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DESORPTION/IONIZATION ON SILICON MASS SPECTROMETRY (DIOS MS) OF SMALL MOLECULES AND PEPTIDES: SAMPLE HANDLING, PREPARATION AND STORAGE EFFECTS ON PERFORMANCE

Grace M. Credo[1], Hillary B. Hewitson[1], Kenneth J Fountain[2], Martin Gilar[2], Jeffrey W. Finch[2], Chris L. Stumpf[2], Christopher C. Benevides[2], Edouard S.P. Bouvier[1], Bruce Jon Compton[1], Zhouxin Shen[3], Gary Siuzdak[4]: [1]Advanced Separations Technology, [2]Waters Corporation, Milford, MA; [3]Mass Consortium Corporation, San Diego, CA; [4]The Scripps Research Institute, La Jolla, CA

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OVERVIEW

Previous work has demonstrated the utility of DIOS in small molecule and peptide detection.[1-3] Using sample substrates manufactured in-house,

- reproducible DIOS chips are produced,
- the chips are shown to be sensitive to contamination,
- a consistent cleaning procedure is demonstrated,
- small molecules and protein digests are detected without matrix-related peaks, and
- speed of analysis, stability, shelf life, dynamic range, sensitivity, and quantitation are explored.

INTRODUCTION

DIOS is a relatively new technique based on the laser desorption of an analyte applied directly (no matrix) to a porous silicon surface. Like matrix-assisted laser desorption ionization (MALDI), the advantages of DIOS include little to no fragmentation of the analyte (easier sample identification), rapid sample preparation/analysis for high-throughput applications, and the ability to analyze complex mixtures. However, since MALDI utilizes an organic acid co-crystallized with the analyte of interest, interference peaks are common below 700 amu. The co-crystallization procedure, which requires that the appropriate matrix be identified, can introduce obstacles to the analysis of peptides above 1000 amu as well. With the elimination of matrix-related ions and sample preparation effects, DIOS can facilitate the identification of analytes that are not easily detected by MALDI.

EXPERIMENTAL

MS: Waters® Micromass® MALDI-TOF mass spectrometer, MALDI LR™ or MALDI R™ model, reflectron mode, Rs(FWHM) > 10,000. Real-time data selection was off. Laser power set to constant value. Automated sample acquisition and manual data processing performed using MassLynx™ 4.0.

Substrate Preparation: The silicon substrate was diced (SiliconQuest, Santa Clara, CA) prior to etching to fit in a custom chip holder. The chip and holder were engineered to the same dimensions as a MALDI plate. An optical mask with a 96-well pattern plate was used to etch low resistivity n-type silicon (~0.010 ohm-cm, As-doped, SiliconQuest) with the following etching conditions: ethanolic 25% HF solution, 6 mA/cm^2, 50 mW/cm^2, 2 min. Chips were oxidized in an ozone stream (30 sec) and then dipped in an aqueous 5% HF solution prior to analysis or storage. [Etching conditions similar to ref. 2]

Sample Preparation: Small molecules were dissolved in methanol (MeOH) and diluted with 50:50 MeOH:Water (with and without 5 mM ammonium citrate (AC). HPLC-purified protein digests in aqueous 0.1% trifluoroacetic acid (TFA) were diluted with 5 mM AC in MeOH:Water. Analytes were manually deposited on the 2 mm porous silicon spots by pipette (0.5 mL in MeOH:Water) and air-dried (2-5 min.). Contact with glass containers was avoided to reduce potassium and sodium levels.

SEM: Selected wafers were cleaved, mounted with carbon tape and carbon paint and gold sputter-coated on the edge prior to analysis (JEOL JSM 5600).

REFERENCES

1) J. J. Thomas, Z. Shen, J. E. Crowell, M. G. Finn, G. Siuzdak, Proc. Natl. Acad. Sci. 2001, 98, 4932-4937.
2) Z. Shen, J. J. Thomas, C. Averbuj, K. M. Broo, M. Engelhard, J. E. Crowell, M. G. Finn, G. Siuzdak, Anal. Chem. 2001, 73, 612-619.
3) J. Wei, J. M. Buriak, G. Siuzdak, Nature 1999, 399, 243-246.
4) E. P. Go, Z. Shen, K. Harris, G. Siuzdak, submitted.

