Designation: E3269 – 21

Standard Test Method for Determination of the Mass Fraction of Particle-Bound Gold in Colloidal Gold Suspensions[1]

This standard is issued under the fixed designation E3269; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method describes the use of inductively coupled plasma optical emission spectrometry (ICP-OES; also includes ICP-AES, where AES is atomic emission spectrometry) or inductively coupled plasma mass spectrometry (ICP-MS) for the determination of the mass fraction of particle bound gold (Au) in colloidal Au suspensions. Particle bound Au is defined as the mass of Au associated with the nanoparticle (NP) fraction and strongly adsorbed to the particle surface. Unbound Au is the fraction of Au in the native suspension not associated with the Au nanoparticle fraction that is, the dissolved Au existing in solution as a complex or free ion. The mass fraction of particle bound Au is determined by subtracting the mass fraction of unbound Au measured in acidified subsamples of the particle-free supernatant from the total Au mass fraction measured in acid-digested subsamples of the colloidal Au suspension. The particle-free supernatant is obtained after centrifugation of the colloidal Au suspension. This standard prescribes the use of an appropriate internal standard and calibration using either external standardization or single-point standard additions.

1.2 Colloidal gold suspensions with AuNP diameters ranging from 1 nm to 100 nm can be determined with this method.

1.3 The standard is not limited to particles with a uniform Au composition and may be applicable to a core-shell particle with a Au shell treatment.

1.4 This standard is specific to Au. The method may be applicable to other elements measurable by ICP-OES or ICP-MS but is limited to nanoparticles that are not reactive in aqueous suspension.

1.5 No detailed instructions for operating instrumentation are provided because of differences among various makes and models. Instead, the analyst shall follow the instructions provided by the manufacturer of their particular ICP-OES, ICP-MS or centrifuge instrument, especially with regard to optimization of the instrument settings.

1.6 The values stated in SI units are to be regarded as standard. No other units of measurements are included in this standard.

1.7 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.8 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D1129 Terminology Relating to Water

D1193 Specification for Reagent Water

D4210 Practice for Intralaboratory Quality Control Procedures and a Discussion on Reporting Low-Level Data (Withdrawn 2002)[3]

D5673 Test Method for Elements in Water by Inductively Coupled Plasma—Mass Spectrometry

D7035 Test Method for Determination of Metals and Metalloids in Airborne Particulate Matter by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES)

D7439 Test Method for Determination of Elements in Airborne Particulate Matter by Inductively Coupled Plasma–Mass Spectrometry

E691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method

E1613 Test Method for Determination of Lead by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES), Flame Atomic Absorption Spectrometry

[1] This test method is under the jurisdiction of ASTM Committee E56 on Nanotechnology and is the direct responsibility of Subcommittee E56.02 on Physical and Chemical Characterization.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[3] The last approved version of this historical standard is referenced on www.astm.org.

(FAAS), or Graphite Furnace Atomic Absorption Spectrometry (GFAAS) Techniques (Withdrawn 2021)[3]

2.2 *ISO Standards:*[4]

ISO Guide 30:2015 Reference materials — Selected terms and definitions

ISO 11885:2007 Water quality — Determination of selected elements by inductively coupled plasma optical emission spectrometry (ICP-OES)

ISO 15202-3:2004 Workplace air — Determination of metals and metalloids in airborne particulate matter by inductively coupled plasma atomic emission spectrometry — Part 3: Analysis

ISO 22036:2008 Soil quality — Determination of trace elements in extracts of soil by inductively coupled plasma — atomic emission spectrometry (ICP-AES)

ISO/TS 19590:2017(E) Nanotechnologies — Size distribution and concentration of inorganic nanoparticles in aqueous media via single particle inductively coupled plasma mass spectrometry

3. Terminology

3.1 *Definitions:*

3.1.1 *blank test solution, n*—solution prepared in the same way as the test sample solution but omitting the test portion. **ISO 22036:2008**

3.1.2 *calibration blank, n*—volume of water containing the same acid matrix and internal standard as the calibration standards, but without the addition of any stock or intermediate standard solution. **adapted from D5673**

3.1.3 *calibration standards, n*—series of known standard solutions used by the analyst for calibration of the instrument (that is, preparation of the analytical curve). **D5673**

3.1.3.1 *Discussion*—Matrix matching to the test sample solution is used in the preparation of calibration standards and calibration blank.

