Designation: ~~F2103 – 11~~ F2103 – 18

Standard Guide for Characterization and Testing of Chitosan Salts as Starting Materials Intended for Use in Biomedical and Tissue-Engineered Medical Product Applications[1]

This standard is issued under the fixed designation F2103; 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

Biopolymers from marine sources have been studied and used in commercial applications and product development for a number of years. Chitosan, a linear polysaccharide consisting of glucosamine and *N*-acetyl glucosamine derived mainly from crustacean shells, has been used in many technical applications such as water purification (as a flocculant), in cosmetics, and recently as a proposed fat-binding weight control product. In solution, the cationic nature of chitosan gives this polymer a mucoadhesive property. Chitosan and its salts can be used as a matrix or scaffold material as well as in non-parenteral delivery systems for challenging drugs. Chitosan salts have been shown to increase the transport of polar drugs across the nasal epithelial surface. The purpose of this guide is to identify key parameters relevant for the functionality and characterization of chitosan and chitosan salts for the development of new commercial applications of chitosan salts for the biomedical and pharmaceutical industries.

1. Scope

1.1 This guide covers the evaluation of chitosan salts suitable for use in biomedical or pharmaceutical applications, or both, including, but not limited to, tissue-engineered medical products (TEMPS).

1.2 This guide addresses key parameters relevant for the functionality, characterization, and purity of chitosan salts.

1.3 As with any material, some characteristics of chitosan may be altered by processing techniques (such as molding, extrusion, machining, assembly, sterilization, and so forth) required for the production of a specific part or device. Therefore, properties of fabricated forms of this polymer should be evaluated using test methods that are appropriate to ensure safety and efficacy.

1.4 **Warning—**Mercury has been designated by 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 EPA's 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 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~~ safety, health, and ~~health~~environmental practices and determine the applicability of regulatory limitations prior to use.*

1.7 *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.*

[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.

Current edition approved ~~March 1, 2011~~June 1, 2018. Published ~~March 2011~~August 2018. Originally approved in 2001. Last previous edition approved in ~~2007~~2011 as ~~F2103 – 01~~F2103 – 11.~~(2007)$^{\varepsilon 2}$~~. DOI: ~~10.1520/F2103-11.~~10.1520/F2103-18.

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D2196 Test Methods for Rheological Properties of Non-Newtonian Materials by Rotational Viscometer
F619 Practice for Extraction of Medical Plastics
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 Screening of Implant 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)[3]
F1439 Guide for Performance of Lifetime Bioassay for the Tumorigenic Potential of Implant Materials
F1903 Practice for Testing For Biological 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)[3]
F1906 Practice for Evaluation of Immune Responses In Biocompatibility Testing Using ELISA Tests, Lymphocyte Proliferation, and Cell Migration (Withdrawn 2011)[3]
F2260 Test Method for Determining Degree of Deacetylation in Chitosan Salts by Proton Nuclear Magnetic Resonance (^{1}H NMR) Spectroscopy
F2602 Test Method for Determining the Molar Mass of Chitosan and Chitosan Salts by Size Exclusion Chromatography with Multi-angle Light Scattering Detection (SEC-MALS)

2.2 *Ph. Eur. Document:*

Ph. Eur.[4] Monograph Chitosan Chloride, Nov. 2000

2.3 *ISO Documents:*[5]

