solution and 50 mL of brine. After drying over $MgSO_4$, the solvent was removed by rotary evaporation. The product was chromatographed on a column of silica gel (60–200 mesh, dried at 175 °C). Elution with 10% ethyl acetate in hexane gave 0.64 g (85% yield) of a clear oil. It had the following data: ^{1}H-NMR, δ 0.77 (3 H, d, J = 6.9 Hz), 0.92 (3 H, d, J = 7.5 Hz), 1.1–1.35 (m), 1.5–1.78 (m), 1.9–2.05 (m), 1.98 (3 H, s), 5.25 (1 H, d, J = 2.7 Hz) ppm; ^{13}C-NMR, δ 17.36, 20.64, 21.06, 27.43, 27.61, 30.90, 33.41, 34.90, 35.20, 38.85, 121.59, 147.82, 169.17 ppm; MS, [M$^+$] 208 amu. This material was used directly in the next step.

In a 100-mL round-bottom flask equipped with a water condenser and magnetic stirrer blanketed with N_2 were placed 2-acetoxy-4,8-dimethylbicyclo[3.3.1]non-2-ene (0.64 g, 3.1 mmol), *N*-bromosuccinimide (0.55 g, 3.1 mmol), and benzoyl peroxide (0.1 mg) dissolved in 40 mL of CCl_4. This was gently refluxed with stirring for 1 h. The solution was cooled to room temperature and filtered. The residue was washed with CCl_4. The filtrates were combined, and the solvent was removed by rotary evaporation. The yellow residue was dissolved in 12 mL of 95% ethanol and 4 mL of 3 M HCl. The solution was gently refluxed with stirring for 2 h. The solvents were removed by distillation under reduced pressure (using a plastic aspirator). The residue was dissolved in diethyl ether and then dried over $MgSO_4$. The solvent was removed by rotary evaporation. The product was chromatographed on a column of silica gel (60–200 mesh, dried at 175 °C). Elution with 10% ethyl acetate in hexane gave pure product. Enone **10** had the following data: IR (film on NaCl), ν 1666 cm^{-1}; UV, ϵ_{358}^{max} 652, ϵ_{345}^{max} 642, ϵ_{330}^{max} 823, ϵ_{231}^{max} 6563; ^{1}H-NMR, δ 0.80 (3 H, d, J = 6.5 Hz), 1.28–2.63 (9 H, m), 1.96 (3 H, s), 6.08 (1 H, br s) ppm; ^{13}C-NMR, δ 19.5 (q), 22.4 (q), 25.9 (t), 33.0 (d), 35.3 (t), 35.4 (t), 35.7 (d), 47.4 (d), 128.6 (d). 159.0 (s), 200.1 (s) ppm.

Anal. Calcd for $C_{11}H_{16}O_2$ (164.2): C. 80.44; H, 9.82. Found: C, 80.23; H, 9.73.

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Electrospray Mass Spectrometry of Poly(ethylene glycols) with Molecular Weights up to Five Million

Takashi Nohmi and John B. Fenn*

Contribution from the Department of Chemical Engineering, Yale University, New Haven, Connecticut 06520-2159. *Received August 19, 1991*

Abstract: Mass analysis has been carried out on ions formed in an electrospray (ES) ion source from poly(ethylene glycols) (PEGs) with molecular weights (*M*s) ranging from 200 to 5 000 000. The number of charges per unit length of oligomer decreases with increasing molecular weight (*M*) in the range below *M* = 20 000. Above that value the number increases until at *M* = 5 000 000 it slightly exceeds the maximum allowed by the electrostatic model previously proposed. This behavior can be understood in terms of the solution properties of PEG and the two mechanisms that have been proposed for the formation of ions from charged droplets.

I. Introduction

Polymers of ethylene oxide have often been the subject of mass spectrometric studies. Comprising linear chains with little or no branching, looping, or cross-linking, they are quite soluble in a variety of solvents including water, and can be had in a wide range of oligomer sizes. The nominal value of molecular weight for commercially available material is that of the most abundant oligomer in a mixture of oligomers having a distribution of sizes that in principle should be Poisson-like but in practice is somewhat broader. Such mixtures of oligomers are known as "poly(ethylene glycols)" or PEGs when the chain lengths are short enough so that the terminal OH groups have a discernible effect on chemical and physical properties. Materials of higher molecular weight are generally referred to as "poly(ethylene oxides)" or PEOs, a name that relates both to the structural unit of the polymer and to the actual starting material from which it is made. The distinction between PEGs and PEOs is not clearly drawn, but custom seems to put the dividing line at a nominal molecular weight value of about 17 500. By this convention the term PEG properly applies to each of the samples used in our earlier study.[1] In order to avoid an unnecessary and perhaps confusing distinction, we will also refer to the subjects of this report as PEGs even though their molecular weights are such that they would ordinarily be called PEOs.