3.1.4 *certified reference material (CRM), n*—reference material (RM) characterized by a metrologically valid procedure for one or more specified properties, accompanied by an RM certificate that provides the value of the specified property, its associated uncertainty, and a statement of metrological traceability. **ISO Guide 30:2015**

3.1.5 *colloidal suspension, n*—any material in suspension with a nominal particle size less than 100 nm. **D1129**

3.1.5.1 *Discussion*—Other documents define colloidal as a state of subdivision, implying that the molecules or polymolecular particles dispersed in a medium have at least in one direction a dimension roughly between 1 nm and 1 µm, or that in a system discontinuities are found at distances of that order (**1**)[5] or in terms of being significantly affected by Brownian (thermal) motion when suspended in a liquid (**2**).

3.1.6 *continuing calibration blank, n*—a solution containing no analyte added, that is used to verify blank response and freedom from carryover. **E1613**

3.1.7 *continuing calibration verification (CCV), n*—a solution (or set of solutions) of known analyte concentration used to verify freedom from excessive instrumental drift; the concentration is to be near the mid-range of a linear calibration curve and may be one of the actual calibration standards. **adapted from E1613**

3.1.7.1 *Discussion*—The continuing calibration verification must be matrix matched to the acid content present in the test samples. It must be analyzed before and after all samples and at a frequency of not less than every ten samples. The measured value shall fall within ±10 % of the known value.

3.1.8 *Gold (Au) mass fraction, n*—total mass fraction of Au determined in the native suspension following complete digestion or the sum of the mass fraction of Au as determined in the dissolved (unbound state) and particle bound state of a sample. **adapted from ISO 11885:2007**

3.1.9 *intermediate stock standard solution, n*—diluted solution prepared from one or more of the primary stock standard solutions. **D1129**

3.1.10 *internal standard, n*—pure element(s) added in known amount(s) to a solution. **D5673**

3.1.10.1 *Discussion*—The internal standard is used to measure the instrument response relative to the other analytes that are components of the same solution. The internal standard should be an element that is not a sample component.

3.1.11 *laboratory reagent blank, n*—aliquot of reagent water that is treated exactly as a sample including exposure to all labware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. **D1129**

3.1.11.1 *Discussion*—The laboratory reagent blank is used to determine if test method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.1.12 *mass fraction component X, n*—defined as mass of X divided by mass of solution.

3.1.13 *matrix interference, n*—effect of a matrix component that might cause an analytical bias. **adapted from D1129**

3.1.14 *matrix matching, n*—a technique used to minimize the effect of the test sample solution matrix on the analytical results. **ISO 15202-3:2014**

3.1.14.1 *Discussion*—Matrix matching involves preparing calibration standard solutions in which the concentrations of acids and other major solvents and solutes are matched with those in the test solutions. With unknown sample matrices, exact matching is not possible. In this case, the technique of standard additions and the use of an appropriate internal standard help to compensate for multiplicative interference.

3.1.15 *memory effect, n*—signal from an element or isotopes of an element in a previous sample that contribute to the signal measured in a new sample. **adapted from D5673**

3.1.16 *method detection limit (MDL), n*—the minimum concentration of an analyte that can be identified, measured

[4] Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036, http://www.ansi.org.

[5] The boldface numbers in parentheses refer to a list of references at the end of this standard.

and reported with a 99 % confidence that the analyte concentration is greater than zero. This confidence level is determined from analysis of a sample in a given matrix containing the analyte. **D5673**

3.1.16.1 *Discussion*—The MDL is also known as the limit of detection (LOD).

3.1.17 *method quantitation limit (MQL), n*—the minimum concentration of an analyte that can be measured within predefined goals for imprecision and bias, ordinarily taken to be at least ten times the standard deviation of the mean blank signal. **adapted from D7035**

3.1.17.1 *Discussion*—The MQL is also known as the limit of quantitation (LOQ).

3.1.17.2 *Discussion*—Calculation of the MDL and MQL in accordance with Test Method D7035 is prescribed in this standard, though alternative approaches may be used if better suited for the intended purpose of the measurement. Alternative approaches are described in Practice D4210 and Refs (**3 and 4**).

3.1.18 *particle-bound Au, n*—Au associated with the nanoparticle and strongly adsorbed to the particle surface.

