

LASER DESORPTION/IONIZATION MASS SPECTROMETRY USING TUNABLE NANOPOROUS STRUCTURES

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ABSTRACT

The performance of porous silicon and cobalt glancing angle deposited (GLAD) thin films for matrix-free laser desorption/ionization-MS analysis of carbohydrates, peptides and drugs with molecular weights < 2,500 Dalton is presented. The performance of these films for selected peptides and small drugs detection is similar to the best reported for desorption/ionization on electrochemically etched porous silicon with detection limits in the femtomole to sub-femtomole range, and was superior to matrix-assisted laser desorption/ionization (MALDI)-MS in terms of background signal and spot-to-spot reproducibility.

KEYWORDS: GLAD, Mass Spectrometry, Laser Desorption/Ionization, Porous Thin Films.

INTRODUCTION

Laser desorption/ionization (LDI) based on nanoporous semiconductor surfaces is a relatively new matrix-free mass spectrometry approach for small molecule detection. The active surfaces of the target material replaces the function of the organic matrices used in conventional MALDI-MS and the analyte mass spectrum does not suffer from interference by the matrix signal in the low mass region (MW < 1000 Daltons). Desorption/ionization by pulsed laser irradiation on a porous silicon substrate for mass spectrometry (DIOS-MS), introduced by Wei *et al.* in 1999 [1], has proven to be one of the most successful LDI methods for matrix-free analysis of small molecules. Electrochemical etching of silicon has been predominantly used to prepare porous silicon surfaces for DIOS-MS applications. Surfaces prepared by this method, however, suffer from spatial non-uniformity and degradation due to trapped etchants that introduce noise to the mass spectra. Moreover, to obtain reproducible surfaces using electrochemical etching techniques, considerable care is required. In this report, we demonstrate the usability of nanostructured porous silicon and cobalt thin films fabricated by GLAD technique for LDI-MS of small molecules. The GLAD film approach offers advantages over electrochemically etched silicon, MALDI and other approaches [2] in terms of cost, contamination control, target uniformity, and control of morphology.

THEORY

GLAD is a physical vapor deposition technique used to fabricates nano-scaled columnar thin films with controlled morphologies, porosities and thicknesses. The computer controlled GLAD fabrication apparatus regulates the oblique angle at which the vapor flux hits the substrate surface and also determines the rate at which the substrate platform itself rotates. Vertical post GLAD films, Figure 1, were used for our LDI experiments and are generated by rotating the substrate at a very fast rate [3].

EXPERIMENTAL

Nanostructured porous thin films were deposited by electron beam evaporation of purified silicon and cobalt (99.999% pure, 3-6mm pieces, Cerac Inc., Milwaukee, WI) under vacuum (base pressure < 10⁻⁶ Torr) onto 3" diameter single crystal (100) silicon wafer substrates, which were subsequently cleaved by hand into 1 cm x 1 cm squares. The deposition was performed in a custom made high vacuum system (Kurt J. Lesker Co., Clairton, PA) with computer-controlled stepper motors for rotating and tilting the substrate holder. Vertical posts (columns) were deposited at angles of 1°, 70°, or 85° relative to the substrate normal, using a substrate rotation rate of 1.2 RPM and a deposition rate of 0.6 nm/s. The nanostructured GLAD films (~ 1 cm²) were attached to a modified MALDI target plate using a conductive double-sided tape. Peptide and drug samples were prepared in 70% trifluoroacetic acid and 30% acetonitrile, while carbohydrates were prepared in 0.5M sodium chloride. DIOS-MS measurements were made using an Applied Biosystems (Framingham, MA) Voyager STR time-of-flight mass spectrometer operated with a pulsed N₂ laser at 337 nm.

