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Hydroxyapatite filled chitosan-polyacrylic acid polyelectrolyte complexes

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Chitosan (CHI) has been complexed with polyacrylic acid (PAA) in the presence of hydroxyapatite (HAP) to prepare composites intended for bone substitute applications. Thermogravimetric analysis of the composite showed that the presence of HAP had little effect on the degradation profile of the polyelectrolyte complex (PEC), except for the onset of degradation. Optimum compressive strength was observed for 70-weight percentage HAP filled PEC. The morphological analysis revealed that the HAP particles were uniformly distributed in the PEC matrix. The results indicate that HAP filled PEC of CHI and PAA could be prepared following the proposed simple route. © *2003 Kluwer Academic Publishers*

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1. Introduction

Concerns over the use and availability of autogenous or allogenic bone graft materials have prompted studies aimed at development of suitable synthetic bone substitutes [1]. Some of the materials that have been and continue to be extensively investigated are hydroxyapatite (HAP) and a range of materials within the calcium phosphate family. This interest is expected, because the main mineral component of bone is a complex inorganic calcium phosphate compound having apatitic structure [2, 3]. Additionally, HAP is reported to be osteoconductive and directly bond to bone [4, 5] and is considered a precursor for *in vivo* formation of bone. Thus, the good bone compatibility of HAP makes it suitable for the repair or replacement of damaged or diseased bone. However, the poor mechanical and fatigue properties limit its applicability to the low load bearing areas of human skeleton. In addition to this, sintered HAP is extremely hard and brittle which makes its processing difficult. It has been reported that HAP in the form of powders, used for the treatment of bone defects, has problem associated with migration to places other than implanted sites [6, 7]. Hence composite materials of HAP with organic polymers, that can to certain extent reduce the above problems, have become of great interest [8]. A wealth of research has been carried out in this regard and composite materials based on HAP and a variety of polymers have been reported [9–12]. However, in making HAP-polymer composites, the lack of interfacial bonding between the HAP and the polymer matrix still remains an issue of concern [13, 14]. The interfacial bonding between inorganic and organic phase plays an important role in determining the ultimate mechanical properties of the composites. Although no direct bonding between the bone mineral and the collagen exists, they are bound to each other by means of the non-collagenous proteins [15].

We report here a polymer—ceramic composite material that gives promise in combating problem of interfacial interaction. The matrix employed is a polyelectrolyte complex, obtained by ionically crosslinking two highly, but oppositely charged polyelectrolytes, namely chitosan (CHI) and polyacrylic acid (PAA). Chitosan, a cationic polysaccharide, is a (1,4) linked 2-amino-2-deoxy-D glucan and can be prepared by the N-deacetylation of chitin. Chitosan has both reactive amino and hydroxyl groups that can be used to chemically alter its properties under mild reaction conditions. Therefore, there have been many interesting chitosan derivatives for the biomedical applications [16–19]. Efforts for the development of bone graft materials based on chitosan–calcium phosphate and chitosan–HAP were made by several research groups [7, 20, 21]. Cross-linking of chitosan in the chitosan–HAP/chitosan–calcium phosphate system with glutaraldehyde will significantly reduce the biodegradation of chitosan and offer adequate strength to the material. However, residual glutaraldehyde retained in the interstices has been implicated for inflammatory reactions, cytotoxicity and lack of endothelialization [22–25]. Polyacrylic acid is a water soluble and biocompatible polymer and has been extensively studied in the context of biomedical applications [26–28].

In the present study the method adopted is to improve the interface between the HAP and the polymer by making use of the complexation reaction between the two polyions, chitosan and polyacrylic acid. The

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complexing ability of the polyions enables the HAP to be firmly incorporated into the polymer matrix.

2. Materials and methods

2.1. Materials

Chitosan (CHI), obtained from shells of lobsters, was kindly provided by the Central Institute of Fisheries Technology, Cochin, India. It had 70% *N*-deacetylation degree and 1.2×10^6 viscosity average molecular weight measured by Ubbelhode viscometer using Mark-Hauwink equation $[\eta] = (8.93 \times 10^{-4}) \times M^{0.71}$ [29]. Polyacrylic acid (PAA) was prepared by the batch polymerization of acrylic acid (Merck, Mumbai, India) initiated by ammonium persulfate [30]. The PAA thus prepared was determined to have viscosity average molecular weight of 2.9×10^5. Hydroxyapatite for the preparation of the composite was synthesized by precipitation route involving ammoniated calcium nitrate and dihydrogen ammonium phosphate followed by freeze-drying technique [31]. The particle size and particle size distribution of the freeze-dried HAP powder used are given in Table I.

2.2. Preparation of the polyelectrolyte-HAP composites

The solution of 1 wt% CHI (w/v) in 5% aqueous acetic acid dispersed with requisite quantity of HAP was added to a solution of 1 wt% PAA (w/v). The measured pH of the CHI solution and the PAA were 3.75 and 2.65 respectively. The addition of PAA to HAP filled CHI solution was accomplished with constant stirring. The composites were prepared by mixing HAP, CHI and PAA in various weight percentages. The compositions and the designations of the systems studied are given in Table II. The composites thus obtained were separated by filtration and were washed several times with distilled water in order to remove any residual reagents present in the system. The composites were then pressed between filter papers to remove excess water, shaped in the form of sheets and blocks and characterized for their physico-mechanical characteristics. During the preparation of composites, the pH values were monitored with a digital pH meter (Accumet model – 50) with an appropriate electrode.

