

Sizing Nanoparticles and Macromolecules in Liquids

using an Electropray and Scanning Mobility Particle Sizer™ Spectrometer
(a.k.a., ES-SMPS, GEMMA, ES-DMA, ES-IMS, *macrolMS™* Macroion Mobility Spectrometer)

Application Note ES-001

Sizing Particles in Liquids Using Differential Mobility Analysis

ES-SMPS and ES-DMA (a.k.a. *macrolMS™* Macroion Mobility Spectrometer, GEMMA, ES-IMS, nES-GEMMA) are acronyms coined by researchers¹⁻⁸ to describe an [Electrospray Aerosol Generator \(EAG\)](#) paired with a [Scanning Mobility Particle \(SMPS™\) Spectrometer](#) used to perform rapid, high resolution sizing of nanoparticle colloids, proteins and other macromolecules.

The fundamental measurement behind the ES-SMPS technique is Differential Mobility Analysis. Mobility based particle sizing is a classic measurement technique which has been used by investigators for over a century⁹. It has been used for particle size distribution measurements since the late 1970s¹⁰, and has been used to size nanoparticles or macromolecules in solutions for the last 15 years¹¹. Briefly described, the EAG is used to aerosolize the colloidal suspension, and then particles are sized via their electrical mobility (ability to traverse an electric field) resulting in a measurement of electrical mobility diameter (EMD) which is a fundamental function of particle size.

Since introduction by Kaufman in 1996 the ES-SMPS technique is frequently chosen by researchers over more conventional techniques and its use is increasingly widespread. The National Institute of Standards and Technology (NIST) & the National Characterization Laboratory (NCL) issued a Joint Assay Protocol, "PCC-10: Analysis of Gold Nanoparticles by Electropray Differential Mobility Analysis (ES-DMA)" which details a protocol for size analysis of liquid born gold nanoparticles via ES-SMPS.

Benefits of ES-SMPS

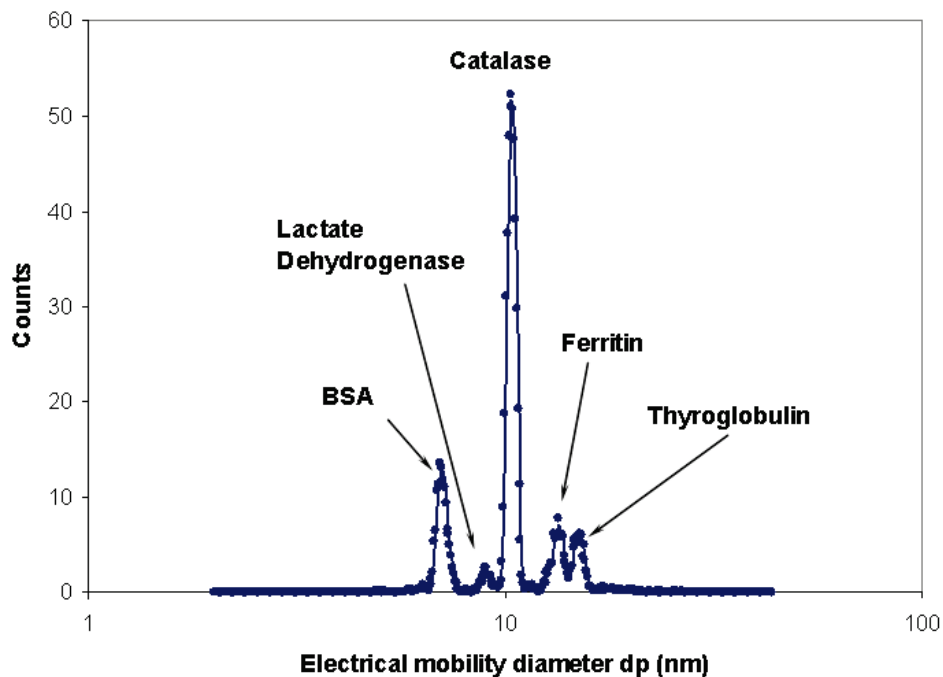
1. Nanoparticle Sizing Accuracy

ES-SMPS has repeatedly shown excellent agreement with more conventional off-line imaging techniques such as Tunneling Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM)¹²⁻¹⁴. Other common techniques like Dynamic Light Scatter (DLS) can have difficulty with nanoparticle size accuracy^{6,15}. Additionally, ES-SMPS does not have any issues with incorrectly representing particle size distributions due to bubbles in the colloid.

2. Nanoparticle Size Resolution

Resolutions of ~3.5 to 10% of the particle size can be achieved²¹. This resolution is comparable to the reported resolution of Size Exclusion Chromatography (SEC)²².