ISO ~~10993~~31-8 ~~Biological Evaluation of Medical Devices~~Quantities and Units — Part 8: Physical Chemistry and Molecular Physics
ISO 13408-1: 1998 Aseptic Processing of Health Care Products — Part 1: General Requirements
ISO ~~10993-1~~22442-1 ~~Biological Evaluation of Medical Devices—Part 1: Evaluation and Testing~~Medical Devices Utilizing Animal Tissues and Their Derivatives — Part 1: Application of Risk Management
ISO ~~10993-3—Part 3:~~22442-2 ~~Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity~~Medical Devices Utilizing Animal Tissues and Their Derivatives — Part 2: Controls On Sourcing, Collection, and Handling
ISO ~~10993-9—Part 9:~~22442-3 ~~Framework for Identification and Quantification of Potential Degradation Products~~Medical Devices Utilizing Animal Tissues and Their Derivatives — Part 3: Validation of the Elimination and/or Inactivation of Viruses and Transmissible Spongiform Encephalopathy (TSE) Agents
~~ISO 10993-17—Part 17: Methods for Establishment of Allowable Limits for Leachable Substances Using Health-Based Risk Assessment~~[5]
ISO ~~13408-1: 1998:~~80000-9:2009 ~~Aseptic Processing of Health Care Products—Part 1: General Requirements~~ Quantities and units — Part 9: Physical Chemistry and Molecular Physics

2.4 *ICH Documents:*[6]

International Conference on Harmonization (1997) Guidance for Industry M3 Nonclinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals 62 FR 62922
International Conference on Harmonization (1996) Guideline for Industry S2A Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals 61 FR 18199
International Conference on Harmonization (1997) Guidance for Industry S2B Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals 62 FR 62472
International Conference on Harmonization (1994) Guideline for Industry S5A Detection of Toxicity to Reproduction for Medicinal Products 59 FR 48746
International Conference on Harmonization (1996) Guidance for Industry S5B Detection of Toxicity to Reproduction for Medicinal Products: Addendum on Toxicity to Male Fertility 61 FR 15360

[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.
[4] Available from EDQM, Publications and Services European Pharmacopoeia, BP 907 226, avenue de Colmar, F-67029 Strasbourg Cedex 1, France.
[5] Available from ~~American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036, http://www.ansi.org.~~International Organization for Standardization (ISO), ISO Central Secretariat, BIBC II, Chemin de Blandonnet 8, CP 401, 1214 Vernier, Geneva, Switzerland, http://www.iso.org.
[6] Available from ICH Secretariat, c/o IFPMA, 30 rue de St-Jean, PO Box 758, 1211 Geneva 13, Switzerland.

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

International Conference on Harmonization (1998) Guidance for Industry S1B Testing for Carcinogenicity of Pharmaceuticals 63 FR 8983

International Conference on Harmonization (1995) Guideline for Industry S1C Dose Selection for Carcinogenicity Studies of Pharmaceuticals 60 FR 11278

International Conference on Harmonization (1997) S1C[R] Guidance for Industry Addendum to Dose Selection for Carcinogenicity Studies of Pharmaceuticals: Addition of a Limit Dose and Related Notes 62 FR 64259

International Conference on Harmonization (ICH) Q1A ICH Harmonized Tripartite Guidance for Stability Testing of New Drug Substances and Products (September 23, 1994)

2.5 *FDA Documents:*[7]

FDA Guideline DHHS

FDA Guideline on Validation of the Limulus Amebocyte Test as an End-Product Endotoxin Test for Human and Animal Parenteral Drugs, Biological Products and Healthcare Products_ DHHS, December 1987

FDA Interim Guidance for Human and Veterinary Drug Products and Biologicals. Kinetic LAL Techniques _DHHS, July 15, 1991

2.6 *ANSI Documents:*[5]

ANSI/AAMI/ISO 11737-1: 1995 Sterilization of Medical Devices—Microbiological Methods—Part 1: Estimation of Bioburden on Product

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

2.7 *AAMI Documents:*[8]

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:~~14160:1998 Sterilization of Single-Use Medical Devices Incorporating Materials of Animal Origin—Validation and Routine Control of Sterilization by Liquid Chemical Sterilants

AAMI ~~ST67/CDV-2: 1999:~~ST67/CDV-2:1999 Sterilization of Medical Devices—Requirements for Products Labeled "Sterile"

2.8 ~~*EN*~~ *United States Pharmacopeia Documents:*[9]

~~EN 12442-1~~USP Chapter <85> ~~Animal Tissues and Their Derivative Utilized in the Manufacture of Medical Devices—Part 1: Analysis and Management of Risk~~Biological Tests and Assays: Bacterial Endotoxins Tests