3.1.19 *primary measurement standard, n*—measurement standard that is designated or widely acknowledged as having the highest metrological qualities and whose property value is accepted without reference to other standards of the same property or quantity, within a specified context. **ISO Guide 30:2015**

3.1.20 *primary stock standard solution, n*—solution used for preparation of the intermediate stock standard solution or calibration standards, containing the analyte of interest at a certified concentration traceable to a primary measurement standard from a recognized Certified Reference Material producer. **adapted from ISO 15202-3:2004**

3.1.21 *spectral interference, n*—an interference caused by the signal from a species other than the analyte of interest. **adapted from D7035**

3.1.22 *standard addition(s), n*—a procedure for the determination of the concentration of a particular species in a sample by adding known amounts of that species to the sample solution and recording the change in signal. **adapted from D1129**

3.1.23 *test sample solution, n*—solution prepared with the fraction (test portion) of the test sample according to the appropriate specifications, such that it can be used for the envisaged measurement. **ISO 11885:2007**

3.1.24 *unbound Au, n*—dissolved Au existing in solution as a complex or free ion.

3.1.24.1 *Discussion*—Unbound Au is the fraction of Au in the native suspension not associated with the Au nanoparticle fraction or strongly adsorbed to the Au nanoparticle surface.

3.1.25 *volume fraction component X, n*—defined as volume of X divided by volume of solution.

4. Summary of Test Method

4.1 Subsamples of Au nanoparticle (AuNP) suspensions are digested with acid, and the Au mass fractions of the resulting solutions are measured. Additional subsamples of the AuNP suspensions are centrifuged to remove the NPs from suspension, and the Au mass fractions of the acid digested particle-free suspension fluid solutions are measured. The mass fraction of AuNPs (bound Au) in the AuNP suspension is calculated as the difference between the Au mass fraction value obtained for the digested samples and the Au mass fraction value obtained for the corresponding acid digested particle-free suspension fluid solutions. This test method describes procedures for the use of ICP-OES or ICP-MS for estimating the Au mass fraction values in the digested suspension and particle-free suspension fluid subsamples. Two methods of quantitation are prescribed: (*1*) external calibration with use of internal standard, and (*2*) single-point standard additions with use of internal standard.

4.2 Basic knowledge of and experience with ICP-OES or ICP-MS and centrifugation are assumed.

5. Significance and Use

5.1 Au nano-objects in various shapes (that is, rods, particles) are increasingly used for a wide variety of applications. Medical applications of AuNPs, such as targeted drug delivery, tumor detection, and treatment are becoming more common (**5**). AuNPs have unique optical properties related to their size and their surface can be readily functionalized. Though Au is recognized to be inert and biocompatible in its bulk form, the behavior of Au nano-objects in biological systems and the environment must be tested to ensure their inertness and safety (**6**). It is important to know whether prepared and stored suspensions of AuNPs contain Au in its bound state (commonly Au (0) and particle adsorbed species) or ionized state (commonly, Au (I) or Au (III)) to attribute the biological response to the appropriate species. Krug, et al., concluded that the significance of toxicity studies is considerably reduced in those cases where the material properties of the nanomaterial suspensions were not characterized prior to and during the study (**7**). Furthermore, the analyte mass fraction of particle bound species is used with knowledge of particle size to compute particle number concentration.

6. Interferences

6.1 Au is an element known to exhibit memory effects and the potential exists for long signal stabilization and wash-out times. Dilute nitric acid (HNO_3) alone (1 % volume fraction HNO_3) is not an appropriate diluent for Au. The use of a dilute acid or acid mixture mimicking that used for Au dissolution, for example hydrochloric acid (HCl) or aqua regia in combination with thiourea, has been shown to be effective in reducing memory effects (**8**).

6.2 *Spectral Interference*—Precautions should be exercised to avoid those interferences normally associated with the determination of Au and prescribed internal standards, copper (Cu) or platinum (Pt) using ICP-OES or Pt using ICP-MS. Blank, sample, and internal standard test solutions shall be used to check for the absence of spectral interference.

