

Sizing Nanoparticles and Macromolecules in Liquids

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

Bibliography

2011

Adsorption and Conformation of Serum Albumin Protein on Gold Nanoparticles Investigated Using Dimensional Measurements and in Situ Spectroscopic Methods

De-Hao Tsai, Frank W. DelRio, Athena M. Keene, Katherine M. Tyner, Robert I. MacCuspie, Tae Joon Cho, Michael R. Zachariah, and Vincent A. Hackley, *Langmuir*, 2011, **27**(6), pp 2464–2477

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 γ value is high, where γ 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°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 $\mu\text{mol/L}$, which is roughly consistent with fluorescence and ATR-FTIR results. The equilibrium binding constant for BSA on AuNPs is calculated by applying the Langmuir equation, with resulting values ranging from 0.51×10^6 to 1.65×10^6 L/mol, 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 α -helix content and more open structures (β -sheet, random, or expanded).

Assembly of a Fragmented Ribonucleotide Reductase by Protein Interaction Domains Derived from a Mobile Genetic Element

Mikael Crona, Connor Moffatt, Nancy C. Friedrich, Anders Hofer, Britt-Marie Sjöberg, and David R. Edgell, *Nucleic Acids Res.* 2011, **39**(4):1381–1389.

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 intron-encoded homing endonucleases and inteins. 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.



TRUST. SCIENCE. INNOVATION.

Combined Electrospray-SMPS and SR-SAXS Investigation of Colloidal Silica Aggregation. Part I. Influence of Starting Material on Gel Morphology

Johnsson AC, Camerani MC, Abbas Z., Department of Chemistry, University of Gothenburg, Göteborg, Sweden, *J Phys Chem B*. 2011 Feb 10, **115(5)**:765-75. Epub 2011 Jan 6.

The slow aggregation of monodisperse, polydisperse, and preaggregated silica nanoparticles was studied with an electrospray-scanning 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.

Formation of Paratacamite Nanomaterials via the Conversion of Aged and Oxidized Copper Nanoparticles in Hydrochloric Acidic Media

Sherrie Elzey, Jonas Baltrusaitis, Shaowei Bian and Vicki H. Grassian, *J. Mater. Chem.*, 2011, **21**, 3162-3169

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 (γ -Cu₂(OH)₃Cl) 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.

Hydrodynamic Fractionation of Finite Size Gold Nanoparticle Clusters

De-Hao Tsai, Tae Joon Cho, Frank W. DelRio, Julian Taurozzi, Michael R. Zachariah, and Vincent A. Hackley, *J. Am. Chem. Soc.*, 2011, **133(23)**, pp 8884–8887

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 MALS 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.

Quantification and Compensation of Nonspecific Analyte Aggregation in Electrospray Sampling

Mingdong Li, Suvajyoti Guha, Rebecca Zangmeister, Michael J. Tarlov & Michael R. Zachariah, *Aerosol Science & Technology*, 2011, **45(7)**:849-860

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. The model 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.

Quantitative Determination of Competitive Molecular Adsorption on Gold Nanoparticles Using Attenuated Total Reflectance–Fourier Transform Infrared Spectroscopy

De-Hao Tsai, Melissa Davila-Morris, Frank W. DelRio, Suvajyoti Guha, Michael R. Zachariah, and Vincent A. Hackley, *Langmuir*, 2011, **27**(15), pp 9302–9313

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 (Mm). Equilibrium binding constants for SH-PEG, obtained using the Langmuir adsorption model, show the binding affinity for SH-PEG is proportional to Mm. 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. Electrostatic 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 Mm 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 Mm values.

Synthesis, Characterization and Particle Size Distribution of TiO₂ Colloidal Nanoparticles

Zareen Abbas, Jenny Perez Holmberg, Anna Karin Hellström, Magnus Hagström, Johan Bergenholtz, Martin Hassellöv and Elisabet Ahlberg, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, Volume **384**, Issues 1-3, 5 July 2011, pp 254-261

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 TiO₂ nanoparticles in the size range of 7-20 nm under well controlled experimental conditions. A simple method based on the hydrolysis of TiCl₄ was used to obtain particles having surfaces free from organics. Stable dispersions of TiO₂ nanoparticles of various sizes were obtained by optimizing the reaction/dialysis time and temperature. The synthesized TiO₂ particles were found to be predominantly of anatase phase and narrow particle size distributions were obtained. The TiO₂ 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 electrostatic 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.

