

Meeting report

Mass Spectrometry of Synthetic-Polymer Mixtures Workshop, Held at the National Institute of Standards and Technology, Gaithersburg, Maryland, USA, 13–14 November 2003

Mass spectrometry is being applied more and more frequently to the analysis of synthetic-polymer mixtures including blends of two or more homopolymers, blends of single homopolymers with different end groups, and copolymers. The NIST "Mass Spectrometry of Synthetic-Polymer Mixtures Workshop" brought together industrial, academic, and government researchers, practitioners, and laboratory managers interested in this burgeoning new field. The central topic was the fundamental aspects of deriving quantitative measures from mass spectral data. This was accomplished via the presentation of new results, discussion of recent trends, and identification of important problems that could limit future progress as well as possible solutions to these problems. The talks focused on measurement methods, including sample preparation, instrument performance, and data analysis. The content of these talks will be reported on here. The visual elements of most of the talks can be found at <http://www.nist.gov/maldi>. In addition to the formal talks, a special session was held where workshop participants presented measurement challenges they have encountered in their own work for comment and discussion by the other workshop participants. This was the second year of the workshop. A report from the first year was published in the January/February 2003 issue of the *Journal of Research of the National Institute of Standards and Technology*, Vol. 108, No. 1, pp 79–85, and is publicly available at <http://www.nist.gov/jres>.

SESSION 1: THE MALDI PROCESS

The first session focused on the matrix-assisted laser desorption/ionization (MALDI) process for creating intact synthetic-polymer macromolecular ions. The first speaker was Prof. Akos Vertes (George Washington University) on the fundamentals of the ionization and desorption mechanisms of MALDI including plume chemistry and ion internal energy. He first discussed experiments comparing nanosecond to picosecond UV laser pulses on pure matrix samples. It was found that the nanosecond laser required half the total fluence of the picosecond laser to produce the same number of molecular matrix ions. The addition of analytes showed that as analyte mass increased, the nanosecond laser produced far more analyte ions than did the picosecond laser. These effects were attributed to temporal overlap between the nanosecond laser and the expanding MALDI plume, which cannot occur when using the picosecond laser. Furthermore, the heavier analytes move slower in the expanding plume increasing the overlap time still further and increasing their ion yield. The addition of laser energy to the expanding plume allows for more sharing of energy

between excited species to create ions. For the picosecond laser most of the laser energy to form ions must be absorbed directly via multiphoton ionization, which is a much less efficient process. The topography of the sample surface was also demonstrated to be important. Pressing matrix powder to form pellets at different pressures formed surfaces of different roughness. Samples pressed under lower pressure had higher fractal dimensions (i.e. were more rough) and produced more molecular matrix ions. This was attributed to the rough edges of the sample surface requiring lower laser energy to desorb into the vacuum. Next Prof. Vertes considered the temperature of the MALDI plume using benzyl-substituted benzylpyridinium salts as 'molecular thermometers'. By measuring the percent of thermometer molecules that fragment as a function of laser power and as a function of matrix, a measure of the internal energy of analyte in the MALDI plume can be made. It was found that the thermometer molecule's internal energy scaled with the laser threshold energy of the matrix. He noted that this seemed to be the opposite trend found in peptide fragmentation studies and suggested that the difference comes from the fact that the benzylpyridinium salts are preformed ions while the peptides must protonate to form an intact molecular ion. This highlighted the subtle interplay between matrix and analyte in the MALDI plume.

