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IJPBS |Volume 3| Issue 3 |JUL-SEP|2013|260-265

Research Article
Pharmaceutical Sciences

SYNTHESIS AND CHARACTERIZATION OF SOME CHITIN CO-(ACETATE/PROPIONATE) COPOLYMERS

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ABSTRACT

Chitin, the natural biocompatible and biodegradable polymer consists of β-(1→4) linked 2-acetamido-2-deoxy-D-glucopyranose repeating units, but has limited pharmaceutical and biomedical applications owing to its insolubility in common solvents, although it is the second most abundant natural polymer in the world synthesized by a large number of living organisms. In the present investigation, a series of chitin acetate/propionate copolymers with different content of propionyl and acetyl groups has been synthesized by the esterification of chitin in the presence of perchloric acid as the catalyst using different ratios of acetic and propionic anhydride under heterogenous conditions. Pure chitin diacetate was also synthesized by using the same reaction and their structures were characterized by FTIR and ^{1}H-NMR spectroscopy. The degree of substitution of propionyl and acetyl groups were 0.05-0.64 and 1.35-1.91, respectively as determined by ^{1}H-NMR spectroscopy. The expected solubility of these derivatives in common solvents would add new dimensions to their applications in the pharmaceutical and biomedical fields.

KEY WORDS

Chitin, Chitin acetate/propionate, Copolymer, Esterification, FTIR, NMR

1. INTRODUCTION

Chitin, a natural polymer, is composed of β-(1→4) linked 2-acetamido-2-deoxy-D-glucopyranose units. It is present in a large number of living organisms such as exoskeleton of arthropods (e.g., crustacean shells of crab, cuttlefish, shrimp), mollusks cell walls of fungi and yeast and in the lower plant kingdom such as in algae. The main commercial sources of chitin production includes crab and shrimp shells which are discarded as a waste material by the canning industries [1]. The formation of supramolecular structure and organization in the form of sheets due to the tendency of chitin molecules to form intermolecular hydrogen bonds by the free hydroxyl groups results in its high insolubility in common solvents [2]. This limitation has restricted the interest of the scientific community towards this otherwise excellent natural polymer, despite the presence of this biodegradable polymer in huge quantity in the nature. The presence of the free hydroxyl groups in the chemical structure of chitin offers possibilities for its chemical modification leading to the development of newer derivatives with potential applications. The substitution of the hydroxyl groups with the hydrophobic groups such as ester may result in the breakdown of supramolecular structure resulting in the solubility of these new compounds in common solvents including water.

Various ester derivatives of chitin such as butyryl, propionyl, formyl, acetyl, have been reported in the literature [3, 4]. These derivatives with the presence of varying amount of corresponding ester residues have been synthesized by using the corresponding acid anhydrides as acylating mixture and methanesulphonic acid as a catalyst. Diacetylchitin, which is prepared under heterogenous conditions

using perchloric acid as a catalyst, does not find much practical applications owing to its limited solubility as similar to solubility of native chitin because of the presence of low number of hydrophobic carbon atoms in the acetyl moiety [3, 5]. To overcome this, mixed esters of chitin with the introduction of the two hydrophobic residues in the structure of chitin, the smaller acetylic and the bulky propylic, may results in the formation of the new derivatives of chitin with improved solubility properties. Several copolymers of chitin with improved solubility such as chitin co-(acetate/octanoate), chitin co-(acetate/butyrate), chitin co-(acetate/hexanoate) and chitin co-(acetate/palmitate) have been reported in the literature [6]. The increase in the acetyl content of chitin co-(acetate/butyrate) copolymer has shown improvement in the mechanical strength of the films cast from them. Therefore, it is expected that the presence of the optimal quantity of acetyl group on the polymer chain might result in the improvement of the mechanical properties of their films and fibers [7]. In the present article, the synthesis of a series of chitin acetate/propionate copolymers with varying content of acetyl residue in the polymer chain has been described and their chemical structure determination by FTIR and ^{1}H-NMR spectroscopy.

2. EXPERIMENTAL

2.1 Materials and methods

Chitin (Himedia, Mumbai), Propionic anhydride (PA) (SRL, Mumbai) and Perchloric acid (Merck, Mumbai) were procured from the commercial sources and used as received. All other reagents /solvents were of suitable analytical grade and used as received.

