

Mass Analysis of Water-Soluble Polymers by Mobility Measurement of Charge-Reduced Ions Generated by Electrosprays

Daniel A. Saucy,^{*,†} Sven Ude,[‡] I. Wuled Lenggoro,[§] and Juan Fernandez de la Mora[‡]

Chemical and Mechanical Engineering Departments, Yale University, 9 Hillhouse Avenue, New Haven, Connecticut 06520-8286, Rohm and Haas Company, 727 Norristown Road, Spring House, Pennsylvania 19477, and Department of Chemical Engineering, Graduate School of Engineering, Hiroshima University, 4-1 Kagamiyama 1 Chome, Higashi-Hiroshima, Hiroshima 739-8527, Japan

Aqueous solutions of poly(ethylene glycol) (PEG) in a 10 mM ammonium acetate buffer are electrosprayed, and the maximum charge state on the resulting gas-phase ions is reduced to unity using a radioactive source. The mobility distribution of these charged particles is then measured in air in a differential mobility analyzer of unusually high resolution. The relation $Z(m)$ between the mobility Z of a polymer molecule and its mass m is determined by means of narrowly distributed PEG mass standards. The molecular weight range of available standards is extended by generating clusters containing from one up to six molecules of the primary PEG standard. The mass at the peak of the distribution of the lowest standard (PEG-4k) is determined by MALDI mass spectrometry and agrees with the manufacturer's value and previous MALDI literature data. The masses for the 50K and 120K standards are found to differ by 8.6 and 6.6%, respectively, from the manufacturer's value. Using known relationships, the particle diameter d of the ions is calculated from the measured mobility. Plots of d versus $m^{1/3}$ give straight lines over the full mass range studied (4000–700 000 Da, particle diameter from 3 to 12 nm), indicating that these PEG particles are indeed spherical and have a density ρ independent of size. The slope of the d versus $m^{1/3}$ curve provides a density $\rho = 1.25 \text{ g/cm}^3$, close to the known bulk density, $\rho_{\text{PEG}} = 1.21 \text{ g/cm}^3$.

One of the goals in polymer characterization is often the determination of the molecular weight distribution (MWD) and from it, various moments or averages. Ideally this distribution is absolute; i.e., the mass axis is true molecular mass. The most common approach to obtaining the MWD is to use gel permeation chromatography (GPC). For successful GPC, the polymer must dissolve in a solvent that is compatible with GPC column packings and avoids adsorption interactions with the column. For copolymers, finding a solvent that meets these requirements is sometimes not possible. Furthermore, unless combined with light

scattering or viscometry, the result will not be an absolute one, but rather, relative to some standard.

More recently, mass spectrometry has become widely applied to determining MWDs. Its critical asset is that the molecular weight axis of the data is absolute. However, the mass spectrometric determination of average molecular weight and MWD of industrially important polymers has been much less successful than that of biopolymers due to several complicating reasons. The electrospray¹ (ES) process yields ions with a range of charge states (z). When this range is convoluted with the broad molecular weight distribution of the polymer to be analyzed, the result is severe spectral overlap since the ions are separated according to their m/z ratio. This overlap often makes it impossible to determine the true MWD of the polymer when the average molecular weight exceeds $\sim 10\,000$ Da. In contrast, since biopolymers are typically nearly monodisperse, the spectral overlap problem is practically nonexistent and multiple charging, rather than being a hindrance, can often be used to advantage for polymers whose mass is beyond the range of the analyzer but whose m/z , because of multiple charging, is not.

Polymer ions formed by electrospray can be stretched and carry a level of charge proportional to their length and, hence, their mass.^{2–4} In contrast, globular proteins sprayed under native conditions can take only a smaller charge, given by Rayleigh's limiting charge on a drop of liquid of the same diameter, which varies as the square root of their volume (mass). That this reduction for spherical ions still leads to severe spectral overlap can be seen in analyses of dendrimers, whose globular form reduces their charge levels drastically with respect to linear polymers. Several generations of PAMAM (polyamidoamine) starburst dendrimers have been investigated mass spectrometrically. A first study with a quadrupole mass spectrometer was able to resolve individual peaks only up to the third generation ($m = 6909 \text{ amu}$),⁵ while a second study with an ion cyclotron resonance

* To whom correspondence should be addressed. DSaucy@Rohmhaas.com.

[†] Rohm and Haas Co.

[‡] Yale University.

[§] Hiroshima University.

(1) Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. K.; Whitehouse, C. *Science* **1989**, *246*, 64–71.

(2) Wong, S. F.; Meng, C. K.; Fenn, J. B. *J. Phys. Chem.* **1988**, *92*, 546–550.

(3) Nohmi, T.; Fenn, J. B. *J. Am. Chem. Soc.* **1992**, *114*, 3241–3246.

(4) Fenn, J. B.; Rosell, J.; Nohmi, T.; Shen, S.; Banks, F. J., Jr. *Biochemical and Biotechnological Applications of Electrospray Ionization Mass Spectrometry*; Snyder, A. P., Ed.; ACS Symposium Series 619; American Chemical Society: Washington DC, 1995; pp 60–80.

mass spectrometer of much higher resolution managed only to resolve individual peaks one generation farther ($m = 14\,214$ amu).⁶ However, spectral congestion was diminished drastically by reducing the charge level, and more significantly, the charge distribution of these ions, to the point where an ion trap analyzer could cleanly distinguish ion peaks of larger but charge-reduced dendrimer ions,⁷ and was only limited by the mass range of the trap, rather than by the onset of spectral overlap.

The alternative of using matrix-assisted laser desorption/ionization (MALDI)⁸ offers the advantage of yielding predominantly singly charged ions. MALDI has, in fact, allowed the analysis of a variety of polymers over a broad range of masses. However, quantification of the wide mass distributions typical of industrial polymers is problematic due to the considerable mass dependence of the ionization yield as well as the detection efficiency.^{8b-e} The latter problem becomes serious at masses above a few hundred thousand, which is a region of keen interest to the polymer industry. In addition, there is typically a loss of resolution arising at large masses, probably due to the increasing nonelectrostatic fraction of the ions' energy, picked up from the matrix during the desorption process.

One approach to eliminating the complexity associated with multiple charging in ES sources is to reduce the maximum charge level of the ions to unity prior to mass analysis. This approach has been used in two rather different forms. Most developed is the elegant method of McLuckey and colleagues,^{9,10} where naturally charged ES cations are collected in a quadrupole trap held at vacuum and then charge-reduced by controlled injection of fluorinated anions. With proper choice of anion, the cation's charge can be reduced without widening its mass distribution through unwanted attachment of the anion to the multiply charged cation. A simpler alternative subjects the ES ions, at atmospheric pressure, to a bipolar population of ions produced by a radioactive source and then injects into a mass spectrometer the resulting singly charged species.¹¹ Both methods have been used to resolve complex mixtures, but applications have been limited by the modest available mass limit of mass spectrometers with electrospray sources ($\sim 20\,000$ Da), a limit which is considerably lower than that of MALDI spectrometers ($\sim 300\,000$ Da).

