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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]

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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 *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:*

D 2196 Test Methods for Rheological Properties of Non-Newtonian Materials by Rotational (Brookfield) Viscometer[2]

F 619 Practice for Extraction of Medical Plastics[3]

F 748 Practice for Selecting Generic Biological Test Methods for Materials and Devices[3]

F 749 Practice for Evaluating Material Extracts by Intracutaneous Injection in the Rabbit[3]

F 756 Practice for Assessment of Hemolytic Properties of Materials[3]

F 763 Practice for Short-Term Screening of Implant Materials[3]

F 813 Practice for Direct Contact Cell Culture Evaluation of Materials for Medical Devices[3]

F 895 Test Method for Agar Diffusion Cell Culture Screening for Cytotoxicity[3]

F 981 Practice for Assessment of Compatibility of Biomaterials for Surgical Implants with Respect to Effect of Materials on Muscle and Bone[3]

F 1251 Terminology Relating to Polymeric Biomaterials in Medical and Surgical Devices[3]

F 1439 Guide for Performance of Lifetime Bioassay for the Tumorigenic Potential of Implant Materials[3]

F 1903 Practice for Testing for Biological Responses to Particles In Vitro[3]

F 1904 Practice for Testing the Biological Responses to Particles In Vivo[3]

F 1905 Practice for Selecting Tests for Determining the Propensity of Materials to Cause Immunotoxicity[3]

F 1906 Practice for Evaluation of Immune Responses in Biocompatibility Testing Using ELISA Tests, Lymphocyte Proliferation, and Cell Migration[3]

2.2 *USP Document:*

USP Monograph USP 24/NF 19<719> Sodium Alginate[4]

[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 Tissue Characterization.

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[2] *Annual Book of ASTM Standards*, Vol 06.01.

[3] *Annual Book of ASTM Standards*, Vol 13.01.

[4] Available from United States Pharmacopeia and National Formulary, U.S. Pharmaceutical Convention, Inc. (USPC), Rockville, MD.

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/DIS 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]

Williams, DF. The Williams Dictionary of Biomaterials. Liverpool University Press, 1999.

3. Terminology

3.1 *Definitions of Terms Specific to This Standard:* (see also Terminology F 1251):

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 *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:

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

[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

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 $\bar{M}_w > \bar{M}_n$ 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.

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 ^{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 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 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: $\log [\eta] = \log K + a(\log M)$, 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

