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SYNTHESIS AND CHARACTERIZATION OF CHITIN co-(ACETATE/SUCCINATE) COPOLYMERS

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ABSTRACT

Chitin, composed of 2-acetamido-2-deoxy-β-D-glucose structural units interlinked through β-(1→4) bridges, is the second most plentiful natural polymer in the world. Crab and shrimp shells are the economic sources of chitin, despite the fact that it is synthesized by a large number of living organisms. The applicability of this biocompatible and biodegradable polymer is limited in the context of pharmaceutical and biomedical area owing to its insolubility in common solvents. In the present investigation, a series of chitin acetate/succinate copolymers with different content of acetyl and succinyl groups has been synthesized by reacting chitin with different ratios of acetic and succinic anhydride in the presence of perchloric acid as the catalyst of the reaction under heterogenous conditions. Pure chitin diacetate was also synthesized by using the same reaction and their structures were confirmed by FTIR and ^{1}H-NMR spectroscopy. The degree of substitution of the acetyl group and succinyl groups were in the range of 0.22-1.34 and 0.64-1.76, respectively as determined by ^{1}H-NMR spectroscopy. The expected solubility of these compounds in common solvents would open new domain of applications in the field of pharmaceutical and biomedical sciences.

KEY WORDS

Chitin, Chitin acetate/succinate, Copolymer, Esterification, FTIR, ^{1}H-NMR

1 INTRODUCTION

Chitin, the natural amino polysaccharide consists of 2-acetamido-2-deoxy-β-D-glucose repeating units connected through β-(1→4) linkage. It is the second most bountiful natural polymer in the world which is synthesized by an extremely large number of living organisms such as arthropods (e.g., crustacean shells of shrimp, crab and cuttlefish), in the cell wall of yeast and fungi as well as in the lower plant and animal kingdoms such as algae, microfauna and planktons etc. Presently, commercial source of chitin is sea food canning industries where it is discarded as a waste material [1-3]. Chitin is biocompatible and biodegradable polymer having good biological properties, but its molecules possess tendency to form intermolecular hydrogen bonding and their organization in the form of sheets results in the formation of supramolecular structure with undesirable biomechanical properties and low solubility in common solvents [4, 5]. This limitation has restricted the interest of the scientific community, particularly pharmaceutical and biomedical scientists, biotechnologist etc. towards this otherwise excellent natural polymer. The presence of free hydroxyl groups in the structure of chitin appears as a boon which offers wide possibilities for its chemical modification leading to the development of newer derivatives with potential novel applications. The intermolecular hydrogen bonding interactions can be weakened by the replacement of the free hydroxyl groups with some hydrophobic residues such as esters thereby allowing easier migration of molecules from crystal lattice making these new compounds more soluble in water and other common solvents.

Various derivatives of chitin such as formyl, acetyl, propionyl, butyryl have been synthesized by various researchers [6, 7]. These derivatives with different degrees of substitution (DS) have been synthesized by using methanesulphonic acid as a catalyst and the corresponding acid anhydrides as acylating reagents.

Diacetylchitin synthesized under heterogenous conditions using perchloric acid as a catalyst of the esterification reaction has shown limited solubility due to lower number of the hydrophobic carbon atoms in the acetyl group, therefore, it does not find much practical applications [6, 8]. To quash this, mixed esters of chitin can be synthesized with the introduction of the two different types of ester groups on the structure of chitin, the bulky succinate and the smaller acetyl, which may result new derivatives with desired solubility profiles. Several mixed ester of chitin with improved solubility such as chitin co-(acetate/octanoate), chitin co-(acetate/propionate), chitin co-(acetate/palmitate), chitin co-(acetate/hexanoate) and chitin co-(acetate/butyrate) have been prepared [9-11]. Moreover, increase in the quantity of the acetyl group content of chitin co-(acetate/butyrate) has shown improvement in the mechanical strength of the films cast from it. Therefore, it is expected that the presence of optimal quantity of acetyl group on the chitin polymer chain may result in the improvement of the mechanical properties of the fibers and films cast from them [12, 13].

The present investigation aims to synthesize a series of chitin acetate/succinate copolymers with varying degree of acetyl and succinyl substitution and determination of their chemical structure by using FTIR and ^{1}H-NMR spectroscopy techniques for practical applications.

