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Synthesis and characterization of chitosan ethers: Hydroxypropyl chitosan and Hydroxyethyl chitosan

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ABSTRACT

The hydroxypropyl chitosan (HPCH) and hydroxyethyl chitosan (HECH) are multifunctional chitosan derivatives with biocompatible and biodegradable properties. Due to their hydroxypropyl and hydroxethyl groups, they have water solubility, moisture retention, and gelling properties. In this study, the chitosan derivatives HPCH and HECH were obtained in two steps alkalisation and etherification. For alkalisation, chitosan was kept in an alkaline medium at -18 □C for 7 days. For etherification; the reaction was carried out for 48 hours by mixing alkaline chitosan with propylene oxide and ethylene oxide separately in a pressure reactor. The structures of the obtained HPCH and HECH were characterised by FT-IR, 1H(13C)-NMR, XRD, and TG analysis methods. Since the degree of deacetylation (DA) of chitosan is 75-85%, the chitosan units contain N-acetyl (-N-(CO)-CH3)) groups in addition to -NH2 functional groups. When the 1H(13C)-NMR spectrum of chitosan was examined, the peak value of these acetyl groups was observed at δ1.89 ppm. When the XRD spectra were examined, it was observed that the strong peak in chitosan at 2θ=20° was weakened in HPCH and HECH. In addition, the thermal stability of HPCH and HECH was found to be higher than chitosan in TG analysis.

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1. Introduction

Chitosan is a natural cationic biopolymer composed of unbranched chains containing both D-glucosamine (deacetylated units) and N-acetyl-D-glucosamine (acetylated units) randomly linked by β (1-4) glycosidic linkages [1-2]. It is obtained by the deacetylation of chitin in some or all acetyl groups using an inorganic base at high temperatures and concentrations (Figure 1) [3]. Chitosan is an amphiphilic natural polysaccharide with excellent physical and biological properties, including biocompatibility, biodegradability, and non-toxicity [4].

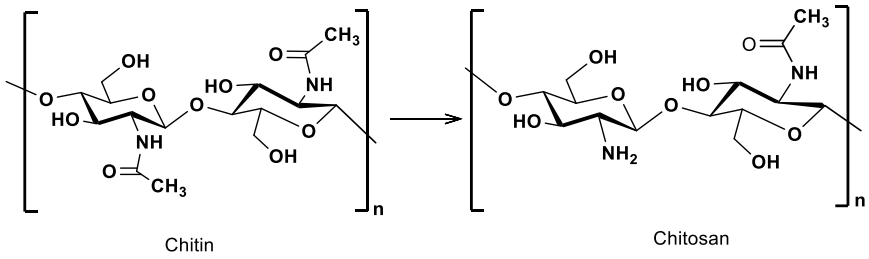

Figure 1. Chemical structure of chitin and chitosan

Chitosan is insoluble in water (pH ~7) due to the presence of amino groups (-NH_2) in its structure, but soluble in nearly all aqueous acidic solutions or mineral acids below pH=6.5 [5]. The presence of these groups makes chitosan a cationic biopolymer [6]. Its applications are limited due to its poor solubility in neutral and basic environments, low porosity, and low mechanical properties [7]. Chitosan derivatives are obtained by incorporating the reactive amino and hydroxyl groups in the structure of chitosan into the structures of molecules, i.e. by chemical modification of chitosan. Hence, the water solubility, biological activities, and mechanical properties of chitosan can be improved [8]. Modification can be achieved by physical or chemical processes such as grafting, cross-linking, composites, incorporation of substituents, etc. [9]. Chitosan and its derivatives are known for their biocompatibility [10], biodegradability [11], antimicrobial and antioxidant properties [12], and anti-tumor activity [13]. They are also hydrophilic, non-toxic, and low-cost, which makes them suitable for various applications in the food industry, biotechnology, biomedical and pharmaceutical industries, cosmetics, and tissue engineering [11, 14-17]. In addition, the extensive use of chitosan in 3D bioprinting applications for tissue regeneration or drug delivery systems is currently being investigated [18-19]. HPCH a kind of water-soluble functional derivative of CH, is obtained by etherification through propylene oxide under alkaline conditions [20-21]. It is used in wound dressings, medical carriers, and tissue engineering due to its biodegradability, non-toxicity, antimicrobial activity, and film-forming properties [22-25]. Compared to chitosan, HPCH exhibits superior properties in terms of hygroscopicity and adsorption [25]. Furthermore, HPCH was found to have higher moisture retention and a better antibacterial effect than chitosan [26]. As another important chitosan derivative, HECH has a multifunctional one that dissolves well in water and has biocompatible and biodegradable properties [27]. It is obtained by reacting chitosan and ethylene oxide under alkaline conditions.

Within the scope of this study, the chitosan derivatives HPCH and HECH were synthesized from chitosan. There are many studies about chitosan in the literature, but studies on HPCH and HECH, which are important derivatives of chitosan, are limited. Therefore, these derivatives were synthesized and their structures were characterized by spectroscopic methods (FT-IR, XRD, NMR), and the surface morphologies and thermal properties were investigated by SEM and DTA-TG analysis, respectively.

