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Layer-by-layer thin film of reduced graphene oxide and gold nanoparticles as an effective sample plate in laser-induced desorption/ionization mass spectrometry

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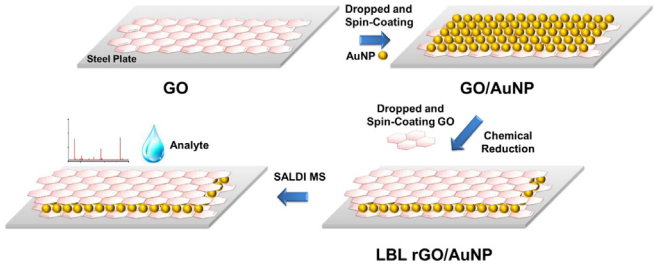
HIGHLIGHTS

- Fabrication of reduced graphene oxide and gold nanoparticles multilayer thin-film.
- The film served as the sample plate and also worked as the matrix in SALDI-TOF MS.
- Preparation of sample by one-step deposition of analytes onto the sample plate.
- Analysis of amino acids, carbohydrates and peptides by using the sample plate.

GRAPHICAL ABSTRACT



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ABSTRACT

This work demonstrated a simple platform for rapid and effective surface-assisted laser desorption/ionization time-of-flight mass spectrometry (SALDI-TOF MS) measurements based on the layer structure of reduced graphene oxide (rGO) and gold nanoparticles. A multi-layer thin film was fabricated by alternate layer-by-layer depositions of rGO and gold nanoparticles (LBL rGO/AuNP). The flat and clean two-dimensional film was served as the sample plate and also functioned as the matrix in SALDI-TOF MS. By simply one-step deposition of analytes onto the LBL rGO/AuNP sample plate, the MS measurements of various homogeneous samples were ready to execute. The optimization of MS signal was reached by the variation of the layer numbers of rGO and gold nanoparticles. Also, the small molecules including amino acids, carbohydrates and peptides were successfully analyzed in SALDI-TOF MS using the LBL rGO/AuNP sample plate. The results showed that the signal intensity, S N^{-1} ratio and reproducibility of SALDI-TOF spectra have been significantly improved in comparison to the uses of gold nanoparticles or α-cyano-4-hydroxy-cinnamic acid (CHCA) as the assisted matrixes. Taking the advantages of the unique properties of rGO and gold nanoparticles, the ready-to-use MS sample plate, which could absorb and dissipate laser energy to analytes quite efficiently and homogeneously, has shown great commercial potentials for MS applications.

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1. Introduction

Surface-assisted laser desorption/ionization time-of-flight mass spectrometry (SALDI-TOF MS) has become one of the most important tools in the field of metabolomics, proteomics and clinic research [1–7]. SALDI-TOF MS with the aid of nanomaterials as matrixes have several advantages, in particular, easy sample preparation, low noise background, high salt tolerance, fast data collection, and functionality for the analysis of small molecules (<500 Da) [8–11]. Recently, several studies have investigated and demonstrated the uses of nanomaterials including metals, metal oxide, silicon and carbon as matrixes in SALDI-TOF MS [12–16]. Among these nanomaterials, gold nanoparticles have been recognized as quite efficient matrixes for desorption/ionization of the carbohydrates, proteins, peptides, amino acids and drugs in SALDI-TOF MS [17–21]. Gold nanoparticles exhibited a broad surface plasmon band from the visible light into the ultraviolet with a high absorption coefficient [22–26]. The strong ultraviolet absorptivity of gold nanoparticles results in high desorption and ionization efficiency because of the increase in photothermal energy transformation during laser irradiation. Also, gold nanoparticles presented the unique physical properties of low heat capacity and high heat conductivity [27,28]. With the low heat capacity of gold nanoparticles, the temperature of analytes and matrixes can be increased rapidly in comparison to other nanomaterials. Additionally, with a high heat conductivity, the rapid heat accumulation and dissipation of gold nanoparticles under laser irradiation have been utilized for desorption and ionization of the analytes in SALDI-TOF MS.

Recent developments in carbon nanomaterials such as carbon nanotube, nanodiamonds, graphene and graphene oxide have been explored as the matrixes in SALDI-TOF MS for the detection of various molecules [29–31]. Reduced graphene oxide (rGO), a reduced form of graphene oxide, was the major form of carbon-based nanomaterials for the use as the matrix in SALDI-TOF MS. The rGO, composed of p-conjugated networks, exhibits many unique properties such as high surface area, large thermal conductivity, superior mechanical properties, and excellent electronic transport properties [32–41]. Therefore, the assisted matrix of rGO not only can adsorb the analytes but also can efficiently absorb and transfer ultraviolet laser energy to analytes for desorption/ionization process. In previous studies, polar and nonpolar compounds including amino acids, polyamines, anticancer drugs, nucleosides, steroids and polycyclic aromatic hydrocarbons have been successfully analyzed with high resolution and sensitivity by using rGO matrix in SALDI-TOF MS [42–45]. A quick and simple detection method of flavonoids and the derivatives of coumarin have been demonstrated by rGO assisted SALDI-TOF MS in the negative ion mode. The rGO film was used as the matrix in SALDI-TOF MS for the analysis of octachlorodibenzo-p-dioxin with detection weight as low as 500 pg. These results have brought new possibilities to utilize rGO as the matrix for sensitive detection of small molecules in SALDI-TOF MS.

