Standard Guide for
Immobilization or Encapsulation of Living Cells or Tissue in Alginate Gels[1]

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INTRODUCTION

Encapsulation in insoluble alginate gel is recognized as a rapid, non-toxic, and versatile method for immobilization of macromolecules and cells. Microencapsulated cells or tissue as artificial organs are under study for treatment of a variety of diseases such as Parkinson's disease, chronic pain, liver failure, hypocalcemia, and, perhaps the most well-known example, immobilization of islets of Langerhans utilized as an artificial pancreas in the treatment of diabetes. Since alginates are a heterogeneous group of polymers with a wide range of functional properties, the success of an immobilization or encapsulation procedure will rely on an appropriate choice of materials and methodology. This must be based on knowledge of the chemical composition of alginate and the correlation between the structure, composition, and functional properties of the polymer, as well as differences in gelation technologies. It is also important to recognize the need for working with highly purified and well-characterized alginates in order to obtain gels with reproducible properties. The aim of this guide is to provide information relevant to the immobilization or encapsulation of living cells and tissue in alginate gels.

1. Scope

1.1 This guide discusses information relevant to the immobilization or encapsulation of living cells or tissue in alginate gels. Immobilized or encapsulated cells are suitable for use in biomedical and pharmaceutical applications, or both, including, but not limited to, Tissue Engineered Medical Products (TEMPs).

1.2 This guide addresses key parameters relevant for successful immobilization and encapsulation in alginate gels.

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

1.4 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory requirements prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

F748 Practice for Selecting Generic Biological Test Methods for Materials and Devices

F1251 Terminology Relating to Polymeric Biomaterials in Medical and Surgical Devices (Withdrawn 2012)[3]

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]

F2064 Guide for Characterization and Testing of Alginates as Starting Materials Intended for Use in Biomedical and Tissue-Engineered Medical Products Application

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[1] This guide is under the jurisdiction of ASTM Committee F04 on Medical and Surgical Materials and Devicesand is the direct responsibility of Subcommittee F04.43 on Cells and Tissue Engineered Constructs 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.

[3] The last approved version of this historical standard is referenced on www.astm.org.

2.2 *USP Document:*

USP Monograph USP 24/NF19 Sodium Alginate[4]

2.3 *Other Referenced Documents:*

EN-ISO 10993 Biological Evaluation of Medical Devices[5]

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

3. Terminology

3.1 *Definitions:*

3.1.1 *alginate, n*—polysaccharide obtained from some of the more common species of marine algae, consisting of an insoluble mix of calcium, magnesium, sodium, and potassium salts.

3.1.1.1 *Discussion*—Alginate exists in brown algae as its most abundant polysaccharide, mainly occurring in the cell walls and intercellular spaces of brown seaweed and kelp. Alginate's 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. Sodium alginate and, in particular, calcium crosslinked alginate gels are used in Tissue Engineered Medical Products (TEMPs) as biomedical matrices, controlled drug delivery systems, and for immobilizing living cells.

3.1.2 *APA bead, n*—alginate-poly-L-lysine-alginate bead.

3.1.3 *encapsulation, n*—a procedure by which biological materials, such as cells, tissues, or proteins, are enclosed within a microscopic or macroscopic semipermeable barrier.

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.

3.1.5 *gel, n*—the three-dimensional network structure arising from intermolecular polymer chain interactions. Such chain interactions may be covalent, ionic, hydrogen bond, or hydrophobic in nature. See also Terminology F1251.

3.1.6 *immobilization, n*—the entrapment of materials, such as cells, tissues, or proteins within, or bound to, a matrix.

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

3.2 Additional definitions regarding alginate may be found in Guide F2064. Additional definitions regarding polymeric biomaterials may be found in Terminology F1251.

4. Significance and Use

4.1 The main use is to immobilize, support, or suspend living cells or tissue in a matrix. The use of an encapsulation/immobilization system may protect cells or tissues from immune rejection. When immobilizing biological material in alginate gels, there are numerous parameters that must be controlled. This guide contains a list of these parameters and describes the methods and types of testing necessary to properly characterize, assess, and ensure consistency in the performance of an encapsulation system using alginate. This guide only covers single gelled beads, coated or not, and not double capsules or other constructs.

