

Reflections on Electrospray Mass Spectrometry of Synthetic Polymers

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Electrospray ions of several samples of poly(ethylene glycol)s (PEGs) were examined and characterized by three different analyzers including two quadrupole mass filters and one ion trap. Also shown are some data obtained by MALDI-TOF. Comparison of the results with those obtained some years ago shows that relatively modest increases in instrument resolving power can greatly change the appearance of a spectrum and increase its information content. However it also seems possible that too much resolving power may actually decrease the accessibility of desired information. Also presented are some results on the effect of polymer solutes on the deformation of droplets subjected to intense electric fields. The observations suggest that such materials may profoundly affect the fundamental processes involved in the formation of solute ions from charged droplets.

1. Introduction

Electrospray ionization mass spectrometry (ESI MS) and polymers are two sides of a coin minted by Malcolm Dole in the mid 1960's. Long interested and active in research on synthetic polymers, he recognized the need for a method of determining the molecular weight (M) distribution of their component oligomers and came up with a provocative approach. A dilute solution of polymer molecules in a volatile solvent would be dispersed by an intense electric field as tiny charged droplets into nitrogen at atmospheric pressure. The first scientific studies of such¹⁾ "Electrospray" dispersion had been carried out by Zeleny early in this century. Evaporation of solvent from such charged droplets would increase the surface charge density until Coulomb repulsion exceeded surface tension. As had been characterized by Rayleigh²⁾ and observed by Zeleny, the resulting instability would disrupt the droplet into a plurality of smaller droplets, each of which would repeat the evaporation-disruption sequence. Dole reasoned that if the original solution were sufficiently dilute, successive repetitions of that sequence would ultimately lead to droplets so small that each one would contain only one molecule of solute. That residual molecule in such an ultimate droplet would retain some of its droplet's charge to become a gaseous ion as the last of the solvent evaporated. Results reported in several of Dole's papers over the next few years seemed to show that this electrospray process could indeed produce gas phase ions from large non-volatile solute molecules.³⁾ However, they did not provide persuasive evidence for the effectiveness of the ionization. Nor did they provide persuasive evidence that his Charged Residue Model (CRM) was an adequate explanation of his results. Part of the problem

was the inadequacy of his apparatus and especially his method of characterizing the ions. More importantly, he did not appreciate the extent to which the ions would be resolved during the adiabatic cooling that occurred in the free jet expansion by which the ions were transferred from the atmospheric pressure at which they were prepared into the vacuum environment of his mass analyzer. Even so, Dole's ideas and experiments were the seeds that ultimately led to all the success that ESI has since achieved. It has often happened in the history of science that errors and misconceptions, even downright wrong ideas, have ultimately led to great advances!

2. The Post Dole Era

Except for some attempts in the early 1970's to reproduce Dole's experiments at Yale, ESI seems to have been ignored by other investigators until the early 1980's when Yamashita and Fenn at Yale revisited Dole's ideas. Instead of trying to produce ions from large molecules they decided to look at solute species small enough for their ions to be "weighed" by an available small quadrupole mass filter with upper limit for m/z of only 400. In 1984 they published two papers showing that intact ions, both negative and positive, could be produced from solutes that though small could not be vaporized without catastrophic decomposition.⁴⁾ A new apparatus was then assembled that contained a quadrupole mass analyzer for which the upper limit of m/z was increased to 1,500. With this new apparatus it was found that ESI could produce intact ions from a variety of species including peptides with M values of at least 1,200. Moreover, the ions of some of those peptides were doubly charged.⁵⁾ In 1984 the results of Wong, Meng, and Fenn with poly(ethylene glycol)s or PEGs provided the first bona fide realization of Dole's original hope that ESI could indeed produce ions from synthetic polymers.⁶⁾ Their results also showed that ions with more than one charge could be produced. PEGs are soluble in various solvents and available over a wide range of nominal molecular weight, *i.e.*, the M value for the most abundant oligo-

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mer in a mixture of oligomers having a distribution of sizes that in principle should be Poisson-like but in practice is somewhat broader. Wong *et al.* also found that the number of charges per ion, increased with the M_n of the parent molecule. Their relatively simple spectra for PEG 1,000 and PEG 1,450 in Fig. 1 show the essential features embodied in most spectra of synthetic polymers comprising mixtures of oligomers of varying sizes. The PEG 1,000 spectrum shows two sets of peaks, I for singly charged ions and II for doubly charged ions. In each set the distribution of peak heights corresponds to the expected distribution of abundance for oligomers having molecular weights (M_n values) ranging from about 700 to 1,400. For PEG 1,450, there are three sets of peaks. The relative abundances for singly charged ions I, as indicated by the peak heights, are much smaller relative to the those with higher charges (II and III) than was the case for PEG 1,000. In each set the most abundant ions were those with M_n values around 1,450, as should be expected. It should be noted that the charges on these PEGs are sodium ions Na^+ which are bound to ether linkage O atoms (*i.e.*, as in $-\text{C}-\text{O}-\text{C}-$) along the polymer spine with an energy of 2.05 eV.⁷ There is enough sodium in the polymer as received to provide all the Na^+ ions in the solution. By adding relatively small amounts of appropriate salts or an acid to the electrospray solution one can produce exactly analogous ions and spectra in which all the charges are due to cations of the added species, *e.g.*, H^+ , K^+ , or Cs^+ .