It was a report from our laboratory on mass spectrometric studies with PEGs that first revealed the remarkable ability of electrospray (ES) ionization to produce ions with extensive multiple charging, up to 23 charges per ion in the case of PEG 17 500.[1] In an attempt to identify an upper limit to the number of charges that a PEG oligomer could hold, that paper also proposed a model by which that maximum number could be calculated. The key assumption of the model is that the number of charges on an oligomer ion is at a maximum when the electrostatic repulsion energy (ERE) of the centermost charge equals the energy with which it is bound to its site. The ERE for any charge on such an ion is simply the pairwise sum of potential energies due to Coulomb repulsion between it and each of the other charges on the ion. The ERE of a system comprising two like charges varies inversely as the distance between them. Consequently, in a linear array of charges the ERE is largest for the centermost charge and is at a minimum for the array as a whole when the charges are equally spaced. The intercharge distances needed for this calculation are obtained from the known geometry of a PEG oligomer in its stretched out or "zig-zag" configuration.[2] In addition the charges are assumed to be bound to oxygen atoms at equidistant intervals along the oligomer backbone. In these experiments the charges were Na^+ ions which we assumed are bound to oxygen atoms with an energy of about 2.05 eV, the value that has been measured for Na^+ and dimethoxyethane, the most similar species for which we could find data.[3]

The experimental results of the earlier investigation indicated that the observed ions contained as few as half the maximum

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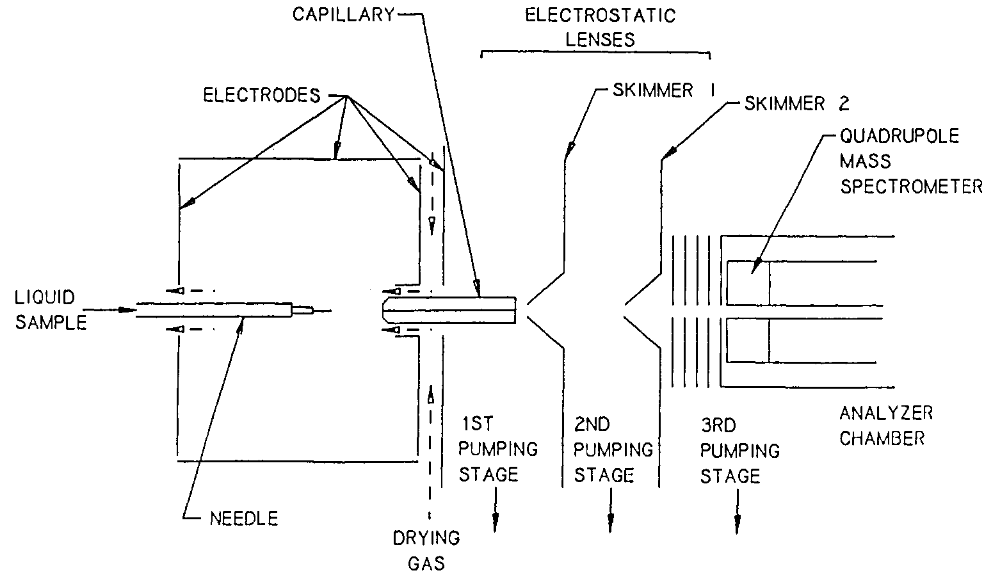

Figure 1. Schematic diagram of apparatus used in this study.

number of charges permitted by the model. Although the model is certainly oversimplified, we believe that most of this discrepancy can be better attributed to the kinetics of ion formation than to the model's overestimation of an oligomer's charge-holding capacity. The widely accepted ion desorption model (IDM) for ion formation, introduced by Iribarne and Thomson,[4] holds that as a charged droplet shrinks due to solvent evaporation, the charge density on its surface, and therefore the field, increase. At some critical diameter the surface field becomes high enough to desorb an analyte ion. As in the case of charges on a PEG molecule, the ERE of a system comprising charges distributed on the surface of a droplet is also at a minimum when the charges are equally spaced. At the time of evaporation, the intercharge distance on the droplet surface must be at least as small as the distance between charges on a multiply charged ion after it desorbs. Thus, the intercharge distance on a desorbed ion, as determined from the mass/charge ratio measured by the mass analyzer, must reflect the charge spacing on the droplet surface at the time the ion desorbs. We conclude that ipso facto the PEG ions can and do desorb before charge spacing on the droplet surface has become small enough to populate a desorbing ion with all the charges it can hold.

Although this explanation of observed results is plausible, it is based on the desorption model for ion formation that is widely but not universally accepted. Some investigators remain persuaded that the charged residue model (CRM) originally proposed by Dole is more defensible.[6] It holds that the combination of solvent evaporation and Coulomb explosions leads to ultimate droplets so small that they contain only one molecule. That molecule becomes an ion by retaining some of the droplet charge as the last of the solvent evaporates.

