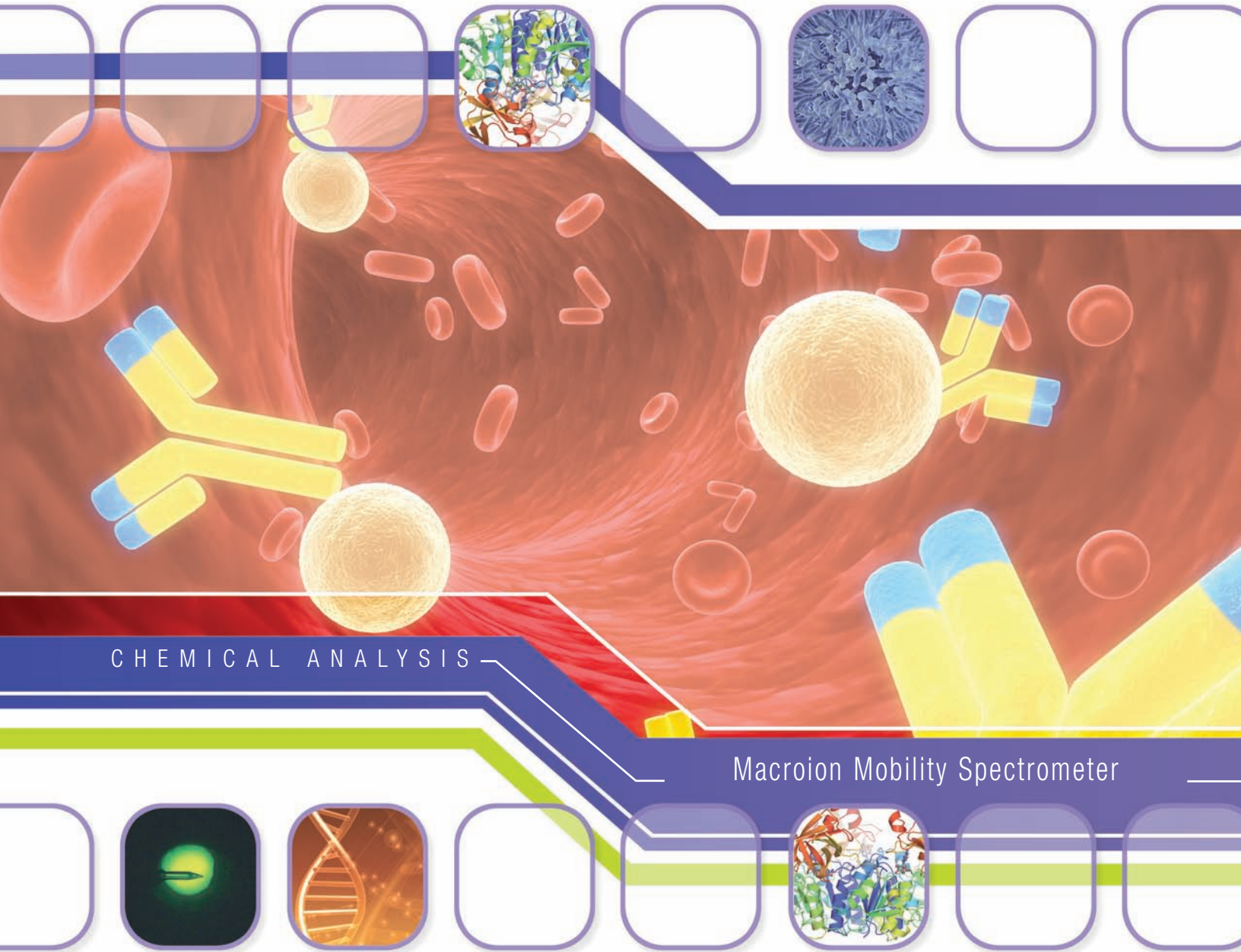


5kDa to > 100 MDa Macromolecule Analysis in Seconds



TRUST. SCIENCE. INNOVATION.



macroIMSTM

The ability to rapidly analyze macromolecules in the mass range of 5 kDa to greater than 100 MDa has been realized with TSI's new macroIMSTM Macroion Mobility Spectrometer. The Model 3980C macroIMS system is an instrument that utilizes:

- a charge-reduced nanoelectrospray ionization source to generate gas-phase macroions
- an ion mobility drift cell to separate these large molecular weight macromolecules
- a new macroion detector to quantify macroions at a given mobility

The macroIMS system operates at near atmospheric pressure, with scan times as fast as 30 seconds. It has demonstrated analysis of complex mixtures of proteins, noncovalent biocomplexes, PEGylated compounds, and other macromolecules.