2.2 Synthesis of chitin co-(acetate/propionate) (CAPC) copolymers

A series of CAPCs were synthesized by the reaction of chitin with the mixture of AA and PA in different proportion. Perchloric acid, a strong catalyst, has been employed for the present reaction carried out under heterogeneous conditions. The mixture of PA and AA was used 5 times in excess of the other ingredients in each reaction to provide the completion of the esterification reaction in accordance with the Le Chatelier's principle. Reagents have been used in the following proportion: chitin/(PA+AA)/perchloric acid = 1/5/1 (mol/mol). Firstly, the acylation mixture was prepared by adding calculated quantity of perchloric acid at -10 °C to the mixture of PA and AA used in the ratio as specified in the **Table 1**. The fresh acylation mixture was added slowly to a conical flask containing chitin powder placed in the ice/sodium chloride bath and transferred to an electronic flask shaker for about half an hour. During this period, the temperature inside the conical flask was maintained at about 0 °C. The reaction was allowed to continue further for 3 hours at room temperature. The raw products of the reaction were washed to remove the excess of the reagents and dried in an oven at about 100 °C. For the preparation of chitin diacetate (DAC), only AA was used in the acylation mixture.

Table 1 Ratio of different reagents in the esterification reaction of chitin

Sr. No.	Chitin	AA	PA	Symbol
1	1.0	3.0	2.0	AA60/PA40
2	1.0	3.5	1.5	AA70/PA30
3	1.0	4.0	1.0	AA80/PA20
4	1.0	4.5	0.5	AA90/PA10
5	1.0	5.0	0	DAC

2.3 Analysis of the obtained products

IR spectrum of chitin, DAC and other products of the reaction were recorded using KBr method on Fourier Transform Infrared Spectrophotometer (IRAffinity-1, Shimadzu Corporation, Japan). The structures of all obtained products were also confirmed by using ^{1}H-NMR spectroscopy. ^{1}H-NMR spectra of the obtained products were recorded on Brucker Ascend 400 spectrometer using DMSO-d_6 as solvent and TMS as reference.

For the calculation of degree of substitution by acetyl and propionyl groups (DS_{Ac} and DS_{Pr}), ^{1}H-NMR spectroscopy was utilized by using the following formula, respectively:

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

$$DS_{Pr} = \frac{1/3 I_{\beta CH_3}}{1/6 I_{H_2-H_6}}$$

Where $I_{\alpha CH3}$ stands for the integral intensity of the signal of methyl protons of acetyl residues at maximum 2.47 ppm, $I_{\beta CH3}$ is the integral intensity of the signal of methyl protons of propionyl residues at maximum 0.91 ppm, and I_{H2-H6} is the integral intensity in the range 3.09–4.62 ppm of the signals of H2–H6 protons of glucosamide ring of the chitin chain [7].

3. RESULTS AND DISCUSSION

3.1 Synthesis of CAPC copolymers

For the synthesis of CAPC copolymers, perchloric acid was used as a catalyst of the reaction as it has been found as a very effective catalyst of esterification reaction of chitin [8] and it has been proved so in our case also with 90-95 % yield of the products. The temperature was kept at about 0 °C in start of the reaction to prevent the degradation of the chitin polymer and later the temperature was allowed to rise to room temperature for the smooth completion of the reaction.

3.2 Analysis of the products of esterification

Analysis of chitin, DAC and other CAPCs was carried out using FTIR and ^{1}H-NMR spectroscopy. The recorded IR spectra of chitin, DAC and CAPCs are presented in the **Figure 1**. The IR spectrum of chitin is characterized by the intense broad band at about 3450 cm^{-1} due to O-H stretching. The peak of N-H stretching of amide group is observed at 3267 cm^{-1} and C-H stretching at 2881 cm^{-1}. The characteristic peaks for amide group i.e. amide I band (C=O stretching) at 1653 cm^{-1} and amide II band (N-H bending) at 1552 cm^{-1} are seen at their respective positions. The band of C-O-C in the glucopyranose ring is present at 1029 cm^{-1} and the specific bands of the β(1→4) glycosidic bridge at 1155, 897 cm^{-1}. The IR spectra of the DAC shows the characteristic C=O stretching at 1750 cm^{-1} and C-O-C stretching at 1262 cm^{-1} due to the newly formed ester groups and the stretching band due to the aliphatic C-H of alkyl is present at 2882, 2942 cm^{-1}. The disappearance of the peak at 3450 cm^{-1} is shows the substitution of the –OH groups by the ester groups.