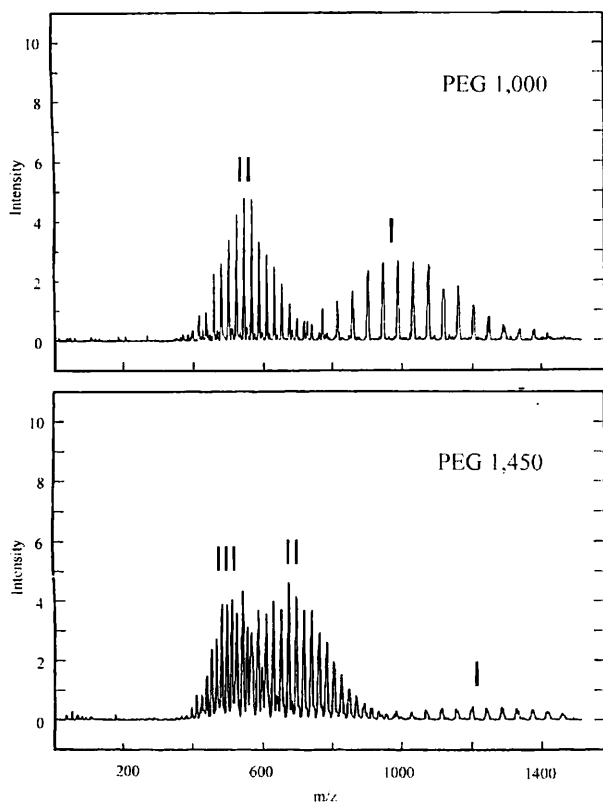


Fig. 1. ESI mass spectra for PEGs 1,000 and 1,450. ESI mass spectra for PEGs 1,000 and 1,450 at a concentration of 0.05 gm/L in 12:1 methanol-water taken with a VG quadrupole mass analyzer (Model 1,212).

The trend hinted at in Fig. 1 is that as nominal M_n increases, the number of different oligomers, the number of monomers per oligomer, and the number of charges per oligomer all increase. These trends result in an increase in peak density that is clearly evident in Fig. 2 for PEG 3,350, the largest one for which individual peaks could be resolved with the VG 1212 mass analyzer used in those first PEG studies. In that spectrum there are no discernible peaks for ions with one or two charges. The most abundant ions have five, followed by those with six and four charges with triply charged ions having the lowest abundance. In each of

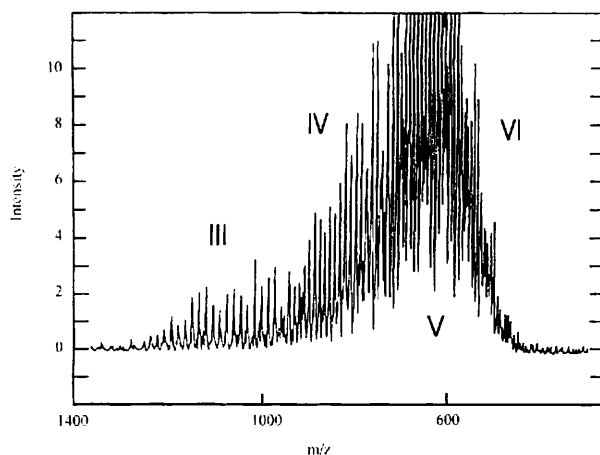


Fig. 2. ESI mass spectrum of PEG 3,350. ESI mass spectrum of PEG 3,350 under conditions identical with those for Fig. 1.

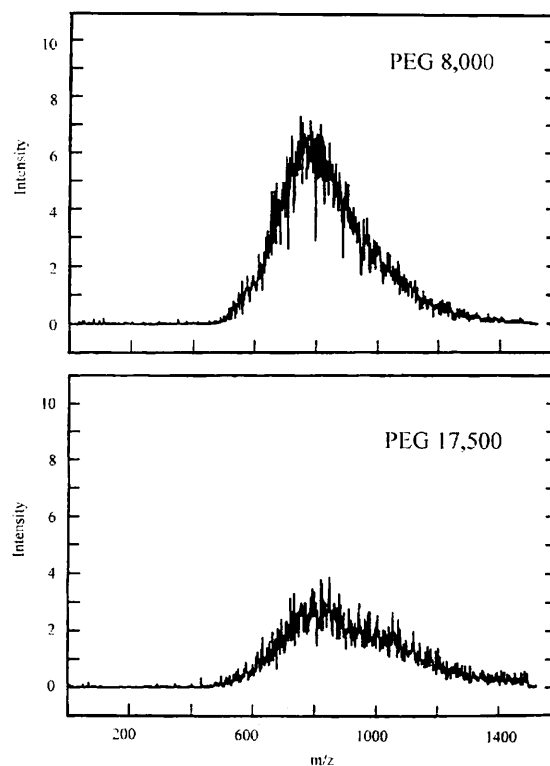


Fig. 3. ESI mass spectra for PEGs 8,000 and 17,500. ESI mass spectra for PEGs 8,000 and 17,500 under conditions identical with those of Figs. 1 and 2. These spectra show the mountain-like wave from that results when the mass analyzer is unable to resolve the individual peaks.

these sets the most abundant ions are those whose m/z values correspond to oligomers with M_r values close to the nominal molecular weight for the sample, *i.e.*, 3,350. The ions with five charges are the most abundant of all.