TABLE I Size distribution of HAP particles

$d_{0.1}$	6.86 μm
$d_{0.5}$	48.52 μm
$d_{0.9}$	120.37 μm

TABLE II Compositions and designations of chitosan-poly (acrylic acid)-hydroxyapatite composites

	Polymer matrix		
Material code	CHI (wt%)	PAA (wt%)	HAP (wt%)
CHI/PAA/40/60	40	60	0
CHI/PAA/50/50	50	50	0
CHI/PAA/60/40	60	40	0
CHI/PAA/HAP 50/50/60	50	50	60
CHI/PAA/HAP 50/50/70	50	50	70
CHI/PAA/HAP 40/60/80	40	60	80
CHI/PAA/HAP 50/50/80	50	50	80
CHI/PAA/HAP 60/40/80	60	40	80
CHI/PAA/HAP 50/50/90	50	50	90
CHI/PAA/HAP 50/50/95	50	50	95

2.3. Characterization of the polyelectrolyte/hydroxyapatite composites

The infrared data of the prepared composites were collected using a Nicolet Inc. (Madison, USA) model Impact 410 FTIR spectrophotometer. Thermogravimetric analysis (TGA) of various composites and individual components were recorded on TA Instruments model SDT 2960 simultaneous DTA-TGA analyzer. A heating ramp of 10°C/min from room temperature to a maximum temperature of 800°C was used. For the evaluation of the compressive strength of the PEC-HAP composites, the samples were prepared by varying the polymer to HAP ratio and shaped in the form of cylindrical blocks. The precipitated composites were filled in Teflon® moulds of height $h = 12$ mm and diameter $\phi = 6$ mm and hand pressed at 30°C. The samples in the moulds were dried under vacuum for 24 h at 60°C. Cylindrical blocks of approximately 12 mm height and 6 mm diameter were thus obtained. The blocks were then characterized for its compressive strength in a Universal Testing Machine (Instron model 1011) at a crosshead speed of 1 mm/min. A minimum of six specimens of each formulation was tested and the average value was taken as the compressive strength of the composite. The fracture morphology of the composite sample was observed under scanning electron microscope (SEM Hitachi model S-2400) after coating the specimen by gold sputtering.

3. Results

3.1. Physical nature

The PEC, CHI/PAA 50/50, was translucent. The addition of HAP, however, made the material opaque.

3.2. pH measurement

The variation in pH with the addition of HAP into the CHI/PAA 50/50 system was noted. The pH values were found to be below 6, which is essential for the complex formation as reported by earlier researchers [32, 33].

3.3. Infra red spectroscopic analysis

Fig. 1a gives the FTIR spectrum of the CHI-PAA 50/50 complex. The co-existence of negatively charged carboxylate ion (—COO^-) near 1700 cm^{-1} and positively charged (—NH_3^+) ion of the chitosan at 1553 cm^{-1}, in the spectra confirms the formation of the polyelectrolyte complex.

Fig. 1b shows the FTIR spectrum of CHI/PAA/HAP 50/50/60. The striking feature of the spectrum is the

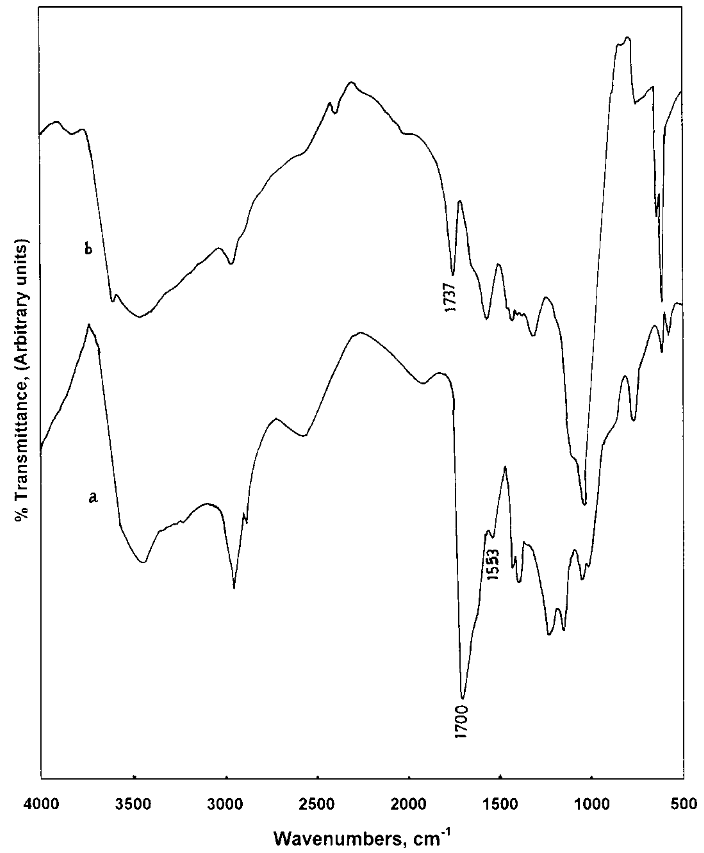


Figure 1 (a) FTIR spectra of chitosan-polyacrylic acid 50/50 complex (CHI/PAA 50/50), showing >C=O stretch at 1700 cm^{-1}, —NH$_3^+$ stretch at 1553 cm^{-1}. (b). FTIR spectra of hydroxyapatite filled chitosan-polyacrylic acid complex (CHI/PAA/HAP 50/50/60), showing >C=O stretch at 1737 cm^{-1}.

