A Novel Method to Prepare Chitosan/Montmorillonite Nanocomposites

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ABSTRACT: We are proposing a novel method to prepare chitosan/montmorillonite nanocomposites. The montmorillonite (MMT) clay was excavated from the mountainside of eastern Taiwan. After being purified, it was incorporated with potassium persulfate (KPS) through the intercalation process. As the KPS-incorporated MMT was dispersed in the acidified aqueous solution of chitosan, the KPS instantly reacted with the chitosan, resulting in the cleavage of polymer chains and exfoliation of MMT as well. After removing the unexfoliated MMT, solutions were cast to form a film of chitosan/MMT nanocomposite. Interestingly, the exfoliated MMT layers were found to flatten out in parallel with the surface, which not only increased the tensile strength of the chitosan film but also hindered degradation in the vitro test. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 98: 2042–2047, 2005

Key words: biomaterials; biopolymers; chitosan; clay; nanocomposites

INTRODUCTION

In recent decades, biopolymers have acquired much attention for their potential application in tissue engineering.[1–3] One of the promising biopolymers is chitosan,[4–6] which is a partially deacetylated derivative of chitin, the most abundant natural polymer next to cellulose. Chitosan with the glucosamine and N-acetylglucosamine units linked by the 1–4 glycosidic bond could be degraded into a smaller size in the presence of potassium persulfate (KPS) in acidic aqueous solution,[7] because the dissociated anionic radical of KPS has a tendency to be attracted to the cationic amino group of the glucosamine ring, resulting in the cleavage of the glycosidic C-O-C bonds.

Montmorillonite (MMT), a clay, is the most widely used silicate in polymer nanocomposites.[8–10] It is constructed of repeating triple-layer sheets composed of two tetrahedral silica sheets fused to an edge-shared octahedral sheet of alumina with a thickness of ~ 1 nm and a length of approximately 100 to several hundred nanometers.[9] Stacking of the layers creates a gap between the layers called the interlayer or gallery. Isomerphic substitution within the layers, such as Si^{4+} by Al^{3+} or Al^{3+} by Mg^{2+}, generates negative charges that are counterbalanced by some cations, such as Na^+ in the gallery.[9,10] Because of the weak interactions between the stacking layers, the cations inside the gallery can be easily exchanged by other cations. The maximum capacity for the cation exchange is known as the cation exchange capacity (CEC) and is expressed as mequiv/100 g.[10]

To prepare chitosan/MMT nanocomposites, in this study MMT was incorporated with KPS in aqueous solution through cationic exchange first and then mixed with the acidified aqueous solution of chitosan. Surprisingly, the resulting mixture has not only rendered the parent chitosan degraded to a smaller size but also rendered MMT exfoliated to thin layers. The composition and structure of prepared chitosan/KPS-MMT nanocomposites were carefully studied by dynamic light scattering (DLS), transmission electron microscopy (TEM), electron spectroscopy for chemical analysis (ESCA), and X-ray diffraction (XRD). Their mechanical properties and biodegradation were also investigated.

EXPERIMENTAL

Materials

MMT employed in this study was extracted from bentonite excavated from the mountainside of the Taidung prefecture located east of Taiwan. The extraction procedure was as follows: Adequate de-ionized water was added to the smashed bentonite with continuous stirring for 30 min. The mixture was then poured to a container and retained for 3 days to fully swell the

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bentonite. After removing the larger particles by centrifugation twice, the slurry was equally divided into top, middle, and bottom sections. Only the middle section was desired and transferred to a freeze-dryer. After being freeze-dried at 3×10^{-2} torr and $-40°C$ for 72 h, the resulting MMT powder was pulverized to a size less than 75 μm. Its CEC determined by the Kjeldahl method[11] was 127 mequiv/100 g.

