Synthesis and Characterization of Aliphatic Ammonium Ionenes: Aqueous Size Exclusion Chromatography for Absolute Molecular Weight Characterization

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ABSTRACT: Cationic aliphatic ammonium polyionenes, specifically 12,12- and 6,12-ionenes, were synthesized using step-growth polymerization and aqueous-based size exclusion chromatography (SEC) coupled with multiangle laser light scattering (MALLS) revealed absolute molecular weight information. The chromatographic separation of cationic polyelectrolytes presents many additional challenges compared to SEC of nonassociating, neutral polymers. Therefore, an aqueous-based SEC-MALLS mobile phase composition was identified to separate these notoriously challenging polyelectrolytes in an effort to achieve absolute molecular weight analysis. Various solvent compositions were evaluated for their ability to solvate and reproducibly separate both 6,12- and 12,-12-ionenes. Dynamic light scattering (DLS) verified the absence of aggregation of polyionenes in preferred mobile phase compositions. The optimum solvent composition comprised a ternary mixture of 54/23/23 water/methanol/glacial acetic acid, 0.54 M NaOAc, at a pH of 4.0. Weight-average molecular weights for the synthesized ammonium 12,12-ionenes ranged from 11 000 to 40 000 g/mol, and ammonium 6,12-ionenes had weight-average molecular weights ranging from 19 000 to 49 900 g/mol. Mark−Houwink parameters were also determined for both the 12,12- and 6,12-ionenes in the optimum mobile phase using an online capillary viscometer.

Introduction

Ammonium ionenes are ion-containing macromolecules containing quaternary nitrogens in the main chain.[1] The typical nomenclature for aliphatic ammonium ionenes is *x*,*y*-ionene, where *x* represents the number of methylene spacer units derived from the ditertiary amine monomer and *y* represents the methylene spacer units in the dihaloalkane monomer (Figure 1). Early efforts for the synthesis of ammonium ionenes involved the homopolymerization of *ω*-halo-alkyl dialkylamines;[1–4] however, more conventional synthetic methodologies for ammonium ionenes involved the copolymerization of dihaloalkanes and ditertiary amines, which Rembaum et al. first reported.[5,6] The ability to easily control charge density and counteranion through monomer selection makes ammonium ionenes an ideal model in the study of well-defined cationic polyelectrolytes. Because of their unique Coulombic interactions, ammonium ionenes were recently explored for several biomedical technologies including antimicrobials,[7,8] gene transfection agents,[9] and polymeric cancer drugs.[10] Osada and co-workers performed cell binding and viability studies and found that ammonium ionenes with lower charge density disrupted cell membranes to a greater extent than structures with higher charge density despite less cell binding.[11] Furthermore, Rembaum reported ionenes as antitumor agents that exhibited selective inhibition of malignant cell growth without affecting normal cells.[10] These examples demonstrate the potential applications of ammonium ionenes as well as the importance of determining their chemo-physical characteristics for conclusive structure−property relationships.

Determination of molecular weight and molecular weight distribution is critical when establishing structure−property−performance relationships. In earlier investigations, a comparison of the amount of nonionic bromine in an ionene solution to the amount of total bromide in the same solution yielded an "average apparent molecular weight".[12] In Rembaum's earlier work, extensive viscosity and light scattering studies were performed on ammonium 3,4- and 6,6-ionenes to determine molecular weights via the Mark−Houwink and Debye relationships, respectively.[13] Absolute molecular weights were determined; however, tedious sample preparation and lengthy experiments were required to complete these calculations. Size exclusion chromatography (SEC), also referred to as gel permeation chromatography (GPC), coupled with multiangle laser light scattering (MALLS) is a well-established method for determining molecular weights of polymers. When MALLS detection is coupled with a concentration detector such as a differential refractometer (dRI), SEC provides a rapid method for determining absolute molecular weights with facile sample preparation. In addition, MALLS provides structural information, such as radii of gyration. Several texts and comprehensive reviews that describe SEC in detail are available.[14–16] Despite the appreciated value of SEC-MALLS and concurrent synthetic investigations of ionenes, SEC analysis for the determination of absolute molecular weights of ammonium ionenes has not been reported earlier. This is partly attributed to the challenges associated with SEC of polyelectrolytes. Difficulties arise from ionic aggregation[17] and non-size exclusion events,[14] such as ion interaction, ion exclusion, and hydrophobic interactions in the stationary phase of the chromatographic column. SEC of cationic polyelectrolytes is especially problematic due to the negative charge that is common on most stationary-phase packing materials and encourages unwanted electrostatic interactions.[18] To the best of our knowledge, earlier SEC analyses of ammonium ionenes were limited to calibrative methods, which provide only standard-equivalent molecular weights. Furthermore, SEC-MALLS determination of absolute molecular weights

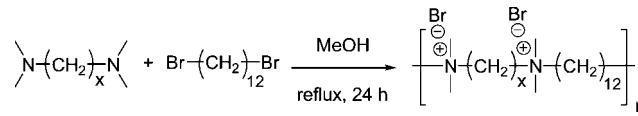

Figure 1. Synthesis of aliphatic ammonium *x*,12-ionenes.

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of ammonium 12,12-ionene compositions was not reported earlier. Kopecká et al. performed SEC on ammonium 5,2- and 10,2-ionenes and determined relative molecular weights based on polyacrylamide standards.[19] Similarly, Reisinger et al. performed SEC on ammonium ionenes using quaternized poly(vinylpyridinium) standards.[20] Although these earlier experiments provided molecular weight trends, absolute molecular weight data are critical for the understanding of fundamental structure−property relationships. Moreover, meaningful molecular weight characterization of branched topologies is difficult using relative calculation methods. Furthermore, our aqueous SEC-MALLS data of ammonium ionenes using two mobile phase compositions that Kopecká and Reisinger reported earlier did not reveal any signals in the chromatograms.