2. Materials and methods

Chitosan (degree of deacetylation 75-85%), Propylene oxide (PO, molecular weight 58.07 g/mol), Ethylene oxide (EO, molecular weight 44 g/mol), sodium hydroxide (NaOH) 99%, hydrochloric acid (HCl) 37%, glacial acetic acid (CH_3COOH), isopropyl alcohol (IPA), ethanol, methanol and acetone were obtained from Sigma Aldrich. All chemicals used in this research were of analytical grade.

2.1. Synthesis of the chitosan derivatives HPCH and HECH

The synthesis of HPCH was carried out according to [28] with some modifications. First, for alkalisation, 0.54 g (0.003mol) of chitosan was mixed with 10 mL of 40% NaOH and kept at -18 ^oC for 7 days. The alkalised chitosan was then brought to room temperature, and 20 mL of IPA was added and stirred for 30 minutes. The mixture was placed in a pressure reactor, 0.94 mL (0.013mol) of PO was added and the reaction was carried out at 40^oC for 48 hours. After 48 hours the solution was brought to room temperature, filtered, and neutralised with HCl, and the final solid was washed several times with anhydrous ethanol and dried at room temperature. The HPCH reaction is shown in Figure 1.

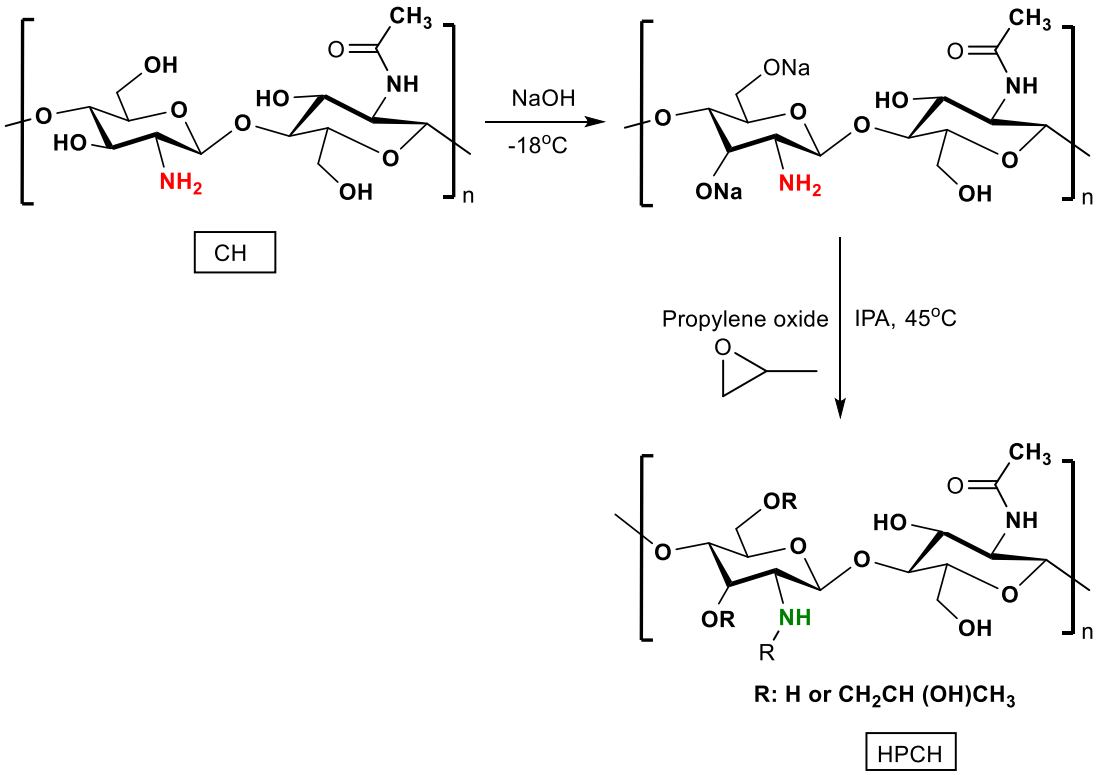

Figure 1. Synthesis reaction of HPCH

The synthesis of HECH was carried out according to [28] with some modifications. First, 0.50 g (0.003mol) of alkalised chitosan and 20 mL of IPA were stirred for 30 minutes. The mixture was placed in a pressure reactor, 0.67 mL (0.013mol) of EO was added and the reaction was carried out at 40°C for 48 hours. The solution was brought to room temperature, filtered, and neutralised with HCl, and the final solid was washed several times with anhydrous ethanol and dried at room temperature. The HPCH reaction is shown in Figure 2.

