Mesoporous Silica for Desorption-Ionization Mass Spectrometry

A.M. Dattelbaum & S. Iyer

Biosciences Division, B-4, Los Alamos National Laboratory
P.O. 1663, Los Alamos, NM 87545
amdattel@lanl.gov; siyer@lanl.gov

ABSTRACT

Here we demonstrate the use of mesoporous silica thin films for matrix free laser desorption/ionization mass spectrometry (MS). In spite of the growing acceptance of matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry for biomolecular analysis, its use in small molecule analysis and tandem MS experiments has been limited. This is chiefly due to interference from matrix molecules and/or issues with uniform energy transfer from matrix to analyte upon UV laser excitation. Further, known matrices are specific for certain molecules, which is an obstacle to rapid analysis of a diverse set of samples. In light of this, recent efforts to develop matrix-free desorption-ionization approaches assume significance. Currently available supports for matrix-free MS include porous silicon and sol-gel-titania films. Mesoporous silica thin films are shown here to be an alternative matrix-free support for mass spectrometry analyses of small molecules (<1000 Da). These films are simple to produce, require no special handling or storage, and are stable at ambient laboratory conditions for at least several months. Mass spectra of several low molecular weight molecules desorbed from mesoporous silica without added matrix will be presented.

Keywords: mesoporous, silica, mass spectrometry, desorption, matrix-free

1 INTRODUCTION

MALDI or Matrix Assisted Laser Desorption Ionization mass spectrometry [1-3] has rapidly gained popularity as a powerful tool for analysis of biomolecules. Although an area of focus in many groups, the exact mechanism of the ionization process is not clear. The use of a matrix to receive the energy from the laser and transfer that to a crystallized analyte has some disadvantages, particularly the generation of background ions that hinders small molecule analysis. The demonstration of porous silicon as a substrate for matrix-free analysis [4] showed that a scaffold with UV absorptivity is an effective tool. Porous silicon however, can be relatively unstable and requires involved handling procedures. Thus, there is a need to develop a more stable platform that will provide the benefits of matrix-free desorption-ionization, while being easier to use. Here we describe the generation of mesoporous thin silica films for desroption-ionization mass spectrometry and discuss preliminary data obtained using this substrate.

2 METHODS

Mesostructured nanocomposite silica thin films were prepared by an evaporation induced self-assembly process [5] . Initially a silica sol was prepared by refluxing a mixture of tetraethylorthosilicate (TEOS, from Aldrich, 60 mL), anhydrous ethanol (Fisher, 60 mL), >18 MΩ deionized water (4.7 mL), and 0.07 N hydrochloric acid (HCl, 0.2 mL) at 60°C. Upon cooling to room temperature, a portion of the above solution was diluted with ethanol, water, and hydrochloric acid (0.07 N). A non-ionic surfactant, $C_{16}H_{33}(OCH_2CH_2)_nOH$; n~10 (technical name: Brij56, Aldrich), was then added to the silica sol solution at ~4.0 wt. %, significantly below the critical micellar concentration (cmc). During the addition of the surfactant, the water, ethanol, and hydrochloric acid concentrations were adjusted to yield the final reactant mixture in the mole ratios of 1 TEOS: 22 C_2H_5OH: 4 H_2O: 0.004 HCl: 0.085

