

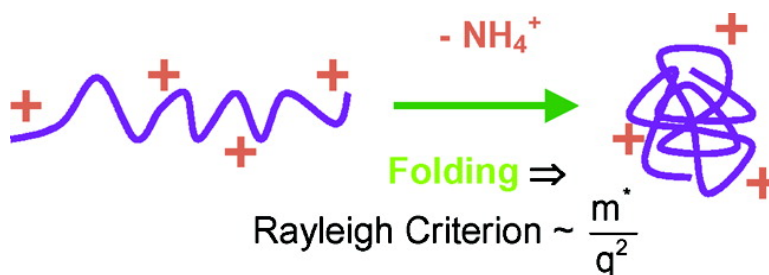
Article

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## Charge-Induced Unfolding of Multiply Charged Polyethylene Glycol Ions

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**Abstract:** The electrical mobility of mass-selected single poly(ethylene glycol) (PEG) chains of mass  $m$  ( $< 14$  kDalton) and charge state  $z$  ( $+1$  to  $+5$ ) reveals a near-spherical shape above a critical mass  $m^*(z) \sim z^2$ . The abrupt unfolding observed at  $m < m^*(z)$  shows that the polymer molecules behave as liquid drops upon reaching the Rayleigh limit, with an apparent surface energy of 0.026 N/m at ion diameters from 1.7 to 3.2 nm. Other nonspherical shape families with structures independent of charge, and with charge-dependent stability domains, are observed. Highly charged ions adopt approximately linear highly stretched configurations where the mobility depends only on  $m/z$ , independently of  $z$ . An operational definition of the surface energy of a single long chain molecule that is computable and agrees with the measured surface energy is provided.

### 1. Introduction

A number of studies have combined the use of ion mobility spectrometry (IMS) and mass spectrometry (MS) in tandem to study the shape of large species in the gas phase, including clusters of carbon<sup>1</sup> and other inorganic materials,<sup>2</sup> as well as synthetic<sup>3</sup> and biological<sup>4</sup> polymers. Although IMS–MS had been used for a long time,<sup>5</sup> its interest has increased greatly when its focus shifted from simple molecules into more complex systems whose shape is no longer uniquely defined by bond arrangement. The use of new ion sources such as electrospray (ES)<sup>6</sup> and matrix-assisted laser desorption ionization (MALDI)<sup>7</sup> has widened drastically the range of species that may be investigated with this tool. Electrospray is especially powerful because it offers the possibility to vary widely the amount of charge that can be placed on an ion, which often has a large effect on its structure.<sup>4a,8</sup> The case of peptides formed by polymerization of a single amino acid (i.e., Ala<sub>n</sub>) is of particular interest because the size and charge variables  $n$  and  $z$  can both

be controlled independently over very wide ranges within an otherwise physicochemically uniform system.<sup>8</sup>

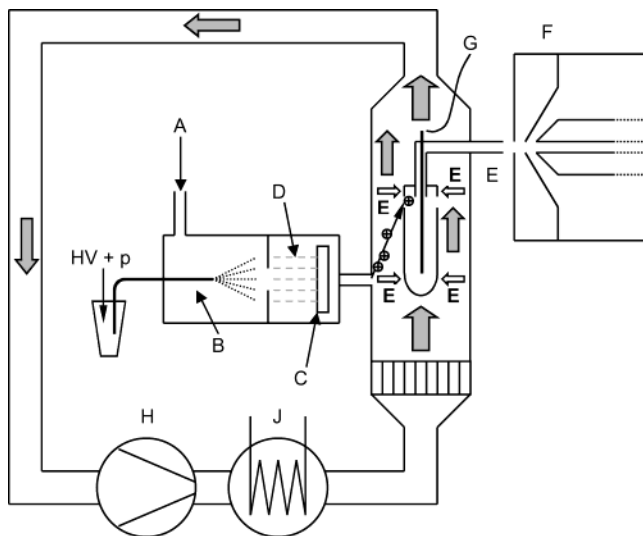
In the present paper, we study the electrical mobility  $Z$  (in air) of mass-selected poly(ethylene glycol) (PEG) chains as a function of the number of charges  $z$  they carry and their mass  $m$ , while covering exhaustively the degree of polymerization  $n$  (past 1000) and  $z$  range, including reduced charge states well below those naturally provided by electrospray, though with the constraint that  $m/z < 3000$  amu. Singly charged cationized ions of PEG<sup>3</sup> and other synthetic polymers<sup>3c</sup> with masses up to about 1000 amu have been previously investigated via IMS–MS. Work on such flexible species has been accompanied by sophisticated calculations of low energy structures, and a simple model for the temperature-dependent cross sections for a given structure has led to excellent agreement between computed and observed cross sections over an impressive temperature range (80 K to 600 K).<sup>3</sup> Some general conclusions on ion structure have also been obtained, showing a tendency for compact shapes, where the oxygen atoms in the polymer closely approach the metal cation, and the ion surface exposes mostly hydrogen (hence, the ion-gas interaction is simply given by that for He–H pairs<sup>3d</sup>). Ion compactness, however, is reduced when either the chain is too long, or the temperature too high. The second effect has been explained through the flexibility of PEG, which permits very many low energy structures, many of them widely open, separated by shallow and readily overcome energy barriers.<sup>3</sup> The first effect has been interpreted as due to the essential role of the cation in grouping the elements of the chain about itself,