2 MATERIALS AND METHODS

2.1 MATERIALS

Chitin and succinic anhydride (SA) from Himedia, India and perchloric acid from Merck, India were procured and used as received. All other reagents/solvents were of analytical reagent grade. DMSO-d_6 and TMS (Tetramethyl silane) were procured from Aldrich, USA and used as solvent and reference standard for the ^{1}H-NMR spectroscopy, respectively.

2.2 SYNTHESIS OF CHITIN co-(ACETATE/SUCCINATE) (CAS) COPOLYMERS

Different CAS copolymers were synthesized by reacting chitin with the mixture of SA and acetic anhydride (AA) in different proportions under heterogeneous conditions in the presence of perchloric acid as a catalyst (Table 1).

TABLE 1: RATIO OF DIFFERENT REAGENTS FOR THE ESTERIFICATION OF CHITIN

Sr. No.	Chitin	AA	SA	Symbol
1	1.0	3.0	2.0	AA60/SA40
2	1.0	3.5	1.5	AA70/SA30
3	1.0	4.0	1.0	AA80/SA20
4	1.0	4.5	0.5	AA90/SA10
5	1.0	5.0	0	CDA

The mixture of SA and AA was used 5 times in excess in each case to conclude the reaction by shifting the equilibrium ahead. Reagents were used in the following proportion: chitin / (SA + AA) / perchloric acid = 1/5/1 (mol/mol). For the preparation of chitin diacetate (CDA), only AA was used in the acylation mixture. Initially, acylation blend was prepared by mixing suitable quantity of perchloric acid at about -10°C with the mixture of SA and AA used in the specified ratio. This fresh acylation mixture was added slowly to chitin powder placed in the ice/sodium chloride bath and then transferred to electronic shaker for agitation at 0 °C for 30 minutes and then for 3 hours at room temperature. The products of reaction was washed to remove any excess of the reagents and dried in an oven at 110 °C [10, 11].

2.3 ANALYSIS OF THE SYNTHESIZED PRODUCTS

IR spectra of chitin, CDA and other synthesized products were recorded using KBr method on Fourier Transform Infrared Spectrophotometer (IRAffinity-1, Shimadzu Corporation, Japan). ^{1}H-NMR spectrum, recorded on Brucker Ascend 400 spectrometer using DMSO-d_6 as solvent and TMS as internal standard, was further analyzed for the confirmation of the chemical structure of all the synthesized products.

The degree of substitution by acetyl and succinyl groups (DS_{Ac} and DS_{Sc}) was calculated based on the results of ^{1}H-NMR spectroscopy using the following formulas, respectively:

$$DS_{Ac} = \frac{1/3I_{\alpha\,CH_3}}{1/6I_{H_2-H_6}}$$

$$DS_{Sc} = \frac{1/2I_{\beta\,CH_2}}{1/6I_{H_2-H_6}}$$

where $I_{\alpha CH3}$ is the integral intensity of the signal of methyl protons of acetyl residues in the range of 2.46-2.52 ppm, $I_{\beta CH2}$ is the integral intensity of the signal of methylene protons of succinyl residues at 2.41 ppm and I_{H2-H6} is the integral intensity of the signals of H2–H6 protons of glucosoamide residues in the range of chemical shift 3.01–4.63 ppm [12].

3.1 SYNTHESIS OF CAS COPOLYMERS

The yield of chitin acetate/succinate copolymers ranged from 85-95% using perchloric acid as catalyst. Earlier workers had reported perchloric acid as an effective catalyst for derivatization of chitin [14] and this fact has proved so in our case also with the appreciable yield of the products. The reaction was performed under controlled temperature as beyond ambient temperature, the deacetylation of chitin starts; hence the temperature was kept at about 0 °C in the starting and then allowed to rise to room temperature for the completion of the reaction.

3.2 ANALYSIS OF THE PRODUCTS OF ESTERIFICATION

Analysis of chitin, CDA and other CASs was carried out using FTIR and ^{1}H-NMR spectroscopy. The chemical structure of chitin, CAS copolymers and CDA are shown in **Figure 1**.

