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Small Molecule TOF MS Detection Using Nanoscale Particles Compared to Analyte Detection Using DIOS Substrates

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Overview

- Previously, porous silicon etched into a silicon substrate has been used to facilitate soft ionization for laser desorption ionization (LDI) TOFMS [1-3] and chemical modification of the surface has recently improved sensitivity [4]
- Particles made of similar material may facilitate the following: ability to use different substrates, higher throughput bulk material manufacturing, increased capacity, lower laser power
- Applying what is known about TOFMS on porous silicon, we explore the following questions for particles:
  - Does surface modification improve performance?
  - Can we obtain improved results as particle size decreases?
  - Does the material matter? Would silica and silicon particles of similar size yield similar results?
  - Can the use of particles decrease the required laser power?
  - How does the particle to analyte ratio affect results?

Low Mass Detection without Matrix Interferences

- Waters MassPREP™ DIOS-target™ plates are an alternative to MALDI with matrix (Fig. 1)
- Optimized for small molecule detection (< 1000 amu)
- The plates can be used in existing MALDI equipment (w/adapter)
- Automated sample analysis is routine using MassLynx™ software

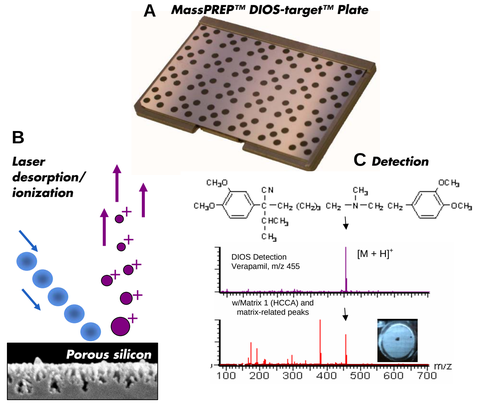

Figure 1. Desorption/ionization on silicon, DIOS: *A) DIOS-target plate with holder, B) sample analysis by LDI, and C) detection of verapamil (125 pg) at same laser power using standard DIOS-target plate compared to MALDI on steel plate using HCCA matrix (purified form available from Waters).*

Methods

Sample Preparation—Silicon particles were physically removed (scraped or sonicated) from DIOS substrates. [5] Other particles were obtained from Sigma. Typical particle concentrations were 1 to 100 mg/mL in methanol (MeOH). Particles were modified [6] by treatment in 10% hydrofluoric acid in ethanol, rinsed in ethanol and then oxidized in 10% HNO_3 in water prior to reacting with fluorinated dimethylchlorosilane [4]. Analytes were dissolved and diluted in MeOH or acetonitrile (ACN). Typical analyte depositions were between 1 and 250 pg. Analytes and analyte/particle solutions were deposited by pipette (1.0 uL) and air-dried. Prototype fluorinated MassPREP DIOS-target Plates were used for comparison.

Analysis—For mass spectrometry analysis, we used a Waters Micromass MALDI-TOF mass spectrometer in reflectron mode with R (FWHM) > 8,000 at m/z 455 and a 337 nm laser pulsed at 5 Hz. Spectra shown are the accumulated signal of at least 100 shots. Each figure presents spectra obtained at the same laser power using the same number of shots, unless otherwise indicated.

Results

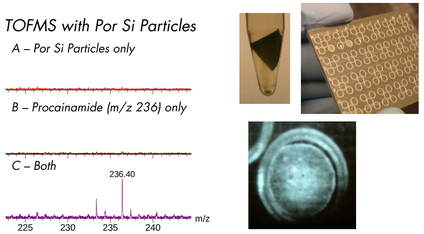

Figure 2. *Proof of principle. MS of control samples and procainamide detection (m/z 236) assisted by particles sonicated from DIOS-target plates.*

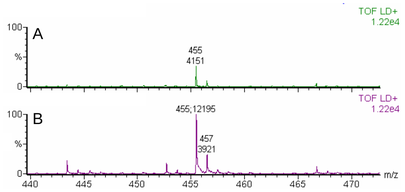

Figure 3. *Surface derivatization. (A) Spectra of verapamil (m/z 455) using ~ 50 nm silicon particles. (B) Significantly improved verapamil detection with fluorinated surface modification.*

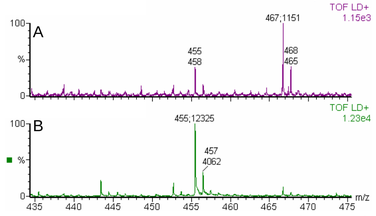

Figure 4. *Silicon particle size. Comparison of spectra from derivatized particles of different size: (A) silicon < 44 um (broad distribution) and (B) silicon ~ 50 nm.*

