Designation: D8526 – 23

Standard Test Method for Analytical Procedure Using Transmission Electron Microscopy for the Determination of the Concentration of Carbon Nanotubes and Carbon Nanotube-containing Particles in Ambient Atmospheres[1]

This standard is issued under the fixed designation D8526; 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 is an analytical procedure using transmission electron microscopy (TEM) for the determination of the concentration of carbon nanotubes and carbon nanotube-containing particles in ambient atmospheres.

1.1.1 This test method is suitable for determination of carbon nanotubes in both ambient (outdoor) and building atmospheres.

1.2 This test method is defined for polycarbonate capillary pore filters through which a known volume of air has been drawn and for blank filters.

1.3 The direct analytical method cannot be used if the general particulate matter loading of the sample collection filter as analyzed exceeds approximately 25 % coverage of the collection filter by particulate matter.

1.4 *Units*—The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.5 *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.*

1.6 *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]

D1193 Specification for Reagent Water

D1356 Terminology Relating to Sampling and Analysis of Atmospheres

D1357 Practice for Planning the Sampling of the Ambient Atmosphere

D5337 Practice for Setting and Verifying the Flow Rate of Personal Sampling Pumps

D6281 Test Method for Airborne Asbestos Concentration in Ambient and Indoor Atmospheres as Determined by Transmission Electron Microscopy Direct Transfer (TEM)

D7712 Terminology for Sampling and Analysis of Asbestos

E2456 Terminology Relating to Nanotechnology

2.2 *NIOSH Standards:*[3]

NIOSH 7400 Asbestos and Other Fibers by PCM

NIOSH 7402 Asbestos by TEM

3. Terminology

3.1 *Definitions*—For definitions of general terms used in this test method, refer to Terminologies D1356, D7712, and E2456.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *analytical sensitivity, n*—the calculated airborne nanotube structure concentration in nanotube structures per liter, equivalent to the counting of one nanotube structure in the analysis.

3.2.2 *carbon nanotube, n*—an allotrope of carbon structurally defined by a size of less than 100 nm in two or more

[1] This test method is under the jurisdiction of ASTM Committee D22 on Air Quality and is the direct responsibility of Subcommittee D22.07 on Sampling, Analysis, Management of Asbestos, and Other Microscopic Particles.

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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] Available from National Institute for Occupational Health and Safety (NIOSH), 400 7th Street S.W., Suite 5W, Washington, D.C. 20024, https://www.cdc.gov/niosh/index.htm.

dimensions and a tubular morphology often comprised of one or more coaxial cylinders of graphene (that is, walls).

3.2.2.1 *Discussion*—This method is also applicable to other micrometer or nanometer size carbon fibers, including amorphous carbon nanotubes, carbon nanofibers, carbon nanorods, cellulose nanofibers, and carbon tubes with diameters greater than or equal to 100 nm.

3.2.3 *carbon nanotube structure, n*—a term applied to isolated carbon nanotubes or to any connected or overlapping grouping of carbon nanotubes or bundles of carbon nanotubes, with or without other non-nanotube particles.

3.2.3.1 *Discussion*—See Annex A1 for terminology related to reported structure types.

4. Summary of Test Method

4.1 A sample of airborne particulate matter is collected by drawing a measured volume of air through a capillary-pore polycarbonate membrane filter of maximum pore size 0.4 µm by means of a battery-powered or mains-powered pump. TEM specimens are prepared from filters by applying a thin film of carbon to the filter surface by vacuum evaporation. Small areas are cut from the carbon-coated filter, supported on TEM specimen grids, and the filter medium is dissolved away by a solvent extraction procedure. This procedure leaves a thin film of carbon that bridges the openings in the TEM specimen grid and that supports each particle from the original filter in its original position. The TEM specimen grids are examined at both low and high magnifications to check that they are suitable for analysis before carrying out a quantitative structure count on randomly-selected grid openings. In the TEM analysis, the elemental composition of a nanotube structure may be confirmed by energy dispersive X-ray analysis (EDXA).

4.2 In addition to isolated single carbon nanotubes, ambient air samples often contain more complex aggregates of single carbon nanotubes, with or without other particles. Some particles are composites of nanotube structures with other materials. Individual carbon nanotubes and these more complex structures are referred to as nanotube structures. Any continuous grouping of particles in which a single carbon nanotube with a length greater than or equal to 250 nm is detected shall be recorded on the count sheet. These will be designated nanotube structures and classified according to the counting rules specified in Annex A1. The number of nanotube structures found on a known area of the microscope sample, together with the equivalent volume of air filtered through this area, is used to calculate the airborne concentration in nanotube structures per liter of air.

