Matrix Optimization of ZnO for Mass Spectrometry Imaging of Small Neurochemicals in Rat and Mouse Brain Tissue

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PURPOSE

Test experimental parameters in order to optimize ZnO nanoparticles as a matrix for detection of low molecular weight neurochemicals in rat and mouse brain tissue.

BACKGROUND

MALDI-MS is an analytical technique used to ionize analyte samples into charged molecules.

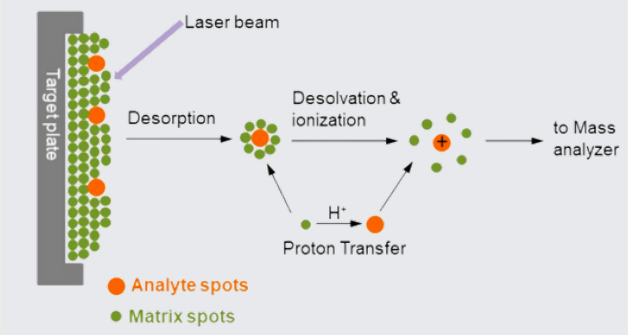
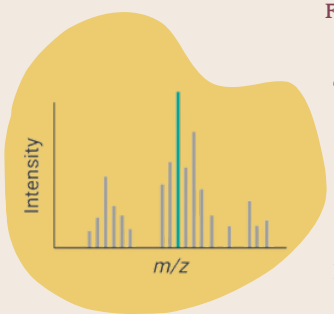


Figure 1. Principle of Matrix Assisted Laser Desorption/Ionization (MALDI-MS)

A brief 350 nm UV laser pulse is applied onto a target plate and absorbed by matrix particles. Vibrational excitation allows analytes embedded in the matrix particles to be carried into the gas phase, where they are ionised by protonation or deprotonation and sent to the **Time of Flight (TOF)** mass analyser.

Analyte ions are accelerated under an applied potential and fixed kinetic energy such that the time it takes for ions to reach the detector direcly correlates to their mass-to-charge ratio. Lighter molecular weight ions reach the detector faster.

Figure 2. SCIEX TOF/TOF 5800 MALDI-MS System



The results are presented on a **mass spectrum** that exhibits intensity of the signal as a function of the m/z of the analyte ions.

INSTRUMENTATION

A brief overview of instruments used and their purpose relative to the goal of the experiment at hand.

- **HTX TM Sprayer :** Instrument used to spray a uniform matrix coating onto conductive ITO glass slides with brain tissue.

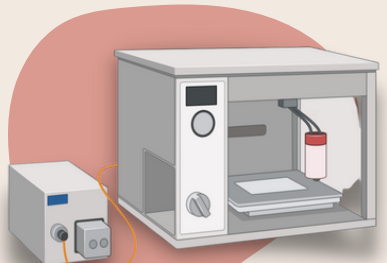


Figure 3. HTX TM Sprayer

- **DeNovix DS-11 Nanodrop :** Instrument used for microvolume analysis of diluted ZnO nanoparticle solutions in order to determine matrix concentrations from UV absorbance.

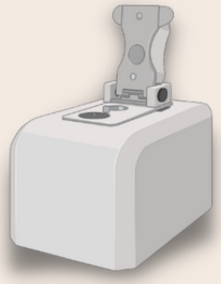


Figure 4. Microvolume UV-Vis Instrument

- **Zetasizer Nano Dynamic Light Scattering Instrument :** Instrument used for determining size distribution of nanoparticles including the formation and disappearance of aggregates in sample

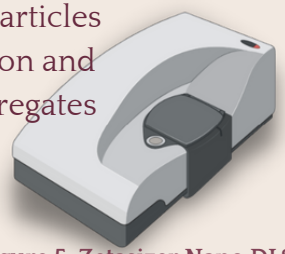


Figure 5. Zetasizer Nano DLS Instrument

EXPERIMENTAL RESULTS

SAMPLE PREPARATION

Stock concentration determined to be 20.6 mg/mL by drying 100 µL of ZnO suspension and monitoring its weight for multiple days.

ZnO nanoparticle solution was **diluted 40x** :

25 µL of stock suspension + 975 µL of MilliQ H20

ZnO nanoparticles have a tendency to form large clusters over time upon exposure to light and heat. These **aggregates** risk to clog the HTMX sprayer, resulting in costly damage.

To prevent this, ZnO nanoparticle solutions were filtered prior to spraying.

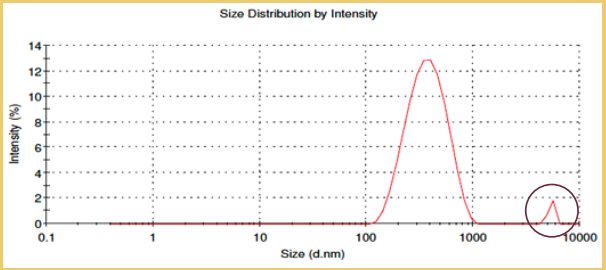


Figure 6. Size distribution by intensity of non-filtered ZnO nanoparticles, aggregates circled

Only 1 mL of solution per filter in order to prevent accumulation of aggregates on film, affecting recovery and reproducibility.