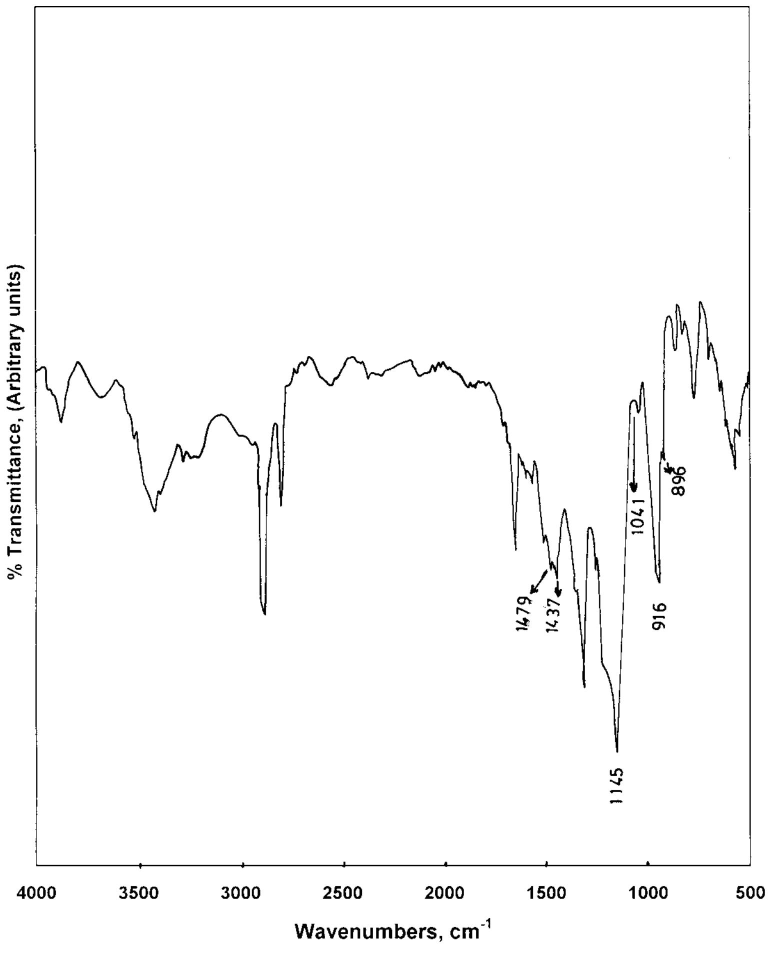


Figure 2 FTIR spectra of hydroxyapatite filled chitosan showing amide I at 1479 cm^{-1}, —NH$_2$ at 1437 cm^{-1} and phosphate peaks of HAP at 1145 cm^{-1}, 1041 cm^{-1}, 916 cm^{-1} and 896 cm^{-1}.

presence of a band at 1737 cm^{-1}. This band could be the one associated with the stretching of >C=O group that appeared around 1724 cm^{-1} in PAA and 1700 cm^{-1} in the CHI-PAA complex.

Fig. 2 gives the FTIR spectra of HAP filled chitosan. This HAP filled chitosan system was fabricated in order to examine the presence of metal chelate complex, which could possibly be formed between the $-NH_2$ groups of chitosan and metal ion present in the HAP. A chemical shift for the $-NH_2$ groups of chitosan, however, has not been detected.

3.4. Thermal analysis

Fig. 3 shows the TGA scans for CHI/PAA 50/50, CHI/PAA/HAP 50/50/80 and CHI/PAA/HAP 50/50/90. The TGA scans of other compositions, namely CHI/PAA 40/60 & CHI/PAA 60/40, are qualitatively similar to that of CHI/PAA 50/50 (and hence are not shown for the sake of clarity). It is found that the sample weight rapidly decreases with increase in temperature, especially in the ranges 60–128°C, 130–240°C and 300–600°C. It is also noted that the temperature at which decomposition commences decreases

