12. Global Requirements for Medical Applications of Chitin and its Derivatives

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1. Introduction

Chitin as well as chitosan are widely used for the design of medical devices. However, an industrial application of mentioned biopolymers as medical device is very rare. Above-mentioned biopolymers possess a huge range of useful properties. Specifically, they are biocompatible, antibacterial and environmentally friendly polyelectrolyte.

Chitin and its derivatives have been designed as:
- ✓ wound dressings - especially for chronic wound treatments;
- ✓ haemostatic topical agents;
- ✓ scaffolds for the regeneration of natural tissue (bone, connective tissue, etc.);
- ✓ sealing for porous vascular prostheses;
- ✓ neurtubes promoting nerve regeneration;
- ✓ blood cholesterol control agent;
- ✓ drug delivery carriers;
- ✓ anti-tumor agent, etc.

2. General requirements

When the design of a medical device and its intended use as a chronic implant require that the prostheses maintain some minimum level of physical, chemical and biological integrity after implantation in living tissue for some time interval, the materials of which the prosthesis is made should be tasted either individually or as part of the finished prosthesis. In the selection of materials to be used in medical device manufacture the first consideration should be fitness for purpose with regard to characteristics and properties of the material, which include chemical, toxicological, physical, morphological and mechanical properties.

As for degradable or semi-degradable medical devices, the basic design criteria for polymers used in the body call for compounds that are biocompatible (definition described in PN-EN ISO 10993-1:2004[1] and PN-EN 14630:2005[2] Standards), processable, sterilizable, and capable of controlled stability or degradation in response to physiological conditions. The reasons to design an medical device that degrades over time often go beyond the obvious desire to eliminate the need for retrieval. For example, the high strength of a stainless-steel neurosurgical implant, highest than surrounding cranial bones, can lead to wide range of complications well-known as "stress shielding," whereas a bioabsorbable or semi-bioreorbable medical devices are able to increase ultimate bone strength by slowly transferring load to the bone as it heal-in.

Chitosan is a linear polysaccharide which is generally prepared by the alkaline or enyzmatic N-deacetylation of chitin. Chitin itself is a naturally occurring polysaccharide which can be obtained from a number of sources, but is generally obtained on an industrial scale from shells of crustaceans. Other sources than are also used for preparation of chitin are fungi and insects.

Chitosan is composed of 1,4-β-linked D-glucosamine (GlcN) and N-acetyl-D-glucosamine (GlcNAc) residues. Chitosan in their standard form, and in particular those of high molecular weight and/or high degrees of N-deacetylation (DD), are practically insoluble in water.

This biopolymer is well-known in the wound management application for its haemostatic properties. Further, it also shows other bioactivities and affect macrophage function that helps in faster wound healing as well as stimulates cell proliferation and histoarchitectural tissue organization. The biological properties including bacteriostatic and fungistatic properties are particularly useful for wound treatment.

Taking into account the well-know history of chitin and chitosan application in wound healing there is no CE- or FDA-certificated wound dressing. The interest of introduction of discussed biopolymers for design wound dressing and other medical devices corresponds to the number of registered patents since 1976 as shown in Figure 1.

The major limitations in the use of chitin and chitosan for designing medical devices are:
- ✓ the collection of raw materials - the most of the raw materials are in the deep sea;
- ✓ difficulty to obtain reproducible products batch after batch with various sources of raw materials;
- ✓ the cost of production is still high although the manufacture efficiency has been improved throughout the years;
- ✓ the absence of the knowledge on the exact physiological mechanism of chitosan sources, required for advanced application in medical devices and pharmaceuticals;
- ✓ the absence of validated process of biopolymers manufacture;
- ✓ the unavailability of a good quality assessment system of chitin and chitosan manufactures (such as ISO 9001:2000 [5] or ISO 13485:2003 [6] Standards);