KPS (reagent grade, Acros) was dissolved into the de-ionized water to prepare the 0.5 wt % KPS solution first. The allocated amount of KPS solution corresponding to the 0.5, 1, 2, 3, 4, and 5 CEC of MMT, respectively, was mixed with 2g MMT to prepare the KPS-MMT. After stirring overnight to complete the intercalation, the KPS-MMT solution was freeze-dried to obtain the KPS-MMT powder. Finally, the KPS-MMT powders were pulverized to a size less than 75 μm.

Chitosan purchased from Acros had a degree of deacetylation of 85%, determined by a colloid titration method.[12] It was dissolved in 0.17M acetic acid by stirring overnight to prepare the 1 wt % solution. 5 phc KPS-MMT powder was then added to the solution. Phc denotes parts of MMT per hundred parts of chitosan by weight. After being heating in a water bath at 60°C for 1h, the chitosan/KPS-MMT solution was separated into two phases. The upper phase was a clear solution, and the bottom phase was a gel-like sediment. After removing the bottom part, the remaining clear solution was concentrated by a rotary evaporator. It should be noted that no phase separation of the chitosan/KPS solution was observed when an equal amount of KPS to that in 5 phc KPS(5CEC)-MMT was mixed with the acidic solution of chitosan. The chitosan/KPS-MMT films were prepared by casting the concentrated solutions into a plastic petri dish and drying in ambient atmosphere for 3 days. To remove the remaining acetic acid and KPS, the cast films were immersed in 0.1N KOH aqueous solution for 1h and then rinsed several times with the de-ionized water. After that, the films were dried to a constant weight at 30°C, 20%RH for at least 3 days before performing the structural analysis as well as the mechanical and degradation tests. A similar procedure to prepare the films was also applied to the acidic solutions of chitosan and KPS-degraded chitosan.

Characterization

The domain size distribution in the clear part of the prepared chitosan/KPS-MMT solution was measured by dynamic light scattering. The measurement was carried out using a Malvern series 4700 apparatus with a 25 mW He-Ne laser as a light source. A Hitachi H-7100 TEM was employed to investigate the exfoliated structure of MMT in the chitosan/KPS-MMT nanocomposite dried from the solution. The speci-
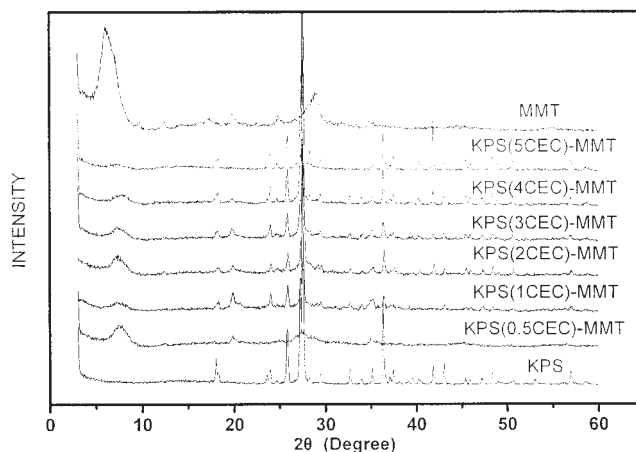

Figure 1 X-ray diffraction patterns of KPS and MMT powders incorporating various CEC of KPS.

mens were prepared by applying a drop of the clear solution onto the carbon-coated copper grid and drying at 60°C. A Philip XRD-500 X-ray diffraction analyzer was employed to investigate the prepared KPS-MMT powder and the cast films of chitosan/KPS-MMT nanocomposites, using nickel-filtered Cu Kα radiation at 30 kV and 20 mA. The elemental analysis of the cast film was conducted using the VG Scientific ESCALAB 250 ESCA system.

The mechanical properties of chitosan/KPS-MMT nanocomposites were tested using an Instron TM model universal testing instrument. The specimens were prepared according to ASTM d 638–77a. All specimens were tested using a crosshead speed of 5 mm/min.

