Materials International
Open Access Journal (ISSN: 2668-5728)

Review
Volume 6, Issue 2, 2024, 16
https://https://doi.org/10.33263/Materials62.016

Multifarious Techniques for Resolving Chitosan's Degree of Deacetylation (DD)

Azuka Nwabike Amitaye [1], Elias Emeka Elemike [2], Terngu Timothy Uzah [2,*]

1. Department of chemistry, Nigeria Maritime University, Okerenkoko, P.M. B. 1005, Warri South-West, Delta State
2. Department of Chemistry, Federal University of Petroleum Resources, P.M.B 1221, Effurun, Delta State

* Correspondence: uzah2t@gmail.com;

Scopus Author ID 57207466454

Received: 13.01.2024; Accepted: 12.05.2024; Published: 10.06.2024

Abstract: Chitosan is a copolymer created from chitin by partial deacetylation. It is a versatile biopolymer due to its many areas of applications. The usefulness of chitosan is hinged on its quality parameters, such as degree of deacetylation (DD), molecular weight, viscosity, turbidity, and other parameters. Still, the DD is the most valuable of them. There are several different techniques for chitosan DD determination, which have been classified into spectroscopic and non-spectroscopic techniques. ^{1}H-NMR is the most precise, accurate, specific, and reliable technique for DD determination, but its expensiveness limits its application in routine analysis. The IR technique is the fastest and simplest for chitosan DD value evaluation. Meanwhile, the concentration of chitosan sample sufficient to elicit absorbance in the IR region ranges from 1.0-10.0 mg/mL$^{-1,}$ which is far smaller than the concentration or amount of chitosan required for DD determination by titrimetric techniques. Titrimetric techniques require the largest sample, while UV spectrophotometry requires the least chitosan sample for DD determination. Although the elemental analysis technique is not as common as IR and the titrimetric techniques, it is simple, cost-effective, and gives reliable DD data. It does not require the use of standard solutions and calibration curve(s) as applicable to the UV-technique. It is a tedious task to select a suitable technique for DD determination. It is, therefore, desirable to consider the time of measurement, precision, and accuracy of measurement results, as well as the cost-effectiveness when choosing a technique to use for DD determination.

Keywords: degree of deacetylation; factors; determination techniques; performance; limitation.

1. Introduction

Chitosan is a family name of biopolymers with DD > 50 %. It is a versatile material for industrial applications. It is used as a biosorbent in water engineering [1-3], as a carrier and releaser in drug formulation, as a preservative and additive in food storage, and value addition [4-6] as a wound healing agent in wound care specimens [7-12], as an additive in the production of high-quality paper [13-15] as defense and/or elicitor of defense against wide range of plants' bacterial and fungal diseases [16-19].

The application of chitosan for these purposes was due to its interesting physico-chemo-biological properties: nontoxicity, biocompatibility, biodegradability, pH sensitivity, reactivity, film-forming capability, eco-friendliness, chelation, adsorption, antimicrobial and antioxidant activities [20-24].

Also, the functional ability of the chitosan molecular chain is another reason for its multifarious applications in many fields. It is a copolymer with amino and hydroxyl functional groups [25-28], as represented in Figure 1.

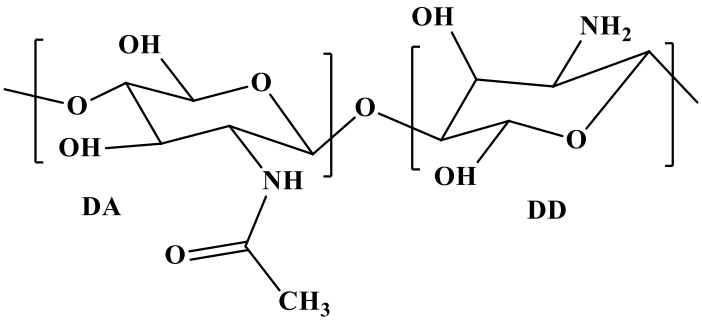


Figure 1. Chitosan's molecular chain [29].

Chitosan is created from chitinous feedstocks such as insects: spiders, cockroaches *Coleoptera Othopteran,* mosquito [30-31]; Arachnids: Octopus, scorpions [31]; Crustaceans: Lobsters, crabs, shrimps, Scampi Krill [31] and the quality of chitosan derived from these sources are different.

Most commercial chitosan are shrimp chitosan due to its high quality. The usefulness of chitosan is hinged on its quality parameters, such as degree of deacetylation, turbidity, protein residue, moisture content, solubility, viscosity, and molecular weight [32-35]. From the quality parameters, DD is the most valuable parameter for grading and classifying chitosan materials because its value influences other chitosan physicochemical and biological properties [32-33]. Table 1 shows the influence of DD on some properties of chitosan.

Table 1. How DD relates with other physical-biochemical properties of chitosan [11, 36].

Properties	**DD**
Physico-chemical	
Solubility	Solubility increases with an increase in DD
Crystallinity	It decreases with an increase in DD
Viscosity	Viscosity relates directly to DD
Biological properties	
Biodegradability	Indirectly proportional
Biocompatibility	Increase with an increase in DD value
Antimicrobial effect	It relates directly to DD
Analgesic	It increases directly with DD
Antioxidant	It relates directly to DD
Mucoadhesion	It relates directly to DD
Heamostatic	It relates directly to DD
Permeation enhancing effect	It relates directly to DD

In addition, other parameters are affected by chitin sources, the age of chitinous material, and the strength of acidic and alkali solutions. For instance, molecular weight varies for different chitinous materials or animal species, as shown in Table 2.

Table 2. Molecular weight variation for chitin created from different grasshopper species (*Orthopteran*) [30].

Orthoptera species	**Molecular weight (kDa)**
Ailopus strepens	5.20
Ailopus simulatrix	5.30
Pyrogomorpha cognata	5.50
Duroniella lacticornis	5.60
Oedipoda caerulescens	6.20
O. miniata	6.80
D. fracta	5.90

However, DD depends essentially on deacetylation reaction conditions and purification method [37-38]. Therefore, DD is a more valuable parameter for the characterization of chitosan before its application.

Nevertheless, diverse techniques abound for chitosan DD measurement, as displayed in Figure 2. There is no universally acceptable technique, which makes it a tedious task for researchers to select the appropriate technique for DD determination. The choice of techniques seems to be random or based on an individual's preference. The DD value is a quality grading parameter for chitosan, so its determination should be standardized.

Moreover, there is a shortage of information comparing commonly used techniques for DD determination, which may affect researchers' choice of technique to adopt. It is therefore necessary to review some of the commonly used techniques to enable researchers to make informed choices on techniques to adopt for the determination of DD value.

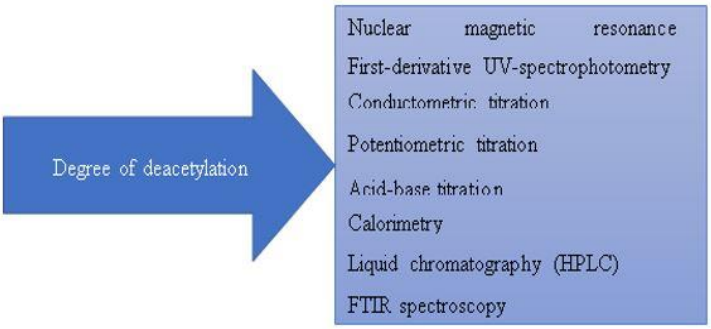


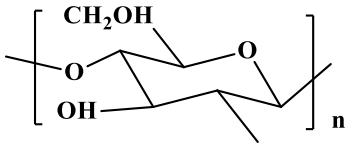
Figure 2. Determination techniques for DD [39].

Therefore, this review covered several techniques for determining chitosan's DD. It described the performances and limitations of different techniques for measuring the DD value of chitosan to afford researchers the requisite bases to make informed selections. The review also covered issues concerning factors that are capable of causing an overestimation of the DD value of chitosan.

2. How Chitosan is Derived from Chitin

Chitosan rarely occurs in nature except in a few fungi species [40-41]. It is mainly derived from chitin by alkaline hydrolysis, as represented in equation 1. The process entails the conversion of acetylamido into amino groups. It is, therefore, a nucleophilic substitution of the acyl group with a hydrogen atom [42]. Since the amides are less reactive, the hydrolysis is performed at very high alkali concentrations and temperatures [43-44]. Overall, acetyl groups are removed, and the process is known as deacetylation. Therefore, deacetylation is the thermo-alkaline hydrolytic reaction.

$$R - NHCOCH_{3\,(aq)} + OH^-_{(aq)} \rightarrow R - NH_{2\,(s)} + CH_3COO^-_{(aq)} \quad (1)$$

Where R = 

n = number of monomer chains in chitosan

Percent acetyl removal or degree of deacetylation is a measure of the extent to which acetyl units are removed from the polymer chain. It is difficult to achieve 100 % removal of acetyl units, but the extent to which it is removed must exceed 50 % for chitosan to form [26,45-47]. Hence, chitosan is obtained from chitin by deacetylation reaction. It is almost impossible

to achieve complete removal of acetyl group from chitin. Consequently, chitosan is created by alkaline hydrolysis of chitin at high alkali concentrations and temperatures.

The DD value of most commercial chitosan occurs in the range of 67.83 ± 1.19 - 96.79 ± 0.34% [48]; 69.8 -76.7% [49]. Values of 85.82 ± 0.51% [50], 79.8% [51], and 94.1% [52] have been reported as well.

3. Variables that Influence DD Value of Chitosan

The DD is the most valuable quality parameter for assessing the usefulness of chitosan. It is the mole fraction of the deacetylated portion of chitosan, as defined by equation 2 [48, 53-55].

$$DD(\%) = \frac{\mu GlcN}{\mu GlcN + \mu GlcNAc} \times 100 \qquad (2)$$

Where μ = number moles

It is closely connected to the degree of acetylation (DA), which is a measure of the acetylated portion of chitosan, by Equation 3

$$DD\ (\%) = 100 - DA \qquad (3)$$

Alternatively, equation 3 can be rewritten as DD = 1-DA, in which case the DA value is not in percentage [52].

The deacetylated portion corresponds to D-glucosamine, an amino-rich subunit in each monomeric unit of chitosan. Any increase in DD value implies an increase in the amino content of chitosan; as such, DD is a measure of the amino content of chitosan. Commercial grade chitosan has different DD values, and those within the range of 55-70 % are low deacetylated chitosan, while 70-85 % is middle deacetylated chitosan. High and ultrahigh deacetylated chitosans have DD values within the range of 85-95 % and 95-100 % respectively [56]. High DD chitosan has a larger number of amino groups than the low DD class and is more reactive as well. This is why chitosan of high DD is a better chelator and biosorbent than chitosan of low DD [21,25,57-58].

Also, chitosan of high DD possesses greater neutralization function against bacteria and fungi as electrostatic interaction between cationic chitosan molecule and anionic species on the microbial surface increases with an increase in DD value [59-63].

Also, the value of DD affects the surface morphology of chitosan. Chitosan with a low DD value has higher surface roughness than chitosan with a high DD value [30,64]. This is not unconnected with the high crystalline index of chitosan of high DD, as molecular chains are in a more organized pattern in the polymer.

Indeed, the DD values of most commercial chitosan are in the range of 70-90% [30,65]. However, chitosan for biomedical applications is expected to have a DD value greater than 95 % [66].

Nevertheless, DD depends essentially on deacetylation reaction conditions and purification method [37-38]. Its value does not depend on the age and type of chitinous feedstock.

