SYNTHESIS AND CHARACTERIZATION OF SEVERAL LAURYL CHITOSAN DERIVATIVES

(Sintesis dan Pencirian Beberapa Terbitan Lauril Kitosan)

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Abstract

Three derivatives of chitosan namely lauryl chitosan, lauryl succinyl chitosan and lauryl carboxymethyl chitosan had been synthesized in this study. The synthesis of lauryl carboxymethyl chitosan and lauryl succinyl chitosan require two steps of reaction whereas for lauryl chitosan only one step was required. Lauryl carboxymethyl chitosan was prepared by reacting chitosan with monochloroacetic acid to form carboxymethyl chitosan. The synthesis of lauryl succinyl chitosan involved the reaction between chitosan with succinic anhydride to form succinyl chitosan. After that, the carboxymethyl chitosan and succinyl chitosan were reacted with dodecanal to form lauryl carboxymethyl chitosan and lauryl succinyl chitosan, respectively. The addition of lauryl group onto the derivative aim at producing chitosan derivatives having both hydrophobic and hydrophillic properties. The chemical structures of the derivatives were characterized by Fourier Transform Infrared (FTIR), ^{1}H Nuclear Magnetic Resonance (^{1}H NMR) and Elemental Analysis (CHNS). In the IR spectrum of carboxymethyl chitosan and succinyl chitosan, peaks at 1645-1630 cm^{-1}, representing C=O were clearly seen. After the substitution of lauryl group on the derivatives, the presence of peaks at 2930-2860 cm^{-1} indicates the C-H stretching of lauryl group. The presence of lauryl group was further supported by the presence of peaks at about 0.8-1.7 ppm in the ^{1}H NMR spectra that assigned for hydrogen that attached to lauryl group. Elemental analysis was used to compare the derivatives prepared with the theoretical values.

Keywords: Hydrophilic, Hydrophobic and Chitosan.

Abstrak

Tiga terbitan kitosan iaitu lauril kitosan, lauril karboksimetil kitosan dan lauril suksinil kitosan telah di sintesis. Proses penyediaan lauril karboksimetil kitosan dan lauril suksinil kitosan melibatkan dua langkah tindak balas manakala lauril kitosan hanya melibatkan satu langkah tindakbalas. Lauril karboksimetil kitosan disediakan dengan mensintesis karboksimetil kitosan terlebih dahulu melalui tindak balas kitosan dengan asid monokloroasetik. Manakala lauril suksinil kitosan pula disediakan dengan mensintesis suksinil kitosan terlebih dahulu melalui tindakbalas di antara kitosan dengan suksinil anhidrida. Kemudian, karboksimetil kitosan dan suksinil kitosan ditindakbalaskan dengan dodekenal untuk menghasilkan lauril karboksimetil kitosan dan lauril suksinil kitosan. Penambahan kumpulan lauril pada terbitan yang terhasil adalah bertujuan untuk menghasilkan terbitan kitosan yang mempunyai kedua-dua sifat iaitu sifat hidrofilik dan hidrofobik. Terbitan kitosan tersebut telah dicirikan dengan menggunakan Spektroskopi Inframerah (FTIR), Resonan Magnetik Nukleus (RMN) dan analisis unsur (CHNS). Berdasarkan data FTIR, bagi terbitan karboksimetil kitosan dan suksinil kitosan, terdapat puncak pada 1645-1630 cm^{-1} yang menunjukkan kehadiran kumpulan C=O. Namun begitu, setelah kemasukkan kumpulan lauril pada terbitan tersebut terdapat kehadiran puncak pada 2930-2860 cm^{-1} yang menunjukkan regangan C-H pada kumpulan lauril. Puncak ini juga hadir pada spektrum FTIR bagi lauril kitosan. Kehadiran kumpulan lauril pada ketiga-tiga terbitan seterusnya disokong dengan kehadiran puncak pada 0.8-1.7 ppm pada spektrum^{1}H- NMR. Analisis CHNS digunakan untuk membandingkan peratus unsur dalam terbitan yang disintesis dengan nilai teori.

Kata kunci: Hidrofilik, hidrofobik dan kitosan.

Introduction

Chitosan were obtained from partial deacetylation of chitin. It compose of β-(1,4)-2-amino-2-deoxy-D-glucopyranose(GLcN) and 2-acetamido-2-deoxy-D-glucopyranose(GLcNAc) residues. Previous studies proved that chitosan have various properties such as antibacterial, non-toxicity, biodegradability, haemocompatible and biocompatibility. All of these properties make it become an attractive biomaterial [1]. However, chitosan has poor solubility in water. In order to improve its water solubility, some chemical modification of chitosan was carried out [2]. Chemical structure of chitosan contains two hydroxyls and one amino group. The general reaction for formation

of lauryl derivatives chitosan was shown in Figure 1 and all chitosan derivatives had been synthesized were listed in Table 1.

In the past, many studies had been focused on improvement of the solubility of chitosan e.g . the insertion of hydrophilic group onto chitosan where the derivatives that had formed such as carboxymethyl chitosan, succinyl chitosan, hydroxylalkyl and many more [2]. Besides that, insertion of the hydrophobic group onto chitosan had formed the derivatives such as N-pentyl-chitosan, N-pentylidene-chitosan and many more[3]. Insertion of both groups also can be done into chitosan [4, 5, 6, 11].

In this study, substitution of both hydrophobic and hydrophilic groups into chitosan structure were performed. The derivatives studied were lauryl chitosan, lauryl carboxymethyl chitosan and lauryl succinyl chitosan. The derivatives were characterized by means of Fourier Transform Infra Red (FTIR) spectroscopy, ^{1}H Nuclear Magnetic Resonance Spetroscopy (^{1}H-NMR) and elemental analysis (CHNS).

