Carbon Nanotubes as Assisted Matrix for Laser Desorption/Ionization Time-of-Flight Mass Spectrometry

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Analysis of low molecular weight compounds with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS) has been developed by using carbon nanotubes obtained from coal by arc discharge as the matrix. The carbon nanotube matrix functions as substrate to trap analytes of peptides, organic compounds, and *β*-cyclodextrin deposited on its surface. It has been found that carbon nanotubes can transfer energy to the analyte under laser irradiation, which makes analytes well desorbed/ionized, and the interference of intrinsic matrix ions can be eliminated. At the same time, the fragmentation of the analyte can be avoided. A good sensitivity and excellent reproducibility of the spectrum signals are achieved. It is believed that this work not only will open a new field for applications of carbon nanotubes, but also will offer a new technique for high-speed analysis of low molecular weight compounds in areas such as metabolism research and characterization of natural products.

The matrix-assisted laser desorption/ionization (MALDI) technique developed by Karas and Hillenkamp[1] and Tanaka et al.[2] have overcome the limitation of laser desorption/ionization of biomolecules and provided a simple analysis approach to high molecular mass species. Typical matrixes used in the analysis of biomolecules include α-cyano-4-hybroxycinnamic acid (CCA), sinapinic acid (SA), 2,5-dihydroxybenzoic (DHB), etc. These matrixes should have efficient absorbance at commonly used laser wavelength (typically for a 337-nm nitrogen laser) and form homogeneous microcrystalline solids with analyte molecules. The soft ionization technique affords little to no fragmentation of analyte. In the past decade, MALDI has been extensively used in mass spectrometric (MS) studies of large polymers, biomolecules,[3−6]

and a variety of thermally labile and fragile, nonvolatile compounds, including peptides and proteins. However, for the low mass analytes (<500 Da), matrix ion interference and detector saturation are unavoidable in MALDI-TOFMS, which makes the characterization of small molecules obscured and difficult.

For this reason, many efforts have been made to eliminate matrix ion interference by using different matrix substances, such as sample substrates,[7−12] high mass molecules,[13,14] surfactant-suppressed matrix,[15] inorganic materials,[2,16−24] and C_{60}[25,26]. Sunner et al.[16] introduced graphite particles and glycerol as a matrix for desorption/ionization of proteins and peptides. In their experiment, the particle was 1000 times larger in size than the 30-nm diameter cobalt particles that Tanaka et al.[2] used, and the presence of glycerol ensures that ion signals are obtained for extended time periods. Another good technique[7] for desorption/ionization of

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small molecules on porous silicon obtained by electrochemical etching has drawn considerable attention, and mass spectra obtained were very "clean", with no or little chemical background.

In 1991, Iijima discovered carbon nanotubes from cathode produced by a carbon-arc discharge method similar to that used for the preparation of fullerenes.[27] Since then, carbon nanotubes have stimulated intense interest and have been extensively studied theoretically and experimentally, and the potential applications of carbon nanotubes as nanoprobes, electrode components, catalyst supports,[28-30] and for hydrogen storage [31,32] have been reported.

In this work, we use carbon nanotubes prepared from coal by an arc discharge method as the matrix in MALDI-TOFMS for analysis of small molecules. The carbon nanotube functions to trap the analyte molecules and acts as an energy receptacle for laser radiation. It has been found that the utility of desorption/ionization on carbon nanotubes for biomolecular analysis can greatly simplify sample preparation and eliminate interference from matrix background ions. This new method has been tested for the efficient analysis of small peptides, organic compounds, and cyclodextrin.

EXPERIMENTAL SECTION

Chemicals and Materials. Matrix α-cyano-4-hydroxycinnamic acid (CCA) was purchased from Sigma (St. Louis, MO). Coal tar binder obtained from the Anshan Iron and Steel Company (Liaoning, China) is a dark, viscous liquid that is a byproduct of the coal carbonization process, and elemental analysis of coal tar binder showed the composition to be carbon 86.2%, hydrogen 5.50%, and other elements (oxygen + nitrogen + sulfur) ∼8.3%. β-Cyclodextrin was purchased from Beckman (Fullerton, CA). N_α-Benzoyl-L-arginine ethylester hydrochloride (BAEE) and N_α-benzoyl-DL- arginine-4-nitroanilide hydrochloride (BAPNA) were obtained from Fluka (Buchs, Switzerland). Peptides of Gly-Gly-Gln-Ala, Tyr-Ala, and Tyr-Ala-Ala were purchased from Serva (Heidelberg, Germany). Other reagents were analytical grade. The water used was obtained from a Milli-Q water purification system (Millipore, Milford, MA).

