

Study on the Properties of Crosslinking of Poly(ethylene oxide) and Hydroxyapatite–Poly(ethylene oxide) Composite

R. Banat, T. Tinçer

Department of Chemistry, Middle East Technical University, 06531 Ankara, Turkey

Received 15 July 2002; accepted 28 December 2002

ABSTRACT: This study covers the crosslinking of poly(ethylene oxide) (PEO) and its composite with calcium hydroxyapatite (HA), their mechanical and swelling properties, and morphology. Sheets of the composites of PEO (two different grades with M_w : 5×10^6 and 2×10^5) and HA and neat PEO were prepared by compression molding. The prepared composite and PEO (0.1-mm-thick) sheets were crosslinked with exposure of UV-irradiation in the presence of a photoinitiator, acetophenone (AP). This simple method for crosslinking, induced by UV-irradiation in the presence of AP, yielded PEO with gel content up to 90%. Gel content, equilibrium swelling ratio, and mechanical and morphological properties of the low molecular weight polyethylene oxide (LMPEO)–HA crosslinked and uncrosslinked composites were evaluated. Although the inclusion of HA into

LMPEO inhibits the extent of crosslinking, the LMPEO–HA composite with 20% HA by weight shows the highest gel content, with appreciable equilibrium swelling and mechanical strength. The growth of HA in simulated body fluid solutions on fractured surfaces of LMPEO and also LMPEO–HA was found to be very favorable within short times. The dimensional stability of these samples was found to be satisfactory after swelling and deposition experiments. The good compatibility between the filler hydroxyapatite and poly(ethylene oxide) makes this composite a useful tissue-adhesive material. © 2003 Wiley Periodicals, Inc. *J Appl Polym Sci* 90: 488–496, 2003

Key words: poly(ethylene oxide); crosslinking; swelling; mechanical properties; morphology

INTRODUCTION

Investigations on the composites of hydroxyapatite (HA) with synthetic polymers have shown that several advantages could be obtained.^{1–8} This led to the development of bone-analogue composites, that is, HA-reinforced polymers. Reinforcing different polymers such as polyethylene,¹ poly(hydroxybutyrate),² poly(lactic acid),^{3,4} poly(glucosyloxyethyl methacrylate) and polyacrylamide gels,⁵ polyurethane sponges adsorbed by HA,⁶ and starch-based thermoplastics⁷ with HA was indeed a subject of concern, for at least 20 years, in development of bone-related biomaterials. Flexible polymeric materials capable of rapid and firm bonding with bone HA are required for orthopedic and oral surgery; moreover, the possibility of reinforcing new emerging polymers has not been fully explored.⁸

Calcium–HA [$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$] is the main inorganic component of bones and teeth of vertebrates. Hydroxyapatite-filled poly(hydroxybutyrate)³ and poly(L-lactide)⁵ were shown to be used as a biomaterial. These composites made from biodegradable polymers and HA were studied to develop biodegradable artificial bone filler (polymer resorbed and replaced by

newly formed bone tissue when incorporated with HA) and seemed to play an active role in new bone formation.^{2,8}

Poly(ethylene oxide) (PEO) is a biocompatible non-ionic water-soluble polymer. Polymer gels including PEO received growing attention for applications in various technologies including medical applications such as wound dressing, controlled release drug systems, and others.⁹ PEO could be crosslinked in a number of ways to obtain a hydrogel polymer. These crosslinking methods include using gamma ray,^{10,11} electron beam,¹² chemical crosslinking, and recently, UV-induced crosslinking of solid PEO under constant flow of argon or in dynamic vacuum, which was the subject of detailed investigation by Doytcheva et al.^{13,14}

Because PEO is a synthetic biocompatible polymer, it possesses hydrogel properties that presumably allow calcification inside the hydrogel, resulting in bone bonding of the polymer. Furthermore, regarding the relevant properties of PEO, it is a tough, high crystalline polymer with a moderate tensile modulus, and high elongation and strength. Although PEO is water soluble, its tensile properties do not exhibit a marked dependency on relative humidity, except at levels greater than 90%.

In the present study the crosslinking of PEO in normal atmospheric conditions without using vacuum or inert gas media is reported as described earlier for

Correspondence to: T. Tinçer (teotin@metu.edu.tr).

TABLE I
Ion Concentrations (mM) of SBF and Human Body Plasma

Ion	Simulated fluid	Blood plasma
Na ⁺	142.0	142.0
K ⁺	5.0	5.0
Mg ²⁺	1.5	1.5
Ca ²⁺	2.5	2.5
Cl ⁻	147.8	103.0
HCO ₃ ⁻	4.2	27.0
HPO ₄ ²⁻	1.0	1.0
SO ₄ ²⁻	0.5	0.5

polyethylene.¹⁵ The second part of this study covers the preparation of a composite of two biocompatible materials [HA and low molecular weight polyethylene oxide (LMPEO)] by compression molding. Mechanical and swelling behaviors of the crosslinked composites are also reported.

