

Preparation, Characterization and Bioactivity Assessment of Chitosan-acetylated Jute Blended Green Films

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Abstract: In recent years, there has been a great demand for artificial materials for biomedical applications, especially to bone growth. In the present study, the *in vitro* bioactivity of chitosan-acetylated jute blended film was investigated through biomimetic growth of bone-like apatite layer formation in simulated body fluid (SBF) on the surface of the film. Chitosan was prepared from crab shells and the blended film was prepared by solvent casting method using 10 % formic acid aqueous solution of in-house chitosan and mercerized, acetylated jute fiber. Water absorption characteristics (swelling test) and tensile strength (Universal testing machine) of the films were determined. Thermal behavior of the films were examined by TGA analysis. The biocompatibility of the chitosan-acetylated jute (CAJ) film was indicated by the formation of hydroxy apatite (HAP) bone-like layer formed on the surface when soaked in SBF solution for 14 days. The topography of the film was characterized by scanning electron microscope (SEM) and Ca/P ratio of the apatite layer was confirmed to be 50:50 by EDX analysis. The results showed that the CAJ film will be a promising bone substituting material.

Keywords: Chitosan, Jute, Biopolymer blend, Hydroxyl apatite, Acetylation

Introduction

Over the past few years, there has been an increasing demand for natural fibers as substitutes for synthetic fibers. The attractive characteristics of natural fibers like jute, sisal, banana and coir have been their availability in nature, light weight, low cost, renewability, biodegradability and thermal insulating properties [1-5]. A number of natural fiber composites and their blends with polymer matrices [6] have been explored for biomedical applications and found to be better alternatives over their synthetic fiber counterparts. Though the natural fibers have many potential applications in various fields, their hydrophilic nature limits the interfacial adhesion of these fibers with polymer matrices like polyester, polypropylene and epoxy resin [7]. Number of studies has reported methods to improve the adhesion between natural fibers and polymers and also their mechanical properties by various chemical modifications (surface treatments) like alkali treatment (mercerization), acetylation, silane treatment, graft copolymerization [8-10].

In the present study, jute fiber (bast fiber) was selected to be blended with chitosan, a biopolymer that is widely prepared from crustacean shells. Chitosan, a natural polysaccharide (poly-2-amino-2-deoxy-b-(1,4)-D-glucopyranose), is derived from chitin which is poly-2-acetamide-2-deoxy-b-(1,4)-D-glucopyranose. Chitin is the most abundant natural polysaccharide obtained from seafood wastes and possesses many desirable properties like, biodegradability, low toxicity, biocompatibility, film forming property, capacity to bind with mammalian and microbial cells, hemostatic, fungistatic, spermicidal, anti-cholesteremic, acceleration of bone formation

and central nervous system depressant [11]. Chitosan and its derivatives have been widely exploited as antitumor, antiulcer, immune-stimulatory, anticoagulant and antimicrobial agents in biomedical and pharmaceutical applications [12]. However, the applications of chitosan films were limited due to its hydrophilic nature, film brittleness and poor mechanical properties. Since both the selected materials, namely, chitosan and jute fiber are hydrophilic in nature, mechanical strength of the blended film would be less and water absorption capacity of the film would be more. However, chemical modification of any one of the materials will help to reduce the hydrophilicity and improve the adhesion between the two. In this context, the present study aimed to prepare blends of chitosan with acetylated jute, since acetylation of jute has been proven to increase its hydrophobicity without much loss in its inherent mechanical strength. Chitosan-acetylated jute blended films (CAJ), reported for the first time, have been assessed for their suitability to biomedical applications through hydroxyl apatite formation in simulated body fluid (SBF) that has similar concentrations to human blood plasma, proposed by Kokubo *et al.* [13,14].

Experimental

Materials

The Jute fiber was supplied by National Jute Board (NJB), Chennai. Chitin and chitosan were prepared from locally available crab shells by conventional methods [15]. Other chemicals such as acetic anhydride, sodium hydroxide, perchloric acid, formic acid and other reagents of laboratory reagent grade supplied by Merck India Ltd were used as received.

