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纳米金淀积的多孔硅靶增强样品的激光解吸/电离质谱信号

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摘要:硅片类型和多孔硅结构的多样性影响了多孔硅表面的激光解吸/离子化质谱(DIOS)(无辅助基质的激光解吸/电离飞行时间质谱(LDI-TOF-MS))数据的重复性和靶的耐储时间。本工作通过在多孔硅的表面淀积金纳米颗粒并将其作为目标靶来增强软物质分子如聚乙二醇和多肽的激光解吸/电离质谱信号。纳米金的淀积钝化了多孔硅表面的 Si-H 活性基团,增加了靶的耐储时间。用场发射扫描电镜表征了多孔硅淀积金纳米颗粒前后的形貌,用 X 射线能量色散光谱法分析金的百分含量,结果表明其含量随沉积时间的延长而增加。激光解吸/电离质谱信号的增强可能是由多孔硅及其支持的金纳米颗粒的光学和物理性质引起的,该类型的样品靶在激光解吸/电离飞行时间质谱的应用上结合了多孔硅和金纳米颗粒的双重优势。

关键词:金纳米颗粒;多孔硅;激光解吸/电离质谱;聚乙二醇;多肽
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Gold Nanoparticle-deposited Porous Silicon as Target to Enhance Laser Desorption/Ionization of Molecules

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Abstract: The diversity of silicon types and porous silicon structures brought many challenges to desorption/ionization on silicon(DIOS) (matrix-free mass spectroscopy) such as poor repeatability/reproducibility in data and short shelf-life of the sample target. The porous silicon layer deposited with gold nanoparticles was studied in this work as a sample target to enhance the laser desorption/ionization(LDI) of polyethylene glycol(PEG) and two peptides (oxytocin and angiomax). Passivation of the surface Si-H species by gold nanoparticles increases the shelf-life of the target. The morphologies of porous silicon before and after deposition of gold nanoparticles were examined by field-emission scanning electron microscopy(FE-SEM). The elemental composition of surface species was analyzed by X-ray energy dispersive spectroscopy(EDS). Effect of the deposition time on the quantity of gold nanoparticles and also on the signal enhancement was investigated. The desorption/ionization enhancement of molecules may be due to the integration of porous silicon and gold nanoparticles with unique optical and physical properties. This type of sample target combines the advantages of both DIOS and gold nanoparticles.

Key words: gold nanoparticles; laser desorption/ionization; peptide; PEG

As a soft ionization mass spectrometric method, matrix assisted laser desorption/ionization(MALDI) has become a preeminent technique in the analysis of a wide variety of compounds including polymers and

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biomacromolecules[1~4]. However, the use of matrices has limited MALDI′s applications in analyses of molecules with low molecular weight because the background of small organic matrices lies in this region and interferes with the analytes[5].

In order to extend MALDI′s applications for small molecules, a significant amount of work has been developed in recent years. Earlier work on this topic was to use metallic powders as inorganic matrices. Tanaka et al.[6] used a suspension of fine cobalt powder (30 nm in diameter) in glycerol as matrices to analyze proteins such as lysozyme. Sunner et al.[7] demonstrated that a suspension of graphite particles (2~150 μm) in glycerol was a good surface assisted laser desorption/ionization (SALDI) method for proteins and peptides. The method seems particularly suited to the analysis of peptide mixtures(for example, proteolytic digestives). Further, Kinumi et al.[8] used metal or metal oxide powder(Al, Mn, Mo, Si, Sn, SnO_2, TiO_2, W, WO_3, Zn or ZnO) as matrices to analyze PEG200 and methylstearate successfully. Since the particle sizes are in the range of a few tens of micrometers, the method can be easily applied to small molecules with a low noise background in the mass range of 0~1 000 Da. DIOS was introduced by Siuzdak′s group in 1999[9]. Porous silicon was fabricated by electrochemical etching in hydrofluoric acid(HF) solution. The porous silicon membrane was used as a matrix-free sample target for small molecules. The microstructures of porous silicon can trap the analyte molecules and efficiently absorb UV laser energy, and then transfer the energy to small molecules to be desorbed and ionized. DIOS simplifies the sample′s preparation because the analyte does not need to be mixed with the matrix for co-crystallization which is otherwise a must for an adequate signal. Hence, the background peaks of DIOS in the low molecular mass region are minimized and the signal-to-noise ratio is enhanced. DIOS is also widely applied to analysis of biological analytes, especially proteins and peptides. However, porous silicon is unstable and it is vulnerable to oxygen and moisture in air, which is a big challenge for DIOS′s applications. Surface oxidation or modification with organic molecules was investigated to stabilize porous silicon and to resist air oxidation and acid/base hydrolysis for a longer shelf-life time[10,11].

