SIZING NANOPARTICLES AND MACROMOLECULES IN LIQUIDS USING AN ELECTROSPRAY AND SCANNING MOBILITY PARTICLE SIZER™ SPECTROMETER

(a.k.a., ES-SMPS, GEMMA, ES-DMA, ES-IMS, *macro*IMS™ Macroion Mobility Spectrometer)

APPLICATION NOTE ES-001 (A4)

Sizing Particles in Liquids Using Differential Mobility Analysis

ES-SMPS and ES-DMA (a.k.a. *macro*IMS™ Macroion Mobility Spectrometer, GEMMA, ES-IMS, nES-GEMMA) are acronyms coined by researchers[1-8] 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.

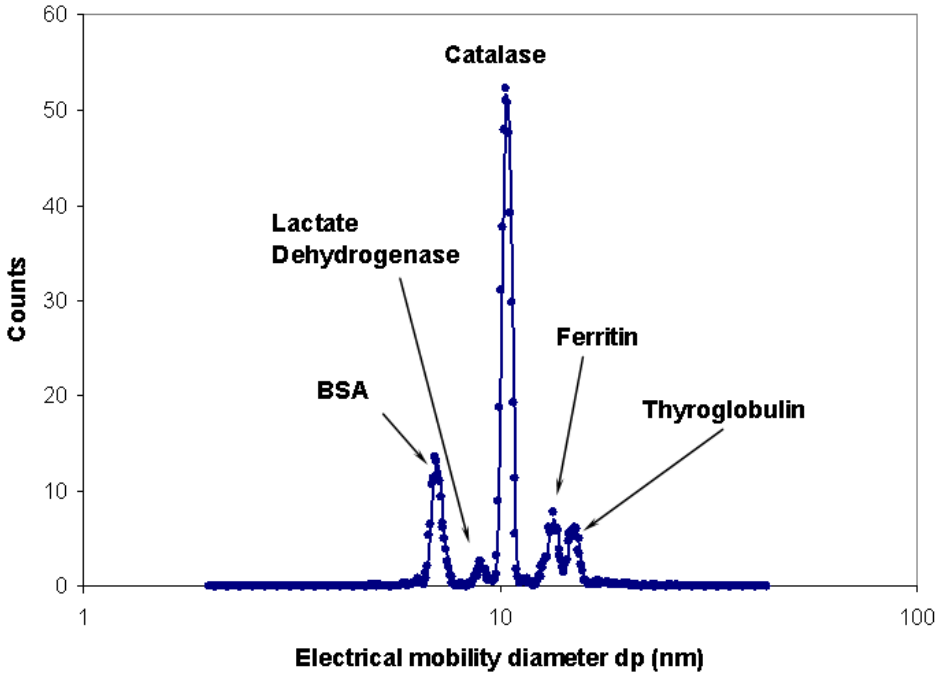
The fundamental measurement behind the ES-SMPS technique is Differential Mobility Analysis. Mobility based particle sizing is a classic measurement technique which has been used by investigators for over a century[9]. It has been used for particle size distribution measurements since the late 1970s[10], and has been used to size nanoparticles or macromolecules in solutions for the last 15 years[11]. 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 Electrospray 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 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 achieved[21]. This resolution is comparable to the reported resolution of Size Exclusion Chromotography (SEC)[22].

