Designation: E3409 – 24

Standard Test Method for Analysis of Liposomal Drug Formulations Using Multidetector Asymmetrical-Flow Field-Flow Fractionation[1]

This standard is issued under the fixed designation E3409; 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 a measurement procedure to reproducibly separate component size populations present within liposomal drug formulations and to characterize their associated size and size distribution. The method can also yield information on the shape and physical stability of the liposomes and is applicable to measurements in the presence of serum proteins. Fractions can be collected for off-line analysis using various techniques not specified in this test method.

1.2 This test method applies to uni-lamellar and multi-lamellar liposomes that are designed for drug delivery and which are dispersed in a native solution that is aqueous in nature. The method is generally applicable over a particle size range (radius) of approximately 10 nm to 250 nm, and for injected lipid mass from 20 µg to 200 µg.

1.3 This test method is based on the multi-detector asymmetrical-flow field-flow fractionation (MD-AF4) technique as configured on a typical commercial instrument platform with online detectors such as multi-angle (static) light scattering (MALS), dynamic light scattering (DLS), ultraviolet-visible (UV-Vis) absorbance, and differential refractive index (dRI) (**1**).[2]

1.4 This method does not address liposome composition. Refer to Test Methods E3297, E3323, or E3324 for lipid quantification.

1.5 *Units*—The values stated in SI units are to be regarded as standard. Where appropriate, cgs units are given in addition to SI.

1.6 *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.*

1.7 *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:*[3]

D1193 Specification for Reagent Water

E1617 Practice for Reporting Particle Size Characterization Data

E3144 Guide for Reporting the Physical and Chemical Characteristics of Nano-Objects

E3206 Guide for Reporting the Physical and Chemical Characteristics of a Collection of Nano-Objects

E3247 Test Method for Measuring the Size of Nanoparticles in Aqueous Media Using Dynamic Light Scattering

E3297 Test Method for Lipid Quantitation in Liposomal Formulations Using High Performance Liquid Chromatography (HPLC) with a Charged Aerosol Detector (CAD)

E3323 Test Method for Lipid Quantitation in Liposomal Formulations Using High Performance Liquid Chromatography (HPLC) with an Evaporative Light-Scattering Detector (ELSD)

E3324 Test Method for Lipid Quantitation in Liposomal Formulations Using Ultra-High-Performance Liquid Chromatography (UHPLC) with Triple Quadrupole Mass Spectrometry (TQMS)

2.2 *ISO Standards:*[4]

ISO/TS 21362 Nanotechnologies—Analysis of Nano-Objects Using Asymmetrical-Flow and Centrifugal Field-Flow Fractionation

ISO 22412 Particle Size Analysis—Dynamic Light Scattering (DLS)

[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 a list of references at the end of this standard.

[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 International Organization for Standardization (ISO), ISO Central Secretariat, Chemin de Blandonnet 8, CP 401, 1214 Vernier, Geneva, Switzerland, https://www.iso.org.

2.3 *Code of Federal Regulations:*[5]
21 CFR § 211.194(a)(2) FDA Current Good Manufacturing Practice for Finished Pharmaceuticals

3. Terminology

3.1 *Definitions:*

3.1.1 *accumulation wall, n*—surface of a field-flow fractionation channel toward which sample components are driven by the applied field acting perpendicular to the channel flow. **ISO/TS 21362**

3.1.1.1 *Discussion*—In asymmetrical-flow field-flow fractionation, the accumulation wall is flat and consists of a replaceable semipermeable membrane on a porous frit substrate.

3.1.2 *asymmetrical-flow field-flow fractionation (AF4), n*—separation technique that uses a cross flow field applied perpendicular to the channel flow to achieve separation based principally on analyte diffusion coefficient or size. **Adapted from ISO/TS 21362**

3.1.3 *band broadening, v*—overall dispersion or widening of an analyte band as the analyte passes through a separation system. **ISO/TS 21362**

3.1.4 *channel, n—in field-flow fractionation*, a thin ribbon-like chamber with a parabolic flow profile required for separation under the influence of a field applied perpendicular to the channel flow. **ISO/TS 21362**

3.1.5 *channel flow,, n—in field-flow fractionation*, the total parabolic laminar-flow of eluent or mobile phase through the channel. **Adapted from ISO/TS 21362**

3.1.5.1 *Discussion*—For purposes of this test method, channel flow and detector flow are equivalent. If the operator uses a post-fractionation split-flow device to divert part of the channel flow to waste (for example, to concentrate the analyte), then the detector flow will be less than the channel flow.

