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Standard Guide for Characterization and Testing of Alginates as Starting Materials Intended for Use in Biomedical and Tissue-Engineered Medical Products Application[1]

This standard is issued under the fixed designation F2064; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

ε[1] NOTE—Mercury warning was editorially added in April 2008.

INTRODUCTION

Alginate has found uses in a variety of products ranging from simple technical applications such as viscosifiers to advanced biomedical matrices providing controlled drug delivery from immobilized living cells. As for most hydrocolloids, the functionality of alginate is related to its chemical and structural composition. The aim of this guide is to identify key parameters relevant for the functionality and characterization of alginates for the development of new commercial applications of alginates for the biomedical and pharmaceutical industries.

1. Scope

1.1 This guide covers the evaluation of alginates 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 alginates.

1.3 As with any material, some characteristics of alginates 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 and are not addressed in this guide.

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 *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 limitations 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

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

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

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 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]

2.2 *USP Document:*
USP Monograph USP 24/NF 19<719> Sodium Alginate[4]

2.3 *ISO Documents:*[5]
ISO 10993 Biological Evaluation of Medical Devices:
ISO 10993-1 Biological Evaluation of Medical Devices—Part 1: Evaluation and Testing
ISO 10993-3 Part 3: Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity
ISO 10993-9—Part 9: Framework for Identification and Quantification of Potential Degradation Products
ISO 10993-17—Part 17: Methods for Establishment of Allowable Limits for Leachable Substances Using Health-Based Risk Assessment
ISO 13408-1: 1998: Aseptic Processing of Health Care Products—Part 1: General Requirements.

2.4 *ICH Documents:*[6]
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 23, 1994)

2.5 *FDA Documents:*[7]
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/ISO 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: Sterilization of Medical Devices—Requirements for Products Labeled "Sterile"
AAMI TIR No. 19—1998: Guidance for ANSI/AAMI/ISO 10993-7: 1995, Biological Evaluation of Medical Devices—Part 7: Ethylene Oxide Sterilization Residuals

2.8 *prEN Documents:*[9]
prEN 12442-1 Animal Tissues and their Derivative Utilized in the Manufacture of Medical Devices—Part 1: Analysis and Management of Risk
prEN –12442 Part 3:Validation of the Elimination and/or Inactivation of Virus and Transmissible Agents

2.9 *Other Documents:*
21CFR184.1724 Listing of Specific Substances Affirmed as GRAS–Sodium Alginate[10]

3. Terminology

3.1 *Definitions of Terms Specific to This Standard:* (see also Terminology F1251):

3.1.1 *alginate, n*—a polysaccharide substance containing calcium, magnesium, sodium, and potassium salts obtained from some of the more common species of marine algae. Alginate exists in brown algae as the most abundant polysaccharide, mainly occurring in the cell walls and intercellular spaces of brown seaweed and kelp. Its main function is to contribute to the strength and flexibility of the seaweed plant. Alginate is classified as a hydrocolloid. The most commonly used alginate is sodium alginate.

3.1.2 *decomposition, n*—structural changes of alginates due to exposure to environmental, chemical or thermal factors, such as temperatures greater than 180°C. Decomposition can result in deleterious changes to the alginate.

3.1.3 *degradation, n*—change in the chemical structure, physical properties, or appearance of a material. Degradation of polysaccharides occurs by means of cleavage of the glycosidic bonds, usually by acid catalyzed hydrolysis. Degradation can also occur thermally. 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.

[3] The last approved version of this historical standard is referenced on www.astm.org.
[4] Available from U.S. Pharmacopeia (USP), 12601 Twinbrook Pkwy., Rockville, MD 20852.
[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] Association for the Advancement of Medical Instrumentation 1110 North 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.
[10] Available from Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402.

3.1.4 *depolymerization, n*—reduction in length of a polymer chain to form shorter polymeric units. Depolymerization may reduce the polymer chain to oligomeric or monomeric units, or both. In alginates, hydrolysis of the glycosidic bonds is the primary mechanism.