6.3 *Matrix Interference*—ICP-OES and ICP-MS are subject to matrix effects and the potential exists for signal drift and

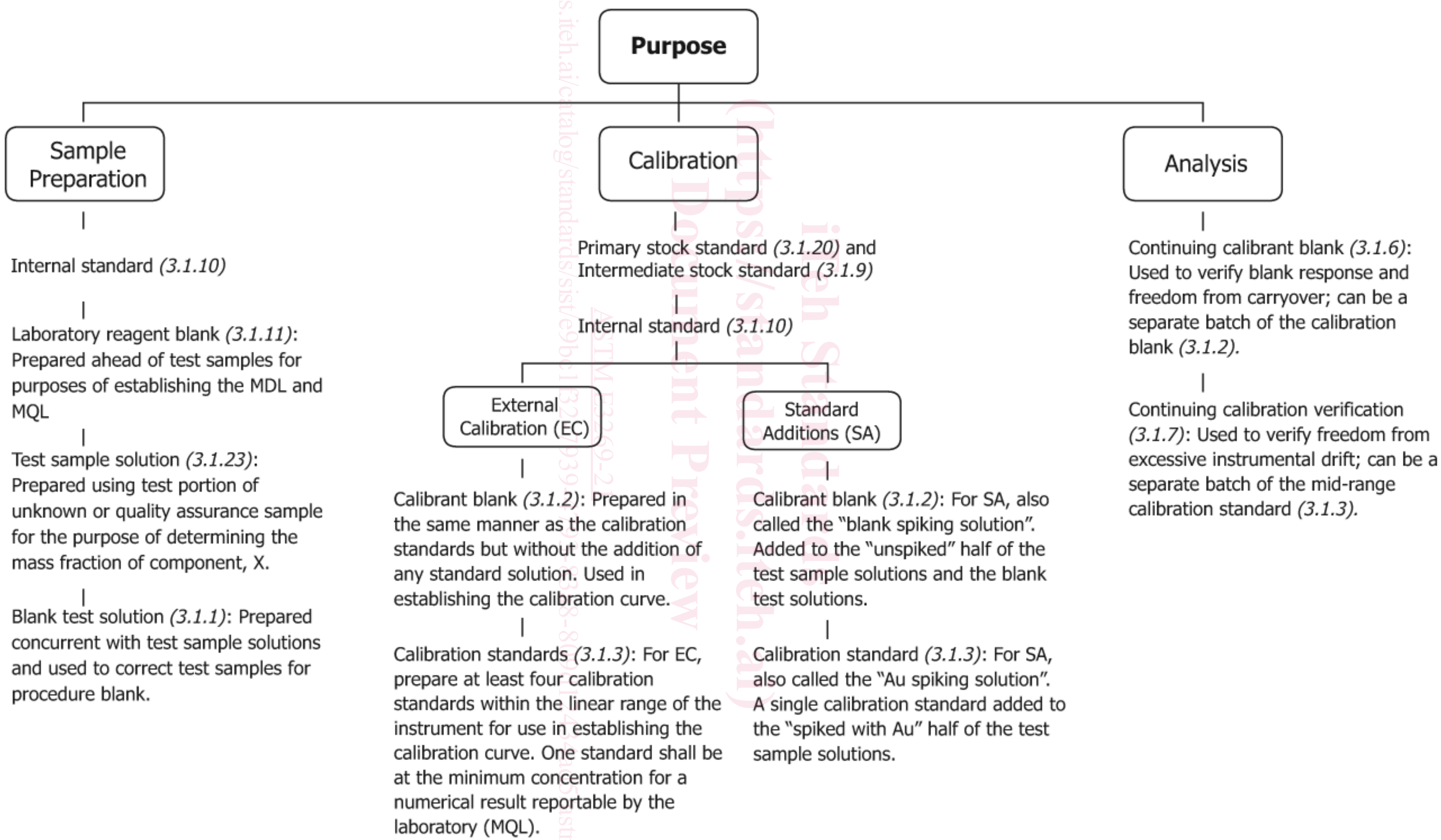
Purpose
Sample Preparation
Calibration
Analysis
Internal standard (3.1.10)
Laboratory reagent blank (3.1.11): Prepared ahead of test samples for purposes of establishing the MDL and MQL
Test sample solution (3.1.23): Prepared using test portion of unknown or quality assurance sample for the purpose of determining the mass fraction of component, X.
Blank test solution (3.1.1): Prepared concurrent with test sample solutions and used to correct test samples for procedure blank.
Primary stock standard (3.1.20) and Intermediate stock standard (3.1.9)
Internal standard (3.1.10)
External Calibration (EC)
Standard Additions (SA)
Calibrant blank (3.1.2): Prepared in the same manner as the calibration standards but without the addition of any standard solution. Used in establishing the calibration curve.
Calibration standards (3.1.3): For EC, prepare at least four calibration standards within the linear range of the instrument for use in establishing the calibration curve. One standard shall be at the minimum concentration for a numerical result reportable by the laboratory (MQL).
Calibrant blank (3.1.2): For SA, also called the "blank spiking solution". Added to the "unspiked" half of the test sample solutions and the blank test solutions.
Calibration standard (3.1.3): For SA, also called the "Au spiking solution". A single calibration standard added to the "spiked with Au" half of the test sample solutions.
Continuing calibrant blank (3.1.6): Used to verify blank response and freedom from carryover; can be a separate batch of the calibration blank (3.1.2).
Continuing calibration verification (3.1.7): Used to verify freedom from excessive instrumental drift; can be a separate batch of the mid-range calibration standard (3.1.3).