For the degradation test in the phosphate buffered saline (PBS) solution, each cast specimen was cut to 10 pieces with a dimension of $2 \times 0.5 \times \sim 0.05$ cm. After being weighed, the specimens were immersed in 50 mL PBS solution controlled at 37°C. At the designated time interval, one of the specimens was removed from the solution and dried at 30°C, 20%RH to a constant weight. Then, the weight reduction was recorded after being dried.

RESULTS AND DISCUSSION

Microstructure

Figure 1 shows the X-ray diffraction pattern of MMT powders incorporated with various amounts of KPS. No diffraction peak from the KPS was observed when the MMT was treated with 0.5 CEC KPS. It strongly suggested that KPS has exchanged with the cations of MMT through the intercalation process. The diffraction peaks from KPS began to appear as its amount was increased to 1 CEC. Presumably, part of the KPS

TABLE I
The Contents of MMT in Chitosan/KPS-MMT Nanocomposites Estimated by the Direct Subtraction Method, by ESCA, and by Eq. (1)

	Contents (wt %)		
Samples, chitosan/	By direct method	By ESCA	$W_{m,s}$ from eq (1)
KPS(0.5CEC)-MMT	1.6	7.5	7.7
KPS(1CEC)-MMT	2.4	9.2	9.9
KPS(2CEC)-MMT	3.1	8.6	11.6
KPS(3CEC)-MMT	3.5	12.0	12.5
KPS(4CEC)-MMT	4.0	18.9	13.6
KPS(5CEC)-MMT	4.2	23.0	14.0

was absorbed to the surface of MMT to form as a crystalline phase when the content was increased.

When 5 phc KPS-MMT powder was added to the acidic solution of chitosan, the chitosan/KPS-MMT solution was separated into two phases after heating at 60°C for 1 h. The upper phase was a clear solution and the bottom phase was a gel-like sediment. The clear solution contained the exfoliated and partially exfoliated MMT, the domains of which have been investigated by dynamic light scattering and will be discussed later. The gel-like portion contained the un-exfoliated MMT. After washing off the remaining chitosan with the acetic aqueous solution and drying, the unexfoliated MMTs were recovered and weighed. The remaining MMTs in the clear part of the solution were

then estimated and recorded in Table I, as noted by the direct (subtraction) method. The results indicated that the more KPS incorporating in the KPS-MMT, the more exfoliated MMT was gained in the clear part of the solutions. It is noteworthy that if MMT was not treated with KPS, it would not suspend in the acidic solution of chitosan and all condensed as a precipitate.

