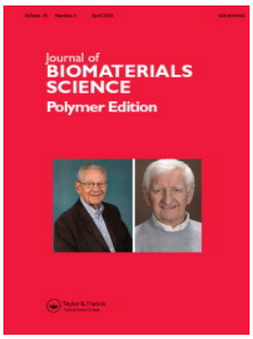

Journal of Biomaterials Science, Polymer Edition

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/tbsp20

Physicochemical modifications in microwave-irradiated chitosan: biopharmaceutical and medical applications

Doaa Elsayed Mahmoud & Nashiru Billa

To cite this article: Doaa Elsayed Mahmoud & Nashiru Billa (2024) Physicochemical modifications in microwave-irradiated chitosan: biopharmaceutical and medical applications, Journal of Biomaterials Science, Polymer Edition, 35:6, 898-915, DOI: 10.1080/09205063.2024.2306695

To link to this article: https://doi.org/10.1080/09205063.2024.2306695

Published online: 29 Jan 2024.

JOURNAL OF BIOMATERIALS SCIENCE, POLYMER EDITION
2024, VOL. 35, NO. 6, 898–915
https://doi.org/10.1080/09205063.2024.2306695

Physicochemical modifications in microwave-irradiated chitosan: biopharmaceutical and medical applications

Doaa Elsayed Mahmoud and Nashiru Billa

Department of Pharmaceutical Sciences, College of Pharmacy, QU Health, Qatar University, Doha, Qatar

ABSTRACT

Biopharmaceutical and biomedical applications of chitosan has evolved exponentially in the past decade, owing to its unique physicochemical properties. However, further applications can be garnered from modified chitosan, specifically, depolymerized chitosan, with potentially useful applications in drug delivery or biomedicine. The use of microwave irradiation in depolymerization of chitosan appears to be more consequential than other methods, and results in modification of key physicochemical properties of chitosan, including molecular weight, viscosity and degree of deacetylation. In-depth review of such microwave-depolymerized chitosan and subsequent potential biopharmaceutical or biomedical applications has not been presented before. Herein, we present a detailed review of key physicochemical changes in chitosan following various depolymerization approaches, with focus on microwave irradiation and how these changes impact relevant biopharmaceutical or biomedical applications.

Abbreviations: MW: Microwave; Mwt: Molecular weight; DD: degree of deacetylation; WB: water bath; APIs: Active pharmaceutical ingredients; NDV: Newcastle disease virus; [η]: Intrinsic viscosity

ARTICLE HISTORY
Received 23 July 2023
Revised Accepted 11 January 2024

KEYWORDS
Chitosan; microwave irradiation; molecular weight; physicochemical; biopharmaceutical, biomedical, applications

1. Introduction

Chitosan is a versatile semisynthetic cationic copolymer, comprising of repeating N-acetyl-D-glucosamine and D-glucosamine subunits, connected through β-,4 glycosidic bonds [1]. It is extracted from eggshells, insects, but mostly from the exoskeletons of crustaceans [2,3]. It is presented as an off-white powder derived from chitin by a process called deacetylation. Chitin is the second most abundant biopolymer following cellulose, and the only polysaccharide that is cationic in nature [4,5]. Unique physicochemical properties akin to chitosan have prompted research interests for the exploration of its use in pharmaceutical, chemical and biomedical fields [1, 6,7], including antitumor, antihyperglycemic, anti-inflammatory, antioxidant, antifungal, wound healing and mucoadhesion [8]. For

CONTACT Nashiru Billa nbilla@qu.edu.qa College of Pharmacy, Qatar University, Doha, Qatar

example, aqueous acidic solutions of chitosan manifests antimicrobial, biocompatible and non-immunogenic properties [1, 9,10]. Furthermore, the amine (-NH_2), primary and secondary hydroxyl (-OH) groups at positions C6, C3, C2 respectively, allow for functionalization with chemical moieties that may impart relevant physicochemical properties to chitosan for further applications (discussed later) [8,11,12,13]. Pharmaceutical or biomedical applications of chitosan is evolving into use of its degradation products. For example, degraded products of chitosan by lysozymes or chitinases are biocompatible and thus have potential biomedical or pharmaceutical applications [14]. Active degradation or depolymerization of chitosan into low Mwt components (oligomers) is being sought through physical means such as microwave irradiation (MW) or chemically. Such physical or chemical depolymerization of chitosan results in changes in physicochemical parameters including molecular weight (Mwt), degree of de-acetylation (DD), viscosity of solutions and crystallinity [4,15,16]. Consequently, reduction in Mwt and increase in DD after physical or chemical depolymerization influences the antibacterial, anticancer or reactive oxygen species (ROS) scavenging activities of chitosan. Reduction in Mwt of chitosan also improves the propensity of drug carrier systems derived as such for cellular uptake and improves the encapsulation efficiency for APIs [17]. There is also improvement in transfection efficiency when degraded chitosan is used as vector for the delivery of genetic material [18]. Various techniques have been employed in the depolymerization of chitosan into lower Mwt entities (oligomers), and these subsequently serve various pharmaceutical and biomedical applications. Chemical depolymerization methods include acid hydrolysis [19], use of sodium nitrite [20], or hydrogen peroxide [21]. Acid hydrolysis can yield low molecular weight products. However it has the disadvantage of slow reaction rates, energy intensiveness, as it demands high temperature and pressure, and the formation of high d-glucosamine monomeric units instead of the desired chitosan sizes [15,22] Moreover, acidic treatment of chitosan is not environmentally friendly, and prone to producing irregular chitosan fragmentations [23]. The use of hydrogen peroxide is relatively more environmentally friendly compared to other chemical methods, however there is a risk of extensive chemical modification, including ring opening, deamination or carboxylation [21,24]. Enzymatic degradation utilizes biological or synthetic enzymes [25], however it is time consuming, not cost-effective and labor intensive [19,22]. On the other hand, physical methods utilize electromagnetic radiation, including microwave (MW) [4,15,24], gamma rays [26], or ultraviolet radiation [27]. Other physical methods include hydrodynamic cavitation, sonication, ultrasound, milling or heating [22]. Each physical method has its constraints. Conventional heating for example is time consuming and causes deamination, dehydration and possible decomposition of the polymer [28]. Ultrasonic cavitation is also time consuming and offers no improvement in the DD compared to MW [29]. Thus, a combination of depolymerization methods may be employed to achieve the most desirable physicochemical features. In comparison to the chemical methods, the physical methods are more reproducible and give better quality oligomers. Physical methods are also amenable for scale-up and environmentally friendly [22]. MW irradiation, in particular, gives the highest yield of degraded products compared to the other physical and chemical methods (90-95% Mwt reduction), and is believed to be efficient in the deacetylation of chitosan, especially, under alkaline conditions or when coupled with H_2O_2 [22].

In this review, we expound on key physicochemical parameters of MW irradiated chitosan degradation products, including, Mwt, DD, rheological properties, and the

impact that these modifications might have on potential biomedical and pharmaceutical applications.

