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Development of Spray Deposition/MALDI-TOFMS and Its Application to the Rapid Screening of Hydrolysis Products Derived from Nitrogen Mustards

Chu-Feng LIN,* Ju-Tsung LIU, and Cheng-Huang LIN*†**

**Department of Chemistry, National Taiwan Normal University, 88 Sec. 4, Tingchow Road, Taipei, Taiwan*
***Forensic Science Center, Military Police Command, Department of Defense, Taipei, Taiwan*

A novel method for preparing samples for use in MALDI-TOFMS (matrix-assisted laser desorption ionization time-of-flight mass spectrometry) is described. Seven hydrolysis products derived from nitrogen mustards and CHCA (α-cyano-4-hydroxycinnamic acid) were selected as model compounds and the matrix, respectively. A capillary atomizer was used for evaporative and spray deposition of the sample/matrix solution, leading to the formation of a freestanding film that coated and accumulated on the MALDI substrate (*i.e.*, sample plate). Compared to the traditional method for MALDI, which involves the production of dried droplets, the surface roughness was reduced, resulting in the accumulation of the sample-doped matrix on the sample plate. This resulted in an increase in the limit of detection of 1 – 2 orders of magnitude. In order to compare the structures of the sample-doped matrices obtained by the traditional dried droplet method *versus* the spray deposition method (developed in this study), the matrices were examined by SEM (scanning electron microscopy). The design of the capillary atomizer and details of the experimental conditions are reported. The application of this method to the above seven degradation products was successful, suggesting that it has great potential for use as a routine monitoring tool.

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Introduction

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOFMS) is a very popular and powerful tool that is routinely used in analyses of biomolecules.[1-9] The analysis of low-mass ($m/z < 500$) molecules by this method has recently been reported.[10-17] For MALDI-TOFMS to be successful, the ratio of sample to matrix is a critical component. While simply applying a matrix solution and an analyte on a sample plate would appear to be an easy task, many variables influence the final results. These include the concentrations of the matrix and analyte, choice of matrices, the condition of the analyte sample, hydrophobicity or hydrophilicity characteristics, the presence of contaminants and whether the matrix and sample solutions are compatible with one another. The dried droplet sample preparation is currently in general use in MALDI-TOFMS. In this procedure, an analyte and matrix solution are premixed in a small tube, or applied directly to the sample plate, allowed to evaporate in air, resulting in the formation of a sample-doped matrix. Detailed procedures can be found in MALDI manuals, but operator skills and experience are also often important factors. It should be noted that some other interesting and potentially useful methods for sample introduction have been reported, which include, atmospheric pressure MALDI,[18,19] desorption/ionization on silicon nanowires,[20] laser-induced acoustic desorption,[21] ESI-MALDI,[22] electrospray sample deposition (ESD),[23] the use of carbon nanotubes[24] and AnchorChip™ sample preparation.[25] In this study, we wish to report on a new method for sample preparation, the spray deposition method, for use in conjunction with MALDI-TOFMS. When the spray deposition method is used, a freestanding film (sample-doped matrix) is created that accumulates on the sample plate, completely coating it, leading to a dramatic improvement in the limit of detection. In contrast, in the traditional method (dried droplet sample preparation), the crystals of the sample-doped matrix are randomly dispersed on the sample plate. SEM (scanning electron microscopy) was used to examine differences between matrices prepared using the 2 methods; these data are included in this report. Furthermore, in an extension of our previous research, dealing with the rapid screening of nerve agent degradation products by MALDI-TOFMS,[17] seven hydrolysis products derived from nitrogen mustards were selected as model samples, and their detection was reexamined using the above mentioned technique. Several nations stock-piled large amounts of munitions containing nitrogen mustard gas during World War II, although none were used in combat. Hence, they are classified as schedule 1 substances by the Chemical Weapons Convention. A number of analytical methods have been developed for their identification, including gas chromatography/mass spectrometry,[26-29] liquid chromatography/mass spectrometry,[30,31] chemiluminescence,[32] capillary electrophoresis,[33] ion trap secondary ion,[34] and MALDI mass spectrometry.[35] In this work, the spray deposition MALDI mass spectrometry was used for the rapid screening of hydrolysis products derived from nitrogen mustards in spiked soil samples.