Gold nanoparticles exhibit many unique and interesting physical and optical properties such as surface plasmon resonance (SPR), surface enhanced Raman scattering (SERS), nonlinear optical properties(NLO), and quantized charging effect[12~14]. Owing to these, gold nanoparticles offer unique advantages to produce analyte ions without the help of organic matrices. Russell et al. used gold nanoparticles(2~10 nm) as selective matrices for the ionization of biomolecules in the region of 500 ~2 500 Da [15]. Tseng et al. demonstrated gold nanoparticles as matrices for analyzing small neutral carbohydrates by SALDI[16]. Russell et al. further developed the capping of gold nanoparticles with an organic self-assembled monolayer for LDI of biomolecules [17]. Compared to other nanoparticle systems, the capped nanoparticles increase ion′s yields, decrease ion′s fragmentation, and extend the useful mass range for analytes.

Trapping of gold nanoparticles in porous silicon has not yet been reported as a sample target to enhance LDI for mass spectrometry. We demonstrate here that gold nanoparticles deposited on porous silicon enhance the laser desorption/ionization-time of flight-mass spectroscopy (LDI-TOF-MS) signal very well. This hybrid target is produced by immersion of freshly etched porous silicon in $HAuCl_4$ solution at room temperature, where the reactive Si-H groups reduce gold ions into gold metal atoms. And at the same time gold atoms are deposited on porous silicon and are nucleated into gold nanoparticles. After deposition, the porous membrane structure is more stable and inert in air. As a target for LDI-TOF-MS, the laser ionizes the analytes efficiently on its surface [16,17]. We use this target to analyze PEG400, PEG2300, oxytocin and angiomax easily without matrices. The method combines the advantages of both DIOS and gold nanoparticles and it could become an alternative matrix-free approach for LDI-TOF-MS.

1 Experimental

1.1 Chemicals and reagents

P-type silicon wafers(single crystals <100>, boron-

doped, 5.0~8.0 Ω·cm, single side polished) were purchased from Guangwei Electronic Material Co., Ltd (Shanghai, P. R. China). Hydrogen tetrachloroaurate(Ⅲ) hydrate was purchased from Shanghai Chemical Reagent Co., Ltd, PEG400 from Sigma-Aldrich, USA and PEG2300 from JenKem Technology Co., Ltd (Beijing, P. R. China). Oxytocin and angiomax were offered by the Institute of Molecular Medicine at Nanjing University. Other reagents were reagent grade or higher and used as received unless otherwise specified. MILLI Q water(>18 MΩ) was used for all experiments.

The analytes, PEG400 and PEG2300, were dissolved in acetonitrile(3 mmol·L^{-1}), and those of oxytocin and angiomax were in water(1 mmol·L^{-1}). A 2 mmol·L^{-1} $HAuCl_4$ solution was freshly prepared by addition of EtOH (1∶4 (*V*/*V*), EtOH/solution) into a 2.5 mmol·L^{-1} $HAuCl_4$ water solution.

