Study on Antithrombosis Dialytic Membrane Prepared by Preirradiation Grafting

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ABSTRACT: A new antithrombosis dialytic membrane with a hydrophilic–hydrophobic microphase structure was prepared by preirradiation grafting of β-hydroxyethyl methacrylate (HEMA) and styrene (St) onto ethylene–vinyl acetate (EVA). The influence of some effects, such as preirradiation dose, dose rate, grafting reaction temperature, reaction time, and monomer component, on the degree of grafting was determined, and the properties of the grafted films were investigated. Compared with the conventional EVA-grafted hydrophilic monomer, the EVA films grafted with HEMA and St have superior antithrombogenicity; the antithrombogenicity and permeability of EVA-*g*-(HEMA-*co*-St) were 30 and 20 times higher than those of the ungrafted films, respectively, when the volume ratio (HEMA versus St) was about 7:3. © 2000 John Wiley & Sons, Inc. J Appl Polym Sci 78: 1321–1327, 2000

Key words: radiation grafting; antithrombosis; ethylene–vinyl acetate; β-hydroxyethyl methacrylate; styrene

INTRODUCTION

Ethylene–vinyl acetate (EVA) has been widely used as a polymeric biomaterial. Because it contains an acetyl group, EVA has good flexibility, a low crystallinity index, especially a loose third structure, and permeability. EVA is suitable to be used in a drug control release device or system.[1–3] However, EVA is hydrophobic; some unexpected reactions, for instance, causing thrombus, inflammation, tumorigenesis, and thrombosis, may happen when it contacts with body fluid. It is important to improve its biocompatibility.

For improving the surface biocompatibility of a biomedical material, preirradiation grafting of a hydrogel has been considered as one of the effective methods.[4] Irradiation grafting of hydrophilic acrylic acid (AAc),[5] acrylate,[6] β-hydroxyethyl methacrylate (HEMA),[7] acrylamide (AAm),[8] and hydrophobic styrene[9] onto a polymer has been reported. But the antithrombogenicity of these grafted membranes cannot be high enough for them to be used for an antithrombosis dialytic membrane.[10,11] In this article, a new antithrombosis polymer dialytic membrane with a hydrophilic–hydrophobic microphase structure was prepared by preirradiation grafting of HEMA and St onto EVA. The effects of the reaction conditions on the degree of grafting were determined, and the properties of the grafted films were investigated.

EXPERIMENTAL

Materials

EVA with a thickness of 85 μm was supplied by the Shanghai Plastic Institute of China (Shang-

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hai, China). Reagents were all of analytical purity grade. The monomer HEMA was distillated in a vacuum before using.

Irradiation

EVA of size 20 × 30 mm was washed with a liquid mixture of alcohol and acetone in a 1:1 ratio and dried at 10 mmHg and 60°C. The samples were preirradiated at a dose rate of 100Gy/s and a dose of 25 kGy by electron beam (0.9 MeV) in a Van de Graff accelerator in air or oxygen at room temperature.

Grafting Reaction

The grafting experiment was performed in a glass ampule having a cock; a solvent consisting of water and alcohol was added first, followed by a mixture of the monomers of HEMA and St in a 7:3 ratio. The irradiated EVA film was immersed in the monomer solution and purged by bubbling nitrogen. The grafting reaction was carried out by placing the ampules in a water bath which was set at the desired temperature. After the grafting reaction, the grafted samples were taken out from the monomer solution and washed with methanol and distilled water to remove the homopolymer. The degree of grafting and absorption of water are defined as

$$G = \frac{W_d - W_0}{W_0} \times 100\%$$

$$H = \frac{W_w - W_d}{W_0} \times 100\%$$

where W_d is the weight of the grafted sample which was in a dry state; W_0, the weight of the ungrafted sample; W_w, the weight of the grafted sample after equilibration in water; G, the degree of grafting; and H, the degree of water adsorption.

Antithrombogenicity of the Grafted EVA Films

The degree of thrombus formation of the grafted films was determined as follows[12]:

$$\text{Degree of thrombus formation} = (W_{tf}/W_{og}) \times 100\%$$

where W_{og} is the weight of the cruor formed on the surface of glass in 60 min and W_{tf}, the weight of the cruor formed on the surface of grafted film in the desired number of minutes.