4.2 The alginate gelation technology covered by this guide may allow the formulation of cells and tissues into biomedical devices for use as tissue engineered medical products or drug delivery devices. These products may be appropriate for implantation 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.

5. Gelation Techniques

5.1 Most methods for encapsulation of cells or tissue in alginate gels basically involve two main steps. The first step is the formation of an internal phase where the alginate solution containing biological materials is dispersed into small droplets. In the second step, droplets are solidified by gelling or forming a membrane at the droplet surface.

5.2 The most simple and common way to produce small beads or capsules is by forming droplets of a solution of sodium alginate containing the desired biological material (cells, tissues, or other macromolecules) and then exposing them to a gelling bath. A gelling bath may be a solution containing divalent cross-linking cations such as Ca^{2+}, Sr^{2+}, or Ba^{2+}. Monovalent cations and Mg^{2+} ions do not induce gelation (**1**).[7]

5.3 *Concentration of Ions:*

5.3.1 The concentration of gelling ions used must be determined based upon factors such as desired gel strength, type of alginate used (G- or M-rich), and isotonicity of the gelling solutions. Calcium ion concentrations of from 50 to 150 mm are often used.

5.3.2 Other gelling ions may be used, such as Ba^{2+} or Sr^{2+}. The concentration of Ba^{2+} in the gelling solution must be determined based upon the desired characteristics of the final gel and on regulatory and toxicological considerations as Ba^{2+} can induce toxic effects in cells.

5.3.3 *Concentration of Non-gelling Ions*—Various additives present in the gelling solution that do not participate in the formation of cross-links constitute non-gelling ions. These ions may be Na^+, which can be used to produce homogeneous gels (see 7.1), ions present in cell culture medium (if present in the gelling bath), and others.

6. Formation of Beads

6.1 Bead size is one of the most important parameters of alginate gel beads and capsules in biomedical applications. The appropriate size will often be a compromise. The bead itself must be large enough to contain the biological material. Larger

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[4] Available from U.S. Pharmacopeia (USP), 12601 Twinbrook Pkwy., Rockville, MD 20852-1790, http://www.usp.org.

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

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

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

beads are also easier to handle during washing or other treatments. In many applications involving cells, the cells should be homogeneously distributed within the internal capsular matrix. When generating beads, the desired mean size and acceptable size distribution should be accounted for. The size of the beads is primarily controlled by regulating droplet formation.

6.2 *Droplet Size*—Droplet size is dependent upon several factors: The size of the material to be immobilized or encapsulated (that is, single cells or cell aggregates such as pancreatic islets), the technique used to generate droplets (that is, pipette or syringe, coaxial air flow, electrostatic generator, jet-cutter, and so forth) and the viscosity of the alginate solution. Generally, for biomedical applications, droplet size is regulated to give a gelled bead having a diameter of <200 to 1000 µm. Per unit volume, smaller beads yield a larger surface area to transplant volume, a ratio that results in enhanced survival of tissue due to better nutritional and oxygen supply. Various techniques can be used to form droplets as described in more detail by Dulieu et al. (**2**). These include, but are not limited to:

6.2.1 *Extrusion through a Needle*—Beads can be made by dripping an alginate solution from a syringe with appropriate diameter needle directly into a gelling bath. While this method does not require any instrumentation, the size and size distribution of the produced beads are difficult to control.

6.2.2 *Coaxial Air or Liquid Flow*—The coaxial air jet system is a simple way of generating small beads (down to around 400 µm), although the size distribution will normally be larger as compared to an electrostatic system. In this system, a coaxial air stream is used to pull droplets from a needle tip into a gelling bath (Fig. 1).

6.2.3 *Electrostatic Potential*—An electrostatic potential can be used to pull droplets from a needle tip into a gelling bath. The primary effect on droplet formation by the electrostatic potential is to direct charged molecules to the surface of the droplet to counteract surface tension (**2**). Using this type of instrument, beads below 200 µm and with a small size distribution may be generated. The desired bead size is obtained simply by adjusting the voltage (electrostatic potential) of the instrument. The principle for making smaller beads by electrostatic potential bead generators is shown in Fig. 1.

6.2.4 *Vibrating Capillary Jet Breakage*—A vibrating nozzle generates drops from a pressurized vessel.