EXPERIMENTAL

Materials

Poly(ethylene oxide) with two different molecular weights [Aldrich (Milwaukee, WI) low molecular weight polyethylene oxide (LMPEO) and high molecular weight polyethylene oxide (HMPEO) with $M_v = 5 \times 10^5$ and 2×10^6 , respectively] in powder form was used. Hydroxyapatite [HA; Ca₁₀(PO₄)₆(OH)₂] was prepared by the nitrate-diammonium hydrogen phosphate process, by using Merck-grade chemicals (Darmstadt, Germany), as illustrated in the literature.¹⁶

Simulated body fluid (SBF) solution was prepared according to the method of Kukubo,¹⁷ in which the ion concentrations and the chemicals used to prepare a liter solution are as presented in Tables I and II. SBF solution was prepared by dissolving appropriate quantities of the chemicals in deionized water in the order given in Table II. Chemicals were added, one by one after each reagent was completely dissolved in 800 mL water, then the volume was adjusted to one liter. Fractured surfaces of crosslinked and composite PEO were employed as substrates for the growth of HA from SBF at 36.5°C, and also the swelling experiments of the LMPEO-HA composite sample were carried out in SBF to study the morphological changes.

Preparation of thin sheets of PEO and composites

A fine powder consisting of low and high molecular weight PEO was compression molded into a 0.1-cm-thick sheet of rectangular shape (2 × 14 cm), between two steel plates at 120°C for 15 min under pressure of 20,000 psi. The mold was immediately cooled under

normal atmospheric pressure to the ambient temperature.

A mixture of LMPEO (2×10^5) with HA (at different wt %) was thoroughly ground and homogenized in an agate mortar. This mixture of LMPEO-HA was then compression molded as described above. The attempts to prepare high molecular weight PEO-HA composites failed because of the difficulty in managing the flow properties of HMPEO in compression molding. These composites were found to possess uneven distribution of HA within the matrix, easily observed even by the naked eye. Therefore no composites were prepared from HMPEO.

Crosslinking process, gel fraction determination, and equilibrium swelling

Polymer and composite molds were put into small sealed polyethylene bags, in the presence of a few drops of photoinitiator, acetophenone (AP), at 40°C in a dark place overnight for complete swelling.¹⁵ AP-treated samples were then exposed to UV-radiation of UV lamp (Blak-Ray-Longwave UV 315–400 nm, Upland, CA) from 1 to 50 h. The irradiated samples were then left to dry at room temperature before other tests.

Soxhlet extraction apparatus was used to determine the gel content of the PEO using methylene chloride as a good solvent of PEO. Irradiated samples were extracted for at least 24 h. The gel content was calculated according to the following equation:

$$\text{Gel content (\%)} = \frac{\text{Weight of extracted sample}}{\text{Initial weight of sample}} \times 100$$

The equilibrium degree of swelling ratio (ES) was determined in deionized water at room temperature. Small rectangular pieces of PEO and PEO-HA samples were equilibrated in distilled water for 4 days. Samples were then removed from the water, confined with filter paper in 10 s, and weighed immediately. The equilibrium degree of swelling was calculated from the following equation:

TABLE II
Chemicals for Preparing 1 L SBF

Chemical	Amount (g)
NaCl	7.996
NaHCO ₃	0.350
KCl	0.224
K ₂ HPO ₄ · 3H ₂ O	0.228
MgCl ₂ · 6H ₂ O	0.305
1N HCl	40 mL
CaCl ₂	0.278
Na ₂ SO ₄	0.071
NH ₂ (CH ₂ OH) ₃	6.057

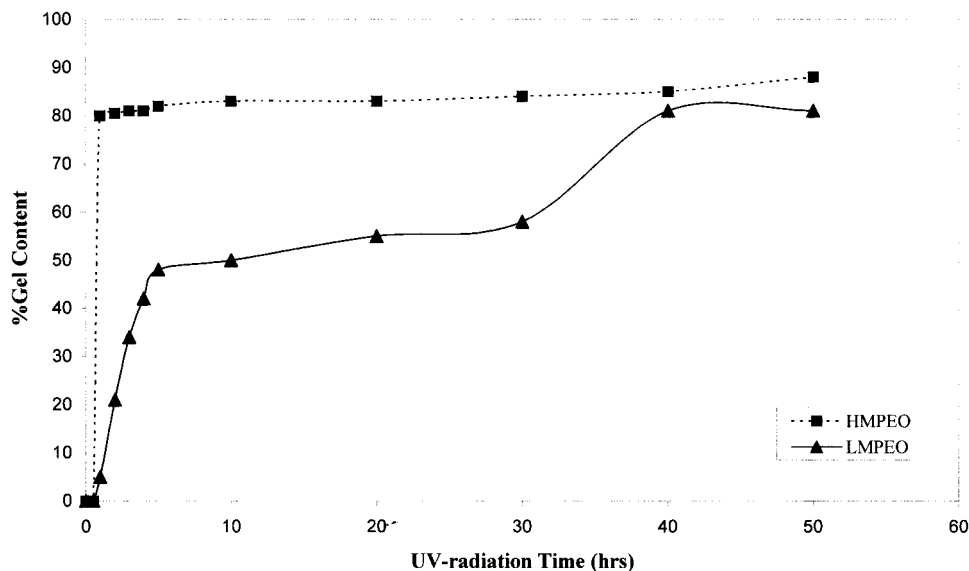


Figure 1 Variation of gel content of PEO versus UV-irradiation time.

$$ES = \frac{\text{Weight of swollen sample}}{\text{Weight of dry sample}}$$

During the gel content and ES experiments no observable loss of HA from the composite matrix was present.