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**Figure 1.** Experimental setup of the DMA-MS system. A: Inlet for air to carry the ions into the DMA. B: Electrospray. C: Radioactive neutralizer. D: Radiation. E: ion outlet from DMA to mass spectrometer. F: API 365 mass spectrometer. G: DMA electrometer electrode. H: Pump for recirculation of air. J: Heat exchanger for cooling of air. The DMA is located on the right branch of the recirculating loop. The gas is laminarized at the bottom, flows around a bullet shaped central electrode into the annular region between two cylinders, where ions are injected through the outer cylinder. Ions are separated in space by the electric field  $E$  according to their mobilities, and only those with a pre-selected  $Z$  value are sampled through a slit on the inner electrode and sent to the mass spectrometer.

whereby this compacting mechanism loses effectiveness when more than about 8 oxygen atoms are present on a singly charged chain.<sup>3</sup> For sodiated PEGs, one would therefore be led to expect low-density structures at  $m/z$  values above about 400 amu. Computations with neutral chains have similarly shown rather spread out structures.<sup>3c</sup> These conclusions,<sup>3</sup> however, must be somehow restricted (perhaps to moderately small chains), as indicated by the conflicting observation that singly charged ions of both proteins<sup>9</sup> and PEG<sup>10</sup> with masses above several thousands have mobilities (in air) close to those expected for spheres with bulk density. This simple result is of course to be expected for a fragment of a deformable material large enough for its density to reach the bulk asymptote, since capillary forces tend to minimize the area. One should therefore expect a transition from some of the loose structures reported for small ions by Bowers and colleagues<sup>3</sup> to the dense and approximately spherical shapes seen for larger ions.<sup>10</sup>

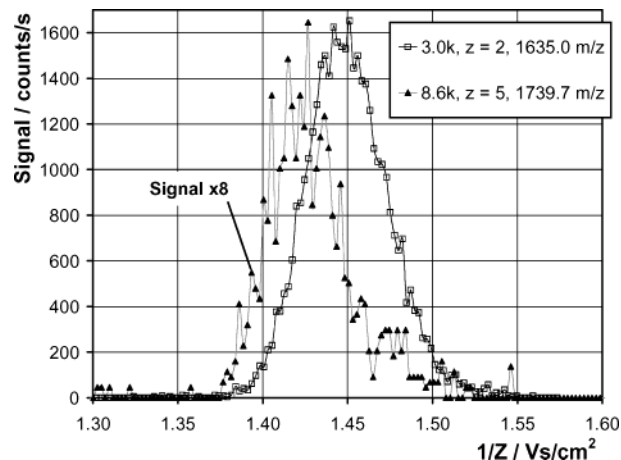
## 2. Experimental

The overall setup is sketched in Figure 1. Multiply charged electrospray ions of Polyethylene Glycol (PEG) were generated from a 50/50 water/methanol solution seeded with 10 mM Ammonium Acetate. A stream of dry air (15 lit/min) entrained them rapidly through a chamber irradiated by  $\alpha$  particles, where the original charge on the ions could be controllably reduced.<sup>9,10</sup> They then went into a differential mobility analyzer (DMA<sup>11</sup>) with unusually high resolution, whose output of mobility-selected particles was blown (15 lit/min) through a stainless steel tube (ID 0.23") into the close vicinity of the inlet hole leading (through the curtain plate) into the vacuum system of a quadrupole mass spectrometer (Applied Biosystems/MDS Sciex API 365).

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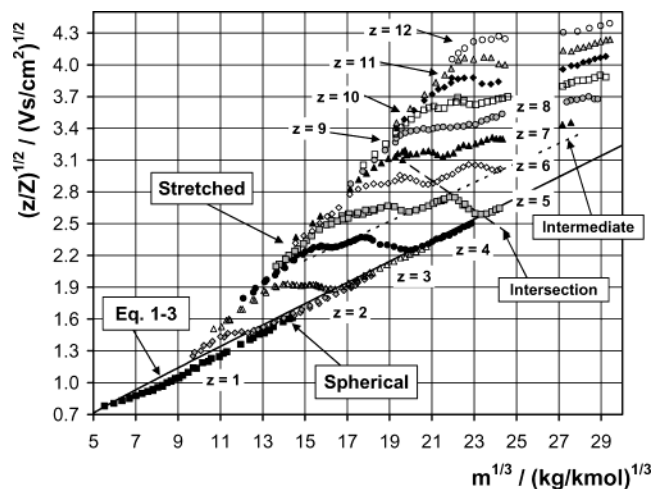
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**Figure 2.** Mass selected mobility spectra of PEG, for  $z = 2$  with  $m/z = 1635.0$ , and  $z = 5$  with  $m/z = 1739.7$ .