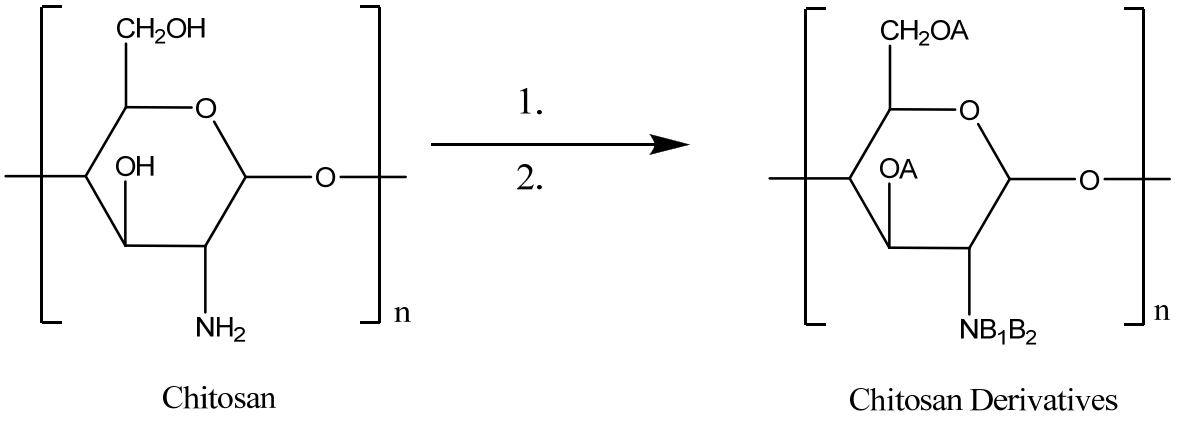

Figure 1: General reaction for formation of lauryl chitosan derivatives

Derivatives of chitosan	Reaction step	A	B_1	B_2
Lauryl Chitosan	Lauryl aldehyde	-H	-H	$-CH_2(CH_2)_{10}CH_3$
Lauryl Carboxymethyl Chitosan	1. Monochloacetic acid 2. Lauryl aldehyde	$-CH_2COOH$	-H	$-CH_2(CH_2)_{10}CH_3$
Lauryl Succinyl Chitosan	1. Succinic anhydride 2. Lauryl aldehyde	$-C(OH)H(CH_2)_{10}CH_3$	$-COCH_2CH_2COOH$	-H

Table 1: All chitosan derivatives synthesized

Materials and methods

Reagents

Chitosan powder was supplied by Chito-chem Sdn Bhd., Malaysia. All commercially available solvents and reagents were used without further purification.

Preparation of chitosan derivatives

Preparation of Lauryl Chitosan

Lauryl chitosan was prepared according to the Muzzarelli et al. [14] and Ramos et al. method [6]. Chitosan (1g) was suspended in a water-methanol 1:1 mixture (100 mL), lauryl aldehyde (1.5g) was added and stirred for 30 min. Reduction was carried out with sodium borohydride solution (0.5 g dissolve in 10 ml of water) for 2h with mechanical stirring. The preparation was left overnight. The reaction mixture was then neutralized with HCl 5M solution and the lauryl chitosan was precipitated with methanol. The precipitate was filtered and washed with 90% methanol/water, methanol, hexane and acetone.

Preparation of Lauryl Carboxymethyl Chitosan

Carboxymethyl chitosan was prepared was prepared according to oleh Chen & Park method [7]. Chitosan (10g), sodium hydroxide (10g), isopropanol (50 mL) and water (50 mL) were added into a flask to swell and alkalize at a 50°C for 1 h. The monochloroacetic acid (15 g) was dissolved in isopropanol (20 mL), added into the reaction mixture drop-wise for 30 min and reacted for 4 h at the same temperature, then stopped by adding 70% ethyl alcohol (200 mL). The solid was filtered and rinsed in 70%, 80%, 90% ethyl alcohol, and dried at room temperature. The product was Na salt Carboxymethyl Chitosan. (Na-CC). Addition of lauryl group to Carboxymethyl Chitosan was done according to the method preparation of lauryl chitosan.

Preparation of Lauryl Succinyl Chitosan

Succinyl chitosan (SC) was prepared according to Zhu et al. method [8]. However, the solvent had been changed from acetone to ethanol during the rinsing process of precipitates. 1 g of chitosan was dissolved into 200 mL distilled water and then transferred into a flask. Succinic anhydride (0.2 g) was dissolve in acetone (20 mL) and added into the flask by drop-wise for 30 min at room temperature, and then the reaction was left for 4 h at 40°C. The reaction mixture was cooled to room temperature. The mixture precipitated in an excess of ethanol, was filtered to remove the solvent, and then washed with 70%, 80%, 90% ethanol, and dried at room temperature. Addition of lauryl group to succinyl chitosan was done according to the method preparation of lauryl chitosan.

Result and discussion

Synthesis of chitosan derivatives

The introduction of a hydrophobic alkyl chain onto chitosan and its derivatives leads to the formation of chitosan derivatives with two group which are hydrophilic and hydrophobic group. In this study, the introduction of lauryl group where C12 chain gives rise to formation of lauryl chitosan (LC), lauryl carboxymethyl chitosan (LCC) and lauryl succinyl chitosan (LSC). All of these derivatives, namely carboxymethyl and succinyl act as hydrophilic moities while lauryl groups as hydrophobic one.

Characterization of derivatives

Structure of chitosan and its derivatives were confirmed by FTIR spectra. The FTIR spectra of chitosan, carboxymethyl chitosan (CC), succinyl chitosan (SC), lauryl chitosan (LC), lauryl carboxymethyl chitosan (LCC) and lauryl succinyl chitosan (LSC) were given in Fig. 2a ,2b, 2c,2d, 2e and 2f. The main bands observed in the infrared spectrum of chitosan (Fig. 2a) were: (i) a broad band due to the stretching of O-H and N-H bond centred at 3429 cm^{-1}, (ii) a band centred at 2923 cm^{-1} corresponding to the stretching of C-H bonds; (iii) a band centred at 1642 cm^{-1} which is attributed to the stretching of C-O bonds of the acetamide groups, named as amide I band; (iv) a band at 1377 cm^{-1} due to the symmetric deformation of CH_3; (vi) the amide III band at 1321cm^{-1}; (vii) the band corresponding to the polysaccharide skeleton, including the vibrations of the glycoside bonds, C-O and C-O-C stretching, in the range 1148–896 cm^{-1} [9].

Based on the IR spectra of carboxymethyl chitosan (Fig. 2b), it was found that absorption bands appear at 1634 cm^{-1} and 1406 cm^{-1} while for succinyl chitosan (Fig. 2c) absorption bands appear at 1646 cm^{-1} and 1402 cm^{-1}. Both bands are due to asymmetric and symmetric stretching vibration of C=O, indicating a successful substitution of carboxyl groups. Stretching vibration of C=O for succinyl chitosan are higher compared to carboxymethyl chitosan because the carbonyl group in the succinyl chitosan are in the form of amide while carbonyl group in the carboxymethyl chitosan are in the form of carboxylate salts [10].

