Table of Contents

Antibody Aggregation


This book describes how to address the analysis of aggregates and particles in protein pharmaceuticals, provides a comprehensive overview of current methods and integrated approaches used to quantify and characterize aggregates and particles, and discusses regulatory requirements. Analytical methods covered in the book include separation, light scattering, microscopy, and spectroscopy.


The biopharmaceutical industry characterizes and quantifies aggregation of protein therapeutics using multiple analytical techniques to cross-validate results. Here, we demonstrate the use of electrospray–differential mobility analysis (ES–DMA), a gas-phase and atmospheric pressure ion-mobility method for characterizing protein aggregates. Two immunoglobulin Gs are systematically heat treated to induce aggregation and characterized using size-exclusion chromatography (SEC) and ES–DMA. Although ES–DMA is a gas-phase characterization method, we find that aggregation kinetic rate constants determined by ES–DMA is in good agreement with those determined by SEC. ES–DMA appears to have a higher resolution and lower limit of detection as compared with SEC. Thus, ES–DMA can potentially become an important orthogonal tool for characterization of nascent protein aggregates in the biopharmaceutical industry.
Adsorbed proteins on walls of glass capillaries used for electrospray (ES) can desorb and potentially affect size distributions and, thus, quantification of aggregates of proteins. In this study we use differential mobility analysis (DMA) to investigate the size distribution of various proteins eluting from bare and passivated glass capillaries. We found no significant differences in aggregate distributions from unpassivated capillaries at 'steady state' when compared to aggregate distributions from passivated capillaries implying that desorbing proteins do not influence protein aggregate distribution. Surface passivation with gelatin was found to be considerably more effective in limiting adsorption of two antibodies (Rituxan and polyclonal human IgG) compared to passivation with BSA. Gelatin passivation was also found to be stable for a few days and from a pH range of 4.8-9.0. © Published by Elsevier Inc.

Electrospray (ES) sources are commonly used to introduce nonvolatile materials (e.g., nanoparticles, proteins, etc.) to the gas phase for characterization by mass spectrometry or ion mobility. Recent studies in our group using ES ion mobility to characterize protein aggregation in solution have raised the question as to whether the ES itself induces aggregation and thus corrupts the results. In this article, we develop a statistical model to determine the extent to which the ES process induces the formation of dimers and higher-order aggregates. The model is validated through ES differential mobility experiments using gold nanoparticles. The results show that the extent of droplet-induced aggregation is quite severe and previously reported cutoff criterion is inadequate. We use the model in conjunction with experiment to show the true dimer concentration in a protein solution as a function of concentration. Themodel is extendable to any ES source analytical system and to higher aggregation states. For users only interested in implementation of the theory, we provide a section that summarizes the relevant formulas.

Here we describe the use of electrospray differential mobility analysis (ES-DMA), also known a Os-phase electrophoretic mobility molecular analysis as a method for measuring low-order soluble aggregates of proteins in solution. We demonstrate proof of concept with IgG antibodies. In ES-DMA, aqueous Solutions of the antibody protein are electrosprayed and the various aerosolized species are separated according to their electrophoretic mobility using a differential mobility analyzer. In this way, complete size distributions of protein species present from 3 to 250 nm can be obtained with the current set up, including distinct peaks for IgG monomers to pentamers. The sizes of the IgG and IgG aggregates measured by DMA were found to be in good agreement with those calculated from simple models, which take the structural dimensions of IgG from protein crystallographic data. The dependence of IgG aggregation on the solution concentration and ionic strength was also examined, and the portion of aggregates containing chemically crosslinked antibodies was quantified. These results indicate that ES-DMA holds potential as a measurement tool to study protein aggregation phenomena such as those associated with antibody reagent manufacturing and protein therapeutics.

**Instrumentation and Technique**

Little has been published on the details of design and fabrication of differential mobility analyzers (DMAs) classifying 1 nm particles with resolving powers of 30 or more. These DMAs must operate at Reynolds numbers Re >> 2000 (supercritical), requiring laminarizers and diffusers that have tended to make them large and heavy. Here we discuss design and fabrication criteria as well as observed performance for several variants of a miniature supercritical DMA weighing 2.7 kg. In spite of the narrow working sections associated to small electrode radii of R1=4 mm and R2=6 or 7 mm (gap=R1−R2=Δ2 or 3 mm), a large enough Re (hence resolution Δr down to 1 nm diameter particles) is achieved via a diffuser enabling up to transonic sheath gas speeds. Several axial distances l between the inlet and outlet aerosol slits have been tested to facilitate classification of particles with diameters above 20 nm. Δr is limited by concentricity errors, magnified by the small gaps used. Nonetheless, short models with l/Δr=2 achieve consistently Δr=4. This is not the case for 4. This performance is with 1 nm particles, and would probably be better with larger particles. While prior long supercritical DMAs have used slightly conical inner electrodes to accelerate and stabilize the flow, a cylindrical version of our long DMAs shows no signs of turbulent transition. © 2012 Elsevier Ltd. All rights reserved.
We have developed a simple, fast, and accurate method to measure the absolute number concentration of nanoparticles in solution. The method combines electrospray differential mobility analysis (ES-DMA) with a statistical analysis of droplet-induced oligomer formation. A key feature of the method is that it allows determination of the absolute number concentration of particles by knowing only the droplet size generated from a particular ES source, thereby eliminating the need for sample-specific calibration standards or detailed analysis of transport losses. The approach was validated by comparing the total number concentration of monodispersed Au nanoparticles determined by ES-DMA with UV/vis measurements. We also show that this approach is valid for protein molecules by quantifying the absolute number concentration of Rituxan monoclonal antibody in solution. The methodology is applicable for quantification of any electrospray process coupled to an analytical tool that can distinguish monomers from higher order oligomers. The only requirement is that the droplet size distribution be evaluated. For users only interested in implementation of the theory, we provide a section that summarizes the relevant formulas. This method eliminates the need for sample-specific calibration standards or detailed analysis of transport losses.

The transfer functions and penetrations of five differential mobility analyzers (DMAs) for sub-2 nm particle classification were evaluated in this study. These DMAs include the TSI nanoDMA, the Caltech radial DMA (RDMA) and nanoRDMA, the Grimm nanoDMA, and the Karlsruhe-Vienna DMA. Measurements were done using tetra-alkyl ammonium ion standards with mobility diameters of 1.16, 1.47, and 1.70 nm. These monomobile ions were generated by electrospray followed by high resolution mobility classification. Measurements were focused at an aerosol-to-sheath flow ratio of 0.1. A data inversion routine was developed to obtain the true transfer function for each test DMA, and these measured transfer functions were compared with theory. DMA penetration efficiencies were also measured. An approximate model for diffusional deposition, based on the modified Gormley and Kennedy equation using an effective length, is given for each test DMA. These results quantitatively characterize the performance of the test DMAs in classifying sub-2 nm particles and can be readily used for DMA data inversion.

Differential mobility analysis (DMA) is a technique suited for size analysis as well as preparative collection of airborne nanosized airborne particles. In the recent decade, the analysis of intact viruses, proteins, DNA fragments, polymers, and inorganic nanoparticles was possible when combining this method with a nano-electrospray charge-reduction source for producing aerosols from a sample solution/suspensions. Mass analysis of high molecular weight noncovalent complexes is also possible with this methodology due to the linear correlation of the electrophoretic mobility diameter and the molecular mass. In this work, we present the analysis (size and molecular mass) of high molecular weight multimers (noncovalent functional homocomplex) of jack bean urease in a mass range from 275 kDa up to 2.5 MDa, with mainly present tri- and hexamers but also higher oligomers of the 91 kDa monomer subunit. In a second experiment, the size analysis of intact very-low-density (similar to 35 nm), low-density (similar to 22 nm) and high-density lipoparticles (similar to 10 nm), which are heterocomplexes consisting of cholesterol, lipids, and proteins in different ratios, is presented. Results from mobility analysis were in excellent agreement with particle diameters found in literature. The last presented experiment demonstrates size analysis of a rod-like virus and selective sampling of a selected size fraction of electrosprayed, singly-charged tobacco mosaic virus particles. Sampling and subsequent transmission electron microscopic investigations of a specific size fraction (40 nm electrophoretic mobility diameter) revealed the folding of virus particles during the electrospray and charge reduction (electrical stress) as well as solvent evaporation (mechanical stress) process, leading to an observed geometry of 150 (length) x 35 (width) nm (average cylindrical geometry of unsprayed intact virus 300 x 18 nm).

Recently it has been demonstrated that nanoelectrospray (nES) in conjunction with macro-ion mobility spectrometry (macroIMS) and condensed particle detection can be used to size various types of nanoparticles, including large biomolecules (proteins, DNA, etc.), having electrophoretic mobility diameters ranging from 3 nm to well over 100 nm. The technique is extremely sensitive; however, it lacks specificity as a result of the detector used. To explore the possibility to overcome this limitation, we demonstrate the direct coupling of the nES-macroIMS system to an inductively coupled plasma mass spectrometer (ICPMS). Technical challenges involving the coupling of the air-based nES-macroIMS with the argon-based ICPMS are addressed and overcome. The resulting novel hyphenated technique is used to determine the elemental composition of nanoparticles resulting from the electrospaying of solutions containing inorganic salts and acids (CsI and dimethylarsinic acid). Even though the sensitivity of the used ICPMS does not allow for the simultaneous sizing of proteins and the determination of their metal, metalloid, or halogen content, we have shown that it is feasible to detect and accurately size proteins at femto-mole levels by adding CsI to their solutions and detecting the resulting Cs adducts. This is also possible with DNA molecules. A linear relationship between protein amount and ICPMS response for Cs-I33(+) is observed, thus hinting at the possibility of further developing the technique for quantitative analysis of large biomolecules.


To clarify how the E. coli class Ia RNR is regulated and what role large complexes have in this regulation, we have studied wild-type and mutant forms of this enzyme with EMMA, surface plasmon resonance (SPR) biosensor analysis, and enzyme activity assays. Moreover, the substrate specificity of the E. coli enzyme was for the first time studied with all four substrates present simultaneously. The conclusion from this study was that the E. coli enzyme is in equilibrium between an active α6β2 octamer formed in the presence of ATP, dTTP, or dGTP with similar substrate specificity as other class Ia RNRs and an inactive α4β4 octamer formed in the presence of dATP or effector combinations of ATP + dTTP/dGTP. The E. coli RNR is therefore different from the mouse enzyme, which forms an active α6β2 octamer in the presence of ATP (or effector combinations of ATP + dTTP/dGTP) and an inactive α4β2 octamer in the presence of dATP (17). Contrary to current models for the mouse enzyme (22), the specificity site in the E. coli enzyme seems to have a central role in the overall activity regulation because the inactive complex is formed regardless whether ATP or dATP occupies the overall activity site as long as a deoxyribonucleotide occupies the specificity site (an inactive complex is not formed when ATP binds both allosteric sites). However, dATP still is the main regulator of overall activity at physiologically relevant concentrations of nucleotides, which is in common with the mouse enzyme.


As previously demonstrated by the technique of gas-phase electrophoretic mobility molecular analyzer (GEMMA) introduced by Kaufman and colleagues, differential mobility analysis (DMA) of charge-reduced electrospray ions in the gas phase is a useful complement to MS for studying large proteins and their weakly bound complexes. Several limitations of GEMMA, the solutions for which have the potential to greatly improve its performance, are discussed here, including DMA resolution and transmission. A quantitative theory of charge reduction kinetics for dried multiply charged globular proteins at atmospheric pressures is also presented, showing that the charge reduction time must be carefully chosen to maximize a singly charged ion signal, while avoiding survival of contaminating multiply charged species. Because charge reduction limits the range of masses analyzable by MS, we also consider the potential of a parallel-plate DMA coupled in series to an MS for DMA-MS studies without charge reduction.
This study explores the potential of a novel electrospray based method, termed gas-phase electrophoretic mobility molecular analysis (GEMMA), allowing the molecular mass determination of peptides, proteins and noncovalent biocomplexes up to 2 MDa (dimer of immunoglobulin M). The macromolecular ions were formed by nano electrospray ionization (ESI) in the 'cone jet mode'. The multiple charged state of the monodisperse droplets/ions generated was reduced by means of bipolar ionized air (generated by an alpha-particle source) to yield exclusively singly charged positive and negative ions as well as neutrals. These ions are separated subsequently at atmospheric pressure using a nano differential mobility analyzer according to their electrophoretic mobility in air. Finally, the ions are detected using a standard condensation particle counter. Data were expressed as electrophoretic mobility diameters by applying the Millikan equation. The measured electrophoretic mobility diameters, or Millikan diameters, of 32 well-defined proteins were plotted against their molecular weights in the range 3.5 to 1920 kDa and exhibited an excellent squared correlation coefficient ($r^2 = 0.999$). This finding allowed the exact molecular weight determination of large (glyco)proteins and noncovalent biocomplexes by means of this new technique with a mass accuracy of ±5.6% up to 2 MDa at the femtosecond level. From the molecular masses of the weakly bound, large protein complexes thus obtained, the binding stoichiometry of the intact complex and the complex stability as a function of pH, for example, can be derived. Examples of specific protein complexes, such as the avidin or catalase homo-tetramer, are used to illustrate the potential of the technique for characterization of high-mass biospecific complexes. A discussion of current and future applications of charge-reduced nano ESI GEMMA, such as chemical reaction monitoring (reduction process of immunoglobulin G) or size determination of an intact virus, a supramolecular complex, and monitoring of partial dissociation of a human rhinovirus, is provided. Copyright © 2001 John Wiley & Sons, Ltd.

“The proteins alpha-lactalbumin (14 KDa), conalbumin (77 KDa), and ferritin (460 KDa) were dissolved in 20 mM NH4OAc along with controlled amounts of sucrose. The solution was electrosprayed into 160 nm diameter droplets in a charge-neutralized electrospray [O.-R. Chen, D.Y. Pui, S.L. Kaufman, J. Aerosol Sci. 26 (1995) 963], at concentrations such that most droplets contained no more than one protein molecule. The electrophoretic-mobility diameter spectra of the aerosol formed on droplet evaporation were measured using a differential mobility analyzer with a condensation type particle detector [S.L. Kaufman, J.W. Skogen, F.D. Dorman, F. Zarrin, K.C. Lewis, Anal. Chem. 68 (1996) 1895, 3703]. Peaks were observed corresponding to residues from droplets containing only sucrose and from droplets containing both sucrose and single protein molecules. For the peaks corresponding to the protein molecules, an increase in particle diameter with increasing sucrose concentration was observed, consistent with a simple model in which a 'crust' of sucrose is formed around the protein molecule as the liquid in the surrounding droplet evaporates. Spectra at high sucrose concentrations, where the pure-sucrose particles were larger than the protein molecules without sucrose, showed a peak at the diameter corresponding to the uncoated protein molecules. This somewhat surprising result appears to show that there is more than one process by which macromolecules or their ions leave the associated droplets in charge-neutralized electrospray. ©2000 Elsevier Science B.V. All rights reserved.

