Separation and Speciation of Nanoparticles with different Field-Flow Fractionation Techniques

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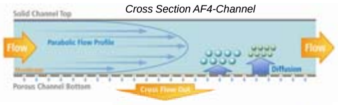
Introduction

In recent times the nanotechnology has developed to an important topic in nearly all areas of science. The change of the scientific interests towards particular material in nanoscale goes hand in hand with a new view on many scientific problems. There is a huge influence of the particle size and shape on the later properties of nanomaterials. In environmental science it is already known that particular and polymeric structures are involved in a huge variety of processes [1-3]. In addition, the boost of nanotechnology and the resulting application in many products are accompanied by the accumulation of such species in the environment. A very miscellaneous analytical technique is the Field-Flow Fractionation (FFF) which became the most important tool for nanoparticle and polymer separation in the last few years. In FFF, the separation is realized without a stationary phase inside an empty channel by an external separation field [4]. The Asymmetric Flow FFF (AF4) and the Centrifugal FFF (CF3) are the most important sub-techniques for particles separation. In AF4, a cross flow field is used which offers a separation according to the diffusion coefficient of the analyte, which is related to the Hydrodynamic Diameter (D_h) [5]. In CF3 a centrifugal field is used which enables to separate structures with the same Hydrodynamic Volume according to differences in density [6].

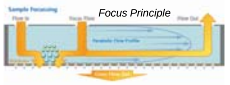
Asymmetric Flow FFF (AF4)

- Cross-Flow field for separation
- Particles are forced towards channel bottom (accumulation wall)
- Laminar flow with parabolic flow profile inside the channel
- Diffusion of particles leads to arrangement in layers of different flow velocity
  → Separation according to **Hydrodynamic Diameter** ($D \sim 1/R_h$)
- Cross-Flow gradient of any shape for „tailor made" separation
- Focus + Slot Technology for concentration of the sample prior and during separation

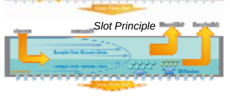
Cross Section AF4-Channel



Focus Principle



Slot Principle



Centrifugal FFF (CF3)

- Centrifugal field for separation (up to 4000 rpm)
- Centrifugal force pushes molecules towards channel bottom
- In addition: Diffusion of particles leads to arrangement in layers of different flow velocity
  → Separation according to **Hydrodynamic Diameter** ($D \sim 1/R_h$)
  AND according to **differences in Density**
  - Centrifugal gradient of any shape for „tailor made" separation

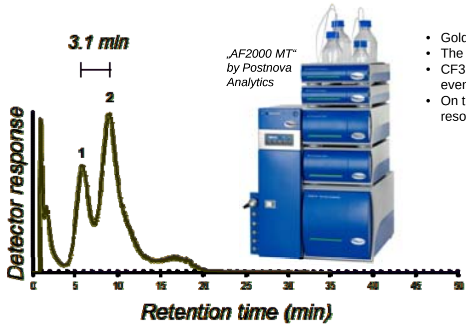
Cross Section CF3-Channel

Comparison of AF4 and CF3

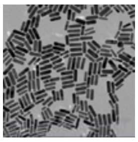
Separation of Gold Rods with different Shape

- Gold rods of different size were separated with both FFF methods and UV detection.
- The results show that both methods can be used for successful Gold particle separation.
- CF3 offers a better resolution for high density particles and the possibility to separate even particles of the same hydrodynamic diameter but different density.
- On the contrary AF4 separates strictly according to hydrodynamic size and offers better resolution for low density particles.