Macromolecules that have molecular weights on the order of kilodaltons or megadaltons are large enough to have mobility diameters in the nanometer size range. Macroion mobility spectrometry is based on TSI's established technology for mobility sizing of nanoparticles. The macroIMS system measures the mobility diameter of singly-charged macromolecules (or nanoparticles) in the size range of 2.5 to 165 nm. Due to a strong correlation between macroion size and molecular weight, a size spectrum and/or mass spectrum can be generated.



5kDa to > 100MDa

Operation

The macrolMS system, at the front-end, uses nanoelectrospray ionization (nanoESI) to convert solution-phase analytes into gas-phase (macro) ions. The nanoESI is accomplished using a fused silica capillary with a 25 μm ID tip, where the applied HV and sheath gas are optimized for a stable Taylor cone. Multiply-charged ions which are inherently created from the nanoESI are then charge-reduced with bipolar air molecules (generated from Po210 alpha emitting source). Charge reduction changes the macroions into primarily neutral and singly-charged macromolecules. The resulting +1 charge state macroions from this Model 3480C Charge-reduced ESI source are then transported to a Model 3085C Ion Mobility Drift Cell.

The ion mobility drift cell separates macroions. The macroions in the drift cell are under the influence of an electric field and are in a laminar flow of air at atmospheric pressure. The separation is accomplished in an annulus of concentric tubes where macroions with the appropriate mobility are selected into a slit on the central tube for detection (see flow path diagram above). Macroions that do not have the appropriate mobility for selection are lost to the walls of the drift cell. The electric field in the drift cell is created by applying a voltage to the central tube. Changing the electric field in the drift cell influences the trajectory of charged macromolecules, and in this manner different mobility macroions are selected for detection.

The Model 3776C Macroion Detector is used to quantify macromolecules at a given mobility. The detector works by condensing a vapor onto the macromolecules forming liquid droplets. These droplets are large enough to be counted individually when they pass through a laser and photodetector assembly. The Model 3776C has a lower size limit of 2.5 nm (\sim 5 kDa).

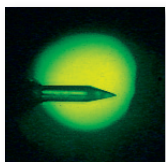
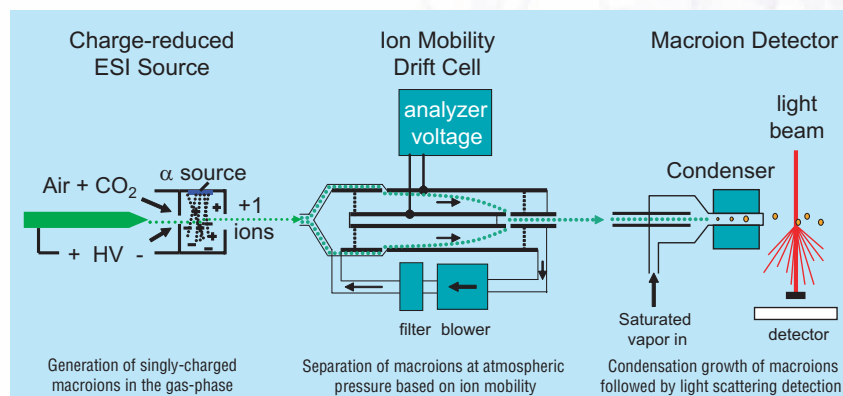
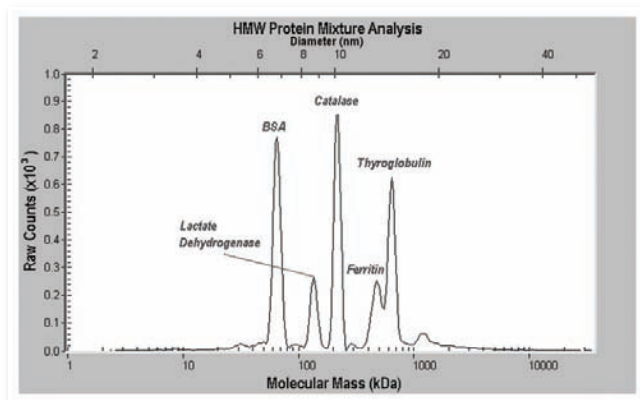


Image of stable cone-jet mode as seen through viewing port on the Charge-reduced ESI Source.