“Aerosol particle detection and sizing techniques have recently been extended downwards to a size range commensurate with the size of many important biomolecules. Although such large molecules do not naturally occur individually as aerosol particles, electrospray-drying makes it feasible to generate an aerosol of isolated single macromolecules from macromolecule solutions. The combination of this electrospray generation with the improved aerosol techniques makes possible a new method of size analysis for biomolecules. We present characteristics of this new system and review some recent results. ©1998 Elsevier Science Ltd. All rights reserved.
Lipoproteins


BACKGROUND: It is critical to develop new metrics to determine whether HDL is cardioprotective in humans. One promising approach is HDL particle concentration (HDL-P), the size and concentration of HDL in plasma. However, the 2 methods currently used to determine HDL-P yield concentrations that differ >5-fold. We therefore developed and validated an improved approach to quantify HDL-P, termed calibrated ion mobility analysis (calibrated IMA).

METHODS: HDL was isolated from plasma by ultracentrifugation, introduced into the gas phase with electrospray ionization, separated by size, and quantified by particle counting. We used a calibration curve constructed with purified proteins to correct for the ionization efficiency of HDL particles.

RESULTS: The concentrations of gold nanoparticles and reconstituted HDLs measured by calibrated IMA were indistinguishable from concentrations determined by orthogonal methods. In plasma of control (n = 40) and cerebrovascular disease (n = 40) participants, 3 subspecies of HDL were reproducibility measured, with an estimated total HDL-P of 13.4 (2.4) μmol/L. HDL-C accounted for 48% of the variance in HDL-P. HDL-P was significantly lower in participants with cerebrovascular disease (P = 0.002), and this difference remained significant after adjustment for HDL cholesterol concentrations (P = 0.02).

CONCLUSIONS: Calibrated IMA accurately determined the concentration of gold nanoparticles and synthetic HDL, strongly suggesting that the method could accurately quantify HDL particle concentration. The estimated stoichiometry of apolipoprotein A-I determined by calibrated IMA was 3–4 per HDL particle, in agreement with current structural models. Furthermore, HDL-P was associated with cardiovascular disease status in a clinical population independently of HDL cholesterol.


OBJECTIVE—whereas epidemiologic studies show that levels of low-density-lipoprotein cholesterol (LDL-C) and high-density-lipoprotein cholesterol (HDL-C) predict incident cardiovascular disease (CVD), there is limited evidence relating lipoprotein subfractions and composite measures of subfractions to risk for CVD in prospective cohort studies.

METHODS AND RESULTS—we tested whether combinations of lipoprotein subfractions independently predict CVD in a prospective cohort of 4,594 initially healthy men and women (the Malmö Diet and Cancer Study, mean follow-up 12.2 years, 377 incident cardiovascular events). Plasma lipoproteins and lipoprotein subfractions were measured at baseline with a novel, high resolution ion mobility technique. Principal component analysis (PCA) of subfraction concentrations identified three major independent (i.e., zero correlation) components of CVD risk, one representing LDL-associated risk, a second representing HDL-associated protection, and the third representing a pattern of increased large HDL, increased small/medium LDL, and increased triglycerides. The last corresponds to the previously described “atherogenic lipoprotein phenotype.” Several genes that may underlie this phenotype—CETP, LIPC, GALNT2, MLXIPL, APOA1/A5, LPL—are suggested by SNPs associated with the combination of small/medium LDL and large HDL.


BACKGROUND: Current methods for measuring the concentrations of lipoprotein particles and their distributions in particle subpopulations are not standardized. We describe here and validate a new gas-phase differential electrophoretic macromolecular mobility-based method (ion mobility, or IM) for direct quantification of lipoprotein particles, from small, dense HDL to large, buoyant, very-low-density lipoprotein (VLDL).

METHODS: After an ultracentrifugation step to remove albumin, we determined the size and concentrations of lipoprotein particles in serum samples using IM. Scan time is 2 min and covers a particle range of 17.2-540.0 Å. After scanning, data are pooled by introducing HDL into the gas phase with electrospray ionization, separated by size, and quantified by particle counting. IM results were correlated with those of standard methods for cholesterol and apolipoprotein analysis.

RESULTS: Intra- and interassay coefficients of variation for LDL particle size were <10%. The intra- and interassay variation for LDL and HDL particle subfraction measurements was <20%. IM-measured non-HDL correlated well with apolipoprotein B (r = 0.92).

CONCLUSIONS: The IM method provides accurate, reproducible, direct determination of size and concentration for a broad range of lipoprotein particles. Use of this methodology in studies of patients with cardiovascular disease and other pathologic states will permit testing of its clinical utility for risk assessment and management of these conditions.
Self-assembly of purified apolipoproteins and phospholipids results in the formation of nanometer-sized lipoprotein complexes, referred to as nanolipoprotein particles (NLPs). These bilayer constructs are fully soluble in aqueous environments and hold great promise as a model system to aid in solubilizing membrane proteins. Size variability in the self-assembly process has been recognized for some time, yet limited studies have been conducted to examine this phenomenon. Understanding the source of this heterogeneity may lead to methods to mitigate heterogeneity or to control NLP size, which may be important for tailoring NLPs for specific membrane proteins. Here, we have used atomic force microscopy, ion mobility spectrometry, and transmission electron microscopy to quantify NLP size distributions on the single-particle scale, specifically focusing on assemblies with 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and a recombinant apolipoprotein E variant containing the N-terminal 22 kDa fragment (E422k). Four discrete sizes of E422k/DMPC NLPs were identified by all three techniques, with diameters centered at ∼14.5, 19, 23.5, and 28 nm. Computer simulations suggest that these sizes are related to the structure and number of E422k lipoproteins surrounding the NLPs and particles with an odd number of lipoproteins are consistent with the double-belt model, in which at least one lipoprotein adopts a hairpin structure.

Nanoparticles


The successful application of differential mobility analysis for the characterization and manipulation of nanoparticles at atmospheric pressure has given rise to further development of this technique. The parallel differential mobility analyzer provides the possibility to simultaneously measure a size spectrum of nanoparticles and select a particular set of nanoparticles with a defined size for collection (as well as enrichment) and further orthogonal analysis (as for example electron microscopy, atomic force microscopy or mass spectrometry). Performing a high resolution measurement of electrical mobility diameters allows molecular weight determination of species with ultrahigh molecular masses in the mega Dalton range (e.g. protein complexes). The precise size measurement of the human rhinovirus has confirmed the potential of this technique to analyze even intact infectious human-pathogenic viruses. Moreover, the real-time measurement of nanoparticle occurrence in an urban environment confirms the versatility of the method presented here and its applicability also in other areas of importance. ©2012 Chinese Society of Particuology and Institute of Process Engineering, Chinese Academy of Sciences. Published by Elsevier B.V. All rights reserved.


Biodegradable nanoparticles (NPs) and hence, for example, NPs prepared from glutaraldehyde cross-linked gelatin (gelatin NPs) are lately receiving increased attention in various fields such as pharmaceutical technology and nutraceutics as biocompatible carriers for hardly water soluble drugs, molecules intended for sustained release or targeted transport. However, in vivo application of such materials requires a thoroughly characterization of corresponding particles. In a previous manuscript, we demonstrated the applicability of chip electrophoresis for the separation of gelatin NPs from NP building blocks. Following our previous results, we intensified our efforts in the characterization of gelatin NPs by electrophoresis in the liquid (capillary and chip format) and the gas phase (gas phase electrophoretic mobility molecular analysis). In doing so, we demonstrated differences between nominally comparable (from the concentration of initially employed material for cross-linking) gelatin NP preparation batches concerning (i) the amount of obtained NPs, (ii) the degree of NP cross-linking, (iii) the amount of NP building blocks present within samples, and (iv) the electrophoretic mobility diameter of NPs. Differences were even more pronounced when NP preparations from batches with different content of initially employed gelatin were compared. Additionally, we compared three setups for the removal of low molecular weight components from samples after fluorescence labeling of NPs. In overall, the combination of the three employed analytical methods for gelatin NP characterization—CE in the capillary and the chip format as well as gas phase electrophoretic mobility molecular analysis—allows a more thoroughly characterization of NP containing samples.
Gold nanoparticles (GNPs) are popular colloidal substrates in various sensor, imaging, and nanomedicine applications. In separation science, they have raised some interest as a support for sample preparation. Reasons for their popularity are their low cost, ability for size-controlled synthesis with well-defined narrow nanoparticle size distributions, as well as straightforward surface functionalization by self-assembling (thiol-containing) molecules on the surface, which allows flexible introduction of functionalities for the selective capture of analytes. Most commonly, the method of first choice for size determination is dynamic light scattering (DLS). However, DLS has some serious shortcomings, and results from DLS may be misleading. For this reason, in this contribution several distinct complementary nanoparticle sizing methodologies were utilized and compared to characterize citrate-capped GNPs of different diameters in the range of 13–26 nm. Weaknesses and strengths of DLS, transmission electron microscopy, asymmetrical-flow field-flow fractionation and nanoelectrospray gas-phase electrophoretic mobility molecular analysis are discussed and the results comparatively assessed. Furthermore, the distinct GNP's were characterized by measuring their zeta-potential and surface plasmon resonance spectra. Overall, the combination of methods for GNP characterization gives a more realistic and comprehensive picture of their real physicochemical properties, (hydrodynamic) diameter, and size distribution.


We report a high-resolution and traceable method to quantify the drug loading on nanoparticle-based cancer therapeutics, and demonstrate this method using a model cisplatin functionalized dendron-gold nanoparticle (AuNP) conjugate. Electrospray differential mobility analysis (ES-DMA) provides upstream size classification based on the electrical mobility of AuNP conjugates in aerosol form following electrospray conversion from the aqueous suspension. A condensation particle counter (CPC) and inductively coupled mass spectrometer (ICP-MS) provide the principal downstream quantification. CPC and ICP-MS yield complementary number-based and elemental mass-based particle size distributions, respectively. Conjugation using three different dendron formulations was differentiated based on changes in the mean mobility particle size. The subsequent cisplatin complexation to the dendron conjugates was quantified by coupling ES-DMA with ICP-MS. Discrete AuNP clusters (e.g., dimers, trimers) could be resolved from the relative quantity of atoms (i.e., Au and Pt) per particle after separation by ES-DMA. Surface density of cisplatin on Au was shown to be proportional to the density of carboxylic groups present and was independent of the state of AuNP clustering. Additionally, we found that colloidal stability of the conjugate is inversely proportional to the surface loading of cisplatin. This study demonstrates a prototype methodology to provide traceable quantification and to determine other important formulation factors relevant to therapeutic performance.


We report the development of a hyphenated instrument with the capacity to quantitatively characterize aqueous suspended gold nanoparticles (AuNPs) based on a combination of gas-phase size separation, particle counting, and elemental analysis. A customized electrospray-differential mobility analyzer (ES-DMA) was used to achieve real-time upstream size discrimination. A condensation particle counter and inductively coupled plasma mass spectrometer (ICP-MS) were employed as downstream detectors, providing information on number density and elemental composition, respectively, of aerosolized AuNPs versus the upstream size selected by ES-DMA. A gas-exchange device was designed and optimized to improve the conversion of air flow (from the electrospray) to argon flow required to sustain the ICP-MS plasma, the key compatibility issue for instrumental hyphenation. Our work provides the proof of concept and a working prototype for utilizing this construct to successfully measure (1) number- and mass-based distributions; (2) elemental compositions of nanoparticles classified by size, where the size classification and elemental analysis are performed within a single experiment; (3) particle concentrations in both solution (before size discrimination) and aerosol (after size discrimination) phases; and (4) the number of atoms per nanoparticle or the nanoparticle density.


The combined use of nanoelectrospray ion mobility spectrometry (IMS) with off-line inductively coupled plasma mass spectrometry (ICP-MS) for determining engineered nanoparticles (NPs) in aqueous solution is demonstrated here for the first time. The resulting analytical technique exhibits capabilities for determining the size and metal content of NPs and can also in principle be used for determining NP concentration. The resolving power exhibited by IMS along with the high sensitivity offered by single particle mode ICP-MS are the main features that make this combination attractive for analysing NPs.
We report on a systematic investigation of molecular conjugation of tumor necrosis factor-alpha (TNF) protein onto gold nanoparticles (AuNPs) and the subsequent binding behavior to its antibody (anti-TNF). We employ a combination of physical and spectroscopic characterization methods, including electrospray-differential mobility analysis, dynamic light scattering, polyacrylamide gel electrophoresis, attenuated total reflectance-Fourier transform infrared spectroscopy, fluorescence assay, and enzyme-linked immunosorbent assay. The native TNF used in this study exists in the active homotrimer configuration prior to conjugation. After binding to AuNPs, the maximum surface density of TNF is (0.09 ± 0.02) nm⁻² with a binding constant of 3 x 10⁶ (mol L⁻¹)⁻¹. Dodecyl sulfate ions induce desorption of monomeric TNF from the AuNP surface, indicating a relatively weak intermolecular binding within the AuNP-bound TNF trimers. Anti-TNF binds to both TNF-conjugated and citrate-stabilized AuNPs, showing that non-specific binding is significant. Based on the number of anti-TNF molecules adsorbed, a substantially higher binding affinity was observed for the TNF-conjugated surface. The inclusion of thiolated polyethylene glycol (SH-PEG) on the AuNPs inhibits the binding of anti-TNF, and the amount of inhibition is related to the number ratio of surface bound SH-PEG to TNF and the way in which the ligands are introduced. This study highlights the challenges in quantitatively characterizing complex hybrid nanoscale conjugates, and provides insight on TNF-AuNP formation and activity.

The adsorption and conformation of bovine serum albumin (BSA) on gold nanoparticles (AuNPs) were interrogated both qualitatively and quantitatively via complementary physicochemical characterization methods. Dynamic light scattering (DLS), asymmetric-flow field flow fractionation (AFFF), fluorescence spectrometry, and attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy were combined to characterize BSA-AuNP conjugates under fluid conditions, while conjugates in the aerosol state were characterized by electrospray-differential mobility analysis (ES-DMA). The presence of unbound BSA molecules interferes with DLS analysis of the conjugates, particularly as the AuNP size decreases (i.e., below 30 nm in diameter). Under conditions where the gamma value is high, where gamma is defined as the ratio of scattering intensity by AuNPs to the scattering intensity by unbound BSA, DLS size results are consistent with results obtained after fractionation by AFFF. Additionally, the AuNP hydrodynamic size exhibits a greater proportional increase due to BSA conjugation at pH values below 2.5 compared with less acidic pH values (3.4-7.3), corresponding with the reversibly denatured (E or F form) conformation of BSA below pH 2.5. Over the pH range from 3.4 to 7.3, the hydrodynamic size of the conjugate is nearly constant, suggesting conformational stability over this range. Because of the difference in the measurement environment, a larger increase of AuNP size is observed following BSA conjugation when measured in the wet state (i.e., by DLS and AFFF) compared to the dry state (by ES-DMA). Molecular surface density for BSA is estimated based on ES-DMA and fluorescence measurements. Results from the two techniques are consistent and similar, but slightly higher for ES-DMA, with an average adsorbate density of 0.015 nm⁻². Moreover, from the change of particle size, we determine the extent of adsorption for BSA on AuNPs using DLS and ES-DMA at 21 degrees C, which show that increasing the concentration of BSA increases the measured change in AuNP size. Using ES-DMA, we observe that the BSA surface density reaches 90% of saturation at a solution phase concentration between 10 and 30 mu mol/L, which is roughly consistent with fluorescence and AM-FTIR results. The equilibrium binding constant for BSA on AuNPs is calculated by applying the Langmuir equation, with resulting values ranging from 0.51 x 10⁶ to 1.65 x 10⁶ L/mmol, suggesting a strong affinity due to bonding between the single free exterior thiol on N-form BSA (associated with a cysteine residue) and the AuNP surface. Moreover, the adsorption interaction induces a conformational change in BSA secondary structure, resulting in less a-helix content and more open structures (beta-sheet, random, or expanded).