† To whom correspondence should be addressed.
E-mail: chenglin@ntnu.edu.tw

Experimental

Instrumentation

The linear type of time-of-flight mass spectrometer (TOFMS), which was a modified Wiley-McLaren design (R. M. Jordan Co., Grass Valley, CA), laser (355 nm radiation generated from a Nd:YAG laser (Spectraphysics GCR-170, Mountain View, CA)) and the data-acquisition system used were similar to that described previously[17] and are abbreviated herein. A thermal type field emission scanning electron microscope (SEM), JSM-7000F, Japan Electron Optics Laboratory Co., Ltd. (JEOL) was used for surface observation.

Reagents

All chemicals used were of analytical grade. α-Cyano-4-hydroxycinnamic acid (CHCA; M_w, 189.17), as purchased from Aldrich (St. Louis, MO). Soil samples (collected from a military shooting range located in Kinmen County, Taiwan) and seven hydrolysis products derived from nitrogen mustards, including dimethylethanolamine (DMEA), diethylethanolamine (DEEA), methyldiethanolamine (MDEA), ethyldiethanolamine (EDEA), dipropylethanolamine (DPEA), diisopropylethanolamine (DIPEA) and triethanolamine (TEA) were generously donated by the Military Police Command, Forensic Science Center, Taiwan. All other chemicals were of analytical grade, and were obtained from commercial sources.

Sample/matrix preparation

Dried droplet. Ten milligrams of CHCA were dissolved in 1.0 mL of a water/acetonitrile (v/v, 50/50) solution. Seven model samples (hydrolysis products of nitrogen mustards) were diluted with methanol to various concentrations to obtain a final concentration. A 2.0-μL aliquot of the CHCA solution was premixed with 2.0 μL of the sample solution. A 1.0-μL aliquot of the sample/matrix solution was then removed and dried on the sample plate, resulting in the deposition of a solid mass of sample-doped matrix crystals. If an additional droplet was added to the solid deposit, the crystals dissolved and collapsed. This points out the difficulties associated with the preparation of an acceptable sample/matrix preparation.

Spray deposition. A sample/matrix solution was prepared by mixing 30 μL of a CHCA solution with 30 μL of the sample solution (seven hydrolysis products derived from nitrogen mustards: 0.1 μg of each in 1 mL methanol). The resulting sample/CHCA solution was placed in a tube, and then ejected into a vacuum chamber by means of a capillary atomizer (described below). As a result, the aerosols underwent evaporation, and finely divided particles of the sample-doped matrix uniformly coated the sample plate.

Spiking and extraction of soil samples. Two types of soil samples were used, one collected from a military shooting range and the other from a university campus. A 1.0-g sample of each soil sample was spiked with seven model samples (0.1 μg of each diluted in 1.0 mL methanol); the mixture was subjected to shaking for 20 min, followed by evaporation of the methanol at room temperature. A 1.0-mL aliquot of methanol was added to a spiked soil sample; the mixture subjected to shaking for 20 min and then allowed to stand for 15 min. The upper layer was collected and centrifuged for 30 s at 5000 rpm. The upper layer was collected again and filtered through a 0.45-μm filter. The filtrate was collected, transferred to a clean tube and used in subsequent experiments.

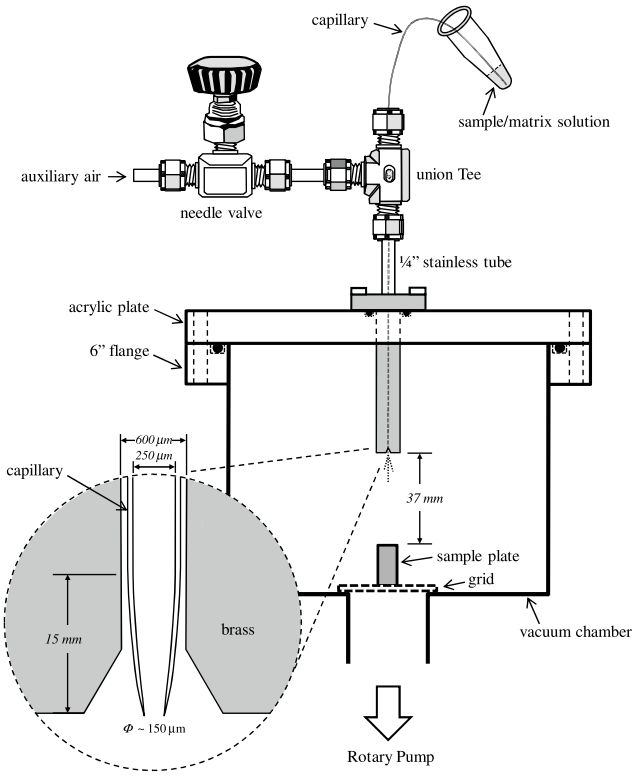

Fig. 1 Schematic diagram of the capillary atomizer used for SD (spray deposition)/MALDI-TOFMS.