FIG. 1 Type and Purpose of Solutions Used in This Test Method

multiplicative interferences (signal enhancement or depression). Properly chosen internal standards can reduce bias from these types of interferences. The method of standard additions in combination with an internal standard is the most effective means to reduce systematic error caused by the matrix.

7. Apparatus

7.1 Clean, *low-density polyethylene bottles (LDPE)*, 0.05 L capacity with polypropylene screw cap closure or similar clean, metal-free polymer labware that is unreactive in concentrated aqua regia.

7.2 Adjustable *pipets* capable of delivering liquid volumes in the range 0.00015 L to 0.025 L.

7.3 *Analytical balance*, capable of weighing to 1×10^{-7} kg

7.4 *Vortex mixer.*

7.5 *Bath sonicator.*

7.6 *ICP-OES instrument.*

NOTE 1—Differences exist among various models and manufacturers of instruments. It is the responsibility of the analyst to verify that the instrument configuration and operating conditions used satisfy the analytical requirements of this method and to maintain quality control data confirming instrument performance and analytical results.

7.7 *ICP-MS instrument* (see Note 1).

7.8 *Centrifuge* (see Note 1).

7.8.1 The centrifuge must produce sufficient force to completely remove AuNPs from suspension for the given nominal particle size distribution of the sample. A high-speed centrifuge with relative centrifugal force (RCF) of 20 000 × g or greater (× g, Earth's gravitational acceleration = 9.81 m/s) is needed

NOTE 2—Falabella, et al., pelletized 5 nm, 20 nm and 60 nm AuNPs by centrifuging at 1570 rad s^{-1} (15 000 r·min^{-1}), 523 rad s^{-1} (5000 r·min^{-1}), and 262 rad s^{-1} (2000 r·min^{-1}), respectively for 20 min using a Beckman CoulterXL-A ultracentrifuge with a titanium 4 place rotor (**9**), but centrifugation conditions strongly depend on the geometry of the centrifuge (**10**). Longer centrifugation times and an RCF greater than 150 000 × g are generally needed to achieve sedimentation of AuNPs with diameters < 5 nm. Eq. 1 in Section 13.1 can be used for guidance.

NOTE 3—The centrifuge should have a cooling option capable of maintaining samples at 20 °C for the duration of the centrifugation.

8. Reagents and Materials

8.1 *Purity of Reagents*—Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society.[6] Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

8.2 *Purity of Water*—Unless otherwise indicated, references to water shall conform to the resistivity requirement of 18 MΩ·cm for Type I water in Specification D1193.

8.3 *Concentrated HNO_3* (trace metal grade).

8.4 *Concentrated HCl* (trace metal grade).

8.4.1 *HCl diluent solution* 5 % volume fraction HCl.

NOTE 4—This diluent solution is used to improve the chemical stability of sample and standard solutions as well as improve the stability of the ICP-OES signal profile and reduce the wash out time of the Au signal from the ICP-OES sample introduction system.

8.4.1.1 Prepare the 5 % volume fraction HCl diluent solution as follows: dilute concentrated HCl with water by a volumetric factor of 20.

8.5 *Crystalline Thiourea:*

8.5.1 *Thiourea diluent solution* 0.5 % mass concentration thiourea in 2.4 % volume fraction HCl and 0.4 % volume fraction HNO_3.

NOTE 5—This diluent solution is used to improve the chemical stability of sample and standard solutions as well as improve the stability of the ICP-MS signal profile and reduce wash out time of the Au signal from the ICP-MS sample introduction system.