The slow aggregation of monodisperse, polydisperse, and preaggregated silica nanoparticles was studied with an electrosprayscanning mobility particle sizer (ES-SMPS) and time-resolved synchrotron radiation -small-angle X-ray scattering (SR-SAXS). Aggregation was induced by varying the NaCl concentration to obtain a fixed gelation time of ~40 min. The combination of these techniques provides a unique tool to monitor and resolve the aggregate development in detail. The monodisperse spherical particles were converted to dimers, trimers, and eventually larger clusters as the aggregation proceeded, while the polydisperse spherical particles formed large clusters at an early stage. The initial particle shape and polydispersity had profound effects on the morphology of the aggregates; spherical primary particles produced compact spherical clusters, whereas the preaggregated dispersions formed open, elongated aggregates. All dispersions produced gels that contained free primary particles well past the point of gelation. The stability of the aggregates and the gel morphology were interpreted by relating to the structure of porous gel layers around the particles.

Nanoparticles and nanostructured aggregates of paratacamite are prepared in acidic solutions through the conversion of copper-based nanoparticles. Aged and oxidized copper nanoparticles with an average primary particle size of ~15 nm, when combined with hydrochloric acid solutions in the range of 0.025 to 0.1 M, show interesting behavior yielding both a change in nanoparticle primary size, as measured by an electrospray scanning mobility particle sizer, and in chemical composition to produce a copper chloride hydroxide mineral identified as paratacamite (y-Cu₂(OH)₂Cl) by powder X-ray diffraction of the dehydrated solid sample. Taken together, these data suggest that paratacamite nanoparticles in solution can aggregate to yield microporous paratacamite materials. Microporous paratacamite was characterized by several techniques including X-ray diffraction, transmission electron microscopy, energy dispersive X-ray analysis, electron energy loss spectroscopy, X-ray photoelectron spectroscopy and surface area measurements. Oxidation of these copper-based nanoparticles with molecular oxygen and the role of the oxidized layer in the formation of paratacamite have been investigated. Comparison to microscale copper particles showed there is unique oxidation behavior of nanoscale copper particles that results in unique reaction chemistry of oxidized nanoscale copper particles with hydrochloric acid solutions to form paratacamite. This study provides a new route for the formation of paratacamite nanomaterials that can be used in a wide range of chemically interesting applications including hydrogen storage materials and as a heterogeneous catalyst for the synthesis of green solvents such as dimethyl and diethyl carbonates. Additionally, this study suggests a potentially new pathway for the degradation of art objects and ancient artifacts as well as other cultural heritage materials containing small copper particles that has not been previously considered.


We demonstrate a high-resolution in situ experimental method for performing simultaneous size classification and characterization of functional gold nanoparticle clusters (GNCs) based on asymmetric flow field flow fractionation (AFFF). Field emission scanning electron microscopy, atomic force microscopy, multi-angle light scattering (MALS), and in situ ultraviolet-visible optical spectroscopy provide complementary data and imagery confirming the cluster state (e.g., dimer, trimer, tetramer), packing structure, and purity of fractionated populations. An orthogonal analysis of GNC size distributions is obtained using electrospray-differential mobility analysis (ES-DMA). We find a linear correlation between the normalized MALCS intensity (measured during AFFF elution) and the corresponding number concentration (measured by ES-DMA), establishing the capacity for AFFF to quantify the absolute number concentration of GNCs. The results and corresponding methodology summarized here provide the proof of concept for general applications involving the formation, isolation, and in situ analysis of both functional and adventitious nanoparticle clusters of finite size.


Here we investigate the parameters that govern the yield and selectivity of small clusters composed of nanoparticles using a Monte Carlo simulation that accounts for spatial and dimensional distributions in droplet and nanoparticle density and size. Clustering nanoparticles presents a powerful paradigm with which to access properties not otherwise available using individual molecules, individual nanoparticles or bulk materials. However, the governing parameters that precisely tune the yield and selectivity of clusters fabricated via an electrospray droplet evaporation method followed by purification with differential mobility analysis (DMA) remain poorly understood. We find that the product of the electrospray droplet mean diameter to the third power and nanoparticle concentration governs the yield of individual clusters, while the ratio of the nanoparticle standard deviation to the mean diameter governs the selectivity. The resulting, easily accessible correlations may be used to minimize undesirable clustering, such as protein aggregation in the biopharmaceutical industry, and maximize the yield of a particular type of cluster for nanotechnology and energy applications.


The electrophoretic mobility of charged, airborne nanoparticles (NPs) or macromolecules and their specific complexes opens new avenues for their analysis and handling. The newly developed parallel differential mobility analyzer in combination with an electrospray particle sampler enables not only the characterization of bio-NPs, but even their sampling while preserving their bioactivity (e.g., the enzyme activity of galactosidase). Precondition for the applicability of this technique is a well-defined charging status of the NPs in question. This charge conditioning can be achieved by means of a radioactive source, Po-210, even if the yield in terms of charged particles is low for sub-20-nm particles and the aging of the source influences the size spectra measured. Nevertheless, this technique enables size-defined sampling and enrichment, combined with real-time measurement of the size of both NPs and viruses. Furthermore, it allows determination of the number of attached biospecific antibodies, thereby providing information about the surface coverage of viruses by antibodies. © 2010 Elsevier Ltd. All rights reserved.
Surface-sensitive quantitative studies of competitive molecular adsorption on nanoparticles were conducted using a modified attenuated total reflectance–Fourier transform infrared (ATR-FTIR) spectroscopy method. Adsorption isotherms for thiolated poly(ethylene glycol) [(SH-PEG) on gold nanoparticles (AuNPs) as a function of molecular mass (1.5, and 20 kDa) were characterized. We find that surface density of SH-PEG on AuNPs is inversely proportional to the molecular mass (\( M_m \)). Equilibrium binding constants for SH-PEG, obtained using the Langmuir adsorption model, show the binding affinity for SH-PEG is proportional to \( M_m \). Simultaneous competitive adsorption between mercaptopropionic acid (MPA) and 5 kDa SH-PEG (SH-PEG5K) was investigated, and we find that MPA concentration is the dominant factor influencing the surface density of both SH-PEG5K and MPA, whereas the concentration of SH-PEG5K affects only SH-PEG5K surface density. Electrospay differential mobility analysis (ES-DMA) was employed as an orthogonal characterization technique. ES-DMA results are consistent with the results obtained by ATR-FTIR, confirming our conclusions about the adsorption process in this system. Ligand displacement competitive adsorption, where the displacing molecular species is added after completion of the ligand surface binding, was also interrogated by ATR-FTIR. Results indicate that for SH-PEG increasing \( M_m \) yields greater stability on AuNPs when measured against displacement by bovine serum albumin (BSA) as a model serum protein. In addition, the binding affinity of BSA to AuNPs is inhibited for SH-PEG conjugated AuNPs, an effect that is enhanced at higher SH-PEG \( M_m \) values.

Nanoparticles of controlled size, well defined shape, pure phase and of clean surfaces are ideal model systems to investigate surface/interfacial reactions. In this study we have explored the possibility of synthesizing TiO\(_2\) nanoparticles in the size range of 7-20 nm under well controlled experimental conditions. A simple method based on the hydrolysis of TiCl\(_4\) was used to obtain particles having surfaces free from organics. Stable dispersions of TiO\(_2\) nanoparticles of various sizes were obtained by optimizing the reaction/dialysis time and temperature. The synthesized TiO\(_2\) particles were found to be predominantly of anatase phase and narrow particle size distributions were obtained. The TiO\(_2\) particles were characterized with respect to their phase, size and shape by X-ray diffraction (XRD) and transmission electron microscopy (TEM), respectively. Particle size distribution in a colloidal dispersion was obtained by the electrospay scanning mobility particle sizer (ES-SMPS) method and compared with an average particle size determined from dynamic light scattering (DLS). The average particle sizes obtained by the DLS and ES-SMPS methods were in good agreement, while a primary particle size of ~4 nm was found in X-ray diffraction irrespective of the particle size in solution. Early stages of the nucleation process were monitored by the ES-SMPS method. These results show that small particles of 4–5 nm are initially formed and it is highly likely that large particles are formed due to aggregation of primary particles.

More information characterizing the biological responses to nanoparticles is needed to allow the U.S. Food and Drug Administration to evaluate the safety and effectiveness of products with nano-scale components. The potential cytotoxicity and inflammatory responses of Au NPs (60 nm, NIST standard reference materials) were investigated in murine macrophages. Cytotoxicity was evaluated by MTT and LDH assays. Cytokines (IL-6, TNF-\( \alpha \)), nitric oxide, and ROS were assayed to assess inflammatory responses. Morphological appearance and localization of particles were examined by high resolution illumination microscopy, transmission electron microscopy (TEM), and scanning TEM coupled with EDX spectroscopy. Results showed no cytotoxicity and no elevated production of proinflammatory mediators; however, imaging analyses demonstrated cellular uptake of Au NPs and localization within intracellular vacuoles. These results suggest that 60 nm Au NPs, under the exposure conditions tested, are not cytotoxic, nor elicit pro-inflammatory responses. The localization of Au NPs in intracellular vacuoles suggests endosomal containment and an uptake mechanism involving endocytosis.

The increasing use of manufactured nanoparticles ensures these materials will make their way into the environment. Silver nanoparticles in particular, due to use in a wide range of applications, have the potential to get into water systems, e.g., drinking water systems, ground water systems, estuaries, and/or lakes. One important question is what is the chemical and physical state of these nanoparticles in water? Are they present as isolated particles, agglomerates or dissolved ions, as this will dictate their fate and transport. Furthermore, does the chemical and physical state of the nanoparticles change as a function of size or differ from micron-sized particles of similar composition? In this study, an electrospray atomizer coupled to a scanning mobility particle sizer (ES-SMPS) is used to investigate the state of silver nanoparticles in water and aqueous nitric acid environments. Over the range of pH values investigated, 0.5 to 6.5, silver nanoparticles with a bimodal primary particle size distribution with the most intense peak at 5.0 ± 7.4 nm, as determined from transmission electron microscopy (TEM), show distinct size distributions indicating agglomeration between pH 6.5 and 3 and isolated nanoparticles at pH values from 2.5 to 1. At the lowest pH investigated, pH 0.5, there are no peaks detected by the SMPS, indicating complete nanoparticle dissolution. Further analysis of the solution shows dissolved Ag ions at a pH of 0.5. Interestingly, silver nanoparticle dissolution shows size dependent behavior as larger, micron-sized silver particles show no dissolution at this pH. Environmental implications of these results are discussed.


This document describes a protocol for size analysis of citrate-stabilized gold nanoparticles using electrospray differential mobility analysis (ES-DMA). The procedures and parameters as defined in this protocol are appropriate for particles in the range from 5 nm to about 400 nm. Nanoparticles are centrifuged to remove excess citrate stabilizer and are re-suspended in ammonium acetate solution for ES-DMA analysis. A number average diameter is calculated from particle mobility measurements. NIST reference materials 8011, 8012 and 8013 (nominally 10 nm, 30 nm and 60 nm, respectively) were used to develop and demonstrate the assay; modification of protocol parameters may be necessary to optimize the methodology for specific gold nanoparticle formulations other than the reference materials used here. The assay requires 900 μL of nanoparticle solution of the following concentrations: ~10 nm particles at [~5 x 10^{12} particles/mL], ~30 nm particles at [~2 x 10^{11} particles/mL], and ~60 nm particles at [~3 x 10^{10} particles/mL]. In any case, the particle number concentration of the test sample should not exceed ~2 x 10^{13} particles/mL for citrate-stabilized gold.


Competitive adsorption kinetics between thiolated polyethylene glycol (SH-PEG) and mercaptopropionic acid (MPA) on gold nanoparticles (Au-NPs) were studied using a prototype physical characterization approach that combines dynamic light scattering (DLS) and electrospray differential mobility analysis (ES-DMA). The change in hydrodynamic particle size (intensity average) due to the formation of SH-PEG coatings on Au-NPs was measured by DLS in both two-component (Au-NP + MPA or Au-NP + SH-PEG) and three-component (Au-NP + MPA + SH-PEG) systems. ES-DMA was employed to quantify the surface coverage of SH-PEG and establish a correlation between surface coverage and the change in particle size measured by DLS. A change in the equilibrium binding constant for SH-PEG on Au-NPs at various concentrations of SH-PEG and MPA showed that the presence of MPA reduced the binding affinity of SH-PEG to the Au-NP surface. Kinetic studies showed that SH-PEG was desorbed from the Au-NP surface following a second-order desorption model after subsequently introducing MPA. The desorption rate constant of SH-PEG from the Au-NP surface by MPA displacement was strongly affected by the concentration of MPA and the excess SH-PEG in solution.
An aerosolization technique has been developed to measure liquid-borne nanoparticles down to 30 nm and applied to evaluate retention efficiencies of liquid filters. This technique involves dispersing nanoparticle suspensions into air-borne form and measuring the size and concentration by a differential mobility analyzer coupled to a condensation particle counter. Polystyrene latex particles larger and smaller than 70 nm in diameter were dispersed by a constant output atomizer, COA, and an electrospray aerosol generator, ES, respectively, to avoid the interference from residue particles. With the ES, residue particles can be controlled to be less than 10 nm, allowing latex particles as small as 30 nm to be clearly distinguished from the size distribution measurements. Calibrations with 30, 50, 125, and 200 nm latex particles showed that liquid-borne and air-borne particle concentrations are proportionally related. This provides an effective way to quantify liquid-borne particles as small as 30 nm, which cannot be analyzed by state-of-the-art liquid particle counters. An application of this technique is to evaluate the nanoparticle retention performance of liquid filters. Both 200 and 400 nm rated Nuclepore filters were challenged with latex particles of different sizes, and retention efficiency as a function of particle size was determined by comparing the particle concentrations upstream and downstream of the tested filters. The results are comparable with the nominal pore size stated by the manufacturer if sieving is the dominant filtration mechanism and demonstrate the feasibility of using the aerosolization technique to evaluate the retention efficiency of filters against nanoparticles in liquids.

In this study, the dissolution of copper nanoparticles in aqueous low-pH suspensions is examined. The dissolution phenomenon is examined using both bulk measurements of copper ion production, as detected by inductively coupled plasma-optical emission spectroscopy (ICP/OES), and a decrease in nanoparticle size using particle-sizing instruments. For size measurements, an electrospray atomizer coupled to a scanning mobility particle sizer (ES-SMPS) was used to monitor changes in the particle size distribution (PSD) of the copper nanoparticles as they dissolved in hydrochloric acid solution in real time. Measured PSDs show interesting changes during the dissolution process, including a change in modality (mono to multi) with time. Although there may be several causes for the observed modality changes upon dissolution, it is clear that only through direct measurements of nanoparticles and nanoparticle PSDs can these dynamic details be captured as these particles change size, thus providing important insights into nanoscale processes.

