



Standard Test Method for Measurement of Airborne Metal and Metal Oxide Nanoparticle Surface Area Concentration in Inhalation Exposure Chambers using Krypton Gas Adsorption¹

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1. Scope

1.1 This test method covers determination of surface area of airborne metal and metal oxide nanoparticles in inhalation exposure chambers for inhalation toxicology studies. Surface area may be measured by gas adsorption methods using adsorbates such as nitrogen, krypton, and argon (Brunauer et al., 1938; Anderson, 1975; Gregg and Sing, 1982) (**1, 2, 3**)² or by ion attachment and mobility-based methods (Ku and Maynard, 2005) (**4**). This test method is specific to the measurement of surface area by gas adsorption by krypton gas adsorption. The test method permits the use of any modern commercial krypton adsorption instruments but strictly defines the sample collection, outgassing, and analysis procedures for metal and metal oxide nanoparticles. Use of krypton is required due to the low overall surface area of particle-laden samples and the need to accurately measure the background surface area of the filter used for sample collection. Instrument-reported values of surface area based on the multipoint Brunauer, Emmett and Teller (BET) equation (Brunauer et al., 1938; Anderson, 1975; Gregg and Sing, 1982) (**1, 2, 3**) are used to calculate surface area of airborne nanoparticles collected on a filter.

1.2 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard. State all numerical values in terms of SI units unless specific instrumentation software reports surface area using alternate units.

1.3 *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 and health practices and determine the applicability of regulatory limitations prior to use.*

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

2. Referenced Documents

2.1 ASTM Standards:³

B922 Test Method for Metal Powder Specific Surface Area by Physical Adsorption

C1274 Test Method for Advanced Ceramic Specific Surface Area by Physical Adsorption

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

E2456 Terminology Relating to Nanotechnology

2.2 ISO Standards:⁴

ISO 9277 Determination of the Specific Surface Area of Solids by Gas Adsorption using the BET Method

ISO 18757 Fine Ceramics (Advanced Ceramics, Advanced Technical Ceramics)—Determination of Specific surface Area of Ceramic Powders by Gas Adsorption using the BET Method

3. Terminology

3.1 *Definitions*—For additional definitions related to nanotechnology, see Terminology **E2456**.

3.1.1 *nanoparticles, n*—in nanotechnology, a sub-classification of ultrafine particle with lengths in two or three dimensions greater than 0.001 micrometre (1 nanometre) and smaller than about 0.1 micrometre (100 nanometres) and which may or may not exhibit a size-related intensive property.

E2456

3.1.2 *adsorbate, n*—material that has been retained by the process of adsorption.

B922

3.1.3 *adsorbent, n*—any solid having the ability to concentrate or collect significant quantities of other substances on its surface.

B922

3.1.4 *adsorption, n*—a process in which fluid molecules are concentrated or collected on a surface by chemical or physical forces, or both.

B922

³ 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.

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

3.1.5 *BET-constant, n*—an indication of the magnitude of the adsorbent/adsorbate interactions in the first adsorbed layer.

3.1.6 *outgassing, n*—the evolution of gas from a material in a vacuum or inert gas flow, at or above ambient temperature.

B922

3.1.7 *physical adsorption (van der Waals adsorption), n*—the binding of an adsorbate to the surface of a solid by forces whose energy levels approximate those of condensation.

B922

3.1.8 *surface area, n*—the total area of the surface of a powder or solid including both external and accessible internal surfaces (from voids, cracks, open porosity, and fissures); the area may be calculated by the BET equation from gas adsorption data obtained under specific conditions; it is useful to express this value as the specific surface area, for example, surface area per unit mass of sample (m^2/kg).

B922

3.1.9 *surface area (BET), n*—the total surface area of a solid calculated by the BET equation, from gas adsorption data obtained under specific conditions.

3.1.10 *surface area, specific, n*—the area, per unit mass of a granular or powdered or formed porous solid, of all external plus internal surfaces that are accessible to a penetrating gas or liquid.

B922

4. Summary of Test Method

4.1 An appropriate filter is pre-weighed to the nearest 1×10^{-8} kg (0.01 mg), outgassed, and the background surface area measured prior to nanoparticle collection in an inhalation exposure chamber. A sufficient amount of nanoparticles (to provide at least the minimum surface area required for reliable results for the instrument used) are collected on the filter, the filter with particles is post-weighed, outgassed, and total surface area measured. The surface area concentration of the airborne nanoparticles in the exposure chamber is estimated by subtracting the background filter surface area from the total surface area of the filter with nanoparticles and normalized by the volume of air sampled, with the final result expressed as m^2/m^3 (LeBouf et al., 2011) (5).

