# Swelling Behavior of Hydroxyapatite-Filled Chitosan–Poly(acrylic acid) Polyelectrolyte Complexes

**G. S. Sailaja, P. Ramesh, H. K. Varma**

*Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Poojappura, Thiruvananthapuram 695 012, India*

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**ABSTRACT:** A family of hydroxyapatite (HAP)-filled chitosan (CHI)–poly(acrylic acid) (PAA) polyelectrolyte complexes was prepared for the development of a degradable biocompatible organic matrix with nascent HAP that will degrade in vivo over a period of time. The effects of complexation on the degradation profile of the composites as well as the interaction between the CHI–PAA matrix and HAP in the composite system were evaluated by studying the swelling behavior of these composites in phosphate-buffered saline (PBS) by varying their CHI–PAA ratio and HAP content. All composite systems showed a general trend of three stages of swelling with the variation in the degree of equilibrium swelling. The percentage weight gain initially decreased in a linear way with increases in the HAP weight percentages, leading to a first equilibrium swelling, represented by the plateau; further increased to a greater extent; and finally stabilized. The CHI/PAA/HAP composites were stable in PBS up to a period of more than 45 days whereas the 50/50 CHI/PAA control sample showed a single equilibrium attained after a period of 288 h. Further exposure of the specimen to the medium led to its disintegration. It was also observed that, even though CHI and PAA were capable of binding HAP, because of the lack of efficient binding, the integrity of the CHI–HAP and PAA–HAP composites were lost within 48 h. The 50/50/80 CHI/PAA/HAP composition showed the minimum amount of swelling in the series.
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**Key words:** composites; hydroxyapatite; polyelectrolytes; swelling

## INTRODUCTION

Hydroxyapatite (HAP) is the clinically established material for the repair and reconstruction of hard tissues because of its inherent osteoconductive behavior and its ability to integrate with bone.[1,2] Sintering is an inevitable part of conventional processing techniques while preparing HAP in desired implantable contours. However, HAP as nascent particles shows a higher resorption rate in vivo.[3] Du et al. prepared a three-dimensional matrix of collagen and nanoparticle-sized HAP for organ culture,[4] and Yamaguchi et al. developed a coprecipitation method to prepare HAP/chitosan (CHI) composites.[5] Other investigations also reported on different composite systems prepared from HAP in the nascent form with organic matrices to increase the resorption rate and thereby in vivo bone formation.[6,7] Among them, CHI–HAP and poly(acrylic acid)–HAP (PAA–HAP) systems attracted increased attention because of their specific interactions with HAP.[5,8] In addition, a CHI/PAA polyelectrolyte system was studied as a degradable matrix for drug delivery applications.[9]

Considering these factors, we developed a class of HAP-filled polyelectrolyte complex (PEC) composite systems consisting of an organic matrix of CHI and PAA with HAP. Chitosan [(1,4)-linked 2-amino-2-deoxy-D glucan], a cationic polysaccharide obtained by the N-deacetylation of chitin, is a biofunctional polymer; its derivatives have been studied for a number of biomedical applications, including wound dressings, drug delivery systems, and scaffolds for tissue engineering.[10–12] Chitosan contains reactive amino as well as hydroxyl groups in its chemical structure; the amino group easily forms PECs with the reactive carboxyl group of PAA, which is a water-soluble and biocompatible anionic polyelectrolyte. The composite systems for the present study were prepared by complexing the HAP-filled CHI system with PAA. The preliminary evaluation of the composites including IR spectra, thermal degradation profiles, morphology, and compressive strength properties were reported previously.[13] In the present study, we report the swelling behavior of the composite systems in phosphate-buffered saline (PBS) with varying weight percentages of HAP in different CHI–PAA ratios. We primarily intended to ascertain the in vitro behavior of this class of composite materials prior to functional evaluation in an animal model.