Here we demonstrate a rapid and quantitative means to characterize the size and packing structure of small clusters of nanoparticles in colloidal suspension. Clustering and aggregation play importantroles in a wide variety of phenomena of both scientific and technical importance, yet characterizing the packing of nanoparticles within small clusters and predicting their aerodynamic size remains challenging because available techniques lack adequate resolution and sensitivity for clusters smaller than 100 nm (optical techniques), perturb the packing arrangement (electron microscopies), or provide only an ensemble average (light scattering techniques). In this article, we use electrospray-differential mobility analysis (ES-DMA), a technique that exerts electrical and drag forces on the clusters, to determine the size and packing of small clusters. We provide an analytical model to determine the mobility size of various packing geometries based on the projected area of the clusters. Data for clusters aggregated from nominally 10 nm gold particles and nonenveloped viruses of various sizes show good agreement between measured and predicted cluster sizes for close-packed spheres.

Nanoparticles, the building blocks of many engineered nanomaterials, can make their way into the environment or into organisms, either accidentally or purposefully. The intent of this study is to provide some insight into the complex environmental, health, and safety issues associated with engineered nanomaterials. In particular, here the state of commercially manufactured silver nanoparticles—i.e., will silver nanoparticles be present as isolated particles, agglomerates, or dissolved ions—in two simulated biological media is explored. Two different commercially manufactured silver nanoparticle samples, one that has been surface modified with a thick polymer coating to render them more water-soluble and the other, with a sub-nanometer surface layer, are studied. The experimental results and the extended DLVO model calculations show that silver nanoparticles have a propensity to settle out in high ionic strength media independent of surface modification. Furthermore, single nanoparticles as well as aggregates/agglomerates are present together in these solutions. Silver ion release in these simulated biological buffers with pHs of 4.5 and 7.4 is negligible after 96 h.
Quantitative techniques are essential to analyze the structure of soft multicomponent nanobioclusters. Here, we combine electrospray differential mobility analysis (ES-DMA), which rapidly measures the size of the entire cluster, with transmission electron microscopy (TEM), which detects the hard components, to determine the presence and composition of the softer components. Coupling analysis of TEM and ES-DMA derived data requires the creation and use of analytical models to determine the size and number of constituents in nanoparticle complexes from the difference between the two measurements. Previous ES-DMA analyses have been limited to clusters of identical spherical particles. Here, we dramatically extend the ES-DMA analysis framework to accommodate more challenging geometries, including protein corona-coated nanorods, clusters composed of heterogeneously sized nanospheres, and nanobioclusters composed of both nanospheres and nanorods. The latter is critical to determining the number of quantum dots attached to lambda (λ) phage, a key element of a rapid method to detect bacterial pathogens in environmental and clinical samples.


We demonstrate the utility of electrospray gas-phase ion-mobility analysis as a new method to investigate nanoparticle flocculation, or aggregation. An nanoparticle (Au-NP) solutions were sampled via electrospray (ES), followed by differential ion-mobility analysis (DMA) to determine the particle mobility distribution. Multimodal size distributions obtained with ES-DMA indicated the presence of single Au-NPs (monomer) as well as larger Au-NP clusters such as dimers, trimers, and tetratomers under specific solution conditions. The fraction of each aggregate species as a function of time was quantitatively characterized, from which the degree of aggregation, aggregation rate, and stability ratio at different ionic strengths were determined. The latter enabled the extraction of a surface potential (or surface charge density) of 64 ± 2 mV for 10 nm Au-NPs, which is in agreement with values obtained from other methods, thus validating our approach. Our results show that ES-DMA is a valuable tool for quantitatively probing the early stages of colloidal aggregation or as a preparatory tool for the size election of aggregates.


A charge reduction (CR) interface for electrospray ionization that permits simultaneous analysis of nanoscale solutions by multiple detection methods was characterized. In the direct infusion configuration, a constant flow of analyte solution undergoes electrospray ionization (ESI). The charged aerosol is sampled directly into the atmospheric pressure inlet of a quadrupole time-of-flight mass spectrometer and into a CR device followed by a differential mobility analyzer (DMA) and condensation particle counter (CPC). In the plug injection configuration, analyte solution is injected into a liquid chromatograph. The effluent is split to an evaporative light scattering detector (ELSD) and the ESI interface. The charged aerosol is then sampled through the CR device directly into the CPC. Performance characteristics of the two configurations were studied with sucrose and protein solutions. When a liquid flow rate in the low microliter per minute range was used, the reconstructed droplet size distribution from the ESI interface had an average diameter of 184 ± 2 mV with a geometric standard deviation of 1.4. For the first configuration, the linear working range was wider for ESI-MS than the CR device-DMA-CPC. For the second configuration, the detection efficiency, defined as the fraction of molecules flowing through the ESI interface that are ultimately detected by the CPC, was on the order of 10⁻⁶. Simultaneous measurements with the ELSD and CPC were consistent with analyte molecular size and may provide a means of estimating the size of unknown particles.


The first characterization of the length distribution of single-walled carbon nanotubes (SWCNT) dispersed in a liquid by electrospray differential mobility analysis (ES-DMA) is presented. Although an understanding of geometric properties of SWCNTs, including length, diameter, aspect ratio, and chirality, is essential for commercial applications, rapid characterization of nanotube length distributions remains challenging. Here the use of ES-DMA to obtain length distributions of DNA-wrapped SWCNTs dispersed in aqueous solutions is demonstrated. Lengths measured by ES-DMA compare favorably with those obtained from multilange light scattering, dynamic light scattering, field flow fractionation with UV/vis detection, and atomic force microscopy, validating ES-DMA as a technique to measure SWCNTs of <250 nm in length. The nanotubes are previously purified and dispersed by wrapping with oligomeric DNA in aqueous solution and centrifuging to remove bundles and amorphous carbon. These dispersions are particularly attractive due to their amenability to bulk processing, ease of storage, high concentration, compatibility with biological and high-throughput manufacturing environments, and for their potential applications ranging from electronics and hydrogen-storage vessels to anticancer agents.
Airborne single-wall carbon nanotubes (SWCNTs) have a high tendency to agglomerate due to strong interparticle attractive forces. The SWCNT agglomerates generally have complex morphologies with an intricate network of bundles of nanotubes and nanoropes, which limits their usefulness in many applications. It is thus desirable to produce SWCNT aerosol particles that have well-defined, unagglomerated fibrous morphologies. We present a method to generate unagglomerated, fibrous particles of SWCNT aerosols using capillary electrospray of aqueous suspensions. The effects of the operating parameters of capillary electrospray such as strength of buffer solution, capillary diameter, flow rate, and colloidal particle concentration on the size distributions of SWCNT aerosols were investigated. Results showed that electrospray from a suspension of higher nanotube concentration produced a bimodal distribution of SWCNT aerosols. Monodisperse SWCNT aerosols below 100 nm were mostly non-agglomerated single fibers, while polydisperse aerosols larger than 100 nm had two distinct morphologies: a ribbon shape and the long, straight fiber. Possible mechanisms are suggested to explain the formation of the different shapes, which could be used to produce SWCNT aerosols with different morphologies.


Magnetic nanoparticles have been proposed for use as biomedical purposes to a large extent for several years. The development of techniques that could selectively deliver drug molecules to the diseased site, without a concurrent increase in its level in healthy tissues, is currently one of the most active areas of cancer research. The conjugate carboxymethyl starch (CMS)/SPIO nanoparticles were prepared by chemical reaction. Several parameters including the drug/polymer ratios in range of 1:14 were examined to optimize formulation. The size distribution and morphology of nanoparticles and in vitro release profile in phosphate buffer medium (pH 7.4) during 12 h were then investigated. The magnetic NPs prepared in this study were spherical with a relatively mono-dispersed size distribution. The conjugate carboxymethyl starch (CMS)/SPIO nanoparticles were exhaustively studied as controlled-release systems for parenteral administration of a model drug 5-aminosalicylic acid (mesalazine) and analyzed using various release kinetic studies.


The slow aggregation process of a concentrated silica dispersion (Bindzil 40/220) in the presence of alkali chlorides (LiCl, NaCl, KCl, RbCl, and CsCl) was investigated by means of mobility measurements. At intervals during the aggregation, particles and aggregates were transferred from the liquid phase to the gas phase via electrospray (ES) and subsequently size selected and counted using a scanning mobility particle sizer (SMPS). This method enables the acquisition of particle and aggregate size distributions with a time resolution of minutes. To our knowledge, this is the first time that the method has been applied to study the process of colloidal aggregation. The obtained results indicate that, independent of the type of counterion, a sufficient dilution of the formed gel will cause the particles to redisperse. Hence, the silica particles are, at least initially, reversibly aggregated. The reversibility of the aggregation indicates additional non-DLVO repulsive steric interactions that are likely due to the presence of a gel layer at the surface. The size of the disintegrating aggregates was monitored as a function of the time after dilution. It was found that the most stable aggregates were formed by the ions that adsorb most strongly on the particle surface. This attractive effect was ascribed to an ion–ion correlation interaction.


We present results of a systematic examination of functionalized gold nanoparticles (Au-NPs) by electrospray-differential mobility analysis (ES-DMA). Commercially available, citrate-stabilized Au colloid solutions (10–60 nm) were sized using ES-DMA, from which changes in particle size of less than 0.3 nm were readily discerned. It was found that the formation of salt particles and the coating of Au-NPs by salt during the electrospray process can interfere with the mobility analysis, which required the development of sample preparation and data correction protocols to extract correct values for the Au-NP size. Formation of self-assembled monolayers (SAMs) of alkanethiol molecules on the Au-NP surface was detected from a change in particle mobility, which could be modeled to extract the surface packing density of SAMs. A gas-phase temperature-programmed desorption (TPD) kinetic study of SAMs on Au-NPs found the data to be consistent with a second-order Arrhenius-based rate law, yielding an Arrhenius factor of $1.0 \times 10^{15}$ s$^{-1}$ and an activation energy $\sim$105 kJ/mol. For the size range of SAM-modified Au-NP we considered, the effect of surface curvature on the energetics of binding of carboxylic acid terminated SAMs is evidently negligible, with binding energies determined by TPD agreeing with those reported for the same SAMs on planar surfaces. This study suggests that the ES-DMA can be added to the tool set of characterization methods used to study the structure and properties of coated nanoparticles.

In this work, the structural properties of silver nanoparticle agglomerates generated using condensation and evaporation method in an electric tube furnace followed by a coagulation process are analyzed using Transmission Electron Microscopy (TEM). Agglomerates with mobility diameters of 80, 120, and 150 nm are sampled using the electrostatic method and then imaged by TEM. The primary particle diameter of silver agglomerates was 13.8 nm with a standard deviation of 2.5 nm. We obtained the relationship between the projected area equivalent diameter ($d_{pa}$) and the mobility diameter ($d_{m}$), i.e., $d_{pa} = 0.92 \times d_{m}$ for particles from 80 to 150 nm. We obtained fractal dimensions of silver agglomerates using three different methods: (1) $D_f = 1.84 \times 0.03$, $1.75 \times 0.06$, and $1.74 \times 0.03$ for $d_{m} = 80, 120, \text{and} 150 \text{nm}$, respectively from projected TEM images using a box counting algorithm; (2) Fractal dimension ($D_f$) = 1.47 based on maximum projected length from projected TEM images using an empirical equation proposed by Koylu et al. (1995) Combust Flame 100:621-633; and (3) mass fractal-like dimension ($D_{ma}$) = 1.71 theoretically derived from the mobility analysis proposed by Lall and Friedlander (2006) Aerosol Sci 37:260-271. We also compared the number of primary particles in agglomerate and found that the number of primary particles obtained from the projected surface area using an empirical equation proposed by Koylu et al. (1995) Combust Flame 100:621-633 is larger than that from using the relationship, $d_{ma} = 0.92 \times d_{m}$ or from using the mobility analysis.


It is clear if one peruses the pages of *The Journal of Physical Chemistry* and other journals of the American Chemical Society that in the years around the beginning of the twenty first century, and in particular the year two thousand eight, there is a great deal of interest in the physical chemistry of nanoparticles. In this article, the focus is on some of the interesting and often not well understood size-dependent properties and surface chemistry of metal and metal oxide nanoparticles in gas and liquid phase environments. Challenges that remain and suggestions for future research needs are also presented at the end of this article.


Nanometre-sized particles (1-100 nm) have unique properties receiving growing attention in wide areas of research. Here, a convenient method to deposit size-selected nanoparticles on surfaces by means of electrospraying colloidal suspensions in the aerosol phase is presented. We demonstrate the deposition of individual nanoparticles and the feasibility of this method in seeding gold particles for nanostructure growth. An advantage of the present method is the easy set-up and operation, using only commercially available machinery and substances. Problems regarding low deposition rates and colloidal remnants are approached, e.g. the aerosol flow is examined in a differential mobility analyzer. This method is not material dependent and could be extended to deposit any colloidal particle.


Here we present a method to determine the surface coverage or surface density of biological molecules conjugated to nanoparticle surfaces. Electrospray-differential mobility analysis (ES-DMA) is used to determine a coating thickness by measuring the change in the size of gold nanoparticles before and after modification with thiol-derivatized single-stranded DNA. The DNA surface coverage is then obtained from the coating thickness through the use of a simple random coil model. The method requires neither fluorescent tagging nor calibration curves. We believe ES-DMA to be a broadly applicable nanometrology tool for the characterization of biologically conjugated nanoparticles.

The surface area of nanosized agglomerates is of great importance as the reactivity and health effects of such particles are highly dependent on surface area. Changes in surface area through sintering during nanoparticle synthesis processes are also of interest for precision control of synthesised particles. Unfortunately, information on particle surface area and surface area dynamics is not readily obtainable through traditional particle mobility sizing techniques. In this study, we have experimentally determined the mobility diameter of transition regime agglomerates with 3, 4, and 5 primary particles. Agglomerates were produced by spray drying well-characterised polystyrene latex particles with diameters of 55, 67, 76, and 99 nm. Tandem differential mobility analysis was used to determine agglomerate mobility diameter by selecting monodisperse agglomerates with the same number of primary particles in the first DMA, and subsequently completely sintering the agglomerates in a furnace aerosol reactor. The size distribution of the completely sintered particles was measured by an SMPS system, which allowed for the determination of the number of primary particles in the agglomerates. A simple power law regression was used to express mobility diameter as a function of primary particle size and the number of primary particles, and had an excellent correlation (R^2 = 0.9971) with the experimental data. A scaling exponent was determined from the experimental data to relate measured mobility diameter to surface area for agglomerates. Using this relationship, the sintering characteristics of agglomerates were also examined for varying furnace temperatures and residence times. The sintering data agreed well with the geometric sintering model (GSM) model proposed by Cho &amp; Biswas (2006a) as well as with the model proposed Koch &amp; Friedlander (1990) for sintering by viscous flow.


Superparamagnetic iron oxide nanoparticles coated with dextran were functionalized with negatively charged functionalities in order to connect specific peptide labels by electrostatic interactions. Peptide binding on the nanoparticles was indicated by HR-TEM an electrospray scanning mobility particle size, and fluorescence measurements.


