J. Biomater. Sci. Polymer Edn, Vol. 8, No. 5, pp. 335–347 (1997)

Adsorption of plasma proteins to DMAA hydrogels obtained by ionizing radiation and its relationship with blood compatibility

ALVARO A. A. DE QUEIROZ[1]*, SANDRA C. CASTRO[2] and OLGA Z. HIGA[3]

[1]*Escola Federal de Engenharia de Itajubá, Instituto de Ciências, PO Box 50, Itajubá, MG, Brazil*
[2]*Universidade Estadual de Campinas, PO Box 6165, SP, Brazil*
[3]*Instituto de Pesquisas Energéticas e Nucleares, PO Box 11049, Pinheiros, SP, Brazil*

Received 22 September 1994; accepted 23 June 1995

Abstract—The interaction of plasma proteins such as albumin, γ-globulin, and fibrinogen with the surface of graft copolymers DMAA-G-PTFE, DMAA-G-PETFE, and DMAA-G-PE obtained by radiation graft polymerization was studied. The adsorption of serum proteins was affected by the hydrophilicity of the graft copolymers. Increased albumin adsorption and decreased fibrinogen and γ-globulin adsorption with increasing grafting levels was shown. A certain range of degrees of grafting showed an improved blood compatibility of the polymeric surfaces due to the existence of a hydrophilic/hydrophobic balance on the polymers. The results suggest that the DMAA-G-PTFE, DMAA-G-PETFE, and DMAA-G-PE graft copolymers can be used as biomaterials for long-term use in cardiovascular systems.

Key words: Grafting; blood compatibility; plasma proteins; protein adsorption; polymerization; hydrogel; hydrophilicity.

INTRODUCTION

The majority of implants used in medicine today are made from polymers [1, 2]. Polymeric materials that are used in contact with biological fluids are known as biomaterials which include biocompatible and functional polymers. The former include two kinds of polymeric materials: the hemocompatible (blood compatible); and the tissue-compatible (for soft and hard tissues) polymers [3]. The pioneering work by Witcherle and Lim [4] showed that hydrophilic gels, the hydrogels, can be used as biological materials. Hoffman [5] and Williams [6] reported that the interaction between polymeric surfaces, as hydrogels, and blood components, cells, and proteins, are the base for the design of hydrogels as antithrombogenic materials.

Since hemocompatibility mainly depends on the surface properties of the material, the use of the radiation grafting technique can be a convenient and effective way to produce an antithrombogenic surface [7]. Okana *et al.* [8, 9] showed the necessity of the existence of microdomains or hydrophilic and hydrophobic balance in polymeric interface and biological fluids for the obtention of antithrombogenic surfaces.

The enormous potential of acrylic acid hydrogels [10] and their derivatives, poly(hydroxiethylmethacrylate) (PHEMA) [11], poly(methylmethacrylate) (PMMA) [12], and polyacrylamide [13] is well known for biomedical applications.

* To whom correspondence should be sent at: Escola Federal de Engenharia de Itajubá – EFEI, Campus Prof. José Rodrigues Seabra, Av. BPS, 1303, Bairro Pinheirinho, 37500–000, Itajubá, MG, Brazil.

Otsuhata *et al.* studied the graft parameters of the hydrophilic monomer *N,N'*-dimethylacrylamide (DMAA) onto poly(tetrafluoroethylene) (PTFE) and poly-(ethylene-co-tetrafluoethylene) (PETFE) and made an attempt to determine the relationship between antithrombogenicity and surface texture of the grafted copolymers [14]. Therefore, this work is not conclusive about the factor which can intrinsically contribute to the hemocompatibility of these materials.

When a polymeric material is placed in contact with blood, the first event to occur is the adsorption of proteins onto the surface. If the material is considered to have a microphase separated structure composed of hydrophilic and hydrophobic microdomains, the albumin and γ-globulin (proteins of the plasma blood) are selectively adsorbed in the regions described above [15].