The increasing congestion of peaks with increasing M_r in ESI MS spectra for synthetic polymers, revealed in these simple examples, imposes an upper limit on the nominal molecular weight of such polymers for which detailed information on composition can be obtained by this technique. Thus, as the spectra in Fig. 3 clearly show, our VG1212 analyzer could not resolve any individual peaks for PEGs having nominal M_r values of 8,000 and 17,500. However, one can make the reasonable assumption that the m/z value of the maximum in what looks like a very noisy wave form in the spectra of Fig. 3 can be taken as the m/z value of the most abundant oligomer with the most probable number of charges. That assumption allowed Wong *et al.* to conclude that the most abundant oligomer ion in PEG 8,000 had ten charges and in PEG 17,500 had 23 charges. Unfortunately, one cannot reverse this logic and determine the nominal M_r value from the m/z value of the maximum in a spectrum like those in Fig. 3. For any m/z value near the maxima of such spectra there are so many different combinations of oligomer M_r and charge number that there is no way of selecting the right one for the sample being interrogated.

In later experiments with that same VG1212 analyzer, Nohmi and Fenn found similarly plausible but indirect evidence that intact ions with several thousand charges could be formed from PEG oligomers with nominal M_r values up to 5,000,000 or more.⁸¹ They also made use of an electrostatic model proposed by Wong *et al.* for determining the maximum number of charges that could be retained by a gaseous oligomer ion of PEG. They were thus able to conclude from their data that the ions of very large analyte species are most likely formed by the Dole's CRM whereas the smaller oligomers were most likely formed by an Ion Evaporation Mechanism (IEM) that had been introduced by Iribarne and Thomson.⁹¹ They argued that before Dole's sequence of evaporation and disruption steps reached its ultimate stage of one solute molecule per charged droplet, the field at the droplet surface would become strong enough to "lift", *i.e.*, "evaporate", a surface ion into the ambient gas. Many investigators think this IEM model applies in many more cases under more conditions than does Dole's Charged Residue Model (CRM). The results of Nohmi and Fenn suggest that the CRM applies for very large PEGs.

Meanwhile, the upper limit of M_r for molecules that ESI can transform from solutes in solution to intact molecules in the gas phase, keeps going up. Recent experiments by others have shown that ESI can produce intact ions of DNA with M_r values of over 100 million, but the resolution with which those ions can be mass-analyzed is limited.^{100, 111}

3. The Problem with Synthetic Polymers

Unfortunately, the multiple-charging ability of ESI that greatly extends the effective mass range of mass analyzers is also the feature that greatly limits its

usefulness in the analysis of synthetic polymers. Most such polymers comprise mixtures of large oligomers with a fairly broad distribution of M_r 's. Each large oligomer in turn gives rise to ions with a broad distribution of charge states. The superposition of these two distributions produces spectra that are extremely congested as shown in the spectra of Fig. 3 obtained with the VG1212 analyzer mentioned earlier for solutions containing 0.05 gm/L of (a) PEG 8,000 and (b) PEG 17,500 in 1 : 1 methanol water. Indeed one of the reviewers of a manuscript showing these spectra insisted that they were not mass spectra of PEG oligomers but simply of dirt in the system! The clear message seemed to be that because of extreme peak congestion, resolving ESI mass spectra of synthetic polymers with M_r values much above 20,000 or so, would require mass analyzers of much higher resolving power than could be provided by those available at modest cost.

The advent of "off-the-shelf" availability for Fourier transform ion cyclotron resonance mass spectrometers (FTICR MS) at gradually decreasing prices seemed to promise a solution to the resolving power problem. So far, however, that possibility remains more of a hope than a promise. O'Connor and McLafferty¹²⁾ have recently studied PEGs with nominal M_r 's from 4,000 to 23,000. Although they were able to obtain spectra with isotopically resolved peaks for all oligomers in a few minutes, the detailed interpretation of those spectra turned out to be a very challenging problem. Deconvolution by available computer algorithms doesn't work very well and the human equivalent is extremely laborious. Moreover, there are substantial mass discrimination problems because, among other reasons, trapping a truly representative sample in the ICR cell is very difficult to achieve. Perhaps some new and clever algorithms will emerge that are "smart" enough to extract the desired information from these very congested FTICR MS spectra. As of now, however, ESI MS instruments with the highest available resolving power cannot readily provide M_r distributions for the oligomers in synthetic polymers with nominal M_r values above 25,000 or so. Thus the question becomes whether there other approaches by which MS can be persuaded to provide the desired M_r information for poly-disperse synthetic polymers. Several such possibilities will be examined in what follows.

4. Decreasing Charge Multiplicity

In the perspective of the preceding paragraphs it seems clear that an ability to produce ions from these polymers with less extensive multiple charging should help. Indeed, ion production by bombardment of sample material with fast atoms as in FAB, or ions as in (SIMS), or laser photons as in MALDI, can produce ions from large species with very few charges. Unfortunately, the ions produced by these desorption techniques have such high mass/charge ratios that their cyclotron frequencies are too low to provide high resolution spectra with FTICR MS. Thus the question becomes whether other methods of mass analysis can provide the resolving power needed for ions with high mass/charge (m/z) values. It has emerged that the classic time-of-flight (TOF) approach lends itself partic-