6.2.5 *Rotating Capillary Jet Breakage*—Bead generation is achieved by cutting a solid jet of fluid coming out of a nozzle by means of a rotating cutting device. The fluid is cut into cylindrical segments that then form beads due to surface tension while falling into a gelling bath.

6.2.6 *Emulsification Methods.*

6.3 *Type of Solvent (that is, Cell Growth 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. When using droplet generators, size and sphericity of the beads will, therefore, depend on the viscosity of the alginate solution and the distance the droplets fall before reaching the gelling solution. In addition, the final size of the beads will be dependent of the gelling conditions used.

6.4 *Concentration of Biological Material (Cells or Others)*—In applications involving immobilization of cells diffusion properties of different molecules within the beads will also depend strongly on the load of cells. As a consequence of diffusion limitations cells surrounded by other cells within the gel network may, therefore, be strongly influenced by the metabolism of the surrounding cells. As a result surrounded cells may be trapped in a micro-environment lacking essential nutrients like oxygen. This may typically result in cell death in the center of the beads with an outer rim of viable cells.

6.5 *Presence of Impurities*—Several authors (**3, 4**) found that perfectly spherical and smooth alginate beads could only be formed by using a highly purified alginate.

7. Final Capsule or Bead Properties

7.1 *Homogeneity of Beads:*

7.1.1 It has been shown that the properties of the gel strongly depend upon the method of preparation. When a gel

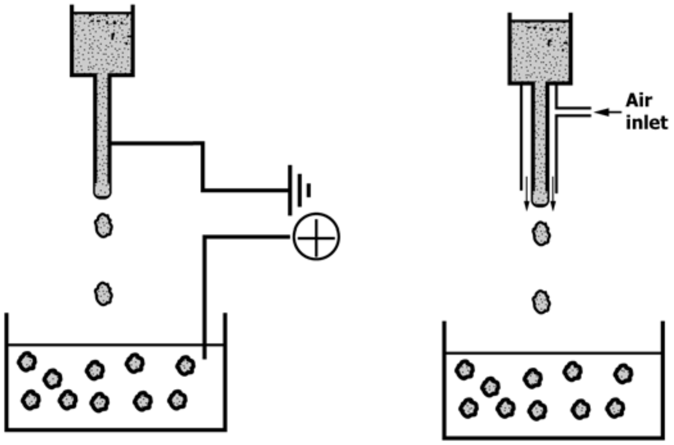

FIG. 1 Principle of Electrostatic (left) and Coaxial Air Flow (right) Bead Generators

bead is formed by diffusion of calcium ions into droplets of alginate solution, a non-uniform distribution of polymer in the bead is obtained. This can be explained by differences in the diffusion rate of the gelling ions into the bead relative to the diffusion rate of alginate molecules towards the gelling zone (**5**).

7.1.2 Another factor that affects homogeneity is the presence of non-gelling ions like Na^+ or Mg^{2+}. Such ions will compete with the gelling ions during the gelling process, resulting in more homogeneous bead. More homogeneous beads will also be mechanically stronger and have a higher porosity than more inhomogeneous beads. For example, adding sodium chloride together with calcium chloride results in the formation of a more homogeneous gel bead. Maximum homogeneity is reached with a high molecular weight alginate gelled with high concentrations of both gelling and non-gelling ions.

7.2 *Gel Porosity and Diffusion:*

7.2.1 For many applications, particularly when capsules are used to limit or restrict certain solutes, for example antibodies for rejection, knowledge about the diffusion characteristics, pore size and pore size distribution is important. Electron microscopy and inverse size exclusion chromatography has been used to study porosity of alginate gels (**6, 7, 8**). It has been found that pore size may range in size from 5 to 200 nm in diameter (**9**).

7.2.2 Diffusion of large molecules, such as proteins, requires a more open pore structure. Therefore, the gel network may restrict the transport of larger molecules. The highest diffusion rate of proteins, indicating the most open pore structures, is found when gels are made using high G alginates (**10, 7**). Diffusion coefficients also increase when lowering the alginate concentration.

7.2.3 Protein diffusion is faster for homogeneous beads than for inhomogeneous beads where the alginate is concentrated at the surface (**7**).