Though many investigators have published the results of protein adsorption onto polymeric surfaces [16, 17], the factors influencing the adsorption are not documented in detail. In the present paper we studied the adsorption of plasma proteins on the graft copolymers poly(*N,N'*-dimethylacrylamide-graft-tetrafluoroethylene) (DMAA-G-PTFE), poly(*N,N'*-dimethylacrylamide-graft-tetrafluoroethylene-co-ethlene) (DMAA-G-PETFE), and poly(*N,N'*-dimethylacrylamide-graft-ethylene) (DMAA-G-PE) and its relationship with grafting degree and antithrombogenicity.

EXPERIMENTAL

Materials

N,N'-dimethylacrylamide (DMAA) suplied by Wako Pure Chemical Industries Ltd was used as received. Poly(tetrafluoroethylene) (PTFE), poly(ethylene-co-tetrafluoroethylene) (PETFE), and low density polyethylene films (PE) of 100, 50, and 100 μm thickness, respectively, were used in the graft polymerization after being washed with detergent, rinsed with ethanol, distilled water, and dried under vacuum at room temperature.

Bovine serum albumin (BSA), γ-globulin, and fibrinogen were purchased from Sigma Co. The fibrinogen was 95% clottable, the albumin was 99% pure and they were used without further purification. Other chemicals were reagent grade and used without further purification.

Graft polymerization

The direct radiation grafting method was used as a technique. A glass ampoule containing the DMAA in ethylacetate (30% v/v) and polymeric films (PTFE, PETFE, PE) (4 × 4 cm) was connected to a vacuum system and was evacuated by a freeze–thaw cycle which was repeated five times. The glass ampoules were then subjected to γ-rays from a ^{60}Co source at a dose rate of 0.468 kGy h^{-1} and a total dose of 0.4–6.0 kGy.

The grafted films were washed throughly with hot distilled water and soaked overnight in water to extract the residual monomer and the homopolymer occluded in the films. The films were then dried under vacuum at room temperature for 24 h and weighed. The degree of grafting was determined by the percentage increase in weight as follows:

$$\text{Degree of grafting (\%)} = \frac{W_g - W_0}{W_0} \times 100$$

where W_g and W_0 represent the weights of grafted and initial films, respectively.

Surface characterization

Water sorption. Known weights of the clean and dry grafted films were immersed in distilled water at 25°C until equilibrium was reached (24 h in most cases). Then the films were removed, blotted quickly with absorbent paper to remove the water attached on its surface and weighed. The water-uptake percent was calculated as follows:

$$\text{Water uptake (\%)} = \frac{W_{\text{wet}} - W_{\text{dry}}}{W_{\text{wet}}} \times 100$$

where W_{wet} and W_{dry} represent the weights of wet and grafted films, respectively.

Contact angle. The grafted and virgin films were conditioned at the equilibrium humidity of the instrument. The water contact angle was measured by putting a sessile drop of bidistilled and deionized water on air-side surface of the polymeric films. Each value was taken as the average of five readings.

Photoelectron spectroscopy. The graft copolymers were analysed by X-ray photoelectron spectroscopy (XPS). X-ray photoelectron spectra were obtained using an ESCA-36 spectrophotometer (McPherson Co) using AlK_α X-radiation. The base pressure in the sample chamber was of the order of 10^{-7}–10^{-8} Torr. The curve fitting was carried out using a nonlinear least-squares-curve fitting program with a Gaussian/Lorentzian product function. The C1*s* binding energy was taken as 284.6 eV for calibration purposes.

In vitro *tests*

Protein adsorption. In order to quantify the surface concentrations of albumin (BSA), γ-globulin, and fibrinogen adhering to the samples, the proteins were labeled with ^{125}I by the chloramine T method [18]. The radioactivity of the labeled proteins was 30 μCi mg^{-1}.