PEG samples with average molecular weights of 50.1, 22.8, 12.6, 4.12, 1.08, 0.44, and 0.194 kDalton and narrow size distribution ( $\Delta m/m$  less than 0.15) were from Polymer Labs (Amherst, MA). Samples with wider mass distributions were purchased from Union Carbide (4.6 and 1.45 kDalton) and Fluka (3, 6, and 10 kDalton). Another narrow noncommercial sample was kindly supplied by Dr. D. Saucy of Rohm & Haas (8.6 kDalton). PEG concentrations in solution varied from  $10^{-4}$  to  $5 \times 10^{-3}$  molar. The spraying tip was a sharpened silica needle (20  $\mu\text{m}$  ID from Polymicro Technologies). The DMA combines a high-speed stream of dry air with an electric field in the annular region between two concentric cylinders, which separates the ions in space (rather than the more conventional separation in time) according to their electrical mobility  $Z$ . The gas flow was re-circulated into the DMA through a pump (Ametek model 116765-13), a cooling circuit and a HEPA filter. All the lines in this gas loop were made of either metal or Teflon (2.5 cm ID). The bottled airflow going into the electrospray source and then into the DMA was regulated upstream through a rotameter. Although the measurement of the mobility depends on the gas flow rate, which cannot be measured with high accuracy, at a fixed gas flow rate  $Z$  is strictly proportional to the voltage difference in the DMA, and can therefore be determined accurately given a suitable mobility standard. Mobilities for the PEGs were determined with the same molecular standard ( $A^+(AB)$ ): A = Tetraheptyl Ammonium, B = Bromide) used by Gamero and Fernández de la Mora,<sup>12</sup> both at the beginning and the end of every series (and occasionally between for experiments conducted over an entire day). As the air flow rate sometimes drifted slightly over the day (from 0 to 2%), linear voltage corrections with time were used for every measurement point to account for the shift in mobility. The operating temperature was 303 K, while the standard mobility was measured at 293 K. On the reasonable assumption that both the standard and the PEG ions behave as hard objects, the data reported would automatically be corrected to their standard value at 1 atm and 293 K. The DMA resolution was limited by the high flow rate of the ion stream to about  $\text{fwhm} = 4.1\%$ . The ion outlet of the DMA was kept (with batteries) at a voltage of 84.8V above the curtain plate, which was maintained at 204V above ground. The MS was operated without curtain gas flow. Mobility spectra were taken by scanning the voltage difference  $V$  between the inner and outer DMA cylinders while monitoring a single mass with a mass width of 0.6 amu. Mass-selected mobility spectra are shown in Figure 2 for typical situations with a strong and a weak ion signal. Although earlier studies with PEG from either MALDI or ES sources have shown primarily sodiated ions,<sup>3</sup> our buffer yields almost exclusively ammoniated ions at all masses and all charge states. A QqTOF system, with a mass

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**Figure 3.** Compilation of all the  $Z(m,z)$  data obtained for PEG, shown in coordinates such that both axes are linear with molecule diameter. For all charge states, particles with masses smaller than a critical value lose the spherical shape. The solid line is for spherical particles with the bulk density of PEG and an accommodation coefficient  $\alpha = 0.91$ .

resolution of approximately 10 000, was used in separate experiments (without charge reduction) to verify the identity of the ammoniated PEG molecules by measuring the exact  $m/z$  values below about 2000. There was no evidence of the presence of sodium or other inorganic ions in the charged clusters. Monitored masses were therefore selected from the natural sequence of  $(\text{NH}_4^+)_z\text{HO}(\text{CH}_2\text{O})_n\text{H}$ . The charge state could be inferred unambiguously from the sequence (for the charge states presented here), so that these measurements yield the electrical mobility  $Z$  as a function of  $m$  (or  $n$ ) and  $z$ .

### 3. Results

Figure 3 shows data of PEG mobility  $Z$  as a function of ion mass  $m$  and charge state  $z$ . The most notable feature is that the data for all charge states collapse at large enough masses into a common asymptote, labeled “Spherical”. The selection of the variables  $(z/Z)^{1/2}$  and  $m^{1/3}$  is motivated by the fact that, for a sphere of diameter  $d$ , both  $(z/Z)^{1/2}$  and  $m^{1/3}$  are linear with  $d$  (eqs (1, 3) when  $m \gg m_g^{13,14}$ ), so that a spherical shape should yield a straight line

$$Z = \frac{3(2\pi)^{-1/2} q(RT/\mu)^{1/2}}{2(1 + \alpha\pi/8) p(d + d_g)^2}; \mu = \frac{mm_g}{m + m_g}; m = \frac{\pi}{6}\rho d^3 \quad (1, 3)$$

where  $R$  is the gas constant,  $T$  the temperature,  $m_g$  the mass of the surrounding gas molecules,  $q$  the net charge,  $p$  the pressure, and  $d_g$  the effective diameter of the gas molecules. The accommodation coefficient  $\alpha$  represents the fraction of the incoming gas molecules that are adsorbed on the surface of the PEG ion. The continuous line drawn in Figure 3 is based on eqs (1–3) with the literature values  $\alpha = 0.91$ ,  $d_g = 0.36$  nm<sup>13,14</sup> and  $\rho = 1.13$  g/cm<sup>3</sup> for a glass.<sup>15</sup> The fit to the data is not exact due to ambiguities in the quantities  $\alpha$ ,  $d_g$ ,  $\rho$  (in particular,  $\alpha$  is known to decrease at smaller sizes,<sup>13</sup> which explains the slight curvature downward observed in the data toward the lower left of the figure). While the agreement with the theoretical line gives confidence to the approximately spherical shape of these

