METHOD PROTOCOL- ANALYSIS TECHNIQUE S.E.M, T.E.M OR ICP-MS

SUMMARY

This document aims to guide and indicate the main steps to achieve the correct analysis of our nano samples using SEM, TEM, or ICP-MS techniques.

DEFINITIONS

Technique S.E.M: Analytical technique to perform analysis on a wide range of materials, at high magnifications, and to produce high-resolution images.

Technique T.E.M: Analytical technique used to visualize the smallest structures in matter. Unlike optical microscopes, which rely on light in the visible spectrum, TEM can reveal stunning detail at the atomic scale by magnifying nanometer structures up to 50 million times.

Technique ICP-MS: Analytical technique that can be used to measure elements at trace levels in biological fluids.

Nano Particles: A nanoparticle or ultrafine particle is a particle of matter 1 to 100 nanometers in diameter. The term is sometimes used for larger particles, up to 500 nm, or fibers and tubes that are less than 100 nm in only two directions.

Flow/Flakes: Appearance of material is fluid or flakes. In a general meaning, a nanoflake is a flake with at least one nanometric dimension. A flake is not necessarily perfectly flat but is characterized by a plate-like form or structure.

Eppendorf: A biotechnology company that develops, produces, and sells laboratory systems for liquid, cell, and sample handling. Their equipment and instruments are the most renowned on the market to ensure the results of testing nanomaterials.

Powder: A powder is a dry, bulk solid composed of many very fine particles that may flow freely when shaken or tilted. Powders are a special sub-class of granular materials, although the terms powder and granular are sometimes used to distinguish separate classes of material.
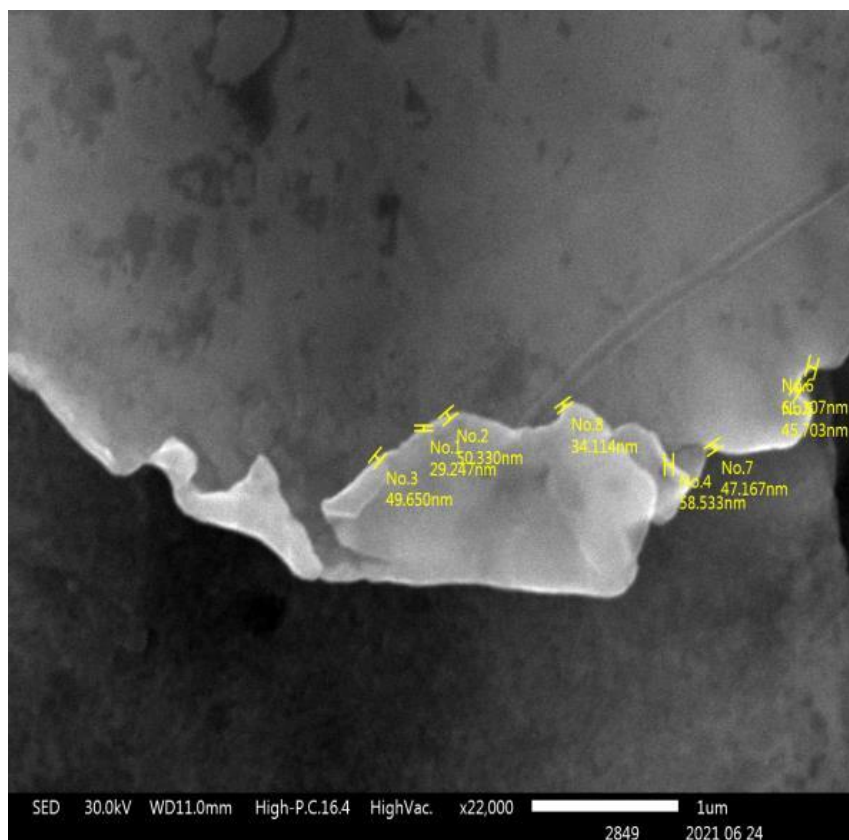
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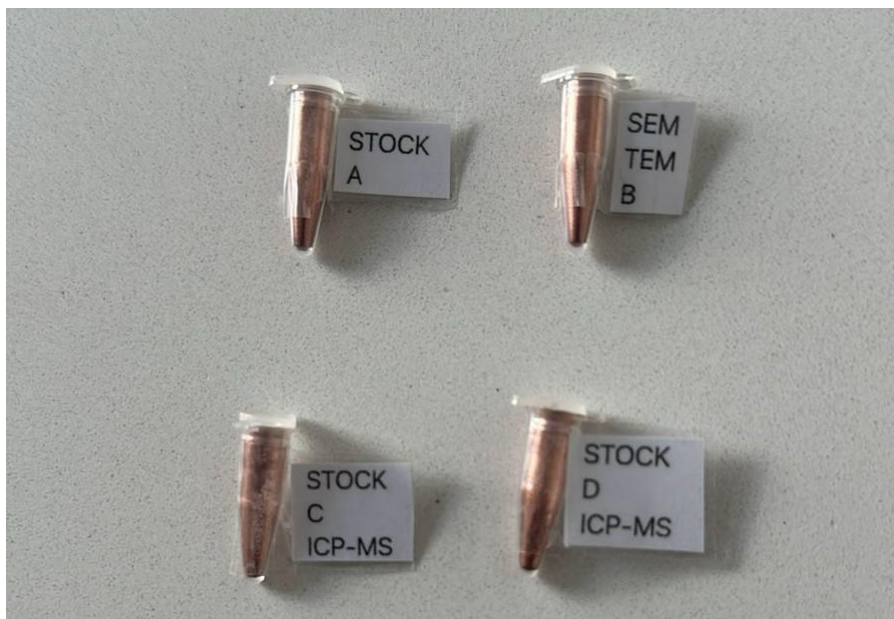
Technique S.E.M:

