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Standard Guide for Characterization and Standardization of Polymerizable Collagen-Based Products and Associated Collagen-Cell Interactions[1]

This standard is issued under the fixed designation F3089; 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.

INTRODUCTION

The collagen family of proteins represents the major structural and mechanical component of the *in-vivo* extracellular matrix of human tissues and organs. Type I collagen is the most abundant and as such, it is an ideal candidate for medical materials, tissue-engineered medical products, delivery of therapeutic cells/molecules, and *in-vitro* cell/tissue culture applications. Furthermore, it is now evident that specific collagen material properties, including microstructure, mechanical integrity ~~(stiffness),~~ (stiffness, strength), cell adhesion, immunogenicity, and ~~biodegradation~~ resorption (degradation) are major determinants of the interfacial properties between cells and collagen-based materials, including guidance of fundamental cell behaviors that contribute to recapitulation and/or restoration of tissue structure and function. Advanced understanding of collagen self-assembly, as occurs *in vivo* and *in vitro*, is contributing to a rapid expansion of commercial and laboratory-produced purified collagen formulations that polymerize (self-assemble) or exhibit ~~solution to gel (matrix) transition.~~ transitions from solution to semi-solid material (for example, gel, scaffold). Most recent developments have focused on polymerizable collagen ~~polymer formulations with tunable features to~~ formulations that support the rational design and custom fabrication of collagen polymeric materials for improved tissue ~~integration and~~ integration, guidance of cell ~~fate.~~ fate, and tissue response outcomes. Unfortunately, the term "collagen" is applied generally to describe various collagen types and formulations (soluble, insoluble, monomeric, gelatin/peptides, oligomeric, tropocollagen, atelocollagen) that vary significantly in their molecular compositions, ~~self-assembly~~polymerization capacity and properties, and ability to interact with cells. As such, the need exists for an expanded set of characterization and standardization strategies to facilitate comparison, safety and efficiency testing, and translation of the next generation polymerizable collagen ~~polymer~~ formulations and associated ~~self-assembled collagen-based~~collagen polymeric materials produced with these formulations.

1. Scope

1.1 This guide is intended to provide characteristics, properties, test methods, and standardization approaches for evaluation and identification of specific polymerizable collagen formulations and collagen polymeric materials produced with these formulations.

1.2 This guide ~~for characterizing polymerizable collagens is intended to provide characteristics, properties, test methods, and standardization approaches for use by producers, manufacturers, and researchers to identify specific collagen polymer formulations~~

[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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and associated self-assembled collagen-based products produced with these formulations. This guide will focus on the characterization of polymer forms of Typefocuses on characterization of purified polymerizable forms of type I collagen, which is the most abundant collagen in mammalian connective tissues and organs, including skin, bone, tendon, and blood vessels. Type Polymerizable type I collagen may be derived from a variety of sources including, but not limited to, animal or cadaveric tissues, cell culture, recombinant, recombinant cell culture, and chemical synthesis. This guide is intended to focus on purified Type I collagen polymers as a starting material for wound and hemostatic dressings, surgical implants, substrates for tissue-engineered medical products (TEMPs), delivery vehicles for therapeutic cells or molecules, and 3D in-vitro tissue systems for basic research, drug development, and toxicity testing. Polymerizable or self-assembly implies that the collagen composition exhibits spontaneous macromolecular assembly from its components in the absence of the addition of exogenous factors including cross-linking agents. Self-assembling collagen polymers may include, but are not limited to: (1) tissue-derived atelocollagens, monomers, and oligomers; (2) collagen proteins and peptides produced using recombinant technology; and (3) chemically synthesized collagen mimetic peptides. It should be noted that the format of associated self-assembled collagen-based products also will vary and may include injectable solutions that polymerize in situ as well as preformed sheets, particles, spheres, fibers, sponges, matrices/gels, coatings, films, and other forms. This guide may serve as a template for characterization and standardization of other fibrillar collagen types that demonstrate polymerization or self-assembly.

1.2.1 This guide covers evaluation of polymerizable collagens and collagen polymeric materials prepared from polymerizable collagens for use as a starting material for wound and hemostatic dressings, surgical implants, substrates for tissue-engineered medical products (TEMPs), delivery vehicles for therapeutic cells or molecules, and 3D *in-vitro* tissue systems for basic research, diagnostics, drug development, and toxicity testing. Most collagen products on the market today are regulated as devices since their primary intended purpose is not achieved through chemical action within or on the body. However, a medical product comprising polymerizable collagens or collagen polymeric materials may be regulated as a device, biologic, drug, or combination product depending on its intended use and primary mode of action.

1.2.2 Polymerizable collagen or collagen self-assembly implies that the collagen composition exhibits spontaneous macromolecular assembly from its components without the addition of exogenous factors such as cross-linking agents. Polymerizable collagens may include but are not limited to: (*1*) tissue-derived monomeric collagens, including tropocollagen or atelocollagen, and oligomeric collagens; (*2*) collagen proteins and peptides produced through *in vitro* cell culture, with or without using recombinant technology; and (*3*) chemically synthesized collagen mimetic peptides. It should be noted that the format of collagen polymeric material products also will vary and may include injectable solutions that polymerize *in situ* as well as preformed sheets, particles, spheres, fibers, sponges, matrices/gels, coatings, films, and other forms.

1.2.3 This guide may serve as a template for characterization and standardization of type I fibrillar collagen or other collagen types that demonstrate polymerization or self-assembly.

1.3 The ability of self-assembled collagen This guide does not provide a significant basis for assessing the biological safety (biocompatibility) of polymerizable collagens and collagen polymeric materials. While the ability of collagen polymeric materials to guide cellular responses through provision of cellular adhesion and proteolytic domains as well as physical constraints (for example, structural, cell-matrix traction force) has been well documented through extensive clinical (1, 2)and basic research studies (**31-5**),[2] 4).users are directed to the ISO 10993 series for evaluating biological risks of medical devices. The biocompatibility and appropriateness of use for a specific application(s)application is the responsibility of the product manufacturer.