1.2 Preparation of porous silicon(PSi) and deposition of gold nanoparticles

Prior to the etching procedure, the single side polished silicon wafers were cut into 18×18 mm size and boiled in 3∶1(*V*/*V*) H_2SO_4/30%H_2O_2 for 30 min(Note: piranha solution reacts violently with organic materials and should be handled with extreme care), then rinsed with copious amounts of water. After dried under a mild stream of nitrogen, the silicon wafers were electrochemically etched in HF solution(1∶3(*V*/*V*), 40%HF/DMF) at a constant current density of 4 mA·cm^{-2} for 60 min. After etching, the wafers were rinsed with copious amounts of EtOH, and dried by nitrogen. The freshly etched porous silicon was immersed in 2 mmol·L^{-1} $HAuCl_4$ solution at room temperature for 20 min. Gold ions were reduced into gold metal atoms by the reactive Si-H species and nucleated into nanoparticles. After rinsing and drying, the chips were stored in a desiccator and then used as the matrix-free targets for LDI-TOF-MS measurements.

1.3 SEM and EDS

Field-emission scanning electron microscope (FE-SEM, Hitachi S-4800) with a point-to-point resolution of 2 nm was used to observe the morphology of samples at an accelerating voltage of 15.0 kV. The high resolution was obtained by using a Schottky field emission source, a beam booster maintaining the high beam energy throughout the microscope column, an electromagnetic multihole beam aperture, and a magnetic field lens. The EDS equipped in FE-SEM was used to identify surface elements.

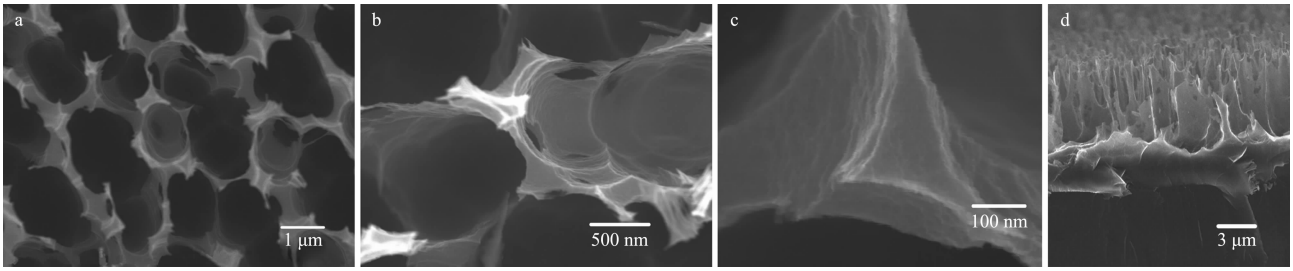
1.4 Laser desorption/ionization mass spectrometry

The mass spectra of analytes were measured by a LDI-TOF mass spectrometer (AutoflexII TOF/TOF, Bruker Daltonics, Germany) operating in a positive reflectron or linear mode. The hybrid gold nanoparticle/porous silicon target was adhered to a modified sample target plate by using conductive carbon tape. The analyte sample solutions were spotted directly onto the hybrid target without any matrix. After dried in air, the target was introduced into the mass spectrometer. The instrument was equipped with a delayed ion extraction device with 100 ns delay time and a pulsed nitrogen laser operated at 337 nm. The laser power was adjusted slightly above the threshold for the desorption/ionization process. All spectra were used at an accelerating voltage of 20 kV and a low-mass gate of 5000 Da. Spectra were collected by averaging 50 or 100 discrete laser shots. All data were processed using Bruker Daltonics FlexAnalysis 2.4 without baseline correction and Gaussian smoothing.