In the subject study mass analysis was carried out for ES ions from PEG oligomers in the molecular weight range from 20 000 to 5 000 000. The results show differences and similarities in the behavior of these larger molecules relative to that of their smaller namesakes and provide some insight on the mechanism of ion formation. In particular, with respect to the issue of the IDM versus the CRM, the former emerges as the most probable mechanism in most cases, but the latter best accounts for ion formation from the largest molecules.

II. Experimental Apparatus and Procedures

The ES mass spectrometer is shown schematically in Figure 1. It differs from the one used in the previous study in that it has three stages of pumping instead of two. The first stage receives the ion bearing gas from the ES source expanding as a free jet from the exit of the glass capillary. It is exhausted by a pair of mechanical rotary pumps with an effective speed of about 500 L/min. The first skimmer is located within 3 or 4 mm of the capillary exit, upstream of the Mach disk so that the entering flow is oblivious to conditions beyond the jet boundary even at ambient pressures as high as several Torr. The insertion of this new stage makes possible an increase in capillary diameter from 0.2 mm to 0.75 mm. The resulting increase of gas flow, and therefore ion current, from the ionization chamber, has increased the analytical sensitivity of the system by as much as 100-fold. Otherwise, this three-stage system functions and behaves in pretty much the same way as did the former two-stage apparatus whose operation has been previously described in detail.[1,5]

Some of the commercial grade PEG samples were kindly donated by Union Carbide. Others were obtained from Aldrich. Experiments were also carried out with samples from TsoHaas (Tokyo) processed by chromatographic fractionation. They had a much narrower spread of oligomer sizes than did the regular commercial grades with which most measurements were made. Unless otherwise noted, the solvent was a 50:50 mixture of methanol (Baker Analytical) and water (deionized, filtered, and distilled). Typically, a sample comprising 20 μL of a solution containing from 0.001 to 0.05 g/L was injected at a rate of 3 μL/min so that several spectra could be obtained even though our printer required 1 or 2 min to record a scan over the whole range of m/z, from 0 to 1500 for our quadrupole analyzer (VG 1212).

One matter of concern is whether the electrospray process can degrade or depolymerize large PEG oligomers. To answer that question, we electrosprayed a solution of PEG with a nominal M of 900 000 at a concentration of 0.005 g/L, the maximum concentration at which a stable spray could be maintained in the "rainbow mode" (i.e., the preferred mode for ion formation that will be described later). The spray was produced on the bench in the open, the droplets and/or ions being trapped on the surface of a plane counter electrode at distance of 3 cm from the tip of the spray needle that was at 4 kV relative to the electrode. Spraying continued for 60 h at a flow rate of 3 μL/min, thus processing 10 mL of solution and depositing about 0.000 05 g of sample that was washed off the electrode with deionized water. This "sprayed" PEG along with some "unsprayed" material from the same original sample, was returned to the supplier (TsoHaas) for chromatographic analysis in an HPLC column packed with TSK gel 5000PW having an exclusion limit of 2 500 000 for water-soluble polymers in an aqueous mobile phase. Both samples had the same characteristics: M_w of 860 000, M_v of 990 000, and M_w/M_v of 1.17, indicating a much narrower size distribution than is found in standard commercial material for which M_w/M_v is usually greater than 2.0. This result provided some assurance that the process of forming ions from PEG by ES did not cause degradation or change in M. There remains the question of whether, in the ESMS measurements, changes could have occurred in the ions during transit from the spray chamber to the mass analyzer. We think that possibility is remote because the ions are treated very gently during a transit time

