Applied Surface Science 256 (2009) 1309–1312

Contents lists available at ScienceDirect

Applied Surface Science

journal homepage: www.elsevier.com/locate/apsusc

Cobalt coated substrate for matrix-free analysis of small molecules by laser desorption/ionization mass spectrometry

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ARTICLE INFO

Article history:
Available online 20 October 2009

Keywords:
Cobalt-coated substrate
Matrix-free target
Laser
Desorption/ionization
Small molecule analysis

ABSTRACT

Small molecule analysis is one of the most challenging issues in matrix-assisted laser desorption/ionization (MALDI) mass spectrometry. We have developed a cobalt coated substrate as a target for matrix-free analysis of small molecules in laser desorption/ionization mass spectrometry. Cobalt coating of 60–70 nm thickness has been characterized by scanning electron microscopy, energy dispersive X-ray analysis, X-ray diffraction, and laser induced breakdown spectroscopy. This target facilitates hundreds of samples to be spotted and analyzed without mixing any matrices, in a very short time. This can save a lot of time and money and can be a very practical approach for the analysis of small molecules by laser desorption/ionization mass spectrometry.

1. Introduction

Matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS) is a very versatile tool for the analysis of large, non-volatile compounds since its introduction in 1990 [1,2]. In this method, sample preparation and introduction plays a very critical role in obtaining a suitable signal. It requires sample and matrix to be mixed at certain molar ratio before applying onto a MALDI target. Small organic dyes are generally used as a matrix to absorb laser energy during the ionization. Those matrices, generally, have molecular weights less than 300 Da and during the ionization, variety of matrix-related ions around 500 Da mass range are produced. Therefore, it has been a challenge to use MALDI for the analysis of low-molecular weight samples ($m/z < 500$). In addition, the necessity of obtaining uniform sample-matrix crystallization on the target before introducing to the system and cleaning of the left over on the target after the analysis are some of the important steps of the MALDI method by using stainless steel or gold coated MALDI targets.

Analysis of small molecules by matrix-assisted laser desorption/ionization have been investigated in detail [3]. There are several studies in the literature for the development of modified and coated surfaces to suppress or remove the matrix signal for the analysis of small molecules by laser desorption/ionization mass spectrometry.

Zenobi et al. [4,5] have reported an effective use of two-phase matrix consisting of graphite particulates and liquid matrix for laser desorption/ionization time of flight mass spectrometry system. It was concluded that thermal desorption of the liquid matrix and analyte occurs by graphite particles acting as an energy transfer medium through the absorption of the UV radiation. Hillenkamp et al. [6] have systematically studied variety of particle suspension matrices for analysis of peptides and proteins by laser desorption/ionization. Han and Sunner [7] have used a thin layer of activated carbon particles immobilized on aluminum substrate for mass spectrometric analysis of organic compounds and peptides. Detection limits varying from attomoles to femtomoles concentrations have been obtained for crystal violet and bradykinin, respectively.

Silicon surface [8–10] and silicon nanopowder (5–50 nm) [11] have been widely used as a matrix for the analysis of small molecules in laser desorption/ionization mass spectrometry. Chen and Wu [12] have used planar silica surfaces as a sample target for the analysis of small organic compounds by laser desorption/ionization mass spectrometry where they have observed very little interference from matrix. The effect of structure and physical and chemical properties of porous silicon surface to be used as a target material by a laser desorption/ionization mass spectrometry have been investigated by Siuzdak et al. [13]. Besides, several other surfaces such as nanoparticle platinum surfaces [14], self-assembled germanium nanodots grown on a silicon substrate [15], and titania nanotube arrays [16] have also been used as a target for laser desorption/ionization mass spectrometry.