4.2.1 For this method, a minimum length of 250 nm has been defined as the shortest carbon nanotube to be incorporated in the reported test results. However, fibers identified as shorter than 250 nm may be reported separately by the analyst.

4.3 The upper range of concentrations that can be determined by this test method is 7000 structures per square millimeter. The air concentration represented by this value is a function of the volume of air sampled.

5. Significance and Use

5.1 This test method is applicable to the measurement of airborne carbon nanotubes in a wide range of ambient air situations and for evaluation of any atmosphere for carbon nanotube structures. Single carbon nanotube structures in ambient atmospheres have diameters below the resolution limit of the light microscope. This test method is based on transmission electron microscopy, which has adequate resolution to allow detection of small thin single carbon nanotubes and is currently a reliable technique capable of unequivocal identification of the majority of nanotube structures. Carbon nanotubes are often found, not as single carbon nanotubes, but as very complex, aggregated structures, which may or may not be aggregated with other particles.

5.2 This test method applies to the analysis of a single filter and describes the precision attributable to measurements for a single filter. Multiple air samples are usually necessary to characterize airborne nanotube structure concentrations across time and space. The number of samples necessary for this purpose is proportional to the variation in measurement across samples, which may be greater than the variation in measurement for a single sample.

6. Apparatus

6.1 *Air Sampling Equipment and Consumable Supplies:*

6.1.1 *Carbon Rods,* for use in vacuum coating unit during carbon coating of filters.

6.1.1.1 Use of carbon fiber type evaporators should be avoided since they may not be capable of preparing clean laboratory blanks and may introduce carbon fibers during the coating process.

6.1.2 *Filter Cassette,* 25 mm to 50 mm diameter, commercially manufactured, non-reusable, three-piece cassettes, with cowls in front of the filter surface, used for sample collection. Electrically conductive cowls are preferred.

6.1.2.1 Cassette should be loaded with a capillary pore polycarbonate filter of maximum pore size 0.4 µm. Back filter with a 5 µm pore size mixed cellulose ester (MCE) filter and support it by a cellulose back-up pad. Apply a shrink cellulose band or adhesive tape when the filters are in position to prevent air leakage. Ensure that the filters are tightly clamped in the assembly so that significant air leakage around the filter cannot occur.

6.1.2.2 A cassette with the same design, filter media, and pore size as the cassettes to be used for collecting air samples should be reserved and used exclusively for adjusting the flow rate of sampling equipment.

6.1.2.3 It is recommended that representative filters from the filter lot be analyzed as described in Section 12 for the presence of nanotube structures before any are used for air sample collection.

6.1.2.4 Alternatively, a cassette as described above loaded with a 0.45 µm pore size (or less) MCE filter in place of the polycarbonate filter (commonly used for asbestos TEM air clearance sampling) may be used if standard MCE filter preparation techniques, such as those described by NIOSH (Method 7402) or Test Method D6281 without plasma etching, are used to prepare the TEM grids.

6.1.2.5 Millette et al. (**1**)[4] have shown that 0.45 µm pore size filters are effective at collecting airborne structures shorter than the pore size designation and NIOSH has accepted the use of MCE filters for nanotube examinations. See NIOSH Manual of Analytical Methods Chapters AE, FP, FI, and CN (**2-5**). However, a relative collection efficiency comparing PC and MCE air filters has not been performed at this time for carbon nanotubes and a direct comparison between results obtained from PC versus MCE filters may not be reasonable.

6.1.3 *Flow Meter*—Calibrated flow meter with an appropriate range for the sampling flow rate used. Flow meter shall be calibrated to a primary standard (for example, bubble meters/burette or near-frictionless flow meters) as described in Practice D5337.

6.1.4 *Sampling Pump,* capable of a flow rate sufficient to achieve the desired analytical sensitivity. The sampling pump used shall provide a stable air flow through the filter. A constant flow of critical orifice-controlled pump meets the requirements. Use flexible tubing to connect the filter cassette to the sampling pump.