Permeability of the Grafted Films

Methylene blue and sucrose were used as model permeant solutions. The diffusivities of the permeant solution across the grafted films were measured at 37°C with side-by-side™ diffusion cells.[13] The permeant solution and deionized water were added to donor and receptor cells, respectively; the grafted films were placed between the halves of the permeation cell with an exposed area of 3.14 cm^2. The solution was withdrawn for the concentration to be measured at 662 nm with a UV-visible spectrophotometer. The permeability coefficient of the grafted films was given by the equation

$$Pm' = \frac{2.303dV'}{A(1 + V'/V)} \times \log \frac{C_0}{[C_0 - (1 + V'/V)C_t]}$$

where d is the membrane thickness; A, the area of the membrane; C_0, the concentration at time = 0; C_t, the concentration at time = t in the membrane; V, the volume of deionized water; and V', the volume of the permeant solution.

RESULTS AND DISCUSSION

Determination of Active Species

A preirradiation grafting reaction can be initiated either by radicals or peroxides. These two kinds of active species have great differences in their initial activity. We determined the active species by ESR.

The sample which was kept in a solid carbon dioxide–alcohol atmospheric condition was irradiated and had an ESR determination after irradiation at −78°C. It was shown that there were radicals in the sample under this condition. We also irradiated the sample at room temperature and atmosphere. The ESR determination was performed at room temperature; there were no radical signal. On the other hand, the sample irradiated at room temperature caused the DPPH solution change its color at 85°C, but it did not do so at room temperature, indicating that the radicals caused by radiation are captured by O_2 or other chemical species at room temperature and atmosphere. We concluded that the active species initiating the grafting reaction are peroxides.

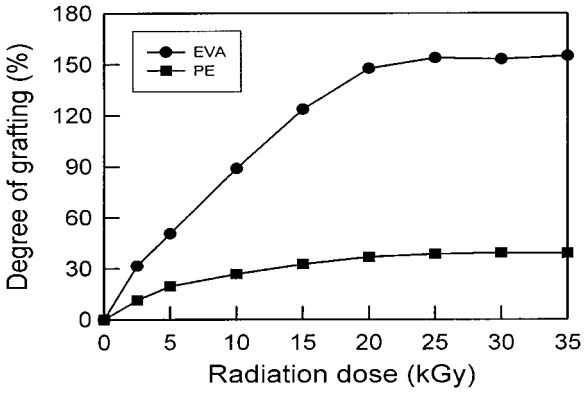

Figure 1 Effect of radiation dose on grafting reaction. Reaction time: 8 h; temperature: 85°C; monomer concentration: 15% ratio of HEMA/St: 7:3.

Because of the good permeability for gas, an EVA disc in the atmosphere contained enough O_2. The molecular chains in the amorphous region can rotate and the radicals are very active at room temperature which is well above the glass transition temperature of EVA. These radicals decay mainly through following reactions:

$$2P\cdot \xrightarrow{v} P - P$$

$$P\cdot + O_2 \xrightarrow{v'} PO_2\cdot$$

where v is the reaction rate; $P\cdot$, the polymer radical; P—P, the polymer; and $PO_2\cdot$, the peroxide radical. The active energy of these two reactions are low so that the reaction rates are decided mainly by the probability of molecular collision. The diffuse rates of O_2 are higher than those of $P\cdot$ so $v' \gg v$ and most of the radicals are captured by O_2 and become peroxides or oxides. The radicals in the crystalline region migrate irregularly by extrication of H. If the radicals reach the crystalline surface, they will decay as they are in an amorphous region.

Influence of Preirradiation Dose and Dose Rate on Grafting Reaction

Figure 1 shows that the degree of grafting is directly proportional to the preirradiation dose in the low-dose region and tends to a constant when radiation dose is over 20 kGy. The degrees of grafting are not affected by the preirradiation dose rate as shown in Figure 2.