A technique for generating charged aerosols of polystyrene (pSty) with narrow size distributions has been developed. It is based on electrospraying commercial narrow mass standards of pSty dissolved in 1-methyl-2-pyrrolidone (NMP) seeded with the newly synthesized salt dimethyl ammonium formate. This salt imparts a much larger electrical conductivity than previously known NMP electrolytes, leading to higher quality sprays with greatly reduced attachment of impurities. Controlling the solute concentration enables forming polystyrene particles containing from one up to more than ten single polystyrene molecules, whereby 4 mass standards with molecular weights from 9200 up to 96,000 g/mol yield particles covering densely the diameter range from 3 to 11 nm. Combined mobility and mass measurement with a differential mobility analyzer and a mass spectrometer in tandem are carried out with a pSty sample 9200 amu in molecular weight. They fix directly the mass versus mobility relation near 9200 amu, and indirectly for the other standards and their clusters. The apparent particle density resulting from mobility versus mass data agrees with the bulk density of the polymer, indicating that the particles are dense and spherical. Although these standards have been studied only in gaseous suspension, their injection in liquids such as water where pSty is insoluble should keep them spherical. © 2005 Elsevier Inc. All rights reserved.


The potential of the electrospray technique in analyzing the structure of nonspherical colloidal particles that are below 100 nm in volume-equivalent diameter was demonstrated by online size measurement using a differential mobility analyzer (DMA) with a condensation nucleus counter (CNC) system. The measured mobility of gold nanorods was confirmed by electron microscope images and the theoretical calculation of particle mobility using the dynamic shape factor and slip correction factor. To evaluate the mobility, rod particles were modeled as both a cylinder and a prolate spheroid. This study also showed that the organic surfactant coated on rod particles might be removed and that the rod particles became spherical upon the elevation of the ambient temperature during the gas-phase dispersion of colloidal nanoparticles. Moreover, the thickness of the surfactants coated on rod particles was estimated by comparing the theoretically and experimentally obtained mobilities.
Cluster ions and residue nanoparticles with sizes below 30 nm were generated by electrospraying (ES) and drying droplets of pure water, tap water, and aqueous solutions of salts. The mobility spectra of the cluster ions between 9.1 and $9.3 \times 10^{-5} \text{m}^2/(\text{Vs})$ were measured using a differential mobility analyzer (DMA) operated at room temperature and atmospheric pressure. A modified Faraday cup and a condensation nucleus counter were used for detection. The concentrations of total residue/contaminants in the water were determined as a function of sizes of measured aerosol particles and of the initial droplets. Method detection limits were at sub-ppb level for pure water and sub-ppm level for tap water. ES/DMA is capable of simultaneously measuring the mobility distribution of cluster ions and concentration of total residue present in water samples.

The goal of this work was to develop a simple technique for sizing colloidal particles by means of electrospray and aerosol techniques. Size distribution of different types of colloids (oxides, metals, and polymers) such as silica, gold, palladium, and polystyrene latex particles, with different nominal sizes below 100 nm was determined online. Nanometer-sized particles were dispersed into the gas phase as an aerosol via electrosprays operating in the cone-jet mode of a colloidal solution followed by a charge reduction of the sprayed droplets to unity and subsequent evaporation of the solvent. The size distribution of the generated aerosol particles was then determined by a differential mobility analyzer combined with a condensation nucleus/particle counter. For comparison, particle sizes were determined by electron microscopy (EM) using the samples which were obtained by (i) naturally dried sols and (ii) on-line deposited on a substrate during electrospraying. The proposed technique is capable of detecting the degree of dispersity of all colloid samples, and the measured values were comparable to results obtained by EM and dynamic light scattering. The results clearly show that the method described here constitutes a convenient, reliable, and rapid tool for the size determination of colloidal nanoparticles.

The goal of this work was to develop a simple technique for sizing colloidal particles by means of electrospray and aerosol techniques. Size distribution of different types of colloids (oxides, metals, and polymers) such as silica, gold, palladium, and polystyrene latex particles, with different nominal sizes below 100 nm was determined online. Nanometer-sized particles were dispersed into the gas phase as an aerosol via electrosprays operating in the cone-jet mode of a colloidal solution followed by a charge reduction of the sprayed droplets to unity and subsequent evaporation of the solvent. The size distribution of the generated aerosol particles was then determined by a differential mobility analyzer combined with a condensation nucleus/particle counter. For comparison, particle sizes were determined by electron microscopy (EM) using the samples which were obtained by (i) naturally dried sols and (ii) on-line deposited on a substrate during electrospraying. The proposed technique is capable of detecting the degree of dispersity of all colloid samples, and the measured values were comparable to results obtained by EM and dynamic light scattering. The results clearly show that the method described here constitutes a convenient, reliable, and rapid tool for the size determination of colloidal nanoparticles.

**Nucleic Acids**

Chimeric RNA/DNA oligonucleotides have been shown to promote single nucleotide exchange in genomic DNA. A chimeric molecule was designed to introduce an A to C nucleotide conversion at the Ser365 position of the rat factor IX gene. The oligonucleotides were encapsulated in positive, neutral, and negatively charged liposomes containing galactocerebroside or complexed with lactosylated polyethyleneimine. The formulations were evaluated for stability and efficiency in targeting hepatocytes via the asialoglycoprotein receptor. Physical characterization and electron microscopy revealed that the oligonucleotides were efficiently encapsulated within the liposomes, with the positive and negative formulations remaining stable for at least 1 month. Transfection efficiencies in isolated rat hepatocytes approached 100% with each of the formulations. However, the negative liposomes and 25-kDa lactosylated polyethyleneimine provided the most intense nuclear fluorescence with the fluorescein-labeled oligonucleotides. The lactosylated polyethyleneimine and the three different liposomal formulations resulted in A to C conversion efficiencies of 19–24%. In addition, lactosylated polyethyleneimine was also highly effective in transfecting plasmid DNA into isolated hepatocytes. The results suggest that both the liposomal and polyethyleneimine formulations are simple to prepare and stable and give reliable, reproducible results. They provide efficient delivery systems to hepatocytes for the introduction or repair of genetic mutations by the chimeric RNA/DNA oligonucleotides.
A scanning mobility particle sizer (SMPS) allows size separation of gas phase particles according to their electrophoretic mobilities. The addition of an electrospray source (ES) recently allowed extension of SMPS analysis to the macromolecular range. We demonstrate here the application of ES-SMPS to nucleic acids analysis. Single- and double-stranded DNA molecules ranging from 6.1 kDa (single-stranded DNA 20 nucleotides in length) to 300 kDa (500 base-pair double-stranded DNA) were separated and detected by ES-SMPS at the picomole to femtomole levels. The measured electrophoretic mobility diameters were found to correlate with the analytes’ molecular weights, while the peak areas could yield quantitative information. No fragmentation of DNA was observed under the conditions employed. Different apparent densities were observed for single-stranded and double-stranded DNAs, showing a different behavior for each type of biomolecule. The total analysis time was about 3 min/spectrum. Further optimization of ES-SMPS is expected to make it a fast and sensitive technique for biopolymer characterization.

Other Analytes

Hyaluronan (HA) is a linear non-sulfated polysaccharide mainly found in the extracellular matrix. The size of HA can vary from a few disaccharides up to at least 25,000 units, reaching molecular weights of $10 \times 10^3$ kDa. HA has many biological functions, and both its size and tissue concentration play an important role in many physiological and pathological processes. It is relatively easy to determine the HA concentration using enzyme-linked binding protein assays, but the molecular weight of HA has so far been shown to be a more challenging task to measure. Here, we present a method for size determination of HA using gas-phase electrophoretic mobility molecular analysis (GEMMA), which utilizes the electrophoretic mobility of molecules in air to estimate the molecular weight of the analyte. We show that this method gives reliable molecular weight estimations of HA in the range of 30–2400 kDa, which covers almost its whole biological range. The average measuring time for one GEMMA spectrum is between 5 and 10 min using only 6 pg of HA. In addition, the peak area in a GEMMA spectrum can be used to estimate the HA concentration in the sample. The high sensitivity and small sample volumes make GEMMA an excellent tool for both size determinations and estimation of concentration of samples with low HA concentration, as is the case for HA extracted from small tissue samples.

Polymers


One of the most prominent polymer group applied for drug conjugation is poly(ethylene) glycol (PEG). Since drug production is subjected to strict restrictions on the part of the FDA and EMEA, also PEG has to be characterized accurately. Particularly its molecular mass distribution (MMD) and polydispersity can result in unrequested inhomogeneous final products. Therefore evaluation of PEG before applying it to drug conjugation is essential. In this study a new analytical method for size and molecular mass determination based on electrophoretic mobility called GEMMA is used to characterize linear PEGs with two differing terminating functional groups. To confirm the data acquired by GEMMA a second, well-established method for molecular weight determination, MALDI-TOF MS (matrix-assisted laser desorption ionization time-of-flight mass spectrometry), was applied. Utilizing these two analytical approaches four monomethoxylated PEG-succinimidyl succinate (mPEG-SS) derivatives were investigated in terms of polydispersity and MMD. Although based on differing principles, both analytical methods yield comparable results. All obtained MMD maxima for the mPEG-SS batches lie within the company stated specifications, MMD ±10% (based on MALDI-TOF MS data). For mPEG-SS 2K a polydispersity of 1.02 and for mPEG-SS 5K, 10K and 20K a polydispersity of 1.01 were determined from GEMMA as well as from MALDI-TOF MS data and are in agreement with the company’s data (based on GPC data), namely 1.05-1.10.
In this work we present the characterization of PAMAM dendrimers from generation two (G2) up to ten (G10) with a focus on the G5–G10 dendrimers with matrix-assisted laser desorption/ionization linear mass spectrometry (MALDI–MS) and nanoelectrospray gas-phase electrophoretic mobility molecular analysis (nES–GEMMA). For the first time the molecular masses of high-mass dendrimers G8–G10 were determined by MALDI–MS and nES–GEMMA, techniques which are based on different physicochemical principles. Obtained experimental data allows the determination of the molecular mass (up to 580 kDa with a precision below ±0.9%), of the spherical size (from 3.3 to 14.0 nm with a precision of ±0.2 nm) and the calculation of their densities. Amounts in the nanogram range were sufficient for an analysis that could be performed within several minutes. The results based on these methods for high-generation dendrimers exhibited an excellent correlation and were compared with published data using techniques based on different principles.

The electrical mobility of mass-selected single poly(ethylene glycol) (PEG) chains of mass m (<14 kDa) and charge state z (+1 to +5) reveal a near-spherical shape above a critical mass m(z) approximately 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.

Aqueous solutions of poly(ethylene glycol) (PEG) in a 10 mM ammonium acetate buffer are electrosprayed, and the maximum charge state on the resulting gas-phase ions is reduced to unity using a radioactive source. The mobility distribution of these charged particles is then measured in air in a differential mobility analyzer of unusually high resolution. The relation Z(m) between the mobility Z of a polymer molecule and its mass m is determined by means of narrowly distributed PEG mass standards. The molecular weight range of available standards is extended by generating clusters containing from one up to six molecules of the primary PEG standard. The mass at the peak of the distribution of the lowest standard (PEG-4k) is determined by MALDI mass spectrometry and agrees with the manufacturer’s value and previous MALDI literature data. The masses for the 50K and 120K standards are found to differ by 8.6 and 6.6%, respectively, from the manufacturer’s value. Using known relationships, the particle diameter d of the ions is calculated from the measured mobility. Plots of d versus m^{1/3} give straight lines over the full mass range studied (4000–700 000 Da, particle diameter from 3 to 12 nm), indicating that these PEG particles are indeed spherical and have a density p independent of size. The slope of the d versus m^{1/3} curve provides a density ρ = 1.25 g/cm^3, close to the known bulk density, ρ_{PEG} = 1.21 g/cm^3.

1-Methyl-2-pyrrolidone (NMP) seeded with 5% trifluoroacetic acid is identified as a singular buffer, polar enough to produce fine electrospray drops, yet having excellent solubility for many industrial polymers such as polystyrene (PSR) and poly(methyl methacrylate) (PMMA). Four PSR mass standards (M = 9.2, 34.5, 68, and 170 kDa) with narrow mass distributions are electrosprayed from their solutions in this buffer. The high charge on the resulting ions is reduced to unity with a radioactive source, whereby their electrical mobility distributions, determined by a differential mobility analyzer, yield unambiguously their size distribution. Each standard produces (at high solution concentration) several mobility peaks associated with the formation of particles containing from one to six polymer molecules, used to establish a relation Z(M) between electrical mobility Z and polymer mass. Within the indeterminacy given by inaccuracies in the nominal masses of the standards, this relation indicates that the polymers form spherical balls with a density close to the bulk density of polystyrene, as seen previously with poly(ethylene glycol) chains. Good mobility spectra from the same buffer are also obtained for PMMA (M = 49 kDa). Because NMP is less conductive and contains more involatile impurities than common aqueous buffers, the electrospray ions formed tend to carry a small contaminant crust, which distorts the inferred mass distribution unless a high spray quality is achieved.


Protein Analysis


A fast and accurate assay to determine the absolute concentration of proteins is described based on direct measurement of droplet entrapped oligomer formation in electrospray. Here we demonstrate the approach using electrospray differential mobility analysis (ES-DMA), which can distinguish monomers and dimers from higher order oligomers. A key feature of the method is that it allows determination of the absolute number concentration of proteins eliminating the need for protein-specific calibration. The method was demonstrated by measuring the concentration of a NIST Standard Reference Material 927e (bovine serum albumin), a high-purity immunoglobulin G 1x, and a formulated Rituximab. The method may be applied to any electrospray source, regardless of diagnostic tool (e.g., MS or ion mobility, etc.), provided the electrospray is operated in a droplet-fission mode.


Analysis of the size and mass of nanoparticles, whether they are natural biomacromolecular or synthetic supramolecular assemblies, is an important step in the characterization of such molecular species. In recent years, electrospray ionization (ESI) has emerged as a technology through which particles with masses up to 100 MDa can be ionized and transferred into the gas phase, preparing them for accurate mass analysis. Here we review currently used methodologies, with a clear focus on native mass spectrometry (MS). Additional complementary methodologies are also covered, including ion-mobility analysis, nanomechanical mass sensors, and charge-detection MS. The literature discussed clearly demonstrates the great potential of ESI-based methodologies for the size and mass analysis of nanoparticles, including very large naturally occurring protein assemblies. The analytical approaches discussed are powerful tools in not only structural biology, but also nanotechnology.


Noroviruses are members of the Caliciviridae family of positive sense RNA viruses. In humans Noroviruses cause rapid onset diarrhea and vomiting. Currently Norovirus infection is responsible for 21 million gastroenteritis yearly cases in the USA. Nevertheless, despite the obvious public health and socio-economic relevance, no effective vaccines/antivirals are yet available to treat Norovirus infection. Since the activity of RNA-dependent RNA polymerase (RdRp) plays a key role in genome replication and in the synthesis/amplification of subgenomic RNA, the enzyme is considered a promising target for antiviral drug development. In this context, following the identification of suramin and NF023 as Norovirus RdRp inhibitors, we analyzed the potential inhibitory role of naphthalene disulfonate (NAF2), a fragment derived from these two molecules. Although NAF2, tested in enzymatic polymerase inhibition assays, displayed low activity against RdRp (IC50=14μM), the crystal structure of human Norovirus RdRp revealed a thumb domain NAF2 binding site that differs from that characterized for NF023/suramin. To further map the new potential inhibitory site, we focused on the structurally related molecule pyridoxal-5′-phosphate-6-(2′-naphthylazo-6′-nitro-4′,8′-disulfonate) tetrasodium salt (PPNDS). PPNDS displayed below-micromolar inhibitory activity versus human Norovirus RdRp [IC50=0.45μM], similarly to suramin and NF023. Inspection of the crystal structure of the RdRp/PPNDS complex showed that the inhibitor bound to the NAF2 thumb domain site, highlighting the relevance of such new binding site for exploiting Norovirus RdRp inhibitors.


