

Electrospray Drying & Deposition of Nanomaterial - Analysis by Atomic Force Microscopy

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Abstract

We explore the use of an electrospray source run in jet-cone mode to dry and deposit nanometer size macromolecules in water suspensions and/or solutions on sample substrates for size analysis by Atomic Force Microscopy (AFM). AFM is an excellent particle sizing technique, which gives a topographical mapping of particles adhered to atomically flat substrates even for particles of just a few nanometers in size. However, it has been observed that the size/shape of macromolecules that were naturally dried on AFM substrate surfaces depend on the drying process, and aggregation or addition of impurities makes it difficult to obtain size information on the single molecule level. We explore the idea of using an electrospray source to dry macromolecule samples in solution and deposit them on AFM substrates, creating a standardized method of measuring macromolecules in liquid samples. Electrosprays may be used to minimize the effects of impurities and dry/deposit single macromolecules since they can be controlled to produce droplets of sizes just slightly larger (tens-hundreds of nanometers) than the macromolecule (a few nanometers). Figure 1 illustrates the advantages of drying macromolecules with nanometric size droplets. For a 1 ppm (volume) concentration of impurities, typical for bi-distilled waters manipulated with careful handling procedures, droplets larger than 0.1 μm generate residues greater than 1 nm (sphere equivalent diameter), which would clearly affect size measurements for macromolecules on the order of a few nanometers. Unwanted effects of impurities are minimized for smaller droplets. Smaller size droplets also increase the production of dried 'monomers' (or single particles / macromolecules) as opposed to clustering of the macromolecule itself (dimers, trimers, etc.). The concentration necessary to produce one analyte per droplet, plotted in Fig. 1 B, is related to the production rate of dried single macromolecule monomers (as opposed to clusters), or the signal in 'electrospray-AFM'; so smaller droplets maximize signal. We present experimental evidence showing that electrospray as a technique for drying and depositing macromolecules on AFM surface substrates is a promising. Further work is needed to prove whether or not electrospray-AFM gives accurate size analysis of macromolecules of a few nanometers in size, and we discuss some problems incurred in depositing highly charged electrospray nano-products on AFM substrates and their solutions.

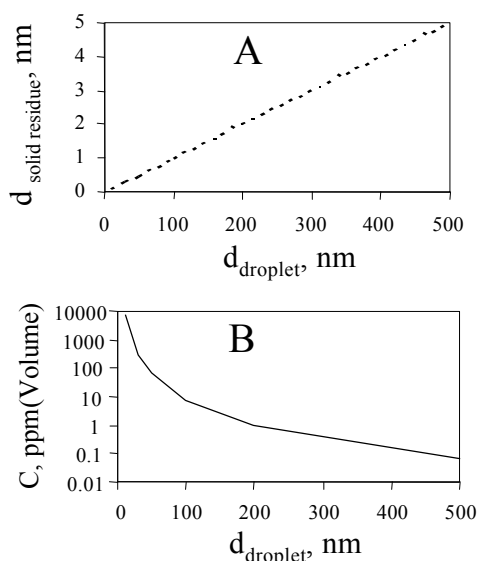


Figure 1: The estimated diameter of a solid residue from a 1 ppm (volume) impurity (A). The concentration of 2 nm particles in solution which gives 1 particle/droplet (B).

1. Introduction

Various macromolecules of a few nanometers in size are readily prepared or captured in liquid samples, including biological samples, polymers, humic acids, and the first material formed during phase change reactions that occur in combustion processes of gas phase hydrocarbons in slightly fuel rich conditions, typical of modern engines and industrial burners. There is considerable interest in being able to measure the size of such material. Only recent state-of-the-art particle sizing techniques and/or chemical analytical techniques are sensitive to nanometer size material, one of which is Atomic Force Microscopy (AFM). AFM gives size/shape information in relatively high resolution (angstrom resolution in height and nanometer resolution in the x-y plane) in the form of a three-dimensional topological mapping of the sample deposited on an atomically smooth substrate surface, typically cleaved mica or highly ordered pyrolytic graphite (HOPG).

