Development of a mass spectrometry cartridge for plasma protein analysis with integrated antibody column and spray substrate

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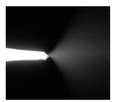
Overview

- 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 human plasma.
- Significantly improved the detection limits for the protein analysis by using carbon nanotube coated porous polyethylene (CNT-PE) spray substrates.
- Three applications of this cartridge will be described: (1) identification of apolipoprotein C1 (ApoC1) T45S variant, (2) detection of hemoglobin and glycated hemoglobin (HbA_{1c}), and (3) detection of transthyretin mutants from human plasma samples.

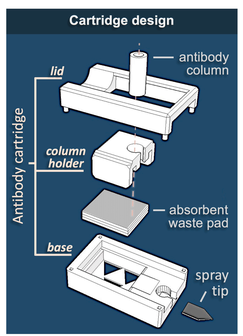
Introduction

- Clinical diagnostic tests of protein markers play a critical role in detection, diagnosis, and treatment of disease.
- Mass spec based assays could be routinely performed at or near the point of care, if its procedures could be dramatically simplified.
- To improve the ionization efficiency of target proteins, a carbon nanotubes coated porous polyethylene (CNT-PE) spray substrate was developed.
- Improving detection limits of proteins from complex samples requires matrix removal and/or concentration of the analyte.
- A 3D-printed mass spectrometry cartridge with an integrated antibody column was developed for the selective and sensitive detection of target proteins.

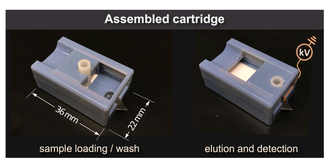


Picture of cone-jet generated from spray substrate

Methods



- A 3D printed antibody cartridge was developed.
- The cartridge consists of a lid, an antibody column, a column holder, and a base. All parts can be assembled together. (LWH: 36mm x 22mm x 15mm)
- Antibody were coupled to carboxyl latex beads and then coated to a glass fiber membrane.
- Mass spectrometer: Thermo Scientific Q-Exactive Focus
- Carbon nanotubes coated porous polyethylene spray tip.



Results

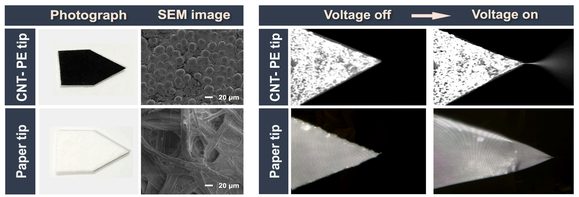
Spray substrates

Paper tip: untreated chromatography paper

CNT-PE tip: CNT-coated porous polyethylene

Taylor cone

The Taylor cone generated from the CNT-PE tip was significantly smaller compared to paper tip.



Detection limits of proteins

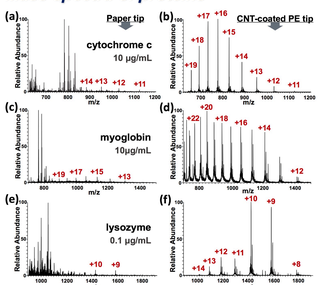
CNT-PE substrate vs Paper tip

- lower detection limits
- higher signal intensities
- better signal/noise ratios

Limits of detection for three proteins by paper spray using paper and CNT-PE tips.

Proteins	Limits of detection (μg/mL)	
	Paper tip	CNT-PE tip
Cytochrome c	3	0.01
Myoglobin	5	0.1
Lysozyme	0.1	0.0001

Mass spectra of proteins



Procedure of the antibody cartridge

Procedure

Sample loading / wash: 200 μL diluted human plasma sample was added to the antibody column. the target proteins were retained on the antibody column while the excess matrix was absorbed onto the absorbent waste pad. A wash step was performed to eliminate the matrix and PBS buffer residuals from the antibody column.