Lauryl group was attached covalently to the amino groups of chitosan and carboxymethyl chitosan while for succinyl chitosan, lauryl group was attached at the hydroxyl group. The IR spectra for native chitosan and the derivatives were compared. Substitution of lauryl group onto chitosan, carboxymethyl chitosan or succinyl chitosan leads to two absorption band around 2923 and 2855cm^{-1} for C-H stretching. Besides that, bands where observed at 1527 cm^{-1}, 1530 cm^{-1} and due to deformation for amines. It proves that the substitution of chitosan by lauryl group occurred at N position for chitosan and carboxymethyl chitosan [10].

In addition, by comparing IR spectrum of lauryl chitosan(Fig. 2d), lauryl-CC (Fig. 2e) and lauryl-SC (Fig. 2f) with IR spectrum of chitosan (Fig. 2a), carboxymethyl chitosan (Fig. 2b), succinyl chitosan (Fig. 2c), the two peaks at 1074 and 1030 cm^{-1} in the derivatives were attributed to the methyl rocking and C-CH_3 stretching vibration respectively of the lauryl group. All of these are evidences suggest that the hydrophilic group and hydrophobic group were introduced to chitosan [4].

The ^{1}H NMR spectra of CC, LCC, SC, LSC and LC were given in Fig. 3a, 3b, 3c, 3d and 3e. ^{1}H NMR spectra of lauryl chitosan (Fig. 3e): ^{1}H NMR ($CDCl_3$), δ=4.81(H1), δ=3.11(H2), δ= 3.40-3.66 (H3, H4, H5, H6), δ= 2.01 (NCOC$\underline{H}_3$), δ= 0.89 (-NH-R-C$\underline{H}_3$;R=alkyl chain), δ =1.27-1.65 (-(C$\underline{H}_2$)$_n$-CH_3) [10, 12, 4, 13]. ^{1}H NMR spectra of carboxymethyl chitosan (Fig. 3a): ^{1}H NMR (D_2O), δ=4.80(H1), δ=3.12(H2), δ= 3.69-3.86 (H3, H4, H5, H6), δ= 2.01 (NCOC$\underline{H}_3$), δ= 3.69-3.86 (O-CH_2-COO^-). ^{1}H NMR spectra of lauryl carboxymethyl chitosan (Fig. 3b), there was a peak as follows: ^{1}H NMR ($CDCl_3$), δ= 0.88 (-NH-R-C$\underline{H}_3$;R=alkyl chain), δ =1.26-1.62 (NH-(C$\underline{H}_2$)$_{11}$-CH_3)) [10, 12, 4, 13].

While ^{1}H NMR spectra of succinyl chitosan (Fig. 3c): ^{1}H NMR (D_2O), δ=4.88(H1), δ=3.12(H2), δ= 3.66-3.82 (H3, H4, H5, H6), δ= 1.92 (NCOC$\underline{H}_3$), δ= 2.45-2.55(NCO-C$\underline{H}_2$-COOH). ^{1}H NMR spectra of lauryl succinyl chitosan (Fig. 3d), there was a peak as follows: ^{1}H NMR ($CDCl_3$), δ= 0.89 (-O-R-C$\underline{H}_3$;R=rantai alkil), δ =1.26-1.60 (O-CH(OH)(C$\underline{H}_2$)$_{10}$-CH_3), δ =3.56 (O-CH(OH)-R)) [10, 12, 4, 13]. ^{1}H-NMR data showed that the, lauryl group bound to N atom for lauryl chitosan and lauryl carboxymethyl chitosan. While lauryl group bound to O atom for lauryl succinyl chitosan.

The degrees of substitution were calculated by comparing the C and N ratio obtained from element analysis in each derivative. The increase in the C/N ratio indicates the increasing carbon in monosaccharides include one nitrogen. For example, in the case of carboxymethylation, carboxymethyl group includes 2 carbons; therefore, the degree of carboxymethylation was estimated from the increasing-molar ratio/2 [11]. While, the substitution of lauryl group involve 12 carbons; so the degree of laurylation was estimated from the increasing-molar ratio/12. The degrees of carboxymethylation, succinylation and laurylation were shown in Table 2. Between all the three derivatives, lauryl chitosan show the highest degree of substitution (DS). This is because; lauryl chitosan only involved one step of preparation while lauryl carboxymethyl chitosan and lauryl succinyl chitosan involve two step of reaction. Besides that, the amount of carbon added to the lauryl carboxymethyl chitosan and lauryl succinyl chitosan was higher than the lauryl chitosan.

Table 2: Elemental Analyses and the Degree of Substitution (DS) of Chitosan Derivatives

Derivatives	Found % C	N	C/N	DS
Chitosan	38.51	7.24	6.20	
Carboxymethyl Chitosan	28.90	5.33	6.33	0.03
Succinyl Chitosan	36.08	5.47	7.69	0.37
Lauryl Chitosan	40.60	5.18	9.14	0.25
Lauryl Carboxymethyl Chitosan	31.44	4.56	8.04	0.14
Lauryl Succinyl chitosan	32.27	4.35	8.65	0.08

Conclusion

Three derivatives of chitosan had been synthesized namely lauryl chitosan, lauryl succinyl chitosan and lauryl carboxymethyl chitosan by introducing lauryl group into chitosan, carboxmethyl chitosan or succinyl chitosan. The chemical structures of the three derivatives were characterized by FTIR, ^{1}H NMR and elemental analysis. IR studies confirmed the appearance of C=O and lauryl group at chitosan derivatives. It also supported by ^{1}H-NMR data. These indicate hydrophobic and hydrophilic group was attached to the chitosan derivatives.