2 Results and discussion

2.1 Morphologies of porous silicon

FE-SEM was employed to determine the topographical parameters of porous silicon such as pore size, surface porosity and the depth of porous layer. The SEM images of porous silicon are shown in Fig. 1a~1d. The porous silicon layer is a homogeneous planar beehive with 0.5~4 μm diameter macro pores randomly distributed on the whole surface (Fig.1a~b). By imaging its inside-structures, a hierarchical architecture structure can be found, where the frames of porous silicon and the bottom of macro pores consist of nano-porous structures with 3~10 nm diameter pores inside (Fig.1c). The cross-section of porous silicon in Fig.1d shows that the depth of the macropore layer is about 6~8 μm.



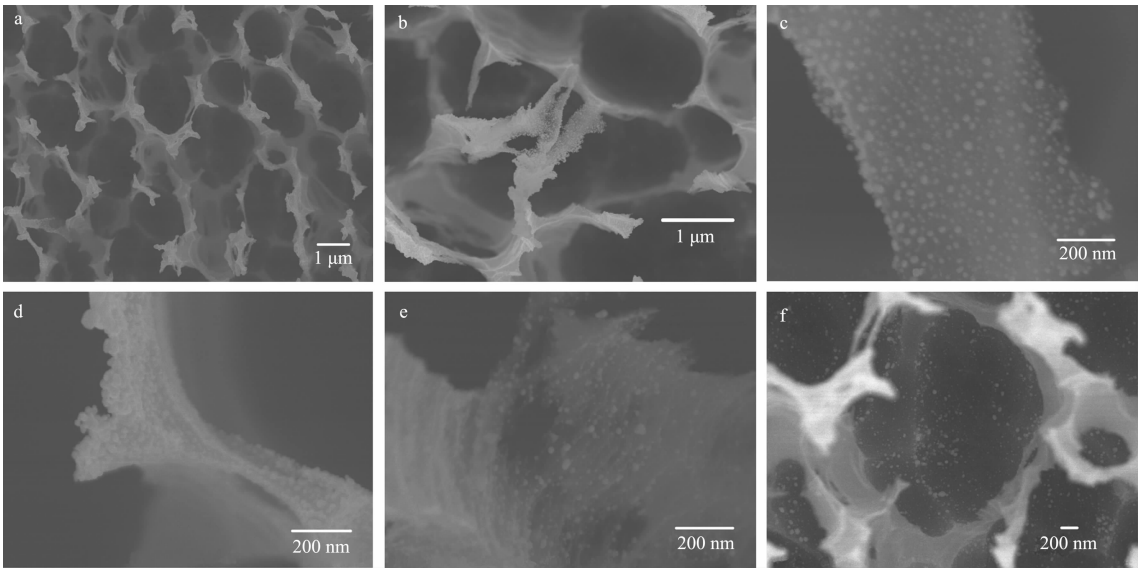
(a), (b) and (c) porous silicon images from lower to higher amplifications; (d) a cross-section structure.

Fig.1 SEM micrographs of porous silicon

2.2 Morphologies of gold nanoparticles deposited on porous silicon

The freshly etched porous silicon is unstable because the metastable Si-H species can be oxidized by oxygen and moisture in air. The Si-H species can reduce gold ions from $HAuCl_4$ solution into gold metal atoms and then gold atoms subsequently nucleates into nanoparticles. However, the porous silicon surface is hydrophobic and it can not be easily wetted by water. So, gold nanoparicles can not be uniformly deposited on porous silicon surfaces. In order to make the deposition easier, EtOH was added in the $HAuCl_4$ water solution(1:4(*V*/*V*), EtOH/solution) before the freshly etched porous silicon was immersed. Our observation confirms that gold nanoparticles can be homogeneously deposited on the porous silicon surface(Fig.2c and 2d) with this formulation. After deposition, the PSi structure was still kept intact without any damages and the sample can be stored for a long time over 3 months. The size of nanoparticles is about 10~30 nm. The particles on the porous silicon frames are obviously denser than those on the pore walls and at the pore bottom(Fig.2e and 2f). It might be caused by the concentration gradients of the $HAuCl_4$ solution from the top frame to the bottom of the pores. It is reasonable to conclude that the concentration of $HAuCl_4$ at the pore bottom is lower than that near the top frame region. Hence, less gold nanoparticles were deposited at the bottom.

