Single- and Few-Chain Polystyrene Particles by Electrospray[†]

Reinhard Festag,[‡] Spiro D. Alexandratos,[‡] Kelsey D. Cook,[‡] David C. Joy,[§,⊥] Brian Annis,[‡,||] and Bernhard Wunderlich*,[‡,||]

Department of Chemistry and Department of Biochemistry and Cellular and Molecular Biology, The University of Tennessee, Knoxville, Tennessee 37996-1600, and Chemistry and Analytical Sciences Division and High Temperature Materials Laboratory, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831

Received February 18, 1997; Revised Manuscript Received June 27, 1997[⊗]

ABSTRACT: An electrospray method is used for the conversion of isolated molecules of atactic polystyrene of high molar mass in solution to solid, single-molecule particles. With this process it is possible to produce not only single molecules but also clusters of several molecules. Particle size and shape distributions were characterized using scanning force and scanning electron microscopy. The size of the clusters of more than one molecule can be tailored to ones needs by controlling the spray conditions.

Introduction

Isolated polymer particles consisting of only one polymer chain have potential for applications including use as molecular mass and size standards or as biological tracers. From a more theoretical point of view, isolated single-molecule particles can contribute to an improved understanding of nano- or microphases, because each particle can be considered to be a distinct phase. Fundamental questions of polymer science like the physical effects of molecular entanglement and disentanglement on viscosity, glass transition, and crystallization behavior can be addressed by comparing the properties of single-molecule particles with those of multiple-molecules and bulk phases. This paper describes a method to obtain single- and multiple-molecule particles.

Most knowledge about properties of single polymer chains is restricted to the behavior of isolated chains in dilute solutions.[1] In this case the molecules are isolated, but the behavior and the physical properties are influenced by interactions with the solvent. Starting in the 1950s attempts to isolate single-chain particles arose from the search for a reliable method to determine the molecular mass distribution of polymers with high molar masses (above 10^6 Da). The standard method, size exclusion or gel permeation chromatography (GPC), was not widely available at that time and could not be applied to the highest molar masses. Accordingly, it was of interest to investigate the imaging of single-molecule particles by electron microscopy, thereby enabling measurement of the geometric dimensions of the particles and calculation of the molecular mass. Isolated single-chain polymer particles were obtained using several different methods, all relying on isolation of the molecules from dilute solutions. Once the polymer is dissolved, the rapid addition of a bad solvent can lead to precipitation of isolated single-chain polymer particles.[2−5] Alternatively, the solution can be rapidly frozen and the solvent removed subsequently by sublimation, leaving isolated single-chain particles.[6−8] In both cases the particles have to be below their glass transition temperature (T_g) to avoid complications from agglomeration of the resulting particles. A variety of spray methods have also been used to generate isolated particles. These rely on the evaporation of the solvent from droplets of dilute solutions containing single polymer molecules. Sufficiently small droplets can be produced by nebulization,[9−12] which has been very effective in producing single molecules. An alternative spraying technique, electrospray (ES)[13] employs an electrical field to facilitate the production of very fine droplets. Spraying methods have been applied to a variety of different polymers, including polystyrene,[11,14] polyethylene,[12] poly(methyl methacrylate),[8] several other polyacrylates,[11] polyisobutene,[11] and natural rubber.[7] Once again, care must be taken to avoid agglomeration of the isolated particles.

Owing to the experimental difficulty of the preparation techniques, none of these methods of preparing single-molecule particles has come into wide use as a method of determining molecular masses. However, spray methods, in particular electrospray (ES),[13,15−17] have encountered wide use for molecular sampling in mass spectrometry. Dole and co-workers pioneered ES mass spectrometry in studies of polystyrene,[18] but today ES is used widely as a “soft” mass spectrometric probe of biomolecules with high molecular mass.[16,19,20]

This work seeks to exploit the properties of ES as a potential source of single- and few-chain particles, to fill the gap between the single-chain particles and bulk material. Furthermore, polymer particles produced by electrospray are visualized for the first time. Although the wide use of ES mass spectrometry led to several investigations of the droplet formation and fission process,[21−23] so far no microscopic investigation of the resulting particles is known.

