Investigation of thin ZnO layers in view of laser desorption-ionization

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Abstract. Thin zinc oxide films (ZnO) were developed as a matrix-free platform for surface assisted laser desorption-ionization (SALDI) time-of-flight mass spectrometry. The ZnO films were deposited by RF magnetron sputtering of ZnO ceramic targets in Ar atmospheres on monocrystalline silicon. The generation under UV (355 nm) laser irradiation of positive ions of atenolol, reserpine and gramicidin S from the ZnO layers deposited was studied. All analytes tested were detected as protonated molecules with no or very structure-specific fragmentation. The mass spectra obtained showed low levels of chemical background noise. All ZnO films studied exhibited high stability and good reproducibility. The detection limits for test analytes are in the 10 femtomol range.

1. Introduction
Surface assisted laser desorption ionization (SALDI) is a novel method of ionization of organic and bioorganic compounds which has been under intensive development over the last decade. In SALDI, the ionized species are formed in a gas phase from analytes deposited on the surface of a specially designed SALDI-active substrate which is irradiated by a pulsed laser. This process does not require the entrainment action of an added matrix compound for desorption [1]. A variety of inorganic materials in the form of powders or monolithic plates have been studied as a possible platform for SALDI; it was found that only a few selected substrates have the ability to strongly promote ion formation. One of the well-known examples of such a substrate is porous silicon obtained by electrochemical etching [2]. In particular, porous silicon is used for small-molecule detection in DIOS (desorption/ionization on silicon), a variant of SALDI [3]. It was found recently that thin nanocrystalline and/or amorphous silicon films prepared by RF magnetron sputtering are very

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effective SALDI substrates [4]. When compared to porous silicon, deposited films have a number of advantages, such as easy and controllable method of fabrication, greater uniformity over large areas, higher purity and good reproducibility. However, the amorphous Si substrates were found to be unstable in ambient air.

The present work is focused on examining thin zinc oxide films (ZnO) as a platform for SALDI time-of-flight mass spectrometry.

2. Experimental

We used a laboratory-built, linear, turbopumped time-of-flight mass spectrometer with a 0.70 m flight tube. The ion acceleration voltage was 16.0 kV; the ions were detected by a 40-mm diameter chevron multichannel plate (MCP) assembly. The detection system was adjusted so as to obtain a direct reading of the ions number hitting the detector. The signal from the MCP was recorded by an ADC digitizer with 1 Gs/s sampling rate and ability to store mass spectra at a rate of up to 1 kHz. A diode-pumped YAG laser with near-diffraction limited beam quality 0.5 ns pulse duration was operated at 300 Hz. A frequency-tripling crystal yielded 355 nm laser pulses of about 100 µJ. The laser pulse energy was varied in the range of 10-30 µJ using an attenuator.

In order to utilize the analyte more efficiently, the laser focus was scanned over a 1×2 mm² sample deposition area using a computer-controlled dual-mirror scanner. The laser focus moved approximately 0.08 mm between successive laser shots; about 300 individual mass spectra were obtained from different points on the probed area of the SALDI substrate and were accumulated by the ADC digitizer during one “scanning cycle”.

Thin films of ZnO were deposited by RF 13.56 MHz magnetron sputtering of ZnO ceramic targets in Ar atmosphere (0.5 Pa) on monocrystalline silicon (for SALDI and X-ray diffraction (XRD) measurements) and glass substrates (for optical measurement) held at 500ºC. The distance between the target and the substrates was 7.5 cm. The vacuum chamber was evacuated to a residual gas pressure of 2×10⁻⁴ Pa. Sputtering was carried out at RF power of 180 W. The thickness of the films deposited measured by a “Talystep” profilometer was about 0.5 µm.

The XRD spectra were obtained using a DRON 3 spectrometer with CuKα radiation: λ CuK₁ = 1.540560 Å and λ CuK₂ = 1.544426 Å (intensity half that of λ CuK₁).

The SALDI substrates used in the present work were approximately 5×5 mm² and were attached to the instrument’s stainless steel insertion probe.

All chemicals were obtained commercially and used without further purification. Atenolol (Mr 266.3 g/mol), reserpine (Mr 608.7 g/mol) and gramicidin S (Mr 1140.7 g/mol) used as test analytes were dissolved in acetonitrile-water at a concentration of 50 to 1000 ng/ml. A volume in amount of 1-4 μl of analyte solution was spotted on the surface of a ZnO film, air-dried and then analyzed.

3. Results and discussion

3.1. ZnO films characterization

The XRD spectrum of the ZnO films deposited was measured. The reflection corresponding to the (002) plane is observed which demonstrates the preferential orientation of the grains in the ZnO films. The grains size was calculated using the Debye-Scherrer formula and was about 160 nm. The transmission of all samples was about 92-93% at wavelengths higher than 600 nm, assuming a value of 100% for the transmission of the glass substrate. The absorption coefficient, α, was calculated. The formula for direct allowed transitions was used to obtain the optical gap, E₉₅, whose value was 3.27 eV, typical for ZnO.

3.2. Mass spectra

Atenolol, reserpine and gramicidin S were selected as test analytes because of their high proton affinities, differences in molecular weights and their widespread use as drugs, so that we could obtain
a good indication of the usefulness of the ZnO assisted laser desorption ionization method.