Results and Discussion

Capillary atomizer

The spray deposition technique can be considered to be an extension of vacuum deposition, a well-known technique. Figure 1 shows a schematic diagram of a capillary atomizer used in the SD (spray deposition)/MALDI-TOFMS. The sample/matrix solution was placed in a micro centrifuge tube. A normal GC column (29 cm in length; o.d./i.d., 375/250 μm) was used for the spray and deposition stages. A portion of the capillary was heated to melting with a high-temperature burner (>1200°C), and then quickly pulled until separation occurred. The tip size and shape could be observed by a microscope. Based on trial and error tests, optimum results were obtained when the size of the tip size was 120 to 150 μm. For a comparison, various untreated capillaries, including i.d. 50, 75 and 250 μm, respectively, were examined, and the findings showed that a narrow tip (similar to a venturi structure) is necessary. The capillary-based tip was inserted into a tunnel (ϕ = 0.6 mm), where auxiliary air was supplied from the outside. Aided by the auxiliary air and attractive forces, the sample/matrix solution was emitted from the capillary tip, resulting in the formation of an aerosol. The flow rate of the auxiliary air could be controlled by a needle valve. During the spray process, the aerosols evaporated and the resulting finely divided solids (sample-doped matrix) accumulated on and coat the MALDI sample plate. The sample plate, which was located downstream, was 12 mm in height and 6.35 mm in diameter. The optimal distance from the tip to the surface of the sample plate was determined to be 37 – 40 mm. Although the sample plate is unlike the general commercial standard MALDI plate with ~100 sample spots, a 100 sample spot experiment is also possible if a

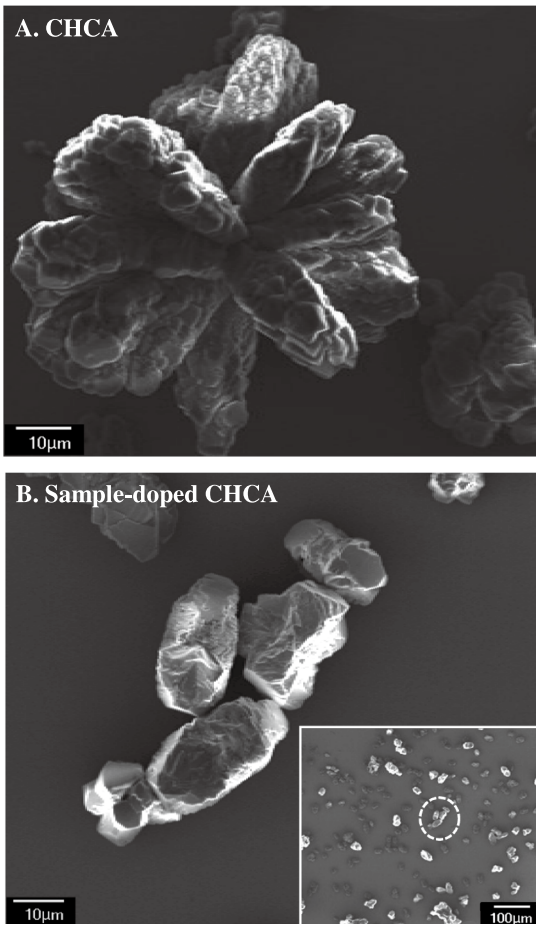

Fig. 2 A, a typical photograph of CHCA crystals obtained by the traditional method, dried droplet sample preparation (CHCA concentration level: 10 mg in 1.0 mL water–acetonitrile solution; v/v, 50/50). B, a photograph of sample-doped CHCA matrix, obtained by the same method. The inset shows an extended range; unit scale up to 100 μm.

stepper motor is used. In order to maintain low-pressure conditions, a 6″ flange-based vacuum chamber (i.d., 95 mm; depth, 75 mm) was connected to a rotary pump and a vacuum gauge, attached to the chamber, was used for monitoring (omitted in Fig. 1). It should be noted that, if the sample solution and matrix solution were sprayed separately, leading to the formation of alternating layers, no signal improvement occurred. A transparent acrylic plate (diameter, 6″; thickness, ~10 mm) was used as a cover, thus allowing the progress of the deposition to be observed. The optimized chamber pressure should be maintained below 60 mmHg, and the injection volume of sample/matrix solution was estimated to be 8 μL for each injection (injection rate, ~40 μL/s). In the case of 60 μL of the sample/matrix solution, a portion of solid-deposits was deposited on the sample plate, and some were lost by the pumping system; the thickness of the deposited material was estimated to be ~10 μm. The plate, coated with the sample-doped matrix was directly used in subsequent MALDI-TOFMS experiments.