Mechanical properties

The tensile properties of compression-molded 0.1-cm-thick samples of neat, crosslinked PEO and composites were investigated by a Lloyd testing machine (LS 5000, Farham, UK) on dumbbell-shape standard samples cut from the molds. The crosshead speed and gauge length measurements were 5 cm/min and 3.5 cm, respectively. Tests were performed at room temperature and the average of three testing samples was reported.

Scanning electron microscopy

The tensile fractured surfaces of PEO and PEO-filled HA composites were studied at various magnifications, after gold plating, by using a JEOL JSM-6400 scanning electron microscope (JEOL, Peabody, MA).

Energy-dispersive X-ray analysis (EDXA) was also carried out on the PEO-HA composite samples. The sharp increase in Ca and P atom intensities with a (Ca/P) ratio of 1.67 proved the presence of the HA, before and after simulated body fluid (SBF) treatment.

RESULTS AND DISCUSSION

Gel content and equilibrium degree of swelling

Variations of the gel content of PEO for two different molecular weights with respect to the time of UV-

irradiation are given in Figure 1. It is evident that the gel content of the HMPEO ($M_v = 5 \times 10^6$) reached the maximum gel content within very short times (i.e., within 1 h of UV-irradiation time), and there was almost no change in the gel content of HMPEO afterward. The gel content for the LMPEO, on the other hand, increased linearly within the initial 5 h of UV-irradiation, but showed no significant change for the next 30 h. The gel content finally reached 80 and 90% for the irradiation times of 40 and 50 h, respectively. Further UV-irradiation, more than 50 h, caused deterioration of thin PEO films, yellowing with increased brittleness, and no improvement in other studied properties.

Equilibrium degree of swelling (ES) showed a maximum for HMPEO within a short UV-irradiation time, after which the ES decreased sharply, as shown in Figure 2. This value was higher than that of the ES value of LMPEO. As the UV-irradiation continued, the decrease in the swelling degree for both PEOs approached a limiting value (i.e., <3). The decrease in ES of the LMPEO was significantly slower than the HMPEO counterpart. This agrees well with the variation of gel content. The high extent of crosslinking in the PEO matrix ($>80\%$) inevitably restricts the polymer to swell the small molecules, thus substantially decreasing the ES value.

The change of gel content and ES of the LMPEO-HA composite with respect to the weight percentage of HA for 50 h of UV-irradiation is given in Figure 3. In all cases the inclusion of HA into PEO was obviously found to reduce the gel content. The gel content of the composites was found to be comparably lower than that of pure LMPEO gel content of 50 h of UV-irradiation (e.g., the 5% HA composite was the lowest among all composites). However, it increased with the filler content, reach-

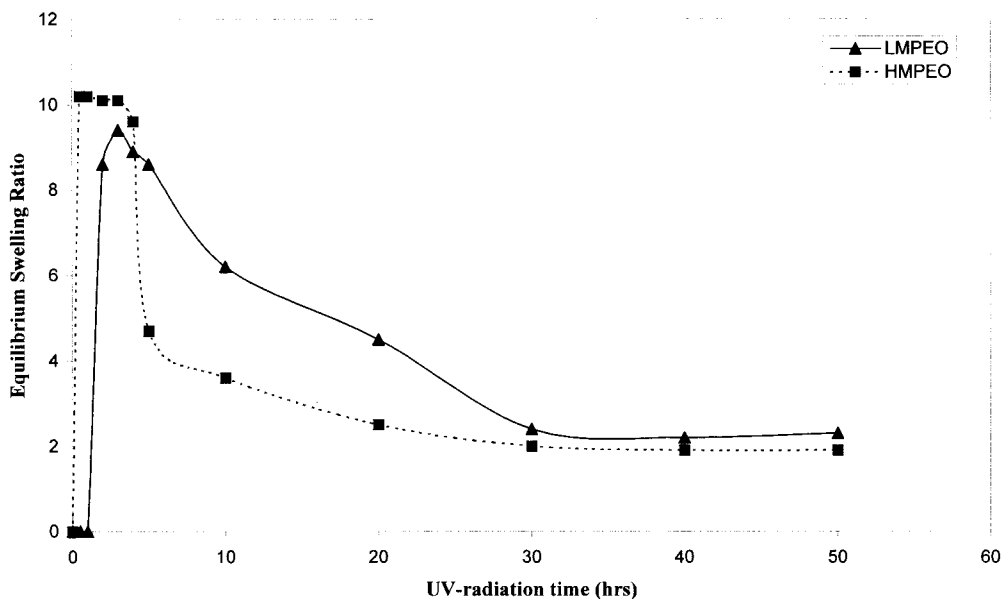


Figure 2 Change in equilibrium degree of swelling (ES) of PEO versus UV-irradiation time.

ing a maximum at 20% HA. Although the variation of ES and gel content with the filler HA showed the same trend with a notable difference in gel contents of unfilled and filled LMPEO, the ES ratios were found to be very similar. The ES values of composites varied between 2.1 and 2.3, and were not significantly different from the ES of the unfilled PEO results for 50 h of UV-irradiation. Therefore the variation in addition of HA seemed not to affect the ES. The optimum value of the equilibrium swelling appeared at 20% HA within this range. It can be concluded that the presence of HA inhibited the crosslinking process of PEO but, on the other hand, its

presence did not cause any change in ES compared with that of pure crosslinked LMPEO.