PEG ions, the most striking observation is the fact that the data for the various charge states fall almost exactly on top of each other. The small  $z$  effect observed in this asymptote is a systematic increase of the drag (size) at increasing  $z$ , but this shift is rather small and decreases rapidly with increasing mass at all  $z$ . We attribute this slight  $z$  influence to the extra interaction between the ions and the dipole they induce in the bath gas molecules (polarization effect). One would be hard pressed to explain this continuous and  $z$ -independent asymptote if each ion mass had its own peculiar configuration. Rather our data show a strong tendency for the shape of these ions to be almost spherical independently of mass and charge, at least for sufficiently large masses or sufficiently small charge states. The tendency to form approximately spherical particles of bulk density has been observed previously for singly charged PEG ions over the mass range 4000 amu <  $m$  < 750 000 amu,<sup>10,16</sup> though with lower precision in the assignment of the mass (determined by Gel Permeation Chromatography). We should clarify here that we use the term “almost spherical” rather than “spherical” because shape inference is based on mobility measurements, and mobility is not very sensitive to slight departures away from a spherical shape. The reason is that both the drag per unit wetted area, and the wetted area (at fixed volume) take extreme values for a spherical geometry.<sup>17</sup> Hence, moderate departures  $\xi$  away from a spherical shape (say an ellipsoid of revolution with an axis ratio  $1 + \xi$ ), with small  $\xi$ , yield, for a fixed molecular volume, changes in the mobility only of order  $\xi^2$ . Consequently, even if one could ensure that the mobility differed by only 1% from that of a perfect sphere, the parameter  $\xi$  could still be in the range of 10%.

The experimental data therefore strongly indicate that when the charge on the PEG ions is reduced (by exposing them to  $\alpha$  particle radiation after drying), they spontaneously assume a nearly spherical shape when the charge is reduced to a critical value (different for each value of  $m$ ). Since the data shown in Figure 3 were obtained only by varying the one experimental parameter of the exposure to neutralizing radiation, and observing the shift in ion mobility, we can only conclude that the change in shape occurs in the gas phase as a result of the charge reduction.

**Nonspherical Shapes.** The assignment of shapes to the various nonspherical configurations seen will be considered in a future communication. We can nonetheless advance here some general qualitative observations.

The various families of shapes terminate on the left in the high-charge region referred to as “stretched” in Figure 3. In this region, the electrical mobility  $Z$  is almost exactly a function of  $m/z$  only, as can be seen in Figure 4. This peculiar behavior is readily associated to a highly stretched linear structure. In the simplest model for the mobility of a long straight chain, its drag coefficient  $\beta$  is linear with the chain length and hence directly proportional to its mass  $m$ , with a certain proportionality constant  $\kappa$ :  $\beta = \kappa m$ . Because  $Z = q/\beta$ , the relation  $1/Z = (\kappa/e) m/z$  follows. This rough model already captures the observed dependence only on the ratio  $m/z$ , which is unique to the case of linear structures. The data in Figure 4 also reveal an approximately linear dependence of  $1/Z$  on  $m/z$ , as the model,

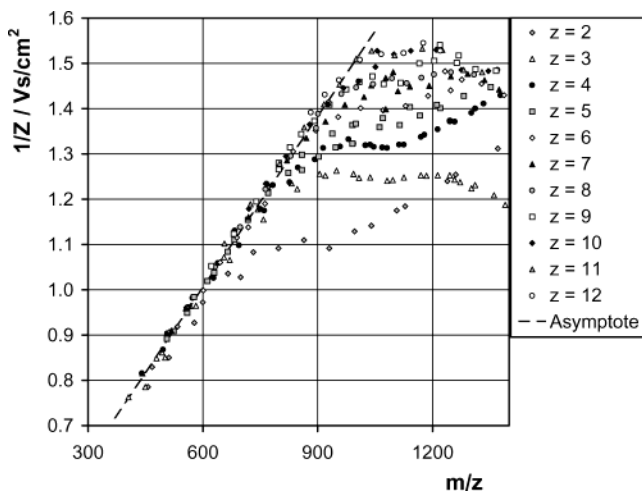
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**Figure 4.** Same data as those in Figure 3 (excluding those for  $z = 1$ ) represented with different variables, showing that high charge states have mobilities that depend strictly on  $m/z$ , independently of  $z$ . This behavior is a strong indication that their shapes are linear, corresponding to ions fully stretched due to Coulombic repulsion.

though the observed straight line does not go as predicted through the origin of coordinates. This deficiency is readily corrected by accounting for the additional drag due to polarization of the carrier gas. The electrical mobility of a point charge in the polarization limit,  $Z_{\text{pol}}$  is independent of its charge.<sup>5</sup> This means that the drag coefficient is directly proportional to charge. The total polarization drag coefficient on the chain is therefore proportional to the total ion charge  $ze$ , whereby, because drag contributions are approximately additive, the mobility of the composite may be written

$$1/Z = 1/Z_{\text{pol}} + (\kappa/e) m/z$$

with the same dependence on  $m/z$  as before, but with a vertical shift more in agreement with the data.