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Methods

Chemical Treatments on Jute Fiber

Mergerization

Mergerization is the process of removal of lignin and other waxy materials from natural fibers. 5 g of powdered jute fiber was treated with 20 % NaOH (250 ml) at 70 °C for about 2 hours with vigorous stirring. Mercerized fibers were filtered and thoroughly washed with water until pH=7 and then dried. Mercerized jute fibers were characterized by FT-IR.

Acetylation of Jute Fiber

About 2 g of mercerized jute fiber was soaked in distilled water for one hour, filtered and placed in a 500 ml round bottom flask containing the acetylating solution. Acetylating solution consisted of 10 ml of acetic anhydride, 100 ml of toluene, and 20 mg of perchloric acid as a catalyst. The reaction mass was heated to 60 °C and the temperature was maintained for 3 hours with vigorous stirring. Then the fiber was washed periodically with water until pH=7. Finally the fiber was dried at room temperature. Acetylated jute fibers were characterized by FT-IR.

Preparation of Chitosan from Crab Shells

Extraction of Chitin from Crab Shells

Coarse crab (*Portunus haanii*) shells collected from the local market were cleaned with water by stirring and filtered off after 20 minutes. This process was repeated until sand and other soils were removed. The cleaned shells were dried overnight in the oven at 80 °C. 570 g of dried crab shells were powdered and heated at 60-70 °C for half an hour with 2 % NaOH solution. The solution was filtered off and the process was repeated until the filtrate was colorless. The shells were washed with distilled water till neutral reactions. Then 7 % HCl was slowly added to the shells and the mixture was stirred at room temperature until effervescence stopped and washed with distilled water until pH=7. The product was dried overnight in an oven at 60 °C.

Preparation of Chitosan from Chitin

150 g of chitin was placed in a round bottom flask and 50 % sodium hydroxide solution was added to it. The mixture was heated at 125 °C for one hour. It was allowed to cool, filtered and the residue was washed with water to neutral reaction. The solid residue was dried in an oven at 60 °C.

Preparation of Chitosan-Jute Blended Films

Chitosan was dissolved and the jute fibers were dispersed in 10 % formic acid separately. Chitosan and jute blended films were prepared by solvent casting method [16]. CAJ film was prepared by blending acetylated jute fiber with prepared chitosan powder. Different ratios of CAJ films were prepared (CJ25, CJ50 and CJ75) and characterized. The number in the blended films denotes the composition of jute fiber and rest of the blend was chitosan.

Characterization of Chitin, Chitosan and Chitosan-jute Films

Fourier Transform-Infrared Spectroscopy (FT-IR)

FT-IR analysis was performed using a Perkin-Elmer spectrum

RX1 instrument for synthesized chitosan and acetylated jute fibers in the range of 4000-400 cm⁻¹. Spectra were integrated by taking the area under the curve between the limits of the peaks of interest.

Determination of Degree of Deacetylation of Chitin (% DD)

The elemental analysis is simple and fastest method to determine DD value of chitin. Percentage of degree of deacetylation of chitin was calculated from the percentage of C, N and H obtained from the CHN analysis for the prepared chitosan using the following equation [17],

$$DD = \left(1 - \frac{C/N - 5.14}{1.72}\right) \times 100 \% \quad (1)$$

where, C/N is the ratio (w/w) of carbon and nitrogen present in chitosan.