2. Microwave irradiation mechanism in depolymerization of chitosan

Concerted efforts aimed at understanding depolymerization mechanisms imposed by MW irradiation of chitosan have led to several proposals. One proposal suggests that MW degradation occurs *via* two possible mechanisms. First is a mechanical induction initiated by the electromagnetic field generated by the of MW energy, which induces molecular vibrational motions, causing molecules to oscillate [28]. The molecular oscillations generate shear forces strong enough to cause chain scission. The second mechanism suggests that heat generated from molecular oscillations induces hydrolysis, albeit it contributes to a lesser extent to the Mwt reduction process [28]. A further proposal suggests that the MW radiation energy cleaves the glycosidic (-C-O-C-) bond between the glucosamine subunits, leading to depolymerization. The cleavage also reduces the acetyl density within the degradation products and increases the concentration of amino and glucosamine subunits (increase in DD) [16,30]. Such degradation mechanism also accounts for higher DD within a short reaction time [30]. Galema et al. [31] and Shaio et al. [21] present a comprehensive explanation governing MW depolymerization of chitosan into degradation products, where they propose that the MW energy cleaves the chitosan polymer chains *via* dielectric heating. In this regard, induction of rotational movements and electrical migration within charged particles causes temporal polarization. Rapid reversals in polarization of the particles due to MW energy causes friction and the generation of heat within the molecules. Furthermore, because of the synchrony between MW and molecular rotational energies of the polar moieties within chitosan, the MW energy is rapidly absorbed by polar bonds, such as the C-O-C bonds. A similar proposal was put forward by Basit et al. [30], who observed, through vibrational analysis, that the scission induced by MW irradiation does not alter the main chain structure. They reported that scission also occurred at the amide moiety, whereby, an increase in free amino groups results in increase in deacetylation (DD), (Figure 1). Hence, it can be deduced from the aforementioned mechanisms that MW radiation is a favorable approach for chitosan fragmentation, with minimal distortion to the polymer backbone, except for the increase in DD, which is a desirable feature responsible for many of the pharmaceutical and biomedical applications of chitosan.

Hydrogen peroxide (H_2O_2) is often used in conjunction with MW irradiation to augment the degradation of chitosan, whereby it can initiate radical reactions to form hydroxyl radicals and superoxide anions (Figure 2). These products will then attack the glycosidic bond of chitosan resulting in cleavage. [24]

3. Physicochemical properties of microwave degraded chitosan

Following chemical, physical or enzymatic degradation of chitosan, the products retain varying degrees of the parent chitosan properties. The ultimate properties displayed by the degraded polymer are crucial in subsequent applications. In the following sections, we review key physicochemical properties of chitosan degraded by MW irradiation.

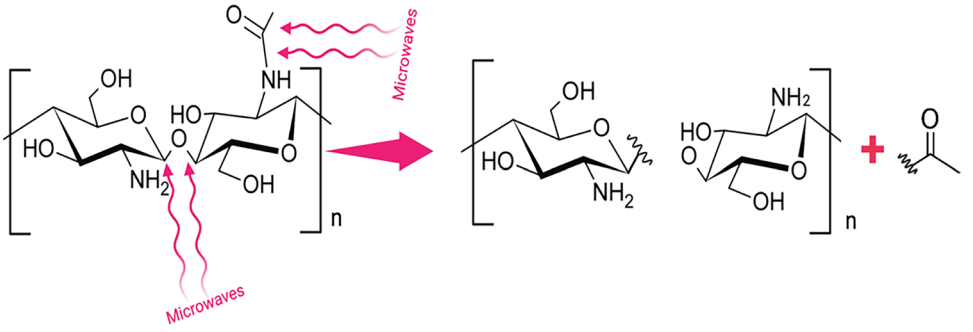

Figure 1. Schematic of chitosan depolymerization by MW irradiation.

i) $H_2O_2 \rightarrow H^+ + COO^-$

ii) $H_2O_2 \rightarrow HO^{\cdot} + O_2^{-\cdot} + H_2O$

Figure 2. hydrogen peroxide decomposition reaction and generation of hydroxyl radical and a superoxide anion.

3.1. Reduction in molecular weight (Mwt)

The processing of chitin into chitosan and subsequent reduction in Mwt by MW radiation provides scope for a wide range of applications. In this regard, reduction in Mwt is a significant physicochemical property related to most of the functionalities of chitosan [15,32]. Variation in Mwt of commercial chitosan is attributable to several factors, including DD, preparation method, and source of chitin raw material [4,15]. Depolymerization of chitosan by variable MW power and duration has led to the production of oligosaccharides with significantly lower Mwt, that, in some studies, reached between 1-3 kDa from an initial 50-190 kDa [15,16,21,24,28,33–35], (Table 1). The Mwt is often determined *via* viscometric analysis or gel permeation chromatography. Other methods include mass spectrometry (MS), Matrix-assisted laser desorption/ionization coupled with time-of-flight mass spectrometry (MALDI-TOF), dynamic light scattering (DLS), tandem mass spectrometry (MS/MS), cryoscopy, osmometry, ebulliometry, end-group analysis, and ultracentrifugation [41]. As discussed in Sections 1 and 2, MW irradiation enables production of lower Mwt products with minimal destruction to the main structure over shorter time periods

compared to other physical methods [15,24]. However, MW reduction rates diminishes as the Mwt of parent chitosan falls below 20 kDa [24]. This is attributed to the non-linear relationship between the degree of depolymerization of chitosan and time, below a certain Mwt threshold. Depolymerization below this threshold requires a higher MW energy input [22]. Depolymerization of chitosan results in a reduction of acetyl and increase in amine functional groups. These in turn improve the solubility of the degraded chitosan [30]. Furthermore, the lone pair of electrons within the amine groups is accountable for affinity with water. It attracts the adjacent protons to form ammonium cations, which collectively increases the positive charge density on the degraded chitosan. Subsequently, this positive charge density forms intermolecular bonds with water, which contributes to the improved solubility of the degraded chitosan [30]. In an application of MW depolymerized chitosan, curcumin was encapsulated in nanoparticles for the treatment of skin burn wounds [30]. The nanoparticle formulation showed improvement in solubility for curcumin and enhanced antibacterial effect against *S. aureus* and *P. aeruginosa* bacteria. The formulation also promoted skin cell migration within 24 hr on HDFa skin cell lines [30]. In another study, Li et al. [24] attributed an improvement in solubility of depolymerized chitosan to a decrease in crystallinity and intermolecular interactions after degradation. Improvement in antifungal properties of depolymerized chitosan (92 kDa) against *B. cinerea*, *P. italicum*, *P. digitatum* and *B. lecanidion* as opposed to the parent chitosan (357.3 kDa) was also reported [42]. The impact of depolymerized chitosan on wound healing and regenerative medicine has also shown that lower Mwt outperforms high Mwt chitosan in the induction of collagen deposition, reduction in the expression of inflammation markers such as the nuclear factor of kappa B (NF- κB), and stimulating anti-inflammatory and pro-regenerative markers such as arginase-1 [43]. In contrast, high Mwt chitosan in another *in vivo* rat model study generated advanced granulation tissue, more epithelial tissues, faster wound closure and better re-epithelialization than the lower Mwt chitosan [44]. Such discrepancies in reported applications of depolymerized chitosan can be related to the source of chitosan or the simultaneous use of more than one depolymerization method.

3.2. Degree of deacetylation

The degree of de-acetylation (DD) is an important parameter that distinguishes chitosan from chitin. It is expressed as the percentage of the molar fraction of d-glucosamine units in chitosan copolymers, that is consisting of both d-glucosamine and n-acetyl-glucosamine [45]. It also identifies the density of free amino groups formed after elimination of acetyl groups of N-acetyl-D-glucosamine subunits. The versatility in the applications of chitosan relies significantly on the amino groups [45,46]. A DD of 55-70% more within chitin is considered as 'low DD of chitosan'. 85%-95% is considered 'high DD', and has good water solubility, and a 100% DD is -although difficult to achieve - 'ultra-high' [47]. DD may be ascertained by ^{1}H NMR, UV-vis spectrometry, infrared spectrometry, or titration. However H^{1} NMR is most sensitive and reliable [45, 48]. As described in Section 2, MW irradiation increases the DD in chitosan, which subsequently impacts solubility, Mwt, charge density,

Table 1. Summary of studies using microwave irradiation for the treatment of chitosan, and the impact on key physicochemical properties.