*Correspondence to:* H. K. Varma (varma@sctimst.ac.in).
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**TABLE I**
**Size Distribution of HAP Particles**

| | |
|---|---|
| $d_{0.1}$ | 6.86 $\mu$m |
| $d_{0.5}$ | 48.52 $\mu$m |
| $d_{0.9}$ | 120.37 $\mu$m |

## EXPERIMENTAL

Chitosan with a 70% degree of N-deacetylation was kindly provided by Central Institute of Fisheries Technology (Kochi, India). The viscosity-average molecular weight was measured by an Ubbelohde viscometer using the Mark–Houwink equation: $[\eta] = 8.93 \times 10^{-4} M^{0.71}$ and was calculated as $1.2 \times 10^6$.[14] Poly(acrylic acid) was prepared by batch polymerization of acrylic acid (Merck, Mumbai, India) using ammonium persulfate as an initiator.[15] The viscosity-average molecular weight was determined as $2.9 \times 10^5$. Hydroxyapatite was synthesized in the laboratory by the precipitation method using ammoniated calcium nitrate and dihydrogen ammonium phosphate followed by the freeze-drying technique, the details of which are reported elsewhere.[16] The particle size and particle size distribution of the freeze-dried HAP powder are given in Table I.

### Preparation of CHI–PAA–HAP composites

The composites were prepared according to procedures reported earlier.[13] Briefly, a uniform dispersion of requisite quantities of HAP in 1 wt % CHI (w/v) in 5% acetic acid was prepared and complexed with 1 wt % PAA (w/v). By varying the amount of HAP and the CHI/PAA ratio, a series of compositions were prepared. The compositions and the designations of the systems are given in Table II. The composites thus prepared were separated by filtration and washed thoroughly with distilled water in order to remove any residual reagents. The composites were then wet pressed at 20 kg/cm$^2$ into thin sheets and dried to constant weight. To study the effective binding of the HAP particles to the CHI/PAA matrix, we used 50/50 CHI/PAA, 20/80 CHI/HAP, and 20/80 PAA/HAP control samples.

**TABLE II**
**Compositions and Designations of Chitosan–Poly(acrylic acid) Polyelectrolyte Complexes and Chitosan–Poly(acrylic acid)–Hydroxyapatite Composites**

| Material code | Polymer matrix | | HAP |
|---|---|---|---|
| | CHI (wt %) | PAA (wt %) | (wt %) |
| CHI–PAA 40/60 | 40 | 60 | 0 |
| CHI–PAA 50/50 | 50 | 50 | 0 |
| CHI–PAA 60/40 | 60 | 40 | 0 |
| CHI–HAP 20/80 | 20 | — | 80 |
| 1 PAA–HAP 20/80 | — | 20 | 80 |
| CHI–PAA–HAP 50/50/40 | 50 | 50 | 40 |
| CHI–PAA–HAP 50/50/60 | 50 | 50 | 60 |
| CHI–PAA–HAP 50/50/80 | 50 | 50 | 80 |
| CHI–PAA–HAP 40/60/40 | 40 | 60 | 40 |
| CHI–PAA–HAP 40/60/60 | 40 | 60 | 60 |
| CHI–PAA–HAP 40/60/80 | 40 | 60 | 80 |
| CHI–PAA–HAP 60/40/40 | 60 | 40 | 40 |
| CHI–PAA–HAP 60/40/60 | 60 | 40 | 60 |
| CHI–PAA–HAP 60/40/80 | 60 | 40 | 80 |