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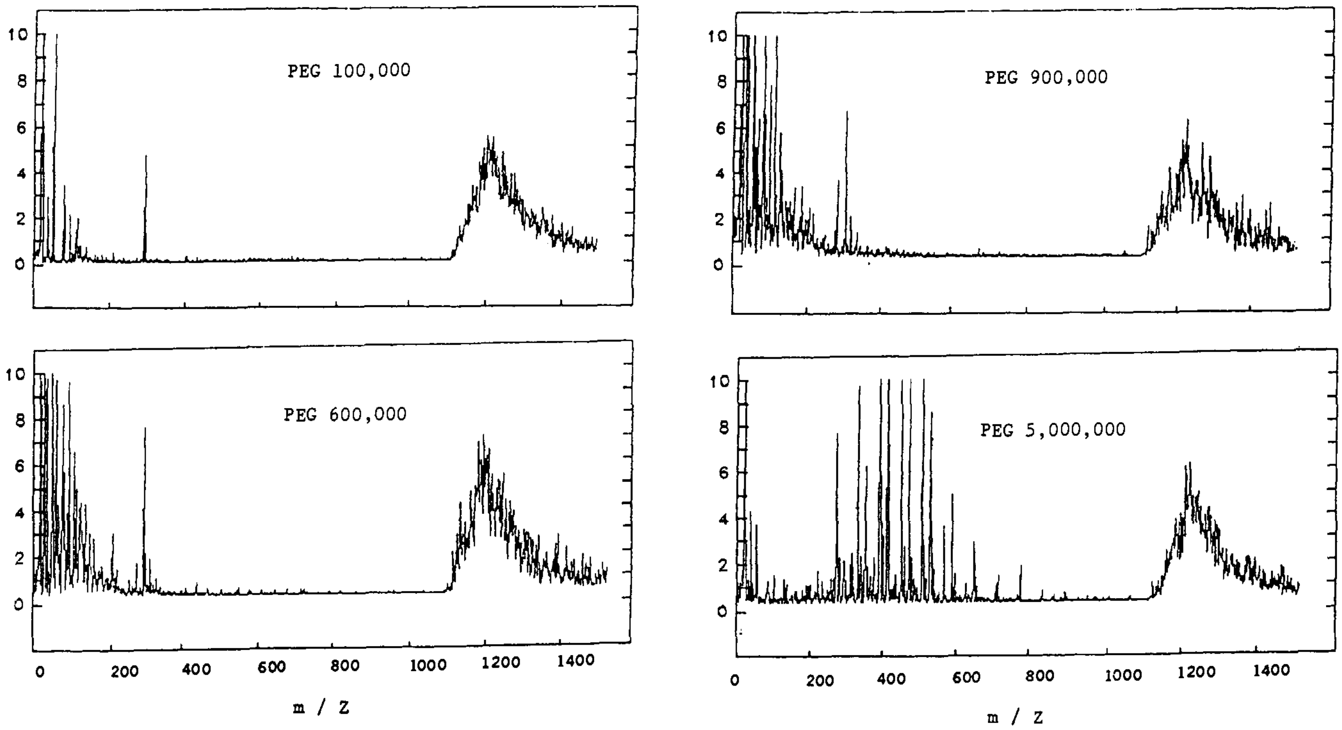

Figure 2. Representative mass spectra of electrospray ions obtained with PEG samples having relatively large molecular weights.

from the spray chamber to the analyzer that is less than 0.1 of the transit time from the needle to the trap electrode in the bench experiment. The evidence for lack of decomposition would have been even more reassuring if we had collected sample at the entrance to the analyzer in the ESMS apparatus. Such collection was not feasible because the analyte ion current at that point was so small, less than 1 nA, that to collect an equivalent amount of sample could have required as much as 10 000 h!

III. Results and Discussion

It will be useful to start with a brief review of observations we have made on the behavior of liquid emerging from the injection needle in the orientation of Figure 1. With no voltage difference between the needle and the end wall of the chamber, the liquid forms a growing droplet that leaves the tip in a vertical trajectory when its weight becomes large enough to overcome the surface tension that attaches it to the needle. When a dc voltage is applied to the needle, raising its potential relative to the end wall, the resulting field at the needle tip deposits net charge on the droplet and provides it with a component of horizontal velocity toward the end wall and the entrance of the glass capillary. As the applied voltage increases at a fixed liquid flow rate, the droplets decrease in size and increase in both frequency and horizontal velocity. When the applied voltage becomes large enough, the droplets form at a very rapid rate with trajectories for which the component of vertical velocity is negligible. As the voltage increases further, the droplets become so small and leave so frequently and rapidly in diverging trajectories that they constitute a fine conical spray or mist in what we call the "fog" mode of operation. A slight further increase in voltage causes a sudden transition to a "rainbow mode" in which the needle current increases by as much as a factor of 2. In addition, the spray exhibits colors characteristic of higher order Tyndall spectra (HOTS), indicating that the droplets are fairly monodisperse with diameters of 2 μm or less. Still further increases in voltage lead to a corona discharge in the bath gas and collapse of the spray.

Microscopic examination, first carried out by Zeleny,[7] reveals that the liquid emerging from the source needle forms a conical meniscus now known as a "Taylor cone".[8] Extensive recent studies by Tang and Gomez[9] clearly show a continuous filament or jet of liquid emerging from the tip of that cone. As do their uncharged counterparts, analyzed long ago by Rayleigh,[10] these charged jets also develop "varicose waves" on their surfaces that grow in amplitude until they disrupt the jet into a stream of droplets having diameters about 1.9 times that of the jet. Driven by Coulomb forces, this stream of charged droplets rapidly disperses into the conical spray characteristic of the fog and rainbow modes just described.

Once established, the rainbow mode can be sustained at voltage differences less than those required to start it. However, at these lower voltages, a slight disturbance, e.g., blowing on the needle tip, will make the spray revert to the fog mode. On the other hand, under some conditions relating to liquid properties and flow rate, rainbow mode operation cannot be induced at any voltage. To be noted is that rainbow mode operation provides the highest yield of solute ions. Consequently, it is important to identify and understand the conditions needed to make it occur.