The IR spectra of other CAPC copolymers recorded in the range of 4000-400 cm^{-1} are also presented in **Figure 1**. In all spectra, there is no intense absorption present at about 3450 cm^{-1}, while the C=O stretching at 1742 cm^{-1}, C-O-C stretching at 1258 cm^{-1} of the newly formed ester groups shows the substitution of the hydroxyl group. The intensity of the bands in the range of aliphatic C-H stretching 2950-2800 cm^{-1} decreases with the decrease in the propyl content of the product. In all the spectra of the obtained products including DAC, the specific bands of the β(1→4) glycosidic bridge at 1155, 897 cm^{-1}, the bands of amide group at 1653, 1552 cm^{-1} and C-O-C stretching of glucopyranose ring at 1029 cm^{-1} remains unaffected showing that structure of chitin chain is not destroyed by the employed reaction conditions and also there is no change in the degree of acetylation of the initial chitin material.

In the ^{1}H-NMR spectrum of chitin, the signals of methyl protons of acetamide group at 2.3 ppm, overlapped signals of H2-H6 protons of polysaccharide chain in the range of 3.2-4.1 ppm and the H1 proton signal at 4.5 ppm is presented in the literature [7]. In **Figure 2**, the ^{1}H-NMR spectrum of one of the obtained CAPCs (AA70/ PA30) is shown. The signals of the protons belonging to the polysaccharide residues are present in the range of 3.06-5.19 ppm. The signal of β-CH_3 of the introduced propionyl residue is present at the maximum at about 0.90 ppm. The signals of the protons of α-CH_3 of introduced acetyl residue and α-CH_2 of propionyl residue overlaps with the signal of the DMSO-d_6 (solvent) at the maximum at 2.50 ppm. The –CH_3 protons acetamide group of the polymer chain is present at 2.31-2.35 ppm. The following signals corresponds to the protons of the glucopyranose ring of chitin: H2 at 3.06-3.20 ppm, H3 at 4.41-4.45 ppm, H4 at 4.54-4.62 ppm, H5 at 4.22 ppm, H6 at 4.02 ppm and H1 at 5.15-5.19 ppm. The proton signal of –NH of the acetamide group appear at the maximum 7.95 ppm.