Determination of Molecular Weight of Prepared Chitosan

Chitosan was dissolved in acetic acid (0.1 M) and sodium chloride (0.2 M) mixture with 1:1 ratio and different concentrations of chitosan solution was prepared. The Ostwald's Viscometer was used to determine the flow time of solvent and chitosan solution. Viscosity average Molecular weight (M_v) of chitosan was determined by Mark-Howink's equation,

$$\eta_i = \kappa (M_v)^\alpha; \text{ where, } \kappa = 1.81 \times 10^{-3} \text{ and } \alpha = 0.93.$$

Water Absorption Studies (Swelling test)

The water absorption studies were carried out for CAJ film. The pre-weighed (dry weight) CAJ film was immersed in water. After a specified period of time, samples were removed, the excess water was dried with filter paper and the weight of swollen samples were recorded (wet weight) and then samples were returned to the water. This operation was performed at intervals of 24 hours until no weight change was observed. Even though this procedure is unsophisticated and simple to perform and it generally yields good, reproducible results. Finally the extent of water absorption was calculated as follows:

$$\text{Extent of water absorption} = \frac{\text{Difference in weight} \times 100}{\text{initial weight}}$$

Mechanical Properties by UTM Analysis

The mechanical properties (tensile strength) of the prepared CAJ film was determined at 25 °C using HOUNSFIELD Universal Testing Machine (UTM) possessing a load cell of 5 kN at room temperature. The gauge length and diameter of all specimens were 50 mm and 5 mm respectively with the thickness of 300 microns (ASTM D638 Type V).

Thermo Gravimetric Analysis (TGA-DSC)

This is a technique in which the change in the weight of a substance with the temperature over a period of time is followed. Thermal stability and decomposition temperature can be assessed easily. The thermo gravimetric analysis was used to analyze the stability of the polymer with the heating rate of 20 °C per minute under nitrogen atmosphere with a

SDT Q 600 US analyzer (ASTM E1131). TGA was performed in the temperature range from room temperature to 800 °C, using alumina as the reference material on platinum pans and calcium sulphate as standard.

Scanning Electron Microscope (SEM) and Energy Dispersive X-ray Spectroscopy (EDX)

Scanning electron microscope (SEM) was utilized mainly for the investigation of the surface morphology of the blended films. SEM micrographs of the gold coated samples were obtained with the help of HITACHI S-3400 with associated energy dispersive X-ray analyzer. The CAJ film was analyzed using the energy dispersive x-ray unit (EDX) in live time of 100 seconds, AC Voltage of 15.0 kV and take off angle of 34.6 deg. The data was utilized to determine the elemental characterization of the hydroxyl apatite layer on the CAJ film.

Bioactivity Test with Prepared Simulated Body Fluid (SBF)

Kokubo and his colleagues developed a solution called simulated body fluid (SBF) that which has inorganic ions similar to that of human extra cellular body fluid in order to reproduce the formation of apatite (calcium apatite, $\text{Ca}_5(\text{PO}_4)_3\text{OH}$) layer on bioactive materials *in vitro*. This fluid is used not only for the evaluation of bioactivity of man-made materials but also coating of apatite powders on various materials under biomimetic conditions. The SBF solution was prepared by mixing NaCl (7.996 g), NaHCO_3 (0.35 g), KCl (0.224 g), $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$ (0.228 g), $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (0.305 g), HCl, CaCl_2 (0.278 g), Na_2SO_4 (0.071 g) and $(\text{CH}_2\text{OH})_3\text{CNH}_2$ (6.057 g). Required amount of standardised HCl was added to adjust pH to 7.25 and then made up to 1L with distilled water. The blended films to be tested were soaked in the prepared SBF solution for 14 days for the formation of bone-like apatite layer on their surfaces and the layer formation was characterized by scanning electron microscope (SEM).

Results and Discussion

FT-IR Analysis

FTIR Spectrum of Chitosan

The FT-IR spectrum of synthesized chitosan is shown in Figure 1. An intense broad band with the maxima at 3433 cm^{-1} , in the higher region was due to OH stretching vibration of chitin and that of water. The peaks at 2928 cm^{-1} and 2850 cm^{-1} were due to CH_2 vibrations. The peak at 1630 cm^{-1} and the shoulder in its lower energy portion were due to C=O vibration, OH–H bending mode and NH bending mode. The peaks at 1433 cm^{-1} and 1384 cm^{-1} were due to CH_2 bending vibrations. The C-O vibrations produce a group of peaks/shoulders in the region around 1200 cm^{-1} and 1000 cm^{-1} .