8.5.1.1 Prepare the thiourea diluent solution as follows: Add 5.00 g of crystalline thiourea to a 1 L, tared, clean fluorinated ethylene propylene (FEP) bottle followed by 0.5 L of water. Cap and mix to dissolve the thiourea. Add 0.022 L concentrated HCl. Cap and mix. Add 0.0004 L concentrated HNO_3. Dilute to a final volume of 1.00 L with water. Cap and mix. The solution is stable for one week.

8.6 *Au primary stock standard solution,* NIST Standard Reference Material (SRM) 3121 Au Standard Solution, or any similar Certified Reference Material (CRM) solution with certified Au mass fraction greater than or equal to 0.001 g/g in dilute HCl.

NOTE 6—When diluted by a factor of 100, the solution should not contain a level of Cu or Pt or the chosen internal standard that is detectable by ICP-OES.

NOTE 7—When diluted by a factor of 30 000, the solution should not contain a level of Pt or the chosen internal standard that is detectable by ICP-MS.

8.7 *Cu primary stock standard solution or chosen internal standard for ICP-OES* shall contain a known mass fraction greater than or equal to 0.001 g/g in dilute HCl or HNO_3.

NOTE 8—When diluted by a volumetric factor of 20, the solution must not contain Au at a level that is detectable by ICP-OES.

8.8 *Pt primary stock standard solution or chosen internal standard for ICP-MS* shall contain a known mass fraction greater than or equal to 0.001 g/g in dilute HCl or HNO_3.

NOTE 9—When diluted by a volumetric factor of 30 000, the solution should not contain Au at a level that is detectable by ICP-MS.

8.9 *Quality assurance sample,* an RM or CRM consisting of a suspension of AuNPs similar to the suspensions to be analyzed and having an assigned value and uncertainty for the mass fraction of particle-bound Au.

9. Hazards

9.1 Concentrated HCl and HNO_3 are corrosive. The vapor of each is an irritant. HNO_3 acid is oxidizing. When mixed in an approximate 3:1 molar ratio of concentrated HCl:HNO_3, aqua regia is formed. Aqua regia solutions are extremely

[6] *ACS Reagent Chemicals, Specifications and Procedures for Reagents and Standard-Grade Reference Materials*, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

corrosive and may result in explosion (when mixed with organic compounds) and skin burns if not handled with extreme caution. Avoid exposure by contact with the skin or eyes, or by inhalation of fumes. Use suitable personal protective equipment (including impermeable gloves, safety goggles, and laboratory coat) as established by a hazard assessment when working with concentrated acids and aqua regia. Open-vessel sample dissolution should be performed in a fume hood.

9.1.1 When preparing aqua regia, add HNO_3 to HCl slowly.

9.1.1.1 An exothermic reaction will occur upon mixing the two acids.

9.2 The diluent, 5 % volume fraction HCl is corrosive. Avoid exposure by contact with skin or by inhalation. Use suitable personal protective equipment (gloves, safety goggles, and laboratory coat) as established by a hazard assessment for HCl.

9.3 The diluent, 0.5 % mass concentration thiourea in 2.4 % volume fraction HCl and 0.4 % volume fraction HNO_3 is corrosive. Avoid exposure by contact with skin or by inhalation. Use suitable personal protective equipment (gloves, safety goggles, and laboratory coat) as established by a hazard assessment for HCl, HNO_3, and thiourea.

10. Preparation of Calibration Standards

10.1 Prepare intermediate stock standard solutions of Au and the appropriate internal standard from the primary stock standard solutions as required using the appropriate diluent solution.

NOTE 10—For improved accuracy and precision, the user should prepare calibration and internal standard solutions on a mass fraction basis.

10.1.1 Cu or Pt or other appropriate element chosen on the basis of a performed correlation study should be used as the internal standard for ICP-OES analysis. The HCl diluent solution (see 8.4.1) should be used in the preparation of the intermediate Au and internal standard stock standard solutions for ICP-OES analysis.

10.1.2 Pt or other appropriate element chosen on the basis of a performed correlation study should be used as the internal standard for ICP-MS analysis. The thiourea acid diluent solution (see 8.5.1) should be used in the preparation of intermediate stock standard Au and Pt solutions for ICP-MS analysis.