Clinical Trials/ Year(s) approved	Method used in addition to MW irradiation	MW power	MW reaction time (minutes)	Molecular weight (Mwt) reduction	Effect on DD%	Effect on Viscosity	Chemical/ Structural Changes
Cheng et al. 2020 [16]	None. Only compared MW to water bath (WB) method)	Equivalent to 90 °C	0 – 240 mins	Increased first then decreased. Final Mwt after 240 mins ≈ $3x10^5$ Da	Increased within 60 mins from ≈ 60% to 73.86% After 240 mins = 79.15%	Viscosity reduced with the increase in MW exposure.	No significant changes
Mahdy Samar et al. 2013 [4]	NaOH ((30%, 40% and 50%) solution.	1400 W	10 mins	Ranged from 866.03 to 4467.05 KDa	Highest was 95.19% (using NaOH 50%)	NR	NR
Li et al. 2012 [24]	0.3% H_2O_2. Compared MW to WB	100, 200, 400, 600, 800, and 1000 W at 70 °C	20 mins	Significant reduction with the increase in reaction time (From ≈ $2.3x10^4$ to $1.5x10^4$	NR	Lower viscosity with the increase in MW exposure.	No significant change in chitosan backbone
Mecwan et al. 2011 [33]	None.	700 W	6, 18, and 30 mins	Decreased significantly from $4.40x10^5$ Da to $2.38x10^5$ Da after 30 mins.	No significant change (from 87.74% to a range of 87.58 to 88.18%)	Decreased by 45.78% after 30 mins treatment	NR
Jafari et al. 2021 [34]	1% H_2O_2	400, 600, 800 and 1000 W at 80 °C	20 mins	From 50–190 kDa to 1-3 KDa, 3-5 KDa, and 4-8 KDa.	No significant difference (from 89.6% to 88.9, 88.8, and 88.6)	Chitosan showed non-newton flow. Viscosity was influenced by the differences in MW.	Chitosan skeleton remained unaffected
Wasikiewicz et al. 2013 [28]	None. compared MW to WB at 89 °C	100 W (2.46 GHz)	80 mins	Reduced from $219.40x10^3$ g/mol at time 0, to 31.36 g/mol	No significant change	NR	No alteration
Zhang et al. 2017 [17]	1-3% H_2O_2, 40% NaOH	2450 MHz and 650 W	30-120s	Reduced from 8712.25 kDa to 124.25 kDa	Increased from 52.14% to 90.58	NR	Structural changes attributed to H_2O_2 use with MW.
Shao et al. 2003 [21]	15% H_2O_2	700W	4 mins	Reduced from $2.2x10^5$ to 900-1000 g/mol	91.2% initially. Change in DD% was NR.	NR	No structural changes
Li et al. 2021 [36]	None	2.45 GHz	10-70 mins	Reduced from 540 KDa to ≤ 10 KDa	Increased from 97.45% to 99.71 with the increase in degradation time.	With the increase in degradation time, viscosity continued to decrease. After 30 mins, e viscosity slowly decreased.	No significant change

(*Continued*)

Table 1. Continued.

Clinical Trials/ Year(s) approved	Method used in addition to MW irradiation	MW power	MW reaction time (minutes)	Molecular weight (Mwt) reduction	Effect on DD%	Effect on Viscosity	Chemical/ Structural Changes
Xing et al. 2005 [37]	NaCl, KCl, and $CaCl_2$ salts	480–800 W	0.5–25 min	Reduced from 560 KDa to ~$10x10^4$ under MW alone, and to ~ $3x10^4$ under MW + inorganic salts.	85% initially. Change in DD% was NR	Decreased with time under MW irradiation + salts	NR
Sun et al. 2006 [38]	30% H_2O_2	NR	15–54 mins	Reduced to ~ 2880 Da	NR	NR	Oxidation of C–O in chitosan oligomers formed aldehyde group (CHO)
He et al. 2016 [15]	30% H_2O_2	600 W at 70 °C	5-240 mins	degraded to 1000 to 10,000 g/mol.	≥90% (deacetylated using alkali solution)	NR	No changes despite H_2O_2 use.
Sun et al. 2007 [39]	30% H_2O_2	800 W	4–14 mins	Reduced to 1130, 2430 and 4350 Da	NR	NR	COO group produced from the oxidation by H_2O_2
Journot et al. 2020 [40]	None	Equivalent to 100 °C	19 mins	Reduced from 90,000 kDa to 12.6 ± 0.6 kDa.	No change (85%)	NR	No degradation side products
Basit et al. 2020 [30]	0.9% NaCl	800 W	5 and 8 mins	Significantly reduced from 30,665 ± 245 Da to 15,223 ± 182 Da within 9 mins	Increased from 81.29 ± 6.9% to 91.41 ± 8.2%	Significantly lower solution viscosity within 9 mins (from 13.466 ± 2.690 to 2.009 ± 0.0164).	NR

'NR': not reported; 'mins': minutes; 'GHz': Gigahertz; 'MHz': Megahertz; 'NaCl': sodium chloride; 'KCl': potassium chloride, '$CaCl_2$': calcium chloride.

biocompatibility and cellular uptake of formed carrier systems such as nanoparticles. The DD in depolymerized chitosan is directly related to its hydrophilicity, which is why there is improvement in solubility. A DD of less than 70% makes it difficult to dissolve chitosan in acetic acid [49]. In the same vein, high DD is directly proportional to the solubility of chitosan in water [50]. The swelling capacity of chitosan after MW treatment was actually found to decrease significantly from 120% to 80%, even though the DD correlates with the increase in amine groups and thus more hydrophilic. A possible explanation is that the increase in amino groups also results in strong intermolecular bonding, which, in turn, impedes interactions with water [49]. Similar observations were made by Mecwan et al. where the swelling ratio of depolymerized chitosan after MW treatment reduced by 36.75% after 30 mins [33]. Increase in DD improves the interactions of chitosan at biological interfaces and enhances cellular uptake of submicron particles. This is due to the positive charge within the amine moiety on the depolymerized chitosan, whilst the plasma membrane possess a negative charge due to sialic acid moieties [51,52]. The positive charge density also prevents opsonization by endosomes and promotes uptake through various pathways (Figure 3), including pinocytosis (clathrin, cadherin or caveolin-mediated uptake), or phagocytosis, which involves the uptake of chitosan nanoparticles with sizes above 250 nm. Adhesion of chitosan to cellular surfaces serves several biomedical applications and is influenced by the DD [53]. For example, mucoadhesion has been used to prolong the gastrointestinal transit of dosage forms [54–58]. Furthermore, parent chitosan with low DD exhibited low levels of cellular adhesion on C28/I2 human chondrocytes and NIH/3T3 mouse fibroblasts. This decrease in propensity for cellular adhesion was also attributed to the higher composition of acetyl groups within parent chitosan comprised of low DD, which also correlates to reduced positive charge and higher hydrophobicity [59].

