Characterization of Nonionic and Cationic Amine-Functional Polymers by Aqueous SEC-MALLS

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SYNOPSIS

Molecular weight, root-mean-square radius, and conformational information obtained from aqueous size exclusion chromatography (SEC) and multiangle laser light-scattering (MALLS) detection are examined for amine-functional polymers. These include homopolymers and copolymers of poly(ethenylformamide) and cationic poly(vinylamine). Light-scattering studies for poly(ethenylformamide) employing 15 angles of detection are used to calculate molecular weights as high as 700,000 daltons with a root-mean-square radius of nearly 50 nm. Calculated conformational coefficients indicate a random-coil configuration at low molecular weights and a more compacted conformation at high molecular weights. Molecular weights and root-mean-square radius values for cationic poly(vinylamine) are determined using a three-angle MALLS detector. Root-mean-square radius is strongly dependent on the ionic strength of the mobile phase. Cationic poly(vinylamine) exhibits a rod-like conformation at both low and high ionic strength. Explanations are proposed for this behavior. © 1996 John Wiley & Sons, Inc.

INTRODUCTION

N-Ethenylformamide (NEF) is a new, water-soluble monomer used in the polymerization of amine functional polymers such as poly(ethenylformamide), or PEF, which is a precursor for poly(vinylamine). Poly(vinylamine) contains primary amine functionality pendant to the polymer backbone and can exist as the free amine or cationic salt. This makes the polymer suitable for applications such as wastewater treatment, paper coatings, personal care products, textiles, and adhesives. NEF is very useful as the starting point for the synthesis of poly(vinylamine) because vinylamines are not stable and do not polymerize.

NEF (structure shown in Fig. 1) is readily polymerized by free radical initiators using precipitation, aqueous solution, or inverse emulsion polymerization. Depending on the polymerization process employed, the molecular weight of the resulting PEF can range from less than 50,000 daltons to greater than one million daltons. Aqueous solution or sol-

vent precipitation polymerization is usually used to synthesize homopolymers of PEF in the 50,000 to 500,000 molecular weight range with polydispersities of 2.0 to 3.5. High molecular weight homopolymers of PEF of one million daltons or greater require preparation by inverse emulsion polymerization. NEF monomer can also be copolymerized with a variety of unsaturated monomers such as vinyl acetate, acrylamide, butyl acrylate, and methyl methacrylate. Copolymerization with vinyl acetate allows, via base hydrolysis, the synthesis of copolymers of vinyl alcohol.[1]

Water-soluble homopolymers of PEF may be hydrolyzed with either strong acids or bases as shown in Figure 1. Poly(vinylamine) in the free base form may be prepared by adding an equivalent amount of base for the desired degree of hydrolysis. Poly(vinylamine) hydrochloride salt (PVam-HCl) may be prepared by adding an amount of acid equivalent to the desired degree of hydrolysis, resulting in a cationic polymer. The physical properties and performance of PEF, free base poly(vinylamine), and cationic PVAm-HCl are strongly dependent on the molecular weight and molecular weight distribution. For example, the relationship of solution viscosity
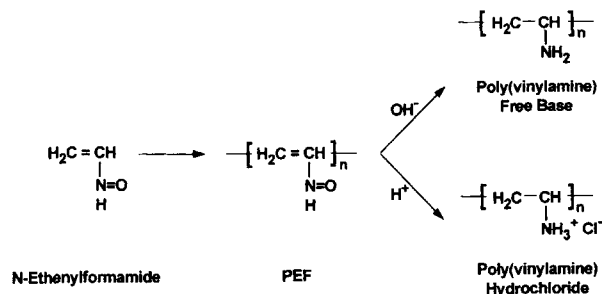
Figure 1 Polymerization scheme of N-ethenylformamide.

and molecular weight of PEF is linear over a wide range.[1] In applications such as waste-water treatment, the efficiency of particulate flocculation is strongly dependent on the molecular weight of PVAm-HCl. Thus, it is critical that sensitive and reliable methods such as aqueous size exclusion chromatography (SEC) be employed for molecular weight analysis. In addition to molecular weight, the determination of polymer size is an important physical property measurement. For example, size determination is very useful for studying the diffusion of a polymer through a porous substrate, the effect on solution viscosity or thickening, and the sensitivity of an ionic polymer, such as PVAm-HCl, to various salt conditions. Light scattering, particularly the angular variation of scattered light, is the principal means for characterizing polymer size in solution. The use of aqueous SEC coupled to multiangle laser light-scattering (MALLS) detection is a powerful tool for the measurement of absolute molecular weight and size of amine-functional polymers.

MALLS has added a new dimension to the characterization of PEF and cationic PVAm-HCl over other techniques such low-angle laser light scattering (LALLS) and differential viscometry. As will be described, SEC-MALLS provides absolute molecular weight, size, and conformational information. MALLS is a primary technique because it can be used to determine molecular weight and size in solution, independent of elution volume and without the need for column calibration. MALLS measures the angular dependence of scattered light that directly results in the determination of polymer size. This unique capability distinguishes the technique from a single, angular measurement such as LALLS, which provides only molecular weight and no size or conformational information.

