NIST - NCL Joint Assay Protocol, PCC-11

Method for Determination of the Mass Fraction of Particle-Bound Gold in Suspensions of Gold Nanoparticles

National Institute of Standards and Technology

Chemical Science and Technology Laboratory

Gaithersburg, MD 20899

Nanotechnology Characterization Laboratory
Frederick National Laboratory for Cancer Research
Leidos Biomedical Research, Inc.
Frederick, MD 21702
(301) 846-6939
ncl@mail.nih.gov
http://www.ncl.cancer.gov

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.

Method written by:

Michael R. Winchester

>Analytical Chemistry Division
>Chemical Science and Technology Laboratory
>National Institute of Standards and Technology

1. Introduction

This document provides a protocol for measuring the mass fraction values of gold (Au) nanoparticles in suspensions of Au nanoparticles similar to National Institute of Standards and Technology (NIST) Reference Materials, RM 8011 Gold Nanoparticles Nominal 10 nm Diameter, RM 8012 Gold Nanoparticles Nominal 30 nm Diameter, and RM 8013 Gold Nanoparticles Nominal 60 nm Diameter. The mass fraction of Au nanoparticles in a given suspension is defined as the mass of Au bound into nanoparticles per mass of suspension. Bound Au is distinguished from Au dissolved in the solution matrix of the suspension.

The protocol described here can be briefly summarized as follows: Samples of Au nanoparticle suspensions are digested with acid, and the Au mass fractions of the resulting solutions are measured. The Au nanoparticles are removed from other samples of the suspensions, and the Au mass fractions of the particle-free solutions are measured. The mass fraction of Au nanoparticles in each suspension is calculated as the difference between the value obtained for the corresponding digested samples and the value obtained for the corresponding particle-free solutions.

This protocol is based upon the use of inductively coupled plasma optical emission spectrometry (ICP-OES) for estimating the Au mass fraction values in the digested suspension and particle-free solution samples. The method of standard additions is used for calibration in the analysis of the digested samples, while a calibration equation is developed by means of prepared calibration samples for the analysis of the particle-free samples. This protocol is based upon the use of centrifugation for the removal of the nanoparticles from the suspensions. Basic knowledge of and experience with ICP-OES and centrifugation are assumed.

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   Deionized Water

        2.1.2   Concentrated nitric acid (HNO_3) – reagent grade or better

2.1.3 Concentrated hydrochloric acid (HCl) – reagent grade or better

2.1.4 *5 % (volume fraction)[†] hydrochloric acid* – concentrated HCl diluted with deionized water by a volumetric factor of 20

2.1.5 *Gold stock solution* – NIST Standard Reference Material® (SRM®) 3121 Gold Standard Solution, or any similar Certified Reference Material (CRM) solution with certified Au mass fraction greater than or equal to 1 mg/g[(‡)] in dilute HCl or HNO_3. When diluted by a volumetric factor of 100 with 5 % (*volume fraction*) HCl, the solution must not contain a level of Cu that is detectable by ICP-OES.

2.1.6 *Copper stock solution* – shall contain a known mass fraction of Cu greater than or equal to 1 mg/g in dilute HCl or HNO_3. It is only necessary to know the Cu mass fraction approximately. When diluted with 5 % (*volume fraction*) HCl by a volumetric factor of 20, the solution must not contain Au at a level that is detectable by ICP-OES.

2.1.7 *Control sample* – NIST RM 8011 Gold Nanoparticles Nominal 10 nm Diameter, RM 8012 Gold Nanoparticles Nominal 30 nm Diameter, or RM 8013 Gold Nanoparticles Nominal 60 nm Diameter, or any other RM or CRM consisting of a suspension of Au nanoparticles similar to the suspensions to be analyzed and having an assigned value and uncertainty for the mass fraction of Au nanoparticles.