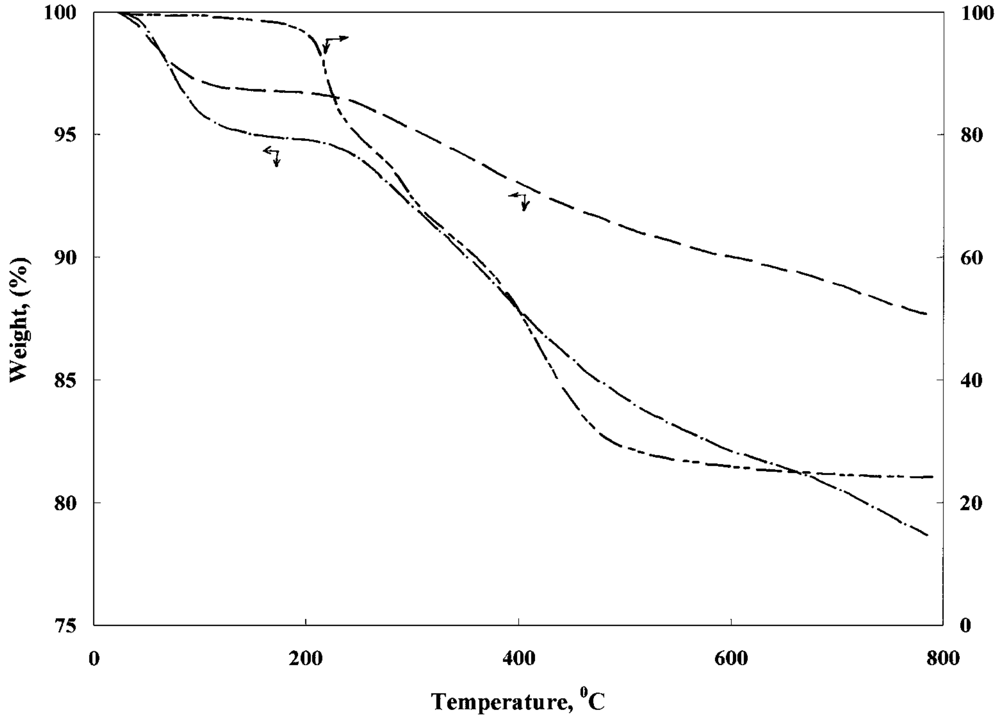


Figure 3 TGA thermograms of CHI/PAA 50/50 without HAP (————), CHI/PAA 50/50 with 80 wt% HAP (CHI/PAA/HAP 50/50/80) (—·—) and CHI/PAA 50/50 with 90 wt% HAP (CHI/PAA/HAP 50/50/90) (— — —).

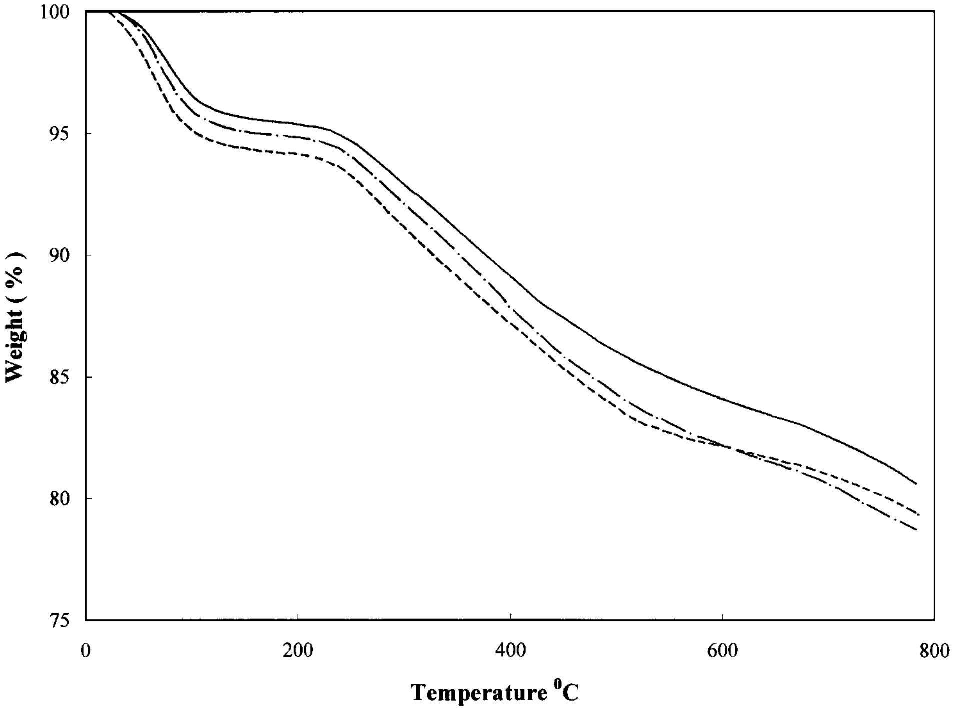


Figure 4 TGA thermograms of PEC with 80 wt% HAP for different PEC compositions CHI/PAA/HAP 40/60/80 (——), CHI/PAA/HAP 50/50/80 (— · —) and CHI/PAA/HAP 60/40//80 (– – – –).

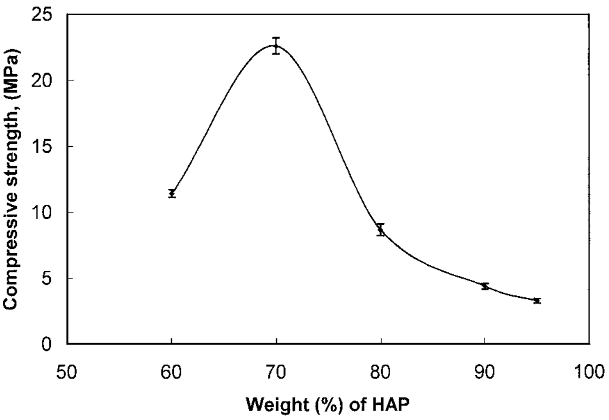


Figure 5 Compressive strength as a function of HAP composition.

as the amount of HAP in the composite increases. The other decomposition regions of the thermograms, however, were not significantly altered.

Fig. 4 shows the TGA scans of CHI/PAA/HAP 40/60/80, CHI/PAA/HAP 50/50/80 and CHI/PAA/HAP 60/40/80. For the same weight percentage of HAP loaded, it is evident from the thermogram that the degradation temperature of the composite increased with increase in the PAA content.

3.5. Compressive strength

The measurements of the compressive strength of the PEC-HAP composites were conducted to examine the mechanical performance of the composites. The compressive strength as a function of HAP composition is shown in Fig. 5. A maximum compressive strength value of 22.3 MPa was obtained at 70 wt%. HAP loading. The compressive strength, however, showed a decreasing trend on further addition of HAP.

