Effect of Gamma Irradiation on the Interpenetrating Networks of Gelatin and Polyacrylonitrile: Aspect of Crosslinking Using Microhardness and Crosslink Density Measurements

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ABSTRACT: The present article reports the effect of gamma irradiation on the hardness behavior of the interpenetrating polymer networks (IPNs) of gelatin and polyacrylonitrile (PAN). Various compositions of gluteraldehyde-crosslinked gelatin and *N*, *N*′-methylene bis acrylamide (MBA)-crosslinked PAN were prepared and investigated for microhardness studies. The pre- and post-irradiated IPNs were characterized for their crosslinking density, determined with swelling ratio measurements. It was found that the crosslinked IPNs get further hardened because of radiational hardening at specific doses in the range from 2 to 250 kGy. The role of acrylonitrile and crosslinker (MBA) in the IPNs, as a consequence of irradiation, has also been explained. A fair consistency has been observed between the microhardness results and crosslinking density measurements. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 101: 2581–2586, 2006

Key words: gelatin; polyacrylonitrile (PAN); crosslinking; irradiation and microhardness

INTRODUCTION

Radiation processing has been demonstrated on a commercial scale to be a very effective means of improving end use properties of various polymers. Important properties of polymer materials such as mechanical, thermal stability, chemical resistance, melt flow processability, and surface properties can be significantly improved by radiation processing.[1] In the biomedical applications, polymers, especially those that are based on hydrogel materials, are in the front line area because of their unusual biocompatibility. Hydrogels are polymer materials that exhibit the ability to swell in water, i.e., they retain a significant fraction of water within themselves without undergoing dissolution.[2] The amount of water imbibed depends on the degree of crosslinking of polymer chains. The higher the degree of crosslinking, the smaller is the amount of water absorption. The hydrogel network can also be formed by radiation crosslinking. Hydrogels show excellent biocompatibility and can be used for many medical applications such as wound dressing, controlled release of drugs, enzyme supports, etc.[3,4]

An effective route to design mechanically strong polymeric matrices has been through the preparation of interpenetrating polymer networks (IPNs), which are defined as a physical mixture of at least two polymers that have been synthesized or crosslinked in the presence of the other with no significant degree of covalent bonds between them.[5] The literature richly documents various polymer blends or IPNs, which have been undertaken for study of mechanical properties such as hardness.

Although a large variety of materials have been prepared,[6–8] however, IPNs made of natural and synthetic polymers are relatively less reported.[9] These materials, which combine the mechanical properties of the synthetic component with biological properties of the natural one, could result in a novel class of hard biomaterials finding wide spectrum of possible applications in hard tissue replacements.[10]

The selection of gelatin as one of the components of the IPN lies in its non-toxicity, non-irritantness, biodegradability, and biocompatibility, which make it a material of first choice for many biomedical applications.[11] The mechanical stiffness and strength depends on the formation of covalent crosslink between the molecules in polymers.[12] Moreover, its hydrophilicity in crosslinked state imparts biocompatibility to it. The

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other component of the proposed IPN is polyacrylonitrile (PAN), which is semicrystalline vinylic homopolymer. PAN is one of the versatile polymers that is widely used for making membranes that offer good resistant to a wide range of solvents.[13] PAN shows good mechanical strength as film and is thermally stable. The microhardness is found to be a non-destructive means of characterizing the mechanical performance of polymers.[14] This technique has also been utilized to study the radiational crosslinking in the polymeric materials. The crosslinking density can be calculated by measuring the swelling ratio of IPNs.[15]

Thus, realizing the significance of IPNs in biomaterial science, the present article aims at studying the effect of gamma irradiation on the water sorption capacity and microhardness of the IPNs of gelatin and polyacrylonitrile. This would also enable to detect the extent of radiation crosslinking in the IPNs. Moreover, the study would correlate the crosslinking behavior of the IPNs to their microhardness and water sorption capacity.

EXPERIMENTAL

Materials

Gelatin (Type A, isoelectric point 7.6), used as a preformed biopolymer, was obtained from Qualigens, Mumbai, India and was used as received. Acrylonitrile was supplied by Research Lab, Mumbai, India and freed from the inhibitor by successive washing the monomer thrice with 10% NaOH, 10% H_2SO_4, and distilled water, and finally distilling the washed monomer under vacuum. Glutaraldehyde and *N*, *N*′-methylene bisacrylamide were obtained from Loba Chemie, Mumbai, India and used as crosslinkers for gelatin and polyacrylonitrile (PAN), respectively. Potassium persulphate (KPS) and potassium metabisulphite (KMBS), employed as polymerization initiator, and activator, respectively, were obtained from Loba Chemie, India and were used as received. Triple distilled water was used throughout the investigation.

