



APPLICATION NOTE

Efficient Analysis of Non-Polar Environmental Contaminants by MALDI-TOF MS with Graphene as Matrix

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Abstract

In this Application Note, we describe, for the first time, the rapid analysis of hydrophobic compounds present in environmental contaminants, which includes polycyclic aromatic hydrocarbons (PAHs) and estrogen, by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) with the use of graphene as matrix. MALDI-TOF MS with conventional matrix has limitations in analyzing low-polarity compounds owing to their difficulty in ionization. We demonstrate that compared with conventional matrix, graphene displays higher desorption/ionization efficiencies for PAHs, and no fragment ions are observed. The method also holds potential in quantitative analysis. In addition, the ionization signal increases with the increasing number of benzene rings in the PAHs, suggesting that graphene binds to PAHs via π – π stacking interactions. Furthermore, graphene as adsorbent for solid-phase extraction of coronene from river water sample displays good performance with a detection limit of 10^{-7} M. This work provides a novel and convenient method for analyzing low-polarity environmental contaminants by MALDI-TOF MS.

Key words: Graphene, MALDI-TOF, Polycyclic aromatic hydrocarbon, Environmental contaminant

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants whose carcinogenicity and estrogenicity have been extensively studied [1–3]. In order to assess the risk of PAHs in the environment, various methods for the determination of PAHs have been used. Gas

chromatography-mass spectrometry (GC-MS) is an excellent and powerful tool for the identification of PAHs because of the high separation efficiency afforded by GC [4]. However, PAHs with molecular masses greater than 300 Da are difficult to characterize and detect by GC-MS [5]. In addition, partial decomposition of unstable PAH species was observed. Liquid chromatography-atmospheric pressure chemical ionization mass spectrometry (LC-APCI) (and atmospheric pressure photoionization (APPI)-MS) were successfully used to determine high-molecular mass PAHs from environmental samples [6–8]. However, there are few reports about the detection of PAHs using electrospray ionization mass spectrometry (ESI-MS) owing to the difficulty in ionizing compounds of low polarity except in some reports where metal cations were used to assist ionization [9, 10].

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Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) [11] is widely used for analyzing proteins, peptides, oligodeoxynucleotides, etc. However, it is difficult to analyze small molecules with conventional matrix due to matrix ion interference. Recently, porous silicon [12–15], matrix with high molecular weight [16, 17], surfactant-suppressed matrix [18], and inorganic materials [19–21] have been successfully employed in the analysis of small molecules. However, there are no reports about the analysis of nonpolar PAHs by MALDI-TOF due to their great difficulty in ionization, though metal-assisted LDI analysis of nonpolar polyethylene was reported [22]. Our recent study revealed graphene as MALDI matrix exhibited good performance in the characterization of small molecules, and the method bears excellent ability in ionizing non-polar compounds due to hydrophobic interaction between graphene and analytes [23]. We reason that graphene might also serve as a good matrix for analyzing non-polar toxic environmental compounds. In this study, PAHs and estrogen were successfully detected for the first time using graphene as matrix. Moreover, solid-phase extraction of PAH in river water at low concentration was achieved which implicated the great potential of using graphene as MALDI matrix for the analysis of non-polar environmental contaminants.

Experimental

Materials

Trifluoroacetic acid (TFA), 1-phenyloctadecane, 2,5-dihydroxybenzoic acid (DHB), and α -cyano-4-hydroxycinnamic acid (CHCA) were purchased from Fluka (St. Louis, MO, USA). Coronene, 1-nitronaphthalene, and estrogen were all obtained from Sigma. Ethanol and acetonitrile were of HPLC grade, and all other reagents were of analytical grade.

Preparation of Graphene

Graphene was prepared using previously described procedures [24]. The obtained graphene was characterized by transmission electron microscopy (TEM) analysis using a JEM-2010 transmission electron microscope (JEOL, Tokyo, Japan) at an accelerating voltage of 100 kV and by powder X-ray diffraction on a Bruker D8-Advance X-ray powder diffractometer (Madison, WI, USA).

Preparation of Analyte Solutions

PAHs were dissolved in dichloromethane or *N,N*-dimethylformamide at a concentration of 1 mg/mL each to give stock solutions, and the mixture solutions were prepared by diluting the stock solutions with dichloromethane until the final concentrations of analytes reached 100 μ g/mL each. Sample solutions at other concentrations were obtained by

step dilution. All stock solutions were kept at ~ 4 °C for further use.

