Asymmetrical Flow Field-Flow Fractionation coupled with ICP-MS for multi element analysis of plasma proteins.

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Introduction

Albumin and transferring are two main plasma proteins that are known to bind and transfer metal ions in the biological fluids. Metal-protein complexes are involved in many health studies such as metal-based drugs for cancer or diabetes treatments [1,2]. Chromatography techniques hyphenated to ICP-MS and MALDI-TOF-MS have been used to investigate metal speciation and metal binding kinetics in plasma and serum proteins [1,2]. Field-Flow Fractionation (FFF) is a chromatography like technique that uses an external field for separation. In FFF the separation takes place in an open channel (no stationary phase). As a result of sample-field Interaction, different sample species (different size classes) form different sample zones which are swept out of the channel at different speeds. The order of separation in FFF is from small to large. The main advantages of FFF over other chromatography techniques are higher sample recovery and being able to use non-denaturing and less destructive carriers. In this study Asymmetrical Flow FFF (AF4) was used to separate mixture of human serum albumin and transferrin. The FFF system was interfaced with ICP-MS to obtain metals distributions in the protein mixture. The results demonstrated that AF4-ICP-MS is a non-destructive and element selective detection technique for plasma proteins such as serum albumin and transferrin. This is mostly important for applications in which the two plasma proteins are acting as binders of the same element (Vanadium in diabetes studies).

Separation Principle

- Application of various separation fields
- Particles are forced towards channel bottom (accumulation wall)
- Laminar flow (parabolic flow profile) inside the channel
- Diffusion of particles leads to arrangement in layers (different flow velocity)

Separation according to molar mass and size: Flow FFF (AF4)
- and chemical composition (Thermal FFF), TF3
- and density (Centrifugal and Gravitational FFF),CF3 and GF3

FFF-Techniques - A schematic overview

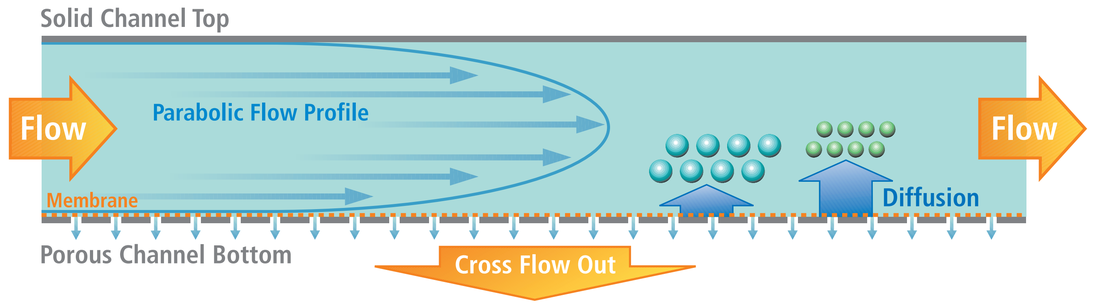
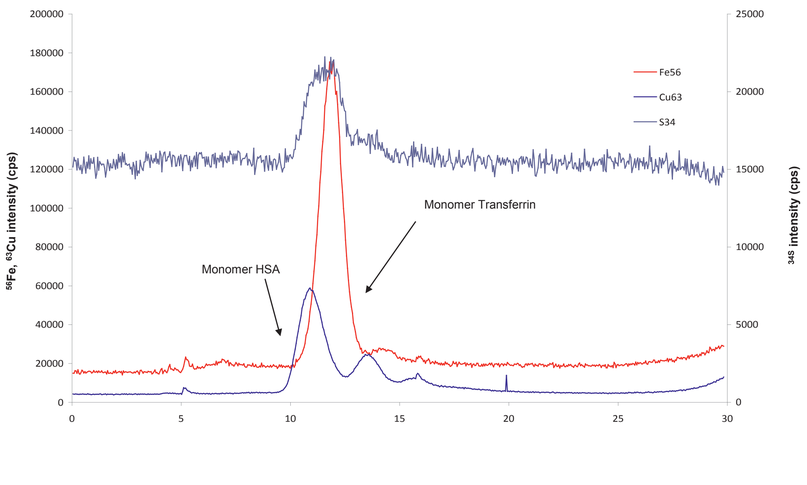


