



Designation: E3269 – 21

Standard Test Method for Determination of the Mass Fraction of Particle-Bound Gold in Colloidal Gold Suspensions¹

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 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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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

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 polymeric particles dispersed in a medium have at least in one direction a dimension roughly between 1 nm and 1 μ m, 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 detected, 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 (HNO₃) alone (1 % volume fraction HNO₃) 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 interad

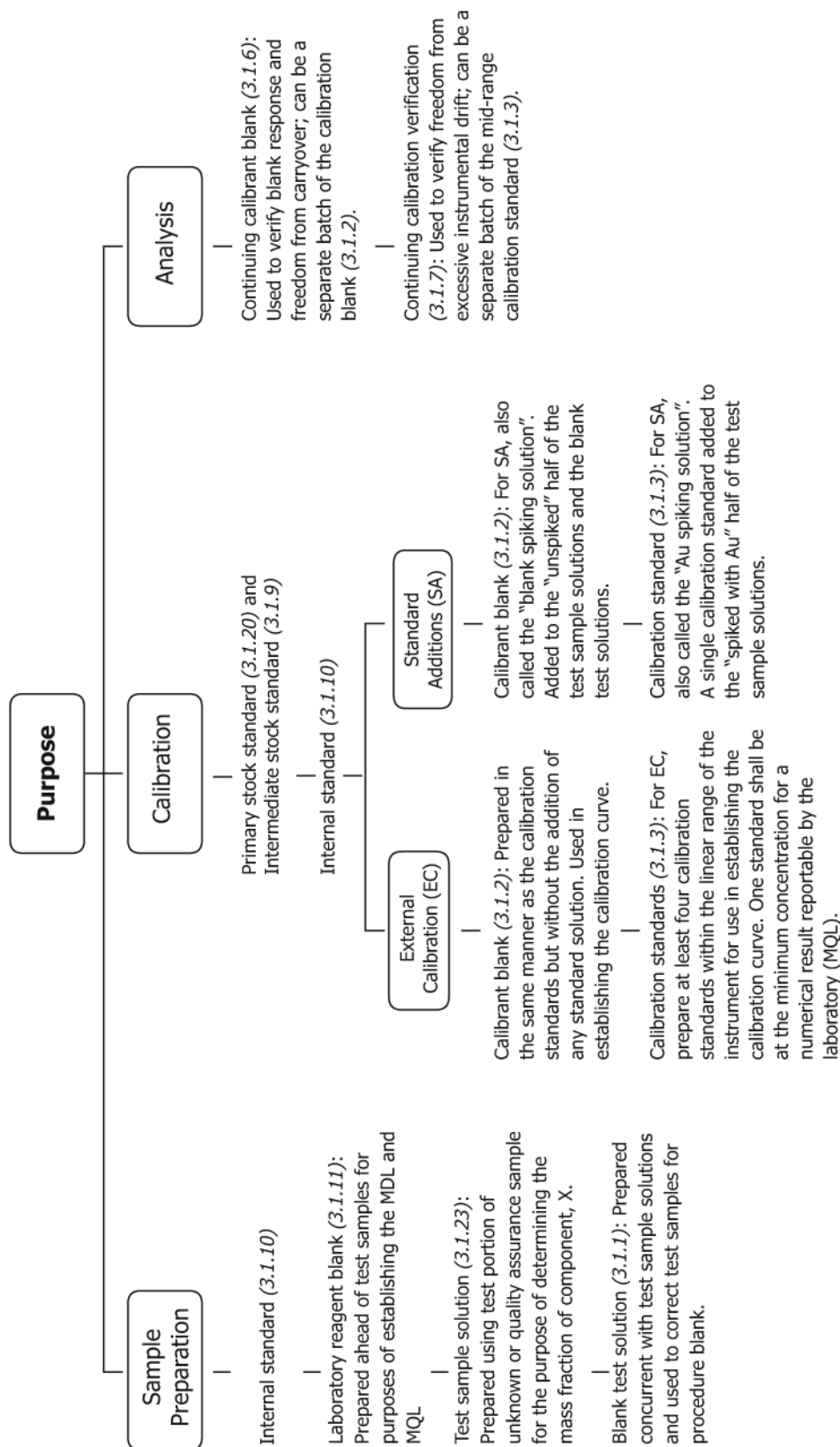


FIG. 1 Type and Purpose of Solutions Used in This Test Method