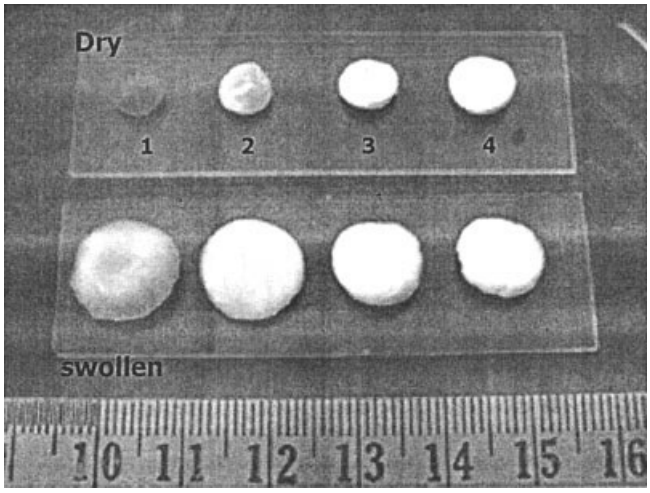



**Figure 1** A set of representative systems before and after 7 days of swelling in PBS: (1) 50/50 CHI/PAA, (2) 50/50/40 CHI/PAA/HAP, (3) 50/50/60 CHI/PAA/HAP, and (4) 50/50/80 CHI/PAA/HAP.

### Swelling in PBS

Circular specimens for the swelling studies were prepared by using a circular cutting die with a diameter of 12 mm. Four samples from each composition were prepared. PBS with a pH of 7.32 at 37°C was prepared. Each of these specimens was immersed in 10 mL of PBS in air-tight containers and kept in an incubator at 37 ± 1°C. The weight of the samples and the pH were

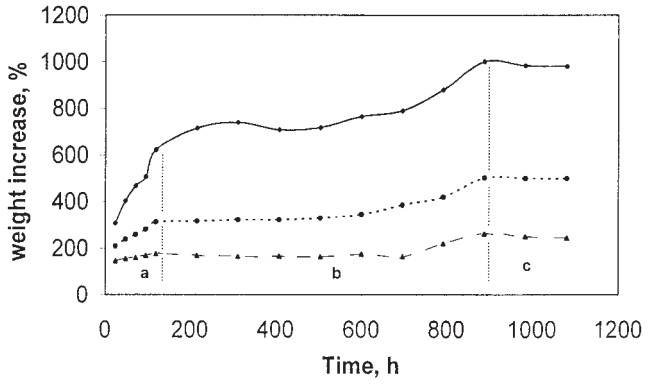



**Figure 2** The swelling behavior of 40/60 CHI/PAA at (—) 40, (- - -) 60, and (– –) 80 wt % filled HAP.

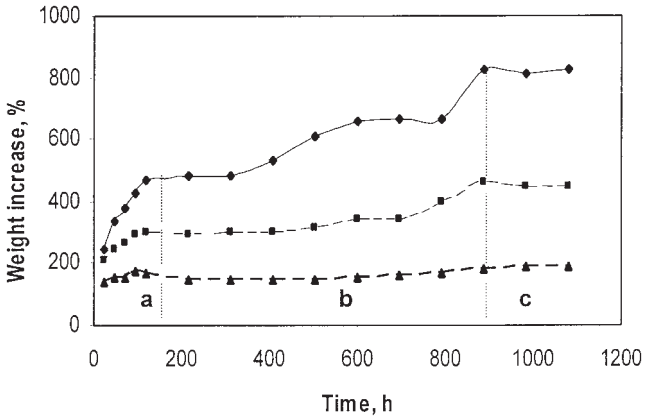



**Figure 3** The swelling behavior of 50/50 CHI/PAA at (—) 40, (- - -) 60, and (– –) 80 wt % filled HAP.

recorded at 24-h intervals for the first 4 days and then at 96-h intervals for the next 40 days.

## RESULTS

The equilibrium swelling degree of the specimens was calculated with the following equation:

$$\text{swelling degree } (\%) = [(W_w - W_d)/W_d] \times 100$$

where $W_d$ and $W_w$ are the weights of the dry and swollen samples, respectively.