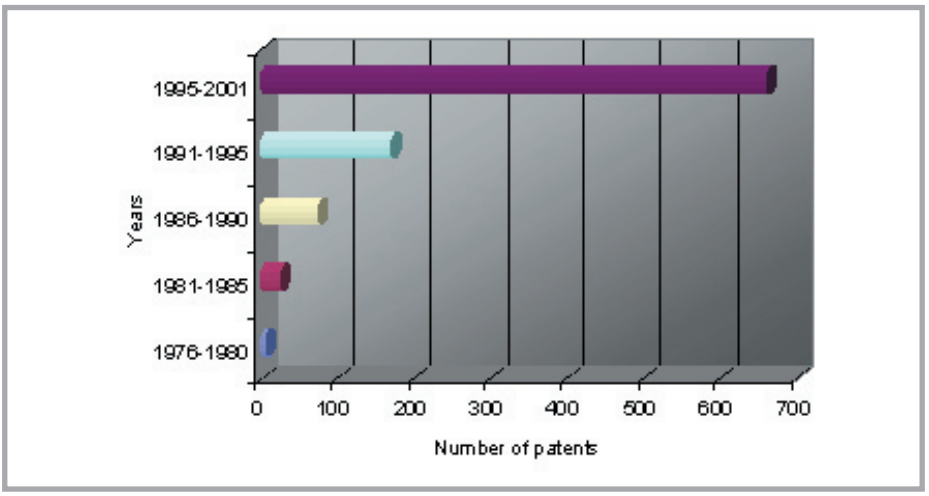

Figure 1. *Number of registered US patents searched by phrase 'chitosan + medical' in US Patent & Trademark Office database [3,4].*

- ✓ no standardization of product quality and product assay methods for chitin and chitosan.

To guarantee reproducible processing of chitin and chitosan, both understanding and control of the important parameters are needed as well as experimental methods for their routine basis for biological, physical and chemical characterization.

Realizing the above-described difficulties encountered in a few ASTM Standards for Tissue Engineered Medical Product (TEMP) by ASTM F04 Division IV that incorporates below issues:

- ✓ patient safety;
- ✓ function relation;
- ✓ reproducible results;
- ✓ realistic assessment(s);

Above issues should be affect:

- ✓ the improvement of manufacturing efficiency;
- ✓ the significant reduction in costs of development and manufacture;
- ✓ the improvement of clinical effectiveness;
- ✓ reduced regulatory hurdles and timelines.

The most interested Standards that describe the requirements for chitosan are:

1) F2103 Standard - *Standard Guide for Characterization and Testing of Chitosan Salts as Starting Materials Intended for Use in Biomedical and Tissue-Engineered Medical Product Applications*. This guide covers the evaluation of chitosan salts

suitable for use in biomedical or pharmaceutical applications, or both, including, but not limited to, tissue-engineered medical products (TEMPs) [7].

2) F2260-03 Standard – *Test Method for the Determination of the Degree of Deacetylation of Chitosan Salts by Proton Nuclear Magnetic Resonance (1H NMR) Spectroscopy*. This test method covers the determination of DD 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) [8].
3) WK965 Standard - *Test Method for the Determination of the Molecular Weight of CHITOSAN and CHITOSAN Salts by Size Exclusion Chromatography with Multi-Angle Light Scattering Detection (SEC-MALS)*. This test method covers the determination of the molecular weight of chitosan and chitosan salts by size exclusion chromatography with multi-angle light scattering detection (SEC-MALS)[9].

3. Characterization of chitosan for medicinal applications (medical devices)

There is necessary to prepare several tests, grouped in preliminary, confirmatory and other tests, for characterization of chitosan used for medical devices manufacture (Figure 2a – 2b) [10]. There is helpful to take into the consideration European Pharmacopoeia as well as requirements of ISO 10993-18:2005 [11] and ISO/DIS 10993-19 [12] Standards for the determination of range of analytical characterization of chitosan.

3.1. Preliminary tests

3.1.1. Moisture content and form identification

Usually moisture content of chitosan vary from 5.0% to 15.0% changing with humidity and form of chitosan (flakes or powder). The form of the biopolymer is able to give suitable information for quality.

