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Standard Guide for Characterization and Testing of Biomaterial Scaffolds Used in Tissue-Engineered Medical Products[1]

This standard is issued under the fixed designation F 2150; 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.

~~ε[1] NOTE—The designation, year date, and footnote 1 were editorially corrected in June 2002.~~

1. Scope

1.1 This guide is a resource of currently available test methods for the characterization of the compositional and structural aspects of biomaterial scaffolds used to develop and manufacture tissue-engineered medical products (TEMPs).

1.2 The test methods contained herein guide characterization of the bulk physical, chemical, mechanical, and surface properties of a scaffold construct. Such properties may be important for the success of a TEMP, especially if they affect cell retention, activity and organization, the delivery of bioactive agents, or the biocompatibility and bioactivity within the final product.

~~1.3This guide may be used as guidance in the selection of appropriate test methods for the generation of a raw material or original equipment manufacture (OEM) specification. This guide also may be used to characterize the scaffold component of a finished medical product.~~

~~1.4This guide addresses natural, synthetic, or combination scaffold materials with or without bioactive agents or biological activity. This guide does not address the characterization or release profiles of any biomolecules, cells, drugs, or bioactive agents that are used in combination with the scaffold.~~

~~1.5~~

1.3 This guide may be used in the selection of appropriate test methods for the generation of an original equipment manufacture (OEM) specification. This guide also may be used to characterize the scaffold component of a finished medical product.

1.4 This guide is intended to be utilized in conjunction with appropriate characterization(s) and evaluation(s) of any raw or starting material(s) utilized in the fabrication of the scaffold, such as described in Guide F 2027.

1.5 This guide addresses natural, synthetic, or combination scaffold materials with or without bioactive agents or biological activity. This guide does not address the characterization or release profiles of any biomolecules, cells, drugs, or bioactive agents that are used in combination with the scaffold. A determination of the suitability of a particular starting material and/or finished scaffold structure to a specific cell type and/or tissue engineering application is essential, but will require additional *in vitro* and/or *in vivo* evaluations considered to be outside the scope of this guide.

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 requirements prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:* [2]

D 412~~Test Methods for Vulcanized Rubber and Thermoplastic Rubbers and Thermoplastic Elastomers—Tension~~ Test Methods for Vulcanized Rubber and Thermoplastic ElastomersTension

D 570 Test Method for Water Absorption of Plastics

D 638 Test Method for Tensile Properties of Plastics

D 648 Test Method for Deflection Temperature of Plastics Under Flexural Load in the Edgewise Position

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

D 695 Test Method for Compressive Properties of Rigid Plastics

~~[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 F04.43 on Tissue Engineered Biomaterials.~~

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[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.

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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, Vol 09.01.~~volume information, refer to the standard's Document Summary page on the ASTM website.