Figure 2 shows the distributions of domain size measured from dynamic light scattering of the acidic solutions of chitosan, KPS-degraded chitosan, and chitosan/KPS-MMT nanocomposites with MMT incorporating various CEC of KPS. The domain size distribution of pure chitosan is broad, ranging from 50 to 900 nm. It shifted to 50 ~ 350 nm range when the chitosan was degraded by KPS. When the acidic solution of chitosan was treated with KPS(0.5CEC)-MMT, the clear phase had two narrow distribution peaks of domain size: one is located in 200 ~ 500nm range and the other is approximately at 100 nm. As the amount of KPS to incorporate with MMT was increased to 2 CEC, the left peak shifted to 30 nm, which is similar to the size of fully-exfoliated MMT observed by TEM (see Fig. 3a). Although the right peak did not apparently change its position, its area became relatively smaller. It is worth noting here that when the acidic solution of chitosan was treated with KPS-MMT, the clear phase contained chitosan, degraded chitosan, and exfoliated and partially exfoliated MMT. Because the domain sizes of the two peaks are similar to the sizes of the exfoliated and partially exfoliated MMT observed by
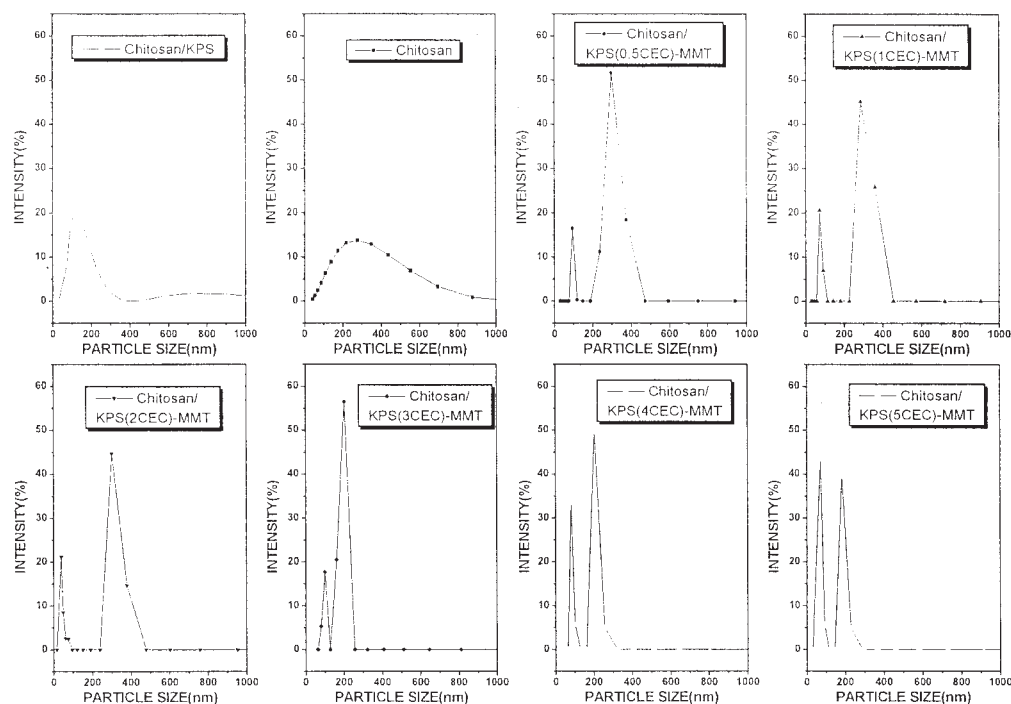

Figure 2 Domain size distributions measured from dynamic light scattering of the acidic solutions of chitosan, KPS-degraded chitosan, and chitosan/KPS-MMT nanocomposites with MMT incorporating various CEC of KPS.
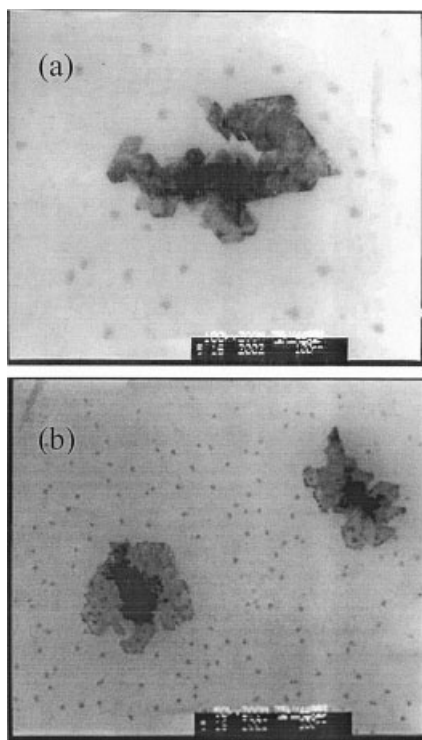
Figure 3 TEM micrographs of (a) chitosan/KPS(2CEC)-MMT ($\times$ 100,000) and (b) chitosan/KPS(4CEC)-MMT ($\times$ 50,000).