Polymer size is often referred to as the "radius of gyration." However, true polymer size is more correctly defined as the root-mean-square (RMS) radius, typically the z-average when a distribution of molecular sizes is present. The RMS radius describes the size of a polymer in solution independent of its shape (see next section). Once molecular weight and RMS radius of a polymer are determined over a distribution, it then becomes possible to examine conformation or shape.[2]

This work is intended to demonstrate the usefulness of MALLS technology for this important class of water-soluble polymers based on NEF. Applications of aqueous SEC-MALLS for characterizing molecular weight, RMS radius, and conformation of PEF and homopolymers and copolymers of cationic poly(vinylamine) are presented. The use of two types of MALLS detectors (15 angles and 3 angles of detection) are discussed. SEC of free base poly(vinylamine) is not presented and is reserved for future discussions.

MALLS THEORY FOR MOLECULAR WEIGHT AND SIZE

For solvated molecules at low concentrations, the weight-average molecular weight (M_w) and the z-average mean square radius can be determined from the expanded form of the Rayleigh equation:

$$Kc/R(\theta) = (1/M_w)[1 + (16\pi^2/3\lambda_o^2)$$
$$\times \langle r_g^2 \rangle \sin^2(\theta/2)] + 2A_2c \quad (1)$$

where $R(\theta)$ is the light scattered by the solution at an angle θ in excess of that scattered by the pure solvent divided by the incident light intensity, c is the molar concentration, λ_o is the vacuum wavelength of the incident light, $\langle r_g^2 \rangle$ is the mean-square radius of gyration, and A_2 is the second virial coefficient. The quantity $\langle r_g^2 \rangle^{1/2}$, the root-mean-square (RMS) radius, can be defined as:

$$\langle r_g^2 \rangle^{1/2} = [(1/N) \sum_{i=1}^{N} \langle r_i^2 \rangle]^{1/2} \quad (2)$$

where $\langle r_i^2 \rangle$ is the average square distance of the ith mass element from the center of gravity over all conformations. The optical constant, K, is given as:

$$K = 4\pi^2 n_o^2 (dn/dc)^2/[\lambda_o^4 N_A] \quad (3)$$

where n_o is the refractive index of the solvent, dn/dc is the specific refractive index increment of the polymer at the same wavelength of light as the light-scattering instrument, and N_A is Avogadro's number.

Molecular weights are calculated across all increments of a chromatogram using the Debye plot of $R(\theta)/Kc$ vs. $\sin^2(\theta/2)$. The y-intercept is the weight-average molecular weight, M_w, and the slope is $\langle r_g^2 \rangle$. Under conditions normally used in SEC, the quantity $2A_2c$ is usually $\ll 1$ and can be ignored. Once the concentration, c_i, molecular weight, M_i, and $(RMS)_i$ radius have been determined for each slice along the entire chromatogram, the molecular weight and RMS radius moments can be calculated using the normal relationships for number, weight, and z-averages.[3] In reality, each slice of the chromatogram contains a slight polydisperse mixture of species, and the quantities calculated for each slice are really the weight-average molecular weight (M_w) and z-average RMS radius. This tends to promote, for example, a slight overestimation of the number-average molecular weight (M_n) of the distribution. However, due to the excellent resolution of today's high performance SEC columns, this overestimation is small and can usually be neglected.

EXPERIMENTAL PROCEDURES

Figure 2 is a schematic of the instrument setup used for aqueous SEC-MALLS measurements of PEF and PVAm-HCl. The MALLS photometer was configured between the SEC instrument and a refractive index (RI) detector, used for measuring concentration. For studies involving PEF, the SEC consists of a Waters 150C GPC interfaced to a Waters Model 410 RI detector (Waters Corporation, Milford, MA) and a Wyatt Technology Model Dawn-F or Mini-Dawn MALLS photometer (Wyatt Technology Corporation, Santa Barbara, CA). The Dawn-F utilizes a 10 milliwatt, 488 nm argon-ion laser with vertically polarized light and up to 15 fixed angles of detection (35.5 to 144.5°). A 488 nm wavelength provides increased signal intensity compared to the standard 632 nm wavelength laser provided with the photometer (signal intensity is inversely related to the fourth power of λ). A set of Toyo Soda TSK-PW columns (Phenomenex, Torrance, CA) were used with an aqueous mobile phase of 0.05 M sodium nitrate prepared in Milli-Q water (Millipore Corporation, Bedford, MA) for the separation of PEF. The SEC was operated at 35°C with a flow rate of 1.0 mL/min. Molecular weight, RMS radius, and molecular weight distribution were calculated using ASTRA-EASI software from Wyatt Technology. Specific refractive index increment values (dn/dc) were measured on a Wyatt Technology Optilab Model 903 Photometer (λ = 488 nm).
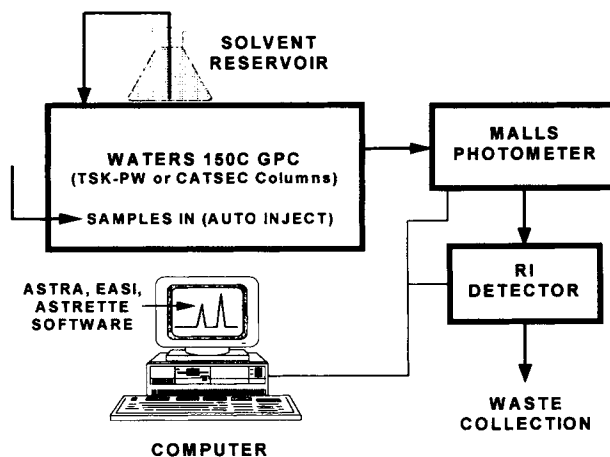

Figure 2 Experimental setup.

