NIST - NCL Joint Assay Protocol, PCC-10

Analysis of Gold Nanoparticles by Electrospray Differential Mobility Analysis (ES-DMA)

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This protocol assumes an intermediate level of scientific competency with regard to techniques, instrumentation, and safety procedures. Rudimentary assay details have been omitted for the sake of brevity.

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1. Introduction

This document describes a protocol for size analysis of citrate-stabilized gold nanoparticles using electrospray differential mobility analysis (ES-DMA). The procedures and parameters as defined in this protocol are appropriate for particles in the range from 5 nm to about 400 nm. Nanoparticles are centrifuged to remove excess citrate stabilizer and are re-suspended in ammonium acetate solution for ES-DMA analysis. A number average diameter is calculated from particle mobility measurements. NIST reference materials 8011, 8012 and 8013 (nominally 10 nm, 30 nm and 60 nm, respectively) were used to develop and demonstrate the assay; modification of protocol parameters may be necessary to optimize the methodology for specific gold nanoparticle formulations other than the reference materials used here. The assay requires 900 µL of nanoparticle solution of the following concentrations: $\approx$ 10 nm particles at [$\approx 5 \times 10^{12}$ particles/mL], $\approx$ 30 nm particles at [$\approx 2 \times 10^{11}$ particles/mL], and $\approx$ 60 nm particles at [$\approx 3 \times 10^{10}$ particles/mL]. In any case, the particle number concentration of the test sample should not exceed $\approx 2 \times 10^{13}$ particles/mL for citrate-stabilized gold.

2. Reagents and Equipment

CAUTION: PERSONAL PROTECTION EQUIPMENT SUCH AS SAFETY GOGGLES, LAB COAT, AND RUBBER GLOVES (LATEX OR NITRILE) MUST BE USED WHEN OPERATING UNDER THIS PROTOCOL.

    2.1   Reagents

        2.1.1   Citrate stabilized nanoparticles:

               $\approx$ 10 nm particles at [$\approx 5 \times 10^{12}$ particles/mL]

               $\approx$ 30 nm particles at [$\approx 2 \times 10^{11}$ particles/mL]

               $\approx$ 60 nm particles at [$\approx 3 \times 10^{10}$ particles/mL]

        2.1.2   Ammonium acetate (99.9%)

        2.1.3   Ammonium hydroxide solution (28-30%)

        2.1.4   Acetic acid (glacial, 99.8%)

        2.1.5   Purified water (18 MΩ·cm resistivity)

    2.2   Equipment

2.2.1   Pipettes covering a range from 0.05 mL to 1 mL

2.2.2   Low retention (such as DNA low binding) 1.5 mL microcentrifuge tubes

2.2.3   Microcentrifuge

2.2.4   Vortex mixer

2.2.5   Commercially available electrospray differential mobility analyzer with condensation particle counter (CPC)

3.   Reagent Preparation

3.1   Ammonium Acetate Buffer [2 mmol/L stock solution]

Dissolve 0.077 g of ammonium acetate (99%) in 0.3 L of 18 MΩ·cm water. Stir to dissolve ammonium acetate. Adjust pH using ammonium hydroxide and/or acetic acid to achieve pH = 8. Transfer volume to a 0.5 L volumetric flask. Add additional 18 MΩ·cm water to bring total volume to 0.5 L.

4.   Preparation of Samples

4.1   Vortex nanoparticle sample to disperse colloids.

4.2   Transfer 900 μL of sample into low binding microcentrifuge tube. *Note: 900 μL aliquots are required for each sample. If more than one sample is to be analyzed, increase the number of aliquots accordingly.*

4.3   Centrifuge each vial containing 10 nm particles for 40 min at 13.2 krpm (30 nm or 60 nm particles for 12 minutes at 7.6 krpm). *Note: Check clarity of supernatant to ensure complete isolation of nanoparticles from supernatant.*