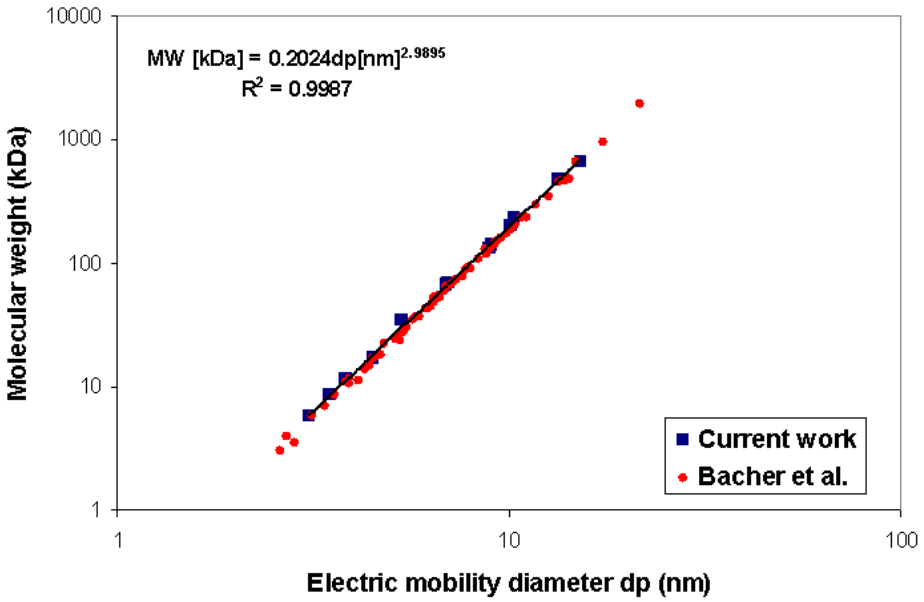


ES-SMPS data of the High Molecular Weight calibration standard. All five components are revealed. Wang X, Tan P, and Kaufman S 2006, "Comparison of Ultrafine Condensation Particle Counters for the *macro*IMS Macroion Mobility Spectrometer"

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 at 0.2 nm at 10 nm and 0.5 nm at 30 nm[16]. This degree of resolution is difficult for other techniques to achieve[3]. 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 distributions[2]. More conventional techniques like DLS are not capable of resolving aggregate concentrations within a multimodal distribution[8]. 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 12MDa[22-26]. Because of this strong correlation, the ES-SMPS (a.k.a. *macro*IMS™ 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 macromolecules[25], and chromatography suffers from unwanted column interactions which degrade MW accuracy. The mass accuracy of ES-SMPS has been found to be ±6%. As a result, researchers routinely use ES-SMPS instrumentation to analyze large biological macromolecules like lipoproteins in high throughput environments[26].



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 *macro*IMS Macroion Mobility Spectrometer"

6. **Single Particle Measurement**
   ES-SMPS is a discreet technique: the diameters of individual nanoparticles or macromolecules are measured[11]. 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 aggregation[15]. 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 methods[2]. ES-SMPS has also been shown to be more sensitive to studying large protein complexes versus IMS or MS[26].

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 repeatability [27].

9. **Small Sample Volume**
   As little as 20 µL 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, SiO_2, TiO_2, 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 time[2].

Nanoparticle Dissolution: Similarly, ES-SMPS has also been used to investigate nanoparticle dissolution in aqueous suspensions[2].

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 coatings[17].

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 low[36]. 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 size[36].

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 sheathe 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 electrospray[21].

Contact Information

If you are interested in more information on the ES-SMPS technique using the Model 3480 Electrospray 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.

References

[1]Hildebrandt N, Hermsdoft D, Signorell R, Schmitz SA, Diederishsen U, 2007,"Superparamagnetic iron oxide nanoparticles functionalized with peptides by electrostatic interactions," *ARKIVOC*, **2007**(v)79-90.

[2]Elzey S, Grassian VH, 2010, "Nanoparticle Dissolution from the Particle Perspective: Insights from Particle Sizing Measurements," *Langmuir*, **26(15)**:12505–12508.

[3]Abbas Z, Holmberg JP, Hellström AK, Hagström M, Bergenholtz J, Hassellöv M, Ahlberg E, 2011, "Synthesis, characterization and particle size distribution of TiO2 colloidal nanoparticles," *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, **384(1-3)**:254-261.

[4]Johnsson AC, Camerani MC, Abbas Z, 2011, "Combined electrospray-SMPS and SR-SAXS investigation of colloidal silica aggregation. Part I. Influence of starting material on gel morphology," *J Phys Chem B.*, **115(5)**:765-75.

[5]Pease LF, Tsai DH, Zangmeister RA, Zachariah MR, and Tarlov MJ, 2010, "Analysis of Gold Nanoparticles by Electrospray Differential Mobility Analysis (ES-DMA)," NIST-NCL Joint Assay Protocol, PCC-10, Version 1.1.

[6]Lenggoro W, Xia B, Okuyama K, de la Mora JF, 2002, "Sizing of Colloidal Nanoparticles by Electrospray and Differential Mobility Analyzer Methods," *Langmuir*,**18(12)**:4584–4591.