4.2 *Multipoint BET Analyses*—Volume of gas adsorbed at 77 K (liquid nitrogen temperature) is determined as 10^{-6} m^3 (cm^3) corrected to standard temperature and pressure for a minimum of five relative pressures within the linear BET transformation range of the physical adsorption isotherm characteristic of the filter and/or nanoparticle. The linear range is that which results in a least squares correlation coefficient of 0.999 or greater for the relationship between BET transformation and relative pressure. Typically, the linear range includes relative pressures between 0.05 and 0.30.

4.3 It is important to use an analytical balance to determine the sample mass. The physical adsorption instrument measures the total amount of gas adsorbed onto the sample under analysis. The sample mass is then used to normalize the measured adsorption results. Any error in the sample mass will affect the final BET surface area.

4.4 Calculations are based on the BET equation, as required by the instrument being used for the determination. The

instrument pressure tolerance (pressure range that must be maintained within a sample cell to accept a valid data point) is 6.6 Pa. In this standard, the cross-sectional area for the krypton adsorbate is taken to be 2.02×10^{-19} m^2 (ISO 9277); however, some instrument software may use a different default value. As such, the cross-sectional area of the krypton adsorbate used in calculations should be reported with the BET surface area results.

5. Significance and Use

5.1 A tiered strategy for characterization of nanoparticle properties is necessary to draw meaningful conclusions concerning dose-response relationships observed during inhalation toxicology experiments. This tiered strategy includes characterization of nanoparticles *as produced* (that is, measured as the bulk material sold by the supplier) and *as administered* (that is, measured at the point of delivery to a test subject) (Oberdorster et al., 2005) (6).

5.2 Test Methods **B922** and **C1274** and ISO Standards 9277 and 18757 exist for determination of the *as produced* surface area of bulk metal and metal oxide powders. During the delivery of metal and metal oxide nanoparticles in inhalation exposure chambers, the material properties may undergo change and therefore have properties that differ from the material *as produced*. This test method describes the determination of the *as administered* surface area of airborne metal and metal oxide nanoparticles in inhalation exposure chambers for inhalation toxicology studies.

6. Interferences

6.1 This test method can be used to determine the internal and external surface of nanoparticles only after the surfaces have been cleaned of any physically adsorbed molecules (for example, water or volatile organic compounds) which prevent physical adsorption of the gas probe molecules used to measure surface area. Therefore, it is necessary to remove these adsorbed contaminants prior to surface area analysis (Anderson, 1975; Gregg and Sing, 1982) (2, 3). Outgassing is performed by evacuating the sample (typically at 10^{-1} Pa) and can be accelerated by using elevated temperatures, provided no irreversible sample changes occur. Outgassing is complete when duplicate surface area analyses produce results within expected instrument repeatability limits.

7. Apparatus

7.1 Commercial instruments employing low temperature (77 K) krypton adsorption are available from several manufacturers for the measurement of specific surface area by physical adsorption. Use of krypton is required due to the low overall surface area of particle-laden samples and the need to accurately measure the background surface area of the filter used for sample collection. Some instruments are automated versions of the classical vacuum apparatus. Others make use of balanced adsorption technology. Additionally, commercial instruments are available which measure physical adsorption based on the dynamic flow method.

7.2 *Analytical Balance*, having a sensitivity of 1×10^{-8} kg.

7.3 *Degassing Equipment*, capable of maintaining a sample degas temperature of $120 \pm 10^\circ\text{C}$.

7.4 *Sampling pump*, calibrated and capable of maintaining constant flow.

7.5 *Pellet style glass sample cell*, minimum internal diameter 9 mm.

7.6 *Static charge neutralizer*, properly operating.

NOTE 1—Use caution with static charge neutralizers as static discharge could be an ignition source for certain types of filters that contain flammable constituents (for example, nitrocellulose).

8. Reagents and Materials

8.1 *Liquid Nitrogen*.

8.2 *Krypton*, 99.999 mole percent, with the sum of N_2 , O_2 , Ar, CO_2 , hydrocarbons (as CH_4), and H_2O totaling less than 10 ppm, dry and oil-free, cylinder, or other source of purified krypton.

8.3 *Helium*, 99.99 mole percent, with the sum of N_2 , O_2 , Ar, CO_2 , hydrocarbons (as CH_4), and H_2O totaling less than 10 ppm, dry and oil-free, cylinder, or other source of purified helium, if needed for determination of void space above sample.

8.4 *Track-etched polycarbonate (TEPC) filters*, 0.037 m (37 mm) diameter, 4×10^{-7} m (0.4 μm) pore size.