Sample Preparation for MALDI-TOF MS

CHCA matrix was prepared as a saturated solution in water/acetonitrile (2/1, vol/vol) containing 0.1% TFA. Graphene (1 mg) was dispersed in a 1-mL solution of ethanol and sonicated for 3 min, and a 1- μ L suspension was quickly pipetted onto the sample target. It was left in air at room temperature for 5–10 min to form a thin layer, and a 0.5- μ L solution of analyte was then pipetted onto the matrix layer and left in air for 5–10 min for evaporation of solvent and for further analysis by MALDI-TOF MS.

Solid-Phase Extraction (SPE) Prior to MALDI-TOF MS Analysis with Graphene as Adsorbent and Matrix

Two mg graphene was rinsed twice with acetonitrile and water and suspended in 0.3 mL of methanol in water (1/1, vol/vol). After sonication for 3 min, 2 μ L of the suspension was pipetted immediately into 500 μ L of water from Santa Ana River spiked with 8.3×10^{-7} M of coronene, 1.0×10^{-6} M of chrysene, and 1.5×10^{-5} M of anthracene in *N,N*-dimethylformamide. The mixture was then sonicated for 10 min. After centrifugation at 13,000 rpm for 10 min, the supernatant was removed, and the pellet of graphene on which the analytes were enriched was resuspended in a 2- μ L solution of methanol in H₂O (1/1, vol/vol). Finally, about 1 μ L of the graphene suspension was pipetted onto the sample target. The sample target was again left at ambient temperature for 10–15 min for evaporation of solvent and for further analysis by MALDI-TOF MS.

Mass Spectrometry

Laser desorption/ionization and matrix-assisted laser desorption/ionization mass spectra were acquired on a Voyager DE STR MALDI-TOF mass spectrometer (Applied Biosystems, Framingham, MA, USA) in positive reflectron mode. The mass spectrometer was equipped with a pulsed nitrogen laser operated at 337 nm with 3 ns-duration pulses. The acceleration voltage, grid voltage, and delayed extraction time were set as 20 kV, 65%, and 190 ns, respectively. Each mass spectrum was acquired from an average of 100 laser shots.

Results and Discussion

As described in our previous study, graphene has a nearly transparent flake-like shape with characteristic crumpled silk waves [24]. This single-layer morphology of graphene provides good efficiency in energy transfer, minimal interference of matrix-related ions and high surface area, thereby enabling small molecules to be analyzed by MALDI-TOF MS [23]. Moreover, much higher sensitivity was obtained for compounds with lower polarity [23], suggesting that graphene may serve as

a good matrix for MALDI-MS analysis of nonpolar environmental contaminants including PAHs. These compounds are difficult to be analyzed by ESI-MS or traditional MALDI-MS owing to their difficulty in gaining or losing protons.

A series of PAH compounds including benzo[*a*]pyrene, anthracene, chrysene, benzo[*a*]anthracene, benzo[*b*]fluoranthene, and coronene were chosen as representative PAHs; all of them are environmental contaminants. For comparison, we first employed conventional CHCA matrix for the detection of these compounds. Not surprisingly, strong background interferences were present while CHCA was used as matrix, which greatly obscured the detection of low-molecular weight compounds as depicted in Figure 1a. As a result, none of the PAHs except coronene could be detected. However, with graphene as matrix, strong signal could be observed without matrix ion interference (Figures 1b and 2).

We also observed that coronene was ionized without protonation; instead it lost an electron to render the $M^{+•}$ ion upon ionization. This could be attributed to two factors: One is the nanosheet structure of graphene with numerous aromatic rings, which can facilitate energy transfer to PAHs via π - π stacking; the other is strong hydrophobic interaction between graphene and analytes. Thus, the graphene matrix functions as a substrate to trap analytes efficiently, and it enhances the ionization of analytes upon exposure to laser irradiation. Interestingly, the signal intensity increases steadily with the increasing number of benzene rings in individual PAH compounds as depicted in Figure 2. Among these compounds, coronene, which has the largest number of benzene rings, yielded the strongest signal, whereas benzene, which contains only one aromatic ring, was not detectable.

The lower signal observed for those compounds with smaller number of benzene rings could potentially be attributed to the higher volatility of these compounds, which may lead to sample loss under vacuum. To examine this possibility, we analyzed, by MALDI-TOF MS using graphene as matrix, 1-phenyloctadecane. This compound possesses a long hydrocarbon chain on the benzene ring and has a boiling point of 408 °C, which is much

higher than those of 1-nitronaphthalene and anthracene (Table S1). It turned out that 1-phenyloctadecane cannot be detected by MALDI-MS using graphene as matrix (spectrum shown in Figure S2), whereas 1-nitronaphthalene and anthracene could be detected (Figure 2). This result supports our deduced mechanism that π - π stacking and hydrophobic interactions between graphene and analytes are important for the ionization.