FT-IR Spectrum of Raw, Mercerized and Acetylated Jute Fibers

The FT-IR spectrum of raw jute was shown in Figure 2.

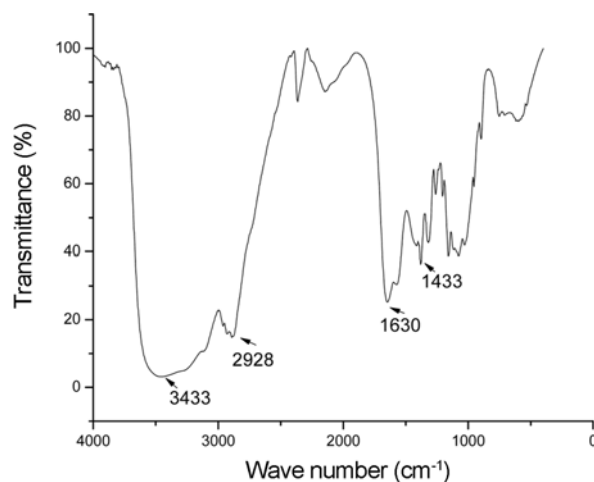


Figure 1. FT-IR spectrum of chitosan.

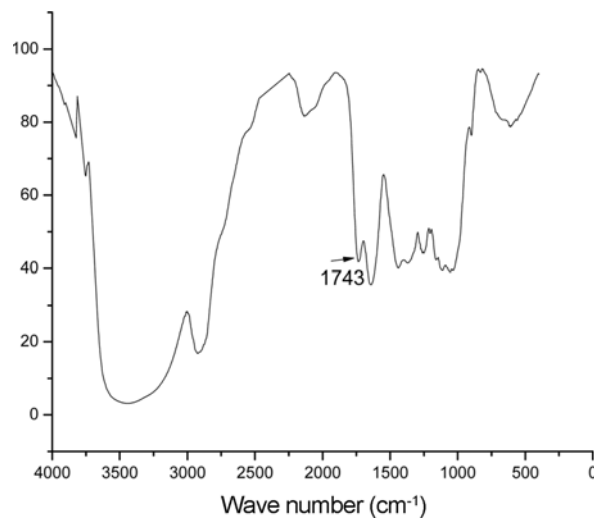


Figure 2. FT-IR spectrum of raw jute fiber.

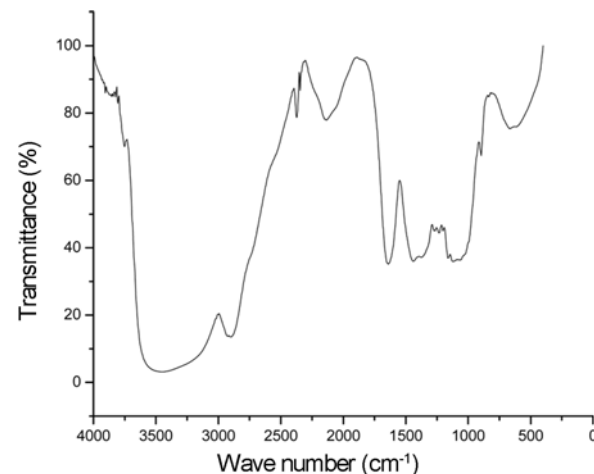


Figure 3. FT-IR spectrum of Mercerized jute fiber.