3.6. Morphological analysis

Figs 6–10 show the SEM fracture micrographs of CHI/PAA 50/50, CHI/PAA/HAP 50/50/60, CHI/PAA/HAP 50/50/80, CHI/PAA/HAP 50/50/90, and CHI/PAA/HAP 50/50/95. The unfilled PEC (Fig. 6) appears highly non-uniform in nature having a number of micro-cracks and flaws. In CHI/PAA/HAP 50/50/60 (Fig. 7), the HAP particles are distributed throughout the polymer matrix. With increase in HAP

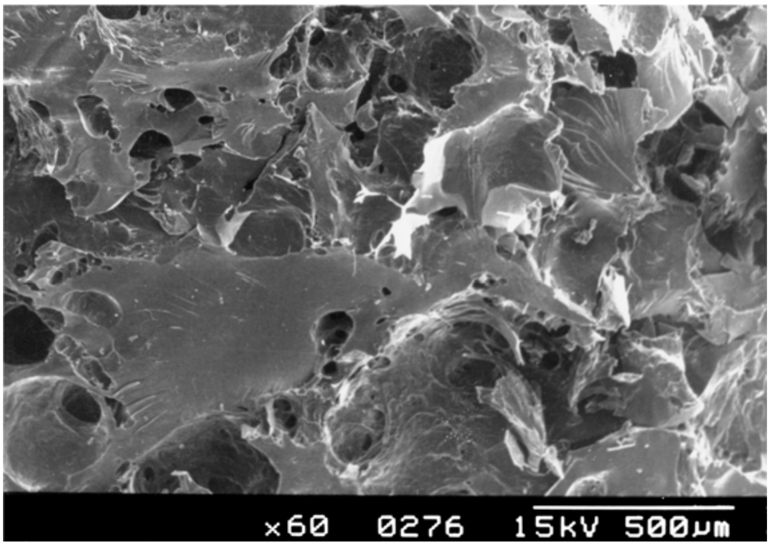


Figure 6 Fracturograph of CHI/PAA 50/50 without HAP (CHI/PAA 50/50).

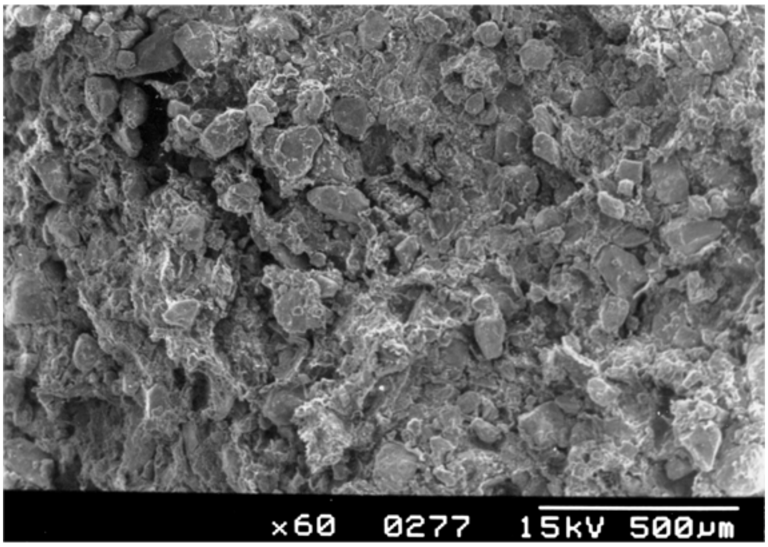


Figure 7 Fracturograph of CHI/PAA 50/50 with 60 wt% HAP (CHI/PAA/HAP 50/50/60).

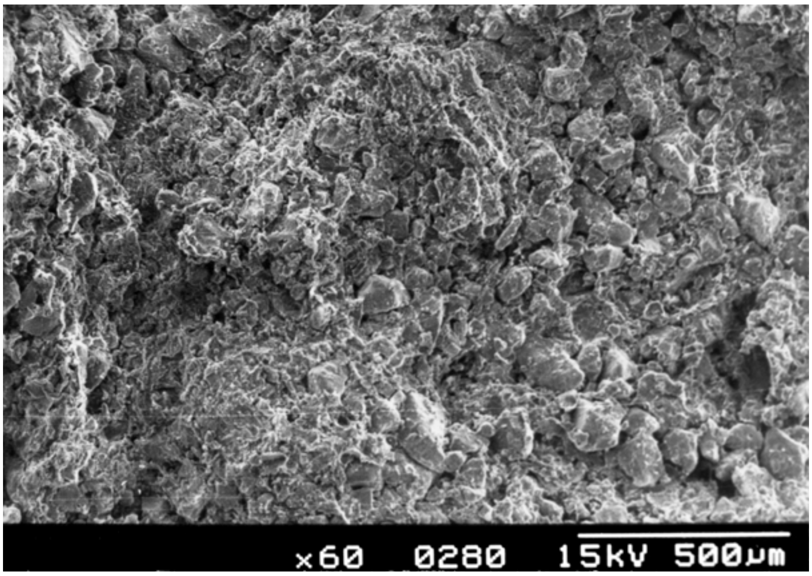


Figure 8 Fracturograph of CHI/PAA 50/50 with 80 wt% HAP (CHI/PAA/HAP 50/50/80).