Mechanical properties

In Figures 4–6, the variation of tensile strength, strain at break, and stress at yield relative to UV-irradiation time, respectively, are given. The tensile strength of HMPEO was found to be higher than that of LMPEO (Fig. 4), attributed to the longer polymer chains, which produce a higher possibility of the physical entanglements in addition to the UV-induced crosslinking.

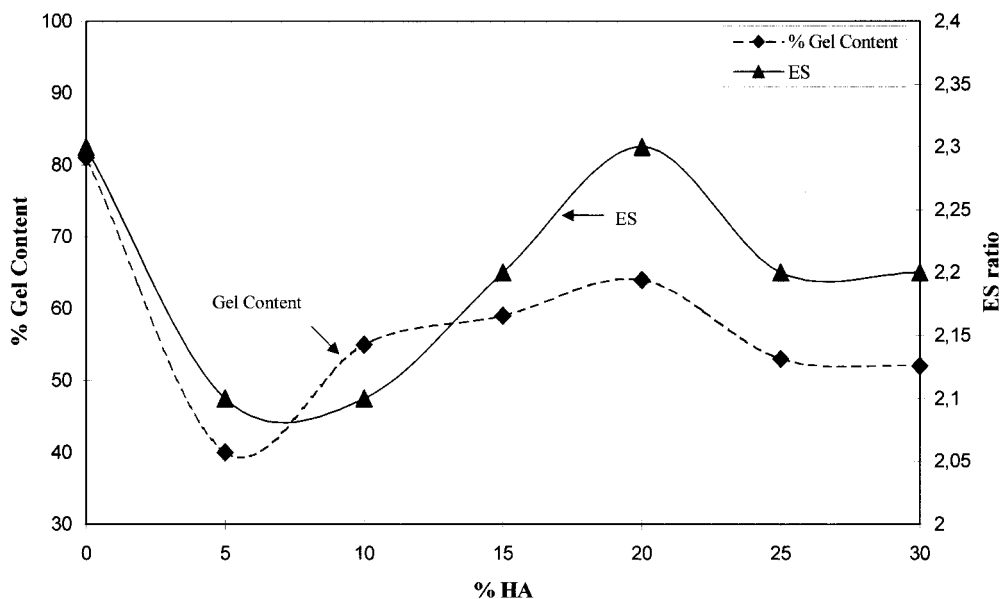


Figure 3 Variation of gel content and equilibrium degree of swelling for 50-h UV-irradiated PEO-HA with respect to wt % of HA.

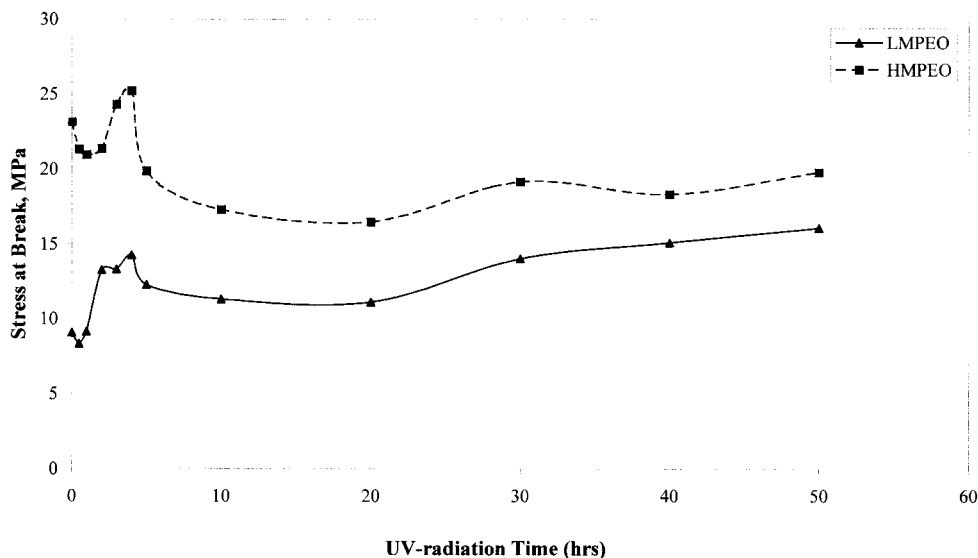


Figure 4 Variation of stress at break of both PEOs with respect to UV-irradiation time.

HMPEO is obviously stronger than LMPEO. During the crosslinking process, the ultimate tensile strength of LMPEO more nearly approximated that of HMPEO but never reached the same strength. At the early stages of UV-irradiation, the initial behavior in ultimate tensile strengths of both PEOs, characterized by a minimum and maximum followed by a decrease (higher in HMPEO), can be attributed to the chain scission, reorientation of chains, crosslinking, and finally reaching an equilibrium. The increasing trend in tensile strength of LMPEO with UV-irradiation time revealed that, although the chain scission become competitive with the crosslinking process, it did not predominate over crosslinking. Furthermore, for HMPEO, it can be said that, although the material was

almost 90% crosslinked, the stress-transferring chains were mostly destroyed after 5 h of UV-irradiation, given that the ultimate stress never reached 20 MPa or higher.