D 747 Test Method for Apparent Bending Modulus of Plastics by Means of a Cantilever Beam
D 790 Test Methods for Flexural Properties of Unreinforced and Reinforced Plastics and Electrical Insulating Materials
D 792 Test Methods for Density and Specific Gravity (Relative Density) of Plastics by Displacement
D 882 Test Method for Tensile Properties of Thin Plastic Sheeting
D 1042 Test Method for Linear Dimensional Changes of Plastics Under Accelerated Service Conditions
D 1238 Test Method for Melt Flow Rates of Thermoplastics by Extrusion Plastometer
D 1388 Test Method for Stiffness of Fabrics
D 1621 Test Method for Compressive Properties ~~of~~Of Rigid Cellular Plastics
D 1623 Test Method for Tensile and Tensile Adhesion Properties of Rigid Cellular Plastics
D 1708~~Test Method for Tensile Properties of Plastics by Use of Microtensile Specimens~~[3]
~~D1898Practice for Sampling of Plastics~~ Test Method for Tensile Properties of Plastics by Use of Microtensile Specimens
D 2857 Practice for Dilute Solution Viscosity of Polymers
D 2873 Test Method for Interior Porosity of Poly(Vinyl Chloride) (PVC) Resins by Mercury Intrusion Porosimetry
D 2990 Test Methods for Tensile, Compressive, and Flexural Creep and Creep-Rupture of Plastics
D 3016 Practice for Use of Liquid Exclusion Chromatography Terms and Relationships
D 3039/D 3039M Test Method for Tensile Properties of Polymer Matrix Composite Materials
D 3417 Test Method for Enthalpies of Fusion and Crystallization of Polymers by Differential Scanning Calorimetry (DSC)
D 3418~~Test Method for Transition Temperatures of Polymers by Differential Scanning Calorimetry~~[6] Test Method for Transition Temperatures and Enthalpies of Fusion and Crystallization of Polymers by Differential Scanning Calorimetry
D 4001 Test Method for Determination of Weight-Average Molecular Weight of Polymers ~~by~~By Light Scattering
D 4404 Test Method for Determination of Pore Volume and Pore Volume Distribution of Soil and Rock by Mercury Intrusion Porosimetry
D 4603 Test Method for Determining Inherent Viscosity of Poly(Ethylene Terephthalate) (PET) by Glass Capillary Viscometer
D 5226 Practice for Dissolving Polymer Materials
D 5296 Test Method for Molecular Weight Averages and Molecular Weight Distribution of Polystyrene by High Performance Size-Exclusion Chromatography
D 5732 Test Method for Stiffness of Nonwoven Fabrics Using the Cantilever Test
D 6125 Test Method for Bending Resistance of Paper and Paperboard (Gurley Type Tester)
D 6420 Test Method for Determination of Gaseous Organic Compounds by Direct Interface Gas Chromatography-Mass Spectrometry
D 6474 Test Method for Determining Molecular Weight Distribution and Molecular Weight Averages of Polyolefins by High Temperature Gel Permeation Chromatography
D 6539 Test Method for Measurement of Pneumatic Permeability of Partially Saturated Porous Materials by Flowing Air
D 6579 Practice for Molecular Weight Averages and Molecular Weight Distribution of Hydrocarbon and Terpene Resins by Size-Exclusion Chromatography
E 128 Test Method for Maximum Pore Diameter and Permeability of Rigid Porous Filters for Laboratory Use
E 177 Practice for Use of the Terms Precision and Bias in ASTM Test Methods
E 456 Terminology ~~for~~ Relating to Quality and Statistics
E 473 Terminology Relating to Thermal Analysis and Rheology
E 691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method
E 793 Test Method for Enthalpies of Fusion and Crystallization by Differential Scanning Calorimetry
E 794 Test Method for Melting ~~and~~And Crystallization Temperatures By Thermal Analysis
E 967~~Practice~~ Test Method for Temperature Calibration of Differential Scanning Calorimeters and Differential Thermal Analyzers
E 968 Practice for Heat Flow Calibration of Differential Scanning Calorimeters
E 996 Practice for Reporting Data in Auger Electron Spectroscopy and ~~X-Ray~~X-ray Photoelectron Spectroscopy
E 1078 Guide for ~~Procedures for~~ Specimen Preparation and Mounting in Surface Analysis
E 1142 Terminology Relating to Thermophysical Properties
E 1294 Test Method for Pore Size Characteristics of Membrane Filters Using Automated Liquid Porosimeter
E 1298 Guide for Determination of Purity, Impurities, and Contaminants in Biological Drug Products
E 1356 Test Method for Assignment of the Glass Transition Temperatures by Differential Scanning Calorimetry ~~or Differential Thermal Analysis~~[16]
E 1642 Practice for General Techniques of Gas Chromatography Infrared (GC/IR) Analysis
E 1829~~Guide for Handling Specimens Prior to Surface Analysis~~[17] Guide for Handling Specimens Prior to Surface Analysis
E 1994 Practice for Use of Process Oriented AOQL and LTPD Sampling Plans
F 151 Test Method for Residual Solvents in Flexible Barrier Materials
F 316 Test Methods for Pore Size Characteristics of Membrane Filters by Bubble Point and Mean Flow Pore Test
F 748 Practice for Selecting Generic Biological Test Methods for Materials and Devices