Alkali concentration and deacetylation time are key moderators of DD value in chitosan. The value of DD increases as the concentration of alkali increases, but care more not to sacrifice molecular weight to have a higher DD value as depolymerization of chitosan occurs at very high alkali concentrations [30, 37, 66-69]. Depolymerization means a reduction in the molecular chain and weight of chitosan, which might be unsuitable for application in water treatment technology but is sought-after for biomedical and pharmaceutical applications. An

alkali concentration exceeding 60 % (for NaOH) will not only deacetylate chitin but also depolymerize it to a low molecular weight.

In addition to the concentration effect, the time duration of deacetylation, at defined alkali concentration, directly relates to a degree of deacetylation as this will afford greater penetration of alkali into the polymer to exert removal of more acetyl group from the chitosan precursor. Hence, the DD value is a function of reaction time. The polymer chain's DD value and amino group content increase as the reaction progresses to equilibrium [30, 70].

Temperature is another factor that can alter the DD value of chitosan. Chitosan specimens derived by deacetylation under different temperature conditions differ in their DD values even though other reaction variables are the same. These factors affect the DD value of chitosan in the ranked order of reaction time > alkali (NaOH) concentration > reaction temperature [47].

4. Techniques for Determination of DD Value

Different techniques are available for determining DD of chitosan and are classified into spectroscopic and non-spectroscopic techniques. The spectroscopic techniques are ^{1}H-NMR, ^{13}C-NMR, ^{15}N-NMR, infrared (IR) spectroscopy, ultra-violet (UV) /first-order derivative UV-spectrophotometry [33,48,39,52,55,71, 72, 73, 74, 75, 76, 77, 78, 79, 80].

The non-spectroscopic techniques for DD determination are conventional titrimetric and destructive techniques. The titrimetric techniques included acid-base titration [33, 81], conductometric titration [82], and potentiometric titration [30, 83], while the destructive techniques are elemental analyses, which involve the use of CHN analyzer [30], hydrolytic decomposition of chitosan by acid or enzyme followed by liquid chromatography [84], and thermal decomposition of chitosan followed by differential scanning calorimetry (DSC) [85].

It is difficult to choose appropriate techniques for DD determination as very few literature covered issues on performances, accuracies, and limitations of known techniques. It is imperative, therefore, to review some of the readily used techniques to reveal their performance, advantages, limitations, and disadvantages. Among the spectroscopic techniques, ^{1}H-NMR, IR, and UV/first-order derivative UV-spectrophotometry are the more commonly used compared to ^{13}C-NMR and ^{15}N-NMR, which require sophisticated instrumentations that make them highly expensive [86]. The ^{13}C-NMR and ^{15}N-NMR techniques are non-destructive to the sample, unlike ^{1}H-NMR, UV-spectrophotometric, and titrimetric techniques, which require sample transformation before analysis. However, solid samples are analyzed using ^{13}C-NMR and ^{15}N-NMR techniques; as such, it is unnecessary to transform samples into solution form before their analysis [86].

Besides, ^{1}H-NMR is the most sensitive of all the techniques mentioned above for DD determination. It is usually used to standardize other techniques [48, 87]. It requires 5 mg of sample to record its spectrum, unlike ^{13}C-NMR and ^{15}N-NMR, which require about 300 and 200 mg of chitosan sample, respectively [88-89].

However, only a small sample concentration (0.1-0.5 mg/L^{-1}) is required for DD determination by UV [80]. The concentrations of chitosan samples required for DD determination by other spectroscopic techniques are greater than that required for UV analytical techniques. As such, a wider range of DD is analyzed using the UV technique.

Meanwhile, a sample range of 0.5-2 mg is sufficient to determine the DD of chitosan by elemental analyses [88-89]. The sample size for DD determination by titrimetric techniques is expected to be larger than that required for spectroscopic and elemental analysis techniques.

Moreover, the high-performance liquid chromatographic (HPLC) technique is less popular among researchers than titrimetric techniques [90]. Titrimetric techniques are popular among researchers from developing nations due to their cost-effectiveness, simplicity, accessibility of their requisite apparatus and reagents, and accuracy [33].

The ^{1}H-NMR spectroscopy is the most precise, accurate, specific, and reliable technique for DD determination, but its expensiveness limits its application in routine analysis [81]. As such, it is not as common as titrimetric and the FT-IR technique among researchers, but it is mainly used by researchers from the United States of America, where ^{1}H-NMR is the standard testing technique for DD of chitosan [91-92].

However, comparable results have been reported among some techniques, as shown in Tables 3, 4, and 5.

Table 3. Results of DD determination by three analytical techniques [33].

	DD value of chitosan (%) by		
Chitosan sample	**^{1}H-NMR technique**	**UV/first-order derivative**	**Titrimetric technique**
CHS_1	93.55 ± 0.15	93.4 ± 0.35	93.19 ± 0.70
CHS_2	92.85 ± 0.13	92.6 ± 0.33	92.5 ± 0.73

CHS_1 and CHS_2 are two different chitosan of molecular weights 1000 Da and 3000 Da, respectively.

Table 4. Comparison of DD values of chitosan obtained by ^{1}H-NMR, Acid-base titration, UV, and FT-IR techniques [48].

	Analytic techniques				
Chitosan sample	**^{1}H-NMR**	**Acid-base titration**		**UV-Vis**	**FTIR**
		(by equation 11)	**(by equation 8)**		
CHS_3	79.72 ± 0.05	76.65 ± 0.48	74.67 ± 1.80	80.14 ± 0.27	76.61 ± 1.92
CHS_4	81.23 ± 0.19	81.47 ± 0.91	77.28 ± 1.51	82.55 ± 1.58	77.84 ± 0.28
CHS_5	89.44 ± 0.32	87.67 ± 1.41	85.59 ± 0.35	90.55 ± 0.92	85.61 ± 2.04

Table 5. Determination of DD by different analytical techniques [93].

Chitosan sample	**Analytical techniques**			
	^{1}H-NMR	IR	Elemental analysis (EA)	Acid-base titration
CHS_6	95.00	65.00±3.00	85.00±3.00	75.00±2.50

Nevertheless, ^{1}H-NMR and UV techniques give more precise and accurate DD data than other techniques [86]. The data in Tables 3 and 4 revealed that the UV/first-order derivative technique is more sensitive and accurate than titrimetric and IR techniques. Similar observations had been made elsewhere [70, 86].

Currently, there is no universally accepted technique for evaluating the DD value of chitosan. Analytical techniques are selected based on organizational specifications, accessibility, and cost [33]. The ^{1}H-NMR is the standard and acceptable testing technique by the U.S. Pharmacopoeia [91, 94], while UV spectrophotometric and titrimetric techniques are recommended by the European and Chinese Pharmacopoeia, respectively [95-96].

4.1. Determination of DD value by ^{1}H-NMR technique.

This is the most accurate but also one of the more expensive techniques for DD value evaluation [33, 67]. Due to its expensiveness, fewer studies are available on its use compared to techniques such as FTIR and titration [48, 76, 97]. However, it is not as expensive as ^{13}C-NMR and ^{15}N-NMR techniques, which require sophisticated instrumentation. This is one reason ^{13}C-NMR and ^{15}N-NMR instrumentations are not available in many countries. The ^{1}H-NMR technique is, therefore, an unsuitable technique for routine determination of DD.

Besides the high cost of instrumentation, the use of the ^{1}H-NMR technique is further limited by the high cost of deuterated solvents: deuterium chloride (DCl), deuterium water

(D_2O), and equipment maintenance [98]. Also, the entire experimentation process requires extensive time, especially for sample preparation [98].

Nevertheless, DD determination by ^{1}H-NMR technique gives the most precise and accurate data [91, 94]. It is highly sensitive to sample composition as it records signals or peaks from trace impurities in the sample. Hence, the ^{1}H-NMR spectrum has many signals or peaks, unlike the ^{15}N-NMR spectrum, which displays just two peaks [86, 99-100].

The ^{1}H-NMR spectroscopic technique entails scanning the sample solution over a chemical shift range δ 0–5 ppm to generate a proton NMR spectrum of chitosan in Figure 3. The proton spectrum is clear and easy to interpret, and the results obtained are highly reproducible [33, 48].

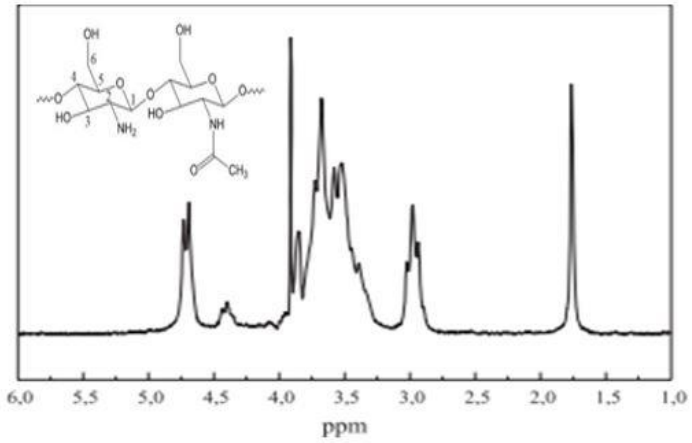


Figure 3. Proton spectrum of chitosan in D_2O [101].

From the proton spectrum, the chemical shift positions, integration values (integrals under characteristic signals), and relative numbers of protons in the polymer are derived for the computation of the DD value using equation 4 [80, 88].

Based on literature, protons attached to C_2–C_6 of the sugar ring structure of chitosan occur in the range 2.6–4.2 ppm [102], which corresponds to the shift position of protons (7H) bordering the oxygen atoms of the sugar structure, the shift position for functional groups (–OH, and $–NH_2$ moieties) appear close to the D_2O resonance at 4.80 ppm while shift position for methyl (CH_3) protons occurs in the range 1.90 –2.10 ppm [33] as displayed in Table 6.

Table 6. Chemical shift positions of protons in chitosan molecule (δ, D_2O, 25°C) [33].

Residue	**Proton's shift positions**			
	H_1	**H_2**	**$H_{2\text{-}6}$**	**Acetyl-H**
GlcNAc	4.55-4.65		2.60-4.20	1.92
GlcN	5.15	3.10-3.20	2.60-4.20	

GlcNAc = N-acetyl-D-glucosamine and GlcN = D-glucosamine

Finally, the integral value of protons is extracted from the spectrum and then substituted as required by equation 4 [94] to obtain the DD value for a triplicate study (n = 3) summarized in Table 7.

$$DD(\%) = \left[1 - \left(\frac{6 \times A_2}{3 \times A_1}\right)\right] \times 100 \qquad (4)$$

Where A_1 = Summation of protons' integral values from positions C_2-C_6 on sugar structure while A_2 = Integral value of three (CH_3) protons from acetyl group of N-acetyl-D-glucosamine.

Table 7. Results of DD determination by ^{1}H-NMR technique [33].

Chitosan sample	A_1	A_2	DD (%)	$\overline{DD}$ (%)	RSD (%)
CHS_{7a}	30.30		93.40		
CHS_{7b}	31.06	1.00	93.56	93.55	0.15
CHS_{7c}	31.70		93.69		

RSD: Relative standard deviation.

4.2. Determination of DD by titrimetric technique.

It is an effective technique for the determination of DD value of chitosan. It is one of the most common techniques for DD resolution. The technique is simple, inexpensive, precise, accurate, and adaptable to chitosan's derivatives [33, 81, 103].

Due to its accuracy, fastness, simplicity, and adaptability, the Chinese Pharmacopeia chooses the technique as a standard DD measurement testing technique [96].

The technique entails three principal steps. The first step is the dissolution of chitosan, which was previously dried to a constant weight. The dissolution is achieved using either deionized water or HCl as solvent. In the case of deionized water, little drops of HCl are usually added to facilitate the dissolution process [83, 96]. Under this condition, it is significant to consider the water percentage of chitosan (W) in the course of computation of its DD value by equation 9.