Tanaka et al. [1] have reported that ultra fine cobalt powder mixed with glycerol could be used as a matrix for the analysis of

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doi:10.1016/j.apsusc.2009.10.030

various compounds by laser desorption/ionization mass spectrometry. Later, they won the Nobel Prize (2002) in Chemistry for developing this unique method for mass spectrometric analysis of biological samples. This novel method has not yet been used as much as organic matrices introduced by Hillenkamp and Karas [2]. Yalcin et al. [17] have demonstrated the use of cobalt powder in direct analysis of polyethylene in matrix-free media. Detachment of fine cobalt particles from the sample plate, which could possibly damage the mass spectrometry, was reported to be one of the limitations of this method. Use of solid substrate such as silicon [8–10] or additives to suppress/eliminate the matrix signal [18] in small molecule analysis by MALDI mass spectrometry has also been studied.

In this study, a thin-layer cobalt coated silica substrate has been developed and used as a target for matrix-free analysis of small organic molecules by laser desorption/ionization mass spectrometry. The thickness of the cobalt layer on silica substrate has been measured as 60–70 nm. Using this target in laser desorption/ionization mass spectrometry, high number of samples can be analyzed in a very short time without using any matrices. This can save time, money, and labor. To our knowledge, it is the first time that a cobalt coated substrate is used to analyze small molecules by laser desorption/ionization mass spectrometry since Tanaka's work concerning the use of cobalt.

2. Experimental

All chemicals were purchased from Sigma Chemical (St. Louis, MO, USA) and used without purification. Peptide and amino acid samples were prepared in ultra pure water at a concentration of 1.0 μM. 1 μL of sample solution was transferred onto the cobalt coated substrate for laser desorption/ionization mass spectrometric analysis.

Sputter deposition technique has been used to prepare thin film onto silica surfaces. Silica surface has been treated with a mixture of sulfuric acid and hydrogen peroxide solution to remove most organic residues from the silicon surface.

The thickness of the cobalt layer coated on to silicon substrate has been determined from its slice by scanning electron microscope, SEM (Philips XL-30S FEG), and SEM-EDX system has been used to determine the chemical composition.

All mass spectra were obtained using Bruker Reflex III MALDI-TOF mass spectrometer and Voyager-DE PRO MALDI-TOF (Applied Biosystem, USA). Both instruments employed a 337 nm pulsed nitrogen laser to generate ions. The laser power was adjusted slightly above the threshold for desorption/ionization process. All spectra were the results of signal averaging of 50 and 100 discrete laser shots.

Laser induced plasma was formed on the coated substrate surface by focusing a pulsed laser (Nd:YAG, 10 ns, 10 Hz, Spectra Physics) operating at its second harmonic wavelength, 532 nm, with 27 mJ/pulse laser energy. An echelle type spectrograph equipped with an intensified CCD (Andor Scientific) detector was used to monitor plasma emission produced on the cobalt coated substrate between 200 and 800 nm spectral window.

3. Results and discussion

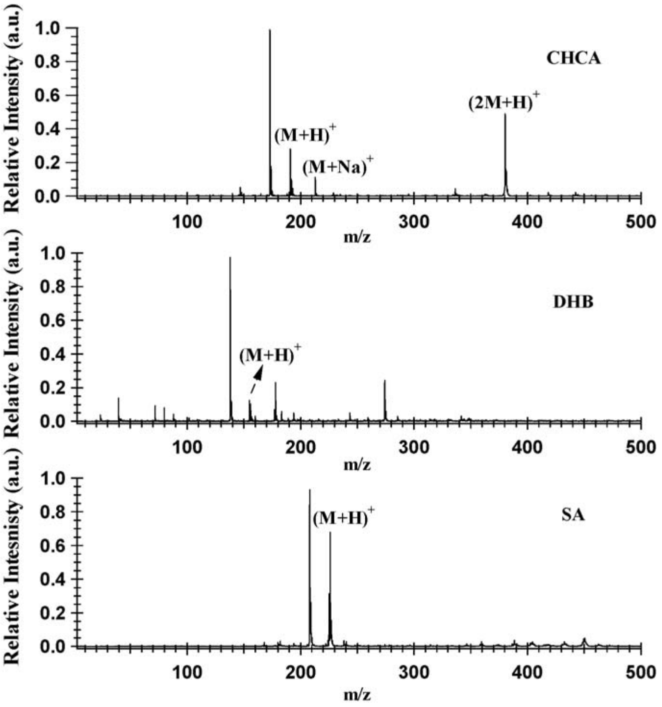

Fig. 1. Mass spectrum of common MALDI matrices.