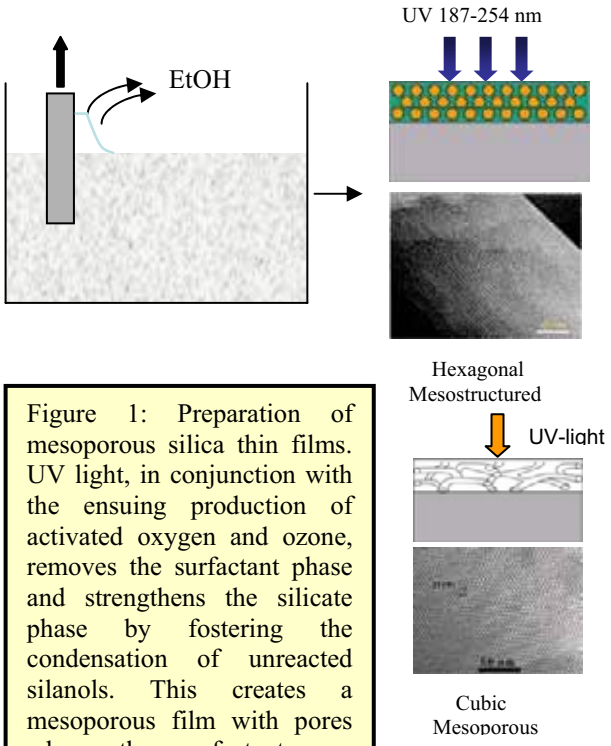

Figure 1: Preparation of mesoporous silica thin films. UV light, in conjunction with the ensuing production of activated oxygen and ozone, removes the surfactant phase and strengthens the silicate phase by fostering the condensation of unreacted silanols. This creates a mesoporous film with pores where the surfactant was destroyed.

Brij56. Mesostructured thin films were deposited onto freshly oxidized single crystal silicon (100) with native oxide overlayer (SiO_2/Si) by withdrawing the substrate from the 4% wt. Brij56/TEOS solution at 5-20 cm/min. All films were aged for at least 24 hrs prior to subsequent patterning or self-assembly experiments.

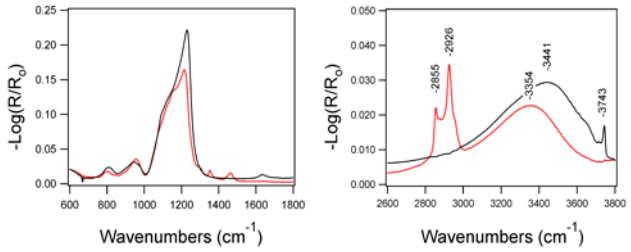

Figure 2: Post UV-light exposure (2hr): Near-complete template removal (2800-3000 cm^{-1}) is seen. So is silicate condensation (1000-1300 cm^{-1}) and the presence of channel wall silanols. There is a change in H-bonding interactions and the surface is rendered hydrophilic [Θ(H2O) < 10 °)

A self-assembled monolayer (SAM) of (heptadecfluoro-1,1-2,2-tetrahydrodecyl)-trichlorosilane, a fluorinated silane, was then deposited onto the mesostructured thin film by immersion in a 0.05 vol % hexadecane solution for 20 min followed by rinsing in chloroform. The SAM-functionalized thin film was subjected to masked deep-uv light to selectively remove the organic material from specific local regions of the film as described previously [6]. This process results in mesoporous regions that can be used as sample wells for spotting analytes in organic or aqueous solution without significant spreading of the dissolved material to neighboring spots (Figure 3).

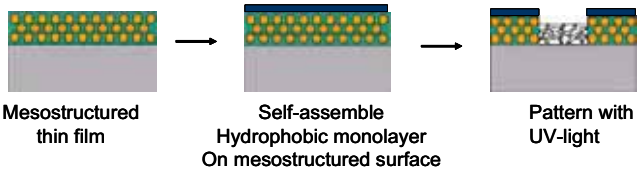

Figure 3: Silanization enhances hydrophobic/hydrophilic character to enhance spotting of organic solvents

Mass spectrometry was performed on a Voyager DE-STR (ABI, Framingham, MA) Time-of-Flight mass spectrometer. Mesoporous silica films were mounted on MALDI probes using double sided adhesive tape. Samples were spotted directly on the sample wells described above. When desired, 0.1% TFA or 50 mM ammonium citrate was added as a proton source. Samples were allowed to dry in air or under a nitrogen stream and MS analysis was performed in reflector mode.

3 RESULTS

A wide range of samples were analyzed using the mesoporous substrate. Our results show that the mesoporous substrate serves as an effective desorption-ionization platform. The films are easy to mount on any standard MALDI plate and our initial results show that the films are stable for over six months in ambient laboratory conditions. The samples chosen to test the film in this study were a mix of organic and inorganic compounds. The spectra in Figure 4 show the analysis of C60 and its derivatives that thus far give the best signal intensities of all compounds tested. These samples were spotted directly on the film without addition of any acid as a proton source.