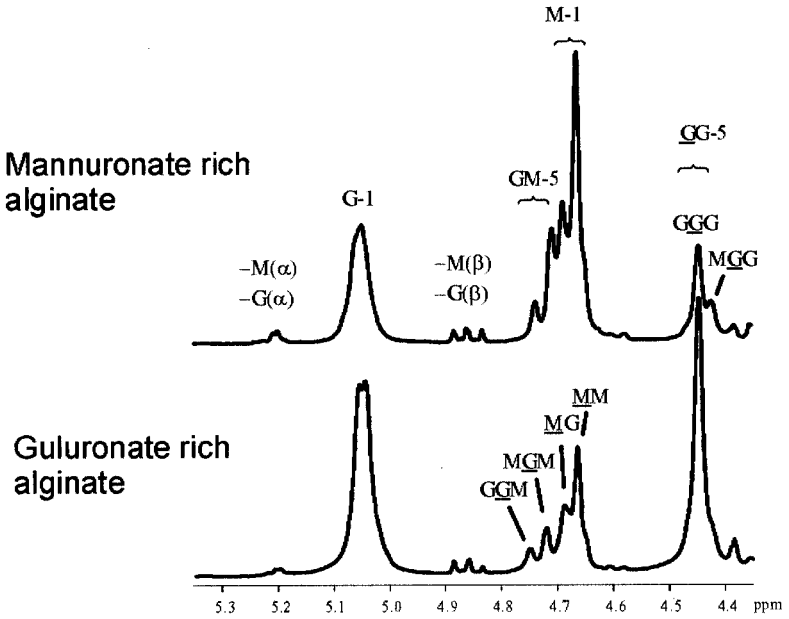

FIG. 1 Typical[1] H NMR of Sodium Alginate

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 D 2196). 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, corrected for dry matter content) appropriate for the end use. For example, if the sample has a suspected molecular weight above about 50 000 g/mol prepare a 1 % (w/w) solution; if the suspected molecular weight is less than about 50 000 g/mol, then prepare a 10 % (w/w) solution. The viscosity is measured using a rotational viscometer (for example, Brookfield type) at 20 ± 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 24/NF19, 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 molecular weight alginate present in a sample of low molecular weight 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, BioWhittakers Kinetic-QCL™ methodology, or other equivalent assay). The kinetic assay measures the amount of time required to reach a predetermined optical density (kinetic turbidimetric) or color intensity (kinetic chromogenic), sometimes called the onset optical density or reaction optical density. The FDA currently defines linearity as a correlation coefficient of $\geq$0.980 (Food and Drug Administration. 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). It is important that operators of the LAL method are qualified and that each new lot of reagents is validated. Positive product controls (PPC) must be added to test inhibition in the sample. Recovery of the known added amount of endotoxin standard must be obtained for a valid assay. It is recommended that endotoxin measurements be performed using an initial 0.1 % concentration of sodium alginate and 3 dilution ranges (for example, 20×, 50×, and 100×). Calcium binding by alginate may produce interference in the assay. Magnesium may be added to reverse this inhibition. The endotoxin level in alginate will ultimately be critical to its use in biomedical applications where they are regulatory limits to the amount of endotoxin that can be implanted into humans. Relevant FDA guidance for allowable levels and information regarding validation of endotoxin assays should be consulted if human trials are contemplated (Interim guidance for human and veterinary drug products and biologicals. Kinetic LAL techniques. DHHS. July 15, 1991).

5.3.2 *Protein Content*—Protein content in sodium alginate should be assayed using an appropriate method having sufficient sensitivity to detect low levels of contamination. One method, although not the only suitable one, is the fluorescence-based NanoOrange™ Protein Quantification method developed and supplied by Molecular Probes. This method is able to quantitate protein content as low as 10 ng/mL. The protein content should be assayed using a 1 % (w/w) alginate solution corrected for moisture. It is important to confirm that the method chosen is insensitive to materials present in the sample and to validate it against a reference method on a one-time basis. It is the responsibility of the end user to evaluate the alginate product for the presence of specific proteins that could cause undesirable immunological or tissue reactions.

5.3.3 *Heavy Metal Content by the USP Method*—This test is provided to demonstrate that the content of heavy metal impurities does not exceed a limit in the individual product specification in terms of ppm lead in the test substance. Under the specified test conditions, the limit is determined by a concomitant visual comparison of metals that are colored by sulfide ion with a control prepared from a standard lead solution. Substances that typically respond to this test are lead, mercury, bismuth, arsenic, antimony, tin, cadmium, silver, copper, and molybdenum. This method is based on <231> Heavy Metals, USP 24/NF 19.

5.3.4 *Microbiological Safety*—The presence of bacteria, yeast, and mold are also impurities that can arise in an alginate sample. The presence of bacteria may also contribute to the presence of endotoxins. The following Microbiological Tests in USP 24 are of particular relevance: Microbial Limit Tests <61>, Sterility Tests <71>, Sterilization and Sterility Assurance of Compendial Articles <12211>, and the Biological Tests and Assays: Bacterial Endotoxins Tests <85>. The user should also consider other relevant standards, such as, but not limited to, Association for the Advancement of Medical Instrumentation (AAMI) standards and international standards, of which the following are examples: 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; ISO 13408-1: 1998: Aseptic processing of health care products—Part 1: general

requirements. Membrane filtration can be used for the determination of bacteria, yeast, and mold in alginate samples. The alginate salt is first dissolved in sterile, deionized water, then filtered using sterile techniques through a 0.45-µm membrane filter. The filters are subsequently incubated on Tryptic Soya Agar to determine the presence of bacteria, and on sabouraud dextrose agar to determine the presence of yeast and mold. If alginate products are intended to serve as a barrier to microorganisms, this function will need to be validated with specific experiments.

