Field-Flow Fractionation – A powerful Equipment for Investigation of the toxic Potential of Nanoparticles

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Introduction

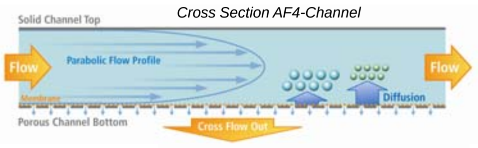
In recent times, small material in nano-scale is very frequently used in numerous products. Beside the huge variety of advantages there are also more and more concerns about potential toxicity of small species which have the ability to enter different regions of the human body. E.g., the decreased size of silver nanoparticles leads to a large active surface with a correspondingly strong ion release which is known to be antibacterial. Unfortunately, nano-silver can be accumulated in the human tissue. In addition, such small particles are able to conquer the barrier between the blood and the brain or the placenta. Silver is suspected to damage genetic material [1]. Animal experiments have shown that silver can have toxic effects on liver [2], lung and nerve cells [3]. For this reason an exact knowledge about the size, the size-distribution, the shape and the composition of nanoparticles is essential to appaise the toxicity. Despite the actuality of the topic there are only a few methods for the characterization of nanomaterial available. A size separation prior the detection is essential for the correct analysis because nanoparticles can be very heterogeneous systems, containing various species which differ in size and shape. Moreover, the analytes are mostly accompanied by complex matrices. This additionally complicates the characterization process.

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]. Moreover, the hyphenation with element specific detectors, like e.g. ICP-MS, allows the determination of the element concentration and ratio across the size distribution.

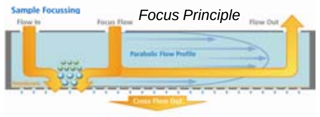
Asymmetric Flow FFF (AF4)

- 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

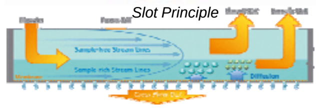
Cross Section AF4-Channel



Focus Principle

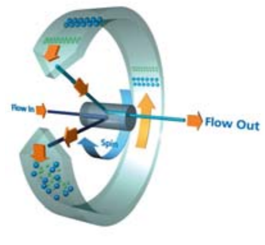


Slot Principle

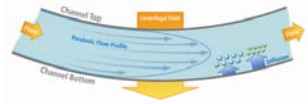


Centrifugal FFF (CF3)

- 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



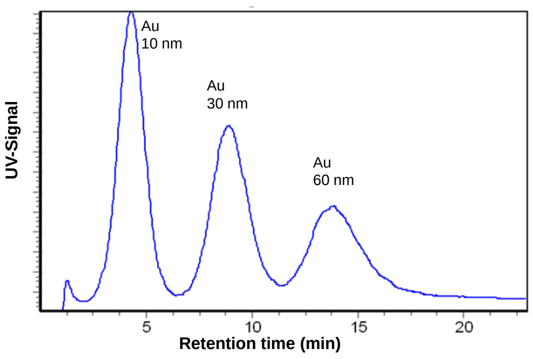
Cross Section CF3-Channel



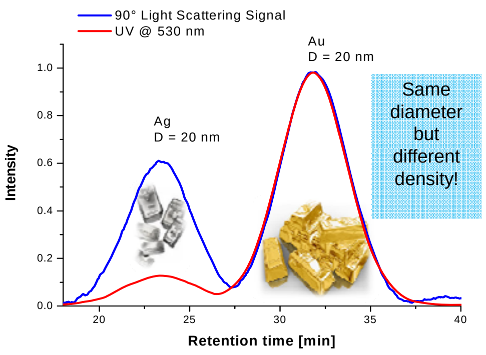
Separation, Quantification and Speciation of Nanoparticles

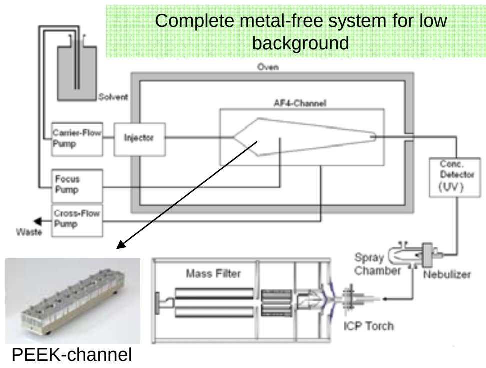
Characterization of a Mixture of NIST Metal-*Standards with different Methods*

