Synthesis of N-Benzyl N'-Methylchitosan by Simultaneous Alkylation of Methanal and Benzaldehyde: Investigation of Chemical Structure and Composition

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Received: September 26, 2019; Published: November 07, 2019

Abstract

The Schiff base was synthesized by simultaneous reaction of chitosan, a natural polyaminosaccharide with methanal and benzaldehyde. N-benzyl N'-methyl derivative of solubility increased chitosan was obtained by reduction of product in the presence of NaBH4. The mechanism of the reaction and structure of an intermediate stage products of the process have been identified by some spectroscopic methods. It has been determined that, carbocations contains >C=N- chromophore groups are formed at intermediate stage. After reduction double bond is replaced with single bond. The composition, degree of crystallinity and molecular electron spectrum of the main product has been characterized by means of FT-IR, XRD, UV-Vis and 1H, 13C NMR methods. It has been shown that, unlike chitosan, N-benzyl N'-methylchitosan has 14 - 17% more crystallinity. Inclusion of hydrophobic methyl- and benzyl radicals into chitosan macromolecule cause reduction in intermolecular interaction and hydrogen bond. This leads to an increase in the polarization degree of functional groups of product in a polar environment and better solubility.

Keywords: Chitosan; Alkylation; Schiff Base; Methanal; Benzaldehyde; N-Benzyl N'-Methylchitosan

Introduction

Chitosan, a linear cationite type natural polyaminosaccharide, is obtained by N-deacetylation of chitin shells of shrimp [1-3]. Chitin is separated from thick rigid shell of some insects, molluscs, especially crustaceans. Chitosan can be considered as copolymer of chitin and chitosan. The main macromolecular chain consists of β-(1,4)-2-amino-2-deoxy-D-glucosamine and β-(1,4)-N-acetyl D-glucosamine remnants [4-7].

Depending on deacetylating rate the amount of free amino groups can be 80 - 85%. The non-toxicity, biodiversity, biodegradation property of chitosan stimulate its use in controlled separation of drugs in medicine and biotechnology and stabilization of antibacterial metal nanoparticles as a carrier and stabilizing matrix [8-12]. Strong hydrogen communication and electrostatic interaction between the functional groups in chitosan macromolecule prevents its ionization with water molecules. In this regard, lack of water-solubility of chitosan limits its free use.

Citation: Shamo Zokhrab Tapdigov. “Synthesis of N-Benzyl N'-Methylchitosan by Simultaneous Alkylation of Methanal and Benzaldehyde: Investigation of Chemical Structure and Composition”. EC Pharmacology and Toxicology 7.12 (2019): 01-12.
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By carrying out chemical modifications like grafting copolymerization, acoustic, alkylation, carboxymethylating, N-phosphonomethylating and etc. of chitosan macromolecule, new chitosan derivatives which soluble in water or aqueous buffers have been synthesized [13-17]. In terms of molecular structure such modifications can be easy to handle and small molecule drugs can easily interact by electrostatic or hydrogen bonding. Reaction ability of primary amine, primary alcohol and secondary alcohol groups on chitosan macromolecule are known. Based on this, a large number of new materials can be found by N-alkyl and N-arylating, quaternization and obtaining grafting derivatives of chitosan in various studies [18-24]. Muzarelli and Tanfani [25] have been synthesized risen water and buffer soluble N-trimethyl chitosan chloride by methylation and then quaternization of chitosan with formaldehyde.

In other studies [26] in order to obtain trimethyl ammonium salt of chitosan a new method have been proposed by using dimethyl carbonate as a quaternizing reagent in ionic-liquid environment. Consequently, derivatives, thermal stability and water solubility are more compared to chitosan, have been synthesized.

Tommeraas, et al. developed fluorescent chitosan by synthesizing Schiff base with 9-anthraldehyde subsequently reducing it with sodium cyanoborohydride [27]. The Schiff base intermediates in synthesis of alkylated chitosan themselves enjoy antioxidant activity. Guo., et al. on synthesis of N-arylidene chitosans with derivatives of benzaldehyde found that the antioxidant activity was equivalent to chitosan [28]. The Schiff bases of chitosan can be used to improve the properties of chitosan concerning to the chelation of metal ions, production of an analytical reagent for determination of metal ions, preparation of modified electrodes, protection of amino group, etc [29]. Chitosan-Schiff bases with salicylaldehyde derivatives and N-(4-pyridimethylidene) chitosan have been prepared with this view [30,31]. The methoxyphenyl aldehydes as vanillin, o-vanillin, syringaldehyde, and veratraldehyde react with chitosan under normal as well as reducing conditions to impart insolubility and other characteristics to chitosan. The films obtained from veratraldehyde are insoluble, biodegradable, and mechanically resistant [32].