6. Product Development Considerations

6.1 *Type of Solvent (for example, Medium or Water)*—The conformation of the alginate molecule will vary with changes in the ionic strength of the solute. Therefore, the apparent viscosity of an alginate solution may change depending upon whether the alginate is dissolved in water or in a salt-containing medium.

6.2 *Stability of Alginate*—For alginate, the most relevant stability-indicating parameters are those related to the functionality of the polymer. Dependent upon what function the alginate will have in the final formulation, parameters such as viscosity (apparent and intrinsic), and molecular weight should be evaluated during a stability study. Storage conditions are of importance, especially for alginate solutions. International Conference on Harmonization (ICH) guidance documents should be consulted for information on stability testing of pharmaceuticals (that is, ICH Q1A ICH Harmonized Tripartite Guideline for Stability testing of New Drug Substances and Products, September 23, 1994).

6.3 *Methods of Sterilization*—Sterilization is intended for the final application or formulation. If sterilization of the alginate is required, then there are several alternative methods available. However, the listing of alternative sterilization methods does not imply that commercial suppliers of alginate need provide a sterile product. Alginate powder can be sterilized by gamma irradiation (with subsequent degradation of the alginate chain resulting in a reduction in molecular weight) or by ethylene oxide. Solutions of alginate may be (*1*) filter sterilized if the viscosity of the alginate solution permits, (*2*) gamma-irradiated with a resulting loss in viscosity (molecular weight), and (*3*) autoclaved (which also reduced the viscosity of the solution). Selection of the method of sterilization will depend upon the viscosity or molecular weight needs of the final application. Use of ethylene oxide will also require testing for residuals. The reader should refer to the relevant standards regarding the sterilization of healthcare products by radiation, steam, and ethylene oxide gas, such as AAMI TIR No. 19—1998: Guidance for ANSI/AAMI/ISO 10993-7: 1995, Biological evaluation of medical devices—Part 7: Ethylene oxide sterilization residues; 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.”

7. Safety and Toxicology Aspects of Alginate

7.1 Sodium alginate is listed on the list of materials affirmed generally recognized as safe (GRAS) by the US Food and Drug Administration (FDA) (21CFR184.1724). This permits sodium alginate (but not other salts such as magnesium) to be used in foods as a thickener or gelling agent, but does not indicate approval for the use of alginate in pharmaceutical or biomedical applications, or both.

7.2 The safety of alginate in biomedical and pharmaceutical applications and in tissue-engineered medical products (TEMPS) should be established in accordance with current guidelines such as ISO 10993 and Practice F 748. Suppliers of alginate may have such documentation on file. Preclinical safety studies specific to the clinical application under consideration must also be in accordance with 21CFR312.

7.3 *Biocompatibility*:

7.3.1 Biomaterials are materials of natural or manmade origin that are used to direct, supplement, or replace the functions of living tissues. These materials may be considered biocompatible if the materials perform with an appropriate host response in a specific application (Williams 1999).

7.3.2 Many materials have been shown to produce a well-characterized level of biological response following long-term clinical use in laboratory animals. When new applications of a material, or modifications to the material or physical forms of the material are being considered, then the recommendations and test methods of the following standards should be considered: Practices F 748, F 619, F 749, F 756, F 763, F 813, F 981, F1903, F1904, F1905, and F1906; Guide F 1439 as well as Test Method F 895 and ISO 10993-1, ISO/DIS 10993-9, Part 9, ISO/DIS 10993-17—Part 17, prEN 12442-1—Part 1, prEN 12442-3—Part 3. Additional guidance can be obtained in ICH S2B Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals, July 1997, as well as ISO 10993–3—Part 3: Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity.