It must be added that the impact of MW irradiation on DD is debatable. Li et al. used MV-induced plasma desorption/ionization technology at 2.45 GHz on chitosan, which saw as expected, a reduction in Mwt from 540 KDa to 10 KDa, and an increase in DD with increase in duration of exposure [36]. On the other hand, MW enabled depolymerization of chitosan with the aid of 1% H_2O_2 at 400, 600, 800 or 1000 W for 20 min showed no significant difference in DD between parent (89.6%) and treated (ranging from 88.6-88.9%) chitosan. This could be due the interference by H_2O_2 on MW irradiation [34]. However, similar observations were made by Mecwan et al. without H_2O_2. In that study, the duration of exposure ranged between 6 to 30 min at 700 W, and yet, no difference in DD was observed (87.74% in parent and 87.58-88.18% in treated chitosan) [33]. Further examples of such discrepancies are presented in Table 1. Thus, further work is warranted on a better understanding the influence of MW radiation on the DD, including the utilization of more robust analytical techniques.

3.3. Rheological properties (viscosity)

Viscosity is also an important rheological parameter that confers to chitosan its unique bio-adhesive properties. It can be determined using a variety of viscometers,

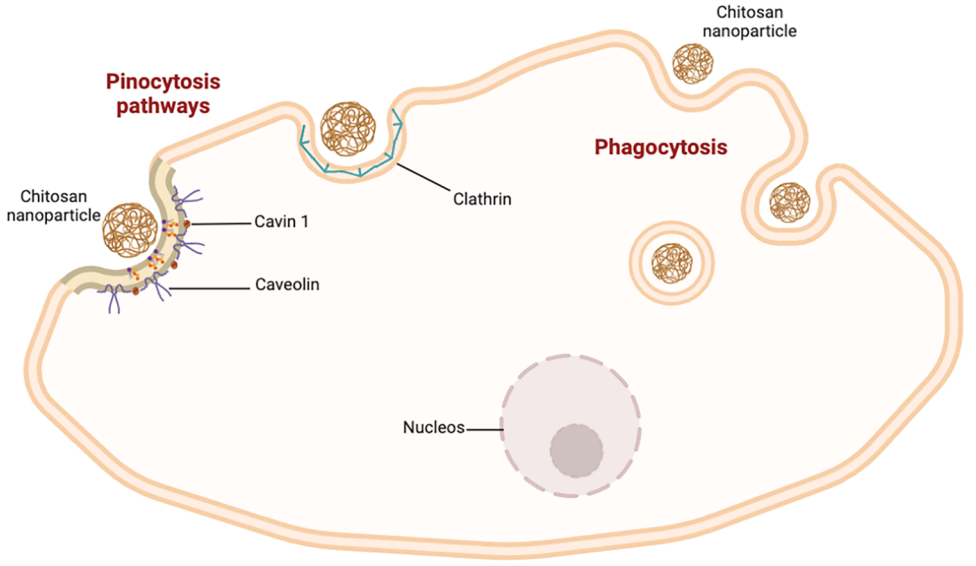

Figure 3. Schematic of various modes of cellular uptake of submicron chitosan particles.

including Ubbelohde viscometer [33], Cannon-Fenske capillary viscometer [5, 17], falling-ball viscometer [37], chip viscometer and rolling-ball viscometer [34], Fungilab rotational viscometer [60] or using more advanced rheometers [16], which are programmable and give viscosity readings at variable shear rates and shear stresses [61]. The intrinsic viscosity of polymer solutions is related to their Mwt *via* the Mark–Houwink–Sakurada equations [8].

$$[\eta] = \mathrm{K}(\mathrm{Mwt})^{\mathrm{a}}. \tag{1}$$

Where η is the solution intrinsic viscosity in ml/g, K and α are constants. K and α are dependent on the temperature and the polymer-solvent system [8,16]. Solutions of parent or depolymerized chitosan demonstrate pseudoplastic behavior (Figure 4). Rheological behavior of chitosan solutions are also affected by polymer Mwt, DD, polymer concentration, and ionic strength of solvent [5,34]. High chitosan polymer concentrations or Mwt exhibit shear rate-dependent apparent viscosity. Furthermore, shear rate-dependent apparent viscosity and zero constant shear viscosity differ in high and low chitosan Mwt and depolymerized chitosan [34]. Low Mwt chitosan and by extension, depolymerized chitosan, exhibits lower viscosity reduction rates compared to higher polymer Mwt at the same shear stresses [62].

Low viscosity chitosan solutions have a wide range of biomedical and pharmaceutical applications [8,24]. MW irradiated chitosan yields polymer solutions with lower viscosity compared to parent (Table 1). Polymer solutions may be used as suspending agents, as they increase the viscosity in pharmaceutical suspensions [63]. Low viscosity chitosan solutions have better bactericidal and bacteriostatic activity against *Staphylococcus epidermidis*. This effect is concentration-dependent, with higher concentration being more effective in reducing the level of colony-forming unit (CFU)

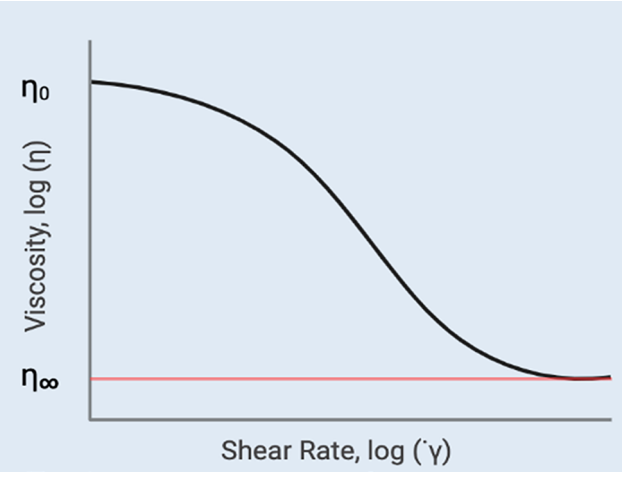

Figure 4. Shear thinning phenomenon in chitosan fluids. Black is shear thinning phenomenon and red line is viscosity at infinite shear rate.

and reducing the formation of *S. epidermidis* biofilms more successfully [64]. Higher viscosity polymer solutions also have some useful applications in regenerative medicine and wound healing, because they promote prolonged retention on biological surfaces [44]. Microwave heating ensures homogenous dissipation of heating within reaction contents and thus finds use in high viscosity solutions (chitosan as an example). For, example Dadhich et al. succeeded in synthesizing uniform reaction products of N-methylene phosphonic chitosan *via* MW irradiation, with yields of 55-60% in 10 mins compared to non-MW methods [65]. N-methylene phosphonic chitosan is used as a drug carrier, and biomedically as macrophages stimulant in tuberculosis [66].

3.4. Chemical and morphological changes due to MW degradation

3.4.1. Chemical structure

Chitin is the source from which chitosan is derived, and has three distinct polymorphic forms, alpha (α), beta (β) and gamma (γ), with the α being the most abundant and identified with three distinct FTIR peaks at1650, 1620 and 1550 cm^{-1} [67]. α-chitin consists of repeating polymer units that contain acetyl groups in opposite sides, and that alternate their position to each monomer (Figure 5A), whilst β-chitin has the same acetyl groups but alternating their position to each two monomers (Figure 5B) [68]. Similarly, chitosan has three configurations (α, β or γ) based on deacetylation of parent chitin. Although β-chitosan (parallel structure) is the least abundant, it has more favorable physicochemical properties than α-chitosan (anti-parallel structure), possibly because β-chitosan has weaker intermolecular forces and thus, presents as amorphous configuration [15,69]. In addition, β-chitosan undergoes higher intra-crystalline swelling, which leads to improved solubility in appropriate aqueous solvents [15,70].

