Designation: F2347 – 03

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

This standard is issued under the fixed designation F2347; 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

Hyaluronan, which in this guide will encompass hyaluronic acid, hyaluronate, and its salt forms, is the simplest of the glycosaminoglycans. Hyaluronan is soluble in water and forms highly viscous solutions. Hyaluronan is found in ubiquitously in the body as part of the extracellular matrix of tissues, with high concentrations in the synovial fluid, vitreous humor, and skin, as well as in cartilage. Hyaluronan has found uses in a variety of products ranging from viscosupplements (treatment of osteoarthritis), adhesion prevention (prevention of post-surgical adhesions), viscoelastics (ocular protection), and dermal implants (lip augmentation and wrinkle removal). New applications, such as scaffolds for tissue engineering, are emerging. The aim of this guide is to identify key parameters relevant to the characterization of hyaluronan for the development of new commercial applications of hyaluronan for the biomedical and pharmaceutical industries.

1. Scope

1.1 This guide covers the evaluation of hyaluronan 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 to the characterization and purity of hyaluronan.

1.3 As with any material, some characteristics of hyaluronan may be altered by processing techniques, such as cross-linking and sterilization, required for the production of a specific formulation or device. Therefore, properties of fabricated forms of this polymer should be evaluated using test methods that are appropriate to ensure safety and efficacy and are not addressed in this guide.

1.4 *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]

D2196 Test Methods for Rheological Properties of Non-Newtonian Materials by Rotational (Brookfield type) 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

[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 Nov. 1, 2003. Published December 2003. DOI: 10.1520/F2347-03.

[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.

Materials on Muscle and Bone

F1251 Terminology Relating to Polymeric Biomaterials in Medical and Surgical Devices

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

F1906 Practice for Evaluation of Immune Responses In Biocompatibility Testing Using ELISA Tests, Lymphocyte Proliferation, and Cell Migration

2.2 *USP Documents:*[3]

USP <61> Microbial Limit Tests

USP <71> Sterility Tests

USP <85> Bacterial Endotoxins Tests

USP <231> Heavy Metals

USP <731> Loss on Drying

USP <1211> Sterilization and Sterility Assurance of Compendial Articles

2.3 *EP Documents:*[4]

EP Monograph 1472 Sodium Hyaluronate

EP 2.6.1 Sterility

2.4 *Other Referenced Documents:*

ISO 10993 Biological Evaluation of Medical Devices[5]

ISO 10993-1 Biological Evaluation of Medical Devices—Part 1: Evaluation and Testing

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

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

ISO 10993-17 Biological Evaluation of Medical Devices—Part 17: Establishment of Allowable Limits for Leachable Substances

ISO 14160-1998 Sterilization of Single-Use Medical Devices Incorporating Materials of Animal Origin—Validation and Routine Control of Sterilization by Liquid Chemical Sterilants[5]

ISO 11737-1: 1995 Sterilization of Medical Devices—Microbiological Methods—Part 1: Estimation of Population of Microorganisms on Products[5]

ISO 11737-2: 1998 Sterilization of Medical Devices—Microbiological Methods—Part 2: Tests of Sterility Performed in the Validation of a Sterilization Process[5]

ISO 13408-1: 1998 Aseptic Processing of Health Care Products—Part 1: General Requirements[5]

ISO EN 12442-1 Animal Tissues and Their Derivative Utilized in the Manufacture of Medical Devices—Part 1: Analysis and Management of Risk[5]

ISO EN 12442-3 Animal Tissues and Their Derivative Utilized in the Manufacture of Medical Devices—Part 3: Validation of the Elimination and/or inactivation of Virus and Transmissible Agents[5]

International Conference on Harmonization (ICH) S2B Genotoxicity A Standard Battery for Genotoxicity Testing of Pharmaceuticals (July 1997)[6]

International Conference on Harmonization (ICH) Q1A ICH Harmonized Tripartite Guidance for Stability Testing of New Drug Substances and Products (September 2001, Revision 1)[6]

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[7]

FDA Interim Guidance for Human and Veterinary Drug Products and Biologicals, Kinetic LAL Techniques, DHHS, July 15, 1991[7]