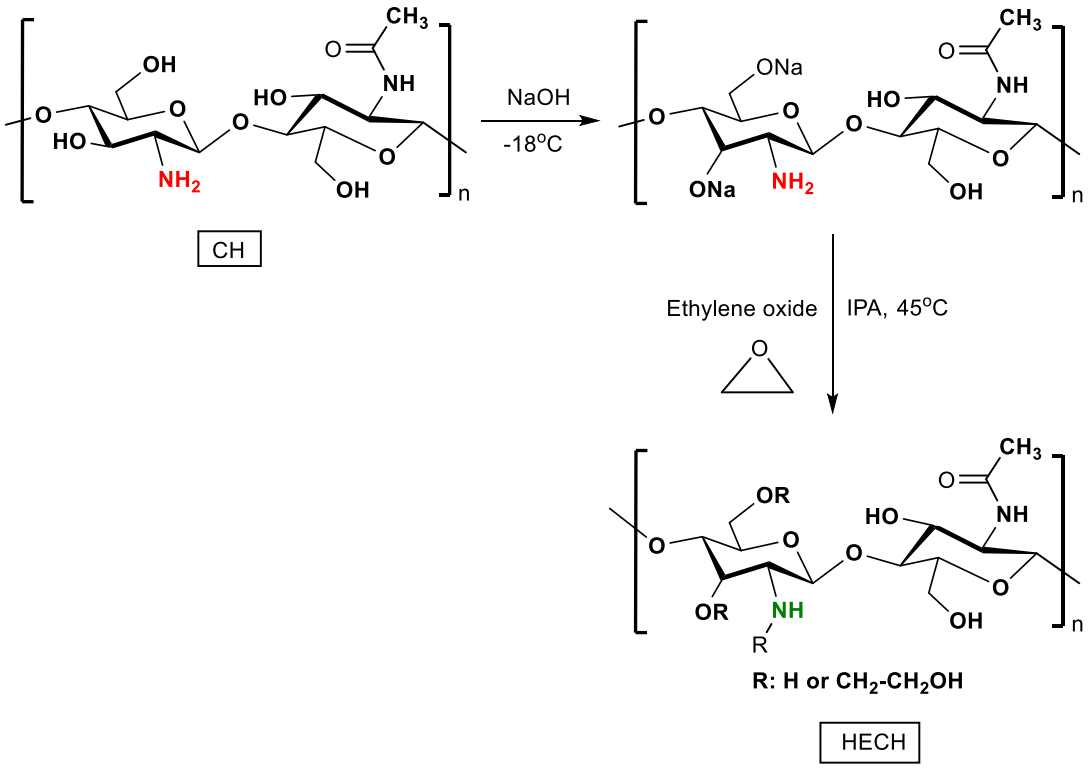

Figure 2. Synthesis reaction of HECH

2.2. Characterization

The morphology of the samples was characterized using a scanning electron microscope (SEM) (JEAL / NEOSCOPE JCM-5000) at EHT = 20 kV. Fourier Transform Infrared (FT-IR) spectra of the samples were recorded from 4000 to 400 cm^{-1} using a Perkin Elmer Spectrum 400 infrared spectrophotometer with ATR apparatus. $^{1}H(^{13}C)$-NMR spectra of the samples were recorded at 30 °C using a Bruker-200 MHz Varian spectrometer (90° pulse and 16 scans). The samples were dissolved in deuterium oxide (D_2O) at a concentration of 25-30 mg/600 µL. Chemical shifts were reported as ppm and the results were calibrated against the residual solvent signal of D_2O (δ 4.8 ppm) as an internal standard. X-ray diffraction (XRD) patterns of the samples were analyzed using an XRD diffractometer (Philips X'Pert PRO) operating with CuKα radiation, the voltage of 40 kV, and current of 30 mA at monochromatic radiation (λ=154060 nm). All samples were scanned from 10 °C to 90 °C at a scan speed of 5° 2θ /min with a step size of 0.02°. The thermal behavior of the samples was measured using a TG-DTA (SEIKO II, Seiko, Japan). The samples (15±5 mg) were placed in a ceramic dish and heated from 30 °C to 600 °C at a heating rate of 20 °C/min under a nitrogen atmosphere (20 mL/min).

3. Result and discussion

3.1. SEM of CH Derivatives

SEM analysis of chitosan involves examination of the surface morphology. SEM micrographs can reveal the structure and distribution of chitosan particles. As shown in Figure 3, the surface of chitosan was smooth and had no fibrous structure. The SEM micrographs of HPCH and HECH showed a rougher and grained surface with an increase in the pore structure of chitosan. This may be due to the hydroxypropyl and hydroxyethyl reactions of chitosan.