Three filter pore sizes, **0.45 µm, 0.65 µm and 1.0 µm**, were assessed based on nanoparticle retention, background signal, reproducibility and ability to sift out large aggregates.

Comparison of **calibration curve data** from UV-Vis testing for non-filtered ZnO and filtered ZnO solutions allowed for the calculation of % recovery.

FILTER PROPERTIES	RESULTS
IC Millex, Hydrophilic PTFE Membrane **0.45 µm,** syringe filter (25 mm)	• **Average np size = 135.7 d.nm** • **Percent np recovery = 4%** • Minimal background signal from filter material • Consistent size distribution without aggregates but low UV absorbance
Millipore Mixed Cellulose Esters Membrane **0.65 µm,** vacuum filter (25 mm)	• **Average np size = 270.1 d.nm** • **Percent np recovery = 85%** • Significant background signal but no signal overlap with analytes • Inconvenient method with risk of procedural error • Consistent size distribution without aggregates
Cytvia Whatman Hydrophobic PTFE Membrane **I.0 µm,** syringe filter (I3 mm)	• **Average np size = 243.5 d.nm** • **Percent np recovery = 92%** • Minimal background signal from filter material • Inconsistent filtrate colours and np size distribution despite consistent preparation

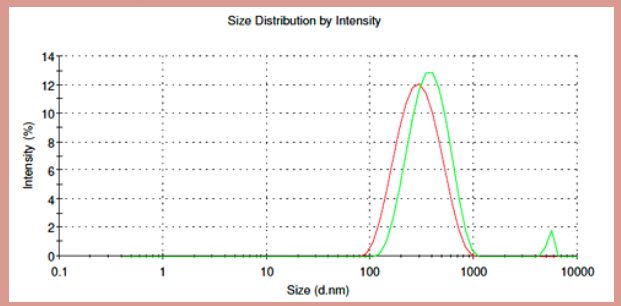


Figure 7. Size distribution by intensity of non-filtered ZnO nanoparticles (light green) vs 0.65 µm filtered ZnO nanoparticles (red)

SAMPLE CONCENTRATION

Matrix concentration previously optimized at 1 mg/mL dissolved in 50% ACN for non-filtered ZnO nanoparticles.

Matrix concentration of filtered nanoparticles found to be optimal between **2-5 mg/mL** through performance of numerous spot tests while observing signal intensity changes of known neurochemical standard.

NUMBER OF PASSES & SIGNAL STRENGTH

HTX TM Sprayer Settings

- **Solvent :** 50% ACN
- **Pressure :** 10 psi
- **Flow Rate:** 0.05 mL/min
- **Temperature:** 65°C
- **Matrix Conc.:** 5 mg/mL
- **Velocity:** 1200 mm/min

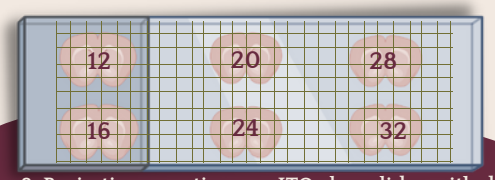


Figure 8. Brain tissue sections on ITO glass slides with digits corresponding to number of passes of matrix coated on tissue and grid showing nozzle pathway with 3 mm spacing

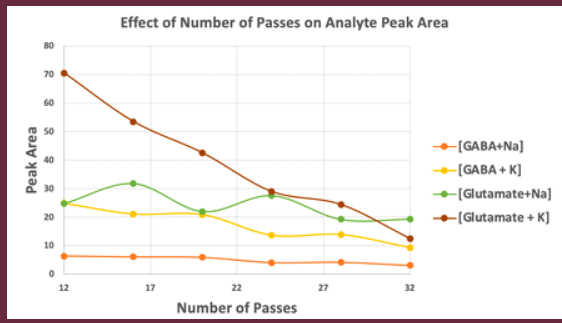


Figure 9. The effect of the number of passes of 5 mg/mL ZnO on various analyte signals

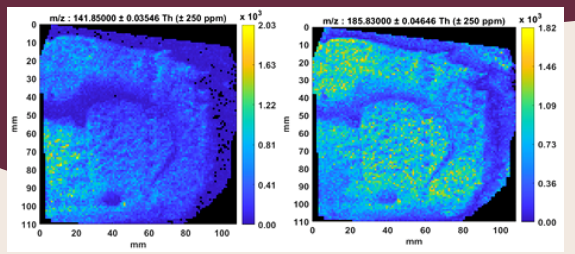


Figure 10. Heat maps showing regions of high intensity for [GABA + K] (left) and [Glutamate + K] (right) for 5 mg/mL of ZnO and 12 passes

FUTURE WORK

More testing to be done in order to further optimize ZnO for concentrations < 5 mg/mL to ensure reproducibility with sprayer and apply developed methodology to answer biochemical research questions.