Designation: E3143 – 18b (Reapproved 2023)

Standard Practice for Performing Cryo-Transmission Electron Microscopy of Liposomes[1]

This standard is issued under the fixed designation E3143; 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 practice covers procedures for vitrifying and recording images of a suspension of liposomes with a cryo-transmission electron microscope (cryo-TEM) for the purpose of evaluating their shape, size distribution and lamellarity for quality assessment. The sample is vitrified in liquid ethane onto specially prepared holey, ultra-thin, or continuous carbon TEM grids, and imaged in a cryo-holder placed in a cryo-TEM.

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

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

1.4 *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]

E456 Terminology Relating to Quality and Statistics

2.2 *ISO Standards:*[3]

13322-1 Particle Size Analysis – Image Analysis Methods – Static Image Analysis Methods

3. Terminology

3.1 *Definitions:*

3.1.1 *anti-contaminator, n*—a specially designed device built into the column of a cryo-transmission electron microscope designed to pull contamination produced by outgassing within the column away from the frozen specimen during imaging. The device is cooled with liquid nitrogen to a temperature below that of the specimen creating a colder surface for contamination to build on.

3.1.2 *carbon evaporator, n*—a device used to evaporate carbon in a high vacuum chamber generally by applying current through two carbon rods pressed against one another, with one rod being sharpened in order to provide resistance to the current; this causes the rods to heat up and evaporate carbon. The same device can also be used as a *glow discharge device* (3.1.15) in order to *glow discharge* surfaces.

3.1.3 *continuous carbon grid, n*—a copper electron microscope grid coated with a self-supporting layer of continuous carbon over the square mesh of the grid.

3.1.4 *copper electron microscope grid, n—commonly referred to as an "EM grid,"* a thin 3 mm diameter copper foil disk, usually manufactured with a pattern of square holes called a 'mesh' through which imaging is conducted in an electron microscope. The number, pattern, and shape of the holes can vary depending on imaging conditions and sample requirements.

3.1.5 *cryo-grid storage device, n*—any device used to store cryo-EM grids indefinitely in a liquid nitrogen dewar.

3.1.6 *cryo-TEM, n—also referred to simply as cryo-EM, or cryo electron microscopy*, the process of imaging frozen hydrated, vitrified nanomaterial using a cryo-transmission electron microscope.

3.1.7 *cryo-TEM holder, n*—a liquid nitrogen refrigerated device specifically designed to hold and maintain a prepared grid containing a frozen, hydrated, vitrified specimen in a cryo-TEM while imaging is conducted.

3.1.8 *cryo-TEM plunger, n*—a device used to vitrify a sample onto a holey, ultra-thin, or continuous carbon grid for cryo-TEM by plunging the grid containing a thin layer of aqueous sample into an ethane slush or other cryogen(s). There

[1] This practice 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] Available from International Organization for Standardization (ISO), ISO Central Secretariat, BIBC II, Chemin de Blandonnet 8, CP 401, 1214 Vernier, Geneva, Switzerland, http://www.iso.org.

are many types of plunger designs both homemade and commercial. The simplest is the homemade guillotine type (see Fig. 3(*B*), 8.3).

3.1.9 *cryo-transmission electron microscope, n*—a specially designed *transmission electron microscope* (see 3.1.25) capable of imaging frozen vitrified specimens under low electron dose conditions in order to prevent beam-induced specimen damage.

3.1.10 *electron micrograph, n*—the individual image recorded by the electron microscope as data are collected.

3.1.11 *electron tomography, n*—the process of using images derived from an electron microscope in which the images were recorded of a sample through an incremental series of tilt angles that are then used with image analysis software to reconstruct the structure of the specimen in 3D.

3.1.12 *ethane, n*—chemical formula C_2H_6. A colorless, odorless flammable gas.

3.1.13 *forceps, n*—a very fine pointed pair of tweezers or pincer used to pick up electron microscope grids.

3.1.14 *glow discharge, n*—a plasma generated by passing an electric current through a low-pressure gas environment within a chamber, usually a bell jar and with respect to this practice, is used to clean and statically charge the carbon surface coating of copper electron microscope grids in order to make them hydrophilic so that they will wet during sample application.

3.1.15 *glow discharge device, n*—a device, such as a *carbon evaporator* (see 3.1.2), designed to generate a glow discharge plasma.