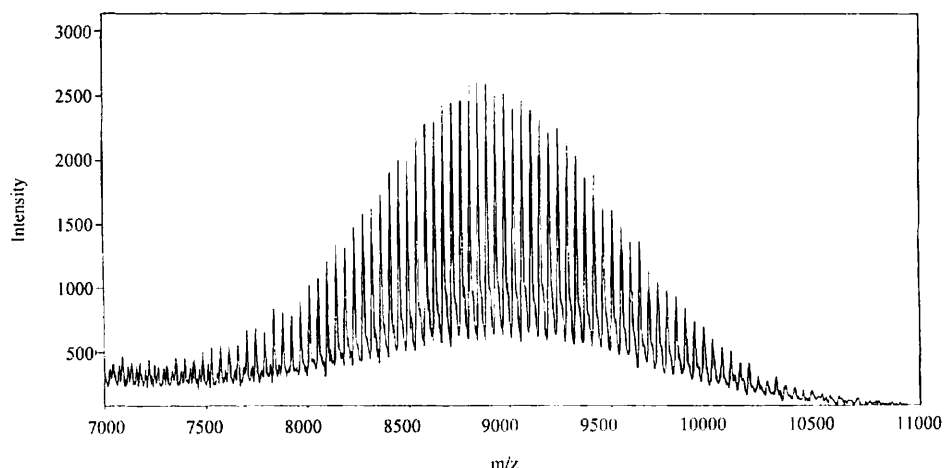


Fig. 4. MALDI-TOF mass spectrum of PEG 6,000. MALDI-TOF mass spectrum of PEG 6,000 obtained with perceptive voyager system using alpha-cyano-4-hydroxydynamic acid as the matrix material.

ularly well to MALDI so that a number of groups are pursuing this option. In some preliminary experiments with a Perceptive Voyager we obtained some spectra, of which an example for PEG 6,000 is shown in Fig. 4. We gave up further experiments when we found that the signal strength decreased as the nominal M_n value increased and became too small to be useful for PEGs of 20,000 or more. Others have found in general that for polymers comprising oligomers with highly disperse M_n 's the relative abundance of oligomer ions does not correlate reliably with the relative abundance of their parent oligomer molecules in the sample. A possible reason may be that too few oligomers of substrate are involved in each desorption-ionization event (*i.e.*, encounter of a packet of photons or particles with a packet of substrate sample oligomers in the surface). Thus, the packet of substrate oligomers may not be representative of the sample as a whole. Similarly, unless all the molecules in the substrate packet are ionized with equal probability, the relative abundances of the resulting ions may not be representative of the abundance of their parent oligomers in the desorbed packet of substrate molecules. For whatever reason, investigators have found that as the nominal M_n values of synthetic polymers increase, the substrate composition deduced from MALDI mass spectra departs increasingly from the actual composition.

It has thus become increasingly clear that in the mass spectrometry of large synthetic polymers polydispersity is a fundamental and formidable problem. When ionization is by ES the spectra have so many peaks so close together that resolving and/or interpreting their pattern becomes extremely difficult, even nearly impossible, in the present state of the art. When ionization is by desorption, from a surface as in FAB, SIMS, and MALDI, the resulting spectra do not faithfully represent the composition of the sample. For these reasons many investigators are resorting to "fractionation" of the original sample, *e.g.*, by gel permeation chromatography, so that each "fraction" of the sample that is ionized will have a dispersity sufficiently low so that the important peaks its mass spectrum are readily resolvable. However, the increase in experimental complication and data interpretation involved in this

option are such that the search goes on for MS methods that can provide the desired information from unfractionated material.

Another possible method of obtaining ions with lower charge multiplicity is to remove some charges from the large ES ions that we already know how to produce, thus reaching a middle ground in which ions have enough charges to exploit the high resolving power and precision of FTICR MS but not so many as to result in hopeless congestion. Removal of charges from multiply charged ions has been observed and is being extensively studied by many investigators with techniques that include charge exchange with other species, neutralization with ions or charges of the opposite sign, and "brute force" removal by energetic collisions with neutral species. A particularly simple and inviting approach is to apply a potential difference between the orifice (through which ion bearing gas in an ESI MS instrument enters the vacuum system of the mass analyzer) and the first collimating orifice or skimmer that passes some of that gas into the high vacuum chamber housing the mass analyzer. The resulting axial field in the free jet accelerates ions but not neutral molecules bringing about ion-neutral collisions with sufficient energy to strip the ion of one or more of its charges. Musselman *et al.* were the first to demonstrate such charge stripping.¹³⁾ McEwen *et al.* showed that it could simplify somewhat the ESI MS spectrum of PEG 3,000.¹⁴⁾ Even so it would seem that neither this approach nor any of the other charge removal techniques are yet in a stage of development that is close to becoming a useful tool in the ESI MS analysis of synthetic polymers.

5. Decreasing MS Resolving Power

We have noted that the very high resolving power of FTICR MS results in high peak congestion of an ESI spectra in part because it produces a separate peak for each molecule that differs from other molecules by as little as the single unit of m/z caused by a difference of one in the atomic isotope content of that molecule. That observation leads naturally to the question of whether reducing the effective resolving power of a mass analyzer could simplify the spectrum enough to