Early in this study we made a most provocative finding. Solutions of the large PEG molecules would not electrospray in the rainbow mode unless the solute concentration was less than some critical value that decreased with increasing molecular weight. Indeed, when the molecular weight reached 5 000 000, the highest value for which we had samples, the critical concentration was so low that most of the nascent droplets contained only a single molecule. Flash photography with nanosecond pulses from a Nd-Yag laser indicated that under these circumstance the diameters of the nascent droplets were about 2.8 μm. Some of the implications of this one-molecule-per-drop scenario will emerge in later discussion. Meanwhile we simply note that all the data reported here were obtained with ES in the rainbow mode.

Figure 2 shows mass spectra obtained with PEG samples having nominal molecular weights of 100 000, 600 000, 900 000, and 5 000 000. The concentrations in the injected solutions were respectively 5.0, 4.2, 2.8, and 0.2 nmol/L. The spectra comprise relatively broad "noisy" bands of the kind reported in the previous paper for PEGs 8000 and 17 500. As pointed out then, a PEG sample comprises a mixture of oligomers, each of which can have a variety of charge states. The number of possible combinations of oligomer size and charge state increases rapidly with increasing

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nominal molecular weight. Already at 8000 there are as many as five or more different ions within an interval of one unit on the m/z scale. Few quadrupole mass analyzers, especially not ours, can resolve such spectral congestion in this m/z range. Even so, as the previous paper shows, useful information can be obtained from such spectra, unresolved though they may be. One fruitful approximation is to assume that the m/z value of the band maximum corresponds to the most probable charge state of the most abundant oligomer, i.e., the one whose molecular weight equals the nominal value for the sample. Many of the conclusions drawn here, in common with those of the earlier paper, rest upon that quite reasonable assumption.

The spectrum in the lower right-hand panel was obtained with PEG 5 000 000 at a concentration of 1.0 mg or 0.2 nmol/L, corresponding to about 1.2 molecules in an average droplet for which the diameter when initially formed is 2.8 μm. The ability to obtain spectra with the indicated signal/noise at such low molar concentrations is probably due to the extensive multiple charging in the ions of such large oligomers. If we make the reasonable assumption, already mentioned, that the m/z value at the peak of the band corresponds to that of the most abundant oligomer with the most probable charge state, we conclude that a PEG oligomer with a molecular weight of 5 000 000 must have about 4200 charges. In all the spectra of Figure 2, the signal amplitude, the signal/noise, and the mass/charge (m/z) values are about the same even though the molecular weights cover a 50-fold range. This observation indicates that a current of ions with 83 charges from an injection flux of N molecules/s for PEG 100 000 provides about the same detector signal as ions with 4100 charges from a flux of $N/25$ molecules/s of PEG 5 000 000. The most plausible explanation is that the multiplier response (number of secondary electrons per incident ion) increases markedly with charge on an incident ion. The alternative explanation, that overall ionization and transport efficiency for PEG oligomers has a similarly strong dependence on molecular weight, seems much less tenable.

Also noteworthy in all the spectra is the abundance of extraneous peaks in the m/z range below 800, especially in the case of PEG 5 000 000. To be remembered is that ions in the ES droplets have the strongest affinity for the most polar and polarizable species. In most experiments these charge-collecting species are the species of interest, i.e., analyte molecules. When they are present at customary concentrations, they sequester all the available charges so that other species do not appear in the spectrum. As noted earlier, it is not possible to operate the spray in the desirable "rainbow mode" with solutions of the very large PEG oligomers unless they are present in such low concentrations that they cannot soak up all the avilable charge. Consequently, even trace amounts of nonanalyte species will be ionized with relatively high efficiency and give rise to extraneous peaks in the spectrum, sometimes referred to as "chemical noise". It thus becomes extremely difficult, if not impossible, to obtain and maintain solutions so free of such trace species that the only ions in the spectrum will be those due to analyte and solvent species. Even impurities in the PEG samples, which would not give rise to ions at higher concentrations of analyte, will produce lots of background peaks at very low analyte concentrations. The relatively larger amount of such chemical noise in the case of PEG 5 000 000 may also reflect a difference in ion formation mechanism, a possibility that will be discussed later.

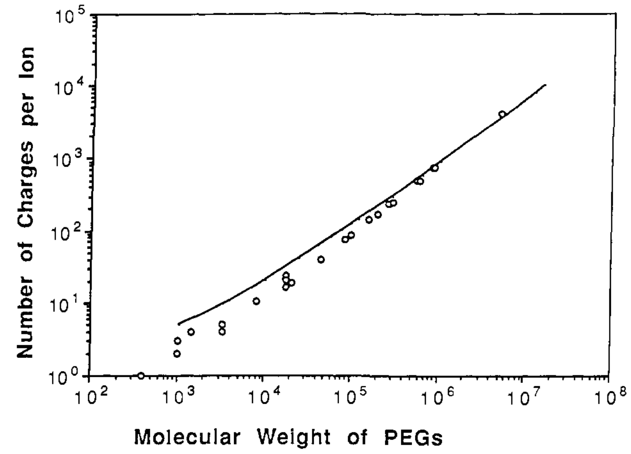

Figure 3. Relation between the number of charges per oligomer ion and its molecular weight. The solid curve shows the predictions of the electrostatic model. The points are experimental values.