Experimental Section

An amorphous polymer, polystyrene (PS), was chosen so that the shape of the collected particles would not be affected by crystallization. The polystyrene had a mass-average molar mass M_w of 330000 Da and a relatively low polydispersity (M_w/M_n) of 1.26. The critical overlap concentration for a polymer solution is under discussion in the literature,[24] but concentrations chosen by us were well below all published values for molecular overlap to assure separate molecules in the initial polymer solution.

[†] A preliminary version of this paper was presented at the SPIE meeting *Space Processing of Materials*, Denver, 1996 (*Proc. SPIE* **1966**, *3809*, 155).

[‡] Department of Chemistry, The University of Tennessee.

[§] Department of Biochemistry and Cellular and Molecular Biology, The University of Tennessee.

[||] Chemistry and Analytical Sciences Division, Oak Ridge National Laboratory.

[⊥] High Temperature Materials Laboratory, Oak Ridge National Laboratory.

[⊗] Abstract published in *Advance ACS Abstracts,* September 1, 1997.

S0024-9297(97)00211-8 CCC: $14.00

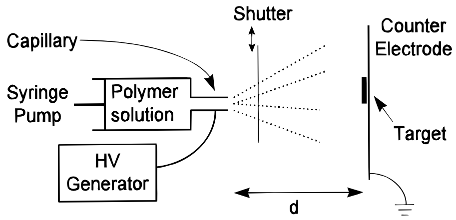

Figure 1. Sketch of the experimental setup.

Several aspects need to be considered in selecting a suitable electrospray solvent. Analyte solubility is a minimum requirement, but electrospray properties (volatility and stability) are also of concern. In the electrospray of PS, it has generally been observed that polymer ionization results from formation of alkali metal adducts.[25-27] Although adduct abundance is enhanced by the addition of salts, adventitious salt (present as a contaminant in the sample and/or solvent) may suffice, given the sensitivity of the method. Because sustained positive ion electrospray requires oxidation of some solution species, electrochemistry may also play a role in the ionization[28] (*via* direct analyte electrochemistry or secondary reactions following solvent electrolysis). Ion/ion repulsion will favor isolation of analyte molecules if charge is preferentially deposited on the analyte. By all these criteria, chloroform is a good choice for the electrospray of PS. It is a good solvent for the polymer, is reasonably volatile, and should have relatively low salt contamination, and its ionization potential (IP; 11.37 eV) is higher than that expected for PS (8−9 eV), on the basis of the IPs of small organics that may reasonably be a mimic PS.[29]

To avoid intermolecular contact and entanglement, any method intended for the isolation of single macromolecules must assure that the molecules remain isolated from each other for as long as they are flexible (mobile). The ES process commences with the generation of small, charged droplets by spraying liquids through biased metal capillaries. While the droplet drifts toward a counter electrode, the solvent evaporates and the charge density in the shrinking droplet increases due to the relative involatility of charge carriers. As droplets reach the “Rayleigh stability limit”, the force from the electrostatic repulsion overcomes the surface tension, promoting subdivision into smaller droplets. Ultimately “naked” (unsolvated) ions should result. In ES mass spectrometry these ions are sampled by a controlled leak into a mass analyzer, but for the present experiments, the ions are collected for microscopic evaluation, using the experimental setup sketched in Figure 1.

Investigations with high-speed photography and laser light scattering have revealed that the “parent” droplet generally loses only around 2% of its mass, but 15% of the charge in a Rayleigh fission.[23,30] In addition to the main residual droplet, several small offspring droplets result with radii approximately one-tenth of the parent droplet.[22,31] Because of their small size, the offspring droplets evaporate very rapidly, leading to dry ions. To avoid entanglements of the molecular chains after impact, the particles should be practically dry when reaching the counter electrode. Thus, the drift time to the counter electrode must be sufficient to evaporate even the biggest sprayed droplets. The minimum time necessary for the evaporation can be calculated[32] and compared with expected drift times for travel from the capillary tip to the planar counter electrode (see Figure 1) at a given applied voltage and distance, *d*, between the tip and the counter electrode.[33] For typical tip-to-plate distances of a few centimeters, a drift time on the order of several milliseconds results.[33] The time necessary to evaporate a droplet of given radius is linearly dependent of the vapor pressure[32] and varies therefore greatly. A droplet with a radius in the micrometer range of a solvent with high vapor pressure, such as chloroform, should evaporate completely in less than 1 ms. Thus, *d* was set to ≈4 cm.