Preparation of Carbon Nanotubes. The proximate and ultimate analysis of the coal sample can be found elsewhere.[33] The coal sample without any pretreatment was crushed and sieved to 150 μm, and fully dried at 380 K for 12 h before use. Then the coal powder was finely mixed with coal tar binder in a weight ratio of 1:4, and subsequently pressed at ∼10 to 20 MPa to form coal rods. The coal rods were put into an electric furnace to be carbonized under flowing N_2 to make electrodes. The furnace was ramped at 3 K/min to 773 K and was kept at that temperature for 1 h to obtain hollow semi-char rod, which was further carbonized

at 10 K/min to 1173 K, at which the rod was further heated for 4 h. Finally, carbon rods with an outer diameter of 9 mm being used as anode were obtained. The cathode was made from a high-purity graphite rod with diameter of 16 mm and length of 30 mm. The arcing experiment lasted ∼20 min. After arc discharge, a deposited carbon rod was built up on the cathode. After the carbon nanotube sample was collected from the arc reactor, it was directly used as a matrix without purification treatment. The soft material containing carbon nanotubes on the cathode was characterized by scanning electronic microscopy (SEM, JEOL JSM-5600LV) and transmission electronic microscopy (TEM, JEM-2000EX) operated at 100 kV.

Preparation of Sample. Matrix CCA was prepared at a concentration of 0.1 M in a solution with a 1:3 volumetric ratio of water to acetone. The matrix of the carbon nanotubes was prepared according to the following procedure: 2 mg of carbon nanotube was suspended in 1 mL of ethanol and sonicated for 3 min, in which the carbon nanotube can be well-dispersed. Then, ∼1 μL of the carbon nanotube suspension solution was pipetted into the probe well immediately, where the ethanol was evaporated in a few seconds. A 0.5 μL portion of sample solution, such as small peptides, organic compounds and cyclodextrin, was deposited on the carbon nanotube layer and dried in hot air. Sample solutions were prepared by dissolving peptides, N_α-benzoyl-L-arginine ethyl ester hydrochloride (BAEE) and β-cyclodextrin in water/methanol (1:1, v/v).

MALDI-TOF-MS Instrument. The mass spectrometric experiments were performed using delayed extraction in positive ion mode on a time-of-flight mass spectrometer (Biflex II, Bruker, Germany) with a 1.7-m flight tube. Desorption/ionization was obtained by using a 337-nm nitrogen laser with a 3-ns pulse width. Available accelerating potential is in the range of +20/-20 kV. The analytical range of laser fluence was adjusted to slightly above the threshold to obtain good resolution and signal-to-noise ratios, and mass calibration was achieved using an external standard.

RESULTS AND DISCUSSION

In our experiment, carbon nanotube obtained from coal by arc discharge was used for assisted-matrix desorption/ionization mass spectrometry. Figure 1 shows the TEM image of single piece of carbon nanotubes. It can be seen that at high magnification, the carbon nanotube appears to have rod morphology, with an outer diameter of ∼20 nm and an inner diameter of ∼2 nm, and it seems that the carbon nanotubes in our case are multiwall carbon nanotubes that consist of several cylindrical graphitic sheets.

Figure 2 shows the mass spectra of BAEE on MALDI-TOFMS by using a traditional organic matrix of CCA and a matrix of carbon nanotubes, respectively. In a comparison of the two cases in Figure 2, carbon nanotube have an excellent ability for desorption/ionization of BAEE. From Figure 2a, we can find that although BAEE is desorbed/ionized as $[M - Cl]^+$ with the matrix of CCA, various signal peaks were observed, which obscured the low mass molecule detection. As the carbon nanotube was applied for desorption/ionization of BAEE as mass spectrum shown in Figure 2b, matrix ion interference has been completely eliminated, and little or no fragmentation peak of the analyte can be observed.