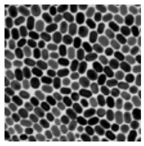
Asymmetric Flow FFF



„AF2000 MT" by Postnova Analytics



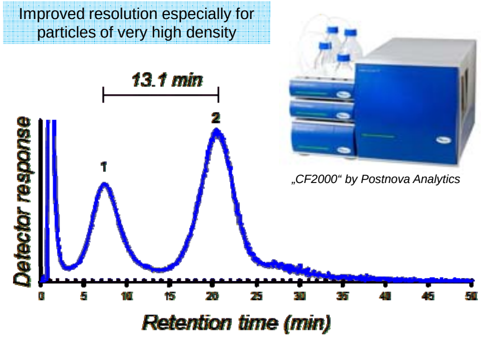
Peak 1 Axial = 10 nm, Legth = 35 nm



Peak 2 Axial = 25 nm, Legth = 35 nm

Centrifugal FFF

Improved resolution especially for particles of very high density



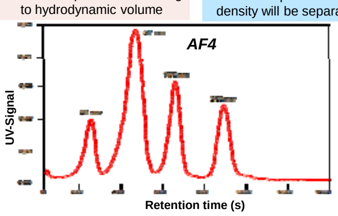
„CF2000" by Postnova Analytics

Validation of FFF methods

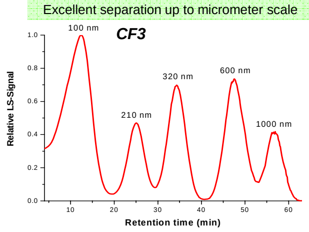
Separation of Latex Particles with defined Size

Universal separation according to hydrodynamic volume

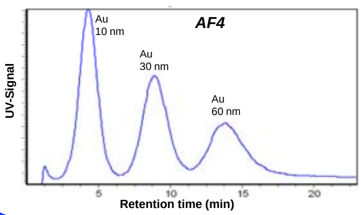
Also small species with low density will be separated

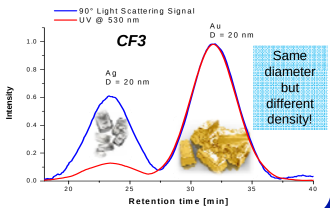


Excellent separation up to micrometer scale



Separation of a Mixture of NIST Metal-Standards

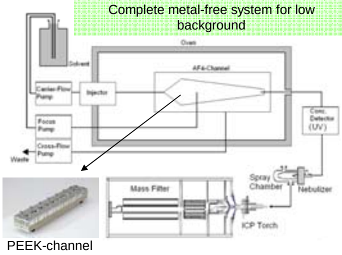




Same diameter but different density!

AF4-ICP-MS - Setup

Complete metal-free system for low background



PEEK-channel

Flow-Scheme of FFF-ICP-MS-Hyphenation

Analysis of Gold Standards with AF4-ICP-MS

- Flow rates in FFF are in the optimal range which is required for ICP-MS-Hyphenation (0.3 – 0.7 mL/min)
- Easy connection by PEEK capillary
- Addition of internal standard possible by T-piece

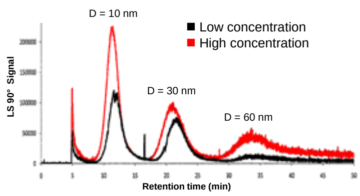


Figure:

Mixture of three NIST gold standards separated with AF4. Low and high concentrations were detected with ICP-MS

AF4-ICP-MS Applications

Quantification and Speciation of different Elements in Bovine Serum Albumin (BSA):

- *BSA was separated by AF4-UV-ICP-MS*
- *Different Elements are associated with the protein and can be detected by ICP-MS*
- *Even sulfur content can be analyzed*

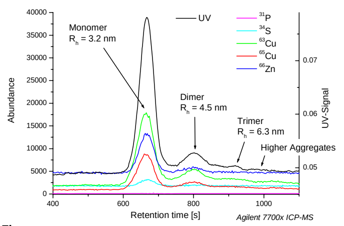


Figure:

Multi Element-based fractogram (counts versus time) of Bovine Serum Albumin (BSA) obtained from Asymmetrical Flow FFF-ICP-MS system. The UV response (black line) represents the mass concentration of the BSA species. The profiles of ^{66}Zn, ^{65}Cu, ^{63}Cu and ^{34}S follow that of UV, indicating the association of these elements with the protein structure. No BSA-bound Phosphorus was detected.