RESULTS AND DISCUSSION

Desorption/Ionization on Porous Silicon

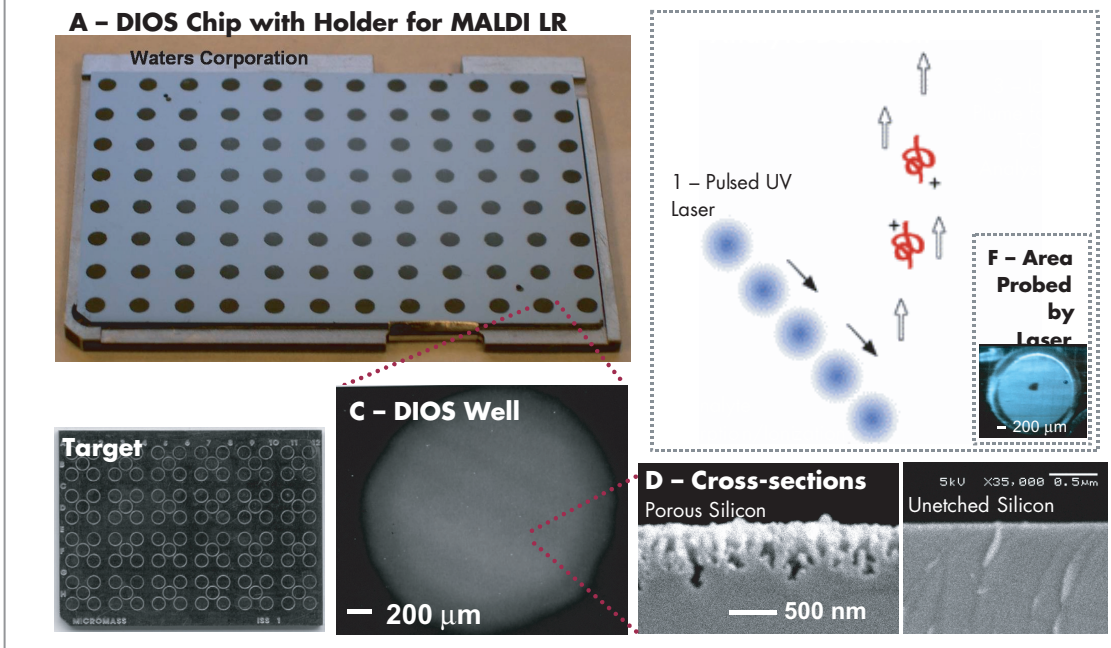


Figure 1. Pictures of the substrate (A—photo, C—microscope, D—SEM), (B) design template, (E) steps in analyte detection. Our photo-etching procedure eliminates discoloration (etching) of the areas surrounding the wells. Using SEM, we observe no etching of the masked silicon. (F) Photo of organic matrix on a steel target plate after laser ablation (F), where the dark area in the center is indicative of the region probed by several laser shots (high 50% on MALDI LR). This dark spot is 1% of the total well area.

Analyte Detection and Speed of Analysis

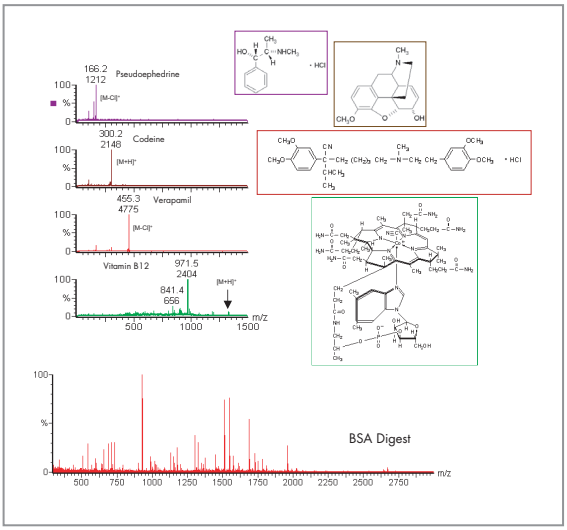
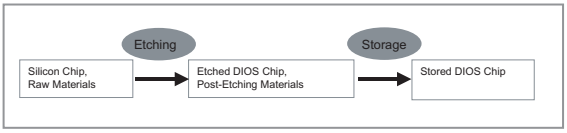


Figure 2. Representative spectra, 20 shots at 4 sec/sample for an analysis speed of less than 7 minutes per 96-well plate. LR, Laser=Low, 30%.(top) Small molecules, 5 ng/µL (MeOH:Water). Peaks are labeled with m/z values and counts. (bottom) BSA digest, 0.5 pmol/µL (5mM AC in MeOH:Water).