NOTE 11—A second set of intermediate stock standard solutions prepared by serial dilution of the first set of intermediate stock standard solutions may be required for ICP-MS analysis to attain the appropriate concentration range for preparation of the final set of calibration standard solutions used to establish the calibration curve.

10.2 *Preparation of Calibration Standards for Calibration by External Calibration:*

10.2.1 From the primary stock or intermediate stock standards, prepare a fresh set of calibration solutions containing a known amount of Au, covering the anticipated range of concentrations for the diluted test samples but within the linear range of the instrument, using the appropriate diluent and matching the acid concentrations in the test samples to the extent possible.

NOTE 12—If a rough estimate of the Au mass fraction in the test sample is unavailable, preliminary analyses should be performed to obtain a rough estimate.

10.2.2 Prepare a calibration blank solution in the same manner as the calibration solutions but without the addition of any stock or intermediate standard solution.

NOTE 13—The calibration blank solution is used in establishing the calibration curve.

10.2.3 Known amount of internal standard (prepared as in accordance with 10.1) should be added to the calibration blank and each calibration solution at a concentration level of similar nominal concentration to the test samples and within the linear range of the instrument. Record the mass fraction of internal standard in the calibration blank and each calibration solution.

10.2.4 At least four calibration standards shall be used to establish the calibration curve. One standard shall be at the minimum concentration for a numerical result reportable by the laboratory. This approach permits corrective actions if results of the continuing calibration blank exceeds this concentration.

10.3 *Preparation of Calibration Solutions for Calibration by Single-Point Standard Additions:*

10.3.1 *Au Spiking Solution*—From the stock or intermediate stock standard, prepare a fresh standard solution containing a known mass fraction of Au. The mass fraction of Au in the spiking solution should be approximately equal to three times the average of the estimated mass fraction values of Au in the suspensions to be analyzed. After the Au spike is added, the Au mass fraction of the samples should be increased by at least a factor of two. The diluted standard solution will hereafter be referred to as the "Au spiking solution."

NOTE 14—If a rough estimate of the Au mass fraction in the test sample is unavailable, preliminary analyses should be performed to obtain a rough estimate.

10.3.2 *Blank Spiking Solution*—Prepare a calibration blank solution in the same manner as the Au spiking solution but without the addition of any stock or intermediate standard solution. This blank solution will hereafter be referred to as the "blank spiking solution."

NOTE 15—The "blank spiking solution" can additionally be used as a continuing calibration blank which is used to verify blank response and freedom from carryover.

11. Sampling

11.1 *Resuspension:*

11.1.1 If the sample was stored in a refrigerator, allow it to come to room temperature. Prior to opening the sample container, make sure the suspension is thoroughly mixed. Invert the container multiple times to ensure complete resuspension of any settled material. Vortexing and sonication should be used: vortex for 30 s and sonicate for 1 min in a bath sonicator.

11.2 *Sub-Sampling:*

11.2.1 Analytical balance linearity and accuracy shall be checked daily with calibrated ASTM Class 4 masses.

11.2.2 Immediately following resuspension (11.1), gravimetrically aliquot each of a predetermined number of 0.2 g to 1 g test sample portions from each ampoule or sample bottle of

each unknown sample and quality assurance sample into a separate, clean, labeled, pre-weighed LDPE plastic bottle. Record the mass of each test sample portion. Test sample portion masses are determined by the difference between the mass of the clean, LDPE plastic bottle with the test portion and the mass of the empty bottle.

NOTE 16—Unknown test sample portions and quality assurance test sample portions are collectively referred to as test samples.

11.2.3 Prepare ten laboratory reagent blank solutions in total and one blank test solution for every three to five test samples, by adding 0.2 g to 1 g subsample of water to separate, clean, labeled, LDPE plastic bottles.

NOTE 17—Laboratory reagent blank solutions are used for determining the MDL and MQL. Blank test solutions are used for determining the correction for procedure blank. Both laboratory reagent blanks and blank test solutions must contain all reagents in the same volumes used in processing test samples and must be carried through the same preparation steps as test samples. Blank test solutions must be processed concurrent with test samples; laboratory reagent blanks can be prepared ahead of test samples for purposes of establishing acceptable analytical conditions.

12. Test Sample Preparation for Determination of Au Mass Fraction in Colloidal Suspension

12.1 *Digestion:*

12.1.1 To each blank test solution and test sample, add concentrated HCl and concentrated HNO_3 in a volume ratio of 3:1 HCl:HNO_3. Add HCl first, immediately followed by 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 NPs.