Homopolymers and copolymers of PVAm-HCl were characterized using a Wyatt Technology Mini-Dawn MALLS photometer under conditions similar to those described above. The Mini-Dawn utilizes three angles of detection (45, 90, and 135°) with a 690 nm, 20 milliwatt photodiode laser. A set of CATSEC columns (SynChrom, Lafayette, IN) were used for the separation of PVAm-HCl polymers at a flow rate of 0.4 mL/min at 35°C. The mobile phase consists of various concentrations of sodium nitrate from 0.005 to 0.30M with 0.1% trifluoroacetic acid (TFA) at pH = 2.5. Different salt concentrations were used to examine the effect on polymer RMS radius. Molecular weight and RMS radius calculations were calculated using ASTRETTE software from Wyatt Technology. Specific refractive index increment values were measured on an Optilab Model 903, which uses a wavelength of 690 nm, the same as the Mini-Dawn.

Both the Dawn-F and Mini-Dawn require instrument calibration and normalization of the photodiode detectors that surround the flow cell and monitor light intensity at a given angle. Instrument calibration is required because no measure of the transmitted laser light intensity through the sample is possible due to the flow-cell configuration. Because the photodiode detectors exhibit different gains, normalization is necessary.[3] Normalization must be performed with an isotropic scatterer, usually a low molecular weight polymer. A monodisperse, polysaccharide standard (pullulan) with a molecular weight of 23,700 daltons and polydispersity of 1.07 was used (Polymer Laboratories, Amherst, MA). It is important that these normalization constants be calculated using an RMS radius value of 5 nm for this polysaccharide standard.

Table I Comparison of Molecular Weights of Polysaccharide Standards using the MALLS Dawn-F

M_w, Vendor	M_w, MALLS	M_n, MALLS	M_w/M_n, MALLS
5800	6200	5900	1.04
12,200	12,600	12,100	1.04
23,700	23,500	22,000	1.06
48,000	51,700	49,700	1.04
100,000	105,000	100,000	1.05
186,000	207,000	195,000	1.06
380,000	391,000	365,000	1.07
853,000	857,000	793,000	1.09

dn/dc = 0.143 mL/g (supplied by Wyatt Technology).

Eight polysaccharide standards ranging in molecular weight from 5800 to 853,000 daltons were used to check the accuracy of the MALLS system. Molecular weight results were compared to those supplied by the vendor (Polymer Laboratories) and are summarized in Table I. There is excellent agreement of the SEC-MALLS with the vendor-supplied M_w values. The difference is 8% or less for all molecular weights except for the 186,000 dalton standard, in which case, the difference is 11% between the MALLS and vendor M_w values. The precision of our SEC-MALLS measurements using Airvol® poly(vinyl alcohol), or PVA, is ±3.6% for M_n and ±1.1% for M_w.

RESULTS AND DISCUSSION

Characterization of PEF Using the Dawn-F

The specific refractive index increment (dn/dc) is the one external value that must be measured or known before molecular weight distribution data can be determined from MALLS. The value for dn/dc must be measured at the same wavelength and mobile phase conditions as that of the MALLS experiments. PEF exhibits a value for dn/dc of 0.155 mL/g (measured on the Optilab 903). As a point for comparison, fully hydrolyzed poly(vinyl alcohol) exhibits a similar value of 0.150 mL/g under the same conditions. The magnitude of dn/dc suggests that PEF should exhibit reasonably good light scattering intensity signals even at low molecular weights.

The use of an aqueous-based mobile phase for measuring light scattering can prove quite challenging because water is a notorious scatterer of contaminants and dust, especially at low angles. In this regard, light-scattering measurements in organic solvents such as toluene or tetrahydrofuran are considerably easier to carry out. The use of an aqueous mobile phase requires proper filtering and preparation for light-scattering analyses such as MALLS. A convenient way to examine the light-scattering response from each angle of detection is shown in Figure 3. The chromatograms for a PEF (M_w = 84,400 daltons) from all 15 angles of detection (including the RI chromatogram) are overlaid as a function of detector angle. Detector number 4 is 35.5°, the lowest accessible angle on the Dawn-F for the cell configuration used (K5 type). Detector number 11 corresponds to 90° and detector number 18 is 144.5°. Note the low degree of baseline noise (low amount of extraneous scattering) in the chromatogram at 35.5°, as shown in Figure 4. This angle is the most sensitive to excess scatter. For this work, our mobile phase was filtered through a 0.22 micron membrane and was allowed to recycle through the SEC during times when MALLS measurements were not being made. This example illustrates the importance of using clean, prefiltered solvents for performing MALLS measurements under aqueous conditions. With proper care and patience, it is possible to obtain chromatographic light-scattering re-
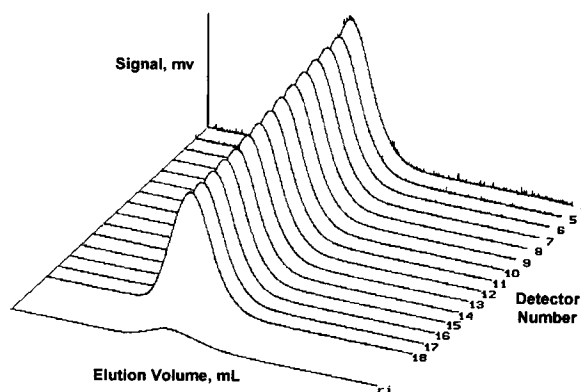