Sample Handling and Preparation

The materials used in the production of in-house DIOS chips, before and after etching as well as before and after storage, were evaluated in order to identify the source of observed contaminants in the mass spectra.



By plotting the intensity of a major contaminant peak, 551 m/z, as a function of plate position, it was apparent that the 551 peak was related to handling. Although this initially suggested glove-related contamination, the true source of the 551 peak was indirect contact with human skin via contaminated gloves. Direct contact with skin produced a 551 peak with much higher intensity. This and other contaminants can interfere with effective ionization of the analyte.

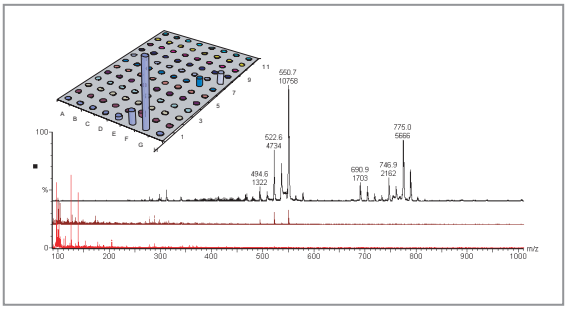


Figure 3. Handling-related contamination. Inset: Peak intensity as a function of well position. The 551 peak occurs most intensely at the edge of chip. MALDI LR, 100 shots, Laser = Low 50%.

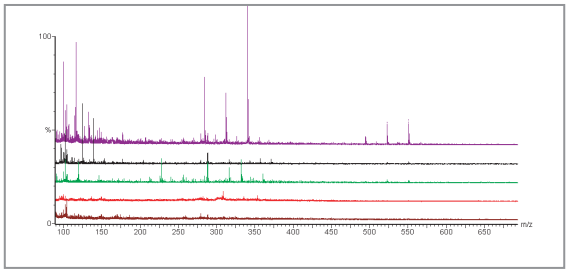


Figure 4. Detection of process-related contamination extracts, compared to a bare well. LR, 100 shots.

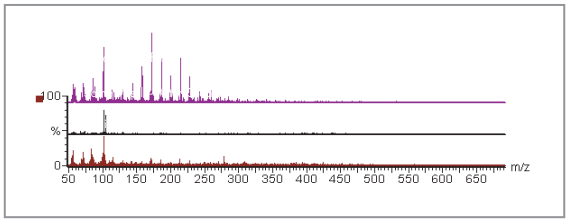


Figure 5. Removal of contamination with overnight solvent soak. Example with storage pouch. LR, 100 shots.

Storage, Stability, Shelf Life

Contaminants introduced during the manufacturing process can be eliminated by overnight solvent soak in ethanol (EtOH) or isopropyl alcohol (IPA) in a Teflon bottle. Since the unprotected hydride-terminated surface oxidizes with time, we store etched chips in IPA. DIOS performance is preserved when the surface is protected in this manner. Storing in a dessicator with activated charcoal or in an argon-filled bag does not eliminate background signal from the DIOS surface prior to analysis. However, we have stored chips in dessicators and sealed bags (three months, so far) and then prepared them for analysis by solvent soak. After soaking, we achieved similar codeine quantitation results with freshly etched and one month old DIOS chips (stored in air).

Dynamic Range and Sensitivity

The linear response range is instrument-limited to two orders of magnitude (circled below), with detector saturation at higher concentrations (dotted line, below). The linear range varies with analyte. Instrument settings, such as the laser power, affected the standard deviation of the intensity, but not the linear range. Limits of detection (S/N=3) and limits of quantitation (S/N=10) are achieved below 10 ng/mL (=10 pg/mL or 5 pg=17 fmol loading) for codeine, but the instrument response is not linear in this region.

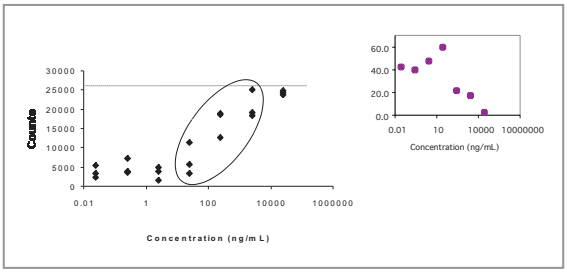


Figure 6. Dynamic range for codeine detection. Inset: %RSD's, typical for MALDI without an internal standard.

Internal Standard and Quantitation

We evaluated codeine detection in the linear response region using deuterated codeine as a 1:1 internal standard. The %RSD's when compared to an internal standard are lower than 10%. The use of an internal standard is known to improve quantitative detection in MALDI and has been recently explored in DIOS [4].