Another general observation from Figure 3 is that there are several asymptotic curves analogous to that corresponding to spheres, already discussed. This point becomes clearer after noticing that all the data lying to the right of the fully stretched asymptote in Figure 3 contain two kinds of segments. Some have a positive slope, comparable in magnitude to that of the spherical asymptote. We interpret them as corresponding to discrete segments of several *shape families*, analogous in most respects to the line for spheres. There are in addition short segments with negative slope, which correspond to *transitions* between two different *shape families*. A most revealing observation is that several discrete segments corresponding to the same shape family exist at different charge states over a domain of masses well beyond their stability range for a single charge state. This is evidently the case for the spherical geometry, and simply means that the shape is charge-independent, though only within a finite and charge-dependent stability range. A similarly clear situation can be seen for the first nonspherical shape adopted below the critical mass where the spheres become unstable, whose mobility data form a line almost parallel to the spherical asymptote and above it. This locus, marked by a dashed line labeled “intermediate” in Figure 3, is clearly defined by the last few data past the kink to the right of the  $z = 6$  line, and the data following immediately below the end of the transition away from the sphere at  $z = 4$  and  $z = 5$ . These segments evidently

**Table 1.** Critical Mass  $m^*$  and  $m^*/z^2$  as a Function of the Number of Charges  $z$

no. of charges $z$	2	3	4	5
$m^*/\text{amu}$	1991	4435	8024	12666
$m^*/z^2 \text{ amu}$	497.7	492.8	501.5	506.6

continue each other, though, unlike the sphere asymptote, the corresponding domains of stability are not broad enough to overlap. The existence of other shape families more distant from the sphere is evident at  $z = 6$  and at higher charge states, though the lack of high mass data limits our ability to affirm that these shape families extend through several charge states. Another shape family closer to the sphere and encompassing several charge states can be guessed for  $z = 3$  and 4 (perhaps also 2), though with rather narrow segments of stability within each  $z$ .

Pending a detailed modeling of the equilibrium shapes of PEG chains with Coulombic destabilization, some preliminary conclusions can be established from these few qualitative observations. The first relates to the possible existence in the gas phase of the two structures previously reported in solution and denoted “zigzag” and “meandering”.<sup>18</sup> The length and diameter of one ethoxy unit considered as a cylinder are 0.35 and 0.25 nm for the zigzag chain, and 0.2 and 0.4 nm for the meandering configuration.<sup>18</sup> The zigzag structure is highly stretched, and may coincide with the fully stretched configuration observed here at high charge levels. The meandering structure is more compact, as the chain coils to form a shorter and wider cylinder. But it is still a cylinder, whereby  $1/Z$  would scale as  $m/z$ . It cannot therefore correspond to any of the shape families seen in Figure 3 (other than the fully stretched). It is also unlikely that the chain arrangement in the shape families observed (spherical or not) might consist of compact combinations of several meandering coils with sharper bends here and there, as in proteins. Such structures would tend to lead to shapes with idiosyncrasies characteristic of each mass, not to just a few shape families at all masses. We note finally that the various nonspherical shape families found must correspond to geometries that (like the sphere and unlike a cylinder) must be relatively invariant with mass.

**Stability Boundary.** We now consider the reasons why a drastic change of conformation away from a nearly spherical shape arises for each  $z$  below a certain critical mass  $m^*(z)$ , and what determines that critical mass  $m^*(z)$  (Although the discussion considers a critical mass  $m^*$ , in the experiment it is actually  $z$  which varies while the mass  $m$  remains fixed. The critical relations  $m^*(z)$  and  $z^*(m)$  are nonetheless equivalent ways of characterizing the phenomenon). Table 1 collects critical masses  $m^*$  as a function of  $z$ , where  $m^*$  is defined experimentally by the intersection between the straight lines formed by taking a few data points in the  $Z(m)$  curves before and after the transition (as illustrated in the figure for  $z = 5$  by the dashed line labeled “intersection”). The Table includes also the ratio  $m^*/z^2$ , which turns out to be constant within the uncertainties involved in defining  $m^*$  from the data. A quadratic relation between  $m^*$  and  $z$  would in fact be expected for the loss of stability of a charged drop of a conducting liquid with surface tension  $\gamma$ , for

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which Rayleigh's criterion<sup>19</sup> yields

$$\frac{m^*}{z^2} = \frac{e^2 \rho}{\gamma \epsilon_0} \frac{1}{48\pi} \quad (4)$$

where  $e$  is the elementary charge and  $\epsilon_0$  the electric permittivity of vacuum. Using literature data for the surface tension of PEG,  $\gamma = 42.5$  mN/m,<sup>15</sup> the expected ratio (4) would be  $m^*/z^2 = 330$  amu. This is substantially smaller than the experimental value shown in the table, apparently implying that the group  $\gamma/\rho$  takes the actual value  $2.33 \times 10^{-5}$  m<sup>3</sup>/s<sup>2</sup> rather than the bulk value ( $3.43 \times 10^{-5}$  m<sup>3</sup>/s<sup>2</sup>). Note that the values of  $m$  and  $z$  reported are known essentially exactly.