~~EN 12442-Part 3:~~USP Chapter <161> ~~Validation of the Elimination and/or Inactivation of Virus and Transmissible Agents~~Microbial Limit Tests

USP36-NF31 Elemental Impurities

USP Chapter <24>

USP Chapter <71> Sterility Tests

USP Chapter <1211> Sterilization and Sterility Assurance of Compendial Articles

<731>USP 24/NF19

2.9 *NIST Document:*[10]

NIST Special Publication 811 Guide for the Use of the International System of Units (SI)

2.10 *U.S. Code of Federal Regulations:*[11]

21CFR312: Title 21— Code of Federal Regulations, Part 312 Investigational New Drug Application

3. Terminology

3.1 *Definitions:*

3.1.1 *chitosan, n*—a linear polysaccharide consisting of β(1→4) linked 2-acetamido-2-deoxy-D-glucopyranose (GlcNAc) and 2-amino-2-deoxy-D-glucopyranose (GlcN).

3.1.1.1 *Discussion*—

Chitosan is a polysaccharide derived by *N*-deacetylation of chitin.

3.1.2 *decomposition, n*—structural changes of chitosans as a result of exposure to environmental, chemical, or thermal factors, such as temperatures greater than 200°C.

[7] Available from Food and Drug Administration (FDA), 5600 Fishers Ln., Rockville, MD 20857, http://www.fda.gov.

[8] Association for the Advancement of Medical Instrumentation, 111 N. Glebe Rd., Suite 220, Arlington, VA 22201–4795.

[9] Available from ~~European Committee for Standardization, CEN Management Centre, 36 rue de Stassart, B-1050 Brussels, Belgium.~~U.S. Pharmacopeial Convention (USP), 12601 Twinbrook Pkwy., Rockville, MD 20852-1790, http://www.usp.org.

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

[11] Available from U.S. Government Printing Office, Superintendent of Documents, 732 N. Capitol St., NW, Washington, DC 20401-0001, http://www.access.gpo.gov.

3.1.2.1 *Discussion—*

Decomposition can result in deleterious changes to the chitosan.

3.1.3 *degradation, n*—change in the chemical structure, physical properties, or appearance of a material.

3.1.3.1 *Discussion—*

Degradation of polysaccharides occurs by means of cleavage of the glycosidic bonds, usually by acid —catalyzed hydrolysis. Degradation can also occur thermally. Note that degradation is not synonymous with decomposition. Degradation is often used as a synonym for depolymerization when referring to polymers.

3.1.4 *degree of deacetylation, n*—the fraction or percentage of glucosamine units (deacetylated monomers) in a chitosan polymer molecule.

3.1.5 *depolymerization, n*—reduction in length of a polymer chain to form shorter polymeric units.

3.1.5.1 *Discussion—*

Depolymerization may reduce the polymer chain to oligomeric or monomeric units, or both. In chitosan, hydrolysis of the glycosidic bonds is the primary mechanism.

3.1.6 *dispersity, n*—measure of the heterogeneity of sizes of molecules or particles in a mixture, calculated by the ratio of M^-_w/M^-_n.

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—*

Though endotoxins are pyrogens, not all pyrogens are endotoxins. Endotoxins are specifically detected through a Limulus Amebocyte Lysate (LAL) test.