Figure 1 illustrates a set of representative systems before and after swelling for 7 days, and it imparts information about the nature as well as the extent of swelling of the different systems. The swelling behavior of 40/60 CHI/PAA filled with 40, 60, and 80 wt % HAP is shown in Figure 2. Note that the percentage increase in weight initially decreases with increases in the HAP content in a linear way, leading to equilibrium swelling, which is represented by the plateau; then, it increases to a greater extent and finally stabilizes. The different regions of the swelling profile are

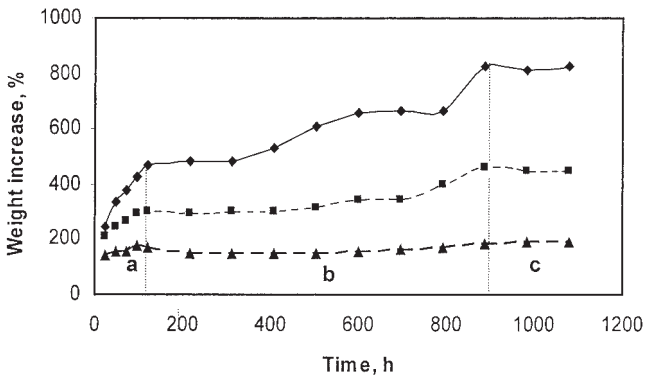



**Figure 4** The swelling behavior of 60/40 CHI/PAA at (—) 40, (- - -) 60, and (– –) 80 wt % filled HAP.

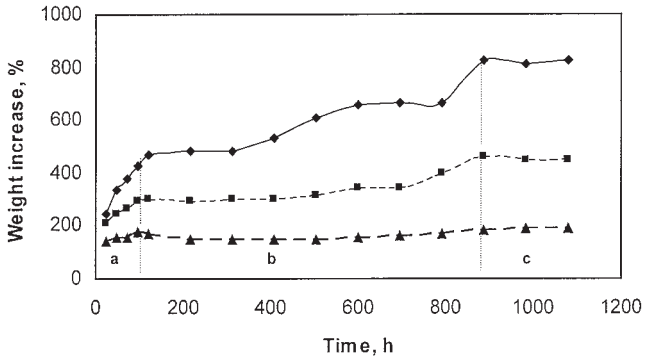



**Figure 5** The swelling behavior of (—) 60/40/40, (– –) 40/60/40, and (- - -) 50/50/40 CHI/PAA/HAP.

represented by points a, b, and c. Similar behavior is observed in the case of 50/50 CHI/PAA and 60/40 CHI/PAA at same weight percentages of HAP, as shown in Figures 3 and 4, respectively.

The swelling behaviors of 40/60, 50/50, and 60/40 CHI/PAA filled with 40 wt % HAP are compared in Figure 5. It is evident that the composite with a higher percentage of PAA (40/60 CHI/PAA) swells initially to a higher level, reaches equilibrium, again increases to a certain extent, and finally arrives at the second-stage equilibrium. However, 60/40 CHI/PAA shows a higher extent of swelling during the second equilibrium. The 50/50 CHI/PAA composition initially shows a moderate rate of swelling and then the rate of increase is minimum compared to the other two compositions, especially between 500 and 1080 h of swelling. Similar swelling profiles are expressed by 60 and 80 wt % HAP-filled CHI/PAA systems, as shown in Figures 6 and 7, respectively.

While comparing the swelling behavior of the composite systems with those of the control samples shown in Figure 8, we observed that the control samples retained their integrity only for a time period of less than 15 days. The incorporation of HAP in the