The carbon nanotubes' ability for desorption/ionization of analytes was further tested with a mixture sample. Figure 3 shows the mass spectrum on MALDI-TOFMS for a mixture of three small

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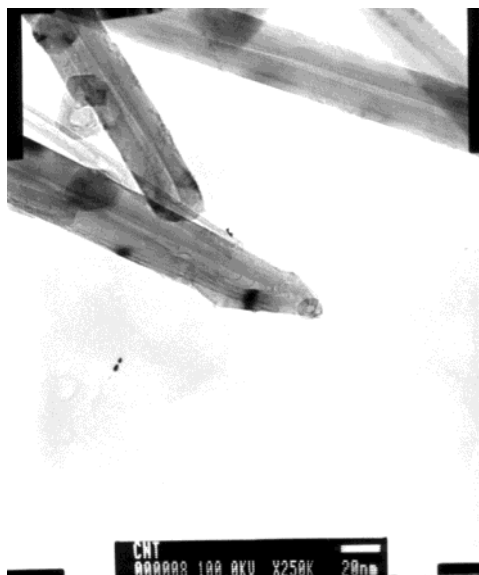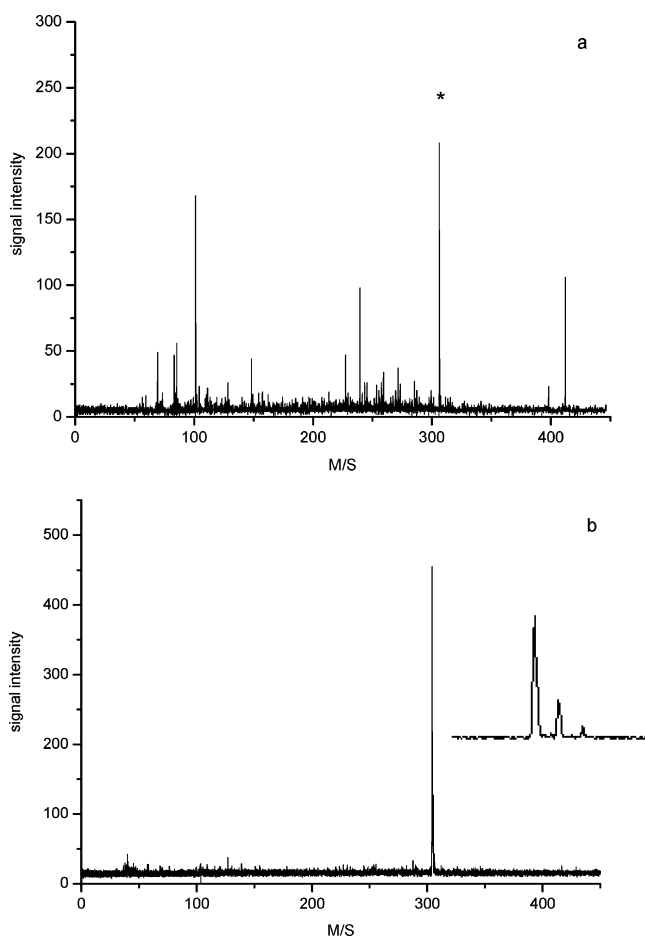**Figure 1.** TEM image (×200000) of pieces of carbon nanotubes.

Figure 2. Mass spectra of N_α-benzoyl-L-arginine ethylester hydrochloride (3 mg/mL) at m/z 307.87 $[M - Cl]^+$ obtained by using matrixes of (a) CCA and (b) carbon nanotubes. 50 pulsed laser shots were applied under laser power set at 195 μJ.

Figure 3. Mass spectra on carbon nanotube of a mixture of three peptides, Tyr-Ala (2.0 mg/mL) at m/z 240.86 $[M + Na]^+$ and 256.83 $[M + K]^+$, Tyr-Ala-Ala (1.0 mg/mL) at m/z 274.63 $[M + Na]^+$ and 289.73 $[M + K]^+$, Gly-Gly-Gln-Ala (1.5 mg/mL) at m/z 354.64 $[M + Na]^+$ and 370.58 $[M + K]^+$, and N_α-benzoyl-L-arginine ethylester hydrochloride (0.1 mg/mL) at m/z 307.87 $[M - Cl]^+$. 30 pulsed laser shots were applied under laser power set at 200 μJ.