RESULTS AND DISCUSSION

Figure 1 shows SEM images of Si films deposited at 70° and 85°. From the SEM images, we estimated films deposited at 70° contain pores less than 5 nm in size and films grown at 85° had pore sizes of tens of nanometers, giving a low film

density of ~30% of bulk, while films deposited at 1° (not shown) are composed of essentially solid structures. Peptides, drugs and carbohydrates in the mass range of 150 – 2500 Da were analyzed using the Si and Co GLAD films. The best performance for Si was obtained with 500 nm thick films deposited at 85° deposition angle [4]. 250 nm and 500 nm Co films deposited at 85° showed no significant difference in terms of performance. Figure 2 compares mass spectra of a mixture of verapamil, bradykinin fragment 1-5 and angiotensin detected using conventional MALDI matrix and 500 nm Si GLAD film. The performance of the films was superior to MALDI-MS for analysis of mixtures of small molecules in terms of background chemical noise, detection limits and spot to spot reproducibility. Low molecular weight carbohydrates were also easily detected with minimal sample preparation and low background signals, Figure 3. We examined the spot-to-spot reproducibility of signals in our films by measuring the signal intensity (100 shots/spectrum) for verapamil and bradykinin fragment 1-5 on 15 and 21 different Co and Si targets, respectively. The average signal intensity of 50 fmol verapamil on Co films was $90 \pm 14\%$ (n=15) and that of 5 pmol verapamil on Si films was $79 \pm 13\%$ (n=21). From single shot measurements (data not shown) there was no indication of mute spots typically seen in MALDI, presumably due to uniform distribution of sample on the nanostructured films. The limit of detection for verapamil was found to be 6.25 fmol for Co GLAD films (Figure 4) and 1 fmol for the Si films. Figure 5 shows detection of femtomole amounts of peptides with calculated detection limits (S/N = 10) of 87 atomol– 243 atomol on Si films.

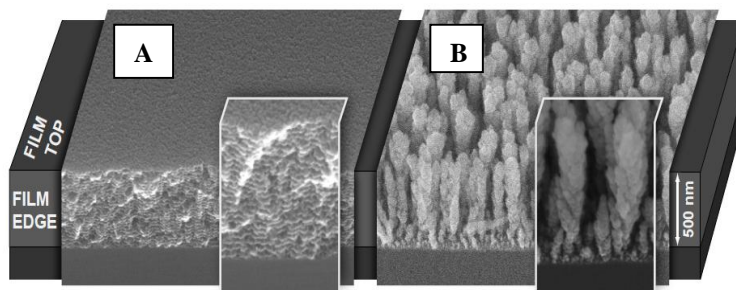


Fig. 1. SEM of 500 nm thick Si GLAD films grown for LDI studies at 70° (A) and (B) 85°.

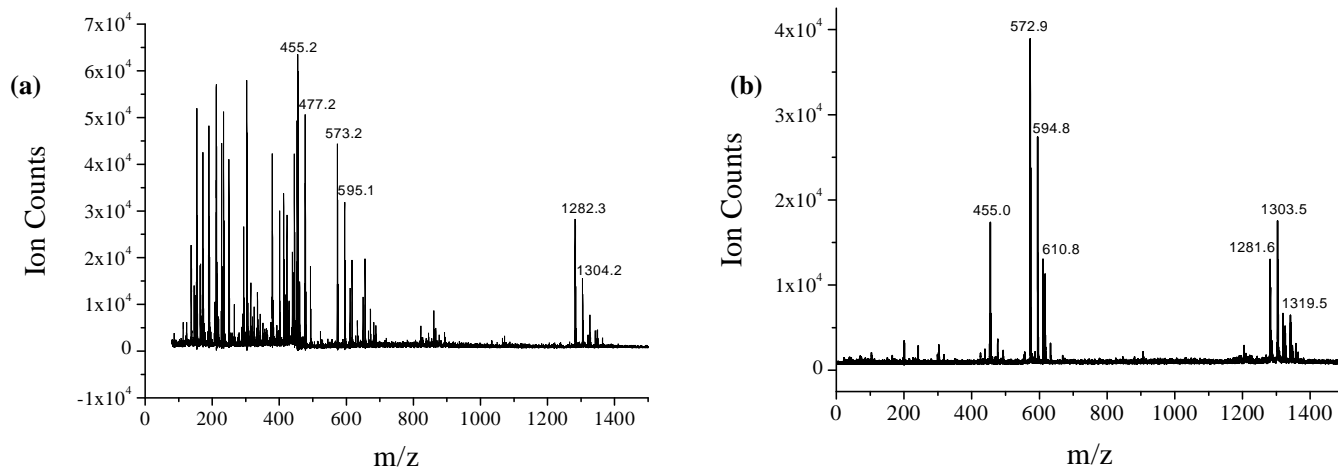


Figure 2 Spectra of a mixture of verapamil, bradykinin fragment 1-5 and angiotensin using (a) MALDI matrix cyano-4-hydroxycinnamic acid and (b) 500nm Si GLAD film.