Figure 1: Flow Field-Flow Fractionation (AF4) channel cross section

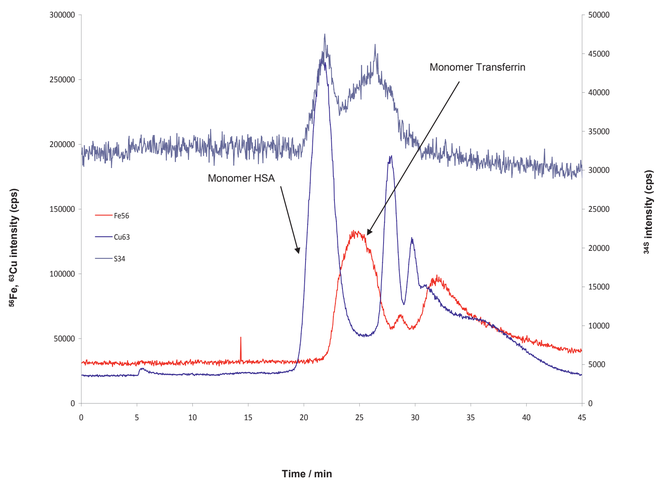
Parameters to choose from: flow rate, separation force and gradient, temperature, focus & outlet splitting technology, fraction collection, pre-purification and up-concentration.

AF4 ICP-MS Size Determination, Distribution and Inorganic Species Identification and Quantification

Inorganic and Metal Content Determination in Biological Samples Can Provide Quantitative and Forensic Information Concerning the History and Condition of the Protein.



FFF-ICP-MS fractograms obtained for mixtures of HSA and transferrin 1 g L-1 of each protein, 350 micron channel spacer



Increased Separation 2.5 g L-1 of each protein, 500 micron channel spacer. The spacer will improve the resolution but will also increase the run time

Postnova Analytics AF2000 MT AF4

Injection volume	21.8 µL
Buffer / eluent	100 mM ammonium acetate, pH7, filtered with Postnova 0.1µm pore size filter
Channel thickness	500 µm
Detector flow rate	0.5 mL/min
Focus time	4 min
Focus flow rate during Injection-focusing step	4.3 mL/min
Carrier flow rate during injection-focusing step	0.2 mL/min
Transition time focuselution	0.2 min
Cross flow	4 mL/min constant for 21 min; linear decay in 5 min to 0.07 mL/min; 0.07 mL/min constant for 5 min; no cross flow for 5 min

Agilent 7700x ICP-MS

RF power	1500 W
Sampling depth	7.2 mm
Carrier gas flow rate	12 L/min
Dilution gas flow rate	Off
Sampler / Skimmer cones	Ni/Ni
Nebulizer	MicroMist
Spray chamber temperature	2°C
Helium cell gas flow rate	9 mL/min
Monitored isotopes and integration time	$^{34}S^{a}$, $^{56}Fe^{b}$, $^{57}Fe^{a}$, $^{63}Cu^{a}$, $^{65}Cu^{a}$, $^{66}Zn^{a}$ [a] = 0.5 sec / mass; [b] = 0.1 sec / mass

Agilent G1315B UV DAD

Monitored wavelength	280 ± 50 nm

Field-Flow Fractionation ICP-MS Benefits

- Determination of molecular weight parameters giving information about plasma protein properties (which metals are present during medication or the source of nucleation of protein aggregates during processing)
- Identification of metal uptake in plasma enables individual patient physiology
- Investigation and optimization of therapeutic protein and antibody production
- Applicable for detection of protein contamination
- Quality control of therapeutic protein and antibodies

Conclusions

This work demonstrates that FFF-ICP-MS is a promising tool for the non-denaturing and element-selective detection of proteins such as human albumin and transferrin, which are involved in many health studies. This is particularly attractive for applications in which the two plasma proteins act as binders of the same element (e.g. vanadium in Diabetes studies). The further improvement in selectivity and multi-elemental capability offered by ICP-MS detection guarantee the future application of FFF-ICP-MS to the S/metal speciation of more complex mixtures of metal-containing proteins in biological samples. The simultaneous on-line combination of this hyphenated technique to UV-Vis/MALS offers a simple and straightforward approach to tentative verification of protein identity, which is particularly useful for routine bio-measurements.