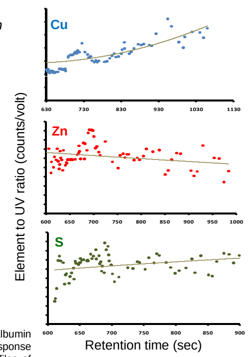


Figure :

Element to UV response ratio versus retention time for Cu, Zn and S. The line in each graph is the best regression fit to the data points. The ratio of each element to UV response represents the amount of the element per BSA mass. There was a significant increase in the copper ratio indicating specific association of copper with the aggregated BSA species. However the ratios of zinc and sulfur did not show a significant change indicating that there was no selective interaction between Zn/S and BSA aggregates.

Analysis of Nanoparticles in River and Brackish Water Samples:

- *Different samples of natural and brackish water were analyzed by AF4-UV-ICP-MS*
- *The traces of Zn, Fe and Al were detected with the aim to visualize the size-dependent adsorption of the elements*
- *The sorption of Al can be correlated with change of salinity*
- *Cationic aluminum-hydroxide ions are toxic for fish, e.g. salmon*
- *AF4-ICP-MS offers possibility to track the sorption of Al on the particle surface for different salinities or pH*
- *Retention-time was transferred into size information by calibration with standards of known size*
- *The Focus technology of AF4 allows to pre-concentrate the highly diluted water samples → Injection volumes of 1 mL and above will not lead to additional peak broadening*

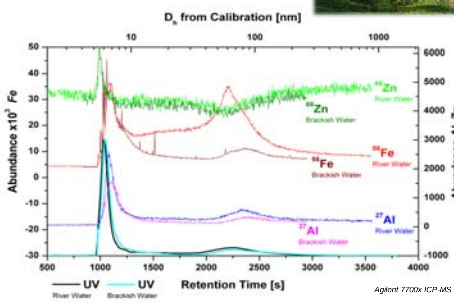


Figure:

The element traces indicate that the sorption tendency of the elements is highly related to the particle size. The UV-trace delivers information about all UV-active particular and macromolecular species and their concentration in the water samples. Two main particle fractions of low and high size are visible. ICP-MS proves a strong affinity of iron and aluminum to the larger particles. The high UV-intensity of the smaller material may be due to the presence of organic macromolecular species. A change of the salinity leads to a dramatic decrease of the concentration of adsorbed iron and aluminum on the particles above 50 nm. The change of iron with salinity indicates that the larger species are not from iron (e.g. hematite) while the peak at 8 nm does not change with the salt content which indicates solid iron particles.

Conclusions

The presented work illustrates the broad applicability of the different FFF-techniques for characterization of nanomaterial from different origin in various matrices. A hyphenation with numerous detectors and spectrometers like e.g. Multi Angle Light Scattering (MALS), Fluorescence or ICP-MS is uncomplicated. The obtained results offer very detailed information about the mostly very complex nanosamples. Challenging studies of complicate environmental migration processes, new knowledge about the aggregation and complexation behavior of proteins and, of course, a high resolution size separation can be realized by FFF. Until now, there are no alternative methods which would offer similar possibilities. As a consequence, FFFF is more and more developing to be the most frequently used analytical technique for separation and characterization of particles and macromolecules.

Ref.

[1] R. Beckett in: *The Role of Particulate Matter in the Transport and Fate of Pollulants*, B. T. Hart (ed.), Water Studies Centre, Chrisholm Institute of Technology, Melburne, Australia, 1986.
[2] W. Stumm, *Chemistry of the Solid-Water Interface*, Wiley (ed.), New York, 1992.
[3] B. Stolpe, L. Guo, A. M. Shiller, M. Hassellöv, *Mar Chem*, 2010, 118, 119-128.
[4] J. C. Giddings, *Sep Sci*, 1966, 1, 123-125.
[5] K. G. Wahlund, J. C. Giddings, *Anal. Chem.* 1987, 59, 1332.
[6] B. N. Barmann, J. C. Giddings, *Anal. Chem.* 1995, 67, 3861.