We next assessed whether MALDI-MS with the use of graphene as matrix can be employed for quantitative analysis. Owing to the structure similarity between coronene and benzo[*a*]pyrene, we selected, in this experiment, these two compounds as model analyte and internal standard, respectively. To this end, we first prepared a series of solutions by varying the concentrations of coronene from 50 to 200 $\mu\text{g/mL}$, while keeping the concentration of benzo[*a*]pyrene constant (100 $\mu\text{g/mL}$). We then subjected the solutions to MALDI-TOF MS analyses with graphene as matrix and plotted peak area ratios of the analyte over internal standard versus the corresponding molar ratios of these two compounds. As displayed in Figure S3, the calibration curves for coronene analysis is linear with an R^2 value of 0.984. This result, therefore, shows that it is feasible to employ the graphene-based MALDI-MS method for quantitative analysis.

Graphene for Solid-Phase Extraction of Coronene from River Water

Graphene is an electron-rich hydrophobic material with high surface area. From the above promising results of using graphene as matrix for PAH analysis, we reason that it may serve as suitable material for solid-phase extraction of PAHs from environmental samples and for the subsequent MALDI-MS analysis. To test this, we used graphene for the solid-phase extraction of river water spiked with coronene, chrysene, and anthracene at the concentrations of 8.3×10^{-7} , 1.0×10^{-6} and 1.5×10^{-5} M, respectively. For comparison, the river water without spiking was analyzed and the background was much cleaner using the solid-phase extraction (SPE) procedure,

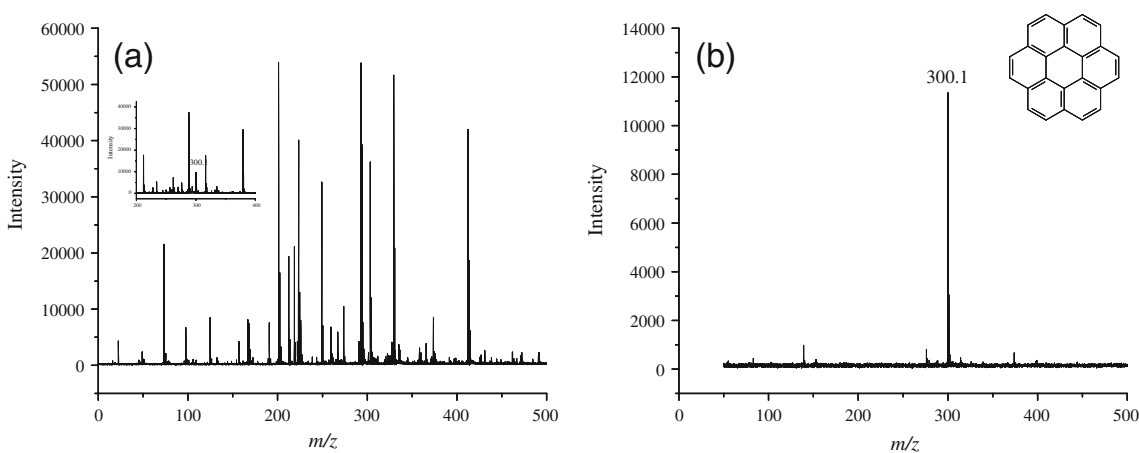


Figure 1. MALDI-TOF mass spectra of coronene in the LDI mode with the use of CHCA (a) or graphene (b) as matrix. A 0.5- μL sample solution was deposited onto the sample target and the concentration of coronene was 100 $\mu\text{g/mL}$ (m/z 300.1, $M^{+•}$)