N_α-benzoyl-L-arginine ethylester hydrochloride (BAEE) was ionized as $[M - Cl]^+$. Intrestingly, metal ions are the main chemical adduct for small peptide analysis in MALDI with a carbon nanotube as matrix, and $[M + H]^+$ peaks of the three peptides are not observed in the mass spectra, which is similar to the result obtained from small peptide analysis[22] using the silical gel as the matrix. Further comparison of ion signal intensity of peptides with BAEE showed that the detection sensitivity of BAEE is ∼10 times higher than that of the peptides, which may be the result of the different desorption/ionization process of BAEE from peptides. BAEE is desorbed and ionized more efficiently by chloride ion that was just lost from the molecule, but peptides are desorbed and ionized by formation of adducts with metal ions. Clearly, analysis of a mixture of small molecules using MALDI MS with an organic matrix could be achieved under suitable conditions, but the matrix interference remains a real problem, as shown in Figure 2a. However, this problem can be solved by desorption/ionization mass spectrometry with carbon nanotube as matrix.

Another example is the analysis of β-cyclodextrin by MALDI-TOFMS with a matrix of carbon nanotubes; and the obtained mass spectrum is shown in Figure 4. It can be seen that β-cyclodextrin is ionized as a sodium adduct $[M + Na]^+$. Obviously, MALDI-TOFMS with a matrix of carbon nanotubes has great potential for application in the detection of a variety of low molecular weight species.

In the setting of laser power, very good signal-to-background ratio is observed on the carbon nanotube matrix. When laser power was set at 180 μJ (the changeable values from 150 to 250 μJ in the Biflex II TOF instrument), the mass signal of compound BAEE is easily obtained with the carbon nanotube matrix. However, at the same laser power, no signal of the compound BAEE and matrix molecules could be obtained with CCA and DHB as matrix. And in order to obtain the ion signal of the BAEE using organic matrix CCA, laser power should increase to about 186 μJ. Subsequently, compound BAPNA was also applied to examine the laser power threshold for desorption/ionization of

peptides, Tyr-Ala, Tyr-Ala-Ala, and Gly-Gly-Gln-Ala, and N_α-benzoyl-L-arginine ethylester hydrochloride. Similarly, all components in the mixture were simultaneously ionized and desorbed on the matrix of the carbon nanotube. Three peptides were ionized as sodium adduct $[M + Na]^+$ and potassium adduct $[M + K]^+$, but
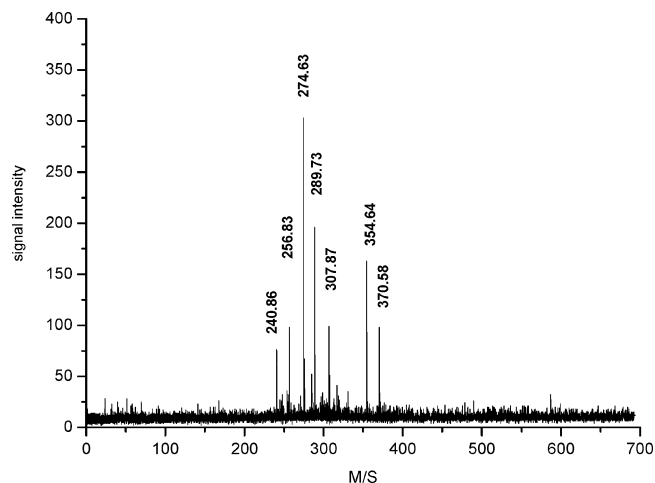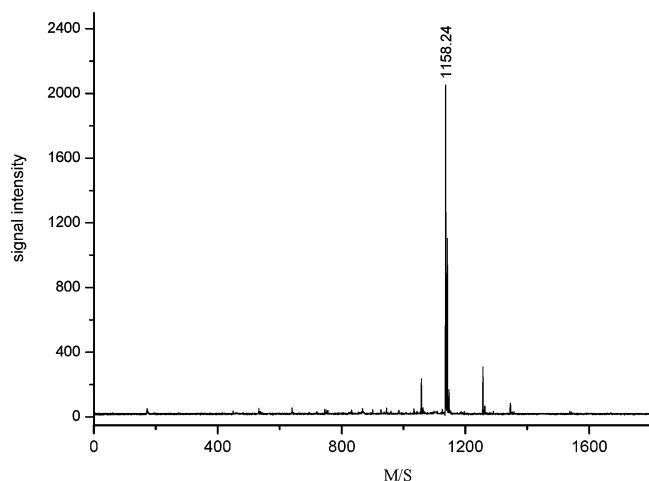
Figure 4. Mass spectrum of β-cyclodextrin on carbon nanotube at m/z 1158.24 $[M + Na]^+$. 30 pulsed laser shots were applied under laser power set at 210 μJ.
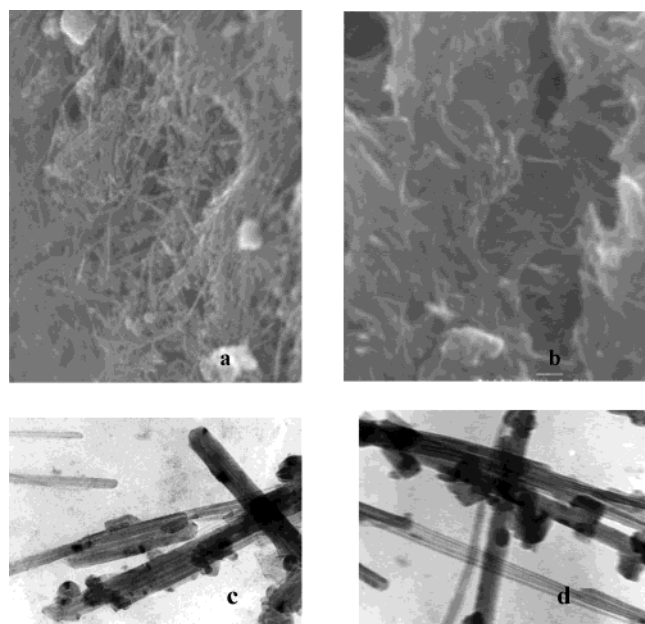