The same kinds of considerations leading to the existence of charge-independent spherical shapes and their limited charge-dependent stability range can help rationalize the observed nonspherical shape families, where an almost fixed nonspherical geometry is formed at different masses and charge states. By analogy with the behavior of an exploding drop past the Rayleigh limit, one could imagine that the polymer molecule would, at  $m < m^*$ , form two daughter drops, which cannot fly away because they are part of a unique chain. In this model, each of the two daughters would lose stability again at a second critical mass  $m^{**}/z^2 = 1/2 m^*/z^2$ . The experimental  $m^{**}/m^*$  ratios are 0.78, 0.63, and 0.57 for  $z = 6, 5$  and  $4$  respectively, always larger than the  $1/2$  ratio in this model. This is to be expected because the two linked spheres interact with each other and this would reduce the charge each can hold relative to the noninteracting case. This model has evidently no pretension to be realistic, but it illustrates one possible mechanism by which a liquid volume constrained to remain in one piece could, past the Rayleigh limit, adopt a nonspherical shape independently of its charge and volume. A more realistic model enabling a fixed nonspherical shape at various charges and volumes is introduced below (for the special case of a sphere). It considers a dielectric liquid whose net charge is concentrated at an interior point. Upon reaching the Rayleigh limit this system would be unable to shed charge because all its surface charge is polarization charge. It would therefore remain in one piece, and evolve into a nonspherical shape that one could presumably determine from purely continuum considerations.

#### 4. Discussion

The two sharpest experimental findings made are the existence of a domain of almost spherical ion shapes, and another of nonspherical configurations, with a sharp instability boundary between the two. Note that the behavior displayed (both on shape and stability boundary) depends only on macroscopic quantities such as  $z$  and  $m$ . It is hence natural to seek its explanation in terms of macroscopic considerations. These considerations are closely related to those making a spherical drop stable below the Rayleigh limit but not above it. There are however several important differences between our single polymer molecules and a liquid drop.

In the discussion below, we characterize the behavior of single PEG ions in terms of macroscopic properties of glass transition temperature and surface tension. We recognize that there is little theoretical support for assuming that these properties can be

applied to individual molecules. However, as the analysis shows, the behavior of these molecules as they lose their charge seems to be well-explained by using such a model. The analysis will show that the apparent surface tension of a single molecule is significantly lower than that of a bulk droplet. However, the fact that the apparent surface tension does not appear to be a function of size over the wide range of molecular sizes, which we investigated, suggests that this difference may be due to the fact that it is an individual molecule, rather than a multi-molecular cluster or droplet. We will comment further on the implication of our terminology in the Conclusion section, and will subsequently introduce a more precise operational definition of the surface energy (rather than the surface tension).

The most obvious difference between a single molecule and a liquid drop is that all the atoms of the polymer "drop" are held together by covalent bonds, while, in liquid drops, the molecules have greater freedom to move around by diffusion. Perhaps a more serious obstacle to the drop analogy is that the PEG samples sprayed are solid at room temperature for  $m > 600$  amu. Taken together, these may suggest that the individual molecule is not as free to assume a spherical shape when the charge is depleted enough to energetically allow it, as would be a liquid drop.

First, we shall argue that these differences do not disqualify the sphere as the proper equilibrium shape prior to the onset of instability. The reason is that, when the loss of one charge leads to a change of stability enabling compaction, a considerable amount of potential energy (surface area) is released from the stretched into the collapsed ion, and this provides the necessary heating for the polymer to "melt" and relax into an energetically favorable spherical shape. Although we use the term "melt" here, making the analogy to a bulk solid/liquid phase transformation, what we really mean is that the molecule is vibrationally heated sufficiently to allow it relax into a lower energy (spherical) shape.

Now, if the ion "melts" upon charge reduction, Rayleigh's criterion applies to it through a certain cooling period thereafter, until it becomes "glassy" (i.e., more rigid) again. Because the surface tension increases at decreasing temperature, the ion should recover the spherical shape provided it is below the Rayleigh limit based on the value of the surface tension at the glass transition temperature. One possible interpretation of our data is therefore that the measured surface tension does not correspond to room temperature but to a higher temperature, and this is why it is smaller than expected. But another interpretation is possible because the bulk glass transition temperature for PEG is really not too far from our operating temperature of around 30 °C (42–47 °C at 5470 amu; 47–55 °C at 12 400 amu). Hence, even if the PEG ions were spherical at the glass transition point, the resulting solid would still behave as a viscous material, and could flow away from a spherical shape if the solid had a smaller surface energy than the liquid. For a glassy state, the relevant parameter  $\gamma$  governing the energy of the ion would be the surface energy rather than the surface tension. We have no data for the bulk surface energy of solid PEG (either single or many molecules) but a reasonable interpretation of our observations is simply that this quantity is precisely what our experiment measures.