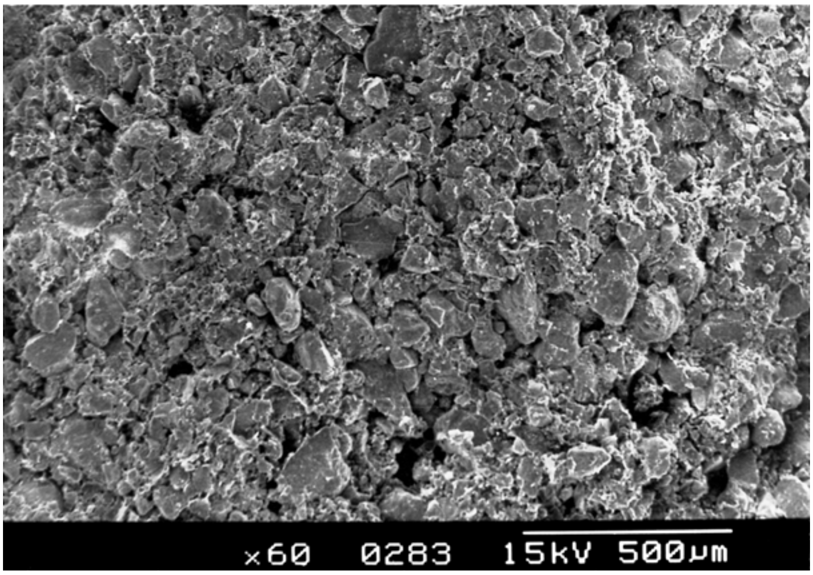


Figure 9 Fracturograph of CHI/PAA 50/50 with 90 wt% HAP (CHI/PAA/HAP 50/50/90).

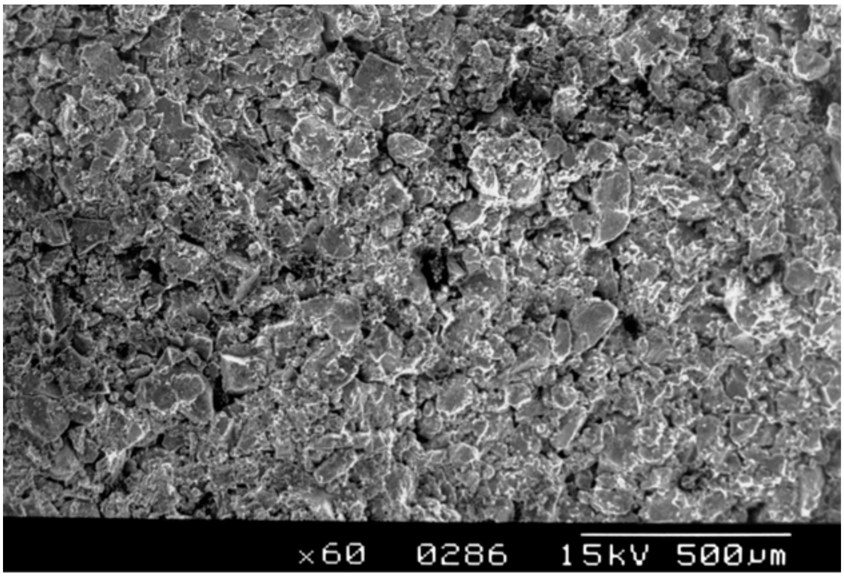
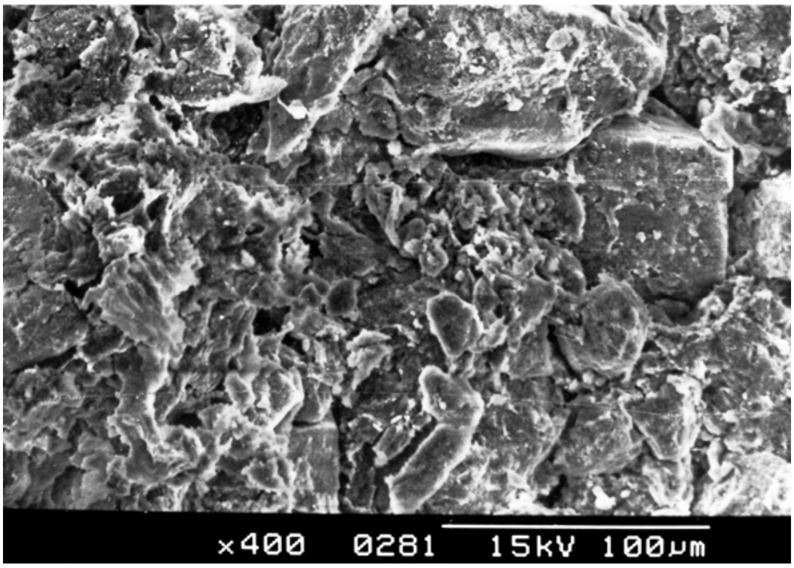
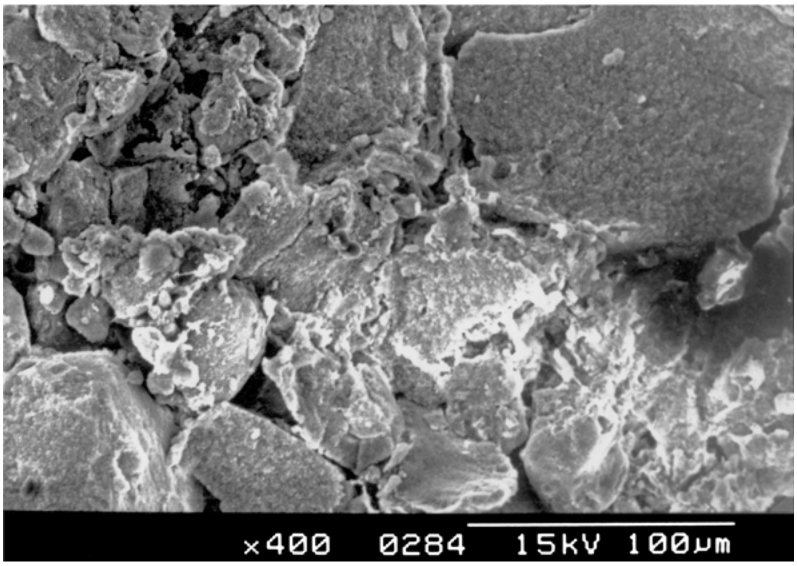


Figure 10 Fracturograph of CHI/PAA 50/50 with 95 wt% HAP (CHI/PAA/HAP 50/50/95).