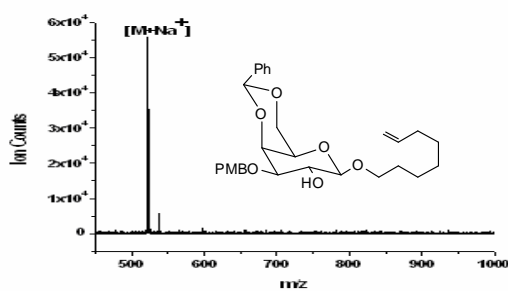


Figure 3. Mass Spectrum of 1.25 pmol carbohydrate (498 MW) on 250 nm Cobalt GLAD Film.

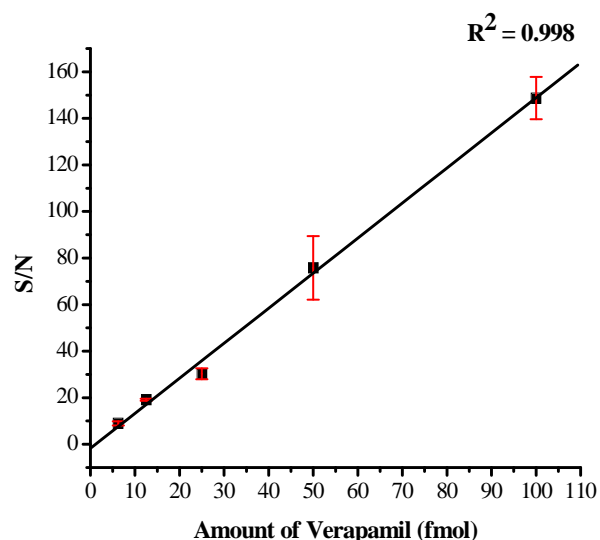


Figure 4. Plot of S/N vs amount of verapamil on 250nm Co GLAD film.

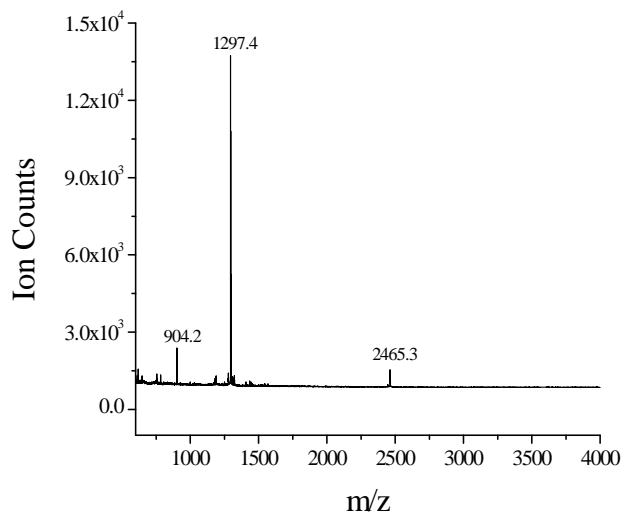


Figure 5. Mass spectrum of a mixture of 6.5 fmol des-arg⁹-bradykinin, 13 fmol angiotensin-I and 10 fmol ACTH fragment (18-39) on a 500 nm thick Si GLAD film.

CONCLUSION

We demonstrated the use of silicon and cobalt GLAD films for matrix-free detection/identification of small carbohydrates, drugs and for proteomics application. GLAD allows the fabrication of large surface area Si and Co thin films with controlled pore size and porosity in a single-step deposition process. Moreover, the vacuum preparation of GLAD films avoids the contamination issues involved in electrochemical etching and exposure to wet solutions. The ability to deposit a range of materials with a varying optical and thermal properties by the GLAD technique may allow for optimization of these parameters for even more efficient LDI-MS detection of small molecules. The fabrication of reproducible films makes GLAD an ideal alternative for preparation of LDI substrates. The lack of background chemical noise in the lower range of mass spectra also serves to promote GLAD films as a replacement for organic MALDI matrices.

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