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

1.4 **Warning—***Mercury has been designated by the Environmental Protection Agency (EPA) and many state agencies as a hazardous material that can cause central nervous system, kidney, and liver damage. Mercury, or its vapor, may be hazardous to health and corrosive to materials. Caution should be taken when handling mercury and mercury-containing products. See the applicable product Material Safety Data Sheet (MSDS) for details and the EPA website (http://www.epa.gov/mercury/faq.htm) for additional information. Users should be aware that selling mercury or mercury-containing products, or both, in your state may be prohibited by state law.*

1.5 The following precautionary caveat pertains only to the test method portion, SectionSections 56 and 7, of this guide.guide: *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 safety, health, and healthenvironmental practices and determine the applicability of regulatory limitations prior to use.*

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

1.6 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[3]

~~E1298~~E4 ~~Guide for Determination of Purity, Impurities, and Contaminants in Biological Drug Products~~Practices for Force Calibration and Verification of Testing Machines ~~(Withdrawn 2014)~~

F619 Practice for Extraction of Materials Used in Medical Devices

F720 Practice for Testing Guinea Pigs for Contact Allergens: Guinea Pig Maximization Test

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

F749 Practice for Evaluating Material Extracts by Intracutaneous Injection in the Rabbit

F756 Practice for Assessment of Hemolytic Properties of Materials

F763 Practice for Short-Term Intramuscular Screening of Implantable Medical Device Materials

F813 Practice for Direct Contact Cell Culture Evaluation of Materials for Medical Devices

F895 Test Method for Agar Diffusion Cell Culture Screening for Cytotoxicity

F981 Practice for Assessment of Compatibility of Biomaterials for Surgical Implants with Respect to Effect of Materials on Muscle and Insertion into Bone

~~F1251 Terminology Relating to Polymeric Biomaterials in Medical and Surgical Devices (Withdrawn 2012)[4]~~

F1439 Guide for Performance of Lifetime Bioassay for the Tumorigenic Potential of Implant Materials

F1903 Practice for Testing for Cellular Responses to Particles ***in vitro***

F1904 Practice for Testing the Biological Responses to Particles ***in vivo***

~~F1905 Practice For Selecting Tests for Determining the Propensity of Materials to Cause Immunotoxicity (Withdrawn 2011)[4]~~

~~F1906 Practice for Evaluation of Immune Responses In Biocompatibility Testing Using ELISA Tests, Lymphocyte Proliferation, and Cell Migration (Withdrawn 2011)[4]~~

F1983 Practice for Assessment of Selected Tissue Effects of Absorbable Biomaterials for Implant Applications

~~F2148~~F2914 ~~Practice~~Guide for ~~Evaluation of Delayed Contact Hypersensitivity Using the Murine Local Lymph Node Assay (LLNA)~~Identification of Shelf-life Test Attributes for Endovascular Devices

2.2 *ISO Standards:*[4]

ISO 5725-1 Accuracy (Trueness and Precision) of Measurement Methods and Results—Part 1: General Principles and Definitions

ISO 5725-2 Accuracy (Trueness and Precision) of Measurement Methods and Results—Part 2: Basic Method for the Determination of Repeatability and Reproducibility of a Standard Measurement Method

ISO 5725-3 Accuracy (Trueness and Precision) of Measurement Methods and Results—Part 3: Intermediate Measures of the Precision of a Standard Measurement Method

ISO 5725-4 Accuracy (Trueness and Precision) of Measurement Methods and Results—Part 4: Basic Methods for the Determination of the Trueness of a Standard Measurement Method

ISO 5725-5 Accuracy (Trueness and Precision) of Measurement Methods and Results—Part 5: Alternative Methods for the Determination of the Precision of a Standard Measurement Method

ISO 5725-6 Accuracy (Trueness and Precision) of Measurement Methods and Results—Part 6: Use in Practice of Accuracy Values

ISO ~~10993–1~~10993-1 Biological Evaluation of Medical Devices—Part 1: Evaluation and Testing with a Risk Management Process

ISO ~~10993–3~~10993-3 Biological Evaluation of Medical Devices—Part 3: Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity

ISO 10993-4 Biological Evaluation of Medical Devices—Part 4: Selection of Tests for Interactions with Blood

ISO 10993-5 Biological Evaluation of Medical Devices—Part 5: Tests for In Vitro Cytotoxicity

ISO 10993-6 Biological Evaluation of Medical Devices—Part 6: Tests for Local Effects after Implantation

ISO 10993-7 Biological Evaluation of Medical Devices—Part 7: Ethylene Oxide Sterilization Residuals

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

ISO ~~10993–10~~10993-10 Biological Evaluation of Medical Devices—Part 10: Tests for ~~Irritation and Delayed-Type Hypersensitivity~~ Skin Sensitization

[3] 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.

[4] Available from International Organization for Standardization (ISO), 1, ch. de la Voie-Creuse, CP 56, CH-1211 Geneva 20, Switzerland, http://www.iso.org.