ES-SMPS data of the High Molecular Weight calibration standard. All five components are revealed. Wang X, Tan P, and Kaufman S 2006, "Comparison of Ultrafine Condensation Particle Counters for the *macrolMS* Macroion Mobility Spectrometer"

3. Sensitivity to Small Changes in Size

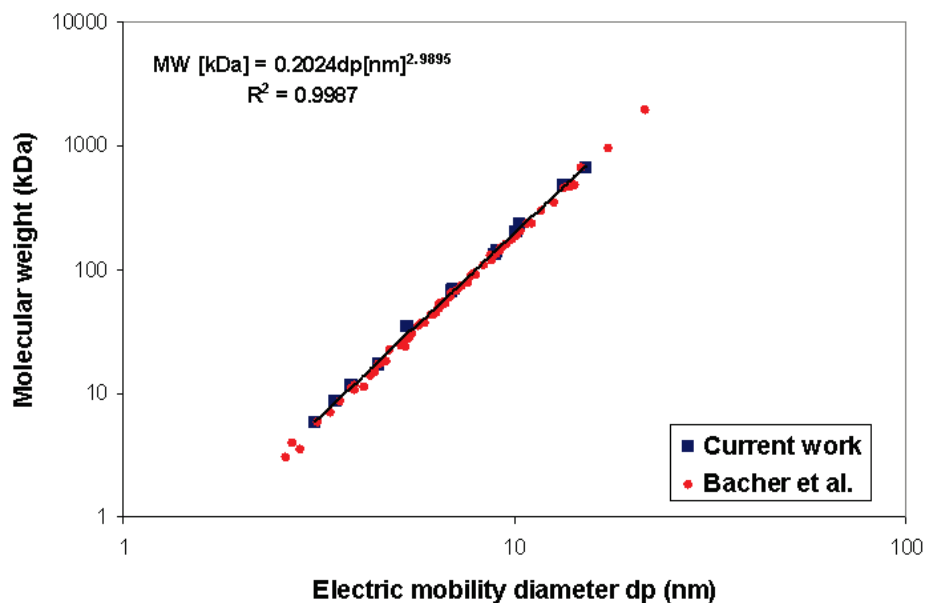
The ES-SMPS can easily resolve two peaks <1 nm apart. Researchers have noted the technique is capable of measuring changes in nanoparticle size distributions as small as 0.2 nm at 10 nm and 0.5 nm at 30 nm¹⁶. This degree of resolution is difficult for other techniques to achieve³. The ability to detect small changes to particle size over time has made the instrument valuable in studying the kinetics of colloidal solutions^{2,17-20}.

4. Measurement of Multimodal Size Distributions

ES-SMPS can measure accurately, the particle size distribution of a multimodal size distribution. As such, with a single scan, ES-SMPS is useful to determine the degree of aggregation in nanoparticle colloids. The proportion of aggregation state (dimers, trimers, tetramers) can be identified rapidly from the multimode size distributions². More conventional techniques like DLS are not capable of resolving aggregate concentrations within a multimodal distribution⁸. The presence of just a few large particles in a DLS sample can strongly affect the measurement.

5. Measurement of Molecular Mass/Molecular Weight (MW)

The EMD is correlated with the molecular mass of macromolecules over 4 orders of magnitude—up to 12MDa²²⁻²⁶. Because of this strong correlation, the ES-SMPS (a.k.a. *macrolMS*TM Macroion Mobility Spectrometer, GEMMA, nES-GEMMA, ES-IMS,) technique is used widely to measure the molecular mass and molecular weight of larger proteins. High resolution structural analysis techniques such as NMR and X-ray crystallography have difficulty sizing larger macromolecules²⁵, and chromatography suffers from unwanted column interactions which degrade MW accuracy. The mass accuracy of ES-SMPS has been found to be $\pm 6\%$. As a result, researchers routinely use ES-SMPS instrumentation to analyze large biological macromolecules like lipoproteins in high throughput environments²⁶.



Protein mobility diameter vs. molecular weight. Note that the power index is very close to 3, indicating proteins particles are compact. Wang X, Tan P, and Kaufman S 2006, "Comparison of Ultrafine Condensation Particle Counters for the *macrol*MS Macroion Mobility Spectrometer"

6. Single Particle Measurement

ES-SMPS is a discrete technique: the diameters of individual nanoparticles or macromolecules are measured¹¹. As a result, the method is capable of measuring subtle irregularities in colloidal solutions and is capable of detecting small changes in the nanoparticle size distribution due to mechanisms like dissolution or aggregation¹⁵. This can be a challenge for more conventional techniques like Dynamic Light Scatter (DLS), because the method requires an assumption be made about the peak form and number of peaks.

7. High Sensitivity

As previously mentioned, The ES-SMPS measurement has been shown to be useful for detecting small aggregates in colloidal solutions that can be a challenge with other methods². ES-SMPS has also been shown to be more sensitive to studying large protein complexes versus IMS or MS²⁶.

8. High Precision and Repeatability

A mature technology and extensive engineering and manufacturing experience have resulted in reliable ES-SMPS instrumentation which has been noted to exhibit a high degree of precision and repeatability²⁷.

9. Small Sample Volume

As little as 20 μ L of solution is needed per analysis. A much smaller amount of sample is actually consumed—approximately 2 ng. DLS and MALLS (Multi-Angle Laser Light Scattering) require a much larger amount of sample.

10. Large Measurement Samples

For every measurement sample, a significant number of particles are sized--providing a very representative size distribution. This is extremely difficult to achieve with other techniques that have comparable size resolution like TEM or SEM. With these methods, typically only a fraction of the particles are evaluated—perhaps a few hundred at most.