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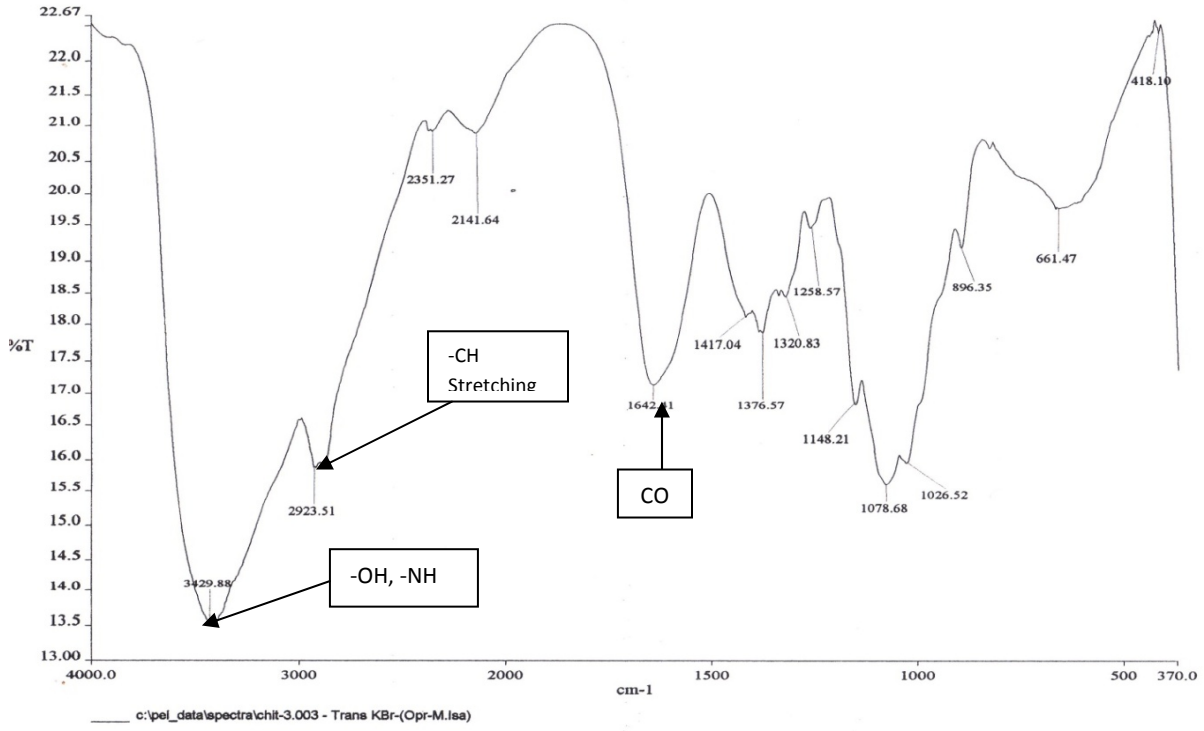