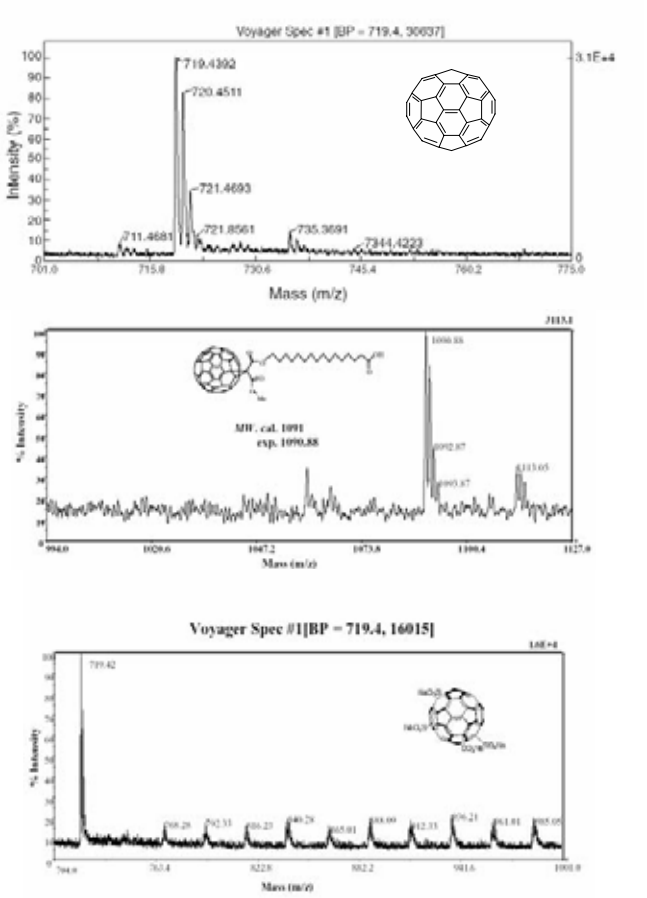

Figure 4: Laser desorption ionization mass spectra of C60 and its derivatives on mesoporous silica. Structure of analyzed molecules are shown as insets in each spectrum.

A significant portion of the research interests in our laboratory is focused on protein and peptide analysis. The ability to analyze small peptides and single amino acids without interference from matrix peaks can enhance the

power of MS and tandem MS approaches on a MALDI platform. Thus, our next step was to assay for the ability to desorb single amino acids. Tryptophan was chosen as a model amino acid and was spotted in a o.1% TFA solution. The resultant spectrum is seen in Figure 5.

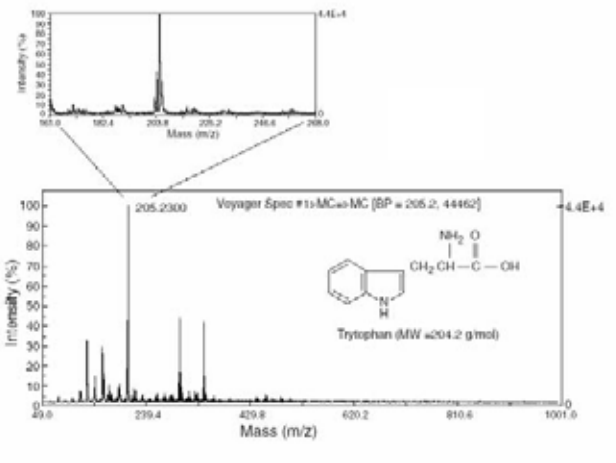

Figure 5: Matrix-free analysis of tryptophan. The major peak is seen in zoom mode in the inset to visualize the C isotopic envelope.

In similar fashion peptide standards were evaluated. Angiotensin II and Bradykinin fragments were analyzed in reflector, positive ion mode. The spectra shown in Figures 6 & 7 demonstrate the ability of this platform to desorb small peptides. These samples were spotted in aqueous mixtures with 0.1 % TFA. Although the resolution of these spectra is acceptable, the signal intensities we obtained were lower than that seen with the traditional matrix-assisted ionization approach. Our current efforts are focused on improving signal intensities by evaluating alternative sample deposition methods. In preliminary experiments, the use of small amounts of organic solvents appears to improve sample spread and also intensity of desorbed ions (data not shown).