(a)



(b)

Figure 11 (a) Fracturograph of CHI/PAA 50/50 with 80 wt% HAP (CHI/PAA/HAP 50/50/80) at high magnification. (b) Fracturograph of CHI/PAA 50/50 with 90 wt% HAP (CHI/PAA/HAP 50/50/90) at high magnification.

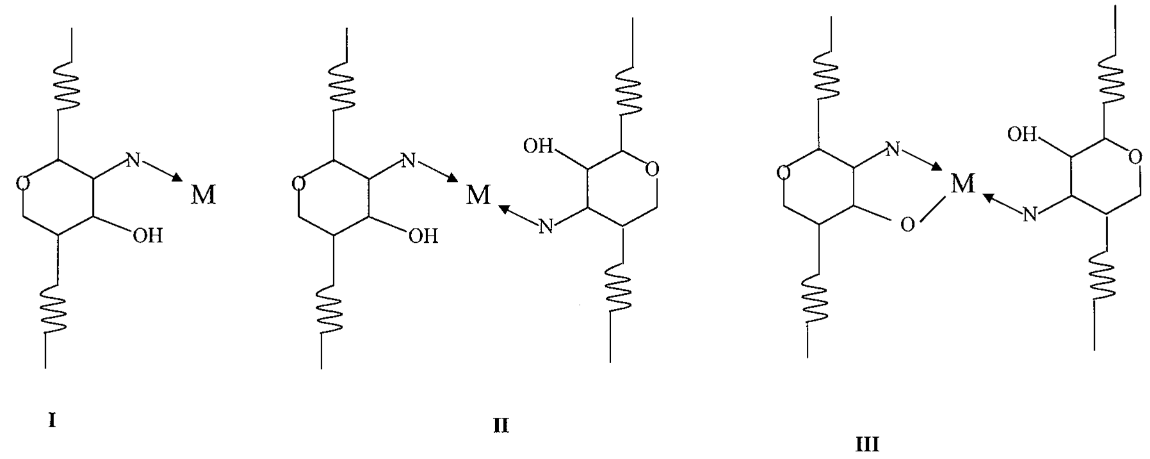
loading, (Figs 8–10) the ratio of polymer to the filler decreases to an extent that the polymer present becomes insufficient to hold the HAP particles together. This is further evident from the poor mechanical properties exhibited by CHI/PAA/HAP 50/50/80, CHI/PAA/HAP 50/50/90, and CHI/PAA/HAP 50/50/95. Fig. 11a and b shows the high magnification fracture surfaces of CHI/PAA/HAP 50/50/80 and CHI/PAA/HAP 50/50/90 respectively. In Fig. 11a, it can be seen that the HAP particles are well embedded in the matrix while in Fig. 11b; the HAP particles are seen as detached from the matrix with cracks and voids in between.

4. Discussion

4.1. Infra red spectroscopic analysis

The FTIR spectra depicted in the Fig. 1 confirm the presence of complex formation between the chitosan and polyacrylic acid entities. This observation is in accordance with that reported by Nam and Lee [34]. In their studies on the properties of PAA-CHI complex membranes prepared by blending two polymers in different ratios, the formation of the complex was confirmed by the presence of both the negatively charged ($-COO^-$) ion and the positively charged ($-NH_3^+$) ion in the complex membrane. Various other investigators have also reported that the complex formation between the polyanion PAA and polycation CHI is essentially a result of electrostatic interaction between oppositely charged polyions [32–36] and hence has not been dealt in detail in the present context of investigation.

In the present study, PAA has been added into HAP-CHI system. It has been reported that the presence of organic acid could dissociate the basic HAP and could form organic acid chelated calcium ions [37]. Chitosan can act as an active polymeric chelating ligand for certain transition metals like Co (II) and Zn (II) ions [38–40]. The possible mechanisms for the chelation of chitosan with the metal ions proposed by these researchers are depicted below.



Scheme 1 The possible metal chitosan chelates. (Ref. [38])

As can be seen from the Scheme 1 the complex formation occur either with amino nitrogen donation or with participation of both amino and hydroxyl oxygen. Hence a chemical interaction due to the co-ordination bond formation between the Ca^{2+} ions of HAP and $-NH_2$ groups of chitosan molecule should exist in the present system of study. A chemical shift for the $-NH_2$ groups of chitosan, however, has not been detected in the FTIR spectra of HAP filled chitosan system (Fig. 2). Consequently, it should be concluded that the co-ordination complex that was bound to form was only a metastable one and was not strong enough to generate a shift in the IR peaks by means of chemical bonding change, probably because of the weaker complex formation activity of Ca^{2+} ions when compared to that of the transition metal ions.