Moreover, β-chitin was shown to present superior antibacterial agent, scaffold in orthopedic, wound dressing, and antiviral agent properties [15,71]. For example, a significant reduction in the Mwt (1000 to 10,000 g/mol) by MW irradiation improved the solubility of β-chitosan and enhanced its inhibitory and antiviral activity against Newcastle disease virus (NDV) in comparison to α-chitosan. No change in the configurations of both chitosans was observed after Mwt reduction using MW irradiation [15].

From Table 1, it can be inferred that in all cases of MW treatment the main chain remained identical to the parent (Table 1). However, when MW irradiation is used in conjunction with chemicals such as H_2O_2, some changes, including formation of carboxyl (-COOH) groups and ring opening do occur. In addition, deamination is observed with use of H_2O_2 in conjunction with MW irradiation. However, the deamination is partially inhibited with the concurrent use of MW irradiation in comparison to using H_2O_2 only [24]. Similar findings were reported by Zhang et al. in the utilization of chitosan degraded by MW irradiation and H_2O_2 on antibacterial activity, and structural properties of chitosan before and after degradation. They also reported the formation of carboxyl groups and changes in the reducing end residue structure [17]. However, other reports indicate that concurrent use of H_2O_2 with MW irradiation resulted in no changes in the chemical structures [15,21,38]. Such differences in observed phenomena may be related to the duration of exposure and/or concentration of chitosan and H_2O_2 used during the irradiation. Notwithstanding, further and more in depth characterization studies will be needed for better understanding of the reason behind the contrasting findings as these relate to biomedical and pharmaceutical applications of chitosan.

3.4.2. Morphology and crystallinity

Crystallographic and morphologic analyses on chitosan after MW degradation may provide valuable insights on physicochemical properties and possible pharmaceutical and

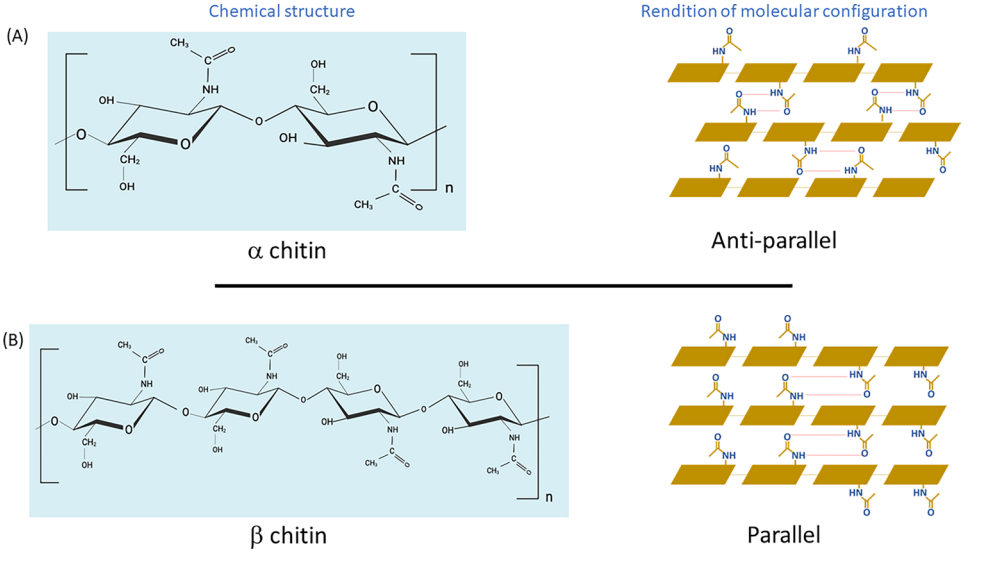

Figure 5. Configuration in alpha and beta chitin (chitosan).

biomedical applications. X-ray diffractometry (XRD) is a non-destructive physical analytical procedure that gives information on the crystalline state of material [72]. Parent chitosan is semicrystalline [73], but tends to initially become more crystalline with increased MW energy. This is because of the preferential degradation by MW energy at the amorphous domains of chitosan during depolymerization. The side chemical moieties impede degradation within the main chains, however, with increase in exposure time, crystallinity tends to decrease due to destruction of structure by MW energy [24]. Decrease in crystallinity of chitosan due to MW is further reported by other studies [16,17,24, 33,34]. It is important to recognize that other factors may also affect the crystallinity of chitosan such as the source of chitin and reaction conditions, including temperature, concentrations, and ratios of chitin to alkali [33,74]. Scanning electron microscopic (SEM) images on MW irradiated chitosan indicate that deacetylation occurs in the first 10 mins, and limited to the outer surfaces of the polymer particles and then gradually traversing to the interior. SEM also reveals tight fiber bundles and no pores within the polymer particles during the early stages of MW irradiation (Figure 6). Significant changes occur after 10 mins, with particles transforming to more porous structure with fiber fragmentation. These observations reinforces the acceleration of deacetylation process seen in the MW treated chitosan when compared to conventional heating [16].

4. Chitosan nanoparticles

One of the most consequential applications of chitosan in drug delivery systems is in the use of nanotechnology to encapsulate APIs. This is because of the myriad of fascinating properties that chitosan nanoparticles present, such as its ability to encapsulate and courier various APIs, including chemotherapeutics, nucleic acids and genes across epithelia [1]. Application of MW irradiation provides an added dimension to enhancing relevant physicochemical properties of chitosan, which ultimately, may find significant application in pharmaceutical and biomedical applications. Herein, depolymerization of chitosan by MW radiation has been used to reduce the Mwt of chitosan, increase the positive charge and subsequently used to formulate chitosan nanoparticle carriers [30,75,76]. The effect of MW radiation on chitosan which is subsequently used to formulate nanoparticles is demonstrated in Figure 7. Basit et al. [30] reported reduced chitosan viscosity and decreased Mwt, which subsequently led to the production of smaller nano-sized particles (170.7 ± 2.9 and 223.7 ± 9.3 nm) with higher positive charge (+46 mV). Recall that high positive charge improves association with negatively charged epithelia (Section 3.2).

MW depolymerization of chitosan also resulted in the production of uniformly sized nanoparticles as displayed by a polydispersity index (PDI) that ranged from 0.27 to 0.5 (low PDI, monodisperse nanoparticles [77]), compared to a PDI of 1.0 in the parent chitosan. In addition, MW modified chitosan used to nano-encapsulate curcumin showed good antibacterial activity against P. *aeruginosa* and *S. aureus*, cytotoxicity and cell mobilization in skin and wound healing applications. The same workers achieved a 99% encapsulation efficiency of curcumin from MW treated chitosan [30]. Furthermore, Kocak et al. [75] reported that the formulation of chitosan nanoparticles by MW-assisted ionic gelation showed an evidently decreased

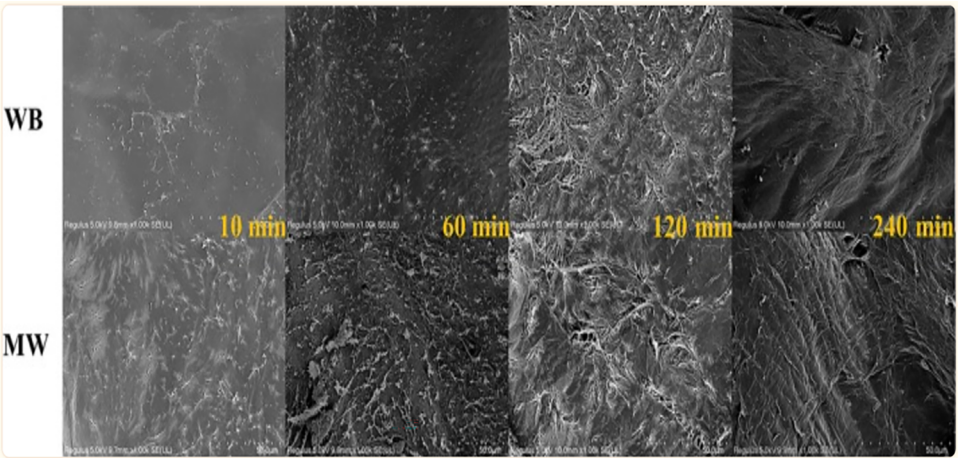

Figure 6. SEM photographs of MW heated chitosan compared to WB heating at different reaction times (adapted from [16]).