ISO 10993-11 Biological Evaluation of Medical Devices—Part 11: Tests for Systemic Toxicity
ISO 10993-12 Biological Evaluation of Medical Devices—Part 12: Sample Preparation and Reference Materials
ISO 10993-13 Biological Evaluation of Medical Devices—Part 13: Identification and Quantification of Degradation Products from Polymeric Medical Devices
ISO ~~10993–17~~10993-17 ~~Methods for~~ Biological Evaluation of Medical Devices—Part 17: Establishment of Allowable Limits for Leachable Substances ~~Using Health-Based Risk Assessment~~
ISO 10993-18 Biological Evaluation of Medical Devices—Part 18: Chemical Characterization of Materials
ISO 10993-20 Biological Evaluation of Medical Devices—Part 20: Principles and Methods for Immunotoxicology Testing of Medical Devices
ISO ~~13408–1~~13408-1 Aseptic Processing of Health Care Products—Part 1: General Requirements
ISO 14971 Medical Devices—Application of Risk Management to Medical Devices
ISO ~~22442–1~~22442-1 Medical Devices Utilizing Animal Tissues and ~~their Derivatives Utilized in the Manufacture of Medical Devices—Part 1: Analysis and Management of Risk~~Their Derivatives—Part 1: Application of Risk Management
ISO ~~22442–2~~22442-2 Medical Devices Utilizing Animal Tissues and ~~their Derivatives Utilized in the Manufacture of Medical Devices—Part~~ Their Derivatives—Part 2: Controls on Sourcing, Collection, and Handling
ISO ~~22442–3~~22442-3 Medical Devices Utilizing Animal Tissues and ~~their Derivatives Utilized in the Manufacture of Medical Devices—Part 3: Validation and~~Their Derivatives—Part 3: Validation of the Elimination and/or Inactivation of ~~Virus and Transmissable~~ Viruses and Transmissible Spongiform Encephalopathy (TSE) Agents
ISO/TR 22442-4 Medical Devices Utilizing Animal Tissues and Their Derivatives—Part 4: Principles for Elimination and/or Inactivation of Transmissible Spongiform Encephalopathy (TSE) Agents and Validation Assays for Those Processes

2.3 *U.S. and European Pharmacopeia Documents:*[5]

~~United States Pharmacopeia (USP), Edition XXX (30)~~U.S. Pharmacopeia (USP) General Chapters
<61> Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests
<71> Sterility Tests
<85> Bacterial Endotoxins Test
<161> Transfusion and Infusion Assemblies and Similar Medical Devices
<232> Elemental Impurities—Limits
<233> Elemental Impurities—Procedures
<791> pH
~~USP 30/NF 19~~<1050> Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin
<1058> Analytical Instrument Qualification
<1225> Validation of Compendial Procedures
<1211> Sterilization and Sterility Assurance of Compendial Articles
European Pharmacopeia ~~5.0~~ 11.0

2.4 *Code of Federal Regulations:*[6]

9 CFR Part 113 Standard Requirements
21 CFR Part 312 Investigational New Drug Application
21 CFR 610.13(b) Rabbit Pyrogen Assay
21 CFR Part 812 Investigational Device Exemption
21 CFR Part 820 Quality System Regulation
~~Federal Register Vol. 43 No. 141, Friday, July 21, 1978~~
21 CFR Parts 207, 807, and 1271 Human Cells, Tissues and Cellular and Tissue-Based Products, Establishment Registration and Listing
21 CFR Part 1271, Subpart C Donor Eligibility
Federal Register Vol. 43 No. 141, Friday, July 21, 1978
Federal Register, Vol. 66 No. 13, Jan. 19, 2001/Rules and Regulations, p. 5447
Federal Register, Vol. 72 No. 8, Jan. 12, 2007, pp. 1581–1619, Proposed Rule: Use of Materials Derived from Cattle in Medical Products Intended for Use in Humans and Drugs Intended for Use in Ruminants
~~21 CFR Part 1271, Part C Suitability Determination for Donors of Human Cell and Tissue-based Products, Proposed Rule~~
Current Good Tissue Practice for Manufacturers of Human Cellular and Tissue-Based Products Inspection and Enforcement. Proposed Rule. Federal Register/Vol. 66, No. 5/January 8, 2001/Proposed Rules, pp. ~~1552-1559~~1552–1559
~~Guidance for Screening and Testing of Donors of Human Tissue Intended for Transplantation Availability. Federal Register/Vol. 62, No. 145/July 29, 1997/Notices Draft Guidance for Preclinical and Clinical Investigations of Urethral Bulking Agents used in the Treatment of Urinary Incontinence. November 29, 1995. (ODE/DRARD/ULDB), Document No. 850~~

[5] ~~Available~~ U.S Pharmacopeia available from U.S. Pharmacopeia (USP), 12601 Twinbrook Pkwy., Rockville, MD 20852-1790, http://www.usp.org. European Pharmacopeia available from EDQM Council of Europe, 7 allée Kastner, CS 30026, F-67081 Strasbourg, France, Tel. +33 3 88 41 30 30, http://pheur.edqm.eu.

[6] 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.

Guidance for Industry and for FDA Reviewers Medical Devices Containing Materials Derived from Animal Sources (Except for *In Vitro* Diagnostic Devices), November 6, 1998, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Devices and Radiological Health

CFR 610.13(b) Rabbit Pyrogen Assay

2.5 *ICH Documents:*[7]

ICH M3M3(R2) Guidance for Industry M3 Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorizations for Pharmaceuticals 62 FR 62922 (1997)(2009)

ICH S2AQ1A Guideline for Industry S2A Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals 61 FR 18199 (1996)Stability Testing of New Drug Substances and Products

ICH S2BQ2 Guidance for Industry S2B Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals 62 FR 62472 (1997)Validation of Analytical Procedures: Text and Methodology

ICH S5AQ3A Guideline for Industry S5A Detection of Toxicity to Reproduction for Medicinal Products 59 FR 48746 (1994)Impurities in New Drug Substances

ICH S5BQ3B Guidance for Industry S5B Detection of Toxicity to Reproduction for Medicinal Products: Addendum on Toxicity to Male Fertility 61 FR 15360 (1996)Impurities in New Drug Products

ICH Q3C Guideline for Residual Solvents

ICH Q3D Guideline for Elemental Impurities

ICH Q5A Quality of Biotechnological Products: Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin

ICH S1A Guideline for Industry S1A The Need for Long-term Rodent Carcinogenicity Studies of Pharmaceuticals 61 FR 8153 (1996)

ICH S1B Guidance for Industry S1B Testing for Carcinogenicity of Pharmaceuticals 63 FR 8983 (1998)