The problems discussed above with mass spectrometric approaches¹² can be avoided by using chromatographic methods such as GPC or by combining field fractionation with optical techniques. However, none of these approaches are fully satisfac-

tory in terms of both resolution or ability to yield absolute mass. Figure 1 shows MALDI and GPC spectra of a polyurethane polymer (polyPEG-9k) formed by linking a variable number ($n = 1\sim 10$) of primary poly(ethylene glycol) (PEG) blocks, each with a narrowly distributed average molecular weight of ~ 9000 amu. The MALDI-MS spectrum of Figure 1a shows well resolved peaks beyond the 7-mer, while the GPC spectrum has a broadening mechanism that limits its resolving power to the 4-mer.

Another most promising technique introduced several years ago by Kaufman and colleagues¹³⁻¹⁵ measures electrical mobility rather than mass. Their approach, gas-phase electrical mobility mass analysis (GEMMA), demonstrated the ability and utility of reducing the charge on electrosprayed ions, the same technique later used to advantage with a mass spectrometer by McLuckey et al.^{9,10} and Scalf et al.¹¹ Mobility measurement in a gas has several important advantages: (1) it is fast; (2) the mobility Z can be related from first principles to particle size and shape; and (3) single ion sensitivity is available via condensation nucleus counter detectors.^{13,16} Figure 2 shows the electrical mobility spectrum obtained with the commercial GEMMA system (TSI Inc., Shoreview, MN) for a polyPEG-9k sample very similar to that shown in Figure 1. The ions are represented in terms of mobility diameter rather than mobility. One can clearly see five well-defined peaks, with hints of one or two more, which might be resolvable with more closely spaced data points. The resolution is clearly much better than that of GPC and increases rather than decreases with mass. It is, in fact, not controlled in this case by fundamental shortcomings of mobility analysis per se, but rather by the particular mobility analyzer used. Since these limitations can be readily overcome by available instrumentation,¹⁷⁻²⁰ it is evident that mobility analysis in the gas phase offers the potential for resolution much improved over that available by GPC.

Kaufman and colleagues¹³⁻¹⁵ noted a number of other analytical advantages associated with their GEMMA approach. Most important is that the measured signal for each ion is related only to its concentration in the sample solution because the ionization process does not favor any species over another, as would often be the case in ES ionization. To achieve this result, they use water with very low levels of nonvolatile residues, make it highly conducting by addition of ammonium acetate (so that electrospray drops are small), and use sample concentrations such that each initial ES droplet contains at most one sample molecule. They have further argued that their charge-reducing technique acts so fast as to preclude secondary atomization via Coulomb explosions and ion evaporation. Hence, each initial ES drop that contains an analyte molecule yields an analyte ion via the charge residue mechanism, irrespective of the characteristics of the analyte

- (5) Schwartz, B. L.; Rockwood, A. L.; Smith, R. D.; Tomalia, D. A.; Spindler, R. *Rapid Commun. Mass Spectrom.* **1995**, *9*, 1552-1555.
- (6) Tolic, L. P.; Anderson, G. A.; Smith, R. D.; Brothers, H. M.; Spindler, R.; Tomalia, D. A. *Int. J. Mass Spectrom. Ion Processes* **1997**, *165*, 405-418.
- (7) McLuckey, S. A.; Asano, K. G.; Schaaff, T. G.; Stephenson, J. L. *Int. J. Mass Spectrom.* **2000**, *196*, 419-437.
- (8) (a) Bahr, U.; Deppe, A.; Karas, M.; Hillenkamp, F.; Giessmann, U. *Anal. Chem.* **1992**, *64*, 2866-2869. (b) McEwen, C. P.; Peacock, P. M. *Anal. Chem.* **2002**, *74*, 2743-2748. (c) Byrd, H. C. M.; McEwen, C. N. *Anal. Chem.* **2000**, *72*, 4568-4576. (d) McEwen, C. N.; Jackson, C.; Larsen, B. C. *Int. J. Mass Spectrom. Ion Processes* **1997**, *160*, 387-394. (e) Malvagna, P.; Impallomeni, G.; Cozzolino, R.; Spina, E.; Garozzo, D. *Rapid Commun. Mass Spectrom.* **2002**, *16*, 1599-1603.
- (9) McLuckey, S. A.; Wu, J.; Bundy, J. L.; Stephenson, J. L.; Hurst, G. B. *Anal. Chem.* **2002**, *74*, 976-984.
- (10) Stephenson, J. L.; McLuckey, S. A. *J. Am. Soc. Mass Spectrom.* **1998**, *9*, 957-965.
- (11) Scalf, M.; Westphall, M. S.; Krause, J.; Kaufman, S. L.; Smith, L. M. *Science* **1999**, *283*, 194-197.
- (12) Benincasa, M.; Caldwell, K. D. *J. Chromatogr., A* **2001**, *925*, 159-169.

- (13) Kaufman, S. L.; Skogen, J. W.; Dorman, F. D.; Zarrin, F.; Lewis, L. C. *Anal. Chem.* **1996**, *68*, 1895-1904.
- (14) Mouradian, S.; Skogen, J. W.; Dorman, F. D.; Zarrin, F.; Kaufman, S. L.; Smith, L. M. *Anal. Chem.* **1997**, *69*, 919-925.
- (15) Kaufman, S. L. *Anal. Chim. Acta* **2000**, *406*, 3-10.
- (16) Gamero-Castaño, M.; Fernandez de la Mora, J. *J. Aerosol Sci.* **2000**, *31*, 757-772.
- (17) Rosell, J.; Loscertales, I. G.; Bingham, D.; Fernández de la Mora, J. *J. Aerosol Sci.* **1996**, *27*, 695-719.
- (18) de Juan, L.; Fernández de la Mora, J. *J. Aerosol Sci.* **1998**, *29*, 617-626.
- (19) Eichler, T. A Differential Mobility analyzer for ions and nanoparticles: Laminar flow at high Reynolds numbers. Senior Graduation Thesis, presented to Fachhochschule Offenburg, Germany, May 1997.
- (20) Fernández de la Mora, J.; de Juan, L.; Eichler, T.; Rosell, J. *Trends Anal. Chem.* **1998**, *17*, 328-339.