Preparation of IPN

IPNs were prepared by redox polymerization method as reported elsewhere.[16] In a typical experiment, into a 10 mL of gelatin solution (40% (w/v)), prepared in distilled water, were added 1.64 g of acrylonitrile (AN), 0.02 g of MBA, 0.1 g of gluteraldehyde and 1 mL each of potassium metabisulphite (0.09 g) and potassium persulphate (0.01 g). The reaction mixture taken in a rectangular glass pellet ($0.06 \times 0.035 \times 0.005$ m^3) was kept at room temperature (30°C) for 48 h so that the whole mass could be converted into a semi-solid IPN with smooth surface. The prepared IPN was subjected to purification as described shortly.

Purification of IPN

The prepared IPNs were purified by equilibrating them in bidistilled water for 1 day till the swelling medium did not show any test of leached out chemicals such as gelatin, acrylonitrile, and crosslinking agent. To test their presence in the outer solutions, aliquots were withdrawn and analyzed for gelatin by biurette test, and acrylonitrile and crosslinker by polymerization test, respectively. After equilibration of the IPNs for 1 day, no more chemicals were found to be present in the outer solution, which obviously confirms their complete expulsion from the IPNs. These purified IPNs were cut into equal sized square pieces and were stored in air-tight polyethylene bags.

Drying of IPNs

The purified IPN samples were dried by placing the samples at 50°C for 1 week till a constant weight of the dried IPN was recorded.

It is worth mentioning here that in forthcoming sections of the paper, the compositions of IPNs will be expressed as weight fractions (g/g) of total weight of specimen.

Preparation of pure gelatin film

For preparing the film of pure gelatin, its solution (40% (w/v)) was poured into a rectangular glass mold ($0.06 \times 0.035 \times 0.005$ m^3) that was kept in the oven at room temperature for 1 week. The dried gelatin films/pellets were pealed out and stored in air-tight polyethylene bags.

Preparation of polyacrylonitrile film

To prepare pure PAN film, the polymer was dissolved in DMF solution (50% (w/v)) and the solution was poured into glass pellet. Upon drying the solution at room temperature for 1 week, a thin film of PAN was obtained. The prepared films were stored in polyethylene bags.

Gamma irradiation

The gamma irradiation of the square shaped (0.01×0.01 m^2), 0.001 m thick, specimens were carried out with Co^{60} Gamma Chamber-900 (irradiation source). Samples were irradiated with various doses ranging from 2 to 250 kGy (2, 4, 6, 8, 10, 50, 100, 150, 200, 250 kGy). The average irradiation dose rate was 3.5 kGy/h.

TABLE I
Network Parameters of the IPNs of Different Compositions

Dose (kGy)	AN Concentration						MBA Concentration					
	0.162 g/g			0.279 g/g			0.0017 g/g			0.0034 g/g		
	SR	M_c	$q \times 10^{-4}$	SR	M_c	$q \times 10^{-4}$	SR	M_c	$q \times 10^{-4}$	SR	M_c	$q \times 10^{-4}$
0	8.8	11056	51.1	7.0	4323	124.9	10.0	7819	69.1	7	4323	124.9
2	11.6	38803	13.9	17.4	50795	10.6	11.3	16468	32.8	17.4	50795	10.6
4	10.3	24687	21.9	9.6	10833	49.8	12.2	19676	27.4	9.6	10833	49.8
6	10.8	14380	37.6	15.2	36267	14.9	6.2	3625	148.9	15.2	36267	14.9
8	15.7	39768	13.6	13.7	27950	19.3	5.3	2403	224.7	13.7	27950	19.3
10	13	23873	22.9	13.7	26820	20.1	6.4	3863	139.0	13.7	26820	20.1
50	12.4	21190	25.4	14.4	30410	17.8	6.4	3863	139.0	14.4	30410	17.8
100	8.8	8601	62.7	8.8	8366	64.5	6.2	3646	148.1	8.7	8366	64.5
150	5.7	3009.6	179.4	6.5	3944	136.9	4.0	1258	429.0	6.5	3944	136.9
200	5.5	2739	197.7	6.4	3863	139.0	3.9	1221	442.0	6.4	3863	139.0
250	4.6	1695	318.5	3.6	956	564.5	4.2	1357	397.6	3.6	956	564.5