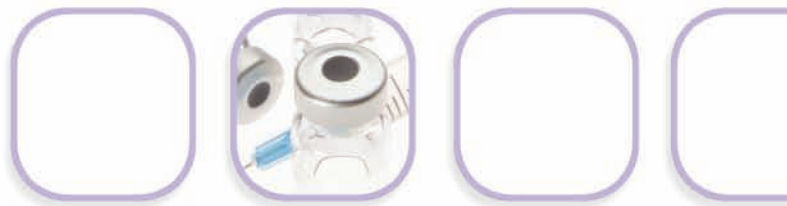
How macrolMS Works



Simplified schematic of the macrolMS system. The Ion Mobility Drift Cell filters singly-charged macroions within a narrow mobility for detection.



MacrolMS spectrum of High Molecular Weight (HMW) protein mixture standard (Amersham Biosciences, Product # 17-0445-01). Respective peaks represent mixture components ranging from Bovine Serum Albumin (BSA) at 67kDa, to Thyroglobulin at 669kDa.

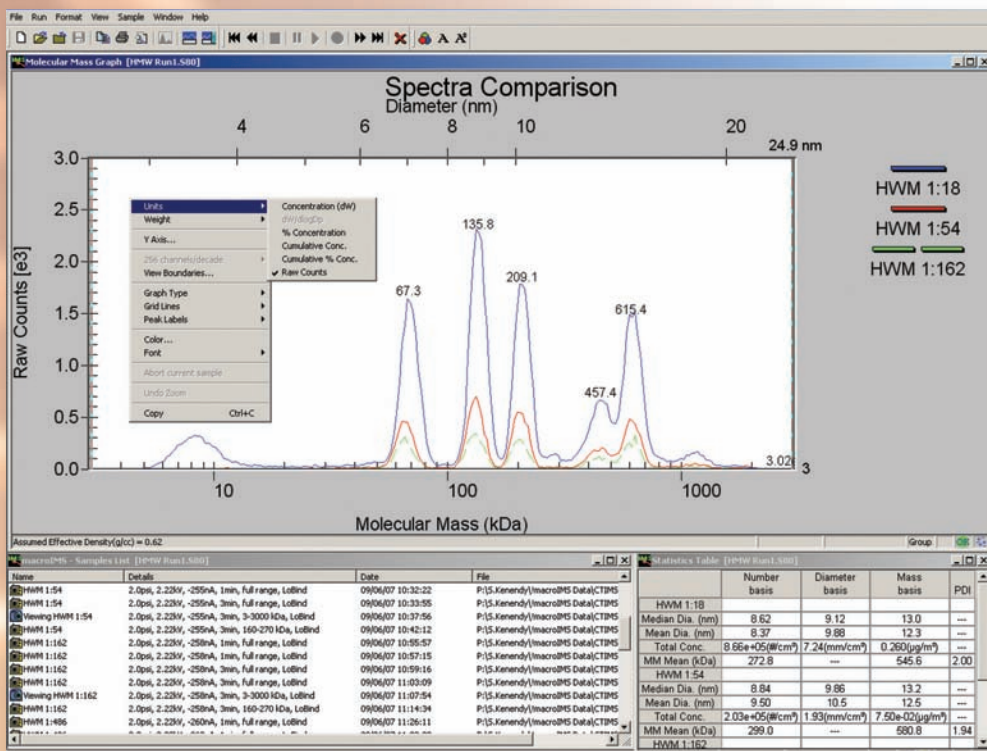


Analyze Proteins in a

Software

TSI's macroIMS software is an easy to use Windows-based package that allows the user to acquire raw data, size spectra, mass spectra, mass average spectra, number average spectra, concentration and cumulative concentration spectra and polydispersity index. Data may be acquired in single scan or average modes, both with user defined scan times and scan range parameters. The software allows the user to focus the scan window on an area of interest in order to improve count statistics when at low concentrations. In addition the program allows for spectral overlay of samples to provide sample comparison with linear y-axis as well as logarithmic y-axis settings. Savitzky-Golay data smoothing may be applied to sample spectra to minimize noise influence by performing local