In order to perform equilibrium experiments, pre-equilibrated samples were introduced into teflon tubes which contained 4 ml sodium phosphate buffer (pH 7.4, ionic strength = 0.01 M) (PBS) at 37°C, before being exposed to the protein solution. Any air bubbles which would adhere to the sample were removed by allowing the samples to cross the air/buffer interface several times. Aliquots (4 ml) of the labeled BSA or fibrinogen solution were then introduced into the tubes.

After the protein solution had remained in contact with the samples for 2 h at room temperature, the adsorption was terminated by dilution of the labeled protein into the tubes with PBS (in order to avoid contact of the samples with the protein solution/air interface where some denaturation can take place). The samples were further gently rinsed until the radioactivity of the surface remained constant. The amount of adsorded proteins was determined by gamma radiation counting, using a gamma counter (Beckman Gamma 4000).

Blood compatibility assessment. Blood compatibility of the grafted and ungrafted polymers was evaluated by the open-static platelet adhesion test with whole human blood [19]. The test was performed by depositing 2 ml of fresh blood onto each of the five test surfaces. After contact times of 180 s, the surfaces were washed with saline under carefully controlled conditions to remove all of the blood components which did not adhere.

After fixation with glutaraldehyde, platelet counts were performed by SEM. The average number of adhered platelets was obtained from five photographs of different surface areas (1 cm^2) of the same sample.

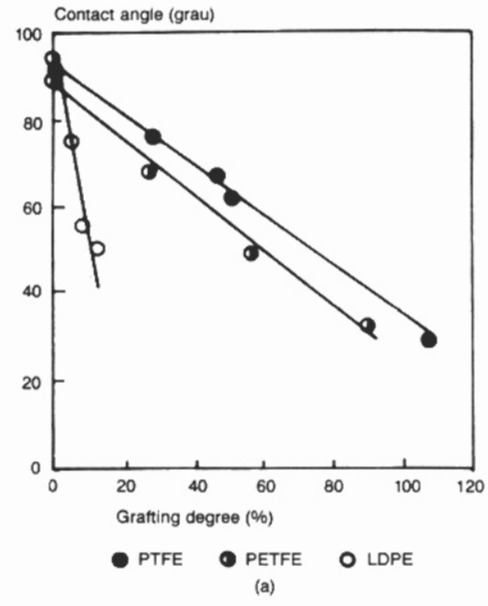


Figure 1. Effect of grafting degree on the contact angle of the PTFE, PETFE and PE films.

RESULTS AND DISCUSSION

The hydrophilicity of the samples after the grafting process was measured using the contact angle (Fig. 1) which decreased with the grafting degree, indicating the appearance of hydrophylic regions on PTFE, PETFE, and PE which were originally hydrophobic. The PTFE, PETFE, and PE unmodified surfaces showed minor contact angles of 100 deg. Figure 2 shows the 01*s* spectra of the ungrafted and grafted films. The presence of the oxygen peak with binding energies of 532.8 eV for the ungrafted PTFE, PETFE, and PE films is characteristic of the C=O groups on the polymeric surfaces and indicates a probable oxidation process of the films during their industrial manufacturing. The presence of oxygen on PTFE, PETFE, and PE matrices was not observed for other analytical techniques such as attenuated total reflectance infrared spectroscopy (ATR-IR).

The grafting of DMAA onto PE was found to be easier than that onto PTFE and PETFE (Table 1). This is due to the higher radiation-chemical yield of radicals derived from C—H bonds [20].

The swelling behavior of grafted films are presented in Fig. 3. All samples show a degree of swelling which increases with an increase in percent grafting. The plateau attained for PE at a higher grafting degree may indicate the cross-linking of the grafted polyDMAA chains.

The distribution of the grafted polyDMAA chains in the region near the surface was investigated by XPS.