Solutions were pumped at 2.6 μL min^{-1} from a 500 μL glass syringe with a Harvard Apparatus syringe pump. A sharpened stainless steel capillary with an inner diameter of 0.17 mm was biased (+8−10 kV) using a Spellman high voltage DC power supply. The counter-electrode was an 8 × 8 cm stainless steel plate maintained at ground potential. Small, 5 × 5 × 0.5 mm, graphite or mica plates were placed as targets on the counter electrode for subsequent microscopic investigations. The graphite or mica was freshly cleaved before the experiments and was mounted with a small (≈1 mm wide) strip of double-sided, adhesive tape which was applied at one edge of the target. A grounded metal shutter between the tip and counter electrode was opened at the beginning and closed at the end of the spraying to avoid the collection of material during the onset and close of the spraying process. The spraying time ranged between 4 and 10 min, chosen in a way to deposit enough material for microscopic investigations, while staying well below the quantity needed for a monolayer, so that the particles remained separated from each other on the target. Under these conditions, surface coverage can be estimated at less than 1% of a monolayer. The sprayed targets were stored in a dust-free enclosure at room temperature and ambient atmospheric conditions until microscopic investigation.

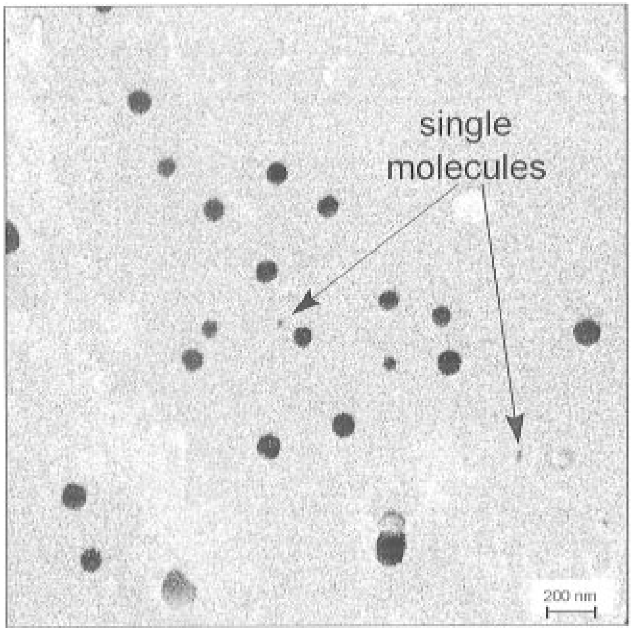

Figure 2. Scanning electron micrograph of electrosprayed PS. Molar mass = 330 000 Da, concentration 1 × 10^{-4} wt % in chloroform, sprayed for 5 min on graphite, original magnification 50 000×.

Scanning force microscopy (SFM) was done with a Nanoscope III from Digital Instruments. The instrument could be operated in either the normal contact mode or in the tapping mode.[34-36] The tip radii were 25 nm for the contact mode and 10 nm for the tapping mode. The force of the tip in the contact mode was experimentally determined to 12 nN. The electron micrographs were obtained with a Hitachi S-4500 scanning electron microscope.

Results and Discussion

Figure 2 shows PS particles collected during 5 min of electrospray of a 1 × 10^{-4} wt % solution. Practically all of the particles are well separated from each other and have the round shape expected for an amorphous polymer. Clearly two different kinds of particles can be distinguished: those with a diameter between 80 and 120 nm and much smaller ones of about 20 nm. Twenty electron micrographs of different regions of the target were obtained. Generally speaking the distribution of particles, as well as the relative amount of small and big particles, were fairly uniform over the whole target. However, as visible in Figure 2, there are areas with only large particles. In order to determine the size of the small particles as precisely as possible, pictures were