F 1249 Test Method for Water Vapor Transmission Rate Through Plastic Film and Sheeting Using a Modulated Infrared Sensor
F 1251 Terminology Relating to Polymeric Biomaterials in Medical and Surgical Devices
F 1634 Practice for In-Vitro Environmental Conditioning of Polymer Matrix Composite Materials and Implant Devices
F 1635 ~~Test Method for In Vitro Degradation Testing of Poly (L-lactic Acid) Resin and Fabricated Form for Surgical Implants[20]~~ Test Method for ***in vitro*** Degradation Testing of Hydrolytically Degradable Polymer Resins and Fabricated Forms for Surgical Implants
F 1884 Test Methods for Determining Residual Solvents in Packaging Materials
F 1980 ~~Guide for Accelerated Aging of Sterile Medical Device Packages[11]~~ Guide for Accelerated Aging of Sterile Barrier Systems for Medical Devices
F 1983 Practice for Assessment of Compatibility of Absorbable/Resorbable Biomaterials for Implant Applications
F 2025 Practice for Gravimetric Measurement of Polymeric Components for Wear Assessment
F 2027 ~~Guide for Characterization and Testing of Substrate Materials for Tissue-Engineered Medical Products[20]~~ Guide for Characterization and Testing of Substrate Materials for Tissue-Engineered Medical Products
F 2312 Terminology Relating to Tissue Engineered Medical Products
F 2450 Guide for Assessing Microstructure of Polymeric Scaffolds for Use in Tissue Engineered Medical Products
G 120 Practice for Determination of Soluble Residual Contamination ~~in Materials and Components~~ by Soxhlet Extraction

2.2 *AAMI Standards:*[3]
AAMI STBK9-1 Sterilization—Part 1: Sterilization in Health Care Facilities
AAMI STBK9-2 Sterilization—Part 2: Sterilization Equipment
AAMI STBK9-3 Sterilization—Part 3: Industrial Process Control

2.3 *ANSI Standards:*[4]
ANSI/ISO/ASQ Q9000-2000: Quality Management Systems—Fundamentals and Vocabulary
ANSI/ISO/ASQ Q9001-2000: Quality Management Systems: Requirements

2.4 *British Standards Institute:*[4]
~~British~~EN 1244–1 British Standard—Animal Tissues and Their Derivatives Utilized in the Manufacture of Medical Devices—Part 1: Analysis and Management of Risk ~~(EN 12442-1)[22]~~
EN 12442–2 British Standard—Animal Tissues and Their Derivatives Utilized in the Manufacture of Medical Devices—Part 2: Controls on Sourcing, Collection, and Handling ~~(EN 12442-2)[22]~~
EN 12442–3 British Standard—Animal Tissues and Their Derivatives Utilized in the Manufacture of Medical Devices—Part 3: Validation of the Elimination and/or Inactivation of Viruses and Transmissible Agents ~~(EN 12442-3)[22]~~

2.5 ~~*ISO Standards:*~~ *ISO Standards:*[4]
ISO 31-8 Physical Chemistry and Molecular Physics—Part 8: Quantities and Units
ISO ~~1133-1991~~ 1133 Determination of the Melt-Mass Flow Rate (MFR) and the Melt Volume-Flow Rate (MVR) of Thermoplastics
ISO 10993-9 Biological Evaluation of Medical Devices—Part 9: Degradation of Materials Related to Biological Testing
ISO 10993-13 Biological Evaluation of Medical Devices—Part 13: Identification and Quantification of Degradation Products from Polymers
ISO 10993-14 Biological Evaluation of Medical Devices—Part 14: Identification and Quantification of Degradation Products from Ceramics
ISO 10993-15 Biological Evaluation of Medical Devices—Part 15: Identification and Quantification of Degradation Products from Coated and Uncoated Metals and Alloys
ISO 11357-1 Plastics—Differential Scanning Calorimetry (DSC)—Part 1: General Principles
ISO 11357-2 Plastics—Differential Scanning Calorimetry (DSC)—Part 2: Determination of Glass Transition Temperature