SEM observation

Figure 2A shows a typical photograph of CHCA crystals obtained by the traditional method (dried droplet sample preparation); CHCA concentration level: 10 mg in 1.0 mL water–acetonitrile solution; v/v, 50/50. As can be seen, crystal growth was excellent and clear; the diameters of the crystals were estimated to be 70 – 80 μm. In contrast, Fig. 2B shows a photograph of a sample-doped CHCA matrix, also obtained by the traditional method. In this case, CHCA is the host, whereas the samples can be considered to be impurities, and as a result the growth of CHCA crystals was suppressed. As shown in Fig. 2B, the size of the crystals were decreased to 10 – 25 μm. In Fig. 2B, the inset shows a larger scale; the unit scale is expanded from 10 to 100 μm. For a comparison, the same crystals are indicated by a white broken circle. In this case, the numbers of such small sample-doped CHCA crystals are estimated to be ~320 grains/mm^2. In other words, only ~17% of the available area is covered with sample-doped CHCA. In a typical UV laser (N_2 laser, 337 nm) used in most commercial MALDI instruments, the spot radius of the laser focusing zone is roughly only ~5 μm. Such a 17% cover ratio is too low to permit high sensitivity and acceptable reproducibility to be obtained. This explains why, in MALDI experiments, it is necessary to produce good crystal shapes for laser shooting. However, when the spray deposition method was used, and cover ratio could be dramatically improved, approaching ~100%. Figure 3 shows a typical photograph of sample-doped CHCA obtained by the spray deposition method. As could be seen in this large scale, a freestanding film is produced, which coats the entire sample plate. For a comparison, the inset shows a smaller scale, scaled down to 100 nm. Since the surface roughness was substantially reduced and the cover ratio of the sample-doped CHCA was significantly increased, a 1 – 2 order of magnitude improvement in the limit of detection could easily be achieved. When a cylindrical lens was used, even more improvement could be achieved.

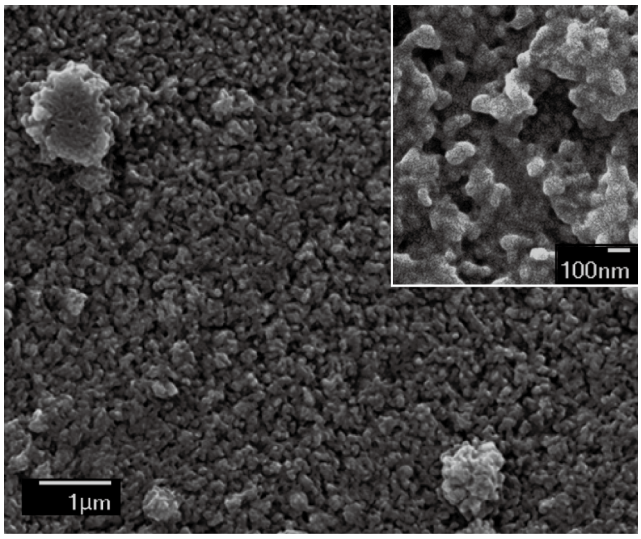

Fig. 3 Typical photograph of sample-doped CHCA, obtained by the spray-deposition method. The inset shows a smaller scale; unit scale down to 100 nm.

Applications

Figure 4 shows typical mass spectra obtained using the dried droplet sample preparation method (mass spectrum a) and the spray deposition method (mass spectrum b), respectively, by a cylindrical lens (focus length, 20 cm); the chemical structures and abbreviations of the 7 test samples are also shown in Fig. 4. The signal intensities were obtained from 50 different laser shots/sample spots. In the case of the dried droplet sample

Table 1 Signal intensity (mV) and limit of detection (LOD, $S/N = 3$) for 7 hydrolysis products derived from nitrogen mustards by the dried droplet and spray deposition methods, respectively