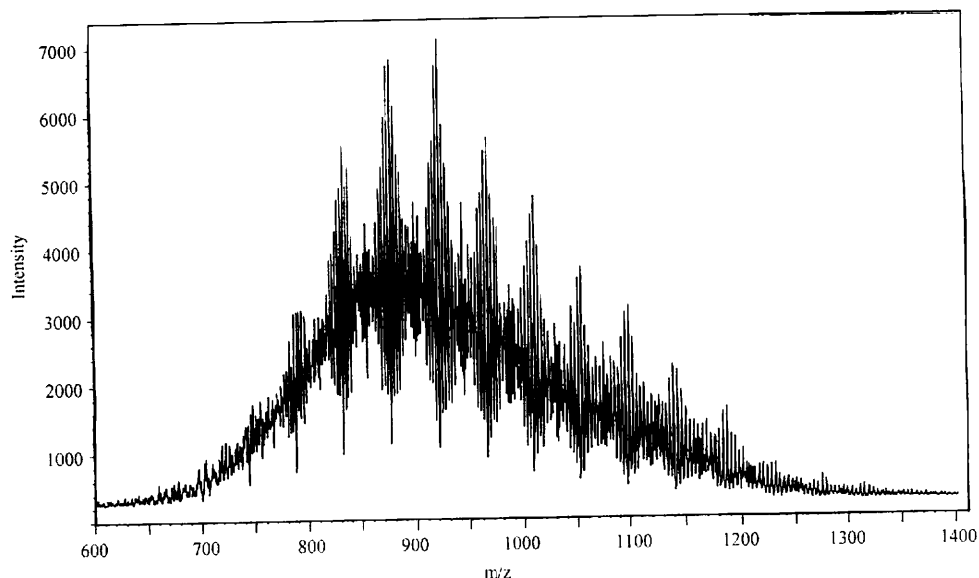


Fig. 5. ESI mass spectrum of PEG 10,000. ESI mass spectrum of PEG 10,000 at a concentration of 0.030 g/L in 1:1 methanol-water obtained with Delsi-Nermag quadrupole analyzer (Model 3010) having an effective resolving power of 1,500.

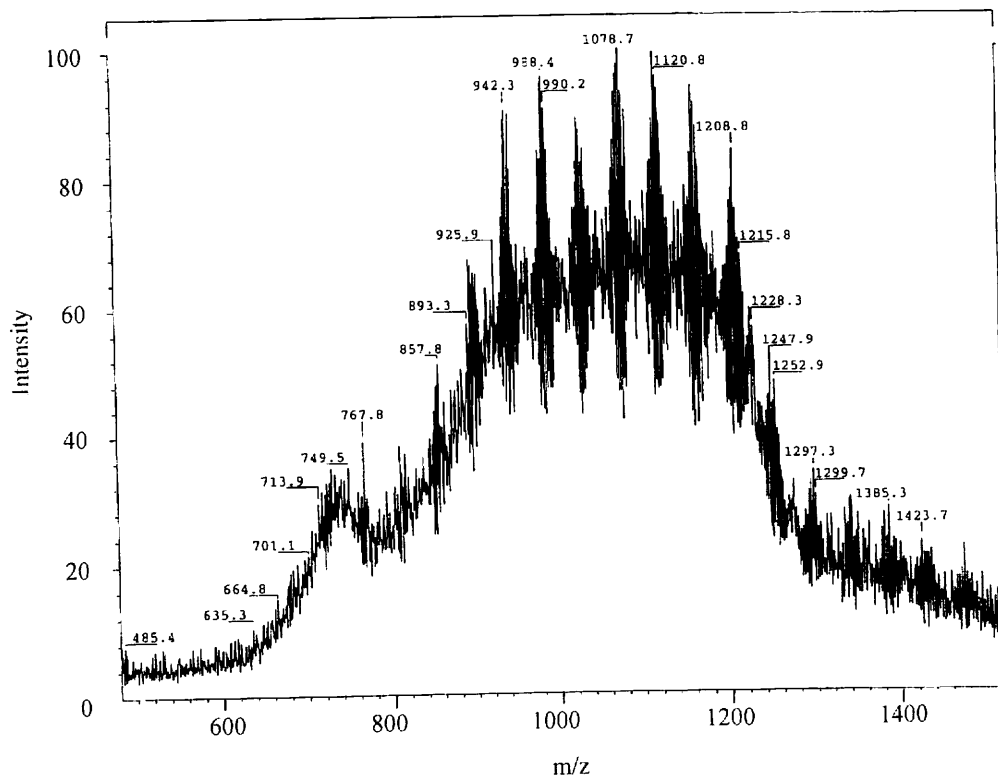


Fig. 6. ESI mass spectrum of PEG 20,000. ESI mass spectrum of PEG 20,000 at a concentration of 0.05 g/L in 1:1 methanol-water obtained with a Thermoquest TSQ7000 MS quadrupole analyzer.

facilitate its interpretation but not enough to prevent the needed information from being obtained. Some of our recent experimental results invited consideration of this possibility.

With a Delsi-Nermag 3010 triple quadrupole instrument having an effective resolving power of unity for m/z values around 1,500, several times better than the VG1212 it replaced, we repeated some of the earlier ESI MS experiments of Wong *et al.* on PEGs. Figure 5 shows the spectrum obtained with a solution of 30 mg/L of PEG 10,000 in 1:1 methanol-water containing

0.33 mM KOH. The mean M_r value of this analyte is somewhat larger than that of the PEG 8,000 in the spectrum of Fig. 3 but the resulting spectrum shown in Fig. 5 reveals a much more distinct and intriguing pattern. A very similar pattern appears in a spectrum for PEG 20,000 shown in Fig. 6 and obtained with a Thermoquest TSQ 7000, a quadrupole analyzer having a somewhat higher resolving power than the Nermag. McEwen and co-workers have found similar patterns.¹⁵⁾ The regular distinct sharp spikes shown in these spectra are 44 units apart on the m/z scale of the abscissa.