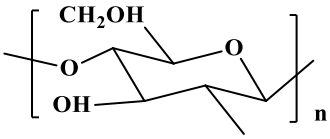
Usually, 0.5 g of chitosan is solubilized in deionized water (32 mL) by constant stirring with a gradient mixer. A solution of HCl (18 mL and 0.3 M) is added to facilitate the complete dissolution of chitosan [83, 96].

However, when chitosan is dissolved using an aqueous HCl solution, the water percentage factor (W) becomes insignificant and unnecessary for DD computation by equation 9 [48, 103-104]. Thus, equation 9 is redefined as equation 10, without the water percentage component '100-W' [104-105].

The challenge associated with the use of deionized water as a solvent is the formation of precipitate during titration at pH >7, but no precipitation issue is associated with using HCl as a solvent [48]. By implication, chitosan in solution exists in ammonium form due to the protonation of its amino group represented in equation 5:

$$R - NH_{2\,(aq)} + H^{+}_{(aq)} \rightarrow R - NH^{+}_{3\,(aq)} \qquad (5)$$

Where:

R = 

n = number of repeating units of glucosamine of chitosan

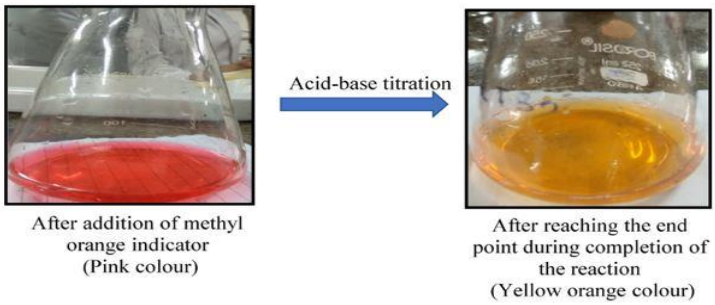


Figure 4. Color transition during acid-base titration.

The next step is the back-titration of the chitosan solution using NaOH of equimolar concentration with the HCl. Before the titrimetric process, 2-3 drops of 1 % aqueous methyl orange indicator are added to the solution and then back titrated to the end. The endpoint is marked by a color transition from pink to yellow, as displayed in Figure 4 [81, 105].

Color transition during acid-base titration (Figure 4) from pink to yellow-orange due to conversion of NH_3^+ to NH_2 via abstraction of H^+ by OH^- of alkali [81] is represented in equation 7.

$$HCl_{(aq)} + NaOH_{(q)} \rightarrow NaCl_{aq} + H_2O_{(l)} \quad (6)$$

$$R\text{-}NH_{3\,(aq)}^{+} + OH_{(aq)}^{-} \rightarrow R - NH_{2\,(aq)} + H_2O_{\,(l)} \quad (7)$$

These reactions manifest conspicuously when data from the process are plotted to obtain the titration curve, as in Figure 5. The curve has two inflection points at V_1 and V_2.

The DD value of chitosan is calculated based on inflection point V_2 or the difference between the two points of inflections on the curve, which is V_2-V_1. By point V_2, which is the total volume of NaOH (V_b), the DD value is computable by equation 8.

Alternatively, if the volume differences (V_2-V_1), which correspond to the volume of NaOH required for complete reconversion of NH_3^+ to NH_2, are considered, then equation 11 is apt [48, 83, 96]. The volume difference between points V_2 and V_1 on the titration curve is equivalent to the amount of HCl required for amine group protonation [48, 83].

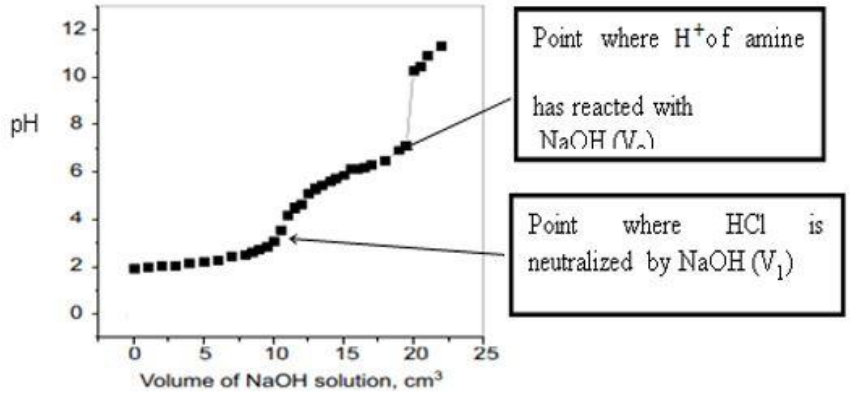


Figure 5. Titration curve based on DD determination by back titration [48].

The first point of maximum is equivalent to the amount of NaOH required for HCl neutralization, while the second point is equivalent to the amount of NaOH required for deprotonation of NH_3^+ into NH_2 [48].

$$DD\ (\%) = \frac{NH_2\%}{9.94\ \%} \times 100 \quad (8)$$

$$NH_2\% = \frac{(C_a \times V_a - C_b \times V_b) \times 0.016}{G \times (100 - W)} \times 100 \quad (9)$$

$$NH_2\% = \frac{(C_a \times V_a - C_b \times V_b) \times 0.016}{G} \times 100 \quad (10)$$

Where C_a = concentration of standardized aqueous HCl solution; C_b = concentration of standardized aqueous NaOH solution; V_a = Volume of HCl used; V_b = Volume of NaOH required; G = weight of chitosan sample used (g); W = water percent of chitosan (that is for aqueous solution of chitosan sample). The factor '0.016' is the molecular weight of NH_2 (g) in 1 mL of HCl (0.1 mol·dm^{-3}), while 9.94 % is the theoretical equivalent mass of NH_2 groups and W is the water percentage of the chitosan solution.

Therefore, eliminating water from the chitosan sample is essential to achieve precision in results before dissolution in the solvent.

$$DD\ (\%)\ = 100 - \left[2.03\ \times \frac{V_2 - V_1}{G + 0.0042 \times (V_2 - V_1)}\right] \times 100 \qquad (11)$$

Where: G = weight of chitosan sample; V_1 = Volume of NaOH (0.1 mol·dm^{-3}) required to achieve the first deflection point; V_2 = Volume of NaOH (0.1 mol·dm^{-3}) required to achieve the second deflection point. Meanwhile, the factors '2.03' is the coefficient due to chitin's molecular weight (in terms of the monomeric unit), and '0.0042' is the coefficient due to the difference in molecular weights of chitin and chitosan (in terms of the monomeric unit).

Comparatively, the DD value computed by equations 8 and 11 yields comparable results, but the results obtained by equation 11 are closer to those obtained by ^{1}H-NMR for an identical sample, as presented in Table 4.

Equation 12 is a more simplified expression for DD computation than equations 8 and 11, which require lengthy calculations. Nevertheless, the suitability and reliability of equation 12 hinge on using equimolar concentrations of acid and base [81]. Also, chitosan solution is prepared in aqueous HCl as a solvent, and the computation by equation 12 is based on inflection point V_1 on the titrimetric curve (Figure 5), which corresponds to the volume of NaOH required (V_r) for complete neutralization of residual HCl. the volume of chitosan solution (V_3) titrated is significant as well [81].

$$DD\ (\%) = \frac{(V_3 - V_r) \times 16}{V_3 \times 9.94 \times G} \times 100 \qquad (12)$$

Where G = weight of dried chitosan; V_3 = volume of chitosan solution used (mL); V_r = Volume of (0.1 N) NaOH required. The factor '9.94' is the theoretical value of %the NH_2 group (that is $^{16}/_{161} \times 100 = 9.94$) while '16' is the molecular weight of NH_2.

As in Table 8, the degree of deacetylation is inversely related to the volume of NaOH required to neutralize residual HCl. This implies that V_r (volume of NaOH for neutralization effect) will be higher for chitosan samples of low DD value and vice versa [81]. This is because high deacetylated chitosan has more amino content, exhibits less acidic character and, as such, uptake a lot of protons from HCl. Thus, a larger volume of NaOH is required for highly deacetylated chitosan than is required for low deacetylated chitosan. In other words, high-deacetylated chitosan has more amino groups that can react with HCl to form a protonated amino (NH_3^+) group; hence, a small amount of (residual) HCl is available for neutralization by NaOH.

Table 8. Relationship between volumes of NaOH required for residual HCl neutralization and DD values.

Chitosan Sample	Chitosan solution used (mL)	Volume of NaOH (Mean ± SD)	DD value (%)
CHS_8	30	16.93 ± 0.23	77.04 ± 1.36
CHS_9		15.53 ± 0.31	81.71 ± 1.73
CHS_{10}		13.77 ± 0.25	91.68 ± 1.42

SD = Standard deviation, CHS = Chitosan sample

The titration technique is simple, cheap, and gives reliable results, but it is time-consuming, especially during sample preparation and the titration process [33, 48,106].

Moreover, the technique is unsuitable for determining the low amino content's DD value of chitosan because the amino group may not be accessible by titration [86]. Likewise, it is not suitable for chitosan of high molecular weight as the formation of a viscous solution gives overestimated values for DD [107].

Besides, the results obtained by acid-base titration are not as precise as those obtained by ^{1}H-NMR and UV spectrophotometric techniques due to precipitate formation that causes

high variability in the results of DD determination by acid-base titration [107], as reflected in the large standard deviation values of DD in tables 3, 4 and 8.

In addition to precipitate formation, the results of DD determination by titrimetric techniques are controlled by temperature, pH ionic strength of solvents, and protein impurity [80, 86, 108]. The detection of titer value is a potential and frequent cause of variability in DD results by acid-base titrimetric technique [86]; it can be avoided by careful experimentation and utilization of visuals to enhance endpoint detection.

4.3. Determination of DD value by UV technique.

This technique gives DD data consistent with those obtained by the ^{1}H-NMR technique, as reported in Tables 3 and 4. The technique is simple and easy to use, and the maintenance cost is lower than that of other techniques like ^{1}H-NMR and other NMRs. Like the ^{1}H-NMR technique, the accuracy of data and minimal deviations among experimental results make the UV technique handy for validating other techniques [70, 80, 86].

The UV- technique involves the construction of two calibration curves, obtained by plots of absorption maxima against concentrations of standard N-acetyl-D-glucosamine and D-glucosamine, respectively [48, 109]. The corresponding concentrations of GlcNAc and GlcN in the chitosan sample are extrapolated from the curves and then substituted into equation 13 to obtain the DD value.

However, absorbance varies for different concentrations of a solvent, just as the position of absorption maxima is not the same for different concentration values of a solvent. The dominant absorption maximum of GlcNAc in 0.3 M HCl occurs at 204 nm, but in 0.1 M HCl, it occurs at 201 nm [33, 48].

The practice of constructing two calibration curves based on the absorbance of standard solutions of GlcNAc and GlcN as the UV-first-order-derivative technique has superseded a function of their concentrations. By UV-first-order derivative, the phenomenon of spectra interference associated with the direct use of absorbances of standard solutions is eliminated. The absorption maximum of GlcNAc in 0.3 M HCl occurs at 204 nm, which is the same absorption maximum for GlcN in 0.3M HCl solution [33, 110].

However, when phosphoric acid is used as a solvent, the dominant absorption of GlcNAc occurs at 203 nm [48]. Spectra interference results in a marginal increase in absorption maximum due to coincidental or simultaneous absorptions of GlcNAc and GlcN in 0.3 M HCl at 204 nm [33].

Spectra interference (overlap) associated with a multi-component matrix is avoidable by running baseline absorption at different wavelengths where the interfering signal is absent. For GlcNAc in 0.3 M HCl solution, the baseline absorption at 202 nm is applicable since, at this wavelength, absorption is not feasible in standard D-Glucosamine [33]. Therefore, constructing a first-order UV calibration curve compensates for the phenomenon of spectra interference associated with primary absorbance measurement. As such, there is no need to construct dual calibration curves. The process entails the conversion of the absorption maxima (absorbance, A) values for various concentration values of standard GlcNAc solution at 204 nm and 202 nm into differential form, that is $\Delta A/\Delta\lambda$, which is followed by the construction of first-order UV calibration curve using resultant differential values on the ordinate axe and concentration values of standard GlcNAc on the abscissa axis as displayed in Figure 6.