TEM, the two peaks were believed to be contributed by their individual aggregated domains with the degraded chitosans. When the amount of KPS reached 3 CEC, the right peak rapidly shifted to 200nm whereas the left peak shifted to the right. At this stage, a large amount of chitosan was degraded concurrent with the further exfoliation of MMT. When the amount of KPS reached 5 CEC, the right peak stayed at $\sim$ 200 nm with the intensity reduced whereas the left peak shifted back to 60 nm from 100 nm.

Figure 3 shows the TEM micrographs of chitosan/KPS(2CEC)-MMT and chitosan/KPS(4CEC)-MMT. The partially exfoliated MMT exhibited a structure composed of stacked layers, where each layer had a regular shape and translucent appearance, whereas the exfoliated MMT exhibited an undefined shape with a size range of 15 $\sim$ 25 nm. Besides, most of the exfoliated and partially exfoliated MMTs were observed with the layers flattening out in parallel to the surface. It can also be concluded that when the exfoliation of MMT was more complete, the exfoliated particles were smaller in size and more in quantity.

Figure 4 shows the ESCA survey-scan spectra from the surfaces of cast films of chitosan, KPS-degraded chitosan, and various chitosan/KPS-MMT nanocomposites. Only C, O, N, Si, and Al atoms were detected, and their relative molar fractions in the surfaces could be measured from the spectra. Since carbon atoms

were only from the chitosan and silicon from MMT, we could calculate the weight ratio of MMT in the surfaces based on their chemical structures. The results are also listed in Table I. The chemical structure of MMT was acquired as $M_{0.33}(Al_{1.67}Mg_{0.33})Si_4O_{10}(OH)_2$ by fitting the X-ray diffraction data of MMT (see Fig. 1) with the Rietveld method,[13,14] where M is the monovalent cation absorbed to the interlayer region to balance the electric charge. The density of MMT could also be estimated as 2.1 g/cm^3 from the structural unit.[15]

The measured weight ratio of MMT in the surfaces of chitosan/KPS-MMT cast films by ESCA was greater than that in the clear solution estimated by the direct subtraction method, as shown in Table I. It was partly due to the fact that the measurements by ESCA were on the two-dimensional surface. Assuming that the KPS has been completely removed and MMT was evenly dispersed in the chitosan matrix, the weight percentage $W_{m,s}\%$ of MMT in the surface of chitosan/KPS-MMT nanocomposites might be estimated by

$$W_{m,s} = \frac{(w_m/\rho_m)^{2/3}\rho_m}{(w_m/\rho_m)^{2/3}\rho_m + (w_c/\rho_c)^{2/3}\rho_c} \times 100 \quad (1)$$

where w_m and w_c are the weight fractions of MMT and chitosan in the nanocomposites; and ρ_m and ρ_c are the density of MMT and chitosan, respectively. Since w_m and w_c can be obtained from the direct subtraction method shown in Table I and ρ_c has a value of 0.942 g/cm^3 by referring to ref. 16, $W_{m,s}$ was then calculated and is listed in Table I. It can be seen that, except for
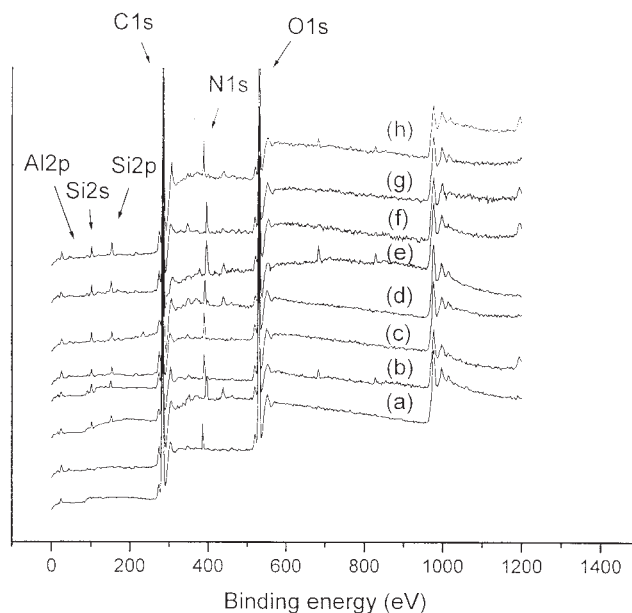

