Development of a disposable cartridge with integrated antibody for protein detection by paper spray mass spectrometry

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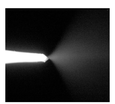
Overview

- A novel antibody cartridge performs extraction, preconcentration, and sample ionization.
- Selective enrichment of target protein from larger sample volumes and removal of the matrix from complex samples such as raw urine.
- Significantly improved the detection limits for the protein analysis by using carbon nanotube (CNT) coated/ dispersed paper/ polyethylene spray substrates.
- Detection limit of cytochrome c in raw urine was lower to 10 ng/mL, a linear calibration curve for cytochrome c from the LOD to 20 μg/mL.

Introduction

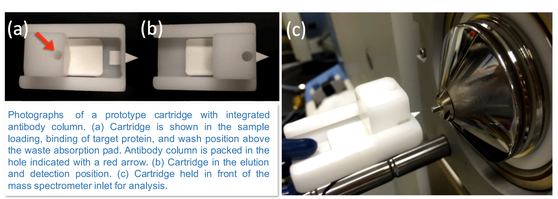
- Paper spray mass spectrometry is a method for performing rapid, direct analysis of samples spotted on paper or other porous substrate.
- Targeted quantitative analysis of drugs and drug metabolites directly from dried biofluids.
- Paper spray simplify and expand the utility of mass spectrometric assays.
- For protein targets, paper spray method shown very poor detection limits (around 1 μg/mL for relatively small proteins in neat solution).
- Simply increasing the sample amount does not significantly increase signal intensity or improve detection limits for direct paper spray analysis.
- Improving detection limits requires matrix removal and/or concentration of the analyte.



Picture of cone-jet generated from paper

Methods

- The cartridges were made from Delrin® plastic on a milling machine.
- MS analysis was performed using Thermo Scientific Q-Exactive Focus mass spectrometer.
- The cartridge consisted of two parts, a bottom part (LWH: 40mm x 26mm x 6mm) and a top part (LWH: 14mm x 22mm x 13mm) can be assembled together using a tongue and groove.
- The antibody was coupled to GE protein G mag sepharose (magnetic beads) prior to use.
- Porous polyethylene: pore size 7~12 μm
- Carbon nanotubes: single-walled, 0.7~1.3 nm diameter, 0.45~2.3 μm length.

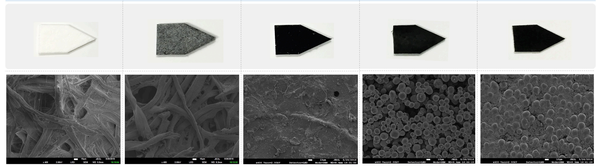


Photographs of a prototype cartridge with integrated antibody column. (a) Cartridge is shown in the sample loading, binding of target protein, and wash position above the waste absorption pad. Antibody column is packed in the hole indicated with a red arrow. (b) Cartridge in the elution and detection position. (c) Cartridge held in front of the mass spectrometer inlet for analysis.

Results

Spray substrates

Chromatography Paper			Porous Polyethylene	
Untreated	CNT-dispersed	CNT-coated	CNT-dispersed	CNT-coated



Photographs and SEM images (×400) of spray substrates

Procedure

CNT-coated: ~100 μL CNT slurry (carbon nanotubes dispersed in methanol, 1 mg/mL) was added onto the upper surface of spray substrates. Allow to dry, and then polish the surface to remove large CNT particles.

CNT-dispersed: the spray substrates were immerged into CNT slurry (carbon nanotubes dispersed in methanol, 1 mg/mL). Sonication treatment for 30 min, and then allow to dry.

Limits of detection

Limits of detection for three proteins by paper spray using paper and polyethylene tips.

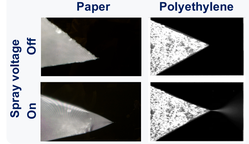
Spray substrates	Cytochrome c LOD (μg/mL)	Myoglobin LOD (μg/mL)	Lysozyme LOD (ng/mL)
Untreated 31ET paper	3	5	100
CNT-dispersed paper	1	0.8	10
CNT-coated paper	0.5	1	100
CNT-dispersed polyethylene	0.05	0.2	0.3
CNT-coated polyethylene	0.01	0.1	0.1

By using the CNT treated polyethylene spray tip, the LODs of all three proteins improved significantly.

