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Standard Guide for Characterization and Testing of Hyaluronan as Starting Materials Intended for Use in Biomedical and Tissue Engineered Medical Product Applications¹

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

1.5 *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*

appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.

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:²

E1953 Practice for Description of Thermal Analysis and Rheology Apparatus

E2975 Test Method for Calibration or Calibration Verification of Constant Speed Concentric Cylinder Rotational Viscometers

F619 Practice for Extraction of Materials Used in Medical Devices

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

¹ This guide is under the jurisdiction of ASTM Committee F04 on Medical and Surgical Materials and Devices and is the direct responsibility of Subcommittee F04.42 on Biomaterials and Biomolecules for TEMPs.

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² For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at www.astm.org/contact. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

- 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 Muscle and Bone Tissue Responses to Long-Term Implantable Materials Used in Medical Devices
- F1439** Guide for Performance of Lifetime Bioassay for the Tumorigenic Potential of Implant Materials

2.2 *USP Documents*:³

- USP <61>** Microbiological Examination of Nonsterile Products—Microbial Enumeration Tests
- USP <71>** Sterility Tests
- USP <85>** Bacterial Endotoxins
- USP <231>** Heavy Metals
- USP <232>** Elemental Impurities—Limits
- USP <233>** Elemental Impurities—Procedures
- USP <731>** Loss on Drying
- USP <1211>** Sterilization and Sterility Assurance of Compensial Articles

2.3 *EP Documents*:⁴

- EP Monograph 1472** Sodium Hyaluronate
- EP 2.6.1** Sterility
- EP 2.2.32** Loss on Drying

2.4 *ISO Documents*:⁵

- ISO 10993** Biological Evaluation of Medical Devices
- ISO 10993-1** Biological Evaluation of Medical Devices—Part 1: Evaluation and Testing Within a Risk Management Process
- 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** Sterilization of Health Care Products—Liquid Chemical Sterilizing Agents for Single-Use Medical Devices Utilizing Animal Tissues and Their Derivatives—Requirements for Characterization, Development, Validation and Routine Control of a Sterilization Process for Medical Devices
- ISO 11737-1** Sterilization of Health Care Products—Microbiological Methods—Part 1: Determination of a Population of Microorganisms on Products
- ISO 11737-2** Sterilization of Health Care Products—Microbiological Methods—Part 2: Tests of Sterility Performed in the Definition, Validation and Maintenance of a Sterilization Process

- ISO 11737-3** Sterilization of Health Care Products—Microbiological Methods—Part 3: Bacterial Endotoxin Testing
- ISO 13408-1** Aseptic Processing of Health Care Products—Part 1: General Requirements
- ISO 22442-1** Medical Devices Utilizing Animal Tissues and Their Derivatives—Part 1: Application of Risk Management
- ISO 22442-3** 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

2.5 *Other Referenced Documents*:

- ICH S2** Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use⁶
- ICH Q1A** Stability Testing of New Drug Substances and Products⁶
- ANSI/AAMI ST67:2019** Sterilization of Health Care Products—Requirements and Guidance for Selecting a Sterility Assurance Level (SAL) for Products Labeled “Sterile”⁵

3. Terminology

3.1 *Definitions*:

- 3.1.1 *decomposition*, *n*—structural changes of hyaluronan due to exposure to environmental, chemical, or thermal factors.
 - 3.1.1.1 *Discussion*—Decomposition may occur at temperatures as low as 121 °C during autoclaving. Decomposition can result in deleterious changes to the hyaluronan.
- 3.1.2 *degradation*, *n*—change in the chemical structure, physical properties, or appearance of a material.
 - 3.1.2.1 *Discussion*—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.
- 3.1.3 *depolymerization*, *n*—reduction in length of a polymer chain to form shorter polymeric units.
 - 3.1.3.1 *Discussion*—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.
- 3.1.4 *endotoxin*, *n*—pyrogenic high molar mass lipopolysaccharide (LPS) complex associated with the cell wall of gram-negative bacteria.
 - 3.1.4.1 *Discussion*—Though endotoxins are pyrogens, not all pyrogens are endotoxins. Endotoxins are specifically detected through a Limulus Amebocyte Lysate (LAL) test.

³ Available from U.S. Pharmacopeia (USP), 12601 Twinbrook Pkwy., Rockville, MD 20852, <http://www.usp.org>.

⁴ Available from European Directorate for the Quality of Medicines (EDQM), <http://pheur.edqm.eu/home>.

⁵ Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036, <http://webstore.ansi.org>.

⁶ Available from International Conference on Harmonization (ICH) Secretariat, c/o IFPMA, 30 rue de St-Jean, P.O. Box 758, 1211 Geneva 13, Switzerland, <http://www.ich.org>.

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

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

3.1.7 *molecular mass average (molecular weight average)*, *n*—the given molecular weight (M_w) of hyaluronan will always represent an average of all of the molecules in the population. The most common ways to express the M_w are as the number average (n) and the weight average (w). The two averages are defined by the following equations:

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

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 $w > n$ is always valid. The coefficient w / n is referred to as the polydispersity index, and will typically be in the range 1.2 to 3.0 for commercial hyaluronan.

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

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 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 Monograph 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 from the European Pharmacopoeia.⁷

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).⁸

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

⁷ EDQM, European Pharmacopoeia, Council of Europe, Avenue de l'Europe F-67075 Strasbourg Cedex, France, www.edqm.eu.

⁸ The boldface numbers in parentheses refer to the list of references at the end of this standard.