The nano copper flake samples, prepared in Micro-Eppendorf, have been sent as follows:

* One sample dispersed in stock solution (A) for use if necessary.

* Another sample with dispersed material in Micro-Eppendorf (B), ready to be analyzed in SEM.This solution is in isopropanol. For SEM application, a drop of the solution on an activated carbon surface is sufficient; there is no need to coat it with gold for analysis.

It's important to note that in this methodology, the focus is on analyzing the thickness of the copper sheets. I have attached an image from the analysis conducted for reference:

Dispersion suggestion for handling:

- *The sample was diluted in 30% benzyl alcohol (in ethanol), diluted to an optimum concentration for NTA software and then sonicated for ~10 minutes with a sonication probe.*
- *The sample was analyzed with the Nano Sight LM10HSB equipped with a 405nm laser module.*
- *To remove the biggest over-micron size particles and aggregates, the sample was filtered by 0.2um syringe filter with a PTFE membrane.*
- *For all measurements viscosity was adjusted to 2.17cP at 23 degrees Celsius.*
- *Additional, I recommend extra sonication.*

METHOD PROTOCOL- ANALYSISTECHNIQUE S.E.M,T.E.M OR ICP-MS

Please take into consideration that the nano copper flake sheets are coated with a material containing both organic and inorganic substances.Therefore, for the purity grade, you should only consider the intensity in relation to the typical minerals found in samples.
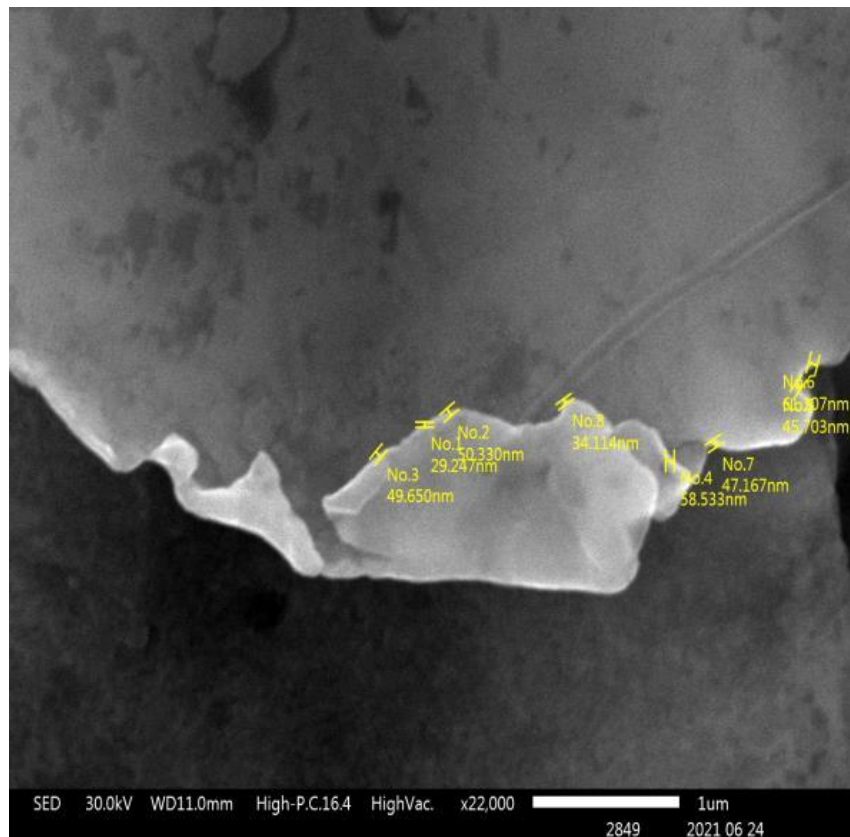
<u>TechniqueT.E.M:</u>

The nano copper flake samples, prepared in Micro-Eppendorf, have been sent as follows:

* One sample dispersed in stock solution (A) for use if necessary.