Microhardness measurements

The irradiated specimens were indented at room temperature by a mhp-160 microhardness tester with a Vicker's diamond pyramidal indenter having a square base and 136° pyramidal angle, attached to a Carl Zeiss NU2 universal research microscope. The Vickers hardness number, H_v, was calculated from the relation

$$H_v = \frac{1.854 \times L}{d^2} \text{ kg/mm}^2 \quad (1)$$

where L is the load (kg) and d is the diagonal of indentation in mm. For each test, the duration was 30 s. For each load, at least five indentations were made at different points of the specimen, and the average H_v was computed. During the test, the specimens were kept strictly horizontal and rigid.

Water sorption measurements

To evaluate water uptake potential of IPNs, a gravimetric procedure was adopted as described elsewhere.[17] In brief, pre-weighed and irradiated pieces of IPNs were placed in a water reservoir and allowed to swell up to equilibrium swelling. The swollen pieces were taken out and gently pressed in between two filter papers to remove excess water and finally weighed on a sensitive electronic balance (APX-203; Denver Instruments GmbH, Germany). The swelling ratio was calculated by the following equation

$$\text{Swelling Ratio} = \frac{\text{Weight of Swollen IPNs}}{\text{Weight of Dry IPNs}} \quad (2)$$

Network parameters

One of the most important structural parameters characterizing a crosslinked network is the average molecular mass between crosslinks (M_c) and crosslink density (q). The magnitude of M_c and q significantly affects the physical and mechanical properties of the end polymer. Equilibrium swelling is widely used to determine M_c and q. The values of these structural parameters may be calculated by the following eqs. as given by Flory and Rehner,[18]

$$M_c = \frac{-V_1 d_p (V_s^{1/3} - V_{s/2})}{\ln(1-V_s) + V_s + \chi V_s^2} \quad (3)$$

$$q = M_o / M_c \quad (4)$$

where V is the molar volume of water (18 mL mol^{-1}), d_p is the IPN density (0.44 g cm^{-3}); V_s is the volume fraction of polymer in the IPN approximately equal to 1/(1 + Swelling Ratio)[15]; χ is the Flory–Huggins interaction parameter between PAN and water (0.50)[19]; and M_o is the molar mass of repeat unit of polyacrylonitrile. The values of M_c and q have been calculated for different IPN compositions and were summarized in Table I.

RESULTS AND DISCUSSION

Radiation effect on pure PAN and gelatin

Figure 1 shows the effect of various doses of gamma irradiation ranging from 0 to 250 kGy respectively, on the surface microhardness of pure gelatin and pure PAN at the load of 60 g.

It is clear from the figure that the micohardness increases with increase in the radiation dose up to 250 kGy for PAN. The increase in microhardness for irradiated samples when compared with non-irradiated (0 kGy) confirms crosslinking in the PAN. The radiational crosslinking begins at 2 kGy, and gets stabilized with further increase in the dose up to 50 kGy. The increase in the dose beyond 50 kGy further increases

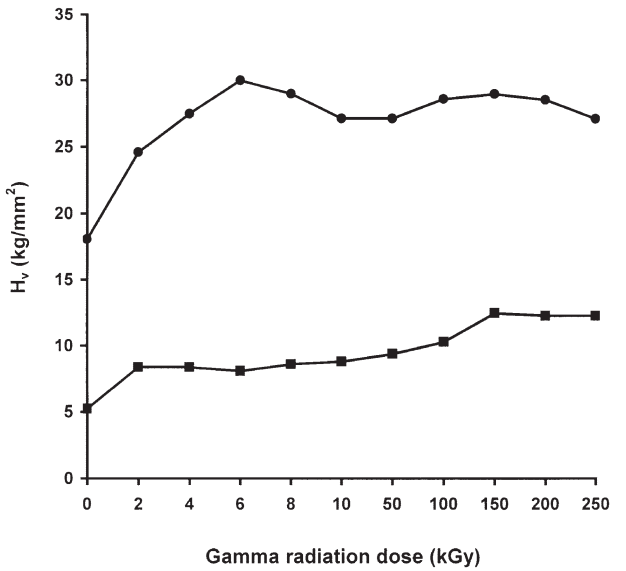

Figure 1 Variation of H_v with various doses of gamma radiation at the load of 60 g for pure gelatin and pure polyacrylonitrile ([●, PGEL; ■, PAN).

crosslinking between the PAN chains up to the dose of 150 kGy. Beyond this the crosslinking effect gets stabilized up to the dose of 250 kGy. Thus, there is a clear indication of the predominance of crosslinking in pure PAN as a result of gamma irradiation.