polynomial regression of the spectra to smooth the points. Smoothing may be performed from 2nd order to 6th order polynomial smoothing with user defined point distribution. Additionally, the software provides an array of analysis tools to determine peak intensity, location and concentration based on line integration with graphical grid display options. Spectra may be inter-converted between line, bar, and area plots for analysis. The robust analytical software allows nearly limitless user control of sample parameters and sample labeling for increased data organization when obtaining an abundance of samples and has the ability to export raw data to ASCII files for import into other spectral analysis and peak fitting software.



Software Features

- Overlay and compare spectra
- Smooth data using Savitzky-Golay
- Print spectra with settings reported
- Calculate Mw, Mn, and PDI

MacroIMS software user interface. Displays real-time spectra and multiple sample spectra overlay (top), multi-sample list display (bottom left), data acquisition-Mw, Mn, PDI-display (bottom right) and easy to navigate drop down data display options (within spectra window, top).

Whole New Way!

Applications

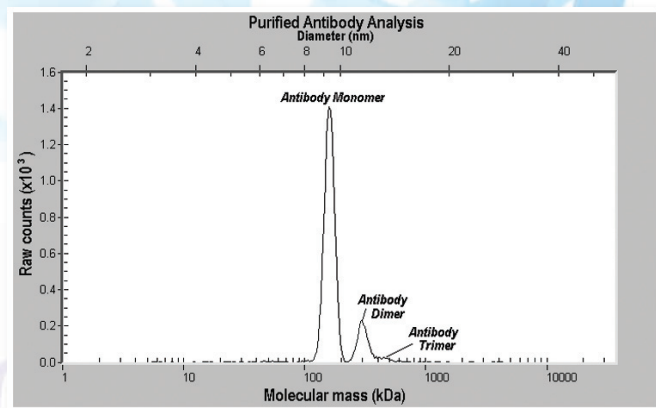
TSI's macrolMS system builds on the Gas-phase Electrophoretic Mobility Macromolecule Analysis (GEMMA) technique that has been successfully applied to:

- 1) Protein and mixture separations (Bacher, G. et al. J. Mass Spectrom. 36:1038-1052, 2001)
- 2) Lipoprotein analysis (Benner et al. US Patent App. 20030136680, July 2003)
- 3) Virus identification (C.H. Wick et al. Tox. Meth. 9:265-273, 1999)
- 4) Structural biology investigations (Henry C.M. From Proteomics to Structural Bio, September 29, 2003. C&EN Magazine)

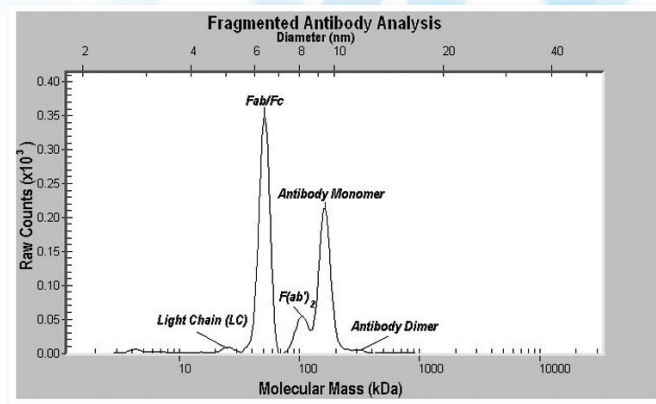
The power of the macrolMS system is its ability to analyze macromolecules that are too large for mass spectrometry (MS), but too small for direct light scattering detection. For instance, protein complexes can consist of proteins as large as 13 MDa, which is much larger than a

MS can analyze, but can be measured by the macrolMS system (see application 4 above). Furthermore, viruses, which are too small to detect with laser light-scattering detectors, and too large to be analyzed intact with MS (normally for MS, viruses would require protein extraction and/or digestion) can be analyzed in whole by the macrolMS system (see application 3 above).

