

Application Note

Wyatt Technology Corporation

Characterization of surface modified gold nanoparticles with asymmetrical-flow field-flow fractionation (AF4) coupled to ICP-MS

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Summary

Engineered Nanoparticles (ENPs) are coming into widespread use and so have become the subject of scientific interest in terms of possible toxic effects on humans and the environment. One key challenge is detection and identification of metallic ENPs in a complex fluid matrix.

In this study, a mixture of gold nanoparticles was characterized via Hollow-Fiber Flow Field-Fractionation (HF5) coupled to single-particle-inductively coupled plasma mass spectrometry (sp-ICP-MS). This approach is able to monitor changes of particle size distribution and recovery caused by variation of ionic strength in the carrier solution of the separation. The analysis yields valuable information on the particle size and the aggregation behavior of a suspension at different ionic strengths.

Thus, sp-ICP-MS complements direct sizing detectors like multi-angle light scattering (MALS) or dynamic light scattering (DLS) to analyze nanoparticles separated by FFF.



The Wyatt Technology Eclipse™ DualTec FFF system can be coupled with a DAWN® HELEOS® II MALS and Optilab® RI detectors to characterize particle size and aggregation.



I. Introduction

Evaluation of the possible toxic effects of engineered nanoparticles (ENP) on biological systems depends on characterization of their fate in the matrices in which they occur in the environment. Particles can change size by dissolution or aggregation, and may evolve so far that they cannot be considered 'nano' any more. This variability represents a challenge for standard analytical tools for size characterization. Additional challenges are 1) the need for particle suspensions to be analyzed in a matrix which is as close as possible to the real conditions in the environment (surface water, sea water, sewage sludge, etc.) and 2) at a low concentration representative of the environment, in the low ppb range.

In this study, we show that Flow-FFF coupled to inductively coupled plasma mass spectrometry (ICP-MS) is able to monitor changes of particle size distribution and recovery caused by variation of ionic strength in the carrier solution of the separation. Specifically, single-particle-ICP-MS (sp-ICP-MS) is used over a complete Flow-FFF separation experiment, demonstrating it to be an efficient tool to obtain size information where other methods (light scattering, size from retention time via calibration) cannot be applied.

II. Materials and Methods

A mix of two gold nanoparticles with a diameter of 15 nm (INM, Saarbrücken, Germany) and 60 nm (NIST 8013, Gaithersburg, Maryland, United States) was used. Both types of nanoparticles initially were citrate-reduced gold particles in aqueous solution (0.01 % HAuCl₄), of which the 15 nm particles were modified with (11-mercaptoundecyl)-tetra(ethylene glycol) covalently bonded by the thiol group to the particle surface. Three different carrier solutions for the FFF-separation were prepared: 0.02% sodium-dodecylsulfate (SDS), and the same SDS with the addition of 0.1% and 0.5% NaCl.

Instrumentation

Flow-FFF

A Wyatt Technology **Eclipse™ DualTec** equipped with a Hollow-Fiber channel (HF5) was coupled to the Thermo Scientific™ iCAP Qc™ (Fig. 1).

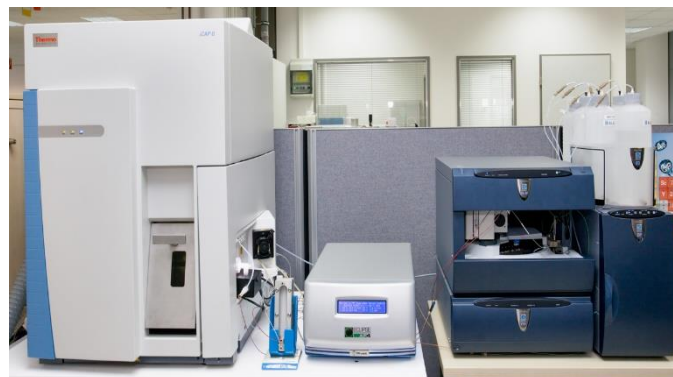


Figure 1: Wyatt Technology Eclipse coupled to a Thermo Scientific iCAP Qc.

Mobile phase was delivered to the Eclipse by means of a Thermo Scientific ICS-5000™. Injections were performed using the DS-DP ICS-5000 autosampler. The Eclipse splits and controls the inject flow and cross-flow using needle valves and flowmeters.

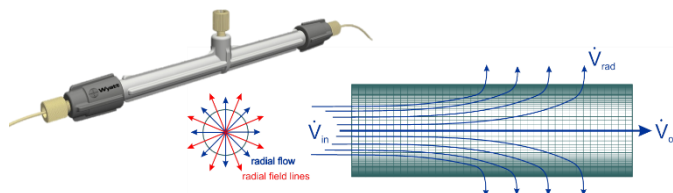


Figure 2: Hollow-Fiber Field-Flow Fractionation channel (HF5) from Wyatt Technology and principle of cross-flow generation in the cylindrical geometry.