Subsequently, the DD value of chitosan solution is evaluated by measurement of the absorbance differential of GlcNAc in chitosan solution (with 0.3 M HCl as solvent).

Extrapolate the concentration value from the first-order UV curve using the value of absorbance differential obtained for the sample [95]. The concentration value extrapolated from the first-order UV calibration curve corresponds to the concentration of GlcNAc (C) in the chitosan sample, which is then substituted into equation 14 to obtain the DD value.

$$DD\ (\%) = \frac{[GlcN]}{[GlcN]+[GlcNAc]} \times 100 \quad (13)$$

$$DD\ (\%) = \frac{C_{sample}-C}{C_{sample}-\frac{42}{221.21}C} \times 100 \quad (14)$$

Where C_{sample} = chitosan's concentration in the analyte solution (µg/mL); C = concentration of GlcNAc in the analyte solution (µg/mL). Factors '221.21' are the molecular weight of GlcNAc in chitosan, and '42' is the molecular weight difference between GlcNAc and GlcN.

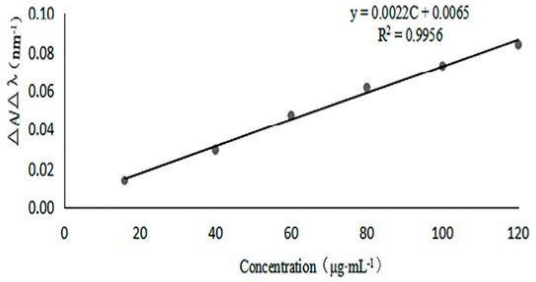


Figure 6. Calibration curve of first-order UV derivative of GlcNAc in 0.3 M HCl [33]. Where A_λ = absorbance at specific wavelength; $\Delta A = A_{204nm} - A_{202nm}$, $\Delta\lambda$ = 2 nm.

Moreover, water is not a suitable solvent for DD determination by UV technique as no absorption bands are observed for GlcNAc and chitosan in water in the wavelength range of 200-400 nm. As such, water is unsuitable for UV spectroscopic analysis of chitosan solution [33]. The insolubility of chitosan in water may account for this observation, even as deionized water does not absorb radiation in the range of 200-400 nm of the electromagnetic spectrum.

Nevertheless, maximum absorption bands are observed in the wavelength region of 200-215 nm for GlcN and GlcNAc in acetic (CH_3COOH), hydrochloric (HCl), or phosphoric (H_3PO_4) acid as solvent [33].

The first-order UV derivative technique yields reproducible results, but the process is highly sensitive to impurities) in chitosan samples [48]. Humidity and impurities such as mineral ions and residual proteins do not affect the results of DD determination by technique, unlike in the case of ^{1}H-NMR and IR techniques though, change in solvent concentration affects the accuracy of absorbance readings [86] as such consistency in solvent concentration is essential for accurate DD determination.

Although a UV-spectrometer is readily available, its application for DD determination is limited by the high cost of standard chemicals for calibration purposes. The technique is laborious and time-consuming, requiring mathematical conversions of absorbance into differential form for first-order UV technique and construction of standard calibration curves UV technique [33].

4.4. Determination of DD value by IR spectroscopic technique.

The IR division of the electromagnetic spectrum stretches from 2.5 to 400 µm, but the portion useful for DD determination ranges from 2.5 to 25 µm (that is 4000 to 400 cm^{-1}) [111].

The IR spectrum of chitin/chitosan is usually observed between 4000 and 1200 cm^{-1} [47, 91, 112,113].

The IR technique is usually used for comparative studies of DD of chitosan from different sources due to its simplicity and fastness [47]. However, it is more suited for crystalline samples as sharper peaks are recorded for crystalline samples compared to amorphous samples [86]. It is a non-destructive technique as the sample's integrity is unaffected or unaltered in the process, unlike DD determination by elemental analyses and other destructive techniques [47].

The technique does not require the construction of calibration curves for standards, as in the case of DD determination using the UV spectrophotometric technique. The IR technique is highly sensitive to the presence of any impurities in the sample. This is why the IR spectrum has many signals or peaks. The slightest variation in sample composition results in different spectra and absorbance. Even variation in the sample's moisture level alters the accuracy and precision of DD data by IR technique [86]. As such, the sample for IR analysis is dehydrated and scanned in the solid state to eliminate interferences and enhance the precision of results.

DD determination requires measurement of absorbances (A_λ) of characteristic bands from the IR spectrum of chitosan and substitution same into any of the expressions in equations 15, 16, and 17 [47, 87, 114]. These equations have been derived and validated by DD values obtained by the ^{1}H-NMR technique.

The concentration of chitosan sample that is sufficient to elicit absorption in the IR region ranges from 1.0-10.0 mg/mL^{-1} [86], which is far smaller than the concentration or amount of chitosan required for DD determination by titrimetric techniques. Titrimetric techniques require the largest amount of chitosan sample, while the UV spectrophotometric technique requires the least amount of chitosan sample for DD determination.

Usually, the procedure entails sample preparation and scanning in the IR region to obtain a spectrum of chitosan. Demoisturization of chitosan sample and potassium bromide (KBr) is the first step in sample preparation. Thereafter, the dried sample and KBr are blended in a ratio of 1:100 by weight (g) in agate mortar using a pestle for 10 min and then compacted into disk form [48, 115-117]. The disk sample is further dehydrated before it is scanned in IR light at various wave numbers to obtain a spectrum of the chitosan sample [48].

The DD value is obtained by substituting respective absorbance values (A) of various reference bands and/or characteristic peaks of the IR spectrum in Figure 7 [87, 118] into any equations 15, 16, 18, or 19.

However, the DD value achieved by equation 15 is more reliable [47] and also closest to the DD value achieved by the ^{1}H-NMR technique, as reported in Table 9.

Equation 15 shows that the DD value of chitosan is obtained by taking the absorbances at 1655 cm^{-1} (absorption for amide-1) and 3450 cm^{-1} (absorption for hydroxyl group). Amide and hydroxyl groups' absorptions depend on the sample's deacetylated or acetylated [55]. So, the absorption of the amido group is greater than the absorption of the hydroxyl group for low-deacetylated chitosan compared to high-deacetylated chitosan. By implication, lower absorbance at 1655 cm^{-1} translates to a larger DD value.

Similarly, the absorption of the amide-1 group is lower than the absorption of the hydroxyl group for the chitosan sample compared to the chitin sample. In all, the absorbance of the amide-1 group decreases with an increase in the DD value of chitosan [30].

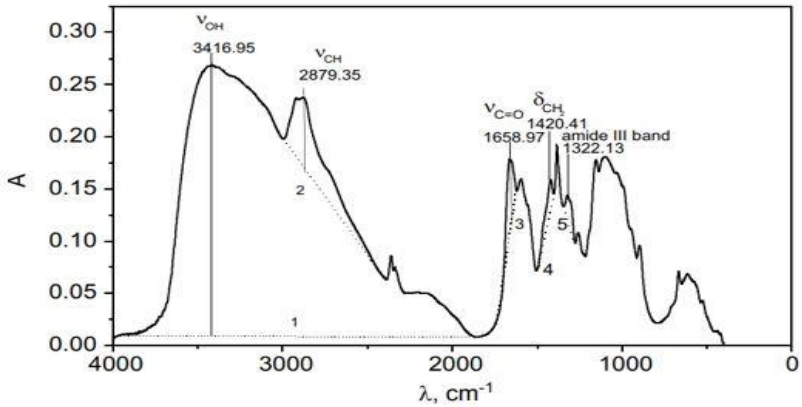


Figure 7. IR spectrum of chitosan [87, 118].

Where: A_{3450}, A_{2870}, A_{1655}, A_{1420}, and A_{1320} are absorbance values at baseline 1, 2, 3, 4, and 5, respectively [47].

$$DD\ (\%) = 100 - \left[\frac{A_{1655}}{A_{3450}} \times \frac{100}{1.33}\right] \quad (15)$$

A_{3450} = absorbance at baseline 1 for hydroxyl group absorption [119]. This absorption serves as an internal standard to correct differences in chitosan powder concentration [70], while A_{1655} = absorbance at baseline 3 for the amide group, which measures the polymer's acetyl group [115, 120-121]. The '1.33' factor in equation 15 is the quotient from 'A_{1655}/A_{3450}' for a fully acetylated compound [48, 87, 91, 114].

$$DD\ (\%) = 100 - \left[\frac{A_{1655}}{A_{3450}} \times 115\right] \quad (16)$$

$$DD\ (\%) = 100 - \left[\frac{A_{1320}}{A_{1420}} \times \frac{0.03822}{0.03133}\right]. \quad (17)$$

$$DD\ (\%) = 100 - \left[\frac{A_{1320}}{A_{1420}} \times 115\right] \quad (18)$$

The DD values obtained by equations 15 - 18 are different because varied baselines were used in the computations [37, 122]. However, the difference in the DD values computed by equations 15 and 18 is not significant.

Although some studies have revealed that DD data from IR is reproducible [47], this was not the case, as quantitative reproducibility of the spectra has been adjudged to be operator-dependent [48]. Also, establishing a baseline for individual reference bands depends on the dexterity of the IR operator.

Another factor that affects the quantitative reproducibility of DD values by IR technique is humidity, which interferes with the vibrational energies of NH_2 and OH groups [123]. For instance, the signal intensity of the reference band at 3450 cm^{-1} changes with humidity; thus, extra carefulness is required to achieve quantitative reproducibility of the spectra [48]. The humidity effect alters both the position and intensity of signals as the water content of the sample changes [76]. Therefore, proper sample preparation is paramount to ensure that the sample is moisture-free before scanning in the IR range.

The reproducibility of DD data from the IR technique is also influenced by the choice of baseline peaks, instrument type, and operation conditions [116, 122].

Although the signal peak at 2870 cm^{-1} does not change with humidity, the use of absorbance at 2870 cm $^{-1}$ instead of 1655 cm^{-1} (as in equation 19) gave DD data that are not indifferent to those obtained by ^{1}H-NMR [47, 48, 124].

$$DD\ (\%) = 100 - \left[\frac{A_{2870}}{A_{3450}} \times \frac{100}{1.33}\right] \quad (19)$$

In like manner, absorption intensity at 1320 cm^{-1} and 1420 cm^{-1} are independent of humidity and, as such, do not vary with change in the sample's moisture content [48].

Nevertheless, equation 15 gives data closest to DD data obtained by ^{1}H-NMR, whereas equation 17 gives unreliable data, as revealed by DD data reported in Table 9. [48].

Conversely, the latest studies have revealed that reliable DD data can be obtained by equation 17 [47]. So, the unreliable results reported by [48, 125] might be due to computational or systematic errors.

Table 9. Comparison of DD values derived by ^{1}H NMR and IR techniques [48, 125].