NOTE 2—Other filter types and sizes of filters may be used provided that their background weight, surface area, pressure drop, collection efficiency, and physical integrity have been characterized (LeBouf et al., 2011) (5).

NOTE 3—The 0.037 m diameter, 4×10^{-7} m pore size TEPC filter will collect 20 nm to 100 nm particles with $\geq 97\%$ efficiency at a flow rate of 0.002 m^3/min (LeBouf et al., 2011; Liu et al., 1983) (5, 7).

8.5 *Plastic filter cassette sampler*, 0.037 m diameter.

9. Hazards

9.1 Precautions applying to the use of liquid nitrogen and compressed gases and handling of powdered nanomaterials should be observed.

10. Procedure

10.1 *Calibration and Standardization*:

10.1.1 Follow manufacturer's instructions for instrument calibration. Verify proper operational performance of the instrument using an acceptable reference material. Examples of available reference materials are provided in Table 1. Instrument manufacturers may also produce reference materials.

TABLE 1 Available Powder Reference Materials

Reference Material ^a	Powder	Adsorbate	BET Specific Surface Area (m^2/g)
BAM-PM-101	Silicon dioxide	Krypton	0.177
BAM-PM-102	α -Alumina	Nitrogen	5.41
BAM-PM-104	α -Alumina	Nitrogen	79.8
BAM-P105	Nanoporous glass	Nitrogen	198.5
NIST 1898	Titanium dioxide	Nitrogen	55.55
NIST 1900	Silicon nitride	Nitrogen	2.79
NIST 2206	Nanoporous glass	Nitrogen	10.99
NIST 2207	Nanoporous glass	Nitrogen	177.8

^aBAM = Bundesanstalt für Materialforschung und -prüfung; NIST = National Institute of Standards and Technology

10.2 Background Filter Surface Area:

NOTE 4—As an alternative to determining the background surface area for each TEPC filter sample, an average background surface area can be determined from a representative sample of filters from each lot. The between-lot filter surface area variability for TEPC filters (0.037 m diameter, 4×10^{-7} m pore size) accounted for 65 % of the total variability whereas the within lot-filter variability accounted for 35 % of the total variability in one laboratory. The within lot filter repeatability standard deviation has been determined to be 0.03 (pooled relative standard deviation) in that same laboratory.

10.2.1 The user must verify the background surface area for the particular type and lot of filter used.

10.2.2 Handle a filter on the edges only using metal tweezers, pass through a static charge neutralizer, and record the mass reading on a calibrated analytical balance capable of reading to 1×10^{-8} kg.

NOTE 5—If desired, a control filter can be weighed and handled in exactly the same manner as the experimental filter to verify that the filter handling steps do not result in gravimetric errors.

10.2.3 Equilibrate the filter in the same temperature- and humidity-controlled environment as the balance prior to weighing.

10.2.4 Wearing clean nitrile gloves roll the filter into a cylinder having a diameter narrow enough to insert it into the glass sample cell.

NOTE 6—The filter can be wrapped around a clean glass rod to obtain cylindrical shape. If helpful, pass the filter through a static charge neutralizer before inserting it into the glass sample cell.

10.2.5 Attach the prepared sample cell to the outgassing port of the instrument. Secure heating mantle or oven around the sample cell.

10.2.6 Outgas the sample for 18 to 24 hours at 393 K (120°C) under light vacuum (1 to 2 Pa).

10.2.7 Remove sample cell from heating mantle or oven and cool to ambient temperature. Remove and seal the sample cell according to the manufacturer's instructions.

10.2.8 Attach the appropriately prepared sample holder to surface area analyzer instrument analysis port according to manufacturer's instructions. Include any required hardware.

10.2.9 Use at least five adsorption points in the linear BET transformation range of the isotherm, that is, relative pressure (p/p_0) from 0.05 to 0.30). If necessary, input the blank filter weight determined in 10.2.2.

10.2.10 Perform an analysis using the following instrument settings: pressure tolerance of 6.6 Pa and cross-sectional area of a krypton adsorbate molecule of 2.02×10^{-19} m^2 .

10.2.11 When the analysis is completed and the sample has warmed to ambient temperature, remove and seal the sample cell.

10.2.12 Pass the sample cell through a static charge neutralizer and carefully remove the blank filter from the sample cell.

NOTE 7—A wire with metal hook can be used to facilitate the removal of the filter.

10.2.13 Handle the filter on the edges only using metal tweezers, pass through a static charge neutralizer and place in bottom piece of 0.037 m plastic cassette sampler.