2.2 Equipment

2.2.1 ICP-OES instrument

2.2.2 Centrifuge

2.2.3 Analytical balance with a readability less than or equal to 1 mg

2.2.4 Pipets and other volumetric ware capable of delivering liquid volumes in the range 0.15 mL to 25 mL, inclusively

2.2.5 Clean, plastic (e.g., high-density polyethylene) bottles

---

[†] Throughout this document, acid volume fraction, indicated by some authors as "(*v/v*)," is defined as volume of concentrated acid divided by volume of solution.
[‡] The chemical compositions of suspensions and some solutions referred to in this document are expressed in mass fraction units (i.e., mass per mass) rather than concentration units (i.e., mass per volume). If the density of a given suspension or solution is known, it can be used to convert between the two.

3. ICP-OES Instrument Setup

    3.1   Prepare the ICP-OES instrument for analysis according to the instrument manufacturer's instructions.

    3.2   Selections of nebulizer and spray chamber types are not critical. They should be made by the user based upon availability and experience with the particular ICP-OES instrument.

    3.3   Develop a method for this application in the instrument's software package:

        3.3.1   Enable measurement of emission intensities at the Au (I) 242.795 nm and Cu (I) 221.458 nm wavelengths.

        3.3.2   Selections of gas flow rates, viewing mode (axial vs. radial), and other method details are unlikely to be critical. They should be made by the user based upon experience with the particular ICP-OES instrument.

        3.3.3   Set the background subtraction points for the Au and Cu peaks while running a test solution containing a Au mass fraction of 2 mg/kg and a Cu mass fraction of 30 mg/kg prepared by dilution of the Au stock solution and the Cu stock solution with 5 % (*volume fraction*) HCl.

        3.3.4   Run the test solution prepared in the preceding step to ensure signal stability, complete washout of Au and Cu from the sample introduction system between samples, and absence of spectral interferences.

    3.4   Check the linearity of the instrument responses:

        3.4.1   Prepare a set of at least three calibration solutions with Au mass fractions covering the range 0 mg/kg to 20 mg/kg, inclusively, and Cu mass fractions covering the range 0 mg/kg to 100 mg/kg, inclusively. These solutions should be prepared by dilution of the Au stock solution and the Cu stock solution with 5 % (*volume fraction*) HCl.

        3.4.2   Run the calibration solutions on the ICP-OES instrument using the developed method.

        3.4.3   Plot the recorded emission intensities against the known Au and Cu mass fractions to ensure that the instrument response is linear for each element.

        3.4.4   Estimate the calibration equation and record the instrument sensitivity (i.e., the slope of the calibration line) for each element.

3.4.5   If the response of the instrument for either element is nonlinear, make appropriate adjustments to the method to obtain linearity.

3.4.6   If the *y*-intercept of either calibration equation is abnormally large for the ICP-OES instrument being used, make appropriate adjustments to the background subtraction points or other method details to obtain typically small *y*-intercepts.

4. Determination of Total Au Mass Fraction Values

4.1   Preliminary Preparations

4.1.1   Estimate roughly the total Au mass fraction values for the Au nanoparticle suspensions (NOTE: This step is unnecessary if rough estimates are already available):

4.1.1.1 Obtain a 1 g sample of each suspension to be measured, including the control.

4.1.1.2 Add 1.5 mL concentrated HCl and 0.15 mL concentrated HNO_3 to each sample to digest the Au nanoparticles. Allow each mixture to sit capped, with occasional agitation, for at least 1 h.

4.1.1.3 Dilute each digested sample with 25 mL deionized water.

4.1.1.4 Run the digested, diluted samples on the ICP-OES instrument using the method developed in Step 3.3.

4.1.1.5 Using the calibration equations developed in Step 3.4 and the dilution factors pertaining to the solution preparations, calculate the approximate total Au mass fraction of each Au nanoparticle suspension.

4.1.1.6 Ensure that Cu is undetectable in each solution.

4.1.2   Au spiking solution

4.1.2.1 Prepare a spiking solution of Au by dilution of the Au stock solution with 5 % (*volume fraction*) HCl. The mass fraction of Au should be approximately equal to the average of the estimated mass fraction values of total Au in the suspensions to be analyzed

(from Step 4.1.1.5). The diluted solution will hereafter be referred to as the "Au spiking solution."

