Designation: ~~F2064 – 14~~ F2064 – 17

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

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.*

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]

~~D2196~~E2975 Test ~~Methods for Rheological Properties of Non-Newtonian Materials by Rotational Viscometer~~Method for Calibration or Calibration Verification of Concentric Cylinder Rotational Viscometers

F619 Practice for Extraction of Medical Plastics

[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 ~~Oct. 1, 2014~~March 1, 2017. Published ~~February 2015~~April 2017. Originally approved in 2000. Last previous edition approved in ~~2000~~2014 as ~~F2064 – 00 (2006)~~F2064 – 14.~~ε1~~. DOI: ~~10.1520/F2064-14.~~10.1520/F2064-17.

[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.

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]
F2259 Test Method for Determining the Chemical Composition and Sequence in Alginate by Proton Nuclear Magnetic Resonance (^{1}H NMR) Spectroscopy
F2315 Guide for Immobilization or Encapsulation of Living Cells or Tissue in Alginate Gels
F2605 Test Method for Determining the Molar Mass of Sodium Alginate by Size Exclusion Chromatography with Multi-angle Light Scattering Detection (SEC-MALS)

2.2 *USP Document:*[4]
USP Monograph USP 35/NF 30 Sodium Alginate

2.3 *ISO Documents:*[5]
ISO 31-8 Quantities and units — Part 8: Physical chemistry and molecular physics
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) S2 Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use
International Conference on Harmonization (ICH) Q1A ICH Harmonized Tripartite Guidance for Stability Testing of New Drug Substances and Products (2003)

2.5 *FDA Documents:*[7]
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: 2006 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: 2011 Sterilization of Health Care Products—Requirements and Guidance for Selecting a Sterility Assurance Level (SAL) 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

[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.

2.8 *National Institute of Standards and Technology:*[9]
NIST SP811 Special Publication: Guide for the Use of the International System of Units
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.

3.1.4 *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 *G*—abbreviation for α-L-guluronic acid, one of the two monomers making up the alginate polysaccharide molecule. G-rich alginate has a greater than 50 % content of guluronate residues in the polymer chain. G-block refers to a homopolymeric block of G residues.

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

3.1.8 *M*—abbreviation for ß-D-mannuronic acid, one of the two monomers making up the alginate polysaccharide chain. M-rich alginate has a greater than 50% content of mannuronate residues in the polymer chain.

3.1.9 *molar mass average, n*—the given mass-average molar mass (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:

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

where:

N_i = number of molecules having a specific molar mass, M_i, and
w_i = mass of molecules having a specific molar mass, M_i.

In a polydisperse molecular population the relation $\bar{M}_w > \bar{M}_n$ is always valid. The coefficient $\bar{M}_w/\bar{M}_n$ is referred to as the polydispersity index, and will typically be in the range from 1.5 to 3.0 for commercial alginates.

[9] Available from National Institute of Standards and Technology (NIST), 100 Bureau Dr., Stop 1070, Gaithersburg, MD 20899-1070, http://physics.nist.gov/cuu/Units/bibliography.html.

[10] Available from Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402.

3.1.9.1 *Discussion*—

The term molecular weight (abbreviated MS) is obsolete and should be replaced by the SI (Système Internationale) equivalent of either relative molecular mass (Mr), 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 grams/mole. For polymers and other macromolecules, use of the symbols Mw, Mn, and Mz 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 SP811.

3.1.10 *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. Further guidance for immobilizing or encapsulating living cells or tissue in alginate gels can be found in Guide F2315.

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 35/NF30.

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. Identity of sodium alginate can be assessed by Fourier transform infrared spectroscopy (FT-IR).

5.1.2.1 *Alginate as a powder*—In attenuated total reflectance (ATR), an infrared beam enters a diamond crystal. Internal reflection within the crystal creates an evanescent wave. The wave continues beyond the crystal surface and into the sample that is held in close contact to the crystal surface. The penetration depth of the beam is of the order of a few microns. The beam is reflected several times within the crystal and carries spectral information from the sample into the detector. The sample is analyzed as a powder. Apply a powder sample of alginate to the FT-IR ATR crystal and follow the instrument manufacturer's procedure for recording spectra. Record the IR spectrum of the crystal without sample (CO_2 and H_2O correction), then record the IR spectrum of the sample using 4 scans at a speed of 0.2 cm^{-1}/s and a resolution of 4 cm^{-1} from 4000 cm^{-1} to 650 cm^{-1}. A typical FT-IR ATR spectrum of sodium alginate is shown in Fig. 1.