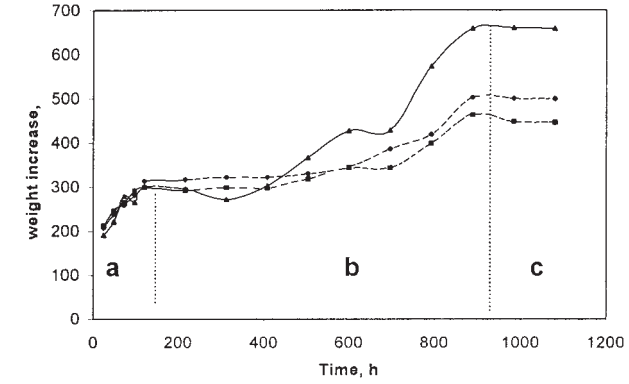



**Figure 6** The swelling behavior of (—) 60/40/60, (– –) 40/60/60, and (- - -) 50/50/60 CHI/PAA/HAP.

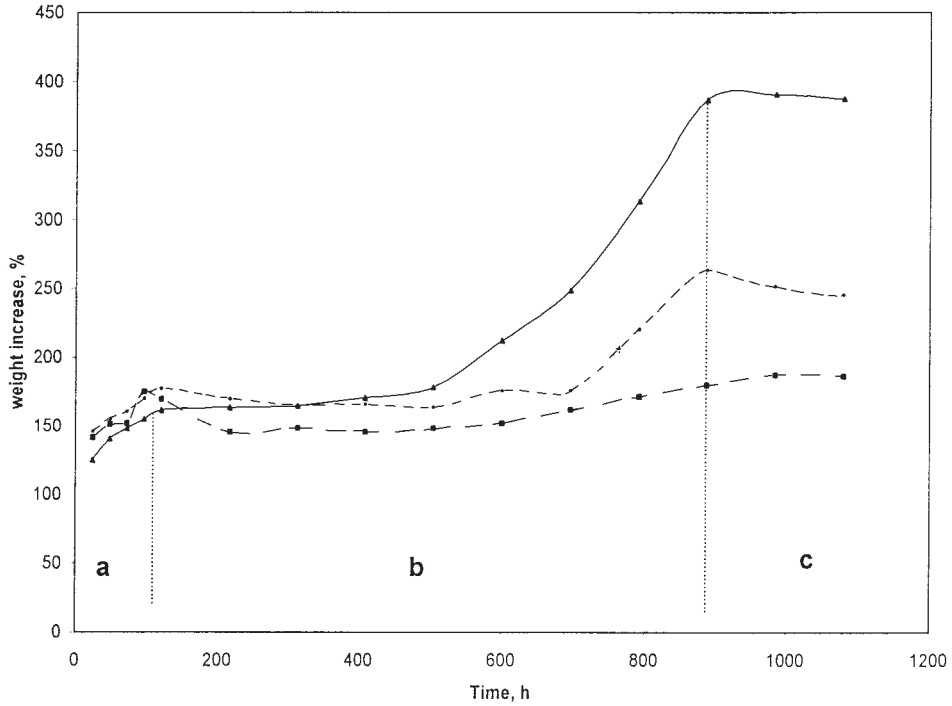



**Figure 7** The swelling behavior of (—) 40/60/80, (- - -) 40/60/80, and (– –) 50/60/80 CHI/PAA/HAP.

PEC matrix enables the system to retain its integrity for longer time periods with restricted swelling. We also observed that CHI/HAP as well as CHI/PAA systems disintegrated within 48 h. At 24 h, the percentages of swelling of the CHI/HAP and PAA/HAP systems were 157.31 and 93.99%, respectively.

## DISCUSSION

The degree of swelling of a hydrogel is favorably influenced by the osmotic potential, strong interactions with water, high free volume, high chain flexibility, and low crosslink density.[17] Because swelling leads to a less entropically desirable configuration, when the water enters the matrix, the chains extend a restrictive force and equilibrium swelling is reached when the restrictive force balances the osmotic force. The study of the degree of equilibrium swelling of the HAP-filled CHI–PAA matrix is obviously helpful in understanding the basic features of the composite systems, like the extent of complexation as well as the interaction between the PEC matrix and HAP. All systems under study show a general trend, regardless of the variation in the CHI/PAA ratio or the weight percent of HAP incorporated. The initial linear increase up to 200 h in all cases could be particularly assigned because of the basic water absorption tendency of the polyelectrolyte system. CHI–PAA is a hydrogel and thus shows the inherent property of spontaneous water uptake from the medium. The initial linearity in the swelling behavior of the CHI–PAA system is in accordance with other reports.[12]