4.1.2.2 Calculate the actual mass fraction of Au in the Au spiking solution, F_{sp}, using the known Au mass fraction value of the Au stock solution and the balance weighings made during dilution.

4.1.2.3 Estimate the standard uncertainty associated with the calculated F_{sp} value, $u(F_{sp})$. To accomplish this, assume that the *relative* standard uncertainty of the calculated value is equivalent to the *relative* standard uncertainty associated with the known Au mass fraction of the Au stock solution. This approach is justified, because the uncertainties in the balance measurements are usually at least several times smaller than the uncertainty in the Au mass fraction of the Au stock solution.

4.1.3 Cu spiking solution

4.1.3.1 Based upon the sensitivities of the ICP-OES instrument for Au and Cu (from Step 3.4.4) and the average of the estimated total Au mass fraction values for the nanoparticle suspensions (from Step 4.1.1.5), calculate the approximate mass of Cu that would need to be added to a 1 g sample of the suspensions to obtain a Cu emission intensity approximately twice the expected Au emission intensity.

4.1.3.2 Dilute a portion of the Cu stock solution with 5 % (*volume fraction*) HCl to obtain a solution with a Cu mass fraction that will allow the mass of Cu calculated in the preceding step to be delivered in 1 g of solution. The diluted solution will hereafter be referred to as the "Cu spiking solution."

4.2 Sample Preparation

4.2.1 Immediately prior to opening an ampoule or bottle containing a suspension of Au nanoparticles, make sure that the suspension is thoroughly mixed. Sonication may be employed.

4.2.2   Gravimetrically aliquot each of a predetermined number of 1 g samples from each ampoule or sample bottle into a separate, clean, plastic bottle. Record the mass of each aliquot transferred. Each bottle into which an aliquot is transferred shall be labeled with the identification of the ampoule or sample bottle from which that aliquot was derived, the identification of the aliquot (e.g., "a," "b," "c," …), and the phrase "not spiked with Au."

4.2.3   To each bottle containing an aliquot, add 1.5 mL concentrated HCl and 0.15 mL concentrated HNO_3. Cap each bottle and agitate gently. The samples should be allowed to sit capped, with occasional agitation, for at least 1 h to ensure complete digestion of the nanoparticles.

4.2.4   Gravimetrically aliquot 1 g of Cu spiking solution into each bottle and record the mass of each aliquot. Mix thoroughly.

4.2.5   Gravimetrically aliquot approximately half of each solution spiked with Cu into another separate, clean, plastic bottle labeled with the corresponding ampoule or sample bottle identification, the corresponding aliquot identification (e.g., "a," "b," "c," …), and the phrase "spiked with Au." Record the mass of each aliquot transferred.

4.2.6   Gravimetrically aliquot 1 g of Au spiking solution into each bottle labeled with the phrase "spiked with Au," and record the mass of each aliquot. Add 1 mL of 5 % (*volume fraction*) HCl to each bottle labeled with the phrase "not spiked with Au."

4.2.7   Dilute each bottle with 13.5 mL deionized water.

4.3   Control and Blank Sample Preparations

4.3.1   Repeat Steps 4.2.1 through 4.2.7 for the control.

4.3.2   A blank solution consisting of 5 % (*volume fraction*) HCl must be prepared. The volume of this solution must be sufficiently large to comply with Step 4.4.1 below.

4.4   ICP-OES Measurements

4.4.1   Label and fill one autosampler tube with blank solution for each pair of "spiked with Au" and "not spiked with Au" sample solutions. This

provides an independent blank for each Au nanoparticle suspension under test, as well as for the control sample.

4.4.2 Label and fill one autosampler tube with each "spiked with Au" sample solution. Also, label and fill one autosampler tube with each "not spiked with Au" sample solution.