Compared research shows that, effective alkyl or arylating of the amino groups of chitosan was carried out by different methods. Inclusion of hydrophobic fragments into chitosan chain increase its antioxidativity and solubility in organic solvents and paralelly changes the thermal properties. In most alkyl or arylating reagents are used in homo form. In our current research, the simultaneous inclusion reaction of both alkyl-methyl and the aril-benzyl groups into chitosan macromolecule have been studied. The chemical mechanism of the reaction has been studied and the molecular structure has been confirmed by spectroscopic techniques.

**Figure 1:** Structure of chitosan.
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As well as, surface morphology and thermal stability have been determined, molecular structure of main product and intermediate compounds obtained in reaction have been characterized by the application of highly sensitive UV-Vis and NMR spectroscopic methods.

Experimental

Materials

Chitosan \( M_n = 35 \text{kDa} \) (deacetylating degree 85 - 87%), formaldehyde (≥ 99.0%), benzaldehyde purified by redistillation (≥ 99.5%), NaBH\(_4\) (purum p.a., ≥ 96%), acetate acid (Glacial), ethanol (95%), acetone (residue analysis, ≥ 99.9%), diethyl ether (contains 1 ppm BHT as inhibitor, anhydrous, ≥99.7%) and others reagent from Sigma-Aldrich.

Synthesis of N-benzyl N’-methylchitosan

The synthesis of N-methyl, N-benzaldehyde chitosan was conducted in two stages - initially by aryl-so-alkylation and then by the reduction process. Synthesis was performed on the known methodology [33] based on Schiff reaction. 1.5g of chitosan is suspended in 60 ml 2% CH\(_3\)COOH solution (containing 7.29 mmol -NH\(_2\) group). 0.81 ml formaldehyde and 0.74 ml benzaldehyde mixture is added intensively to the solution in the form of drops - twice more than the equivalent molar ratio. After 30 minutes the color of the solution varies from light brown to milky white suspension. The mixing is continued in an inert N\(_2\) environment for 4 hr. At the end of the solution is turned into a visco-flowing gel. After the gel was grinded and waited for 12 hours, 0.36 g NaBH\(_4\) was dissolved in 8 ml water and added to the solution by dropping over 2 - 4 hours under intensive mixing. At that time, the pH of the solution is contained 4.0 - 4.5. After 4 hours, the pH of the solution is brought to 10 by added 1M NaOH, then the gel is formed. Gel is first washed with distilled water then with ethanol, collapsed in acetone, and extracted with diethyl ether at Soxlet for 2 days. Then, gel was dried overnight until constant weight at 40 - 50°C after extraction, and freeze dried in vacuum for 24 hrs.

Spectroscopic characterization of N-benzyl N’-methylchitosan

IR spectra of samples were studied in 4000 - 500 cm\(^{-1}\) area with 4 cm\(^{-1}\) imaging potential with KBr with its pressed mixtures in the AVATAR 370 (Thermo Nicolet Corporation, USA). X-ray diffractograms of chitosan and reaction products are outlined on the German production Bruker Advance D8 equipment. Diffractograms comprised with (2θ) 0.020 imaging scattering rates ranged from 3 to 800, and scanning speeds 2.0 min\(^{-1}\), accelerated tension 40 kV and intensity 35 mA. \(^1\)H and \(^{13}\)C NMR spectra of initial polymer, intermediate and final product were outlined at 300.13 MHz and 75.47 MHz 300K on the Bruker Avance 300. Deuteriumized D\(_2\)O and d-acetate acid were used as solvent. The concentration of samples were taken in the range of 10 - 25 mg/ml. The UV-Visible analysis of the samples was carried out using UV-Visible Spectrophotometer (Perkin Elmer Lambda-850) the absorbance mode in the wavelength choice of 200 - 800 nm.

