Designation: F 1635 – 04

Standard Test Method for *in vitro* Degradation Testing of Hydrolytically Degradable Polymer Resins and Fabricated Forms for Surgical Implants[1]

This standard is issued under the fixed designation F 1635; 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 covers *in vitro* degradation of hydrolytically degradable polymers (HDP) intended for use in surgical implants.

1.2 The requirements of this test method apply to HDPs in various forms:

1.2.1 Virgin polymer resins, or

1.2.2 Any form fabricated from virgin polymer such as a semi-finished component of a finished product, a finished product, which may include packaged and sterilized implants, or a specially fabricated test specimen.

1.3 This test method has no provisions for mechanical loading, fluid flow, or other dynamic challenges.

1.4 *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.*

2. Referenced Documents

2.1 *ASTM Standards:* [2]

D 638 Test Method for Tensile Properties of Plastics

D 671 Test Method for Flexural Fatigue of Plastics by Constant-Amplitude-of-Force[3]

D 695 Test Method for Compressive Properties of Rigid Plastics

D 747 Test Method for Apparent Bending Modulus of Plastics by Means of a Cantilever Beam

D 790 Test Method for Flexural Properties of Unreinforced and Reinforced Plastics and Electrical Insulating Materials

D 882 Test Method for Tensile Properties of Thin Plastic Sheeting

D 1708 Test Method for Tensile Properties of Plastics by Use of Microtensile Specimens

D 1822 Test Method for Tensile-Impact Energy to Break Plastics and Electrical Insulating Materials

D 2857 Test Method for Dilute Solution Viscosity of Polymers

F 748 Practice for Selecting Generic Biological Test Methods for Materials and Devices

2.2 *Other Referenced Standard:*

ISO 10993-9:1999 Biological Evaluation of Medical Devices—Part 9 Framework for Identification and Quantification of Potential Degradation Products[4]

3. Terminology

3.1 *Definitions:*

3.1.1 *resin*—any polymer that is a basic material for plastics.[5]

3.1.2 *hydrolytically degradable polymer (HDP)*—any polymeric material in which the primary mechanism of chemical degradation in the body is by hydrolysis (water reacting with the polymer resulting in cleavage of the chain).

4. Summary of Test Method

4.1 Samples of polymer resins, semi-finished components, finished surgical implants, or specially designed test specimens fabricated from those resins are placed in buffered saline solution at physiologic temperatures. Samples are periodically removed and tested for various material or mechanical properties at specified intervals. The required test intervals vary greatly depending on the specific polymeric composition. For example, poly(*l*-lactide) and poly(*e*-caprolactone) degrade very slowly and can require two or more years for complete degradation. Polymers based substantially on glycolide can completely degrade in two to three months depending on the

[1] This test method is under the jurisdiction of ASTM Committee F04 on Medical and Surgical Materials and Devices and is the direct responsibility of Subcommittee F04.15 on Material Test Methods.

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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] Withdrawn.

[4] Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036.

[5] *Polymer Technology Dictionary*, Tony Whelan ed., Chapman & Hall, 1994.

exact composition and on the size of the specimen. Degradation time is also strongly affected by specimen size, polymer molecular weight, and crystallinity.

5. Significance and Use

5.1 This test method is intended to help assess the biodegradation rates (that is, the mass loss rate) and changes in material or structural properties, or both, of HDP materials used in surgical implants. Polymers that are known to degrade primarily by hydrolysis include but are not limited to homopolymers and copolymers of *l*-lactide, *d*-lactide, *d,l*-lactide glycolide, caprolactone, and *p*-dioxanone.[6]

5.2 This test method may not be appropriate for all types of implant applications or for all known absorbable polymers. The user is cautioned to consider the appropriateness of the test method in view of the materials being tested and their potential application (see X1.1.1).

5.3 Since it is well known that mechanical loading can increase the degradation rate of absorbable polymers, the presence and extent of such loading needs to be considered when comparing *in vitro* behavior with that expected or observed *in vivo*. Where feasible, it is recommended during degradation testing to simulate the *in vivo* loading conditions expected in the intended application. The nature and frequency of the applied mechanical load must be considered on a case by case basis, with specifics beyond the scope of this test method.