While AFM is a powerful sizing technique for macromolecule samples in the size range of several to tens of nanometers (similar to electron microscopic techniques), some difficulties exist in depositing samples in a concentration appropriate for accurate analysis on to the sample surface, especially for water samples when the analyte is in the smallest size range. Natural drying techniques do not work well for material in this size range because air drying requires a long time, during which dust particles from the drying environment can be introduced, and depending on the characteristics of the material in solution, complete removal of the water layer may require high vacuum [1]. Also, impurities present in the solvent, may attach to the particles rather than evaporate with the solvent, which may significantly alter the size of the material sampled for molecular clusters of only a few nanometers in size. In general, structures in liquid samples observed by AFM depend strongly on the drying technique and conditions, as we saw in our own experiments and is reported by others [2]. Here, we explore the idea of electrospray drying and deposition as a technique, which could offer a standard method for preparing substrates for AFM analysis of analyte material in liquid suspensions. We describe the results of experiments depositing dried electrospray products on the surface of mica and HOPG substrates.

2. Electrospray as a nano-scale spray dryer

In this section, we describe what is currently known about the use of electrosprays to dry nanometric materials in solvents for size analysis by other diagnostics, namely differential mobility analysis (DMA) and mass spectrometry (MS). By applying a voltage difference between the fluid pushed through a capillary tube and a counter electrode, an electrospray is established, and the physical description of this spray depends both on the fluid and electric fields affecting the jet flow [3, 4]. A particular mode of electrospray operation [4], referred to as Taylor or cone-jet has been well-studied both because of its stability and its ability to produce quite monodisperse droplets of controllable size. A successful example of how electrosprays are used as a nano-spray dryer for macromolecules with sizes on the order of a few to tens of nanometers is its coupling with dynamic mobility analyzers to analyze proteins and biological macromolecules [5], colloidal nanoparticles [6], and combustion generated material trapped in condensed water samples [7]. The analytical strategy of these works to accurately determine the size distribution of nano-material in solution or suspension is to use the electrospray to create droplets slightly larger than the analyte of interest and spray a solution containing the analyte in a concentration such that each droplet contains approximately one particle.

A. Droplets produced by electrospray: Main, satellites, daughter drops from Coulomb fissions

Droplets produced by electrospray running in jet-cone mode are highly charged. They may be of three types, defined by the physical nature of their origins: 1) highly monodisperse main droplets, for which scaling laws exist relating the droplet diameter to the liquid flow rate of the spray and the liquid conductivity, 2) satellite droplets, smaller droplets formed during jet breakup, which migrate to the periphery of the spray as the spray progresses, and 3) daughter droplets of Coulomb fissions [8-11]. Scaling laws, which are supported by experimental observations [9, 12-17], exist that predict the diameter of the ‘main’ droplets produced by electrospraying highly conductive liquids in cone-jet mode ($K > 1 \mu\text{S/cm}$) [12, 13]:

$$d_{\text{maindrop}} = G(\epsilon) \frac{Q\epsilon\epsilon_0}{K} \quad (1)$$

where ϵ , and K are the dielectric constant and conductivity of the solution being sprayed, and ϵ_0 is the electrical permittivity of vacuum.

The main droplets are naturally separated from the smaller satellite droplets as the spray progresses because the space charge of the spray causes the smaller satellites, with higher electric mobility to migrate to the spray cloud periphery [8, 10]. As a result, by sampling only from the spray core, these smaller satellites can be eliminated, passing only the monodisperse main droplets, whose diameter is a function of the controllable parameters Q and K, to the AFM sample substrate.

The third type of droplet in electrosprays is the daughter droplets formed during Coulomb fissions. Neutral solvent evaporation reduces the size of the droplets, while the charge remains constant until they approach the Rayleigh charge limit for their size, causing the droplet to divide by Coulomb fission, thus destroying the high monodispersity of the spray [9, 11, 18]. These secondary droplet divisions occur downstream of the jet breakup, and the diameter and charge of daughter droplets born from Coulomb fissions depend on the properties of the liquid drop and its surrounding electric field [11]. Coulomb fissions will continue to occur until the droplets are small enough ($d < 35$ nm) so that the electric field surrounding the droplet is high enough (approximately > 1 V/nm) to stimulate direct ion evaporation, which reduces both mass/size and charge, keeping the droplet well beneath the Rayleigh charge limit [9, 18-20].

