Supplement to PCC-8 & PCC-9

Metal Quantitation by Inductively Coupled Plasma-Mass Spectrometry

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Please cite this protocol as:

Clogston JD, Hansen M, Supplement to PCC-8 & PCC-9: Metal Quantitation by Inductively Coupled Plasma-Mass Spectrometry. https://ncl.cancer.gov/resources/assay-cascade-protocols

DOI: 10.17917/B68Q-T959

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 MΩ-cm de-ionized water.

The Periodic Table of the Elements

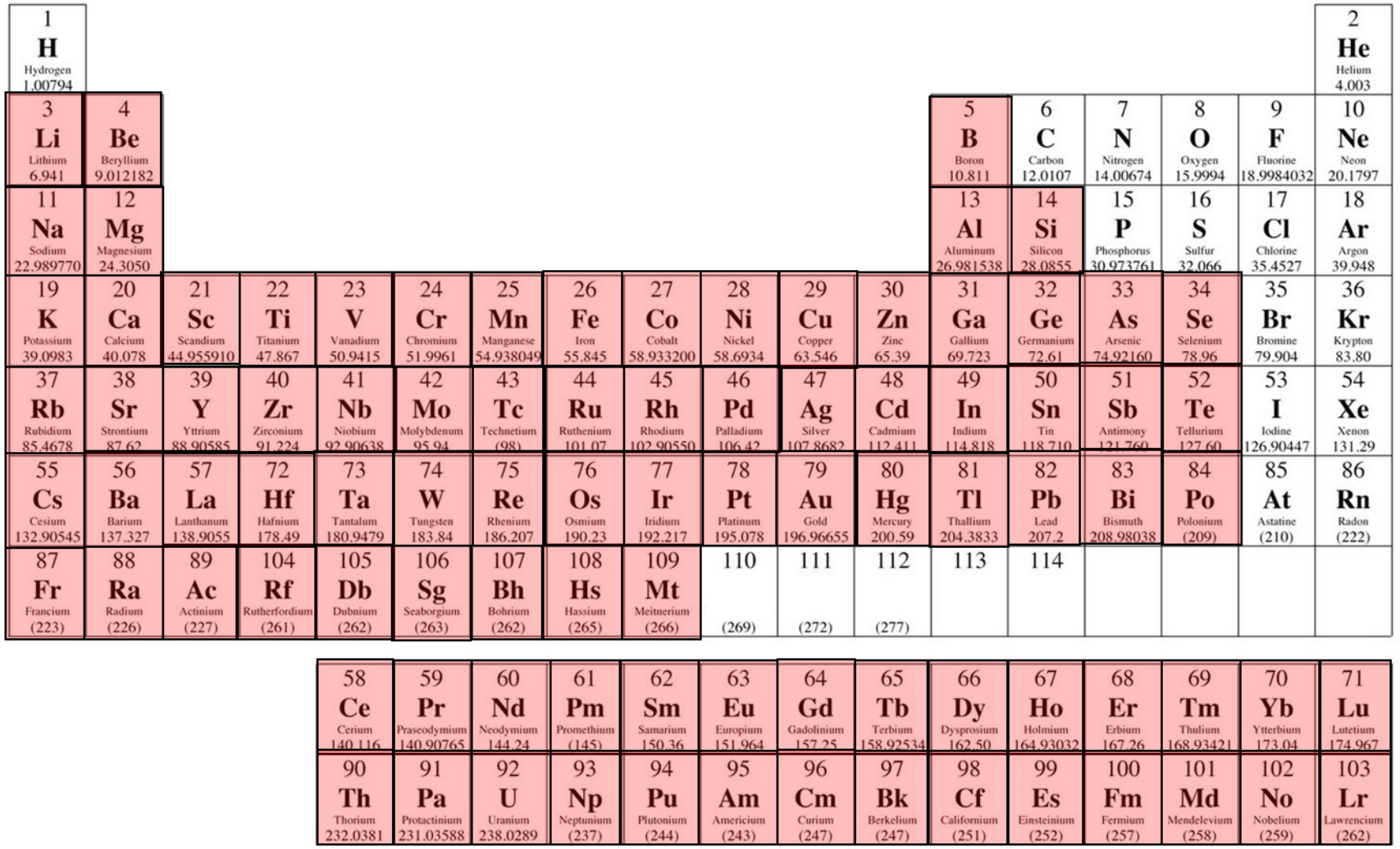

Figure 1. Red-highlighted elements represent those elements that can be quantified by ICP-MS.

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,

$$\frac{Concentration\ measured}{Theoretical\ concentration} * 100$$

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_2O_2 (5:1)	HNO_3, is not sufficient to digest even with microwave assistance. Addition of H_2O_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_2O_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