The change of the degrees of grafting means a change of peroxides. Peroxides are produced by following reactions:

$$P \xrightarrow{V_1} P\cdot \qquad v_1 = G_p . D \qquad (1)$$

$$P\cdot + O_2 \xrightarrow{K_2} POO\cdot \begin{cases} \nearrow POOP \text{ or } POOH \\ \searrow oxides \end{cases}$$

$$v_2 = K_2[P\cdot]\,[O_2] \qquad (2)$$

$$2P\cdot \xrightarrow{K_3} P — P \qquad v_3 = K_3[P\cdot]^2 \qquad (3)$$

Because the diffusion rate of O_2 is much higher than that of $P\cdot$, $V_2 \gg V_3$ and reaction (3) can be neglected. There are many O_2 molecules in the sample due to the good permeability of EVA for gas and reaction (2) requires very low active energy, so that V_2 is very large and it is possible that $V_2 \gg V_1$. As a result, peroxides are controlled mainly by reaction (1). So,

$$\frac{d[POO\cdot]}{dt} = V_1 = G_P . \dot{D}$$

$$\int_0^t d[POO\cdot] = \int_0^t G_P . \dot{D}\, dt$$

$$[POO\cdot] = G_P \cdot D$$

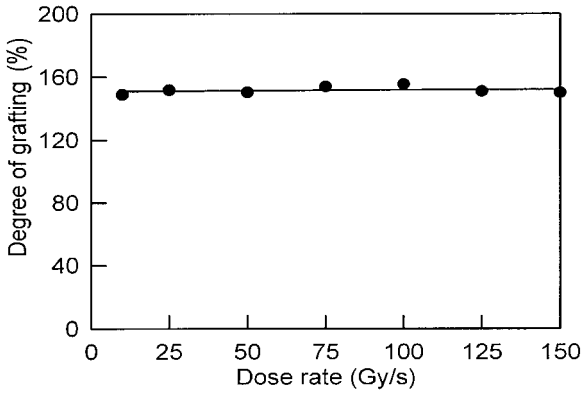

Figure 2 Effect of radiation dose rate on the grafting reaction (same conditions as in Fig. 1).

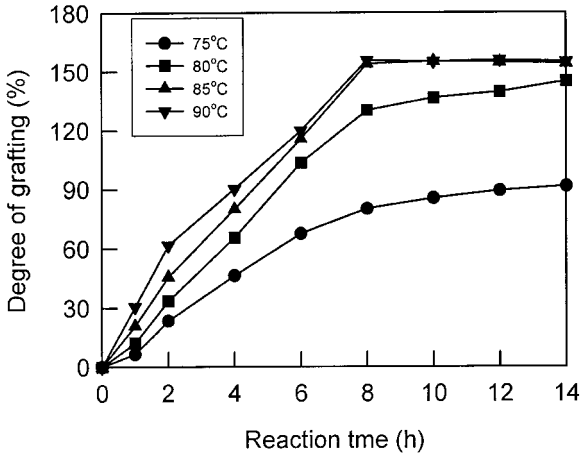

Figure 3 Kinetics of grafting reaction. Monomer concentration: 15% HEMA/St: 7/3.

where G_p is value of the products' polymeric free radicals caused by radiation; Ḋ the radiation dose rate; and D, the radiation dose, indicating that the products of the polymeric free radicals are dependent on the dose but are independent of the dose rate. Because there are few peroxides in the EVA sample at a low dose and radiation degradation of the peroxides can be neglected, the products of the peroxides are directly proportional to the dose. Increase of peroxides makes the radiation degradation rate of the peroxides increase as does the dose accumulation. The products of the peroxides level off when an equilibrium between the radiation-producing and degradation of peroxides is reached.

Influence of Reaction Conditions on the Grafting Reaction

The reaction time and grafting temperature play a very important and complex role in the grafting reaction. It is an important way to investigate the relationship among the time, temperature, and degree of grafting for obtaining information about the kinetics of the grafting reaction. Figure 3 shows the kinetics curves of the grafting reaction at different temperatures. The degree of grafting is proportional to the reaction time in the initial stage; the reaction rate is constant. The reaction rate is controlled by monomer diffusion in the grafting reaction. In the case of a lower change ratio of the monomer, the monomer concentration is nearly constant, so the diffusion rate and the reaction rate are also constant. The reaction rate decreases with the proceeding of the reaction and, finally, it tends to zero. Because the active centers caused by preirradiation are gradually consumed until they are exhausted, the degree of grafting tends to saturate.