AF4 – Size Separation



CF3 – Separation according to Size and Density





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

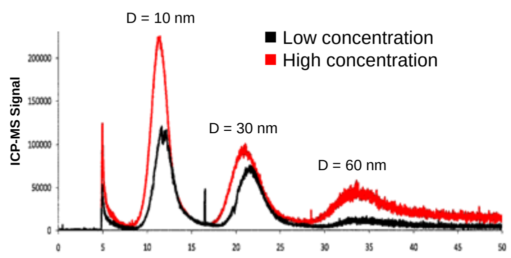


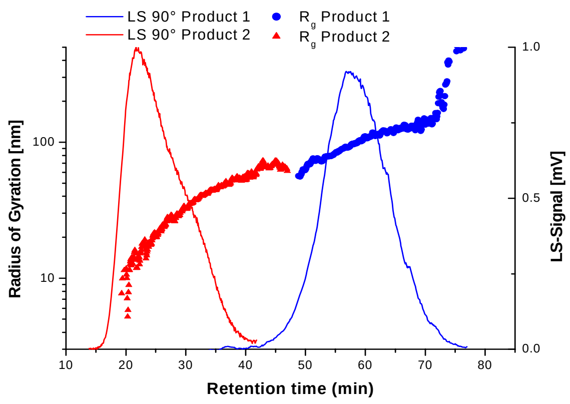
Figure:

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

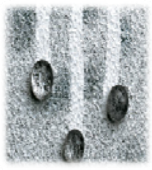
Nano Science in Consumer Products

Particles in Window Cleaner separated by AF4

- *Nanoparticle coating on glass surface avoids permanent attachment of dust/dirt (Lotus-Effect)*
- *In this example the knowledge of the exact particle size is essential for the product characteristics and potential harms*



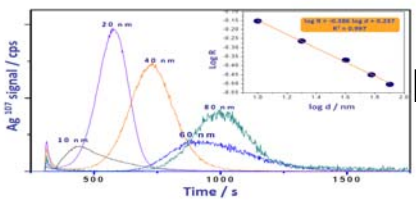
Polydisperse particle distributions demonstrate the separation power of AF4 over a wide size range!



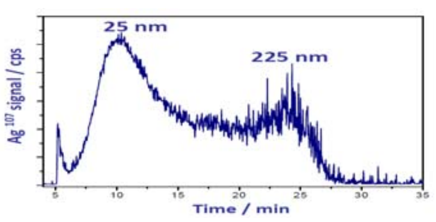
Silver Nanoparticles in commercial Desinfectant Spray

(Data kindly provided by Dr. Francisco Laborda, University of Zaragoza, Pedro Cerbuna 12, 50009 Zaragoza/Spain)

- *The toxicity of particulate silver depends on the size*
- *AF4-ICP-MS offers now the possibility to combine the size information with speciation and quantification of different elements*
- *If the particle size is too low a migration in the human body is possible*



Calibration curve for used AF4 cross-flow gradient – Separation of Ag standards with known diameter



Nanoparticles and nerve cell (Source: Kopp online 2009)

Nanoparticles in Environment

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*

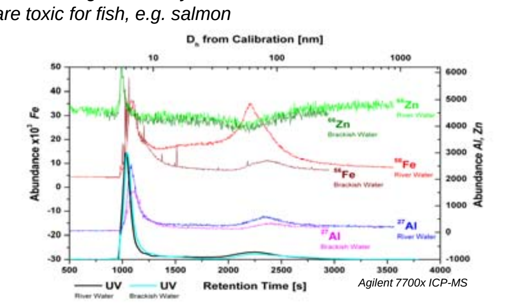


Figure 1:
Visualization of variable adsorption in dependence on salinity of the aqueous environment
Large Species not from Hematite (Fe_2O_3), iron only adsorbed on surface.
More free (toxic) Al in brackish water