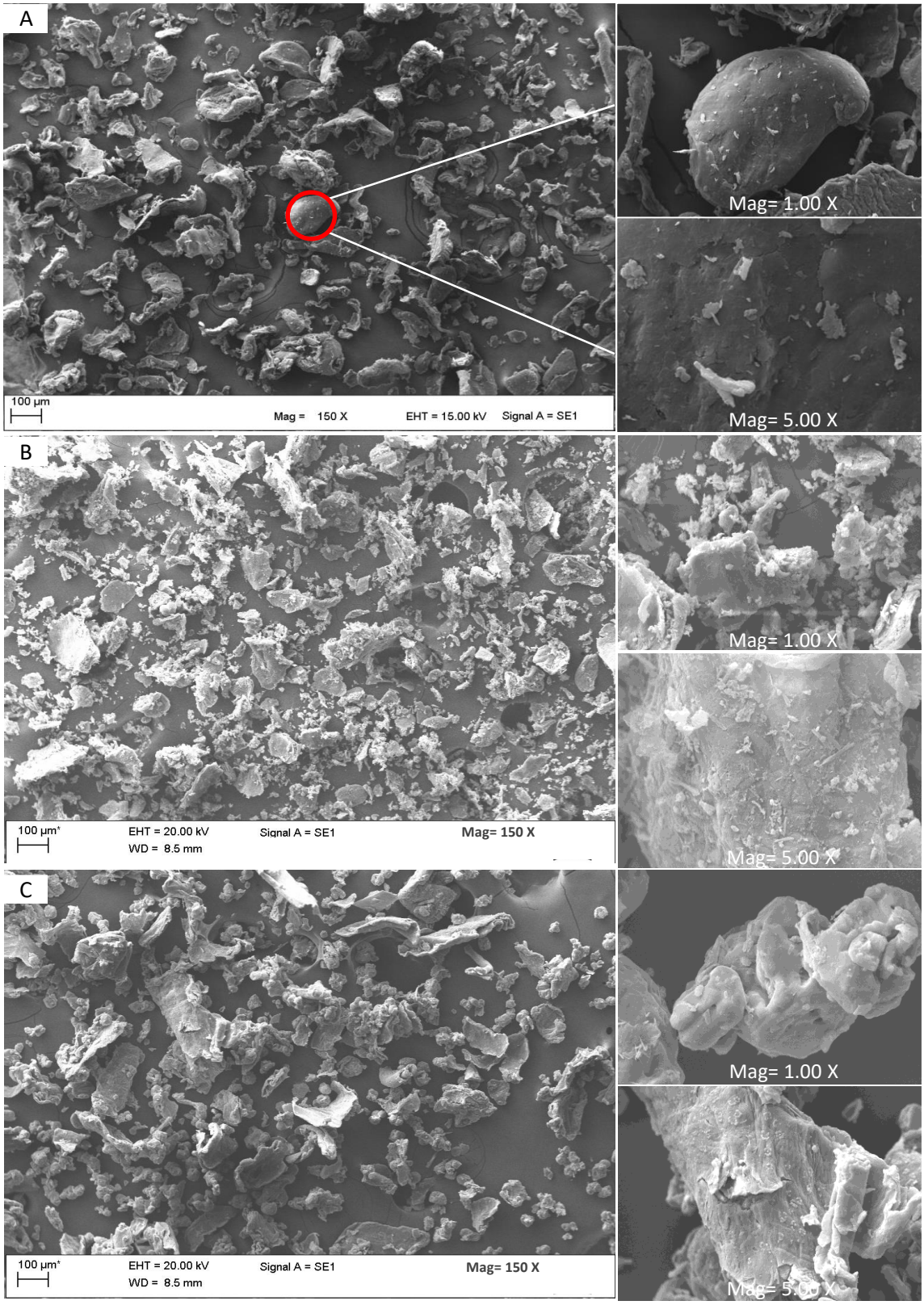

Figure 3. SEM images of CH (A), HPCH (B) and HECH (C) with a magnification of 150X, 1000X and 5000X

3.2. FTIR spectrums of CH Derivatives

FTIR spectra of CH, HPCH and HECH were shown in Figure 4. The broad bands at 3281-3386 cm^{-1} correspond to molecular hydrogen bonding and N-H and O-H vibrations [29]. A bands in the range of 2920-2868 cm^{-1} are due to the symmetric and asymmetric stretching of the amides. The band at 1643 cm^{-1} shows the C-O stretches of amides I and the band at 1325 cm^{-1} shows the C-N stretches of amides III. The peak at 1620 cm^{-1} is caused by the N-H bending of a protonated amine group ($-NH_2$). The peak at 1587 cm^{-1} corresponds to the N-H bending of amide I. The CH_2 bending and the CH_3 symmetric deformations were confirmed by the presence of bands at 1423 and 1371 cm^{-1}, respectively [30-31]. The band at 1149 cm^{-1} corresponds to the asymmetric stretching of the C-O-C bridge, and the bands at 1066 and 1024 cm^{-1} correspond to the stretching of the primary and secondary OH groups [23]. The small signal at 1256 cm^{-1} indicates the bending vibrations of the OH groups present in chitosan [32]. In the FT-IR spectrum of HPCH and HECH, the peak intensities at 2890 cm^{-1}, and 1423 cm^{-1} were increased ascribing to stretching vibration of C-H, indicating the presence of more methylene groups in the structure. The peaks at 1066 cm^{-1}, and 1024 cm^{-1} were notably increased due to the hydroxypropyl and hydroxyethyl groups being replaced by both hydroxyl groups and amino groups of CH [28].