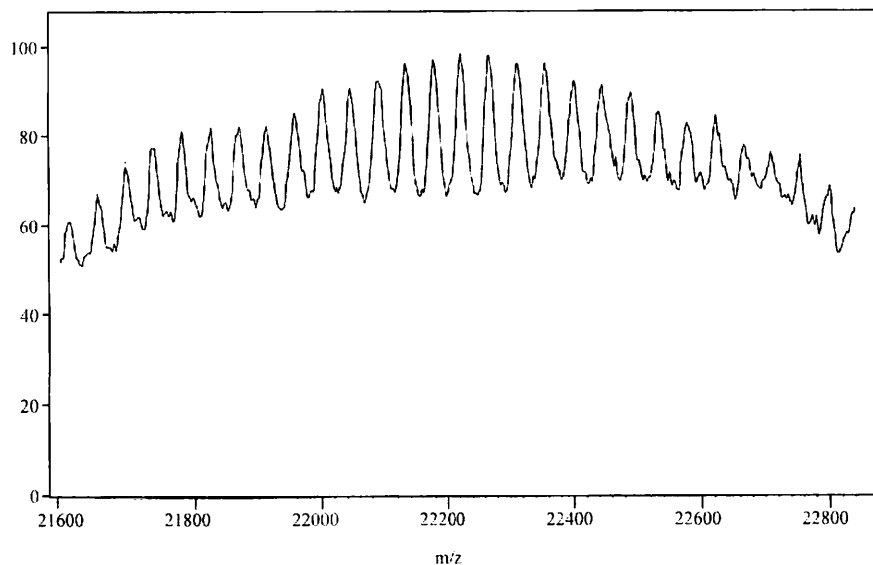


Fig. 7. Deconvolution of ESI mass spectrum of Fig. 6.

In a table of m/z values for all possible combinations of charge state and M_n values it becomes clear that there is an exact superposition of m/z values for several such combinations at intervals of 44 units of m/z . Thus, the difference between the m/z values of the spikes in Figs. 5 and 6 is exactly equal to the M_n value of the ethylene oxide monomers from which the oligomers are formed. Similar spectra for poly(vinyl acetate) show similar spikes separated on the m/z scale by an amount numerically identical with the M_n value of the vinyl acetate monomer! We have not yet had a chance to characterize more fully this "resonance" phenomenon by experiments with other polymers but it seems highly likely that it should be general. It would seem to follow that the "spike-spacing" in ESI mass spectra of homopolymers obtained with analyzers of modest resolution can identify the M_n value of the monomer, almost by simple inspection of the spectrum! What happens with polymers comprising more than one monomer species should be interesting to investigate.

In fact, much more information can be extracted from spectra like those in Figs. 5 and 6. Figure 7 shows the result of deconvoluting the spectrum of Fig. 6 with the algorithm included in the software supplied with the TSQ 7000. Such algorithms are now available in the operating system software supplied with many if not most commercial mass spectrometers equipped with ESI sources. Such software did not work very well with FTICR mass spectra obtained by O'Connor and McLafferty,¹²⁾ probably because the very high resolution of their analyzer produced an additional peak for each different combination of isotope atoms in every oligomer. The very high peak congestion that resulted probably confused the deconvolution algorithm. In other words, there is such a thing as too much resolving power!

It is noteworthy that the spectrum in Fig. 6 shows a secondary maximum at an m/z value near 740 that is absent in the spectrum of Fig. 5. It turns out that the relevant difference between the conditions for the spectra of Figs. 5 and 6 is not that the M_n values are different. Rather it is that the adduct charge for the

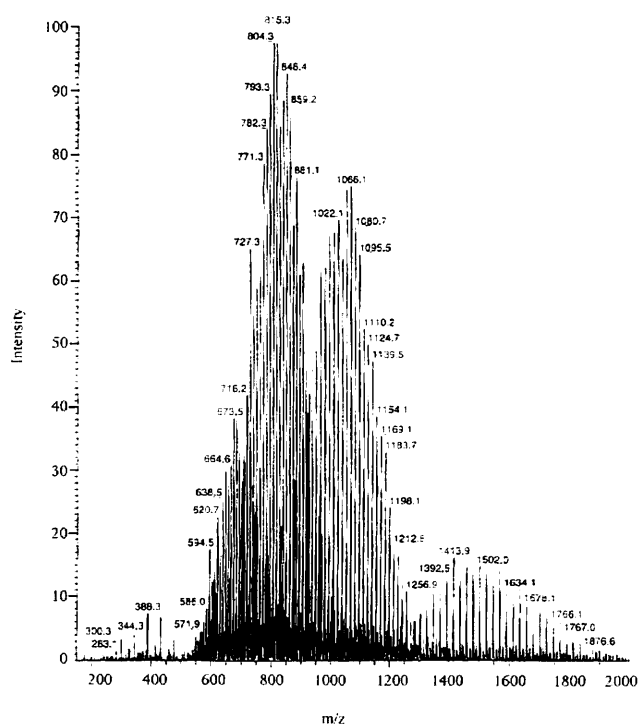


Fig. 8. ESI mass spectrum of PEG 4,000. ESI mass spectrum of PEG 4,000 at a concentration of 0.05 g/L in 1:1 methanol-water obtained with a Thermoquest LCQ MS at resolving power of about 8,600.