5.4 Absorbable devices subjected to flow conditions (for example, vascular stents) may degrade more rapidly than the same device maintained under static degradation test conditions. In specific cases it may be possible to predict the flow conditions that an implant will be subjected to *in vivo* and replicate them *in vitro*. However, details regarding appropriate flow modeling are beyond the scope of this test method.

5.5 Sterilization of HDP materials should be expected to cause changes in molecular weight or structure, or both, of the polymers. This can affect the initial mechanical and physical properties of a material or device, as well as its subsequent rate of degradation. Therefore, if a test is intended to be representative of actual performance *in vivo*, specimens shall be packaged and sterilized in a manner consistent with that of the final device. Non-sterilized specimens may be included for comparative purposes.

6. Materials and Apparatus

6.1 *Physiologic Soaking Solution*—A phosphate-buffered saline (PBS) solution shall be used. The pH of the solution shall be maintained at 7.4 ± 0.2 (see X1.3) unless it is determined through documented literature or self-advised study that the pH should be different due to the physiological conditions of the intended application (this may require use of an alternate buffer system). Limited excursions outside of the specified pH range are tolerable provided the time weighted average pH after buffer replenishment is maintained within this range (see X1.3.1). The ionic concentration should be in the physiological range for the intended application (for example, a solution that contains 0.1 M phosphate buffer and 0.1 M NaCl would be appropriate for most tissue or blood contact devices). The solution-to-HDP mass ratio shall be as high as practical. Although there is some experience with ratios as low as 20:1, the experimenter is cautioned that at lower ratios (that is, less buffering capacity) the solution pH may change more quickly. In accordance with 9.1.3 and X1.4, aging/testing is to be terminated if the solution temperature or pH are allowed to drift outside of the specified ranges. Higher solution/specimen ratios (for example, 100:1) will be more likely to facilitate maintenance of stable aging conditions.

6.1.1 Over the course of the study, the pH should be monitored frequently and the solution shall be changed periodically in order to maintain the pH within the acceptable limits. Refer to X1.5 for additional information.

6.1.2 Other physiologic solutions, such as bovine serum, may be substituted provided the solution is properly buffered. An anti-microbial additive should be used to inhibit the growth of microorganisms in the solution during the test period but the investigator must demonstrate through literature reference or experimentation that the chosen antimicrobial does not affect the degradation rate. Section X1.6 provides additional information. The appropriate MSDS should always be consulted concerning toxicity, safe use, and disposal of such additives.

6.2 *Sample Container*—A self-contained, inert container (bottle, jar, vial, and so forth) capable of holding the test sample and the required volume of physiologic soaking solution (see X1.7). Multiple samples may be stored in the same container provided that suitable sample separation is maintained to allow fluid access to each sample surface and to preclude sample-to-sample contact. Each container must be sealable against solution loss by evaporation.

6.3 *Constant Temperature Bath or Oven*—An aqueous bath or heated air oven capable of maintaining the samples and containers at physiologic temperatures, 37 ± 2°C, for the specified testing periods.

6.4 *pH Meter*—A pH metering device sensitive in the physiological range (pH 6 to pH 8) with a precision of 0.02 or better.

6.5 *Balance*—A calibrated weighing device capable of measuring the weight of a sample to a precision of 0.1 % of its initial weight. A balance having precision to 0.05 % or 0.01 % will facilitate establishment of an appropriate specimen drying period.

6.6 *Other*—Additional equipment as deemed appropriate by the specific test method.

7. Sampling

7.1 *Weight Loss*—A minimum of three samples shall be tested per time period.

7.2 *Molecular Weight*—A minimum of three samples shall be tested per time period.

7.3 *Mechanical Testing*—A minimum of six samples shall be tested per time period.

NOTE 1—Statistical significance may require more than the minimum number of samples to be tested.

7.4 *Solution Temperature and pH*—Soaking solutions shall be tested on a periodic basis throughout the test duration. The

[6] *Handbook of Biodegradable Polymers*, A.J. Domb ed., Harwood Academic Publishers, 1997.

required test period is dependent on the degradation rate of the test polymer, the solution/specimen mass ratio, and the solution's buffering capacity; once per week is generally practical and suggested. In cases where no prior knowledge of the degradation rate is available, it is suggested that the pH be tested at least daily until a baseline is established. This increased sampling frequency may need to be repeated during periods of elevated mass loss (that is, pH change).

