Lessons Learned from NIST's Two Recent Polymer MS Interlaboratory Comparisons

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Purpose for Interlaboratory Comparison

- **Compare data among laboratories as a check on technique consistency.**
- **Obtain some measure of current experimental practice.**
- **Compare aggregate MS data from many labs with measures of the same properties by classical methods, i.e. "benchmarking".**
- **Begin a dialog in the synthetic polymer MS community on issues related to the quantitative measurement of single-chain properties by MS.**
- **NIST has a national mandate to produce measurement standards and to monitor the US Measurement System.**

Participation

- First Interlaboratory Comparison
  - 20 Laboratories returned data
    - 10 Industrial Labs
    - 4 Government Labs
    - 6 Academic Labs
  - 8 Countries Represented
- Second Interlaboratory Comparison
  - 14 Laboratories returned data
    - 5 Industrial Labs
    - 5 Government Labs
    - 4 Academic Labs
  - 7 Countries Represented

Results from First Interlaboratory Comparison of Polystyrene 7000 u to obtain Molecular Mass Distribution

Comparison of MALDI with Classical Methods

- Light Scattering gives **M_w = 7300 u**
  uncertainty including type A & type B ±600 u
- NMR gives **M_n = 7050 u**
  uncertainty including type A & type B ±400 u
- MALDI Interlaboratory Data Average
  M_w = 6700 u type A uncertainty ±85 u
  M_n = 6600 u type A uncertainty ±125 u

Data Analysis

- Three outliers: All due to data analysis errors
- Weakness of analysis software
  - Background/Baseline Subtraction
  - Peak Integration
  - Smoothing
- M_n computed by participants at times did not agree with the M_n we computed from their data
- These errors are easily preventable by more careful use of data analysis software programs.

For All Moments

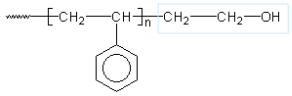
- The variance within each lab is less than the variance among labs.
- There is no statistically significant difference between retinoic acid and dithranol as matrices.
- There is no statistically significant difference between moments for data run in linear or reflectron mode.
- There is a statistically significant difference between instruments.

Conclusions

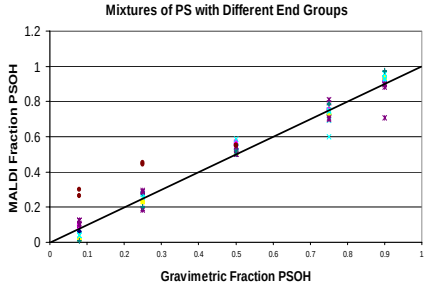
- MALDI seems to be consistently lower than classical methods by about 3% to 5%.
- There seems to be differences that are easily corrected by improved calibration and analysis methods.
- The overall agreement between labs is very good for this polymer in this mass range.
- Differences between MALDI procedures, (matrix, instrument, etc.) can often be seen statistically but were generally small.

Results from Second Interlaboratory Comparison on Mixtures of Polystyrene with Different End Groups – A Study of Mass Fraction

One PS had $-CH_2CH_2OH$ as an end group (shown below outlined in light blue). The second PS had -H as an end group.



Comparison of MALDI-TOF-MS Results with Gravimetric Results



Some Further Results

- Lab-to-Lab variation is significant
- Calibration Problems with about 30% of Labs
  - Identification of end groups
- Instrument variation is significant
  - S/N variation
  - Some Instruments had problems with the recipe

Conclusions

- Overall MALDI is in Good Agreement with Gravimetric Methods
- Strong Variation amongst Labs
- Calibration again an important issue
- Optimization of Instrument for S/N

Lessons Learned

1) The instrument must be optimized for best signal to noise in order to identify the minor components in a mixture. At this time there seems to be no generally-accepted, systematic procedure or set of necessary and sufficient criteria to optimize MALDI-TOF mass spectrometers.

2) There is a need for generally-accepted practices for data analysis. This includes, but is not necessarily limited to, baseline subtraction, and peak integration. *These procedures need to be supported by statistical theory in order that they may also provide meaningful uncertainties.*

3) Instrument mass calibration must be carefully performed. This is best done under measurement conditions as close to the analyte measurement conditions as is feasible. *Biomacromolecules may not be the best choice for calibration when the analyte to be measured is a synthetic polymer due to the large differences in operating parameters required for these classes of sample.*

References

Guttman, C.M.; Wetzel, S.J.; Blair, W.R.; Fanconi, B.M.; Girard, J.E.; Goldschmidt, R.J.; Wallace, W.E.; VanderHart, D.L. *Anal. Chem.*, 2001, **73**, 1252-1262.

Guttman, C.M.; Wetzel, S.J.; Flynn, K.R.; Fanconi, B.M.; J.; Wallace, W.E.; VanderHart, D.L. *Anal. Chem.*, 2005, in press.

Other Outputs

A. See our web site:

www.nist.gov/maldi

For our Polymer MALDI Recipes page, *MassSpectator* our online peak integration tool, and announcements about our Fall 2005 Workshop (Nov. 9-10).

B. ASTM Standard Test Method to Determine the Molecular Mass Distribution of Polystyrene using MALDI Approved as ASTM D7134
This standard uses the *Lessons Learned* from the interlaboratory comparison studies.