The capsid of hepatitis B virus (HBV) is a major viral antigen and important diagnostic indicator. HBV capsids have prominent protrusions (‘spikes’) on their surface and are unique in having either T = 3 or T = 4 icosahedral symmetry. Mouse monoclonal and also human polyclonal antibodies bind either near the spike apices (historically the ‘α-determinant’) or in the ‘floor’ regions between them (the ‘β-determinant’). Native mass spectrometry (MS) and gas-phase electrophoretic mobility molecular analysis (GEMMA) were used to monitor the titration of HBV capsids with the antigen-binding domain (Fab) of mAb 3120, which has long defined the β-determinant. Both methods readily distinguished Fab binding to the two capsid morphologies and could provide accurate masses and dimensions for these large immune complexes, which range up to ~8 MDa. As such, native MS and GEMMA provide valuable alternatives to a more time-consuming cryo-electron microscopy analysis for preliminary characterisation of virus-antibody complexes.
Understanding competitive adsorption-desorption of proteins onto surfaces is an important area of research in food processing and biomedical engineering. Here, we demonstrate, how electrospray-differential mobility analysis that has been traditionally used for characterizing bionanoparticles, can be used for quantifying complex competitive adsorption-desorption of oligomeric proteins or multiprotein systems using monomers and dimers of IgM as a model example onto silica and modified silica surfaces. Using ES-DMA, we show that IgM dimers show a preference to stay adsorbed to different surfaces although monomers adsorb more easily and desorption rates of monomers and dimers of IgM are surface-type-dependent and are not significantly affected by shear. We anticipate that this demonstration will make ES-DMA a popular "label-free" method for studying multicomponent multi-oligomeric protein adsorption to different surfaces in the future. Published by Elsevier Inc.


The biopharmaceutical industry characterizes and quantifies aggregation of protein therapeutics using multiple analytical techniques to cross-validate results. Here, we demonstrate the use of electrospray – differential mobility analysis (ES-DMA), a gas-phase and atmospheric pressure ion-mobility method for characterizing protein aggregates. Two immunoglobulin Gs are systematically heat treated to induce aggregation and characterized using size-exclusion chromatography (SEC) and ES-DMA. Although ES-DMA is a gas-phase characterization method, we find that aggregation kinetic rate constants determined by ES-DMA is in good agreement with those determined by SEC. ES-DMA appears to have a higher resolution and lower limit of detection as compared with SEC. Thus, ES-DMA can potentially become an important orthogonal tool for characterization of nascent protein aggregates in the biopharmaceutical industry.


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"Electrospray Ionization–Ion Mobility Spectrometry Identified Monoclonal Antibodies that Bind Exclusively to Either the Monomeric or a Dimeric Form of Prostate Specific Antigen." R.C. Blake and D.A. Blake, Anal. Chem., 84(15): 6899-6906 (2012)

Macroion mobility spectrometry was used to distinguish between a monoclonal antibody (clone M612165) that bound exclusively to monomeric prostate specific antigen and a different monoclonal antibody (clone M612166) that bound exclusively to a dimeric form of the antigen that only comprised 6.8% of the total protein. In the presence of excess antigen, the mobility spectrum of M612165 was replaced by a composite spectrum that represented a mixture of antibodies that included either one or two equivalents of the protein antigen. In similar circumstances, the mobility spectrum of M612166 was replaced by a composite spectrum that represented a mixture of antibodies that included either two or four equivalents of the protein antigen. When exposed to either of the two antibodies, the mobility spectrum of the prostate specific antigen showed a concomitant decrease in the monomeric antigen in one case and in the dimeric antigen in the other case. While sensitive kinetic exclusion assays demonstrated large differences in the antigen binding behavior of the two antibodies, these functional studies alone were insufficient to reveal the likely structural origins of the observed differences. Macroion mobility measurements were shown to be a useful and informative complement to functional studies in understanding complex macromolecular interactions.

Ribonucleotide reductase (RNR) is a critical enzyme of nucleotide metabolism, synthesizing precursors for DNA replication and repair. In prokaryotic genomes, RNR genes are commonly targeted by mobile genetic elements, including free standing and intronencoded homing endonucleases and intiends. Here, we describe a unique molecular solution to assemble a functional product from the RNR large subunit gene, *nrdA* that has been fragmented into two smaller genes by the insertion of *mobE*, a mobile endonuclease. We show that unique sequences that originated during the *mobE* insertion and that are present as C- and N-terminal tails on the split *NrdA*-a and *NrdA*-b polypeptides, are absolutely essential for enzymatic activity. Our data are consistent with the tails functioning as protein interaction domains to assemble the tetrameric (*NrdA*-a/*NrdA*-b)2 large subunit necessary for a functional RNR holoenzyme. The tails represent a solution distinct from RNA and protein splicing or programmed DNA rearrangements to restore function from a fragmented coding region and may represent a general mechanism to neutralize fragmentation of essential genes by mobile genetic elements.


The combination of ion mobility separation with mass spectrometry is an emergent and powerful structural biology tool, capable of simultaneously assessing the structure, topology, dynamics, and composition of large protein assemblies within complex mixtures. An integral part of the ion mobility-mass spectrometry measurement is the ionization of intact multiprotein complexes and their removal from bulk solvent. This process, during which a substantial portion of protein structure and organization is likely to be preserved, imposes a foreign environment on proteins that may cause structural rearrangements to occur. Thus, a general means must be identified to stabilize protein structures in the absence of bulk solvent. Our approach to this problem involves the protection of protein complex structure through the addition of salts in solution prior to desorption/ionization. Anionic components of the added salts bind to the complex either in solution or during the electrospray process, and those that remain bound in the gas phase tend to have high gas phase acidities. The resulting 'shell' of counterions is able to carry away excess energy from the protein complex ion upon activation and can result in significant structural stabilization of the gas-phase protein assembly overall. By using ion mobility-mass spectrometry, we observe both the dissociation and unfolding transitions for four tetrameric protein complexes bound to populations of 12 different anions using collisional activation. The data presented here quantifies, for the first time, the influence of a large range of counterions on gas-phase protein structure and allows us to rank and classify counterions as structure stabilizers in the absence of bulk solvent. Our measurements indicate that tartrate, citrate, chloride, and nitrate anions are among the strongest stabilizers of gas-phase protein structure identified in this screen. The rank order determined by our data is substantially different when compared to the known Hofmeister salt series in solution. While this is an expected outcome of our work, due to the diminished influence of anion and protein solvation by water, our data correlates well to expected anion binding in solution and highlights the fact that both hydration layer and anion protein binding effects are critical for Hofmeister-type stabilization in solution. Finally, we present a detailed mechanism of action for counterion stabilization of proteins and their complexes in the gas-phase, which indicates that anions must bind with high affinity, but must dissociate readily from the protein in order to be an effective stabilizer. Anion-resolved data acquired for smaller protein systems allows us to classify anions into three categories based on their ability to stabilize protein and protein complex structure in the absence of bulk solvent.


We quantify the adsorption and desorption of a monoclonal immunoglobulin-G antibody, rituxamab (RmAb), on silica capillary surfaces using electrospray-differential mobility analysis (ES-DMA). We first develop a theory to calculate coverages and desorption rate constants from the ES-DMA data for proteins adsorbing on glass capillaries used to electrospray protein solutions. This model is then used to study the adsorption of RmAb on a bare silica capillary surface. A concentration-independent coverage of approximate to 4.0 ng/m2 is found for RmAb concentrations ranging from 0.01 to 0.1 mg/mL. A study of RmAb adsorption to bare silica as a function of pH shows maximum adsorption at its isoelectric point (pI of pH 8.5) consistent with literature. The desorption rate constants are determined to be approximate to 10^−5 s−1, consistent with previously reported values, thus suggesting that shear forces in the capillary may not have a considerable effect on desorption. We anticipate that this study will allow ES-DMA to be used as a "label free" tool to study adsorption of oligometric and multicomponent protein systems onto fused silica as well as other surface modifications.
The determination of protein assembly size and relative molecular mass is currently of great importance in biochemical analysis. In particular, the technique of nanoelectrospray (nES) with a gas-phase electrophoretic mobility molecular analyzer (GEMMA) has received increased attention for such measurements. However, in order for the GEMMA technique to gain broader acceptance in protein analysis, it must be further evaluated and compared with other established biochemical protocols. In the present study, nES-GEMMA was evaluated for the analysis of a set of protein and protein complexes involved in the Sec and the bacterial type III secretion pathway of enteropathogenic Escherichia coli bacteria. The same set of proteins, isolated and purified using standard biochemical protocols, were also analyzed using multi-angle laser light scattering (MALLS) and quasi-elastic light scattering (QELS), following size exclusion chromatography. This allowed for direct comparisons between the three techniques. It was found that nES-GEMMA, in comparison to the more established MALLS and QELS techniques, offers several complementary advantages. It requires considerably less amount of material, i.e., nanogram vs. milligram amounts, and time per sample analysis, i.e., few minutes vs. tens of minutes. Whereas the determined size and relative molecular mass are similar between the compared methods, the electrophoretic diameters determined using nES-GEMMA seem to be systematically smaller compared to the hydrodynamic diameter derived by QELS. Some of the GEMMA technique disadvantages include its narrow dynamic range, limited by the fact that at elevated protein concentrations there is increased potential for the occurrence of nES-induced oligomers. Thus, it is preferred to analyze dilute protein solutions because non-specific oligomers are less likely to occur whereas biospecific oligomers remain detected. To further understand the formation of nES-oligomers, the effect of buffer concentration on their formation was evaluated. Also, nES-GEMMA is not compatible with all the buffers commonly used with MALLS and QELS. Overall, however, the nES-GEMMA technique shows promise as a high-throughput proteomics/protein structure tool.
We investigate whether “supercharging” reagents able to shift the charge state distributions (CSDs) of electrosprayed protein ions upward also influence gas-phase protein structure. A differential mobility analyzer and a mass spectrometer are combined in series (DMA-MS) to measure the mass and mobility of monomer and multimeric phosphorylase B ions (monomer molecular weight similar to 97 kDa) in atmospheric pressure air. Proteins are electrosprayed from charge-reducing triethylammonium formate in water (pH = 6.8) with and without the addition of the supercharging reagent tetramethylene sulfone (sulfolane). Because the DMA measures ion mobility prior to collisional heating or dechllusterling, it probes the structure of supercharged protein ions immediately following solvent (water) evaporation. As in prior studies, the addition of sulfolane is found to drastically increase both the mean and maximum charge state of phosphorylase B ions. Ions from all protein n-mers were found to yield mobilities that, for a given charge state, were similar to 6-10% higher in the absence of sulfolane. We find that the mobility decrease which arises with sulfolane is substantially smaller than that typically observed for folded-to-unfolded transitions in protein ions (where a similar to 60% decrease in mobility is typical), suggesting that supercharging reagents do not cause structural protein modifications in solution as large as reported by Williams and colleagues [E. R. Williams et al., J. Am. Soc. Mass Spectrom., 2010, 21, 1762-1774]. In fact, the measurements described here indicate that the modest mobihility decrease observed can be partly attributed to sulfolane trapping within the protein ions during DMA measurements, and probably also in solution. As the most abundant peaks in measured mass-mobility spectra for ions produced with and without sulfolane correspond to non-covalently bound phosphorylase B dimers, we find that in spite of a change in mobility/cross section, sulfolane addition does not substantially alter the structure of non-covalently bound protein complexes involved gas-phase.

We find evidence for a direct transition of insulin monomers into amyloid fibrils without measurable concentrations of oligomers or protofibrils, suggesting that fibrillogenesis may occur directly from assembly of denaturing insulin monomers rather than by successive transitions through protofibril nuclei. To support our finding, we obtain size distributions using electrospray differential mobility analysis (ES-DMA), which provides excellent resolution to clearly distinguish among small oligomers and rapidly generates statistically significant size distributions. The distributions detect an absence of significant peaks between 6 nm and 17 nm as the monomer reacts into fibers—exactly the size range observed by others for small-angle-neutron-scattering-measured intermediates and for circular supramolecular structures. They report concentrations in the nanomolar range, whereas our limit of detection remains three-orders-of-magnitude lower (<5 pmol/L). This finding, along with the lack of significant increases in the β-sheet content of monomers using circular dichroism, suggests monomers do not first structurally rearrange and accumulate in a β-rich state but react and reorganize at the growing fiber’s tip. These results quantitatively inform reaction-based theories of amyloid fiber formation and have implications for neurodegenerative, protein conformation, and imbalances including Alzheimer’s disease and bovine spongiform encephalopathy.

The demand for analysis of nanosized particles and assemblies of biologic and inorganic origin has increased in the recent decade together with the growing development of biotechnology and nanotechnology. Recent developments of electrostatic differential mobility analysis (DMA) provide an excellent characterization tool in the nanometer size range. With an increasing number of available nano-DMA (nDMA) systems, the question of data comparability and implementation of possible calibration procedures arise. Here we present analysis of proteins in a range between 3 nm (5.7 kDa) and 15 nm (660 kDa) with five different nDMA systems. Results show differences in the obtained sizes up to 15% between different nDMA systems, which consequently leads to the conclusion that a calibration procedure for each nDMA is necessary when applying such systems for the analysis of nanoparticles with respect to size and molecular mass.

Mass spectrometry (MS) has advanced to analyze ever-larger biomolecules with the invention of soft ionization techniques like electrospray ionization (ESI). Although ESI has provided a method of generating ions of high mass, mass spectrometers generally suffer both lower sensitivity and lower resolution as the mass-to-charge ratio of an ion increases. To extend the mass range of ionized macromolecules beyond the limits of MS, macromolecules mobility spectrometry utilizes ion mobility sizing to characterize charge-reduced ESI-generated macromolecules from >5 kDa to beyond megadalton masses. One prominent application of macromolecule mobility spectrometry, highlighted here, is the high sensitivity analysis of intact proteins, antibodies, and conjugates in which molecular masses range from antibody light-chain fragments to high mass immunoglobulin multimers.

The measurement of large biomolecules has benefited tremendously from the development of ESI coupled to gas-phase analyzers such as mass spectrometers and ion mobility spectrometers. The role of multislitunit assemblies and aggregation in normal cellular processes and diseases warrants a practical method for the study of large macromolecular complexes. X-ray crystallography and NMR spectroscopy provide unrivaled high-resolution structural information. However, protein crystallization is traditionally time consuming; NMR is limited by the size of the protein target; and compared with MS, both methods require large quantities of purified analyte.


How does the development of mass spectrometry (MS) for the study of protein complexes, intact in the gas phase, contribute to the twin fields of genomics and proteomics? Foremost, it is important to recognize the fact that the vast majority of proteins do not exist as single entities in the cell, but rather interact noncovalently with additional copies of the same protein and/or other proteins. Furthermore, additional interactions can occur with nucleic acids, ligands, cofactors, or metal ions, such that the functional form of many proteins is rarely the simple monomeric state. As such, while traditionally genomics and proteomics has focused on determining which proteins are encoded by the genetic material, in order to understand their function, their interaction to form protein complexes must also be investigated.