Compound	DMEA	DEEA	MDEA	EDEA	DPEA	DIPEA	TEA
Signal intensity/mV							
a. Dried droplet	3.0 ± 1.0	1.5 ± 0.3	3.0 ± 0.5	3.9 ± 1.7	2.3 ± 0.8	3.6 ± 1.5	2.6 ± 1.2
b. Spray deposition	20.2 ± 3.9	18.1 ± 4.2	18.4 ± 1.3	14.4 ± 1.5	12.3 ± 1.3	12.4 ± 5.0	14.2 ± 2.0
c. Spray deposition	58.3 ± 18.0	61.1 ± 12.0	33.1 ± 10.7	25.2 ± 7.8	37.5 ± 13.5	52.5 ± 8.4	20.8 ± 2.2
Limit of detection							
d. Dried droplet	0.3 ± 0.2	0.6 ± 0.1	0.3 ± 0.1	0.1 ± 0.0	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.2
e. Spray deposition	3.6 ± 0.6	6.5 ± 1.5	5.8 ± 0.4	12.0 ± 1.3	13.0 ± 1.5	11.0 ± 3.3	13.0 ± 1.9
f. Spray deposition	1.3 ± 0.4	1.8 ± 0.5	3.5 ± 1.1	6.8 ± 4.4	4.6 ± 1.5	2.3 ± 0.4	9.0 ± 1.0

The signal intensities were obtained from 50 different laser shots/sample spots; reduplicated experiments, $n = 4$. Circular lens/sample concentration: a, 1.0 μg mL^{-1}; b, 0.1 μg mL^{-1}; d, 1 μg mL^{-1}; e, 1 ng mL^{-1}. Cylindrical lens/sample concentration: c, 0.1 μg mL^{-1}; f, 1 ng mL^{-1}.

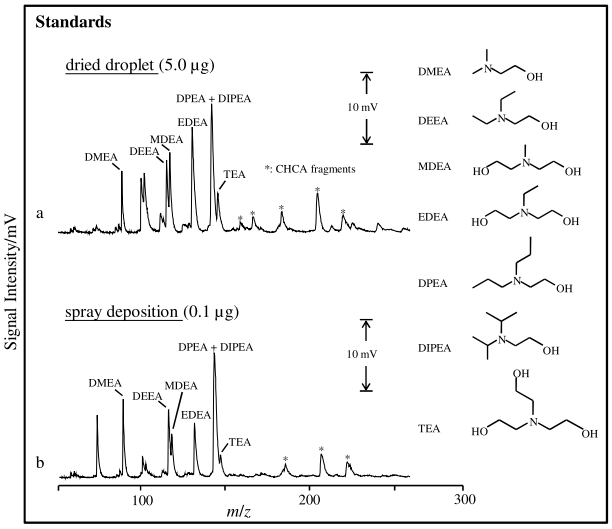

Fig. 4 Typical mass spectra obtained using the dried droplet sample preparation method (mass spectrum, a) and the spray deposition method (mass spectrum, b), respectively, by a cylindrical lens. In mass spectrum, a, the sample/matrix solution was prepared by mixing 2 μL of CHCA solution (10 mg in 1.0 mL water–acetonitrile solution; v/v, 50/50) with 2 μL of sample (each 5.0 μg diluted in 1.0 mL methanol). A 1-μL droplet was used and dried on the sample plate. In mass spectrum, b, the sample/matrix solution was prepared by mixing 30 μL of CHCA solution (10 mg in 1.0 mL water–acetonitrile solution; v/v, 50/50) with 30 μL samples (0.1 μg each in 1.0 mL methanol). The entire 60 μL sample/matrix solution was used for spraying and deposition and portion of the sample coated the plate.

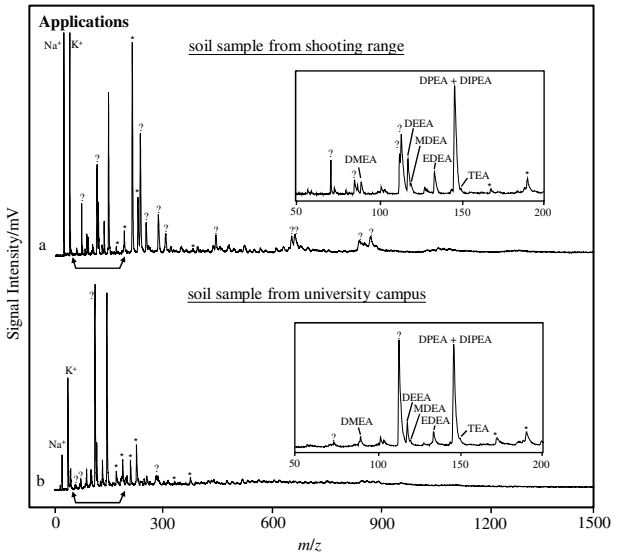

Fig. 5 Mass spectra obtained by SD/MA after spiking soil samples with the seven standards (0.1 μg of each in 1.0 mL methanol, spiked to 1.0 g soil samples). The insets show the extended m/z range from 50 to 200.