6.2 *Equipment for Analysis:*

6.2.1 *Transmission Electron Microscope*—A TEM operating at an accelerating potential of 80 kV or greater, with a resolution better than 1.0 nm, and a magnification range of approximately 300 to 100 000 (or greater) with the ability to obtain a direct screen magnification of about 100 000, shall be used for inspection of carbon nanotube morphology.

6.2.1.1 This magnification may be obtained by supplementary optical enlargement of the fluorescent screen image by use of a binocular or by use of a digital viewing screen.

6.2.1.2 It is also required that the fluorescent screen of the microscope be calibrated such that the lengths and widths of nanotube structures down to 1 mm width (on fluorescent screen) can be estimated in increments of 1 mm regardless of structure orientation. This requirement is often fulfilled through use of calibrated gradations in the form of circles (see Fig. 1) etched into the fluorescent screen or alternatively, through the use of a digital viewing screen.

6.2.1.3 The TEM shall have an illumination and condenser lens system capable of forming an electron probe smaller than 250 nm in diameter.

6.2.1.4 *Energy Dispersive X-ray Analyzer (EDXA)*—The TEM shall be equipped with an energy dispersive X-ray analyzer capable of obtaining a carbon X-ray spectrum and achieving a resolution better than 180 eV (FWHM) on the MnKa peak.

6.2.1.5 The EDXA unit shall provide the means for subtraction of the background, identification of elemental peaks, and calculation of background-subtracted peak areas.

6.2.2 *Carbon Rod Sharpener,* to neck the carbon rods that allow the carbon to be evaporated on to the filters with a minimum of heating.

6.2.3 *Vacuum Coating Unit*—Vacuum coating unit capable of producing a vacuum better than 0.013 Pa, used for vacuum deposition of carbon on the membrane filters.

6.2.3.1 A sample holder is required that will allow a glass microscope slide to be tilted through an angle of approximately 45° and continuously rotated during the coating procedure.

6.2.3.2 A liquid nitrogen trap may be used to minimize the possibility of contamination of the filter surfaces by oil from the pumping system.

6.2.4 *Solvent Washer (Jaffe Washer)*—Allows for dissolution of the filter polymer while leaving an intact evaporated carbon film supporting the nanotube structures and other

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

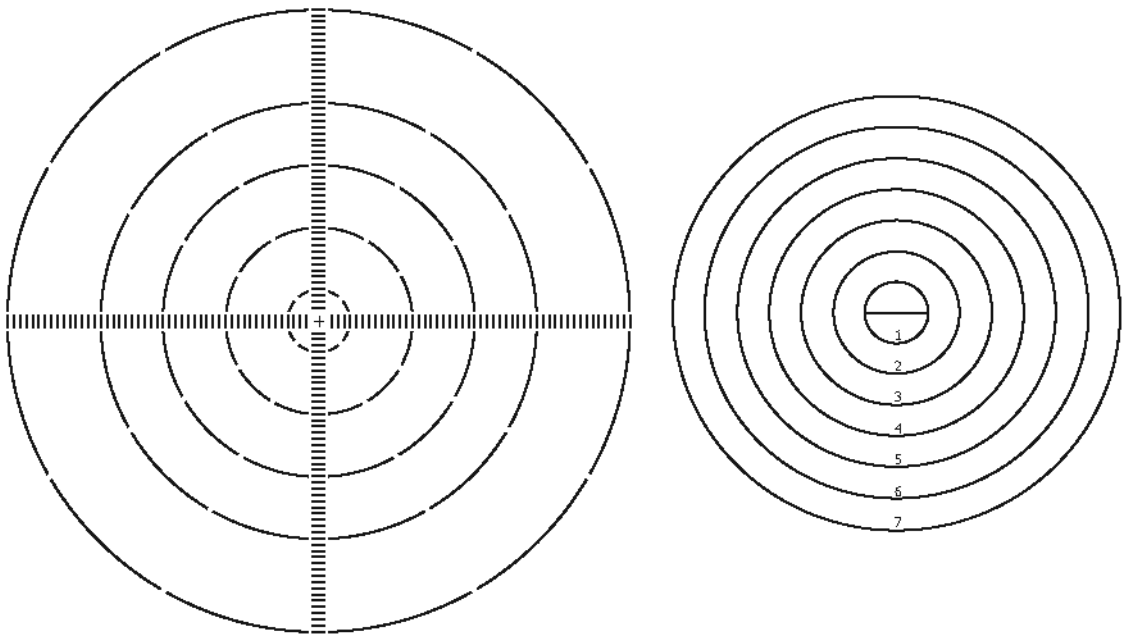

FIG. 1 Examples of Calibration Markings on TEM Viewing Screen: Compton-Feingold Screen (left); Test Method D6281 Screen (right)