Herein, we report aqueous-based SEC-MALLS of 6,12- and 12,12-ammonium ionenes. This article reports the most suitable aqueous SEC mobile-phase composition for obtaining reliable separations of aliphatic ammonium ionenes through elimination of polymer−column interactions and reduction of polymer−polymer aggregation. In addition to absolute molecular weight determination, we also report dilute solution rheology of ammonium ionenes using an online, capillary-based viscometric detector. This complementary detector functions with dRI and MALLS detectors to determine the intrinsic viscosity of the sample across the molecular weight distribution.

Experimental Section

General Methods and Materials. HPLC-grade methanol was obtained from Fischer Scientific and distilled from calcium hydride (reagent grade, 95%), which was obtained from Sigma-Aldrich and used as received. 1,12-Dibromododecane (98%) was obtained from Sigma-Aldrich, recrystallized from ethanol (AAPER Alcohol and Chemical Co.), and dried under reduced pressure. *N,N,N′,N′*-Tetramethyl-1,6-hexanediamine (99%) was obtained from Sigma-Aldrich and distilled from calcium hydride. Dimethylamine (60% in water, ∼11.0 M) was obtained from Fluka and used as received. HPLC-grade tetrahydrofuran (THF), HPLC-grade water, and diethyl ether were obtained from Fischer Scientific and used as received. Sodium acetate (NaOAc) (99.0%), sodium azide (99%), and ACS-grade glacial acetic acid (99.7%) were purchased from Alfa Aesar and used as received.

^{1}H and ^{13}C NMR spectra were recorded on a Varian Inova 400 MHz spectrometer. Chemical shifts are reported in ppm downfield from TMS using the residual protonated solvent as an internal standard (CD_3OD, ^{1}H 4.87 ppm and ^{13}C 77.0 ppm) (D_2O, ^{1}H 4.79 ppm). FAB-MS was obtained on a JEOL HX110 dual focusing mass spectrometer, and FTIR data were recorded on a Perkin-Elmer Spectrum One FT-IR spectrometer with a 1 mW He−Ne laser operating at a wavelength of 633 nm using a Spectrum v5.0.1 software package. In situ FTIR was performed with a Mettler Toledo ReactIR 4000 ATR apparatus equipped with a light conduit and a stainless steel insertion probe with a DiComp (diamond composite) probe tip. IR spectra were collected every minute with 256 scans per spectrum. Reaction analysis was performed with ReactIR 3.1 software provided by Mettler Toledo.

Synthesis of *N,N,N′,N′*-Tetramethyl-1,12-dodecanediamine. 1,12-Dibromododecane (6.00 g, 18.3 mmol) was dissolved in THF (150 mL) in a two-necked, 500 mL round-bottomed flask, and the solution was cooled to −78 °C. Dimethylamine (60% in water, 327 mL, 3.6 mol) was added to the flask, and the solution was magnetically stirred for 30 min. The solution was allowed to warm to room temperature and stir for an additional 24 h. The reaction solvent was removed under reduced pressure, and the subsequent white residue was dissolved in a 2.0 M NaOH aqueous solution (150 mL). Diethyl ether (150 mL) was added to the flask, and the mixture was magnetically stirred for 2 h. The organic layer was collected and concentrated, and a yellow oil was obtained. The crude product was purified via vacuum distillation (100 °C and 150 mTorr) from CaH_2 to provide a clear colorless product (yield 2.3 g, 47%). ^{1}H NMR (400 MHz, CD_3OD): δ 2.26 (t, 4 H), 2.19 (s, 12 H), 1.45 (q, 4 H), 1.28 (s, 16 H). ^{13}C NMR (100 MHz, CD_3OD): δ = 123.8, 59.7, 44.3, 29.6, 29.5, 27.5, 27.2. FAB-MS (*m/z* calculated = 256.48, found = 257.29). FTIR (oil) ν = 2925.1, 2853.2, 2812.8, 2761.1, 1459.2, 1041.9 cm^{-1}.

Preparation of Ammonium Ionene. In a typical synthesis, 1,12-dibromododecane (1.02 g, 3.1 mmol) was transferred into a two-necked, 50 mL round-bottomed flask, which was equipped with a reflux condenser and a mechanical stirrer. Methanol (2.25 g, 70.3 mmol) was added into the flask with a cannula under nitrogen, and the solution was refluxed. *N,N,N′,N′*-Tetramethyl-1,6-hexanediamine (0.53 g, 3.1 mmol) was quickly added into the flask, and additional purging with nitrogen was performed. The reaction was mechanically stirred for 24 h under reflux. Upon completion, the methanol was removed under reduced pressure at 25 °C to yield the polymer product. ^{1}H NMR (400 MHz, D_2O): δ 3.31 (m, 8 H), 3.05 (s, 12 H), 1.75 (m, 8 H), 1.31 (m, 20 H). M_n 14 000−41 500 g/mol, M_w 19 000−49 900 g/mol, PDI 1.31−1.36.

The 12,12-ionene was also prepared as described above. ^{1}H NMR (400 MHz, D_2O): δ 3.31 (m, 8 H), 3.05 (s, 12 H), 1.75 (m, 8 H), 1.31 (m, 32 H). M_n 8000−30 700 g/mol, M_w 11 000−40 000 g/mol, PDI 1.26−1.42.