Figure 5. Morphology of carbon nanotube layer on probe well before and after dropping sample solution. (a, b) SEM image ($\times$5000) of carbon nanotube layer before and after dropping sample solution. (c, d) TEM image ($\times$100000-fold) of carbon nanotube layer before and after deposition of sample solution.

the tested molecule. BAPNA can be well desorbed and ionized with carbon nanotube matrix under laser power at 185 μJ, while 189 μJ with organic matrix of CCA. It was demonstrated that carbon nanotube has a lower laser power threshold for desorption/ionization of small molecules tested.

Successful MALDI experiments for analysis of different classes of small molecules strongly depends on the choice of matrix, sample preparation methods, and homogeneous crystal formed by the matrix with analyte molecules, yet this continues to be an empirical consideration. In our work, the analyte solutions were only dropped onto the carbon nanotube layer on the probe well, and the carbon nanotubes acted as a matrix medium to trap the analyte molecules. Figure 5 shows the SEM and TEM images of the carbon nanotube layer before and after dropping of the sample solution. As shown in Figure 5a, the SEM of the carbon nanotube
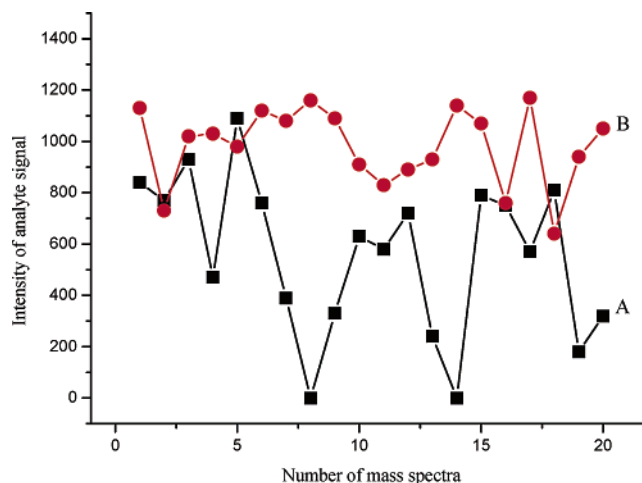

Figure 6. Comparison of intensity of analyte ion signal in the discrete location of probe well with the matrixes of (A) CCA and (B) carbon nanotubes. 20 pulsed laser shots were applied under laser power set at 205 μJ. Equal concentration of analyte BAEE was used for MS experiments in both cases.

