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Standard Practice for *In-Vitro* Environmental Conditioning of Polymer Matrix Composite Materials and Implant Devices[1]

This standard is issued under the fixed designation F1634; 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 two procedures for conditioning non-absorbable polymer matrix composite (PMC) materials and implant devices in a liquid environment to obtain a state of saturation.

1.2 The purpose of this practice is to standardize methods and reporting procedures for conditioning PMC materials and implant devices (PMC specimens) in a user selected liquid environment prior to conducting subsequent tests. It is not the purpose of this practice to determine the diffusion coefficients or actual saturation levels of a given liquid into the materials and devices. For these determinations, other procedures, such as Test Method D5229/D5229M, may be followed.

1.3 Diffusion of liquid into a solid material is a slow process. While the time necessary to achieve saturation at 37°C may be sufficiently short for thin specimens, such as fracture fixation plates, it may be prohibitively long in thick sections, such as femoral components for hip arthroplasty. However, the diffusion process may be accelerated at an elevated temperature. Consequently, two separate procedures (Procedures A and B) are presented in this practice. Procedure A covers exposing the specimen to the desired conditioning environment at 37°C. Procedure B prescribes a method to accelerate the diffusion process by conditioning the specimen at a selected elevated temperature.

1.4 This practice does not specify the test environment to be used for conditioning. However, the pH value of immersion liquid shall be maintained at 7.4 ± 0.2 to simulate the *in vivo* environment.

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

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

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D618 Practice for Conditioning Plastics for Testing

D756 Practice for Determination of Weight and Shape Changes of Plastics Under Accelerated Service Conditions (Withdrawn 1998)[3]

D3878 Terminology for Composite Materials

D5229/D5229M Test Method for Moisture Absorption Properties and Equilibrium Conditioning of Polymer Matrix Composite Materials

3. Terminology

3.1 *Definitions:*

3.1.1 *cumulative moisture content, M_t (%), n*—the amount of absorbed moisture in a material at a given time *t*, expressed as a percentage of the weight of absorbed moisture divided by the initial specimen weight, as follows:

$$M_t, \% = \frac{W_i - W_b}{W_b} \times 100 \quad (1)$$

where:

W_t = current specimen weight, g, and

W_b = initial (baseline) specimen weight at $t = 0$ and standard laboratory atmosphere, g.

3.1.2 *liquid, n*—water, saline solution, calf serum, or any other liquid solution that is used to condition PMC specimens.

3.1.3 *nominal saturated moisture content, M_s (%)*—an approximation of the amount of moisture absorbed by a specimen at saturation, expressed as a percentage of the weight of absorbed moisture at approximate saturation divided by the initial specimen weight, as follows:

[1] This practice 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] The last approved version of this historical standard is referenced on www.astm.org.

$$M_s, \% = \frac{W_s - W_b}{W_b} \times 100 \quad (2)$$

where:

W_s = specimen weight at approximate saturation, g, and
W_b = initial (baseline) specimen weight at $t = 0$ and standard laboratory atmosphere, g.

3.1.4 *standard laboratory atmosphere, n*— a laboratory atmosphere having a temperature of 23 ± 2°C and a relative humidity of 50 ± 10 %.

4. Summary of Test Method

4.1 In Procedure A, a specimen is immersed in a liquid bath at 37 ± 1°C with a pH value of 7.4 ± 0.2.

4.2 In Procedure B, conditioning occurs in a liquid bath at a selected elevated temperature.

4.3 Weight change is monitored over time until specimens reach the nominal moisture saturation content.

4.4 Keep specimens in the conditioning bath for storage prior to subsequent tests.

5. Significance and Use

5.1 The conditioning procedures covered in this practice provide methods for saturating PMC specimens in a liquid environment prior to conducting other tests for property evaluation.

5.2 The conditioning may affect geometric and dimensional changes in specimens. In some severe cases, chemical changes may occur in the fiber, polymer and fiber-polymer interphase and interface.