Results and Discussion

The exchange of hydrogen atoms from 85 - 90% free -NH\(_2\) groups on the content of chitosan is commonly found to be substituted by various alkyl and aromatic radicals based on the Schiff reaction [34,35]. In most cases introducing of alkyl or aryl group into chitosan occur with the addition of the same radicals. Also, the degree of alkylation or arylation of the amine groups ultimately affect the product’s solubility and biological properties, which is explained in various ways by the researchers [36-38]. Initially, the alkylation also aryl exchange process for one protons of amine groups was done in our study. Based on FT-IR, \(^1\)H, \(^{13}\)C NMR, UV-Vis analysis investigations of chitosan, intermediate and final product, the reaction occurs on the following mechanism.

The presence of a chromophore group in the final product shows itself in the color of the substance as well as in the formation of a characteristic stripe of the -HC=N+H- double bond in the spectrum of 1640 cm\(^{-1}\). The peak which belongs to this strip is not observed in the spectrum of chitosan. After a certain period of time, the change in the color of the solution depends on the following equilibrium process.

Depending on the mole ratio of the reaction components, concentration and nature of aldehyde, the average molecular weight of the chitosan and the reaction time the gel formation occurs in different forms [39,40]. It has been determined that, complete replacement of
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**Figure 2:** The initially intermediate product structure of chitosan with methanal and benzaldehyde.

![Structure of initial intermediate product](image)

**Figure 3:** The chemical reaction of reduction processes of quaternized N-benzyl N’-methylchitosan.

![Chemical reaction of reduction processes](image)

Amine groups do not occur in chitosan. Moreover, asetoamide groups in the composition cannot be replaced by alkyl or aromatic radicals. Taking this into consideration, structure for the final product can be shown as follows.

**Figure 4:** Supposed structural composition of N-benzyl N’-methylchitosan.

![Supposed structural composition](image)
After alkylation, FT-IR spectra of product and initial polymer were investigated comparatively. The absorption strips belonging to the polysaccharide groups in the spectrum of the chitosan and N-benzyl N’-methylchitosan groups (C-O-C vibration of asymmetric stretching) in 1157 cm⁻¹, the strips characterizing II and I alcohol groups 1422 cm⁻¹ and 1378 cm⁻¹, as well as 1087 and 1032 cm⁻¹ bond strips (the deformation of C-O bond), are the same [28, 29]. This shows that, there is not any chemical changes that have occurred in these groups.

**Figure 5:** The FT-IR spectrum of chitosan and N-benzyl N’-methylchitosan.

The peaks at 1657 cm⁻¹ (assigned to the axial stretching of >C = O bonds of the acetamide groups, referred to as amide I band), 1571 cm⁻¹ (angular deformation of the -NH₂ group), 1260 cm⁻¹ (bending vibration of C-N band) match with the chitosan groups involved in the chemical modification. The observed peaks in the 3478 cm⁻¹ region belong to tightened vibrations of -OH and -NH₂ groups. After alkylation, the peaks 1592 - 1598 cm⁻¹ belonging to adsorption strip of the N-H bond disappeared, which proves the exchange of protons as a result of methyl or benzyl. Additionally, intensive peaks in the 2875 and 1457 cm⁻¹ region are increasing as compared to chitosan, which corresponds to the asymmetric stretch of C-H bond. The presence of the benzene nucleus in macromolecule can be proved it according to 4 absorption lanes basically in the 2000 - 1400 cm⁻¹ range. C = C bond oscillation of the benzene ring in N-benzyl N’-methylchitosan demonstrate 1583 cm⁻¹, 1466 cm⁻¹ combined frequencies and oberton absorption strips. On the other hand, if benzene nucleus is considered as mono replacement state, benzene rings in 900 - 650 cm⁻¹ area have strong absorption as a result of deformation oscillations of C-H bonds. Such a replacement characteristics in the product appears in 760 and 675 cm⁻¹ adsorption area [41].

The reaction mechanism and molecular structure of product was studied by the UV-Vis electron spectra was performed. It was determined besides the chitosan, and the reduced chitosan derivatives were very poorly soluble in water. High sensitive method has been used in the discovery of the molecular structure of these polymer modifications. The analysis of samples has been studied in the ultraviolet region due to slight solubility. Taking this into account, their solutions were prepared (0.01 - 0.001%) and electron spectra of them were monitored (Figure 6).

Apparently, in the content of initial polysaccharide-chitosan spectrum a broad peak around 208 nm was observed, that belongs to the non-deacetylating fragment, that belongs to the carbodiimide functional group >C=O. With inclusion alkyl - methyl or aryl fragment the structural variation occurs in the polymer chains. This shows itself in the form of a spectrum and in the formation of the second absorp-
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Figure 6: The UV spectrum of chitosan and N-benzyl N’-methylchitosan.