The macrolMS system has proven itself as a novel analytical instrument within the realm of biomedicine as a tool for analysis and visualization of human antibody. TSI's macrolMS allows fast analysis of antibody solutions to assess immunoglobulin integrity. It displays both aggregation and fragmentation products of a soluble antibody sample. This provides major application to the biotechnology industry for immunoglobulin-injectable drug efficacy and long term stability assessment. Aggregation and degradation of antibody products are part of FDA and ICH guideline measurements for biological products and such analyses can be made with the macrolMS system.



MacrolMS spectrum of purified IgG antibody from human serum (Sigma-Aldrich, Product # I4506) with aggregation after storage. Respective peaks display the presence of IgG monomer (150kDa), dimer (300kDa) and trimer (450kDa).



MacrolMS spectrum of IgG antibody from human serum (Sigma-Aldrich, Product # I4506) after fragmentation with the cysteine-endopeptidase Papain. Respective peaks display presence of light chain IgG (25kDa), Fab/Fc fragments (50kDa), $(Fab')_2$ (100kDa) and IgG monomer and dimer (150 & 300kDa respectively).

Applications have also been made in the medical diagnostics field from work by Benner et al. published in U.S. Patent Application 20030136680. In this work, the macrolMS technique was used to separate lipoproteins and do so in a much faster analytical time than gel-based methods. A high-resolution quantitative size spectrum of subfractions in lipoproteins allows better analysis of related illnesses such as heart disease. The fast time response of macrolMS system should permit better prophylaxis.

Analysis of large biocomplexes in the gas phase, and the study of solvent effects on these complexes, are other applications of the macrolMS system. By adding pH and heat gradients to the macrolMS system, applications have been made that explore virus degradation products. Other potential applications, such as protein binding analysis, are also being studied. Binding stoichiometry analysis are also being studied with the macrolMS with work published on covalent (Avidin-Biotin) and non-covalent complexes.