The next speaker was Dr. Richard Knochenmuss (Novartis Pharmaceuticals) who presented details of his two-step theory of the MALDI process. In the first step, which occurs during or shortly after the nanosecond-scale laser pulse, primary ionization of matrix molecules occurs via exciton diffusion and pooling in the solid state and in gas-phase clusters of matrix molecules. In the second step, ion-molecular reactions occur in the expanding MALDI plume. Dr. Knochenmuss submitted that in this second stage the reactions that occurred could be treated using equilibrium gas-phase thermodynamics under conditions of ample in-plume molecular collisions (e.g. at sufficient laser power). This crucial insight can be used to explain matrix or analyte suppression effects where cation affinity would be the critical controlling factor and should be predictable by comparing the difference in Gibbs free energy for cationization between the different species present. In addition it accounts for analyte-analyte suppression effects as well. He showed spectra with matching model calculations where one analyte can fully suppress the ionization of a second analyte either through relative concentration effects or through relative cationization energetics. His model also provided good quantitative agreement with changes in laser pulse length and spot-size (each when the laser pulse is held at constant total energy), changes in matrix to analyte ratio, and with time-delayed two-laser-pulse studies. His current work centers on combining his continuum model theory with molecular dynamics calculations to obtain a more complete picture of MALDI plume chemistry on a molecular scale.

SESSION 2: MASS SPECTROMETRY OF POLYMER MIXTURES

The second session focused on the mass spectrometry of polymer mixtures. The first speaker was Prof. Alan Marshall (Florida State University) on the topic of petroleum and liquefied coal, two of the most complex mixtures to be analyzed by mass spectrometry. He used electrospray ionization (ESI), in both positive and negative modes, to create gas-phase ions out of polar molecules, and field ionization/desorption of non-polar molecules, both of which are found in these mixtures. His mass separation method of choice is Fourier transform mass spectrometry (FTMS) at very high magnetic field strengths. Using such high magnetic fields, a 50% peak-to-valley resolution ($m/\Delta m$) of 350 000 can be achieved in certain cases. This means that no isobaric interferences are present, making exact mass determination of chemical composition unambiguous. As an example, the resolution needs to be in the order of 3.4×10^{-3} u to resolve the mass of three carbon atoms versus one sulfur and four hydrogen atoms in a molecule. He went on to demonstrate the use of the Kendrick mass defect to understand mass spectra with more than 20 000 peaks. The Kendrick mass defect sets the mass of the CH_2 group to be exactly 14 u. This renders clear series of ions that differ in only one methylene group versus those that differ in any other functional group by its difference in mass from 14 u. Ultimately, these methods can be used to determine the relative amount of sulfur and other heteroatoms in the analyte that need to be removed chemically before processing into fuel.

The second speaker in the polymer mixtures session was Dr. William Simonsick (DuPont) on the use of gel permeation chromatography (GPC) separation techniques and mass spectrometry to understand complex polymer mixtures. GPC has the benefit of being readily coupled to ESI sources for mass spectrometry. Using FTMS with sufficiently high resolution, the multiple charging effects of ESI can be overcome and series of the same molecule in different charge states can be deconvoluted. However, quantitation can only be achieved using a quantitative chromatographic detector (UV/visible detection and/or evaporative mass detection) and relating its output to chemical composition information derived from mass spectrometry. Dr. Simonsick submitted that mass spectrometry alone could not provide this quantitative detection of polymer mixtures.

The third speaker in the session was Prof. Kevin Owens (Drexel University) on the use of factorial experimental design to quantitate instrument response to controlled mixtures of polymers. The factors studied were relative quantities of polymers A and B, polymer-to-matrix ratio, and laser intensity. The dependent variables included absolute peak areas of polymers A and B, the A/B peak area ratio, and the peak-area/molar-concentration ratio. Using two narrow mass distribution polyethylene glycols, he found that a combination exists of the four variables studied where mass ratio of the polymers in the mixture was quantitatively measured by MALDI-TOFMS. This was a very important demonstration for the future of MALDI-MS in quantitatively measuring molecular mass distribution of synthetic polymers. He next considered how to optimize the salt concen-

tration. For the specific case of poly(methyl methacrylate), 2,5-dihydroxybenzoic acid matrix, and sodium trifluoroacetate salt as the salt concentration increased the instrument response plateaued off such that addition of more salt did not increase signal intensity. However, this point was not a constant for PMMA but was mass-dependent. This indicates that ionization probability increases with polymer mass. Furthermore, when the matrix was changed to indoleacrylic acid, the ion intensity would plateau and then come down indicating that there is an oversaturation effect in the case. For varying matrix-to-analyte ratios, using dithranol as the matrix, a similar saturation effect was seen with the addition of too much analyte. Clearly, further study is required to understand all the interrelationships between variables. Prof. Owens also cautioned that the matrix materials must be free of matrix salts (primarily of sodium and potassium) before these experiments can be conducted. The presence of alkali metal impurities will alter the amount of cationizing salt that needs to be added to the sample, even if the metal salt to be added is different from the metal contaminating the matrix.