Using Nano-electrospray Ion Mobility Spectrometry (GEMMA) to Determine the Size and Relative Molecular Mass of Proteins and Protein Assemblies: a Comparison with MALLS and QELSE

Kapellios S., Karamanou M. F., Sardis M., Aivaliotis A., Economou S. A., Pergantis, *Analytical Bioanalytical Chemistry*, 2011, **399**:2411-2433

Ozone-driven chemistry is a source of indoor secondary pollutants of potential health concern. This study investigates secondary air pollutants formed from reactions between constituents of household products and ozone. Gas-phase product emissions were introduced along with ozone at constant rates into a 198-L Teflon-lined reaction chamber. Gas-phase concentrations of reactive terpenoids and oxidation products were measured. Formaldehyde was a predominant oxidation byproduct for the three studied products, with yields for most conditions of 20-30% with respect to ozone consumed. Acetaldehyde, acetone, glycolaldehyde, formic acid, and acetic acid were each also detected for two or three of the products. Immediately upon mixing of reactants, a scanning mobility particle sizer detected particle nucleation events that were followed by a significant degree of secondary particle growth. The production of secondary gaseous pollutants and particles depended primarily on the ozone level and was influenced by other parameters such as the air-exchange rate. Hydroxyl radical concentrations in the range 0.04–200 × 10⁵ molecules/cm³ were determined by an indirect method. OH concentrations were observed to vary strongly with residual ozone level in the chamber, which was in the range 1-25 ppb, as is consistent with expectations from a simplified kinetic model. In a separate chamber study, we exposed the dry residue of two products to ozone and observed the formation of gas-phase and particle-phase secondary oxidation products.

2010

Agglomeration, Isolation and Dissolution of Commercially Manufactured Silver Nanoparticles in Aqueous Environments

Sherrie Elzey and Vicki H. Grassian, *Journal of Nanoparticle Research*, 2010, Volume **12**, Number **5**, 1945-1958

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, 0.5–6.5, 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.

Analysis of Gold Nanoparticles by Electrospray Differential Mobility Analysis (ES-DMA)

NIST-NCL Joint Assay Protocol, PCC-10, Version 1.1, 2010

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 μ L of nanoparticle solution of the following concentrations: ~10 nm particles at [$\sim 5 \times 10^{12}$ particles/mL], ~30 nm particles at [$\sim 2 \times 10^{11}$ particles/mL], and ~60 nm particles at [$\sim 3 \times 10^{10}$ particles/mL]. In any case, the particle number concentration of the test sample should not exceed $\sim 2 \times 10^{13}$ particles/mL for citrate-stabilized gold.

Competitive Adsorption of Thiolated Polyethylene Glycol and Mercaptopropionic Acid on Gold Nanoparticles Measured by Physical Characterization Methods

De-Hao Tsai, Frank W. DelRio, Robert I. MacCuspie, Tae Joon Cho, Michael R. Zachariah and Vincent A. Hackley, *Langmuir*, 2010, **26(12)**, pp 10325–10333

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.

GEMMA and MALDI-TOF MS of Reactive PEGs for Pharmaceutical Applications

Kemptner J, Marchetti-Deschmann M, Siekmann J, Turecek PL, Schwarz HP, Allmaier G., *J Pharm Biomed Anal.* 2010 **52(4)**:432-7

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 \pm 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.

Measurement of Retention Efficiency of Filters against Nanoparticles in Liquids using an Aerosolization Technique

Tsz Yan Ling, Jing Wang and David Y. H. Pui, Particle Technology Laboratory, Department of Mechanical Engineering, University of Minnesota, Minneapolis, Minnesota, *Environ. Sci. Technol.*, 2010, **44(2)**, pp 774–779

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.

Nanoparticle Dissolution from the Particle Perspective: Insights from Particle Sizing Measurements

Sherrie Elzey and Vicki H. Grassian, *Langmuir*, 2010, **26(15)**, pp 12505–12508

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.

Optimizing the Yield and Selectivity of High Purity Nanoparticle Clusters

Leonard F. Pease, *Journal of Nanoparticle Research*, 2010, **13(5)**:2157-2172.

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.

Packing and Size Determination of Colloidal Nanoclusters

Leonard F. Pease, III, De-Hao Tsai, Joshua L. Hertz, Rebecca A. Zangmeister, Michael R. Zachariah and Michael J. Tarlov, *Langmuir*, 2010, **26(13)**, pp 11384–11390

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 important roles 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 can 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.