ICH S1C Guideline for Industry S1C Dose Selection for Carcinogenicity Studies of Pharmaceuticals 60 FR 11278 (1995)

ICH S1C(R)S2 Guidance for Industry Addendum to Dose Selection for Carcinogenicity Studies of Pharmaceuticals: Addition of a Limit Dose and Related Notes 62 FR 64259 (1997)on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use

ICH Q1A ICHS5 Harmonized Tripartite Guidance for Stability Testing of New Drug Substances and Products (September 23, 1994)Detection of Reproductive and Developmental Toxicity for Human Pharmaceuticals

2.6 *FDA Documents:*[8]

U.S. Food and Drug Administration (FDA and Committee for Proprietary Medicinal Products (CPMP), 1998(FDA) Center for Devices and Radiological Health (CDRH) and Center for Biologics Evaluation and Research (CBER), 2020 International Conference on Harmonization (ICH), Quality of Biotechnological Products: Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin, Consensus Guideline ICH Viral Safety Document: Step 5Use of International Standard ISO 10993-1, "Biological evaluation of medical devices – Part 1: Evaluation and testing within a risk management process." Guidance for Industry and Food and Drug Administration Staff

FDAU.S. Food and Drug Administration (FDA) Center for Drug Evaluations and Research (CDER), Center for Biologics Evaluation and Research (CBER), Center for Veterinary Medicine (CVM), Center for Devices and Radiological Health (CDRH), Office of Regulatory Affairs (ORA), 2012 Guidance for IndustryIndustry. Pyrogen and Endotoxins Testing: Questions and Answers, DHHS, June 2012Answers

U.S. Food and Drug Administration (FDA) Center for Devices and Radiological Health, 2019 Medical Devices Containing Materials Derived from Animal Sources (Except for In Vitro Diagnostic Devices). Guidance for Industry and for Food and Drug Administration Staff

U.S. Food and Drug Administration (FDA) Center for Biologics Evaluation and Research (CBER), 1993 Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals

U.S. Food and Drug Administration (FDA) Center for Biologics Evaluation and Research (CBER), 1997 Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use, 94D-0259Use

U.S. Food and Drug Administration (FDA) Center for Drug Evaluation and Research (CDER) and Center for Biologics Evaluation and Research (CBER), 2015 Analytical Procedures and Methods Validation for Drugs and Biologics. Guidance for Industry

FDAU.S. Food and Drug Administration (FDA) Division of Small Manufacturers Assistance Office of Training and Assistance Center for Devices and Radiological Health, 1991 Interim Guidance for Human and Veterinary Drug Products and Biologicals, Kinetic LAL techniques, DHHS, July 15, 1991Shelf Life of Medical Devices

[7] Available from International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), ICH Secretariat, c/o IFPMA, 15 ch. Louis-Dunant, 9, chemin des Mines, P.O. Box 195, 1211 Geneva 20, Switzerland, http://www.ich.org.

[8] Available from Food and Drug Administration (FDA), 10903 New Hampshire Ave., Silver Spring, MD 20993-0002, http://www.fda.gov.

2.7 *AAMI Documents:*[9]

ANSI/AAMI/ISO ~~11737-1: 2006~~11737-1:2018 Sterilization of ~~Medical Devices—Microbiological~~Healthcare Products—Microbiological Methods—Part 1: ~~Estimation of Bioburden on Product~~Determination of a Population of Microorganisms on Products

ANSI/AAMI/ISO ~~11737-2: 1998~~11737-2:2009 Sterilization of Medical Devices—Microbiological Methods—Part 2: Tests of Sterility Performed in the ~~Validation~~ Definition, Validation, and Maintenance of a Sterilization Process

AAMI TIR ~~No. 19-1998~~19:1998 Guidance for ANSI/AAMI/ISO 10993-7: 1995, Biological Evaluation of Medical Devices—Part 7: Ethylene Oxide Sterilization Residuals

AAMI/ISO ~~14160-1998~~14160:2011 (R2016) Sterilization of Health Care Products—Liquid Chemical Sterilizing Agents for Single-Use Medical Devices ~~Incorporating Materials of Animal Origin—Validation and~~ Utilizing Animal Tissues and Their Derivatives—Requirements for Characterization, Development, Validation and Routine Control of ~~Sterilization by Liquid Chemical Sterilants~~a Sterilization Process for Medical Devices

AAMI ~~ST67/CDV-2: 1999~~ST67:2019 Sterilization of ~~Medical Devices—Requirements for~~ Health Care Products—Requirements and Guidance for Selecting a Sterility Assurance Level (SAL) for Products Labeled "Sterile"

AAMI ST72:2019 Bacterial Endotoxins—Test Methods, Routine Monitoring, and Alternatives to Batch Testing

2.8 *Other References:*

~~Draft Guidance for Preclinical and Clinical Investigations of Urethral Bulking Agents Used in the Treatment of Urinary Incontinence, November 29, 1995. (ODE/DRARD/ULDB), Document No. 850~~[11]

Council Directive 93/42/EEC, with Respect to Medical Devices Using Tissues of Animal Origin[10]

Commission Directive 2003/32/EC, with Respect to Medical Devices Manufactured Using Tissues of Animal Origin[11]

~~EMEA/410/01-rev.2;~~EMA/410/01-rev.3 Committee for Proprietary Medical Products, Note for Guidance on Minimizing the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medical Products[12]

~~The European Agency for the Evaluation of Medicinal Products, (EMA),~~European Medicines Agency, (EMA/CHMP/CVMP/QWP/850374/2015) ~~Committee for Proprietary Medicinal Products (CPMP) Guidance Document for Decision Trees for the Selection of Sterilization Methods (CPMP/QWP/054/98 corr 2000) and Annex to Note for Guidance on Development Pharmaceutics (CPMP/QWP/155/96)~~Guideline on the Sterilisation of the Medicinal Product, Active Substance, Excipient and Primary Container

Automotive Industry Action Group (AIAG) Measurement Systems Analysis Reference Manual, 4th Edition

National Institute of Standards and Technology (NIST) NIST/SEMATECH e-Handbook of Statistical Methods, http://www.itl.nist.gov/div898/handbook/, Chapter 2: Measurement Process Characterization

3. Terminology

3.1 *Definitions:*

3.1.1 *adventitious ~~agents~~agent, n*—an unintentionally introduced microbiological or other infectious contaminant.