B. How Coulomb fissions affect the size of dried electrospray products

Recent work shows that sprays of high solvent conductivities can be operated such that the original ‘main’ droplets produced at the jet break-up section of the spray are originally in conditions which encourage direct ion evaporation, thus keeping the droplets under the Rayleigh limit and eliminating Coulomb fissions throughout the entire drying process [20]. Electrospray-DMA systems usually use a radioactive source to quickly neutralize the droplets just after they are formed, suppressing Coulomb fissions. If Coulomb fissions are suppressed, the main droplets will all be of roughly the same diameter and their evaporation histories will therefore be the same. If Coulomb fissions are not suppressed, the droplet size distribution will be less monodisperse as the spray evolves and droplets begin to divide and sub-divide. In both cases, if the concentration of the analyte in solution is such that there would be 1 macromolecule/main droplet (main drop diameter calculated by equation 1), then the dried products will be only monomers (the dried macromolecule) with much smaller residues from impurities in the water (produced from empty drops). If the concentration in solution is greater than that which gives 1 macromolecule/main droplet, the dried products will be larger than the macromolecule as a result of clustering of 2 or more macromolecules contained in each droplet.

C. Electrospray-AFM

Summarizing, the idea to use electrospray to dry and deposit macromolecules or nanometric particles in solution is drawn schematically in Fig. 2. Satellite droplets are eliminated naturally by sampling only from the spray core through a small hole in the counter electrode plate. The main droplets produced are made small enough (controlling Q and K of the solution by equation 1) to be slightly larger than the macromolecule and the concentration of macromolecule in solutions is that which gives 1 or less macromolecules per main droplet by the following equation:

$$C = \frac{d_{dry-product}^3}{d_{main-droplet}^3} \quad (2)$$

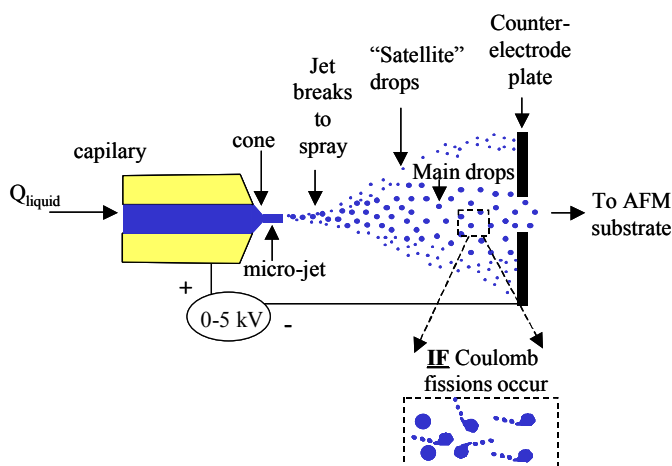
If the main droplets are small enough to suppress Coulomb fissions throughout their evaporation history, only main droplets dry and deposit on the AFM substrate. If, instead, Coulomb fissions are not suppressed, both the main droplets and all daughter droplets born from Coulomb fissions dry and deposit on the AFM surface. In both of these cases, if the concentration is lower or equal to that which gives 1 macromolecule/drop, measuring the size of dried material will give the size of the macromolecule. (Coulomb fissions when the concentration gives 1 macromolecule/drop would only help the drying process by creating smaller droplets.) However, to avoid counting residues of minerals or other solid impurities produced from dried ‘empty’ droplets (that do not contain the analyte macromolecule), we kept the size of the main droplet produced by electrospray smaller than 200 nm, so that the dried products would be smaller than the detection limit of our AFM (See Fig. 1A).