The sample of biopolymer should be suspended in water, filtered and dried for detection of water-soluble contaminants.

3.1.2. Ash and protein content in chitosan

Protein and ash content in chitosan are very important parameters and those should be investigated early in the quality assay protocol. A high quality grade product should have less than 1% of protein as well as ash content.

3.1.3. Insolubility, turbidity, color and UV absorption of chitosan solution

For determination of insolubility, turbidity, coluor and UV absorption of chitosan solution 1% (w/v) solution of dried sample ought to be prepared in 1% acetic acid solution. When the sample is dissolved, the solution is filtered and insolubility of the sample is determined. The insolubility of a good quality chitosan should not exceed 0.1%. If the appearance of the residues is different from that of chitin and chitosan, one may consider analyzing its nature.

The turbidity of 1% sample solution in 1% acetic acid should be less than 15 NTU and should be free from physical contamination by visual examination.

The transmittance should be higher than 90%, both at $\lambda = 400$ nm and $\lambda = 560$ nm. The UV spectrum of chitosan is additionally able to provide the information upon the interference of other chromophores.

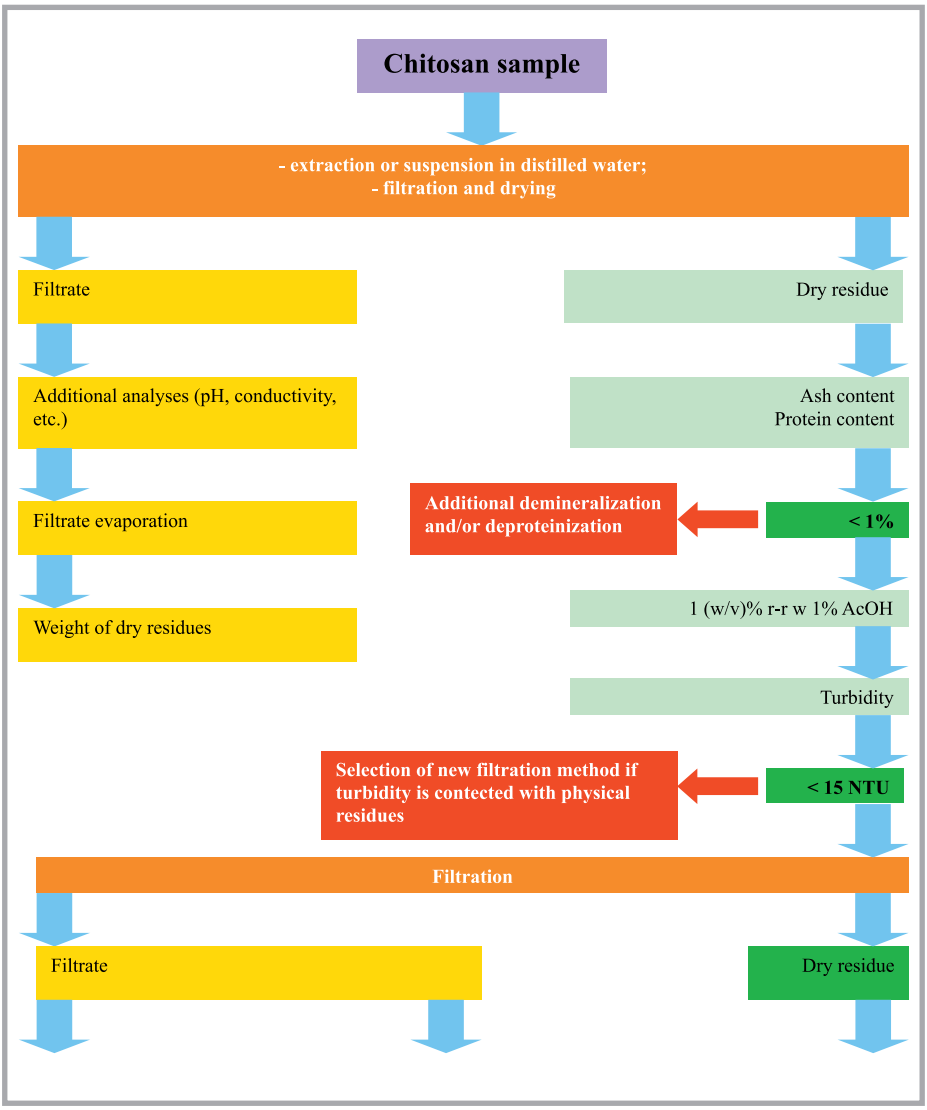

Figure 2a. *Preliminary, confirmation and additional tests for the estimation of chitosan samples used for manufacture of medical device [10].*

