Polymeric Materials Interest Group Workshop: Quantitative Determination of Synthetic Polymer Molecular Mass Distribution

This year's workshop centered around a lively discussion of the preliminary results of an interlaboratory comparison sponsored by National Institute of Standards and Technology and presented by Charles Guttman. This follows from the discussion at the workshop of the 1998 ASMS meeting (Orlando, FL) where it was decided that an interlaboratory comparison using MALDI-TOF instruments to determine a molecular mass distribution would be beneficial to the synthetic polymer mass spectrometry community. Samples of a well-characterized low molecular mass polystyrene were sent to any institution requesting it. A total of 18 institutions responded (10 industry, 5 academic, 3 government). (Results are still being collected for those few who could not finish before the ASMS meeting deadline.)

The polystyrene had a nominal mass of 7000 u. It was synthesized to have a tertiary butyl endgroup at one end and a proton at the other. The tertiary butyl group allowed for NMR characterization of Mn which was found to be (7050 $\pm$ 400) u. The Mw was found to be (7300 $\pm$ 600) u by light scattering. Note that to perform light scattering on such a low molecular weight polymer the change in index of refraction with polymer concentration in solution must be well known and accounted for in the data analysis. FTIR confirmed the presence of the two end groups and no others in measurable amounts. SEC was used to insure vial-to-vial homogeneity in the samples distributed to participants. The workshop attendees spent considerable time discussing the strengths and weaknesses of these "classical" methods in light of their relationship to mass spectrometry.

Each participating laboratory was asked to perform MALDI mass spectrometry using two distinct protocols: one using all-trans retinoic acid with defined concentrations of matrix:analyte:salt (AgTFA) and a second protocol defined by the user as their preferred method for analyzing polystyrene. The overwhelming majority of participants chose dithranol as their second matrix. Each laboratory was asked to do three repeats of each protocol to check for intralaboratory variability. Some participants accounted for the mass of the silver in their calculations others did not. This spawned a discussion within the group where it was decided that the mass of the cation should always be removed before the molecular mass is calculated especially in low mass polymers.

By compiling all the returned data using both protocols into one preliminary analysis it was found that MALDI mass spectrometry returned an Mn of (6600$\pm$ 100) u and an Mw of (6700 $\pm$ 90) u. These numbers were below those of the classical methods but still within the overlapping uncertainty ranges. The statistical uncertainty in the mass spectrometry measurements was very small indicating that from lab to lab reproducibility was extremely good in the reporting of these moments of the molecular mass distribution. Since there has been no comprehensive study of the systematic uncertainty of MALDI mass spectrometry applied to this problem it cannot be said at

this time with confidence whether the mass spectrometry results are systematically or statistically too low. The results for all-trans retinoic acid were systematically higher by about 75 u in Mn than the results from dithranol indicating that the choice of matrix does have a small but measurable effect on the molecular mass determination. There was no statistical difference between the use of linear or reflectron time-of-flight instruments.

The largest variability appeared in the mass calibration of the data. This was surprising because mass calibration is typically thought of as the strength of mass spectrometry. The total mass of the endgroups was 58 u. The data received from the interlaboratory comparison participants ranged from 36 u to 98 u indicating a miscalibration of a much as 40 u. Most participants externally calibrated their instruments with biomolecules using an appropriate matrix before running the polystyrene sample on a separate sample plate. Clearly calibrating in this way can lead to significant errors possibly due to difference is plume velocity and dynamics arising from using a different class of analyte in a different matrix. Workshop discussion here centered around defining a calibration protocol in addition to an analysis protocol in future interlaboratory comparisons. Suggestions for a follow up interlaboratory comparison were also discussed.

In order to facilitate a more general comparison of the data, that is something beyond comparing moments of the distributions, the data from each participant was placed into 11 bins of equal width spanning the entire mass range of interest. By comparing the percent of signal in each bin a statistical measure of the differences in the shapes of the mass distributions could be determined. From this analysis it was apparent that very differently shaped distributions could yield essentially the same moments of the molecular mass distributions. For example, a mass spectrum that failed to display both low and high mass oligomers (that is, no data in the first and last bins) could still produce the same Mn as a mass spectrum clearly showing both low and high mass oligomers. This lead to a discussion that revolved around the difficulty in comparing mass spectral data (which gives a full distribution) to the classical methods which only return one moment of that distribution.

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