Silver Nanoparticles in Simulated Biological Media: a Study of Aggregation, Sedimentation, and Dissolution

Larissa V. Stebounova, Ethan Guio and Vicki H. Grassian, *Journal of Nanoparticle Research*, 2010, **Volume 13, Number 1**, 233-244

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.

Structural Analysis of Soft Multicomponent Nanoparticle Clusters

Leonard F. Pease, Jeremy I. Feldblyum, Silvia H. DePaoli Lacaerda, Yonglin Liu, Angela R. Hight Walker, Rajasekhar Anumolu, Peter B. Yim, Matthew L. Clarke, Hyeong Gon Kang, and Jeesong Hwang, *ACS Nano*, 2010, **4(11)**, pp 6982–6988

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.

Uptake of Gold Nanoparticles in Murine Macrophage Cells without Cytotoxicity or Production of Pro-inflammatory Mediators

Qin Zhang, Victoria M. Hitchins, Amanda M. Schrand, Saber M. Hussain, & Peter L. Goering, *Nanotoxicology*, September 17, 2010

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- α), 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.

2009

Aggregation Kinetics of Colloidal Particles Measured by Gas-Phase Differential Mobility Analysis

D.-H. Tsai, L. F. Pease III, R. A. Zangmeister, M. J. Tarlov and M. R. Zachariah, *Langmuir*, 2009, **25(1)**, pp 140–146

We demonstrate the utility of electrospray gas-phase ion-mobility analysis as a new method to investigate nanoparticle flocculation, or aggregation. Au 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 tetramers 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 good 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 selection of aggregates.

Length Distribution of Single-Walled Carbon Nanotubes in Aqueous Suspension Measured by Electrospray Differential Mobility Analysis

Leonard F. Pease III, De-Hao Tsai, Jeffery A. Fagan, Barry J. Bauer, Rebecca A. Zangmeister, Michael J. Tarlov, Michael R. Zachariah, *Small*, Volume 5, Issue 24, pp. 2894–2901, December 18, 2009

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 multiangle 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.

Morphology of Single-Wall Carbon Nanotube Aggregates Generated by Electro spray of Aqueous Suspensions

Bon Ki Ku and Pramod Kulkarni, *Journal of Nanoparticle Research*, 2009 **11(6)**:1393-1403

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.

Particle Concentration Measurement of Virus Samples using Electro spray Differential Mobility Analysis and Quantitative Amino Acid Analysis

Cole KD, Pease LF 3rd, Tsai DH, Singh T, Lute S, Brorson KA, Wang L., *J Chromatogr A.*, 2009, **1216(30)**:5715-22.

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 ϕ X174). 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.

Quantitative Characterization of Virus-Like Particles by Asymmetrical Flow Field Flow Fractionation, Electro spray Differential Mobility Analysis, and Transmission Electron Microscopy

Leonard F. Pease III, Daniel I. Lipin, De-Hao Tsai, Michael R. Zachariah, Linda H.L. Lua, Michael J. Tarlov, Anton P.J. Middelberg, *Biotechnology and Bioengineering*, 2009, **102(3)**:845 – 855

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. *Biotechnol. Bioeng.* 2009; 102: 845–855. © 2008 Wiley Periodicals, Inc.

Synthesis and Characterization of Superparamagnetic Nanoparticles Coated with Carboxymethyl Starch (CMS) for Magnetic Resonance Imaging Technique

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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 (mesalamine) and analyzed using various release kinetic studies.

2008

Aggregation of Nanosized Colloidal Silica in the Presence of Various Alkali Cations Investigated by the Electrospray Technique

Ann-Catrin J. H. Johnson, Peter Greenwood, Magnus Hagström, Zareen Abbas and Staffan Wall, *Langmuir*, 2008, **24(22)**, pp 12798–12806

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.

Determination of Protein Aggregation with Differential Mobility Analysis: Application to IgG Antibody

http://www.enme.umd.edu/~mrz/pdf_papers/2008_BTech_BEng_Leonard.pdf

Leonard F. Pease III, John T. Elliott, De-Hao Tsai, Michael R. Zachariah, Michael J. Tarlov, *Biotechnology and Bioengineering*, 2008, **101(6)**:1214-1222

Here we describe the use of electrospray differential mobility analysis (ES-DMA), also known as gas-phase electrophoretic mobility molecular analysis (GEMMA), 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.