The percentage strain at break for both PEOs decreased less than 30% after 10 h of UV-irradiation time (Fig. 5). The decrease in the percentage strain at break for HMPEO was sharp, from 500% for nonirradiated to lower than 30% upon crosslinking. However, the initially low percentage strain at break of LMPEO was observed to increase 200% after 4 h of UV-irradiation. The initial increase in the ultimate elongation in LMPEO can be attributed to the low extent of initial increase in crosslinking, which inhibits short-chain slippage at early extension, where the number of en-

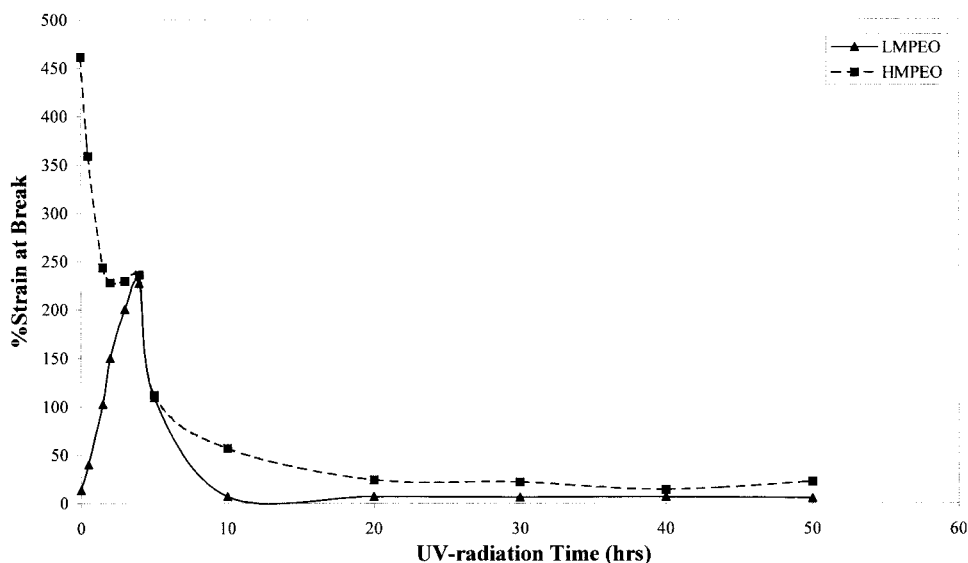


Figure 5 Variation of percentage strain at break of both PEOs versus UV-irradiation time.

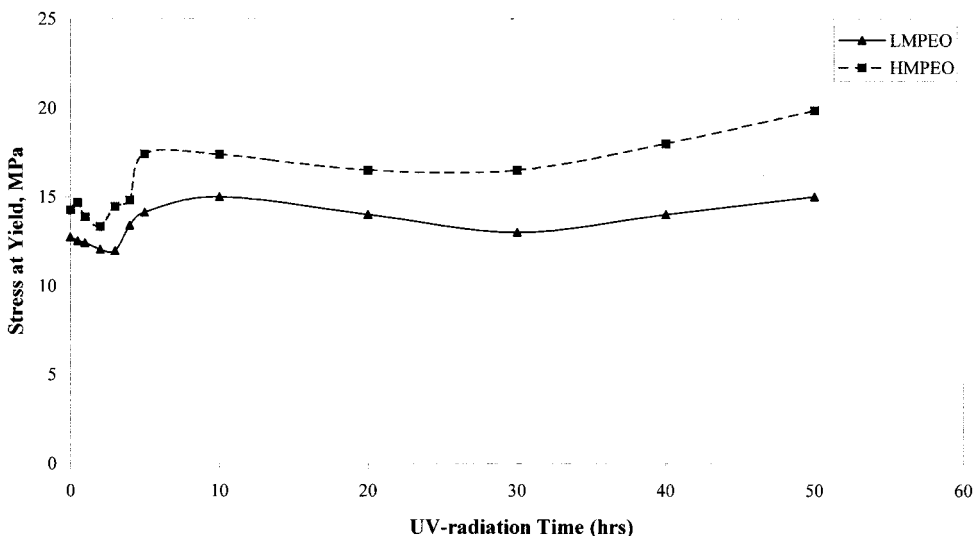


Figure 6 Variation of stress at yield of both PEOs versus UV-irradiation time.

tanglements is lower in this polymer. Finally, strain at break reduced to values around 10% after 10 h of UV-radiation. The sudden decrease in percentage strain at break for both PEOs, especially for the HMPEO, is attributed to the loss of physical entanglements and also to the formation of high gel in the polymer, which gives no chance for chains to elongate.

The yield point was observed in all cases of crosslinked PEO. The yield stress first decreased, however, then recovered and remained almost constant for the rest of UV-irradiation time. The increase in stress at yield was from 15 to 20 MPa for HMPEO and from 12 to 15 MPa for LMPEO with UV-irradiation time (Fig. 6). The yield stress of HMPEO was apparently higher than that of LMPEO, indicating higher modulus compared to that of LMPEO. The Young's modulus

of LMPEO and HMPEO varied between 300 and 500 MPa, in which HMPEO showed a slightly higher value and upon the UV-irradiation crosslinking process was found not to be very influential on the modulus. The observation of yield point, even in extensive crosslinking, indicates that the material still possesses ductile properties rather than brittle behavior.