The relationship between the initial reaction rate of grafting and the reaction temperature is shown in Figure 4. The grafting reaction rate is dependent mainly on the monomer diffusion rate. It is low because of the low diffusion rate of the monomer in the EVA sample at low temperature. As the temperature is increased, the motion of the monomer molecules and the free volume in the EVA sample are increased and the grafting reaction rate is increased. The grafting reaction cannot occur until the temperature is over 70°C. The reason is that few peroxides decompose when the temperature is lower than 70°C, which corresponds with the decomposition of radiation effects of polyethylene in air.[14]

Influence of Monomer Component on Antithrombogenicity of the Grafted Films

1. Degree of water adsorption.

The degree of water adsorption is a symbol of the hydrophilic property of a material. It was one of our major aims to improve the hydrophilic property of EVA. Figure 5 shows the relationship between the monomer component and the degree of water adsorption of EVA film grafted with HEMA and St. The water-adsorption degree of the grafted film decreased as the ratio of St increased.

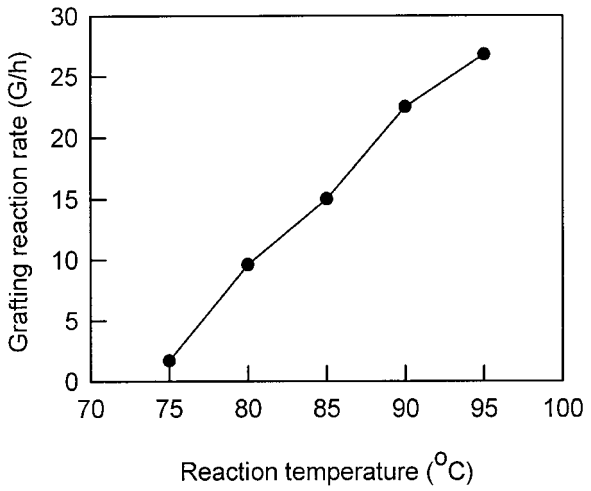

Figure 4 Effect of reaction temperature on the grafting reaction rate (same reaction conditions as in Fig. 3).

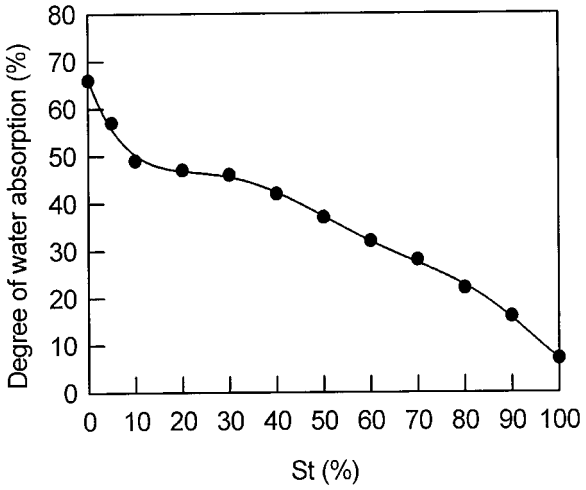

Figure 5 Effect of ratio St/(St + HEMA) on degree of water absorption. Reaction time: 8 h; temperature: 85°C; monomer concentration: 15%.

2. Antithrombogenicity of the grafted films.

The relationship between the component of the grafting monomer and the degree of thrombus formation of the grafted film is shown in Figure 6 (Fig. 6 and the following figures have the same reaction conditions as in Fig. 5). The degree of thrombus formation of the film grafted with a mixture of monomers is lower than that of the film grafted with a single monomer, and it is different with the ratio of the monomers (HEMA/St). The relationship between the grafting degree and the degree of thrombus formation of EVA-*g*-HEMA and EVA-*g*-St are shown in Figures 7 and 8, respectively. Figure 7 shows that the degree of thrombus formation of EVA-*g*-HEMA decreases