7.4 Alginate for use in biomedical and pharmaceutical applications and in tissue-engineered medical products (TEMPS) should ideally be documented in a device or drug master file to which end users may obtain a letter of cross reference from suppliers of alginate. Such a master file should be submitted to the US FDA and to other regulatory authorities, both national and international.

8. Keywords

8.1 alginates; biomedically engineered; tissue-engineered medical products

APPENDIXES

(Nonmandatory Information)

X1. RATIONALE

X1.1 The use of naturally occurring biopolymers for biomedical and pharmaceutical applications and in TEMPS is increasing. This guide is designed to give guidance in the characterization and testing parameters for sodium alginate used in such applications. Knowledge of the physical and chemical properties of the alginate, such as guluronate to mannuronate ratio, G-block size, molecular weight (or viscosity), and so forth, will assist end users in choosing the correct alginate for their particular application. Knowledge of these parameters will also ensure that users can request and obtain similar material from suppliers on reordering. Molecular characterization of alginate will also assist end users in documentation of their formulation or device. Finally, characterization of the alginate will allow the functionality of the alginate to fit the application or end product. Tests outlined in this guide are sufficient for release of alginate to the end user. Other validated tests that would accomplish the same purposes as those set forth in this guide may be substituted. The tests may not be suitable for characterization and functionality of the final product.

X2. BACKGROUND

X2.1 "Alginate" refers to a family of non-branched binary copolymers of 1-4 glycosidically linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues. The relative amount of the two uronic acid monomers and their sequential arrangement along the polymer chain vary widely, depending on the origin of the alginate. The uronic acid residues are distributed along the polymer chain in a pattern of blocks, where homopolymeric blocks of G residues (G-blocks), homopolymeric blocks of M residues (M-blocks), and blocks with alternating sequence of M and G units (MG-blocks) co-exist. Thus, the alginate molecule can not be described by the monomer composition alone. The NMR characterization of the sequence of M and G residues in the alginate chain is needed in order to calculate average block lengths. It has also been shown by NMR spectroscopy that alginate has no regular repeating unit. The length of the polymer chain is rather long in native form, but will decrease during the manufacturing process. Depolymerization is a natural process for alginate. The molecular weight of commercial alginates will seldom be higher that 500 000 g/mol, similar to a degree of polymerization (DP) of approximately 2500.

X2.2 *Raw Materials for Alginate Production*:

X2.2.1 All current industrial manufacture of alginate is based on the extraction of the polymer from brown algae. Alginate may also be synthesized as an exocellular material by some bacteria. It has been found feasible to manufacture certain specialty grades of alginate by fermentation.

X2.2.2 The seaweed grows naturally mainly in the temperate zone, but large amounts are also cultivated in the Far East, off the coast of China, and near Japan, in particular.

X2.2.3 The stiffness of the plant reflects the content of guluronic acid, and in particular, the content of G-blocks. An increase in the G-block length results in an increase in gel strength due to increased cross-linking of alginate molecules by calcium.

X2.3 *Variability in Chemical Composition and Sequential Structure of Alginate*—The variability in chemical composition and sequential structure of alginate is related to the seaweed and kelp species from which the alginate is extracted. Table X2.1 represents some of the differences in alginate composition from various seaweed sources. Table X2.1 indicates that