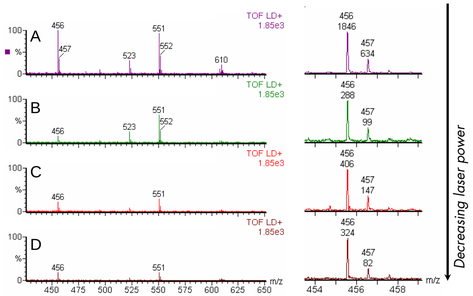

Figure 5. *Laser power (unmodified Si ~ 50 nm particles). Comparison of small molecule mix detection (verapamil at m/z 455, reserpine at m/z 609) using different laser power settings: A) 80% attenuation (low), 50% maximum iris opening, B) low, 40%, C) low, 25% and D) low, 20%. There is a threshold laser energy for analyte detection with significant intensity (> 1000 cts.).*

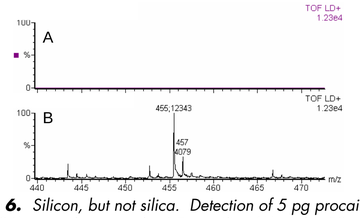

Figure 6. *Silicon, but not silica. Detection of 5 pg procainamide (m/z 236) using derivatized ~ 15 nm silica (A) and ~ 50 nm silicon particles (B). Laser power = low 15%. (No detection of procainamide using silica even at highest laser power).*

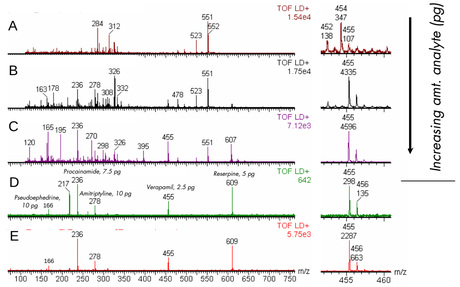

Figure 7. *Laser power and particle to analyte ratio (small molecule mix with components at m/z 166, 236, 278, 455, and 609 in 4:3:4:1:2 ratio). Comparison of (A) background peaks from blank 10 µg derivatized Si (~ 50 nm), (B) particles:verapamil (5 pg) in 3600000:1 ratio, (C) particles:verapamil (45 pg) in 44000:1 ratio. Spectra from DIOS-target: D) 2.5 pg verapamil at low, 13% laser (low signal intensity), and (E) same well at low, 23% laser setting.*

Particles	Unmodified	Chemically Modified
Si (filtered, < 44 um)	X	√ 460 (12190)
Si (#2)	X	√ 2400 (8780)
Si, ~ 50 nm	√ 4150	√ 12330 (30290)
Silica, 3 nm	X	X
SiO_2 10 nm	X	X
SiO_2 15 nm	X	X
Si_3N_4 nanopowder	X (requires higher laser power)	X (requires higher laser power)
fullerite	√ 4170 (10006)	n/a

Table 1. *Summary of small molecule detection using particles (from Sigma) in this study. Red indicates no detection at 80% laser attenuation setting typically used for DIOS-target plates. Green indicates successful detection. The numbers in the table refer to counts of verapamil (m/z 455), which is an indication of the relative peak intensity for 125 shots. The number in parentheses is a contaminant peak at m/z 551 (indicative of chemical noise).*

Discussion

Here we compared particle-assisted LDI with DIOS-target plate performance using an instrument that is well-characterized with respect to DIOS performance. This is significant because previous evaluations (internal) have established that adjustments in laser alignment and angle relative to the DIOS surface can render an instrument DIOS-inactive, even though MALDI results are relatively unaffected. A separate recent study has also indicated that MS results are dependent on laser alignment and angle of incidence, leading to LDI results that can vary from one instrument to another.[7]

Once we established a baseline for comparison using porous silicon substrates for small molecule TOFMS detection, we proceeded with particle-based studies in order to better understand both DIOS-target plate performance and previous work on particle-based LDI. Previous studies [8] have compared DIOS substrates and porous silicon particles for TOFMS; however, recent improvements in making DIOS substrates provide an opportunity to improve silicon and silica particle-based TOFMS. Several studies have reported the use of silica and silicon particles compared to other types of particles, although some of these results involve larger particle sizes that may be ineffective for MS of small molecules[9]. It was hypothesized that using smaller particles increased available surface area for analyte adsorption and would provide benefits to MS analysis by decreasing laser power required to desorb analyte, resulting in less fragmentation and higher sensitivity. However, sources of contamination could adsorb to available surfaces also.

Conclusions

Initial results indicate the following trends:

- *Although particles can be sonicated from porous silicon-based and can be synthesized, commercially available particles provided an initial route for these studies*
- *Silicon particles can be used to detect analyte, with better signal intensity and lower laser power required using chemically modified (fluorinated) particles*
  - *Results analogous to work conducted on DIOS-target plates*
- *Smaller (~ 50 nm) silicon particles performed better than ~ 44 um sized silicon; however, although commercially available silica particles were smaller (~15 nm), they were shown to be ineffective in both unmodified and chemically modified form*
- *Analytes were detected using other materials such as Si_3N_4 nanopowder and fullerite*
- *We observed many background peaks, which made it difficult to provide more quantitative results, particularly regarding particle to analyte ratio*

References

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