Direct determination of Lipoprotein Particle Sizes and Concentrations by Ion Mobility Analysis Caulfield MP, Li S, Lee G, Blanche PJ, Salameh WA, Benner WH, Reitz RE, Krauss RM., *Clin Chem.* 2008, **54(8)**:1307-16.

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 totaling the particle number across a predetermined size range that corresponds to particular lipoprotein subclasses. 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 <1.0%. 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.

Gas-Phase Electrophoretic Molecular Mobility Analysis of Size and Stoichiometry of Complexes of a Common Cold Virus with Antibody and Soluble Receptor Molecules

Christian Laschober, Juergen Wruss, Dieter Blaas, Wladyslaw W. Szymanski, and Günter Allmaier, *Anal. Chem.*, 2008, **80(6)**:2261–2264

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 electrophoretic molecular mobility analyzer (nESI-GEMMA). The virus mass, as determined via nESI-GEMMA, was within instrument accuracy ($\pm 6\%$) close to the theoretical value (8×10^6 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 12.5×10^6 Da corresponding to 30 attached antibodies) and virus–receptor complexes (up to a mass 8.8×10^6 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.

Gas-Phase Ion-Mobility Characterization of SAM-Functionalized Au Nanoparticles

D-H. Tsai, R. A. Zangmeister, L. F. Pease III, M. J. Tarlov and M. R. Zachariah, *Langmuir*, 2008, **24(16)**, pp 8483–8490

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 $1.0 \times 10^{11} \text{ s}^{-1}$ and an activation energy 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.

Nano ES GEMMA and PDMA, New Tools for the Analysis of Nanobioparticles-Protein Complexes, Lipoparticles, and Viruses

Allmaier G, Laschober C, Szymanski WW., *J Am Soc Mass Spectrom.* 2008 **19(8)**:1062-8.

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 (~35 nm), low-density (~22 nm) and high-density lipoparticles (~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).

Nanoelectrospray Ion Mobility Spectrometry Online with Inductively Coupled Plasma-Mass Spectrometry for Sizing Large Proteins, DNA, and Nanoparticles

Chiara Carazzone, Reingard Raml and Spiros A. Pergantis, *Anal. Chem.*, 2008, **80(15)**:5812–5818

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 electrospraying 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 femtomole 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 $^{133}\text{Cs}^+$ is observed, thus hinting at the possibility of further developing the technique for quantitative analysis of large biomolecules.

Oligomerization Status Directs Overall Activity Regulation of the Escherichia coli Class Ia Ribonucleotide Reductase

Reza Rofougaran, Mikael Crona, Munender Vodnala, Britt-Marie Sjöberg and Anders Hofer, *J Biol Chem.* 2008, **283(51)**:35310-8

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 GEMMA, 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 $\alpha_2\beta_2$ complex formed in the presence of ATP, dTTP, or dGTP with similar substrate specificity as other class Ia RNRs and an

inactive $\alpha_4\beta_4$ complex 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 $\alpha_6\beta_2$ octamer in the presence of ATP (or effector combinations of ATP + dTTP/dGTP) and an inactive $\alpha_6\beta_2$ 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 is still the main regulator of overall activity at physiologically relevant concentrations of nucleotides, which is in common with the mouse enzyme.

Quantifying Size Distributions of Nanolipoprotein Particles with Single-Particle Analysis and Molecular Dynamic Simulations

Craig D. Blanchette, Richard Law, W. Henry Benner, Joseph B. Pesavento, Jenny A. Cappuccio, Vicki Walsworth, Edward A. Kuhn, Michele Corzett, Brett A. Chromy, Brent W. Segelke, Matthew A. Coleman, Graham Bench, Paul D. Hoepflich and Todd A. Sulchek, *Journal of Lipid Research*, 2008, **49**:1420-1430

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.

Structural Properties of Silver Nanoparticle Agglomerates Based on Transmission Electron Microscopy: Relationship to Particle Mobility Analysis

Weon Gyu Shin, Jing Wang, Michael Mertler, Bernd Sachweh, Heinz Fissan, David Y H Pui, *Journal of Nanoparticle Research* (2008), **Volume: 11, Issue: 1**, pp 163-173

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., $d_{pa} = 0.92 A 0.03 d_m$ for particles from 80 to 150 nm. We obtained fractal dimensions of silver agglomerates using three different methods: (1) $D_f = 1.84 A 0.03$, $1.75 A 0.06$, and $1.74 A 0.03$ for $d_m = 80$, 120 , and 150 nm, respectively from projected TEM images using a box counting algorithm; (2) fractal dimension (D_{fL}) = 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_{fm}) = 1.71 theoretically derived from the mobility analysis proposed by Lall and Friedlander (2006) *J 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, $d_{pa} = 0.92 A 0.03 d_m$ or from using the mobility analysis.