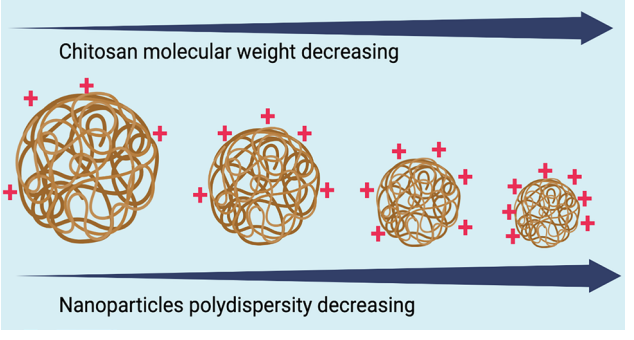

Figure 7. Effect of MW irradiation on chitosan molecular weight, nanoparticle size, polydispersity, surface morphology and charge.

nanoparticle size and reaction time with the help of MW energy. The formulation of a perfectly round small (25 nm sized) nanoparticles required only 1 min, and formed a homogenous dispersion, without affecting the zeta potential significantly [75]. Similar findings were observed by Mostafa et al. [78] in their chitosan/zinc oxide nanocomposites with the assistance of MW, achieving 80 nm and positive zeta potential of +14.2 mV. It is also noteworthy that Raghavendra et al. succeeded in fabricating chitosan-silver nanocomposite films *via* MW in ~ 100 min with a power of only 146 W. The manufactured nanocomposites exerted antibacterial activities against both gram-positive and negative micro-organisms [79].

5. Conclusion

MW irradiation is arguably, the most powerful depolymerization technique for chitosan. It outperforms other degradation approaches due to minimal structural

distortions to the main polymer chain, cost-effectiveness, environmental friendliness and accessibility. It contributes effectively to reducing the Mwt of chitosan and hence reducing the viscosity of resulting solutions, increasing the DD and hence improving the solubility of chitosan. MW degraded chitosan facilitates cellular interactions with cell membranes, consequently improving cellular uptake of submicron/nanoparticles comprised of the former. MW degraded chitosan also imparts ideal biopharmaceutical properties to nanoparticulate drug delivery systems, such as monodispersity, high positive charge and small particle sizes. Thus, we believe that the next frontier in the biomedical and biopharmaceutical applications of chitosan lie in MW derived-depolymerized versions.

Disclosue statement

No potential conflict of interest was reported by the author(s).

Funding

Open Access funding provided by the Qatar National Library.

ORCID

Nashiru Billa http://orcid.org/0000-0002-8496-1882

References

[1] Gaspar VM, Moreira AF, de Melo-Diogo D, et al. Multifunctional nanocarriers for codelivery of nucleic acids and chemotherapeutics to cancer cells. Nanobiomater Med Imaging. 2016;8:163–207. doi: 10.1016/b978-0-323-41736-5.00006-6.

[2] Elieh Ali Komi D, Sharma L, Dela Cruz CS. Chitin and its effects on inflammatory and immune responses. Clin Rev Allergy Immunol. 2017;54(2):213–223. doi: 10.1007/s12016-017-8600-0.

[3] Jiang T, James R, Kumbar SG, et al. Chitosan as a biomaterial. Nat Syn Biomed Polym. 2014;91–113 doi: 10.1016/b978-0-12-396983-5.00005-3.

[4] Mahdy Samar M, El-Kalyoubi MH, Khalaf MM, et al. Physicochemical, functional, antioxidant and antibacterial properties of chitosan extracted from shrimp wastes by microwave technique. Ann Agric Sci. 2013;58(1):33–41. doi: 10.1016/j.aoas.2013.01.006.

[5] Hwang JK, Shin HH. Rheological properties of chitosan solutions. Korea-Aust Rheol J. 2000;12(3/4):175–179.

[6] Zhao D, Yu S, Sun B, et al. Biomedical applications of chitosan and its derivative nanoparticles. Polymers (Basel). 2018;10(4):462. doi: 10.3390/polym10040462.

[7] Mohammed M, Syeda J, Wasan K, et al. An overview of chitosan nanoparticles and its application in non-parenteral drug delivery. Pharmaceutics. 2017;9(4):53. doi: 10.3390/pharmaceutics9040053.

[8] Aranaz I, Alcántara AR, Civera MC, et al. Chitosan: an overview of its properties and applications. Polymers (Basel). 2021;13(19):3256. doi: 10.3390/polym13193256.

[9] Baharlouei P, Rahman A. Chitin and chitosan: prospective biomedical applications in drug delivery, cancer treatment, and wound healing. Mar Drugs. 2022;20(7):460. doi: 10.3390/md20070460.

[10] Confederat LG, Tuchilus CG, Dragan M, et al. Preparation and antimicrobial activity of chitosan and its derivatives: a concise review. Molecules. 2021;26(12):3694. doi: 10.3390/molecules26123694.
[11] Kim I-Y, Seo S-J, Moon H-S, et al. Chitosan and its derivatives for tissue engineering applications. Biotechnol Adv. 2008;26(1):1–21. doi: 10.1016/j.biotechadv.2007.07.009.
[12] Botelho da Silva S, Krolicka M, van den Broek LAM, et al. Water-soluble chitosan derivatives and PH-responsive hydrogels by selective C-6 oxidation mediated by tempo-laccase redox system. Carbohydr Polym. 2018;186:299–309. doi: 10.1016/j.carbpol.2018.01.050.
[13] Luo Y, Wang Q. Recent advances of chitosan and its derivatives for novel applications in food science. J Food Process Beverages. 2013;1(1):1–13.
[14] Rodrigues S, Dionísio M, López C, et al. Biocompatibility of chitosan carriers with application in drug delivery. J Funct Biomater. 2012;3(3):615–641. doi: 10.3390/jfb3030615.
[15] He X, Xing R, Li K, et al. Beta-chitosan extracted from loligo japonica for a potential use to inhibit newcastle disease. Int J Biol Macromol. 2016;82:614–620. doi: 10.1016/j.ijbiomac.2015.10.059.
[16] Cheng J, Zhu H, Huang J, et al. The physicochemical properties of chitosan prepared by microwave heating. Food Sci Nutr. 2020;8(4):1987–1994. doi: 10.1002/fsn3.1486.
[17] Zhang Y, Zhang H, Chen S, et al. Microwave-assisted degradation of chitosan with hydrogen peroxide treatment using box-behnken design for enhanced antibacterial activity. Int J of Food Sci Tech. 2017;53(1):156–165. doi: 10.1111/ijfs.13569.
[18] Jeong SI, Park S-C, Park S-J, et al. One-step synthesis of gene carrier via gamma irradiation and its application in tumor gene therapy. Int J Nanomed. 2018;13:525–536. doi: 10.2147/ijn.s149532.
[19] Gonçalves C, Ferreira N, Lourenço L. Production of low molecular weight chitosan and chitooligosaccharides (COS): a review. Polymers (Basel). 2021;13(15):2466. doi: 10.3390/polym13152466.
[20] Chokradjaroen C, Rujiravanit R, Watthanaphanit A, et al. Enhanced degradation of chitosan by applying plasma treatment in combination with oxidizing agents for potential use as an anticancer agent. Carbohydr Polym. 2017;167:1–11. doi: 10.1016/j.carbpol.2017.03.006.
[21] Shao J, Yang Y, Zhong Q. Studies on preparation of oligoglucosamine by oxidative degradation under microwave irradiation. Polym Degrad Stab. 2003;82(3):395–398. doi: 10.1016/s0141-3910(03)00177-0.
[22] Pandit A, Indurkar A, Deshpande C, et al. A systematic review of physical techniques for chitosan degradation. Carbohydr Polym Technol Appl. 2021;2:100033. doi: 10.1016/j.carpta.2021.100033.
[23] El Knidri H, Belaabed R, Addaou A, et al. Extraction, chemical modification and characterization of chitin and chitosan. Int J Biol Macromol. 2018;120(Pt A):1181–1189. doi: 10.1016/j.ijbiomac.2018.08.139.
[24] Li K, Xing R, Liu S, et al. Microwave-assisted degradation of chitosan for a possible use in inhibiting crop pathogenic fungi. Int J Biol Macromol. 2012;51(5):767–773. doi: 10.1016/j.ijbiomac.2012.07.021.
[25] Konieczna-Molenda A, Fiedorowicz M, Zhong W, et al. Polarized light-stimulated enzymatic hydrolysis of chitin and chitosan. Carbohydr Res. 2008;343(18):3117–3119. doi: 10.1016/j.carres.2008.09.007.
[26] Zhang G, Li X, Xu X, et al. Antimicrobial activities of irradiation-degraded chitosan fragments. Food Biosci. 2019;29:94–101. doi: 10.1016/j.fbio.2019.03.011.
[27] Sionkowska A, Kaczmarek B, Gnatowska M, et al. The influence of UV-irradiation on chitosan modified by the tannic acid addition. J Photochem Photobiol B. 2015;148:333–339. doi: 10.1016/j.jphotobiol.2015.03.028.
[28] Wasikiewicz JM, Yeates SG. "Green" molecular weight degradation of chitosan using microwave irradiation. Polym Degrad Stab. 2013;98(4):863–867. doi: 10.1016/j.polymdegradstab.2012.12.028.