Several investigators have documented interaction of acrylate cements with HAP [41–43] and several theories on the adsorption of carboxylates on the HAP have been postulated. Wilson *et al.* [42] studied the mechanism of polyacrylate onto HAP and found that polyacrylate was irreversibly sorbed, a process which was associated with the displacement of phosphate and $Ca^{2+}$from HAP. The conclusions of this study were found to tally with that hypothesized by Smith [43] who suggested that the polyacrylate adheres to the HAP surface by the formation of chelates between acids and Ca^{2+} ions.

In the present study the explanation for the enhanced shift of $>C=O$ stretch should be because of the formation of chelate structure. The pH of the CHI-HAP system is sufficiently acidic to make the HAP surface more positively charged and hence more attractive to negatively charged carboxylate groups. Now, if the interaction were to take place via hydrogen bonding between the hydroxyl groups on HAP and the COO^- groups in the PEC, then the band would have been occurred in the 1690 cm^{-1}–1695 cm^{-1} region. The enhanced intensities of the band, especially evident from the higher frequency (1737 cm^{-1}) vibration also support the view that the interaction is not accounted for solely by simple complex formation. Clearly the polymeric nature of polyacrylate and the strong co-operative effect between its carboxylate groups is responsible for its strong interaction. The absorption at higher frequency could be attributed to the involvement of calcium and phosphate groups of HAP and the entities such as $-NH_3^+$, $-COOH$ of CHI and PAA respectively. A similar upward shift have been reported in the case of PAA grafted aluminium oxide system, which had occurred due to the complex interaction between the metal ion (Al^{3+}) and the carboxylic group of PAA [44].

The possibility of the replacement of PO_4^{3-}ions of the HAP by the carboxylic group of PAA cannot be ruled out. This possibility is further evident from the acidic pH of the HAP complex indicating that displacement of calcium and phosphate from HAP has occurred by some type of ion-exchange mechanism. In the presence of chitosan containing entities, the reorientation and stabilization of PO_4^{3-}ions would be easier. Such interaction together with a calcium ion bridging could lead to a stable structure. The strong electrical coupling between the metal atom and the polar bands has been attributed to the upward shift [45].

4.2. Thermal analysis

Thermal scans performed on the filled PEC exhibits a multiple step degradation profile. The weight loss is observed at various ranges for the composites CHI/PAA/HAP 50/50, CHI/PAA/HAP 50/50/80 and CHI/PAA/HAP 50/50/90, corresponds to the dehydration of inter-polyeletrolyte ionic bonds, deacetylation of chitosan moieties, and degradation of the polyacrylic chains of the PEC respectively. Peniche *et al.* [36] reported similar observation in their studies on CHI/PAA interpenetrating polymeric networks obtained by radical polymerization. This observation is similar to that reported by Yamaguchi *et al.* [7] and Meenan *et al.* [46] in chitosan and poly (ether ether ketone) matrix respectively. An initial decrease in the onset of decomposition temperature, observed in the case of CHI/PAA 50/50, CHI/PAA/HAP 50/50/80 and CHI/PAA/HAP 50/50/90, corresponds to the loss of water because the weight loss observed here is most pronounced up to 100°C. This relatively small weight loss is assigned to the removal of adsorbed water between 80 and 120°C and to the slow dehydroxylation of HAP material when heated up to 700°C [47]. From the above discussions it could be inferred that HAP does not interfere with the decomposition behaviour of the PEC, except for the onset of degradation.

The decomposition temperature of the PEC composites increases with the increase in the PAA content (Fig. 4). PAA being thermally more stable could modify the decomposition behaviour of the PEC-HAP composite in its favour, making the whole system a thermally stable one. Similar results were reported by Peniche *et al.* [36]. The composition of residue in the thermogram was found to be almost consistent with the HAP content as initially added during the preparation, therefore it is believed that HAP was almost perfectly incorporated into the composites.

4.3. Compressive strength

Among the composites studied, CHI/PAA/HAP 50/50/70 showed the maximum value of compressive strength. In general, the proper stress transfer occurring between the reinforcement and the matrix governs the mechanical characteristics of filled polymers. In the present context, the enhanced strength observed for CHI/PAA/HAP 50/50/70 could probably be due to the complexation of the HAP with the polymer matrix. However, at higher loadings of HAP, the polymer matrix is insufficient to keep the filler particles bound. This results in poor stress transfer which in turn adversely affects the mechanical properties of the composite material.

4.4. Morphological analysis

The microstructure of composites with varying HAP content reflects a more uniform compaction with increase in HAP content. The flaws and micro-cracks seen in the PEC could be attributed to the inherent elastic recovery of the polymer that reduces the stress during the moulding process. The simultaneous complex formation and filler incorporation adopted for the preparation of this composite material offer uniform distribution of the filler in the matrix supported by chemical and mechanical interlocking that is helpful for the efficient stress-transfer in the composite system. The better compaction seen in the case of higher HAP loaded composites favour better load transfer. The interactions between the PEC and HAP offer advantages in processing as well as mechanical properties. The HAP content, when increased to a higher extent, the polymer becomes insufficient to bind the HAP particles and the performance of the composite could be adversely affected as seen in the compressive strength data.

5. Conclusions

The present investigation offers a simple method for the manufacture of a family of novel bioactive composite materials for tissue regeneration. The interaction between the inorganic and the organic phases contributes much in the mechanical performance of the composite. The simultaneous complexation and filler incorporation could favour uniform distribution of HAP in the PEC matrix. HAP loading in the optimum range is preferred since the higher percentage could dilute the chemical interaction and the mechanical interlocking. A better compaction of the composite material with higher HAP loading was effective in minimizing the flaws and micro-cracks. Further increase in the HAP content reduces the compressive strength.

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