3.1.1.1 *Discussion*—

In the production of TEMPs, these agents may be unintentionally introduced into the process stream, the final product, or both.

3.1.2 *atelocollagen, n*—triple helical molecule in which the telopeptide regions have been partially or completely removed from tropocollagen (see Fig. 1). Such preparations are typically the outcome of enzyme-based (for example, pepsin) collagen extraction procedures from tissues.

3.1.3 *biocompatibility, n*—a material may be considered biocompatible if the material performs with an appropriate host response in a specific application (**6**).

3.1.4 *biomaterial, n*—a synthetic or natural substance or composite used for a biological or biomedical application.

3.1.5 *collagen, n*—a family of at least 20 genetically different secreted proteins that serve a predominantly structural function and possess a unique triple helical structure configuration of three polypeptide units known as alpha chains.

[9] Available from Association for the Advancement of Medical Instrumentation (AAMI), 4301 N. Fairfax Dr., Suite 301, Arlington, VA 22203-1633, http://www.aami.org.

[10] Available from Office for Official Publications of the European Communities—European Law, 2, rue Mercier, L-2985, Luxembourg, http://eur-lex.europa.eu/en/'index.htm.

[11] Available from European Medicines Agency ~~(EMEA), 7 Westferry Circus, Canary Wharf, London E14 4HB, U.K., http://www.eudora.org/emea.html, and http://www.emea.europa.eu/pdfs/human/bwp/TSE%20NFG%20410-rev2.pdf.~~(EMA), Domenico Scarlattilaan 6, 1083 HS Amsterdam, The Netherlands, and https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-sterilisation-medicinal-product-active-substance-excipient-primary-container_en.pdf.

[12] Available from European Medicines Agency ~~(EMEA), 7 Westferry Circus, Canary Wharf, London E14 4HB, U.K., http://www.eudora.org/emea.html, and http://www.emea.europa.eu/pdfs/human/qwp/005498en.pdf.~~(EMA), Domenico Scarlattilaan 6, 1083 HS Amsterdam, The Netherlands, and https://www.ema.europa.eu/en/documents/scientific-guideline/minimising-risk-transmitting-animal-spongiform-encephalopathy-agents-human-veterinary-medicinal_en.pdf.

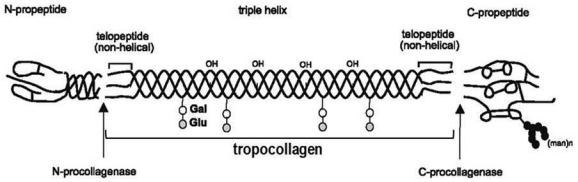

FIG. 1 Schematic of Procollagen Molecule and Associated Propeptide, Telopeptide, and Triple Helical Regions. Enzymatic Removal of Amino- and Carboxy-terminal Propeptide Ends of Procollagen Molecule by Procollagenases Yields Tropocollagen.

~~3.1.4 *collagen mimetic peptides, n*—specific amino acid sequences representing the triple helical portion of collagen, often $-(Pro–Hyp–Gly)_x-$, forms a triple helix conformation that resembles that found in natural collagens.~~

~~3.1.5 *collagen polymer, n*—purified Type I collagen formulation that demonstrates the capacity to self-assemble or polymerize into higher order structures (macromolecular assemblies) in absence of exogenous agents such as cross-linkers.~~

3.1.6 *diffusion, n*—the random thermal motion of atoms, molecules, clusters of atoms, etc., in gases, liquids, and some solids.

3.1.7 *endotoxin, n*—pyrogenic high molar mass lipopolysaccharide (LPS) complex associated with the cell wall of gram-negative bacteria.

3.1.7.1 *Discussion*—

Although endotoxins are pyrogens, not all pyrogens are endotoxins. Endotoxins are specifically detected through a test using the Limulus Amebocyte Lysate (LAL) ~~test (USP<85> Bacterial Endotoxin Tests).~~or recombinant Factor C (rFC) reagents.

3.1.8 *extracellular matrix (ECM), n*—a composite medium, where cells reside, remodel, and interact. ECM promotes cell adhesion, spreading, survival, proliferation, migration, differentiation, and/or other functions over a range of dimensional scales to maintain cell/tissue homeostasis, growth, and remodeling.

3.1.8.1 *Discussion*—

The ECM component of mammalian tissues is produced and assembled by cells and often has collagen as a predominant component.

3.1.9 *fibrillogenesis, n*—the process of tropocollagen monomers assembling into mature fibrils and associated fibril-network structures.

3.1.10 *fibrosis, n*—an *in situ* process of tissue repair resulting in a relatively avascular and collagen rich tissue.

3.1.11 *gel, n*—the three-dimensional network structure arising from intermolecular polymer chain interactions.

3.1.11.1 *Discussion*—

Such chain interactions may be covalent, ionic, hydrogen bond, or hydrophobic in nature.

3.1.12 *mechanotransduction, n*—process by which cells convert mechanical stimuli into a chemical response.

3.1.13 *medical product, n*—any diagnostic or therapeutic treatment that may be regulated as a device, biologic, drug, or combination product.

3.1.14 *microorganism, n*—bacteria, fungi, yeast, mold, viruses, and other infectious agents. ~~However, it should be noted that not all microorganisms are infectious or pathogenic.~~

3.1.14.1 *Discussion*—

However, it should be noted that not all microorganisms are infectious or pathogenic.