[29] Yue W, Yao P, Wei Y, et al. Synergetic effect of ozone and ultrasonic radiation on degradation of chitosan. Polym Degrad Stab. 2008;93(10):1814–1821. doi: 10.1016/j.polymdegradstab.2008.07.010.
[30] Basit HM, Mohd Amin MC, Ng S-F, et al. Formulation and evaluation of microwave-modified chitosan-curcumin nanoparticles—a promising nanomaterials platform for skin tissue regeneration applications following burn wounds. Polymers (Basel). 2020;12(11):2608. doi: 10.3390/polym12112608.
[31] Galema SA. Microwave chemistry. Chem Soc Rev. 1997;26(3):233. doi: 10.1039/cs9972600233.
[32] Czechowska-Biskup R, Wach RA, Rosiak JM, et al. Procedure for determination of the molecular weight of chitosan by viscometry. PCACD. 2018;XXIII:45–54. doi: 10.15259/PCACD.23.004.
[33] Mecwan MM, Rapalo GE, Mishra SR, et al. Effect of molecular weight of chitosan degraded by microwave irradiation on lyophilized scaffold for bone tissue engineering applications. J Biomed Mater Res A. 2011;97(1):66–73. doi: 10.1002/jbm.a.33029.
[34] Jafari H, Delporte C, Bernaerts KV, et al. Development of marine oligosaccharides for potential wound healing biomaterials engineering. Chem Eng J Adv. 2021;7:100113. doi: 10.1016/j.ceja.2021.100113.
[35] Rokhati N, Pramudono B, Istirokhatun T, et al. Microwave irradiation-assisted chitosan hydrolysis using cellulase enzyme. Bull Chem React Eng Catal. 2018;13(3):466–474. doi: 10.9767/bcrec.13.3.2378.466-474.
[36] Li Y, Yang Y, Huang Z, et al. Preparation of low molecular chitosan by microwave-induced plasma desorption/ionization technology. Int J Biol Macromol. 2021;187:441–450. doi: 10.1016/j.ijbiomac.2021.07.122.
[37] Xing R, Liu S, Yu H, et al. Salt-assisted acid hydrolysis of chitosan to oligomers under microwave irradiation. Carbohydr Res. 2005;340(13):2150–2153. doi: 10.1016/j.carres.2005.06.028.
[38] Sun T, Zhou D, Xie J, et al. Preparation of chitosan oligomers and their antioxidant activity. Eur Food Res Technol. 2006;225(3-4):451–456. doi: 10.1007/s00217-006-0439-1.
[39] Sun T, Zhou D, Mao F, et al. Preparation of low-molecular-weight carboxymethyl chitosan and their superoxide anion scavenging activity. Eur Polym J. 2007;43(2):652–656. doi: 10.1016/j.eurpolymj.2006.11.014.
[40] Journot CM, Nicolle L, Lavanchy Y, et al. Selection of water-soluble chitosan by microwave-assisted degradation and PH-controlled precipitation. Polymers (Basel). 2020;12(6):1274. doi: 10.3390/polym12061274.
[41] Tian Y, Kuzimenkova MV, Halle J, et al. Molecular weight determination by counting molecules. J Phys Chem Lett. 2015;6(6):923–927. doi: 10.1021/acs.jpclett.5b00296figure.
[42] Chien P-J, Chou C-C. Antifungal activity of chitosan and its application to control post-harvest quality and fungal rotting of tankan citrus fruit (*Citrus tankan* Hayata). J Sci Food Agric. 2006;86(12):1964–1969. doi: 10.1002/jsfa.2570.
[43] Ribeiro JC, Forte TC, Tavares SJ, et al. The effects of the molecular weight of chitosan on the tissue inflammatory response. J Biomed Mater Res A. 2021;109(12):2556–2569. doi: 10.1002/jbm.a.37250.
[44] Alsarra IA. Chitosan topical gel formulation in the management of burn wounds. Int J Biol Macromol. 2009;45(1):16–21. doi: 10.1016/j.ijbiomac.2009.03.010.
[45] Jiang Y, Fu C, Wu S, et al. Determination of the deacetylation degree of chitooligosaccharides. Mar Drugs. 2017;15(11):332. doi: 10.3390/md15110332.
[46] Mathaba M, Daramola MO. Effect of chitosan's degree of deacetylation on the performance of PES membrane infused with chitosan during AMD treatment. Membranes (Basel). 2020;10(3):52. doi: 10.3390/membranes10030052.
[47] Lv SH. High-performance superplasticizer based on chitosan. Biopolym Biotech Admixtures Eco-Eff Constr Mater. 2016;131–150. doi: 10.1016/b978-0-08-100214-8.00007-5.