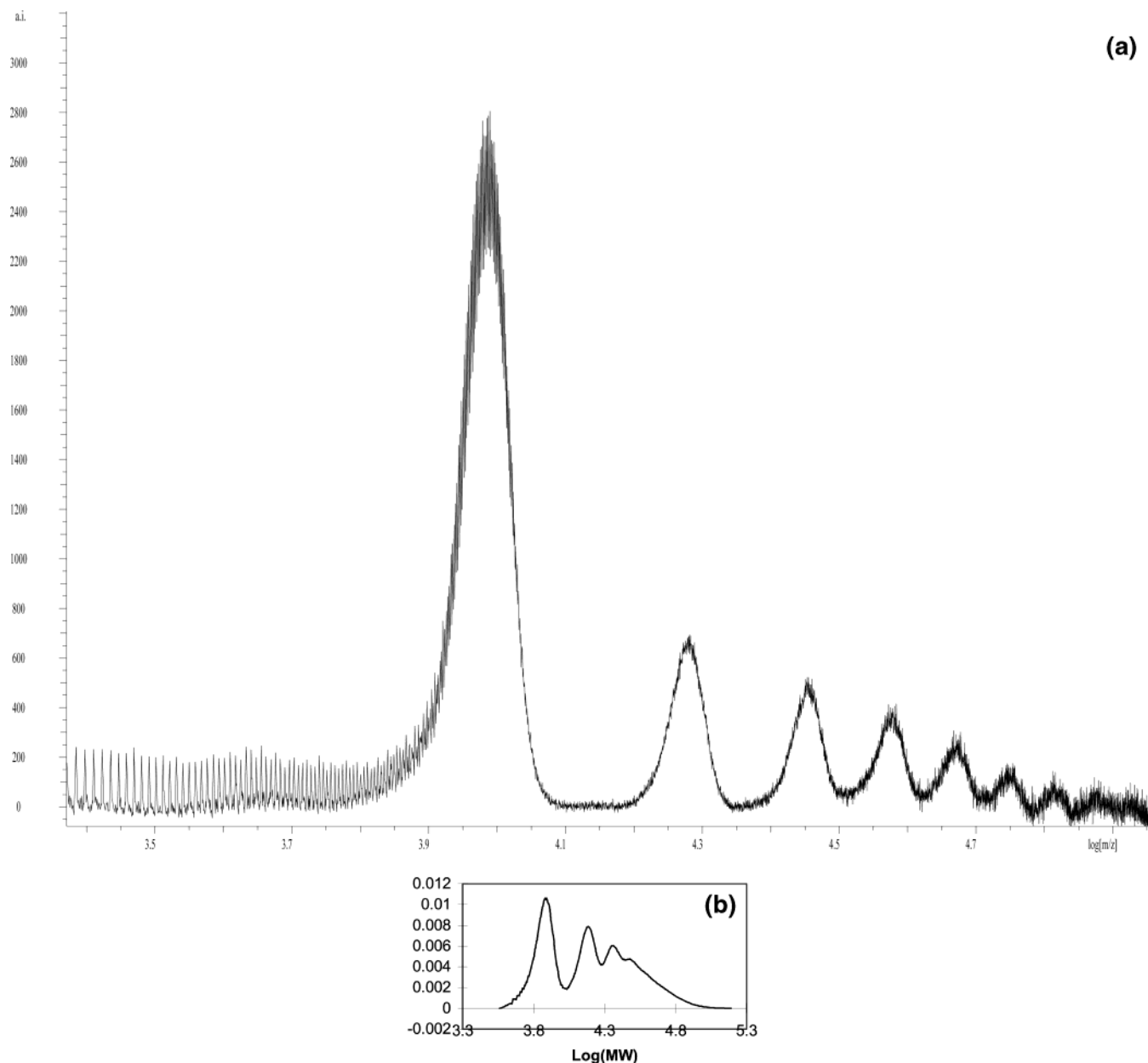
(a)

Figure 1. (a) MALDI-MS spectrum of polyPEG-9k. (b) GPC chromatogram of polyPEG-9k.

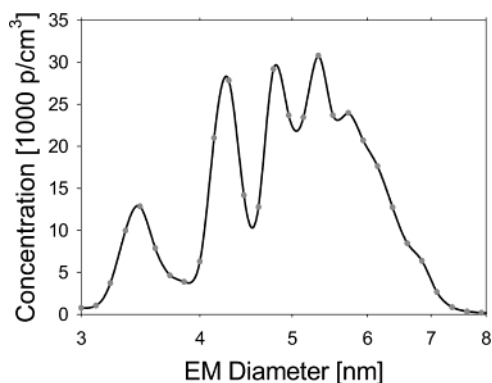


Figure 2. Mobility spectrum of the charge-reduced polyPEG-9k sample, obtained with TSI's commercial GEMMA instrument.

molecule. Although it is difficult to be sure that the neutralization is indeed complete prior to secondary atomization,¹⁵ this ambiguity

does not conflict with the overall picture drawn. Indeed, if the original drops contain only one analyte molecule, there is little harm in letting them explode as they evaporate. In fact, such secondary atomization may be desirable as it reduces the quantity of nonvolatile residue accompanying the analyte molecule.

Given this background, the goal of the present study is to explore in more depth the potential of ES followed by charge reduction and mobility analysis for MWD determination of water-soluble polymers. Using a mobility analyzer of considerably higher resolving power than that of Kaufman et al.,¹³ we will first show that the technique provides effectively the same information as mass spectrometry, since the narrowest available PEG standards are considerably broader than the line width resolvable by our instrument. We will subsequently obtain a reliable relation between mobility and mass by means of commercial mass standards of PEG. This relationship will establish mobility determination as a technique with unique advantages over the common

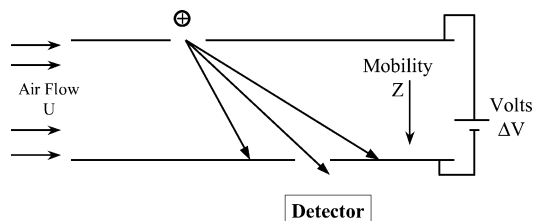


Figure 3. Principle of the operation of the differential mobility analyzer. Ions introduced through a sampling slit in the upper plate are separated in space according to their mobility by the combination of an electric and a flow field orthogonal to each other. Only a narrow mobility range is sampled and detected in the slit on the lower plate.

alternatives described above, at least for polymers soluble in polar liquids such as water. These results were first reported two years ago in a preliminary fashion by Saucy et al.²¹ We shall, finally, describe our efforts to extend the approach to the case of water-insoluble polymers, such as poly(methyl methacrylate) (PMMA). The quality of the spectra obtained in this case has been far less satisfactory due to the difficulty of identifying a solvent of substantial electrical conductivity (in order to produce the required small ES drops) while also being able to dissolve the analyte polymer. Much work remains to be done in this area.