ions of Fig. 6 was Na^+ which is always the case when the PEG samples are dissolved in pure solvent so that the only available cations for adduct charges come from the sodium that is always present in the polymer as received. As noted, the spectrum of Fig. 5 was obtained after KOH was added to the sample solution so that the adduct charges are K^+ . When no KOH was added to the sample solution, the spectrum obtained for PEG 10,000 with the Delsi Nermag analyzer also showed the secondary maximum of Fig. 6. Additional experiments proved beyond any doubt that when Na^+ is the adduct charge, the secondary maximum always

appears. It is always absent when the adduct charge was H^+ , K^+ , or Cs^+ . In other words Na^+ adduction is somehow responsible for that secondary maximum but how or why remain unanswered questions. The take-home message of these experiments seems to be that judicious reduction of the resolving power of the mass analyzer may be able to achieve an optimum value that results in peak patterns of the kind shown in Figs. 5 and 6 which can be resolved with available computer algorithms. We have not yet determined a maximum nominal M_r value up to which this idea can be extended but we are encouraged by the fact that it worked well for a PEG whose nominal M_r value was close to that of the one which gave trouble in ref. 11.

Also to be considered is the seemingly ever increasing resolving power being achieved by the relatively simple and inexpensive quadrupole ion trap. Figure 8 shows a preliminary spectrum of PEG 4,000 obtained with a ThermoQuest LCQ. It seems to show excellent resolution of isotopic peaks for the doubly charged monomer with an M_r value of about 3,000. The magnification of that portion of the spectrum of Fig. 8 between the m/z values of 1,497 and 1,507 clearly shows the ability of an ion trap to resolve the isotopic components of the corresponding peaks in the spectrum of Fig. 8. We have not yet had an opportunity to explore further what can be done with this instrument but with the further increases in resolving power that seem almost inevitable, the ion trap may yet provide the most inexpensive but adequate answers to the most common questions about polymer composition.

6. Variation on a Theme from Fluid Mechanics

Disruptive instabilities of charged liquid droplets have been understood theoretically at least as far back as Rayleigh's paper in 1883²¹ but the first visual evidence of such disruption came in 1992 with the milestone photographs of Tang and Gomez who managed to capture an image of a droplet in the process of disrupting.¹⁶ Their results, along with those obtained earlier by Taflin *et al.*¹⁷ who followed the mass change in an exploding droplet with an electrostatic "balance", showed that the disruption process is so fast that it occurs on a micro- or nanosecond time scale. We thought it might be interesting to carry out the disruption with liquids sufficiently viscous to slow the process down, perhaps to the point where we could obtain photographs at several stages of the process. In some preliminary experiments we have produced a droplet of liquid at the tip of a short vertical length of hypodermic tubing with an od of 0.3 mm. About 3 cm above the tube was a counter electrode comprising a fine mesh disk about 2.5 cm in diameter. A potential difference between the tube and the counter electrode could be varied from zero to 10 kV. Backlighting illumination from a Strobotac flash lamp "blinking" at about 3 kHz passed through a low power microscope that focussed a shadow image of the tube and its droplet on the focal plane of a video camera operated at a framing rate of 30 s. The magnified shadow image was displayed on the screen of a CRT monitor and could be simultaneously recorded on a VCR (video cassette recorder). Incorporated in the circuit was a "grabber"

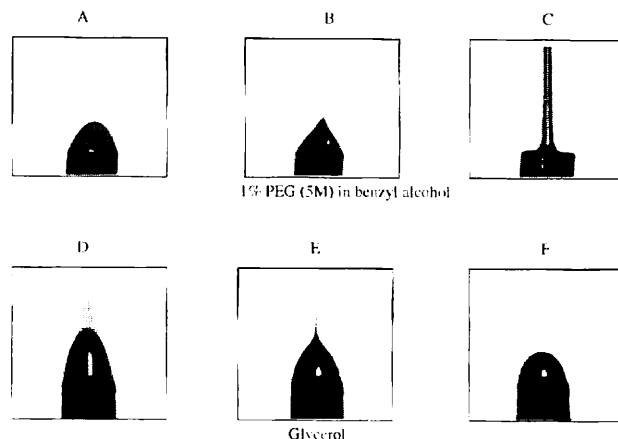


Fig. 9. Microshadowgraph pictures of hemispherical droplet on the tip of a small stainless steel tube for PEG 5,000,000 in benzyl alcohol and glycerol. Microshadowgraph pictures at various stages of distortion by an imposed electrostatic field of a droplet of a one per cent solution of PEG 5,000,000. In Panels A, B, and C the solvent was glycerol. In D, E, and F the solvent was benzyl alcohol.

that during replay of the tape could digitize and send to a desktop computer memory the picture on any single frame that we could select as we scanned the tape one frame at a time. The usual procedure for an experiment was to admit sample liquid through the tube to establish at its tip a droplet with a diameter roughly the same as that of the supporting tube. The flow was then stopped and the potential difference was slowly increased. In some preliminary experiments we compared the behaviour of a droplet of glycerol with one of benzyl alcohol (chosen for its low vapor pressure) containing one per cent of PEG 1,000,000 as a thickener. Both liquids had about the same conductivity and intrinsic viscosity (at zero shear stress) but the PEG-benzyl alcohol solution is non-Newtonian in that its elongational viscosity increases with increasing shear stress. Such non-Newtonian behavior is a well known characteristic of solutions having long chain polymers as solutes. Figure 9 summarizes the results of this comparison. Panel (A) shows a hemispherical "drop" of the PEG solution just after the field has become strong enough for the shape of its meniscus to depart from a perfect hemisphere on its way to becoming a cone. Panel (B) is a "snapshot" taken shortly after the meniscus has assumed a full conical shape with a wispy "jet" of fluid particles emerging from the apex. That wispy jet becomes the solid well-defined jet of Panel (C) which was "snapped" after that jet had removed almost all of the fluid originally in the droplet. Shortly after the picture of Panel (C) the jet stopped abruptly because all the droplet fluid had been removed. If fluid had continued to flow through the tube so as to replenish what was being removed, the cone-jet flow could have been maintained indefinitely. The quite different behaviour of the glycerol droplet is shown in the lower half of Fig. 9. In Panel (D) the droplet is just beginning to reach a conical shape with a wispy jet emerging from its tip similar to the config-

