Chromatographic Fidelity and Matrix /Analyte Solubility in Complex Polymer Systems using HPLC-MALD/I TOF MS

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CHEM 395

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Overview

- **Introduction to MALDI**
- **Objective:**
  - To demonstrate compatibility of matrix/analyte mixtures and to also ascertain the Chromatographic fidelity in MALDI analysis of polymers.
- **Approach:**
  - Coupling of HPLC with MALD/I TOF MS......Hence the name “LC-MALDI”
  - LC-MALDI interfaced using LC-Transform Series 600 (LabConnections, Inc.).
  - Selective use of solvents’ gradient over varying temperature range.
- **Results:**
  - Retention time/scan reproducibility over various samples analyzed and the signal to noise ratio.
  - Capability of functional HPLC separation combined with automated MALDI analysis is demonstrated using K-GH Polyol (thermal dye-receiver polymer).
  - Demonstrated advanced capability for differentiating materials.
Introduction to MALDI

- Since its discovery in the late 1980s, Matrix Assisted Laser Desorption Ionization (MALDI) has emerge as an important soft-ionization MS technique.
- Applications in many biologically related research areas (e.g., protein, peptides, DNA, carbohydrates) and materials-related fields (e.g., synthetic polymers)
- The fundamental mechanisms still remain unknown despite its routine usage
MALDI experiment can be reduce to four different steps:

- **Sample Prep**: Molar ratio of matrix to analyte is large (e.g. between $10^2 : 1$ and $10^6 : 1$)
- **Desorption**: Pulsed laser beam (typically a Nitrogen laser, 337nm)
- **Ionization**: Gas phase ionization reactions (Cationized, protonated, de-protonated or radical species)
- **Mass Analysis**: Time-of-flight (TOF), FTICR, QIT etc.
1) **Sample Preparation**
- Individual solutions of matrix material and analyte
- Solutions are mixed (high matrix-to-analyte ratio)
- Mixture is deposited onto the sample plate (solvent evaporated)
- Analyte is homogeneously embedded within the matrix crystal (solid solution)

2) **Desorption**
- Matrix is desorbed off the surface, transferring the analyte molecules into the gas phase.
- A supersonic expansion from the surface - the velocities of various components of the sample are found to be very similar

3) **Ionization**
- Reactions occur in the dense plume - Gas-phase ionization
- Formation of ions: cationized species, protonated species, de-protonated species, or radical cations

4) **Mass Analysis** - Time-of-Flight Mass Spectrometer…
In linear TOF instrument, ions drift through the flight tube until they reach the detector.

Because ions of different species in a mixture have different masses, they have different velocities based on the following equation:

\[ v = \sqrt{\frac{2KE}{m}} \]

where \( v \) = velocity, \( KE \) = kinetic energy, and \( m \) = mass

\[ t = \sqrt{\frac{m}{2KE}D} \]

where \( D \) is the flight distance

\[ \frac{m}{z} = \frac{2Ut^2}{D^2} \]

where \( z \) = number of charge on an ion, \( U \) = accelerating voltage, \( t \) = flight time
LCMS vs LC-MALDI

**LC/MS**

HPLC Pump → LC Column → Mass Spectrometer

Liquid phase → Gas Phase Ionization

**LC-MALDI**

HPLC Pump → LC Column → Spray interface → Mass Spectrometer

Liquid phase → Solid phase → Gas Phase Ionization
LC-MALDI Interface

Research & Development Laboratories, Eastman Kodak Company, Rochester, New York
LC-Interface Experimental Conditions

**HPLC**

Column: Zorbax Eclipse XDB-C8  
2.1mm x 150mm; 5µm  
A: Water  
B: ACN/IPA (1:1)  
Gradient:  
- 50% B → 100%B in 20 min. at 250uL/min  
- Hold 100% B for 5 min at 250uL/min  
- 100% B → 50% B in 0.1 min at 350uL/min  
- Hold 50% B for 5 min at 350uL/min  
Inj Vol.: 2uL  
Detection: DAD (monitor at 210 nm)

**Matrix Pump**

Pump 1: CHCA matrix 1 mg/mL in ACN:IPA  
Pump 2: ACN:IPA  
Isocratic pumping of 50:50 (pump 1 : pump 2)  
Flow Rate: 100 uL/min

α-cyano-4-hydroxycinnamic acid (CHCA)

**Sprayer**

Temperature gradient: 172 °C at initial conditions to 133 °C at final conditions  
N₂ Gas Nebulization: 30 psi
Example used for Demonstration of Capability

- K-GH Polyol (low MW polymer used in Thermal dye-receiver)

\[
\text{CH}_3 \quad \text{CH}_3
\]

- Important to have correct end-group chemistry
- Multiple end-group combinations have been observed
- Interest in pursuing functional separation
Direct MALDI/TOF MS of K-GH Polyol (no separation)

In-Spec Material

Out of Spec Material
Direct MALD/I TOF MS of K-GH Polyol (no separation)

“In-Spec” Material

“Out of Spec” Material

2371-06938-1a raster 4 (0.603) Sb (30,30.00 ); Cm (1:49)

2371-06937-1raster 41 (5.436) Sb (30,30.00 ); Cm (1:49)

TOF LD+
9.41e4

TOF LD+
5.63e4
Spectral of PMMA 6300 using NaTFA as the cationization reagent with six matrixes (a) RA (b) DCTB (c) IAA (d) CHCA (e) DHB (f) DHBQ

Results

Spectra of PS 7000 using AgTFA as the cationization reagent with six matrices: (a) RA, (b) DCTB, (c) IAA, (d) CHCA, (e) DHB, (f) DHBQ

- **DHBQ**: 2,5-dihydroxy-p-benzoquinone
- **DHB**: 2,5-dihydroxybenzoic acid
- **CHCA**: a-cyano-4-hydroxycinnamic acid
- **IAA**: trans- indoleacrylic acid
- **DCTB**: 2-[(2E)-3-(4-tert-butylphenyl)-2-methylpropenylidene] malononitrile
- **RA**: all-trans-retinoic acid

Results Cont’d

Spectral of PEG 10000 using NaTFA as the cationization reagent with six matrixes (a) RA (b) DCTB (c) IAA (d) CHCA (e) DHB (f) DHBQ

Conclusion

- LC-MALDI retention time reproducibility is within a factor of 3, compared with conventional LC-DAD, despite the solid state transition. *(Within a factor of 2 compared with conventional LC/MS).*

- Demonstrated chromatographic fidelity for LC-MALDI compared to LC-DAD and LC-ELSD.

- Matrix-addition solvent effects were observed which compromised chromatographic fidelity. 3D MALDI Imaging being used to investigate the dynamics of this effect.

- Matching the RTs of the matrix and analyte consistently produced the best MALDI spectra with regard to S/N.

- The results presented here suggest that a good starting point for choosing the appropriate matrix for an “unknown” sample would be to match its relative polarity with that of the matrix.
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CHEM 395 Class of Spring ’07 !!
References


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