Our interpretation of the data therefore suggests three explanations for the apparently low surface tension: that it is

(19) Rayleigh, L. *Philos. Mag.* **1882**, *14*, 184; Rayleigh, L. *The Theory of Sound*; Dover: New York, 1945; §364; p 374; Peters, J. M. H. *Eur. J. Phys.* **1980**, *1*, 143–146.

due to the fact that we are dealing with a single molecule, or that it corresponds to a higher temperature of the molecule at which the shape transition occurs, or that it is better characterized as a surface energy of a semirigid structure (analogous to a bulk glassy state). These interpretations are the only ones we find that are entirely consistent with all observations. Several other conceivable scenarios are analyzed and rejected below.

**Surface Tension Anomaly is Not Due to Shortcomings of Rayleigh's Model.** One could hypothesize that Rayleigh's model is derived for the particular case of a perfect conductor of zero viscosity, and its predictions are therefore inapplicable to our polymer molecules. To reject this notion with greatest generality, we shall first depart from Rayleigh's dynamical analysis, because the liquids under consideration are indeed very far from the inviscid limit he considered. That this difference has no effect on the point of instability is evident from the discussion provided for this problem by Landau and Lifshitz.<sup>20</sup> Their analysis is purely static, and considers the conditions when a slight deformation away from the spherical shape increases or decreases the potential energy (surface + electrostatic). If any such deformation increases the potential energy, the spherical form will be stable, and this is the case below the Rayleigh limit (note that Landau and Lifshitz are not limited to small perturbations, but they consider only ellipsoidal shapes. Yet their analysis is readily generalized for small perturbations by Rayleigh's spherical harmonics expansion method). Rayleigh's conclusion therefore holds irrespective of whether the liquid is inviscid, viscous, Newtonian or not, or even glassy [though in this case the surface energy rather than the surface tension would be the appropriate quantity to use in (4)]. The Rayleigh criterion holds provided that the medium can change shape in order to minimize its energy. This much we can assume of our polymer ions because we see them change shape upon changing their charge.

Having shown the generality of Rayleigh's criterion with respect to the dynamical behavior of the liquid, we must however note that Rayleigh's stability criterion is not universal because the potential energy may not be exactly proportional to the surface, and the electrostatic contribution does depend on the material considered. Rayleigh's drop was a perfect conductor. We have similarly considered the case of a dielectric medium with fixed dielectric constant  $\epsilon$ . In the particular case when the full charge is concentrated in the center of a slightly deformed sphere, instability arises when

$$\frac{z^2 e^2}{8^2 \pi^2 \epsilon_0 \gamma R^3} > \left[ (1 - \epsilon^{-1}) \left( 1 - \frac{15}{3 + 2\epsilon} \right) \right]^{-1} \quad (5)$$

Note that, when  $\epsilon$  is finite and larger than 6, the right-hand side term always exceeds unity. Such a dielectric sphere can therefore hold more charge than a conducting sphere. Hence, if the stability data measured for a dielectric were interpreted based on Rayleigh's result (4) rather than on (5), they would yield an anomalously high surface tension rather than the low value observed. The spherical dielectric model considered is more readily analyzed than the more realistic case where the charges would distribute themselves approximately equidistantly near the drop surface. Note still that, at a high dielectric constant,

the actual position of the net charges is relatively irrelevant because most of the net interior charge  $(1 - 1/\epsilon)$  is neutralized by polarization of the medium and becomes polarization charge on the drop surface. The precise position of the little remaining net interior charge  $(q/\epsilon)$  cannot therefore have the large effects observed on the apparent surface tension. In the limit when the number of charges is large and they get very close to the surface, the behavior would evidently approach that of a conducting droplet where Rayleigh's results hold strictly. Nothing of the sort is seen in our data at increasing  $z$ .

**Surface Tension Anomaly is Not Due to Small Size Effects.**

Another conceivable scenario could be that the very small size of the PEG ions (critical diameters of 1.8 and 3.2 nm for  $z = 2$  and 5, respectively) lowers their glass transition temperature making them "liquid" at room temperature, and also alters their surface tension. The surface tension is believed to vary through the Tolman curvature correction<sup>21</sup> as  $\gamma = \gamma_0(1 + \delta/d)$ , where  $\delta$  is a constant with dimensions of length. A correction due to the large electric field at the surface could also arise. These effects, however, have not been reliably quantified. Furthermore, if the surface tension depended on the drop size as in,<sup>21</sup> the ratio  $m^*/z^2$  would not be constant. We do see a slight variation of  $m^*/z^2$ , equivalent to a 1.8% change in  $\gamma$  when the diameter is almost doubled. Interpreting our  $m^*/z^2$  data in terms of Tolman's correction would yield an asymptote  $m^*/z^2 = 514$  amu at infinite mass, and a bulk value for  $\gamma$  only slightly shifted and still far too small. Within experimental error we can therefore assert that there is no Tolman correction for our data. The possibility that the field and the curvature correction would coincidentally be such as to drop drastically the apparent surface tension equally at all sizes is unlikely, and certainly fails to hold at micron sizes. The possibility that we are observing a strictly size-dependent variation of surface tension must hence be rejected.