As can be seen in Fig. 4 the values of the ratios of the CO/CF and CN/CH peaks of different grafting degrees were calculated and plotted against the degree of grafting. It can be seen that ratios of the CO/CF and CN/CH peaks increased with the degree of grafting and level off at a certain grafting degree. This means that the distribution of grafted chain in the surface increased with increasing degrees of grafting. At a saturated level of CO/CF

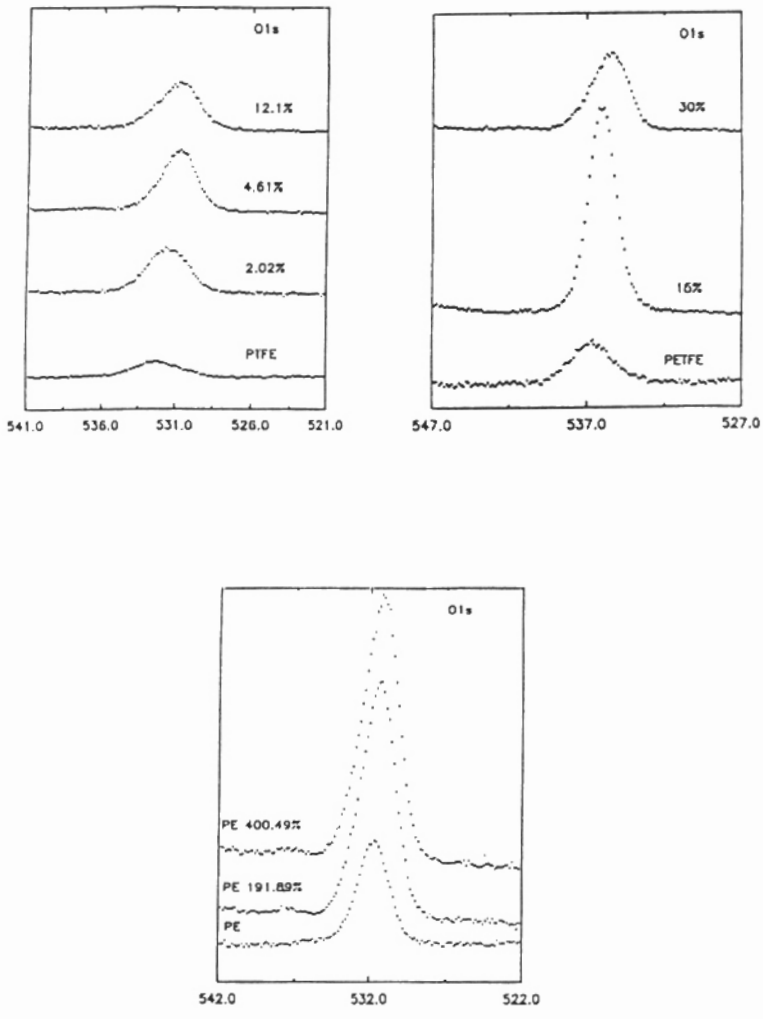


Figure 2. 01*s* XPS spectra for the ungrafted and grafted PTFE, PETFE and PE films. (%): grafting degree.

Table 1.
Influence of the polymeric matrix on the degree of grafting

Polymer	Grafting attained (%)
PTFE	9.85
PETFE	90.56
PE	326.70

Graft conditions: DMAA concentration, 35% in ethyl acetate; Irradiation dose, 0.44 kGy; irradiation dose rate, 0.055 kGy h^{-1}