Chitosan sample	^{1}H-NMR	IR			
		by equation 15	by equation 16	by equation 17	by equation 18
CHS_{11}	79.72 ± 0.05	76.61 ± 1.92	64.49 ± 7.14	-8.50 ± 13.03	-
CHS_{12}	81.27 ± 0.19	77.84 ± 0.28	74.60 ± 3.60	-12.50 ±23.30	-
CHS_{13}	89.44 ± 0.32	85.61 ± 2.04	89.50 ± 5.85	43.86 ± 9.19	-
CHS_{14}	80.95± 1.16	98.69 ± 0.45	-	74.32 ± 1.86	97.99 ± 0.69

4.5. Determination of DD value by elemental analysis (EA).

This destructive technique requires sample combustion at very high temperatures [126-127]. Usually, sample combustion is performed at a temperature of 1000°C to give species that are carried on helium gas flowing at 100 mL/min for elemental analyses [127]. The weight percent of C and N are determined while the DD value is calculated using either equations 20 or 21 [86, 93, 126, 128-132].

$$DD(\%) = 100 - \left[\frac{(C/N - 5.14)}{1.72}\right] \times 100 \qquad (20)$$

$$DD(\%) = \left[6.857 - \frac{(C/N)}{1.7143}\right] \times 100 \qquad (21)$$

Where C = weight percent of carbon; N = Weight percent of nitrogen content in chitosan

Since there are two different nitrogen atoms in chitosan (DD < 100 %), viz: nitrogen atoms of amino and acetamido groups, the levels of these nitrogen atoms vary based on the extent to which the sample had been deacetylated. For fully N-deacetylated chitosan ($C_6H_{11}O_4N$ as repeat unit), percent-nitrogen is 8.696 %, while for completely N-acetylated chitin ($C_8H_{13}O_5N$ as repeat unit), percent-nitrogen is 6.897 % [133-134]. The ratios of carbon to nitrogen (C/N) for fully N-deacetylated chitosan and completely N-acetylated chitin are 5.145 and 6.861, respectively [86, 133]. Thus, the weight percent of carbon in fully N-deacetylated chitosan is approximately 44.741 %, while that of completely N-acetylated chitin is 47.320 %.

Moreover, the weight percent of C and N by elemental analyses have been utilized individually, not as a ratio, to calculate the DD value by equations 22 and 23 [64, 135].

$$DD\ (\%) = \left[\frac{9600}{(364 \times C + 2400)}\right] \times 100 \qquad (22)$$

$$DD\ (\%) = \left[\frac{1400}{(364 \times N)}\right] \times 100 \qquad (23)$$

Although different elemental weight percent was used, the DD values obtained by equations 21 and 22 were statistically similar [30] and had good agreement with those obtained by titrimetric and IR techniques, as revealed by DD data in Table 10.

The determination of DD by the elemental analysis technique is not as common as that of IR and titrimetric techniques, but the technique is simple, cost-effective, and gives reliable DD values. This is because the DD value obtained by this technique is comparable to those obtained by ^{1}H-NMR spectroscopy [93]. Table 10 shows that the DD value obtained by

elemental analysis (EA) is closer to that obtained by ^{1}H-NMR than other techniques like IR and titration.

In addition, DD determination by elemental analyzer does not require the use of standard solutions of GlcNAc and GlcN, which are expensive. Also, the technique is suitable for resolving the entire range of DD for both crystalline and amorphous samples of chitosan.

Table 10. Results of DD determination by different analytical techniques [30, 93].

Chitosan sample	Analytical techniques					
	^{1}H-NMR	FTIR	EA_1	EA_2	EA_3	Acid-base titration
CHS_6	95.0	65.0±3.0	85.0±3.0	-	-	75.0±2.5
CHS_{15}	-	55.80	-	55.80	55.66	54.60
CHS_{16}	-	61.30	-	61.30	60.85	60.50
CHS_{17}	-	85.40	-	85.30	85.85	84.70

Like some spectroscopic techniques, the EA technique is highly sensitive to impurities in chitosan samples, which might cause it to give an overestimated DD value, especially if the impurities are of organic materials and/or carbohydrates besides chitosan [136]. Proteins and pigments are other impurities that may cause an overestimated DD value in a contaminated sample. This is because organic impurities result in a noticeable increase in percent C and N.

Also, it is a long-term technique as it requires sample decomposition before analyses of resulting species [41, 86].

5. Conclusion

Based on the previous, the ^{1}H-NMR is the most reliable of the techniques reviewed in this study. It is also the most expensive and time-consuming technique. Next, in terms of reliability, is the UV/first-order derivative UV spectrophotometry, followed by elemental analyses, titrimetric, and FTIR techniques. The titrimetric technique is simple and cost-effective but time-consuming as well.

The first-order UV-visible spectrophotometric technique is also cheaper than the ^{1}H-NMR technique. Still, it requires a lot of time to construct calibration curves using an expensive standard solution of GlcNAc.

The IR technique is cost-effective relative to ^{1}H-NMR and the fastest among the reviewed techniques. It only required measurements of absorbance ratios of characteristic bands from the IR spectrum of the chitosan sample. However, the reproducibility of DD data by IR technique is highly influenced by the choice of baseline peaks, instrument type, and operation conditions.

It is, indeed, a tedious task to select appropriate techniques for DD determination. It is, therefore, desirable to consider the time of measurement, precision, accuracy of measurement results, and even the cost-effectiveness when choosing a technique to use for DD determination**.**

Funding

This research received no external funding

Acknowledgments

The authors thank Lovely Federal University of Petroleum Resources, Effurun for providing the necessary facilities to carry out this research.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Posada-Velez, M.C.; Pineda-Gomez, P.; Martinez-Hernandez, H.D. Acetylated corn and potato starches as an alternative to the toxic inorganic coagulants/flocculants for wastewater treatment. *Environ. Nanotechnol. Monit. Manag*. **2023**, *20*, 100786, https://doi.org/10.1016/j.enmm.2023.100786.
2. Desbrières, J.; Guibal, E. Chitosan for wastewater treatment. *Polym. Int.* **2018**, *67*, 7–14, https://doi.org/10.1002/pi.5464.
3. Mohammadzadeh Pakdel, P.; Peighambardoust, S.J. Review on recent progress in chitosan-based hydrogels for wastewater treatment application. *Carbohydr. Polym.* **2018**, *201*, 264-279, https://doi.org/10.1016/j.carbpol.2018.08.070.
4. Colijn, I.; Yanat, M.; Terhaerdt, G.; Molenveld, K.; Boeriu, C.G.; Schroën, K. Chitin Nanocrystal Hydrophobicity Adjustment by Fatty Acid Esterification for Improved Polylactic Acid Nanocomposites. *Polymers* **2022**, *14*, 2619, https://doi.org/10.3390/polym14132619.
5. Yanat, M.; Colijn, I.; Schroën, K. Chitin Nanocrystals Provide Antioxidant Activity to Polylactic Acid Films. *Polymers* **2022**, *14*, 2965, https://doi.org/10.3390/polym14142965.
6. Iqbal, Y.; Ahmed, I.; Irfan, M.F.; Chatha, S.A.S.; Zubair, M.; Ullah, A. Recent advances in chitosan-based materials; The synthesis, modifications and biomedical applications. *Carbohydr. Polym.* **2023**, *321*, 121318, https://doi.org/10.1016/j.carbpol.2023.121318.
7. Liu, H.; Wang, C.; Li, C.; Qin, Y.; Wang, Z.; Yang, F.; Li, Z.; Wang, J. A functional chitosan-based hydrogel as a wound dressing and drug delivery system in the treatment of wound healing. *RSC Adv.* **2018**, *8*, 7533–7549, https://doi.org/10.1039/C7RA13510F.
8. Naseri-Nosar, M.; Ziora, Z.M. Wound dressings from naturally-occurring polymers: A review on homopolysaccharide-based composites. *Carbohydr. Polym.* **2018**, *189*, 379-398, https://doi.org/10.1016/j.carbpol.2018.02.003.
9. Hamedi, H.; Moradi, S.; Hudson, S.M.; Tonelli, A.E. Chitosan based hydrogels and their applications for drug delivery in wound dressings: A review. *Carbohydr. Polym.* **2018**, *199*, 445–460, https://doi.org/10.1016/j.carbpol.2018.06.114.
10. Sánchez-Machado, D.I.; López-Cervantes, J.; Martínez-Ibarra, D.M.; Escárcega-Galaz, A.A.; Vega-Cázarez, C.A. The use of chitosan as a skin-regeneration agent in burns injuries: A review. *e-Polymers* **2022**, *22*, 75-86, https://doi.org/10.1515/epoly-2022-0011.
11. Hameed, A.Z.; Raj, S.A.; Kandasamy, J.; Baghdadi, M.A.; Shahzad, M.A. Chitosan: A Sustainable Material for Multifarious Applications. *Polymers* **2022**, *14*, 2335, https://doi.org/10.3390/polym14122335.
12. Shaabani, A.; Bizari, D.; Khoshmohabat, H. PEGylated curcumin-loaded *poly(vinyl alcohol)*/Zwitterionic poly(sulfobetaine vinylimidazole)-grafted chitosan nanofiber as a second-degree burn wound dressing. *Carbohydr. Polym.* **2023**, *321*, 121307, https://doi.org/10.1016/j.carbpol.2023.121307.
13. Yao, X.; Lin, R.; Liang, Y.; Jiao, S.; Zhong, L. Characterization of acetylated starch nanoparticles for potential use as an emulsion stabilizer. *Food Chem.* **2023**, *400*, 133873, https://doi.org/10.1016/j.foodchem.2022.133873.
14. Fitch-Vargas, P.R.; Camacho-Hernández, I.L.; Rodríguez-González, F.J.; Martínez-Bustos, F.; Calderón-Castro, A.; Zazueta-Morales, J.d.J.; Aguilar-Palazuelos, E. Effect of compounding and plastic processing methods on the development of bioplastics based on acetylated starch reinforced with sugarcane bagasse cellulose fibers. *Ind. Crops Prod*. **2023**, *192*, 116084, https://doi.org/10.1016/j.indcrop.2022.116084.
15. Habibie, S.; Hamzah, M.; Anggaravidya, M.; Kalembang, E. The effect of chitosan on physical and mechanical properties of paper. *J. Chem. Eng. Mater. Sci.* **2016**, *7*, 1-10, https://doi.org/10.5897/JCEMS2015.0235.
16. Watcharakitti, J.; Win, E.E.; Nimnuan, J.; Smith, S.M. Modified Starch-Based Adhesives: A Review. *Polymers* **2022**, *14*, 2023, https://doi.org/10.3390/polym14102023.
17. Li, H.; Wang, Y.; Zhao, P.; Guo, L.; Huang, L.; Li, X.; Gao, W. Naturally and chemically acetylated polysaccharides: Structural characteristics, synthesis, activities, and applications in the delivery system: A review. *Carbohydr. Polym.* **2023**, *313*, 120746, https://doi.org/10.1016/j.carbpol.2023.120746.