Aqueous SEC of Ammonium Ionenes. Aqueous-based SEC-MALLS was used to determine absolute molecular weights in acetate buffer solutions. The mobile phase consisted of 0.540 M sodium acetate in a ternary mixture of 54/23/23 water/methanol/glacial acetic acid (v/v/v %). The pH of the resulting solution was 4.0, which was measured using a Thermo Orion 3 Star portable pH meter with a Thermo Orion Triode pH electrode. Sodium azide was added at 200 ppm to the solution as a precaution to prevent bacterial growth in the SEC system. Other compositions that employed different molarities of salt and varying mixtures of water, methanol, and acetic acid are noted in the Discussion section. The mobile phase solutions were vacuum-filtered through NALGENE MF75 Series Media-Plus Filter Units with a minimum pore size of 0.200 μm. Samples were analyzed at 0.8 mL/min through 2× Waters Ultrahydrogel linear columns and 1× Waters Ultrahydrogel 250 column, with all columns measuring 7.8 × 300 mm and equilibrated to 30 °C. SEC instrumentation consisted of a Waters 1515 isocratic HPLC pump, a Waters 717plus Autosampler, a Wyatt miniDAWN multiangle laser light scattering (MALLS) detector operating a He−Ne laser at a wavelength of 690 nm, a Viscotek 270 capillary viscosity detector, and a Waters 2414 differential refractive index detector operating at a wavelength of 880 nm and 35 °C. The only calibration constant, the Wyatt Astra V AUX1, was calculated using a series of aqueous sodium chloride solutions. The accuracy and reproducibility were confirmed with poly(ethylene oxide) and poly(methacrylic acid) sodium salt standards (Polymer Laboratories/Varian Inc.) ranging in molecular weight from 1000 to 1 000 000 g/mol. Samples were processed using the Wyatt Astra V software package. Interdetector volume was determined through the superposition of a narrow polydispersity poly(ethylene oxide) standard. Weight-average molecular weights were determined from light scattering data using the equation

$$\frac{K^*c}{R(\theta)} = \frac{1}{M_w P(\theta)} + 2A_2c \tag{1}$$

where M_w is the weight-average molecular weight, c is the concentration of the polymer, $R(\theta)$ is the measured excess Rayleigh ratio, $P(\theta)$ is the particle scattering function, A_2 is the second virial coefficient of the polymer−solvent system, and K^* is an optical scattering constant, determined using the equation

$$K^* = \frac{4\pi^2 n_0^2 (dn/dc)^2}{\lambda_0^4 N_A} \tag{2}$$

where dn/dc is the specific refractive index increment, n_0 is the refractive index of the solvent, λ_0 is the wavelength of the incident laser in a vacuum, and N_A is Avogadro's number.

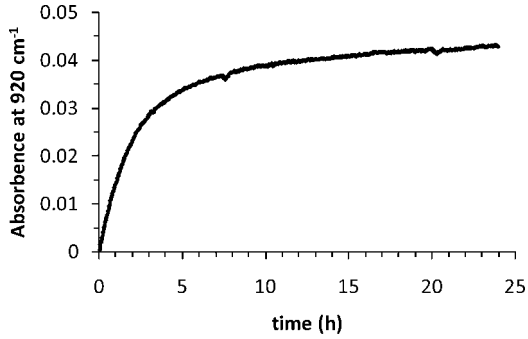

Figure 2. In situ FITR analysis of an ammonium 6,12-ionene polymerization. In situ absorbance at 920 cm^{-1} as a function of reaction time.

Determination of Specific Refractive Index Increments (d*n*/d*c*). A Wyatt OptiRex differential/absolute refractive index detector operating at a wavelength of 690 nm and 30 °C was used for all specific refractive index increment measurements. Polymer samples (0.076−1.512 mg/mL) were allowed to dissolve in the appropriate solvent overnight. Samples were metered at 0.8 mL/min into the dRI detector at 30 °C using a syringe pump and a syringe affixed with a 0.45 μm PTFE syringe filter. The d*n*/d*c* values were determined using the Wyatt Astra V software package.

Dynamic Light Scattering. Dynamic light scattering measurements were performed on a Malvern Zeta Sizer Nano Series Nano-ZS instrument using Dispersion Technology Software (DTS) version 4.20 at a wavelength of 633 nm using a 4.0 mW, solid state He−Ne laser at a scattering angle of 173°. The experiments were performed at a temperature of 25 °C. Polymer samples were prepared at 1 mg/mL and allowed to dissolve in the appropriate solvent overnight. Samples were syringed through 0.45 μm PTFE syringe filters directly into clean cuvettes. Data were observed for the presence or absence of aggregation peaks based on particle diameter size.

Results and Discussion

The syntheses of 6,12-ionenes were performed with commercially available monomers. *N,N,N′,N′*-Tetramethyl-1,12-dodecanediamine was synthesized according to a modified literature procedure from dimethylamine and α,ω-dibromododecane.[21] The synthesis of aliphatic ammonium ionenes was performed via the Menshutkin reaction between the appropriate dihalide and ditertiary amine in a methanolic solution under reflux for 24 h. Reaction progress was analyzed via in situ FTIR spectroscopy, and growth of the $C-N^+$ stretch at 920 cm^{-1} was observed as shown. The polymerization was complete after 20 h, based on the lack of further increase in the $C-N^+$ stretch (Figure 2).[22] Our research group has shown in situ FTIR spectroscopy as a useful technique to monitor reaction progress and determine reaction kinetics.[23,24] Upon removal of methanol, the resulting polymers formed colorless, transparent, and ductile films.