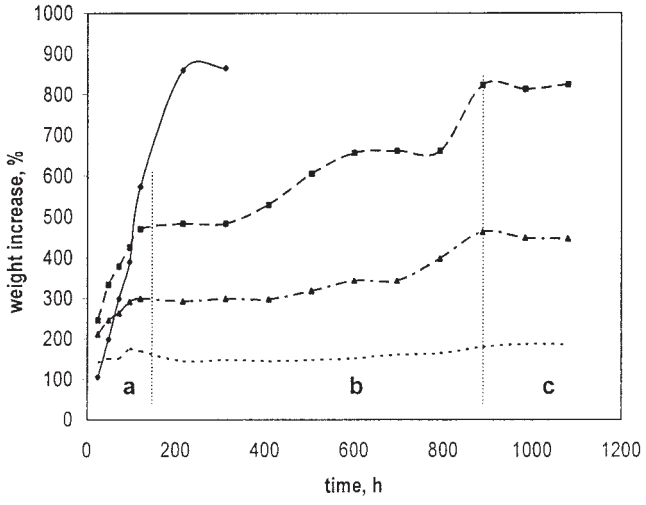



**Figure 8** The swelling behavior of (—) 50/50 CHI/PAA and (– –) 50/50/40, (– · –) 50/50/60, and (- - -) 50/50/80 CHI/PAA/HAP.

In the case of the HAP-filled CHI–PAA system, the equilibrium degree of swelling is considerably restricted because the free volume and chain flexibility are decreased as a result of the electrostatic interactions between polymeric chains and because of the well-entrapped HAP moieties. Figures 2–4 show that, in each of the three kinds of PEC matrixes, the rate of swelling is decreased with increases in the weight percentage of HAP. The compositions with higher HAP content swell to a lower extent. Schematic rep-