particles from the filter surface. One design of a washer that has been found satisfactory for various solvents and filter media is shown in Fig. 2. Use either chloroform or 1-methyl-2-pyrrolidinone for dissolving polycarbonate filters. A mixture of 20 % 1-2-diaminoethane and 80 % 1-methyl-2-pyrrolidinone may also be used to dissolve polycarbonate filters. The higher evaporation rates of chloroform and acetone require that a reservoir of 10 mL to 50 mL of solvent be used, which may need replenishment during the procedure. Because 1-methyl-2-pyrrolidinone has a lower vapor pressure, much smaller volumes of solvent may be used. Use the washer in a fume hood, and keep the petri dishes covered with their lids when specimens are not being inserted or removed during the solvent dissolution. Clean the washer before it is used for each batch of specimens.

6.2.5 *Ultrasonic Bath,* for cleaning of apparatus used for TEM specimen preparation.

6.2.6 *Carbon Grating,* with approximately 2000 parallel lines per millimeter, used to calibrate the magnification of the TEM (see 6.2.1).

6.2.7 *Calibration Specimen Grids*—TEM specimen grids prepared from dispersions of reference carbon nanotubes for comparison of morphology and EDXA analysis.

7. Reagents and Materials

7.1 *Reagents:*

7.1.1 **Warning—**Use the reagents in accordance with the appropriate health and safety regulations. Review their Safety Data Sheets (SDS) before use; avoid skin contact and inhalation via appropriate protective clothing and ventilation.

7.1.2 *Purity of Water*—Water shall be reagent water as defined by Type II of Specification D1193.

7.1.3 *Chloroform,* analytical grade, distilled in glass (preserved with 1 % (v/v) ethanol).

7.1.4 *1-Methyl-2-Pyrrolidinone,* analytical grade (used alone or in combination with 1-2-diaminoethane as an optional alternative to chloroform).

7.1.5 *1-2-Diaminoethane,* analytical grade (used in combination with 1-methyl-2-pyrrolidinone as an optional alternative to chloroform).

7.2 *Materials:*

7.2.1 *Copper Electron Microscope Grids,* TEM grids with grid openings of uniform size such that they meet the requirement of 12.3. Use grids with numerical or alphabetical indexing of individual grid openings to facilitate the relocation of individual grid openings for quality assurance purposes. 200 mesh are commonly used, however 400 mesh may be utilized for reducing the examination time per grid opening as long as they meet the requirements above.

7.2.2 *Carbon Rod Electrodes,* spectrochemically pure for use in the vacuum evaporator during carbon coating of filters.

7.2.3 *Routine Electron Microscopy Tools and Supplies,* such as fine-point tweezers, scalpel holders and blades, microscope slides, double-coated adhesive tape, lens tissue, tungsten filaments, and other routine supplies.

8. Hazards

8.1 Many of the solvents used in the dissolution of filter media during the preparation of TEM grids are known flammable and hazardous materials. Consult the respective Safety Data Sheets (SDS) for information regarding appropriate use.

8.2 Although medical research is ongoing, carbon nanotubes are a suspected inhalation hazard and should be handled with care. Avoid creating dust.

9. Sampling, Test Specimens, and Test Units

9.1 See Terminology D1356 and Practice D1357 for general information on sampling and documents by NIOSH (2.2 and References Section) for information about sampling for airborne particulate.

9.2 Establish the desired range of analytical sensitivity for the analysis prior to sample collection. It is defined as that structure concentration corresponding to the detection of one