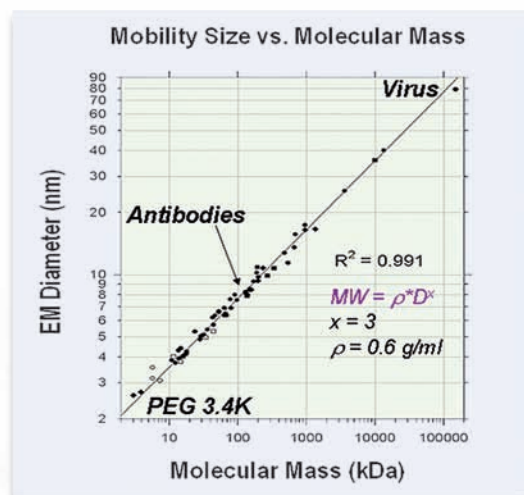




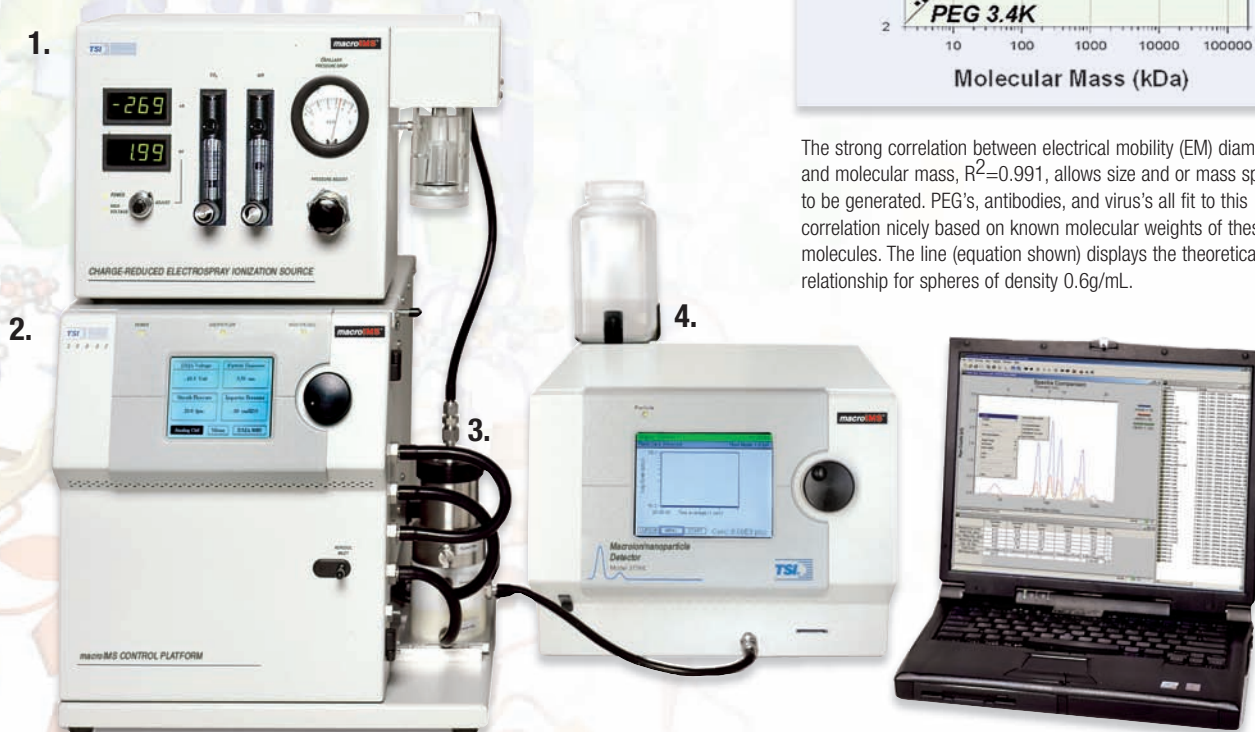
TSI's macroIMS System Offers Novel and Practical Capabilities

Features

- Size and mass analyses of macromolecules, supramolecular complexes, and large biomolecules in the range of 5 kDa to > 100 MDa
- Fast response time of seconds, as compared to half an hour for liquid chromatography
- High sensitivity, as typical protein samples are run at low fmol/μL levels
- Excellent reproducibility
- Small sample consumption (<250 nL/run)
- Dynamic sample concentration range: 10 pM to 100 nM

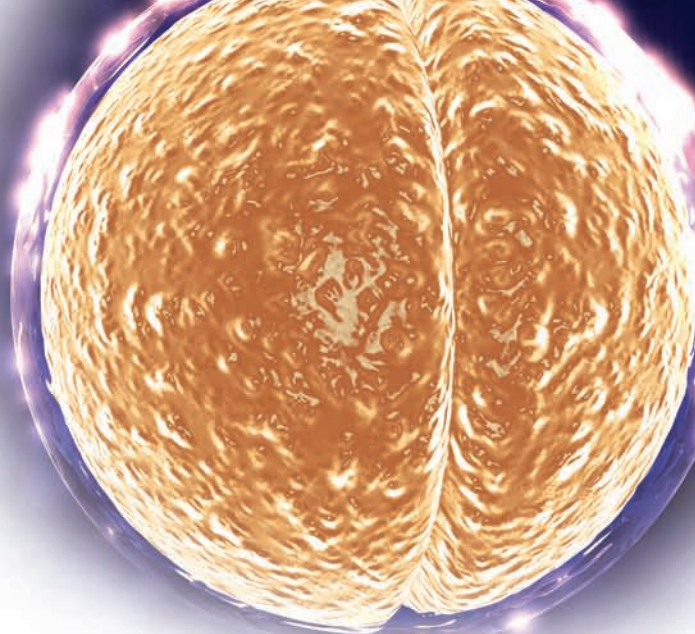


The strong correlation between electrical mobility (EM) diameter and molecular mass, $R^2=0.991$, allows size and or mass spectra to be generated. PEG's, antibodies, and virus's all fit to this correlation nicely based on known molecular weights of these molecules. The line (equation shown) displays the theoretical relationship for spheres of density 0.6g/mL.



The Model 3980C macroIMS, Macroion Mobility Spectrometer system (computer not included). The macroIMS includes the Charge-reduced ESI Source (1), the macroIMS Control Platform (2), the Ion Mobility Drift Cell (3), a Macroion Detector (4), and the MacroIMS Manager Software.