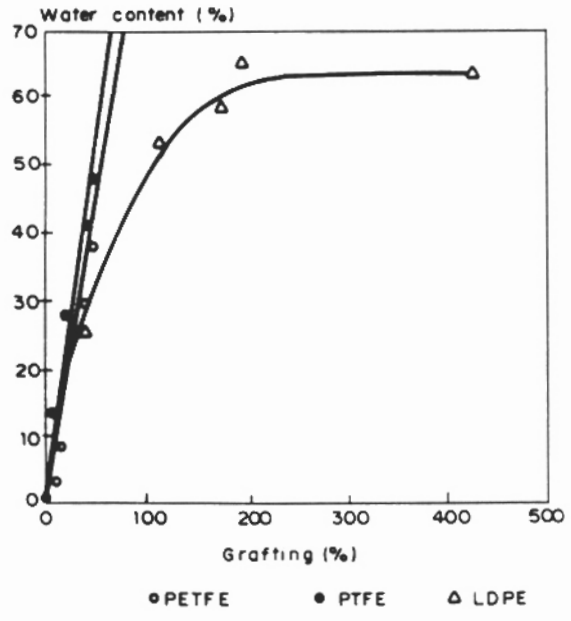


Figure 3. Effect of grafting degree on the water content of DMAA-G-PTFE, DMAA-G-PETFE and DMAA-G-PE hydrogels.

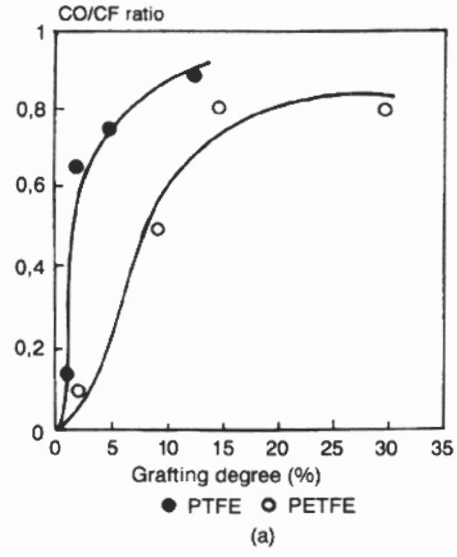
and CN/CH ratios, the distribution of grafted chain may be interpreted as becoming homogeneous on the surface of the polymeric matrices [21].

The hydrophilic character of the graft copolymers, promoted a differentiation in the adsorption of plasma proteins. In Figs 5–7 an adsorption of albumin and a diminution of adsorption degree are observed. That behaviour indicated that the surface of PTFE, PETFE, and PE were effectively covered by the hydrophilic polymer layer, polyDMAA, through which its hydrophilicity increased. However, the adsorption of the γ-globulin would not only be related to the hydrophobicity or hydrophilicity of the polymeric matrix but also to the chemical structure of the copolymer [22].

The blood compatibility of the polymeric materials is a complex problem. On the one hand, protein adsorption is known to be the first stage of blood/surface interaction. Figure 8 represents the main events that take place during the interaction of a polymeric material with blood, and establishes the role of protein adsorption [23].

After the adsorption of proteins onto the polymeric surface, the next event is the adhesion of platelets (blood cell) [24]. Thus, platelet adhesion is promoted when fibrinogen is adsorbed from the blood onto foreign surfaces [25]. The adhesion of platelets in these cases can be explained by assuming that the adsorbed glycoprotein fibrinogen interact with platelets. In the model of Lee and Kim, a glycosyl transferase enzyme located in the platelet membrane form a complex with a glycorprotein containing incomplete heterosaccharides (e.g., fibrinogen) when adsorbed on a foreign surface [26]. On the other hand, reduced platelet adhesion has been reported for polymers which adsorb relative amounts of albumin [27].

From the thrombus formation curves in Fig. 9, the blood compatibility of DMAA-G-PTFE, DMAA-G-PETFE, and DMAA-G-PE was found to be better than the



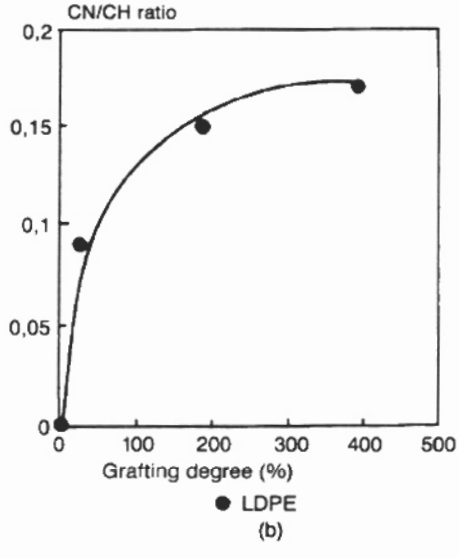


Figure 4. (a) CO/CF ratio for DMAA-G-PTFE, DMAA-G-PETFE systems and (b) CN/CH ratio for DMAA-G-PE, obtained from XPS spectroscopy.