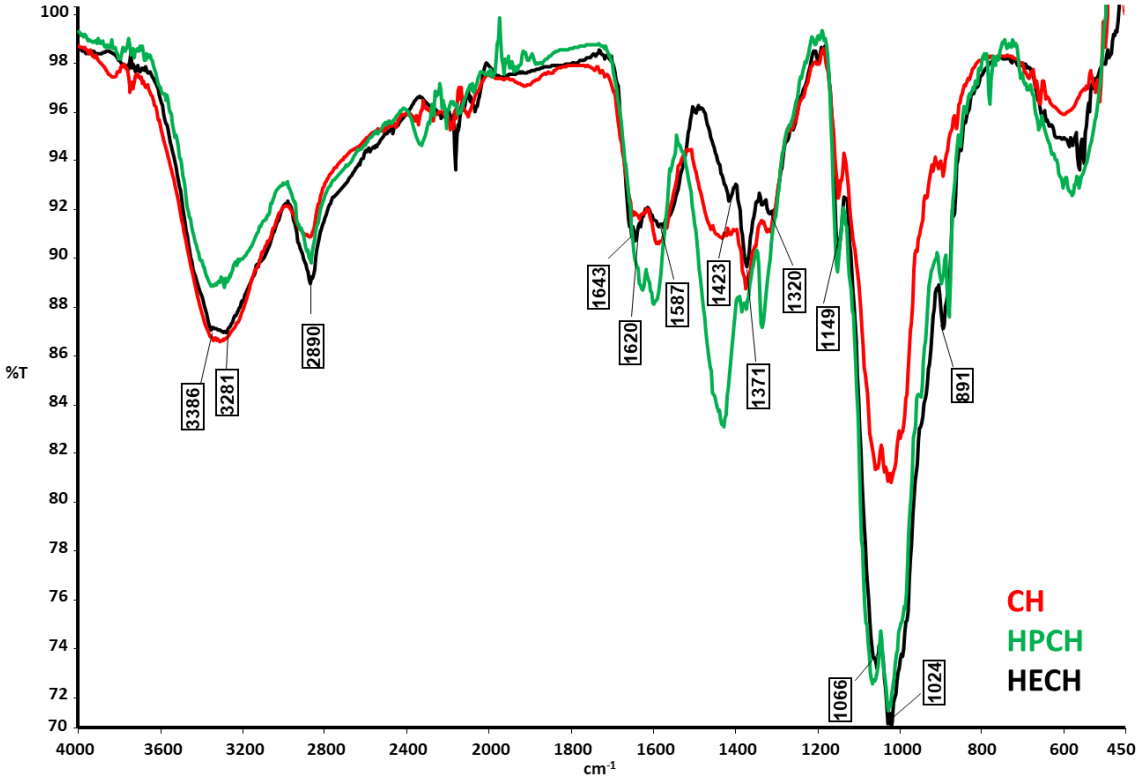

Figure 4. FTIR spectra of CH, HPCH and HECH

3.3. ^{1}H- NMR spectrum of CH Derivatives

The ^{1}H-NMR spectrum of CH was illustrated in Figure 5. The peak at δ 1.89 ppm is due to the H atoms of the acetyl group. As the deacetylation degree of the chitosan used is 75-85%, there are also significant amounts of N-acetyl ($-N-(CO)-CH_3$)) groups in the chitosan units, as well as $-NH_2$ functional groups. As a result of the deacetylation of chitin, it is not possible to obtain chitosan compounds with 100% removal of acetyl groups due to the polymeric structure. The peak at δ3.01 ppm belongs to the hydrogen (C2) of the glucosamine ring and the signals between δ3.56-3.74 ppm belong to the hydrogens at C4, 6, 3, 5 of the main chain in chitosan. The peak at 4.62 is attributed to hydrogen in OH groups [29, 33-36]. The peak at 4.81 ppm is due to hydrogen in the C1 group.

