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EVALUATION OF AN AEROSOL MATRIX DEPOSITION METHOD FOR MALDI ION IMAGING

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OVERVIEW

PURPOSE

- To evaluate the use of a nebulising sprayer for depositing matrix on to thin tissue sections for imaging applications.

METHOD

- A nebulising sprayer which provides independent control over matrix flow rate, temperature, gas pressure and target plate travel velocity.

RESULTS

- The ability of the nebuliser to re-solubise pre-deposited peptides has been used as a sensitivity test.
- The degree of de-localization resulting from matrix deposition has been evaluated.
- The nebuliser has been used to coat tissue samples.

INTRODUCTION

There has been increasing interest over the past several years in using MALDI to produce maps or images of the location of peptides, proteins, drugs and their metabolites within thin tissue sections; the technique is increasingly becoming referred to as MALDI-MSI (matrix assisted laser desorption ionization-mass spectrometry imaging).

An inherent requirement during the sample preparation stage of a MALDI-MSI experiment is the ability to deposit matrix onto the sample in such a manner as to prevent delocalization of the analytes of interest, whilst obtaining sufficient co-crystallization of the matrix and analyte to obtain high quality, reproducible, mass spectra. Common approaches have been the normal dried-droplet method (which maximizes the possibility of co-crystallization due to the large droplet size and its correspondingly long drying time, but has poor spatial resolution due to the large physical dimensions of the droplet) in addition to two other techniques; deposition using a hand held glass spray nebuliser (of the type commonly found in craft shops), and electrospray deposition.

We present early results of an evaluation of a motorized nebulising sprayer and its use for matrix deposition in MALDI-MSI experiments.

EXPERIMENTAL—MS

All mass spectra were obtained using a Waters® Micromass® MALDI micro MX™, axial TOF mass spectrometer. No hardware or software modifications to the instrument were necessary.

NEBULISING SPRAYER

A capillary nebuliser with controllable nozzle temperature and desolvation gas pressure was used for matrix deposition. The velocity of the sample stage beneath the nebuliser in addition to the flow rate may be controlled to give optimization of the matrix deposition process. Deposition was performed at a flow rate of 15 uL/min, with a gas pressure of 12.5 PSI at 20°C unless specified otherwise. The target plate was rastered at a velocity of 40mm/min.

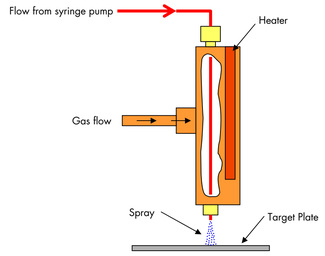

Figure 1 : Schematic of the nebulising sprayer

RESULTS

To test the degree of de-localization resulting from matrix deposition by the nebuliser, alternating tracks of Glu-Fibrinopeptide (M+H 1570.6774 Da) and Bradykinin (M+H 1060.569 Da) were deposited onto two target plates using the nebuliser. The width of the tracks were defined by using a shadow mask placed above the target plates during deposition. The peptides deposited onto target plate 1 were pre-mixed with matrix solution so that matrix and analyte were simultaneously deposited. For target plate 2, the peptides were deposited onto the target plate first, then the entire plate was coated with matrix with the shadow mask removed and with the plate rotated through 90°.

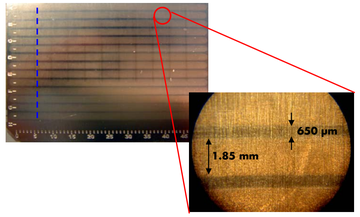

***Figure 2 : Digital photographs of the deposited tracks of* Glu-Fibrinopeptide and Bradykinin**

Figure 2shows a digital photograph of the tracks deposited onto plate 1. Following deposition, line profiles of the intensity of the two peptides as a function of position were produced by acquiring a mass spectrum from individual positions separated by 100um in a line perpendicular to the tracks (as indicated by the blue line in the above figure). This was repeated 20 times for each plate.

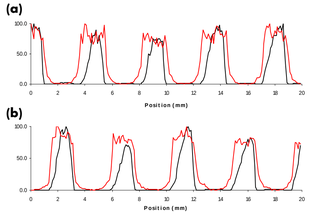

***Figure 3 : Averaged line profiles for* Glu-Fibrinopeptide (top) and Bradykinin (bottom) from plate 1 (black line) and plate 2 (red line)**

Figures 3 (a) and (b) display the averaged line profiles for Glu-Fibrinopeptide and Bradykinin respectively. Both graphs show data from plate 1 (black line) and plate 2 (red line). It is clear that the width of each peak from plate 2 is wider than the corresponding peak from plate 1. This is expected and is the result of the de-localization resulting from the matrix deposition process. What is more important is the degree to which each peptide has become delocalized, as if this is wider than the width of the focused laser beam, this will limit the imaging resolution for any MALDI-MSI experiment. The average FWHM of the line profiles obtained from plate 1 is 1.1mm. This compares with an average FWHM of 1.8mm for plate 2. The spread of each edge is therefore of the order of 350µm. This corresponds to approximately twice the diameter of the focused laser spot in this experiment.