Figure 4 ESCA survey-scan spectra from the surfaces of cast films of (a) chitosan, (b) KPS-degraded chitosan, and chitosan/KPS-MMT nanocomposites with MMT incorporating (c) 0.5, (d) 1, (e) 2, (f) 3, (g) 4, and (h) 5 CEC of KPS.
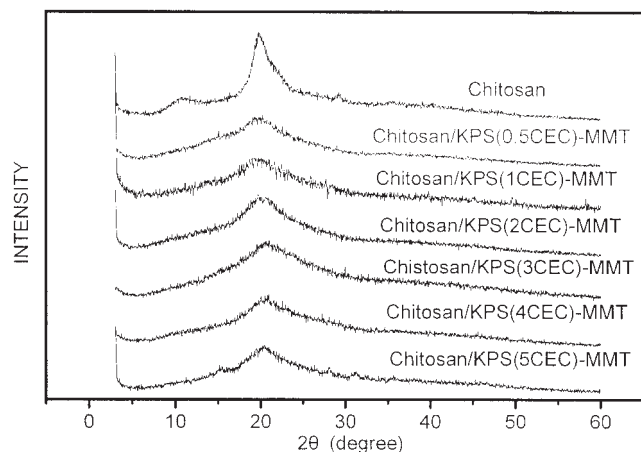
Figure 5 X-ray diffraction patterns of cast films of chitosan and their nanocomposites with various KPS-MMTs.

the chitosan/KPS(2CEC)-MMT sample, the calculated $W_{m,s}$ was roughly matched with the ESCA data until 3 CEC KPS was incorporated with the MMT. By referring to the dynamic light scattering data of the chitosan/KPS(2CEC)-MMT solution shown in Figure 2, we found that some of the MMT had been fully exfoliated but the chitosan was still not substantially degraded. Thus, the anomalous $W_{m,s}$ of the chitosan/KPS(2CEC)-MMT sample might be due to the nonuniform dispersion of MMT in the chitosan matrix. When the KPS was increased to 4 CEC, chitosan was significantly degraded along with more exfoliation of the MMT. The discrepancy between the ESCA data and the estimated value by eq. (1) might be due to the drying process. Because fully exfoliated MMT has a larger surface area per unit volume, it should have a tendency to aggregate on the surface when the surface concentration was increased during drying.

Figure 5 shows the X-ray diffraction patterns of cast films of chitosan and their nanocomposites with KPS-MMT. Chitosan is a partial crystalline polymer. When chitosan was incorporated with KPS-MMT, the diffraction peak of chitosan became broader, indicating that the incorporation of KPS-MMT had interfered with the crystalline structure of chitosan. However, when the amount of KPS incorporating in MMT was increased to 5 CEC, the diffraction peaks at $2\theta = 16$, 28, and 36° from MMT appeared. It is interesting to note that the original characteristic peak of MMT at 6 ~ 7° contributed by the c-axis of the layer structure was undetectable, suggesting that the MMT has been almost fully exfoliated. This is consistent with the results of TEM and ESCA investigations.

Properties

Figure 6 shows the tensile strength and Young's modulus of cast films of chitosan, degraded chitosan, and

chitosan/KPS-MMT nanocomposites with MMT incorporating various CEC of KPS. The KPS-degraded chitosan had rather low tensile strength and Young's modulus compared to the pristine chitosan. When 0.5 CEC KPS was used to incorporate with the MMT, the resulting chitosan/KPS(0.5CEC)-MMT nanocomposite has higher tensile strength but lower Young's modulus than the pristine chitosan. It is noteworthy that there are two contradictory factors affecting the mechanical properties of nanocomposites: one is the decreased molecular weight of chitosan and the other is the exfoliation of MMT. With increasing the amount of KPS incorporating in MMT, more MMT exfoliated along with the degradation of chitosan so that the Young's modulus increased but the tensile strength decreased. Nevertheless, both of them were still much greater than those of the KPS-degraded chitosan.