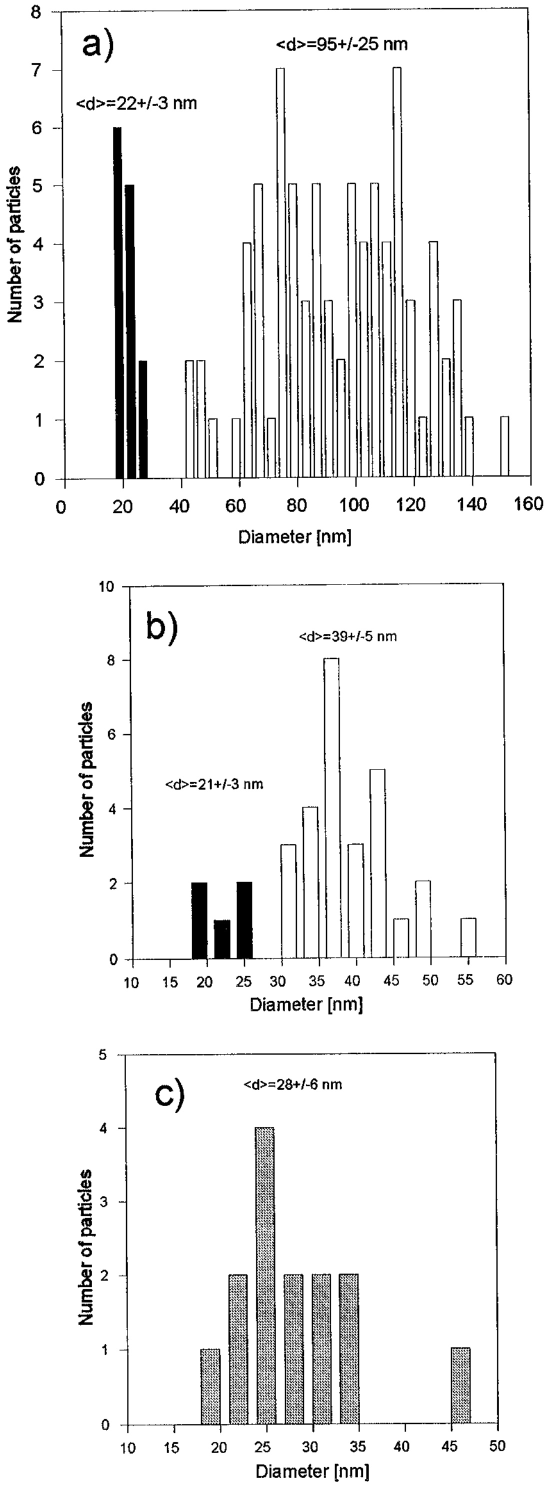

Figure 3. Analysis of the particle size distribution for different concentrations of PS with M_w = 330 000 Da in chloroform. (a) 1×10^{-4} wt %, (b) 1×10^{-5} wt %, (c) 3×10^{-6} wt %.

mainly taken of sections and at magnifications to include at least one of the small particles. Therefore, the relative abundance of the small particles shown in Figure 3a, amounting to a few percent by number, is somewhat exaggerated in the size distribution generated by the measuring and counting of particles in the micrographs. Nevertheless, the bimodal size distribution obtained clearly shows a peak at small diameters with an average diameter of 22 ± 3 nm and a relatively broad distribution of larger particles with an average diameter of 95 ± 25 nm.

The origins of both types of particles have to be determined. Several questions arise in looking at the results: How many polymer chains are in those particles? Why are there two distinctly different sizes? And are the small particles also whole polymer chains, or fragmented ones? To answer these questions, a volume estimate of the particles is necessary which, in turn, requires a knowledge of the particle height. This was obtained for a number of particles with SFM. A typical example of a scanning force micrograph is shown in Figure 4. The small particles observed in Figure 2 are missing, probably because the SFM tip sweeps the very small particles off the picture area. Consistent with this hypothesis, small particles were visible in SFM micrographs obtained when the tapping mode was used with a very low lateral force. The characteristic features of the SFM pictures and the lateral sizes are consistent with the SEM investigations.

To use the SFM data, the observed radius and the tip radius must be considered to determine the particle dimensions. Assuming the particle and the tip are both semispheres, one can easily derive that the particle radius is

$$r_{\text{Particle}} = \sqrt{{r_{\text{Tip}}}^2 + {r_{\text{Observed}}}^2} - r_{\text{Tip}} \qquad (1)$$

For the large particles, diameters of 80−120 nm result, consistent with the SEM observations. More importantly, the particle height is also given by the SFM data. With the tapping SFM the height depression due to the force of the tip is negligible.[34−36] The height equals almost always the radius, with a slight flattening for larger particles. For all calculations half-spheres were thus taken as the particle geometry. This geometry may be the result of the remaining kinetic energy of the particles hitting the target, helped by the remaining solvent and the small particle size which both depress the glass transition temperature.[37]