Fig 2a: FTIR spectrum of Chitosan

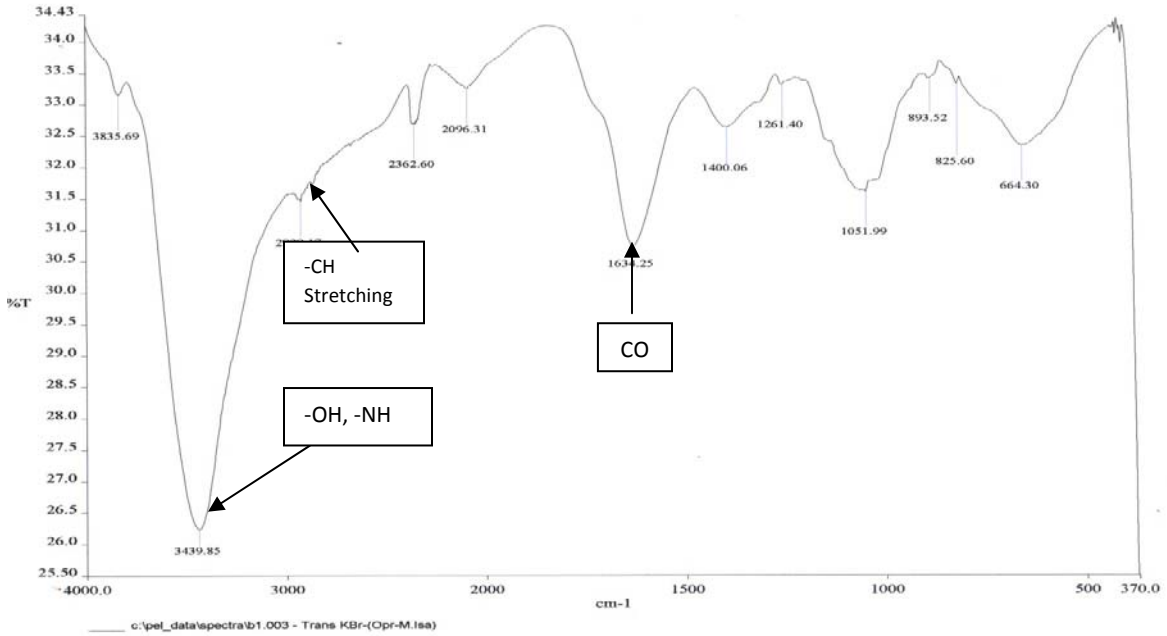

Fig. 2b: FTIR spectrum of Carboxymethyl Chitosan

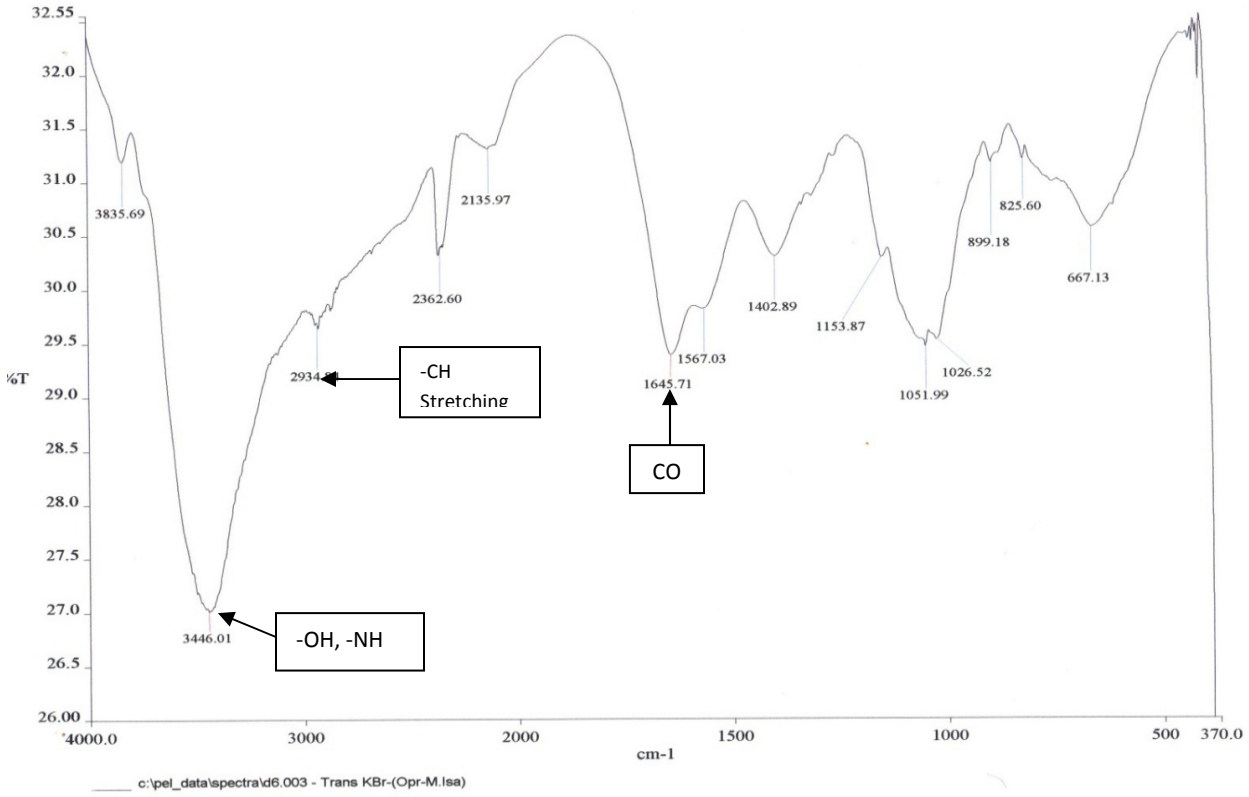

Fig 2c: FTIR spectrum of Succinyl Chitosan

