



Supplement to PCC-8 & PCC-9

Metal Quantitation by Inductively Coupled Plasma-Mass Spectrometry

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1. Introduction

This protocol is meant to serve as a supplement to the NIST-NCL protocols PCC-8, “Determination of gold in rat tissue by inductively coupled plasma-mass spectrometry (ICP-MS)” (http://ncl.cancer.gov/NCL_Method_PCC-8.pdf) and PCC-9, “Determination of gold in rat blood by ICP-MS” (http://ncl.cancer.gov/NCL_Method_PCC-9.pdf). Its purpose is to highlight the ICP-MS capabilities of the Nanotechnology Characterization Laboratory (NCL) and to expand on the detailed protocols offered in PCC-8 and PCC-9 with additional experimental nuances in working with alternate materials or tissues.

ICP-MS is an analytical tool that can be used to provide elemental analysis and can measure the concentration of most metals (see Figure 1). With the current instrumentation, NCL has the ability to measure most transition metals, provided a standard reference material is available.

There will be general issues such as instrumental drift and run-to-run variability, as well as nanoparticle specific issues such as sample and biological digestion, that will require specific optimization for each experiment. This protocol is meant to address as many of these issues as possible and will be updated as the NCL’s experience with nanoparticle-related ICP-MS advances.

2. Reagents and Equipment

Please consult protocols PCC-8 and PCC-9 for a list of reagents and standards commonly used in ICP-MS quantitation. The equipment list below dictates current NCL resources.

Note: The NCL does not endorse any of the suppliers listed below; their inclusion is for informational purposes only. Equivalent supplies from alternate vendors can be substituted.

2.1 Equipment

- 2.1.1 An Agilent 7500cx (Santa Clara, CA) inductively coupled plasma mass spectrometer (ICP-MS) equipped with a quartz, micro-concentric nebulizer interfaced with a quartz, water-cooled double pass spray chamber. Perform the set up and optimization of the ICP-MS daily in accordance with the procedure listed in Appendix A of protocols PCC-8 and PCC-9.
- 2.1.2 A four-place analytical balance, e.g., a Mettler (Columbus, OH) model XP205 analytical balance, for weighing in the preparation of samples and

standards. Verify the calibration of the balance in accordance with the procedure listed in Appendix B of protocols PCC-8 and PCC-9.

- 2.1.3 A microwave digestion system, e.g. a CEM (Matthews, NC) model MARSXpress microwave systems equipped with either 55 or 10 mL PFA microwave vessels for the digestion of samples in accordance with the procedure listed in Appendix C of protocols PCC-8 and PCC-9.
- 2.1.4 High-purity water generation system, e.g. a Millipore Synthesis A10 for generating 18 M Ω -cm de-ionized water.

The Periodic Table of the Elements

1 H Hydrogen 1.00794																	2 He Helium 4.003														
3 Li Lithium 6.941	4 Be Beryllium 9.012182											5 B Boron 10.811	6 C Carbon 12.0107	7 N Nitrogen 14.00674	8 O Oxygen 15.9994	9 F Fluorine 18.9984032	10 Ne Neon 20.1797														
11 Na Sodium 22.989770	12 Mg Magnesium 24.3050											13 Al Aluminum 26.981538	14 Si Silicon 28.0855	15 P Phosphorus 30.973761	16 S Sulfur 32.066	17 Cl Chlorine 35.4527	18 Ar Argon 39.948														
19 K Potassium 39.0983	20 Ca Calcium 40.078	21 Sc Scandium 44.955910	22 Ti Titanium 47.867	23 V Vanadium 50.9415	24 Cr Chromium 51.9961	25 Mn Manganese 54.938049	26 Fe Iron 55.845	27 Co Cobalt 58.933200	28 Ni Nickel 58.6934	29 Cu Copper 63.546	30 Zn Zinc 65.39	31 Ga Gallium 69.723	32 Ge Germanium 72.61	33 As Arsenic 74.92160	34 Se Selenium 78.96	35 Br Bromine 79.904	36 Kr Krypton 83.80														
37 Rb Rubidium 85.4678	38 Sr Strontium 87.62	39 Y Yttrium 88.90585	40 Zr Zirconium 91.224	41 Nb Niobium 92.90638	42 Mo Molybdenum 95.94	43 Tc Technetium (98)	44 Ru Ruthenium 101.07	45 Rh Rhodium 102.90550	46 Pd Palladium 106.42	47 Ag Silver 107.8682	48 Cd Cadmium 112.411	49 In Indium 114.818	50 Sn Tin 118.710	51 Sb Antimony 121.760	52 Te Tellurium 127.60	53 I Iodine 126.90447	54 Xe Xenon 131.29														
55 Cs Cesium 132.90545	56 Ba Barium 137.327	57 La Lanthanum 138.9055	72 Hf Hafnium 178.49	73 Ta Tantalum 180.9479	74 W Tungsten 183.84	75 Re Rhenium 186.207	76 Os Osmium 190.23	77 Ir Iridium 192.217	78 Pt Platinum 195.078	79 Au Gold 196.96655	80 Hg Mercury 200.59	81 Tl Thallium 204.3833	82 Pb Lead 207.2	83 Bi Bismuth 208.98038	84 Po Polonium (209)	85 At Astatine (210)	86 Rn Radon (222)														
87 Fr Francium (223)	88 Ra Radium (226)	89 Ac Actinium (227)	104 Rf Rutherfordium (261)	105 Db Dubnium (262)	106 Sg Seaborgium (263)	107 Bh Bohrium (262)	108 Hs Hassium (265)	109 Mt Meitnerium (266)	110 (269)	111 (272)	112 (277)	113	114																		
																		58 Ce Cerium 140.116	59 Pr Praseodymium 140.90765	60 Nd Neodymium 144.24	61 Pm Promethium (145)	62 Sm Samarium 150.36	63 Eu Europium 151.964	64 Gd Gadolinium 157.25	65 Tb Terbium 158.92534	66 Dy Dysprosium 162.50	67 Ho Holmium 164.93032	68 Er Erbium 167.26	69 Tm Thulium 168.93421	70 Yb Ytterbium 173.04	71 Lu Lutetium 174.967
																		90 Th Thorium 232.0381	91 Pa Protactinium 231.03588	92 U Uranium 238.0289	93 Np Neptunium (237)	94 Pu Plutonium (244)	95 Am Americium (243)	96 Cm Curium (247)	97 Bk Berkelium (247)	98 Cf Californium (251)	99 Es Einsteinium (252)	100 Fm Fermium (257)	101 Md Mendelevium (258)	102 No Nobelium (259)	103 Lr Lawrencium (262)