3. Experimental Conditions

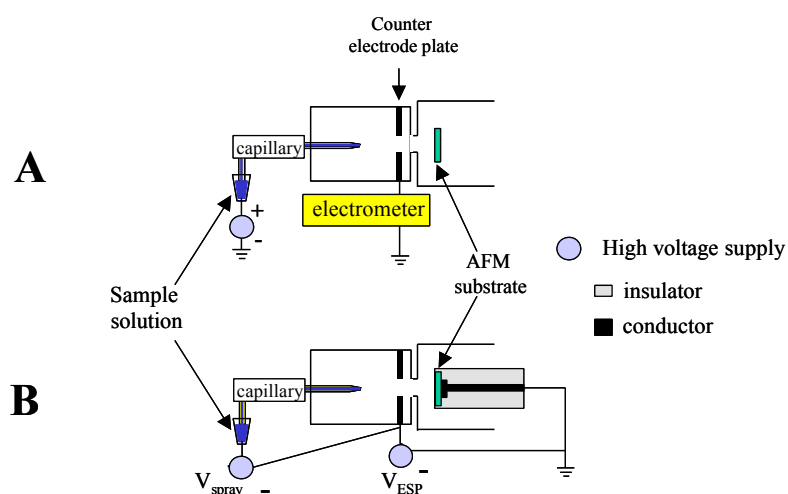
Once the particles have been dried by electrospray, their charge is essentially the same as the droplet, from which it came, just before the last molecule of solvent is evaporated.¹ As a result, the dried particles/macromolecules will have a distribution of multiple charges. The cloud of these multiply charged particles will tend to expand because of their mutual repulsion and space charge similar to the highly charged droplets in the electrospray cloud (shown schematically in Fig. 2). As a result, it was necessary to take care to avoid any unwanted electric fields in the lines in which the particles were drying and being transported to the AFM substrate. Also, the lines were kept short to minimize losses of these highly charged expanding cloud of evaporating droplets/recently dried particles.

Figure 2: Physical aspects of electrospray-drying.

We tested two types of deposition strategies: deposition by diffusion alone (Fig. 3A) and



deposition by diffusion and electrostatic precipitation (ESP) (Fig. 3B). We also tested two types substrates: Mica, which is hydrophylic and non-conductive, and HOPG, which is hydrophobic and conductive. Both Mica and HOPG are cleavable as they are generated in layers. A fresh atomically flat layer was obtained relatively easily by peeling off the top layer of these substrate surfaces with a piece of scotch tape just before mounting them in position for deposition, as verified by AFM observations. HOPG cleavage often produced terraces, but the AFM was able to detect well the



background (zero height) and resolve the height of deposited particles.

Figure 3: Electro spray deposition by diffusion only (A), and by diffusion and ESP (B).

¹ A notable exception is that the charge will be singular if the macromolecule creates ions in solution, and can undergo direct ion evaporation from the droplet. However, large macromolecules that are in the size range amenable to AFM analysis, $d \geq 2$ nm, seem to form ions by the charged residue mechanism described earlier in this paper rather than by direct ion evaporation [21].

We used a Digital Instruments Nanoscope IIIa[®] AFM operated in tapping-mode (to minimize tip-sample surface interactions) to observe particle deposits. AFM provides a three-dimensional topological mapping of the sample surface by scanning over the horizontal x-y plane and monitoring the height of a tip mounted on a cantilever beam vibrating over the surface. A Nanoprobe[®] Super Sharp Silicon probe was used for the tip to reduce probe-sample convolution effects. The particle volume was evaluated using S.P.I.P.[™], an advanced image processing software by ImageMetrology, which allows accurate virtual separation between the sample and the background. An equivalent-spherical diameter (ED) is calculated from the measured particle volume.

The various “modes of operation” of the electrospray [4] were observed by measuring the spray current with an electrometer as a function of applied voltage between the spray tip and the counter electrode plate (Fig. 3A). The current increased in discrete steps, clearly demonstrating the passage from dripping, pulsating, cone-jet and multi-jet spray modes. The spray was also observed by eye with the aid of a microscope. We always ran experiments in jet-cone mode to take advantage of the derived scaling laws predicting droplet size (equation 1). The droplet size was kept small by using highly conductive solvents ($K > 0.2$ S/m) of ammonium acetate in bi-distilled water. The conductivity of sprayed solutions was measured with a RS 180-7127 hand held conductivity meter, and was found to be approximately linear with ammonium acetate concentration in the range of $C = 200$ mM acetate ($K = 2$ S/m) – 20 mM acetate ($K = 0.2$ S/m). The liquid flow rate, Q , was controlled and kept small by forcing the liquid through a silica capillary ($ID = 30$ μm). We measured the pressure, P , applied to the liquid, rather than directly measuring Q , with a mercury manometer. By Poiseuille's law, P is expected to be linearly related to Q , but we found that deposits (near the capillary tip) tend to reduce Q as a function of P (as observed by slight changes in the shape of the cone-jet). Eventually, deposits would totally block the flow, and therefore, it is difficult to estimate well Q from the measured P . We found that blocked capillaries can be cleaned by quickly passing the tip through a blue flame, while forcing some solutin through the capillary. We cleaned the capillary in this way just prior to experiments, but since Q was not directly measured and since the flow resistance increases slowly due to deposits at the capillary tip, for the purpose of this paper, when discussing relative changes in Q , we will report the liquid flow rate in terms of P rather than its absolute value in terms of liquid flow rate.