EXPERIMENTAL SECTION

The analyzer is a differential mobility analyzer (DMA) based on the designs commonly used for particle sizing,²² whose principle of operation is shown schematically in Figure 3. Conceptually, ions are injected through a narrow slit on the upper plate into an air stream moving at speed U . The injecting plate is charged to a voltage V above the lower plate, creating an electric field that drives the ions downward across the air streamlines. Ions of only a narrow mobility range (controlled through the ratio U/V) are sampled through the slit on the lower plate and detected as a current I , providing a mobility spectrum $I(V)$. In practice, rather than a parallel plate arrangement, a concentric cylinder geometry is used, but the principle remains the same. By scanning the voltage difference, ΔV , between the two coaxial cylinders, the mobility, Z , of the ions reaching the detector can be scanned and, thus, allow the determination of the mobility distribution of the ions. The product $Z\Delta V$ is constant for given gas flow rates and is determined for each series of experiments by calibration (at fixed flow rates) with an ion of known electrical mobility, e.g., the dimer ion of tetraheptylammonium bromide ($1/Z = 1.51 \text{ V}\cdot\text{s}/\text{cm}^2$), generated also by electrospray, though without charge reduction.²³

The main feature enabling high resolving power is that the DMA flow is unusually high, with corresponding Reynolds numbers in the range of 10 000, yet, because of suitable fluid dynamic design, the flow remains laminar. The DMA used in these experiments is based on the design of Herrmann et al.,²⁴ though with an extended axial length and a wider line width (full width at half-height $\sim 2.9\%$). It operates laminarly up to Reynolds number

Table 1. Nominal Molecular Weights and Polydispersity Ratios (As Given by the Manufacturer), and Maximum Concentrations of the Sprayed PEG Standards

PEG, g/mol	polydispersity ratio, M_w/M_n	concn, mol/L
4 120	1.02	5×10^{-3}
12 600	1.01	5×10^{-4}
22 800	1.02	2×10^{-4}
50 100	1.02	2×10^{-5}
120 000	1.02	7×10^{-5}

beyond 30 000 as calculated from the mean velocity in the analyzing section and the separation between cylindrical electrodes (0.5 cm).

The detector sensing the mobility-selected particles is an electrometer commercially supplied by Lazcano Inc. (joelaz@arrakis.es) with a response time of $\sim 1 \text{ s}$ and a noise level of $\sim 10^{-16} \text{ A}$. It is connected to a collector wire located immediately downstream from the slit through which the Z -selected particles are sampled. It is biased between -9.5 and -28.5 V with respect to the sampling electrode to facilitate ion collection. This detector is not as sensitive as the single-particle condensation counters used previously¹³ but is adequate for the relatively concentrated samples of interest in industrial polymer analysis. A limitation of this approach is that a spectrum typically needs at least 100 points and therefore takes several hundred times the response time of the electrometer to collect. This time was typically of several minutes in this work, though some spectra taken under special conditions to yield maximum signal/noise ratio took $\sim 1 \text{ h}$. At higher polymer concentrations, a lower electrometer gain with faster response time could be used, though this luxury may not be available when broad distributions are being run.

The electrospray source is similar in design to that of ref 13, though the drops are given ample time to explode and evaporate before the dry particles are charge-reduced. The level of exposure to the $5 \text{ mC } ^{210}\text{Po}$ source is controlled by covering its active region with metal foil. It is regulated so that the relatively narrow PEG sample ceases to display a peak assigned to doubly charged species. The solvent was typically 50/50 (v/v) water/methanol, with 10 mM ammonium acetate. The gas surrounding the ES needle (a silica capillary with o.d. of $360 \mu\text{m}$ and i.d. of $20 \mu\text{m}$ whose tip was sharpened into a cone) was air. We also occasionally used a purely aqueous solution with CO_2 as the background gas in the electrospray chamber with similar results, though neutralization in CO_2 is less effective than in air and requires either larger radiation doses or longer residence times. It is important in all cases not to allow direct penetration of the α radiation into the high-field region in the vicinity of the spraying needle.

The polyPEG-9k sample used for Figure 1 was synthesized at Rohm and Haas. The remaining PEG samples were purchased from Polymer Laboratories Inc. (Amherst, MA). Their nominal molecular weights established by the manufacturer via GPC and light scattering are reported in Table 1. Ammonium acetate was from Fluka.

(21) Saucy, D.; Fernandez de la Mora, J. Measuring molecular weight by analyzing individual polymer chains, Proceedings of the International Gel Permeation Chromatography (GPC) symposium, Las Vegas, NV, October 21–25, 2000.

(22) Knutson, E. O.; Whitby, K. T. *J. Aerosol Sci.* **1975**, *6*, 443–451.

(23) Gamero-Castaño, M.; Fernández de la Mora, J. *Anal. Chim. Acta* **2000**, *406*, 67–91.

(24) Herrmann, W.; Eichler, T.; Bernardo, N.; Fernandez de la Mora, J. Turbulent transition arises at $\text{Re} \sim 35,000$ in a short Vienna-type DMA with a large laminarizing inlet. Abstract presented to the annual meeting of the AAAR, St. Louis, MO, October 6–10, 2000.

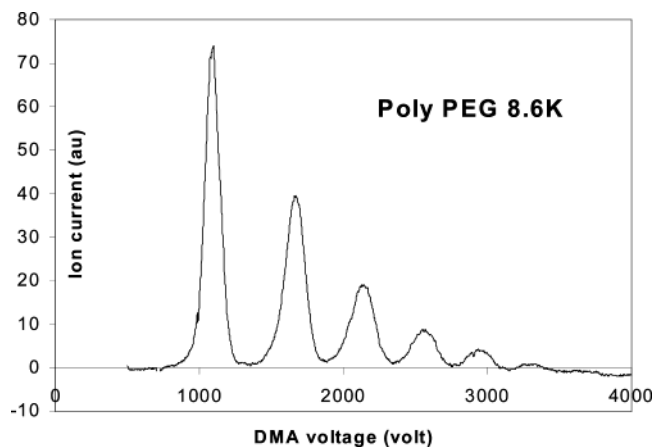


Figure 4. Raw DMA spectrum for charge-reduced polyPEG-9k.

RESULTS

Resolving Power. Figure 4 shows the raw spectrum for the same polyPEG-9k sample used for Figures 1 and 2, taken with the Herrmann DMA. One can see a series of sharp peaks with essentially no baseline noise, very much as in the MALDI spectrum of Figure 1a. The DMA is unable to resolve single-ion peaks as does MALDI-MS at the smallest masses, but it does yield their envelope. The peak broadening, expressed as full width at half-height (fwhh), of the DMA used in this work is 2.9%. To evaluate the effect of this broadening on the observed distribution for a narrow polymer, we can calculate that the expected mobility distribution, absent any broadening, for a polymer of $M_w = 9000$ and $M_w/M_n = 1.01$ has a fwhh of $\sim 15\%$. The MALDI MS data of Figure 1 show fwhh of 19, 14, 12, 11, and 11% for peaks 1–5, respectively. The DMA spectrum of Figure 4 shows corresponding fwhh of 11, 9.6, 9.2, 7.3, 6.3, and 5.8% for $n = 1$ –6, approximately as expected from the MS data and the relation between mobility and mass (see Discussion below). Thus, the broadening introduced by the DMA will not significantly affect the calculated molecular weight distribution for the narrowest available polymer and will be of even less consequence as the polydispersity increases. This result confirms that the limitations previously noted in relation to Figure 2 are incidental to the particular DMA and data acquisition routine used in the current commercial embodiment of GEMMA.

The ability to identify single-ion peaks (i.e., separated by 44 amu), as MS can do, would of course be very helpful here to establish a one-to-one correspondence between Z and m by allowing for simple counting of ethylene glycol units within the unimer envelope, starting from the monomer (1 glycol unit) and counting up. It would have served also for the purpose of establishing the purity of the sample and even to determine the nature of the cations providing the charge. While mass spectrometry has the resolution needed for this approach at low masses, at the molecular weights at which polymer mass distributions are often sought ($m > 10^4$ amu), the resolution of polymer-capable mass spectrometers is also not sufficient to allow individual ion mass determination.