Mass spectrometry (MS) and ion mobility with electrospray ionization (ESI) have the capability to measure and detect large noncovalent protein-ligand and protein-protein complexes. Using an ion mobility method of gas-phase electrophoretic mobility molecular analysis (GEMMA), protein particles representing a range of sizes can be separated by their electrophoretic mobility in air. Highly charged particles produced from a protein complex solution using electrospray can be manipulated to produce singly charged ions, which can be separated and quantified by their electrophoretic mobility. Results from ESI-GEMMA analysis from our laboratory and others were compared with other experimental and theoretically determined parameters, such as molecular mass and cryoelectron microscopy and X-ray crystal structure dimensions. There is a strong correlation between the electrophoretic mobility diameter determined from GEMMA analysis and the molecular mass for protein complexes up to 12 MDa, including the 93 kDa enolase dimer, the 480 kDa ferritin 24-mer complex, the 4.6 MDa cowpea chlorotic mottle virus (CCMV), and the 9 MDa MVP vault assembly. ESI-GEMMA is used to differentiate a number of similarly sized vault complexes that are composed of different N-terminal protein tags on the MVP subunit. The average effective density of the proteins and protein complexes studied was 0.6 g/cm³. Moreover, there is evidence that proteins and protein complexes collapse or become more compact in the gas phase in the absence of water.


Beyond its primary, secondary, and tertiary structures, the quaternary structure of a protein can be defined as its interactions and associations with other proteins, macromolecules, and ligands that conspire to define its biological function. Thus, the structural determination of protein complexes can play an important role in the fundamental understanding of biochemical pathways. Traditionally, researchers have a variety of tools at their disposal to probe and measure such interactions. These tools include ultracentrifugation, light scattering, yeast two-hybrid, surface plasmon resonance, affinity chromatography, and native gel electrophoresis, and the methods that provide an image of the protein complex, such as cryoelectron microscopy, nuclear magnetic resonance (NMR) spectroscopy, and X-ray crystallography. Each of these methods has its advantages and disadvantages, and each provides a defined level of information detail, from low-resolution assembly size information (e.g., dynamic light scattering) to high-resolution structure from NMR and X-ray.
Gas-phase electrophoretic-mobility macromolecule analysis (GEMMA) is a relatively new method to study protein complexes in solution: a diluted protein sample (usually 10 ng/μl) is transmitted into the gas phase by a charged reduced electrospray process (11–12). The generated particles, each containing one protein molecule with a +1 charge, are separated according to size in a differential mobility analyzer and subsequently quantified by a particle counter. In contrast to mass spectrometry, this method is run at atmospheric pressure and measures the diameter of the particle rather than the mass. However, because particle diameter and mass are correlated to each other, the mass can usually be determined with an error of ± 5.6% (12). Electrospray ionization mass spectrometry can be used to obtain exact masses of protein complexes, but the sensitivity is strongly biased toward small protein complexes (13). Taken together, we think the two methods complement each other, with GEMMA providing quantitative information about the protein complexes while mass spectrometry determines the exact size of these complexes. In this study, we analyzed how various nucleotide effectors affect the quaternary structure of mouse ribonucleotide reductase by gel filtration, GEMMA, and mass spectrometry. In agreement with previous studies, we found that nucleotide effectors that bind only to the specificity site induce the formation of R1 dimers. The R1 dimers can interact with the R2 dimer, forming an enzymatically active αβ6 complex. However, in the presence of ATP or dATP both allosteric sites become occupied and then R1 hexamers are formed. These hexamers can interact with the R2 dimer, forming α6β complex. The α6β complex could either be in a hyperactive form in the presence of ATP or in an inactive form in the presence of dATP.


Vaults are 13 million Da ribonucleoprotein particles with a highly conserved structure. Expression and assembly by multimerization of an estimated 96 copies of a single protein, termed the major vault protein (MVP), is sufficient to form the minimal structure and entire exterior shell of the barrel-shaped vault particle. Multiple copies of two additional proteins, VPARP and TEP1, and a small untranslated vault RNA are also associated with vaults. We used the S9 insect cell expression system to form MVP-only recombinant vaults and performed a series of protein-mixing experiments to test whether this particle shell is able to exclude exogenous proteins from interacting with the vault shell. Surprisingly, we found that VPARP and TEP1 are able to incorporate into vaults even after the formation of the MVP vault particle shell is complete. Electrospray molecular mobility analysis and spectroscopic studies of vault-interacting proteins were used to confirm this result. Our results demonstrate that the protein shell of the recombinant vault particle is a dynamic structure and suggest a possible mechanism for in vivo assembly of vault-interacting proteins into preformed vaults. Finally, this study suggests that the vault interior may functionally interact with the cellular milieu.


Mass spectrometry and gas phase ion mobility [gas phase electrophoretic macromolecule analyzer (GEMMA)] with electrospray ionization were used to characterize the structure of the noncovalent 28-subunit 20S proteasome from Methanococcus jannaschii and rabbit. ESI-MS measurements with a quadrupole time-of-flight analyzer of the 192 kDa α7-ring and the intact 690 kDa α2β7-β7α7 are consistent with their expected stoichiometries. Collisionally activated dissociation of the 20S gas phase complex yields loss of individual α-subunits only, and it is generally consistent with the known αβ7-β7α7 architecture. The analysis of the binding of a reversible inhibitor to the 20S proteasome shows the expected stoichiometry of one inhibitor for each β-subunit. Ion mobility measurements of the α7-ring and the α2β7-β7α7 complex yield electrophoretic diameters of 10.9 and 15.1 nm, respectively; these dimensions are similar to those measured by crystallographic methods. Sequestration of multiple apo-myoglobin substrates by a lactacystin-inhibited 20S proteasome is demonstrated by GEMMA experiments. This study suggests that many elements of the gas phase structure of large protein complexes are preserved upon desolvation, and that methods such as mass spectrometry and ion mobility analysis can reveal structural details of the solution protein complex. © 2005 American Society for Mass Spectrometry.


Noncovalently bound macromolecular protein complexes constitute an essential aspect of the living cell and are responsible for many biological processes. This review focuses on the analysis of these important species by electrospray ionisation mass spectrometry. The current range of instrumentation is discussed and the major biological complexes studied to date are highlighted. This review has 108 references.

Bikunin is a glycosylated protein that aggregates extensively during mammalian cell culture, resulting in loss of activity, loss of native secondary structure, and the formation of nonnative disulfide bonds. We investigated the use of high hydrostatic pressure (1000-3000 bar) for the refolding of bikunin aggregates. The refolding yield obtained with pressure-modulated refolding at 2000 bar was 70 (±5%) by reverse-phase chromatography (RP-HPLC), significantly higher than the value of 55 (±6%) (RP-HPLC) obtained with traditional guanidine HCl “dilution-refolding.” In addition, we determined the thermodynamics of pressure-modulated refolding. The change in volume for the transition of aggregate to monomer ΔVrefolding was calculated to be ~28 (±5) mL/mole. Refolding was accompanied by a loss of hydrophobic exposure, resulting in a positive contribution to the ΔVrefolding. These findings suggest that the disruption of electrostatic interactions or the differences in size of solvent-free cavities between the aggregate and the monomer are the prevailing contributions to the negative ΔVrefolding.


Mass spectrometry has grown in recent years to a well-accepted and increasingly important complementary technique in structural biology. Especially electrospray ionization mass spectrometry is well suited for the detection of non-covalent protein complexes and their interactions with DNA, RNA, ligands, and cofactors. Over the last decade, significant advances have been made in the ionization and mass analysis techniques, which makes the investigation of even larger and more heterogeneous intact assemblies feasible. These technological developments have paved the way to study intact non-covalent protein–protein interactions, assembly and disassembly in real time, subunit exchange, cooperativity effects, and effects of cofactors, allowing us a better understanding of proteins in cellular processes. In this review, we describe some of the latest developments and several highlights.


The recent successful use of electrospray gas-phase electrophoretic mobility molecular analysis (GEMMA) to separate globular proteins (mass 6 to 670 kDa) and the excellent correlation found between the electrophoretic mobility diameter (EMD), or Millikan diameter, and the protein mass (S. L. Kaufman et al., 1996, *Anal. Chem.* 68, 1895-1904; 1996, *Anal. Chem.* 68, 3703), prompted the examination of a large protein complex, the 3.6-MDa, heteromultimeric, hexagonal bilayer hemoglobin (Hb) and its subunits from the earthworm Lumbricus terrestris. The native Hb had an EMD of 25.7 nm and the products of its dissociation at pH >8 and <5 were resolved into peaks with EMDs of 10.5, 6.3, 5.0, and 4.2 nm, identified as a dodecamer of globin chains ([a+b+c]3d3, 213 kDa), the disulfide-bonded trimer of globin chains ([a+b+c]52.7 kDa), all the linker chains (L1, 27.5 kDa; L2, 32.1 kDa; L3, 24.9 kDa; L4, 24.1 kDa), and the monomer subunit (chain d, 17 kDa), respectively. Reassembly of the Hb complex was observed on restoring the pH from >8 to 7. The EMDs and the masses of the Hb and its subunits are in excellent agreement with the correlation found earlier, under the assumption of nearly spherical shape with an effective density around 0.7 g/cm³. GEMMA also provided a profile of the Hb completely dissociated in 0.1% SDS; its deconvolution permitted a quantitative determination of the subunit stoichiometry, providing a globin to linker ratio of 3 to 1.


Globular proteins ranging in molecular mass from 5.7 to 669 kDa were separated and analyzed using an aerosol technique based on the electrophoretic mobility of singly-charged molecular ions in air. The ions were produced by electrospraying and drying 100-nm-diameter droplets of a liquid suspension of the proteins, using ionized air to remove the droplet charge due to the spray process. The electrophoretic mobility was measured using a modified commercial continuous-flow differential mobility analyzer operated near atmospheric pressure. An unmodified commercial condensation particle counter was used for detection. The concentrations analyzed ranged from 0.02 to 200 µg of protein/mL of buffer, with a liquid sample flow rate of approximately 50 nL/min. Sampling time of 3 min was used for each complete distribution measured. The electrophoretic mobilities measured were determined entirely from air flow rates, apparatus geometry, and applied potentials. Results were expressed as electrophoretic mobility equivalent diameters using a Millikan formula.
Viruses, VLPs, Vaccines


Commonly used methods for size and shape analysis of bionanoparticles found in vaccines like X-ray crystallography and cryo-electron microscopy are very time-consuming and cost-intensive. The nano-electrospray (nanoES) gas-phase electrophoretic mobility macromolecular analyzer (GEMMA), belonging to the group of ion mobility spectrometers, was used for size determination of vaccine virus particles because it requires less analysis time and investment (no vacuum system). Size exclusion chromatography (SEC) of viral vaccines and production intermediates turned out to be a good purification/isolation method prior to GEMMA, TEM(transmission electron microscopy) and AFM (atomic force microscopy) investigations, as well as providing a GEMMA analysis-compatible buffer. Column materials and different elution buffers were tested for optimal vaccine particle yield. We used a Superdex 200 column with a 50 mM ammonium acetate buffer. In addition, SEC allowed the removal of process-related impurities from the virions of interest. A sample concentrating step or a detergent addition step was also investigated. As a final step of our strategy SEC-purified or untreated vaccine-nanoparticles were further analyzed: (a) by immunological detection with a specific polyclonal antibody (dot blot) to verify the biological functionality, (b) by GEMMA to provide the size of the particles at atmospheric pressure and (c) by AFM and (d) TEM to obtain both size and shape information. The mean diameter of inactivated tick-borne encephalitis virions determined by GEMMA measurement was 46.6 ±0.5 nm, in contrast to AFM and TEM images providing diameters of about 58 ±4 and 52 ±5 nm, respectively.


The capsid of hepatitis B virus (HBV) is a major viral antigen and important diagnostic indicator. HBV capsids have prominent protrusions (‘spikes’) on their surface and are unique in having either T =3 or T =4 icosahedral symmetry. Mouse monoclonal and also human polyclonal antibodies bind either near the spike apices (historically the ‘α-determinant’) or in the ‘floor’ regions between them (the ‘β-determinant’). Native mass spectrometry (MS) and gas-phase electrophoretic mobility molecular analysis (GEMMA) were used to monitor the titration of HBV capsids with the antigen-binding domain (Fab) of mAb 3120, which has long defined the β-determinant. Both methods readily distinguished Fab binding to the two capsid morphologies and could provide accurate masses and dimensions for these large immune complexes, which range up to ~8MDa. As such, nativeMS and GEMMA provide valuable alternatives to a more time-consuming cryoelectron microscopy analysis for preliminary characterisation of virus-antibody complexes.


Influenza virus-like particle vaccines are one of the most promising ways to respond to the threat of future influenza pandemics. VLPs are composed of viral antigens but lack nucleic acids making them non-infectious which limit the risk of recombination with wild-type strains. By taking advantage of the advancements in cell culture technologies, the process from strain identification to manufacturing has the potential to be completed rapidly and easily at large scales. After closely reviewing the current research done on influenza VLPs, it is evident that the development of quantification methods has been consistently overlooked. VLP quantification at all stages of the production process has been left to rely on current influenza quantification methods (i.e. Hemagglutination assay (HA), Single Radial Immunodiffusion assay (SRID), NA enzymatic activity assays, Western blot, Electron Microscopy). These are analytical methods developed decades ago for influenza virions and final bulk influenza vaccines. Although these methods are time-consuming and cumbersome they have been sufficient for the characterization of final purified material. Nevertheless, these analytical methods are impractical for in-line process monitoring because VLP concentration in crude samples generally falls out of the range of detection for these methods. This consequently impedes the development of robust influenza VLP production and purification processes. Thus, development of functional process analytical techniques, applicable at every stage during production, that are compatible with different production platforms is in great need to assess, optimize and exploit the full potential of novel manufacturing platforms.

Human rhinoviruses (HRVs) are valuable tools in the investigation of early viral infection steps due to their far-reaching (although still incomplete) characterization. During endocytosis, native virions first lose one of the four capsid proteins (VP4); corresponding particles sediment at 155S and were termed subviral A particles. Subsequently, the viral RNA genome leaves the viral shell giving rise to empty capsids. In continuation of our previous work with HRV serotype 2 (HRV2) intermediate subviral particles, in which we were able to discriminate by CE even between two intermediates (AI and AII) of virus uncoating, we further concentrated on the characterization of AI particles with the electrophoretic mobility of around -17.2 × 10^{-9} m^2/Vs at 20°C. In the course of our present work we related these particles to virions as previously described at the subviral A stage of uncoating (and as such sedimenting at 155S) by determination of their protein and RNA content—in comparison to native virions AI particles did not include VP4, however, still 93% of their initial RNA content. Binding of an mAb specific for subviral particles demonstrated antigenic rearrangements on the capsid surface at the AI stage. Furthermore, we investigated possible factors stabilizing intermediates of virus uncoating. We could exclude the influence of the previously suspected so-called contaminant of virus preparation on HRV2 subviral particle formation. Instead, we regarded other factors being part of the virus preparation system and found a dependence of AI particle formation on the presence of divalent cations.


