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CHITIN DEPOLYMERIZATION: MEDICAL APPLICATIONS

A. CIUCĂ[1] D. MIHĂILESCU[1]

Abstract: *Chitin, a β(1-4) linked N-acetyl-D-glucosamine polysaccharide, has come to the forefront of biomaterial research as a novel material, being studied for its great biocompatibility and biodegradability properties as well as its great mechanical properties. Potential biomedical application includes surgical threads for sutures and wound-healing materials. The development of these applications is affected by the chitin molecular weight, as such commercial grade chitin requires depolymerization. In this work it was achieved using a 2 GeV deuteron beam to induce a depolymerization from 6.8 to 5.4 ×10^9 g/mole.*

Key words: *chitin, depolymerisation, biomaterials, medical applications.*

1. Introduction

Chitin is a polysaccharide of β-(1→4) N-acetyl-D-glucosamine and is the second most abundant biopolymer in nature [13]. This biopolymer generally serves as support and protection in crustaceans, insects and fungi due to its great mechanical properties [8]. Beside this it was observed that chitin and its derivatives exhibit great biocompatible, biodegradable and non-toxic properties [4] making it ideal for biomedical applications.

As a consequence much research has been conducted in the field of biomaterials using chitin and it was found that chitin and other chitoligosaccharides can be used in surgical sutures [16], wound dressings [10], scaffolds for tissue regenerations [11] and drug delivery vehicles [3].

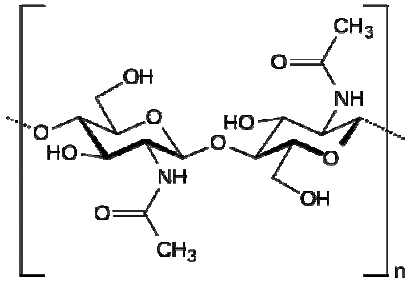

Fig. 1. *Chitin monomer chemical structure.*

2. Objective

Each medical application studied for chitin is characterized by certain critical values for chitin's molecular weight which make it efficient in implementation. As natural occurring chitin is characterised by a high molecular weight, the values, or value ranges necessary can be achieved through depolymerization techniques. These techniques can be either physical [19-9], chemical [5] or enzymatic [14],

[1] Faculty of Physics, "Alexandru Ioan Cuza" University of Iaşi.

each with its advantages and disadvantages. The purpose of this study was to present the molecular weight dependence on the biological properties relevant in biomedical applications and the means to achieve these values. Finally a novel method was used to achieve depolymerization and the results and efficiency of this method is analysed.

2.1. Medical applications

Surgical sutures using chitin were developed from chitin fibres that have shown great mechanical properties as well as great biocompatibility and biodegradability [12]. When compared with other materials used as surgical threads, such as PGA, PDS or catgut, chitin fibres have shown to be more resilient in acidic media such as pancreatic juice or urine [20]. As the threads are comprised of chitin fibres, these first need to be separated from the cross-linked matrix, through solubilisation, after which the fibres form threads through various spinning techniques (wet-spinning, electrospinning) [18]. The molecular weight of the chitin sample is only limited by the solubilisation process as for the manufacturing of threads a high molecular weight chitin is required.

Fig. 2. *HemCon™ chitosan bandage made from shrimp chitin. [26].*

Chitin has also been studied for its tissue regenerative and antibacterial properties which make it useful in wound healing and wound management materials such as plasters and wound-dressings. Animal trials have shown great promise in the use of chitin wound-dressings, with 90% increased healing when using chitin over non-chitin treatments [1]. The antibacterial and antifungal role is related to the interference between the chitin macromolecule and the bacterial wall, which may lead to a leak of intracellular constituents, such as electrolytes, as well as inhibiting bacterial development [6]. The onset of these properties is observed only at molecular weights higher than 10 kDa but high efficiency is only possible at lower molecular weights (<50kDa) [17].