The fourth and final talk in the session was given by Prof. Troy Wood (State University of New York-Buffalo) on the structural analysis of polyaniline by MALDI-MS and ESI-MS. With ESI-MS the sample was found to be a mixture, having four different end groups. To verify the structure, collision-induced dissociation (CID) was necessary to isolate each end group of the various oligomers in the mixture. MALDI of polyaniline was found to give a large number of fragments, owing to the fact that the polymer itself absorbed the UV radiation from the laser. With such a high level of fragmentation, quantitation of relative oligomer concentrations in the mixture becomes impossible due to the possibility of different fragmentation probabilities between them. Solvent-less sample preparation, that is grinding together the polyaniline, matrix and salt, gave less fragmentation but still a considerable amount. When a radical scavenger was added to the mixture the fragmentation was further reduced, but not eliminated. Nevertheless, Prof. Wood concluded that the presence of molecular fragmentation was shown to be a barrier to confidently quantitating the various species in the polyaniline mixture.

SESSION 3: COPOLYMERS

After the lunch break the third session on the topic of copolymers was held. The first speaker was Prof. David Hercules (Vanderbilt University) whose first topic described the use of selective chemical reactions to simplify the mass spectra of copolymers. His example was polyurethane where the more-flexible soft block of the polymer could be 'cut out' of the chain and analyzed both by GPC and MALDI-MS. This allowed for the quantitative measure of at least one aspect of the copolymer: the block length of one of the two blocks. He also discussed analyzing polyimides with a similar method first derivatizing the chain before cutting it at specific sites. His second topic covered discrete mass oligomers of poly(butylene glutamate) that could be synthesized as pure samples of the dimer, tetramer, octomer, 16-mer, 32-mer, and 64-mer. When the last four are mixed in equimolar mixtures the MALDI mass spectrum shows a monotonically decreasing

instrument response indicating that there is a strong bias against the high-mass oligomers under the specific conditions studied. Prof. Hercules suggested that these discrete mass oligomers would work well as synthetic polymer standards for mass spectrometry.

The second talk of the session was given by Prof. Murray Johnston (University of Delaware) on the topic of chemical and instrumental approaches to copolymer characterization. His first example was the random copolymer poly(*para*-methyl styrene-*isobutylene*) where he found that the percent *para*-methyl styrene (pMS) by MALDI mass spectrometry was consistently higher than that found by nuclear magnetic resonance. Using Bernoulli statistics, and adjusting the relative ionization efficiency of the two repeat units in the polymer, this effect was traced to the higher ionization probability of the pMS monomer, thus accounting for over counting of such chains in the mixture. His second example was the random copolymer poly(ethylene-co-carbon monoxide). Since each of the two repeat units have the same nominal mass (28 u) one way to determine the monomer concentration in the polymer was by selective chemical reaction with sodium borohydrate which changes the carbonyl to the poly(vinyl alcohol) repeat unit (CHOH) resulting in a 2 u mass increase in those repeat units. While this effectively separates the masses of the two monomers, the completely reduced product is difficult to detect by MALDI. By turning to high mass resolution instead of selective chemical reaction, Prof. Johnston showed that a more effective approach is to perform FTMS, which has the resolving power to distinguish oligomers with different exact masses and hence different monomer compositions.

In the final talk of this session Prof. Liang Li (University of Alberta) spoke on the characterization of poly(ethylene oxide-co-propylene oxide) copolymers. He showed that when two different cations (Li^+ or Co^{2+}) are used in ESI-MS with CID the fragmentation patterns differ in such a way as to allow determination of general copolymer sequence (e.g. block or random). This was attributed to the size and charge state of the cation playing a part in the location along the chain for fragmentation. In his second example MALDI-MS was used as a method to determine the ratio of ethylene oxide and propylene oxide in a random copolymer. This was successful with the use of standards of known monomer feed ratio concentration and the correlation of this concentration with molecular mass. Prof. Li showed that once a calibration curve is produced the relative monomer concentration of any unknown can be determined simply from its number-average molecular mass.