Variations of the ultimate tensile strength of the LMPEO-HA composite and UV-irradiated composites with respect to filler HA are shown in Figure 7. LMPEO-HA composites were also UV-irradiated for 50 h under the same conditions in the presence of AP. The idea behind this extensive UV-irradiation was to produce a material that would not deteriorate in SBF and also keep HA self-contained when immersed into SBF. The ES experiments revealed that ES ratios of

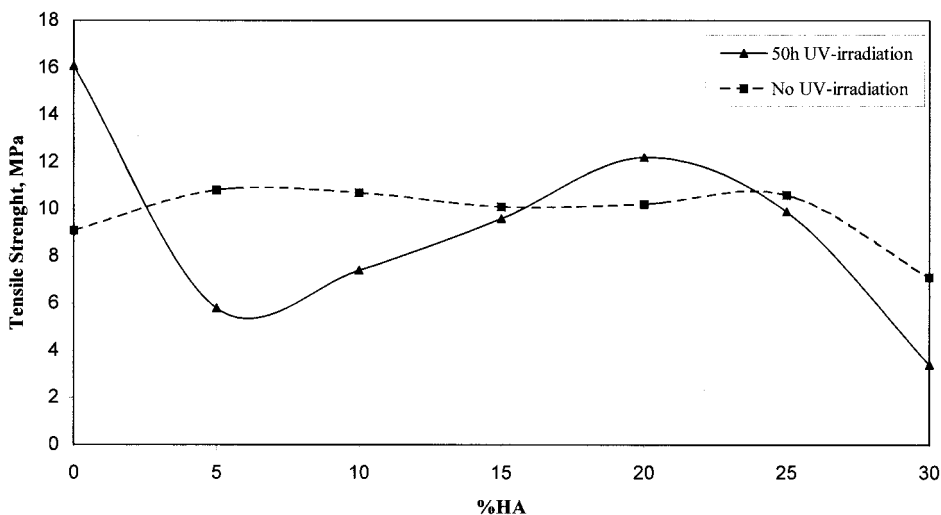


Figure 7 Variation of tensile strength of UV-irradiated (50-h UV-irradiation) and nonirradiated HA-LMPEO composites versus wt % of HA.

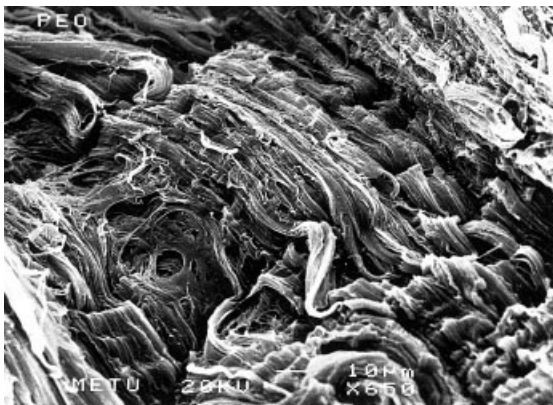


Figure 8 Tensile fractured surface of pure PEO.

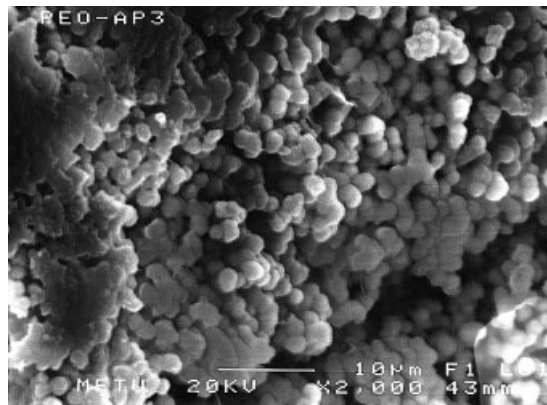


Figure 9 Deposition of HA on crosslinked LMPEO surface (50 h UV-irradiated) after 15-day treatment in simulated body fluid (SBF) solution.

crosslinked LMPEO–HA composites were not very different compared with those of crosslinked LMPEO, as shown in Figure 3.

The tensile strength of the nonirradiated composite samples showed only very slight changes with increasing the filler (HA) content up to 25% HA (Fig. 7). The decrease in tensile strength at 30% HA was the result of increased discontinuity of the LMPEO matrix. These composites failed without any yield point as a consequence of loss matrix continuity by the presence of HA. Although the ultimate strength of these composites is apparently significantly different from that of any composites prepared from polyethylene¹ and poly(hydroxybutyrate),² there was no big change, loss or gain, in the tensile strength with HA content within the range studied. This indicates that LMPEO–HA composites are significantly good mixtures and have appreciably good adherence between LMPEO and HA but no reinforcing effect.