[7]Han B, Lenggoro IW, Choi M, Okuyama K, 2003, "Measurement of cluster ions and residue nanoparticles from water samples with an electrospray/differential mobility analyzer," *Anal Sci*., **19(6)**:843-51.

[8]Pease LF, Tsai DH, Hertz JL, Zangmeister RA, Zachariah MR, Tarlov, MJ, 2010 "Packing and Size Determination of Colloidal Nanoclusters ," *Langmuir*, **26(13)**:11384–11390.

[9]Flagan RC, 1998, "History of Electrical Aerosol Measurements," *Aerosol Science and Technology*, **28(4)**:301-380.

[10]Knutson EO, Whitby KT, 1975 "Aerosol Classification by Electric Mobility: Apparatus, Theory, and Applications," *Journal of Aerosol Science*, **6**:443-451.

[11]Kaufman SL, Skogen JW, Dorman FD, Zarrin F, Lewis KC, 1996, "Macromolecule Analysis Based on Electrophoretic Mobility in Air: Globular Proteins," *Anal. Chem*., 1996, **68**:1895-1904.

[12]Wacaser BA, Deppert K, Böttger M, Bi Z, Adolph D, Dick KA, Karlsson LS, Karlsson MA, 2007, "Electrospraying of colloidal nanoparticles for seeding of nanostructure growth," *Nanotechnology*, **18(10)**:5304-5309.

[13]Grassian VH, 2008, "When Size Really Matters: Size-Dependent Properties and Surface Chemistry of Metal and Metal Oxide Nanoparticles in Gas and Liquid Phase Environments," *J. Phys. Chem. C*, **112(47)**:18303–18313.

[14]Pease LF, Tsai DH, Zangmeister RA, Zachariah MR, Tarlov MJ, 2007, "Quantifying the Surface Coverage of Conjugate Molecules on Functionalized Nanoparticles," *J. Phys. Chem. C*, **111(46)**:17155–17157.

[15]Elzey S, Grassian VH, 2010, "Agglomeration, isolation and dissolution of commercially manufactured silver nanoparticles in aqueous environments," *Journal of Nanoparticle Research*, **12(5)**:1945-1958.

[16]Tsai DH, Zangmeister RA, Pease LF, Tarlov MJ, Zachariah MR, 2008, "Gas-Phase Ion-Mobility Characterization of SAM-Functionalized Au Nanoparticles," *Langmuir*, **24(16)**:pp 8483–8490.

[17]Johnson AC, Greenwood P, Hagstrom M, Abbas Z, Wall S, 2008, "Aggregation of Nanosized Colloidal Silica in the Presence of Various Alkali Cations Investigated by the Electrospray Technique," *Langmuir*, **2(22)**:12798–12806.

[18]Tsai DH, Pease LF, Zangmeister RA, Tarlov MJ, Zachariah MR, 2009, "Aggregation Kinetics of Colloidal Particles Measured by Gas-Phase Differential Mobility Analysis," *Langmuir*, **25(1)**;140–146.

[19]Pease LF, Elliott JT, Tsai DH, Zachariah MR, Tarlov MJ, 2008, "Determination of protein aggregation with differential mobility analysis: Application to IgG antibody," *Biotechnology and Bioengineering*, **101(6)**:1214-1222.

[20]Bacher G, Szymanski WW, Kaufman SL, Zöllner P, Blaas B, Allmaier G, 2001, "Charge-reduced nano electrospray ionization combined with differential mobility analysis of peptides, proteins, glycoproteins, noncovalent protein complexes and viruses," *Journal of Mass Spectrometry*, **36(9)**:1038–1052.

[21]Kinney PD, Pui DYH, 1991, "Use of the Electrostatic Classification Method to Size 0.1um SRM Particles – A Feasibility Study," *Journal of Research of the National Institute of Standards and Technology*, **96(2)**:147-176.

[22]Allmaier G, Laschober C, Szymanski WW, 2008, "Nano ES GEMMA and PDMA, new tools for the analysis of nanobioparticles-protein complexes, lipoparticles, and viruses," *Journal of the American Society for Mass Spectrometry*,**19(8)**:1062-1068.