18. Laura, O.; Beatrice, C.; Cinzia, F.; Luca, R. Chitosan in Agriculture: A New Challenge for Managing Plant Disease. In Biological Activities and Application of Marine Polysaccharides, Emad, A.S., Ed.; IntechOpen, Rijeka, **2017**, 17–36, https://doi.org/10.5772/66840.
19. Mwaheb, M.A.M.A.; Hussain, M.; Tian, J.; Zhang, X.; Hamid, M.I.; El-Kassim, N.A.; Hassan, G.M.; Xiang, M.; Liu, X. Synergetic suppression of soybean cyst nematodes by chitosan and *Hirsutella minnesotensis* via the assembly of the soybean rhizosphere microbial communities. *Biol. Control* **2017**, *115*, 85-94, https://doi.org/10.1016/j.biocontrol.2017.09.011.
20. Hosseinnejad, M.; Jafari, S.M. Evaluation of different factors affecting antimicrobial properties of chitosan. *Int. J. Biol. Macromol.* **2016**, *85*, 467-475, https://doi.org/10.1016/j.ijbiomac.2016.01.022.
21. Sudha, P.N.; Aisverya, S.; Gomathi, T.; Vijayalakshmi, K.; Saranya, M.; Sangeetha, K.; Latha, S.; Thomas, S. Application of Chitin/Chitosan and Its Derivatives as Adsorbents, Coagulants, and Flocculants. In Chitosan: Derivatives, Composites and Applications; Ahmed, S., Ikram, S., Eds.; Scrivener Publishing LLC, **2017**, 453–487, https://doi.org/10.1002/9781119364849.ch17.
22. Yanat, M.; Colijn, I.; de Boer, K.; Schroën, K. Comparison of the Degree of Acetylation of Chitin Nanocrystals Measured by Various Analysis Methods. *Polymers* **2023**, *15*, 294, https://doi.org/10.3390/polym15020294.
23. Mahmoud, M.G.; El Kady, E.M.; Asker, M.S. Chitin, chitosan and glucan, properties and applications. *World J. Agric. Soil Sci*. **2019**, *3*, 1–19, https://doi.org/10.33552/WJASS.2019.03.000553.
24. Kedir, W.M.; Abdi, G.F.; Goro, M.M.; Tolesa, L.D. Pharmaceutical and drug delivery applications of chitosan biopolymer and its modified nanocomposite: A review. *Heliyon* **2022**, *8*, e10196, https://doi.org/10.1016/j.heliyon.2022.e10196.
25. Varun, T.K.; Senani, S.; Jayapal, N.; Chikkerur, J.; Roy, S.; Tekulapally, V.B.; Gautam, M.; Kumar, N. Extraction of chitosan and its oligomers from shrimp shell waste, their characterization and antimicrobial effect. *Vet. World* **2017**, *10*, 170-175, https://doi.org/10.14202%2Fvetworld.2017.170-175.
26. Cheng, J.; Zhu, H.; Huang, J.; Zhao, J.; Yan, B.; Ma, S.; Zhang, H.; Fan, D. The physicochemical properties of chitosan prepared by microwave heating. *Food Sci. Nutr.* **2020**, *8*, 1987-1994, https://doi.org/10.1002/fsn3.1486.
27. Costa, E.M.; Silva, S.; Pina, C.; Tavaria, F.K.; Pintado, M.M. Evaluation and insights into chitosan antimicrobial activity against anaerobic oral pathogens. *Anaerobe* **2012**, *18*, 305-309, https://doi.org/10.1016/j.anaerobe.2012.04.009.
28. Costa, E.M.; Silva, S.; Madureira, A.R.; Cardelle-Cobas, A.; Tavaria, F.K.; Pintado, M.M. A comprehensive study into the impact of a chitosan mouthwash upon oral microorganism's biofilm formation *in vitro*. *Carbohydr. Polym.* **2014**, *101*, 1081-1086, https://doi.org/10.1016/j.carbpol.2013.09.041.
29. Ardean, C.; Davidescu, C.M.; Nemeş, N.S.; Negrea, A.; Ciopec, M.; Duteanu, N.; Negrea, P.; Duda-Seiman, D.; Musta, V. Factors Influencing the Antibacterial Activity of Chitosan and Chitosan Modified by Functionalization. *Int. J. Mol. Sci.* **2021**, *22*, 7449, https://doi.org/10.3390/ijms22147449.
30. Hussain, R.; Iman, M.; Maji, T.K. Determination of Degree of Deacetylation of Chitosan and Their effect on the Release Behavior of Essential Oil from Chitosan and Chitosan-Gelatin Complex Microcapsules. *Int. J. Adv. Eng. Appl.* **2013**, *2*, 4-12.
31. Younes, I.; Rinaudo, M. Chitin and Chitosan Preparation from Marine Sources. Structure, Properties and Applications. *Mar. Drugs* **2015**, *13*, 1133-1174, https://doi.org/10.3390/md13031133.
32. Jana, S.; Saha, A.; Nayak, A.K.; Sen, K.K.; Basu, S.K. Aceclofenac-loaded chitosan-tamarind seed polysaccharide interpenetrating polymeric network microparticles. *Colloids. Surf. B: Biointerfaces* **2013**, *105*, 303–309, https://doi.org/10.1016/j.colsurfb.2013.01.013.
33. Jiang, Y.; Fu, C.; Wu, S.; Liu, G.; Guo, J.; Su, Z. Determination of the Deacetylation Degree of Chitooligosaccharides. *Mar. Drugs* **2017**, *15*, 332, https://doi.org/10.3390/md15110332.
34. Kumar, V.; Sharma, N.; Janghu, P.; Pasrija, R.; Umesh, M.; Chakraborty, P.; Sarojini, S.; Thomas, J. Synthesis and characterization of chitosan nanofibers for wound healing and drug delivery application. *J. Drug Deliv. Sci. Technol.* **2023**, *87*, 104858, https://doi.org/10.1016/j.jddst.2023.104858.
35. Hasan, S.; Boddu, V.M.; Viswanath, D.S.; Ghosh, T.K. Chitin and Chitosan: Science and Engineering; Springer, **2022**.
36. Lv, S.H. 7 - High-performance superplasticizer based on chitosan. In Biopolymers and Biotech Admixtures for Eco-Efficient Construction Materials, Pacheco-Torgal, F., Ivanov, V., Karak, N., Jonkers, H., Eds.; Woodhead Publishing, **2016**; 131–150, https://doi.org/10.1016/B978-0-08-100214-8.00007-5.

37. Baxter, A.; Dillon, M.; Anthony Taylor, K.D.; Roberts, G.A.F. Improved method for i.r. determination of the degree of N-acetylation of chitosan. *Int. J. Biol. Macromol.* **1992**, *14*, 166-169, https://doi.org/10.1016/S0141-8130(05)80007-8.
38. Li, J.; Revol, J.F.; Marchessault, R.H. Effect of degree of deacetylation of chitin on the properties of chitin crystallites. *J. Appl. Polym. Sci.* **1997**, *65*, 373–380, https://doi.org/10.1002/(SICI)1097-4628(19970711)65:2%3C373::AID-APP18%3E3.0.CO;2-0.
39. Cheung, R.C.F.; Ng, T.B.; Wong, J.H.; Chan, W.Y. Chitosan: An Update on Potential Biomedical and Pharmaceutical Applications. *Mar. Drugs* **2015**, *13*, 5156-5186, https://doi.org/10.3390/md13085156.
40. Pochanavanich, P.; Suntornsuk, W. Fungal chitosan production and its characterization. *Lett. Appl. Microbiol.* **2002**, *35*, 17–21, https://doi.org/10.1046/j.1472-765X.2002.01118.x.
41. Banks, I.R.; Specht, C.A.; Donlin, M.J.; Gerik, K.J.; Levitz, S.M.; Lodge, J.K. A Chitin Synthase and Its Regulator Protein Are Critical for Chitosan Production and Growth of the Fungal Pathogen *Cryptococcus neoformans*. *Eukaryot Cell* **2005**, *4*, 1902–1912, https://doi.org/10.1128%2FEC.4.11.1902-1912.2005.
42. Dewick, P.M. Medicinal Natural Products: A Biosynthetic Approach. John Wiley & Sons, **2009**; https://doi.org/10.1002/9780470742761.
43. Karty, J. Organic Chemistry, Principles and Mechanism, 1st Edition. W.W. Norton & Company, **2014**.
44. Ng, C.-H.; Hein, S.; Ogawa, K.; Chandrkrachang, S.; Stevens, W.F. Distribution of D-glucosamine moieties in heterogeneously deacetylated cuttlefish chitin. *Carbohydr. Polym.* **2007**, *69*, 382–390, https://doi.org/10.1016/j.carbpol.2006.12.021.
45. Rinaudo, M. Main properties and current applications of some polysaccharides as biomaterials. *Polym. Int.* **2008**, *57*, 397–430, https://doi.org/10.1002/pi.2378.
46. Raafat, D.; Sahl, H.-G. Chitosan and its antimicrobial potential - a critical literature survey. *Microb. Biotechnol.* **2009**, *2*, 186-201, https://doi.org/10.1111/j.1751-7915.2008.00080.x.
47. Boukhlifi, F. Quantitative Analysis by IR: Determination of Chitin/Chitosan DD. In Modern Spectroscopic Techniques and Applications, Maaz, K., Gustavo Morari do, N., Marwa, E.-A., Eds.; IntechOpen: Rijeka, **2020**; 107-131, https://doi.org/10.5772/intechopen.89708.
48. Czechowska-Biskup, R.; Jarosińska, D.; Rokita, B.; Ulański, P.; Rosiak, J.M. Determination of degree of deacetylation of chitosan -comparision of methods. *Prog. Chem. Appl.Chitin Deriv.* **2012**, *17*, 5-20.
49. Aiba, S.-i. Studies on chitosan: 1. Determination of the degree of *N*-acetylation of chitosan by ultraviolet spectrophotometry and gel permeation chromatography. *Int. J. Biol. Macromol.* **1986**, *8*, 173–176, https://doi.org/10.1016/0141-8130(86)90022-x.
50. Tan, C.H.; He, W.X.; Meng, H.Y.; Huang, X.G. A new method based on fiber-optic sensing for the determination of deacetylation degree of chitosans. *Carbohydr. Res.* **2012**, *348*, 64-68, https://doi.org/10.1016/j.carres.2011.04.047.
51. Wu, T.; Zivanovic, S. Determination of the degree of acetylation (DA) of chitin and chitosan by an improved first derivative UV method. *Carbohydr. Polym.* **2008**, *73*, 248–253, https://doi.org/10.1016/j.carbpol.2007.11.024.
52. Dash, M.; Chiellini, F.; Fernandez, E.G.; Piras, A.M.; Chiellini, E. Statistical approach to the spectroscopic determination of the deacetylation degree of chitins and chitosans. *Carbohydr. Polym.* **2011**, *86*, 65-71, https://doi.org/10.1016/j.carbpol.2011.04.010.
53. Dharani, M.; Balasubramanian, S. Synthesis, characterization and application of acryloyl chitosan anchored copolymer towards algae flocculation. *Carbohydr. Polym.* **2016**, *152*, 459-467, https://doi.org/10.1016/j.carbpol.2016.07.031.
54. Arata Badano, J.; Vanden Braber, N.; Rossi, Y.; Díaz Vergara, L.; Bohl, L.; Porporatto, C.; Falcone, R.D.; Montenegro, M. Physicochemical, *in vitro* antioxidant and cytotoxic properties of water-soluble chitosan-lactose derivatives. *Carbohydr. Polym.* **2019**, *224*, 115158, https://doi.org/10.1016/j.carbpol.2019.115158.
55. Shigemasa, Y.; Matsuura, H.; Sashiwa, H.; Saimoto, H. Evaluation of different absorbance ratios from infrared spectroscopy for analyzing the degree of deacetylation in chitin. *Int. J. Biol. Macromol.* **1996**, *18*, 237-242, https://doi.org/10.1016/0141-8130(95)01079-3.
56. Yadav, M.; Kaushik, B.; Rao, G.K.; Srivastava, C.M.; Vaya, D. Advances and challenges in the use of chitosan and its derivatives in biomedical fields: A review. *Carbohydr. Polym. Technol. Appl.* **2023**, *5*, 100323, https://doi.org/10.1016/j.carpta.2023.100323.
57. Mohamed, R.R. 3 - Chitin and chitosan as adsorbents. In Natural Polymers-Based Green Adsorbents for Water Treatment, Kalia, S., Ed.; Elsevier, **2021**; 73-91, https://doi.org/10.1016/B978-0-12-820541-9.00004-1.