The structures of ammonium 6,12- and 12,12-ionenes were confirmed via ^1H NMR spectroscopy. Both ionene structures exhibited similar chemical shifts, differing only in their relative peak integrations. A singlet at δ 3.05 ppm corresponded to the methyl groups bonded to quaternized nitrogen atoms (CH_3-N^+). A broad resonance centered at δ 3.31 ppm corresponded to the methylene directly bonded to quaternized nitrogen atoms ($-CH_2-N^+$). The methylene units beta to the quaternized nitrogen atoms had a broad resonance centered at δ 1.75 ppm ($-CH_2CH_2N^+$). A broad resonance centered at δ 1.31 ppm corresponded to the methylene spacer units three or more units away from the quaternized nitrogen atoms ($-CH_2-CH_2-$).

Successful SEC of polyelectrolytes requires the elimination of polymer−polymer and polymer−stationary phase interactions. It is widely recognized that electrostatic interactions between polymer chains or between the polymer and the stationary phase are effectively eliminated with an increase in ionic strength of the mobile phase.[25] Thus, sodium acetate was used in these studies; however, as the ionic strength of the solvent increased, it was presumed that hydrophobic interactions become more prevalent; thus, in order to overcome both polymer−polymer and polymer−stationary phase hydrophobic interactions, methanol was added to the mobile phase and glacial acetic acid was also determined to improve SEC separations. The acetic acid likely functioned as both an organic modifier to eliminate hydrophobic interactions and a proton donor to increase the ionic strength of the solvent. An increase in the ionic strength of the solvent is known to shield electrostatic interactions due to the reduction in the Debye screening length.[25] As this occurs, the persistence length of the polymer decreases, resulting in a more random coil-like conformation of the polymer chain, similar to neutral polymers.[26] Random coils are the preferred conformation for reliable SEC since chain-extended conformations fail to sample the available pore volume, resulting in non-Gaussian distributions.[14] Decreasing the Debye screening length also helps to eliminate electrostatic interaction between the polyelectrolyte and the stationary phase, which can delay the elution of polyelectrolytes leading to tailing in chromatograms.[27] Charge screening also functions to eliminate ion-exclusion effects, which prevent polyelectrolyte chains from sampling smaller pore sizes leading to premature sample elution. The ionic strength that is necessary for reducing these effects depends on the chemo-physical properties of a given polymer/solvent/column system.

Ionic aggregation is generally considered detrimental to successful SEC since larger aggregates may impede instrumentation flow, elute prematurely, and/or skew measurements of the molecular weight distribution.[27] In order to determine whether the ionenes were aggregating in solution, DLS was first performed in a variety of potential mobile phase compositions. Ammonium 12,12-ionene samples that were dissolved in the mobile phase (54/23/23 v/v/v % water/methanol/glacial acetic acid, 0.54 M NaOAc, pH 4.0) showed insignificant aggregation behavior (Figure 3), and this observation suggested that the polymer−solvent composition fulfilled one of the requirements necessary for reliable SEC. Other solvent compositions consisting of lower amounts of organic cosolvent, lower sodium acetate concentrations, and/or higher pH exhibited notable aggregation; thus, these compositions were eliminated as mobile phases for the SEC of aliphatic ammonium ionenes. As shown in Figure 4, a decrease in the amount of methanol and glacial acetic, from 23 to 17 vol %, resulted in bimodal DLS curves. Also, completely eliminating glacial acetic acid from the mobile phase composition promoted polymer aggregation (Figure 5). These mobile phase compositions were not pursued as possible mobile phases for the SEC of ammonium ionenes since they encouraged aggregation.

The specific refractive index increment (d*n*/d*c*) corresponds to the dependence of the solution's refractive index on solute concentration.[14] The d*n*/d*c* of a polymer depends on the chemical composition of solute, solvent used to dissolve the solute, and wavelength of the incident laser.[28] The d*n*/d*c* of a polymer is usually considered as a constant in a given solvent; however, the contribution of end groups becomes significant at lower molecular weights, leading to dissimilar chemical compositions.[29] In the SEC-MALLS analysis of neutral polymers, d*n*/d*c* values are readily calculated online using 100% mass recovery methods. However, counterion dissociation and non-quantitative sample recovery prevent online determination for SEC analysis of polyelectrolytes. Therefore, individual offline batch-mode measurements must be performed to determine accurate d*n*/d*c* values. In this study, an offline determination was performed on the ammonium ionenes to obtain accurate

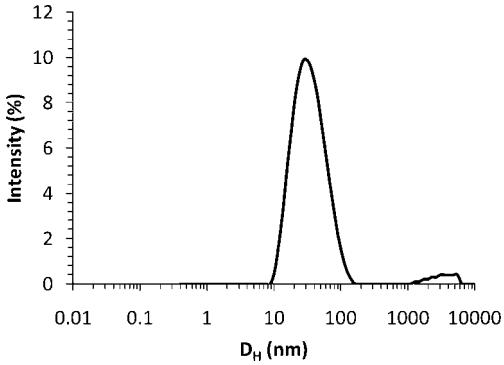

Figure 3. DLS analysis of ammonium 12,12-ionene (sample 6) in 54/23/23 (v/v/v %) water/methanol/glacial acetic acid, 0.54 M NaOAc, pH 4.0.