7.2.4 Porosity of an alginate bead may also be reduced by partially drying. Beads made with a high G-content alginate will swell only slightly when returned to water, and the resulting increased alginate concentration will reduce the average pore size.

7.2.5 The gel network to a lesser extent influences diffusion properties of smaller molecules. Diffusion coefficients of molecules such as glucose and ethanol are typically as high as about 90 % of the diffusion coefficient in water. Tanaka et al. (**11**) found no reduction in diffusion coefficients for solutes with $Mw < 2 \times 10^4$ in calcium alginate gel beads as compared with free diffusion in water.

7.2.6 Diffusion within the gel network is not solely dependent upon porosity. Since the gel matrix is negatively charged, electrostatic forces between the gel network and ionic substrates should also be considered (**12**). For example, the rate for BSA (bovine serum albumin) diffusion out of alginate beads increased with increasing pH (**7**), presumably due to the negative charge on the protein as the pH increased. The negative charged of the alginate matrix is also responsible for a difference between influx and efflux of molecules. At pH 7, most proteins are negatively charged and will therefore not easily diffuse into the matrix. When such proteins are immobilized in a gel, the repulsive forces result in an efflux that is greater than their free molecular diffusion rate (**13**).

7.2.7 There may be other contributing factors to diffusion of molecules through, into, or out of, the gel. Diffusion of drugs, or other molecules of interest, or the molecular weight cut-off of the gel network itself, needs to be experimentally determined, if important for the functionality of the TEMP.

7.3 *Gel Strength and Stability:*

7.3.1 Mechanical properties of alginate beads will to a large extent vary with the alginate composition (**10**). The highest mechanical strength is found when the G-content is more than about 70 % and average length of G blocks (NG > 1) of about 15. For molecular weights above a certain value, the mechanical strength is determined mainly by chemical composition and block structure, and is therefore independent of the molecular weight. However, low molecular weight alginates are often preferred in biomedical applications because they are easier to sterilize by membrane filtration. Below a certain critical molecular weight the gel forming ability is reduced. This effect will also be dependent of the alginate concentration because of polymer coil overlap.

7.3.2 The alginate gel as an immobilization matrix is sensitive to chelating compounds such as phosphate, lactate and citrate, presence of anti-gelling cations such as Na^+ or Mg^{2+}. To avoid this, gel beads may be kept in a medium containing a few millimolar free calcium ions and by keeping the $Na^+ : Ca^{2+}$ ratio less than 25:1 for high G alginates and 3:1 for low G alginates (**10**). An alternative is also to replace Ca^{2+} with other divalent cations with a higher affinity for alginate. There has been found a correlation between mechanical gel strength and affinity for cations (**8**). It was found that gel strength decreased in the following orders: $Pb^{2+} > Cu^{2+} = Ba^{2+} > Sr^{2+} > Cd^{2+} > Ca^{2+} > Zn^{2+} > Co^{2+} > Ni^{2+}$. However, in applications involving immobilization of living cells only Sr^{2+}, Ba^{2+}, and Ca^{2+} are considered non-toxic enough for these purposes (**13**).

7.4 *Coating of Alginate Gel Beads:*

7.4.1 As alginates may form strong complexes with polycations such as chitosan or polypeptides, or synthetic polymers such as polyethylenimine they may be used to stabilize the gel. When used as coating materials, such complexes may also be used to reduce the porosity. Alginate gels have been found stable in a range of organic solvents and are therefore, in contrast to other hydrogels, potentially useful in applications involving entrapment of enzymes in non-aqueous systems (**13**).

7.4.2 The high porosity of the alginate network has promoted the development of coating techniques. Non-coated beads may also be weaker than coated beads for some *in vivo* applications (**14, 15**). The most commonly used materials for coating of alginate beads are polypetides like poly-L-lysine (PLL) (**16, 17, 18, 19, 20, 21, 22, 23, 24, 25**) and poly-L-ornithine (PLO) (**26**), but other polycations like chitosan is also commonly used (**27, 28-30, 31, 32, 33, 34**).