3 RESULTS AND DISCUSSION

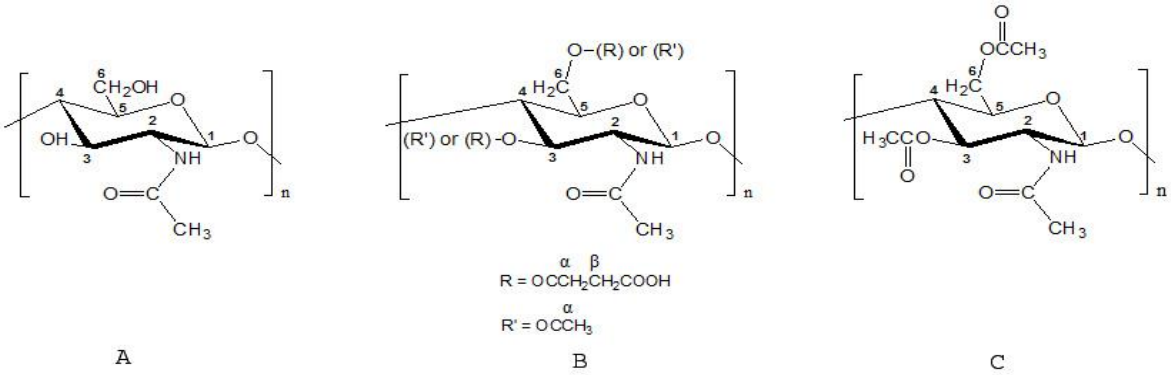

Figure 1: Chemical structure of Chitin (A), CAS copolymers (B) and CDA (C)

The obtained IR spectra of chitin, CDA and other CAS copolymers are presented in the **Figure 2**. The IR spectrum of chitin is characterized by the intense broad band in the region 3480-3260 cm^{-1} (O-H stretching band). The characteristic bands of the amide group present in the structure of chitin was observed at 1653 cm^{-1} and 1552 cm^{-1} for C=O stretching (amide I band) and N-H bending (amide II band), respectively. The peak of N-H stretching of amide group is observed at 3267 cm^{-1} and C-H stretching is present at 2881 cm^{-1}. The particular bands of the β(1→4) glycosidic bridge are present at 1155, 897 cm^{-1} and C-O-C stretching band of the glucopyranose ring at 1029 cm^{-1}. In the IR spectra of CDA, no peak is observed at about 3450 cm^{-1} showing the conversion of –OH groups of chitin into ester groups. The IR spectra of the CDA also shows the characteristic peaks of the ester i.e., C=O stretching at 1748 cm^{-1} and C-O-C stretching at 1261 cm^{-1} which arises due to the presence of the newly introduced acetate groups in place of the hydroxyl groups with the disappearance of the peak at about 3450 cm^{-1} and absorption at 2939 cm^{-1} corresponds to the C-H stretching of the aliphatic alkyl groups.

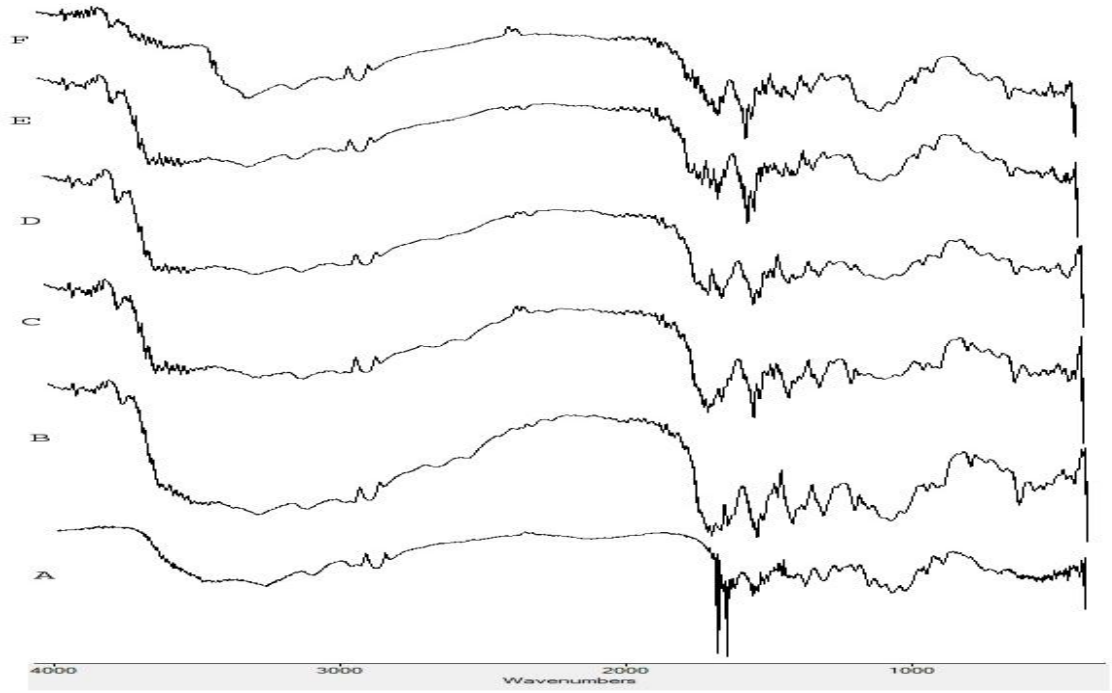

Figure 2: IR spectra of chitin (A), AA60/SA40 (B), AA70/SA30 (C), AA80/SA20 (D), AA90/SA10 (E) and CDA (F)