During infection, enteroviruses, such as human rhinoviruses (HRVs), convert from the native, infective form with a sedimentation coefficient of 150S to empty subviral particles sedimenting at 80S (B particles). B particles lack viral capsid protein 4 (VP4) and the single-stranded RNA genome. On the way to this end stage, a metastable intermediate particle is observed in the cell early after infection. This subviral A particle still contains the RNA but lacks VP4 and sediments at 155S. Native (150S) HRV serotype 2 (HRV2) as well as its empty (80S) capsid have been well characterized by capillary electrophoresis. In the present paper, we demonstrate separation of at least two forms of subviral A particles on the midway between native virions and empty 80S capsids by CE. For one of these intermediates, we established a reproducible way for its preparation and characterized this particle in terms of its electrophoretic mobility and its appearance in transmission electron microscopy (TEM). Furthermore, the conversion of this intermediate to 80S particles was investigated. Gas-phase electrophoretic mobility molecular analysis (GEMMA) yielded additional insights into sample composition. More data on particle characterization including its protein composition and RNA content (for unambiguous identification of the detected intermediate as subviral A particle) will be presented in the second part of the publication.


This review critically examines an emerging tool to measure viral clearance from biomanufacturing streams, monitor assembly of viruses and virus-like particles, rapidly identify viruses from biological milieu, assay virus neutralization, and prepare bionanoconjugates for bacterial detection. Electrospray differential mobility analysis (ES-DMA) is a tool of choice to simultaneously determine viral size and concentration because it provides full multimodal size distributions with subnanometer precision from individual capsid proteins to intact viral particles. The review contrasts ES-DMA to similar tools and highlights expected growth areas including at-line process sensing as a process analytical technology (PAT), bioseparating as a distinct unit operation, monitoring viral reactions, and interrogating virus host protein interactions.


The technique of electrospray differential mobility analysis (ES-DMA) was examined as a potential potency assay for routine virus particle analysis in biomanufacturing environments (e.g., evaluation of vaccines and gene delivery products for lot release) in the context of the International Committee of Harmonisation (ICH) Q2 guidelines. ES-DMA is a rapid particle sizing method capable of characterizing certain aspects of the structure (such as capsid proteins) and obtaining complete size distributions of viruses and virus-like particles. It was shown that ES-DMA can distinguish intact virus particles from degraded particles and measure the concentration of virus particles when calibrated with nanoparticles of known concentration. The technique has a measurement uncertainty of approximate to 120%, is linear over nearly 3 orders of magnitude, and has a lower limit of detection of approximate to 10^9 particles/mL. This quantitative assay was demonstrated for non-enveloped viruses. It is expected that ES-DMA will be a useful method for applications involving production and quality control of vaccines and gene therapy vectors for human use. ©2011 Published by Elsevier B.V.

The electrophoretic mobility of charged, airborne nanoparticles (NPs) or macromolecules and their specific complexes opens new avenues for their analysis and handling. The newly developed parallel differential mobility analyzer in combination with an electrostatic particle sampler enables not only the characterization of bio-NPs, but even their sampling while preserving their bioactivity (e.g., the enzyme activity of galactosidase). Precondition for the applicability of this technique is a well-defined charging status of the NPs in question. This charge conditioning can be achieved by means of a radioactive source, Po-210, even if the yield in terms of charged particles is low for sub-20 nm particles and the aging of the source influences the size spectra measured. Nevertheless, this technique enables size-defined sampling and enrichment, combined with real-time measurement of the size of both NPs and viruses. Furthermore, it allows determination of the number of attached biospecific antibodies, thereby providing information about the surface coverage of viruses by antibodies. ©2010 Elsevier Ltd. All rights reserved.


We present a rapid and quantitative method to physically characterize the structure and stability of viruses. Electrospray differential mobility analysis (ES-DMA) is used to determine the size of capsomers (i.e., hexons) and complete capsids. We demonstrate how to convert the measured mobility size into the icosahedral dimensions of a virus, which for PR772 become 68.4 nm for vertex-to-vertex, 54.4 nm for face-to-facet, and 58.2 nm for edge-to-edge lengths, in reasonable agreement with dimensions from transmission electron microscopy for other members of the family Tectiviridae (e.g., PRD1). These results indicate ES-DMA’s mobility diameter most closely approximates the edge-to-edge length. Using PR772’s edge length (36.0 nm) and the size of the major capsid hexon (approximate to 8.4 nm) from ES-DMA with icosahedral geometry, PR772’s T = 25 symmetry is confirmed and the number of proteins in the capsid shell is determined. We also demonstrate the use of ES-DMA to monitor the temporal disintegration of PR772, the thermal degradation of PP7, and the appearance of degradation products, essential to viral stability assays. These results lay groundwork essential for the use of ES-DMA for a variety of applications including monitoring of vaccine and gene therapy vector products, confirmation of viral inactivation, and theoretical studies of self-assembling macromolecular structures.


We have combined ion mobility spectrometry mass spectrometry with tandem mass spectrometry to characterise large, non-covalently bound macromolecular complexes in terms of mass, shape (cross-sectional area) and stability (dissociation) in a single experiment. The results indicate that the quaternary architecture of a complex influences its residual shape following removal of a single subunit by collision-induced dissociation tandem mass spectrometry. Complexes whose subunits are bound to several neighbouring subunits to create a ring-like three-dimensional (3D) architecture undergo significant collapse upon dissociation. In contrast, subunits which have only a single neighbouring subunit within a complex retain much of their original shape upon complex dissociation. Specifically, we have determined the architecture of two transient, on-pathway intermediates observed during in vitro viral capsid assembly. Knowledge of the mass, stoichiometry and cross-sectional area of each viral assembly intermediate allowed us to model a range of potential structures based on the known X-ray structure of the coat protein building blocks. Comparing the cross-sectional areas of these potential architectures before and after dissociation provided tangible evidence for the assignment of the topologies of the complexes, which have been found to encompass both the 3-fold and the 5-fold symmetry axes of the final icosahedral viral shell. Such insights provide unique information about virus assembly pathways that could allow the design of anti-viral therapeutics directed at the assembly step. This methodology can be readily applied to the structural characterisation of many other non-covalently bound macromolecular complexes and their assembly pathways. Copyright © 2010 John Wiley & Sons, Ltd.
Aerosolization of bacteriophage MS2 virions by nebulization and charge-reduced electrospray were compared during testing of three filter media. Sample swatches were taken from a surgical mask, N95 filtering-facepiece respirator (FFR), and N100 FFR for use in repeated, short-duration (15 min) penetration tests with bacteriophage MS2 aerosolized by nebulizer and electrospray. Evaluated were (1) the virus suspension preparation protocol, (2) resulting particle size distribution, count stability, and count variability, and (3) the ability to generate culturable MS2 virions. While preparation of the electrospray bacteriophage suspension required additional purification and concentration steps and took more time than the nebulization protocol, it resulted in a much higher titer suspension. The nebulizer produced a polydisperse aerosol; conversely, the electrospray produced a relatively monodisperse aerosol with a count peak at the mobility size of the single virion. The nebulized aerosol particle count was 2.8 times as variable as the electrosprayed aerosol particle count although neither aerosolization method maintained a constant count over repeated 15-minute filter tests. No differences in filter penetration were observed between nebulized and electrosprayed MS2 aerosol particles. Electrosprayed dextrose particles, used as an inert aerosol particle comparator, exhibited higher penetration than MS2 particles in two of the three filter samples, which can be attributed, at least partially, to the difference in dielectric properties of dextrose and virus particles. Both aerosolization methods generated culturable MS2 virions with the electrospray producing an airborne concentration approximately 20-fold higher than the nebulizer. In general, the electrospray produced cleaner, more stable, and more viable bacteriophage aerosol particles compared to conventional nebulization. The findings of this study are expected to assist researchers in selecting appropriate generation methods when using viable virus-based challenge aerosol particles.

Biological electrospray techniques are rapidly becoming a promising means for controlling living organisms in applications ranging from mass spectrometry to developmental biology. We investigated the generation characteristics of airborne MS2 bacteriophage particles <30 nm in size, using an electrospray technique. A suspension containing bacteriophage MS2 was sprayed in cone-jet mode using a specially designed electrospray system with a point-to-orifice-plate configuration mentioned in previous studies based on a charge reduced electrospray size spectrometry. The highly charged droplets were discharged rapidly into a radioactive neutralizer of Po(210). The electrosprayed airborne MS2 particles (23.8 ±0.49 nm GMD) maintained their monodisperse size distribution with good stability and uniformity for > 1 h. Compared with the generation characteristics observed using the previous nebulization process (51.5 ±0.86 nm GMD), this electrospray technique produced nonagglomerated particles, resulting in a narrow size range of generated particles. The total MS2 particle number concentration and GMD increased with changes in the suspension flow rate from 5 to 25 μL/h. As the applied voltage increased in cone-jet mode, the GMD and culturable bacteriophage concentration decreased slightly. Our investigation shows that the electrospray process, driven by high-intensity electric fields, can be used for nanometer-sized living organisms.

Virus reference materials are needed to develop and calibrate detection devices and instruments. We used electrospray differential mobility analysis (ES-DMA) and quantitative amino acid analysis (AAA) to determine the particle concentration of three small model viruses (bacteriophages MS2, PP7, and φX174). The biological activity, purity, and aggregation of the virus samples were measured using plaque assays, denaturing gel electrophoresis, and size-exclusion chromatography. ES-DMA was developed to count the virus particles using gold nanoparticles as internal standards. ES-DMA additionally provides quantitative measurement of the size and extent of aggregation in the virus samples. Quantitative AAA was also used to determine the mass of the viral proteins in the pure virus samples. The samples were hydrolyzed and the masses of the well-recovered amino acids were used to calculate the equivalent concentration of viral particles in the samples. The concentration of the virus samples determined by ES-DMA was in good agreement with the concentration predicted by AAA for these purified samples. The advantages and limitations of ES-DMA and AAA to characterize virus reference materials are discussed.

Here we characterize virus-like particles (VLPs) by three very distinct, orthogonal, and quantitative techniques: electrospray differential mobility analysis (ES-DMA), asymmetric flow field-flow fractionation with multi-angle light scattering detection (AFFFF-MALS) and transmission electron microscopy (TEM). VLPs are biomolecular particles assembled from viral proteins with applications ranging from synthetic vaccines to vectors for delivery, of gene and drug therapies. VLPs may have polydispersed, multimodal size distributions, where the size distribution can be altered by subtle changes in the production process. These three techniques detect subtle size differences in VLPs derived from the non-enveloped murine polyomavirus (MPV) following: (i) functionalization of the surface of VLPs with an influenza viral peptide fragment; (ii) packaging of foreign protein internally within the VLPs; and (iii) packaging of genomic DNA internally within the VLPs. These results demonstrate that ES-DMA and AFFFF-MALS are able to quantitatively determine VLP size distributions with greater rapidity and statistical significance than TEM, providing useful technologies for product development and process analytics.


Attachment of a nonaggregating monoclonal antibody and of a soluble recombinant receptor molecule to the icosahedral nonenveloped human rhinovirus serotype 2 was studied with a nanoelectrospray ionization gas-phase electrospray molecular mobility analyzer (nESI-GEMMA). The virus mass, as determined via nESI-GEMMA, was within instrument accuracy (±6%) close to the theoretical value (8 x 10^6 Da) calculated from the sum of all constituents of one virus particle (60 copies of each of the four viral capsid proteins, the RNA genome, and one copy of the RNA-linked protein VpG). The formation of virus-antibody complexes of different stoichiometries (up to a mass 12.5 x 10^6 Da corresponding to 30 attached antibodies) and virus-receptor complexes (up to a mass 8.8 x 10^6 Da corresponding to 12 attached receptor molecules) was monitored. Via the volume derived from the electrophoretic mobility diameter (EMD), the stoichiometry of the HRV complexes was calculated. The accuracy of the EMD was within ±0.5 nm, which corresponds to an accuracy of ±4 antibodies and ±5 receptor molecules in the respective complexes. For the first time, we here demonstrate the use of nESI-GEMMA for the analysis of the size and stoichiometry of biomolecules in high-order complexes in real time under normal pressure conditions.


The ability to analyze and identify large macromolecular complexes whose molecular weight is beyond the analyzable range of mass spectrometry is of great interest. The size of such complexes makes them suitable for analysis via mobility size spectrometry. In this work, charge reduced electrospray size spectrometry was used for the analysis of bacteriophage viruses with total molecular masses ranging from 3.6 MDa up to the gigadalton range. The electrospray source used was operated in “cone jet” mode with a mean droplet diameter of 170.56 nm. Bacteriophage MS2 was found to have a mobility diameter of 24.13 ± 0.06 nm and remain highly viable after the electrospray process. Larger bacteriophages T2 and T4 have lengths greater than the diameter of the electrospray jet and droplets; thus, they could not be completely enclosed and were found to fragment at the virus capsid head-tail noncovalent interface during either the jet formation or jet breakup process. No viable T2 or T4 virions were detectable after being electrosprayed. While the exact mechanism of fragmentation could not be determined, it is proposed here that macromolecular fragmentation at noncovalent interfaces occurs due to mechanically and electrically induced stresses during jet formation and jet breakup. Bacteriophage T4 capsid heads were found to be statistically significantly larger than bacteriophage T2 capsid heads, with a mean peak diameter of 88.32 ± 0.12 nm for T4 and 87.03 ± 0.18 nm for T2. While capsid head fragments were detectable, tail and tail-fiber fragments could not be detected by size spectrometric analysis. This is attributed to the fact that the contractile tails of bacteriophage T2 and T4 virions mechanically deform to a varying degree while confined within the smaller jet and droplets. Further evidence of contractile tail deformation during the electrospray process was found by measuring the size spectrum of bacteriophage, which has a noncontractile tail. Bacteriophage A had two distinct peaks in its size spectrum, one corresponding to the capsid head and the other corresponding to the tail fragment. Size spectrometry was also used for rapid quantification of virus concentrations, thus demonstrating its full capabilities in the analysis of large macromolecular complexes.

Characterizing supramolecular interactions offers significant challenges using NMR or crystallographic techniques either because of size limitations or the difficulty in forming suitable crystals, while mass spectrometry is largely limited to low resolution mass information. Here we report gas phase measurements of intact virus particles using electrospray ion mobility spectrometry with an accuracy in radial measurements that were sufficient to differentiate closely related species. In addition, measured diameters indicate that icosahedral virus particles retain their structure in the gas phase as well as undergoing a slight compaction in the absence of solvent. Analysis of the human pathogen adenovirus represents the largest and most sophisticated biomolecular complex detected in the gas phase to date. These results, on a diverse set of viral systems, suggest that ion mobility spectrometry may have broad applications for the analysis of biological complexes.


A new physically based methodology – The Integrated Virus Detection System (IVDS) – was used to characterize a high concentration, 10.2 mg protein/ml, sample preparation of MS2 Bacteriophage with a reported $10^{14}$ pfu/ml (DPM14) virus count in a common TNME buffer. Virus counts were made using the IVDS instrument following serial dilution. Results indicated virus counts of $1.5 \times 10^5$ for the neat sample (DPM14), followed by $6.5 \times 10^4$ viruses (DPM13), $1.2 \times 10^4$ viruses (DPM12), $9.3 \times 10^2$ viruses (DPM11), 88 viruses (DPM10) and 5 viruses (DPM9) respectively. Lower concentrations display a consistent multiplier and were consistent with target dilutions. Increases in virus concentration appear to decrease the multiplier, a variation is considered to be due to aggregation. Results demonstrate a consistent and simple to use methodology. Results further indicate that the IVDS instrument can be used for characterization of other virus preparations with equal ease and similar results.