5.3 Caution must be taken if Procedure B (10.2, Procedure B—Accelerated Moisture Saturation at Elevated Temperature) is followed to condition PMC specimens at an elevated temperature. Physical and chemical reactions in materials are normally temperature dependent. An increase in experimental temperature may accelerate a desirable moisture diffusion process. However, elevated temperatures above 37°C may also cause undesirable reactions that do not represent appropriate responses of materials at 37°C. Consequently, a pilot study is recommended in Procedure B to determine if a selected elevated temperature can be used for accelerated conditioning. If the properties of materials are determined to be irreversibly affected by this pilot study at the selected elevated temperature, then either an appropriate lower elevated temperature should be determined by repeating the pilot study, or Procedure B should not be used.

6. Apparatus

6.1 *Balance*—An analytical balance capable of measuring weight of specimens to within a resolution of at least 0.005 % of the total specimen weight.

6.2 *Conditioning Bath*—A temperature-controlled liquid bath shall be capable of maintaining the required temperature to within ±1°C. The bath shall be monitored either on an automated continuous basis or on a manual basis at regular intervals.

6.3 *Specimen Bag*—A sealable, flexible, moisture-proof bag made of material suitable for exposure to specimens that have been removed from the conditioning bath for cooling prior to weighing. Bags that meet the requirement of MIL-B-131 have been found to be satisfactory for use in such applications.

6.4 *Absorbent Cloth*—Clean, non-linting absorbent cloth for use in wiping excess liquid from surface of specimens.

6.5 *Gloves*—Clean, non-linting gloves for use when handling specimens.

6.6 *pH Measurement System*—An analytical system capable of measuring pH to within ±0.1.

6.7 *Differential Scanning Calorimeter*—An analytical system capable of heating a specimen at a controlled rate while measuring heat input and temperature.

7. Sampling and Test Specimens

7.1 *Preparation*—Precaution shall be taken to avoid the entrapment of moisture in uneven surfaces, or delamination due to inappropriate machining and manufacturing processes.

7.2 *Labeling*—Label the specimen so as to be distinct from each other in a manner that will both be unaffected by the test and not influence the test and, furthermore, will not be removed during conditioning.

8. Measurements of Test Specimens

8.1 The following measurements shall be made on specimens prior to immersion, after conditioning at the end of a test procedure, and at any intermediate stage as prescribed in the test procedures:

8.1.1 *Weight*—The weight within 0.005 % of specimen weight.

8.1.2 Characteristic dimensions of specimens may be measured as a function of immersion time to determine the amount of swelling induced by moisture absorption.

9. Visual Examination

9.1 Noticeable qualitative changes in surfaces, outline, and general appearance of the test specimen shall be recorded after each stage of the testing procedure. These changes include color, surface irregularities, odor, surface voids, delamination and cracking. The immersion liquid should also be observed for evidence of material that has leached from specimens or holders, and evidence of bacterial or fungal contamination. If bacterial or fungal contamination is found, specimens should be removed from the solution, washed with detergent and water, rinsed, and placed in fresh solution. If contamination is a recurring problem, antibacterial or antifungal agents must be added to the solution; minimal amounts should be used as they may affect specimen properties.

10. Procedures

10.1 *Procedure A—Moisture Saturation Determination at 37°C:*

10.1.1 *Specimen Preconditioning*—Bring the test specimens to a uniform 23 ± 2°C after manufacturing process.

10.1.2 *Moisture Absorption:*

10.1.2.1 Record the initial (baseline) weight, W_b.

10.1.2.2 Place the specimen in the conditioning bath, which has previously reached the specified temperature 37 ± 1°C. The pH value of immersion liquid used shall be maintained at 7.4 ± 0.2 throughout the conditioning process and monitored at least once a week. If the solution pH falls outside the designated range, the solution should be changed. The pH should not be maintained by repeatedly adding buffer to the same solution. This will change solution composition and may affect specimen properties. Evaporation losses should be made up with sterile deionized water if saline, serum, plasma, or other hydrous medium is used as the conditioning environment.

10.1.2.3 Monitor the weight gain of specimens over time. A suggested schedule is to weigh each specimen every 24 h for the first 120 h, then every 96 h.

10.1.2.4 At the end of each time interval, remove the specimens from the conditioning bath and place them in the specimen bag. Seal the bag and allow the specimens to come to laboratory standard temperature. Remove the specimens from the bag and wipe the specimens free of surface moisture with an absorbent lint-free cloth. Wait for 10 min and measure the weight of specimens to the required precision, and W_t, along with the corresponding total elapsed time and the time interval since the previous measurement.