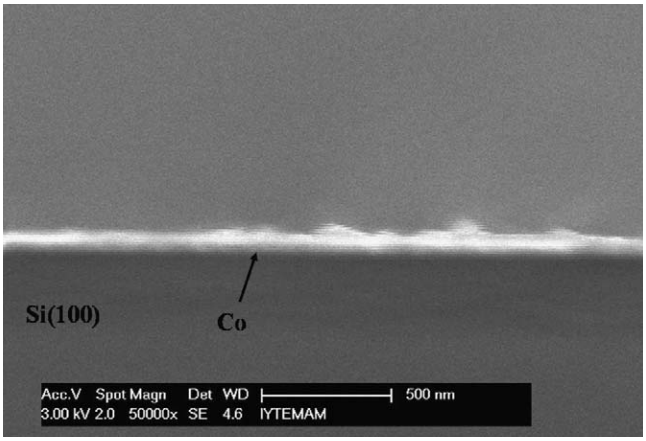

Fig. 2. SEM image from a slice of cobalt coated substrate.

In MALDI, analyte signal can easily be suppressed by matrix-related and cluster ion signals due to high concentration of matrix-to-analyte ratio. Therefore, MALDI-TOF is not a useful and a practical method for analysis of small molecules. Fig. 1 shows the MALDI-TOF mass spectra of common matrices, α-cyano-4-hydroxy-cinnamic acid, CHCA, 2,5-dihydroxybenzoic acid, DHB and sinapinic acid (SA) used for biological sample analysis. Generally, strong matrix signal and some impurities below 500 Da mass range can be easily seen in the spectra. In order to eliminate the matrix signal from the spectra, we have developed a method which utilizes a cobalt coated substrate as a target. Here, cobalt was coated onto silicon wafer substrate by using sputter coating. Coating smoothness and strong adsorption onto the substrate make this technique a very powerful one for coating technology. Fig. 2 shows SEM image of cobalt coated silicon substrate. Coating of uniform cobalt layer can be easily seen. Thickness of the cobalt layer was measured as between 60 and 70 nm after 1 h deposition. Elemental analysis of the coated substrate was also performed through energy dispersive X-ray (EDAX) analysis. Atomic percentage compositions of cobalt, silicon, and oxygen were measured as 64.42%, 32.06%, and 3.53%, respectively.

Crystal structure of cobalt coated layer has been characterized by X-ray diffraction measurements. Fig. 3 shows the XRD spectra of thin cobalt layer. In this figure, cobalt layer shows the peak at $2\theta = 44.53^\circ$ corresponds to (0 0 2) hcp structure. Spectral composition of the cobalt coated substrate has been identified by laser

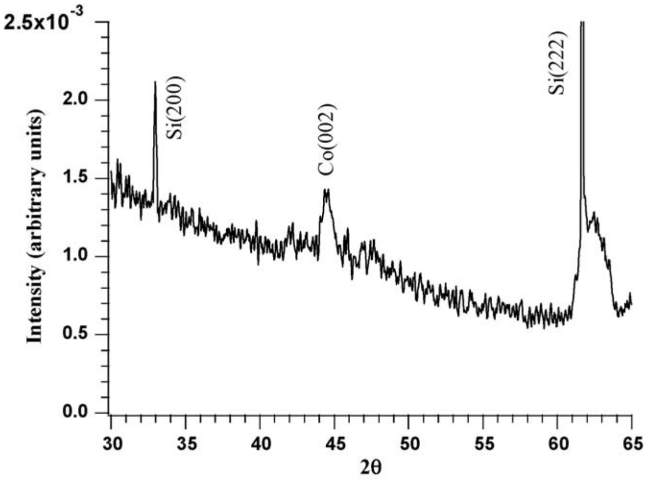

Fig. 3. XRD spectrum of cobalt coated substrate.