Since the polymer is relatively monodisperse, different particle sizes must originate from different numbers of polymer chains in the particle. The number of polymer chains in a particle can be calculated assuming that the particle density is the same as the bulk density (ρ), as established in the literature for particles produced with different techniques.[7,8,38] On the basis of these assumptions, the number of molecules in a particle can be estimated from

$$n = \frac{2\pi}{3}\frac{\rho r^3}{m_u M_w} \qquad (2)$$

where m_u is the atomic mass unit (1.66×10^{-27} kg). Because of possible deviation of the large particles from semispherical, eq 2 gives only an upper limit for the number of chains in the large particles. Data calculated from the radii of figures such as Figure 1 are presented in Figure 3a. The smaller particles are represented by the black bars and contain on the large-diameter side at most 10 polymer chains. On the small-diameter side (18 nm) there must be less than two molecules in a particle. On the basis of these calculations and because smaller particles have not been found, we expect most of the small particles to be single-chain particles derived from the "offspring droplets" of the normal ES process.

The remaining question is the origin of the big particles. A big particle with an average diameter of 95 nm consists of about 400 polymer chains. This is roughly the number of chains expected in the initial

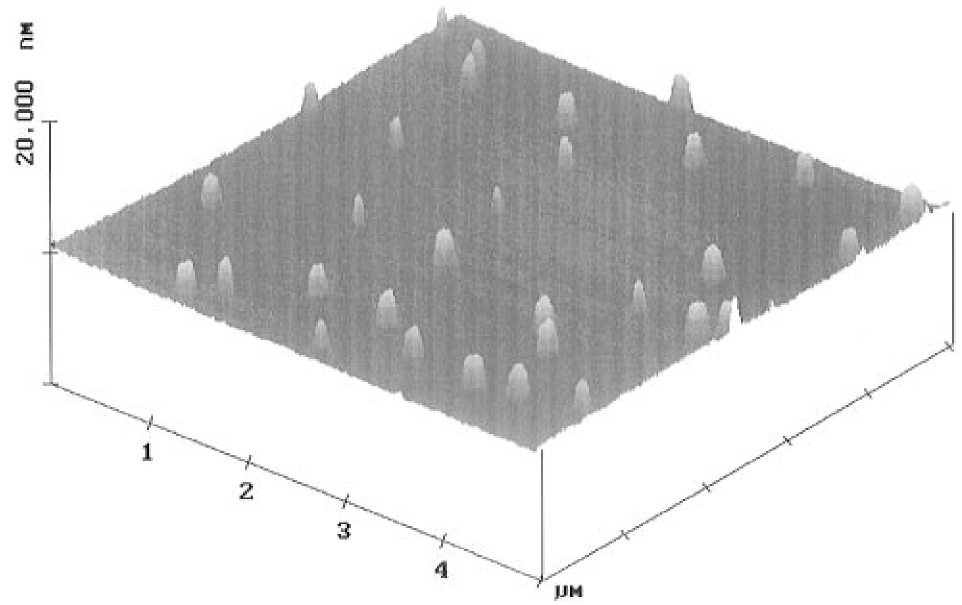

Figure 4. SFM scan of electrosprayed PS (M_w = 330 000 Da, concentration 1 × 10^{-4} wt % in chloroform, sprayed for 10 min on mica).

spray droplets prior to subdivision. At an initial concentration of 1 × 10^{-4} wt % a droplet with 400 chains would have a radius of 3.3 μm. Such a radius is consistent with literature data about ES droplet formation.[23,31] This means that the larger particles on the target contain most of the initial polymer chains of the droplet. We have reasoned that this failure to partition the polymer molecules among the offspring droplets occurs when entanglements impede the droplet fission process. Once entangled, a disentanglement is not possible during the fission because the energy of a fission process is not sufficient to separate the entangled coil.[39]