3.1.15 *permeability, n*—a measure of the ability of porous materials to transmit fluids; the rate of flow of a liquid through a porous material.

3.1.16 *polymerization, n*—a chemical reaction in which two or more molecules combine to form larger molecules that contain repeating structural units.

3.1.17 *procollagen, n*—collagen molecule comprising three hydroxylated prototcollagen (alpha) chains; amino- and carboxy-terminal propeptide ends are intact (Fig. 1).

3.1.18 *propeptides, n*—amino- and carboxy-terminal nontriple-helical domains of individual collagen protocollagen (alpha) chains that direct triple-helix folding and formation of procollagen molecules (Fig. 1); propeptide removal is required for collagen fibrillogenesis and self-assembly.

3.1.19 *protocollagen, n*—single collagen alpha polypeptide chain as produced by ribosomes.

3.1.20 ~~*recombinant collagen protein/peptide,*~~ *scaffold, n*—~~collagen or collagen-like polypeptide produced by recombinant methods, such as by expression of a nucleotide sequence encoding the protein or peptide in a microorganism, insect, plant, or animal host. Such compositions often comprise Gly-X-Y triplets where Gly is the amino acid glycine and X and Y can be the same or different, are often proline or hydroxyproline, but can be any known amino acid.~~a two- or three-dimensional structural matrix that provides a conductive surface that enables the attachment, survival, proliferation, migration, and/or differentiation of local or transplanted cells, and thereby facilitates the distribution of a tissue formation response throughout a desired surface or tissue volume. Medically, scaffolds may be used to replace, repair, augment, or regenerate tissues.

3.1.21 *self-assembly, n*—the process by which a complex macromolecule (as collagen) or a supramolecular system (as a virus) spontaneously assembles itself from its components.

3.1.22 *solution, n*—a type of homogenous mixture in which atoms, ions, or molecules (the solute) are distributed uniformly throughout another substance (the solvent) and which does not separate upon standing.

3.1.23 *sterilization, n*—the destruction or removal of all microorganisms in or about an object (for example, by chemical agents, electron beam, gamma irradiation, or filtration).

3.1.23.1 *Discussion—*

If the medical product collagen permits, terminal sterilization is preferential to reliance on aseptic processing.

3.1.24 *stiffness, n*—a general term describing the extent to which a material resists deformation in response to an applied force; specific measures of stiffness depend upon the material loading format (for example, tension, compression, shear, bending).

3.1.25 *suspension, n*—the dispersion of a solid through a liquid with a particle size large enough to be detected by purely optical ~~means and which separates or settles upon standing.~~means.

3.1.26 *telopeptide, n*—amino- and carboxy-terminal nontriple-helical domains of tropocollagen strands known to be important to fibrillogenesis and intermolecular cross-link formation (Fig. 1).

3.1.27 *tissue engineered medical product (TEMP), n*—a manufactured or manipulated article that consists of cells, with or without a synthetic and/or naturally derived scaffold, used for repair, replacement, restoration, or regeneration of the structure or function of a recipient's cells, tissues, or organs.

3.1.28 *tissue regeneration, n*—an *in-situ* process of tissue repair where there is a partial or complete restoration of normal tissue structure and function.

3.1.29 *tissue repair, n*—a process of partial or complete restoration of tissue structure and/or function.

3.1.30 *tropocollagen, n*—collagen molecule comprising three alpha chains with amino- and carboxy-terminal propeptide ends removed (Fig. 1); carboxy- and amino-terminal non-helical telopeptide ends are intact; able to undergo self-assembly into fibrillar matrix.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *adhesion, n*—steady or firm attachment; in the context of collagen, adhesion refers to the ability of cells to physically attach or bind to collagen molecules and macromolecular assemblies of collagen via cell surface proteins like integrins.

3.2.2 *collagen mimetic peptides, n*—specific amino acid sequences representing the triple helical portion of collagen, often –(Pro–Hyp–Gly)x–, forms a triple helix conformation that resembles that found in natural collagens.

3.2.3 *collagen polymeric material, n*—a composition formed by polymerization or self-assembly and consisting essentially of repeating collagen structural units.

3.2.4 *degradation, n*—change in chemical, physical, or molecular structure or appearance (that is, gross morphology) of ~~material; degradation of collagen under physiologic conditions involves site-specific cleavage within the central triple helical region by proteolytic enzymes known as collagenases. Collagenases are members of the larger family of proteases known as matrix metalloproteases.~~material.

3.2.4.1 *Discussion—*

Degradation of collagen under physiologic conditions involves site-specific cleavage within the central triple helical region by proteolytic enzymes known as collagenases. Collagenases are members of the larger family of proteases known as matrix metalloproteases.

3.2.5 *matrix, n*—loose meshwork within which cells are embedded or arrangement of connected things. In the context of collagen, matrix refers to a composite material comprised of an insoluble collagen-fibril network or amorphous nanostructure surrounded by an interstitial fluid phase.

3.2.6 *monomer, n*—individual tropocollagen molecule (Fig. 1).

3.2.7 *oligomer, n*—two or more tropocollagen molecules covalently attached by a naturally occurring intermolecular cross-link.

3.2.8 *polymerizable collagen, n*—purified type I collagen formulation that demonstrates the capacity to self-assemble or polymerize into higher order structures (macromolecular assemblies) in absence of exogenous agents such as cross-linkers.

3.2.9 *recombinant collagen protein/peptide, n*—collagen or collagen-like polypeptide produced by recombinant methods, such as by expression of a nucleotide sequence encoding the protein or peptide in a microorganism, insect, plant, or animal host. Such compositions often comprise Gly-X-Y triplets where Gly is the amino acid glycine and X and Y can be the same or different, are often proline or hydroxyproline, but can be any known amino acid.

3.2.10 *resorption, n*—removal by gradual breakdown into component materials; a loss of substance by lysis, or by physiologic or pathologic means.