[48] Czechowska-Biskup R, Jarosińska D, Rokita B, et al. Determination of degree of deacetylation of chitosan - comparision of methods. Prog Chem Appl Chitin Deriv. 2012;17:5–20.
[49] Wenling C, Duohui J, Jiamou L, et al. Effects of the degree of deacetylation on the physicochemical properties and Schwann cell affinity of chitosan films. J Biomater Appl. 2005;20(2):157–177. doi: 10.1177/0885328205049897.
[50] Aizat MA, Aziz F. Chitosan nanocomposite application in wastewater treatments. Nanotechnology in Water and Wastewater Treatment. 2019;243–265. doi: 10.1016/b978-0-12-813902-8.00012-5.
[51] Nishino M, Matsuzaki I, Musangile FY, et al. Measurement and visualization of cell membrane surface charge in fixed cultured cells related with cell morphology. PLoS One. 2020;15(7):e0236373. doi: 10.1371/journal.pone.0236373.
[52] Cook GMW. Glycobiology of the cell surface: its debt to cell electrophoresis 1940-65. Electrophoresis. 2016;37(11):1399–1406. doi: 10.1002/elps.201500476.
[53] Aibani N, Rai R, Patel P, et al. Chitosan nanoparticles at the biological interface: implications for drug delivery. Pharmaceutics. 2021;13(10):1686. doi: 10.3390/pharmaceutics13101686.
[54] Sabra R, Billa N. Gastrointestinal delivery of apis from chitosan nanoparticles. Chitin and Chitosan - Physicochemical Properties and Industrial Applications [Working Title]. 2020; London: IntechOpen. doi: 10.5772/intechopen.95363.
[55] Tan SL, Billa N. Improved bioavailability of poorly soluble drugs through gastrointestinal muco-adhesion of lipid nanoparticles. Pharmaceutics. 2021;13(11):1817. doi: 10.3390/pharmaceutics13111817.
[56] Tan JS, Roberts C, Billa N. Pharmacokinetics and tissue distribution of an orally administered mucoadhesive chitosan-coated amphotericin B-loaded nanostructured lipid carrier (NLC) in rats. J Biomater Sci Polym Ed. 2019;31(2):141–154. doi: 10.1080/09205063.2019.1680926.
[57] Chuah LH, Roberts CJ, Billa N, et al. Using nanoparticle tracking analysis (NTA) to decipher mucoadhesion propensity of curcumin-containing chitosan nanoparticles and curcumin release. J Dispers Sci Technol. 2014;35(9):1201–1207. doi: 10.1080/01932691.2013.800458.
[58] Ling JT, Roberts CJ, Billa N. Antifungal and mucoadhesive properties of an orally administered chitosan-coated amphotericin B nanostructured lipid carrier (NLC). AAPS PharmSciTech. 2019;20(3):136. doi: 10.1208/s12249-019-1346-7.
[59] Lim SM, Song DK, Cho KJ, et al. Cell adhesion and degradation behaviors of acetylated chitosan films 3rd Kuala Lumpur International Conference on Biomedical Engineering 2006. p. 2007;94–7. doi: 10.1007/978-3-540-68017-8_24.
[60] Sebastian J, Rouissi T, Brar SK, et al. Microwave-assisted extraction of chitosan from rhizopus oryzae NRRL 1526 biomass. Carbohydr Polym. 2019;219:431–440. doi: 10.1016/j.carbpol.2019.05.047.
[61] Mercan H. Thermophysical and rheological properties of hybrid nanofluids. Hybrid Nanofluids for Convection Heat Transfer. Academic Press; 2020;101–142. doi: 10.1016/b978-0-12-819280-1.00003-3.
[62] Luo Q, Wang Y, Han Q, et al. Comparison of the physicochemical, rheological, and morphologic properties of chitosan from four insects. Carbohydr Polym. 2019;209:266–275. doi: 10.1016/j.carbpol.2019.01.030.
[63] Deb PK, Kokaz SF, Abed SN, et al. Pharmaceutical and biomedical applications of polymers. Basic Fundam Drug Deliv. 2019;203–267 doi: 10.1016/b978-0-12-817909-3.00006-6.
[64] Dragland IS, Rukke HV, Stenhagen IS, et al. Antibacterial and antibiofilm effect of low viscosity chitosan against *staphylococcus epidermidis*. Int J Microbiol. 2016;2016:9159761–9159767. doi: 10.1155/2016/9159761.

[65] Dadhich P, Das B, Dhara S. Microwave assisted rapid synthesis of N-methylene phosphonic chitosan via mannich-type reaction. Carbohydr Polym. 2015;133:345–352. doi: 10.1016/j.carbpol.2015.06.105.
[66] Rando DG, Brandt CA, Ferreira EI. Use of N-methylene phosphonic chitosan to obtain an isoniazid prodrug. Rev Bras Cienc Farm. 2004;40(3): 335–344. doi: 10.1590/S1516-93322004000300009.
[67] Kaya M, Lelešius E, Nagrockaitė R, et al. Differentiations of chitin content and surface morphologies of chitins extracted from male and female grasshopper species. PLoS One. 2015;10(1):e0115531. doi: 10.1371/journal.pone.0115531.
[68] Rufato B, Galdino K, Ody JS, et al. Hydrogels based on chitosan and chitosan derivatives for biomedical applications. In: Hydrogels - smart materials for biomedical applications. London: IntechOpen, 2019. doi: 10.5772/intechopen.81811.
[69] Jung J, Zhao Y. Impact of the structural differences between α- and β-chitosan on their depolymerizing reaction and antibacterial activity. J Agric Food Chem. 2013;61(37):8783–8789. doi: 10.1021/jf4018965.
[70] Roy JC, Salaün F, Giraud S, et al. Solubility of chitin: solvents, solution behaviors and their related mechanisms. Solubility Polysacc. London: IntechOpen; 2017. doi: 10.5772/intechopen.71385.
[71] Lin S-B, Chen S-H, Peng K-C. Preparation of antibacterial chito-oligosaccharide by altering the degree of deacetylation of β-chitosan in a *Trichoderma harzianum* chitinase-hydrolysing process. J Sci Food Agric. 2008;89(2):238–244. doi: 10.1002/jsfa.3432.
[72] Raja PB, Munusamy KR, Perumal V, et al. Characterization of nanomaterial used in nanobioremediation. In: Nano-bioremediation : fundamentals and applications. Amsterdam: Elsevier BV; 2022; 57–83. doi: 10.1016/b978-0-12-823962-9.00037-4.
[73] Croisier F, Jérôme C. Chitosan-based biomaterials for tissue engineering. Eur Polym J. 2013;49(4):780–792. doi: 10.1016/j.eurpolymj.2012.12.009.
[74] Jampafuang Y, Tongta A, Waiprib Y. Impact of crystalline structural differences between α- and β-chitosan on their nanoparticle formation via ionic gelation and superoxide radical scavenging activities. Polymers (Basel). 2019;11(12):2010. doi: 10.3390/polym11122010.
[75] Kocak N, Sahin M, Akin I, et al. Microwave assisted synthesis of chitosan nanoparticles. J Macromol Sci, Part A. 2011;48(10):776–779. doi: 10.1080/10601325.2011.603613.
[76] Hiep NT, Khon HC, Niem VV, et al. Microwave-assisted synthesis of chitosan/polyvinyl alcohol silver nanoparticles gel for wound dressing applications. Int J Polym Sci. 2016;2016:1–11. doi: 10.1155/2016/1584046.
[77] Osmani RA, Thirumaleshwar S, Bhosale RR, et al. Nanosponges: the spanking accession in drug delivery- An updated comprehensive review. Der Pharmacia Sinica. 2014;5(6): 7–21.
[78] Mostafa MH, Elsawy MA, Darwish MSA, et al. Microwave-assisted preparation of chitosan/zno nanocomposite and its application in dye removal. Mater Chem Phys. 2020;248:122914. doi: 10.1016/j.matchemphys.2020.122914.
[79] Raghavendra GM, Jung J, Kim D, et al. Microwave assisted antibacterial chitosan–silver nanocomposite films. Int J Biol Macromol. 2016;84:281–288. doi: 10.1016/j.ijbiomac.2015.12.026.