AAMI TIR No. 7: 1999 Chemical Sterilants and High Level Disinfectants: A Guide to Selection and Use[8]

AAMI ST67/CDV-2: 1999 Sterilization of Medical Devices—Requirements for Products Labeled "Sterile"[8]

21 CFR 312 FDA Title 21, Food and Drugs, Investigational New Drug Applications[9]

3. Terminology

3.1 *Definitions:*

3.1.1 *hyaluronan, n*—a polysaccharide with a disaccharide repeating unit composed of D-glucuronic acid and *N*-acetyl-D-glucosamine in β-(1→3) linkage. Each disaccharide unit is attached to the next by β-(1→4) bonds. Hyaluronan is a linear polymer. Other common names are hyaluronic acid and sodium hyaluronate.

3.1.2 *hydrocolloid, n*—a water-soluble polymer of colloidal nature when hydrated.

3.1.3 *molecular mass average (molecular weight average), n*—the given molecular weight (Mw) of hyaluronan will always represent an average of all of the molecules in the population. The most common ways to express the Mw are as the number average ($\overline{M}_n$) and the weight average ($\overline{M}_w$). The two averages are defined by the following equations:

$$\overline{M}_n = \frac{\sum_i N_i M_i}{\sum_i N_i} \quad \text{and} \quad \overline{M}_w = \frac{\sum_i w_i M_i}{\sum_i w_i} = \frac{\sum_i N_i M_i^2}{\sum_i N_i M_i}$$

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.

[3] Available from U.S. Pharmacopeia (USP), 12601 Twinbrook Pkwy., Rockville, MD 20852.

[4] Available from European Directorate for the Quality of Medicines (EDQM), Council of Europe, BP 907, 67029 Strasbourg, France.

[5] Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036.

[6] Available from ICH Secretariat, c/o IFPMA, 30 rue de St-Jean, P.O. Box 758, 1211 Geneva 13, Switzerland.

[7] Available from U.S. Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857-0001.

[8] Available from Association for the Advancement of Medical Instrumentation, 1110 North Glebe Rd., Suite 220, Arlington, VA 22201-4795.

[9] Available from Standardization Documents Order Desk, DODSSP, Bldg. 4, Section D, 700 Robbins Ave., Philadelphia, PA 19111-5098

In a polydisperse molecular population the relation $\overline{M}_w > \overline{M}_n$ is always valid. The coefficient $\overline{M}_w / \overline{M}_n$ is referred to as the polydispersity index, and will typically be in the range 1.2 to 3.0 for commercial hyaluronan.

3.1.4 *depolymerization*, *n*—reduction in length of a polymer chain to form shorter polymeric units. Depolymerization may reduce the polymer chain to smaller molecular weight polymers, oligomeric, or monomeric units, or combination thereof. In hyaluronan, acid hydrolysis of the glycosidic bonds is the primary mechanism.

3.1.5 *degradation*, *n*—change in the chemical structure, physical properties or appearance of a material. Degradation of polysaccharides occurs via cleavage of the glycosidic bonds, usually by acid catalyzed hydrolysis. Degradation can also occur thermally and by alkali. It is important to note that degradation is not synonymous with decomposition. Degradation is often used as a synonym for depolymerization when referring to polymers. Degradation (depolymerization) of hyaluronan may also occur enzymatically by the action of hyaluronidases.

3.1.6 *decomposition*, *n*—structural changes of hyaluronan due to exposure to environmental, chemical, or thermal factors. Decomposition may occur at temperatures as low as 121°C during autoclaving. Decomposition can result in deleterious changes to the hyaluronan.

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

3.1.8 *endotoxin*, *n*—a high molecular weight lipopolysaccharide (LPS) complex associated with the cell wall of gram-negative bacteria that is pyrogenic in humans. Though endotoxins are pyrogens, not all pyrogens are endotoxins.

3.1.9 *non-animal derived*, *n*—a term describing the absence of any animal-derived tissue, proteins, or products in the manufacturing process.

4. Significance and Use

4.1 This guide contains a listing of those characterization parameters that are directly related to the functionality of hyaluronan. This guide can be used as an aid in the selection and characterization of the appropriate hyaluronan for a particular application. This guide 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 hyaluronan. It may have use in the regulation of these devices by appropriate authorities.