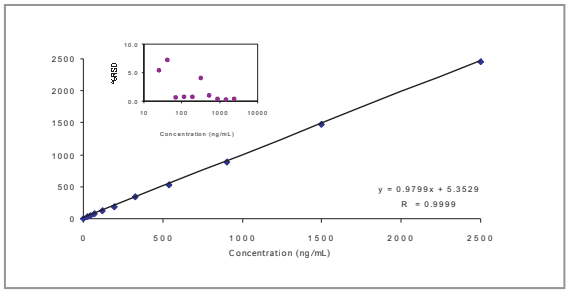


Figure 7. Codeine response compared to deuterated codeine internal standard. Inset: % RSD's with IS.

"Real World" Sample

Reproducibility and Repeatability

One of the key advantages of DIOS is the absence of matrix and crystallization effects. In order to determine reproducibility with DIOS, we compared signal intensity levels for analyte wells on the same chip, compared average values on three chips, and compared these values on two instruments. Well-to-well and chip-to-chip performance were fairly reproducible, with %RSD's usually less than 50%. Instrument performance was not directly comparable due to discrepancies in laser settings (focusing, power).

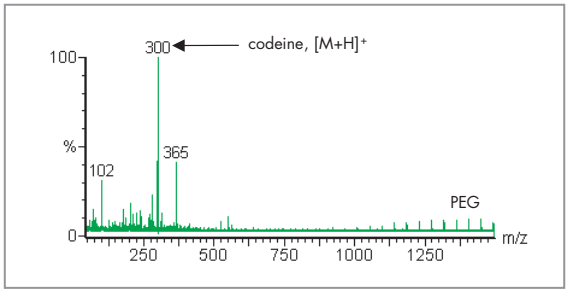


Figure 8. Codeine detection in a diluted preparation of medicated syrup (10,000x in MeOH:Water).

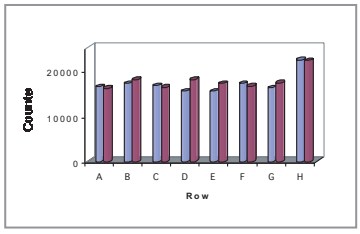
A—Well Performance:

Ranitidine, 250 ng/mL

RSD=12% (n=16)

100 shots, MALDI R,

Laser=15%



B—Chip-to-Chip Performance: Twelve Small Molecules, 250 ng/mL

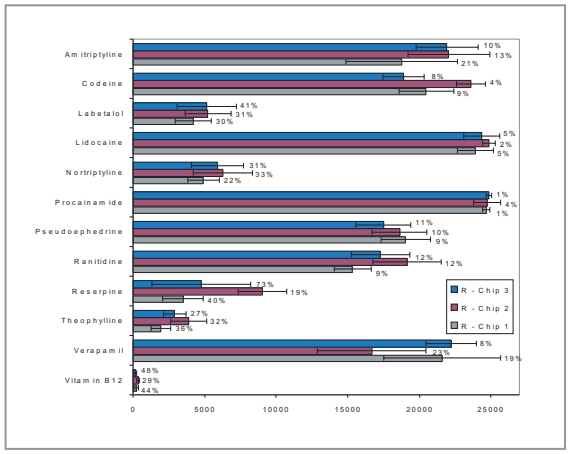


Figure 9. A) Well-to-well intensity. B) Chip-to-chip average intensities, with standard deviation and % relative standard deviation (RSD), n =16 on one DIOS chip. For each analyte, the three bar colors represent individual DIOS chips (three chips per analyte). Note: Theophylline loaded at 5000 ng/mL.

Conclusions

- Reproducible DIOS chips are produced with porous silicon formation confined to patterned well regions
- As in MALDI, contaminants can compete for or suppress ionization during the desorption/ionization process and degrade performance
- An overnight solvent soak (IPA, EtOH) yields a clean DIOS chip
  - We can eliminate manufacturing-related and storage-related contamination
  - Primary contaminants appear to be from indirect skin contact or hydrocarbons
- DIOS can be used as an identification and analysis (qualitative and quantitative) tool for small molecules and small peptides
  - Cleaning and preparation optimized for small molecules (<1000 amu)
  - Some samples and solvents introduce contamination and will require pre-analysis treatment
  - Quantitative analysis with %RSD < 5% requires an internal standard
- With appropriate laser settings and homogeneous sample deposition, well-to-well and chip-to-chip performance is reproducible

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