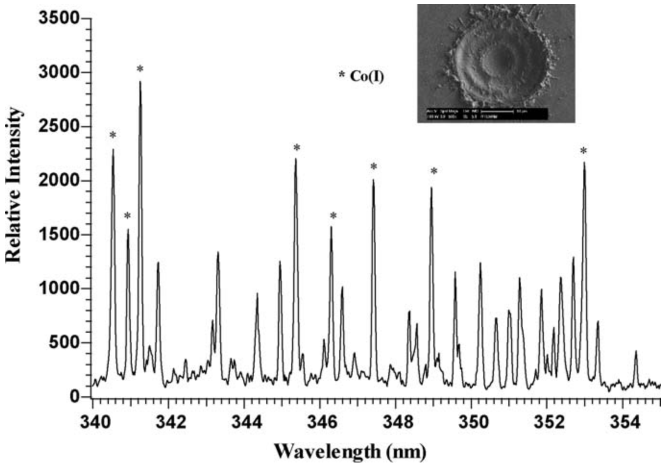

Fig. 4. LIBS spectra and SEM picture of the crater produced from a single laser shot of 27 mJ energy.

induced breakdown spectroscopy (LIBS). LIBS is one of the powerful techniques for the determination of the elemental composition of solids, liquids, and gases with the added benefits of performing multi-element analysis. Fig. 4 shows neutral atomic cobalt lines Co(I) between 340 and 354 nm spectral range as a result of focusing a high-powered laser pulse on cobalt coated substrate. Inset is the crater produced from a single laser shot of 27 mJ pulse energy. It is clearly shown that the substrate is coated with cobalt metal and no other elemental contamination is observed.

After the characterization studies were performed, use of cobalt coated substrate as a target for analysis of amino acids and peptides in laser desorption/ionization mass spectrometry has been tested. Mass spectra of glutamic acid with and without cobalt coated substrate are shown in Fig. 5. Strong analyte signal from the cobalt coated substrate has been observed while no analyte signal from un-coated substrate has been detected. The reason for this improvement is thought to be that cobalt coated onto the substrate absorbs multiple photons of the UV laser causing thermal desorption of the analyte through rapid heating as explained previously by some other groups [4,5,17]. Here, the difference of our work comes from the use of cobalt coated substrate as a target rather than use of powdered particles that range between 2 and 100 μm in size. To our knowledge, this is the first time that a cobalt coated substrate is used to analyze small molecules by laser desorption/ionization mass spectrometry since Tanaka's work.