Mass Determination from Mobility Measurements. Given our lack of a tandem DMA-MS system to determine directly the mass of the species whose mobility is measured, we will rely initially on commercial standards of PEG, whose nominal masses

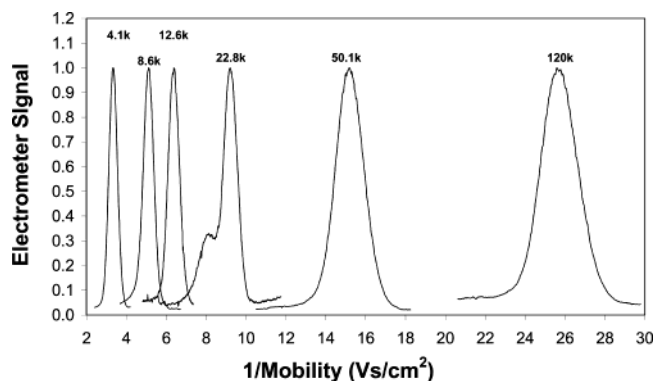


Figure 5. Mobility spectra for charge-reduced ions of PEG samples with nominal molecular masses of 4.12, 8.6, 12.6, 22.8, 50.1, and 120 kDa (left to right). The solutions used are sufficiently dilute to exhibit little clustering.

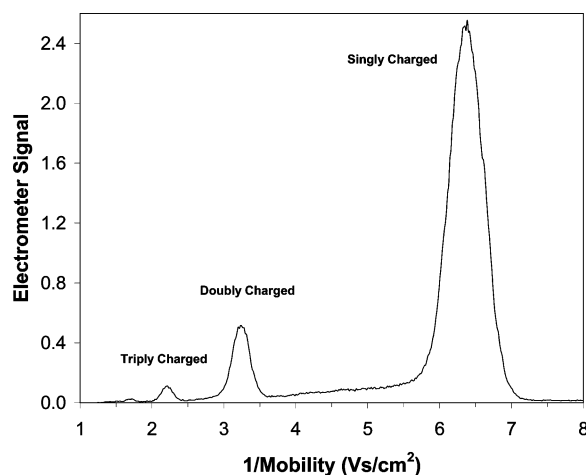


Figure 6. Mobility spectra of PEG-12.6k at low neutralization, exhibiting a doubly charged peak at half the mobility of the main peak and a small triply charged peak.

are reported in Table 1. When sprayed at concentrations low enough to yield no more than one polymer chain per droplet, single-chain ions are produced. Figure 5, obtained under such conditions, gives a qualitative impression on the narrowness of the PEG samples used as mass standards. Figure 6 shows mobility spectra of just the PEG-12.6k sample under conditions of less neutralization than used for Figure 5. The primary 12.6K PEG peak is seen at ~ 6.4 V·s/cm², which is the same mobility as the peak seen in Figure 5 for this standard. In addition, a second, lower intensity peak at 3.2 V·s/cm² is observed under these less neutralizing conditions. This peak, then, has twice the mobility of the primary peak. Because we cannot have half the mass, we must have twice the charge. Hence, this peak is the doubly charged particle, which is present because of incomplete charge reduction. In addition, there is a weak third peak at ~ 2.1 V·s/cm², i.e., one-third of the 6.4 V·s/cm², and is thus the result of triple charging.

To maximize the information obtainable from the five individual PEG standards, we have sprayed not only dilute PEG solutions but also more concentrated ones, where multiple PEG molecules are trapped inside a given drop and end up forming physically bound particles containing numerous single molecules. The gradual appearance of such cluster ions containing more than one PEG molecule can be seen in Figure 7, where peaks from the

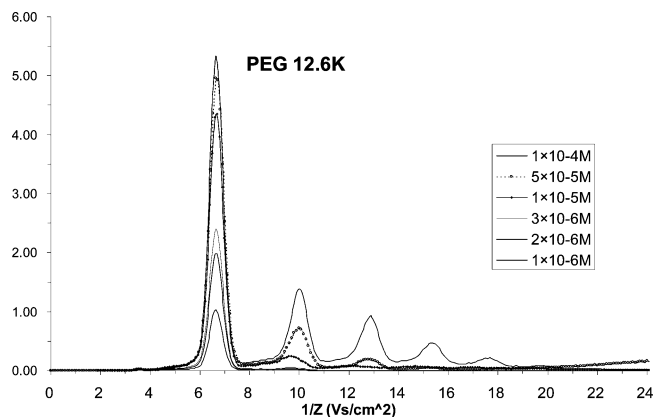


Figure 7. Effect of varying the solution concentration of PEG-12.6K on the resulting degree of clustering. The detector is a condensation nucleus counter, operated in a fashion such that it is insensitive to the ions from the neutralizer and considerably more sensitive than the electrometer for particles larger than 1.5 nm.

unimer (one polymer chain) up to the hexamer (six chains) can be recognized as the sample concentration is increased from 1×10^{-6} to 1×10^{-4} M. Such a clustering process not only magnifies greatly the effective number of mass standards available it also has the advantage that, although the mass m_1 of a given primary standard is slightly ambiguous, the masses $n \times m_1$ associated with its clusters are exact multiples of m_1 . There is no ambiguity in the degree of clustering n , as it follows simply by counting peaks from left to right in mobility spectra such as those shown in Figure 7. Clusters beyond the hexamer are formed in many cases, but the corresponding n -mers can no longer be recognized as individual peaks in the spectrum.

It is worth noting that these clusters are formed by individual molecules, whose masses are distributed over a certain range. The grouping of n such molecules, each of which has a certain mass distribution, produces combined distributions with a mean mass equal to n times the mean of the primary distribution and a relative standard deviation smaller (by a factor $n^{1/2}$) than that of the primary distribution. This relative narrowing of the distributions at increasing degree of clustering n has been discussed in relation to the MALDI spectra of Figure 1 and the DMA spectra of Figure 4. It ensures that the quality of the mass standard improves rather than declines with the degree of clustering.