3.1.5 *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.

3.1.6 *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 (Mw) of an alginate 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 $(\bar{M}_n)$ and the weight average $(\bar{M}_w)$. The two averages are defined by the following equations:

$$\bar{M}_n = \frac{\sum_i N_i M_i}{\sum_i N_i} \quad \text{and} \quad \bar{M}_w = \frac{\sum_i w_i M_i}{\sum_i w_i} = \frac{\sum_i N_i M_i^2}{\sum_i N_i M_i} \qquad (1)$$

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 from 1.5 to 3.0 for commercial alginates.

3.1.8 *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 alginate. This guide can be used as an aid in the selection and characterization of the appropriate alginate 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 alginate. It may have use in the regulation of these devices by appropriate authorities.

4.2 The alginate covered by this guide may be gelled, 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 alginate, physical and chemical characterization and testing, impurities profile, and performance-related tests.

5. Chemical and Physical Test Methods

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

5.1.1 Sodium alginate monograph USP 24/NF19.

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 an alginate film from a 0.25 % (w/v) solution of sodium alginate by drying approximately 500 µL of the sample onto a disposable IR card 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} , % transmission mode. Label the peaks. Typical frequencies (cm^{-1}) for sodium alginate are 3375-3390 (b), 1613 (s), 1416 (s), 1320 (w), 1125, 1089, 1031 (s), 948 (m), 903 (m), and 811 (m). The peak designators are: sh: sharp; s: strong; m: medium; w: weak; and b: broad.

5.2 *Physical and chemical characterization of alginate:*

5.2.1 The composition and sequential structure of alginate can be a key functional attribute of any alginate. Variations in the composition or the sequential structure, or both, may, but not necessarily, cause differences in performance of an alginate in a particular end use. This information may be determined by the following method: High-resolution ^1H and ^{13}C-nuclear magnetic resonance spectroscopy (NMR). Sodium alginate should be dissolved in D_2O and partially degraded to a degree of depolymerization of 20 to 30 using mild acid hydrolysis before recording proton or carbon NMR spectra (Grasdalen, H., Larsen, B., and Smidsrød, O., Carbohydr. Res., 68, 23-31, 1979). Techniques have been developed to determine the monad frequencies F_G (fraction of guluronate residues) and F_M (fraction of mannuronate residues), the four nearest neighboring (diad) frequencies (F_{GG}, F_{GM}, F_{MG}, and F_{MM}) and the eight next nearest neighboring (triad) frequencies (F_{GGG}, F_{GGM}, F_{GMM}, F_{GMG}, F_{MGM}, F_{MGG}, F_{MMG}, and F_{MMM}). A typical ^1H-NMR spectrum of alginate is shown as follows. Alginate is characterized by calculating parameters such as M/G ratio, G-content, consecutive number of G monomers (that is, G>1), and average length of blocks of consecutive G monomers.

5.2.2 Molecular mass (molecular weight) of an alginate 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 alginates are polydisperse with respect to molecular weight (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