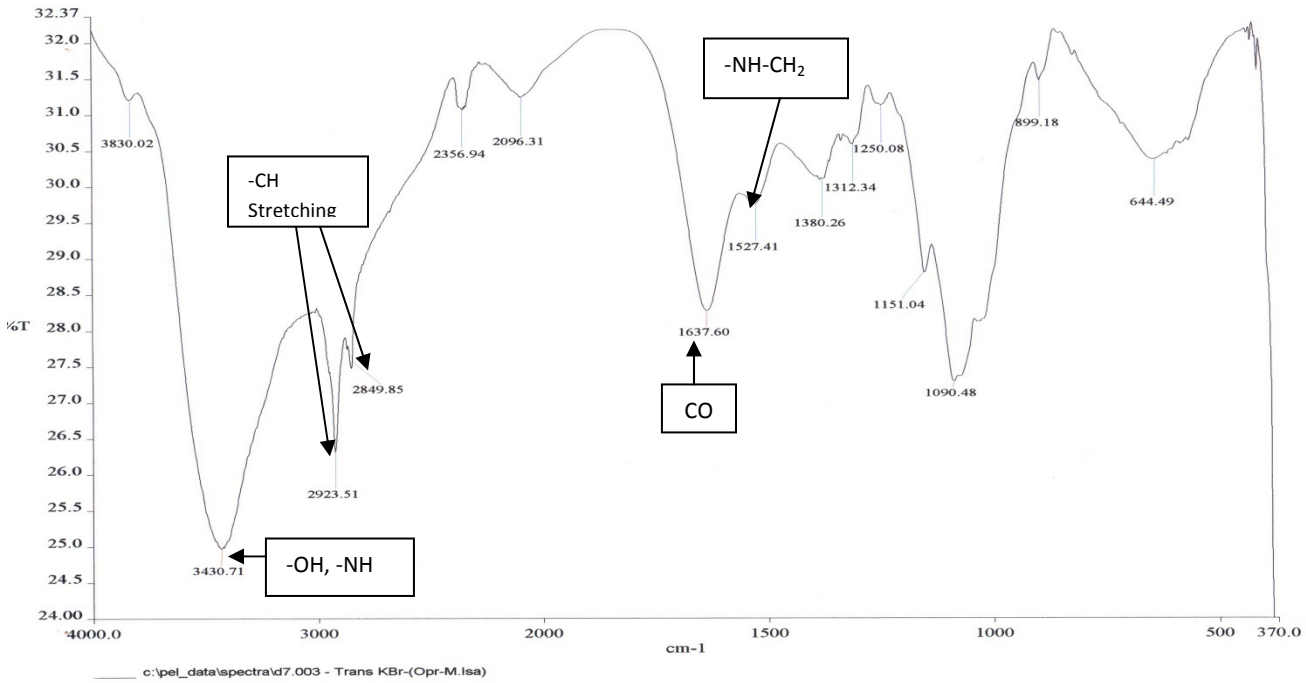

Fig 2d: FTIR spectrum of Lauryl Chitosan

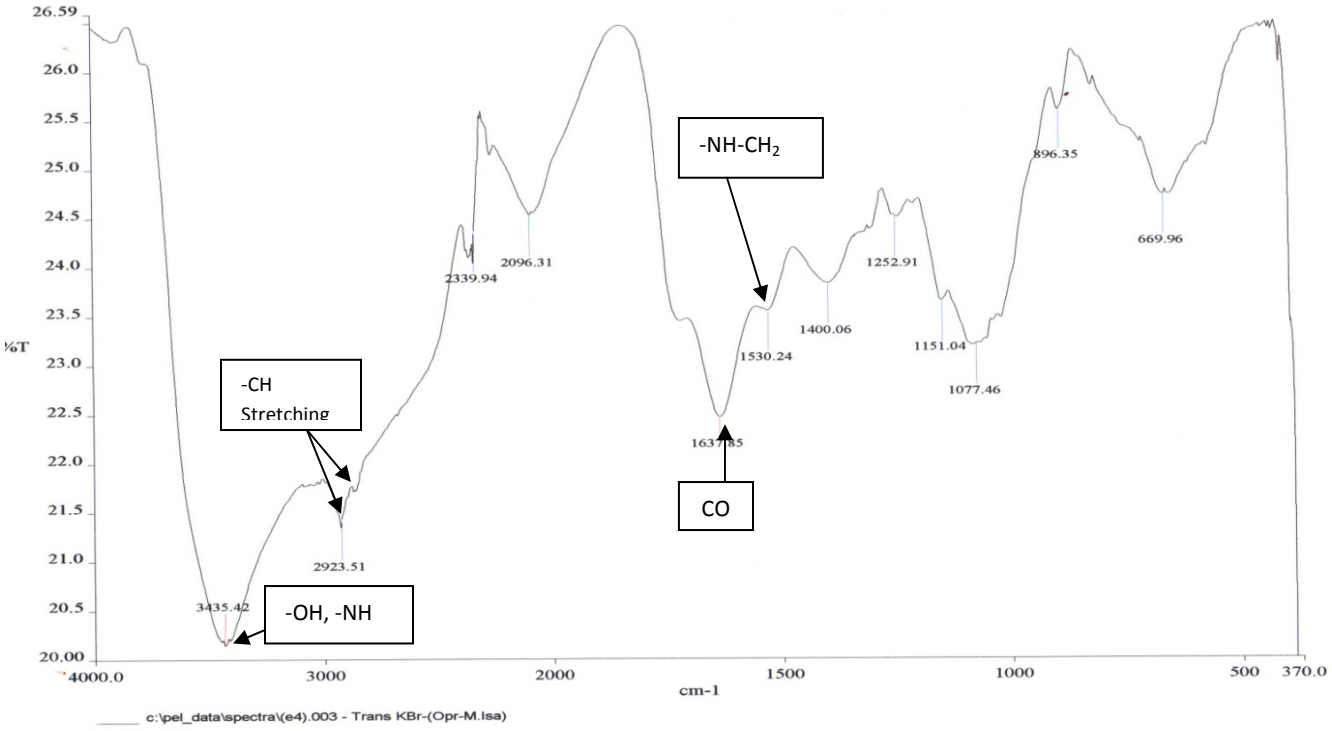

Fig 2e: FTIR spectrum of lauryl carboxymethyl chitosan

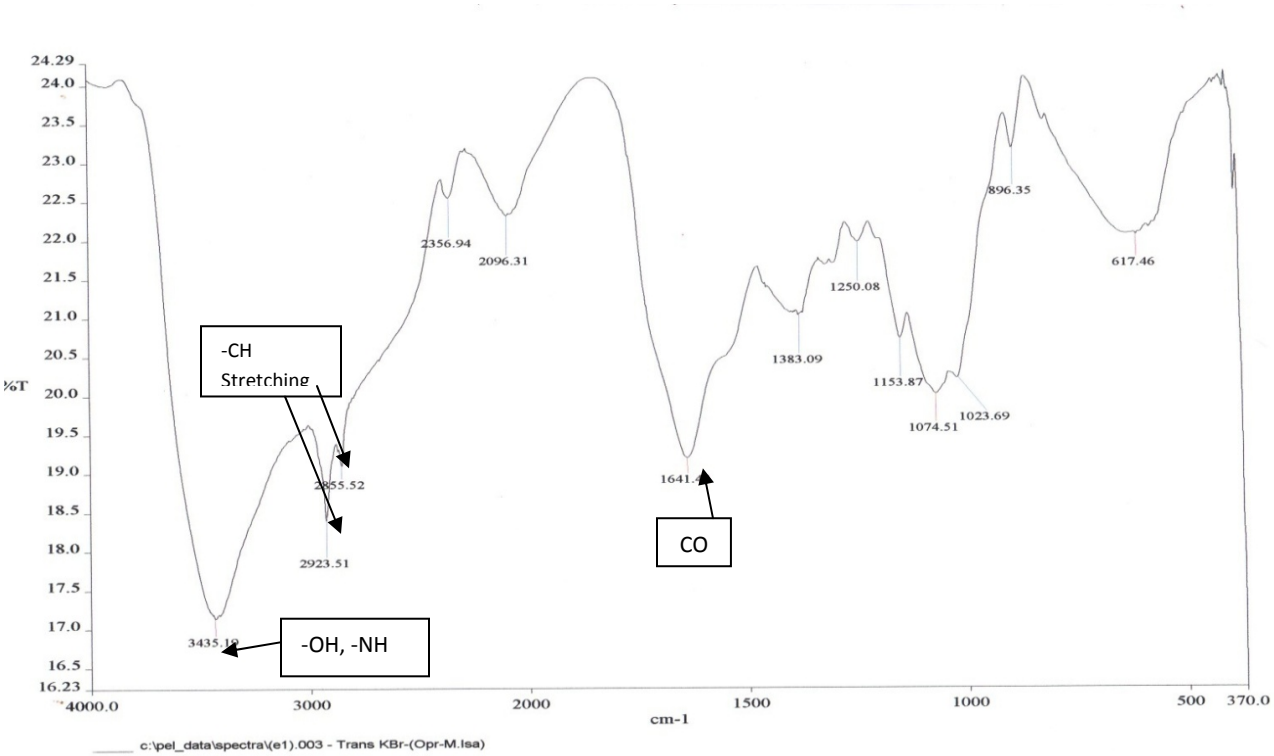

Fig 2f: FTIR spectrum of lauryl succinyl chitosan