Complexes, and Beyond



Bibliography

Andrien BA, Patel R, Willard L, Alford R, Tan P, An Investigation of Protein Therapeutics Including Antibodies and Antibody Conjugates with Ion mobility Spectrometry, Presented at *American Society for Mass Spectrometry 55th Annual Meeting*, 2007.

Basa LJ, Lancaster K, Shyong B, Tan P, Katta V, Stoichiometry of Antibody and Complexes Measured by Macroion Mobility Spectrometry (IMS), Presented at *American Society for Mass Spectrometry 55th Annual Meeting*, 2007.

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

Loo JA, Kaddis CS, Native Protein MS and Ion Mobility: Large Flying Proteins with ESI, *Analytical Chemistry*, 1778-1784, 2007.

Muller R, Laschober C, Szymanski WW, Allmaier G, Determination of Molecular Weight, Particle Size, and Density of High Number Generation PAMAM Dendrimers Using MALDI-TOF-MS and nES-GEMMA, *Macromolecules*, 40(15), 5599-5605, 2007.

Tan P, Kennedy S, Zerrath A, Mass Analysis from Kilodaltons to Megadaltons Using Macroion Mobility Spectrometry, *Current Trends in Mass Spectrometry*, 26-29, 2007.

Hogan CJ Jr, Kettleton EM, Ramaswami B, Chen D, Biswas P, Charge Reduced Electrospray Size Spectrometry of Mega- and Gigadalton Complexes: Whole Viruses and Virus Fragments, *Analytical Chemistry*, 78, 844-852, 2006.

Rofougaran R, Vodnala M, Hofer A, Enzymatically Active Mammalian Ribonucleotide Reductase Exists Primarily as an a6b2 Octamer, *Journal of Biological Chemistry*, 281(38), 27705-27711, 2006.

Loo JA, Berhane B, Kaddis CS, Wooding KM, Xie Y, Kaufman SL, Chernushevich IV, Electrospray Ionization Mass Spectrometry and Ion Mobility Analysis of the 20S Proteasome Complex, *J. Am. Soc. Mass Spectrom.* 16, 998-1008, 2005.

Ku BK, de la Mora JF, Saucy DA, Alexander, JN, Mass distribution measurement of water-insoluble polymers by charge-reduced electrospray mobility analysis, *Anal. Chem.* 76, 814-822, 2004.

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

Seefeldt MB, Ouyang J, Froland WA, Carpenter JF, Randolph TW, High-pressure Refolding of Bikunin: Efficacy and Thermodynamics, *Protein Science*, 13, 2639-2650, 2004.

Benner WH, Krauss RM, Blanche PJ, Ion mobility analysis of biological particles, US Patent Application, 20030136680, 2003.

Loo JA, Kaufman SL, Chernushevich I, Analysis of Large Supramolecular Protein Complexes by Mass Spectrometry and Gas-Phase Mobility, Presented at *American Society for Mass Spectrometry, Annual Meeting*, Montreal, 2003.

Lenggoro IW, Xia B, Okuyama K, de la Mora JF, Sizing of colloidal nanoparticles by electrospray and differential mobility analyzer methods, *Langmuir*. 18, 4584-4591, 2002.

Shang TQ, Johnston MV, Quantitative Protein Characterization with Gas-Phase Electromobility, Proc. *50th ASMS Conference on Mass Spectrometry and Allied Topics*, Orlando, FL, 2002.

Allmaier G, Bacher G, Zehl M, Sutton C, Kaufman S, Szymanski WW, Gas-Phase Electrophoretic Molecular Mobility Analysis at Atmospheric Pressure of High Mass Hetero- and Homo-Noncovalent Biocomplexes in Comparison with UV MALDI MS Analysis, Proc. *49th ASMS Conference on Mass Spectrometry and Allied Topics*, Chicago, IL., 2001.

Bacher G, Szymanski WW, Kaufman SL, Zoellner P, Blaas D, Allmaier G, Charge-Reduced Nano Electrospray Ionization Combined with Differential Mobility Analysis of Peptides, Proteins, Glycoproteins, Noncovalent Protein Complexes and Viruses, *J. Mass Spectrom.* 36(9), 1038-1052, 2001.