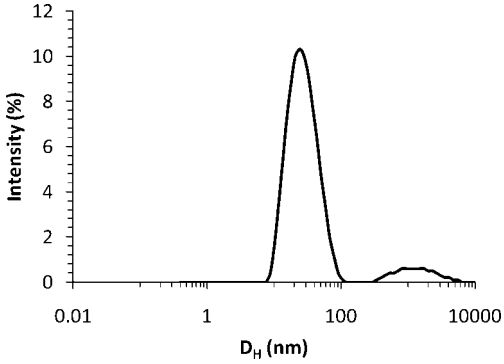

Figure 4. DLS analysis of ammonium 12,12-ionene (sample 6) in 66/17/17 water/methanol/acetic acid (v/v/v %), 0.42 M NaOAc, and pH 4.0.

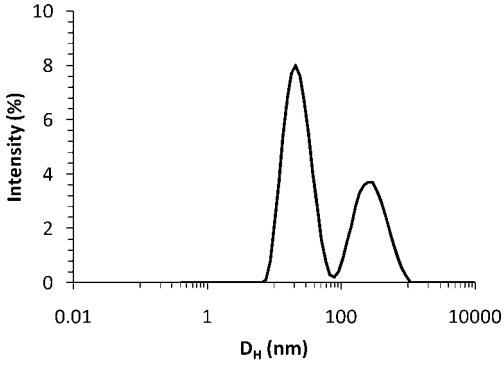

Figure 5. DLS analysis of ammonium 12,12-ionene (sample 6) in 80/20 water/methanol (v/v/v %), 0.50 M NaOAc, at pH 8.49.

d*n*/d*c* values. Various polymer concentrations were prepared ranging from 0.076 to 1.512 mg/mL. Differential refractive index data were obtained for each solution and plotted versus concentration using the Wyatt Astra V software package. The slope of the linear fit is the d*n*/d*c* of the polymer for this particular solvent. A typical d*n*/d*c* plot for an ammonium 12,12-ionene (sample 5) in the mobile phase, 54/23/23 (v/v/v %) water/methanol/glacial acetic acid, 0.54 M NaOAc, pH 4.0, is shown in Figure 6. In this study, all molecular weights of each ionene analyzed had similar d*n*/d*c* values within experimental error. The d*n*/d*c* values for ammonium 12,12- and 6,12-ionenes in this mobile phase are shown in Table 1.

Absolute moments of the molecular weight distribution were determined for ammonium 6,12- and 12,12-ionenes using 54/23/23 (v/v/v %) water/methanol/glacial acetic acid, 0.54 M NaOAc, pH 4.0, and are summarized in Table 2. Ammonium 12,12-ionene prepared from various dihalide:diamine monomer

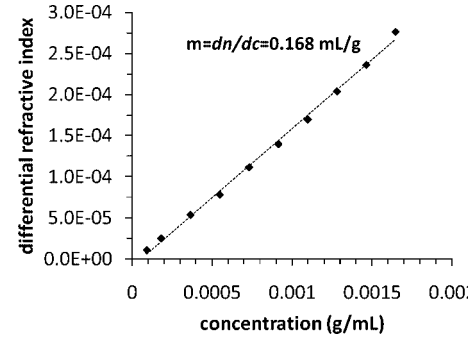

Figure 6. Typical d*n*/d*c* plot for an ammonium 12,12-ionene in 54/23/23 (v/v/v %) water/methanol/glacial acetic acid, 0.54 M NaOAc, pH 4.0. The d*n*/d*c* was determined to be 0.168 mL/g in this example.

Table 1. d*n*/d*c* Values for Aliphatic Ammonium Ionenes

	d*n*/d*c*[a] (mL/g)
ammonium 12,12-ionene (sample 5)	0.168
ammonium 6,12-ionene (sample 8)	0.154

[a] Each d*n*/d*c* was measured using 11 concentrations varying from 0.076 to 1.55 mg/mL in the mobile phase, 54/23/23 (v/v/v%) water/methanol/glacial acetic acid, 0.54 M NaOAc, pH 4.0.

Table 2. Molecular Weight Characterization for Aliphatic Ammonium Ionenes

sample	ammonium	momomer stoichiometry (diamine:dihalide)	M_n[a] (g/mol)	M_w[a] (g/mol)	M_w/M_n[a]	M_w/M_n[b]
1	12,12	1:1.10[c]	3 500	4 300	1.26	2.11
2	12,12	1:1.07[c]	9 700	12 300	1.27	2.50
3	12,12	1:1.05[c]	11 600	14 600	1.26	2.45
4	12,12	1:1.03[c]	14 000	17 800	1.27	2.36
5	12,12	1:1[c]	18 700	24 900	1.33	2.15
6	12,12	1:1[d]	20 600	26 000	1.26	2.03
7	12,12	1:1[e]	30 700	39 600	1.29	2.28
8	6,12	1:1[d]	10 700	14 300	1.34	2.15
9	6,12	1:1[e]	30 500	43 300	1.42	1.74

[a] Molecular weights determined via SEC-MALLS in 54/23/23 (v/v/v %) water/methanol/glacial acetic acid, 0.54 M NaOAc, pH 4.0. [b] Molecular weight distributions determined using poly(ethylene oxide) equivalent molecular weights. [c] Ionene synthesis performed at 20 wt % solids. [d] Ionene synthesis performed at 30 wt % solids. [e] Ionene synthesis performed at 50 wt % solids.

stoichiometries had number-average molecular weights between 8000 and 30 700 g/mol and weight-average molecular weights between 11 000 and 40 000 g/mol. Ammonium 6,12-ionene prepared from various dihalide:diamine molar ratios had number-average molecular weights between 14 000 and 41 500 g/mol and weight-average molecular weights between 19 000 and 49 900 g/mol. As expected for a step-growth polymerization, molecular weights for ammonium ionenes increased as the molar ratios of the two difunctional monomers approached 1:1.

