 M NaCl.

Moreover, the unimodal GPC traces shown in Figure 6 suggest a high blocking efficiency for the  $P(KSPMA_6-stat-HEMA_{29})$ macro-CTA because there is no evidence of any unreacted macro-CTA. The high molecular weight shoulder that becomes more prominent at higher conversion is due to a small amount of a dimethacrylate impurity ( $\sim$ 0.26 mol %) present in the HPMA monomer.<sup>28</sup> The evolution of hydrodynamic particle diameter with time was monitored by DLS for the same RAFT aqueous dispersion polymerization of HPMA at 70 °C using the P-(KSPMA<sub>6</sub>-stat-HEMA<sub>29</sub>) macro-CTA (Figure 7). Initially, no particles could be detected (up to 20% monomer conversion). Then nucleation occurs as the PHPMA chains become sufficiently hydrophobic to overcome the unfavorable electrostatics and induce block copolymer self-assembly: at this point, the formation of colloidal particles in the reaction solution is also confirmed visually by the increase in turbidity. The mean intensity-average diameter reported by DLS gradually increased from around 50 to 59 nm as the HPMA polymerization proceeded to full conversion within 2 to 3 h at 70 °C. The final sterically stabilized nanoparticles have a relatively low polydispersity of 0.05. A closer inspection of Figures 4 and 7 confirms that the onset of nucleation at around 45 min corresponds to an enhanced rate of polymerization. (Note the change in slope in the semilogarithmic kinetic plot.) Similar observations have been reported by Blanazs and co-workers<sup>28</sup>

for a RAFT aqueous dispersion polymerization formulation based on a nonionic macro-CTA. The most likely explanation is that, once the block copolymer nanoparticles are formed, some of the unreacted HPMA monomer dissolved in the continuous phase becomes solubilized within the PHPMA micelle cores, leading to a relatively high local monomer concentration and hence a faster rate of polymerization.<sup>28</sup>

A series of P(KSPMA<sub>x</sub>-stat-HEMA<sub>y</sub>)-PHPMA<sub>500</sub> diblock copolymer dispersions prepared using four different macro-CTAs were characterized by aqueous electrophoresis (Figure 8). As expected, nanoparticles prepared using the PKSPMA homopolymer macro-CTA (x = 34, y = 0) exhibited the most negative zeta potentials (-55 to -60 mV), with little or no pH dependence over the pH range studied. However, gradually diluting the anionic charge density within the coronal chains using the nonionic HEMA comonomer allows the zeta potential of these nanoparticles to be systematically adjusted. Moreover, just six KSPMA comonomer units per stabilizer chain produce a zeta potential of -30 to -35 mV, again exhibiting almost no pH dependence.

A second strategy was also explored to modulate the coronal charge density in these block copolymer nanoparticles (Scheme 2): HPMA was polymerized using a binary mixture of two macro-CTAs. From an entropic viewpoint, this approach should produce block copolymer nanoparticles with mixed coronas. Hence the nonionic



**Figure 10.** Transmission electron micrographs obtained for the RAFT aqueous dispersion polymerization of HPMA at 70 °C using *x*PKSPMA<sub>34</sub> + *y*PGMA<sub>60</sub> binary mixtures (where the *x/y* molar ratio is 1:1, 3:7, 1:4, 1:9, and 1:19) under the following conditions: (a-e) zero salt, 10 wt %; (f-j) 0.20 M NaCl, 10 wt %; and (k-o) 0.20 M NaCl, 20 wt %.

 $PGMA_{60}$  stabilizer chains should dilute the lateral electrostatic repulsions between the highly anionic PKSPMA<sub>34</sub> chains within the coronal layer, facilitating polymerization-induced self-assembly. The proportion of PKSPMA macro-CTA chains was systematically varied from 5 to 50 mol %, and the mean DP of the coreforming PHPMA chains was fixed at 500 at 10 w/w % solids in the presence of 0.20 M NaCl. This approach also provided good control over the electrophoretic footprint exhibited by the block copolymer nanoparticles (Figure S6). At present, we cannot explain the remarkable nonlinear nature of the zeta potential curves observed in this case, although it is worth emphasizing that these data sets were replicated three times using two different Nanosizer instruments, with  $10^{-3}$  M NaCl being used as a background electrolyte in each case.

To verify that true entropic mixing of the two types of stabilizer chains had occurred with the coronal layer, we examined the raw mobility data obtained at pH 4.25 prior to calculating the zeta potentials. As expected, the nonionic PGMA<sub>60</sub>-PHPMA<sub>500</sub> block copolymer nanoparticles prepared as a reference exhibited essentially zero mobility. In contrast, PKSPMA34-PHPMA500 nanoparticles exhibited a highly negative mobility of almost  $-4 \mu m cm/V s$  whereas the mixed-corona (1 PKSPMA<sub>34</sub> + 4 PGMA<sub>60</sub>)-PHPMA<sub>500</sub> nanoparticles prepared using 20 mol % PKSPMA34 macro-CTA had an intermediate negative mobility of around  $-2 \,\mu m \, cm/V \, s$ (Figure 9). Moreover, this binary mixture strategy not only afforded control over the electrophoretic footprint of the nanoparticles but also enabled higher-order morphologies to be accessed (Figure 10). Thus, HPMA polymerizations performed at a solids content of 10 wt % in the presence of 0.20 M NaCl using 5 to 20 mol % PKSPMA macro-CTA led to the formation of block copolymer vesicles (Figure 10h-i). TEM studies also confirmed the presence of some short worms (formed by the partial fusion of two or more spheres) when using a 30 mol % PKSPMA stabilizer (Figure 10g), whereas only spheres were observed when 50 mol % PKSPMA stabilizer was employed (Figure 10f). Finally,

regardless of the precise binary mixture of anionic/nonionic macro-CTAs employed, HPMA polymerizations conducted at a higher solids content (20 wt %) always resulted in the formation of block copolymer vesicles (Figure 10k–o).

# CONCLUSIONS

A range of anionic polyelectrolyte-stabilized diblock copolymer nanoparticles were prepared via RAFT aqueous dispersion polymerization of 2-hydroxypropyl methacrylate at 70 °C using a PKSPMA-based macro-CTA. The strongly anionic character of the PKSPMA stabilizer hinders polymerization-induced self-assembly due to the strong lateral repulsive forces between adjacent chains, which leads to either no particle formation or the formation of loose block copolymer aggregates comprising hydrated PHPMA chains. This problem can be overcome by the addition of salt, which screens these repulsive forces and leads to the formation of significantly denser, more well defined nanoparticles. Alternatively, the anionic charge density can be diluted by statistically copolymerizing KSPMA with a nonionic comonomer such as 2-hydroxyethyl methacrylate. The latter approach allows the electrophoretic behavior of these anionic nanoparticles to be precisely tuned. A third strategy for promoting in situ self-assembly involves diluting the anionic PKSPMA macro-CTA with a nonionic poly-(glycerol monomethacrylate) macro-CTA to modulate the electrostatic repulsive forces. This approach enables higher-order morphologies such as vesicles to be obtained, particularly for syntheses conducted in the presence of salt in more concentrated solution.

# ASSOCIATED CONTENT

**Supporting Information.** Additional TEM images, DLS diameters (and polydispersities), and aqueous electrophoresis data obtained for these anionic diblock copolymer nanoparticles. This material is available free of charge via the Internet at http://pubs.acs.org.

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