Mouradian S, Skogen JW, Dorman FD, Zarrin F, Kaufman SL, Smith LM, DNA Analysis using an Electrospray Scanning Mobility Particle Sizer, *Anal. Chem.* 69, 919-925, 1997.

Specifications

3980C macroIMS System

Operational

Mass Range	5 kDa to >100 MDa
Size Range	2.5 nm to 165 nm
Limit of Detection	<10 amol/uL (pM) for standard proteins Low ng/mL for 200 kDa proteins
Dynamic Concentration Range	~10 pM to >100,000 pM
Display Resolution	>256 channels per decade of size
Mass Resolution	R~7-10 m/Δm; Δm = FWHM
Mass Accuracy	+/- 5%
Intensity Reproducibility	+/- 5%
Sample Consumption	<250 nL / spectrum
Sensitivity	<10 amol/uL at 180 second scan times
Scan Time	30 seconds to 300 seconds user defined
Sample Flow Rate	50-100 nL/min
Minimum Sample Requirements	20 uL
Typical Sample Volume	50 uL
Vial Size	1.5 mL
Liquid Conductivity	0.2 S/m nominal
System Operating Pressure	Atmospheric

Physical

Alpha Source	Po-210; 5 millicurie
Power Requirements	100-230 VAC, 50/60 Hz
Footprint Area	24 x 32 in. (61 x 155 cm)
Total Weight	60 lbs

Auxiliary

PC Requirements	Intel Pentium 4 or equivalent Processor 256 MB RAM or more 9-pin Serial RS232 Port or USB
Operating System	Windows XP or 2000
Gas Requirements	CO ₂ – 15 psig; Filtered Dry Air – 25 psig

Specifications are subject to change without notice. TSI, the TSI logo and macroIMS are trademarks of TSI Incorporated. Microsoft Windows is a trademark of Microsoft Corporation.

Some macroIMS components include Aerosol Neutralizers, which contain a radioactive source. TSI is authorized by the United States Nuclear Regulatory Commission to distribute these and other Aerosol Neutralizers. If your location is within the United States, no other federal license is required. Check local regulations for your own protection. End-user name and address are required. Aerosol Neutralizers are shipped separately from other system components. To order a macroIMS system without a neutralizer, contact your TSI Representative.

Technology used in the macroIMS system is protected by the United States Patent Numbers 4,790,650; 5,118,959; 6,230,572; 5,076,097 and 5,247,842. A portion of this system was developed in cooperation with the California Institute of Technology and AEA Technology.

TSI Incorporated serves a global market by investigating, identifying and solving measurement problems. As an industry leader in the design and production of precision instruments, TSI partners with research institutions and customers around the world to set the standard for measurements relating to aerosol science, air flow, health and safety, indoor air quality, fluid dynamics and biohazard detection. With headquarters based in the U.S. and field offices throughout Europe and Asia, TSI has established a worldwide presence in the markets we serve. Every day, our dedicated employees turn research into reality.

TSI Incorporated - 500 Cardigan Road, Shoreview, MN 55126-3996 USA			
USA	Tel: +1 800 874 2811	E-mail: chem@tsi.com	Website: www.tsi.com
UK	Tel: +44 149 4 459200	E-mail: tsiuk@tsi.com	Website: www.tsiinc.co.uk
France	Tel: +33 491 95 21 90	E-mail: tsifrance@tsi.com	Website: www.tsiinc.fr
Germany	Tel: +49 241 523030	E-mail: tsigmbh@tsi.com	Website: www.tsiinc.de
India	Tel: +91 80 41132470	E-mail: tsi-india@tsi.com	
China	Tel: +86 10 8260 1595	E-mail: tsibeijing@tsi.com	



TRUST. SCIENCE. INNOVATION.

To Order

macroIMS Macroion Mobility Spectrometer

Specify	Description
3980C	macroIMS Macroion Mobility Spectrometer system

Featuring:

3480C	Charge-reduced ESI Source
3080C	macroIMS Control Platform
3085C	Ion Mobility Drift Cell
3776C	Macroion / nanoparticle Detector
390063C	macroIMS software
1035989C	macroIMS integration and accessory kit

Optional

3074B	Filtered Air Supply
-------	---------------------

Contact your local TSI Distributor or visit our website chem.tsi.com for more detailed specifications.