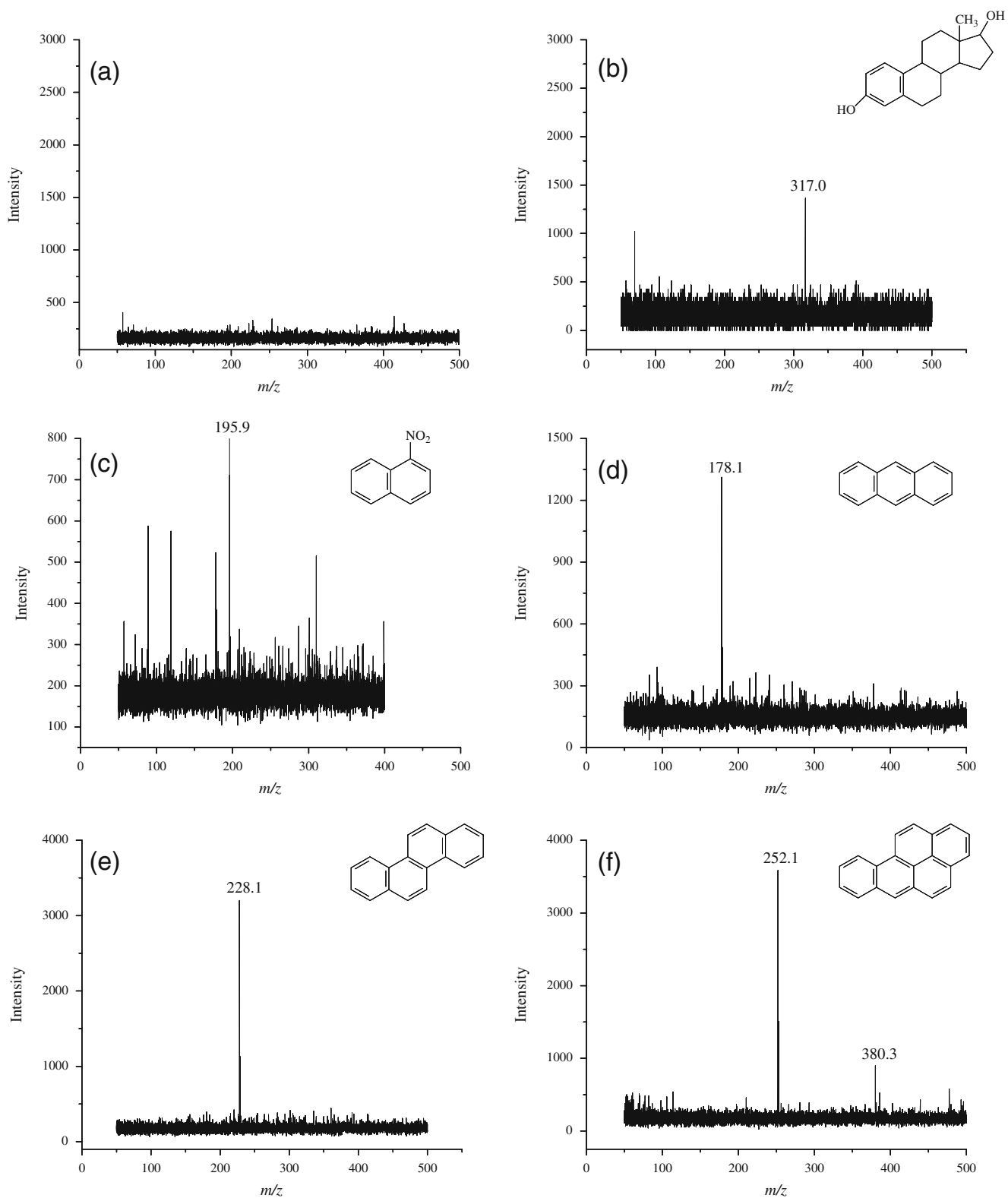


Figure 2. MALDI-TOF mass spectrum of (a) benzene; (b) estrogen (m/z 317.0, $[M+2Na - H]^+$); (c) nitronaphthalene (m/z 195.9, $[M+Na]^+$); (d) anthracene (m/z 177.7, M^+); (e) chrysene (m/z 228.0, M^+); (f) benzo[*a*]pyrene (m/z 251.9, M^+ ; the ion of m/z 380 was introduced by impurity present in DMF solvent). The concentrations of all analytes were 100 $\mu\text{g/mL}$, and graphene was used as matrix

demonstrating that the SPE method could remove most polar contaminants in the river water. After spiked with target PAHs followed with SPE enrichment, graphene was directly analyzed using MALDI-TOF MS and the analyte signals could be readily observed (Figure S4b). It revealed that the method is especially useful for sample concentration and cleanup. Therefore, graphene's dual capability as SPE adsorbent and MALDI matrix offered a powerful and rapid technique for the detection of PAHs in complicated environmental samples. Obviously, MALDI-TOF MS with the use of graphene as matrix has great potential for application in the detection of a variety of low-molecular mass, nonpolar compounds in environmental samples while simplifying sample preparation.

Conclusions

PAHs are widespread environmental pollutants, and analytical methods are needed for their detection. Owing to their difficulty in being protonated or deprotonated, these compounds are not amenable to ESI-MS or, with the use of conventional matrix, MALDI-MS analysis. Results from this study revealed that these compounds could be analyzed by MALDI-TOF MS with the use of graphene as matrix. Thus, it opens a new analytical avenue for this important group of compounds.

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