2.6 *U.S. Code of Federal Regulations:*[5]
21 CFR Part 58 Title 21—Food And Drug Administration, Part 58—Good Laboratory Practice For Nonclinical Laboratory Studies
~~Title 21—Food and Drugs Services, Part 820—Quality System Regulation (21 CFR Part 820)~~ 21 CFR Part 820 Title 21—Food and Drugs Services, Part 820—Quality System Regulation

2.7 *U.S. Pharmacopeia (USP) Standards:*
~~Source: General Tests and Assays—USP24/NF19, Jan. 1, 2000~~ [6]

~~[3] Annual Book of ASTM Standards, Vol 08.01.~~
[3] Available from the Association for the Advancement of Medical Instrumentation, 1110 N. Glebe Rd., Suite 220, Arlington, VA 22201-4795.
~~[4] Annual Book of ASTM Standards, Vol 07.01.~~
[4] Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036, http://www.ansi.org.
~~[5] Discontinued 1998; see 1997 *Annual Book of ASTM Standards* , Vol 08.01.~~
[5] Available from U.S. Government Printing Office Superintendent of Documents, 732 N. Capitol St., NW, Mail Stop: SDE, Washington, DC 20401, http://www.access.gpo.gov.
~~[6] Annual Book of ASTM Standards, Vol 08.02.~~

Source: General Tests and Assays—USP30/NF25, May 1, 2007

2.8 *NIST Document:*[7]

NIST SP811 Special Publication SP811: Guide for the Use of the International System of Units (SI)

2.9 *Other Documents/Web Sites:*

U.S. Food & Drug Administration (FDA) Center for Devices & Radiologic Health (CDRH), Consensus Standards Database[8]

FDA-CDRH Guidance Documents Database[9]

FDA-CDRH Premarket Approval (PMA) Database[10]

FDA-CDRH 510(k) (Premarket Notification) Database[11]

3. Terminology

3.1 Unless provided otherwise in 3.2, terminology shall be in conformance with Terminology~~ies~~ies F 1251 and F 2312.

3.2 *Definitions:*

3.2.1 *bioactive agents*, *n*—any molecular component in, on, or within the interstices of a device that ~~is intended to elicit~~elicits a desired tissue or cell response. Growth factors, antibiotics, and antimicrobials are typical examples of bioactive agents. Device structural components or degradation byproducts that evoke limited localized bioactivity are not included.

3.2.2 *pores*, *n*—an inherent or induced network of channels and open spaces within an otherwise solid structure.

3.2.3 *porometry*, *n*—the determination of the distribution of pore diameters relative to direction of fluid flow by the displacement of a wetting liquid as a function of pressure.

3.2.4 *porosimetry*, *n*—the determination of pore volume and pore size distribution through the use of a nonwetting liquid (typically mercury) intrusion into a porous material as a function of pressure.

3.2.5 *porosity*, *n*—property of a solid which contains an inherent or induced network of channels and open spaces. Porosity can be measured by the ratio of pore (void) volume to the apparent (total) volume of a porous material and is commonly expressed as a percentage.

~~3.2.6*scaffold*, *n*—a support, delivery vehicle, or matrix for facilitating the migration, binding, or transport of cells or bioactive molecules used to replace, repair, or regenerate tissues.~~

~~3.2.6.1*Discussion*—ASTM Committee F04 is continuing to refine definitions for the terms tissue engineering, tissue-engineered medical products (TEMPs), and scaffold. Final definitions will be from consideration of Committee F04 and other resources such as *The Williams Dictionary of Biomaterials* (**9**) and will be balloted at a later date.~~

4. Summary of Guide

4.1 The physicochemical and three-dimensional characteristics of the scaffold material are expected to influence the properties of TEMPs. It is the intent of this guide to provide a compendium of materials characterization techniques for properties that may be related directly to the functionality of scaffolds for TEMPs.

~~4.2Numerous general areas of characterization also should be considered when developing a scaffold for TEMPs. Among these are~~ 4.2 Other characterizations for scaffolds utilized in TEMPs may include compositional identity, physical and chemical properties or characteristics, viable sterilization techniques, degradability/resorbability, and mechanical properties.