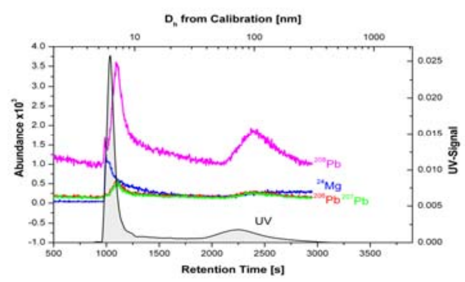
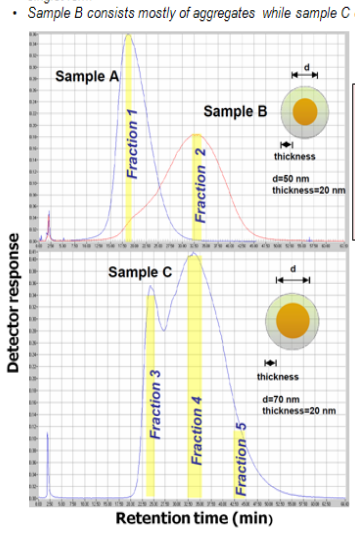


Figure 2:
High lead content in both particle fractions of the water sample
Toxicity may be increased by accumulation of lead on surface of nanoparticles

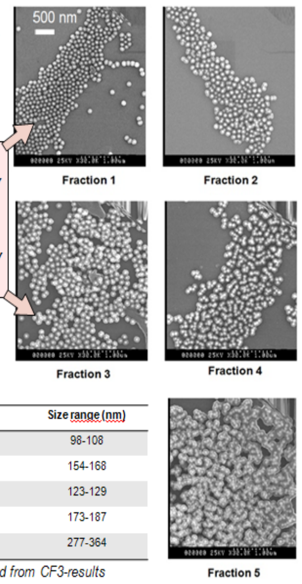
Separation of synthetic Nanoparticles with Centrifugal FFF

Gold Nano-Particles with Silica Coating

- Fractograms obtained by CF3 with UV detection
- Sample A is more mono-dispersed with the majority of the particles in the singlet form
- Sample B consists mostly of aggregates while sample C contains both



Electron Microscopy confirms the results obtained by CF3!



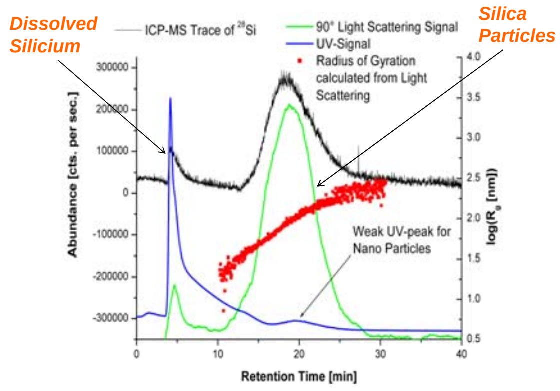
Fraction	Size range (nm)
1	98-108
2	154-188
3	123-129
4	173-187
5	277-364

Size calculated from CF3-results

Silica Particles as Drug Carrier

In pharmaceutical science nanoparticles are more and more used in special applications. Silica nanoparticles are used e.g. as oral drug delivery carrier [7-8] or for modification of labeling agents [9-10].
A very important point of interest in this context is the exact knowledge about the size and size distribution of the used nano-material. In the displayed example synthetic silica particles were separated with AF4 connected to UV, Light Scattering and ICP-MS detection.

- *Dissolved silicium as well as a broad distributed particle fraction of silica was identified in the sample*
- *Dissolved silicium has to be removed to provide pure particle material for medical use*



(Data kindly provided by Dr. Francesco Cubadda, Istituto Superiore di Sanità, Rome, Italy)

Conclusions

It was shown that FFF is the method of choice for the characterization of nanomaterial due to its unique capabilities like broad separation range, short analysis times and the huge flexibility regarding to different eluents or samples. The hyphenation with numerous detectors and spectrometers like e.g. Multi Angle Light Scattering (MALS), UV or ICP-MS is uncomplicated and offers additional information. The nano technology has already a fixed position in our daily life. Therefore everything should be done to ensure that maximum benefit can be gained from the new nanomaterial and unnecessary risks are avoided

Ref.

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