In pure gelatin, the microhardness increases with increasing dose up to 6 kGy and thereafter it slightly decreases upto 50 kGy and tends to attain saturation in the dose range of 50–250 kGy. However, the decrease in the value of H_v up to the dose of 50 kGy is still higher than the corresponding H_v value for nonirradiated specimen (0 kGy). Thus, in pure gelatin specimens, the overall effect of gamma irradiation is to induce crosslinking in the gelatin. However, the degree of crosslinking varies with the dose.

Figure 1 also indicates that the microhardness of pure gelatin is quite greater than that of pure polyacrylonitrile, both in unirradiated and irradiated states. The reason for the observed higher level of microhardness of gelatin may be attributed to the greater extent of intermolecular interaction between the functionals of pure gelatin chains. On the other hand, in polyacrylonitrile molecules, only nitrile (—CN) groups are present along the polymer chain, which obviously produce less association between the polymer chains and thus may cause lower hardness.

Variation of acrylonitrile

Figure 2 and Table I exhibit the variation of gamma irradiation dose with microhardness (H_v) and crosslinking density calculated on the basis of swelling ratio measurement for various specimens of PAN–gelatin with increasing concentration of AN. For IPN specimen with 0.162 g/g acrylonitrile (AN), the value of H_v decreases as well as increases at different doses in the range of 2–250 kGy. The observed results clearly reveal that at lower AN content (0.162 g/g) the irradiated specimens of IPN show greater hardness with increasing radiation dose beyond 2 kGy. The obtained values of hardness (H_v) continuously increases up to 100 kGy with maximum value at 50 kGy. The observed findings may be explained by the fact that at the low level of crosslink density, the PAN forms longer chains between the crosslinks, which can easily be rearranged to develop small crystalline regions within the IPNs. These crystallites result in increasing hardness although the crosslink density is appreciably reduced.

The radiation dose–H_v profile for IPN specimens with higher acrylonitrile content (0.279 g/g) clearly indicates that in the whole range of irradiated dose (2 to 250 kGy) the overall hardness of the irradiated IPN is always smaller than that of the unirradiated one. This type of behavior is quite unusual and may be explained by the reasons given later.

Since in redox polymerization the molecular weight of resulting polymer varies linearly with the employed monomer concentration, at higher concentration of acrylonitrile the IPN obtained will contain higher molecular weight polyacrylonitrile chains. Thus, IPN shows a greater hardness, which may be due to the higher molecular weight polyacrylonitrile present in the unirradiated IPN. Upon irradiation of the specimen, greater number of acrylonitrile molecules of polyacrylonitrile chain interact with the ionizing radiation and give rise to bond scissioning and

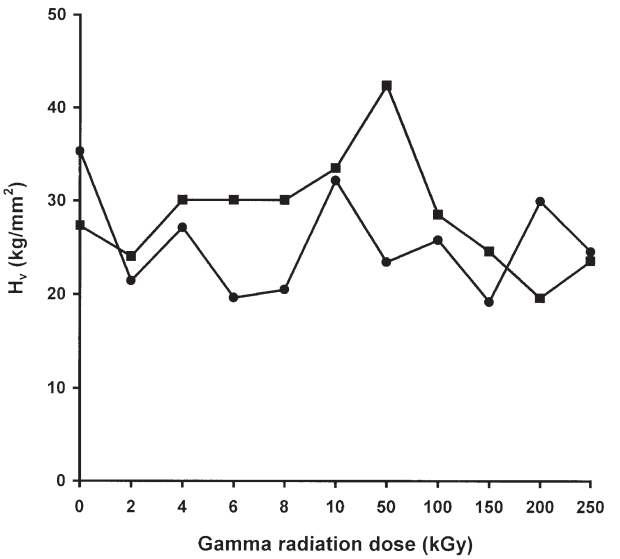

Figure 2 Variation of H_v with various doses of gamma radiation at the load of 60 g for different contents of AN in the IPN (●, 0.279 g/g; ■, 0.162 g/g).