8. Sample and Test Specimen

8.1 All test samples shall be representative of the material under evaluation.

8.1.1 For most HDP resins, inter-lot variations in the molecular weight and residual monomer content can be significant. Since these factors can strongly affect degradation rates, molecular weight (or inherent viscosity) and residual monomer content of the source resin and fabricated test parts need to be understood.

8.1.2 Where evaluation aims allow, it is recommended that samples comparing variations in design be produced from the same material lot (or batch) and under the same fabrication conditions.

8.1.3 When testing for inter-lot variability in degradation rate (for example, for process validation purposes), a minimum of three resin lots should be used.

8.2 If a test is intended to be representative of actual performance *in vivo*, specimens shall be packaged and sterilized in a manner consistent with that of the final device. Unsterilized control specimens may be included for comparative purposes showing the effects of sterilization.

9. Procedure

9.1 *Test A, Weight Loss*:

9.1.1 Test samples, in either resin or fabricated form, shall be weighed to a precision of 0.1 % of the total sample weight prior to placement in the physiological solution. Samples shall be dried to a constant weight before initial weighing (see Note 2 and X1.8). Drying conditions, including final relative humidity (if applicable), shall be reported and may include the use of a desiccator, partial vacuum, or elevated temperatures (see Note 3).

9.1.2 Test samples shall be fully immersed in the physiological solution for a specified period of time as discussed in 4.1 (for example, 1 week, 2 weeks, and so forth).

9.1.3 Upon completion of the specified time period, each sample shall be removed, gently rinsed with sufficient distilled water to remove saline, placed in a tared container, and dried to a constant weight (see Note 2 and X1.8). The weight shall be recorded to a precision of 0.1 % of the original total sample weight.

NOTE 2—Drying to a constant weight may be quantified as less than 0.1 % weight change over a period of 48 h, or less than 0.05 % change in 24 h if the balance used is capable of such precision. Section X1.8 provides additional information.

NOTE 3—Elevated temperatures may be used to assist drying of the sample provided that the temperature used does not induce material or chemical changes in the sample. Vacuum drying with a dry gas purge can alternately be used without concern for material degradation. The drying conditions used for the samples prior to aging and for the samples retrieved at each test interval shall be identical. The actual drying conditions used are to be reported.

9.1.4 After weighing, the samples shall not be returned to the physiological solution and shall be retired from the study.

9.2 *Test B, Molecular Weight*:

9.2.1 Prior to placement of samples in the physiological solution, determine the inherent viscosity (logarithmic viscosity number) of representative samples using Test Method D 2857 in a solvent appropriate for the test polymer and at a temperature sufficient to allow adequate solubility and temperature control. For example, poly(*l*-lactide) IV should be determined in chloroform at 25°C. The sample dilution ratio (mg/cm^3) and test temperature shall be reported. Alternative means of molecular weight determination such as size exclusion chromatography may be used when feasible.

9.2.2 Test samples shall be fully immersed in the physiological solution for the specified period of time (for example, 1 week, 3 weeks, 52 weeks, and so forth).

9.2.3 Samples shall be removed at each specified time period throughout the duration of the test, dried as in 9.1.1, and tested for inherent viscosity as above. For polymers that undergo very rapid degradation the molecular weight may change significantly during the drying procedure, causing an overestimate of the degradation rate. Therefore the user should exercise caution in interpretation of this data. This caution does not generally apply to mass loss measurements, since continued degradation after the samples are placed in tared containers will not affect the sample mass unless the degradation products are volatile. For rapidly degrading HDP materials, alternative procedures such as vacuum drying should be considered.