The tensile strength of crosslinked LMPEO–HA composites showed a similar variation with gel content and ES (as given in Fig. 3), reaching a maximum value (12 MPa) at 20% HA. The tensile strength of the 20% HA composite irradiated for 50 h was the highest obtained value, which was still a little lower than that of pure LMPEO crosslinked (15 MPa) for 50 h. Although the addition of HA slightly improved the ultimate tensile strength of PEO (from 9 to 11 MPa), the UV-irradiation caused a drastic drop in the tensile strength, especially in low filler-containing composites. This decrease had almost the same trend with inhibition of gel formation in LMPEO–HA composites. The highest gel content coincides with the maximum tensile strength of UV-irradiated LMPEO–HA composites. The corresponding strain at break values were found to decrease with the addition of HA for crosslinked and noncrosslinked composites, but varied insignificantly between 5 and 3%, and also found to be almost ineffective with the UV-irradiation.

Scanning electron microscopy

The tensile fractured surface of pure LMPEO is shown in Figure 8 with apparent fibrillar morphology of LMPEO upon uniaxial extension. This ductile fracture with some fibrillation was not present when PEO was crosslinked. The fractured surface of crosslinked PEOs showed mostly a brittle appearance with some extent of ductility, but no fibrillar extensions.

The deposition of HA in SBF solution on the surface of crosslinked LMPEO, shown in Figure 9, was proved by using EDX analysis, which gave a Ca/P ratio of 1.67. The formation of nodular HA after 15 days of SBF treatment was observed on the surface of HA–PEO composite. SEM micrographs revealed that PEO matrix containing HA enabled the deposition of HA in an acceptably short time.

The fractured surface of 10% HA-filled crosslinked LMPEO revealed a homogeneous distribution of HA fine particles within the PEO matrix (Fig. 10). There was no fibrillar morphology of PEO, but brittle morphology, when HA was involved in the LMPEO matrix. In addition to these there were no void or free spaces attributed

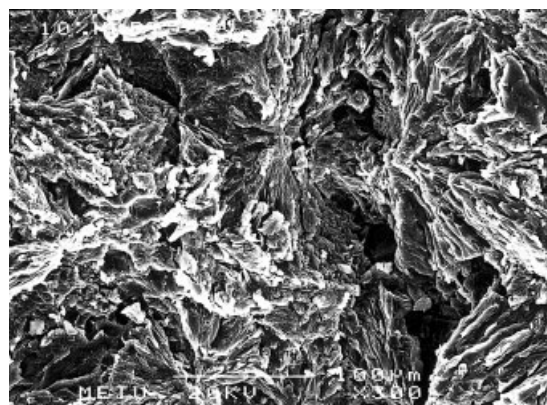


Figure 10 Tensile fractured surface of crosslinked composite of 10% HA–LMPEO.

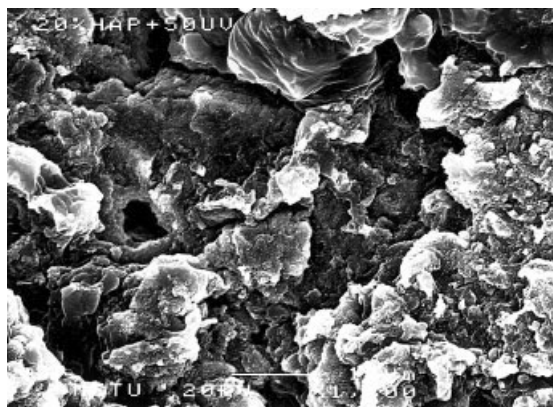


Figure 11 Tensile fractured surface of crosslinked composite of 20% HA-LMPEO.

to the possible weak interaction between HA and PEO, as observed in the HA-PEO matrix.¹ The tensile fractured surface of 20% HA-filled LMPEO also showed a similar view of the distribution of HA within the LMPEO matrix with higher magnification (Fig. 11). There was an increase in the number of spaces occupied by the filler (HA) that had an average particle size less than 5 μm , clearly revealing that the phase continuity was still present. The grinding and mixing process followed by melt compression molding appeared to be successful because no voids or individual particles of HA separated from the polymer matrix were observed at these moderate magnifications.

The tensile fractured samples of 10 and 20% HA crosslinked LMPEO composites after immersing into refreshed SBF for 10 days followed by drying in vacuum at 40°C (well below the T_m of PEO) for 15 h are given in Figures 12 and 13, respectively. The change in crystalline spherulite morphology of PEO was observed in 10% HA-LMPEO composite after swelling in the physiological solution (Fig. 12 compared with Fig. 10). The fractured surface of the SBF-treated sample of 20% HA-filled crosslinked LMPEO (Fig. 13)

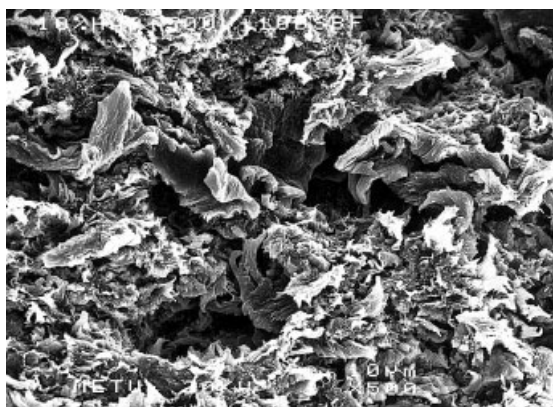


Figure 12 Tensile fractured surface of crosslinked composite of 10% HA-LMPEO after 10-day treatment in SBF solution.