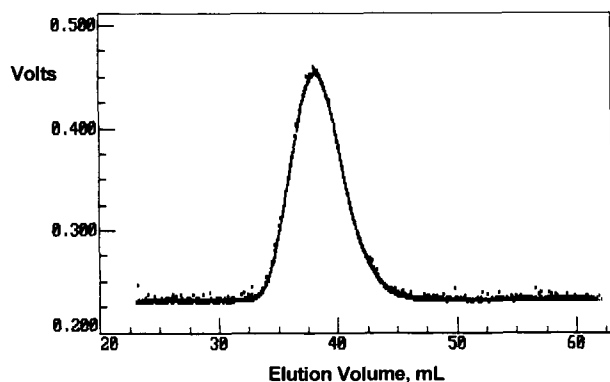**Figure 3** MALLS chromatograms for PEF.

Figure 4 35.5° Chromatogram for PEF in 0.05M sodium nitrate.

Figure 5 Debye plot for PEF.

sults as good as those from organic-based SEC-MALLS.

It is also important to note in Figure 3 that the chromatograms exhibit a small, but gradual increase in intensity with decreasing detector angle (decreasing detector number). It is this angular variation that enables determination of the RMS radius. Molecular weights are determined from a Debye Plot across all increments of the chromatogram, as shown in Figure 5, for PEF with a molecular weight of 118,000 daltons.

Calculation of molecular weights using SEC-MALLS does not necessarily require input of an exact value for the mass of polymer injected if the dn/dc value is known. This procedure is known as the "dn/dc Method in the ASTRA software. Although it does not require prior knowledge of total injected mass, it does require values for the RI detector instrument constant and the dn/dc value of the polymer. The polymer concentration is calculated from the sample RI detector signal, the RI detector constant, and the value of dn/dc. Another procedure known as the "Mass Method" calculates the concentration of polymer in each slice from the known injected mass for the peak, the sample RI detector signal, and the volume of the slice. The RI detector constant is used to calculate a value for dn/dc of the polymer sample. This value is subsequently used in the calculations for molecular weight. One other procedure is available in the ASTRA software known as the "Combined Method." The Combined Method uses the injected mass to calculate the concentration for each data slice and the value of dn/dc to calculate molecular weights. The RI detector constant is not used.[3]

The Mass Method will calculate an accurate value for molecular weight and dn/dc if the total mass of polymer injected is accurately known and totally
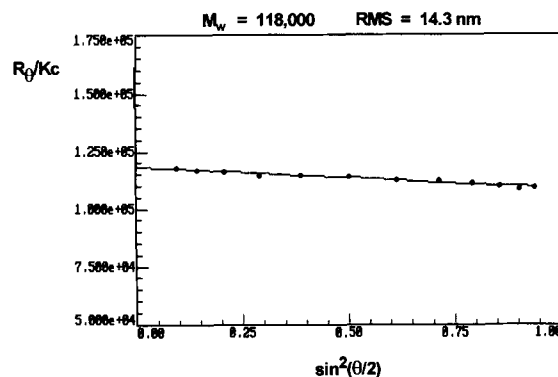
elutes through the SEC columns. However, these conditions may not always be applicable and the Mass Method should be used with some caution, especially for polydisperse samples. On the other hand, the dn/dc Method can prove quite useful because it calculates a theoretical mass eluted through the columns (and detectors). Percent recovery of the polymer through the SEC can then be determined. Figure 6 exhibits RI and 90° chromatograms for a PEF with the limits (shown as vertical arrows to the left and right of the chromatograms) for the molecular weight calculations. Over these limits, a theoretical mass injected was calculated as 0.57 mg using a dn/dc = 0.155. The actual mass was 0.59 mg, determined from the known concentration of the PEF solution and the injected volume of 0.200 mL. The percent recovery was 103%. However, if this PEF was initially present as a solution in which the concentration of PEF was not precisely known (for example, approximately 20% weight solids), we would still be able to calculate molecular weight values using dn/dc = 0.155. This procedure can also prove very useful when measuring the molecular
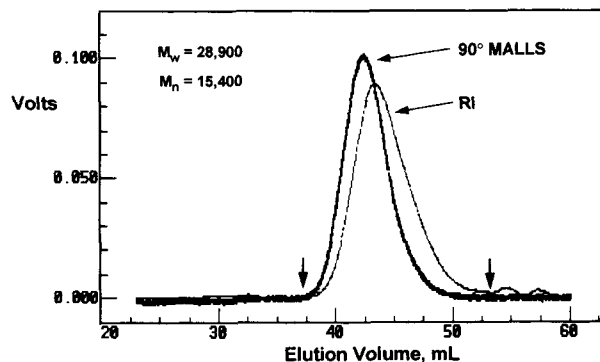

Figure 6 RI and 90° chromatograms of PEF with calculation limits (shown as vertical arrows).

weight of PEF in a formulation in which the exact concentration of the polymer is unknown.