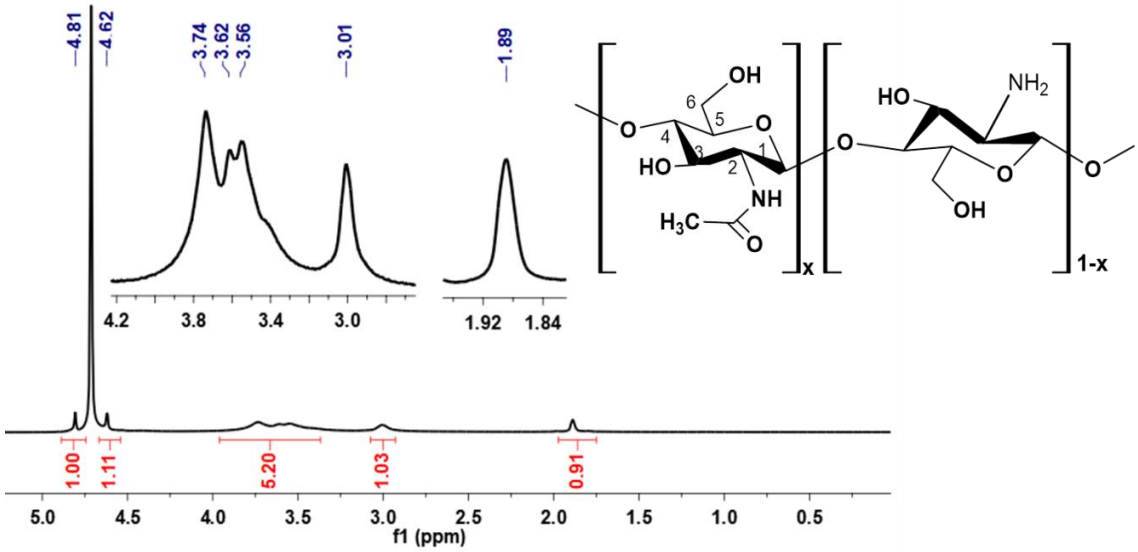

Figure 5. ^{1}H NMR of CH

The ^{1}H NMR spectrum of HPCH is given in Figure 6. The peak at δ1.13 ppm is attributed to the H protons of methyl group (H9). This is evidence that hydroxypropyl is incorporated into the chitosan ring [37-38]. Protons (H3-H8) of HPCH were observed at δ 3.36-3.91 ppm, and the peak at δ 2.03 ppm indicates the methyl hydrogen (H10) of N-acetylglucosamine. The peak at δ 2.68 ppm indicates the hydrogen (H2) of the glucosamine ring.

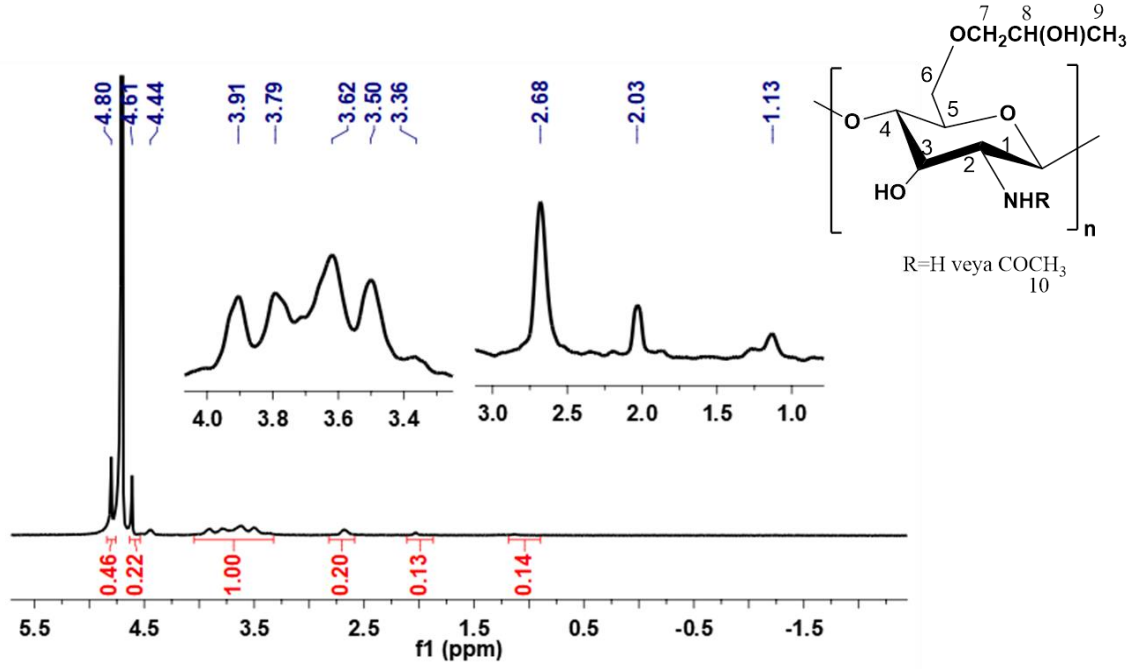

Figure 6. ^{1}H NMR of HPCH

The ^{1}H NMR spectrum of HECH is presented in Figure 7, the ring protons (H4,5,6) were considered to resonate between δ 3.91-3.30 ppm. The peak at δ 2.64 ppm indicates the hydrogen (C2) of the glucosamine ring.