**Surface Tension Anomaly is Not Due to Hysteretic Phenomena.** Another conceivable explanation for the seemingly anomalous  $\gamma$  value found could be traced to the fact that our experiment starts not with a spherical ion that loses stability upon increasing its charge, but rather with an initially highly charged nonspherical ion which becomes spherical upon losing some charge. One could argue that the critical charges governing these two rather different transitions are not the same. Because we cannot in our system increase the charge, it would seem that a complete interpretation of our observations could not be given without first establishing the shape of the ions at a charge slightly larger than that turning them into spheres, and then analyzing the stability of this geometry at diminishing charge. However, this alternative scenario can also be rejected. Consider for instance an ion with a mass such that  $m^{1/3} = 19$  in Figure 3, which remains slightly nonspherical at  $z = 4$ . When its charge is reduced from  $z = 5$  to  $z = 4$ , its mobility changes drastically, and so must its shape. A lot of potential (surface) energy must then be released into vibrations, leading to melting (as a reference, a sphere of bulk density and with that mass would have a diameter of 2.7 nm, and would change its Coulombic energy by 4.8 eV on going from  $z = 5$  to  $z = 4$ ). The corresponding temperature change for a specific heat capacity of  $cp$  [J/molK] =  $0.138T + 14.066$ ,<sup>22</sup> where mol refers to the

(20) Landau, L.; Lifshitz *Electrodynamique des milieux continus*, MIR, Moscow, 1990 (problem 5.6).

(21) Tolman, R. C. *J. Chem. Phys.* **1948**, *16*, 758–774; Tolman, R. C. *J. Chem. Phys.* **1949**, *17*, 333–337.

monomer mass, is 24.7 K). If the surface tension of this molten polymer molecule were that of the bulk, then a spherical shape would be stable for this mass and charge. And if the polymer then adopted a rigid structure upon cooling, it would preserve its spherical shape. But it does not. Hence, either the surface tension of the liquid is smaller than that of the bulk, or the  $z = 5$  liquid drop becomes spherical after losing a charge, but then loses that shape to become slightly (but significantly) non-spherical upon solidifying.

## 5. Conclusions

A study of the electrical mobility of PEG ions has covered systematically almost all masses and charge states accessible with charge-reduced electrospray at  $m/z < 3000$  Dalton. Ions with a mass larger than a charge-dependent critical value  $m^*(z)$  take nearly spherical configurations, with cross sections that, at fixed  $m$ , are almost independent of  $z$ . A variety of conformations are observed, with a sharp transition at the point where near spherical shapes lose stability. This transition is governed by the same relation  $m^* \sim z^2$  as the Rayleigh instability of a charged liquid drop, with an apparent surface tension  $\gamma$  of 0.026 N/m, substantially smaller than the surface tension for the bulk polymer material in liquid form. A number of possible explanations for this apparent anomaly have been considered, leading to the conclusion that either the ions are liquid (possibly due to heating from elastic energy release upon reducing the charge) and the anomalous surface tension measured is their actual surface tension at the glass transition temperature, or they are glassy but can flow within experimental times, whereby the measured apparent surface tension is the surface energy of the ions. Alternatively, the low apparent surface tension obtained could be due to the fact that we are dealing with a single molecule rather than a multi-molecule drop or cluster. Either way, the experimental technique introduced enables the measurement of the surface energy of single molecules, and the ambiguity remaining can be resolved by undertaking similar studies at variable temperature.

Despite the imprecision of the macroscopic terms “surface tension” or “surface energy” used here to describe a single molecule, note that a surface energy can be defined operationally for an isolated single molecule or a cluster, by assigning initial positions and velocities to the various atoms, as commonly done in dynamic simulations. After a brief initial relaxation process, the total initial energy  $E$  (a conserved quantity) will oscillate between the kinetic energy  $E_k$  (unambiguously defined from the instantaneous velocities of all atoms) and the potential energy  $E_p = E - E_k$ . The latter needs to be known only up to an

arbitrary constant. Following this initial relaxation, one can keep track of the temperature (given by the total kinetic energy in the center of mass system), the potential energy, as well as the surface area (suitably defined) associated to the various shapes taken by the cluster as it vibrates. Our data of a constant apparent surface tension give strong empirical basis to the notion that there will be a linear relation between potential energy and surface area, as is generally the case for large enough liquidlike systems. We can therefore provide an operational definition of the surface energy as the slope of the potential energy versus surface area curve in the vicinity of the potential energy minimum. This slope is not only well defined (for large enough systems) and computable, but is also the same quantity that determines the stability of a charged deformable sphere (as shown when arguing that the surface tension anomaly is not due to shortcomings of Rayleigh’s model). Temperature control can evidently be exerted through the initial conditions, while suitable generalizations to the case of charged and rotating clusters can be provided. Hence, this “surface energy” is both measurable and computable for clusters and large single molecules. A computation based on the hypothesis of an isolated cluster is of course different from the behavior of the cluster in a bath gas at constant temperature and pressure. But the isolation hypothesis is a widely used and computationally far more convenient model. The fact that the cluster cools as it vibrates does not preclude a proper determination of the temperature, which for small amplitude vibrations can be defined from the kinetic energy at the bottom of the potential energy well (the vicinity of the spherical shape in our case).

Since this article was submitted for publication, we have become aware of a very interesting study by Williams and colleagues,<sup>23</sup> in which the rich history of the “liquid drop model” is first reviewed and then employed to illuminate their study on the dissociation of multiply charged peptide clusters.

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