4.4   Remove 850 μL of clear supernatant by pipet, leaving dark red nanoparticles in bottom of microcentrifuge tube.

4.5   Vortex remaining 50 μL of solution to re-suspend sample.

4.6   Add 500 μL of 2 mmol/L ammonium acetate solution to each sample vial.

4.7   Vortex to mix.

5.   Analysis of Sample by ES-DMA used the following conditions:

5.1   Capillary diameter: nominally 25 μm

5.2   Electrospray voltage: 1.67 kV to 2.78 kV as necessary to achieve stable Taylor cone

5.3 CO_2 pressure and flow rate: 6.89×10^4 Pa (10 psi) and 0.2 L/min

5.4   Air pressure and flow rate: 2.55×10^4 Pa (3.7 psi) and 1.0 L/min

5.5   Sheath/carrier gas flow rate: 30 L/min for $\approx$ 10 nm and $\approx$ 30 nm particles; 10 L/min for $\approx$ 60 nm particles

5.6   Exit flow rate from DMA: 1.0 L/min

5.7   Flow entering the condensation particle counter (supplemented by filtered air): 1.5 L/min

5.8   The baseline cut off value was set at 30 counts to ensure clear separation between peaks and to account for baseline noise.

6. Data Analysis

6.1   Nanoparticle diameter calculation

DMA voltages are converted to equivalent diameters using the following relationship supplied by the ES-DMA vendor and parameter values unique to our instrumentation:

$$\frac{d}{C_c(d)} = \frac{2neV_{DMA}L}{3\mu q_{sh} \ln(r_2/r_1)},$$

Note: The following parameter values should be adjusted appropriately for the ES-DMA system used for analysis.

     Inner radius of the DMA (r_1) = 0.937 cm

     Outer radius of the DMA (r_2)= 1.905 cm

     Length of the DMA (L) = 4.987 cm

     Flow rate of the sheath gas of the DMA (q_{sh}) = 30 L/min

     Viscosity of air (μ) = 1.81×10^{-5} Pa s

     Number (n) of elementary charges (e) on a particle = 1

     Cunningham slip correction factor:

$$C_c = 1 + Kn[\alpha + \beta \exp(\frac{-\gamma}{Kn})],$$

$$Kn = 2\lambda/d$$

$$\alpha = 1.257$$

$$\beta = 0.40$$

$$\gamma = 1.110$$

The gas mean free path at room temperature (λ) = 66 nm.

6.2   Correction for size dependent particle charge distribution

The neutralization process imparts a Boltzmann charge distribution on the particles that is size dependent. The raw counts from the CPC represent only positively charged particles (+1). The total counts are obtained by dividing the raw counts for the positively charged particles by a correction factor that represents the proportion of particles with a +1 charge. The following correction factor was used to elucidate the number of particles at each size measured before neutralization as specified by the commercial vendor:

$$f = 10^{\sum_{i=0}^{5} a_i \left[\log\left(d_p/1nm\right)\right]^i}$$

d_p = the diameter of the particle

$$a_o = -2.3484$$

$$a_1 = 0.6044$$

$$a_2 = 0.4800$$

$$a_3 = 0.0013$$

$$a_4 = -0.1553$$

$$a_5 = 0.0320$$

6.3   Correction for dissolved salts

To account for the thickness of residual nonvolatile salts encrusted on the surface of the particles, the mode of the salt peak, d_{salt}, was determined and subtracted from the subsequent particle size using the following equation:

$$d_{p,corrected} = \sqrt[3]{d_p{}^3 - d_{salt}{}^3} \; .$$

This relationship also suggests a limitation of the protocol when d_{salt} and d_p become too closely spaced. Below $d_p \approx 30$ nm, the salt correction becomes increasingly significant. In this range, it is necessary that d_{salt} and d_p are sufficiently well separated to allow for an accurate determination of the salt correction and to avoid having the residual salt particles contribute to the gold particle peak. As a rule of thumb, d_{salt} should be no larger than roughly half the value of d_p for gold particles smaller than 30 nm.

6.4 Calculation of the number average diameter

The number average diameter was then calculated with the following equation:

$$\bar{d} = \frac{\sum_i d_i N_i}{\sum_i N_i} \; .$$

7. References

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8. Abbreviations

cm   centimeter

CO_2	carbon dioxide
CPC	condensation particle counter
ES-DMA	electrospray differential mobility analysis
g	gram
krpm	kilo-rotations per minute
kV	kilovolts
L	liter
mg	milligram
min	minutes
mL	milliliter
mmol	millimoles
$M\Omega$	megaohm
μL	microliter
μm	micrometer
nm	nanometer
NIST	National Institute of Standards and Technology
Pa	Pascal
psi	pounds per square inch