Separation was achieved using a single hollow-fiber with 800 μm diameter made of polyethersulfone with 10 kDa cutoff and mounted in a specifically designed cartridge (Wyatt Technology), which creates a cylindrical channel shown in Figure 2. The channel has a 90 μl volume resulting in low sample dilution. It is disposable and therefore easy to replace at the end of its lifetime, which can reach 100 injections.

Appropriate flow conditions were chosen to result in baseline resolution of the two peaks. The method starts with two minutes of solvent to check the baseline signals. Then one minute of focus is followed by two minutes focus+inject. Elution included a decrease of the



cross-flow from 0.2 mL/min to 0.08 mL/min in 10 minutes, followed by 5 minutes elution without cross-flow. Then the inject line was activated to flush the injection flow path. Detector flow rate was 0.5 mL/min in all experiments.

ICP-MS and sp-ICP-MS

An iCAP Qc with PFA-ST nebulizer was used for all ICP-MS and sp-ICP-MS determinations. Dwell time was 100 ms for ICP-MS and 0.005 ms for sp-ISP-MS. For the sp-ICP-MS mode, the injected sample was diluted with a factor of approx. 1000.

Data Analysis

Thermo Scientific Qtegra™ Intelligent Scientific Data System software was used throughout for iCAP Q control and all data acquisition. Thermo Scientific Chromleon™ software was used to drive the Wyatt Eclipse and ICS-5000 in a single method. Wyatt Technology ISIS™ software was used to optimize the FFF separation conditions and to calculate theoretical retention time of the particles given their known size.

III. Results and Discussion

Figure 3 shows the fractogram in 0.02% SDS. It has four distinct peaks, the void peak with a retention time corresponding to the channel volume, the peak of the 15 nm Au particle, followed by the 60 nm particle, and a fourth peak eluting after activation of the flow through the injector at the end of the run.

Focus time was intentionally set to 2 minutes to generate a void peak. Doubling the focusing time reduces the void peak by a factor of 15 (Figure 4).

Retention time t_r calculated from FFF theory using ISIS software is 220 and 510 s for 15 nm and 60 nm particles, respectively. It cannot be predicted which sizes are contained in the void peak; this peak can consist of oversized particles from steric elution as well as small particles.

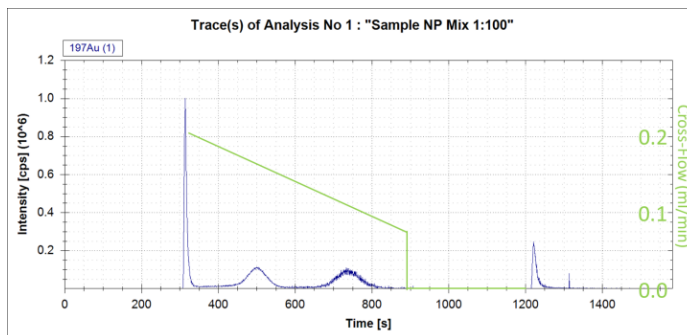


Figure 3: ^{197}Au trace showing fractionation of two nanoparticles with 15 nm and 60 nm diameter, focus time 2 minutes.

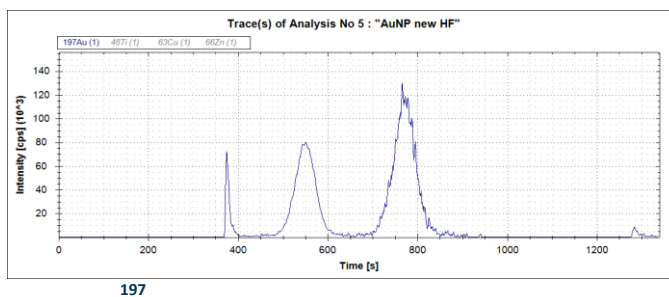


Figure 4: Au trace showing fractionation of two nanoparticles with 15 nm and 60 nm diameter, focus time 4 minutes.

It is well known that increase of ionic strength will destabilize the Au nanoparticle suspension. We tested the effect of adding 0.1% and 0.5% NaCl to the carrier solution. The 15 and 60 nm peaks were greatly diminished while the void peak, corresponding to aggregates, increased.

