# OVERVIEW ON MOLECULAR WEIGHT AND N-ACETYL GLUSCOSAMINE/ GLUCOSAMINE UNITS DISTRIBUTION OF ORIGINAL AND DEGRADED CHITIN AND CHITOSAN

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## Abstract

Main and side chain scission in two copolymers, chitin and chitosan, cause change in two types of distribution as follows: (1) the distribution of glucosamine and N-acetyl gluscosamine units or sequence; and (2) the distribution of molecular weight or polydispersity. This study provides a brief overview of published data on two types of the distribution, sequence and polydispersity. The literature data on sequence are limited only for two or three units those are neighbors and located side by side. Chitosans with sequence of random, block or alternated block were obtained in deacetylation and acetylation processes. A clear conclusion can not be given using the available data on sequence.

Contradict results have been reported on molecular weight distribution of chitin and chitosan obtained from chemical methods (acid or alkali hydrolysis and oxidative degradation). Enzymatic degradation did not obey any typical modes of chain scission. Molecular weight distribution of chitosans obtained from mechanical techniques (microfluidization or sonication) in solutions was narrower than that of original one. Degradation of chitosan in solution by conventional and laser light resulted in a wider molecular weight distribution. In general: (1) the larger macromolecules were preferentially cleaved in chemical and physical techniques; (2) the polydispersity and sequence of resulting samples obtained from degradation processes depend on the source; procedure of extraction and purification; the reaction conditions; and the nature of chemical and biochemical reagents.

## Introduction

Chitin and chitosan are two copolymers composed of two units: D-glucosamine; and N-acetyl-D-glucosamine. The number of N-acetylated units or degree of N-acetylation, DA, distributed in the copolymers is used to differentiate chitin from chitosan. Usually the copolymer with DA smaller than 50% is called chitosan. In other word, chitin consists of β- (1-4) linkage with major residues of N-acetyl-glucosamine and chitosan consists of β- (1-4) linkage with major residues of glucosamine. Naturally occurring chitin and chitosan are polydisperse in terms of molecular weight and degree of acetylation i.e. individual chains possess different glucosamine or N-acetyl glucosamine contents and different molecular weights. Thus, chemical structures of the two copolymers are changed by varying in distribution of the two units along macromolecule chains. Physical, chemical and biological properties of chitin and chitosan depend strongly on DA, the sequence and the polydispersity [1-4]. The efficiency, performance and many applications of the two copolymers are based on the properties, which are influenced by DA, sequence and polydispersity [4-9].

Main chain scission (depolymerization) process usually carried out to control degree of polymerization (molecular weight). Side chain reaction (deacetylation or reacetylation) is performed to control DA. Both reactions are carried out to obtain desirable properties, to increase the efficiency and performance and applications. The objective of this study was an overview of the literature data on sequence of two monomer units and polydispersity of both original chitin and chitosan and the samples obtained from degradation processes.

**Evaluation of Literature Data on Sequence and Polydispersity of Chitin and Chitosan**

*Chemical Methods*

The sequence of 2- acetamido- 2- deoxy- β- D- glucopyranose (GlcNAc) and 2- amino-2- deoxy- β- D- glucopyranose (GlcN) residues of chitosans prepared under homogeneous (DA= 46- 94) and heterogeneous conditions of N-deacetylation for two or three neighbors units which located side by side (diad and triad) were determined by $^{13}C$ NMR spectroscopy [10]. They obtained Bernoullian random distributions for diad and triad frequencies. Wu and Bough [7] reported that the polydispersity, $M_w/ M_n$, of chitosan in deacetylation process ($C_{NaOH}$= 50%; T = 100°C; t = 0 - 5 h.), slightly increased as a function of time (from 4.63 to 5.28). While Knaul et al. [8] reported that molecular weight and polydispersity, $M_w/ M_n$, of chitosan (DA=29) in deacetylation process ($C_{NaOH}$= 50%; T = 100°C) decreased. The difference in polydispersity of decatylated chitosans reported by different groups could be due to deacetylation conditions (concentration of NaOH; temperature; and reaction time), and the source of original chitins. There is a possibility in reduction of polydispersity by removing small macromolecules, which are soluble in water and may be drained out during washing operation [8]. A reduction of molecular weight from 600 kDa to 500 kDa was observed in preparation of 100% deacetylated chitosan from 78% deacetylated chitosan in alkali deacetylation process [3]. To avoid main chain scission reaction in deacetylation process (alkali treatment), the experiment should be carried out at temperature as low as and at time course as short as possible [3]. Wu and Bough [7] also determined the polydispersity, $M_w/ M_n$, of several chitosans samples produced in several manufacturers. The range of their polydispersities was between 2.18 and 9.81. The broad range of variation for polydispersity could be due to wide values of polydispersity for origin chitin/ chitosan and different production conditions.