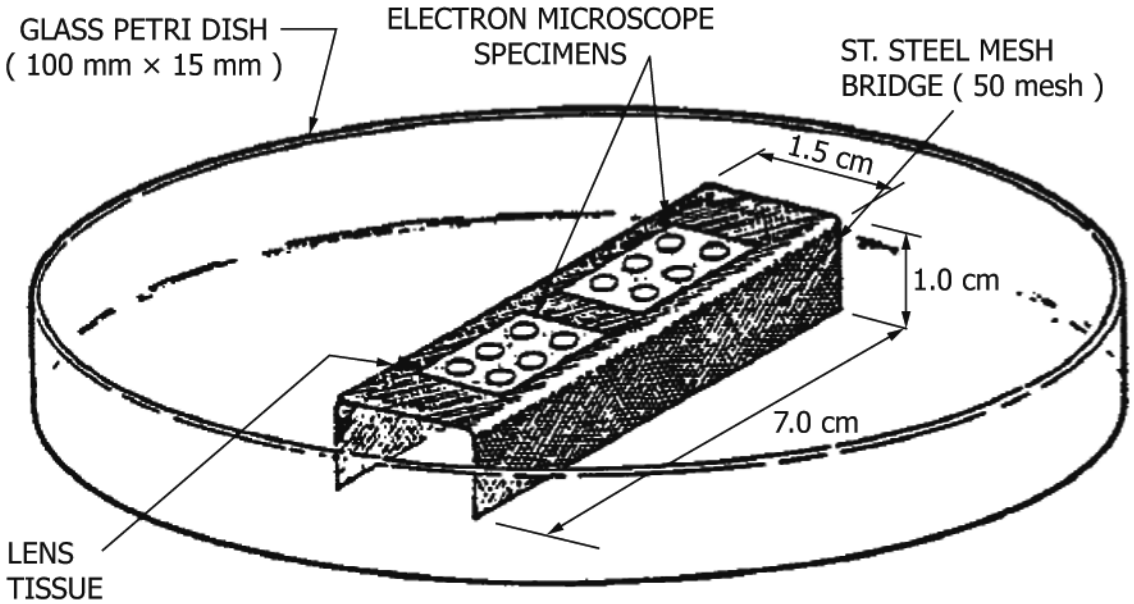

FIG. 2 Example of Design of Solvent Washer (Jaffe Washer)

structure in the analysis. For direct transfer methods of TEM specimen preparation the analytical sensitivity is a function of the volume of air sampled, the active area of the collection filter, and the area of the TEM specimen over which structures are counted. Select the sampling rate and the period of sampling to yield the required analytical sensitivity, as detailed in Table 1.

9.2.1 Measure the sampling flow-rate, both at the beginning and end of the sampling period, using a calibrated flow meter (6.1.3) and a cassette with the same media type and pore size as that used during the sampling period (6.1.2.2). Use the mean value of these two measurements to calculate the total air volume sampled. If the difference in flow rate at the beginning and end of the sampling period is greater than 10 %, the result should be labeled as suspect or void due to sampling errors.

9.2.2 Collect air samples using cassettes (6.1.2), monitoring sampling pumps on a periodic basis during the entire sampling time. Place a cap over the open end of the cassette after sampling, and store the cassette with the filter face-upwards for return to the laboratory. Include blank field filters, as described in 12.7, and process them through the remaining analytical procedures along with the samples.

9.2.3 Determine the analytical sensitivity *S* in structures per liter as described in 13.3.

9.2.4 To achieve a particular analytical sensitivity when the total airborne dust levels are high, it may be necessary to collect low volumes of air and examine many grid openings.

10. Preparation of Apparatus

10.1 The ability to meet the blank sample criteria is dependent on the cleanliness of equipment and supplies. Consider all supplies, such as microscope slides and glassware, as potential sources of carbon nanotube contamination. Wash all glassware before it is used. Wash any tools or glassware that come into contact with the air sampling filters or TEM specimen preparations, both before use and between handling of individual samples. Use disposable supplies whenever possible.

10.2 *Cleaning of Sample Cassette*—Nanotube structures can adhere to the exterior surfaces of air sampling cassettes and these particles can inadvertently be transferred to the sample during handling. To prevent this possibility of contamination, and after ensuring that the cassette is tightly sealed, wipe the exterior surfaces of each sampling cassette before the cassette is taken into the clean facility or laminar flow hood.

10.3 *Direct Preparation of TEM Specimens from Polycarbonate Filters:*

10.3.1 *Selection of Filter Area for Carbon Coating*—Use a cleaned microscope slide to support representative portions of polycarbonate filter during the carbon evaporation. Use double-coated adhesive tape to hold the edges of filter portions to the glass slide. Take care not to stretch the polycarbonate filters during handling. Remove the polycarbonate filter from the sampling cassette, using freshly-cleaned tweezers, and place it onto a second cleaned glass microscope slide that is used as a cutting surface. Cut the filter by rocking the blade from the point, using a freshly-cleaned curved scalpel blade, pressing it into contact with the filter. Repeat the process as necessary. Several such portions may be mounted on the same microscope slide. Wash and dry the scalpel blade and tweezers between the handling of each filter. Identify the filter portions by writing on the glass slide.