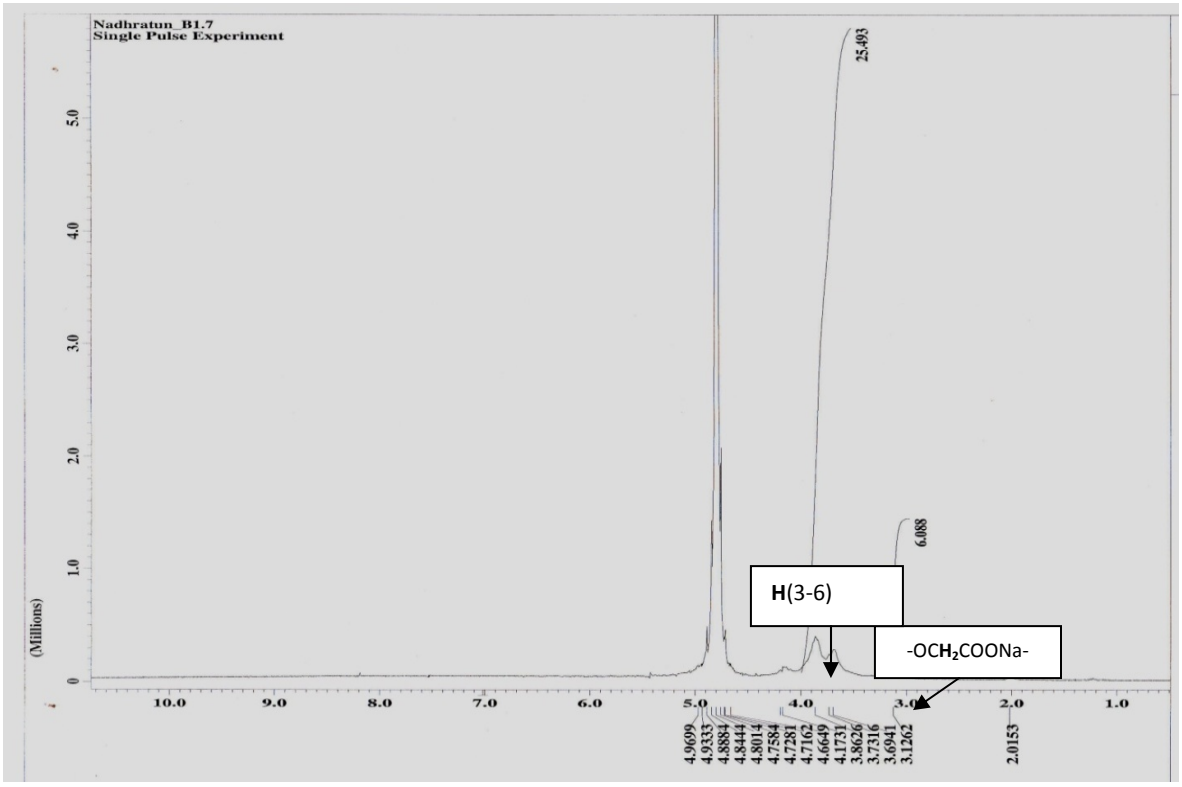

Fig 3a: NMR spectrum of carboxymethyl chitosan

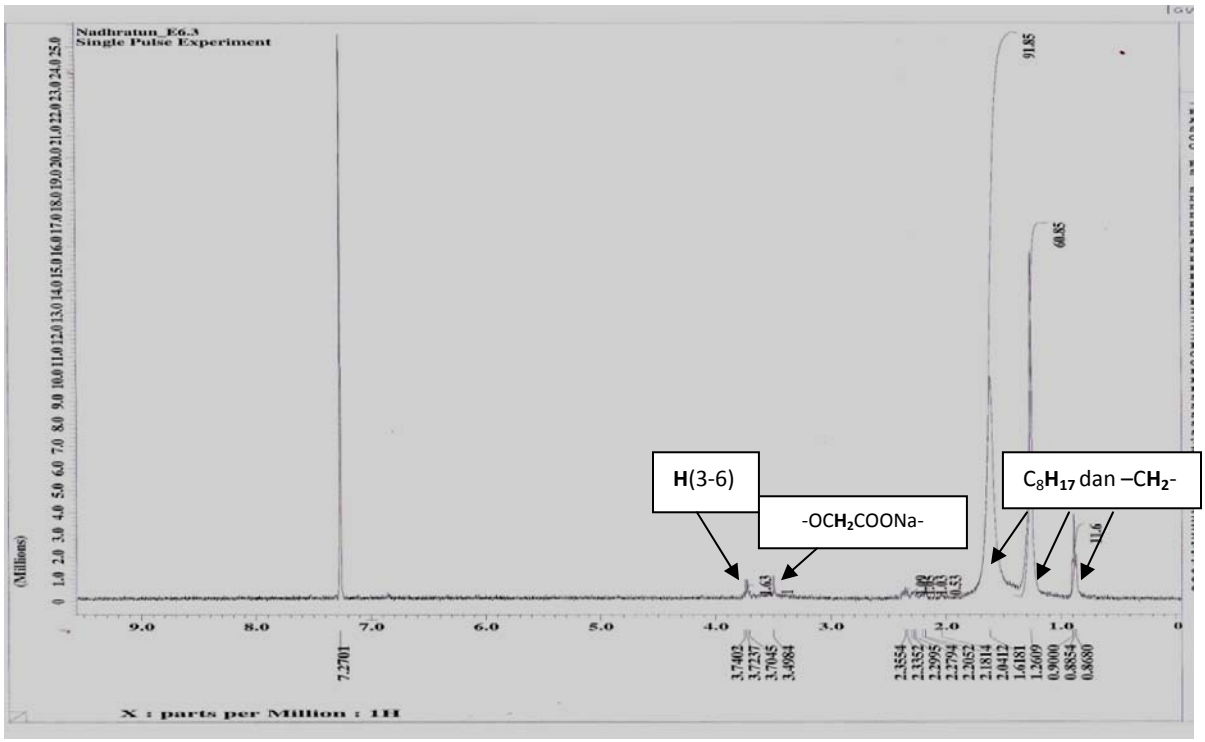

Fig 3b: NMR spectrum of lauryl carboxymethyl chitosan

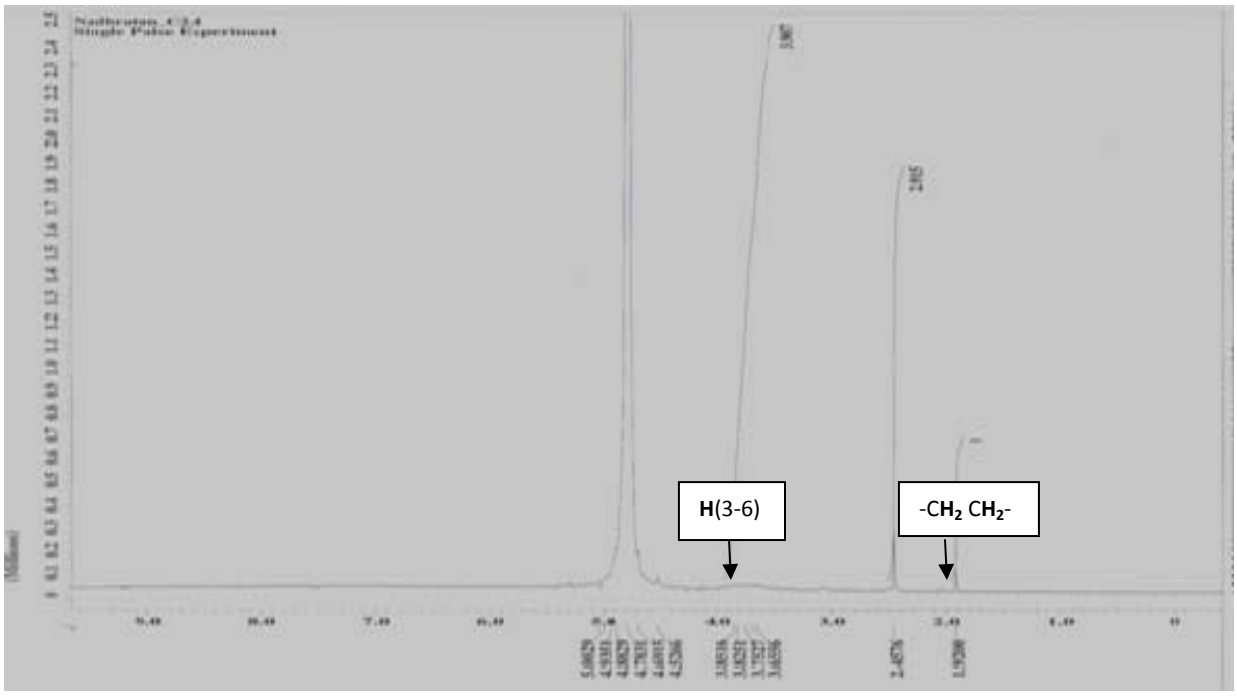