The SEC chromatogram for a typical ammonium 12,12-ionene in the mobile phase (54/23/23 (v/v/v %) water/methanol/glacial acetic acid, 0.54 M NaOAc, pH 4.0) including the dRI trace, MALLS trace, and molecular weight as a function of the molecular weight distribution is shown in Figure 7. The monomodal, symmetrical peak in the MALLS and dRI chromatograms demonstrated reliable sample separation and provided additional support for the absence of polymer aggregation in solution. Thus, in addition to fulfilling the nonaggregating requirement, this mobile phase successfully reduced polymer−stationary phase interactions, which indicated that the ionic strength of the tested mobile phase was sufficient to discourage any electrostatic interactions that would otherwise lead to ion interaction or ion exclusion effects. When the organic content of the solvent was decreased, poor SEC separation was observed.

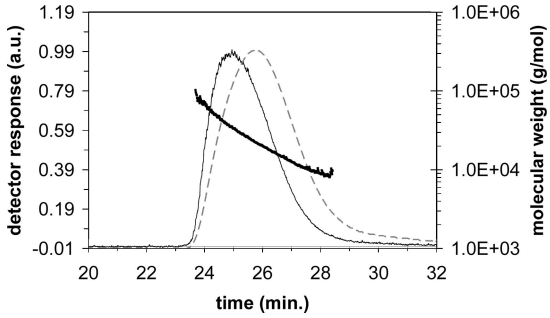

Figure 7. SEC chromatogram of ammonium 12,12-ionene (sample 5) showing the RI trace (dotted gray), MALLS trace (solid black), and molecular weight (bold black line) in 54/23/23 (v/v/v %) water/methanol/glacial acetic acid, 0.54 M NaOAc, pH 4.0.

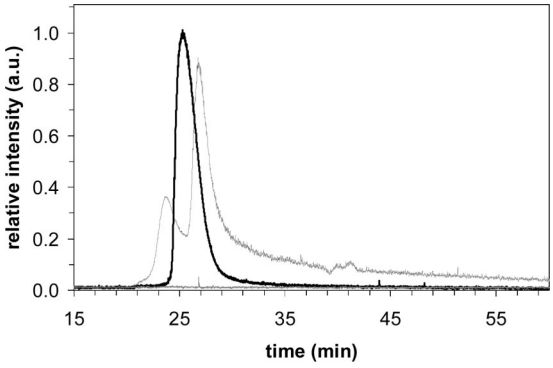

Figure 8. SEC chromatogram of ammonium 12,12-ionene (sample 6) showing the influence of the water/organic solvent ratio and NaOAc molarity on SEC separation. The three ratios were 74/8/18 water/methanol/acetic acid (v/v/v %), 0.57 M NaOAc, pH 4.0 (light gray), 66/17/17 water/methanol/acetic acid (v/v/v %), 0.42 M NaOAc, pH 4.0 (dark gray), and 54/23/23 (v/v/v %) water/methanol/glacial acetic acid, 0.54 M NaOAc, pH 4.0 (bold black).

In the case of the 12,12-ionene, decreasing the relative amount of methanol and glacial acetic acid, both from 23 to 17 vol %, resulted in bimodality and tailing in the SEC chromatograms (Figure 8). Similar to the 12,12-ionene, SEC-MALLS of the ammonium 6,12-ionene in the same mobile phase also showed monomodal peaks (Figure 9). However, unlike the 12,12-ionene, the asymmetric 6,12-ionene also showed monomodal peaks in the mobile phase, 66/17/17 (v/v/v %) water/MeOH/AcOH, 0.42 M NaOAc, pH 4.0 solvent composition (Figure 10). Although this mobile phase works well for the ammonium 6,12-ionene, the mobile phase, 54/23/23 (v/v/v %) water/methanol/glacial acetic acid, 0.54 M NaOAc, pH 4.0, is more versatile since it provides reliable separations for both of the ionenes tested.

Curiously, SEC-MALLS-obtained molecular weight distributions for all ammonium ionene polymers ranged from 1.26 to 1.42. The expected molecular weight distribution for conventional step-growth polymerization to form linear products is ~2.0 at high monomer conversion; however, the molecular weight distributions for ammonium ionenes were unexpectedly low. It is important to note that only the SEC-MALLS molecular weight distributions were low. We also calculated molecular weight distributions using poly(ethylene oxide)-equivalent molecular weights from dRI measurements and observed more reasonable values ranging from 1.74 to 2.50 (Table 2). Molecular weight distributions less than 2.0 in step-growth polymerizations can occur because of fractionation in the purification process or sample exclusion within the chromatography columns.[14] We excluded the possibility of fractionation since we solution-cast ionenes directly from the reaction mixture. Furthermore, samples were analyzed a second time using columns with a higher resolution (PL aquagel-OH 30 and PL aquagel-OH 40 measuring

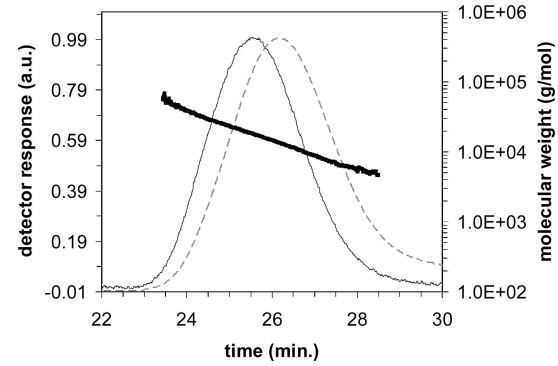

Figure 9. SEC chromatogram of ammonium 6,12-ionene (sample 8) showing the RI trace (dotted gray), MALLS trace (solid black), and molecular weight (bold black line) in the mobile phase (54/23/23 (v/v/v %) water/methanol/glacial acetic acid, 0.54 M NaOAc, pH 4.0).