4.4.3 Run all of the "spiked with Au," "not spiked with Au," and blank solutions on the ICP-OES instrument using the method developed in Step 3.3. It is best to utilize a randomized complete block sequence for a total of at least three runs per solution. In other words, the solutions should be run once each in random order, then again once each in a different random order, and so forth, until each solution has been run the desired number of times. (NOTE: Some ICP-OES autosamplers do not allow repeat measurements from the same sample tube. In such cases, multiple tubes will need to be filled for each solution in Steps 4.4.1 and 4.4.2.)

4.4.4 Download the Au and Cu emission intensity data to a spreadsheet (or to another appropriate data reduction tool) for processing.

4.5 Calculations

4.5.1 Au nanoparticle suspensions under test

4.5.1.1 For each ith pair of "spiked with Au" and "not spiked with Au" samples prepared from a particular Au nanoparticle suspension under test, calculate a separate value of Q_i as follows:

$$Q_i = R_{u,i} \left(\frac{m_{sp,i} / m_{spsolu,i}}{R_{sp,i} - R_{u,i}} \right) \left(\frac{m_{solu,i}}{m_{sample,i}} \right) \qquad \text{Eq. 1}$$

where:

$m_{sample,i}$ is the mass of the aliquot initially taken from the suspension

$m_{solu,i}$ is the mass of the solution after the additions of concentrated HCl, concentrated HNO_3, and Cu spiking solution

$m_{spsolu,i}$ is the mass of the portion of this solution that was spiked with Au spiking solution

$m_{sp,i}$ is the mass of Au spiking solution added to this portion

$R_{sp,i}$ is the ratio of the blank-corrected Au emission intensity to that of Cu for this portion

$R_{u,i}$ is the same quantity for the other portion (i.e., the portion not spiked with Au spiking solution).

4.5.1.2 For each Au nanoparticle suspension, compute the average, $\overline{Q}$, and the standard uncertainty of the mean, $u(\overline{Q})$, of all values of Q_i. The standard uncertainty of the mean is defined as the standard deviation of the set of Q_i values divided by the square root of the number of Q_i values in the set.

4.5.1.3 Calculate the mass fraction of total Au in each Au nanoparticle suspension, F_{total}, as:

$$F_{total} = \overline{Q} F_{sp}$$

Eq. 2

4.5.1.4 Estimate the standard uncertainty of each calculated F_{total} value, $u(F_{total})$ as the square root of the sum of the squares of $u(F_{sp})$ and the corresponding value of $u(\overline{Q})$.

4.5.2   Control sample

4.5.2.1 Repeat Step 4.5.1 for the control sample preparations.

5. Determination of Dissolved Au Mass Fraction Values

5.1   Removal of Nanoparticles from Au Nanoparticle Suspensions

5.1.1   Obtain one 5 mL aliquot of each Au nanoparticle suspension under test. (NOTE: The available centrifuge and centrifuge tubes may necessitate a different aliquot volume.)

5.1.2   Centrifuge each aliquot for at least one hour at a gravitational force that would be expected to remove completely the Au nanoparticles, given the

nominal particle size distribution. Decant or siphon off each supernatant solution.

5.1.3 Dilute 0.3 mL of each supernatant solution with 5 mL of 5 % (*volume fraction*) HCl, and run each diluted solution on the ICP-OES instrument using the method developed in Step 3.3. Record the Au emission intensity for each diluted solution.

5.1.4 Iteratively repeat Steps 5.1.2 and 5.1.3 using the remaining portion of each undiluted supernatant solution until the level of Au becomes undetectable or until no significant change is noted for three successive Au emission intensity measurements. In some cases, it may be necessary to increase the gravitational force and/or centrifugation time to remove completely the Au nanoparticles.

5.2 Removal of Nanoparticles from the Control Sample

5.2.1 Repeat Steps 5.1.1 through 5.1.4 for the control.

5.3 Determination of Au Mass Fraction Values in Supernatant Solutions from Au Nanoparticle Suspensions (NOTE: If Au is undetectable in all supernatant solutions from Steps 5.1 and 5.2, proceed directly to Step 6.)