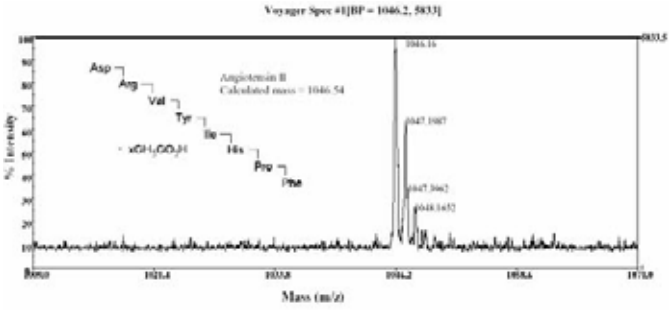

Figure 6: Mass spectrum of Angiotensin II spotted with 0.1% TFA.

In summary, here we have demonstrated the utility of mesoporous silica thin films as an effective substrate for laser desorption ionization mass spectrometry.

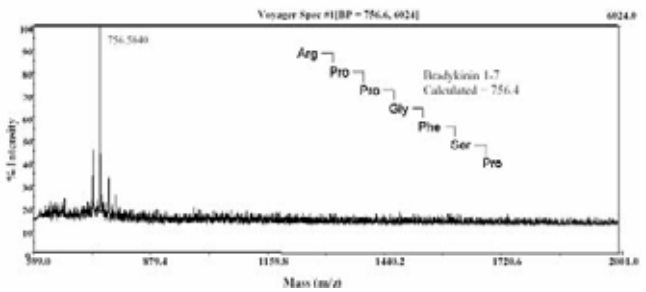

Figure 7: Mass spectrum of Bradykinin 1-7 in 0.1% TFA.

The use of matrix-free approaches greatly simplifies these analyses and facilitates the coupling of high-throughput separation systems to MALDI-type analyses. The stability of these films in ambient conditions will enable the storage of samples in an archival system.

4 ACKNOWLEDGMENTS

This work is supported by Technology Maturation funds from the LANL TTO. LANL is operated by the University of California for the United States Department of Energy.

5 REFERENCES

[1] K. Tanaka, Waki, H., Ido, Y., Akita, S., Yoshida, Y., Yoshida, T., Protein And Polymer Analyses Up To M/Z 100,000 By Laser Ionization Time-Of-Flight Mass Spectrometry, Rapid Communications In Mass Spectrometry Vol.2 (1988) 51-153.

[2] M. Karas, D. Bachmann, U. Bahr And F. Hillenkamp, Matrix-Assisted Ultraviolet-Laser Desorption Of Nonvolatile Compounds, International Journal Of Mass Spectrometry And Ion Processes 78 (1987) 53-68.

[3] F. Hillenkamp, M. Karas, D. Holtkamp And P. Klusener, Energy Deposition In Ultraviolet Laser Desorption Mass Spectrometry Of Biomolecules., International Journal Of Mass Spectrometry And Ion Processes 69 (1986) 265-76.

[4] Z. Shen, J.J. Thomas, C. Averbuj, K.M. Broo, M. Engelhard, J.E. Crowell, M.G. Finn And G. Siuzdak, Porous Silicon As A Versatile Platform For Laser Desorption/Ionization Mass Spectrometry, Anal Chem 73 (2001) 612-9.

[5] Y.F.G. Lu, R.; Drewien, C. A.; Anderson, M. T.; Brinker, C. J.; Gong, W. L.; Guo, Y. X.; Soyez, H.; Dunn, B.; Huang, M. H.; Zink, J. I., Nature 389 (1997) 364.

[6] M.L.Amweg. A. M. Dattelbaum, C. Yee, L. E. Ecke, A. P. Shreve, A. N. Parikh., Photochemical Pattern Transfer And Enhancement Of Thin Film Silica Mesophases., Nano Letters 3 (2003) 719.