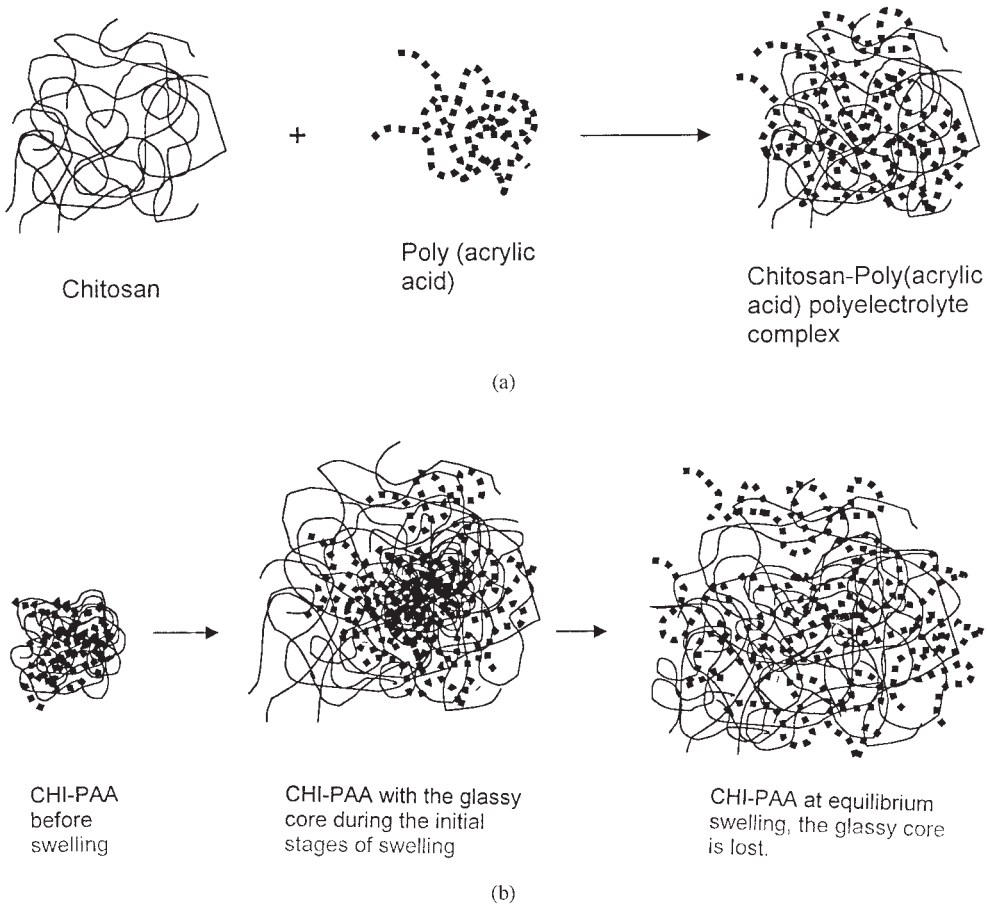


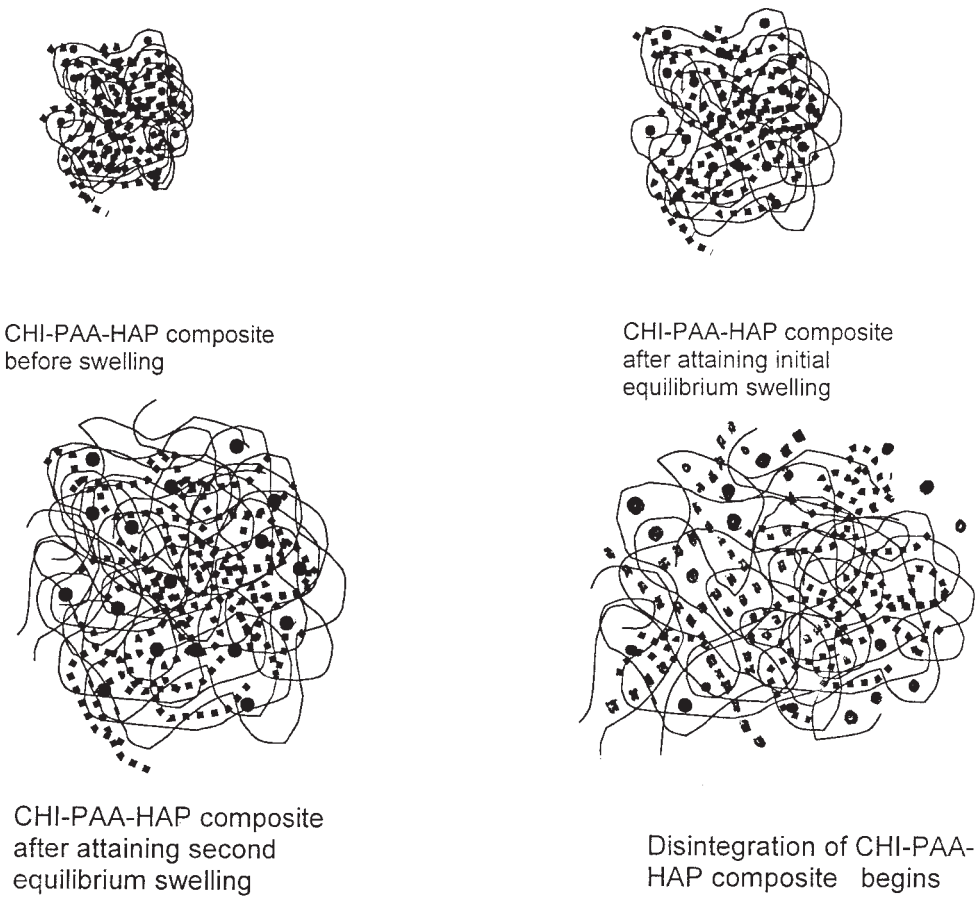

**Figure 9** Schematic representations of (a) the formation of the CHI–PAA polyelectrolyte complex, (b) its swelling behavior, and (c) the swelling behavior of the HAP-filled CHI–PAA polyelectrolyte complex.