10.2.14 Insert the top piece of plastic cassette sampler into bottom and press together using a pneumatic press to apply an

even and repeatable pressure across the surface of the cassette to seal. Place shrink seal around the seam of the plastic cassette sampler.

NOTE 8—It is very important that the plastic cassette pieces are well sealed. Bypass leakage can result in up to a 4 % loss of particles and therefore underestimation of the concentration of the aerosol sampled (Baron, 2002) (8).

10.3 Sampling:

10.3.1 The aerosol in the inhalation exposure chamber should be mixed uniformly using a fan (Chen et al., 2006) (9) or other appropriate means to ensure a representative sample. Alternatively, repeat samples can be collected from the exposure chamber atmosphere to evaluate aerosol mixing.

10.3.2 Position the 0.037 m plastic cassette sampler using either configuration shown in Fig. 1 and pull air through the filter at an appropriate flow rate using a calibrated sampling pump (capable of 0.001 to 0.010 m³/min flow rate) for a known amount of time. Record both the flow rate and time to calculate the volume of sampled air.

NOTE 9—If the configuration in Fig. 1b is used to collect aerosol from the inhalation exposure chamber, use less than 0.7 m of tubing from the inlet to the 0.037 m plastic cassette sampler to avoid nanoparticle concentration loss in the tubing of up to 10 % (Jankovic et al., 2010) (10).

10.3.3 The cumulative amount of material collected must exceed at least 0.02 m² nanoparticle surface area to overcome the minimum surface area of TEPC filters to provide reliable results. If the amount of material collected is less than 0.02 m², the nanoparticle surface area cannot be distinguished from the TEPC filter surface area. If the amount of material collected is so high that loose particles are visible on the surface of the filter, particles may be lost from the filter during folding (section 10.4.2) which would result in an underestimation of airborne nanoparticle surface area.

10.4 Analysis of Airborne Nanoparticle Surface Area:

10.4.1 Equilibrate the filter in the 0.037 m plastic cassette sampler in the same temperature- and humidity-controlled environment as the balance prior to weighing.

10.4.2 Remove the filter from the 0.037 m plastic cassette sampler using metal tweezers, pass the filter through a static charge neutralizer, and weigh.

10.4.3 Using tweezers, carefully fold the particle-laden filter as shown in Fig. 2 and insert into the glass sample cell.

10.4.4 Attach the prepared sample cell to the outgassing port of instrument. Secure the heating mantle or oven around the sample cell.

10.4.5 Outgass the sample cell for 18 hours at 393 K under light vacuum (1 to 2 Pa).

10.4.6 Remove the glass sample cell from the heating mantle or oven and cool to ambient temperature. Remove and seal the sample cell according to manufacturer’s instructions.

10.4.7 Securely attach the appropriately prepared glass sample cell to the instrument analysis port according to manufacturer’s instructions. Include any required hardware.

10.4.8 Use at least five adsorption points in the linear BET transformation range of the isotherm ($p/p_0 = 0.05$ to 0.30). If necessary, input the sample weight determined in 10.4.1.

10.4.9 Perform the analysis using the following instrument settings: pressure tolerance of 6.6 Pa and value of 2.02×10^{-19} m² for the cross-sectional area of a krypton molecule.

10.4.10 When the analysis has finished and the sample has warmed to ambient temperature, remove and seal the sample cell.

11. Calculations

11.1 If the instrument reports the results as specific surface area, the surface area of nanoparticles on the filter is calculated as:

$$SA_{np} = (SSA_s \cdot M_s) - (SSA_b \cdot M_b) \tag{1}$$

where:

- SA_{np} = surface area of nanoparticles, m²,
- SSA_s = specific surface area of sample (filter + nanoparticles), m²/kg,
- M_s = mass of sample (filter + nanoparticles), kg,
- SSA_b = background specific surface area of filter, m²/kg, and
- M_b = background mass of filter, kg.

NOTE 10—Some instrument software may report specific surface area as m²/g, instead of using correct SI units (m²/kg).

11.2 If the instrument reports results as surface area, the surface area of nanoparticles on the filter is calculated as:

$$SA_{np} = SA_s - SA_b \tag{2}$$

where:

- SA_{np} = surface area of nanoparticles, m²,
- SA_s = surface area of sample (filter + nanoparticles), m², and
- SA_b = background surface area of filter, m².

11.3 Normalize the calculated value of SA_{np} (m²) to the volume of air sampled from the inhalation chamber as described in 10.3.2 (m³), with the final result expressed as m²/m³.