3.1.8 *molecular mass average (molecular weight average), n*—the given molecular weight ~~(Mw)~~(M) of a chitosan will always represent an average of all of the molecules in the population. The most common ways to express the ~~Mw~~M are as the number average (M^-_n) and the weight average (M^-_w). The two averages are defined by the following equations:

$$\overline{M}_n = \frac{\sum_i N_i M_i}{\sum_i N_i}$$

and

$$\overline{M}_w = \frac{\sum_i W_i M_i}{\sum_i W_i} = \frac{\sum_i N_i M_i^2}{\sum_i N_i M_i}$$

where:

N_i = number of molecules having a specific molecular weight M_i and

~~w_i = weight of molecules having a specific molecular weight M_i. In a polydisperse molecular population the relation $M^-_w > M^-_n$ is always valid. The coefficient M^-_w/M^-_n is referred to as the polydispersity index, and will typically be in the range 1.5 to 3.0 for commercial chitosans.~~

W_i = weight of molecules having a specific molecular weight M_i. In a disperse molecular population the relation $M^-_w > M^-_n$ is always valid. The ratio M^-_w/M^-_n is referred to as the dispersity, and will typically be in the range from 1.5 to 3.0 for commercial chitosans.

NOTE 1—The term molecular weight (abbreviated MW) is obsolete and should be replaced by the SI equivalent of either relative molecular mass (M_r), which reflects the dimensionless ratio of the mass of a single molecule to an atomic mass unit (see ISO 31-8), or molar mass (M), which refers to the mass of a mole of a substance and is typically expressed as g/mol. For polymers and other macromolecules, use of the symbols M_w,M_n, and M_z continue, referring to mass-average molar mass, number-average molar mass, and z-average molar mass, respectively. For more information regarding proper utilization of SI units, see NIST Special Publication 811.

3.1.9 *pyrogen, n*—any substance that produces fever when administered parenterally.

4. Significance and Use

4.1 This guide contains a listing of those characterization parameters that are directly related to the functionality of chitosan. This guide can be used as an aid in the selection and characterization of the appropriate chitosan or chitosan salt for a particular application. This standard is intended to give guidance in the methods and types of testing necessary to properly characterize, assess, and ensure consistency in the performance of a particular chitosan. It may have use in the regulation of devices containing chitosan by appropriate authorities.

4.2 The chitosan salts covered by this guide may be gelled, extruded, or otherwise formulated into biomedical devices for use as tissue-engineered medical products or drug delivery devices for implantation as determined to be appropriate, based on supporting biocompatibility and physical test data. Recommendations in this guide should not be interpreted as a guarantee of clinical success in any tissue-engineered medical product or drug delivery application.

4.3 To ensure that the material supplied satisfies requirements for use in TEMPs, several general areas of characterization should be considered. These include identity of chitosan, physical and chemical characterization and testing, impurities profile, and performance-related tests.

5. Chemical and Physical Test Methods

5.1 *Identity of Chitosan*—The identity of chitosan and chitosan salts can be established by several methods including, but not limited to the following:

5.1.1 Chitosan chloride monograph Ph. Eur.

5.1.2 *Fourier Transform Infrared Spectroscopy (FT-IR)*—Almost all organic chemical compounds absorb infrared radiation at frequencies characteristic for the functional groups in the compound. A FT-IR spectrum will show absorption bands relating to bond stretching and bending and can therefore serve as a unique fingerprint of a specific compound. Cast a chitosan film from a 0.25 % ~~(w/v)~~(weight/volume) solution of chitosan ~~(in~~in 1 % (volume/volume) acetic ~~acid)~~acid or chitosan salt (dissolved in water) by drying approximately 500 µL of the sample onto a disposable IR card[12] for 3 to 4 h at 60°C. Record a background spectrum between 4000 and 400 cm-1 using 128 scans at a resolution of 4 cm^{-1}. Record the IR spectrum of a dried blank IR card, then record the IR spectrum of the sample using 128 scans at a resolution of 4 cm^{-1}, percent transmission mode. Label the peaks. Typical representative frequencies (cm^{-1}) for chitosan are as follows:

[12] No suitable commercially available IR cards are available for the IR analysis of chitosan glutamate salt. Alternative methods are under investigation.