resentations of the swelling behavior of the CHI–PAA complex and the HAP-filled CHI–PAA system are presented in Figure 9. At the initial equilibrium, the HAP moieties and surrounding polymer matrix are in metastable equilibrium because of sufficient interaction as well as interlocking between them. On further exposure to the swelling medium, solvation energy dominates the PEC–HAP interaction and leads to the second-stage swelling, which is more time dependent than the first-stage swelling. The interaction between HAP and the macromolecular network disappears slowly, leaving a gelled mass with poor mechanical properties, which leads to the second stabilization period, as represented in Figure 9. On continued exposure to PBS, the composites begin to disintegrate.

Comparing the percentages of weight increases shown by 40/60, 50/50, and 60/40 CHI–PAA at the same weight percentage of HAP loading, 50/50 CHI–PAA shows minimum swelling in comparison to the other two (Figs. 5–7). From this it can be inferred that the extent of complexation plays a significant role in the rate of swelling. The 50/50 composition has maximum complexation and hence minimum swelling of 600, 450, and 150% at 40, 60, and 80 wt % HAP loading, respectively.

The first-stage equilibrium swelling is maximum for 40/60 CHI–PAA, whereas after the second stage the equilibrium swelling is higher for 60/40 CHI–PAA. Because the PAA content is lower for the 60/40 complex, it is expected to show the least swelling. It could therefore be inferred that the complexation is lower in the 60/40 system than in 40/60 one; hence, after the initial stabilization period, it swells to a higher extent than the higher PAA-containing composition, irrespective of the HAP content. This also strongly supports the idea that the complexation has a very significant role in controlling the rate of swelling of the composite systems.

When examining the swelling profile of the control samples (CHI/HAP and PAA–HAP), it is obvious that, even though CHI and PAA can bind HAP to a certain extent, the bond that is formed is not sufficient to hold HAP particles for a longer time, which is hardly less than 48 h in PBS. The swelling profile of 50/50 CHI/PAA shows that there is only one equilibrium, it is attained after a period of 288 h, and further




(c)

**Figure 9** (*Continued*)

exposure to the medium leads to its disintegration. Because the PEC formed by the complexation is a type of hydrogel, its swelling behavior is dependent on the structure of polymeric networks that have been created by the complexation of the polyelectrolytes.[18] The clustering of the chains, the inhomogenities, and the network defects in the system may also contribute to the nature of swelling of this system. At the initial stages of swelling, the chains between the crosslinks change into an elongated configuration while an opposite elastic force tries to retract the chains; then, over a period of time when these two opposite forces become equal, equilibrium swelling is achieved.[17] From the results it is obvious that the 50/50 CHI/PAA compositions filled with 40, 60, and 80 wt % HAP showed lower swelling in the series and 50/50/80 CHI/PAA/HAP had the minimum swelling. This is partially attributed to the effective complexation that is occurring in 50/50 CHI/PAA and partly due to the higher HAP content in the specimen.

## CONCLUSION

The simultaneous complexation and filler incorporation procedure followed in this study is a very efficient method that can be adopted to prepare nascent HAP-containing degradable composite systems for hard tissue substitute applications. The extent of complexation played a major role in the degree of swelling of the composite systems. The interaction between the PEC matrix and HAP had a significant influence on controlling the rate of swelling. The composite systems showed two-stage swelling behavior. The first-stage swelling was due to the tendency of the macromolecular networks to assume an elongated configuration and was proportional to the PAA content in the system, which then stabilized as a result of increased filler–polymer interactions. The second-stage swelling arose as a result of continued exposure to the medium, wherein the solvation energy overcame the complexation force and the HAP–PEC interactions. The 50/50 CHI/PAA compositions filled with 40, 60, and 80 wt % HAP showed lower swelling, and the lowest was for 50/50/80 CHI/PAA/HAP.

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