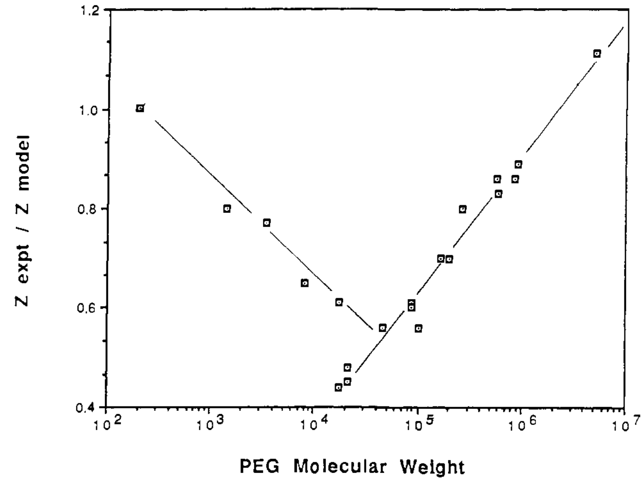

Figure 4. Relation between the nominal molecular weight of PEG samples and the ratio of the number of charges actually found on oligomer ions to the number predicted by the electrostatic model.

Figure 3 compares, at various molecular weights along the abscissa, the number of charges per oligomer ion shown by the ordinate values of the indicated data points. This number of charges Z is equal to $M/(m/Z - 23)$, where M is the nominal molecular weight of the solute (that of the most abundant oligomer) and m/Z is the scale value for the peak of the spectral band. It represents the ratio of the ion's mass m to its number of charges Z and is, of course, equal to m for the singly charged ions with which most mass spectrometrists are familiar. The number 23 accounts for the mass of each adduct charge, assumed to be Na^+ for these large PEG ions as it clearly is for the smaller ones.[1] The solid line shows predictions according to the model set forth in the previous paper. Data from that study as well as this one are included in the plot. Because they embrace such a large range of values, logarithmic scales were used for both coordinates. The greater apparent scatter in the values at the lower end of the scale reflects the effects of changes from point to point in concentration, solvent composition, and/or the amount of cosolute NaOH. In spite of scale "compression" and data scatter, it is clear that the discrepancy between the experimental and predicted values decreases with increasing molecular weight. Indeed, for the oligomers with a nominal molecular weight of 5 000 000, the number of charges observed (4200) exceeds the maximum possible number of charges predicted by that model (3760).

Because the ordinate scale in Figure 3 is logarithmic, the differences between the observed and model values for the number of charges on an oligomer are so compressed that any "fine structure" in the dependence of Z upon M is not apparent to the eye. The nature of this dependence emerges clearly in Figure 4 which shows for the same data the molecular weight dependence of the ratio Z_{expt}/Z_{model}, the fraction of the possible number of charges (according to the model) that are actually found on the desorbed (mass analyzed) ions. On this semilog plot the data points seem to fall on or close to two straight lines that intersect at a molecular weight somewhere between 20 000 and 30 000. The branch for low molecular weights shows a monotonic decrease (from a value that must be unity for the limiting case of oligomers so small that they can hold only one charge) to the intersection point value of about 0.55. The branch for high molecular weights starts at a low value of about 0.47 and increases linearly to a value of 1.11 for oligomers with a molecular weight of 5 000 000, the largest we tried. This behavior, shown in Figure 4, raises some questions that we will now attempt to address.

1. First we note that the value of 1.11 for $Z_{\mathrm{expt}}/Z_{\mathrm{model}}$ of the largest oligomers indicates that they are retaining more charges than the model allows. We think this apparent anomaly is probably due to the model's neglect of possible shielding of the Coulomb interaction between charges. The values of dielectric constant for methyl and ethyl ethers, structurally similar to unit lengths of PEG oligomer, are relatively low, 5.02 and 4.34, respectively, at room temperature. But they are enough larger than unity so that there should be a small but finite attenuation of Coulomb repulsion between charges along the oligomer spine, especially between pairs that are far apart. Of course, wiggling of the chain due to Brownian motion, another factor ignored by the model, would tend to decrease the time-averaged distance between charges and thus decrease the charge capacity. However, the substantial tensile stress on the oligomer spine due to Coulomb repulsion should minimize the amplitudes of any such Brownian wiggles. In our view, therefore, the increase in charge-holding capacity due to shielding is probably greater than any decrease due to Brownian motion and accounts for the apparent "excess" of charge on the oligomers of PEG 5 000 000.