3.1.6 *channel spacer, n—in asymmetrical-flow field-flow fractionation*, a thin plastic film with a cut-out that defines the nominal thickness and lateral dimensions of a channel. **Adapted from ISO/TS 21362**

3.1.6.1 *Discussion*—The geometry of the channel is typically defined by a trapezoid shape with breadth typically ca. 20 mm to 25 mm, length typically ca. 100 mm to 300 mm and height typically between 190 µm and 500 µm. The nominal channel height and lateral dimensions can also be defined using fixed-height channels that do not require a spacer.

3.1.7 *channel thickness (height), w, n—in field-flow fractionation*, the nominal vertical separation between the depletion wall and the accumulation wall.

3.1.7.1 *Discussion*—The effective thickness can vary depending on the membrane used and the degree of compression of the membrane as well as other factors.

3.1.8 *cross flow, n—in asymmetrical-flow field-flow fractionation*, the flow field applied perpendicular to the channel flow to achieve separation of analytes. **ISO/TS 21362**

3.1.9 *detector flow, n—in field-flow fractionation*, that portion of mobile phase that exits the channel and enters the detectors. **Adapted from ISO/TS 21362**

3.1.9.1 *Discussion*—While typically detector flow and channel flow are identical, in some configurations channel flow can be split before entering the detectors, for example, to concentrate the analyte by purging a portion of the analyte-free mobile phase.

3.1.10 *elution, n—in field-flow fractionation*, a process by which analytes in the mobile phase, or eluent, are transported through, and exit from, the fractionation channel. **ISO/TS 21362**

3.1.11 *elution time, t, n—in field-flow fractionation*, the elapsed time beginning with the initiation of elution following sample injection and excluding pre-elution steps such as focusing, relaxation or transitions.

3.1.11.1 *Discussion*—Retention and elution share an equivalent timeline and may be used interchangeably. However, retention is principally used in reference to specific retained species (for example, an analyte) eluting more slowly than the unretained tracer (that is, the void peak) due to their specific properties and interaction with the applied field. A graphical representation of a fractogram generally shows elution time or retention time on the (horizontal) x-axis, after the time associated with pre-elution steps has been subtracted.

3.1.12 *field-flow fractionation, n*—separation technique where a field is applied to a liquid passing along a narrow channel to induce separation of analytes present in the liquid, dependent on their differing mobility under the force exerted by the field. **Adapted from ISO/TS 21362**

3.1.13 *focusing, v—in asymmetrical-flow field-flow fractionation*, a process by which, during and after sample injection, a counter-balanced flow entering from opposite directions in the channel is applied to focus the sample components into a thin band close to the inlet port and near the accumulation wall. **Adapted from ISO/TS 21362**

3.1.13.1 *Discussion*—While conventional channels require a focusing step, there are channel designs, such as the frit-inlet channel, that do not require focusing. Focusing is also associated with the process of sample relaxation.

3.1.14 *fractogram, n—in field-flow fractionation*, a two-dimensional graphical representation of data derived from an experiment, typically with one or more detector signals on the vertical y-axis and time on the horizontal x-axis. **Adapted from ISO/TS 21362**

3.1.15 *hydrodynamic radius, R_h, n*—the sphere-equivalent ensemble average radius that reflects the central tendency of the underlying population of particles as determined by dynamic light scattering. **Adapted from Test Method E3247**

3.1.15.1 *Discussion*—The hydrodynamic radius can be obtained from different computational methods, including, for instance, the cumulants method combined with the Stokes-Einstein equation. Note that when obtained from cumulants the average hydrodynamic radius is often referred to in the literature as the z-average (z-avg) size, a legacy term. This measurand can be scattering-angle-dependent and can be influenced by other factors besides size.

[5] Available from U.S. Government Publishing Office (GPO), 732 N. Capitol St., NW, Washington, DC 20401, http://www.gpo.gov.