Figure 1. Red-highlighted elements represent those elements that can be quantified by ICP-MS.

3. Digestion of Nanomaterials and Biologicals Containing Nanomaterials

For most nanoparticle samples in non-biological matrix, it is sufficient to use only the appropriate concentrated acids for digestion (Table 1). This can be confirmed, generally, by dissolving multiple samples and checking for any variance in their concentrations. Should the samples appear (both visibly and through varying concentrations) to not completely digest, samples can then be subjected to microwave digestion. Table 1 lists several elements used in nanoformulation and offers an acid or acid mixture for the nanomaterial digestion.

For biological samples and certain nanoparticle samples (e.g. cerium), the NCL routinely follows the microwave digestion protocol written by Yu, Wood, and Long (NIST) as outlined in PCC-8 and PCC-9. The NCL has worked with a variety of biological samples, including liver, spleen, kidney, colon, lungs, feces, urine, bile, blood, and plasma. Table 2 offers some tips for proper digestion of these matrices.

The NIST protocol also offers procedures for the set-up and operation of the ICP-MS instrument, proper balance technique, and calculation of the measured metal content. Caution should be used in the handling of all nanomaterials, biologicals and acidic reagents.

4. Additional Considerations:

- 4.1 Run a semi-quantitative analysis of the sample initially, just to estimate approximate sample concentration and to survey and scan for any other metals present in the sample.
- 4.2 Calibration Curve
 - 4.2.1 Run the calibration curve using NIST SRM for the analyte at both the beginning and end of the run. This will help correct for instrumental drift and anomalies in the calibration standards (long term detector fluctuations).
- 4.3 Internal Standard
 - 4.3.1 Run an internal standard to help account for fluctuations from individual measurement to individual measurement (short term detector fluctuations). This internal standard is a different metal SRM than the one that is being analyzed for in the sample.

4.4 Controls

- 4.4.1 Run various concentrations along the calibration range. This will help ensure the accuracy of the calibration standards and the efficacy of the digestion procedure.

Currently, the only appropriate nanoparticle controls available are the NIST gold nanoparticle standards, NIST RMs 8011, 8012, and 8013; however these may not be entirely appropriate for a given sample. Although NIST does not sell other nanoparticle reference materials, these standards can still prove useful in validating the calibration curve.

4.5 Spiking Experiments

- 4.5.1 Spiking Internal Standards

To assess accuracy in sample preparation, an internal standard consisting of a separate, non-interfering element (both different from the analyte and internal standard that is being T'ed in prior to nebulization) may be added prior to digestion. This standard is useful for tracking any errors associated from dilution of the sample, either by weighing or pipetting. By calculating a percent recovery,

$$\left(\frac{\text{Concentration Measured}}{\text{Theoretical Concentration}} \times 100 \right)$$

the error associated with the ICP-MS measurement can be further expanded.