SESSION 4: INSTRUMENTATION AND METHODOLOGY

The first speaker in this session was Prof. Robert Cotter (Johns Hopkins Medical School) on 'Miniaturized Time-of-Flight Mass Spectrometers with Broad Mass Range', a topic of particular importance to polymer mass spectrometrists where materials with broad mass distributions are often encountered. Prof. Cotter first described how simultaneous space and time focusing of the ions coming from the ion source is the prime requirement in obtaining high resolution in time-

of-flight mass separation. He outlined three approaches to improving mass resolution: fixed-delay pulsed ion extraction, mass-correlated ion acceleration, and non-linear-field TOF mass separation. For the first case, he showed how the tuning of the relative lengths and the ion extraction delay time in a dual-stage ion source can place the time focus of the ions at the detector provided the path length of the mass spectrometer is small (on the order of centimeters). He went on to show how properly ramping in time the applied voltage to the source, termed 'mass correlated acceleration', could provide excellent resolution over a wide mass range. The key to TOF mass spectrometer performance lies as much in the source (to produce ions of a narrow energy and spatial distribution) as it does in the details of the ion separation geometry. It was shown that the simple 'end cap reflectron' geometry with its natural non-linear (square-root) field shape can yield excellent resolution with short flight distances provided the ion source has the proper configuration. This could be further improved by using time-varying non-linear ion extraction fields as well.

The final speaker in this session was Dr. Charles Guttman (National Institute of Standards and Technology) on the second NIST interlaboratory comparison, an activity that arose out of a consensus suggestion made by industry at the June 2002 American Society for Mass Spectrometry meeting. This 'round robin' uses two polystyrenes of nearly equal molecular mass distribution each with butyl initiation but one terminated with a proton and the other with ethylene glycol. This gives nominal mass shift between the two series of 44 u, yielding well-separated peaks with only moderate mass resolution required. These two polymers were mixed gravimetrically in mass ratios that were unknown to the participants whose task it was to determine the mass ratio of each polymer in the five mixtures provided. Samples of each polymer component were also provided. The two major challenges are the possibility of end-group effects in the charging on the polystyrenes with silver cations (which the participants could do little about), and issues of data processing due to poor signal-to-noise when one component of the mixture is in the minority (defined as about 10% by mass fraction of the mixture). Dr. Guttman commented that this last scenario seems borne out by the preliminary results. There was a greater variability between participants in cases where one constituent was in the minority in the mixture than in cases where the components were in more or less equal concentration.

LESSONS LEARNED

The lessons learned from the workshop can be grouped into several themes. First, the MALDI and ESI processes are subtle and will require both experimentation and theory to understand fully. Full understanding in this case means the ability to rationally choose matrices, cationizing agents, their ratios compared to the analyte, as well as a sample preparation technique to perform a quantitative, or semiquantitative, mass spectrometry experiment. Such effects as preferential ionization and fragmentation can severely limit reasonable determination of quantitative measures from the data. Second, for complex mixtures, high-resolution mass spectrometry

(either Fourier transform or time-of-flight) is a powerful tool to identify all the components of the mixture; however, quantitation of the amounts of each species in the mixtures requires prior chemical speciation by either chromatographic separation or selective chemical reaction. Third, data analysis remains a stumbling block where unbiased, robust means are required to distill mass spectra down to their essential information content. Especially needed are methods that can work in a high-throughput setting. Along the same lines, the use of chemometrics (e.g. application of the Kendrick mass defect or of Bernoulli distributions) is required to convert the information distilled from the mass spectrum into usable knowledge.

Lastly, the third annual quantitative polymer MS workshop is planned for the fall of 2004 to be held in cooperation with the American Society for Mass Spectrometry.

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