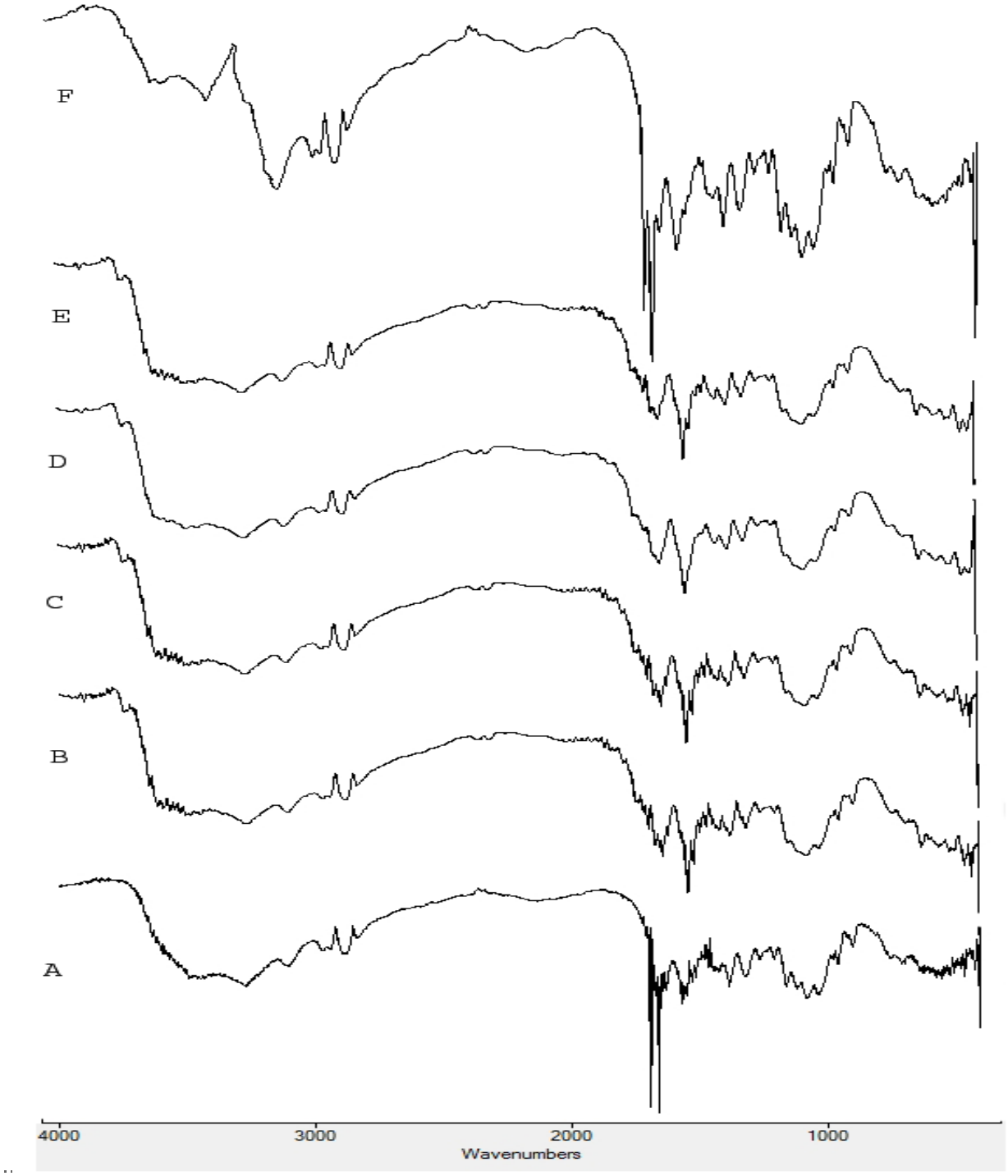

Figure 1. IR spectra of chitin (A), CAPCs: AA60/PA40 (B), AA70/PA30 (C), AA80/ PA20 (D), AA90/PA10 (E) and DAC (F)