5.1.2.2 *Alginate film*—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.

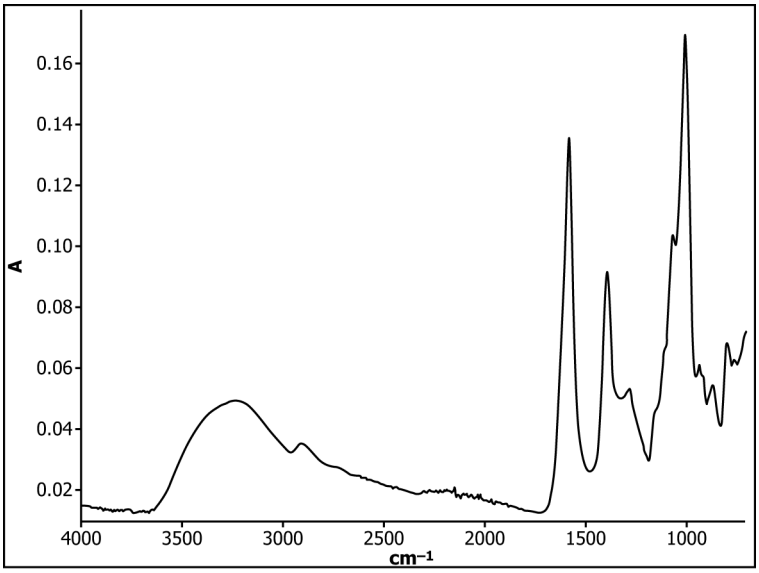

FIG. 1 Typical FT-IR ATR Spectrum of Sodium Alginate

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 ^{1}H 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 ^{1}H-NMR spectrum of alginate is shown in Fig. 2. 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. Test Method F2259 gives guidance on determining the chemical composition and sequence of alginate by proton NMR.

5.2.2 Molar mass (molecular weight; typically expressed as grams/mole) 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 molar mass directly or indirectly may be necessary. Commercial alginates are polydisperse with respect to molar mass (M_w). Molar mass may be expressed as the number average (M_N) or the weight average (M_W). Molar mass may be determined by methods such as, but not limited, to the following:

5.2.2.1 *Molar Mass 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 molar mass 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 molar mass of a polymer through the Mark-Houwink-Sakurada (MHS) equation: $[\eta] = KM^a$, where K is a constant, M is the viscosity derived average molar mass, 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 molar mass can be determined if K and a are accurately known for the sample: $\log [\eta] = \log K + a(\log M)$, where M is the molar mass. 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 *Molar Mass and Polydispersity Determination by Size Exclusion Chromatography With Multiple Angle Light Scattering Detection (SEC-MALS)*—As there are no alginate standards currently available, refractive index detectors can not be adequately calibrated. It is not sufficient to only use pullulan or other polysaccharide standards as a calibration material. Therefore, the method of choice is to use refractive index coupled to multiple angle light scattering detection (MALS). For separation of the alginate into different molar mass 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:

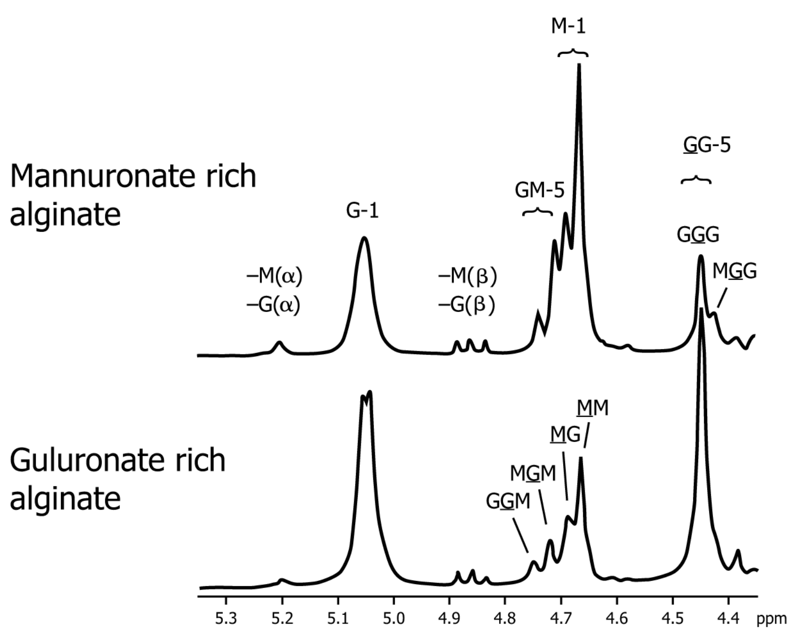

FIG. 2 Typical ^{1} H NMR of Sodium Alginate

(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 5000, and TSK 6000 columns. Test Method F2605 gives guidance in determining the molar mass of sodium alginate by SEC-MALS.