Chitosans with different DA, $M_w$, and MWD were obtained by alkali hydrolysis ($C_{NAOH}$ = 50%, 100-140 °C, 1- 12h) of chitin with DA= 0.83. This reaction yielded chitosans with non-Gaussian and broad MWD [9]. This result indicates that the deacetylation was a random process and the polydispersity increased. The increase in polydispersity depends on deacetylation conditions (concentration of NaOH, temperature and time) and source of chitins (crab, Shrimp, Squid pen) [11]. Vårum et al. [10, 12] reported that the distribution of N-acetyl units in diads and triads samples was random. These samples obtained from either homogenous (acetylation) or heterogeneous (deacetylation) reactions of partially N-acetyl chitosan (DA< 0.3). $^1H$ NMR spectroscopy was used to determine the sequence of co-units along macromolecules chains. While Brugnerotto et al. [13, 14] noticed that the distribution of diads and triads for heterogeneous samples was block with a higher frequency of GlcNAc- GlcNAc, whereas they reported alternated block distribution with a higher frequency of GlcNac- GlcN- GlcNAc for homogeneous samples.

Deacetylation of chitin resulted random distribution of N-acetyl glucosamine and glucosamine units [15]. Deacetylation of chitin with amorphous region under heterogeneous conditions resulted in block copolymers of N- acetyl-D- glucosamine and D-glucosamine units [15]. The solubility test was used to determine the type of distribution. The sample with random distribution was water soluble, whereas block copolymer was not.

The sequence of two units located side by side (diad) and prepared by N-deacetylation under homogenous conditions yielded random distribution of the N-acetyl groups. Samples prepared under heterogeneous conditions had a frequency of GlcNAc-GlcNAc slightly higher than for a random (Bernoullian) distribution. Chitosans with DA of 0.29 in 50% NaOH aqueous solution were

deacetylated by heating at 100 °C. Sequence analysis was performed on purified samples containing single components [16, 17].

A reduction of molecular weight from 600 kDa to 500 kDa was observed in preparation of 100% deacetylated chitosan from 78% deacetylated chitosan in alkali deacetylation process [3]. The difference in polydispersity of decatylated chitosans reported by different groups could be due to deacetylation conditions (concentration of alkali, temperature and reaction time) and original chitins. To avoid main chain scission reaction in deacetylation process (alkali treatment), the experiment should be carried out at temperature as low as and at time course as short as possible [3]. Wu and Bough [7] also determined the polydispersity, $M_w/ M_n$, of several chitosans samples produced in several manufacturers. The range of their polydispersities was between 2.18 and 9.81. The broad range of variation for polydispersity could be due to wide values of the polydispersity for origin chitin/ chitosan and different production conditions.
The polydispersity of resulting chitosans obtained from oxidative degradation of partially deacetylated chitosan with low concentrations of hydrogen peroxide decreased from 3.3 to ≈ 2 within a short time [18]. The polydispersity of the resulting samples increased as a function of time for longer term. These authors indicated that the degradation process was a combination of random and chain end scissions. The depolymerization of chitosan by nitrous acid or sodium nitrite followed by deaminative cleavage [19]. The compositional heterogeneity of the degraded chitosan by nitrous acid was shown to be Bernoullian (random) distribution of acetylated and deacetylated units similar to what reported by Vårum, Ottøy, and Smidsrød [1]. A series of chitosan samples with molecular weights ranging from 880 to 90 kDa were obtained in degradation process with sodium per-borate ($NaBO_3$). The distribution of chitosan samples increased as illustrated in SEC/HPLC chromatograms [2]. The DA of chitosan samples was not changed significantly.
A derivative of chitosan, N-Succinyl-chitosan, was depolymerized with 7.5 M HCl at room temperature or 3.3 M HCl at 40 °C. The molecular weight ($M_w$) of the products were determined by size-exclusion chromatography–multi angle light scattering (SEC–MALS) and viscometry [20]. $M_w$ and MWD ($M_w/ M_n$) of the fragments decreased from (310 kDa and 2.1) to (70 kDa and 1.6); or (28 kDa and 1.3), respectively. The chemical structure of the fragment was principally similar to the original one as examined by $^1$H NMR spectroscopy [see Table 1, Ref. 20].