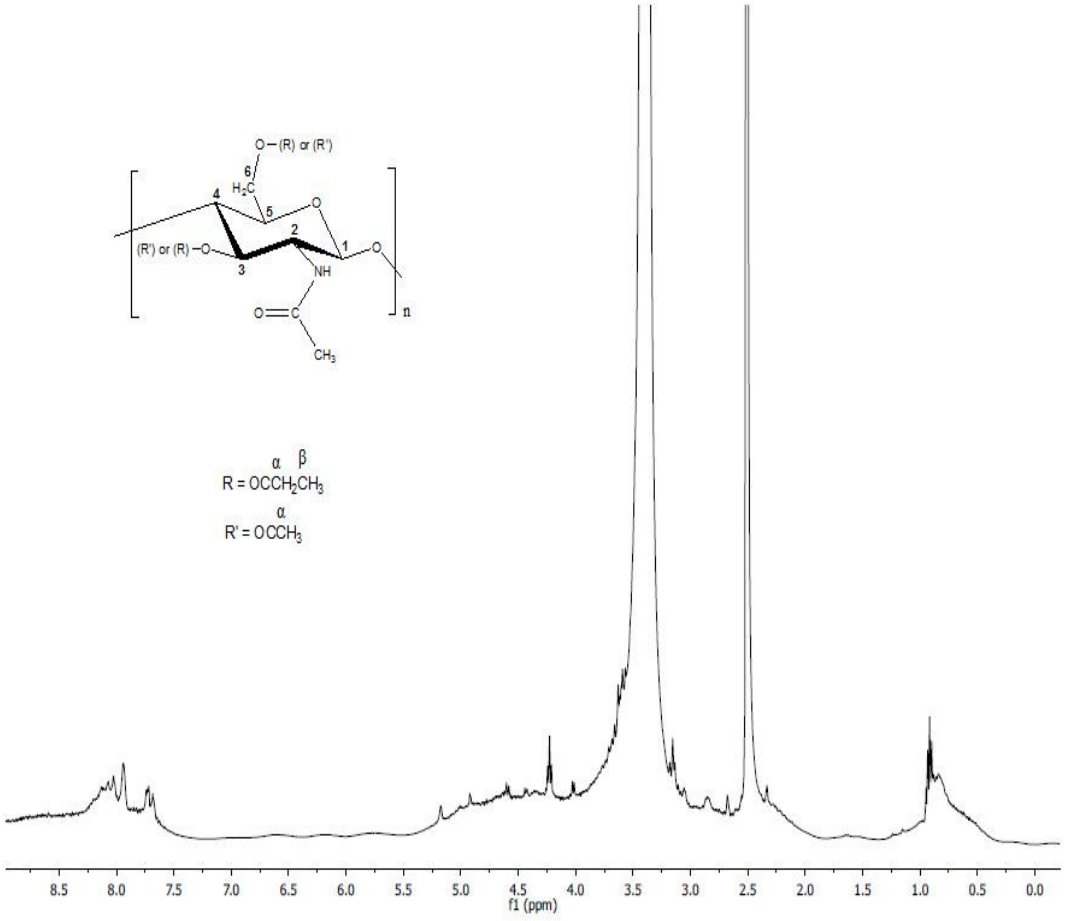

Figure 2. ^{1}H-NMR spectrum of one of the synthesized CAPCs (AA70/ PA30)

The presence of the signals corresponding to the signals of methyl protons and –NH proton of acetamide group, ring protons of the glucopyranose ring of the chitin polymer backbone is in confirmation with the results of the IR spectroscopy showing that the basic structure of the native chitin is not affected under the employed conditions of the reaction. The presence of the signals for the acetyl and propionyl groups confirms the substitution of the hydroxyl group of the chitin. From the integration of the signals corresponding to the β-CH_3 of the propionyl group, α-CH_3 of the acetyl group and H2-H6 of the glucopyranose ring in the range of 3.09-4.62 ppm, the DS_{Pr} and DS_{Ac} were calculated. ^{1}H-NMR spectrum of other CAPCs synthesized in the reaction was recorded as before and based on these spectra, the determined values of the DS_{Pr} and DS_{Ac} and corresponding theoretical values are presented in the **Table 2**.

Table 2. Degree of substitution by acetyl and propionyl groups on the chitin backbone

Sr. No.	Symbol of CAPC	DS_d based on ^{1}H-NMR spectroscopy			Theoretical DS	
		DS_{Ac}	DS_{Pr}	Total DS	DS_{Ac}	DS_{Pr}
1	AA60/PA40	1.35	0.64	1.99	1.20	0.80
2	AA70/PA30	1.55	0.43	1.98	1.40	0.60
3	AA80/PA20	1.72	0.26	1.98	1.60	0.40
4	AA90/PA10	1.91	0.05	1.96	1.80	0.20
5	DAC	1.99	0	1.99	2.00	0

Results of the ^{1}H-NMR investigation shows that the employed reaction conditions for the esterification of the chitin results in the completion of the reaction in the presence of perchloric acid as a catalyst with the substitution of the hydroxyl groups by ester groups and the final products *i.e.* DAC and other CAPCs were obtained with the total DS about 2. The slightly lower value of the total DS may be due to the experimental errors. The content of the acetyl groups in the synthesized CAPCs was found to be more than the theoretical values based on the composition of the acylation mixture, which shows the higher reactivity of the acetic anhydride in comparison to propionic anhydride and this is in confirmation with the already published literature [9, 10]. These results confirm the structural changes caused by the reaction and these are also in line with the results of the IR spectroscopy.

4. CONCLUSION

Perchloric acid is again proved as an effective catalyst of the chitin esterification reaction and DAC, a series of CAPCs with varying chemical structure has been synthesized in good yield. The summary DS of the propionyl group and acetyl group were in the range of 0.05-0.64 and 1.35-1.91, respectively. Chemical structure of the obtained CAPCs has been confirmed by using IR and ^{1}H-NMR spectroscopy. The expected solubility in common organic solvents might result in the preparation of various types of materials such as fiber, film etc. for biomedical and pharmaceutical applications.

5. ACKNOWLEDGEMENT

The authors are highly grateful to the Haryana state technical education society, Panchkula, India for providing the financial assistance.

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