When Size Really Matters: Size-Dependent Properties and Surface Chemistry of Metal and Metal Oxide Nanoparticles in Gas and Liquid Phase Environments

Vicki H. Grassian, *J. Phys. Chem. C*, 2008, **112(47)**:18303–18313

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.

2007

Determination of Molecular Weight, Particle Size, and Density of High Number Generation PAMAM Dendrimers Using MALDI-TOF-MS and nES-GEMMA

Roland Müller, Christian Laschober, Wladyslaw W. Szymanski, and Günter Allmaier, *Macromolecules*, 2007, **40(15)**:5599–5605

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 nano-electrospray 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 $\pm 0.9\%$), 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.

Electrospraying of Colloidal Nanoparticles for Seeding of Nanostructure Growth

P H Michael Böttger, Zhaoxia Bi, David Adolph, Kimberly A Dick, Lisa S Karlsson, Martin N A Karlsson, Brent A Wacaser and Knut Deppert, *Nanotechnology*, 2007, **18(10)**:5304-5309

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 analyser. This method is not material dependent and could be extended to deposit any colloidal particle.

Native Protein MS and Ion Mobility: Large Flying Proteins with ESI

Catherine S. Kaddis and Joseph A. Loo, *Anal. Chem.*, 2007, **79(5)**:1778–1784

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 multisubunit 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.

Protein Complexes in the Gas Phase: Technology for Structural Genomics and Proteomics

Justin L. P. Benesch, Brandon T. Ruotolo, Douglas A. Simmons, and Carol V. Robinson U.K., *Chem. Rev.*, 2007, **107(8)**:3544–3567

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.

Quantifying the Surface Coverage of Conjugate Molecules on Functionalized Nanoparticles

Leonard F. Pease III, De-Hao Tsai, Rebecca A. Zangmeister, Michael R. Zachariah, and Michael J. Tarlov, *J. Phys. Chem. C*, 2007, **111(46)**, pp 17155–17157

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.

Sizing Large Proteins and Protein Complexes by Electrospray Ionization Mass Spectrometry and Ion Mobility

Catherine S. Kaddis, Shirley H. Lomeli, Sheng Yin, Beniam Berhane, Marcin I. Apostol, Valerie A. Kickhoefer, Leonard H. Rome, and Joseph A. Loo, *J Am Soc Mass Spectrom.* 2007, **18(7)**:1206–1216.

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 termed GEMMA (Gas-Phase Electrophoretic Mobility Molecular Analysis), 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 to 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/cm^3 . Moreover, there is evidence that proteins and protein complexes collapse or become more compact in the gas phase in the absence of water.

Structural Analysis of Soft Multicomponent Nanoparticle Clusters

Leonard F. Pease, Jeremy I. Feldblyum, Silvia H. DePaoli Lacaerda, Yonglin Liu, Angela R. Hight Walker, Rajasekhar Anumolu, Peter B. Yim, Matthew L. Clarke, Hyeong Gon Kang, and Jeeseong Hwang, *ACS Nano*, 2010, **4(11)**, pp 6982–6988

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.

Study of the Mobility, Surface Area, and Sintering Behavior of Agglomerates in the Transition Regime by Tandem Differential Mobility Analysis

Cho, Kuk, Hogan, Christopher, Biswas, Pratim, *Journal of Nanoparticle Research*, 2007, **9(6)**:1003-1012.

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 & Biswas (2006a) as well as with the model proposed Koch & Friedlander (1990) for sintering by viscous flow.

Superparamagnetic Iron Oxide Nanoparticles Functionalized with Peptides by Electrostatic Interactions

Nicole Hildebrandt, Dana Hermsdoft, Ruth Signorell, Stephan A. Schmitz, and Ulf Diederichsen, *ARKIVOC*, 2007(v)79-90

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.