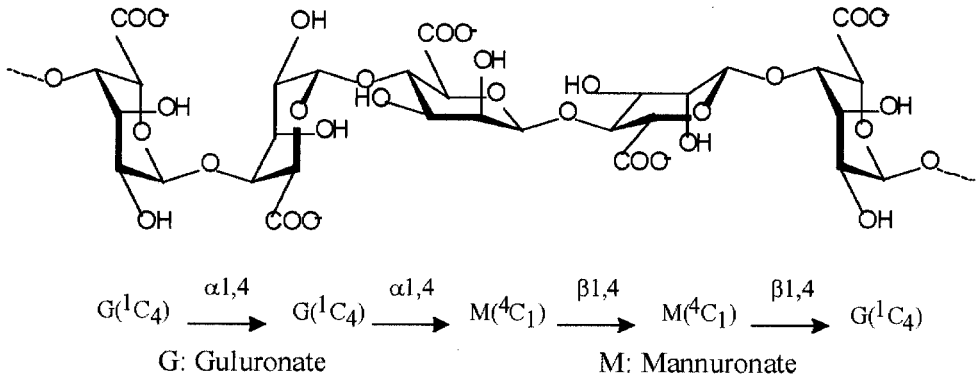

FIG. X2.1 Alginate Chain

TABLE X2.1 Differences in Alginate Composition from Various Seaweed Sources

Seaweed	M/G	%M	%G	%MM	%GG
Laminaria hyperborea (stem)	0.45	30	70	18	58
Laminaria hyperborea (leaf)	1.22	55	45	36	26
Laminaria digitata	1.22	55	45	39	29
Macrocystis pyrifera	1.50	60	40	40	20
Lessonia nigrescens	1.50	60	40	43	23
Ascophyllum nodosum	1.86	65	35	56	26
Laminaria japonica	1.86	65	35	48	18
Durvillea antarcitica	2.45	71	29	58	16
Durvillea potarum	3.33	77	23	69	13

there is a range of compositions that must be defined and described for alginate utilized in biomedical and pharmaceutical applications. The range in chemical composition and sequential structure can be broad or narrow depending upon the end use.

X2.4 *Functional Properties and Applications of Alginate*:

X2.4.1 The functional properties of alginate of primary importance for most biomedical applications are the viscoelastic ones. Solubility, swellability, and film-forming properties are other characteristics exploited in biomedical and pharmaceutical applications.

X2.4.2 Gelling properties are a function of the M/G composition and the sequential structure of M and G along the alginate chain. Consecutive guluronic monomers form a G-block, which represents areas within the alginate molecule able to cross-link with multivalent cations. In practice, calcium is most often used as the cross-linking cation.

X2.4.3 Thickening (viscosifying) properties of alginate are a function of the molecular weight and the conformation of the alginate molecule in solution. Interaction with other molecules in the solution as well as competition for water at high alginate concentrations affect the flow properties of alginate solutions. Calcium or other cross-linking materials present in small quantities artificially increase the measured viscosity because of aggregate formation. This results in solutions with thixotropic flow properties. A sequestrant that binds the cross-linking agent can be added to avoid measuring an artificially high viscosity.

X2.4.4 Both gelling and thickening properties of alginate are dependent upon the order in which the different materials are added.

X2.4.5 Solubility of alginate is related to the rate of dissociation of the alginate molecule. At pH <3, both M- and G-structures will precipitate as alginic acid, while alternating structures will still remain in solution even when fully protonated.

X2.4.6 Sellability of alginate is related to the rate of hydration, and it will depend strongly on the form in which alginate interacts with the solute (water). Cross-linked alginates will, for instance, swell slower than pure sodium alginate.

X2.4.7 Films can be formed from alginate solutions simply by evaporation of the solvent. The molecular weight of alginate needs to be above a certain lower limit in order to achieve film formation and avoid brittleness. Films can be formed easily in situ by spraying an alginate solution onto a binding surface.

X2.4.8 *Degradation*—As described in 3.1.3, degradation of alginate occurs by means of cleavage of the glycosidic bond. Compared with other sugars, glycosidic bonds involving uronic acids such as M and G are quite resistant to hydrolysis in very strong acids, (that is, conditions normally used to convert polysaccharides into monosaccharides). The degradation rate is directly proportional to the concentration of protons below about pH 1. However, at pH values near the pK of alginates (pH 1-4), the degradation rate is less dependent on pH. In this range, the protonated (-COOH) form of M and G contributes to the hydrolysis by intramolecular catalysis in addition to that caused by the free H ions. For this reason, alginate is less stable than some other polymers (for example, methylcellulose) between pH 1-5. Optimum stability is normally obtained at pH 7-8. For higher pH values, other degradation processes come into play. In most cases, degradation results in a decrease in the solution viscosity (η). The polymer concentration and the viscosifying power of the molecules involved determine the viscosity.