4. Results/Discussion

Figure 4 shows a particle size distributions observed of dried electrospray products deposited by diffusion alone on Mica. The electrospray solution for these tests was 8.2×10^{-5} volume fraction of sucrose in a solvent of 20 mM ammonium acetate in bi-distilled water. The liquid conductivity was $K = 0.2$ S/m, and the pressure applied to force the liquid flow through the capillary was 0.1 bar.

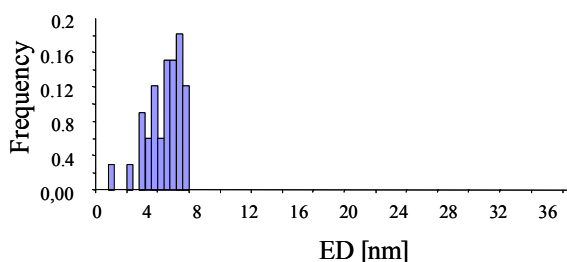


Figure 4: Counted size distribution of particles deposited on Mica by diffusion only. The spray solution was 8.2×10^{-5} sucrose (vol. fraction) in 20 mM acetate in water. $P = 0.1$ bar.

We had problems repeating these results using Mica surfaces such that depositions ranged from no deposition at all to about 30 particles deposited in 500 nm \times 500 nm, which is a count density that is very well quantified by AFM. These inconsistencies were not associated with problems of spray instability, as the independent measure of the spray current was constant from day to day as measured by the electrometer shown in fig. 3 A. We tried passing the Mica under a Corona negative ion source to neutralize its surface as suggested in an article describing ESP deposition for particles [22], but we were not able to find a procedure, which gave repeatable deposition. We suspect that the reason for the inconsistent deposition is related to the fact that the dried particles are highly charged, and the charge density on

the Mica surface after cleavage is unpredictable. Since Mica is a non-conductor, charges on its surface resulting from the cleavage will be discharged only by ambient air ions of opposite charge that diffuse to its surface. Furthermore, once some charged particles deposit on its surface, the non-conducting Mica builds up an electric field on its surface which prevents further deposition.

Figure 5 A shows the deposition on an HOPG surface for the same experimental conditions previously measured on the Mica substrate. We used electrostatic precipitation (as shown schematically in Fig. 3 B) to focus the particles to the substrate surface. By connecting the substrate surface to electrical ground the surface charge remains neutral even as highly charged particles deposit. The size distribution observed on HOPG is significantly wider and contained about 10 times as many particles than those observed on Mica, confirming that charge build up on the Mica surface likely explains the previous observations. While mica is an excellent substrate for collecting nanoparticles at high temperatures on its surface by thermophoresis [23], it is not adapt for collection of the highly charged particles produced by naturally dried electrosprays because it is non-conducting.