Weight-fraction molecular weight distribution curves for three molecular weights of PEF are shown in Figure 7. The lowest molecular weight PEF exhibits M_w = 84,400 daltons with M_w/M_n = 1.9 (polydispersity). The two higher molecular weight PEF samples exhibit M_w = 293,000 and 366,000 daltons, each with M_w/M_n = 4.4. Polydispersity values of PEF generally range from 1.5 to 5.0, which are fairly typical of free radical polymerizations.

The data in Table II summarize M_w and RMS radius values for eight different molecular weights of PEF ranging from approximately 40,000 daltons to 700,000 daltons. The data show the expected trend in which RMS radius increases with increasing molecular weight from approximately 9 to 48 nm.

RMS radius data for low molecular weight polymers are particularly challenging because they exhibit little or no angular variation in scattered light intensity. The data in Table II raise the question as to how small an RMS radius can be measured for PEF. The minimum radius that can be determined by light scattering is $(\lambda/20)/2$, where $\lambda = \lambda_o/n_o$.[4] For our work, n_o = 1.332 (the refractive index of the aqueous mobile phase) and λ_o = 488 (the laser wavelength). From this calculation, the lowest measured value for the RMS radius should be 9 nm. It was found that a PEF with M_w = 40,600 daltons was the lowest molecular weight that would yield an acceptable value for the RMS radius. Lower molecular weights yielded either zero values or significantly higher values than 9 nm for RMS radius. At these low molecular weights, there is little or no angular variation in scattered light intensity, which yields erroneous values for an RMS radius. Our lowest measured value for an RMS radius of 9.5 nm is in good agreement with the calculated lower limit of 9.0 nm.
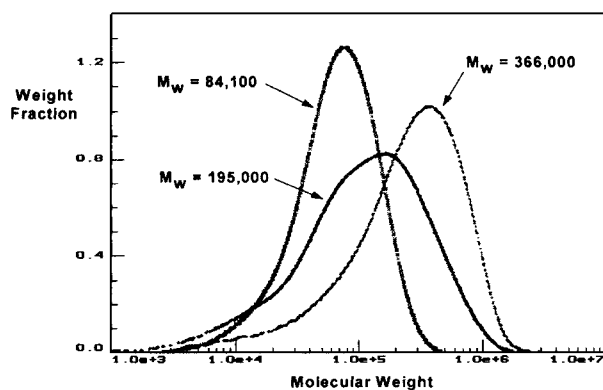

Figure 7   Molecular weight distributions of PEF.

Table II   MALLS Data for PEF in 0.05 M Sodium Nitrate

M_w	RMS Radius, nm	α
40,600	9.5	0.54
84,400	15.3	0.50
145,000	19.2	0.42
195,000	23.2	0.39
222,000	25.3	0.37
276,000	27.8	0.43
366,000	30.3	0.37
700,000	48.3	0.35

The measurement of molecular weight and RMS radius provides the means to examine the conformational characteristics of a polymer using the relationship:

$$RMS = kM^{\alpha}, \tag{4}$$

where α is the conformational coefficient and k is the proportionality constant. It is known that α = 1.0 for a rigid rods, 0.5 for random coils in a theta solvent, and 0.33 for a sphere. Flory has shown that the value for α of a polymer in a good solvent is 0.55–0.60.[5] The conformational coefficient is determined from the slope of a log–log plot of RMS radius vs. molecular weight.

The data in Table II also include the measured conformational values for PEF as a function of molecular weight for each sample. At low molecular weight ($<$ 100,000 daltons), the α value suggests a random coil configuration for PEF. The 0.05M sodium nitrate aqueous mobile phase solution is closer to that of a theta solvent for PEF than that of a thermodynamically good solvent. The data show a trend in which α decreases with increasing molecular weight of PEF. This suggests a more compact or tightly coiled structure at higher molecular weight, possibly due to branching. A plot of log(RMS) vs. log (molecular weight) for a medium weight PEF is shown in Figure 8 (M_w = 222,000 daltons, RMS = 25.3 nm). For this molecular weight of PEF, the slope corresponds to an α value of 0.37 or that of a conformation intermediate between a sphere and a random coil in a theta solvent, probably as a consequence of polymer branching. Polymer conformation data for PEF and several other common, water-soluble polymers that we have examined by aqueous SEC-MALLS are summarized in Table III. Fully hydrolyzed poly(vinyl alcohol), partially hydrolyzed poly(vinyl alcohol), poly(ethylene oxide), polysaccharide, along with PEF exhibit values typ-
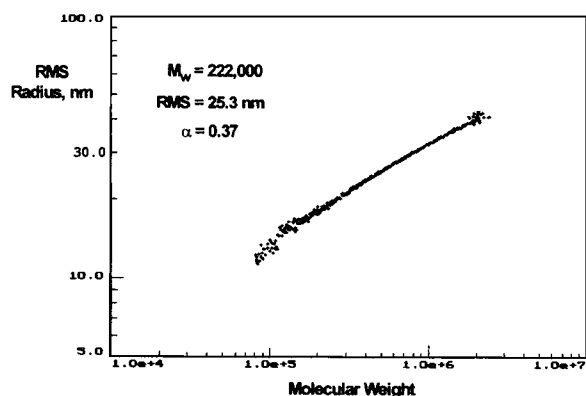
Figure 8 Conformational plot of PEF.