A constant shape and density for the particles formed is vital to being able to deduce mass from the measured mobility since Z is a function of cross-sectional area, which depends on shape and volume, which in turn depends on density. These properties of the particle may depend on the process leading to its formation. If a drop containing several PEG molecules is first charge-reduced and then dries (the presumed scenario in the neutralizer of ref 13), the various polymer molecules remain in solution and are entangled together irrespective of whether they are joined chemically (as in the polyPEG chains of Figure 1) or not. Furthermore, such a chain remains inside the droplet to the end and is therefore subject to strong compressive capillary forces that tend to make it spherical. But the steps in the process are quite different when the drops dry first, and it is the dry ions that are then charge-reduced. It has become clear from the work of Fenn and colleagues²⁻⁴ that multiply charged PEG-containing droplets must be highly stretched before complete evaporation

as they contain far more charge than could be held by a spherical drop of solvent having the same volume as the polymer particle.²⁵ If two or more PEG molecules were contained in one drop, then the same Coulombic forces stretching a single multiply charged chain would tend to extend and perhaps shear apart a group of several of them. The earlier observation by Meng²⁶ of clusters of multiply charged peptides produced in electrospray mass spectrometry (without charge reduction), as well as our own observation of cluster formation, shows that such physically bound, multiply charged clusters can remain intact against Coulombic stretching. In the case of peptides, Kebarle²⁷ has rationalized this observation as resulting from hydrogen bonding. In any case, once a multiply charged and stretched PEG molecule (or cluster) is charge-reduced, one would expect it to coil up into a relatively compact ball. At room temperature, PEG is well below its glass transition temperature of $\sim 69^\circ\text{C}$ ²⁸ and would ordinarily not have the required mobility to be able to minimize its surface energy by taking a spherical shape. But a highly stretched molecule has considerable potential energy which, when released into kinetic energy, should provide more than enough heating to enable such a shape rearrangement. For this reason, one could reasonably expect that the collapse of a stretched PEG molecule would be able to produce a spherical particle as dense (or possibly more dense) as one precipitating out of solution at room temperature. In either case, the PEG particles should be expected to form spherical balls with bulk densities corresponding to a glassy or crystalline state, independently of whether they consist of single or multiple polymer chains.

In the hypothesis that the particles are spherical and much smaller than the gas mean free path, their mobility Z and mass m can be readily inferred from each other because both m and Z are simply related to the particle diameter d :^{20,29}

$$d = (6m/\pi\rho)^{1/3} \quad (1)$$

$$Z = \frac{3q}{2p(d + d_g)^2} \frac{(kT/2\pi m_g)^{1/2}}{(1 + \pi\alpha/8)} \quad (2)$$

where ρ is the density of the sphere, q its electrical charge, p , T , and m_g are the pressure, absolute temperature, and molecular mass of the suspending gas, k is Boltzmann's constant. d_g is the effective gas molecule diameter for gas-particle collisions, and α is the corresponding accommodation coefficient. α is zero for elastic collisions and unity for completely inelastic collisions. A variety of measurements for small particles in air are compatible with eq 2 with $\alpha = 0.91$,³⁰ while a limited number of recent studies report d_g values of ~ 0.5 nm.^{20,29}

Reported values for the bulk density of PEG are 1.204 g/m³ at an average molecular mass of 3400 g/mol,³¹ and 1.21 g/cm³ at 6000 g/mol³² at 298 °C. It hence seems reasonable to take the

(25) Fernandez de la Mora, J. *Anal. Chim. Acta* **2000**, *406*, 93–104.

(26) Meng, C. K. Multiple and fractional charging of solute molecules in electrospray ionization. Ph.D. Thesis, Yale University, 1988.

(27) Kebarle, P. J. *Mass Spectrom.* **2000**, *35*, 804–817.

(28) Measured at Rohm and Haas by DSC on 9000 molecular weight sample.

(29) Tammet, H. J. *Aerosol Sci.* **1995**, *26*, 459–475.

(30) Friedlander, S. K. *Dust, Smoke and Haze*; Wiley: New York, 1977.

(31) Aldrich, *Catalog Handbook of Fine Chemicals*; Aldrich Chemical Co., Inc.: Milwaukee, WI, 1994; p 1162.

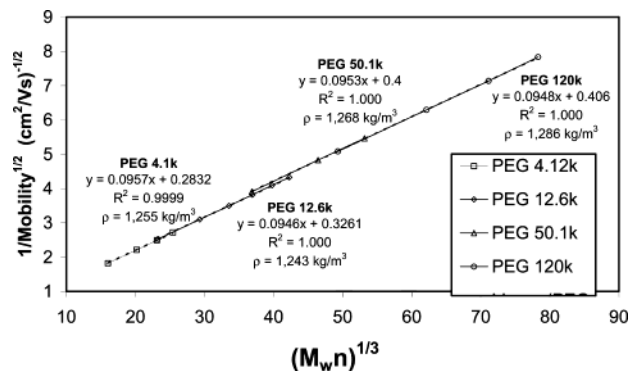


Figure 8. Mobility vs the particle masses provided by the supplier, for four of the PEG samples. Each data series contains points for various degrees of clustering of a given primary PEG sample.

density of our PEG particles to be 1.21 g/cm³ independent of molecular weight (see ref 33 for more detail). Despite the uncertainties still remaining in the values of ρ , d_g , and α , it follows from eqs 1 and 2 that the quantities $m^{1/3}$ and $Z^{-1/2}$ are both linear in d and should hence yield straight lines when plotted against each other:

$$Z^{-1/2} = A + Bm^{1/3} \quad (3a)$$

where the constants A and B can, in principle, be obtained by calibration, as well as estimated from the information just given. Note, however, that eq 2 holds only for particles much smaller than the gas mean free path in the analyzer (~ 67 nm) and thus cannot be expected to be accurate at masses of several megadaltons, where particle diameters will be 13 nm or more. The error is in fact several percent for our largest PEG particles and will be corrected below by making use of the complete expression relating Z and d over the full particle size range.³⁰ A common procedure with larger particles is to invert the relation $Z(d)$ to express the measured mobility in terms of a so-called mobility diameter $d(Z)$. This can be done purely algebraically so that the generalization of our procedure to the megadalton range would simply plot $d(Z)$ versus $m^{1/3}$ and still be represented by a straight line within the whole range of masses of interest to industrial polymer analysis.

Figure 8 uses the representation of eq 3a to plot the measured mobilities for single-chain particles and clusters against the nominal masses provided by the manufacturer, as listed in Table 1. Note that all the series of clusters resulting from a given mass standard group together in straight lines, as discussed above. The data for PEG-4k and PEG-12k overlap almost exactly, defining a single straight line, but the cluster series for PEG-50k and PEG-120k, while also falling on their own straight lines, depart slightly from each other and from the line defined by the lighter PEGs.

DISCUSSION

Since Z is measured directly using the same mobility calibration for all the PEGs, the small discrepancy seen in Figure 8

between the light and heavy PEGs could be due to slight inaccuracies in the nominal masses used or differences in the density. We expect no difference in the density between the PEG-50k unimer and the nearly equal mass tetramer of PEG-12k. Hence, the likely explanation for the discrepancy is a slight error in the nominal mass. This hypothesis is tested in Figure 9 where the masses of PEG-50k and PEG-120k are readjusted from the manufacturer's value (by 8.6 and 6.6%, respectively) such that the corresponding unimer data points fall on the linear regression line obtained in Figure 9 for the two lighter PEGs. Once this adjustment is made to the two unimer masses, the full cluster series for the two heavy PEGs fall on the expected line, lending credibility to the hypothesis that the nominal manufacturer masses differ by several percent from the actual masses at the peaks of their distributions. In fact, Figure 9 shows several duplicate data sets, i.e., unimer plus clusters, based on each of the four unimer standards. That all of these points fall on a single line when the masses are adjusted further supports the contention that the mismatch seen in Figure 8 is due to molecular weight error rather than experimental error in determining the mobility.

