In MALDI mass spectrometry the peaks observed in the resulting mass spectrum is dependent on the details of sample preparation. These details include the selection of the matrix, solvents, deposition method, and matrix-to-analyte ratio, among others. These choices affect the selectivity of the experiment, resulting in biases toward certain analytes. In a MALDI imaging experiment, there can be hundreds of analytes belonging to different classes of compounds and the tissues introduced during sample preparation can result in a number of analytes of interest not being detected [1]. Due to the clear utility of MALDI imaging on complex biological samples, a better understanding of the effect of sample preparation parameters on the resulting MALDI imaging experiment is essential. Electrospray deposition (ESD) allows for a homogeneous and reproducible application of matrix to a sample, making it well suited for these studies.

Methods

Samples were prepared in two steps: the application of the analyte followed by the application of the matrix (as a mixture with a cationization agent, when one is necessary). For samples with the same analyte, a charger was shown, as in Figure 1, was dipped into the analyte dissolved in a 90:10 methanol/water solution. The sample was then applied to a Kipmeico once to remove excess solution, then re-applied onto the target plate. Analyte was also applied by the dry drop technique and by ESD for other samples. For all samples, the analyte was first applied and allowed to dry completely, before the matrix was applied by ESD [3]. All matrices, cationization reagents and solvents were used as received. All spectra were collected using FlexAnalysis (version 3.4). Images were collected with FlexImaging (version 4.0). All spectra were collected in linear mode using a BioSavanna II laser with a repetition rate of 100 Hz.

Results

Effect of Spraying Parameters

In Figure 6, four matrix/cationization agent systems were sprayed on identically prepared sets of analytes. While using the same cation reagent together, they are all detected, however, due to their different matrix/drift time, there are several combinations of analytes and matrix systems which do not produce signal. Neutron detection is not observed when both matrices are used. Polyethylene glycol is deposited only in the difference system (as ACN is used for cationization), and the polyethylene glycol is detected over a much larger area when dimethyl is applied as compared to CHCA.

Conclusion

The data presented here demonstrates the dependence of a MALDI imaging experiment on many sample preparation and sampling parameters which interact in complex ways. The spray time used will determine the amount of matrix solution being deposited, which dictates the ratios between matrix, analyte and cationization reagent (if present). The spray time also determines the time in which an analyte can interact with the matrix and cationization reagent within the droplet. The change in the matrix deposition will also affect the properties of the matrix and the resulting solid sample [3]. The selection of the matrix and cationization reagent also impacts the selectivity of an imaging experiment. The selection of an acetonitrile matrix would improve the ionization efficiency of basic analytes and vice versa. Analytes with an affinity for particular cations would have different signals as the identity, amount, and distribution of the matrix changes. Some heterogeneity is expected from sample to sample due to the application of analyte by the dry drop technique, but when saved as the matrix was changed. In three of the samples, the early laser shots produce no analyte signal. In figures 10 and 12, signal is immediately seen. Depending on which range of laser shots is selected to create an image, very different images would be observed.

References

1. Amstalden van Hove, E.R., Smith, D.F., Heeren, R.M.A. A concise review of mass spectrometry imaging, with an affinity for particular cations would have different signals as the identity, amount, and distribution of the matrix changes. Some heterogeneity is expected from sample to sample due to the application of analyte by the dry drop technique, but when saved as the matrix was changed. In three of the samples, the early laser shots produce no analyte signal. In figures 10 and 12, signal is immediately seen. Depending on which range of laser shots is selected to create an image, very different images would be observed.

References