Table III Conformational Coefficients for PEF and Selected Nonionic Water-Soluble Polymers in 0.05 M Sodium Nitrate

Polymer	α
PEF	0.54–0.35
Fully hydrolyzed PVA	0.50
Partially hydrolyzed PVA	0.48
Poly(ethylene oxide)	0.49
Poly(saccharide)	0.56
Dextran	0.38

ical for random coil polymers. The α value of 0.38 for the commercial dextran is consistent with the fact that this material is highly branched. Similar values have also been reported using aqueous SEC-MALLS.[6]

Copolymers of PEF and PVA can be readily chromatographed and analyzed by MALLS detection as in the case for PEF homopolymers. RI and 90° chromatograms of a poly(vinyl alcohol)-PEF copolymer (12 mol % PEF) with $M_w = 137{,}000$ daltons and RMS = 24.3 nm are shown in Figure 9. The measured conformational coefficient for this sample is 0.44, somewhat less than that observed for low molecular weight PEF homopolymer and partially hydrolyzed PVA. This result suggests a more tightly coiled conformation.

Characterization of Cationic Poly(vinylamine) Using the Mini-Dawn

Cationic polymers, such as the acid-hydrolyzed product of PEF, PVAm-HCl, can be readily characterized by aqueous SEC-MALLS. As a polyelectrolyte, PVAm-HCl and other cationic polymers will elute without adsorption on SynChrom CATSEC columns using the proper mobile phase, ionic strength, and pH. CATSEC columns contain a silica-based support with crosslinked poly(ethylene imine) bound on the packing particle surface. This imparts a cationic surface charge on the packing that can be "controlled" with the proper level of salt in the mobile phase. Proper SEC of cationic polymers requires that polymer/solvent/packing interactions such as ion exchange, ion exclusion, adsorption, and partition be eliminated or significantly minimized.[7] The ionic strength of the mobile phase can be optimized to effect separation by size alone and will directly affect the elution volume and the RMS radius value of the polymer. The use of CAT-

SEC columns for cationic polymers has been previously reported by this author.[8,9]

The accuracy of the Mini-Dawn was examined by characterizing the molecular weight of a series of cationic, poly(2-vinyl pyridines) from American Polymer Standards Corporation. A cationic, poly(allylamine) hydrochloride was also analyzed, because this polymer had previously been characterized by SEC with on-line, differential viscometry detection.[8] The data for poly(2-vinyl pyridine) and poly(allylamine) are summarized in Tables IV and V, respectively.

The poly(2-vinyl pyridine) standards do not exhibit as narrow a molecular weight distribution as the polysaccharide standards. Weight-average molecular weights calculated by MALLS using the Mini-Dawn generally show good agreement with the vendor-supplied molecular weights. In particular, for the standards less than 10,000 daltons the agreement is not as good as in the case with the polysaccharide standards. This may be a consequence of the lower limit of detection of MALLS that is dependent upon the dn/dc of the polymer, the injected mass, and the laser wavelength. From our work using SEC-MALLS, 1000 to 2000 daltons is considered to be the lower limit of detection. For the standards
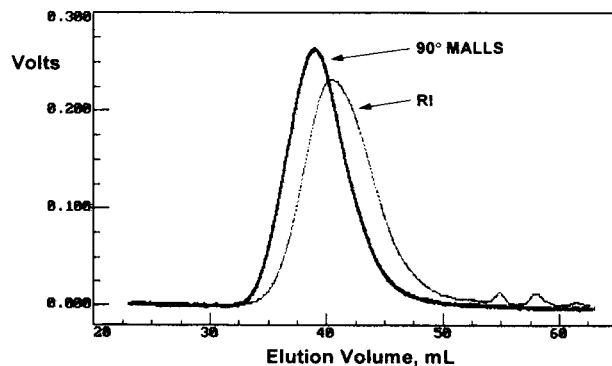

Figure 9 RI and 90° MALLS chromatograms of PVA-PEF copolymer in 0.05 M sodium nitrate.

Table IV Comparison of Cationic Poly(2-vinyl pyridine) Molecular Weights using the MALLS Mini-Dawn

M_w, Vendor	M_w, MALLS	M_n, MALLS	M_w/M_n, MALLS
3300	4600	4000	1.15
8000	9900	8700	1.14
13,100	13,900	11,600	1.20
34,700	36,200	23,600	1.53
105,000	100,000	85,200	1.17
159,000	153,000	141,000	1.09
309,000	285,000	216,000	1.32
460,000	469,000	391,000	1.20

dn/dc = 0.250 mL/g.

greater than 10,000 daltons, the M_w values from MALLS and those supplied by the vendor agree quite well (within 8% or less). The MALLS molecular weights of the poly(allylamine) hydrochloride from the Mini-Dawn are in good agreement with those previously measured by SEC-differential viscometry.

A chromatogram of a PVAm-HCl homopolymer (detector angle = 45°) is shown in Figure 10 using a mobile phase of 0.05 M sodium nitrate with 0.1% TFA. A low degree of excess baseline scatter is readily observed as was the case with the Dawn-F at low scattering angles. This type of behavior is a prerequisite for accurate calculation of molecular weight and RMS radius values due to the use of only three angles of detection compared to 15 angles for the Dawn-F.

