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Sensitivity and intensity enhancement in open air mass spectrometry assisted by a continuous wave infrared laser

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To improve signal-to-noise ratios (SNRs) in open air mass spectrometry, a laser-assisted, direct-analysis-in-real-time (DART) mass spectrometer (LA-DART-MS) was developed by integrating a continuous wave (CW) infrared (IR) laser into an open air DART-MS. The CW IR laser (wavelength of 1070 nm) was used to assist the desorption of analytes and promote the reactivity of protonated water from the DART ion source. Using the LA-DART-MS, SNRs of Rhodamine 6G (R6G), urea, and testosterone were enhanced by factors of 31, 11, and 4, respectively, compared with the conventional DART-MS. The sensitivity enhancement was ascribed to the increased analyte concentration in air and activated protonated water induced by the IR laser irradiation.

1. Introduction

Open air mass spectrometry (MS) experienced drastic development in recent years.[1] To effectively ionize analytes under ambient conditions, a variety of techniques have been developed, including direct analysis in real time (DART),[2,3] atmospheric pressure solid analysis probe,[4] surface desorption atmospheric pressure chemical ionization,[5,6] and desorption electrospray ionization.[7-9] Among these ambient ionization techniques, DART has been intensively examined due to its capability to effectively ionize bio- and organic molecules. Electronically excited helium (He) atoms from the DART ion source react with atmospheric water molecules. Protonated water clusters, $[(H_2O)_nH]^+$, are formed and collide with analytes producing protonated molecules through charge transfers. Setting the DART gas heater to a sufficiently high temperature, the analytes are desorbed and sequentially introduced to a mass spectrometer for analysis.[2,3] Relying on thermal desorption to introduce analytes, DART-MS requires analytes to be volatile.[10] This, together with the low signal-to-noise ratios (SNRs) of analytes at low concentration levels, limits the application of DART-MS.

It was previously reported that lasers were used to combine with other ambient ionization techniques to realize two-step ionization of analytes, such as PAMLDI-MS[11] and ELDI-MS[12], before going into the orifice of MS. In both cases, lasers played an important role of desorbing analytes from the substrates before post-ionization using DART and electrospray.

To address our challenge, a continuous wave (CW) infrared (IR) laser was installed in a conventional DART-MS to assist in the desorption of analytes and the activation of protonated water molecules. The CW laser used in the experiment instead of pulsed laser prevented the analytes from fragmenting. Significantly improved SNRs for R6G, urea, and testosterone were observed in the laser-assisted, direct-analysis-in-real-time mass spectrometer (LA-DART-MS) in open air benefiting from laser-assisted analytes desorption and vibrational excitation of protonated water molecules.

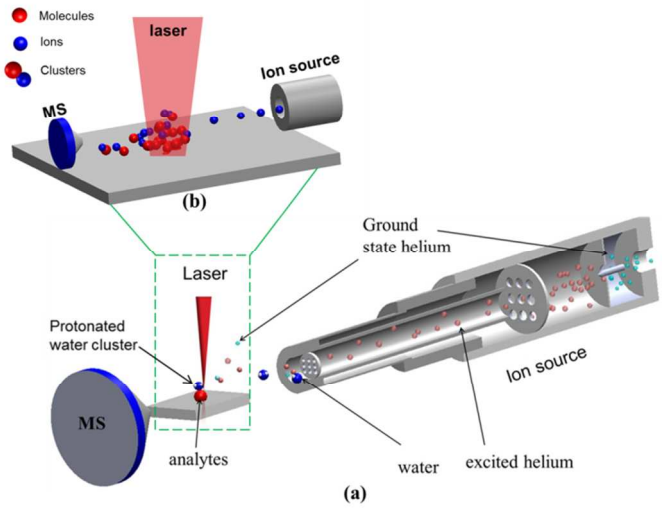

Fig. 1. Experiment setup of LA-DART-MS (a) and desorption and ionization processes of LA-DART-MS (b).