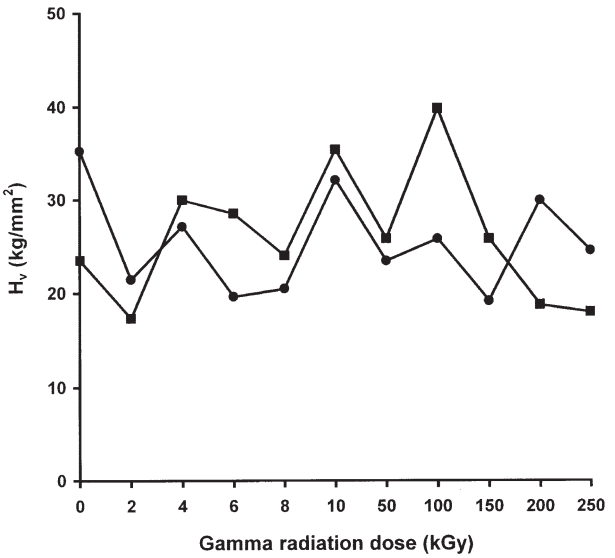

Figure 3 Variation of H_v with various doses of gamma radiation at the load of 60 g for different contents of MBA in the IPN (●, 0.0034 g/g; ■, 0.0017 g/g).

radiational crosslinking effects. The predominance of these two opposing effects depend upon the local concentration of acrylonitrile molecule coming across the incident radiation and, therefore, produces an irregular type of variation in the hardness of the IPN. However, it is important to notice here that in the case of the IPN with low polyacrylonitrile content (0.162 g/g), the random variation in hardness is not visible, which has already been explained on the basis of radiation crosslinking of the IPN.

Variation of mba (crosslinker)

Figure 3 and Table I show the variation of H_v with varying doses of radiation from 0 to 250 kGy for IPN specimens and calculated values of crosslinking density with increasing content of MBA. The profile of variation shown in Figure 3 of IPN specimens with 0.0034 g/g content of MBA (0.279 g/g of AN) is similar to that of the specimens as described in Figure 2. The gamma irradiation of IPN specimens with relatively low content of MBA, i.e., for 0.0017 g/g, initially yields low values of H_v and crosslink density at the dose of 4 kGy. The reasons for the observed decrease in crosslink density and hardness values upon exposure to radiation are same as discussed earlier in the variation of AN. However, the values of H_v and crosslink density increase with higher dose of irradiation beyond 4 kGy when compared with that at 0 kGy. Thus, in general, beyond the dose of 4 kGy, the irradiation causes radiational hardening of IPN. However, the degree of crosslinking enhances as a consequence of varying radiation dose. At the higher dose level (200 and 250 kGy), the H_v values decrease because of bond scissioning and crosslink density value increases because of radiational crosslinking. Here, the remarkable feature is that the hardening trend observed for non-irradiated IPNs with increasing content of MBA gets reversed because of gamma irradiation beyond the dose of 2 kGy. This is because the level of microhardness for IPNs with lower content of crosslinker (0.0017 g/g) is higher than that with higher content (0.0034 g/g). Therefore, the role of MBA as crosslinker can be optimized to yield hardened IPNs as a consequence of irradiation. The results obtained with crosslinking density measurements (Table I) also exhibit the same effect; the crosslinking density of 0.0017 g/g specimen increases with radiation beyond 4 kGy, compared with the non-irradiated (0 kGy) specimens. The maximum enhancement in crosslink density is obtained at the dose of 100 kGy.

A close examination of Table I indicates that in the lower range of irradiation dose (up to 10 kGy), the crosslink density (q) with varying acrylonitrile and MBA concentration varies with almost zig-zag pattern, i.e., with no regular trend. However, in the higher range of applied dose (50 to 250 kGy) the crosslink density increases in both the variations of acrylonitrile and MBA concentrations. The reason for the observed irregular behavior of crosslink density in the lower dose range may be due to the relative dominating effects of radiation crosslinking and bond scissoning, which consequently increase and decrease crosslink density, respectively. However, in the higher range of applied dose, the observed increase in crosslink density may simply be attributed to the greater crosslinking effect of applied radiations.

CONCLUSIONS

The studies related to the effect of gamma irradiation on the microhardness and crosslink density of gelatin–polyacrylonitrile IPN reveal that developed crosslinked IPNs can be further hardened with gamma irradiation doses and the radiational crosslinking of IPNs can be a choice of material as hydrogels for their use in drug delivery system and other biomedical applications. The results obtained with microhardness measurements have a good consistency with the crosslink density measurements. Thus, the microhardness testing proves to be a good tool for investigating the mechanical performance of biopolymeric materials.

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