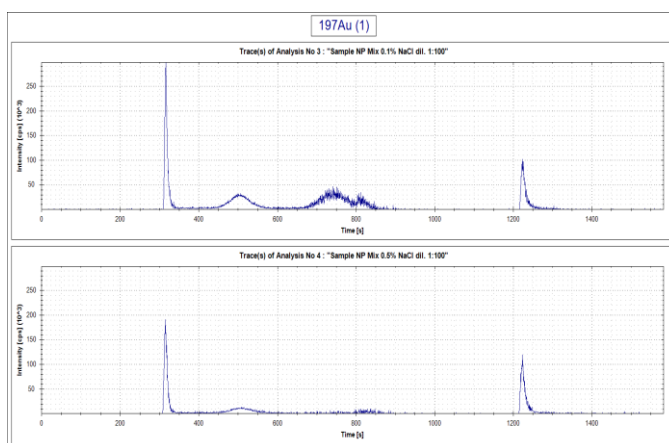


Figure 5: ^{197}Au traces of separation with 0.1% and 0.5% addition of NaCl to 0.02% SDS solution. The distinct peaks are diminished and the void peak increased.



sp-ICP-MS

In order to understand possible changes in particle size by increasing ionic strength, the sp-ICP-MS mode was used during the complete run time of the separation, including the void and the injector-rinse.

The sample load was reduced by a factor of 100 to reach a dilution high enough for single particle events.

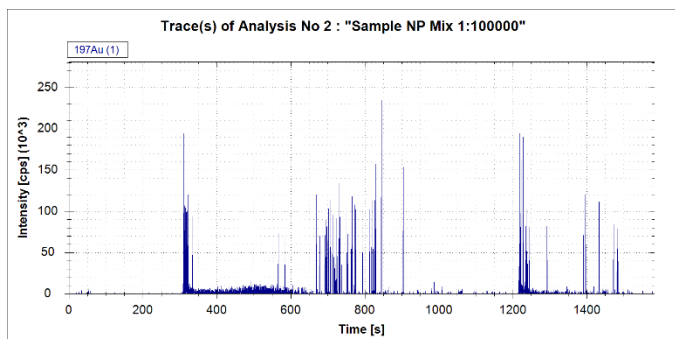


Figure 6: sp-ICP-MS data on separation of the two Au nanoparticles in 0.02% SDS.

The height of spikes can be used as an indication of the corresponding particle size. Assuming a spherical shape, we expect spikes from 15 nm particles to be 64x lower compared to those of the 60 nm particles, which are around 100k counts. Significant further dilution would be required to resolve single 15 nm particles and signal to noise would probably not be sufficient for reliable detection. We can conclude that 60 nm particles are present in the void peak plus oversized particles which must be aggregates.

The injection-rinse peak looks similar to the void peak. We note that it corresponds to a flow-injection measurement of the sample, because it comes from the injector through the channel without focusing and without cross-flow.

Figure 7 shows data on the experiment with addition of 0.5% NaCl. Recovery of 60 nm particles is so low that the sample does not need to be diluted, whereas 15 nm particles are still too concentrated to give single particle events. We see 100k count spikes between 600 and 800 sec, but also a few larger ones. Interestingly, there are larger spikes in the elution+inject peak compared to the void peak. Flow-injection into higher ionic strength conditions leads to aggregation, but not to absorption, because the particles are not concentrated at the membrane.

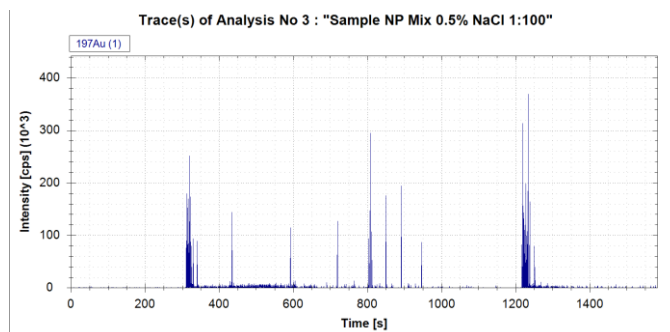


Figure 7: sp-ICP-MS data on separation of the two Au nanoparticles in 0.02% SDS with addition of 0.5% NaCl.

From the results it can be concluded that sample loss in higher ionic strength carrier solutions is caused by formation of aggregates and then absorption of those aggregates on the membrane or other surfaces. This is independent of the different surface coating of the two Au particles.

Retention time of the 60 nm Au particle is considerably smaller than predicted by theory, indicating non-ideal effects which are not influenced by the ionic strength. Therefore, sp-ICP-MS is a useful tool to provide additional size information where it cannot be obtained from FFF retention time, and can detect aggregates in the void and the release peaks.

IV. Conclusions

The results show potential of sp-ICP-MS as a detection mode coupled to Flow-FFF to generate size information, complementing direct sizing detectors such as MALS or DLS instruments. The detection limit depends on size and can vary dramatically with the metal of interest; in unfavorable cases the detection limit can be above the critical size of 100 nm. It is a challenge to find a suitable dilution range allowing detection over a wide range of sizes.