3.1.16 *hang time, v*—the amount of time after blotting of the sample from the prepared grid, prior to plunging the grid into the liquid ethane for vitrification.

3.1.17 *holey carbon grid, n*—a copper electron microscope grid used for cryo-TEM consisting of a thin electron semi-transparent carbon film containing small holes that is suspended over the larger mesh of square holes of the copper electron microscope grid (see Fig. 3(*A*), 8.2.1).

3.1.18 *image analysis, n*—the process of analyzing digital images with computer software for the purpose of extracting meaningful information from the data, for example, a size distribution.

3.1.19 *liposomes, n*—microvesicles composed of a bilayer and/or a concentric series of multiple bilayers separated by aqueous compartments formed by amphipathic molecules such as phospholipids that enclose a central aqueous compartment.

Liposome Drug Products (1)[4]

3.1.20 *liquid ethane, n*—liquefied ethane made by cooling gaseous ethane to the liquid state.

3.1.21 *liquid nitrogen, n*—a cryogen, is the liquid state of nitrogen that boils at –196 °C and is commonly used for extreme cooling. Poses a freezing and suffocation hazard requiring caution when used.

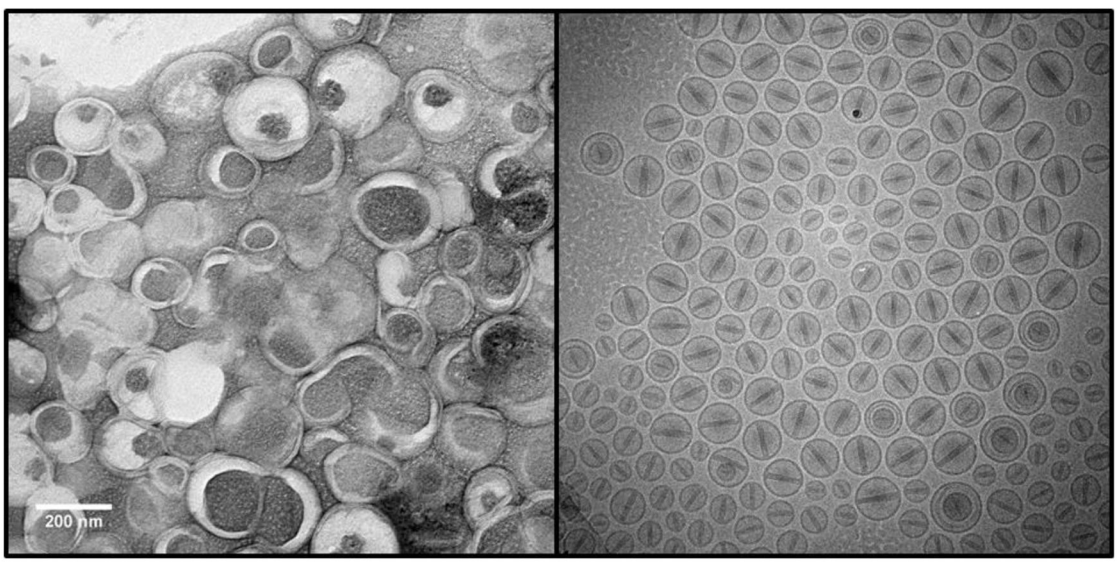
3.1.22 *sample, n*—a small volume of a preparation of liposomes suspended in an aqueous solution.

3.1.23 *specimen, n*—a cryo-TEM grid onto which a sample has been applied and vitrified. The specimen will be placed in a cryo-TEM holder and imaged by cryo-TEM.

3.1.24 *structure, n*—the 3D shape, arrangement, composition, and construction of any element that has physical *x*, *y*, and *z* dimensions.

3.1.25 *transmission electron microscope, n*—a microscope that employs an electron beam and a series of electro-magnetic lenses to illuminate [transmit] through very thin samples and then image these samples to extremely high resolutions and high magnifications.

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



NOTE 1—Both images are shown to the same scale; scale bar is 200 nm.

FIG. 1 Left—An Electron Micrograph of an Air-Dried Liposomal Preparation that has been Negatively Stained with 2 % Uranyl Acetate for Contrast; Right—An Electron Micrograph of the Same Liposomal Preparation Prepared as a Frozen Vitrified Specimen for Cryo-TEM