4.2 The hyaluronan covered by this guide may be gelled, cross-linked, extruded, or otherwise formulated into biomedical devices for use in 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 are: identity of hyaluronan, physical and chemical characterization and testing, impurities profile, and performance-related tests.

5. Chemical and Physical Test Methods

5.1 *Identity of Hyaluronan*—The identity of hyaluronan can be established by several methods including, but not limited to the following:

5.1.1 *Sodium Hyaluronate Monograph EP 1472*.

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. Direct FT-IR analysis of hyaluronan powder is perhaps the easiest technique to perform. One method utilizes a horizontal attenuated total reflectance (HATR) accessory with a zinc-selenium (ZnSe) crystal (or equivalent) having a sample trough and a pressure plate. Record background and sample spectra between 4000 and 600 cm^{-1} at an appropriate resolution. Label the peaks. Typical frequencies (cm^{-1}) for hyaluronan (sodium salt) are 3275-3390 (b), 1615 (s), 1405 (m), 1377 (m), 1150, 1077, 1045 (s), 946 (m), 893 (w). The peak designators are: sh: sharp; s: strong; m: medium; w: weak; b: broad. A typical FT-IR HATR spectrum is shown in Fig. 1. A reference spectrum can be obtained form the European Pharmacopoeia.[10]

5.2 *Physical and Chemical Characterization of Hyaluronan*:

5.2.1 The composition and sequential structure of hyaluronan can be determined by the following method: High-resolution ^1H- and ^{13}C-nuclear magnetic resonance spectroscopy (NMR). Hyaluronan should be dissolved in D_2O. If the resulting solution is viscous, viscosity may be reduced by chemical or enzymatic depolymerization. A typical ^1H-NMR spectrum of hyaluronan is shown below. Hyaluronan is characterized by calculating parameters such as glucuronic acid: N-acetylglucosamine ratio. Some literature references to the determination of composition and structure of hyaluronan are given in the References section (**1-4**).[11]

5.2.2 Molecular mass (molecular weight) of hyaluronan will define certain performance characteristics such as viscosity or gel strength, or both. 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 hyaluronan is polydisperse with respect to molecular weight (M_w). M_w 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:

5.2.2.1 *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

[10] EDQM, European Pharmacopoeia, Council of Europe, B.P. 907, F-67029 Strasbourg France; www.pheur.org

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

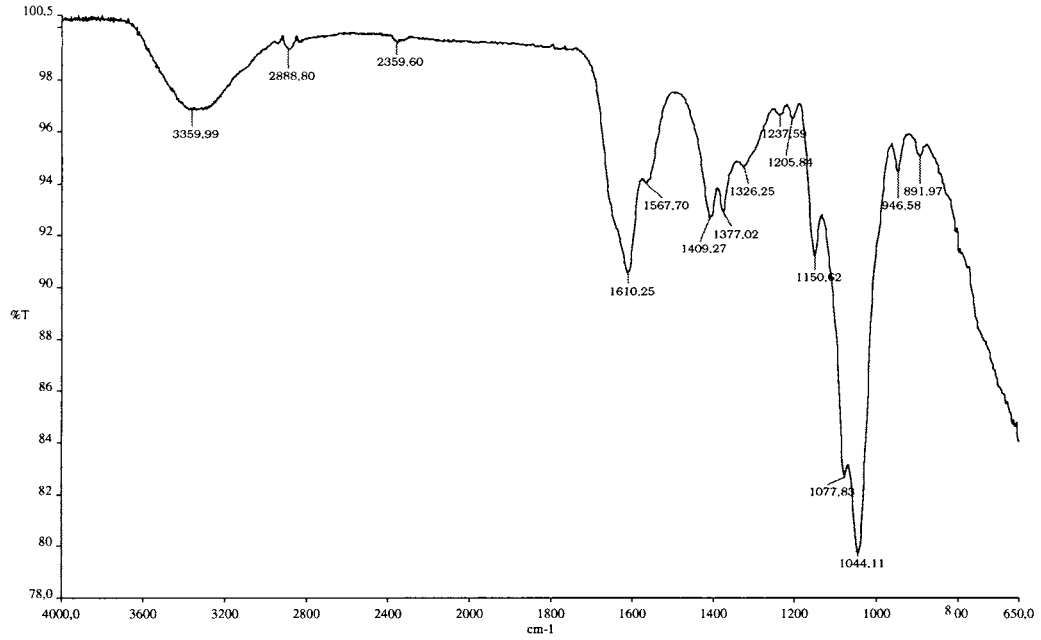

FIG. 1 FT-IR Spectrum of Hyaluronan, Sodium Salt Using Horizontal Attenuated Total Reflectance (HATR)