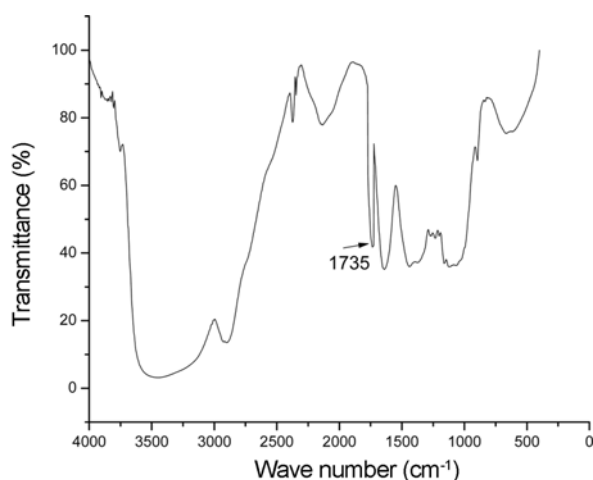


Figure 4. FT-IR spectrum of acetylated jute fiber after mercerization.

The major broad peak showed at 3434 cm^{-1} was due to several OH groups present in the hemicelluloses and cellulose components of jute. The same peak was shown in FT-IR spectrum of mercerized fiber (Figure 3) also. But the peak which was showed at 1743 cm^{-1} due to carbonyl group of one of the hemicelluloses components and the peaks between 1600 cm^{-1} to 1200 cm^{-1} due to aromatic portion of lignin were disappeared in Figure 3. It showed the partly removal of both hemicelluloses and lignin contents (by mercerization) of jute fiber. The presence of carbonyl peak in the acetyl group was shown again at 1735 cm^{-1} in Figure 4. It confirmed the presence of acetyl group (extent of acetylation was done by acid-base titration) on the surface of the jute fiber. Since the extent of reaction is less, small peak for carbonyl group was observed. The peak at 1630 cm^{-1} in Figure 4 showed the interlocked water present in the crystalline cellulose of jute fiber. The peaks between 1600 cm^{-1} to 1100 cm^{-1} showed small amount of aromatic part of lignin present in the acetylated jute fiber.

Degree of Deacetylation of Chitin and Molecular Weight of Chitosan

The elemental composition of synthesized chitosan was found by CHN analyser as 33.32 % carbon, 5.87 % hydrogen and 5.92 % nitrogen. The extent of deacetylation reaction was determined by degree of deacetylation of chitin. Using equation (1), the degree of deacetylation of chitin was calculated to be 71.6 %.

The molecular weight of chitosan was determined by Mark-Hownik's equation and the molecular weight was calculated as $M_v = 7755\text{ g/mole}$.

Water Absorption Studies

The comparative studies of water absorption capacity of CAJ and CRJ (Chitosan-Raw jute) films were shown in Figure 5. In both the films, CJ25 (25 % of chitosan in the

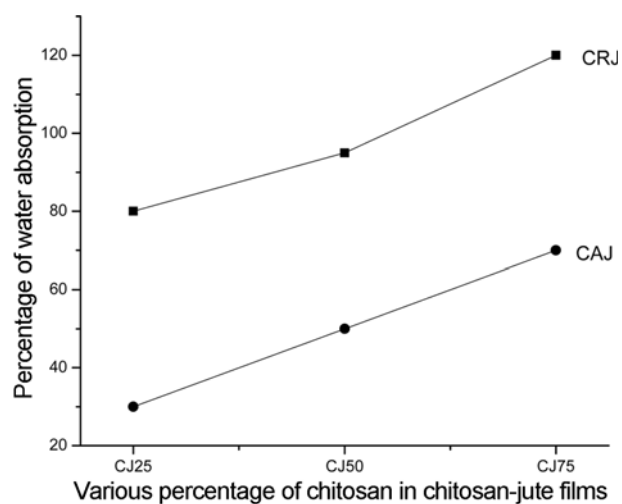


Figure 5. Water absorption studies of CRJ & CAJ films.

blend) had lower water absorption capacity when compared to CJ50 and CJ75. This was expected because of hydrophilic nature of chitosan. The higher water absorption capacity of CJ75 was due to the higher loading of chitosan. Even though same pattern was followed in both the films, the extent of water absorption was reduced to 50 % in CAJ films when compared to the CRJ films because of the acetylation of jute fiber before blending with chitosan. This chemical modification seemed to have reduced the moisture intake of jute fibers and improved the interfacial adhesion of the blending components. Further characterization results have been discussed for the CAJ film with 50 % chitosan and 50 % acetylated jute.