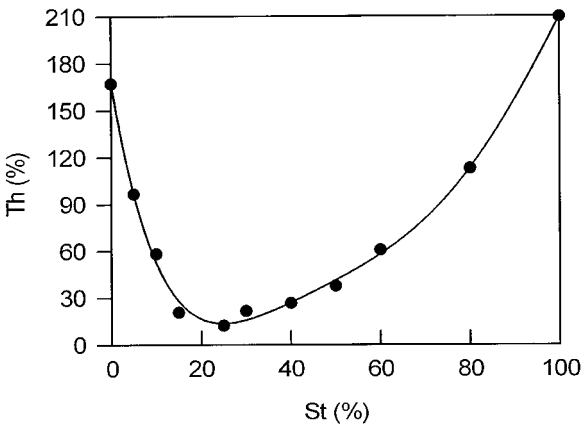

Figure 6 Effect of ratio St/(St + HEMA) on degree of thrombus formation.

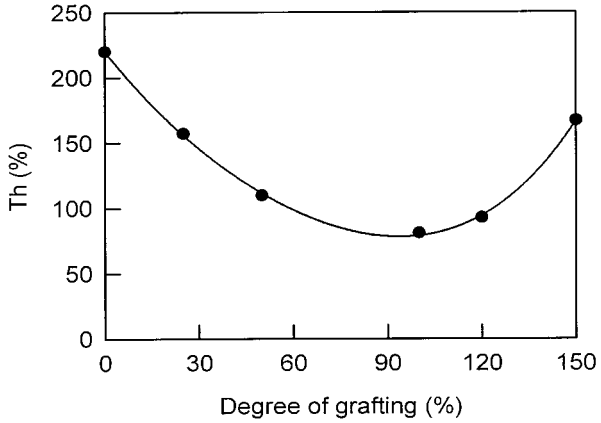

Figure 7 Effect of grafting degree of HEMA on thrombus formation in EVA film.

gradually with increase of the degree of grafting in the lower grafting degree. The degree of thrombus formation is the least when the grafting degree reaches about 100% and then increases as the grafting degree increases, because increase of the water-adsorption degree improves the antithrombogenicity of the grafted film in the lower grafting degree. But when the grafting degree is over 100%, damage to the thrombocytes is enhanced obviously with increase of the water-adsorption degree of the grafted film; the thrombin is stimulated to be released and the degree of thrombus formation increases. Figure 8 shows that the degree of thrombus formation decreases with increase of the grafting degree of St in EVA-*g*-St. Because St gives priority to the adsorption of serum albumen, there was a decrease in the chance of thrombocyte and thrombin contact with

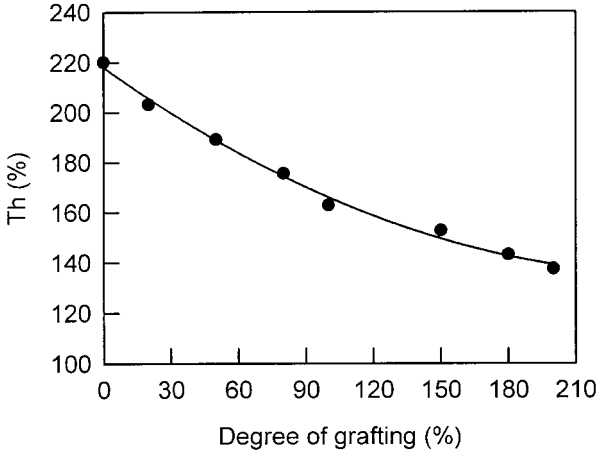

Figure 8 Effect of grafting degree of ST on thrombus formation in EVA film.

the surface of the grafted EVA. However, on the surface of EVA grafted with HEMA and St, there are a hydrophilic grafting chain and a hydrophobic grafting chain. According to the theory of flow-inlay of a biologic membrane, the hydrophilic chain and hydrophobic chain are similar to phosphoprotein and albumin, respectively.[15] The structure of the grafted film with a hydrophilic–hydrophobic microphase structure is similar to that of a natural biologic membrane, so the EVA films grafted with HEMA and St have superior antithrombogenicity. It is clear from figures that the antithrombogenicity of EVA-*g*-(HEMA-*co*-St) was 30 times higher than that of the ungrafted films when the volume ratio of St versus HEMA was about 3:7.