The dn/dc values of PVAm-HCl and PVA-VAm-HCl, determined on the Optilab 903 at 690 nm in 0.05 M NaNO$_3$/0.1% TFA, are 0.214 and 0.162 mL/g, respectively. However, these measurements were not made on dialyzed samples of the PVAm-HCl and PVA-VAm-HCl, and the dn/dc does not reflect the true value of the polymer backbone. This is due to the fact that the original counterions are, for the most part, replaced by the counterions of the supporting electrolyte in the mobile phase, which are present in large excess. This ion exchange modifies
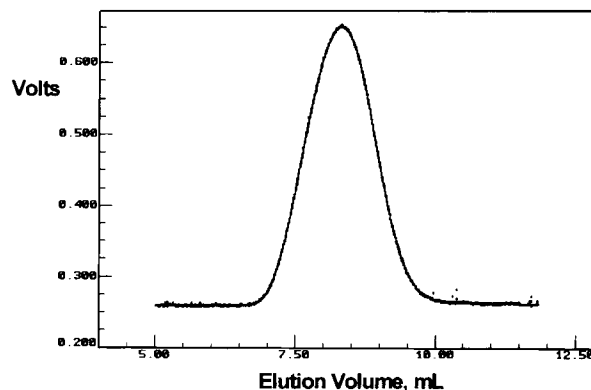
the chemical nature of the polymer in solution and the measured molecular weight can differ considerably from the true value of the polymer chain.[4] If the counterions are chemically the same as the ionic species that make up the solution electrolyte, and if the dn/dc was determined using a polymer solution dialyzed against this salt, then the molecular weight measured is that of the polymer chain and does not include the molecular weight of the associated counterions. Problems arise if the counterions on the chain are different from those in solution and the dn/dc was not determined via dialysis. The molecular weight that is obtained would be an apparent value that would depend on the number of "foreign" counterions associated with the chain and their dn/dc.

One way around this problem is to use the Mass Method for the calculation of molecular weight. This was done for a PVAm-HCl sample and the molecular weight was compared to that of the original PEF from which it was acid hydrolyzed. Figure 11 illustrates the two molecular weight distributions: PEF with M_w = 146,000 from the Dawn-F (using previ-

Table V Comparison of Poly(allylamine) Molecular Weight from SEC-MALLS and Differential Viscometry

	M_w	M_n	M_w/M_n
SEC-MALLS[a]	105,000	60,500	1.7
Differential viscometry	109,000	67,600	1.6

[a] dn/dc = 0.195 mL/g, RMS = 16.6 nm, α = 0.82.

Figure 10 45° Chromatogram of PVAm-HCl from the Mini-Dawn.

ously described conditions) and the corresponding acid hydrolyzed PVAm-HCl from the Mini-Dawn. If the all the amide groups on the PEF were completely hydrolyzed, then a molecular weight of 164,000 daltons would be expected for the PVAm-HCl. In actuality, we measure a molecular weight of 160,000 daltons using the Mass Method for calculation (assuming 100% recovery through the columns). Because a known mass of PVAm-HCl was used, the calculated dn/dc is that of the polymer backbone alone (with Cl^-) and not any associated nitrate (or TFA) counterions. It was determined from carbon-13 NMR analysis that this particular PEF was fully converted to PVAm-HCl via acid hydrolysis. The close agreement of the calculated and theoretical M_w values bear this out.

Figure 12 is a plot of molecular weight (calculated directly using ASTRETTE) vs. elution volume for a PVAm-HCl homopolymer (M_w = 95,000 daltons) for three ionic strength levels (with 0.1% TFA) in the mobile phase. These include no sodium nitrate, 0.02 M sodium nitrate, and 0.20 M sodium nitrate. As the ionic strength of the mobile phase increases, the polycation coil size decreases, and further, suppression of electrostatic double layer effects between the packing and polymer results in an increase in elution volume, as more pore volume in the SEC packing becomes accessible to the polymer. At low ionic strengths, the increased level of electrostatic repulsion between packing and polymer contributes to less accessible pore volume and lower elution volumes. This type of effect has been previously measured using on-line differential viscometry detection
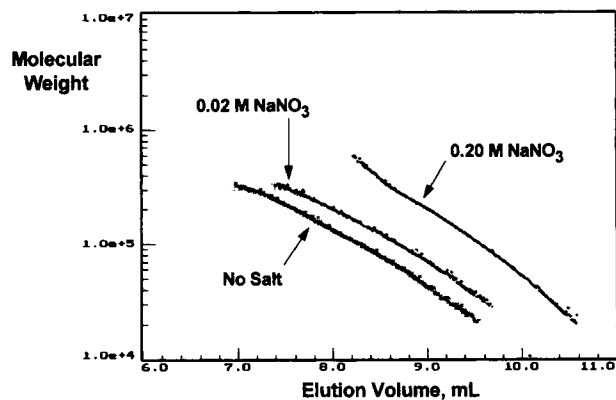

Figure 12   Molecular weight vs. elution volume for PVAm-HCl at various mobile phase ionic strengths.

for cationic polymers.[9] This is the first reported use of MALLS detection for investigating electrostatic effects on cationic vinylamine-based polymers.