10.1.2.5 Return the specimens to the conditioning bath. The specimens shall not be out of the conditioning bath for more than 30 min and shall not be out of the specimen bag for more than 15 min.

10.1.2.6 Calculate cumulative moisture content, M_t (%), using Eq 1 at each time interval and plot versus time.

10.1.2.7 The minimum time, t_{min}, required to reach nominal saturated moisture content, M_s, is the time at which the change in cumulative moisture content from the prior measurement is less than 0.010 % of specimen weight for three consecutive weighings with no less than 96 h of elapsed time between each consecutive weighing.

10.1.2.8 Following moisture saturation within the specified tolerance range, the specimen should be stored in a bath of the same fluid which is to be used for post-conditioning testing until the time the post-conditioning testing is conducted.

10.2 *Procedure B—Accelerated Moisture Saturation at Elevated Temperature:*

10.2.1 *Determination of Accelerated Temperature (T_a) Level:*

10.2.1.1 Specimens should be first saturated in the conditioning environment at 37°C and then evaluated by differential scanning calorimeter (DSC) evaluation over a temperature range of 37 to 120°C. The purpose of this is to determine if a material transition temperature (that is, glass transition or melting temperature), or a degradation temperature (such as an oxidation or thermal decomposition temperature), occurs within this temperature range. If the material is stable within this range, then accelerated conditioning may be conducted at T_a = 95°C in a conditioning environment containing water. If a transition or degradation temperature is found between 37 and 120°C, then the maximum acceleration temperature to be used should be at least 25°C below the lowest transition or decomposition temperature. If nonhydrous solutions are used as the conditioning environment (that is, pure lipid), then a similar procedure can be followed except the temperature would now be governed by the boiling or decomposition temperature of the given environment and specimen combination.

10.2.2 *Effect of Accelerated Temperature Conditioning:*

10.2.2.1 Once T_a is determined from 10.2.1.1, at least five samples representative of the specimen being evaluated should be conditioning at both T_a and 37°C and then tested to check for conditioning temperature induced differences. Because conditioning may influence different material properties of PMC specimens in different ways, the test implemented to check for differences between T_a and 37°C should closely match the intended post-conditioning test that the PMC specimen will be conditioned for. If T_a influences the properties in question, then a lower conditioning temperature must be identified by repeating this procedure at selected lower temperatures in which the property is not influenced by conditioning. If the property is determined to be not influenced by T_a, then T_a can be used to accelerate the conditioning process for subsequent specimens.

10.2.3 *Accelerated Conditioning:*

10.2.3.1 Follow the procedures outlined in 10.1.2 with the exception that the conditioning bath will be maintained at T_a ± 1.0°C. The same time-measurement sequence as described in 10.1.2 should be used to determine the minimum time, t_{min}, when saturation is reached. The bath should be adequately sealed to minimize evaporation loss and periodically monitored to ensure adequate bath depth is maintained for complete immersion of samples. If the bath is physically sealed, a safe mechanism of pressure relief (that is, pressure relief valve or releasable lid seal) must be provided to prevent pressure build-up if the oven is accidentally overheated. Evaporation losses should be made up with sterile deionized water if saline, serum, plasma, or other hydrous medium is used as the conditioning environment.

10.2.3.2 Following moisture saturation within the specified tolerance range, the specimen should be stored in a bath of the same fluid which is to be used for post-conditioning testing until the time the post-conditioning testing is conducted.

11. Selection of Conditioning Procedure

11.1 The choice between procedures should preferably be based on the one that gives the most reproducible results.

12. Report

12.1 Report the following information:

12.1.1 Description of the specimen or device being considered (that is, material name, dimensions, part number, model number, size designation).

12.1.2 The date of issue of this practice and the procedure used.

12.1.3 The date(s) and location(s) of the test.

12.1.4 The name(s) of the test operator(s).

12.1.5 Any variations to this practice, anomalies noticed during testing or equipment problems occurring during testing.

12.1.6 Description of the materials and fabrication method used in preparing the specimen including: cure cycle, consolidation method and a description of equipment used.

12.1.7 Method of preparing the test specimens.

12.1.8 Weight of the specimen following preconditioning prior to moisture conditioning and at the completion of the saturation procedure.

12.1.9 Type of conditioning bath used, liquid used for the test and its average pH value, the average actual test temperature, and measurement time interval.