2006

Charge Reduced Electrospray Size Spectrometry of Mega- and Gigadalton Complexes: Whole Viruses and Virus Fragments

Christopher J. Hogan, Jr., Eric M. Kettleson, Bala Ramaswami, Da-Ren Chen, and Pratim Biswas, *Anal. Chem.*, 2006, **78(3)**, pp 844–852

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 ± 1.02 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 λ , which has a noncontractile tail. Bacteriophage λ 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.

Direct Characterization of Protein Complexes by Electrospray Ionization Mass Spectrometry and Ion Mobility Analysis

Joseph A. Loo, Catherine S. Kaddis, *Book Mass Spectrometry of Protein Interactions*, December 2006

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.

Enzymatically Active Mammalian Ribonucleotide Reductase Exists Primarily as an $\alpha 6\beta 2$ Octamer

Reza Rofougaran, Munender Vodnala and Anders Hofer, *The Journal of Biological Chemistry*, 22006, **81**:27705-27711.

Gas-phase electrophoretic-mobility macromolecule analysis (GEMMA)² is a relatively new method to study protein complexes in solution: a diluted protein sample (usually 10 ng/ μ l) 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 $\pm 5.6\%$ (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 $\alpha 2\beta 2$ 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 $\alpha 6\beta 2$ complexes. The $\alpha 6\beta 2$ complex could either be in a hyperactive form in the presence of ATP or in an inactive form in the presence of dATP.

The Potential of Differential Mobility Analysis Coupled to MS for the Study of Very Large Singly and Multiply Charged Proteins and Protein Complexes in the Gas Phase

Juan Fernández de la Mora Dr, Sven Ude, Bruce A. Thomson, *Biotechnol J*. 2006 **(9)**:988-97.

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.

The Vault Exterior Shell is a Dynamic Structure that Allows Incorporation of Vault Associated Proteins into its Interior

Michael J. Poderycki, Valerie A. Kickhoefer, Catherine S. Kaddis, Sujna Raval-Fernandes, Erik Johansson, Jeffrey I. Zink, Joseph A. Loo, and Leonard H. Rome, *Biochemistry*. 2006, **45**(39):12184-12193.

Vaults are 13 million Dalton 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 Sf9 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 interior. 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 be functionally interactive with the cellular milieu.

2005

Changes in the Shape and Mobility of Colloidal Gold Nanorods with Electrospray and Differential Mobility Analyzer Methods

Dong Keun Song, I. Wuled Lenggoro, Yoshimasa Hayashi, Kikuo Okuyama, and Sang Soo Kim, *Langmuir*, 2005, **21(23)**, pp 10375–10382

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.

Electrospray Ionization Mass Spectrometry and Ion Mobility Analysis of the 20S Proteasome Complex

Joseph A. Loo, Beniam Berhane, Catherine S. Kaddis, Kerry M. Wooding, Yongming Xie, Stanley L. Kaufman and Igor V. Chernushevich, *Journal of the American Society for Mass Spectrometry* 2005, **16(7)**:998-1008

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 *Methanosarcina thermophila* and rabbit. ESI-MS measurements with a quadrupole time-of-flight analyzer of the 192 kDa $\alpha 7$ -ring and the intact 690 kDa $\alpha 7\beta 7\beta 7\alpha 7$ 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 $\alpha 7\beta 7\beta 7\alpha 7$ 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 $\alpha 7$ -ring and the $\alpha 7\beta 7\beta 7\alpha 7$ 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.

2004

Charge-Induced Unfolding of Multiply Charged Polyethylene Glycol Ions

S. Ude, J. Fernández de la Mora, and B. A. Thomson, *J. Am. Chem. Soc.*, 2004, **126(38)**:12184–12190

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

Electrospray Ion Mobility Spectrometry of Intact Viruses

Thomas JJ, Bothner B, Traina J, Benner WH, Siuzdak G, *Spectroscopy*, 2004, **18**:31-36.

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.

Investigation of Intact Protein Complexes by Mass Spectrometry

Albert J.R. Heck and Robert H.H. van den Heuvel, *Mass Spectrometry Reviews*, 2004, **23**:368-389

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.

Mass Analysis of Water-Soluble Polymers by Mobility Measurement of Charge-Reduced Ions Generated by Electrosprays

Saucy DA, Ude S, Lenggoro IW, de la Mora JF, *Anal. Chem.*, 2004, **76**:1045 1053.

