# Characterization of Nanoparticles using ICP-MS: Advantages and Challenges For Nanoparticles in Food

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## Overview

**Purpose:** Demonstrate the potential of FFF-ICP-MS and sp-ICP-MS for the characterization of nanoparticles.

**Methods:** FFF was coupled to ICP-Q-MS, with liquid flows driven using an ICS-5000. For sp-ICP-MS, samples were directly aspirated.

**Results:** FFF was shown to separate Ag and Au nanoparticles based on their diameters. sp-ICP-MS was used to determine the average diameter of Ag nanoparticles.

## Introduction

The need for nanoparticle (NP) characterization has exploded in recent years due the ever increasing use of engineered nanoparticles (EN) in various industries and the consequent studies that investigate the environmental and consumer risk.

Of the methods developed with this goal in mind, Field Flow Fractionation (FFF) coupled to ICP-MS has proved to be one of the most promising. FFF has a separation principle based on the differing mobilities of different particle sizes in a laminar liquid flow contained in a channel between two plates. Smaller particles flow faster through the channel, enabling a separation based on size. FFF is compatible for particle sizes in the low nm to low µm range and is thus perfectly suited to NP separation.

Another promising approach for NP characterization is sp-ICP-MS. Through direct analysis of an appropriately diluted solution containing NPs, the NPs can be counted. If the NPs consist of just one element, peak height, proportional to the size of the NP can be used to determine the NP diameter.

Although both strategies benefit from the high sensitivity of ICP-MS detection, the single particle approach limit of particle size detection is actually governed by signal-to-noise ratio. The more sensitive an instrument, the smaller the particle it can detect.

## Methods

### Sample Preparation

In general NP standards were diluted in water and sonicated for 5 to 15 minutes just prior to analysis.

### Field Flow Fractionation

A Wyatt Technology™ Eclipse® equipped with a short channel (SC) was coupled to the Thermo Scientific™ iCAP Qc™ ICP-MS (Fig. 1). Mobile phase was delivered to the Eclipse chassis using a Thermo Scientific ICS-5000™ and injections were performed using the sample loop of the AS-AP ICS-5000 autosampler. The Eclipse chassis splits the flow appropriately with a series of specially configured valves.

**FIGURE 1. Wyatt Technology Eclipse AF4 with SC coupled to a Thermo Scientific iCAP Qc ICP-MS.**

The FFF membrane used and the separation parameters are shown in Table 1.

**TABLE 1. Field Flow Fractionation Conditions.**

| | Gold NPs | | Silver NPs | |
|---|---|---|---|---|
| **Membrane** | 10 kD RC* | | 10 kD RC* | |
| **Mobile Phase** | Water | | 0.5 mM Amm. Carbonate | |
| **Injection vol. (µL)** | 20 | | 20 | |
| **Detector flow (mL/min)** | 0.5 | | 1.0 | |
| **Focus flow (mL/min)** | 1.5 | | 1.0 | |
| **FFF protocol** | **Time** | **Vx** | **Time** | **Vx** |
| Elution | 1 | 0 | 2 | 0 |
| Inject | 1 | 0 | 1 | 0 |
| Focus and Inject | 5 | 0 | 2 | 0 |
| Focus | 1 | 0 | 5 | 0 |
| Elution | 5 | 1.5 | 40 | 1.0 |
| Elution | 15 | 1.5 | 5 | 0 |
| Elution | 5 | 0.5 | | |
| Elution and Inject | 10 | 0 | 5 | 0 |

*RC – Regenerated Cellulose

### ICP-MS

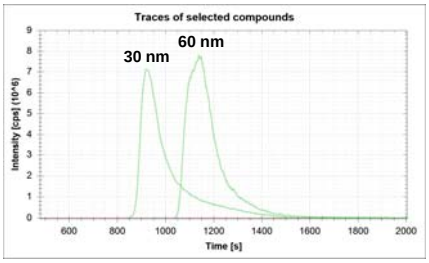
A Thermo Scientific iCAP Qc was used for all experiments. Thermo Scientific Qtegra™ software was used throughout for iCAP Q control and all data acquisition. Thermo Scientific Chromeleon™ software was used to drive the Wyatt Eclipse and ICS-5000 in a single method. ISIS™ (Intelligent Separation Improvement Software) was used to optimize the FFF separation conditions.

## Results

### Field Flow Fractionation

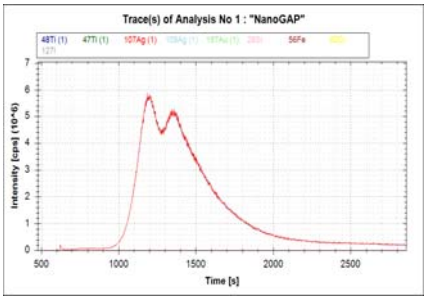
Certified gold NPs with 30 nm (NIST 8012) and 60 nm (NIST 8013) diameters were used to evaluate the NP separation potential of FFF. Table 1 summarizes the channel, membrane and conditions used. Figure 2 shows elution profiles of 30 nm and 60 nm gold NPs.