10.3.2 *Carbon Coating of Filter Portions*—Place the slide holding the filter portions on the rotation-tilting device, approximately 100 mm to 120 mm from the evaporation source, and evacuate the evaporator chamber to a vacuum better than 0.013 Pa. Perform the evaporation of carbon in very short bursts, separated by a few seconds to allow the electrodes to cool.

10.3.2.1 If evaporation of carbon is too rapid, the strips of polycarbonate filter will begin to curl, and cross-linking of the surface will occur. This cross-linking produces a layer of polymer that is relatively insoluble in organic solvents, and it will not be possible to prepare satisfactory TEM specimens. The thickness of carbon required is dependent on the size of particles on the filter, and approximately 30 nm to 50 nm has been found to be satisfactory. If the carbon film is too thin, large particles will break out of the film during the later stages of preparation, and there will be few complete and undamaged grid openings on the specimen. Ensure that the carbon film thickness is the minimum possible while retaining most of the grid openings of the TEM specimen intact.

10.3.3 *Preparation of the Jaffe Washer*—Place several pieces of lens tissue, as shown in Fig. 2, on the stainless steel bridge, and fill the washer (6.2.4) with chloroform to a level

TABLE 1 Examples of the Minimum Number of Grid Openings Required to Achieve a Particular Analytical Sensitivity for a Collection Filter Area of 385 mm² and TEM Grid Openings of 85 mm (0.0072 mm²)

Analytical Sensitivity	Volume of Air Sampled, Liters						
	500	1000	1200	2000	3000	4000	5000
0.1	1066	533	444	267	178	134	107
0.2	533	267	223	134	89	67	54
0.3	356	178	148	89	60	45	36
0.4	267	134	112	67	45	34	27
0.5	214	107	89	54	36	27	22
0.7	153	77	64	39	26	20	16
1.0	107	54	45	27	18	14	11
2.0	54	27	23	14	9	7	6
3.0	36	18	15	9	6	5	4
4.0	27	14	14	7	5	4	4
5.0	22	11	13	6	4	4	4
7.0	16	8	7	4	4	4	4
10.0	11	6	5	4	4	4	4

where the meniscus contacts the underside of the mesh, resulting in saturation of the lens tissue. Alternatively, with or without using lens paper, fill the washer with 1-methyl-2-pyrrolidone or a mixture of 20 % 1,2-diaminoethane and 80 % 1-methyl-2-pyrrolidinone to a level where the meniscus contacts the underside of the mesh. Use of the Jaffe washer and the chosen reagent (6.2.4) shall be in accordance with one of the three procedures below depending on which reagent is used.

10.3.3.1 *Use of the Jaffe Washer with Chloroform*—Cut three 3 mm square pieces of carbon-coated polycarbonate filter from the carbon-coated filter portion, using a curved scalpel blade. Select three squares to represent the center and the outer periphery of the active surface of the filter. Place each square of filter, carbon side up, on a TEM specimen grid, and place the grid and filter onto the saturated lens tissue in the Jaffe washer. Place the three specimen grids from one sample on the same piece of lens tissue. Any number of separate pieces of lens tissue may be placed in the same Jaffe washer. Cover the Jaffe washer with the lid, and allow the washer to stand for at least 8 h. It has been found that some lots of polycarbonate filters will not completely dissolve in the Jaffe washer, even after exposure to chloroform for as long as three days. This problem also occurs if the surface of the filter was overheated during the carbon evaporation.

10.3.3.2 *Use of the Jaffe Washer with 1-Methyl-2-Pyrrolidinone*—Cut three 3 mm square pieces of carbon-coated polycarbonate filter from the carbon-coated filter portion, using a curved scalpel blade. Select three squares to represent the center and the outer periphery of the active surface of the filter. Place each square of filter, carbon side up, on a TEM specimen grid, and place the grid and filter on the stainless steel mesh in the Jaffe washer. Any number of separate grids may be placed in the same Jaffe washer. Cover the Jaffe washer with the lid, and allow the washer to stand for 2 h to 6 h. After dissolution is complete, remove the stainless steel mesh from the Jaffe washer and allow the grids to dry. 1-methyl-2-pyrrolidinone evaporates very slowly. If it is required to dry the grids more rapidly, transfer the stainless steel bridge into another petri dish, and add distilled water until the meniscus contacts the underside of the mesh. After approximately 15 min, remove the mesh and allow the grids to dry. If it is desirable to retain the water-soluble particle species on the TEM grids, ethanol may be used instead of distilled water for the second wash.