2. Experimental

The experiment setup of the LA-DART-MS is shown in Fig. 1. A CW fiber laser (IPG Photonics, YLR-400-AC) with a center wavelength of 1070 nm and linewidth of 5 nm was used as the irradiation source. The laser power density was optimized to 1.74 W/cm^2 to obtain the strongest signal intensity of the mass spectra. The laser beam was defocused to a spot size of 1.1 mm. A DART ion source (DART^{TM}, JEOL USA, Inc.) was used to ionize analytes. The temperature of the DART ion source was optimized for desorbing and ionizing the analytes on glass substrates. The time-of-flight mass spectrometer (TOF-MS) (AccuTOF^{TM}, JEOL USA, Inc.) was used as the analyzer. All mass spectra were acquired in the positive ion mode. The voltages of the outer orifice and inner orifice were set to be 30 and 5 V, respectively. The temperature of the skimmer cone was heated to 80 ºC. The acquisition range was 10-600 m/z with an acquisition duration of 1 min.

Rhodamine 6G (R6G, $C_{28}H_{31}N_2O_3Cl$, Sigma Aldrich, R 4127, ~95%) was dissolved in methanol at a concentration ranging from 10^{-2} to 10^{-7} mol/l. Urine was obtained from a healthy male without any further treatment. Testosterone (Sigma-Aldrich, purity > 98%) was dissolved in methanol at a concentration of 10 µg/ml. A droplet of analyte solution (~ 0.1 ml) was deposited on a glass slide (1×1 cm^2, 26005, TED PELA, Inc.). The samples were dried in air for about 20 min before the MS analyses.

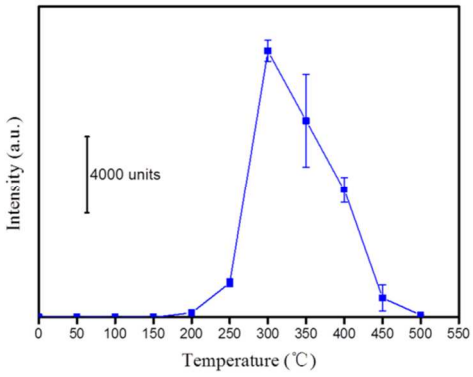

Fig. 2. Temperature dependent DART-MS signal intensity changes of 10^{-7} mol/cm^2 R6G from room temperature to 500 ˚C.

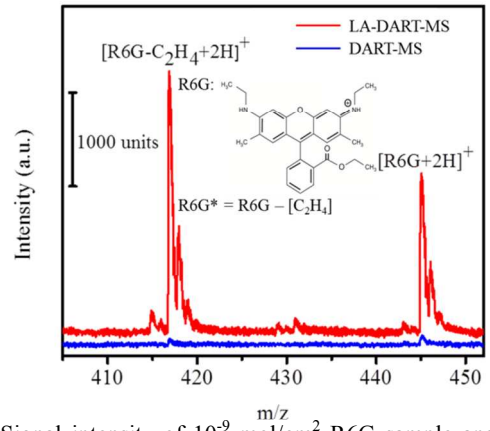

Fig. 3. Signal intensity of 10^{-9} mol/cm^2 R6G sample analyzed by DART-MS and LA-DART-MS.

3. Results and discussion

3.1 Rhodamine 6G

To investigate the influence of ion source temperatures on DART-MS signal intensity, temperature-dependent DART-MS of R6G (with a surface distribution density of 10^{-7} mol/cm^2) is conducted as shown in Fig. 2 The strongest signal intensity is obtained at an ion source temperature of 300 °C. At temperatures below or higher than 300 ˚C, the R6G signal intensity decreases dramatically due to insufficient analyte desorption and analyte decomposition respectively. Based on the results shown in Fig. 2, the ion source temperature is fixed at 300 °C for subsequent studies on R6G.