The uses of either gold nanoparticles or carbon nanomaterials have proven to be effective matrixes for the detection of small molecules in SALDI-TOF MS, but some technical issues should be considered to further develop new nanomaterial-assisted matrixes [46–48]. First, the nanomaterials could be desorbed from the target upon irradiation with pulsed laser light resulted in significant interference to the sample signal in the low mass region. Second, the inhomogeneous distribution of the nanomaterials on the sample wells usually resulted in very poor shot-to-shot and sample-to-sample reproducibility [49–51]. In some recent studies, functionalized nanomaterials and optimized sample preparation methods have been investigated to address the issues [52–56]. These studies suggested that the control of the content and the homogeneity of nanomaterials to form the assistant matrixes on the steel plate was a key step to enhance the sample signal and also reproducibility in SALDI-TOF MS.

In this work, the layer structure of rGO and gold nanoparticles was fabricated for SALDI-TOF MS applications. By alternate layer-by-layer deposition of rGO and gold nanoparticles, a flat and clean two-dimensional film were formed and served as the sample plate in SALDI-TOF MS. The optimization of MS signal was tuned by the variation of layer numbers of rGO and gold nanoparticles. Several of analytes including raffinose, arginine, serine, valine, glucose, ribose, maltose and glutathione were tested to compare their MS detection performance. The comparison with gold nanoparticles and CHCA as the assisted matrixes of SALDI-TOF MS was also performed to confirm that the LBL rGO/AuNP sample plate generated strong signals with high $S\,N^{-1}$ ratio and good reproducibility for the detection of various small molecules.

2. Materials and methods

2.1. Chemicals

Graphite powder, sodium tetra-chloroaurate dehydrate and trisodium citrate were purchased from Sigma–Aldrich. Sodium nitrate and sulfuric acid were purchased from Acros. Acetone, methanol and acetonitrile were purchased from Merck.

2.2. Synthesis of graphene oxide

Graphene oxide was prepared by Hummers method with some modifications [57]. Raw graphite powder (2.5 g) was mixed with of 1.5 g $NaNO_3$ (purity 99%) and 67.5 mL of H_2SO_4 (purity 96%). The mixture was stirred while being cooled in an ice water bath. $KMnO_4$ (9 g, purity 99%) was gradually added within an hour. The mixture was kept in the ice-bath cooling for 2 h and then was allowed to stand for five days at approximately 20 °C with gentle stirring. In order to wash out the excess reactant, 1 L of 5 wt% H_2SO_4 aqueous solution was added to the resultant mixture and stirred for 2 h. Afterward, 30 g of H_2O_2 (30 wt% aqueous solution) was added to reduce the excess $KMnO_4$. Manganese ions from oxidant were facilitated by repeat wash with aqueous solution of 3 wt% H_2SO_4/0.5 wt% H_2O_2 for the removal of ions. Deionized water was added to the final product and then vortex well to make a uniform suspension for the following experiments.

2.3. Preparation of gold nanoparticles

Gold nanoparticles were prepared by citrate reduction of sodium tetra-chloroaurate dehydrate. In the first step, 50 mL of 4 mM trisodium citrate was stirred in a round-bottom flask under reflux. The solution of trisodium citrate was heated to a vigorous boil and then 0.5 mL of 100 mM $NaAuCl_4$ aqueous solution was added to the boiling solution. The solution of trisodium citrate and $NaAuCl_4$ was boiled for additional 3 min. Subsequently, the color of the boiling solution changed from colorless to wine-red. The wine-red solution containing gold nanoparticles was cooled to the room temperature in an ice bath. Finally, the sample was stored at 4 °C for further experiments.

2.4. Fabrication of LBL rGO/AuNP sample plate

A spin-assisted layer-by-layer assembled method was employed to prepare LBL rGO/AuNP sample plate based on the electrostatic interaction. By controlling the speed of the spin coating machine (3000 rpm), graphene oxide layer was deposited onto the steel plate (1 cm × 1 cm) using 100 μL graphene oxide solution. Sequentially, 100 μL of the gold nanoparticle solution was deposited on the graphene oxide thin film to form the first