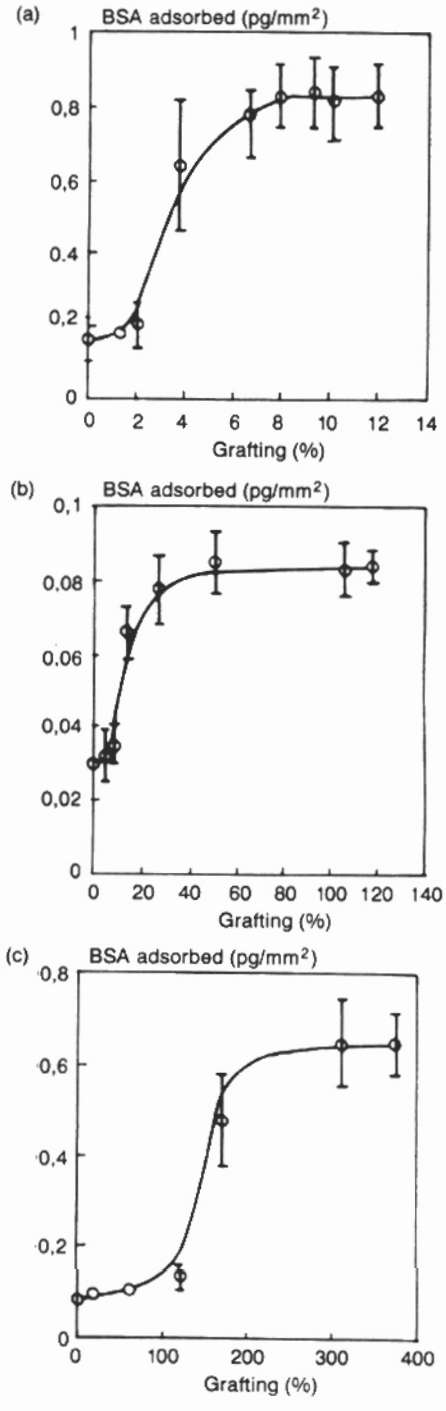


Figure 5. Effect of the grafting degree on albumin adsorption to the graft copolymers: (a) DMAA-G-PTFE, (b) DMAA-G-PETFE and (c) DMAA-G-PE.

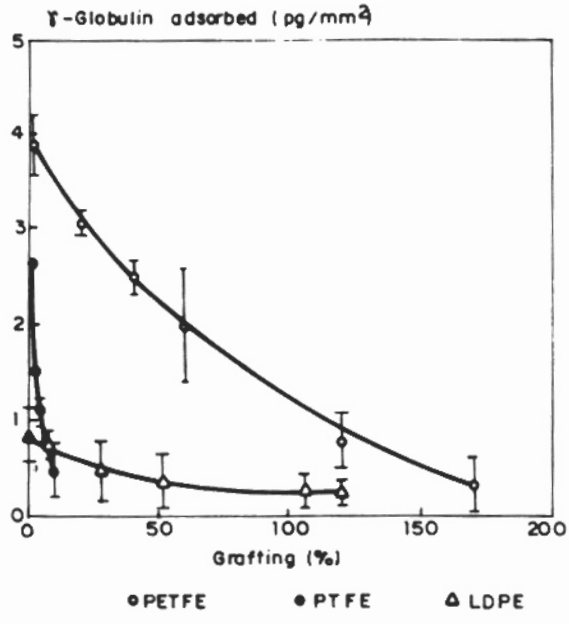


Figure 6. Relationship between the γ-globulin adsorbed onto polymeric surfaces and grafting degree.