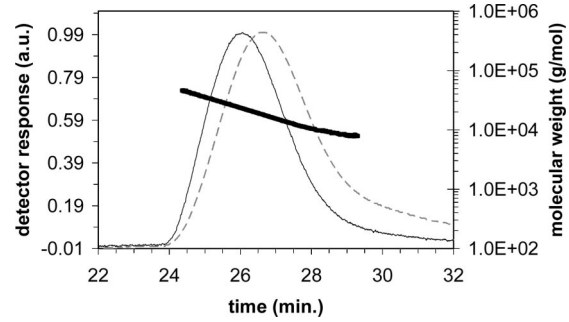

Figure 10. SEC chromatogram of ammonium 6,12-ionene (sample 8) showing the RI trace (dotted gray), MALLS trace (solid black), and molecular weight (bold black line) in 66/17/17 water/MeOH/AcOH (v/v/v %), 0.42 M NaOAc, pH 4.0.

7.5 × 300 mm), and we eliminated the possibility of excluding sample volume from the column pores.

Another explanation for step-growth polymerization leading to narrow molecular weight distributions is different reactivities of monomers relative to polymer chains.[30] Nanda demonstrated that distributions less than 2.0 were observed if reactivity decreased with increasing molecular weight.[31] It is plausible that the rate of addition of neutral monomer to a charged species differs from reaction of two charged species. In addition, aggregation in solution of charged polymers may result in shielding of reactive chain ends. We measured the molecular weights of aliquots obtained during a 24 h polymerization of an ammonium 6,12-ionene and demonstrated that molecular weight increases throughout the polymerization process (Figure 11), and we are currently studying the reaction kinetics to determine whether reactivity of monomers is greater than polymer chains. Wegner has studied the reaction kinetics of aliphatic ammonium ionenes with in situ NMR spectroscopy and demonstrated that monomer reactivity varied depending on the stage of polymerization.[32] For these reasons, reaction kinetics may be one explanation for narrow molecular weight distributions. On the other hand, although we have demonstrated that our mobile phase composition allows for reliable separations of aliphatic ammonium ionenes to achieve appropriate magnitudes of the weight-average molecular weight, this mobile phase may not perfectly separate these polyelectrolyte samples. If any adsorption is occurring between our polymer and the packing material of the SEC columns, imperfect separation will lead to an overestimate of the number-average molecular weight.[14,33] Thus, polydispersity of the sample will be underestimated. To investigate the possibly of sample adsorption, we injected an ammonium 12,12-ionene sample multiple times to evaluate the reproducibility of our SEC separations. As shown in Figure 12,

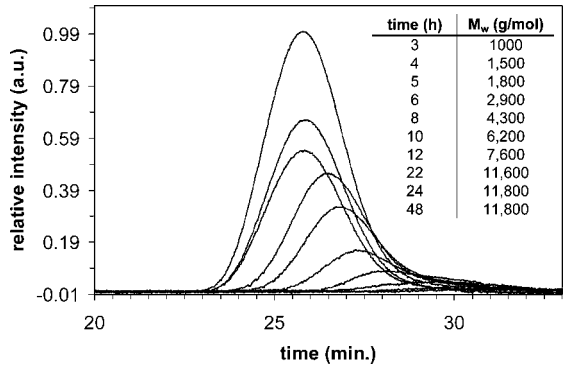

Figure 11. SEC chromatograms (MALLS traces) of aliquots sampled during a 24 h polymerization of ammonium 6,12-ionene showing molecular weight increased during the reaction. Inset table show weight-average molecular weight as a function of reaction time.

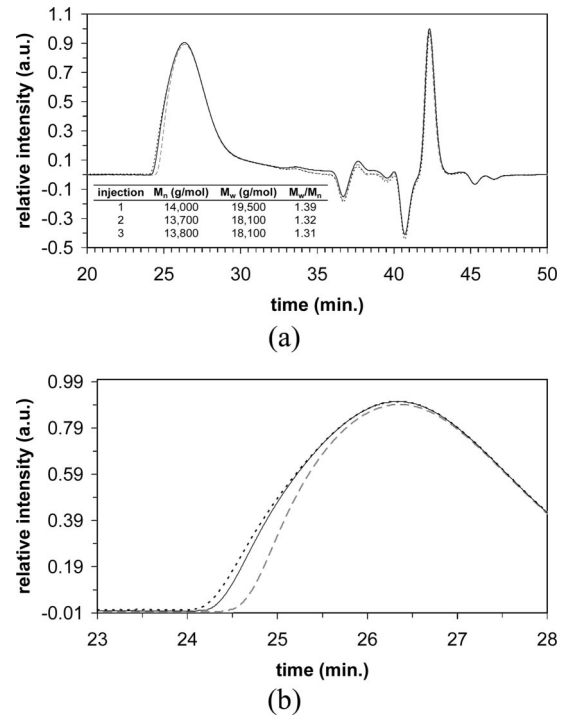

Figure 12. (a) Overlaid SEC chromatograms (dRI traces) of an ammonium 12,12-ionene injected three times in the mobile phase (54/23/23 (v/v/v %) water/methanol/glacial acetic acid, 0.54 M NaOAc, pH 4.0). Inset table shows SEC-MALLS caclulated molecular weights of each injection. (b) Zoomed-in portion of the chromatograms.

the dRI chromatograms do not superimpose perfectly, likely due to slight sample adsorption resulting from hydrophobic interactions between the polymer and the column stationary phase. However, the deviations in retention time associated with adsorption are much smaller when compared to differences in retention times between samples of different molecular weight. Also in Figure 12, the dRI chromatograms do not quite return to baseline, indicative of some sample adsorption.