11. Fast measurement times

ES-SMPS takes only 3 to 5 minutes per sample, which makes it rapid compared to chromatographic separations or ultracentrifugation. The short measurement time required makes this a high-throughput technique.

12. First Principle Measurement

The absolute accuracy of differential mobility analysis comes in part from the fact that the measurement is fundamentally based on physics and math. No size calibration or calibration analytes are required. The measured instrument response is electrical mobility--not light scatter or diffusional movement. Complex data correction algorithms are not needed to measure highly accurate electrical mobility based distributions.

13. Independent of Optical Properties of Material or Solution

Since ES-SMPS does not rely on light scatter to size the particles, the technique is independent of the optical properties of the nanoparticle/macromolecule and solvent.

14. Independent of Sample Temperature

The equations governing the size calculation for the ES-SMPS technique are very weak functions of temperature, so in general, moderate differences in sample temperature will not affect the measurement in any discernable way.

15. Well Understood Technology

The science of mobility-based nanoparticle sizing is very well understood, and the resolution, precision and accuracy achievable via this real-time technique are noteworthy.

Useful Information via ES-SMPS

Size Distribution of Nanoparticles in Solutions

Investigators routinely use ES-SMPS to determine the size distribution of nanoparticles in solution. A wide variety of nanoparticle types have been investigated including Au, Ag, SiO₂, TiO₂, Cu, CNTs, nanopolymers^{28,29}, and viruses. For more detail refer to the partial bibliography of peer reviewed technical papers published using an ES-SMPS based technique.

Molecular Mass of Proteins and other Macromolecules

ES-SMPS has been used to measure the molecular mass of proteins and other macromolecules including biospecific proteins and bacteriophages^{20, 30-34}, and viruses and viral related proteins^{20,30,35}. For more detail refer to the partial bibliography of peer reviewed technical papers published using an ES-SMPS based technique.

Insight into Colloidal Kinetics

Kinetic Evaluations of Aggregation Behavior: ES-SMPS presents a systemic approach to detecting liquid-phase aggregation of nanoparticles. It is used by researchers to investigate the time dependent changes in size distributions of nanoparticle colloids during aggregation^{15,17,18}. Protein aggregation has also been investigated using ES-SMPS^{19,20}. The method is useful for detecting small changes in aggregates as a function of ionic state and reaction time².

Nanoparticle Dissolution: Similarly, ES-SMPS has also been used to investigate nanoparticle dissolution in aqueous suspensions².

Surface Density and Thickness of Nanoparticle Coatings

ES-SMPS has proven to be a useful tool for the study of coatings on nanoparticles. Several investigators have used the technique to investigate coating thickness as a function of synthesis process factors^{1,13,14,16}. ES-SMPS has sufficient resolution to track both the packing density and the desorption kinetics of nanoparticle coatings¹⁷.

ES-SMPS Best Practices

Solution Type

Aqueous colloidal solutions are the best choice. From an application knowledge point of view there are a limited amount of buffer solutions that lend itself to this technique, although many other solutions have the potential of being compatible with this method.

Solution Concentration

A moderately dilute sample needs to be used to ensure that the ES-SMPS process does not induce aggregation. The probability that a droplet would contain two or more particles should be low³⁶. For a

150 nm droplet (approximate droplet size) 140 ug/mL ensures a single particle per droplet. Its good practice to consider a range of solution concentrations to verify that successively smaller mobility diameters are not measured or that peak ratios do not change with subsequent dilutions. This ensures that the measured size distribution is representative of the sample, and if applicable, that the observed dimers and trimers (agglomerates) are intrinsic and not induced. Researchers have investigated and determined that when the ES-SMPS technique is properly executed, particles and aggregates are aerosolized without causing further aggregation^{4,37}.

Size Accuracy

Impurities in the solvent create a thin film of residue on the measured particles. For many applications this slight effect can be neglected. However, for higher precision, the impurity concentration can be estimated using the residue concentration. The residue coating thickness can then be calculated and subtracted off of the measured particle size³⁶.

Size Resolution

The size resolution in the ES-SMPS system is primarily a function of a variable instrument setting (sheath to aerosol flow rate)¹⁰. Greater sheath to aerosol flow ratios in general result in better size resolution. The internal flow system in the TSI ES-SMPS supports sheath flows up to 20 LPM.

Size Range

The typical size range for the ES-SMPS technique is 2.5 to 150 nm. However, if liquid particle sizing of larger particles is required, an atomizer can be used on the front end of the SMPS instead of the electrospray²¹.

Contact Information

If you are interested in more information on the ES-SMPS technique using the [Model 3480 Electro Spray Aerosol Generator \(EAG\)](#) and the [Model 3936 Scanning Mobility Particle \(SMPS™\) Spectrometer](#), or are interested in learning more about how the technique could help with your specific application, visit our website at www.tsi.com, send an e-mail to particle@tsi.com or call 866-266-5919 (+1 651-490-3824) and ask to speak with one of our Particle Instrument application specialists.

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