58. Upadhyay, U.; Sreedhar, I.; Singh, S.A.; Patel, C.M.; Anitha, K.L. Recent advances in heavy metal removal by chitosan based adsorbents. *Carbohydr. Polym.* **2021**, *251*, 117000, https://doi.org/10.1016/j.carbpol.2020.117000.
59. Matica, M.A.; Aachmann, F.L.; Tøndervik, A.; Sletta, H.; Ostafe, V. Chitosan as a Wound Dressing Starting Material: Antimicrobial Properties and Mode of Action. *Int. J. Mol. Sci.* **2019**, *20*, 5889, https://doi.org/10.3390/ijms20235889.
60. Xing, K.; Zhu, X.; Peng, X.; Qin, S. Chitosan antimicrobial and eliciting properties for pest control in agriculture: a review. *Agron. Sustain. Dev.* **2015**, *35*, 569–588, https://doi.org/10.1007/s13593-014-0252-3.
61. Chandrasekaran, M.; Kim, K.D.; Chun, S.C. Antibacterial Activity of Chitosan Nanoparticles: A Review. *Processes* **2020**, *8*, 1173, https://doi.org/10.3390/pr8091173.
62. Kong, M.; Chen, X.G.; Liu, C.S.; Liu, C.G.; Meng, X.H.; Yu, L.J. Antibacterial mechanism of chitosan microspheres in a solid dispersing system against *E. coli*. *Colloids Surf. B Biointerfaces* **2008**, *65*, 197–202, https://doi.org/10.1016/j.colsurfb.2008.04.003.
63. Hao, Y.; Zhang, M.; Wang, L.; Tao, N.; Li, L.; Zhu, W.; Xu, C.; Deng, S.; Wang, Y. Mechanism of antimicrobials immobilized on packaging film inhabiting foodborne pathogens. *LWT* **2022**, *169*, 114037, https://doi.org/10.1016/j.lwt.2022.114037.
64. Gupta, K.C.; Jabrail, F.H. Effects of degree of deacetylation and cross-linking on physical characteristics, swelling and release behavior of chitosan microspheres. *Carbohydr. Polym.* **2006**, *66*, 43–54, https://doi.org/10.1016/j.carbpol.2006.02.019.
65. Mourya, V.K.; Inamdar, N.N.; Choudhari, Y.M. Chitooligosaccharides: Synthesis, characterization and applications. *Polym. Sci. Ser. A* **2011**, *53*, 583–612, https://doi.org/10.1134/S0965545X11070066.
66. Tolaimate, A.; Desbrieres, J.; Rhazi, M.; Alagui, A. Contribution to the preparation of chitins and chitosans with controlled physico-chemical properties. *Polymer* **2003**, *44*, 7939-7952, https://doi.org/10.1016/j.polymer.2003.10.025.
67. Taghizadeh, S.M.; Davari, G. Preparation, characterization, and swelling behaviour of *N*-acetylated and deacetylated chitosan. *Carbohydr. Polym.* **2006**, *64*, 9-15, https://doi.org/10.1016/j.carbpol.2005.10.037.
68. Chebotok, E.N.; Novikov, V.Y.; Konovalova, I.N. Depolymerization of chitin and chitosan in the course of base deacetylation. *Russ. J. Appl. Chem.* **2006**, *79*, 1162-1166, https://doi.org/10.1134/S1070427206070238.
69. Pérez-Álvarez, L.; Ruiz-Rubio, L.; Vilas-Vilela, J.L. Determining the Deacetylation Degree of Chitosan: Opportunities To Learn Instrumental Techniques. *J. Chem. Educ.* **2018**, *95*, 1022–1028, https://doi.org/10.1021/acs.jchemed.7b00902.
70. Khan, T.A.; Peh, K.K.; Ch'ng, H.S. Reporting degree of desacetylation values of chitosan: the influence of analytical methods. *J. Pharm. Pharm. Sci.* **2002**, *5*, 205-212.
71. Zając, A.; Hanuza, J.; Wandas, M.; Dymińska, L. Determination of N-acetylation degree in chitosan using Raman spectroscopy. *Spectrochim. Acta - A: Mol. Biomol. Spectrosc.* **2015**, *134*, 114–120, https://doi.org/10.1016/j.saa.2014.06.071.
72. da Silva, R.M.P.; Mano, J.F.; Reis, R.L. Straightforward Determination of the Degree of *N*-Acetylation of Chitosan by Means of First-Derivative UV Spectrophotometry. *Macromol. Chem. Phys.* **2008**, *209*, 1463–1472, https://doi.org/10.1002/macp.200800191.
73. Hein, S.; Ng, C.-H.; Stevens, W.F.; Wang, K. Selection of a practical assay for the determination of the entire range of acetyl content in chitin and chitosan: UV spectrophotometry with phosphoric acid as solvent. *J. Biomed. Mater. Res. Part B* **2008**, *86B*, 558-568, https://doi.org/10.1002/jbm.b.31056.
74. Thevarajah, J.J.; Van Leeuwen, M.P.; Cottet, H.; Castignolles, P.; Gaborieau, M. Determination of the distributions of degrees of acetylation of chitosan. *Int. J. Biol. Macromol.* **2017**, *95*, 40–48, https://doi.org/10.1016/j.ijbiomac.2016.10.056.
75. Dimzon, I.K.D.; Knepper, T.P. Degree of deacetylation of chitosan by infrared spectroscopy and partial least squares. *Int. J. Biol. Macromol.* **2015**, *72*, 939–945, https://doi.org/10.1016/j.ijbiomac.2014.09.050.
76. Lavertu, M.; Xia, Z.; Serreqi, A.N.; Berrada, M.; Rodrigues, A.; Wang, D.; Buschmann, M.D.; Gupta, A. A validated ^{1}H NMR method for the determination of the degree of deacetylation of chitosan. *J. Pharm. Biomed. Anal.* **2003**, *32*, 1149–1158, https://doi.org/10.1016/S0731-7085(03)00155-9.
77. Kumirska, J.; Czerwicka, M.; Kaczyński, Z.; Bychowska, A.; Brzozowski, K.; Thöming, J.; Stepnowski, P. Application of Spectroscopic Methods for Structural Analysis of Chitin and Chitosan. *Mar. Drugs* **2010**, *8*, 1567-1636, https://doi.org/10.3390/md8051567.

78. Vårum, K.M.; Antohonsen, M.W.; Grasdalen, H.; Smidsrød, O. Determination of the degree of *N*-acetylation and the distribution of *N*-acetyl groups in partially *N*-deacetylated chitins (chitosans) by high-field n.m.r. spectroscopy. *Carbohydr. Res.* **1991**, *211*, 17–23, https://doi.org/10.1016/0008-6215(91)84142-2.
79. Lamarque, G.; Viton, C.; Domard, A. Comparative Study of the First Heterogeneous Deacetylation of α- and β-Chitins in a Multistep Process. *Biomacromolecules* **2004**, *5*, 992–1001, https://doi.org/10.1021/bm034498j.
80. Tan, S.C.; Khor, E.; Tan, T.K.; Wong, S.M. The degree of deacetylation of chitosan: advocating the first derivative UV-spectrophotometry method of determination. *Talanta* **1998**, *45*, 713–719, https://doi.org/10.1016/S0039-9140(97)00288-9.
81. Dutta, J.; Priyanka. A facile approach for the determination of degree of deacetylation of chitosan using acid-base titration. *Heliyon* **2022**, *8*, e09924, https://doi.org/10.1016/j.heliyon.2022.e09924.
82. Wang, C.; Yuan, F.; Pan, J.; Jiao, S.; Jin, L.; Cai, H. A novel method for the determination of the degree of deacetylation of chitosan by coulometric titration. *Int. J. Biol. Macromol.* **2014**, *70*, 306–311, https://doi.org/10.1016/j.ijbiomac.2014.07.007.
83. Tolaimate, A.; Desbrières, J.; Rhazi, M.; Alagui, A.; Vincendon, M.; Vottero, P. On the influence of deacetylation process on the physicochemical characteristics of chitosan from squid chitin. *Polymer* **2000**, *41*, 2463–2469, https://doi.org/10.1016/S0032-3861(99)00400-0.
84. Han, Z.; Zeng, Y.; Lu, H.; Zhang, L. Determination of the degree of acetylation and the distribution of acetyl groups in chitosan by HPLC analysis of nitrous acid degraded and PMP labeled products. *Carbohydr. Res.* **2015**, *413*, 75–84, https://doi.org/10.1016/j.carres.2015.03.002.
85. Guinesi, L.S.; Cavalheiro, É.T.G. The use of DSC curves to determine the acetylation degree of chitin/chitosan samples. *Thermochim. Acta* **2006**, *444*, 128–133, https://doi.org/10.1016/j.tca.2006.03.003.
86. Kasaai, M.R. Various Methods for Determination of the Degree of N-Acetylation of Chitin and Chitosan: A Review. *J. Agric. Food Chem.* **2007**, *57*, 1667–1676, https://doi.org/10.1021/jf803001m.
87. Brugnerotto, J.; Lizardi, J.; Goycoolea, F.M.; Argüelles-Monal, W.; Desbrières, J.; Rinaudo, M. An infrared investigation in relation with chitin and chitosan characterization. *Polymer* **2001**, *42*, 3569–3580, https://doi.org/10.1016/S0032-3861(00)00713-8.
88. Hirai, A.; Odani, H.; Nakajima, A. Determination of degree of deacetylation of chitosan by ^{1}H NMR spectroscopy. *Polym. Bull.* **1991**, *26*, 87–94, https://doi.org/10.1007/BF00299352.
89. Ottey, M.H.; Vårum, K.M.; Smidsrød, O. Compositional heterogeneity of heterogeneously deacetylated chitosans. *Carbohydr. Polym.* **1996**, *29*, 17–24, https://doi.org/10.1016/0144-8617(95)00154-9.
90. Nwe, N.; Furuike, T.; Tamura, H. CHITOSAN FROM AQUATIC AND TERRESTRIAL ORGANISMS AND MICROORGANISMS: PRODUCTION, PROPERTIES AND APPLICATIONS. In Biodegradable Materials, Johnson, B.M., Berkel, Z.E., Eds.; Nova Science Publishers, **2011**; 29-48.
91. Kasaai, M.R. A review of several reported procedures to determine the degree of *N*-acetylation for chitin and chitosan using infrared spectroscopy. *Carbohydr. Polym.* **2008**, *71*, 497–508, https://doi.org/10.1016/j.carbpol.2007.07.009.
92. American Standard Test Method Organization. *Patent No. 13.01, F2260-03.* United states of America, **2003**.
93. Abdel-Rahman, R.M.; Hrdina, R.; Abdel-Mohsen, A.M.; Fouda, M.M.G.; Soliman, A.Y.; Mohamed, F.K.; Mohsin, K.; Pinto, T.D. Chitin and chitosan from Brazilian Atlantic Coast: Isolation, characterization and antibacterial activity. *Int. J. Biol. Macromol.* **2015**, *80*, 107-120, https://doi.org/10.1016/j.ijbiomac.2015.06.027.
94. United States Pharmacopeial Convention. *American Pharmacopoeia* (Vol. 36). New York, USA, **2012**: The United States Pharmacopeial convention.
95. European Pharmacopoeia Commission. *European Pharmacopoeia* (Vol. 65). Paris, France, **2013**: European Directorate for the Quality Control of Medicines.
96. Chinese Pharmacopoeia Commission. *Chinese Pharmacopoeia* (Vol. 6). Beijing, China, **2015**: Chemical Industry Press.
97. Vårum, K.M.; Antohonsen, M.W.; Grasdalen, H.; Smidsrød, O. Determination of the degree of *N*-acetylation and the distribution of *N*-acetyl groups in partially *N*-deacetylated chitins (chitosans) by high-field n.m.r. spectroscopy. *Carbohydr. Res.* **1991**, *211*, 17–23, https://doi.org/10.1016/0008-6215(91)84142-2.