This behavior can be utilized to produce particles with a defined number of polymer chains. To demonstrate this, electrospray experiments were performed using different concentrations of polystyrene in the initial solution. In addition to the 10^{-4} wt % used above, concentrations ranging from 3 × 10^{-5} to 3 × 10^{-6} wt % were sprayed. Concentrations lower than 10^{-6} wt % were not useful because the number of particles deposited on the target was too low to be detected. Typical particle-size distributions of the lower concentrations are also shown in Figure 3 (b and c). Figure 3b (concentration 1 × 10^{-5} wt %) shows again a bimodal particle size distribution. The smaller particles have an average size of 21 nm. This is basically unchanged compared to the results obtained at the higher concentration in Figure 3a. For the larger particles, the average size is, however, markedly smaller than that in Figure 3a (an average of 39 nm compared to 95 nm). In the case of the lowest concentration investigated (3 × 10^{-6} wt %), only one maximum can be detected. Here the bimodal distribution collapses to one broad maximum with an average diameter of 29 nm. In this case a generation of big particles is no longer possible because the nascent droplets at the capillary tip contain only a few polymer chains. It is significant that the size of the smallest particles do not depend on the concentration; the particle size distribution always has the same lower limit. This lower limit is outside the demonstrated spatial resolution of the SEM which is 1−1.5 nm under these conditions. Both findings corroborate that the smallest particles are, indeed, single-molecule particles. The mean particle size of the bigger particles decreases with the concentration. This observation has led to a revised electrospray model.[39] With lower concentration, the number of polymer chains in an initial droplet decreases, but the initial droplet size remains constant.[13] This smaller polymer content alone would already lead to smaller particles with a given number of fission steps. In addition, the lower initial concentration allows more fission steps prior to the onset of entanglements. This leads to a further decrease of the polymer content of a droplet at the time it reaches the entanglement onset. A plot of the particle diameter versus concentration permits, thus, targeted production of particles with a distinct number of chains.

The applicability of the electrospray method for the formation of single-chain particles out of high molecular weight polymers is limited due to the necessary solvent evaporation and the resulting entanglements of the polymer. In fact, earlier mass spectrometric measurements of high molecular mass polymers were only successful with concentrations so low that there was only a single molecule in the nascent droplet.[20]

Conclusions

The electrospray method proved to be a versatile tool for isolating single molecules and defined few-molecule clusters. Amorphous polystyrene particles were isolated and investigated. The singular advantage is the possibility to achieve different particle sizes. A tailoring of the particle size is possible by adjusting the initial concentration in the solution.

Acknowledgment. This work was supported by the Microgravity Science and Applications Division of the National Aeronautics and Space Administration, Grant NAG 8-1065. R.F. thanks the German Science Foundation (DFG) for additional support. The assistance of Dr. Thomas G. Thundat of the Life Sciences Division of ORNL with the tapping mode SFM is gratefully acknowledged. The SFM work was supported by the Division of Materials Research, National Science Foundation, Polymers Program, Grant no. DMR 90-00520 and the Division of Materials Sciences, Office of Basic

Energy Sciences, U.S. Department of Energy at ORNL, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy, under contract number DE-AC05-96OR22464.