Elution and detection: The cartridge was positioned in front of the inlet to the mass spectrometer. 80 μL elution buffer /spray solvent was added to the antibody column, recovering the proteins in the elution process, and then onto the spray substrate. Ionization occurs by inducing an electrospray at the sharp tip of the spray substrate near the MS inlet.

Workflow of the antibody cartridge

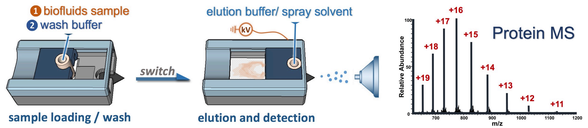


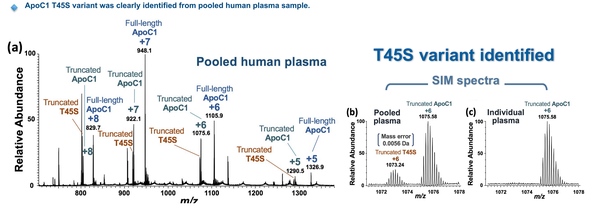
Diagram depicting the workflow for protein analysis with integrated on-cartridge antibody column.

Applications of antibody cartridge to clinically significant problems

I. identification of T45S variant of apolipoprotein C1

Apolipoprotein C1 (ApoC1) T45S variant, encoded by ApoC1 S45 allele, is a naturally occurring amino acid polymorphism that is present in aboriginal North Americans. ApoC1 S45 allele was associated with elevated body mass index with possible extension to diabetes in American Indian or Mexican ancestry.

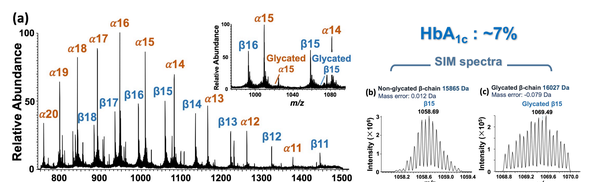
- ApoC1 T45S variant was clearly identified from pooled human plasma sample.



II. relative quantitative of hemoglobin A1C

Relative quantitation of a particular hemoglobin fraction, HbA_{1c}, which consists primarily of Hb in which the N-terminal valine residue of the beta chain has been glycosylated, has been used as a marker of glycemia control in diabetes for decades.

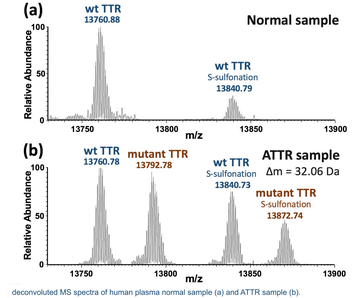
- Hb and glycosylated Hb could be quickly detected from human plasma without performing hemolysis.
- The relative intensity of glycosylated Hb is ~7% compared to unglycated Hb, detected from a nondiabetic individual.



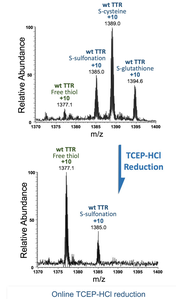
III. identification of sequence variants of transthyretin

Transthyretin (TTR) is a 55kDa homotetramer protein that transports thyroxine and retinol in blood and cerebrospinal fluid. Misfolding and aggregation of serum wild-type (wt) and mutant transthyretin protein (TTR) is the cause of Transthyretin Amyloidosis (ATTR), a severe and fatal disease characterized by abnormal deposits of TTR protein in the form of amyloid in the body's organs and tissues.

- different TTR mutants were clearly identified from clinical human plasma samples.
- online reduction reduced the posttranslational modifications for both TTR and mutant.



deconvoluted MS spectra of human plasma normal sample (a) and ATTR sample (b).



Online TCEP-HCl reduction

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.
- Applications of this cartridge to clinically significant problems:
  - identification of T45S variant of apolipoprotein C1;
  - relative quantitative of hemoglobin A1C;
  - identification of sequence variants of transthyretin.

References

Zhang, C.; Manicke N.E. *Anal. Chem.*, **2015**, *87*, 6212–6219

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