98. Kasaai, M.R. Determination of the degree of *N*-acetylation for chitin and chitosan by various NMR spectroscopy techniques: A review. *Carbohydr. Polym.* **2010**, *79*, 801-810, https://doi.org/10.1016/j.carbpol.2009.10.051.
99. Heux, L.; Brugnerotto, J.; Desbrières, J.; Versali, M.F.; Rinaudo, M. Solid State NMR for Determination of Degree of Acetylation of Chitin and Chitosan. *Biomacromolecules* **2000**, *1*, 746–751, https://doi.org/10.1021/bm000070y.
100. Yu, G.-e.; Morin, F.G.; Nobes, G.A.R.; Marchessault, R.H. Degree of Acetylation of Chitin and Extent of Grafting PHB on Chitosan Determined by Solid State ^{15}N NMR. *Macromolecules* **1999**, *32*, 518–520, https://doi.org/10.1021/ma9813338.
101. de Abreu, F.R.; Campana-Filho, S.P. Preparation and characterization of carboxymethylchitosan. *Polímeros: Ciência e Tecnologia* **2005**, *15*, 79-83.
102. Kim, S.-K. Chitin, Chitosan, Oligosaccharides and Their Derivatives: Biological Activities and Applications, 1st Edition. CRC Press, Boca Raton, **2010**; https://doi.org/10.1201/EBK1439816035.
103. Sivashankari, P.R.; Prabaharan, M. 5 - Deacetylation modification techniques of chitin and chitosan. In Chitosan Based Biomaterials Volume 1, Jennings, J.A., Bumgardner, J.D., Eds.; Woodhead Publishing, **2017**, 117-133, https://doi.org/10.1016/B978-0-08-100230-8.00005-4.
104. Jia, Z.; Shen, D. Effect of reaction temperature and reaction time on the preparation of low-molecular-weight chitosan using phosphoric acid. *Carbohydr. Polym.* **2002**, *49*, 393-396, https://doi.org/10.1016/S0144-8617(02)00026-7.
105. Hossain, S.; Uddin, M.K. ISOLATION AND EXTRACTION OF CHITOSAN FROM SHRIMP SHELLS. *Int. J. Adv. Res.* **2020**, *8*, 657–664, http://dx.doi.org/10.21474/IJAR01/11704.
106. Zhang, Y.; Zhang, X.; Ding, R.; Zhang, J.; Liu, J. Determination of the degree of deacetylation of chitosan by potentiometric titration preceded by enzymatic pretreatment. *Carbohydr. Polym.* **2011**, *83*, 813-817, https://doi.org/10.1016/j.carbpol.2010.08.058.
107. Arcidiacono, S.; Kaplan, D.L. Molecular weight distribution of chitosan isolated from *Mucor rouxii* under different culture and processing conditions. *Biotechnol. Bioeng.* **1992**, *39*, 281–286, https://doi.org/10.1002/bit.260390305.
108. Curotto, E.; Aros, F. Quantitative Determination of Chitosan and the Percentage of Free Amino Groups. *Anal. Biochem.* **1993**, *211*, 240–241, https://doi.org/10.1006/abio.1993.1263.
109. Liu, D.; Wei, Y.; Yao, P.; Jiang, L. Determination of the degree of acetylation of chitosan by UV spectrophotometry using dual standards. *Carbohydr. Res.* **2006**, *341*, 782-785, https://doi.org/10.1016/j.carres.2006.01.008.
110. Jiang, X.; Chen, L.; Zhong, W. A new linear potentiometric titration method for the determination of deacetylation degree of chitosan. *Carbohydr. Polym.* **2003**, *54*, 457-463, https://doi.org/10.1016/j.carbpol.2003.05.004.
111. Jantzen da Silva Lucas, A.; Quadro Oreste, E.; Leão Gouveia Costa, H.; Martín López, H.; Dias Medeiros Saad, C.; Prentice, C. Extraction, physicochemical characterization, and morphological properties of chitin and chitosan from cuticles of edible insects. *Food Chem.* **2020**, *343*, 128550, https://doi.org/10.1016/j.foodchem.2020.128550.
112. Mohan, K.; Ganesan, A.R.; Muralisankar, T.; Jayakumar, R.; Sathishkumar, P.; Uthayakumar, V.; Chandirasekar, R.; Revathi, N. Recent insights into the extraction, characterization, and bioactivities of chitin and chitosan from insects. *Trends Food Sci. Technol.* **2020**, *105*, 17-42, https://doi.org/10.1016/j.tifs.2020.08.016.
113. Saha, J.; Mondal, M.I.H.; Ahmed, F.; Rahman, M. Extraction, characterization and functionality assessment of Aloe vera, chitosan and silk sericin. *Arab. J. Chem.* **2023**, *16*, 105087, https://doi.org/10.1016/j.arabjc.2023.105087.
114. Yen, M.-T.; Mau, J.-L. Physico-chemical characterization of fungal chitosan from shiitake stipes. *LWT-Food Sci. Technol.* **2007**, *40*, 472–479, https://doi.org/10.1016/j.lwt.2006.01.002.
115. Bagheri-Khoulenjani, S.; Taghizadeh, S.M.; Mirzadeh, H. An investigation on the short-term biodegradability of chitosan with various molecular weights and degrees of deacetylation. *Carbohydr. Polym.* **2009**, *78*, 773–778, https://doi.org/10.1016/j.carbpol.2009.06.020.
116. Sabnis, S.; Block, L.H. Improved infrared spectroscopic method for the analysis of degree of *N*-deacetylation of chitosan. *Polym. Bull.* **1997**, *39*, 67-71, https://doi.org/10.1007/s002890050121.

117. Mohammed, M.H.; Williams, P.A.; Tverezovskaya, O. Extraction of chitin from prawn shells and conversion to low molecular mass chitosan. *Food Hydrocoll.* **2013**, *31*, 166-171, https://doi.org/10.1016/j.foodhyd.2012.10.021.
118. Duarte, M.L.; Ferreira, M.C.; Marvão, M.R.; Rocha, J. An optimised method to determine the degree of acetylation of chitin and chitosan by FTIR spectroscopy. *Int. J. Biol. Macromol.* **2012**, *31*, 1-8, https://doi.org/10.1016/S0141-8130(02)00039-9.
119. Rujiravanit, R.; Kantakanun, M.; Chokradjaroen, C.; Vanichvattanadecha, C.; Saito, N. Simultaneous deacetylation and degradation of chitin hydrogel by electrical discharge plasma using low sodium hydroxide concentrations. *Carbohydr. Polym.* **2020**, *228*, 115377, https://doi.org/10.1016/j.carbpol.2019.115377.
120. Jang, M.-K.; Kong, B.-G.; Jeong, Y.-I.; Lee, C.H.; Nah, J.-W. Physicochemical characterization of α-chitin, β-chitin, and γ-chitin separated from natural resources. *J. Polym. Sci., A: Polym. Chem.* **2004**, *42*, 3423–3432, https://doi.org/10.1002/pola.20176.
121. Kaewboonruang, S.; Phatrabuddha, N.; Sawangwong, P.; Pitaksanurat, S. Comparative Studies on the Extraction of Chitin – Chitosan from Golden Apple Snail Shells at the Control Field. *J. Polym. Textile Eng.* **2016**, *3*, 34-41.
122. El Knidri, H.; El Khalfaouy, R.; Laajeb, A.; Addaou, A.; Lahsini, A. Eco-friendly extraction and characterization of chitin and chitosan from the shrimp shell waste via microwave irradiation. *Process Saf. Environ. Prot.* **2016**, *104*, 395-405, https://doi.org/10.1016/j.psep.2016.09.020.
123. Zhang, Y.; Xue, C.; Xue, Y.; Gao, R.; Zhang, X. Determination of the degree of deacetylation of chitin and chitosan by X-ray power diffraction. *Carbohydr. Res.* **2005**, *340*, 1914-1917, https://doi.org/10.1016/j.carres.2005.05.005.
124. Domard, A.; Rinaudo, M. Preparation and characterization of fully deacetylated chitosan. *Int. J. Biol. Macromol.* **1983**, *5*, 49-52, https://doi.org/10.1016/0141-8130(83)90078-8.
125. Sánchez-Machado, D.I.; López-Cervantes, J.; Escárcega-Galaz, A.A.; Campas-Baypoli, O.N.; Martínez-Ibarra, D.M.; Rascón-León, S. Measurement of the degree of deacetylation in chitosan films by FTIR, 1H NMR and UV spectrophotometry. *MethodsX* **2024**, *12*, 102583, https://doi.org/10.2139/ssrn.4609392.
126. Dong, Y.; Xu, C.; Wang, J.; Wu, Y.; Wang, M.; Ruan, Y. Influence of degree of deacetylation on critical concentration of chitosan/dichloroacetic acid liquid-crystalline solution. *J. Appl. Polym. Sci.* **2002**, *83*, 1204–1208, https://doi.org/10.1002/app.2286.
127. Kim, S.S.; Kim, S.H.; Lee, Y.M. Preparation, characterization and properties of β-chitin and *N*-acetylated β-chitin. *J. Polym. Sci. B: Polym. Phys.* **1996**, *34*, 2367–2374, https://doi.org/10.1002/(SICI)1099-0488(199610)34:14%3C2367::AID-POLB6%3E3.0.CO;2-T.
128. Kaya, M.; Seyyar, O.; Baran, T.; Erdoğan, S.; Kar, M. A physicochemical characterization of fully acetylated chitin structure isolated from two spider species: With new surface morphology. *Int. J. Biol. Macromol.* **2014**, *65*, 553-558, https://doi.org/10.1016/j.ijbiomac.2014.02.010.
129. Kaya, M.; Baran, T. Description of a new surface morphology for chitin extracted from wings of cockroach (*Periplaneta americana*). *Int. J. Biol. Macromol.* **2015**, *75*, 7–12, https://doi.org/10.1016/j.ijbiomac.2015.01.015.
130. Kaya, M.; Baran, T.; Erdoğan, S.; Menteş, A.; Aşan Özüsağlam, M.; Çakmak, Y.S. Physicochemical comparison of chitin and chitosan obtained from larvae and adult Colorado potato beetle (*Leptinotarsa decemlineata*). *Mater. Sci. Eng. C* **2014**, *45*, 72-81, https://doi.org/10.1016/j.msec.2014.09.004.
131. Abdou, E.S.; Nagy, K.S.A.; Elsabee, M.Z. Extraction and characterization of chitin and chitosan from local sources. *Bioresour. Technol.* **2008**, *99*, 1359–1367, https://doi.org/10.1016/j.biortech.2007.01.051.
132. Kucukgulmez, A.; Celik, M.; Yanar, Y.; Sen, D.; Polat, H.; Kadak, A.E. Physicochemical characterization of chitosan extracted from *Metapenaeus stebbingi* shells. *Food Chem.* **2011**, *126*, 1144–1148, https://doi.org/10.1016/j.foodchem.2010.11.148.
133. Kasaai, M.R.; Arul, J.; Chin, S.L.; Charlet, G. The use of intense femtosecond laser pulses for the fragmentation of chitosan. *J. Photochem. Photobiol. A Chem.* **1999**, *120*, 201–205, https://doi.org/10.1016/S1010-6030(98)00432-8.
134. Kasaai, M.R.; Charlet, G.; Paquin, P.; Arul, J. Fragmentation of chitosan by microfluidization process. *Innov. Food Sci. Emerg. Technol.* **2003**, *4*, 403-413, https://doi.org/10.1016/S1466-8564(03)00047-X.
135. Weißpflog, J.; Vehlow, D.; Müller, M.; Kohn, B.; Scheler, U.; Boye, S.; Schwarz, S. Characterization of chitosan with different degree of deacetylation and equal viscosity in dissolved and solid state – Insights

by various complimentary methods. *Int. J. Biol. Macromol.* **2021**, *171*, 242-261, https://doi.org/10.1016/j.ijbiomac.2021.01.010.

136. Roberts, G.A.F. Chitin Chemistry. Red Globe Press London, **1992**; 16-350, https://doi.org/10.1007/978-1-349-11545-7.