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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 F1635; 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.

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

~~1.4~~

1.3 This test method provides guidance for mechanical loading or fluid flow, or both, when relevant to the device being evaluated. The specifics of loading type, magnitude, and frequency for a given application are beyond the scope of this test method.

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

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D638 Test Method for Tensile Properties of Plastics

D671 Test Method for Flexural Fatigue of Plastics by Constant-Amplitude-of-Force[3]

D695 Test Method for Compressive Properties of Rigid Plastics

D747 Test Method for Apparent Bending Modulus of Plastics by Means of a Cantilever Beam

D790 Test Methods for Flexural Properties of Unreinforced and Reinforced Plastics and Electrical Insulating Materials

D882 Test Method for Tensile Properties of Thin Plastic Sheeting

D1708 Test Method for Tensile Properties of Plastics by Use of Microtensile Specimens

D1822 Test Method for Tensile-Impact Energy to Break Plastics and Electrical Insulating Materials

D2857 Practice for Dilute Solution Viscosity of Polymers

F748 Practice for Selecting Generic Biological Test Methods for Materials and Devices

2.2 *Other Referenced Standard:*

ISO 31–8 Physical Chemistry and Molecular Physics - Part 8: Quantities and Units

ISO 10993–1 Biological Evaluation of Medical Devices—Part 1 Evaluation and Testing[4]

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

[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, http://www.ansi.org.

NIST Special Publication SP811 Guide for the Use of the International System of Units (SI)[5]

3. Terminology

3.1 *Definitions:*

3.1.1 ~~*resin*—any polymer that is a basic material for plastics.~~ absorbable, *adj— in the body*—an initially distinct foreign material or substance that either directly or through intended degradation can pass through or be assimilated by cells and/or tissue.

NOTE 1—See Appendix X2 for a discussion regarding the usage of absorbable and other related terms.

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).

3.1.3 *resin*—any polymer that is a basic material for plastics.[6]

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

NOTE 2—The term molecular weight (abbreviated MW) is obsolete and should be replaced by the SI (Système Internationale) equivalent of either relative molecular mass (M_r), which reflects the dimensionless ratio of the mass of a single molecule to an atomic mass unit [see ISO 31–8], or molar mass (M), which refers to the mass of a mole of a substance and is typically expressed as grams/mole. For polymers and other macromolecules, use of the symbols M_w, M_n, and M_z continue, referring to mass-average molar mass, number-average molar mass, and z-average molar mass, respectively. For more information regarding proper utilization of SI units, see NIST Special Publication SP811.

5. Significance and Use

5.1 This test method is intended to help assess the ~~biodegradation~~degradation 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.[7]

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~~ .

5.3.1 *Mechanically Unloaded Hydrolytic Evaluation*—Conditioning of a hydrolysable device under mechanically unchallenged hydrolytic conditions at 37°C in buffered saline is a common means to obtain a first approximation of the degradation profile of an absorbable material or device. It does not necessarily represent actual *in vivo* service conditions, which can include mechanical loading in a variety of forms (for example. static tensile, cyclic tensile, shear, bending, and so forth). If the performance of a device under its indicated use includes loading, hydrolytic aging alone is NOT sufficient to fully characterize the device.

5.3.2 *Mechanically Loaded Hydrolytic Evaluation*—The objective of loading is to approximate (at 37°C in buffered saline) the actual expected device service conditions so as to better understand potential physicochemical changes that may occur. Such testing can be considered as necessary if loading can be reasonably expected under *in vivo* service conditions. When feasible, test specimens should be loaded in a manner that simulates *in vivo* conditions, both in magnitude and type of loading. Clinically relevant cyclic load tests may include testing to failure or for a specified number of cycles followed by testing to evaluate physicochemical properties.

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

[5] Available from National Institute of Standards and Technology (NIST), 100 Bureau Dr., Stop 1070, Gaithersburg, MD 20899-1070, at http://physics.nist.gov/cuu/Units/bibliography.html.

[6] ~~*Handbook of Biodegradable Polymers*, A.J. Domb ed., Harwood Academic Publishers, 1997.~~ Polymer Technology Dictionary, Tony Whelan ed., Chapman & Hall, 1994.

~~[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.~~

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

5.3.2.1 *Static Loading*—It is notable that for some polymeric materials it has been shown that a constant load results in the same failure mechanism (for example, creep) and is the worst case when compared to a cyclic load (where the maximum amplitude of the cyclic load is equal to the constant load). Thus, in specific cases it may be acceptable to simplify the test by using a constant load even when the anticipated *in vivo* loading is cyclic. It is encumbent upon the user of this test method to demonstrate through experiment or specific reference that this simplification is applicable to the polymer under investigation and does not alter the failure mode of the test specimen. If such evidence is not available ,it is necessary to recognize that static loading and cyclic loading are measuring different material properties and are not comparable. Using one to replace the other could lead to misinterpretation of the results.

NOTE 3—Caution must be taken to ensure that fixturing does not introduce artifactual performace or degradation issues, or both. An example is the use of rigid foam block, which restricts swelling & expansion and can elevate pull out strength test results from sample compression within the block. Additionally, restricted perfusion due to the closed cell nature of the foam can result in concentration of acidic byproducts that result in accelerated degradation when compared to a normally perfused and buffered *in vivo* condition.

NOTE 4—When performing degradation testing under load, it may be necessary to consider and monitor polymer creep during testing, which may be significant.

5.4 Absorbable devices subjected to flow conditions (for example, vascular stents, particularly those with a drug eluting component) may degrade more rapidly than the same device maintained under static degradation test conditions. When it is feasible to estimate the flow conditions that an implant will be subjected to *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.~~

~~5.4Absorbable 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* the degradation study should be conducted under flow conditions. 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~~molar mass 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*~~Molar Mass—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~~5—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 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~~molar mass and residual monomer content can be significant. Since these factors can strongly affect degradation rates, ~~molecular weight~~molar mass (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~~ Note 6 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~~Note 7).

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~~ Note 6 and X1.8). The weight shall be recorded to a precision of 0.1 % of the original total sample weight.

NOTE ~~2—Drying~~ 6—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~~7—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*~~Test B, Molar Mass:

~~9.2.1Prior to placement of samples in the physiological solution, determine the inherent viscosity (logarithmic viscosity number) of representative samples using Test Method~~

9.2.1 Prior to placement of samples in the physiological solution, determine the molar mass of representative samples using either inherent viscosity (logarithmic viscosity number) testing following the recommendations of Test Method D2857 ~~in a solvent appropriate for the test polymer and at a temperature sufficient to allow adequate solubility and temperature control. For example, poly(~~*or size exclusion chromatography. Testing shall be done in a solvent appropriate for the test polymer and at a temperature sufficient to allow solubility and temperature control. For example, the molar mass of poly(l-lactide) ~~IV~~ should be determined in chloroform at ~~25°C.~~30°C. The sample dilution ratio (mg/cm^3) and test temperature shall be reported. Alternative means of ~~molecular weight~~molar mass 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~~molar mass 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