Designation: F3142 – 24

Standard Guide for Evaluation of *in vitro* Release of Biomolecules from Biomaterials Scaffolds for TEMPs[1]

This standard is issued under the fixed designation F3142; 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 To describe general principles of developing and/or using an *in vitro* assay to evaluate biomolecule release from biomaterials scaffolds for TEMPs, with examples from the literature

1.2 The guide will address scaffolds that do not contain seeded cells; general principles may still apply but may need to be modified if cells are part of the TEMPs.

1.3 *In vitro* release assessment of biomolecules from matrices is a valuable tool for screening biomolecule-scaffold interactions, as well as characterization, and/or quality control.

1.4 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]

F2312 Terminology Relating to Tissue Engineered Medical Products

F2902 Guide for Assessment of Absorbable Polymeric Implants

2.2 *United States Pharmacopeia (USP) General Chapters:*[3]

USP <1> Injections and Implanted Drug Products (Parenterals)—Product Quality Tests

USP <711> Dissolution

USP <724> Drug Release

USP <785> Osmolality and Osmolarity

USP <1001> In Vitro Release Test Methods for Parenteral Drug Preparations

USP <1092> The Dissolution Procedure: Development and Validation

2.3 *European Pharmacopeia (Ph. Eur.):*[4]

Ph. Eur. 2.9.3 Dissolution Test for Solid Dosage Forms

Ph. Eur. 2.9.4 Dissolution Test for Transdermal Patches

Ph. Eur. 2.9.25 Dissolution Test for Medicated Chewing Gums

2.4 *FDA Document:*[5]

FDA Guidance for Industry Bioanalytical Method Validation (May 2018)

2.5 *ICH Documents:*[6]

Q2(R2) Validation of Analytical Procedures

M10 Bioanalytical Method Validation and Study Sample Analysis

3. Terminology

3.1 The present document uses the definitions of Terminology F2312.

4. Significance and Use

4.1 European and U.S. Pharmacopeia provide test methods for measuring release of a drug analyte, and USP <1001> provides a method that is intended to be applicable to implanted products. However, the characterization of biomaterial

[1] This guide is under the jurisdiction of ASTM Committee F04 on Medical and Surgical Materials and Devices and is the direct responsibility of Subcommittee F04.42 on Biomaterials and Biomolecules for TEMPs.

Current edition approved Dec. 1, 2024. Published January 2025. Originally approved in 2016. Last previous edition approved in 2016 as F3142 – 16. DOI: 10.1520/F3142-24.

[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at www.astm.org/contact. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[3] Available from U.S. Pharmacopeial Convention (USP), 12601 Twinbrook Pkwy., Rockville, MD 20852-1790, http://www.usp.org.

[4] Available from EDQM Council of Europe, 7 allée Kastner, CS 30026, F-67081 Strasbourg, France, or visit the website, www.edqm.eu.

[5] Available from Drug Information Branch (HDF-210), Center for Drug Evaluation and Research (CDER), 5600 Fishers Lane, Rockville, MD 20857, or visit the website, http://www.fda.gov/cder/guidance/index.

[6] Available from International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), ICH Secretariat, Route de Pré-Bois, 20, P.O Box 1894, 1215 Geneva, Switzerland, https://www.ich.org.

scaffolds that release biomolecules presents unique challenges not present in other drug or drug-releasing products.

4.2 An appropriately designed *in vitro* release test would be favorable in the early stage of development of biomolecule-releasing scaffolds for TEMPs, as well as in quality control, and may help to reduce the number of animal experiments.

4.3 Appendix X1 provides a tabulated overview of published *in vitro* release studies performed with biomaterial scaffolds loaded with biomolecules.

4.4 One goal of *in vitro* release studies is to simulate the *in vivo* conditions as closely as possible, but with sufficiently simplifying abstraction. The simplification comprises two general aspects: the amount of fluid or release medium in contact with the implant to simulate the physiological environment, and the composition of that release medium.

5. Elements of *in vitro* Release Assays

5.1 *Sample (biomaterial scaffold loaded with biomolecule(s)):*

5.1.1 The sample should be taken from the final product, manufactured by a representative process including packaging and sterilization.

5.1.2 There are several considerations that will influence the selection of the sample dimensions.

5.1.2.1 Vessel size into which the sample can physically fit.

5.1.2.2 Method used to generate a representative sample, where needed, for example, by biopsy punch, cutting with a scalpel blade.