* Another sample with dispersed material in Micro-Eppendorf (B), ready to be analyzed inTEM.This solution is in isopropanol. ForTEM, simply applying a drop of sample B on the grid is enough.

It's important to note that in this methodology, the focus is on analyzing the thickness of the copper sheets. I have attached an image from the analysis conducted for reference:

Dispersion suggestion for handling:

- *The sample was diluted in 30% benzyl alcohol (in ethanol), diluted to an optimum concentration for NTA software and then sonicated for ~10 minutes with a sonication probe.*
- *The sample was analyzed with the Nano Sight LM10HSB equipped with a 405nm laser module.*
- *To remove the biggest over-micron size particles and aggregates, the sample was filtered by 0.2um syringe filter with a PTFE membrane.*
- *For all measurements viscosity was adjusted to 2.17cP at 23 degrees Celsius.*
- *Additional, I recommend extra sonication.*

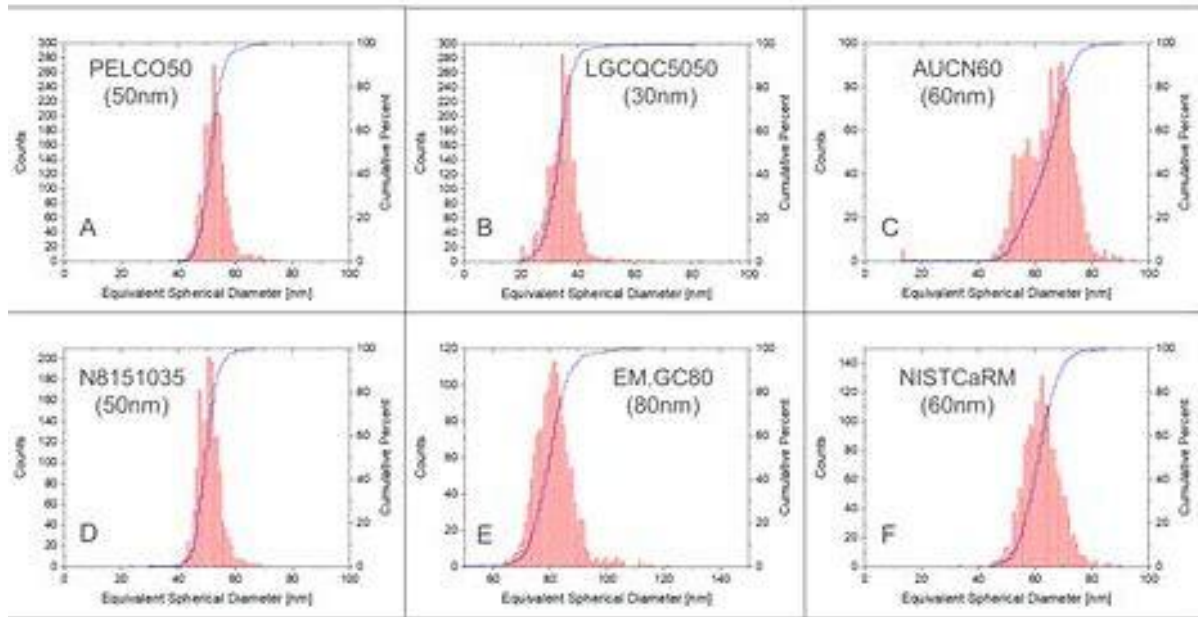# METHOD PROTOCOL- ANALYSISTECHNIQUE S.E.M, T.E.M OR ICP-MS

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<u>Technique ICP-MS:</u>

For the analysis of the sample in relation to its purity, ICP-MS was suggested due to its sensitivity range. To achieve this, a solution of the material in demineralized water was prepared, and two samples were sent:

* A sample in a 1.5ml Eppendorf tube containing nano copper flake (less than one milligram dispersed in solution), ready to be diluted if necessary. It can be used at a ratio of 10 µl in 1 ml.

* A sample ready to be analyzed by ICP-MS, consisting of 10 µl in 1 ml of demineralized water, prepared and ready for analysis.

Dispersion suggestion for handling:

- *The sample was diluted in 30% benzyl alcohol (in ethanol), diluted to an optimum concentration for NTA software and then sonicated for ~10 minutes with a sonication probe.*
- *The sample was analyzed with the Nano Sight LM10HSB equipped with a 405nm laser module.*
- *To remove the biggest over-micron size particles and aggregates, the sample was filtered by 0.2um syringe filter with a PTFE membrane.*
- *For all measurements viscosity was adjusted to 2.17cP at 23 degrees Celsius.*
- *Additional, I recommend extra sonication.*

Please take into consideration that the nano copper flake sheets are coated with a material containing both organic and inorganic substances. Therefore, for the purity grade, you should only consider the intensity in relation to the typical minerals found in samples.