3. Permeability of the grafted films.

The permeability coefficient of the grafted film is an important parameter to evaluate the permeability of the film. Table I indicates the influence of the ratio of the monomer on the permeability coefficient of the grafted films. Compared with the ungrafted film (sample 1), the grafted EVA has a superior permeability coefficient—the permeability of the grafted films becomes higher with increase of the ratio of HEMA. The permeability coefficient of EVA-*g*-HEMA-*co*-St (shown in sample 2) was more than 20 times higher than that of the ungrafted films when the volume ratio of St/HEMA was about 3:7.

Figure 9 shows the microstructures of EVA samples 1, 2, and 3 that were measured by a scanning electron microscope (SEM). There are obviously differences in the morphology of the sample grafted with different monomer components. The surface of the ungrafted EVA film is smooth, and the surface of the grafted EVA appears "bumpy." The photos shown in (B) and (C) indicate that the larger "bumps" develop on the surface of the EVA grafted with HEMA, and smaller and denser "bumps" develop on the surface of EVA grafted with HEMA and St.

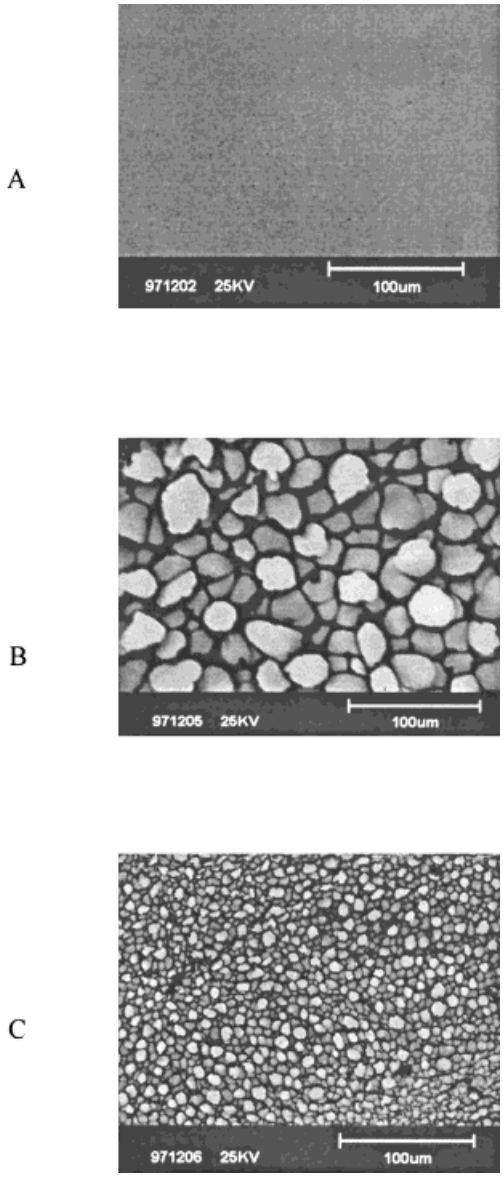

Figure 9 SEM photographs of films: (A) ungrafted EVA film; (B) EVA-*g*-HEMA film; (C) EVA-*g*-HEMA-*co*-St) film. The ratio of HEMA/St is 7:3.

Table I Influence of Monomer Component on Permeability Coefficient of Grafted Films

	Ratio of Monomers (%)		Permeability Coefficient (10^8 cm^2)	
Sample No.	HEMA	St	Methylene Blue	Sucrose
1 (ungrafted)	0	0	2.03	2.98
2	70	30	52.1	67.3
3	100	0	58.6	71.8

CONCLUSIONS

1. The grafting reaction was initiated by peroxides but not by tripped free radicals. The degree of grafting increased as the preirra-

diation dose, reaction time, and grafting temperature increased and it is independent of the preirradiation dose rate.

2. EVA-*g*-(HEMA-*co*-St) has superior hydrophilicity, and the antithrombogenicity and permeability of the grafted films were 30 and 20 times higher than those of the ungrafted films, respectively.

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