Fig 3c: NMR spectrum of succinyl chitosan

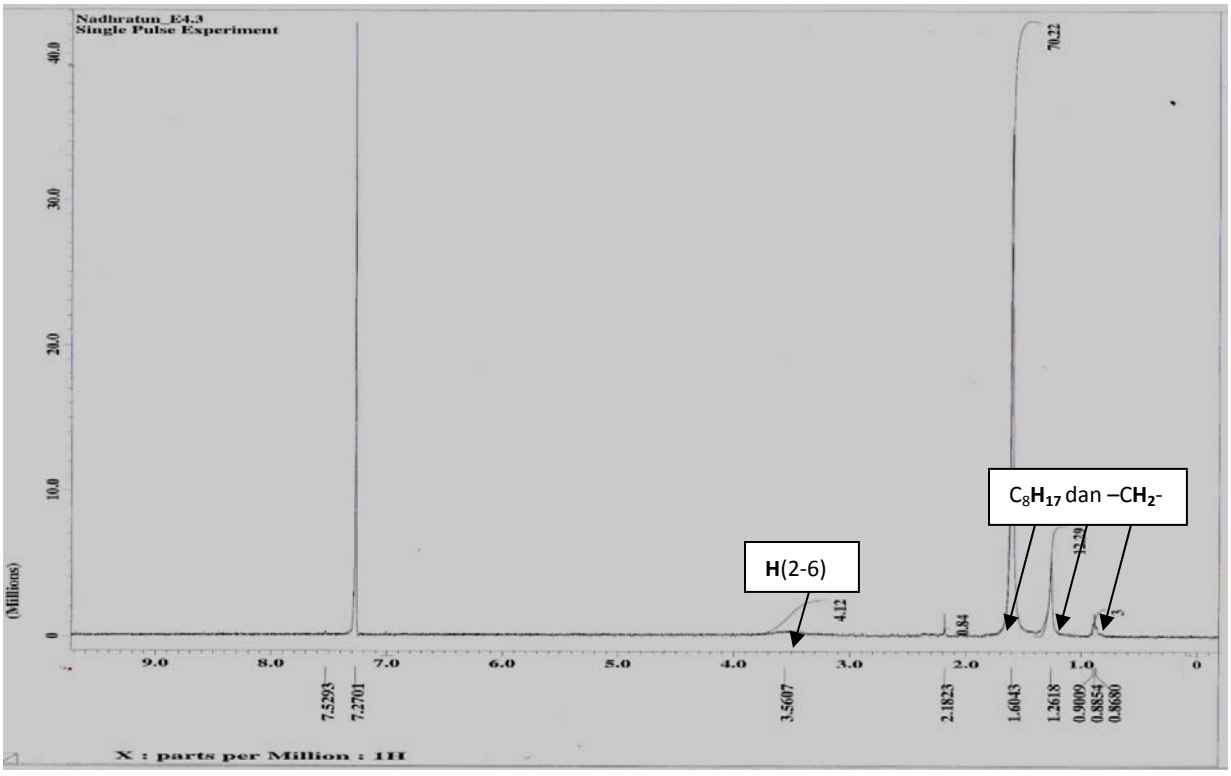

Fig 3d: NMR spectrum of lauryl succinyl chitosan

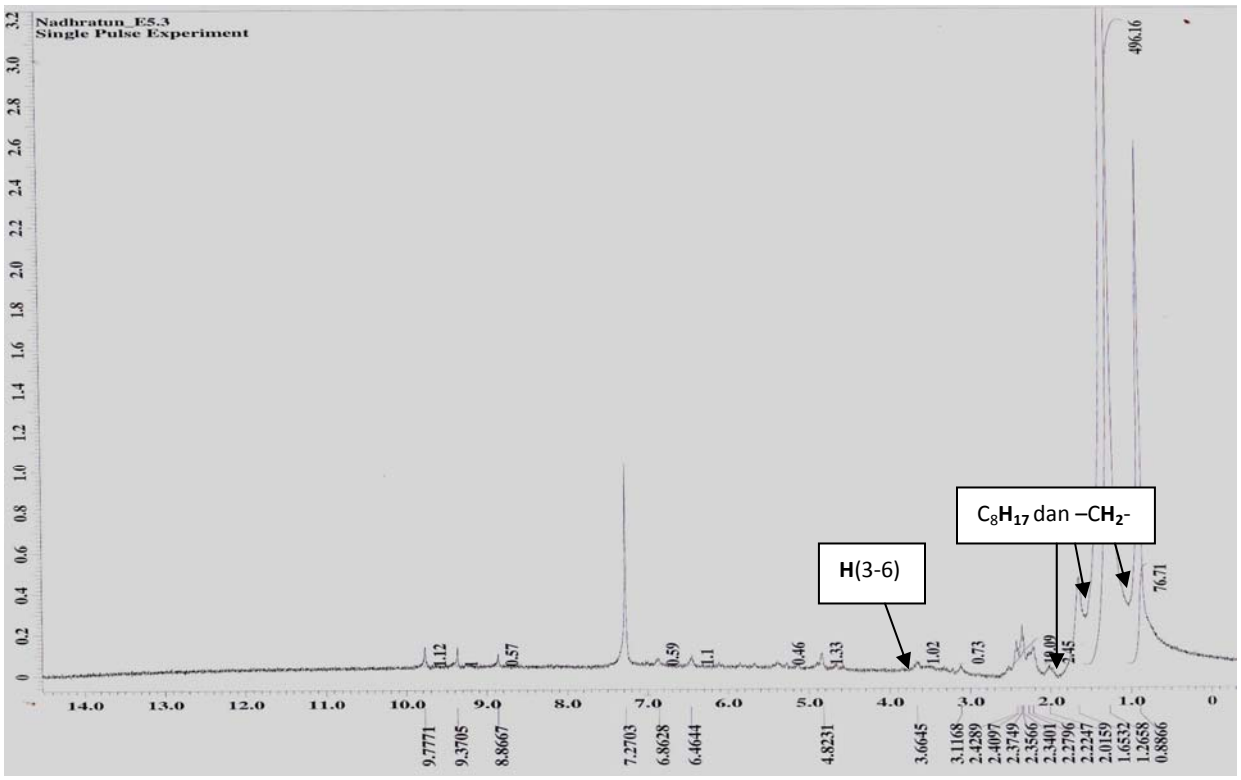

Fig 3e: NMR spectrum of lauryl chitosan