uration in Panel (B) for the PEG solution. In Panel (E) the cone has formed and is emitting a fine jet of liquid from its tip. Shortly after the snapshot of Panel (E) was taken the jet flow stopped and the meniscus "snapped back" to its starting hemispherical shape as shown in Panel (F). This cycle, including the stages of Panels (D), (E), and (F), then repeated as long as fluid remained at the tip. The few experiments carried out indicate that similar differences in behavior occur with other PEGs in water as well as in benzyl alcohol but become less pronounced with decreasing M_n .

We believe the field driven behavior of charged liquid surfaces exhibited in these experiments simulates what goes on in the routine practice of ESI, at the tip of the injection needle and during the disruption of ES droplets by Rayleigh instabilities that arise during solvent evaporation. To the extent that this belief is true these observed differences between Newtonian and non-Newtonian liquids can have strong implications with respect to the practice of ESI MS. It seems highly likely that many analytes, especially those with high molecular weight such as natural and synthetic polymers, may give rise to non-Newtonian behavior of the analyte solution. Even when a non-volatile solute's initial concentration in the sample liquid is very low, evaporation of solvent from a droplet can increase that concentration very rapidly. At half its original diameter a droplet's solute concentration is increased by a factor of eight. At a tenth the increase is a 1,000 fold. In our earlier study of large PEG oligomers as solutes⁶⁹ we found a distinct and as yet unexplained change in the dependence of ion charge on M_n , as M_n went from 10,000 to 30,000. It seems possible that this change might have been due to increased departure of liquid viscosity from Newtonian behavior. We plan further study of this phenomenon.

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References

- 1) J. Zeleny, *J. Phys. Rev.*, **3**, 69 (1914); *ibid.*, **10**, 1 (1917).
- 2) L. Rayleigh, *Phil. Mag.*, **14**, 184 (1882).
- 3) M. Dole, R. L. Hines, L. L. Mack, R. C. Mobley, L. D. Ferguson, and M. B. Alice, *Macromolecules*, **1**, 96 (1968); *J. Chem. Phys.*, **49**, 2240 (1968); L. L. Mach, P. Kralik, A. Rheude, and M. Dole, *ibid.*, **52**, 4977 (1970); M. Dole, "My Life in the Golden Age of America," Vantage Press, New York (1989), p. 169.
- 4) M. Yamashita and J. B. Fenn, *J. Phys. Chem.*, **64**, 2287 (1984); *ibid.*, **64**, 4471 (1984).
- 5) C. M. Whitehouse, M. Yamashita, C. K. Meng, and J. B. Fenn, "Rarefied Gas Dynamics," Vol. 2 (14th International Symposium) (ed. by H. Oguchi), Univ. Tokyo Press (1985), p. 733; C. M. Whitehouse, R. N. Dreyer, M. Yamashita, and J. B. Fenn, *Anal. Chem.*, **57**, 675 (1985); J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong, and C. M. Whitehouse, *Science*, **246**, 64 (1989)
- 6) S. F. Wong, C. K. Meng, and J. B. Fenn, *J. Phys. Chem.*, **92**, 546 (1987).
- 7) R. G. Keese and A. W. Castleman, Jr., *J. Phys. Chem. Ref. Data*, **15**, 1011 (1986).
- 8) T. Nohmi and J. B. Fenn, *J. Am. Chem. Soc.*, **114**, 3241 (1992).
- 9) J. B. Iribarne and B. A. Thomson, *J. Chem. Phys.*, **64**, 2287 (1976); *ibid.*, **71**, 4451 (1979).
- 10) S. D. Fuerstenau and W. H. Benner, *Rapid Commun., Mass Spectrom.*, **9**, 1528 (1995).
- 11) R. D. Chen, X. H. Cheng, D. W. Mitchell, S. A. Hofstadler, Q. Wu, A. L. Rockwood, M. G. Sherman, and R. D. Smith, *Anal. Chem.*, **67**, 1159 (1995); R. D. Smith, X. Cheng, E. Bruce, S. A. Hofstadler, and G. A. Anderson, *Nature*, **369**, 137 (1994).
- 12) P. B. O'Connor and F. W. McLafferty, *J. Am. Chem. Soc.*, **117**, 1286 (1995).
- 13) R. B. Cody, J. Tamura, and B. D. Musselman, *Anal. Chem.*, **64**, 1561 (1992).
- 14) C. N. McEwen, W. J. Simonsick, Jr., and B. S. Larsen, *J. Am. Soc., Mass Spectrom.*, **6**, 906 (1995).
- 15) C. N. McEwen, private communication.
- 16) A. Gomez and K. Tang, *Phys. Fluids*, **6**, 404 (1994).
- 17) D. C. Taffin, T. L. Ward, and E. J. Davis, *Langmuir*, **5**, 376 (1989).

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