The solvent compositions that were employed in previous SEC-dRI investigations of ammonium ionenes were evaluated using our instrumentation.[19,20] In two prior studies, one mobile phase consisted of 0.020 M NaOAc in water with acetic acid added to adjust the pH to 5.2, and a second mobile phase comprised 20/80 acetonitrile/0.5 M sodium sulfate, 0.5 M acetic acid in water. However, peaks corresponding to polymer were not observed in either the RI or MALLS chromatograms in either of these solvents.

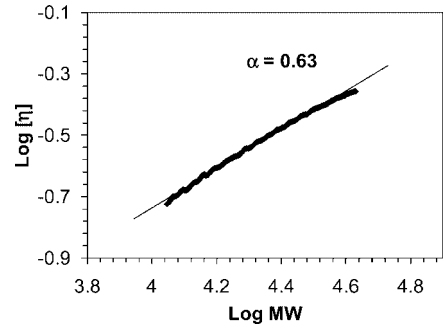

Figure 13. MHS plot for the ammonium 12,12-ionene (sample 5) in the mobile phase, 54/23/23 (v/v/v %) water/methanol/glacial acetic acid, 0.54 M NaOAc, pH 4.0. Measured intrinsic viscosities (black diamonds) were fitted with the logarithmic MHS relationship (dotted line).

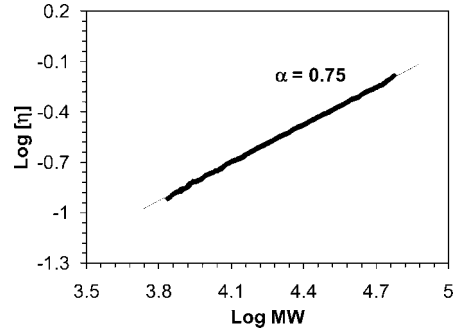

Figure 14. MHS plot for the ammonium 6,12-ionene (sample 8) in the mobile phase, 54/23/23 (v/v/v %) water/methanol/glacial acetic acid, 0.54 M NaOAc, pH 4.0. Measured intrinsic viscosities (black diamonds) were fitted with the logarithmic MHS relationship (dotted line).

Utilizing an online viscosity detector, the intrinsic viscosity was determined across the molecular weight distribution. When plotted as the logarithm of intrinsic viscosity as a function of the logarithm of molecular weight, a linear fit yields constants for the well-known Mark−Houwink−Sakurada (MHS) relationship, which is given as

$$\log [\eta] = \alpha \log M - \log k \qquad (3)$$

where $[\eta]$ is the intrinsic viscosity, and α and k are constants, which depend on the polymer and solvent used at a given temperature.[34] The MHS α parameter provides information about the solvent quality and/or chain stiffness for a polymer in solution. Typically, α values near 0.50 indicate Θ-solvent conditions whereas values near 0.80 indicate good solvent conditions.[30] Data obtained for both the 12,12- and 6,12-ionenes fit well to the linear MHS relationship. As shown in Figure 13, the MHS plot for the ammonium 12,12-ionene (sample 5) in the mobile phase (54/23/23 (v/v/v %) water/methanol/glacial acetic acid, 0.54 M NaOAc, pH 4.0) at 30 °C yielded an α parameter of 0.63 and a k value of 5.3×10^{-4} dL/g. As shown in Figure 14, the MHS plots for the ammonium 6,12-ionene (sample 8) in the same mobile phase at 30 °C yielded an α value of 0.75 and a k value of 1.6×10^{-4} dL/g. The higher α parameter of 0.75 for the 6,12-ionene suggested that the mobile phase was a better solvent for this sample compared to the 12,12-ionene and/or the 6,12-ionene has a stiffer chain conformation in the same solvent. The higher charge density of the 6,12-ionenes may lead to a greater polyelectrolyte effect, which could be a possible explanation for any observed chain stiffness.

Conclusions

Absolute molecular weight characterization of aliphatic ammonium ionenes was successfully accomplished using aqueous-based SEC-MALLS. A suitable mobile phase composition

of 54/23/23 (v/v/v %) water/methanol/acetic acid and 0.54 M NaOAc at pH 4.0 was developed to reduce polymer−polymer and polymer−stationary phase interactions. Using this mobile phase composition, reasonable separations were obtained and accurate measurements of the weight-average molecular weight were achieved. Ammonium 6,12-ionene had number-average molecular weights ranging from 14 000 to 41 500 g/mol and weight-average molecular weights ranging from 19 000 to 49 900 g/mol with molecular weight distributions ranging from 1.31 to 1.42. Ammonium 12,12-ionene had number-average molecular weights ranging from 8000 to 30 700 g/mol and weight-average molecular weights ranging from 11 000 to 40 000 g/mol with molecular weight distributions ranging from 1.26 to 1.42. Additionally, intrinsic viscosity data were collected for ammonium 12,12- and 6,12-ionens as a function of molecular weight distribution to determine MHS parameters.

The apparently low molecular weight distributions are currently being studied in our laboratories. We have eliminated the possibilities of fractionation during purification and pore volume exclusion in the column and thus have concluded that either the reaction mechanism is producing molecular weight distributions lower than 2.0 or, more likely, imperfect SEC separation is leading to an underestimation of the molecular weight distribution. Ongoing work correlating MALDI-TOF measurements to SEC-MALLS data will be reported in the future.

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