4.3 Application of the test methods contained within this guide does not guarantee clinical success of a finished product but will help to ensure consistency in the properties and characterization of a given scaffold material.

4.4 This guide does not suggest that all of the listed tests be conducted. The decision regarding applicability or suitability of any particular test method remains the responsibility of the supplier, user, or regulator of the scaffold material based on applicable regulations, characterizations, and preclinical/clinical testing.

5. Significance and Use

5.1 Scaffolds potentially may be metallic, ceramic, polymeric, natural, or composite materials. Scaffolds are usually porous to some degree, but may be ~~solid or porous,~~ solid. Scaffolds can range from mechanically rigid to~~r~~ gelatinous~~,~~ and can be either absorbable/degradable~~,~~ or nonresorbable/nondegradable. The scaffold may or may not have a surface treatment. Because of this large breadth of possible ~~substrate~~starting materials and scaffold constructions, this guide cannot be considered as exhaustive in

[6] Available from U.S. Pharmacopeia, 12601 Twinbrook Pkwy., Rockville, MD 20852, or through http://www.usp.org/products/USPNF/. The standards will be listed by appropriate USP citation number. Succeeding USP editions alternately may be referenced.

~~[7] Annual Book of ASTM Standards, Vol 15.03.~~

[7] Available from National Institute of Standards and Technology (NIST), 100 Bureau Dr., Stop 1070, Gaithersburg, MD 20899-1070, http://www.nist.gov.

~~[8] Annual Book of ASTM Standards, Vol 04.08.~~

[8] Available from http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfStandards/search.cfm.

~~[9] Annual Book of ASTM Standards, Vol 08.03.~~

[9] Available from http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfggp/search.cfm.

~~[10] Annual Book of ASTM Standards, Vol 07.02.~~

[10] Available from http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMA/pma.cfm.

~~[11] Annual Book of ASTM Standards, Vol 15.09.~~

[11] Available fromit. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMN/pmn.cfm

its listing of potentially applicable tests. A voluntary guidance for the development of tissue-engineered products can be found in Omstead, et al (**1**).[12] Guide F 2027 contains a listing of potentially applicable test methods specific to various starting materials.

5.2 Each TEMP scaffold product is unique and may require testing not within the scope of this guide or other guidance documents. Users of this guide are encouraged to examine the references listed herein and pertinent FDA or other regulatory guidelines or practices, and conduct a literature search to identify other procedures particularly pertinent for evaluation of their specific scaffold material (**2,3,4**). It is the ultimate responsibility of the TEMP scaffold designer to determine the appropriate testing, whether or not it is described in this guide.

~~5.3A listing of potentially applicable substrate specific tests may be found in Guide F2027, with additional tests listed in X1.4 of this guide. Other unique characterization procedures may also be relevant and not covered by this guide.~~

5.3 A listing of potentially applicable tests for characterizing and analyzing the materials utilized to fabricate the scaffold may be found in Guide F 2027. However, conformance of a raw material to this and/or any other compendial standard(s) does not, in itself, ensure that the selected material is suitable or that the provided quality is adequate to meet the needs of a particular application. Thus, other characterization procedures may also be relevant and not covered by this guide.

5.4 The following provides a listing of links to U.S. Food & Drug Administration (FDA)—Center for Devices & Radiologic Health (CDRH) web sites that may potentially contain additional guidance relevant to biomaterial scaffolds covered within this document.

5.4.1 *Recognized FDA-CDRH Consensus Standards Database*:

5.4.1.1 http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfStandards/search.cfm

5.4.1.2 This database provides a resource for locating FDA recognized consensus standards for medical products.

5.4.2 *FDA-CDRH Good Guidance Practice (GGP) Database*:

5.4.2.1 http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfggp/search.cfm

5.4.2.2 This database provides a resource for locating non-binding FDA guidance documents intended for CDRH staff, regulated industry and the public that relate to the processing, content, and evaluation of regulatory submissions, the design, production, manufacturing, and testing of regulated products, and FDA inspection and enforcement procedures.