**FIGURE 2. 197 Au FFF-ICP-MS fractograms of 30 nm and 60 nm gold NPs.**




A slightly different FFF elution program was used to separate PVP-stabilized silver NPs (Nanogap, Spain). The nominal size of the NPs is 42 ± 10 nm but a doublet profile (Fig. 3) clearly indicates a mixture of sizes (or shapes). The fractogram profile is in excellent agreement with a separate study that comprehensively optimized the FFF parameters for this sample[1].

**FIGURE 3. FFF-ICP-MS fractogram of Nanogap® AgNPs.**



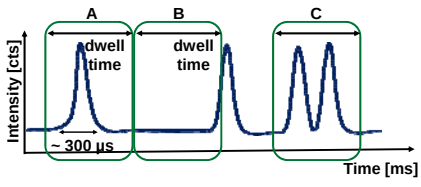

### Single Particle ICP-MS

The underlying principle of sp-ICP-MS lies in the fact that the size of the NP is directly proportional to the intensity of the single particle event (SPE). Smaller NPs (40 nm) generate lower intensity SPEs than the larger NPs (100 nm). The frequency in which SPE are observed is related to the number of particles in solution, thus the concentration of the nanoparticles. The sample is diluted until it was within a suitable range of particles per volume and directly aspirated into the plasma. Data from fast scanning across a single isotope in a predetermined time window (e.g. 3 ms dwell time for 60 s) is exported into a calculation spreadsheet , where the nanoparticle size distribution and concentration is calculated.

The correlation between dwell time and particle events is illustrated in figure 5. A typical nanoparticle event has a duration of 300µs.

- Only one nanoparticle event should be monitored per data point (A)
- Short dwell times lead to incomplete registration of particle events (underestimation of particle size, B)
- Long dwell times lead to registration of two (or more) particle events (overestimation of particle size, C)

**FIGURE 5: Relation between single particle event and dwell time**



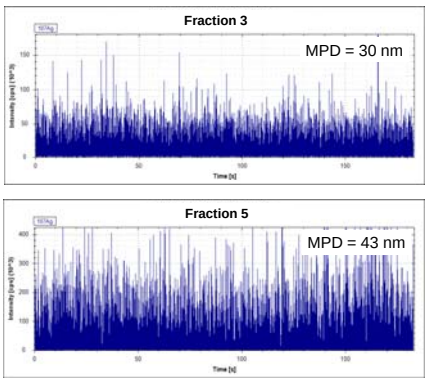

### Analysis of Nanoparticles in Food[2]

Nanoparticle characterization in food faces many challenges due to the presence of complex matrix and the lack of fully developed and validated sample preparation protocols. An approach based on enzymatic extraction, followed by fractionation using FFF and final nanoparticle characterization with spICP-MS was investigated for a chicken meat paste.

Chicken meat paste was spiked with AgNPs and extracted with Proteinase K 1:5 (60 µg enzyme /mg tissue) with incubation for 40 minutes in a water bath (37°C). The extracted sample was analyzed using a similar FFF-ICP-MS setup to that described above and fractions were collected. The observations from the FFF analysis indicated that a significant proportion of approximately 80 % of the nanofraction was recovered from the FFF analysis and that there was a formation of additional peaks in comparison to a standard of AgNPs (or pristine AgNPs). Additionally, the presence of the partially degraded meat matrix had an influence on the elution behavior of the AgNPs following FFF separation. Earlier elution (approx. 2 mins) was observed in comparison to the pristine AgNPs.

Five FFF fractions were collected and analyzed by spICP-MS: Two of the fractions are shown in Figure 6

**FIGURE 6. Analysis of FFF fractions of chicken meat paste spiked with AgNPs with spICP-MS.**




Fraction 3 was collected in the rising slope of the FFF nanoparticle peak at an elution time of 9 minutes and fraction 5 was collected in the tailed elution of the nanaoparticle peak at an elution time of 26 minutes. The fractions were found to have mean particle diameters (MPD) of 30 and 43 nm respectvively. The obtained number-based particle size distributions for each fraction present a more accurate size information than the mass concentration-based size distribution based on FFF calibration. Additionally, an earlier fraction was found to contain non-nano Ag which was most likely ionic Ag bound to organic constituents of the enzymatic meat digestate. This demonstrated that additional information can be obtained by combination of single particle ICP-MS with a separation method like FFF in comparison to bulk analysis, especially for NP analysis in complex matrices.

## Conclusion

- Both FFF-ICP-MS and sp-ICP-MS bring analytical advantages to the characterization of NPs and act as complementary techniques.
- The integrated FFF-ICP-MS package is fully automated with bidirectional control and emergency shut-off features.
- The completely metal free FFF/IC system operates with a single pump and offers a switch option that allows the user to quickly change from FFF to IC.
- The high base sensitivity and low backgrounds of the iCAP Q offer a particular advantage in sp-ICP-MS.

## References

1. K. Loeschner et al., J. Chromat. A 1272 116-25.
2. K. Loeschner et al., Anal. Bioanal. Chem. 405 8185-8195

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