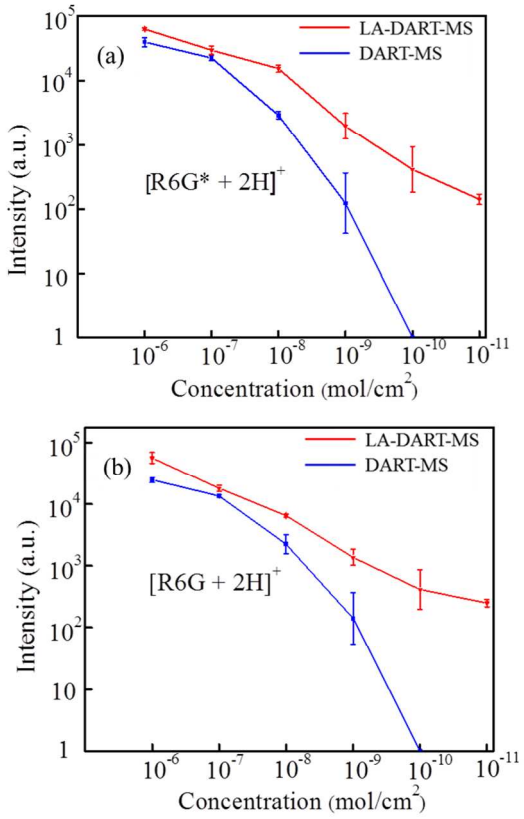

Fig. 4. Signal intensities of two major peaks from R6G samples with different surface concentrations analyzed by DART-MS and LA-DART-MS: (a) protonated R6G* and (b) protonated R6G.

Figure 3 shows the mass spectra of the R6G samples obtained from DART-MS and LA-DART-MS. An average surface distribution concentration of the R6G is 10^{-9} mol/cm^2. Two prominent peaks are observed in both spectra, at 417 and 445, respectively. The peak at 417 is ascribed to a protonated fragmentation (R6G*) of R6G due to the loss of a C_2H_4 radical. The other peak at 445 is the protonated R6G. As shown in Fig. 3, the intensities of both peaks in DART-MS are barely distinguishable from corresponding background noise. However, with the assistance of the CW IR laser, sharp peaks are clearly identified. The SNRs were enhanced by factors (SNRs obtained from LA-DART-MS divided by those obtained from DART-MS) of 31 and 14 for R6G* and R6G, respectively.

The signal intensity changes of R6G* and R6G, along with analyte concentrations, are shown in Fig. 4(a) and (b), respectively. Different concentrations of R6G, from 10^{-6} mol/cm^2 to 10^{-11}mol/cm^2, were used to investigate the sensitivity of the LA-DART-MS (red line) and conventional DART-MS (blue line). In Fig. 4, the signal intensities of LA-DART-MS are obviously higher than those of DART-MS at all analyte concentrations. When the concentrations of R6G decrease to 10^{-10} mol/cm^2, R6G becomes indistinguishable in the conventional DART-MS while still obvious in LA-DART-MS. The lowest R6G concentration which can be detected by LA-DART-MS is 10^{-11} mol/cm^2. This implies a two-magnitude sensitivity improvement in the LA-DART-MS compared to regular DART-MS.

3.2 Urine and Testosterone

To further confirm the capability of LA-DART-MS, analyses of urine and testosterone were performed. The ion source temperature was optimized to 250 ^{o}C for both of them. As shown in Fig. 5(a), the signal of urea was not prominent using DART-MS. By applying the CW IR laser, the signal intensity of urea significantly increased. Figure 5(b) shows the mass spectra of a 10 μg/ml testosterone solution dry deposited on a glass substrate, measured both by DART-MS and LA-DART-MS, respectively. Using DART-MS, the SNR of testosterone was approximately 2.2. However, with laser irradiation, the signal intensity of testosterone increased, leading to an enhanced SNR of 9.5, which could be clearly identified in the mass spectrum.