Tensile Strength Measurements

Tensile strength measurements were carried out for five samples of CAJ film and the average result was 10.68 MPa which revealed the considerable mechanical strength of the film. The tensile strength of CRJ film was 2.567 MPa. The higher tensile strength of CAJ film was due to mercerization process of jute fiber which removed the amorphous materials like wax, lignin and hemicelluloses to major extent from jute fiber and also the acetylation of jute fiber when compared to CRJ film.

Thermal Behavior of CAJ Film

The TGA-DSC curves of chitosan-acetylated jute film were shown in Figure 6. The initial weight loss that started at $140\text{ }^{\circ}\text{C}$ was due to moisture vapourisation. The decomposition of the material started at $295.34\text{ }^{\circ}\text{C}$ as evidenced from the degradation curve and complete degradation of the cellulosic jute and chitosan materials occurred at temperature beyond $368.03\text{ }^{\circ}\text{C}$. It indicated that the chitosan based films can be used at moderately high temperatures up to $250\text{ }^{\circ}\text{C}$ without any loss of material by degradation.

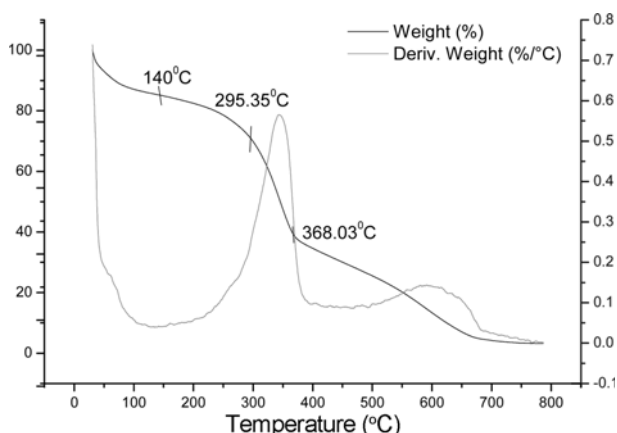


Figure 6. TGA-DSC curve of CAJ film.

Morphological Properties and Bioactivity Test of CAJ Film

The surface morphology of the prepared CAJ film in different resolutions taken by using scanning electron microscope (SEM) was shown in Figure 7(a) and 7(b). The individual lines shown in Figure 7(a) and 7(b) confirmed the presence of jute fiber and the chitosan particles. SEM images showed the complete blending of chitosan, jute fiber and confirmed the smooth, clear morphology of the blended film which was rendered by mercerization process where waxy materials like pectin, part of lignin and hemicelluloses were removed.

The prepared CAJ film was soaked in SBF solution for 14 days. Then it was dried in the desiccator for a day and then

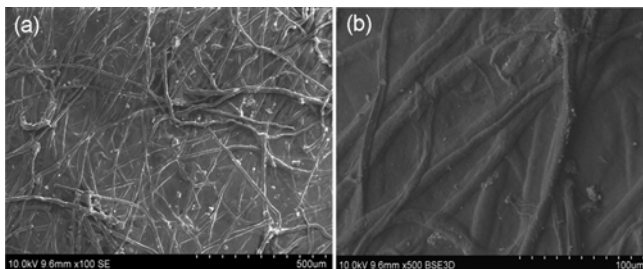


Figure 7. SEM images of CAJ film at various resolutions before soaking in SBF.

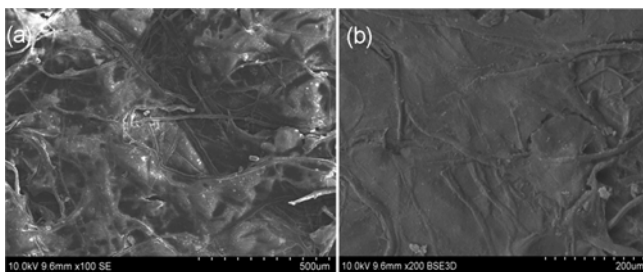


Figure 8. SEM images of CAJ film at various resolutions after soaking in SBF.