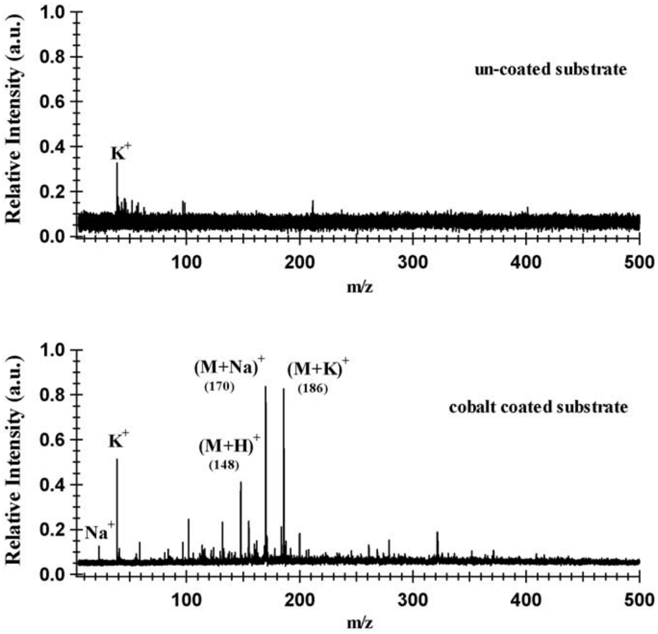

Fig. 5. Mass spectra of glutamic acid: using cobalt coated and un-coated substrate.

Also, in Fig. 5, in addition to sodium and potassium ion signals observed at m/z 23 and m/z 39, analyte–adduct signals, $(M + Na)^+$, $(M + K)^+$, having stronger intensity than protonated analyte signal, $(M + H)^+$ have been observed. The reason for observing strong analyte–adduct signal is due to the presence in sodium and potassium salt on the cobalt coated substrate's surface. Some other researchers have also observed similar behavior when they have used the particle suspended laser desorption/ionization mass spectrometry [1,4,5,19,20]. In order to eliminate these salts from the medium, coated substrate has been cleaned in water using ultrasonic bath before analysis. As it can be seen in Fig. 6,

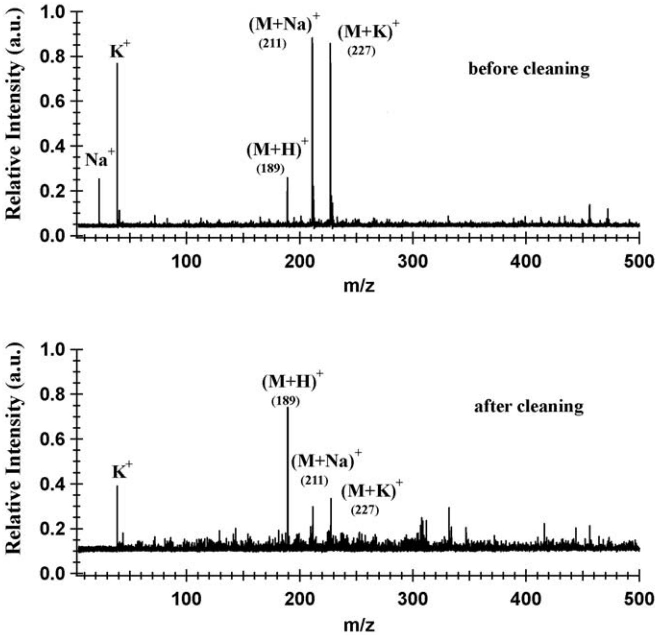

Fig. 6. Mass spectra of alanine–valine before and after cleaning the cobalt coated substrate.

protonated analyte signal has been enhanced after cleaning. However, the signal quality is not as high as expected, for which in the future, some improvement studies need to be carried out.

Close control of the crystal structure and thickness of the cobalt coating might be some of the important parameters in obtaining better analyte signal from the samples which are being investigated in our laboratory.

4. Conclusion

It has been shown that thin-layer cobalt coated silicon substrate can be used as a pre-coated target for laser desorption/ionization mass spectrometry for analysis of small molecules. Using this target, amino acids, peptides, and small organic molecules can be analyzed without using common matrices in a very short time. Use of this target in applications those require high throughput can save time, money, and labor.

To our knowledge, this is the first study where cobalt coated substrate has been used as a matrix-free target for analysis of biologically important small molecules in laser desorption/ionization mass spectrometry since Tanaka's novel application concerning the use of cobalt.

Acknowledgements

This research was supported by the Scientific and Technological Research Council of Turkey, TUBITAK, under project no. 105T384. Authors thank Dr. Serife Yalcin for her assistance in obtaining LIBS data. Authors also thank the research staff of Materials Research Center, IYTE, Turkey.

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