Another class of biomedical applications concerning chitin is in the field of tissue engineering and regeneration, where this polymer acts as scaffold that promotes new tissue formation and cellular differentiation [22]. In this regard chitin is mainly used as porous matrices obtained from hydrogels [25]. Chitin is first solubilized, in DMAc/LiCl, a porosifier is added to the solution that will fill the space in the chitin gel and set the porosity of the formed gel. The gel is poured into moulds and washed with water. As chitin is not soluble in water, but all the other components are, after lyophilisation only the dry porous chitin will remain. These scaffolds have been used in bone and cartilage repair, especially in combination with carboxymethyl (CM) and hydroxyapatite (HA) that increase the calcium uptake of the chitin matrix [11]. As with the previous applications the molecular weight plays and important role, on one hand a lower molecular weight sample will facilitate faster solubilisation while on the other hand a higher molecular weight sample will more easily form gels, due to inter-chain crosslinking. When taking into consideration the anti-inflammatory, anti-bacterial and general increased healing properties of lower molecular weight chitin, the latter is the preferred choice. When chitin scaffolds are used to promote cellular differentiation and proliferation, by using implanted neural

progenitor cells, in traumatic brain injury (TBI) sites the localisation of positive charge along the chitin macromolecule is an advantage [25]. This can be achieved by increasing the degree of deacetylation of chitin, through chemical means. The deacetylation process removes acetyl groups along the chitin chain, leaving behind amine groups which under physiological condition protonate.

Finally a novel biomedical application for chitin and its derivatives is in the field of drug delivery and transportation. Using low molecular weight chitin nanoparticles loaded with vitamins, probiotics, enzymes or antitumor drugs it is possible to achieve transport to the desired tissue and controlled release of the drug [15]. Deacetylation of chitin will increase binding efficiency between the polymer and the active molecule.

2.2. Depolymerization techniques

As we can see low molecular weight chitin is necessary to implement this polymer in biomedical applications, with the possible exception of surgical sutures. As natural occurring chitin is characterized by a high molecular weight (600 kDa) a depolymerization process is required. To induce depolymerization the techniques available can be classified, as stated previously, into three categories: physical, through the use of ultrasounds and ionizing radiation, chemical, by acidic hydrolysis and enzymatic.

Ultrasonic depolymerization is achieved by the formation of cavitation bubbles and the subsequent collapse of these bubbles which releases high amounts of energy that facilitate the degradation process. The degradation also occurs in the absence of cavitation, due to the increased frictional forces between the smaller solvent molecules, accelerated by the ultrasound, and the large polymer macromolecules [7]. The effectiveness of this method depends on both the parameters of the ultrasound as well as the properties of the polymers. A higher intensity (90-100 W) and lower frequency (20-25 kHz) were correlated with an increased degradation rate [7], for high molecular weight chitin. As the irradiation time increases so does the degradation rate, until it reaches saturation due to the decrease in molecular weight, which makes the process less effective. This methods is characterized by ease of implementation and high efficiency.

Another effective depolymerization techniques is through the use of ionizing radiation, specifically gamma radiation from ^{60}Co sources [23]. This method can be implemented either on solubilized or dry chitin. Dose rates of 5 kGy/h in the 5-150 kGy dose range have proven to be very effective in the depolymerization of high molecular weight chitin (270 kDa) [9]. As with ultrasonic treatment, this method becomes less effective as the duration of irradiation increases, due to the decrease in molecular weight. None the less it is possible to achieve high depolymerization rates (from 270 to 19 kDa) with little preparation and low cost.

Chemical depolymerization through the use of acidic treatment is also a very popular technique, characterized by high degradation rates. The treatment is done by placing chitin in dilute solutions of HCl [5] or H_2SO_4 [24] at high temperatures, which leads to the hydrolysis of the glycosidic bonds. The depolymerization is higher the longer the treatment takes place but becomes less effective as the molecular weight reaches 100 kDa, making this process effective only for very high molecular weight samples. Aside from the hydrolysis of the glycosidic bonds, the acidic treatment also induces deacetylation [5], which in some cases is beneficial, especially for medical applications.

Finally we have the enzymatic treatment, which also induced hydrolysis of the

glycosidic bonds but through the use of specific enzymes such as chitinases [2] and lysozyme [14]. These enzymes need to be first extracted from bacteria or fungi cultures, separating and purifying the enzyme before the depolymerization process can be initiated, which makes this technique more elaborate and complex than the other techniques discussed. Enzymatic depolymerization is responsible for the biodegradable nature of chitin and other chitooligosaccharides and is characterized by the highest degradation rate, making possible the synthesis of very low molecular weight oligomers (dimers, trimers, pentamers) [2].