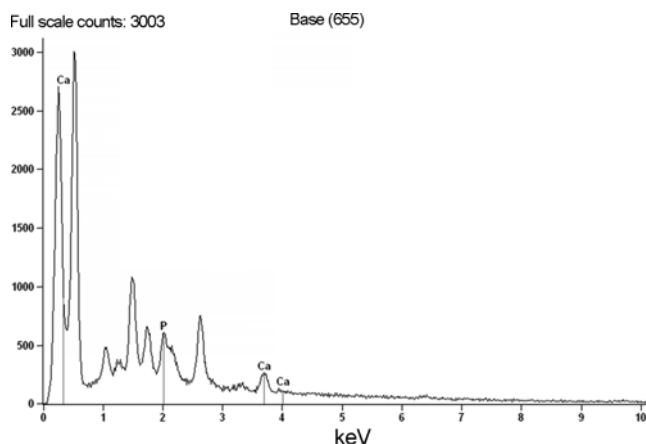


Figure 9. EDX spectrum of CAJ film after soaking in SBF.

Table 1. Quantitative results of EDX analysis

Element	Net counts	Weight (%)	Atom (%)
Phosphorous (P)	2766	37.75	43.97
Calcium (Ca)	2643	62.25	56.03
Total		100	100

the film was subjected to SEM analysis. The various resolutions of SEM images before soaking in SBF were shown in Figure 7(a) and 7(b). Similarly, the various resolutions of SEM images after soaking in SBF were shown in Figure 8(a) and 8(b) which showed the clear deposition of hydroxy apatite layer (HAp) on the surface of the film. The jute fibers were masked by the layer formed on the surface of the film. It indicated the bioactivity of CAJ film.

EDX Analysis of CAJ Film

The EDX spectrum of the hydroxy apatite layer formed on the surface of the CAJ film after soaking in SBF for 14 days was shown in Figure 9. The quantitative results of Ca/P ratio (approximately 1:1) of the hydroxyl apatite layer was shown in Table 1. The significant peaks of calcium and phosphorous in EDX analysis indicated the formation of hydroxyl apatite layer on the surface of the CAJ film. It showed that calcium and phosphate ions of SBF were consumed by the CAJ film for the formation of bone-like apatite layer on its surface.

Conclusion

A green blended film was prepared from biopolymer chitosan and natural fiber jute. A combination of chitosan and a natural fiber with or without surface modification, intended for the proposed use is very few and far between. The choice of acetylated jute as a blend with chitosan was made considering the fact that the former was most likely to enhance the mechanical strength of chitosan film without affecting its biocompatibility. Acetylation treatment was

given to jute fiber to increase the hydrophobicity, tensile strength and smooth surface of the blended film. The stability and enhancement of property due to blending in the CAJ film was evaluated in terms of thermal analysis and tensile strength measurements. The effect of acetylation of jute could be ascertained from the fact that the tensile strength and hydrophobicity of the chitosan-acetylated jute film were doubled and quadrupled respectively, compared to chitosan-raw jute films. Thermal stability data indicated that the CAJ film was stable up to 295 °C and that it decomposed slowly beyond this temperature because of the degradation of jute and chitosan. Bioactivity and suitability of the prepared film for bone repair application has been indicated by the formation of hydroxyl apatite layer over the surface of the film in SBF solution, as confirmed by SEM and EDX analysis.

The results of the study suggested that blending of acetylated jute with in-house chitosan has resulted in enhancement of mechanical strength and the material has also been proved to show considerable biocompatibility. Hence it may be concluded that the prepared acetylated jute blended chitosan film is suitable for exploitation as a scaffolding biomaterial in bone formation for orthopedic applications.

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