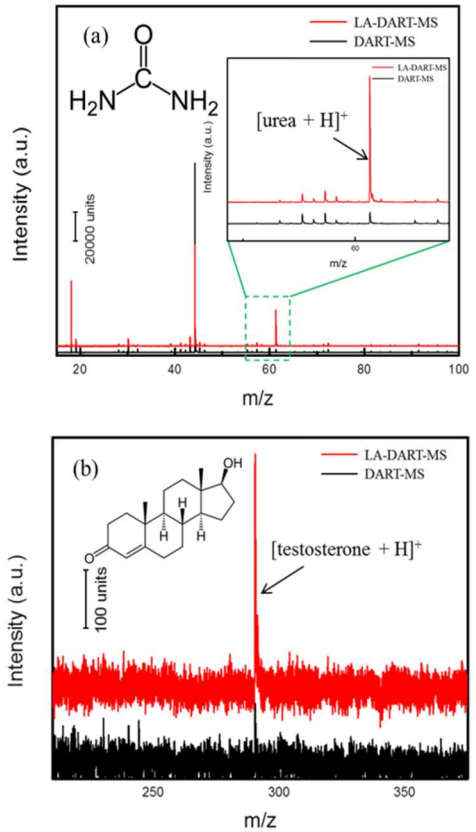

Fig. 5. Mass spectra of analytes on glass substrates: (a) deposited urine solution without any preparation measured by DART and LA-DART-MS and (b) 10 μg/ml deposited testosterone solution measured by DART and LA-DART-MS.

3.3 Mechanisms of the sensitivity improvement

Fig. 1 shows that lasers can irradiate on both analytes and ions from DART when irradiating on a sample substrate. This implies that the signal intensity enhancement in the LA-DART-MS can be twofold: (1) laser-analyte interactions, including laser-induced analyte desorption, and (2) laser-ion interactions, i.e., laser-induced selective excitation of a vibrational OH bending mode. Both interactions are discussed subsequently.

As shown in Fig. 6(a), no signal from 10^{-10} mol/cm^2 R6G was found in the mass spectrum when only laser irradiation was introduced. However, when opening the DART ion source simultaneously with laser irradiation, the signals of the R6G sample were obtained (Fig. 6(b)). Figures 6(a) and (b) imply that CW IR laser irradiation itself cannot achieve efficient ionization of analytes in atmosphere. It can only thermally desorb the neutral analytes without fragmentation and overall temperature increase. Instead, protonated water clusters from the DART ion source can collide with the vaporized analytes by laser desorption, carry them, and thereafter expel ions through charge transfers. Laser-induced vaporization led to increased analyte population in open air, which resulted in increased collisions between the analytes and the protonated water clusters. This process resulted in the increase in SNRs of the mass spectra, i.e., improved MS sensitivity.

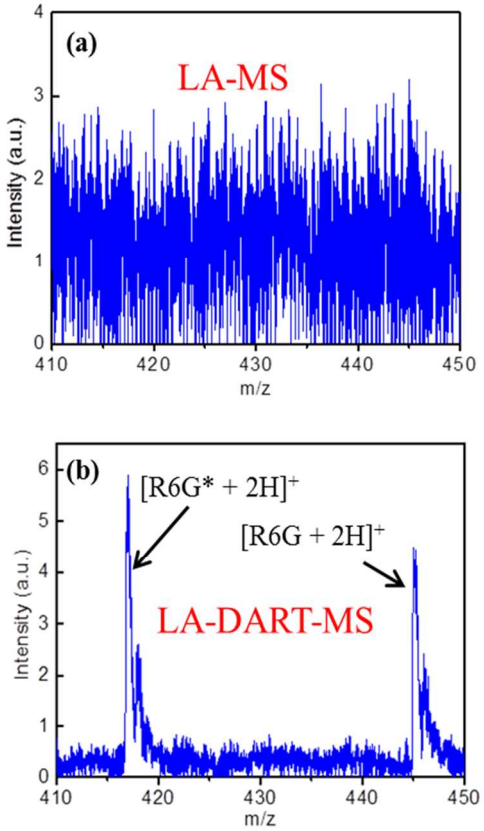

Fig. 6. Mass spectrum of 10^{-10} mol/cm^2 R6G: (a) introduced by laser only and (b) introduced by a laser and DART ion source.