5.1.2.3 Load of the biomolecule, and the concentration in the final setup.

5.2 *Release Medium:*

5.2.1 In order to simulate physiologic conditions at the intended implant site, the release medium should be of appropriate tonicity (osmolality) and pH. Regarding osmolality, the term is used according to USP <785> and as such is a measure of concentration of real solutes, expressed in osmole per [kg] of soluent. Isotonic solutions are iso-osmotic relative to whole blood. Isotonicity is a target attribute for parenteral injections per USP <1>.

NOTE 1—Cells, for example red blood cells, are susceptible to conditions that are outside the isotonic range, by showing swelling through influx of water through the cell membrane (potentially leading to burst) when in a hypotonic environment, or shrinking through efflux of water through the cell membrane when in a hypertonic environment. Regarding pH, the medium should be buffered to prevent pH shifts over time due to the release of the biomolecule and/or degradation of the matrix. pH shifts could change the release mechanism or kinetics.

5.2.2 One medium used very often in the published literature is phosphate-buffered saline (PBS), pH 7.2 or 7.4, in the range of isotonicity (approximately 300 mOsm/kg). In this case, the buffer system is provided by phosphate salts; the tonicity is typically augmented with sodium chloride.

5.2.3 The release medium may be spiked with other components to make it more similar to serum, for example, bovine serum albumin (BSA), or fetal bovine serum (FBS; multicomponent additive). It should be noted that these additives can substantially interfere with the detection method (see 5.7). If protein components are added to the medium (BSA, FBS), evaluation of *in vitro* release will require more specific and/or sophisticated assays (compared to simple A280 UV detection for concentration), as both the biomolecule that is studied and the proteinaceous component(s) of the medium may contribute to the detected signal. The same applies if there are several biomolecules that can be released from the biomaterial scaffold. Further considerations are described in 5.8 (especially 5.8.4 and 5.8.6). It should also be noted that regarding the use of BSA and FBS, it is important to limit the use to a single or limited number of lots to reduce variability in the assay system, especially for studies that require more than a single iteration.

5.2.4 One of the important factors is to determine if there are any solubility issues for the biomolecule, in order to define the most appropriate medium. This primary evaluation can be achieved by exposing the biomolecule to the same experimental conditions without the scaffold present to create a baseline in the candidate medium, and comparing it to a known stable formulation of the biomolecule at the same concentration levels. If the concentrations measured in the candidate medium are substantially the same as in the known stable formulation, it is a good indicator for sufficient solubility and minimum absorption to surfaces in the system (see also 5.3.5).

5.2.5 If the experiment is set up for an extended period of time to simulate the target period of release *in vivo* (that is, several days to weeks or months), consider adding azides or other preservatives to maintain the sterility of the medium and aseptic conditions in the setup. Possible impact of any additive on either the sample or the analytical method should be evaluated.

5.2.6 The ratio of the volume of release medium to biomolecule load (which may correspond to the volume or size of the construct) is important. If there is a requirement to have sink conditions in the experiment (for example, to simulate implantation sites with high fluid circulation), then sink conditions can be achieved by using a large volume of release medium at the beginning of the experiment and extraction of small aliquots, or frequent exchange of the medium. Per USP definition, sink conditions can be maintained at about three times the volume of a saturated solution, and it has been suggested that it should remain below 10 % of saturation concentration in the solution (Reference: USP <1092>). As indicated in 5.2.4, and 5.8.8, the saturation concentration in the candidate medium may need to be experimentally determined.

NOTE 2—Solubility of biomolecules is dependent on pH, salts, and temperature, and may vary widely not only between biomolecules but also for each biomolecule at different conditions (for example, BSA reported to be soluble in water at 40 mg/mL; vendor data sheets for basic fibroblast growth factor (bFGF), nerve growth factor (NGF), and bone morphogenetic protein-2 (BMP-2) recommend reconstitution of lyophilized material to not less than 100ug/mL. Note that lyophilized material is typically formulated and contains some buffer/salt/stabilizers).

5.2.7 The addition of a protease or other enzyme may be considered, if the biomolecule is expected to be tightly bound or incorporated. The presence of the protease or other enzyme may interfere with the assay used to quantify the biomolecule of interest (see 5.2.3). Also, the susceptibility of the biomolecule of interest to the protease or other enzyme must be experimentally evaluated.