Contradict results were obtained for MWD and sequence from chemical degradation methods. This is because the reaction conditions, source of chitin, and the procedure of purification, separation, measurement and analysis of the polymers should affect the polydispersity and sequence of products.

*Biochemical Methods*
Chitinase and chitobiase hydrolyzed chito-oligosaccharides [21]. Chitinase acted as exo-enzyme and produced N, N- diacetyl-chitobiose and N-acetylglucosamine. It did not act on N, N- diacetyl-chitobiose, N, N- diacetyl-chitobitol and N, N, N- triacetyl-chitobiose. Chitobiase completely decomposed chito-oligosaccharides to N-acetylglucosamine. The formation of monomers and different oligosaccharides with different sizes were confirmed with HPLC. In addition, a moderately N-acetylated chitosan (DA= 0.35 and DP = 20) was hydrolyzed with *Bacillus* sp. No. 7-M chitosanase, showing that both the new reducing and non-reducing ends consisted exclusively of D-units [21]. This result indicates that MWD increased by these enzymes [21]. A variety of culture and processing protocols using Mucor rouxi were studied for their effects on molecular weight and molecular weight distribution [22]. They obtained chitosans with molecular weight ranging from 200 to 1400 kDa, DA ranging from 0.08 to 0.40, the polydispersity between 5 and 8. The relatively high polydispersity reflects the heterogeneous distribution of polymer chain lengths, possibly due to the cell wall extraction procedure. Completely deacetyled chitosan was depolymerized by chitsanase [23], and partially acetyled chitosan by chitinase [24, 25] and lysozyme [26, 27].

Partially N-acetylaed chitosans prepared by homogeneous N-deacetylation of chitin (DA= 0.04 to 0.6), were degraded by lysozyme, and resulted a random distribution of acetyl groups [27]. Monomers and oligomers with DP = 2-8 and low molecular weight chitosans were observed in chromatograms of depolymerzed samples of partially N-acetylated chitosans with proteases enzymes (pesin, papain and pronase) [28], indicating that MWD of depolymerized samples was larger than the original ones. The enzymes may play as endo and exo-enzymes.

*Physical Methods*

Sonication and microfluidization techniques yielded a narrower MWD [29, 30] compared to the original ones. Chain scission mostly occurred at the center of macromolecules using the two mechanical modes of actions (sonication and microfluidization). Larger molecules were more susceptible to chain scission than smaller molecules by the two mechanical methods and a photo technique (intense laser pulses) [31]. The polydispersity of the samples resulting from photo-degradation increased with irradiation time [32]. The photodegradtion of chitosan film by conventional ultraviolet light (290- 340 nm) yielded chain scission in backbone of chitosan macromolecule [33]. A broad absorption band was observed in spectra of the resulting samples, suggesting that the polydispersity of degraded samples increased.

## Conclusions

The sequence of two monomer units and the polydispersity for the samples obtained from degradation processes (chemical, biochemical and physical) depends on: the history of origin (chemical structure and morphology); the procedure used for extraction and purification; the affinity of chemical or biochemical reagent to chitin/ chitosan; and process degradation conditions. A few published articles are available on the sequence of the two units of the two copolymers and these are limited only for two or three units those are neighbors and located side by side (diads and triads). Different types of sequences: random; block; or alternated block were obtained from larger macromolecules in deacetylation and acetylation (heterogeneous and homogenous) processes. A clear conclusion can not be given using the available data on sequence.

The polydispersity of resulting samples from chemical degradation increased due to random processes. The level of increasing depends on the reaction conditions. At moderate conditions change in polydispersity was slightly, whereas at extreme conditions was significant. The sequence and polydispersity of resulting samples from biochemical methods (micro-organisms or enzymes) vary depending on the mechanism of enzyme and micro-organism actions (endo-enzymes and exo-enzymes). Mechanical mode of actions (sonication and microfluidization) resulted in narrower distribution and chain scission occurred mainly at the center of macromolecules. The polydispersity of the samples obtained from photodegradation using both conventional and laser light was larger than the original ones.

Mechanical degradation led to a narrow, whereas chemical (hydrolysis and oxidative) degradation yielded broaden molecular weight distribution. Chemical degradation process mainly followed random chain scission model and mechanical degradation obeyed Gaussian model. Single scission model is not applicable for enzymatic or photo degradation.

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