preparation, the sample/matrix solution was prepared by mixing 2 μL of a CHCA solution (10 mg in 1.0 mL water–acetonitrile solution; v/v, 50/50) with 2 μL aliquots of the samples (each 5.0 μg diluted in 1 mL methanol), as described above. Only a 1.0-μL droplet was used and dried on the sample plate. As can be seen, in the case of DMEA (10 ng, average spread on the sample plate), a, 10 mV signal can be obtained. Various samples have different properties, leading to variations in signal intensities. DPEA and DIPEA are isomers, so that only one overlapped peak is observed. Several peaks can be assigned to CHCA fragments, indicated as "*". Other very small peaks correspond to fragmentation products derived from the seven model samples (m/z = 58, β-cleavage of DMEA; m/z = 72, DMEA-OH; m/z = 74, α-cleavage of DEEA; m/z = 86, β-cleavage of DEEA; m/z = 88, β-cleavage of MDEA; m/z = 102, α-cleavage of DPEA/DIPEA; m/z = 104, α-cleavage of DPEA/DIPEA; m/z = 114, β-cleavage of DPEA/DIPEA; m/z = 128, DPEA-OH/DIPEA-OH; m/z = 132, TEA-OH, respectively). In contrast, in the case of the spray deposition sample preparation, the sample/matrix solution was prepared by mixing 30 μL of a CHCA solution (10 mg in 1.0 mL water–acetonitrile solution; v/v, 50/50) with 30 μL of sample solution (0.1 μg of each in 1.0 mL methanol). The entire 60 μL of the sample/matrix solution was used for spraying and deposition. A portion of the sample-doped matrix accumulates on and coats the sample plate, leading to a higher detection sensitivity. As a result, the limits of detection of DMEA, DEEA, MDEA, EDEA, DPEA, DIPEA and TEA were improved by 94-, 97-, 51-, 10-, 32-, 26- and 31-folds, respectively. Furthermore, when a cylindrical lens (focus length, 20 cm) was used, the limits of detection were further improved by 261-, 350-, 85-, 16-, 91-, 126- and 45-folds, respectively. This can be seen from the Table 1 where the peak intensities and limit of detections of the 7 analytes are summarized, based on the dried droplet and spray deposition methods, respectively. Actual samples were investigated by spiking soil samples with the seven model samples. Figure 5 shows the results obtained by the SD/MALDI-TOFMS. In both

cases, numerous peaks correspond to unknown components (indicated as "?") in the soil samples. The insets, above mass spectra a and b, show the extended *m/z* range from 50 to 200. As can be seen from these mass spectra, all of the expected major peaks are observed in the spiked soil extracts. Thus, we conclude that the SD/MALDI-TOFMS, which is simple, sensitive and rapid, has considerable promise for use in rapid drug-screening and is sufficiently reliable to serve as a complementary method in this field.

Conclusion

We developed a novel sample preparation method, spray deposition, for use in conjunction with MALDI-TOFMS. The method is quite simple, rapid and permits a higher sensitivity, and is much simpler than the electrospray sample deposition (ESD) method. This is because, in the ESD method, varying the spray voltage and distance resulted in different crystal sizes and volatilization rates. Under different conditions (such as wet spray, damp spray, mist spray and dry spray, respectively) the images and lifetimes of sample spots deposited by electrospray onto a stainless-steel MALDI plate were varied. In contrast, the spray deposition described herein is much simple and economical, where a high ESI voltage is not needed, the plate is easy to prepare and the deposited sample can be saved, even a long period. Furthermore, the size of the deposited material is estimated to be 2 – 3 mm in diameter and ~10 μm in thickness. Hence, a 1 – 2 order of magnitude improvement in the limit of detection can easily be achieved, and when a cylindrical lens is used, even more improvement can be achieved. For seven model samples, *i.e.* seven hydrolysis products derived from nitrogen mustards, a 1 – 2 order of magnitude improvement in the limit of detection can be achieved, showing that this method can be successfully used for the rapid screening of chemical agent degradation products. Thus, we believe that the SD/MALDI-TOFMS technique described herein has not only great potential for the rapid screening of chemical warfare agents and related compounds, but also has a variety of applications in other, related areas and could potentially be used in practical trace analysis.

Acknowledgements

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