10.3.3.3 *Use of the Jaffe Washer with a Mixture of 20 % 1,2-Diaminoethane and 80 % 1-Methyl-2-Pyrrolidinone*—Cut three 3 mm square pieces of carbon-coated polycarbonate filter from the carbon-coated filter portion, using a curved scalpel blade. Select three squares to represent the center and the outer periphery of the active surface of the filter. Place each square of filter, carbon side up, on a TEM specimen grid, and place the grid and filter on the stainless steel mesh in the Jaffe washer. Any number of separate grids may be placed in the same Jaffe washer. Cover the Jaffe washer with the lid, and allow the washer to stand for 15 min. After dissolution is complete (15 min), remove the stainless steel mesh from the Jaffe washer and transfer the stainless steel bridge into another petri dish, then add distilled water until the meniscus contacts the underside of the mesh. After approximately 15 min, remove the mesh and allow the grids to dry. If it is desirable to retain the water-soluble particle species on the TEM grids, ethanol may be used instead of distilled water for the second wash.

10.3.4 Reproducible results for carbon nanotubes have also been reported when using MCE filters; however, these results have not been validated to determine if short nanotube structures are retained within the dissolved MCE filter during TEM grid preparation. Use of MCE filters instead of PC filters may be acceptable, if comparative analyses are conducted between PC filters prepared according to this method and MCE filters prepared according to standard filter preparation techniques published by NIOSH (**5**).

10.4 *Criteria for Acceptable TEM Specimen Grids*—Valid data cannot be obtained unless the TEM specimens meet specified quality criteria. Examine the TEM specimen grid in the TEM at a magnification sufficiently low (300 to 1000) so that complete grid openings can be inspected. Reject the grid if:

10.4.1 The TEM specimen has not been cleared of filter medium by the filter dissolution step. If the TEM specimen exhibits areas of undissolved filter medium, and if at least two of the three specimen grids are not cleared, either additional solvent washing shall be carried out or new specimens shall be prepared from the filter;

10.4.2 The sample is overloaded with particulate matter. If the specimen grid exhibits more than approximately 25 % obscuration on the majority of the grid openings, designate the specimen as overloaded. While the detection of carbon nanotubes may be possible, the filter cannot be analyzed satisfactorily for purposes of reporting a structure concentration using the direct preparation methods because the grid is too heavily loaded with debris to allow separate examination of individual particles by EDXA, and obscuration of single carbon nanotubes by other particulate matter may lead to underestimation of the nanotube structure count;

10.4.3 The particulate matter deposits on the specimen are not uniformly distributed from one grid opening to the next. If the particulate matter deposits on the specimen are obviously not uniform from one grid opening to the next, designate the specimen as nonuniform. This condition is a function either of the air sampling conditions or of the fundamental nature of the airborne particulate matter. Satisfactory analysis of this filter may not be possible unless a large number of grid openings are examined;

10.4.4 The TEM grid is too heavily loaded with fibrous structures to make an accurate count. Accurate counts cannot be made if the grid has more than approximately 7000 structures/mm^2; or

10.4.5 More than approximately 25 % of the grid openings have broken carbon film over the whole grid opening. Since the breakage of carbon film is usually more frequent in areas of heavy deposit, counting of the intact openings can lead to an underestimate of the structure count.

10.5 If the specimens are rejected because unacceptable numbers of grid openings exhibit broken carbon replica, apply an additional carbon coating to the carbon coated filter, and prepare new specimen grids. The larger particles can often be supported by using a thicker carbon film. If this action does not

produce acceptable specimen grids, this filter cannot be analyzed using the direct preparation methods.

10.6 If one or more of the conditions described in 10.4.1 – 10.4.5 exist, it may not be possible to analyze the sample by this method.