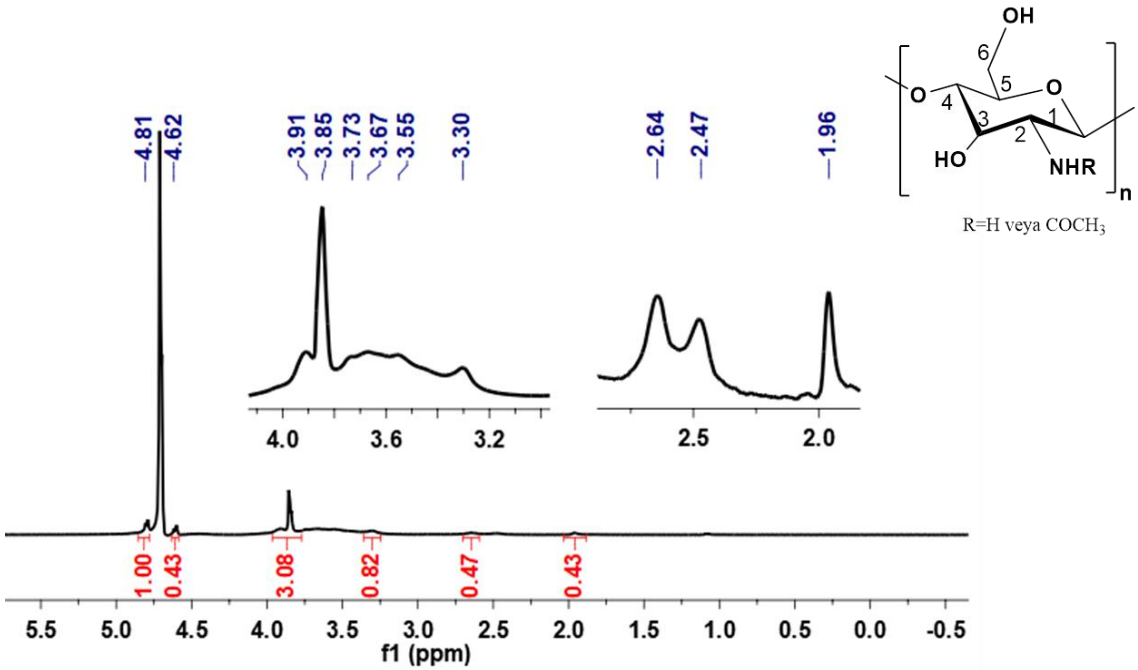

Figure 7. [1]H NMR of HECH

3.4. XRD spectrums of CH Derivatives

XRD patterns of CH, HPCH and HECH were shown in Figure 8. The specific peaks of chitosan are observed as 2θ=15° and 20° and these diffractions correspond to crystalline regions in chitosan [39]. This can be explained by the regularity in the polymer chain structure due to the strong intermolecular hydrogen bonds formed between the hydroxyl and amino groups present in chitosan. The strong peak at 2θ=20° in chitosan was weakened in HPCH and HECH. It can be seen that amorphous regions are replaced by a crystalline structure at 2θ=31° in HPCH, 2θ=27°, 31°, 45°, 56° and 66° in HECH. This can be explained by the incorporation of functional groups into the natural structure of chitosan as a result of the hydroxypropyl, and hydroxyethyl reactions.

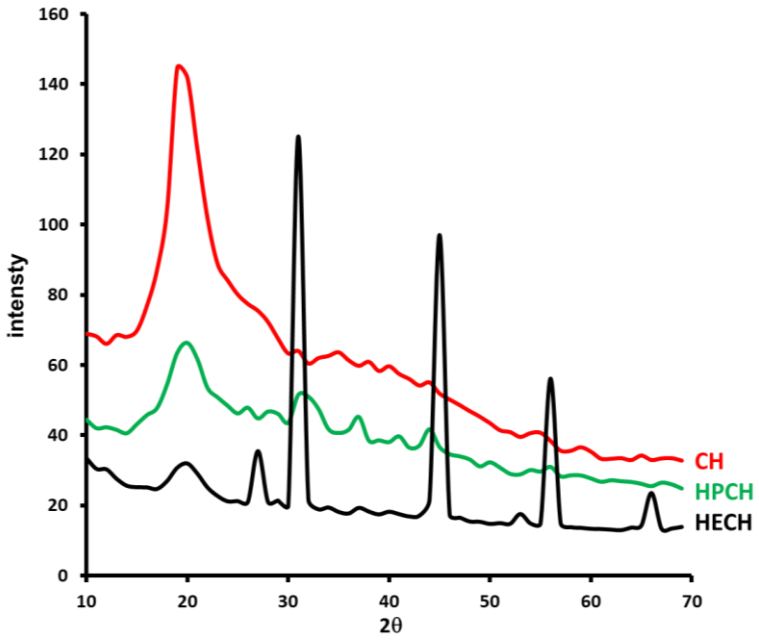

Figure 8. XRD pattern of CH, HPCH and HECH

3.5. TG-DTA spectrums of CH Derivatives

Thermogravimetric analysis (TGA) is used to study the thermal stability or thermal degradation behaviour where the weight loss of the sample is measured continuously. The thermal behaviours of CH, HPCH and HECH were given in Figure 9. CH and its derivatives showed two degradation stages: the first step was occurred around 100 °C and was assigned to the evaporation of the remaining water due to the strong affinity of polysaccharides to water. The initial weight loss at 100 °C was 6.4% in chitosan, 0.1% in HPCH, and 0.2% in HECH. Then, the weight of partially chitosan remained stable about 250 °C followed by a rapid substantial loss of weight [40-41]. The second step occurred around 310 °C in CH and the weight loss was 24.6 %. The second step occurred around 400 °C in HPCH and HECH and the weight loss was 51.0% and 51.1, respectively. The total weight loss at the final temperature (599.9 °C) was 67.9 % in CH. The total weight loss at the final temperature (599.9 °C) was 98.7% and 98.6 % in HPCH and HECH, respectively.