Figure 5 B shows the size distribution of electrospray products deposited for a solution containing the protein lysozyme from chicken egg white for a more conductive ($K=1$ S/m) solution operating in cone-jet mode at much lower liquid flow rate, $P=0.019$ bar. Increasing the conductivity of the liquid reduces the droplet size directly by equation 1. In addition, at higher K , the spray can be operated at much lower flow rate, Q , while still remaining in stable cone-jet mode, which again reduces the main droplet size. Even though the concentration of solid material in fig. 5 B is much higher ($4e^{-3}$) than the sucrose concentration sprayed for fig. 5A ($8e^{-5}$), the residues observed by AFM are smaller, and less polydisperse. The observation that the size distribution of the dried residues is more narrow in Fig. 5B compared to Fig. 5A indicates that the smaller main droplets underwent less Coulomb fissions. The size distribution of Fig. 5 B is still wider than the distributions reported for (much larger) $32\ \mu\text{m}$ electrospray droplets observed by phase doppler anemometry before evaporation (Standard deviation/mean, $\text{St.dev.}/d_{\text{avg.}} = 0.06$) [10] or the size distribution reported for sucrose residues using electrospray-DMA in conditions where Coulomb fissions were suppressed by neutralizing the droplets just after they are formed with a bipolar radioactive charge neutralizer ($d_{\text{avg.}} = 28\ \text{nm}$, $\text{St.dev.}/d_{\text{avg.}} = 0.04$) [14]. Therefore, Figure 5 shows evidence that reducing the droplet size has reduced Coulomb fissions, but not suppressed them.

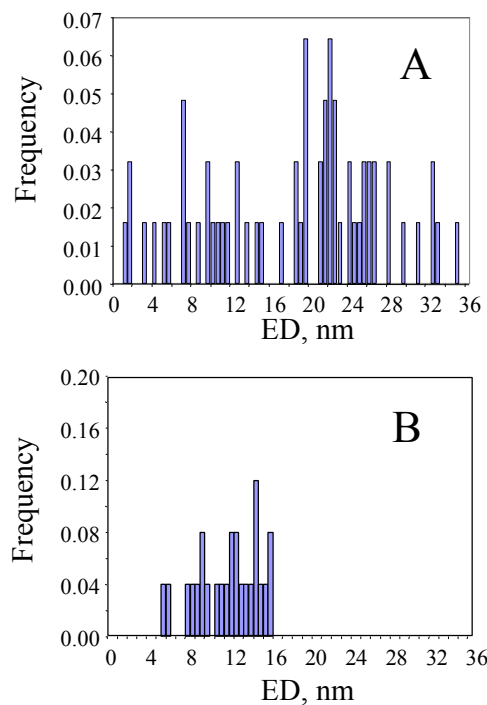


Figure 5: Counted size distributions of particles deposited on HOPG by diffusion and ESP. $8.2e^{-5}$ sucrose in 20 mM acetate in water ($K=0.2$ S/m) $P=0.1$ bar (A), $4.5e^{-3}$ lysozyme in 100 mM acetate ($K=1$ S/m) $P=0.019$ bar (B).

Because of the problems discussed associated with residues building up on the capillary tip, we do not have an accurate measure of Q . From the observed ED for the maximum residue measured by AFM and the known concentration of lysozyme, we calculate from eqn. 2 that a droplet size of approximately 100 nm would give residues of about 16 nm, and the smaller residues probably come from smaller daughter droplets, which have undergone Coulomb fissions. The size of a single lysozyme macromolecule calculated from its known mass (14.3 kDa) and density ($1.3\ \text{g}/\text{cm}^3$ [24]) is 3.5 nm, while that measured by electrospray mobility spectra is 3.9 nm [25, 26]. The size distribution

observed from the conditions sprayed in Fig. 5 B do not quite arrive to the single-molecule size, as the smallest ED observed was slightly larger than 4 nm. Since the concentration was higher than that needed to give 1 macromolecule/droplet, the dried residues counted are clusters of lysozyme molecules. To test whether electrospray-AFM can accurately measure the size of a singly macromolecule, we will reduce the concentration to provide 1 macromolecule/droplet (approximately $5e^{-5}$, for the same spray conditions as Fig. 5B).

5. Conclusions

The work presented here shows that deposition of dried electrospray deposits on conductive surfaces seems a promising technique for preparing AFM samples of nanometric material in liquid samples. An analyzable deposition was found by sampling dried electrospray products using electrostatic precipitation to focus the charged electrospray particles to the surface of HOPG substrates. Further experiments are needed to confirm that electrospray-AFM can accurately measure the size of single macromolecules of just a few nanometers in size. Reducing the droplet size significantly reduces the amount of Coulomb fissions incurred, observed by a drastic narrowing of the particle size distribution counted by AFM of dried residues deposited on HOPG substrates.

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