2. Next we consider how these largest of oligomers acquire a much larger fraction of the charge allowed by the model than do their smaller cousins that desorb before they reach the maximum charge state. We noted earlier that to electrospray solutions of large PEG oligomers we had to decrease their concentrations to very low values, 0.2 nM in the case of PEG 5 000 000. For this concentration the nascent ES droplets, having diameters of 2.8 μm, each contained on average only about 1.4 molecules. Therefore, a majority of the droplets contained only one oligomer molecule. From measurements of the liquid flow rate and the total needle current, we know that there were about 4.4×10^4 charges on each such nascent droplet. The 4200 charges found on the final oligomer ions represent slightly less than 0.10 of this number. Elegant measurements on isolated droplets by Taflin et al.[11] show that for a variety of liquids with low vapor pressure, charged droplets with diameters down to 50 μm or so undergo a Coulomb explosion when their surface charge density reaches about 80% of the Rayleigh limit, a value confirmed by recent in situ observations of droplets in a spray by Gomez and Tang.[9] Moreover, Taflin et al. found that each such explosion resulted in a loss of about 2% of the droplet mass and 15% of its charge. If our much smaller droplets behave in the same way, even after a sequence of 14 such explosions, an unlikely possibility in our view, they would retain some 4500 or so charges, more than enough to saturate the single PEG oligomer they might still contain. We can readily calculate that the diameter of a droplet for which 4500 charges represents 80% of the Rayleigh limit is about 0.3 μm if the surface tension has a value of 36 dyn/cm, our guess for a droplet of water containing some methanol and some PEG which has some surface-active properties. An oligomer with a molecular weight of 5 000 000 is about 40 μm long. To be contained within a volume whose maximum dimension is 0.3 μm, it clearly must be folded into a coil or tangle so that the droplet resembles a piece of composite material comprising fibers dispersed in a matrix. Its effective "tensile strength" should be much greater than the surface tension of the pure liquid so it would not as readily undergo a Coulomb explosion as would a droplet with much smaller solute species. Indeed, it seems likely that even the original droplet, with a diameter of 2.8 μm and containing a 40-μm polymer chain, might resist being torn apart by Coulomb forces. In any case, the most likely scenario for ion formation with these very large oligomers would seem to involve the solvent leaving an analyte molecule trapped in the droplet, rather than an analyte ion leaving a droplet by field-assisted desorption. The mode of solvent departure may be by evaporation of individual molecules, charged or neutral, or by Coulomb ejection of tiny charged droplets, or both. Clearly, however it leaves, the solvent must constitute a vehicle for removal of charge that cannot be retained by the residual oligomer. In sum, the charging of these very large oligomers probably occurs by the charged residue mechanism first invoked by Dole in his pioneering experiments of 2 decades ago.[6]

Additional support for this conclusion can be plausibly inferred from the spectra in Figure 2 that show a much higher abundance of chemical noise in the spectrum for PEG 5 000 000 than for the smaller oligomers. If ions of PEG oligomers form by desorption, then the smaller and less polarizable solute species would remain in the droplet until the amounts of both charge and PEG oligomers had been greatly depleted by desorbing ions. By then the charge remaining on the droplet might not be sufficient for desorption of the smaller species as ions. Evidence from both theory and experiment suggests that small solute species often seem to require higher surface fields for desorption as ions than do PEG oligomers. If they could not desorb as ions, the smaller species would not leave the droplet but would comprise a component of the charged residue after all the solvent evaporated. On the other hand, if a large PEG oligomer forms an ion not by desorption but by remaining in the parent droplet until all the solvent evaporates, in accordance with the CRM, then there might be ample charge and opportunity for smaller trace species to desorb as ions while the evaporation is occurring because the PEG oligomer would not remove charge from the droplet by desorbing. In this perspective, the greater amount of chemical noise in the spectrum for PEG 5 000 000 constitutes additional evidence that the large oligomers do indeed form ions by the charged residue mechanism.

3. We now consider a possible explanation for the decrease in the ratio $Z_{\mathrm{expt}}/Z_{\mathrm{model}}$ from a value of unity for very small oligomers to about 0.5 as the molecular weight increases to values around 20 000. In the perspective of the desorption model for ion formation, this trend indicates that large oligomers leave the surface with a smaller fraction of their charge capacity occupied than do small oligomers. Because the model requires that oligomer charge capacity per unit length also decreases with increasing molecular weight, the trend in Figure 4 indicates that the number of charges *per unit length* of oligomer leaving the surface decreases even more rapidly with increasing molecular weight than the slope of the line would suggest. As noted earlier, the charge spacing on a desorbing ion cannot be smaller than the charge spacing on the droplet surface. Consequently, the field at the droplet surface when a large oligomer ion desorbs must be less than when a small oligomer desorbs. This combination of fewer charges per unit length and smaller surface field means that when large oligomer ions leave the surface they require substantially less electrostatic force per unit length for desorption than do their smaller kinfolk. In other words the trend shown in the low molecular weight regime of Figure 4 strongly indicates that the fugacity or escaping tendency of PEG oligomers in an aqueous solvent increases with increasing molecular weight. Is there other evidence of such a trend in escaping tendency? In a word, yes.