Improved LODs might be attribute to:

- Smaller Taylor cone, higher ionization efficiency
- No binding effect between proteins and substrate
- Lower ion suppression from polyethylene substrate

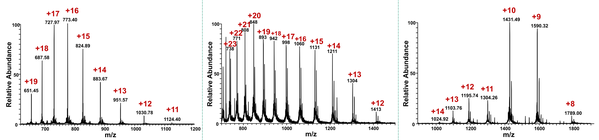
Picture of Taylor cone generated from:



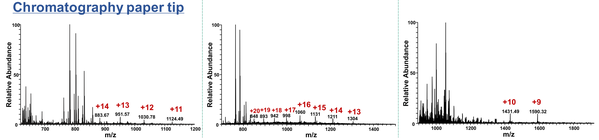
Mass spectra of proteins

Cytochrome C	Myoglobin	Lysozyme
MW: 12358 Da, 10μg/mL	MW:16950 Da, 10μg/mL	MW: 14336 Da, 0.1μg/mL

CNT-coated polyethylene tip *(Higher signal to noise ratio (S/N))*



Chromatography paper tip



Antibody cartridge

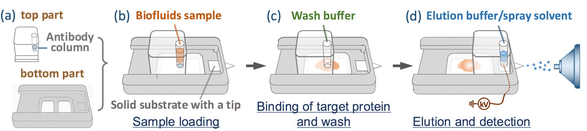


Diagram depicting the workflow for protein analysis with integrated on-cartridge antibody column.

cartridge

- **The bottom part** has two separate recessed regions to hold an absorbent waste pad and the spray substrate.
- **The top part** has a hole bored through it to contain the antibody column. Antibody was coupled to magnetic beads.

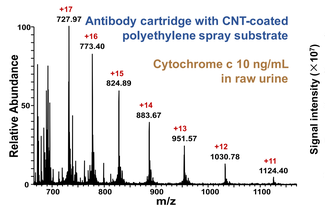
Procedure

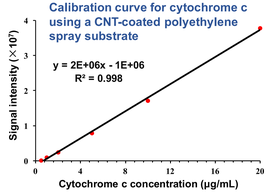
Sample loading: Up to several mL biofluid samples were added to the hole in the top part. The sample wicked through the antibody column and subsequently onto the absorbent pad.

Binding of target protein and wash: the target proteins were retained on the antibody column while the excess matrix was absorbed onto the waste pad. A wash step was performed to eliminate the matrix and PBS buffer residuals in the antibody column.

Elution and detection: The cartridge was positioned in front of the inlet to the mass spectrometer, and 30 μL elution buffer /spray solvent wicked through the SPE column, recovering the proteins in the process, and onto the spray substrate. Ionization occurs by inducing an electrospray at the sharp tip of the spray substrate near the MS inlet.

Quantitative analysis

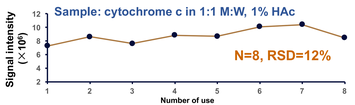




- **LOD of cytochrome c in urine was 10 ng/mL by using the antibody cartridge.**
- **Quantitative performance: linearity from the LOD to 20 μg/mL**

Reuse of CNT coated polyethylene spray substrate

A relative consistent signal intensity of protein sample could be obtained by reuse of a single CNT coated polyethylene spray substrate.



Conclusions

- The aim of this work was to develop a antibody cartridge so that protein extraction, pre-concentration, and ionization could be performed from a single device.
- Compared to chromatography paper substrate, the CNT treated polyethylene spray substrate showed significantly lower LODs for three tested proteins.
- Compared to direct paper spray, the integrated antibody approach:
  - improved the MS signal intensity and detection limits significantly.
  - have lower levels of ionization suppression.
- A 10 ng/mL detection limit of cytochrome c in raw urine could be obtained.

References

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