9.3 *Test C, Mechanical Testing*:

9.3.1 Determine the appropriate mechanical properties of representative samples of resin or fabricated forms using tensile, compressive, torque, bending or other appropriate mechanical tests prior to placement of the samples in the physiological solution (time zero). Relevant ASTM test methods may include one or more of the following:

Test Method D 638
Test Method D 671
Test Method D 695
Test Method D 747
Test Method D 790
Test Method D 882
Test Method D 1708
Test Method D 1822

9.3.2 Fully immerse test samples in the physiological solution at 37°C for the specified period of time (for example, 1 week, 2 weeks, and so forth).

9.3.3 Remove samples at each specified time period throughout the duration of the test and retest using the originally selected mechanical test methods and conditions. Unless otherwise deemed relevant, samples should be tested in a non-dried or wet condition. Section X1.9 provides additional information. Testing conditions, wet versus dry, testing temperature, and so forth, should be reported.

9.3.4 Unless specifically germane to the testing scheme, samples shall be retired after the completion of each test.

9.4 *Other Testing*:

9.4.1 The characterization of other material properties and use of other test methods (for example, thermal properties measured using Differential Scanning Calorimetry) may also be performed at each test interval. Conditioning and testing parameters, as well as test results, should all be recorded and reported.

9.4.2 The degradation products of the HDP under investigation may be analyzed. ISO 10993-9 provides guidelines for identification and quantification of degradation products.

9.4.3 Biological response to HDP materials or their degradation products may be investigated. Practice F 748 provides guidelines for the selection of *in vitro* and *in vivo* biocompatibility tests for medical devices and materials.

10. Test Termination

10.1 Testing of samples shall be terminated when one or more of the following has occurred:

10.1.1 A predetermined end point has been reached, that is, elapsed time (for example, 2 years), percent weight loss, minimum inherent viscosity, percent strength loss, and so forth.

10.1.2 Sample integrity has been compromised by the progression of degradation or by mechanical damage to the point that meaningful and reliable data may no longer be obtained.

10.1.3 The soaking solution temperature or pH has drifted outside of the ranges specified in Section 6. Any sample properties obtained since the last in-range temperature and pH measurements shall be considered invalid and so noted in the study report (see X1.4).

11. Report

11.1 Report the following information:

11.1.1 Test material description, batch or lot number and dimensions (as appropriate).

11.1.2 Solution composition and preparation procedures.

11.1.3 Measurements of solution temperature and pH with time, if applicable.

11.1.4 Sample weights expressed as an average percentage loss, initial and subsequent by time period.

11.1.5 Inherent viscosity, initial and subsequent by time period.

11.1.6 Mechanical properties (tensile strength, compressive strength, stiffness, elongation at break, and so forth) appropriate for tests performed, at time zero and at each time period.

11.1.7 Other material properties measured.

11.1.8 Reason(s) for test termination.

12. Precision

12.1 Intralaboratory and interlaboratory reproducibility has not been systematically determined.

13. Keywords

13.1 absorbable; bioabsorbable; degradation; *in vitro*; hydrolytically degradable polymer; hydrolysis; PLA, poly(*l*-lactic acid); poly(*d*-lactide); poly(*d*,*l*-lactide); PGA, poly(glycolide); poly(caprolactone); poly(*p*-dioxanone); surgical implant

APPENDIX

(Nonmandatory Information)

X1. RATIONALE

X1.1 With the development of absorbable polymers for use in implantable devices, there is a need to define standard testing methods that aid in characterizing material and mechanical properties with time in a simulated physiological environment. This test method is intended only as a framework for assessing degradation of implant materials and devices.

X1.1.1 This test method is written for use in characterizing hydrolytically degradable polymer resins and devices. Given the wide variety of bioabsorbable polymer compositions currently available or under investigation, it is incumbent upon the researcher to show through reference or experimentation that other degradation mechanisms are not dominant for the material and the intended use. For example, certain bio-polymers (for example, collagen based materials such as gelatin) are known to degrade *in vivo* primarily by enzymatic attack and the use of this method would give a serious underestimation of the degradation rate. It has also been hypothesized that enzymatic degradation may play a role in the degradation of some synthetic polymers. *in vitro* studies have shown that in sufficient concentration certain enzymes (for example, esterases) may increase degradation rates of specific polymers with susceptible bonds. However, when comparisons have been made between *in vitro* and *in vivo* degradation rates of equivalent samples of hydrolytically degradable polymers under unloaded conditions, the results have consistently shown that *in vivo* acceleration of degradation is either not present or is within the error of measurement.[6]

X1.2 It is recognized that the use of test coupons or specimens in forms other than final implant configurations may be helpful in assessing relevant polymer properties. For example, rectangular or round rods may be necessary to measure flexural properties, while a screw geometry may be required to evaluate the performance of a specific implantable device. However, specimen size, surface area, and process considerations must be addressed in order to relate *in vitro* degradation of test specimens to *in vivo* behavior of implant devices.