7.4.3 In the production of microcapsules with a coacervate alginate/polycation membrane and a solid alginate gel core, the variations in the procedures and the materials applied are wide. However, there are two principally different procedures: a one-stage procedure where a complex coacervate membrane is formed at the interface between the alginate and polycation solutions when the alginate solution is dropped directly into a solution of polycation. This will give capsules with a complex alginate/polycation membrane surrounding a liquid alginate core. The core is subsequently gelled either by adding calcium chloride to the polycation solution or by treating the liquid core capsules with calcium chloride after the membrane has been formed (**35**). A more common method is to use a two-step procedure where alginate beads are first produced by gelling of alginate droplets in calcium chloride. After gelling, the second step is to transfer the beads into a solution of polycation. This will give a capsule with a 10 to 30 µm polycation membrane formed around the beads. For implantation, it is necessary to apply an additional layer of alginate to the beads covered with cytotoxic and immunogenic polycations like poly-L-lysine in order to avoid rejection due to immunogenic activity towards PLL by the host.

7.4.4 The alginate core may also be dissolved within the capsules by using a calcium chelating agent (citrate or EDTA) or anti-gelling cations. This will give a polyanion-polycation complex that behaves as a semipermeable membrane. This treatment, although frequently used may, however, often damage some of the microcapsules (**36**) and seems to have little advantage as compared to a solid core alginate gel bead.

8. Properties of Alginate

8.1 When immobilizing biological material in alginate gels, the following parameters regarding alginate should be taken into account.

8.1.1 *Alginate Concentration*—The alginate concentration most useful for gelling procedures must be determined based upon the characteristics of the alginate (molecular weight, monomer composition, block structure) and the desired properties of the final product. Useful ranges for alginate concentration are from 0.5 to 4 % aqueous solutions (corrected for dry matter content of the alginate).

8.1.2 *Alginate Mw Distribution*—To ensure that all alginate molecules take part in the gel network, and thereby avoiding low molecular weight material leaking out of the beads, a narrow molecular weight distribution is important (**37**).

8.1.3 *Alginate Composition and Sequential Distribution (M/G)*—The gelling properties of an alginate are highly dependent upon the monomer composition and sequential structure of the polymer. Gel strength will depend upon the guluronic acid content (F_G) and also the average number of consecutive guluronate moieties in G-block structures ($N_G > 1$).

8.2 The mechanical and swelling properties of alginate gel beads are strongly dependent on the monomeric composition, block structure as well as size and size distribution of the alginate molecules. A Ca^{2+} alginate gel will shrink during gelling, and thereby losing water and increase the polymer concentration. Beads made from an alginate with a low G-content will shrink more than beads with a higher G-content. For cells immobilized in alginate beads, shrinkage will increase the relative concentration of cells in immobilized cell matrices.

8.3 *Methods of Sterilization*—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 reduces 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. Sterile solutions or sterile, freeze-dried alginate products may also be commercially available.

8.4 Alginate for use in biomedical and pharmaceutical applications and in Tissue Engineered Medical Products (TEMPs) should ideally be documented in a Drug Master File to which end users may obtain a letter of cross-reference from suppliers of alginate. Such a Drug Master File should be submitted to the US FDA and to other regulatory authorities, both national and international.

9. Biocompatibility of Alginate Gels

9.1 For any immobilization material, a good biocompatibility towards the immobilized cells and for implantation purposes also towards the host is of vital importance. For applications involving implantation the recognition of beads as foreign bodies by the host may result in the beads eventually becomes coated in a fibrous sheath consisting of giant cells and fibrous tissues. This may decrease the mass transfer of nutrients and metabolites, leading to the eventual death of the immobilized cells.

9.2 It has also been demonstrated that smaller beads show higher biocompatibility than larger beads (**24**). This may be due to the fact that smaller beads are less fragile (**38**, **24**). Also larger beads will occupy more space at the site of implantation and this could therefore also possibly represent more stress to the surrounding tissue and thereby promoting adverse host reactions.

9.3 When cells are immobilized in alginate beads or capsules there are several ways in which they may fail as an implantation device:

9.3.1 The strength of the alginate gel may weaken over time if beads are constructed containing too many cells (high cell density). Likewise, if proliferating cells are encapsulated, the gel network may break down after time due to continued cell proliferation. The longevity of an encapsulated cell system, therefore, should be experimentally determined.