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 ρ independent of size. The slope of the d versus $m^{1/3}$ curve provides a density $\rho = 1.25 \text{ g/cm}^3$, close to the known bulk density, $\rho(\text{PEG}) = 1.21 \text{ g/cm}^3$.

Mass Distribution Measurement of Water-Insoluble Polymers by Charge-Reduced Electrospray Mobility Analysis

Ku BK, de la Mora JF, Saucy DA, Alexander, JN, *Anal. Chem.*, 2004, **76**:814 822.

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 ($M = 9.2, 34.5, 68, \text{ and } 170 \text{ kDa}$) 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 ($M = 49 \text{ kDa}$). 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.

2003

Measurement of Cluster Ions and Residue Nanoparticles from Water Samples with an Electrospray/Differential Mobility Analyzer

Han B, Lenggoro IW, Choi M, Okuyama K., *Anal. Sci.* 2003 Jun; **19(6)**:843-51.

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 $9.1 \text{ and } 9.3 \times 10^{-5} \text{ m}^2/(\text{V s})$ 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.

2002

Sizing of Colloidal Nanoparticles by Electrospray and Differential Mobility Analyzer Methods

Wuled Lenggoro, Bin Xia, and Kikuo Okuyama, Juan Fernandez de la Mora, *Langmuir*, 2002, **18(12)**, pp 4584–4591

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.

2001

Charge-Reduced Nano Electrospray Ionization Combined with Differential Mobility Analysis of Peptides, Proteins, Glycoproteins, Noncovalent Protein Complexes and Viruses

Gerold Bacher, Wladyslaw W. Szymanski, Stanley L. Kaufman, Peter Zöllner, Dieter Blaas, Günter Allmaier, *Journal of Mass Spectrometry*. 2001 **36(9)**:1038-52.

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 $\pm 5.6\%$ up to 2 MDa at the femtomole 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.

2000

Electrospray Diagnostics Performed by using Sucrose and Proteins in the Gas-Phase Electrophoretic Mobility Molecular Analyzer (GEMMA)

Kaufman SL, *Anal. Chim. Acta*, 2000, **406**:3-10.

The proteins α -lactalbumin (14 KDa), conalbumin (77 KDa), and ferritin (460 KDa) were dissolved in 20 mM NH_4OAc along with controlled amounts of sucrose. The solution was electrosprayed into 160 nm diameter droplets in a charge-neutralized electrospray [D.-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.

1999

Characterization of Purified MS2 Bacteriophage by the Physical Counting Methodology used in the Integrated Virus Detection System (IVDS)

Wick CH, McCubbin PE, *Toxicological methods*, 1999, **9(4)**:245-252

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 1.5×10^5 for the neat sample (DPM14), followed by 6.5×10^4 viruses (DPM13), 1.2×10^4 viruses (DPM12), 9.3×10^2 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.

1998

Analysis of a 3.6 MDa Hexagonal Bilayer Hemoglobin from Lumbricus Terrestris using an Electrospray Gas Phase Electrophoretic Mobility Molecular Analyzer

Kaufman SL, Kuchumov AR, Kazakevich M, Vinogradov SN, *Anal. Biochem.*, 1998, **259**:195-202.

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.

Analysis of Biomolecules using Electrospray and Nanoparticle Methods: The Gas-Phase Electrophoretic Mobility Molecular Analyzer (GEMMA)

Kaufman SL, *J. Aerosol Sci.*, 1998, **29**:537

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.

1996

Macromolecule Analysis Based on Electrophoretic Mobility in Air: Globular Proteins

Kaufman SL, Skogen JW, Dorman FD, Zarrin F, Lewis KC, *Anal. Chem.*, 1996, **68**:1895-1904.

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.

1986

Particle Size Analysis of Carbon Black

Blackford, David B.; Simons, Gary R., 1986, *Particle and Particle Systems Characterization*, **4(1-4)**:112-117

A new technique is described for measuring the size distribution of submicrometer sized powders, such as carbon black. Powder is first dispersed as an aerosol and the subsequent particle size measured with a differential mobility classifier and an aerosol concentration detector. The technique has been used to provide a size distribution for seven ASTM/D1765 designated grades of carbon black (N110, N234, N299, N330, N650, N683, N762). In addition, four of these samples (N234, N330, N683, N762) are standards designated by ASTM D24 committee on carbon black as Standard Reference Blacks (C3, B3, D3, A3, respectively). This paper will discuss the effectiveness of the dispersion technique and present distribution data on the seven ASTM grades of carbon black.

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