3.2.10.1 *Discussion—*

In situations where there is an inflammatory response to implanted materials, immune cells, including neutrophils, macrophages, lymphocytes, and giant cells, can actively participate in resorption through material phagocytosis and/or proteolysis processes.

3.2.11 *solubility, n*—a measure of the extent to which a material can be ~~dissolved; in the context of collagen polymers, solubility refers to collagen molecules (partial, full, or multiples) or peptides in a solution; further qualification of solubility may include "acid-soluble" and "neutral salt-soluble" which describes compositions that are soluble in dilute acids and neutral salt solutions, respectively.~~dissolved.

3.2.11.1 *Discussion—*

In the context of collagen polymers, solubility refers to collagen molecules (partial, full, or multiples) or peptides in a solution; further qualification of solubility may include "acid-soluble" and "neutral salt-soluble" which describes compositions that are soluble in dilute acids and neutral salt solutions, respectively.

4. Significance and Use

4.1 The objective of this document is to provide guidance in the production, characterization, testing, and standardization of: (~~(a)~~1 ~~collagen polymers as a starting~~) polymerizable collagen starting materials; and (2 ~~material~~) collagen polymeric materials produced with polymerizable collagen formulations, used for surgical implants, substrates for ~~tissue-engineered medical products (TEMPs),~~ TEMPs, vehicles for therapeutic cells and molecules, and 3D *in-vitro* tissue systems for basic research, drug development, and toxicity ~~testing; and~~ testing. ~~(b) self-assembled collagen-based materials produced with collagen polymer formulations.~~ This guide can be used as an aid in the selection, characterization, and standardization of the appropriate polymerizable collagen ~~polymer~~ starting ~~material~~formulations as well as ~~associated self-assembled collagen-based products~~ collagen polymeric materials prepared

from polymerizable collagens for a specific use. Not all tests or parameters are applicable to all uses of ~~collagen.~~collagen and users are expected to select and justify a subset of the tests for characterization purposes.

4.2 This guide can be used by the following types of users:

4.2.1 Manufacturers of polymerizable collagens and collagen polymeric materials who wish to set specifications for their products or provide characterization data for customers or users. They may also use the terminology and characterization sections to specify and differentiate the properties of polymerizable collagens and collagen polymeric materials.

4.2.2 Producers of collagen polymeric materials that use polymerizable collagen as starting materials. Producers may use this guide to evaluate and characterize multiple sources of polymerizable collagen. They may also use this guide to assist with evaluation and comparison of single or multiple sources of polymerizable collagen and collagen polymeric materials.

4.2.3 Researchers may use this guide as a reference for properties and test methods that can be used to reproducibly evaluate polymerizable collagens and collagen polymeric materials.

4.3 The collagen covered by this guide may be used in a broad range of applications, forms, or medical products, for example (but not limited to) wound and hemostatic dressings, surgical implants or ~~injectables,~~ injectables (including *in-situ* forming), hybrid medical devices, ~~tissue-engineered medical products~~TEMPs, injectable (including ~~*(TEMPs),*~~*in-situ* ~~injectable~~forming) or implantable delivery vehicles for therapeutic cells, molecules, and drugs, and 3D *in-vitro* tissue systems or models for basic research, drug development, and toxicity testing. The practical application of ~~the collagen polymers and associated self-assembled collagen-based~~ polymerizable collagens and collagen polymeric materials should be based, among other factors, on biocompatibility, application-specific performance measures, as well as chemical, physical, and biological test data. Recommendations in this guide should not be interpreted as a guarantee of success for any specific research or medical application.

4.4 The following general areas should be considered when determining if the collagen supplied satisfies requirements for use in the above mentioned medical and research applications: source of ~~collagen polymer,~~polymerizable collagen, impurities profile, and comprehensive chemical, physical, and biological characterization and testing.

4.5 The following documents or other relevant ~~guidances~~ guidance documents from appropriate regulatory bodies relating to the production, regulation, and regulatory approval of devices, biologics, drugs, and combination products should be considered when determining if the collagen supplied satisfies requirements for use in medical and research products, including TEMPs, therapeutic delivery vehicles, and 3D *in-vitro* tissue systems:

FDA CFR:
21 CFR 3: Product Jurisdiction:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=3
21 CFR 58: Good Laboratory Practice for Nonclinical Laboratory Studies:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=58

FDA/CDRH CFR and Guidances:
21 CFR Part 803: Medical Device Reporting:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=803
21 CFR 812: Investigational Device Exemptions:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=812
21 CFR 814: Premarket Approval of Medical Devices:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=814
21 CFR 820: Quality System Regulation:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=820
Design Control Guidance for Medical Device Manufacturers:
http://www.fda.gov/cdrh/comp/designgd.pdf
Preproduction Quality Assurance Planning Recommendations for Medical Device Manufacturers (FDA 90-4236):
http://www.fda.gov/cdrh/manual/appende.html
The Review and Inspection of Premarket Approval Applications under the Bioresearch Monitoring Program—Draft Guidance for Industry and FDA Staff:

http://www.fda.gov/cdrh/comp/guidance/1602.pdf

FDA/CDRH Search Engines:
CDRH Guidance Search Engine:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfggp/search.cfm
CDRH Premarket Approval (PMA) Search Engine:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMA/pma.cfm
CDRH 510(k) Search Engine:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMN/pmn.cfm
CDRH Recognized STANDARDS Search Engine:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfStandards/search.cfm