Linear interpolation through all the data of Figure 9 yields the following values

$$A = 0.355 (\text{V}\cdot\text{s})^{-1/2}/\text{cm}$$

$$B = 0.0936 (\text{V}\cdot\text{s})^{-1/2}/\text{cm}(\text{g/mol})^{1/3} \quad (4 \text{ kDa} < m < 0.77 \text{ MDa}) \quad (3b)$$

On the assumption that $\alpha = 0.91$, these values correspond to $d_g = 0.505$ nm and $\rho = 1.325$ g/cm³. This density exceeds by 9.7% the bulk value.

Since the ratio of the diameter of the largest particles to the gas mean free path is of the order of this difference, it suggests that this difference may result from the simplifying assumptions about particle size effects made to obtain eq 2. Thus, we will reinterpret the data in terms of the full relation between Z and d , commonly referred to as the Millikan relation, where the Knudsen number Kn is the ratio between twice the mean free path of the carrier gas and the particle diameter d .³⁰

$$Z = qC(Kn)/3\pi\mu(d + d_g) \quad (4a)$$

$$C(Kn) = 1 + Kn[1.257 + 0.4 \exp(-1.1/Kn)] \quad (4b)$$

$$Kn = \frac{2\mu}{(d + d_g)\rho_g} \left(\frac{\pi m_g}{2kT} \right)^{1/2} \quad (4c)$$

where μ is the viscosity coefficient of the gas and ρ_g is its density. The usual Millikan expression reduces to eq 2 in the free molecule limit, except for the fact that it ignores the finite dimensions of the gas molecules. This deficiency has been averted by simply writing $(d + d_g)$ in place of d . Inverting eq 4a yields d as a function of Z , which then allows d to be used in place of $Z^{1/2}$, to give a linear relationship (eq 5) between d and $m^{1/3}$, as shown in Figure 10.

$$d = d_g + B_m m^{1/3} \quad (5)$$

(32) Windholz, M.; Budavari, S. *The Merck Index*; Merck & Co., Inc.: Cleveland, OH, 1983; p 1092.

(33) Tawfik, W. Y.; Teja, A. S. *Chem. Eng. Sci.* **1989**, *44*, 921–923.

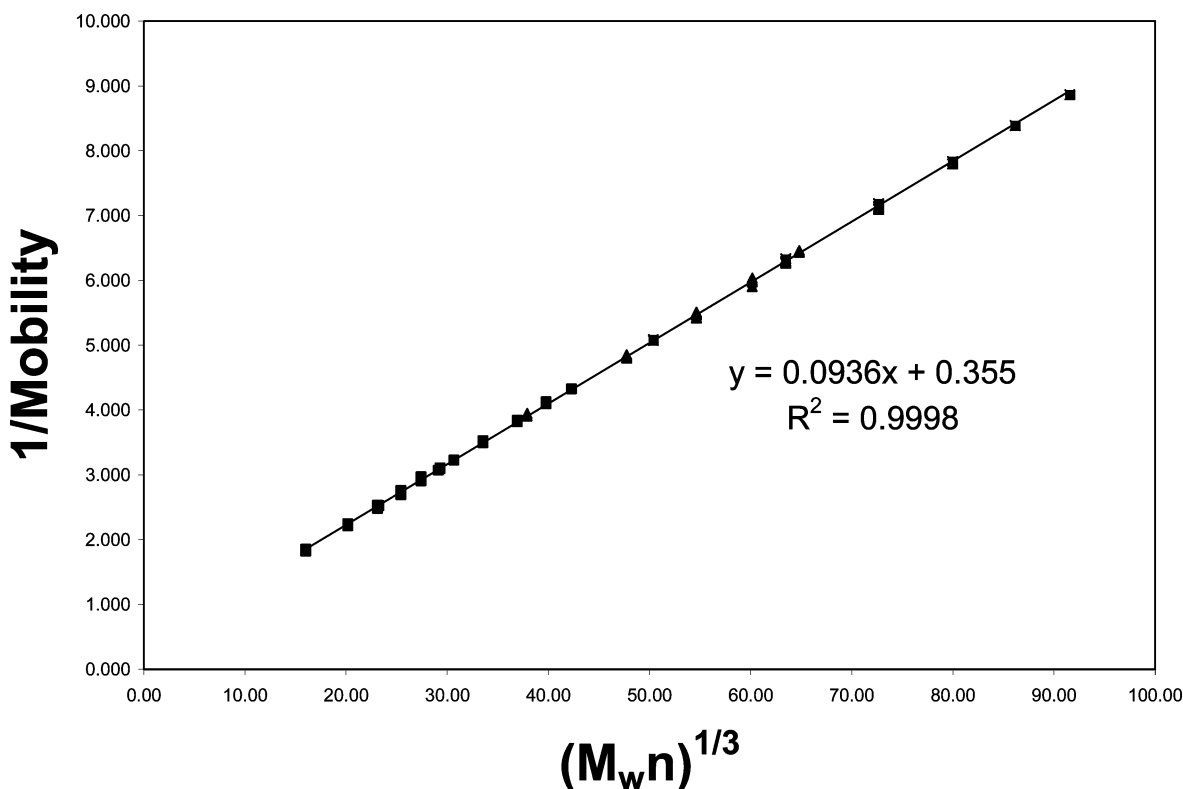


Figure 9. Mobility vs mass for unimers and clusters generated from four individual PEG samples, using PEG masses adjusted as described in the text.

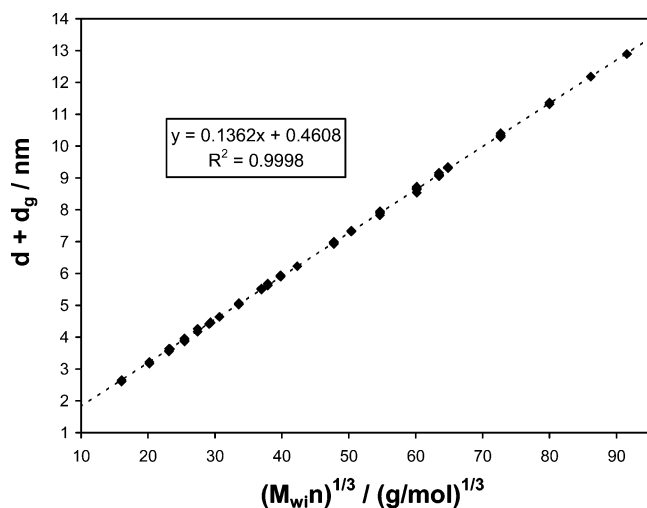


Figure 10. Generalization of Figure 9 to include corrections to the free molecule relation (2) between Z and d , as shown in eq 4a.