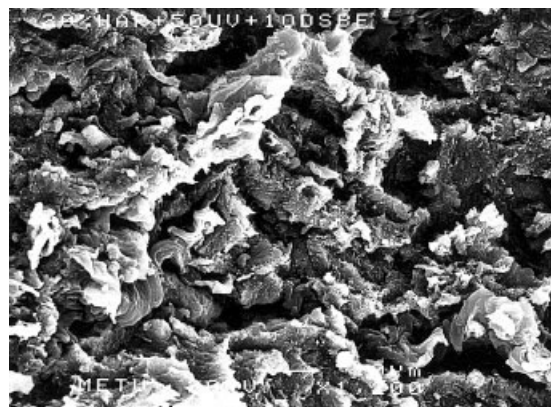


Figure 13 Tensile fractured surface of crosslinked composite of 20% HA-LMPEO after 10-day treatment in SBF solution.

showed almost the same morphology after being immersed into SBF. In both cases, LMPEO was found to be usually contracted and curled after this 10-day treatment. Although a certain extent of deposition of HA on the substrate PEO surface in SBF solution was present there was no clear evidence of a deposition of HA inside the composite matrix.

CONCLUSIONS

A simple and fast method of crosslinking poly(ethylene oxide) was achieved in atmospheric conditions. There is a significant difference in the extent of crosslinking between LMPEO and HMPEO. HMPEO can be crosslinked within a short UV-irradiation time (1 h). However, the crosslinking increases slowly with UV-irradiation time in the case of LMPEO, reaching a maximum value at 50 h.

The addition of HA is apparently inhibits and reduces the crosslinking compared to that of unfilled LMPEO. The highest crosslinking is observed in 20% HA-LMPEO composite at 50 h of UV but the extent of crosslinking is still far below that of LMPEO. Nevertheless, 20% HA-LMPEO composite shows the best mechanical strength with an acceptably good equilibrium swelling ratio. SEM studies reveal that the deposition and growth of HA in SBF on crosslinked PEO and HA-PEO composites seem to be very favorable in short times, showing promise for future applications. Furthermore, the dimensional stability of these samples were found to be good enough after swelling and deposition experiments. The good compatibility between the filler hydroxyapatite and poly(ethylene oxide) makes this composite a useful tissue-adhesive material.

References

1. Wang, M.; Bonfield, W. *Biomaterials* 2001, 22, 1311.
2. Doyle, C.; Tanner, E. T.; Bonfield, W. *Biomaterials* 1991, 12, 841.

3. Higashi, S.; Yamamuro, T.; Nakamura, T. *Biomaterials* 1986, 7, 183.
4. Shikinami, Y.; Hata, K.; Okuno, M. In: *Bioceramics*; Kokubo, T.; Nakamura, T.; Miyaji, F., Eds.; Pergamon: London, UK, 1996; Vol. 9, p. 391.
5. Taguchi, T.; Kishida, A.; Yanagi, M.; Shimotakahara, T.; Aikou, T.; Akashi, M. In: *Bioceramics*; Kokubo, T.; Nakamura, T.; Miyaji, F., Eds.; Pergamon: London, UK, 1996; Vol. 9, p. 375.
6. Yanagi, M.; Kishida, A.; Okadome, M.; Taguchi, T.; Shimotakahara, T.; Aikou, T.; Akashi, M. In: *Bioceramics*; Kokubo, T.; Nakamura, T.; Miyaji, F., Eds.; Pergamon: London, UK, 1996; Vol. 9, p. 415.
7. Reis, L. R.; Cunha, M. A.; Lacerda, S. R.; Fernandes, M. H.; Correia, R.N. In: *Bioceramics*; Kokubo, T.; Nakamura, T.; Miyaji, F., Eds.; Pergamon: London, UK, 1996; Vol. 9, p. 435.
8. Kokubo, T.; Nakamura, T.; Miyaji, F., Eds.; *Bioceramics*, Vol. 9; Pergamon: London, UK, 1996.
9. Bouillot, P.; Vincent, B. *Colloid Polym Sci* 2000, 287, 74.
10. Tranquilan-Aranilla, C.; Yoshii, F.; Dela, A. M.; Makuuchi, K. *Radiat Phys Chem* 1999, 55, 127.
11. Zhang, L.; Zhang, W.; Zhang, Z.; Yu, L.; Zhang, H.; Qi, Y.; Chen, D. *Radiat Phys Chem* 1992, 40, 501.
12. Yoshii, F.; Zhanshan, Y.; Isobe, K.; Shinozaki, K.; Makuuchi, K. *Radiat Phys Chem* 1999, 50, 133.
13. Doytcheva, M.; Doytcheva, D.; Stamenova, R.; Orahovats, A.; Tsvetanov, Ch. B.; Leder, J. *J Appl Polym Sci* 1997, 64, 2299.
14. Doytcheva, M.; Stamenova, R.; Zvetkov, V.; Tsvetanov, Ch. B. *Polymer* 1998, 29, 6715.
15. Batmaz, N.; Tinçer, T.; Akay, G. *Radiat Phys Chem* 1990, 36, 345.
16. Tas, A. C. *Biomaterials* 2001, 21, 1429.
17. Kokubo, T. *J Non-Cryst Solids* 1990, 120, 138.