It has long been known that the solubility of PEGs in aqueous solvents decreases with increasing temperature. This unusual behavior is revealed by the appearance of turbidity when a clear solution being heated reaches a particular temperature. This "consolution" temperature decreases as the PEG molecular weight increases up to values around 50 000 but remains relatively unchanged as the molecular weight is further increased.[12,13] In other words, at a given temperature, large oligomers are less soluble, i.e., are more eager to leave the solution, than are smaller ones. Similarly, the surface activity of aqueous PEG solutions increases with increasing molecular weight up to values of about 10 000. From there on, up to values of several million, the interfacial adsorption characteristics remain approximately constant.[14] The decreasing consolution temperature and the increasing surface activity both strongly suggest that increasing molecular weight in the range below 10 000 should increase the fugacity of PEG oligomers and decrease the work required to remove them from

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the droplet. The slope of the left-hand straight line in Figure 4 provides strong confirmation of this suggestion.

4. There remains the question of why the $Z_{\mathrm{exptl}}/Z_{\mathrm{model}}$ ratio should increase as oligomer molecular weight increases above the 30 000 level. As we just noted, both consolution temperature and surface activity seem to remain relatively constant in this range. A possible clue to this rise is found in the molecular weight dependence of another solution property, the viscosity. This dependence for dilute solutions can be described by an expression of the form:

$$\lambda = A + KM^a$$

where λ is the intrinsic viscosity, A and K are constants, and M is the molecular weight. Faucher and Callard found that for PEG in water at 303 K the exponent a had a value of 0.5 or slightly less at molecular weights below about 3000.[15] Above that value, it becomes 1.0. Thus, along the left-hand line of Figure 4 as molecular weight goes from 1000 to 20 000, the viscosity increases by a factor of about 4. On the right-hand line from 20 000 to 5 000 000, it goes up by a factor of 200. Moreover, evaporation of solvent from the droplet further increases the viscosity by increasing the solute concentration. As solution viscosity increases, the velocity with which oligomers and charges can move around on the droplet surface must decrease. Consequently, it should take longer for an oligomer to position itself so that there is coincidence between the positions of O atom sites and the Na^+ ions on the surface. Moreover, the surface becomes more and more "sticky" as the viscosity goes up so that desorption occurs more slowly even after an oligomer has attached enough charges to achieve lift-off. Meanwhile, evaporation of solvent continues to shrink the droplet and decrease the charge spacing on its surface. These effects combine to slow down the rate of ion desorption relative to the rate at which charge spacing decreases on the droplet surface. Thus, as solution viscosity increases with increasing solute molecular weight, the number of charges on a desorbing ion should be expected to increase. The positive slope of the right-hand straight line in Figure 4 is consistent with this expectation.

The scenario just described seems plausible but is certainly too speculative and qualitative to be above reproach. Even so it may provide a useful perspective from which to view and to reflect on what actually happens in the extremely complex drama that takes place in an ES source between the time sample solution leaves the injection needle tip and solute ions enter the vacuum system. The underlying idea is that ion evaporation from a charged droplet is the process by which most solute species in solution become ions in the gas phase. Since first proposed by Iribarne Thomson,[4] this model has become widely accepted as the mechanism underlying ion formation on all of the ionization techniques that involve dispersing a sample solution into charged droplets. It seems to provide a reasonable basis for explaining most of what we have found in this study of ions formed in an ES source from poly(ethylene glycols) over a wide range of molecular weights. Only for the case of the largest oligomers (M = 5 000 000) does it clearly have to defer to the charged residue model originally proposed by Dole. However, persuasive arguments against the ion evaporation mechanism have been mounted. It may well turn out that an equivalently plausible scenario can be based on the idea that observed solute ions are, in fact, the charged residues of tiny droplets born of Rayleigh instabilities (Coulomb explosions) in parent droplets. This mechanism, favored by Roellgen et al.,[16,17] is similar to the original charged residue model of Dole and, in fact, provides the most reasonable explanation for ion formation with very large molecules. Indeed, the size of a molecule that can be ionized intact by the charged residue mechanism would seem to be limited only by the size of the initial droplet! Unfortunately, to distinguish usefully between individual ion species from parents with ultrahigh mass may require more resolving power than can be achieved with available analyzers except, perhaps, large sector instruments and those based on trapping ions with electrostatic or magnetic fields. Because ES ions from most species, however massive, have enough charge to bring the m/z values into the desired range for ICR machines, mass analysis by FTICR may provide the resolution needed to exploit fully the ability of ES to produce intact ions from ultralarge molecules. It is noteworthy that there are enough charges on large ES ions to allow accurate "weighing" of single ions by FTICR. Thus, there also arises the intriguing prospect of being able to carry out sequence analysis of large biopolymers by stepwise fragmentation of single ions. Many problems must be solved before this prospect can materialize.

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Registry No. PEG, 25322-68-3.

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