References and Notes

(1) Morawetz, H. *Macromolecules in Solution*, 2nd ed.; John Wiley: New York, 1975.
(2) Boyer, R. F.; Heidenreich, R. D. *J. Appl. Phys.* **1945**, *16*, 621.
(3) Bittiger, H.; Husemann, E. *Makromol. Chem.* **1964**, *75*, 222.
(4) Bittiger, H.; Husemann, E. *Makromol. Chem.* **1964**, *80*, 239.
(5) Bittiger, H.; Husemann, E. *Makromol. Chem.* **1966**, *96*, 92.
(6) Ruscher, C. *J. Polym. Sci., Part C* **1964**, *16*, 2923.
(7) Barnikol, I.; Barnikol, W. K.; Beck, A.; Campagnari-Terbojević, M.; Jovanović, N.; Schulz, G. V. *Makromol. Chem.* **1970**, *137*, 111.
(8) Koszterszitz, G.; Barnikol, W. K.; Schulz, G. V. *Makromol. Chem.* **1977**, *178*, 1133.
(9) Richardson, M. J. *J. Polym. Sci., Part C* **1963**, *3*, 21.
(10) Richardson, M. J. *Proc. R. Soc. London, Ser. A* **1964**, *279*, 50.
(11) Quayle, D. V. *Br. Polym. J.* **1969**, *1*, 15.
(12) Furuta, M. *J. Polym. Sci., Polym. Phys. Ed.* **1976**, *14*, 479.
(13) Kebarle, P.; Tang, L. *Anal. Chem.* **1993**, *65*, 972A.
(14) Siegel, B. M.; Johnson, D. H.; Mark, H. *J. Polym. Sci.* **1950**, *5*, 111.
(15) Bailey, A. G. *Electrostatic Spraying of Liquids*; John Wiley: New York, 1988.
(16) Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M. *Science* **1989**, *246*, 64.
(17) Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M. *Mass Spectrom. Rev.* **1990**, *9*, 37.
(18) Dole, M.; Mack, L. L.; Hines, R. L.; Mobley, R. C.; Ferguson, L. D.; Alice, M. B. *J. Chem. Phys.* **1968**, *49*, 2240.
(19) Wong, S. F.; Meng, C. K.; Fenn, J. B. *J. Phys. Chem.* **1988**, *92*, 546.
(20) Nohmi, T.; Fenn, J. B. *J. Am. Chem. Soc.* **1992**, *114*, 3241.
(21) Lüttgens, U.; Röllgen, F. W.; Cook, K. D. Optical Studies of Electrohydrodynamic Disintegration of Liquids in EH and ES Mass Spectrometry. In *Methods and Mechanisms for Producing Ions from Large Molecules*; Standing, G. G., Ens, W., Eds.; Plenum Press: New York, 1991.
(22) Gomez, A.; Tang, K. *Phys. Fluids* **1994**, *6*, 404.
(23) Tang, K.; Gomez, A. *Phys. Fluids* **1994**, *6*, 2317.
(24) Daoud, M.; Jannink, G. *J. Phys. (Paris)* **1976**, *37*, 973.
(25) Deery, M. J.; Jennings, K. R.; Jasieczek, C. B.; Haddleton, D. M.; Jackson, A. T.; Yates, H. T.; Scrivens, J. H. *Rapid Commun. Mass Spectrom.* **1997**, *11*, 57.
(26) Nielen, M. W. F. *Rapid Commun. Mass Spectrom.* **1996**, *10*, 1652.
(27) Jasieczek, C. B.; Buzy, A.; Jennings, K. R. *Rapid Commun. Mass Spectrom.* **1996**, *10*, 509.
(28) VanBerkel, G. J.; McLuckey, S. A.; Glish, G. L. *Anal. Chem.* **1992**, *64*, 1586. See also: VanBerkel, G. J. The Electrolytic Nature of Electrospray. In *Electrospray Ionization Mass Spectrometry*; Cole, R. B., Ed.; John Wiley: New York, 1997; Chapter 2.
(29) Lias, S. G.; Bartmess, J. E.; Liebman, J. F.; Holmes, J. L.; Levin, R. D.; Mallard, W. G. *J. Phys. Chem. Ref. Data* **1988**, *17*, Suppl. 1.
(30) Taflin, D. C.; Ward, T. L.; Davis, E. J. *Langmuir* **1989**, *5*, 376.
(31) Tang, L.; Kebarle, P. *Anal. Chem.* **1993**, *65*, 3654.
(32) Davies, C. N. Evaporation of Airborne Droplets. In *Fundamentals of Aerosol Science*; Shaw, D. T., Ed.; John Wiley: New York, 1978.
(33) Ikonomou, M. G.; Blades, A. T.; Kebarle, P. *Anal. Chem.* **1990**, *62*, 957.
(34) Sarid, D. *Scanning Force Microscopy*; Oxford University Press: New York, 1991.
(35) Wickramasinghe, H. K. *J. Vac. Sci. Technol.* **1990**, *A8*, 363.
(36) Ohnesorge, F.; Binning, G. *Science* **1993**, *260*, 1451.
(37) Gaur, U.; Wunderlich, B. *Macromolecules* **1980**, *13*, 1618.
(38) Bu, H.; Pang, Y.; Song, D.; Yu, T.; Voll, T. M.; Czornyj, G.; Wunderlich, B. *J. Polym. Sci., Part B: Polym. Phys.* **1991**, *29*, 139.
(39) Festag, R.; Alexandratos, S. D.; Joy, D. C.; Wunderlich, B.; Cook, K. D. In *Space Processing of Materials*; Ramachandran, N., Ed. *Proc. SPIE* **1996**, *2809*, 155.

MA970211X