FDA/CBER CFR and Guidances:
21 CFR 312: Investigational New Drug Application:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=312
21 CFR 314: Applications for FDA Approval to Market a New Drug:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=31
21 CFR 610: General Biological Products Standards:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=610
21 CFR 1271: Human Cells, Tissues and Cellular and Tissue-Based Products:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=1271
Cellular & Gene Therapy Guidances and Other Publications:
http://www.fda.gov/cber/genetherapy/gtpubs.htm
Human Tissue Guidances and Other Publications:
http://www.fda.gov/cber/tissue/docs.htm
CBER Product Approval Information:
http://www.fda.gov/cber/efoi/approve.htm
21 CFR 600, 601 BLA Regulations:
http://www.access.gpo.gov/nara/cfr/waisidx_07/21cfrv7_07.html
21 CFR 210, 211 GMP Regulations:
http://www.access.gpo.gov/nara/cfr/waisidx_07/21cfr210_07.html

5. Standardization of Polymerizable Collagens and Collagen Polymeric Material Products

5.1 *Master File and Product Specifications*—For the purposes of standardizing and characterizing polymerizable collagens and collagen polymeric materials prepared from polymerizable collagens, manufacturers should compile specifications and certificates of analysis with information on the important properties and performance parameters described in the following sections. Users of polymerizable collagen may choose to characterize properties when data is not available from the manufacturer. Some properties are important to understand the end performance of the materials, while others are important for the use of the materials. Manufacturers are recommended to notify regular users when revising product specifications. Collagen for use in biomedical and pharmaceutical applications including TEMPs should ideally be documented in a master file to which end users may obtain a letter of cross reference from suppliers of collagen. Such a master file should be submitted to the relevant national and international regulatory authorities. ISO 14971 should also be referenced when appropriate.

5.2 The characterization methods outlined below and in Tables 1 and 2 represent suggested chemical, physical, and biological assays or analyses; however, other validated assays and analyses may be used (7). Method selection will vary depending on the formulation and source of the collagen (for example, tissue-derived molecular collagen or collagen peptides produced synthetically). The user should ensure that the method selected is reliable and commonly accepted for protein, polymer, biological, and biomaterial analyses. In addition, the test should have appropriate dynamic range, detection limits, specificity, and sensitivity.

5.3 *Test Method Development and Validation*—Testing that is performed to demonstrate conformance to specifications should be done, when possible, using well-characterized and reliable methods. Sound and validated scientific methodologies should be applied to ensure production of consistent, accurate, and meaningful results that are insensitive (robust) to changes in environment, equipment and analytical instrumentation, personnel, sampling procedure, and test specimen format. It is beyond the scope of this document to provide references applicable to all the methods herein, but users should be aware of the most common practices.

TABLE 1 Characterization Methods for Type I Polymerizable Collagens

Parameter	Example Methods (not comprehensive or exclusive)	Qualitative	Quantitative
	Physical/Chemical/Biochemical		
Form and Appearance	•Visual inspection	X	
Collagen Concentration or Content	•Spectrophotometric (A_{230}; Sirius Red Assay; Hydroxyproline Assay)		X
	•Gel Electrophoresis	X	X
	•Solids Content (Loss on Drying Methods)		X
pH	•pH meter		X
	•pH indicator	X	
	•pH test papers	X	
Viscosity	•Viscometry		X
	•Rheology		X
Purity, Including Collagen Type Composition	•MS		X
	•FTIR		X
	•Amino Acid Analysis		X
	•ELISA		X
	•Circular Dichroism		X
	•Gel Electrophoresis; Western Blot; Peptide Mapping	X	X
Impurities Profile, Including Heavy Metals Analysis	•GC		X
	•HPLC		X
	•ICP		X
	•Gel Electrophoresis	X	X
	•Specific Chemical Assays	X	X
	•TGA (Thermogravimetric Analysis)		
Degree of Cross-linking (Natural Intermolecular Cross-links or Exogenous Cross-links)	•MS		X
	•HPLC		X
	•DSC		X
	•Gel Electrophoresis	X	X
	•Colorimetric	X	X
	•TGA (Thermogravimetric Analysis)		X
Molecular Mass; Molecular Mass Distribution; Average Polymer Molecular Weight	•Viscometry		X
	•Rheology		X
	•LC		X
	•DLS		X
	•Analytical Ultracentrifugation		X
Enzyme Susceptibility (e.g., trypsin, collagenase)	•Digestion Assay	X	X
Additives (e.g., light/heat stabilizers, viscosity modifiers, antimicrobial agents, cross-linking agents, other biomolecules, drugs)	•HPLC	X	X
	•GC	X	X
	•MS	X	X
Polymerization Kinetics	•Spectrophotometric		X
	•Rheometric		X

5.3.1 *Equipment*—Common practices pertaining to test equipment include routine calibration, more frequent measurement verifications, periodic preventative maintenance, and formal equipment qualifications. An example of these practices is provided in USP <1058>. More specific practices also exist for some applications, such as Practice E4 for force verification of testing machines.

5.3.2 *Personnel and Procedures*—The performance of personnel and the procedures assigned to them is ensured through training and tests to demonstrate proficiency. Computational or analytical tasks should be included alongside operational tasks.

5.3.3 *Control Materials and Calibration Curve*—For methods that are sensitive to interference or background noise, usually analytical or thermal tests, well-characterized control samples are included alongside each batch of test samples to demonstrate ongoing accuracy and precision as far as possible. Similarly, a set of known reference samples may be used to create a calibration curve for each sample batch that can relate direct sensor measurements (such as fluorescence intensity) to an estimate of sample concentration.

5.3.4 *Method Validation*—A test method validation is a formal process of demonstrating the capability of a test method to meet the needs of its intended use.

5.3.4.1 *Analytical Method Guidance*—Guidance for test method validation of analytical methods include ICH Q2, U.S. FDA Guidance for Analytical Procedures and Methods Validation for Drugs and Biologics, and USP <1225>. For analytical methods, these documents generally provide recommendations to document a method's performance in terms of accuracy, specificity, sensitivity, range, linearity, and precision/reproducibility. Method validation includes ongoing activities such as routine system suitability tests.

5.3.4.2 *Physical Method Guidance*—Method validations for physical tests generally focus on characterizing the accuracy and