5.4.2.3 A document within this database possessing content that warrants particular consideration for its potential applicability for tissue engineering scaffolds is *Guidance for the Preparation of a Premarket Notification Application for a Surgical Mesh; Final*.

5.4.3 *FDA-CDRH Premarket Approval (PMA) Database*:

5.4.3.1 http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMA/pma.cfm

5.4.4 *FDA-CDRH 510(k) (Premarket Notification) Database*:

5.4.4.1 http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMN/pmn.cfm

6. Chemical Properties and Tests

NOTE 1—Chemical properties are the chemical composition characteristics of a compound. Chemical tests provide information about the identity or nature of the chemical components of a scaffold. Chemical tests include those that provide information about the nature or size of constituent molecules, the product's purity, and/or the chemical nature of the scaffold surface.

6.1 *Identification of Impurities* :

6.1.1 Chemical impurities are expected and unexpected ~~contamination~~materials that ~~is~~are not part of the intended design of the scaffold. Acceptable levels ~~of impurities~~ are a function of the nature of the ~~contamination~~impurity and the scaffold's intended *in vitro* or *in vivo* application, and may be evaluated by appropriate qualification studies. A more precise definition of both contaminants and impurities and guidance regarding their significance may be found in Guide E 1298.

~~6.1.2Expected impurities of potential biological significance should be monitored through appropriate analytic means. Such typical impurities may include, but are not limited to processing aids or solvents, unreacted cross-linking agents, residual monomers, endotoxins, sterilization residuals, and residual solutions used in the production of collagen, elastin, or other naturally derived products that, by their chemical nature or relative concentrations, carry potential for influencing cell or tissue response.~~

~~6.1.3Impurities may be identified or quantitatively determined by infrared (IR) spectroscopy, nuclear magnetic resonance (NMR), combined gas chromatography/mass spectrometry (GC/MS), or other analytic methods as appropriate.~~

~~6.1.4Generally, impurities are isolated more readily when the scaffold in its entirety can be solvated along with possible contaminants. If the scaffold cannot be dissolved, extraction in appropriate solvents becomes indicated.~~

6.1.2 Expected impurities of potential biological significance should be monitored through appropriate analytic means. Impurities can occur in both synthetic and natural based materials (for example, proteins, such as collagen and elastin; polysaccharides, such as cellulose, alginate, hyaluronan, and chitin based derivatives) and may include, but are not limited to processing aids or solvents, unreacted cross-linking agents, residual monomers, endotoxins, sterilization residuals, and residual solutions that, by their chemical nature or relative concentrations, carry potential for influencing cell or tissue response.

6.1.3 Impurities may be identified or quantitatively determined by infrared (IR) spectroscopy, nuclear magnetic resonance (NMR), combined gas chromatography/mass spectrometry (GC/MS), or other analytic methods as appropriate. Polyacrylamide gel

~~[12] Annual Book of ASTM Standards, Vol 11.03.~~

[12] The boldface numbers in parentheses refer to the list of references at the end of this standard.

electrophoresis is a possible method for assessing the presence of impurities in biologically derived scaffold materials (for example, collagen, hyaluronic acid). Impurities separated within such gels can be detected with Coomassie Blue (as a general protein stain) or silver (as a general protein and carbohydrate stain), and characterized further by immunonblot analysis and/or protein sequencing to identify specific impurities that may possess critical biological activities (for example, elastin immunogenicity, cytokines and growth factors). Once characterized, such impurities can be assessed by other robust and sensitive methods well suited to a manufacturing environment (for example, ELISA for specific substances identified by immunoblot analysis or protein sequencing.)

6.1.4 Generally, impurities are isolated more readily when the scaffold in its entirety can be solvated along with possible contaminants. If the scaffold cannot be dissolved, exhaustive extraction with one or more solvents appropriate to the suspected impurity is necessary.