In order to study the relationship between the immersion time and the amounts of gold nanoparticles de-



(a) and (b), gold nanoparticles deposited on porous silicons with lower amplifications;
(c) and (d), gold nanoparticles on porous silicon with higher amplifications on the top of the frame and inside the pore wall;
(e) and (f), gold nanoparticles at the pore bottom

Fig.2 SEM micrographs of PSi after deposition of gold nanoparticles in 2 mmol·L^{-1} $HAuCl_4$ solution for 20 min at room temperature

posited on the porous silicon, a series of porous silicon immersion time(0~60 min) were followed by EDS for elemental analyses. The gold concentration increases quickly during the first 20 min immersion (Fig.3). After 20 min, the deposition speed slows down and even more, nanoparticles tend to cluster into bigger particles.

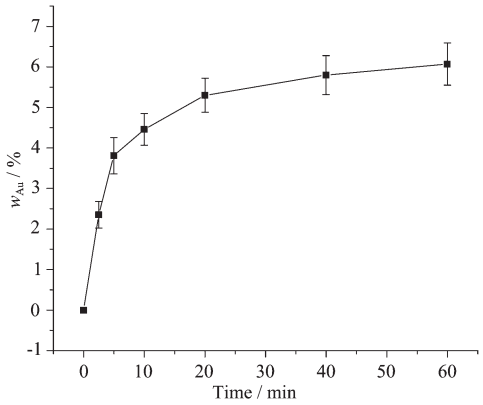


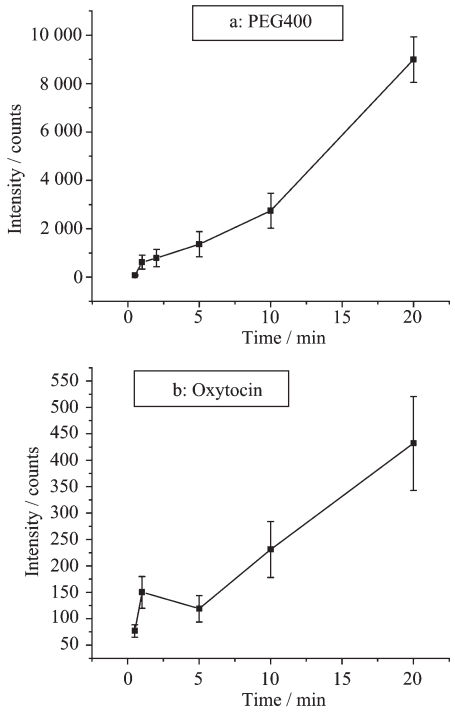
Fig.3　Dependence of the gold concentration on porous silicon surface on the immersion time. Gold was deposited in 2 mmol·L^{-1} $HAuCl_4$ solution for 0~60 min at room temperature. The gold weight percentage was detected by EDS.

The amount of gold nanoparticles deposited on porous silicon increases with increasing the incubation time as shown by EDS measurement in Fig.3. The surface weight percentage of gold increases very fast at the first 10 min, and then it evolves to a saturated surface coverage of about 6% after 20 min.

2.3　Application of gold nanoparticle coated PSi for LDI-TOF-MS

To optimize the chip structure for LDI-TOF-MS measurements, we investigated the signal dependence of PEG400 and oxytocin on the different immersion times in 2 mmol·L^{-1} $HAuCl_4$ solution. The signal intensities of analytes increased obviously in the first 20 min (Fig.4). Combined with the EDS analysis of gold concentrations, the chips with 20 min incubation were chosen as sample targets for mass analyses.