The preliminary study of the ZnO films threshold behavior, i.e., the ion currents dependence on the laser fluence showed that 40 mJ/cm² of laser intensity gives the best performance for all analytes studied. Decreasing the laser fluence leads to a dramatic decrease of the SALDI-signal. As the laser fluence is increase, zinc containing peaks appear in the mass spectra, indicating the destruction of ZnO films. Therefore, all following experiments were carried out at 40 mJ/cm² of laser fluence. Figure 1 shows a typical SALDI mass spectrum of 400 pg of atenolol deposited on the ZnO surface from a 200 ng/ml solution. It is seen that the base peak in the spectrum in figure 1 is associated with the protonated atenolol molecule at m/z=267. No fragment ions are observed in the spectrum. The minor background peaks throughout the mass range result from surface contamination, possibly from vacuum pump oil. K⁺ at m/z=39 and Na⁺ at m/z=23 ions were also observed in the low-mass region (not shown). In general, however, the mass spectra obtained are “clean” with low chemical background. This property of the nc-ZnO surface minimizes the need for sample cleanup.

Figure 2 shows a SALDI mass spectrum of 1 ng (0.9 pmole) of gramicidin S measured from a 500 ng/ml concentration solution of this peptide. The base peak in the mass spectrum is the protonated molecule at m/z = 1141, but there are also sodium [M+Na]⁺ and potassium [M+K]⁺ cation adduct peaks. Similarly to the case of atenolol, no fragment ions are observed in the mass spectrum of gramicidin S. These observations prove that ZnO based SALDI is a soft ionization technique. However, as it was shown earlier [4] SALDI mechanism involves the stage of thermally induced desorption of ions. This can result in at least partial decomposition of thermally labile compounds.

Figure 1. SALDI mass-spectrum of atenolol. Figure 2. SALDI mass-spectrum of gramicidin S.

Reserpine exhibits such behavior. This is illustrated in figure 3, which shows the mass spectrum of 400 pg of reserpine deposited on the ZnO surface from a 200 ng/ml solution. The mass spectrum presented contains a protonated molecule at m/z=609 and a few structure-specific fragment ions. The major fragment ion at m/z = 195 is formed by heterolytic cleavage of the bond between oxygen of the ester group and carbon of the ring accompanied by hydrogen transfer to the oxygen and elimination of the 3,4,5-trimethoxybenzoic acid. Another fragment ion at m/z=397 can be interpreted as a result of the C=O bond cleavage of inside the ester group, as is shown in figure 3.

3.3. Analytical parameters
Based on the actual amount of analyte deposited onto the ZnO surface, it was found that the average number of ions detected per femtomole of atenolol, reserpine and gramicidin S were 50 ions/fmol, 45 ions/fmol and 5 ions/fmol, respectively. With about 100 ions required for reliable compound detection, the detection limits obtained were in the range of 2-20 femtomoles.
A relatively high mass spectrometric selectivity was obtained, as a relatively high resolution, \( m/\Delta m \sim 800 \), was implemented in a simple linear TOF mass spectrometer due to a short desorbing laser pulse and to a well defined ionization zone located on the active ZnO surface.

The SALDI ions signal was measured for different amounts of analytes over a range of 2 orders of magnitude by changing the concentration of the solutions deposited. A typical dependence of the analytical signal on the analyte concentration is illustrated in figure 4 for reserpine. Very similar calibration curves were obtained for atenolol and gramicidin S. It was found that the linearity was better than 0.98 for all three compounds studied. The reproducibility was monitored by measuring the analytical signal using the same experimental conditions but with the different ZnO film samples. Twenty replicate analyses were carried out over a one-week period for each of the compounds studied. The relative standard deviation was less than 15% for all three compounds. This result clearly demonstrates the applicability of ZnO films for quantitative analysis of low molecular weight drugs. However, internal standards are needed for better quantification using the droplet analyte deposition method. In contrast to silicon based SALDI-active substrates, such as porous, electrochemically etched and amorphous silicon, ZnO based SALDI substrates are very stable under atmospheric conditions. There was no significant decrease in the ionization efficiency after a few months storage in air. The substrates were not “cleaned” in any way, except by laser irradiation during the experiments.

4. Conclusions
Thin ZnO films, prepared by an RF method, were investigated as the platform for analysis of organic and bioorganic molecules by SALDI-TOF mass-spectrometry. The suitability of the films prepared for drug detection in the mass range 250-1150 m/z was examined. Atenolol, reserpine and gramicidin S were studied as test analytes.

It was found that the ZnO films are very stable and show good sensitivity in the range of analyte concentrations examined. The mass spectra obtained are “clean” with almost no chemical background and with no or very specific analyte ions fragmentation. The detection limits are in the 2 - 20 femtomol range. Good reproducibility under the same experimental conditions was found for all analytes.

The high purity, the ease of reproducible fabrication, the good analytical parameters, and the ability to analyze samples rapidly make thin ZnO films a potentially powerful tool for the detection of small bioorganic and organic molecules especially in the field of drug analysis by the SALDI method.
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