A fit to the data of Figure 10 yields

$$d_g = 0.453 \text{ nm}$$

$$B_M = 0.1364 \text{ nm}/(\text{g/mol})^{1/3}$$

This slope B_M can be turned into a particle density of $\rho = 1.25 \text{ g/cm}^3$, much closer now to the bulk density of 1.21 g/cm^3 .

The technique we have used to obtain improved masses for the higher PEG standards is in principle a direct mass calibration. It starts with PEG-4k as a primary mass standard, and then it

produces equally reliable secondary mass standards, through its clusters, that extend to the unimer weight of the next higher PEG standard. The clusters of this heavier standard in turn allow further extension of the mass scale in a fashion limited only by the availability of sufficiently pure soluble samples with a sharply recognizable feature that survives through the clustering process. The mobility data demonstrating agreement between the PEG-4k and PEG-12k masses are consistent with the MALDI-MS data of Montaudo et al.³⁴ and Ude et al.³⁵ Our unconventional “balance” uses mobility as its scale, but this does not make it less reliable than any other balance, provided that mobility and mass are related in a unique fashion. This reasonable “proviso” is confirmed directly by our data from the PEG-4k unimer up to the PEG-12k hexamer ($m = 72\,000 \text{ Da}$). Its indirect confirmation up to 0.77 MDa is given by the little scatter seen in Figure 9 for the data of the widely overlapping cluster series of three different PEG standards. Its validity beyond even 0.77 MDa can be reasonably inferred from the present data.

There are a number of polymers that, like PEG, are water-soluble. These can be electrosprayed similarly as PEG from a buffer of water and ammonium acetate to yield comparably small drops. This should make their mass analysis viable by the present technique, provided that a suitable $Z(m)$ calibration is produced. An interesting precedent of this possibility can be found in the work of Kaufman and colleagues with proteins¹³ and nucleic acids.¹⁴ Plotting the protein data of Kaufman et al.¹³ in the representation of Figure 8,²⁰ yields an average density of 1.28

(34) Montaudo, G.; Montaudo, M. S.; Puglisi, C.; Samperi, F. *Macromolecules* **1995**, *28*, 4562–4569.

(35) Ude, S.; Fernández de la Mora, J. *J. Aerosol Sci.* **2003**, 1245–1266.

g/cm³, quite close to the 1.3 g/cm³ typically reported for protein crystals.^{36–38} Nonetheless, $Z^{-1/2}$ versus $m^{1/3}$ plots of such chemically inhomogeneous samples contain substantial scatter, so that relating mobility to mass is in this case more adventurous than for homogeneous polymer chains. An additional difficulty associated with proteins is their tendency to attach water,^{36–38} which may have drastic effects on their mobility and mass. An indication of this problem can be sensed in the large set of mobilities (in air) compiled by Bacher et al.³⁹ for globular proteins with masses ranging from a few thousands to several million daltons. All their data fall surprisingly close to a single line in the representation of Figure 9, in contrast to those of Kaufman et al.,¹³ which show considerable scatter. Perhaps more unsettling is the fact that these new protein data yield a density of 0.6 g/cm³ for protein particles. It is hence clear that relating mobility to mass may be in some cases more difficult than found here for PEG.

For general applicability to the determination of polymer molecular weights, we must either directly determine the mobility versus mass relation for each polymer or transform the relation obtained with one polymer for use with another. The first approach is very limiting in that it requires molecular weight standards of the unknown polymer. The second approach requires that we know the particle density of both the standard and the unknown and that both form spherical particles. The PEG data suggest that the density of the single-chain particle is very similar to that of the bulk polymer, and bulk densities are relatively easy to measure or estimate for most polymers. As discussed previously, the assumption of spherical particles appears to be reasonable in cases where the charge is reduced. Thus, we have the information and criteria for a broadly applicable calibration for this approach.

A distinct advantage that this approach offers over conventional GPC analysis is that it does not require that the polymer pass through an analysis column. Thus, it can avoid the issues of adsorption and shear degradation that may occur in GPC analyses. In addition, its upper size limit is well beyond that of GPC. For example, a 100-MDa PEG particle would have a diameter of ~63 nm, still well within the range of GEMMA. Last, as demonstrated above, using a high-resolution DMA, the molecular weight resolution offered can be significantly better than that available with GPC.

(36) Richards, F. M. In *Protein Folding*; Creighton, T. E., Ed.; W. H. Freeman: New York, 1992; pp 1–58.

(37) Low, B.; Richards, F. M. *J. Am. Chem. Soc.* **1954**, *76*, 2511–2518.

(38) Rupley, J. A.; Gratton, R.; Careri, G. *Trends Biochem. Sci.* **1983**, *8*, 18–22.

(39) Bacher, G.; Szymanski, W. W.; Kaufman, S. L.; Zollner, P.; Blaas, D.; Allmaier, G. *J. Mass Spectrom.* **2001**, *36*, 1038–1052.

WATER-INSOLUBLE POLYMERS

We have investigated in a preliminary fashion the rather different case of polymers such as PMMA, which are insoluble in water. Although soluble in some highly polar solvents, this solubility is often lost when high levels of volatile salts or acids are added in an attempt to raise the conductivity and thus reduce the electrospray droplet size to achieve the single-chain criterion at concentrations that yield sufficient ions for detection. By trial and error we have occasionally succeeded at obtaining a suitable solution by making ternary mixtures of tetrahydrofuran, acetone, and water with ammonium acetate added to provide conductivity. But these mixtures typically produced much larger initial drops than the 10 mM aqueous ammonium acetate buffers used previously to electrospray PEG. As a result, the quality of the PMMA data that we have obtained is considerably worse than that obtained with PEG for two reasons. First, because of the larger droplet sizes, the concentration of polymer must be lower to achieve the single-chain criterion, leading to poorer signal/noise ratios. Second, the larger droplets contain a higher weight of insoluble impurity, which will be included in the dry particle along with the polymer, thus creating a particle that is too large. Such coarse sprays are often suitable for ES-MS work, probably because the transit region from atmospheric pressure to the high-vacuum chamber of the mass spectrometer can be used to accelerate mildly the ions and clear them from physically bound species. This cleaning option, however, is not viable at atmospheric pressure, and cannot therefore be implemented upstream of the DMA. Further complicating this mixed-solute approach, the analysis of each new water-insoluble polymer demands the patient identification of a new solvent mixture. Thus, in the absence of a more universal electrospray-compatible solvent, the analysis of water-insoluble polymers by this approach will be tedious at best.

ACKNOWLEDGMENT

The work at Yale University has been supported by NSF Grant CTS-9871885 and by a gift from Rohm and Haas. I.W.L. is grateful to the NEDO-Nanoparticle Project, JSPS, and Prof. K. Okuyama for their support of this work. We are indebted to Dr. Stan Kaufman of TSI for providing the GEMMA data shown Figure 2. We thank Prof. Jerzy Blawdziewicz for his input on the interpretation of Figure 6. We gratefully acknowledge Dr. Lin Zhu for obtaining the MALDI-MS spectrum of the polyPEG sample.

Received for review February 12, 2003. Accepted November 7, 2003.

AC034138M