The IR spectra of other CAS copolymers recorded in the range of 4000-400 cm^{-1} shows almost no absorption at about 3450 cm^{-1}, while the characteristic peaks of the C=O stretching at 1744 cm^{-1}, C-O-C stretching at 1203 cm^{-1} of the newly introduced ester groups shows the replacement of the hydroxyl groups. The intensity of the peak at 2887 cm^{-1} of aliphatic C-H stretching decreases with the decrease in the succinyl content in the formed product. The O-H stretching due to the carboxylic acid functional group present in the newly introduced succinate moiety is seen as a broad band in the range of 3120-2935 cm^{-1} and its C=O stretching band at 1724 cm^{-1}. In all the spectra of the CAS copolymers and CDA, the bands corresponding to the specific peaks of the β(1→4) glycosidic bridge at 1155, 897 cm^{-1}, the amide group at 1653, 1552 cm^{-1}, C-O-C stretching of glucopyranose ring at 1029 cm^{-1} remains unchanged pointing the native structure of chitin chain is preserved under the applied reaction conditions in the presence of perchloric acid as a catalyst and also there is no change in the degree of acetylation of the initial chitin.

The signals of methyl protons of acetamide group in the ^{1}H-NMR spectrum of chitin is present at 2.3 ppm, while the overlapped signals of H2-H6 protons of polysaccharide chain in the range of 3.2-4.1 ppm and the signal of H1 proton at 4.5 ppm are reported in the literature [12]. **Figure 3** shows the ^{1}H-NMR spectrum of one of the synthesized CAS (AA70/SA30). The signals of the protons belonging to the polysaccharide residues are present in the range of 3.01-5.20 ppm. The signal of protons of β-CH_2 of the newly introduced succinyl group appears at 2.41 ppm, while the signals of the protons of α-CH_3 of newly introduced acetyl group and α-CH_2 of succinyl group overlap with each other in the range of 2.46-2.52 ppm. The methyl protons of the acetamide group of the chitin polymer chain are present at 2.21-2.29 ppm and the signals corresponding to the protons of the glucopyranose ring of CAS copolymers are present at the following positions: H2 at 3.01-3.16 ppm, H3 at 4.42-4.45 ppm, H4 at 4.54-4.63 ppm, H5 at 4.22 ppm, H6 at 4.01 ppm and H1 at 5.14-5.20 ppm. The protons of the –NH group the acetamide moiety of CAS copolymers are present with the maximum at 8.08 ppm, while the proton of the –COOH group of the succinate residue appear at 12.11 ppm.