[23]Kaufman SL, 1998, "Analysis of Biomolecules using Electrospray and Nanoparticle Methods: The Gas-Phase Electrophoretic Mobility Molecular Analyzer (GEMMA)," *J. Aerosol Sci*., **29**:537.

[24]S. Ude, J. Fernández de la Mora, and B. A. Thomson, 2004, "Charge-Induced Unfolding of Multiply Charged Polyethylene Glycol Ions," *J. Am. Chem. Soc.*, 2004, **126(38)**:12184–12190.

[25]Caulfield MP, Li S, Lee G, Blanche PJ, Salameh WA, Benner WH, Reitz RE, Krauss RM, 2008, "Direct determination of lipoprotein particle sizes and concentrations by ion mobility analysis," *Clin Chem*., **54(8)**:1307-16.

[26]Kaddis CS, Lomeli SH, Yin S, Berhane B, Apostol MI, Kickhoefer VA, Rome LH, and Loo JA, 2007, "Sizing Large Proteins and Protein Complexes by Electrospray Ionization Mass Spectrometry and Ion Mobility," *J Am Soc Mass Spectrom*., **18(7)**:1206–1216.

[27]Kapellios S, Karamanou MF, Sardis M, Aivaliotis A, Economou S, Pergantis A, 2011, "Using nanoelectrospray ion mobility spectrometry (GEMMA) to determine the size and relative molecular mass of proteins and protein assemblies: a comparison with MALLS and QELSE," *Analytical Bioanalytical Chemistry*, **399**:2411-2433.

[28]Saucy DA, Ude S, Lenggoro IW, de la Mora JF, 2004, "Mass analysis of water-soluble polymers by mobility measurement of charge-reduced ions generated by electrosprays," *Anal. Chem*., 2004, **76**:1045 1053.

[29]Poderycki MJ, Kickhoefer VA, Kaddis CS, Raval-Fernandes S, Johansson E, Zink JI, Loo JA, and Rome LH, 2006, "The Vault Exterior Shell is a Dynamic Structure that Allows Incorporation of Vault Associated Proteins into its Interior," *Biochemistry*, **45(39)**:12184–12193.

[30]Thomas JJ, Bothner B, Traina J, Benner WH, Siuzdak G, 2004, "Electrospray Ion Mobility Spectrometry of Intact Viruses," *Spectroscopy*, 2004, **18**:31-36.

[31]Loo JA, Berhane B, Kaddis CS, Wooding KM, Xie Y, Kaufman SL and Chernushevich IV, 2005, "Electrospray Ionization Mass Spectrometry and Ion Mobility Analysis of the 20S Proteasome Complex," *Journal of the American Society for Mass Spectrometry* **16(7)**:998-1008.

[32]Kaddis CS and Loo JA, 2007, "Native Protein MS and Ion Mobility: Large Flying Proteins with ESI," *Anal. Chem*., **79(5)**:1778–1784.

[33]Hogan CJ, Kettleson EM, Ramaswami B, Chen DR, and Biswas P, 2006, "Charge Reduced Electrospray Size Spectrometry of Mega- and Gigadalton Complexes: Whole Viruses and Virus Fragments," *Anal. Chem*., 2006, **78(3)**:844–852.

[34]Wick CH, McCubbin PE, 1999, "Characterization of purified MS2 bacteriophage by the physical counting methodology used in the integrated virus detection system (IVDS)," *Toxicological methods*, 1999, **9(4)**:245-252.

[35]Laschober C, Wruss J, Blaas D, Szymanski WW, and Allmaier G, 2008, "Gas-Phase Electrophoretic Molecular Mobility Analysis of Size and Stoichiometry of Complexes of a Common Cold Virus with Antibody and Soluble Receptor Molecules," *Anal. Chem.*, 2008, **80(6)**:2261–2264.

[36]Kaufman SL, 2000,"Electrospray Diagnostics Performed by using Sucrose and Proteins in the Gas-Phase Electrophoretic Mobility Molecular Analyzer (GEMMA)," *Anal. Chim. Acta*, 2000, **406**:3-10.

[37]Li M, Guha S, Zangmeister R, Tarlov MJ and Zachariah MR, 2011, "Quantification and Compensation of Nonspecific Analyte Aggregation in Electrospray Sampling," *Aerosol Science & Technology*, **45(7)**:849-860.