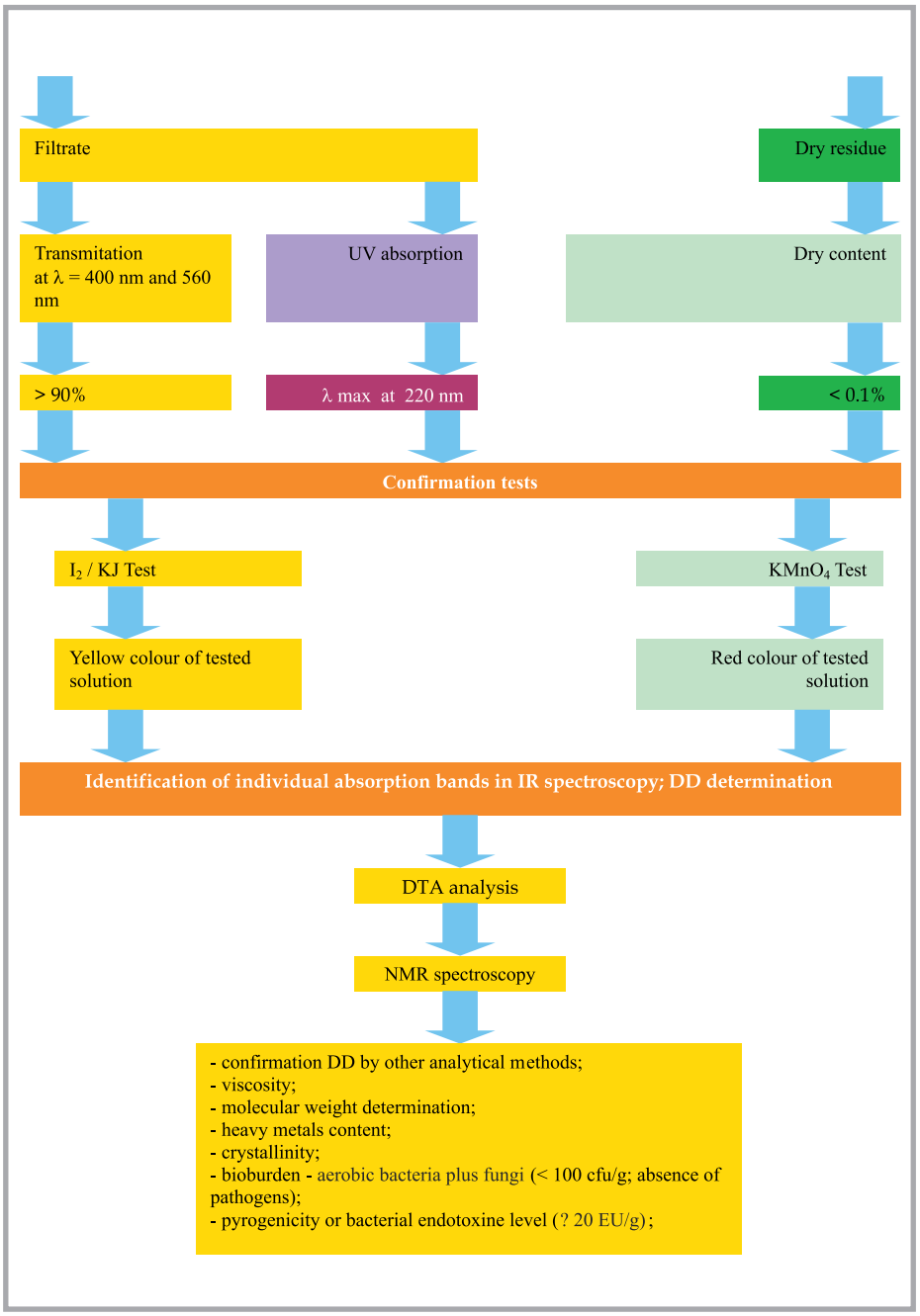

Figure 2b. *Preliminary, confirmation and additional tests for the estimation of chitosan samples used for manufacture of medical device [10].*

If during preliminary tests no significant deviation has been found, then further investigations should be conducted for the confirmatory test.

3.2. Confirmatory test

3.2.1. Chemical identification tests

Chemical identification of sample should be preceded using I_2 and $KMnO_4$ tests that resulted in specific colored reaction for chitosan solution: yellow if I_2 used and red for $KMnO_4$.

3.2.2. Chromatographic and spectroscopic examinations

Acid hydrolysis of chitosan followed by HPLC detection of the amount of acetic acid liberated is able to give acetyl content of chitin/chitosan. Due to the high sensitivity, availability, easiness of method and effectiveness in detection of functional groups IR spectroscopy can give useful information about the acetyl content of chitin/chitosan as well as possible cross-contaminations.

Differential Thermogravimetric Analysis (DTA) results in date of thermal degradation of the sample. Polymer shows increase thermostability compared to GlcNAc. Discussed biopolymer shows its main thermal process from 275 °C to 280 °C respectively.

3.3. Further confirmation tests (optional)

In general, one can assure the confirmatory test for chitosan is complete after colorimetric analysis since above-mentioned series of physical and chemical analysis can clearly investigate the identity of sample. But in certain cases, one might encounter to check the purity of chitosan for very sensitive applications that demand very high purity. Then, NMR spectroscopy will come into consideration as described in ISO 10993-18: 2005[9] and ISO/DIS 10993-19[10] Standards.

3.4. Other characteristics of chitosan

The remaining parameters of chitosan should be taking into the considered. Molecular weight of chitosan is one of the important properties affecting chitosan quality and reproductively due to several medicinal applications requires different range of molecular weights or strictly narrow range of molecular weight.