The UV spectrum shows a transition band in the 225 - 240 nm. Thus, the second intensive peak at the high wavelength is characteristic to the >C= N- chromophore group (also benzene ring) or n→π* transition in it. The breakdown of π bond after reduction affects the electron density of the macromolecule and the characteristic strip of the chromophore group disappears. This proves that the alkylation reaction occurs consistently, and the intermediate carbocation product forms a major mass during the reaction. Also, the electron nature of the >C=O group in the deacetylated chitin residue disrupts due to alkaline substitution occurring in the chitosan macromolecule within or between macromolecules that affects the nature of the electrostatic interactions. As a result this proves that the characteristic strip belonging to this passage is exposed to the chemical sliding with bathochromic shift.

The exact molecular structure of the derivatives from the reaction and the presence of the functional groups were studied using the 1H and 13C NMR spectroscopy. In the following figure, the 1H NMR spectrum of chitosan and the final products N-benzyl N’-methylchitosan and the signals of corresponding peaks are given.

Figure 7: The 1H NMR spectrum of chitosan and N-benzyl N’-methylchitosan.
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As the reaction proceeds gradually, NMR spectra of intermediate products were analyzed and signals of protons and carbons which exposed to basic chemical shifts were registered.

The \(^1\)H and \(^{13}\)C NMR spectrum of chitosan: (300 MHz, D\(_2\)O/d–acetic acid) \(\delta\) 2.08 ppm (-NH\(_2\)), \(\delta\) 2.12 ppm (C\(^6\)-OH), \(\delta\) 1.98 ppm (C\(^3\)-OH), \(\delta\) 4.81 ppm (H-1) the hydrogen of the 1\(^{st}\) carbon in the cycle, \(\delta\) 3.18 ppm (H-2), \(\delta\) 3.79 ppm (H-3), \(\delta\) 3.28 ppm (H-4), \(\delta\) 4.06 ppm (H-5), \(\delta\) 3.63 ppm (-CH\(_2\)- the protons in the C-6). The \(^{13}\)C NMR spectrum: (300 MHz, D\(_2\)O/d–acetic acid) \(\beta\) C-1 \(\delta\) 102.3 ppm, C-2 \(\delta\) 53.4 ppm, C-3 \(\delta\) 68.2 ppm, C-4 \(\delta\) 72.1 ppm, C-5 \(\delta\) 72.6 ppm, C-6 \(\delta\) 59.1 ppm.

After protonation, double bond disappears, the substance is discolored, and chromophore groups are replaced by auxochrome groups. Changes are observed in the common view of the spectrum and in the chemical shift of signals. Because of the exchange of the proton in the amine group with the methyl and benzyl radicals, new chemical signals appear [42].

\[\text{Figure 8: The } ^1\text{H and } ^{13}\text{C NMR spectrum of N-benzyl N'}\text{-methylchitosan: (300 MHz, D}_2\text{O/d–acetic acid).}\]

Weak and strong signals for -NH- are observed at \(\delta\) 7.85 ppm and at \(\delta\) 2.12 ppm respectively. These signals are characteristic for free amino groups and mono replaced NH protons which attached to methyl and phenyl groups. Hydrogens attached to NH in the methyl group are observed at the intermediate \(\delta\) 2.38 ppm level, ortho position protons for the phenyl ring were seen at \(\delta\) 7.09 ppm, meta protons \(\delta\) 7.22 ppm, while para protons appear at \(\delta\) 6.98 ppm with weak signals respectively and chemical shift in other protons are not sharp.

\(^{13}\)C NMR spectrum: (deacetylating fragment) characteristic strong chemical signals are observed for -CH\(_3\) at \(\delta\) 18.37 ppm, for carbon in the carbonyl group at \(\delta\) 171.2 ppm, for carbon typical for the methyl group included with alkylation at \(\delta\) 31.9 ppm (intermediate level), for carbons in the benzene ring at \(\delta\) 128.7 ppm ortho-, meta- and para-positions. Chemical signals in other carbons are identical to chitosan.

The chemical signals of the methyl group and CH groups in benzene ring, as well as the signals of carbons in the methyl group prove that alkylation occurs. When taking aldehyde more than the equimolar ratio (2 - 4 times) during the reaction, the alkylation occurs deeper. Thus, both of the protons in the amine group are substituted by the methyl or benzyl radical, which shows itself in the steps of the process in the NMR results of the products.