layer at 5000-fold magnification before depositing the sample solution appears to have a web morphology. However, the SEM image of the carbon nanotube layer after deposition of the sample solution revealed that the web morphology was masked, as shown in Figure 5b. It is clearly indicated that sample molecules formed microcrystalline solids and absorbed onto the surface of the carbon nanotube layer during the condensation. The carbon nanotube layer before and after deposition of the sample solution was further characterized by TEM image under 100 000-fold magnification, and the obtained results are shown in Figure 5c and d, respectively. By comparison of TEM image results shown in Figure 5c and d, it is clearly indicated that there is almost no change in configuration and shape for a single piece of carbon nanotube before and after the deposition of sample solution on the carbon nanotube layer. It is demonstrated that the carbon nanotube matrix can provide a platform for the formation of crystals of sample molecules, which is completely different from conventional organic matrix that forms crystals with analyte molecules. Thus, the sample preparation using the carbon nanotube matrix in MALDI-TOFMS can greatly be simplified, and laborious effort to adjust the ratio of sample to matrix to get good spectra can be avoided.

The sensitivity of the spectrum signal with carbon nanotube as matrix was investigated with compound BAEE as analyte. It was observed that ~50 amol of BAEE could be well-detected at a laser power 235 μJ by using the carbon nanotube matrix. At a higher laser power, intense ion signals of Na$^+$ and K$^+$ were acquired on MALDI-TOFMS with the carbon nanotube matrix layer, except for the analyte ion signal. However, the signal of 50 amol BAEE was not discerned by using the matrix of CCA due to strong background ion interference. Yet, the detection limit obtained on MALDI with matrixes of the porous silicon[7] and activated carbon[24] are ~700 and 100 amol for the analyte of bradykinin, respectively. It may be expected that the carbon nanotube matrix may improve the detection sensitivity for MALDI-TOFMS analysis of small molecules.

Figure 6 shows a direct comparison of the intensities of analyte ion peaks with MALDI analysis of equal amounts of compound

BAEE by using the CCA and carbon nanotube matrixes. The continuous 20 mass spectra were obtained from a discrete location in the probe well where each mass spectrum was obtained by applying 20 laser shots. The carbon nanotube matrix produces higher signal intensities and shot-to-shot reproducibility of the BAEE signal than the CCA matrix. Chances are that the analyte molecules individually form microcrystals on the carbon nanotube layer, which leads to better homogeneity than formation of crystals of analyte molecules with the conventional organic matrix. The ion signal of analyte BAEE can be enhanced greatly by increasing the laser power value in MALDI with the carbon nanotube matrix, but the reproducibility and mass resolution of analyte BAEE do not change significantly at the laser power values from 190 to 215 μJ. Thus, MALDI with carbon nanotube as matrix may provide a possibility to improve quantitative analysis of small molecules. On the other hand, the resolution of analyte ion peaks is greatly improved for analysis of small molecules on MALDI with carbon nanotube as matrix. For example, the resolution of the ion peak of BAEE for 20 continuous random mass spectra with the carbon matrix ranged from 1800 to 5500; however, the resolution with the CCA matrix ranged from 700 to 3300.

Carbon nanotube can be well-applied to high-speed analysis of small molecule samples as a new matrix medium by laser desorptio/ionization TOFMS. Furthermore, the carbon nanotube surface activity can be modified through derivation with other reactive groups,[34,35] which may extend its application in mass spectrometry. However, the mechanism for desorption and ionization of small molecules on the matrix carbon nanotube is not well understood, and transferring energy from laser irradiation to the sample molecules by carbon nanotube should be one of the most important processes. No observable peak signals were obtained if laser energy from 150 to 180 μJ was radiated on the carbon nanotubes without any sample molecules, which is similar to the result of sample molecules directly placed on the metal target, indicating its good inert nature.

CONCLUSION

Carbon nanotubes, prepared from coal by an arc discharge method, were investigated as the matrix for analysis of small molecules by MALDI-TOFMS. It was observed that a carbon nanotube layer on a probe well before deposition of sample solution appears to have a web morphology, but which was masked by formation of analyte microcrystals after deposition of the sample solution. However, there is almost no change in configuration and shape for a single piece of carbon nanotube before and after the deposition of sample solution on the carbon nanotube layer. The carbon nanotube as matrix provides a lower laser power threshold for desorption/ionization and higher detection sensitivity and mass resolution of small molecules than organic matrixes. This method of desorption/ionization for small molecules can greatly simplify sample preparation, eliminate interference of matrix background ions, and improve shot-to-shot reproducibility.

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