X-ray diffraction is a perfect analytical method to define the crystallinity but IR and NMR spectra are able to provide additional date of sample morphology.
Heavy metal as well as nitrogen, chloride, ammonia contents in chitosan is very important in medical applications. Additionally, extractable residues in non-polar and polar solvent are helpful to identify the characteristic of investigated samples.

Source for manufacture of medical devices should be apyrogenic as well as it should not contain pathogenic contamination. Therefore, it is useful to determine biological contamination (well-know as a bioburden level) defined as a viable aerobic microor-

ganisms, fungi, mould and yeast content. The bioburden level should measure before introduction of new portion of source for production to avoid accidental cross-contamination. The method of bioburden determination is detailed described in European Pharmacopoeia, the 5th Edition.

4. Conclusions

- The limitation of chitin and chitosan use for medical device manufacture is strictly connected with the absence of international standards describing range of requirements, both for manufactures of sources as well as manufactures of medical devices. Above-mentioned fact negatively affected on the possibility of new, innovative medical devices introduction on market.
- Furthermore, the absence of reproductivity of biopolymers, an unavailability of a good quality assessment system of chitin and chitosan manufactures inspires fear against the above-mentioned biopolymers application for medical device production.
- As with any source used for the manufacture of medical device, some characteristics of chitosan may be altered by processing techniques (such as molding, extrusion, machining, assembly, lyophilzation, coating, sealing, cross-linking, sterilization, etc.) 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 [13].

5. References

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2. PN-EN 14630:2005 "Non-active surgical implants - General requirements"
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