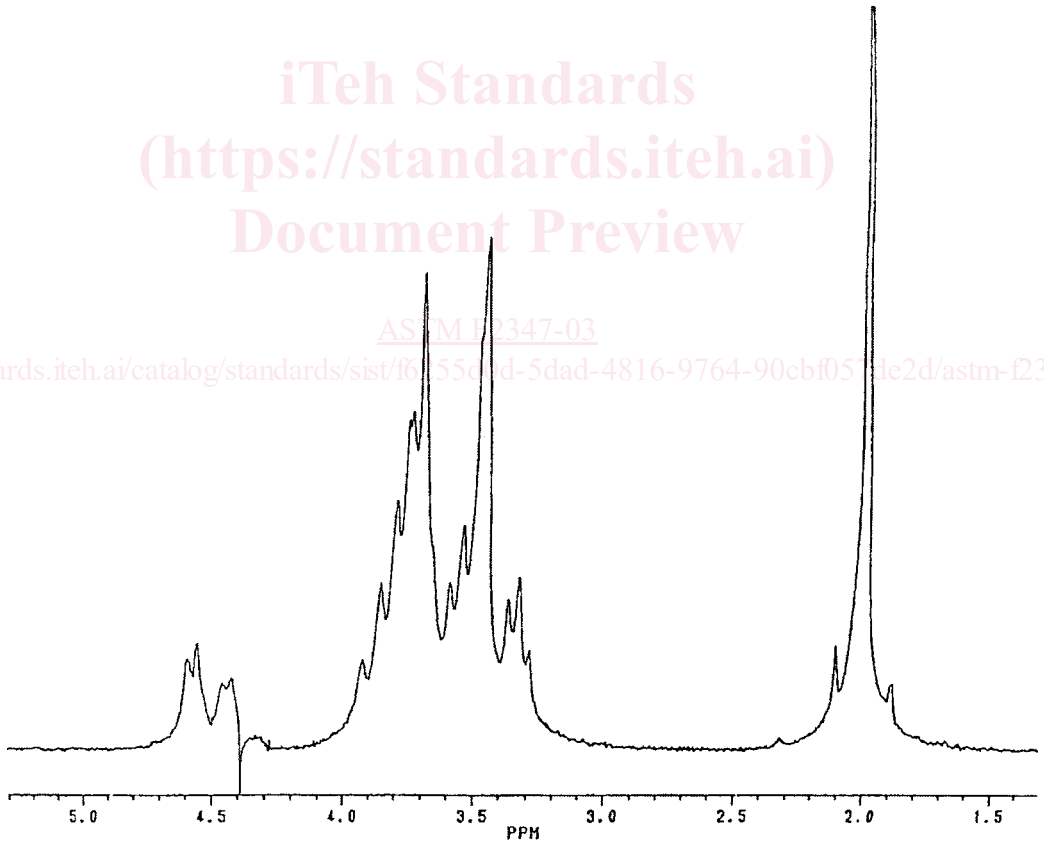

FIG. 2 ^{1}H NMR Spectrum of Hyaluronan from Rooster Comb (Mw ~700 000)

molecular weight of a polymer through the Mark-Houwink-Sakurada (MHS) equation: $[\eta] = KM^{a}$. For hyaluronan, K is 0.00057 and the exponent (a) is 0.75 at the following conditions: 0.15 M NaCl in phosphate buffer, pH 7.5, 20°C (**5**). By measuring the intrinsic viscosity, the viscosity average molecular weight can be determined if K and a are accurately known for the sample: $\log [\eta] = \log K + a (\log M)$, where M is the molecular weight. The intrinsic viscosity is determined by measuring the relative viscosity in an Ubbelohde capillary viscometer. The measurements should be performed in a