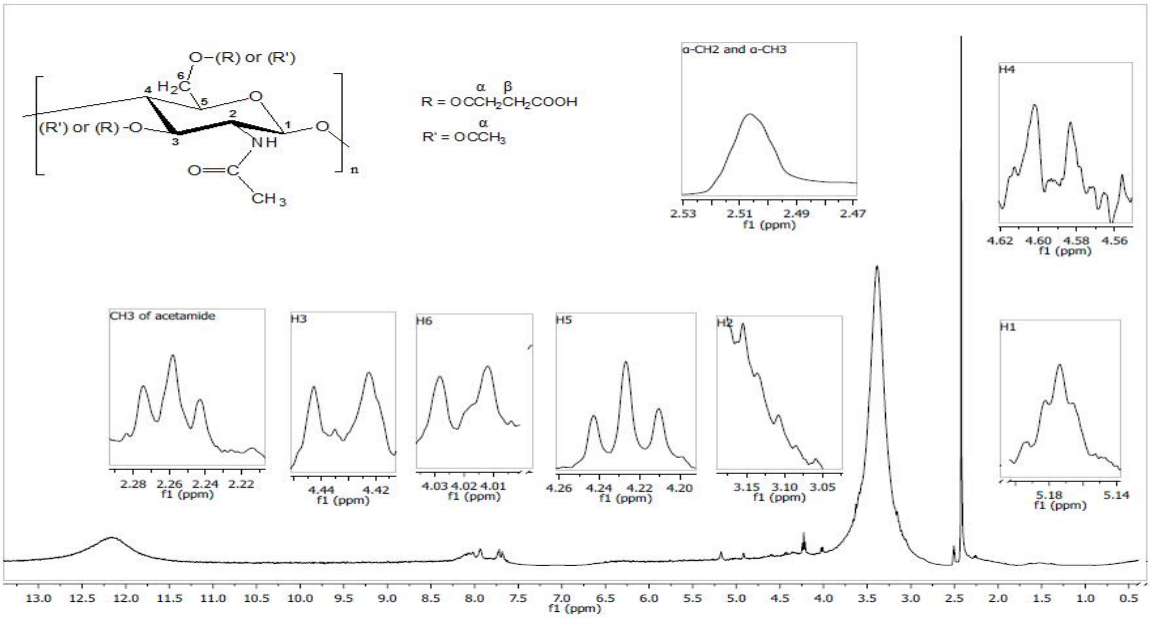

Figure 3: ^{1}H-NMR spectrum of CAS copolymer (AA70/SA30).

The presence of the signals in the ^{1}H-NMR spectrum of CAS copolymers corresponding to the protons of the glucopyranose ring, signals of methyl protons and –NH proton proton of acetamide group of the chitin polymer backbone is in accordance with the results of the IR spectroscopy showing that the basic structure of the chitin chain is preserved under the employed conditions of the reaction. The appearance of the signals for the succinyl and acetyl groups confirms the substitution of the hydroxyl groups of the chitin chain with the ester groups. From the integration of the signals corresponding to the α-CH_3 of the acetyl residue, β-CH_2 of the succinyl residue and H2-H6 of the glucopyranose ring in the range of 3.01-4.61 ppm, the DS_{Ac} and DS_{Sc} were calculated. ^{1}H-NMR spectrum of other synthesized CASs was recorded as before and based on these spectra, the determined values of the DS_{Ac} and DS_{Sc} and their corresponding theoretical values are presented in the Table 2.

TABLE 2: DEGREE OF SUBSTITUTION BY ACETYL AND SUCCINYL GROUPS

Sr. No.	Symbol of CAS copolymer	DS_d based on ^{1}H-NMR spectroscopy			Theoretical DS	
		DS_{Ac}	DS_{Sc}	Total DS	DS_{Ac}	DS_{Sc}
1	AA60/SA40	1.32	0.66	1.98	1.20	0.80
2	AA70/SA30	1.55	0.44	1.99	1.40	0.60
3	AA80/SA20	1.76	0.25	2.01	1.60	0.40
4	AA90/SA10	1.92	0.08	2.00	1.80	0.20
5	CDA	2.00	0	2.00	2.00	0

^{1}H-NMR investigations shows the applied reaction conditions completes the esterification of chitin with the substitution of the hydroxyl groups by ester groups and the final products *i.e.*, CAS copolymers and CDA were obtained in appreciable quantity with the total DS about 2. The slight variation in the value of total DS may be due to the practical errors. Table 2 shows that the content of the acetyl group in the synthesized CAS copolymers was more than the theoretical values based on the proportion of two acylating agents in the reaction mixture, which shows the higher reactivity of the AA in comparison to SA and this is in accordance with the already published experimental outcomes [15, 16]. These results

confirm the changes in the chemical structure of chitin produced by the esterification reaction and these are also in accordance with the results of the IR spectroscopy.

4 CONCLUSIONS

Using the process of chitin esterification in the presence of perchloric acid as a catalyst, a series of CAS copolymers and CDA with varying content of succinyl and acetyl groups has been synthesized in the significant yield, whose chemical structures were confirmed by using IR and ^{1}H-NMR spectroscopy techniques. The presence of the signals corresponding to the basic structure of chitin in the IR and ^{1}H-NMR spectrum of all the synthesized compounds shows that under the applied reaction conditions the native structure of the chitin polymer chain remains unaffected. The DS of the succinyl and acetyl groups in the obtained CAS copolymers were in the range of 0.08-0.66 and 1.32-1.92, respectively with total DS about 2.00. The expected solubility in several common solvents will open new domain of applications in the formulation of film, fiber, foams etc. for pharmaceutical and biomedical applications.

5 ACKNOWLEDGEMENTS

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