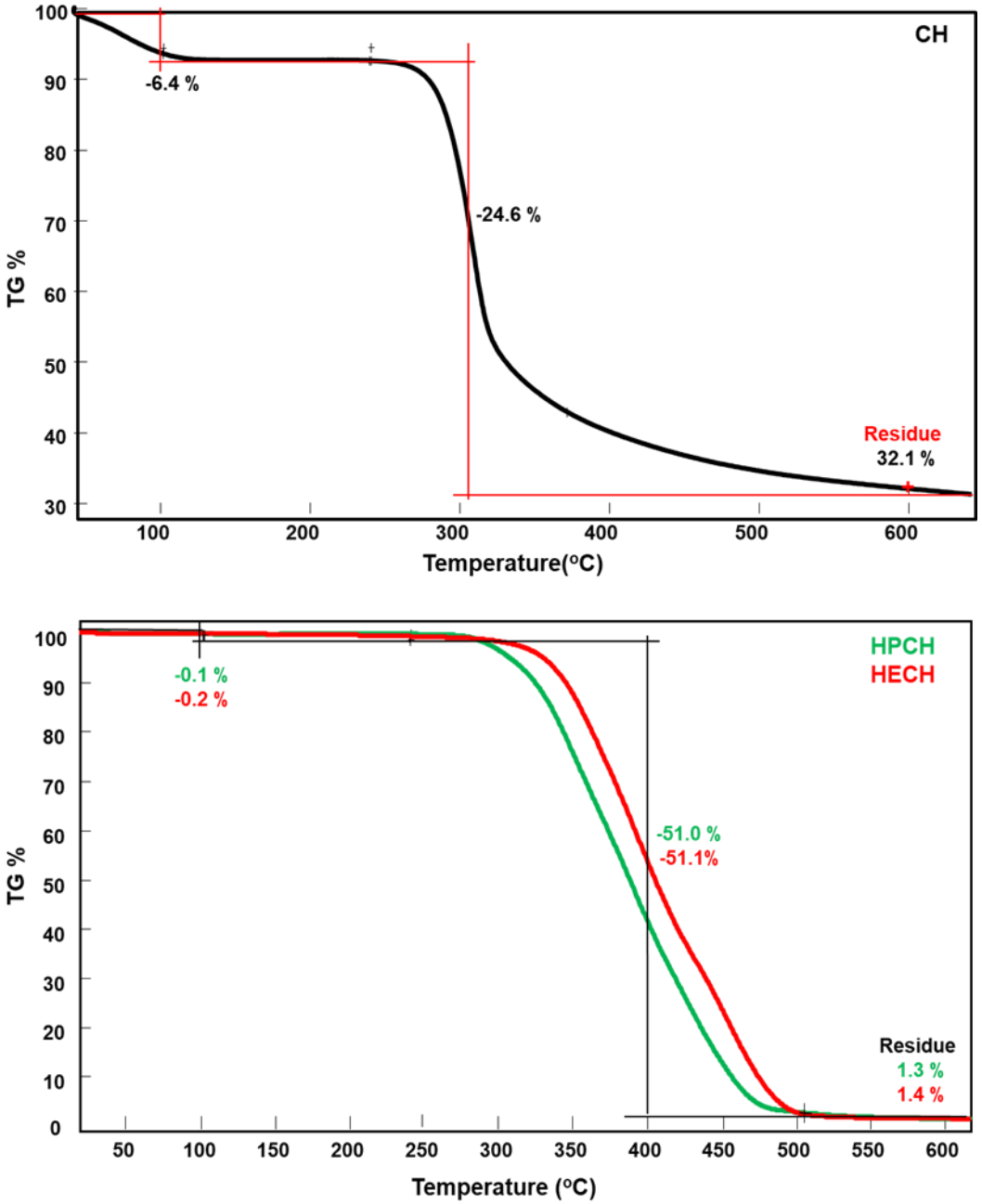

Figure 9. TG of CH, HPCH and HECH

Conclusion

This study successfully synthesized chitosan ethers: HPCH and HECH. Structures of synthesized chitosan derivatives were characterized by FT-IR, XRD, and ^{1}H-NMR spectroscopy, and the surface morphologies and thermal properties were investigated by SEM and DTA-TG analysis, respectively. Chitosan and its derivatives have become promising polymers in multidisciplinary fields due to their distinct properties such as biocompatibility, biodegradability, non-toxicity, and low reactivity. However, there are few studies in the literature on the synthesis of HPCH and HECH, which are important chitosan derivatives. Therefore, this study will contribute to the literature and science.

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