In our experiments, the laser irradiated on the DART ion source together with the laser desorption of the analytes. To investigate the effect of laser interaction with the protonated water clusters from the DART ion source in this process, the laser was shifted slightly from the sample surfaces, as shown in Fig. 7(a), so that it could irradiate only the protonated water clusters without influencing the analytes, thereby excluding the effect on laser-induced analyte desorption. Figure 7(b) implies that a laser interacting with the protonated water clusters from the DART ion source shows a positive effect on the increase in the intensity of the analytes. According to the work by Cody et al.,[2] protonated analytes are formed following four reactions:

$$He\ (2^3S) + H_2O \longrightarrow H_2O^{+\cdot} + He\ (1^1S) + e^- \quad (1)$$

$$H_2O^{+\cdot} + H_2O \longrightarrow H_3O^+ + OH^\cdot \quad (2)$$

$$H_3O^+ + nH_2O \longrightarrow [(H_2O)_{n+1}H]^+ \quad (3)$$

$$[(H_2O)_{n+1}H]^+ + M \longrightarrow MH^+ + nH_2O \quad (4)$$

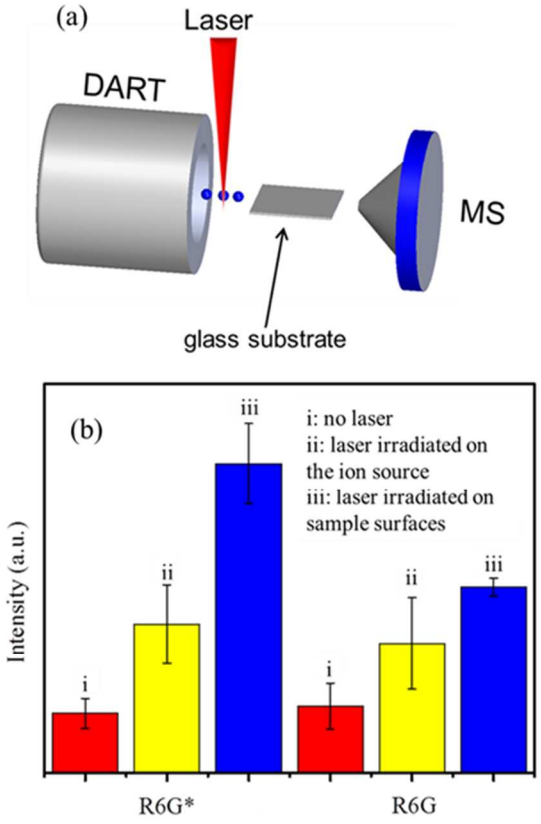

Fig. 7. (a) Experiment schematic of laser irradiating on the ion source and (b) signal intensities of 10^{-8} mol/cm^2 R6G sample analyzed under three conditions: (i) no laser, (ii) laser irradiate on the ion source only, and (iii) laser irradiate on sample surfaces directly.

The efficiency of every reaction determines the overall efficiency of DART ionization. The CW IR laser (1070 nm) used in our experiment increased the reactivity of the four reactions described above by inducing OH bending vibrations (at approximately 1050 nm) of water-based clusters.[12-14] Under irradiation by the CW IR laser, water radicals absorbed energy sufficiently through the OH bending vibrations and subsequently promoted them to a high energy level, which helped in overcoming energy barriers to further reactions. The reactive water-based clusters accelerated the four reactions to form protonated analytes, leading to enhanced ionization efficiency. The SNRs of the analytes in the mass spectra were, therefore, enhanced, leading to enhanced sensitivity and intensity. In this experiment, the reactivity of water-based clusters was enhanced by laser irradiation without increasing the environmental temperature which led to a decrease in intensity with the temperature above the optimized value.

4. Conclusions

The LA-DART-MS was developed and investigated for the enhanced sensitivity and intensity of open air MS. Among the analyses of R6G, urea, and testosterone, the highest enhancement factor was 31 for R6G*. At the same time, LA-DART-MS extended the limit of detection of R6G from 10^{-9} to 10^{-11} mol/cm^2. From our investigation, the sensitivity and intensity enhancement was ascribed to a laser-induced population increase of analytes and laser-induced reactivity enhancement of protonated water clusters from the DART ion source by exciting OH bending vibrations of the water radicals.

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Notes and references

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