(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 molar 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 molar mass among alginate samples. To allow comparison between laboratories, the viscometer used must be calibrated with traceable standards (see Test ~~Methods~~Method ~~D2196~~E2975). 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.

(2) Alginate Concentration—The moisture content of the alginate must be known in order to prepare correct concentrations of alginate.

(3) Ionic strength—The viscosity of an alginate solution is very sensitive to the ionic environment in which the measurement is made. Although any ion can have an impact, multivalent ions other than magnesium will have the most effect. The most important aspect is to keep the ionic content consistent. Typically viscosity measurements are made in deionized water or a standardized ionic environment such as isotonic saline.

(4) Molecular Mass—Viscosity measurements are sensitive to the molecular mass of the alginate. The following is one suggestion concerning the measurement of alginate viscosity, but any appropriate method would apply. To measure the apparent viscosity of sodium alginate, prepare a solution in deionized water with a concentration ~~(w/w,~~ (mass fraction, corrected for dry matter content) appropriate for the end use. For example, if the sample has a suspected molar mass above about ~~50 000 g/mol~~50 kg/mol prepare a 1 % ~~(w/w)~~ (mass fraction) solution; if the suspected molar mass is less than about ~~50 000 g/mol,~~50 kg/mol, then prepare a 10 % ~~(w/w)~~ (mass fraction) solution. The viscosity is measured using a rotational viscometer ~~(for example, Brookfield type)~~ at 20 °C ± 0.2 °C (or other controlled temperature) using the appropriate spindle, spindle rotation speed, and a temperature-controlled water bath.

5.2.2.6 *Dry Matter Content*—Various alginates are supplied with different moisture contents. The dry matter content determination is based upon the removal of water from the sample. Normally with alginate, gravimetric techniques are used. They are adapted directly from <731> USP 35/NF30, Loss on Drying, and utilize a calibrated drying oven at 105 °C.

5.2.2.7 *Ash Content*—The ash content of a sample describes the total amount of inorganic material present. After combustion, the sample contains a mixture of salts. The composition of the ash depends on the temperature used during the combustion of the organic material. For ash content of sodium alginate, a combustion temperature of 800 °C for at least 6 h is recommended.

5.3 *Impurities Profile*—The term impurity relates to the presence of extraneous substances and materials in the alginate powder. Impurities can also arise from the presence of other alginate salts (for example, calcium alginate) or alginic acid in the sodium alginate material. Additionally, and dependent upon the end use, a high molar mass alginate present in a sample of low molar mass could constitute an impurity. Various processing aids, such as, but not limited to, filtering and clarifying agents such as Filter Aid™ may also be used in the manufacture of alginate and could constitute an impurity. If there is a concern for the presence of processing aids or other contaminants associated with alginate, they should be addressed with the supplier. The major impurities of concern include, but are not limited to the following:

5.3.1 *Endotoxin Content*—Endotoxin contamination is difficult to prevent because it is ubiquitous in nature, stable, and small enough to pass through sterilizing filters. There are several tests to determine the presence of endotoxin in the alginate powder. These are the gel clot, endpoint assay and the kinetic assay. The gel clot test is the simplest and easiest of the limulus amebocyte lysate (LAL) test methods, although much less sensitive than the kinetic assay. A firm gel that maintains its integrity upon inverting the tube is scored as a positive test. Anything other than a firm gel is scored as a negative test. The endpoint assay is based on the linear relationship between the endotoxin concentration and the formation of color (chromogenic assay) over a relatively short range of standard dilutions. A standard curve is then constructed by plotting the optical densities of a series of endotoxin standards as a function of the endotoxin concentration.

5.3.1.1 Using linear regression analysis, the standard curve covers an endotoxin range of approximately 1 log (usually 1.0 to 0.1 EU/mL). The most sensitive means of determining the endotoxin content is with a quantitative, kinetic assay. This test utilizes a LAL and a synthetic color producing substrate to detect endotoxin chromogenically (such as, but not limited to, Lonza