5.3.1 Preparation of Supernatant Solutions for Analysis

5.3.1.1 Gravimetrically aliquot 1 g of each supernatant solution containing a detectable amount of Au into a separate, clean, plastic bottle, and record the mass transferred.

5.3.1.2 Spike each aliquot with 1 mL of Cu spiking solution.

5.3.1.3 Dilute each solution with 13 mL of 5 % (*volume fraction*) HCl.

5.3.1.4 Run a small portion of each diluted solution on the ICP-OES instrument and record the Au emission intensities.

5.3.2 Preparation for Calibration

5.3.2.1 Preparation of Secondary Au Stock Solution

5.3.2.1.1 Prepare another Au stock solution by dilution of a portion of the first Au stock solution with 5 % (*volume fraction*) HCl. The new Au stock solution will hereafter be called the "secondary Au stock solution." The Au

mass fraction of this solution should be at a level such that dilution by a factor of 15 will produce a solution that provides a Au emission intensity approximately equal to the average Au emission intensities of the prepared supernatant sample solutions observed in Step 5.3.1.4.

5.3.2.1.2 Calculate the Au mass fraction of the secondary Au stock solution, F_{Au}^{2nd}, from the known Au mass fraction of the Au stock solution and the dilution data.

5.3.2.1.3 Estimate the standard uncertainty associated with the calculated F_{Au}^{2nd} value, $u(F_{Au}^{2nd})$, by assuming that the *relative* standard uncertainty is equivalent to the *relative* standard uncertainty associated with the known Au mass fraction of the Au stock solution.

5.3.2.2 Prepare at least four calibration samples as follows:

5.3.2.2.1 Gravimetrically transfer aliquots of the secondary Au stock solution into separate, clean, plastic bottles. The masses of the aliquots should be designed such that, when diluted by a factor of 15, the Au emission intensities would be expected to bracket the Au intensities of the prepared supernatant solutions observed in Step 5.3.1.4. Record the mass of each aliquot transferred. Also, based upon the known Au mass fraction of the secondary Au stock solution, F_{Au}^{2nd}, calculate the mass of Au transferred in each aliquot.

5.3.2.2.2 Spike each aliquot with 1 mL of Cu spiking solution.

5.3.2.2.3 Dilute each solution with 13 mL of 5 % (*volume fraction*) HCl.

5.3.3 Calibration

5.3.3.1 Run the samples prepared in Step 5.3.1, the calibration samples prepared in Step 5.3.2, and a blank consisting of 5 % (*volume*

fraction) HCl on the ICP-OES instrument using the method developed in Step 3.3. Record the Au and Cu emission intensities.

5.3.3.1 Using linear least squares regression or another appropriate statistical routine, calculate a calibration equation from the recorded emission intensities and the known masses of Au in the calibration solutions. The equation must be computed using the ratios of Au to Cu emission intensities as the ordinate values and the masses of Au in the calibration solutions as the abscissa values.

5.3.4 Calculation of Dissolved Au Mass Fraction Values

5.3.4.1 Compute the mass of Au present in each supernatant solution, M_{Au}, from the observed ratio of the Au to Cu emission intensities for that solution and the calibration equation developed in Step 5.3.3.2.

5.3.4.2 Estimate the *relative* standard uncertainty associated with each M_{Au} value, $u_r(M_{Au})$, using Eq. 3 (**adapted from Ref. 1**):

$$u_r(M_{Au}) = \frac{s_{y/x}}{b(M_{Au})} \sqrt{\frac{1}{p} + \frac{1}{n} + \frac{(y_o - \overline{y})^2}{b^2 \sum_i (x_i - \overline{x})^2}} \qquad \text{Eq. 3}$$

where:

p is the number of observed values of the ratio of Au to Cu emission intensities for the supernatant solution

y_o is the average of those p observed values

n is the number of calibration points

$\overline{x}$ is the average of the abscissa values for the calibration points

$\overline{y}$ is the average of the ordinate values for the calibration points

x_i is the abscissa value of the ith calibration point

b is the y-intercept of the calibration line

The value of $s_{y/x}$ is computed using Eq. 4 (**2**):

$$s_{y/x} = \sqrt{\frac{\sum_{i}(y_i - \hat{y})^2}{n - 2}} \qquad \text{Eq. 4}$$

where each value of the quantity $y_i - \hat{y}$ corresponds to the y-residual for the ith calibration point.