6.1.4.1 *Solvation/Dissolution*—In the absence of known or established dissolution solvents for a particular ~~substrate,~~material, Practice D 5226 may ~~be referred to deliver~~ provide added guidance in identifying suitable potential solvents for dissolving a scaffold material. ~~Note that samples cannot~~ Samples should not be dissolved in analytic solvents that can ~~also~~ be considered as potential contaminants or create analytic interferences.

6.1.4.2 Extraction of residuals may be undertaken by ~~utilization of~~ methods such as Practice G 120. The extract ~~then~~ may then be concentrated and analyzed by appropriate chromatographic analysis.

6.1.5 The amount of any expected impurity should be quantified and the analytic detection limit reported. Both solvated and extracted samples should provide results that specify the amount of expected impurity per mass of test sample in either percentage, ppm (µg/g;mg/kg), ppb (ng/g;µg/kg), or ~~ppb (ng/g;µg/kg)~~other appropriate units.

6.1.6 The following analytic methods may be applicable in the determination and quantification of potential impurities:

6.1.6.1 Gas chromatography (GC) ~~is best~~may be used for the routine detection of volatile relatively low molecular mass (formerly known as molecular weight) impurities or contaminants. Some methods that may prove suitable include Test Methods F 151 and F 1884.

6.1.6.2 Gas chromatography can be coupled with both quantitative and qualitative analytic methods such as ~~infrared spectrophotometry (IR)~~ IR or ~~mass spectroscopy (MS)~~ MS to provide compositional identification while quantitatively detecting low molecular ~~weight~~mass volatile impurities or contaminants. Some particular methods that may prove useful include Test Method D 6420 and Practice E 1642.

6.2 ~~*Molecular Weight Determination*~~ Molar Mass Determination:

~~6.2.1For polymeric materials (synthetic or natural), the molecular weight and molecular weight distribution may be determined through size exclusion chromatography (SEC) or gel permeation chromatography (GPC). Other procedures such as inherent or intrinsic viscosity, light scattering, or membrane osmometry may be used.~~

~~6.2.2In any of the preceding tests, the solvent solubility characteristics of the scaffold will be highly significant in allowing determination of suitable molecular weight test methods. In the absence of known or established dissolution solvents for a particular scaffold substrate, Practice D5226 provides added guidance in identifying suitable potential solvents for dissolving a substrate material.~~

~~6.2.3The following test methods may be applicable in the determining the molecular weight of the fabricated scaffold.~~

~~NOTE2—The following GPC/SEC and IV methods are considered to be suitable for use on linear polymer systems only. Branched polymer systems should use light-scattering techniques.~~ 2—The term molecular weight (abbreviated MW) is obsolete and should be replaced by the SI (Système Internationale) equivalent of either relative molecular mass (Mr), 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 Mw, Mn, and Mz 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 SP811.

6.2.1 For polymeric materials (synthetic or natural), the molar mass and molar mass distribution may be determined through size exclusion chromatography (SEC) or gel permeation chromatography (GPC). Other procedures such as inherent or intrinsic viscosity (both abbreviated with the acronym "IV"), light scattering, or membrane osmometry may be used. For protein impurities, SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) has proven robust and generally applicable. In specific instances, mass spectrometry can provide highly accurate mass determinations as well.

6.2.2 In any of the preceding tests, the solvent solubility characteristics of the scaffold will be highly significant in allowing determination of suitable molar mass test methods. In the absence of known or established dissolution solvents for a particular scaffold construct, Practice D 5226 provides added guidance in identifying suitable potential solvents for dissolving a particular material.

6.2.3 The following test methods may be applicable in the determining the molar mass of the fabricated scaffold.

NOTE 3—The following GPC/SEC and IV methods are considered to be suitable for use on linear polymer systems only. Branched polymer systems should use light-scattering techniques.

6.2.3.1 *Gel Permeation Chromatography (GPC), Also Known as Size Exclusion Chromatography (SEC)*—See Test Methods D 5296 and D 6474 and Practices D 3016 and D 6579.

NOTE~~3—The~~ 4—The SEC solvent system and calibration standard polymer type should be specified with any obtained result.