RMS radius values determined from the Mini-Dawn can be used to ascertain ionic strength effects. Figure 13 is a plot of RMS radius vs. concentration of sodium nitrate for a PVAm-HCl homopolymer, a PVA-VAm-HCl copolymer and a fully hydrolyzed PVA homopolymer (nonionic). The RMS radius for the PVAm-HCl and PVA-VAm-HCl exhibit the expected decrease with increasing ionic strength. The increased salt concentration of the mobile phase contributes to suppression of intramolecular screening and repulsive effects of the cationic PVAm-HCl, resulting in a decreased hydrodynamic size. The PVA-VAm-HCl copolymer, which contains approximately 6 mol % of vinylamine present as the hydrochloride salt,
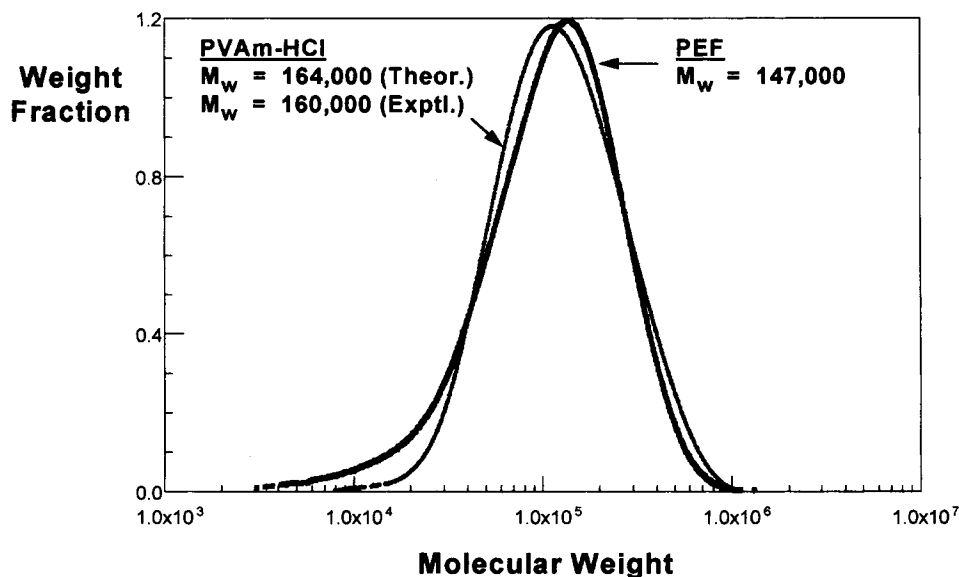

Figure 11   Molecular weight comparison of PVAm-HCl and PEF precursor.

decreases less than the PVAm-HCl due to the lower amount of cationic charge along the polymer backbone. The PVA exhibits virtually no change in RMS radius due to its nonionic character.

Conformational coefficients for PVAm-HCl and PVA-VAm-HCl are summarized in Table VI. The α values for the PVA-VAm-HCl copolymer fall in the range from 0.43-0.62, close to the expected range for a random coil (0.5-0.6). The values for the homopolymer PVAm-HCl (0.64-0.76) are all higher than those for a random coil. These data suggest that the homopolymer exhibits a more rod-like conformation at low and high ionic strengths. However, a more likely explanation of these data may lie in the accuracy of measuring conformational values (and RMS radius values) with the Mini-Dawn. Extrapolating these light-scattering data to zero angle using only three angles can not be applied with the same level of confidence as with the Dawn-F, which utilizes up to 15 angles. The uncertainty arises from the fact that fewer data points are available for the extrapolation and that large polymer molecules exhibit nonlinear behavior due to the significance of higher order terms in the particle-scattering function.[10] These results suggest that further experimentation is required with the Dawn-F. It remains to be determined if the higher number of angles improves the accuracy of conformational measurements, particularly on polyelectrolytes, which are not very high in molecular weight.

SUMMARY

Aqueous SEC-MALLS is a powerful technique for characterizing amine functional polymers because both molecular weight and hydrodynamic size can be directly measured. Molecular weights have been measured from less than 50,000 to over 1,000,000 daltons. Our studies show that PEF exhibits a ran-
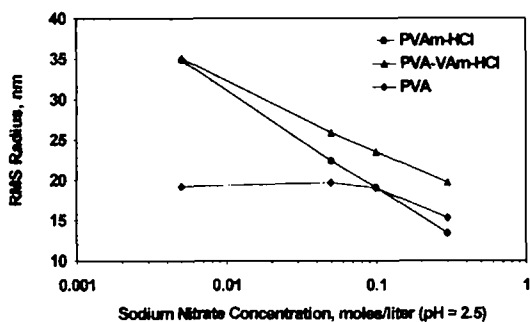
Figure 13 RMS radius vs. ionic strength using the Mini-Dawn.

Table VI   Conformational Coefficients using the Mini-Dawn

Conc. NaNO$_3$	PVAm-HCl	PVA-VAm-HCl
0.005	0.64	0.62
0.050	0.63	0.53
0.100	0.78	0.55
0.300	0.76	0.62
0.500	*	0.43

* Sample precipitated out of solution.

dom coil conformation consistent with those of other water-soluble polymers such as poly(ethylene oxide), poly(vinyl alcohol), and polysaccharide. The RMS radius of cationic PVAm-HCl and cationic PVA-VAm-HCl copolymers is strongly dependent on the ionic strength of the mobile phase and can be measured using the Mini-Dawn (three angles of detection). This work should provide a framework for further study and for improved understanding of molecular weight, size, and conformation of various types of other water-soluble polymers.

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