12.1.10 Specimen weight at each interval.

12.1.11 Plots of percent weight changes versus time, calculated by (Eq 1).

12.1.12 Description of procedures, frequency and reasons for changing immersion liquid.

12.1.13 Any unusual observations described in 9.1.

12.1.14 Mean, standard deviation, number of specimens, coefficient of variation of specimen property measured, number of samples used in the verification test and T_a, if Procedure B is followed.

12.1.15 Any dimension changes during immersion due to swelling (if measured).

13. Keywords

13.1 accelerated conditioning; composite; diffusion; environmental conditioning; fiber; implant devices; moisture absorption; polymer

APPENDIX

(Nonmandatory Information)

X1. RATIONALE

X1.1 The primary reason for developing this practice is to establish methods for *in vitro* environmental conditioning of polymer matrix composite materials and implant devices (PMC specimens) prior to determining their properties and behavior. It is not the purpose of this practice to accurately determine actual percentages of moisture uptake at saturation or diffusion coefficients. Therefore, the PMC specimens do not need to be dried prior to environmental conditioning.

X1.2 On a molecular scale, PMC specimens are porous materials through which components of a surrounding environment, such as water and salt ions, can diffuse. Diffusion into a PMC specimen is a slow, temperature dependent process which may significantly influence material properties. Therefore, testing a PMC specimen in a simulated *in vivo* environment, without first conditioning the material to attain environmental saturation, may lead to erroneous test results regarding the performance of the material or device in the body. This practice presents test methods to condition PMC specimens.

X1.3 Theoretically, an infinite amount of time is necessary to allow a liquid diffusing into a solid to achieve full diffusion equilibrium. Therefore, some approximation to full saturation must be defined for practical use. Weight gain at diffusion equilibrium of water in most engineering thermoplastic polymers is less than 1.0 % by weight. In order to obtain the necessary sensitivity for weight gain measurement, a balance sensitivity is specified in this practice of at least 0.005 wt % of total specimen weight. This sensitivity was selected to provide a sufficient number of possible weight increments in between the baseline weight (W_b) and the approximate saturation weight (W_s) to adequately determine when the specimens are effectively saturated.

X1.4 In 9.1, the conditioning solution should be visually monitored on a periodic basis for evidence of bacterial or fungal contamination. Molecular species released from the bacteria or fungus, or both, may influence the properties of the test specimen. Therefore, the specimens should be removed and washed and the solution should be replenished if contamination is observed.

X1.5 In 10.1.2.2, the pH of the immersion solution is to be monitored at least once per week to ensure it is within the specified range of 7.4 ± 0.02. In buffer solutions, the pH may remain stable for a long period of time, and then abruptly change once all the buffer has been depleted. Also, solution pH may influence the properties of the PMC specimens that will be measured following environmental conditioning. It is therefore important that pH be monitored on a periodic basis throughout the conditioning process.

X1.6 In 10.1.2.2 and 10.2.3.1 it is stated that if hydrous solutions are utilized, then evaporation losses should be made up with deionized water. Evaporative losses from a physiologic solution will be due to loss of water only. The larger molecules and salt ions will remain, thus tending to concentrate the solution. It is therefore important to maintain a nearly constant volume of solution with evaporation losses being made up by adding water.

X1.7 A minimum of 96 h is suggested between consecutive weighings in 10.1.2.3. As the effective saturation level is approached, the rate of weight gain will become very slow well before reaching effective saturation. Time intervals less than 96 h between weighings may therefore lead to erroneous conclusions regarding whether or not effective saturation has been reached.

X1.8 Environmental conditioning of a material to saturation at a temperature above a given thermal transition temperature may influence specimen properties differently than if the material was conditioning to saturation below the transition temperature. Therefore, if accelerated conditioning is to be

utilized, it is important to ensure that the material does not undergo a thermal transition within the temperature range between 37 and 25°C above the desired accelerated temperature. Thus, as indicated in 10.2.1.1, if accelerated conditioning is to be used, the specimen should be checked by differential scanning calorimetry (DSC) for the presence of any thermal transition within the range of 37 to 120°C. The upper limit of 120°C is specified as it is 25°C above 95°C, which is the upper temperature limit for accelerated conditioning in hydrous solutions at atmospheric pressure.