Designation: F 2260 – 03

Standard Test Method for Determining Degree of Deacetylation in Chitosan Salts by Proton Nuclear Magnetic Resonance (^{1}H NMR) Spectroscopy[1]

This standard is issued under the fixed designation F 2260; 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.

1. Scope

1.1 This test method covers the determination of the degree of deacetylation in chitosan and chitosan salts intended for use in biomedical and pharmaceutical applications as well as in Tissue Engineered Medical Products (TEMPs) by high-resolution proton NMR (^{1}H NMR). A guide for the characterization of chitosan salts has been published as Guide F 2103.

1.2 The test method is applicable for determining the degree of deacetylation (% DA) of chitosan chloride and chitosan glutamate salts and is valid for % DA values from 50 up to and including 99. It is simple, rapid, and suitable for routine use. Knowledge of the degree of deacetylation is important for an understanding of the functionality of chitosan salts in TEMP formulations and applications. This test method will assist end users in choosing the correct chitosan for their particular application. Chitosan salts may have utility in drug delivery applications, as a scaffold or matrix material, and in cell and tissue encapsulation applications.

1.3 *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:*

E 386 Practice for Data Presentation Relating to High-Resolution Nuclear Magnetic Resonance (NMR) Spectroscopy[2]

F 2103 Guide for the Characterization and Testing of Chitosan Salts as Starting Material Intended for Use in Biomedical and Tissue Engineered Medical Product Applications[3]

2.2 *United States Pharmacopeia Document:*

USP 24-NF19 <761> Nuclear Magnetic Resonance[4]

2.3 *European Pharmacopoeia Document:*

European Pharmacopoeia Monograph 2002:1774 Chitosan Chloride[5]

3. Terminology

3.1 *Definitions:*

3.1.1 *chitosan*, *n*—a linear polysaccharide consisting of β(1→4) linked 2-acetamido-2-deoxy-D-glucopyranose (GlcNAc) and 2-amino-2-deoxy-D-glucopyranose (GlcN). Chitosan is a polysaccharide derived by *N*-deacetylation of chitin.

3.1.2 *degradation*, *n*—change in the chemical structure, physical properties, or appearance of a material. Degradation of polysaccharides occurs via cleavage of the glycosidic bonds. 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.

3.1.3 *degree of deacetylation*, *n*—the fraction or percentage of glucosamine units (GlcN: deacetylated monomers) in a chitosan polymer molecule.

3.1.4 *depolymerization*, *n*—reduction in the length of a polymer chain to form shorter polymeric units.

4. Significance and Use

4.1 The degree of deacetylation of chitosan salts is an important characterization parameter since the charge density of the chitosan molecule is responsible for potential biological and functional effects.

4.2 The degree of deacetylation (% DA) of water-soluble chitosan salts can be determined by ^{1}H nuclear magnetic resonance spectroscopy (^{1}H NMR). Several workers have reported on the NMR determination of chemical composition and sequential arrangement of monomer units in chitin and

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

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[2] *Annual Book of ASTM Standards*, Vol 03.06.

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

[4] Available from U.S. Pharmacopeia (USP), 12601 Twinbrook Pkwy., Rockville, MD 20852.

[5] Available from European Directorate for the Quality of Medicines (EDQM), Publications and Services, European Pharmacopoeia, BP 907, F-67029 Strasbourg, France.

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