Chitosan Base (as Acetate)		Chitosan Chloride		Chitosan Glutamate	
Bond	Frequency (cm^{-1})	Bond	Frequency (cm^{-1})	Bond	Frequency (cm^{-1})
O-H and N-H	3362b	O-H and N-H	3344b	N-H	1555b
Asymmetric carboxylate anion	1556	$N-H_2$	1605	CH_2-OH	1396
Symmetric carboxylate anion	1406	N-H	1513	C-O-C (glycosidic linkage)	1154
C-O-C (glycosidic linkage)	1153	CH_2-OH	1379	C-O-C (glycosidic ring)	1085s
C-O-C (glycosidic ring)	1083s	C-O-C (glycosidic linkage)	1154	. . .	. . .
. . .	. . .	C-O-C (glycosidic ring)	1086s	. . .	. . .

The peak ~~designators~~designations are: sh: sharp; s: strong; m: medium; w: weak; and b: broad.

5.2 *Physical and Chemical Characterization of Chitosan:*

5.2.1 The composition and sequential structure of chitosan can be a key functional attribute of any chitosan or chitosan salt. Variations in the composition or the sequential structure, or both, may, but not necessarily will, cause differences in performance of a chitosan in a particular end use. This information may be determined by the following method: High-resolution ^{1}H- and ^{13}C-nuclear magnetic resonance spectroscopy (NMR).

5.2.2 The degree of deacetylation of chitosan can be established using a number of techniques including, but not limited to, the following:

5.2.2.1 *High-resolution ^{1}H- and ^{13}C-Nuclear Magnetic Resonance Spectroscopy (NMR)*—Chitosan salts should be dissolved in D_2O and partially degraded to a degree of depolymerization of 20 to 30 using sodium nitrite before recording proton or carbon NMR ~~spectra.~~spectra[13] (see Test Method F2260).

5.2.2.2 ~~*Determination of the Degree of Deacetylation by UV Spectroscopy*~~—This method is based upon that reported by Muzzarelli et al.[14] The method is actually a quantitative measure of the number of amine functional groups in the polymer. The method uses a standard curve produced from varying concentrations of *N*-acetyl glucosamine. The degree of deacetylation is calculated from recordings of the first derivative of the UV spectra of *N*-acetyl glucosamine and of chitosan samples at 202 nm.

5.2.2.3 *Titration*—Methods are based on titrating a known base solution to a known mass of chitosan dissolved in acid. A graph of pH versus added volume of base added will have two inflexion points with the first representing the neutralization of acid and the second the neutralization of the ammonium ion group on the chitosan. The difference in the inflection points is used to calculate the degree of de-acetylation **(1, 2)**[15].

5.2.3 Molecular mass (molecular weight) of a chitosan will define certain performance characteristics such as viscosity. As such and depending on the sensitivity of a particular end use to these variations, determination of molecular mass directly or indirectly may be necessary. Commercial chitosans are ~~polydisperse~~disperse with respect to molecular weight ~~((M).M$_W$).~~ Molecular weight may be expressed as the number average ($M_{~~n~~}$) or the weight average ($M_{~~w~~}$). Molecular weights may be determined by methods such as, but not limited to, the following:

5.2.3.1 *Molecular Weight Determination Based on Intrinsic Viscosity*—The intrinsic viscosity describes a polymer's ability to form viscous solutions in water and is directly proportional to the average molecular weight of the polymer. The intrinsic viscosity is a characteristic of the polymer under specified solvent and temperature conditions. It is independent of concentration. The intrinsic viscosity (η) is directly related to the molecular weight of a polymer through the Mark-Houwink-Sakurada (MHS) equation:

$$[\eta] = KM^{a}$$

where:

K = a constant,
M = viscosity derived average molecular weight, and

[13] Vårum, K. M., Anthonsen, M. W., Grasdalen, H., and Smidsrod, O., *Carbohydrate Research*, Vol 211, 1991, pp. 17–23.
[14] Muzzarelli, R. A. A., Rochetti, R., Stanic, V., and Weckx, M., *Chitin Handbook*, R. A. A. Muzzarelli and M. T. Peters, Ed., Atec Grottammare, 1997.
[15] The boldface numbers in parentheses refer to a list of references at the end of this guide.