After reductions with NaBH\(_4\), the product becomes colorless, it means that the >C = N chromophore bond is protonated and a new view appears in the \(^1\)H and \(^{13}\)C NMR spectrum.

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A detailed comparison of NMR results indicates that alkylation of chitosan with methyl- and benzaldehyde has been occurred chemically. Meanwhile, the analysis of intermediate products indicates that the process is chemically effective.

It is known that, some crystalline phase could be observed in the chitosan macromolecule, which is essentially amorphous. Also, the chemical modification of the polymer macromolecule - the conjunction of alkyl or aromatic groups to the content, later, conversion to a salt form affects its crystallinity. For this purpose, X-ray diffractograms of chitosan and N-benzyl N'-methylchitosan were investigated and the results were given in the following figure.

![Figure 9: X-ray diffractogram of chitosan and N-benzyl N'-methylchitosan.](image)

The two diffraction peaks at 2θ = 10° and 19.7° levels for the chitosan characterized by the crystal domains are shown in the figure. Which, these crystalline phases have been formed due to the hydrogen bonds between amine groups. In N-benzyl N-methylchitosan diffractogram, the peaks are clearly visible. The inclusion of methyl and benzyl groups into chitosan macromolecule leads to an increase of crystallinity up to 28 - 29%. Also, absence of characteristic peaks for hydrogen bond proves that the protons in amine group were exposed to the alkylation also arylation [45].

N-benzyl N'-methylchitosan has the ability to dissolve in water and in a certain pH range, in contrast to the free polymer. Whereas the solubility of the chitosan is made up 2 - 3%, its methyl and benzyl derivative has a solubility of 65 - 78%. Also, chitosan is well-soluble in the pH = 1 - 5 range, and precipitates in pH = 8 - 12. In the obtained product the functional groups produce intensive absorption in the solution, which are soluble in pH = 1 - 10 range. The degree of solubility depends on the amount of aldehyde during the reaction [46,47]. It has been found that the best solubility of N-benzyl N'-methylichitosan is optimum, when the content of methyl and benzene groups totally 60 - 70%. The N-aryl substituted TMC obtained by the reductive alkylation and quaternization sequence such as quaternized N-(4-methylbenzyl) chitosan, N-(4-N,N-dimethylaminobenzyl) chitosan and quaternized N-(4-pyridylmethyl) chitosan have also been prepared and tested for antibacterial activity. These substituents did not impart increase in the antibacterial activity of chitosan backbone [48]. Of these derivatives, quaternized N-(4-N,N-dimethylaminobenzyl) chitosan was investigated for the transfection efficiency using the plasmid DNA encoding green fluorescent protein pEGFP-C2 on human hepatoma cell lines (Huh7 cells), in comparison to TMC and chitosan [49]. The results indicated that the improved gene transfection was due to the hydrophobic group (N,N-dimethylaminobenzyl) substitution on.
chitosan, which promoted the interaction and condensation with DNA, as well as N-quaternization which increased the water solubility. Spectroscopic and thermal analyzes show that after alkylation, the product maintains its basic physical and biological properties characteristic to the chitosan. This allows it to be used in the process of delivery and controlled release of some antibiotics and proteins.

**Conclusion**

The inclusion of hydrophobic methyl and benzyl groups into chitosan macromolecule leads to change of chemical properties. Thus, solubility of non-water soluble chitosan with methyl and benzaldehyde and Schiff bases and their reductions products increases 20 - 23 times in the water environment and at pH = 6 - 10 buffers. Simultaneous inclusion of alkyl and aril groups in macromolecule increases both crystallinity of chitosan up to 22%. As it is known, interaction of soluble chitosan derivatives with drugs and enzymes becomes easier and such type of matrices can be used as a carrier or storage in medicine.

**Acknowledgement**

The authors acknowledge the Science Foundation of “The State Oil Company of the Azerbaijan Republic” (SOCAR), for financial support of the research activities related to project; Grant project 33LR-SOCAR/ANAS.

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**Citation:** Shamo Zokhrab Tapdigov. “Synthesis of N-Benzyl N’-Methylchitosan by Simultaneous Alkylation of Methanal and Benzaldehyde: Investigation of Chemical Structure and Composition”. *EC Pharmacology and Toxicology* 7.12 (2019): 01-12.
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Volume 7 Issue 12 December 2019
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