NOTE 18—The total volume of concentrated acid required is adjusted based on sample size. For example, 0.0003 L HCl and 0.0001 L HNO_3 is sufficient for a 0.2 g subsample of a 50 $mg \cdot kg^{-1}$ AuNP suspension; a 1 g subsample of the same suspension may require 0.001 L HCl and 0.0003 L HNO_3.

12.2 *Preparation for External Calibration with Internal Standard:*

12.2.1 Known amount of internal standard (see prepared as in accordance with 10.1) should be added to each digested test sample and blank test solution. Intensity of the added internal standard signal should be within a factor of 1, but no more than a factor of 2 of the intensity of the Au signal in the test sample. Mix thoroughly.

12.2.2 Quantitatively dilute test samples and blank test solutions in the appropriate diluent (see 8.4.1 and 8.5.1) so that the Au and internal standard signal intensities are within the linear range of the instrument.

NOTE 19—When diluted, test samples, blank test solutions and calibration samples (see 10.2) should have similar nominal internal standard concentration.

NOTE 20—Two serial dilutions of test samples and blank test solutions may be required to stay within the linear range of the instrument. Dilute the first serial dilution in water to dilute the digestion acids and prepare the second serial dilution in the appropriate diluent (see 8.4.1 and 8.5.1).

12.2.3 Compute and record the exact dilution factor and internal standard mass fraction of each test sample and blank test solution.

12.3 *Preparation for Single-Point Standard Additions with Internal Standard:*

12.3.1 In accordance with 12.2.1, add a known amount of the internal standard solution at a single concentration level to each digested test sample and blank test solution. Record the mass of the solution for each after dilution with the internal standard. Mix thoroughly.

12.3.2 Gravimetrically aliquot approximately half of each digested, internal standard diluted test sample and blank test solution into another separate, clean, plastic bottle labeled with the corresponding ampoule or sample bottle identification and the phrase “spiked with Au”, record the mass of each aliquot transferred as $m_{sp\ solu}$. The remaining half is considered the “unspiked” test solution.

12.3.3 Add a known portion of Au spiking solution into each test sample and blank test solution bottle labeled as “spiked with Au” and record the mass of each aliquot as m_{sp}.

12.3.4 Add enough Au spiking solution such that the Au concentration in the spiked test solution is approximately two to three times that of the Au concentration of the unspiked test solution.

12.3.5 Add a portion of the blank spiking solution (see 10.3.2) similar in volume to that of the added Au spiking solution to each unspiked test solution and blank test solution.

12.3.6 Dilute spiked and unspiked test solutions and blank test solutions as in accordance with 12.2.2.

13. Test Sample Preparation for Determination of Unbound Au Mass Fraction in Colloidal Suspension

13.1 *Removal of NPs from AuNP Suspensions:*

13.1.1 Resuspend test and quality assurance samples (collectively referred to as test solutions) in accordance with 11.1 and subsample in accordance with 11.2.

13.1.2 Add appropriately sized subsample aliquot to centrifuge tubes. If dilution is required to obtain a total volume based on the requirements of the centrifuge, adjust the subsample mass so that the test sample is diluted no more than a factor of two. Prepare blank test solutions in a similar manner. Record the mass of the subsample and dilution volume; compute the dilution factor.

13.1.3 Centrifuge each test solution and blank test solution at a relative centrifugal force (RCF) expected to completely remove the AuNPs in a test solution, given the nominal particle size distribution.

NOTE 21—The time, t required for sedimentation of particles of a given diameter, d using an ultracentrifuge of angular velocity, ω can be approximated using Stokes' equation:

$$t = \frac{10 \times \eta \times \ln\left(\frac{r_1}{r_0}\right)}{d^2 \times \omega^2 \times (\rho_p - \rho_L)} \quad (1)$$

where:

- t = time,
- η = the viscosity of the fluid,
- r_1 and r_0 = the radial position of the particle before and after centrifuging,
- d = the diameter of the particle,
- ω = angular velocity, and
- ρ_p and ρ_L = the particle and suspension fluid density, respectively.

NOTE 22—Eq 1 applies only to spherical particles; adjustments are needed for non-spherical particles.

NOTE 23—Livshits, et al., provide a web-based calculator that can be