5.3.4.3 Calculate the dissolved Au mass fraction value of each supernatant solution, $F_{dissolved}$, by dividing the corresponding mass of Au computed in Step 5.3.4.1 by the mass of sample aliquoted in Step 5.3.1.1.

5.3.4.4 Estimate the standard uncertainty associated with each $F_{dissolved}$ value, $u(F_{dissolved})$, by multiplying the value of $F_{dissolved}$ by the corresponding value of $u_r(M_{Au})$ computed in Step 5.3.4.2. This approach is justified, because the uncertainty associated with the measurement of the sample mass in Step 5.3.1.1 is usually negligible.

6. Calculations

6.1 For each Au nanoparticle suspension under test, calculate the mass fraction of Au bound into nanoparticles, F_{bound}, using Eq. 5:

$$F_{bound} = F_{total} - F_{dissolved} \qquad \text{Eq. 5}$$

Assume that $F_{dissolved}$ is zero for any suspension that had an undetectable level of Au in the corresponding particle-free solution.

6.2 For each Au nanoparticle suspension under test, estimate the standard uncertainty of the determined F_{bound} value, $u(F_{bound})$, as the square root of the sum of the squares of the corresponding values of $u(F_{total})$ and $u(F_{dissolved})$. Note that $u(F_{dissolved}) = 0$ for any suspension for which $F_{dissolved} = 0$.

6.3   For each Au nanoparticle suspension under test, compute the expanded
       uncertainty of the determined F_{bound} value, $U(F_{bound})$, by multiplying $u(F_{bound})$ by
       2. The expanded uncertainty estimated in this way will correspond to an interval
       at a level of confidence less than or equal to 95 %. The precise level of
       confidence depends upon the estimation of the effective degrees of freedom,
       which is beyond the scope of this document **(3,4)**.

7. Acceptance Criteria

The results of this analysis for a given Au nanoparticle suspension under test shall be
considered acceptable if the following two criteria are satisfied:

7.1   The mass fraction of Au bound into nanoparticles determined for the control
       agrees statistically with the known value at a double-sided significance level of P
       = 0.05.

7.2   The expanded uncertainty of the mass fraction of Au bound into nanoparticles,
       $U(F_{bound})$, for the unknown sample does not exceed 10 % of the corresponding
       F_{bound} value (or the limit imposed by applicable within-laboratory regulations).

8. References

1. J.C. Miller and J.N. Miller, <u>Statistics for Analytical Chemistry</u>; Halsted Press: New York,
    1984; p. 94.
2. *ibid.*; p. 91.
3. *Guide to the Expression of Uncertainty in Measurement,* ISBN 92-67-10188-9, 1st ed.
    International Organization for Standardization (ISO): Geneva, Switzerland (1993).
4. B.N. Taylor and C.E. Kuyatt, *Guidelines for Evaluating and Expressing the Uncertainty of
    NIST Measurement Results*, NIST Technical Note 1297, U.S. Government Printing Office:
    Washington, DC (1994); http://physics.nist.gov/Pubs/guidelines/TN1297/tn1297s.pdf.

9. Abbreviations

Au	gold
CRM	Certified Reference Material
Cu	copper
g	gram
h	hour
HCl	hydrochloric acid
HNO_3	nitric acid
ICP-OES	inductively couple plasma-optical emission spectroscopy
kg	kilogram
mg	milligram
mL	milliliter
nm	nanometer
NIST	National Institute of Standards and Technology
RM	Reference Material
SRM®	Standard Reference Material®