- 4.5.2 Spiking Analyte and Standard Addition Calibration

When there is insufficient material to create matrix matched calibration standards it becomes important to run spike recovery samples. This works by spiking an unknown sample with a known amount of analyte reference material prior to digestion, in parallel with an unspiked sample. When the concentration from the unknown sample is subtracted from the sample containing the unknown and the spiked analyte, the remainder can be used to determine the spike recovery. This value can be used to expand the error and correct for any signal depression or elevation effects caused by the matrix. In addition, a standard addition calibration curve can be constructed by adding three additional spiked standards of increasing

concentration. The usefulness of an internal calibration curve is that there is no need to make matrix matched standards, as the internal spiking corrects for matrix effects. This method is extremely labor intensive and is not feasible for use when there are numerous unknown samples. However, constructing one standard addition calibration curve as an internal check versus an external calibration curve is a good way to validate the accuracy of your external calibration curve and standards.

4.6 Biologicals

- 4.6.1 Calibration standards and controls should be run “Matrix Matched” to the samples. Changes in acid concentration and matrix content can affect the signal of the analyte.
- 4.6.2 “Quantitative Blanks” should also be prepared when biologicals are used. These are prepared from control animals, and will provide baseline counts of analyte should any element be naturally occurring in matrix.
- 4.6.3 The largest difficulty in dealing with biological samples is ensuring the starting sample is completely homogeneous. Solid tissues or other biological samples need to either be: 1) used in whole or 2) completely homogenized so that a proper random sampling can be conducted. This is important for creating an accurate representation of the concentrations of metals in the sample.

Table 1. Common Acids Used for the Digestion of Nanomaterials.

Element	Digestive Acid(s)	Additional Information
Gold	Aqua Regia (HNO ₃ , HCl; 1:4)	Must use a mixture of HNO ₃ and HCl; solution should become clear to yellow after digestion depending on gold concentration; Gold is “sticky” and will require addition rinses between samples to ensure a sufficiently low background during analysis.
Platinum	Aqua Regia (HNO ₃ , HCl; 1:4)	Must use a mixture of HNO ₃ and HCl; solution should become clear to yellow after digestion depending on gold concentration.
Nickel	HNO ₃	Solution should become clear to light blue depending on concentration; may be significant amount of interference if analyzed at masses 58, 62 amu.
Arsenic	HNO ₃	HNO ₃ is sufficient for digestion; may contain interferences at analyzed mass (75 amu). If digesting with microwave, As will leech into teflon microwave tubes leaving a residual As signal for subsequent samples. Quartz microwave vessels should be used when available.
Titanium	HNO ₃ , HF (5:1)	Solution should become clear. HF is hazardous to both health and ICP-MS instrumentation. Proper health safety must be observed. To protect instrument, sample must be diluted sufficiently prior to analysis ([HF]< 0.25%).
Cobalt	HNO ₃	Solution should become clear to pink upon digestion depending on concentration of analyte.
Gadolinium	HNO ₃	HNO ₃ is sufficient for digestion.
Iron	HNO ₃ , HCl	Will dissolve in HNO ₃ , however digestion is slow and may need microwave assistance. Addition of HCl aids in digestion, and microwave assistance is not needed. Significant interferences arise when analyzing at mass 56 (due to ArO formed in plasma), analysis is generally carried out at mass 57 with concentration kept in ppm range. Contamination of samples with externally introduced Fe is also an issue.
Cerium	HNO ₃ , H ₂ O ₂ (5:1)	HNO ₃ is not sufficient to digest even with microwave assistance. Addition of H ₂ O ₂ along with microwave digestion is necessary for digestion. CeO ₂ and Ce ²⁺ formed readily in plasma, instrument must be tuned to minimize the formation of these species prior to analysis.
Silver	HNO ₃	HNO ₃ is sufficient for digestion. Must avoid the introduction of Halogens during sample processing, otherwise AgX (where x= Cl, Br, I) will form. AgX is insoluble in both acid or water and will not be easily nebulized causing a decrease in measured Ag concentration.

Table 2. Digestion Information for Biological Samples.

Biological	Digestion Information
Liver	Fatty tissue which requires more acid (10 mL minimum) and longer microwave times to digest all of the fats.
Spleen, Kidney, Colon, Lungs	Microwave digest in concentrated acid (acid type depends on metal nanoparticle).
Feces	May require H ₂ O ₂ in addition to acid for digestion. There will be some insoluble inorganics remaining after microwave digestion, but these should not contain analyte.
Urine, Bile	Microwave digestion not necessary, however lack of microwave digestion will require instrument cleaning more often.
Blood, Plasma	Microwave digest in concentrated acid (acid type depends on metal nanoparticle).

5. Abbreviations

ICP-MS	inductively coupled plasma-mass spectrometry
NCL	Nanotechnology Characterization Laboratory
NIST	National Institute of Standards and Technology
PCC	physicochemical characterization
PFA	paraformaldehyde
RMs	Reference Materials
SRM	Standard Reference Material