X1.3 The pH level specified for the buffered saline solution (that is, 7.4 ± 0.2) was selected on the basis of information received from two consultants to the Task Group that this range of pH values was representative of that found in human blood and extra-cellular fluid. For devices intended for use in applications where the fluid environment has a different pH (for example, urethral stents exposed to urine), a different pH

specification may be more appropriate. It is then incumbent upon the researcher to properly document the choice of environmental conditions. The range of ±0.2 should be maintained regardless of the chosen target value of pH.

X1.3.1 For this application, the time weighted average (TWA) pH is computed using the following equation:

$$\text{TWA pH} = \frac{(\text{pH}_1\, t_1) + (\text{pH}_2\, t_2) + (\text{pH}_3\, t_3) + ... + (\text{pH}_n\, t_n)}{(t_1 + t_2 + t_3 + ... + t_n)} \quad \text{(X1.1)}$$

where:

pH = measured pH at the respective sampling point,
t_1 = elapsed time from buffer replenishment, and
t_n = elapsed time from the prior sampling point.

X1.3.2 It is also recommended that the starting pH of the solution be made as close to the upper end of the chosen range as possible since all known HDP systems generate degradation products that are acidic.

X1.3.3 Information regarding the actual impact both alkaline (pH = 10.09) and acidic (pH = 5.25) pH has on the mechanical properties of absorbable sutures (as observed at pH = 7.44) can be found in Chu.[7]

X1.4 Termination of testing, following a significant change in solution temperature or pH, is indicated in 10.1.3 in order to avoid the generation of invalid results once meaningful loss of control over soaking conditions has occurred.

X1.5 A wide variety of PBS compositions is available and in common use. The components are targeted to achieve final solutions that exert near-physiological osmotic pressures of approximately 280 to 300 mOsm. Common buffer concentrations range from approximately 0.01 to 0.1 M with the higher concentrations providing greater buffering capacity. Additional information about the composition and preparation of pH proportioned monobasic-dibasic phosphate buffer solutions may be found in a handbook available from Calbiochem Inc., a division of EMD Biosciences.[8]

X1.6 Addition of sodium azide at a concentration of 0.1 % is common. Other anitimicrobials that are commonly used include penicillin (100 U/mL), streptomycin (100 µg/mL), and amphotericin (0.25 to 2.5 µg/mL). Regardless of the antibiotic or antimicrobial agent(s) that is used, it is incumbent upon the investigator to determine that their use does not affect the degradation rate of the HDP under investigation. These materials may be hazardous and all persons using them should review the MSDS before handling and use all recommended safety precautions.

X1.7 The inert containers used to hold the samples and solution are usually glass or plastic. However, for some (short duration) tests, stainless steel containers may be appropriate.

X1.8 Revision or further specification of requirements for drying to a constant weight are intended to be developed from round robin testing to follow issuance of this test method. The requirements stated in Note 2 are based on experience with extruded 3.2-mm diameter rods of *l*-PLA dried under nominal full vacuum at room temperature. Constant weight was achieved after 3 to 4 days.

X1.9 Task Group members have observed the use of wet versus dry test conditions to result in significant differences in some mechanical property measurements. It is recommended that testing be performed on specimens that are immersed in water at 37°C at the time of testing. Report whichever conditions are actually used.

[7] Chu, C. C., "The Effect of pH on the *in vitro* Degradation of Poly(glycolide lactide) Copolymer Absorbable Sutures," *Journal of Biomedical Materials Research*, 16, 1982, pp. 117-124.

[8] Available from EMD Biosciences, Inc., 10394 Pacific Center Ct., San Diego, CA 92121. http://www.emdbiosciences.com