Based on the interaction mechanism and efficiency of the physical depolymerization techniques a new method was proposed, that may yield low molecular weight chitin. If low energy ionizing gamma radiation is efficient in inducing chitin chain degradation, we analysed the degradation induced by a high energy heavy charged particle beam, specifically a 2 GeV deuteron beam.

3. Materials and Methods

A 5% LiCl/DMAc solution was formed by adding 5 g of LiCl to 100 mL of DMAc which was then sonicated until the LiCl was solubilized. Chitin was purchased from Sigma-Aldrich and irradiated with a 2 GeV deuteron beam at JINR laboratories, Dubna, Russian Federation. Irradiated and non-irradiated chitin samples were added to the 5% LiCl/DMAc solution without any further treatment. The solution was magnetically stirred for 7 days to achieve partial dissolution of chitin. The insoluble material was separated through filtration. Solution concentration was estimated by precipitating the solution in water, washing with water, lyophilisation and then weighting the pure soluble chitin.

Viscosity measurements were carried out using an Ubbelohde viscometer with constant K=0.01004 mm^2/s^2 in a thermostat bath as to ensure a fixed temperature. Measurement were carried out from 25 °C. Different solution concentrations were obtained through dissolution of the stock solution. The viscosity-average molecular weight (M_w) was determined using the Mark-Houwink Eq. (1) where [η] is the intrinsic viscosity, *M* is the viscosity-average molecular weight and *K* and *a* are the Mark-Houwink parameters.

$$[\eta] = KM_w^a \tag{1}$$

The intrinsic viscosity was estimated by fitting the Huggins (2) and Fuoss-Mead (3) equations on the some graph. The Mark-Houwink parameters were used from *Terbojevich* [21], determined by light scattering techniques.

$$\frac{\eta_{sp}}{c} = [\eta] + k_1[\eta] \tag{2}$$

$$\frac{\ln \eta_{rel}}{c} = [\eta] - k_2[\eta] \tag{3}$$

4. Results and Discussions

The intrinsic viscosity was determined in Fig. 3 by plotting Eq. (1), the intercept being its value. In Table 1 we have the values for the Mark-Houwink parameters, determined by *Terbojevich et al.* [21]. As we can see the deuteron beam induced polymerization of the macromolecular chains, expressed through a reduction in viscosity-average molecular weight from 6.9×10^5 to 5.7×10^5 g/mole, equivalent to a decrease from 690 to 570 kDa.

Table 1

intrinsic viscosity and molecular weight values for both chitin samples

Chitin sample	a	K (g/mL)	[η] (mL/g)	$M_w \times 10^{-5}$ (g/mole)
Non-irradiated	0.65	2.4×10^{-4}	2560	6.9
Irradiated			2240	5.7

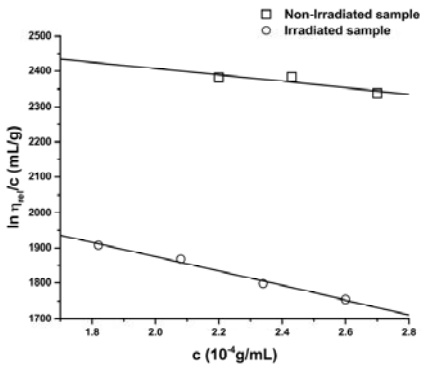

Fig. 3. *Relative viscosity plotted against temperature gives us Eq. (3).*

The high energy deuteron beam was able to induce little depolymerisation in the chitin after one single irradiation. Possible reasons for this small effect may be attributed to the low penetrability of the deuteron beam, which may have caused degradation in the superficial layers of the sample as it was placed under the beam. When using gamma or ultrasonic radiation the sample is irradiated in a solution, which under radiation may facilitate the formation of highly reactive compounds. These compounds are a major part of the depolymerisation process and in their absence the process is diminished.

5. Conclusions

When taking into consideration the high maintenance and cost of deuteron irradiation as well as when comparing this method with the other methods presented in this work we can conclude that the 2 GeV deuteron beam irradiation is not an effective depolymerisation technique for chitin. None the less further research on the nature of the glycosidic bonds and the depolymerization process are necessary as it may provide additional information.

Acknowledgments

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