When the cast film of chitosan was subjected to the degradation test in the PBS solution at 37°C, the apparent degradation began after 5 days of immersion, as shown in Figure 7. It began to accelerate after 25 days. However, the KPS-degraded chitosan lost weight as soon as it was immersed in the PBS solution. Other than that, the degradation behavior was similar to that of the pristine chitosan. The degradation behavior of chitosan/KPS-MMT in PBS solution is quite different from that of the parent chitosan. Because the chitosan has been degraded by the KPS incorporating in MMT, the nanocomposites lose weight as soon as they are immersed in the PBS solution. The degradation rate was then significantly reduced to a constant rate of ~ 0.3 wt % per day after 5 days of immersion. The exfoliated MMT seemed to act like an anchor hindering the structure of chitosan from dissolution to the PBS solution.

CONCLUSIONS

In this study, we have successfully prepared chitosan/ MMT nanocomposites through the reactions of chi-
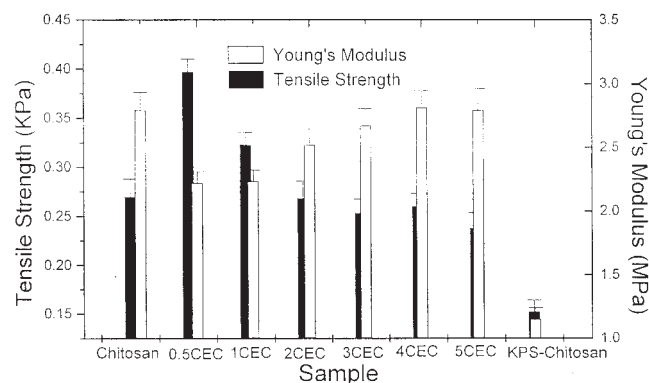

Figure 6 Tensile strength and Young's modulus of cast films of chitosan, KPS-degraded chitosan, and chitosan/ KPS-MMT nanocomposites with MMT incorporating various CEC of KPS.
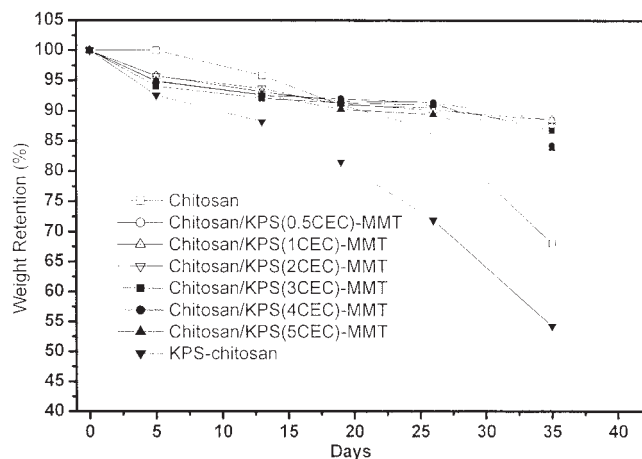
Figure 7 Weight retention of the indicated specimens subjected to the degradation test in PBS solution at 37°C as a function of the immersion days.

tosan in acidic solution with KPS being incorporated with the MMT by the intercalation process. The resulting scission of chitosan by KPS triggered the exfoliation of MMT. The prepared nanocomposite solutions could be cast into films with the exfoliated MMT flat-

tening out on the surface. The latter not only improved the tensile properties of chitosan but also hindered the degradation in the vitro test. Further exploration for possible applications of chitosan/MMT nanocomposite solutions on biomaterials is ongoing.

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