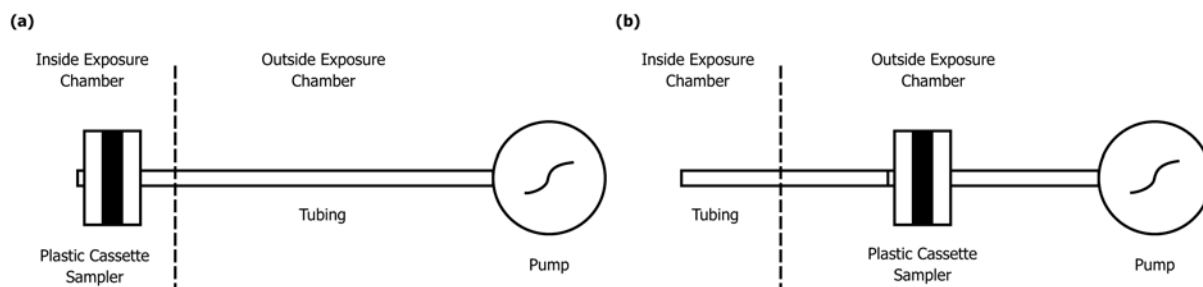


FIG. 1 Inhalation Exposure Chamber Sampling Configurations

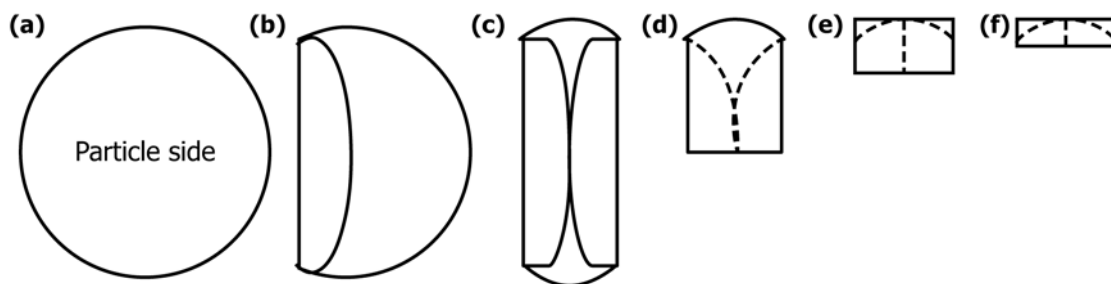


FIG. 2 Procedure for folding filter: (a) nanoparticle-laden filter, (b) fold left edge to center, (c) fold right edge to center, (d) fold bottom edge to top edge, (e) fold bottom edge to top edge again, and (f) fold bottom edge to top edge one more time. Insert folded filter into glass sample cell.

12. Report

12.1 Report the following information:

- 12.1.1 Complete sample identification.
- 12.1.2 Air sample volume—in units of m^3 .
- 12.1.3 Sample outgassing method, including total time and outgassing temperature.
- 12.1.4 Collected isotherm point(s) as volume adsorbed versus relative pressure.
- 12.1.5 Correlation coefficient and BET-constant.
- 12.1.6 Instrument settings: 6.6 Pa pressure tolerance.
- 12.1.7 Cross sectional area of krypton molecule.
- 12.1.8 Results—in units of m^2/m^3 .

13. Precision and Bias

13.1 An interlaboratory study is underway, conducted according to Practice E691. Expected precision will be determined for these materials.

13.2 *Precision*—The repeatability standard deviation and reproducibility standard deviation will be determined upon completion of the interlaboratory study. In one laboratory, BAM-PM-104, an alumina certified reference material for surface area, was aerosolized and collected on filters at five different nominal loadings: 0.005 m^2 ($n = 1$), 0.01 m^2 ($n = 1$),

0.1 m^2 ($n = 4$), 0.15 m^2 ($n = 3$), and 0.35 m^2 ($n = 1$). Across all surface area loadings the repeatability standard deviation was determined to be 0.03 (pooled relative standard deviation). At the two surface area loadings for which multiple samples were collected, the repeatability standard deviations were determined to be 0.05 (0.1 m^2) and 0.01 (0.15 m^2). In that same laboratory, a commercially available titanium dioxide nanopowder (Maier et al., 2006) (11) was aerosolized and collected on filters at a nominal loading of 0.05 m^2 ; the repeatability standard deviation was determined to be 0.03 ($n = 5$). The reproducibility of this test method is being determined and will be available within 5 years of the publication date of this standard.

13.3 *Bias*—No information can be presented on the bias of the procedure in this test method for measuring surface area because there is no accepted reference value based upon first principles.

14. Keywords

14.1 BET surface area; gas sorption analysis; inhalation exposure; krypton; metal and metal oxide nanoparticles; multipoint surface area; outgassing; physical adsorption; surface area

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