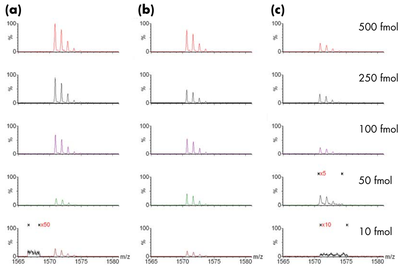

***Figure 4 : Mass spectra from a dilution series of* Glu-Fib. Matrix deposited simultaneously (left) after sample (center) and using nebuliser (right)**

SENSITIVITY

To test the degree to which matrix deposited using the nebuliser can successfully co-crystallize with analytes on a surface, a dilution series of Glu-Fibrinopeptide was spotted three times onto a target plate. The first set of samples were pre-mixed with matrix solution before being deposited using a pipette, the second set were allowed to dry before matrix was added using a pipette and the third set were again allowed to dry, but were then coated with matrix using the nebuliser. These three different deposition methods are designed to compare the ideal situation where 100% of the analyte is pre-dissolved into the matrix, with dried droplet and nebuliser matrix deposition.

Figure 4 compares the signal intensity obtained from the three different data sets. The samples which were pre-mixed with matrix show the most intense signals with good sensitivity and signal to noise for each dilution. In contrast, the samples which had matrix deposited using a pipette show reduced intensity in each case, but still with good sensitivity. The samples coated with matrix from the nebuliser show a further reduction in signal intensity and at a level of 10fmol/µL the peptide can no longer be detected. This is a result of the matrix effectively being deposited too dry—the nebulised spray does not remain liquid on the surface of the sample plate for long enough for the analyte to dissolve into the matrix.

MALDI IMAGING OF RAT BRAIN

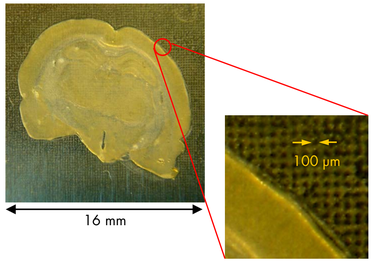

Figure 5 : Digital photographs of a rat brain section

To assess the effectiveness of the nebuliser in depositing matrix onto real tissue samples, it was used to coat thin sections of rat brain from male Sprague-Dawley rats (8weeks) in addition to liver and kidney sections obtained from mouse. The animals were killed by cervical dislocation in accordance with Schedule 1 of the UK Government Animals (Scientific Procedures) Act (1986). Tissue samples were frozen immediately prior to sectioning on a cryo-microtome. The sections were thaw mounted onto stainless steel target plates and then re-frozen until required for analysis.

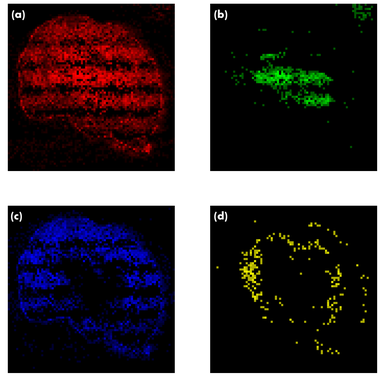

Figure 6 : MALDI ion images obtained from rat brain section for (a) 4996 Da, (b) 6770 Da, (c) 8329 Da and (d) 15,175 Da.

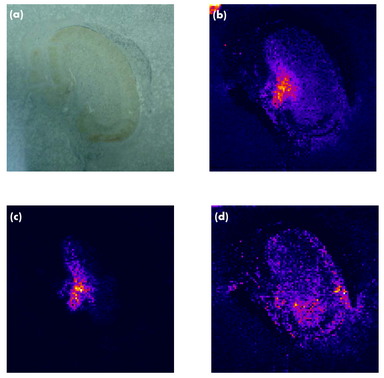

Figure 7 : Digital photograph (a) and ion images obtained from mouse kidney section. (b) Total ion count, (c) 4965 Da, (d) 11,362 Da.

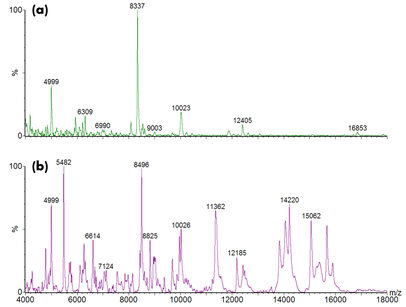

Figure 8: Mass spectrum obtained from rat brain section

Figures 5 and 6 are digital photographs and ion images obtained from a single rat brain section. The ion images were produced by acquiring mass spectra in an 80 by 80 array. The separation between pixels was 200µm, and the focused laser spot in this experiment had a diameter of 100um. The 'striping' visible in the ion images is a result of non-uniform matrix coverage resulting from the nebuliser runs being spaced too far apart in this instance.

Figure 7 contains a digital photograph and ion images obtained from a section (60 µm) of mouse kidney. These images are 100 x 100 pixels and the pixel separation is 150 µm.

Figures 8 (a) and (b) are typical mass spectra obtained from the thin tissue samples. Figure 10 (a) was obtained from a thin section of rat brain whereas figure 10 (b) was acquired from a thin section of mouse liver. The liver sample in particular was found to contain an abundance of proteins.

CONCLUSIONS

- The use of a nebulising sprayer is currently being evaluated as a matrix deposition method for MALDI-MSI.
- The de-localization of peptides resulting from matrix deposition is approximately 375µm.
- The sensitivity of the nebulising technique (its ability to dissolve existing analytes on a surface) is not as good as the dried droplet method, likely due to the short drying time.
- The nebuliser has successfully been used to coat thin tissue sections of brain and kidney and obtain both mass spectra and MALDI ion images.

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