5.2.2.1 *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

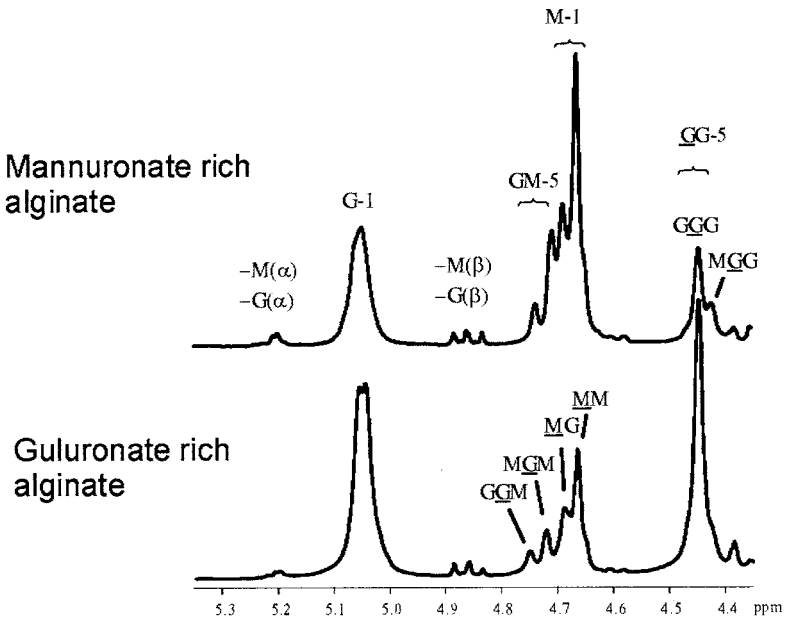

FIG. 1 Typical ^{1} H NMR of Sodium Alginate

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* is a constant, *M* is the viscosity derived average molecular weight, and *a* is an empirical constant describing the conformation of the polymer. For alginate, the exponent (a) is close to unity at an ionic strength of 0.1 (for example, 0.1 *M* NaCl). By measuring the intrinsic viscosity, the viscosity average molecular weight can be determined if K and a are accurately known for the sample: log [η] = log K + a(log *M*), where *M* is the molecular weight. The intrinsic viscosity is determined by measuring the relative viscosity in a Ubbelohde capillary viscometer. The measurements should be performed in a solvent containing 0.1 *M* NaCl (a non-gelling, monovalent salt) at a constant temperature of 20°C, and at a sufficiently low alginate concentration. Automatic operation and data acquisition are preferred.

5.2.2.2 *Molecular Weight and Polydispersity Determination by Size Exclusion Chromatography With Multiple Angle Laser Light Scattering Detection (SEC-MALLS)*—As there are no alginate standards currently available, refractive index detectors can not be adequately calibrated. It is not sufficient to only use pullulan standards as a calibration material. Therefore, the method of choice is to use refractive index coupled to multiple angle laser light scattering detection (MALLS). For separation of the alginate into different molecular weight fractions a hydrophilic column with the appropriate pore size is required. Such columns include, but are not limited to, those mentioned in the techniques as follows: The precision of these techniques must be determined as results can vary by 10 to 20 %. Typical methods using these techniques include, but are not limited to the following:

(*1*) Using 0.01 *M* sodium EDTA/0.05 *M* sodium sulfate, pH 6.0 as the mobile phase with separation using TSK 3000, TSK 4000, and TSK 5000 columns.

(*2*) Using 0.1 *M* $NaNO_3$ (sodium nitrate) as an eluant in combination with a Waters Ultrahydrogel 2000 column in series with an Ultrahydrogel Linear column.

5.2.2.3 *Polydispersity*—Depending on the end use and the sensitivity of the application to the molecular mass, the presence of a wide range of alginate fractions may be an issue. In such cases, calculation of the polydispersity will be important. Typically, this is between 1.5 and 3.0 for commercial alginates.

5.2.2.4 Depending on the final use and the required performance control, other characterization assays can include, but are not limited to the following:

5.2.2.5 *Viscosity in Aqueous Solution*—Viscosity is defined as a liquid's resistance to flow. The molecular mass of an alginate will determine the extent to which it will thicken an aqueous solution. Therefore, a simple viscosity test may yield information on the relative differences in molecular mass among alginate samples. To allow comparison between laboratories, the viscometer used must be calibrated with traceable standards (see Test Methods D2196). The viscosity measured will depend on several parameters related to how the testing is conducted. Important parameters to control include, but are not limited to the following:

(*1*) *Temperature*—The temperature at which the measurement is performed is critical. An increase in temperature will, in almost every case, result in a decrease in the viscosity. Consistent and controlled temperature (that is, with a standard temperature bath) is critical to achieving reproducible results. Typically, the temperature used to measure viscosity can be 20, 25, or 37°C, or a combination thereof.