The mass spectra of analytes are shown in Fig.5. Fig.5a and 5b are the mass spectra of PEG400 and PEG2300, respectively. These spectra demonstrate obvious oligomer distributions. The main oligomer distribution corresponds to the expected polyethylene glycol with adjacent peaks separated by 44 units, i.e., the

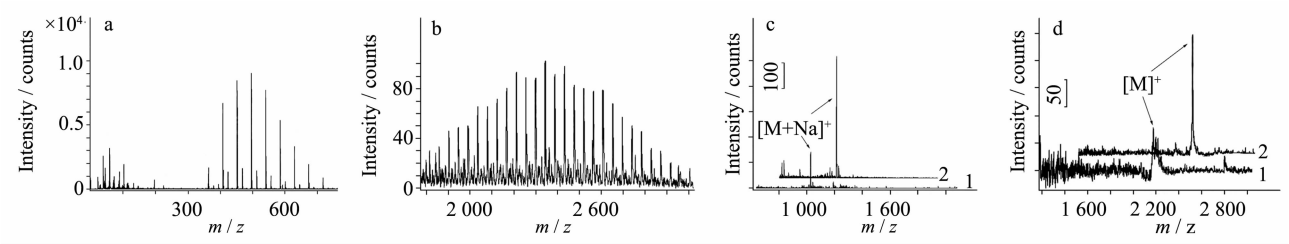


(a) PEG 400; (b) oxytocin

Fig.4　LDI-TOF-MS signal intensity vs deposition time

mass of the repeat unit of CH_2CH_2O. The metal-adduct ions of $[M+Na]^+$ and $[M+K]^+$ are ubiquitous in the mass spectra of PEG polymers. In our measurement, the $[M+Na]^+$ distribution dominates the spectra and the $[M+K]^+$ distribution is weak and even disappears sometimes. In Fig.5a, ions of 197, 394 and 591 *m/z* are produced by gold clusters(Au^+, Au_2^+, Au_3^+). These gold ions are constant and are much weaker than those of analytes, so they can be easily discerned from molecular analytes. The peaks of 22.8, 38.8, 57.9 and 225.2 might be produced by Na^+, K^+, SiO_2^+ and $[Au+Si]^+$. There still have some unknown peaks in the low molecular mass region, which should be assigned to contaminants. However we have not yet detected either PEG400 or PEG2300 on porous silicon, probably due to the silicon type or the porous structure.

The mass spectra of two peptides, oxytocin and angiomax, were also measured. Oxytocin in Fig.5c is in the form of $[M+Na]^+$ and $[M+K]^+$, mainly in $[M+Na]^+$, where spectrum 1 is the signal from DIOS (sample on porous silicon) and 2 from gold coated PSi. DIOS presents only one fifth signal of that from gold coated PSi. Angiomax is observed in the form of $[M^+]$ in Fig.5d,



(a) PEG400; (b), PEG2300; (c) oxytocin (spectrum 1 is from DIOS and 2 from gold coated PSi);
(d) angiomax (spectrum 1 is from DIOS and 2 from gold coated PSi)
The mass spectra of (a)~(c) were analyzed in reflected mode and that of (d) analyzed in linear mode

Fig.5 Mass spectra of analytes using gold nanoparticle coated PSi as sample targets

where spectrum 2 is from gold coated PSi and 1 from DIOS. Obviously DIOS (spectrum 1) presents a weak and wide peak with higher noise background, while spectrum 2 provides a clean and strong signal. All four samples confirm the mass signal enhancement on gold nanoparticle coated PSi.

3 Conclusions

In the report, we present the use of gold nanoparticle deposited porous silicon as sample target to enhance the laser desorption/ionization of molecules for mass analysis without matrix. The approach combines the advantages of both DIOS and gold nanoparticles. Four sample molecules, PEG400, PEG2300, oxytocin and angiomax were analyzed as examples. It enhances the analyte signal remarkably compared to DIOS. Both porous silicon and gold nanoparticles play important roles in laser desorption/ionization of analytes and also contribute to the signal enhancement.

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