11. Calibration and Standardization

11.1 *Calibration of TEM Screen Magnification*—Align the electron microscope according to the specifications of the manufacturer. Initially, and at regular intervals, calibrate the magnifications used for the analysis using a diffraction grating replica. Adjust the specimen height to the eucentric position before carrying out the calibration. Measure the distance on the fluorescent viewing screen or digital screen occupied by a convenient number of repeat distances of the grating image, and calculate the magnification. Repeat the calibration after any instrumental maintenance or change of operating conditions. The magnification of the image on the viewing screen is not the same as that obtained on photographic plates, film or CCD camera sensors. The ratio between these is a constant value for the particular model of TEM. A sample TEM grid of reference carbon nanotubes should be analyzed as part of the calibration process to make sure that carbon nanotubes are observable.

11.2 *Calibration of the EDXA System*—Perform an energy position calibration of the EDXA system for a low-energy and high-energy peak regularly. A sample copper grid of reference carbon nanotubes should be analyzed to make sure that x-ray instrument is calibrated correctly for the peak positions of carbon (0.277 keV) and copper (8.03 keV). The peak centers should be within ±0.01 keV.

12. Procedure

12.1 *Introduction*—The examination consists of a count of nanotube structures that are present on a specified number of grid openings. Carbon nanotubes within structures are classified into groups on the basis of morphological observations and EDXA spectra. In the absence of nanotube structures, the area of the TEM specimen grids to be examined depends on the analytical sensitivity required.

12.2 So that the estimate of the structure density on the sampling filter shall not be based on the small area represented by one specimen grid, examine the grid openings on at least two of the three specimen grids prepared. Then combine the results in the calculation of the structure density. Examine the grid surface at a magnification of at least 40 000 with examination of the suspect structure at magnifications of at least 50 000. Terminate the count at the end of the examination of the grid opening on which the 50th nanotube structure is observed. Otherwise, continue the structure count to that number of grid openings at which the specified analytical sensitivity has been achieved.

12.2.1 The normal range for the number of grid openings examined is from 4 to 40. If insufficient air has been sampled through the filter, the analytical sensitivity (13.3) may require that an impractically large number of grid openings should be examined. When this situation occurs, a larger value of analytical sensitivity may have to be accepted.

12.3 *Measurement of Mean Grid Opening Area*—Measure the mean grid opening area for the type of TEM specimen grids in use. Ensure that the relative standard deviation of the mean of ten openings selected from ten grids is less than 5 %. As an optional procedure, or if the 5 % relative standard deviation criterion cannot be demonstrated, measure the dimensions of each grid opening examined in the TEM at a calibrated magnification.

12.4 *TEM Alignment and Calibration Procedures*—Align the TEM according to instrumental specifications before structure counting is performed. Calibrate the TEM and EDXA system according to the procedures in Section 11.

12.5 *Determination of Stopping Point*—Before structure counting has begun, calculate the area of filter to be examined in order to achieve the selected analytical sensitivity. Determine the maximum number of grid openings to be examined from the formula:

$$K = \frac{A_f}{A_g} \cdot V \cdot S \tag{1}$$

where:

K = number of grid openings to be examined (round K up to the next highest integer),
A_f = area of sample filter exposed to the passage of air (square millimeters),
A_g = mean area of TEM specimen grid openings (square millimeters),
V = volume of air sampled (liters), and
S = desired analytical sensitivity (structures per liter).

12.6 *General Procedure for Structure Counting and Size Analysis*—Two or more specimen grids prepared from the filter will be used in the structure count. Several grid openings from each grid will be selected at random, and the data combined in the calculation of the results. Use a form containing, at a minimum, those elements required in Annex A1 to record the data. Insert the first specimen grid into the TEM.

12.6.1 Insert the grid into the specimen holder in a standard and relocatable orientation with the grid bars parallel and perpendicular to the axis of the specimen holder to facilitate quality assurance measurements that require reexamination of the same grid opening by different microscopists. This will provide scan directions parallel to the edges of the grid opening. Ensure that all microscopists begin scanning at the same starting point on the grid opening and that they use similar scan patterns. This procedure permits rapid relocation of nanotube structures for further examination, if necessary.

12.6.2 Select a typical grid opening and set the screen magnification to the calibrated value (approximately 40 000). Adjust the sample height until the features in the center of the TEM viewing screen are at the eucentric point. Set the goniometer tilt angle to zero. Record the number or letter used to identify the grid in the data recording form. Record the identification of the particular grid opening in the data recording form. Position the specimen so that the grid opening is positioned with one corner visible on the screen. Move the image by adjustment of only one translation control, carefully examining the sample for nanotube structures, until the opposite side of the grid opening is encountered. Move the image by