9.3.2 For beads coated with a polycation, such as poly-L-lysine, which may induce immunologic reactions, it is essential that the polycation again is covered with a non-immunogenic material (that is, alginate). If the second coating is incomplete the cation will be openly exposed to the immune system that will then respond to the antigen. Poly-L-lysine is also highly cytotoxic to surrounding cells.

9.3.3 Presence of defective beads either as a result of inappropriate production methods or harmful handling may expose immobilized materials to the immune system.

9.3.4 Inadequate immobilization methods or cellular growth may as a result give beads with cells present at the bead surface and thereby subjecting them to the immune system.

9.3.5 Secretion of immunogenic materials from the beads may activate the immune system. This may include proteins or other cellular constituents, but also immunogenic coating materials or immunogenic alginates from the beads themselves may be responsible for rejection.

9.4 The safety of alginate gels in biomedical and pharmaceutical applications and in Tissue Engineered Medical Products (TEMPs) should be established according to current guidelines such as EN-ISO 10993 and Practice F748. Suppliers of alginate may have documentation of alginate biocompatibility, safety and toxicology on file. Preclinical safety studies specific to the clinical application under consideration must also be done in accordance with 21 CFR part 312.

9.5 Other guides and practices may also contain relevant information to assist in determining the biological safety of alginate gels, such as Practices F1903, F1904, F1905, and F1906.

APPENDIXES

(Nonmandatory Information)

X1. RATIONALE

X1.1 The use of naturally occurring biopolymers for biomedical and pharmaceutical applications and in Tissue Engineered Medical Products (TEMPs) is increasing. This guide is designed to give guidance in encapsulation/immobilization technologies using alginate.

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 amounts 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 cannot be described by the monomer composition alone. 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 2,500.

X2.2 *Gelling Properties*:

X2.2.1 The uniqueness of alginate as an immobilizing agent in biomedical applications rests in its ability to form heat-stable gels that can be formed and set at room temperatures. Most applications utilize the formation of instantaneous gels by cross-linking alginate with calcium ions. However, alginate forms gels with most di- and multivalent cations. Monovalent cations and Mg^{2+} ions do not induce gelation (**1**), while ions like Ba^{2+} and Sr^{2+} will produce stronger alginate gels than Ca^{2+} (**39**).

X2.2.2 The gelling reaction occurs when divalent cations take part in interchain binding between G-blocks within the alginate molecules giving rise to a three dimensional network in the form of a gel. The gel strength will depend upon the guluronic content (F_G) and also the average number of guluronic acid residues in the G-blocks ($N_G > 1$).

X2.2.3 An increase in the G-block length results in an increase in gel strength due to increased cross-linking of alginate molecules by calcium. Additionally, using alginates

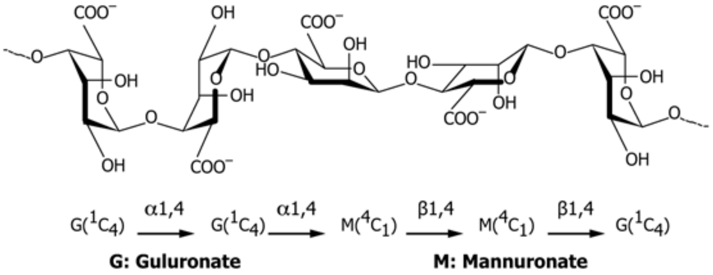

FIG. X2.1 Alginate Structure

with increasing molecular weights will also increase the strength of the gel, at least up to a certain limit of molecular weight.

X2.3 The use of an encapsulation/ immobilization system protects cells or tissue from immune rejection. Compared to other encapsulation techniques such as macrocapsules and intravascular devices, microcapsules hold several advantages. The large surface area of small beads results in enhanced survival of tissue due to better nutrition and oxygen supply, and in addition microcapsules can be implanted with minimal invasive surgery.

X2.4 Further information on alginate and encapsulation technologies may be found at the following, and other, Internet sites. (This information is given for the convenience of the user and does not constitute an endorsement by ASTM of the products named.)

X2.4.1 http://www.fmcbiopolymer.com

X2.4.2 http://www.novamatrix.biz

X2.4.3 http://www.nisco.ch

X2.4.4 http://www.inotechintl.com

X2.4.5 http://www.genialab.com

X2.4.6 http://brg.enitiaa-nantes.fr

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