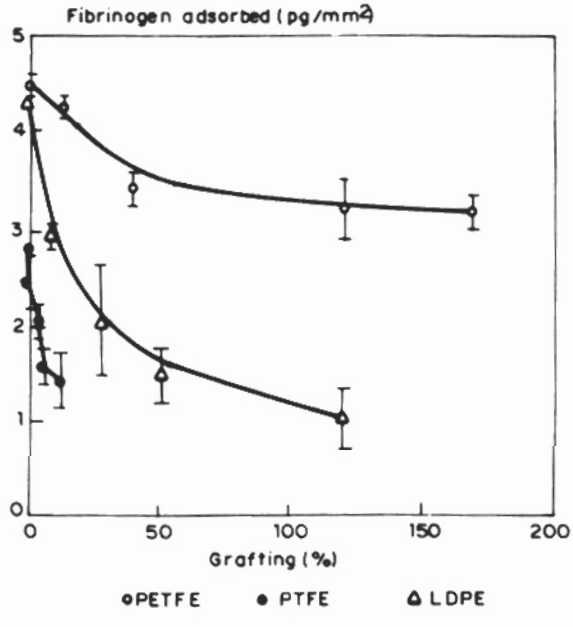


Figure 7. Effect of grafting degree on the fibrinogen adsorbed onto DMAA-G-PTFE, DMAA-G-PETFE and DMAA-G-PE hydrogels.

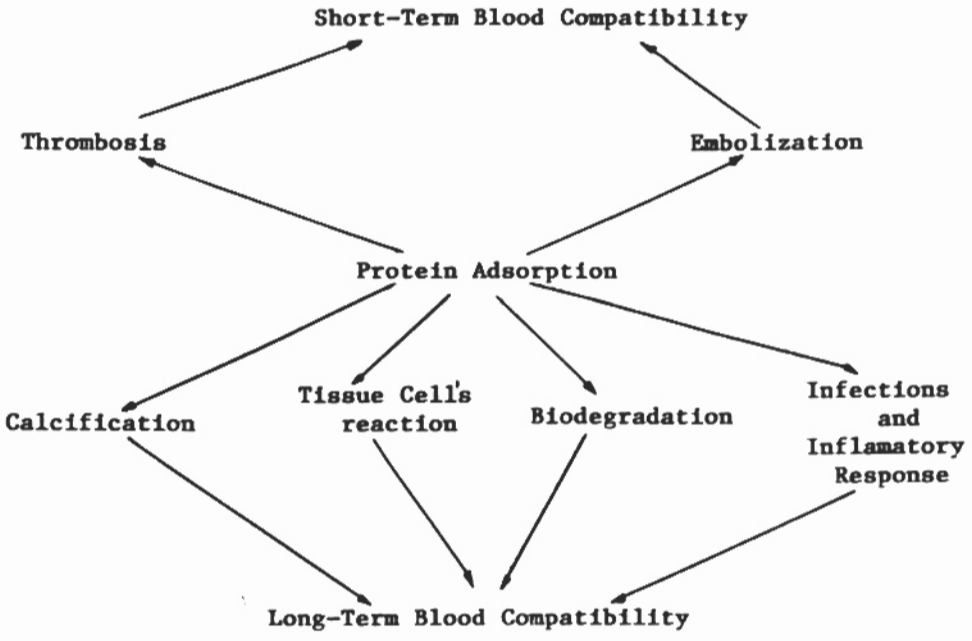


Figure 8. The role of protein adsorption in the blood compatibility of the polymeric surface.

unmodified films until a certain degree of grafting. Above this degree of thrombus, formation on the polymeric surfaces occurred sooner than those nongrafted surfaces (Fig. 9b, c).

There is a range of grafting level for which the coagulation time is higher than others observed for the highest grafting levels. The thrombus formation is increased with the grafting degree. Thus, the thrombogenicity of the copolymers at high graft levels of polyDMAA may be due to a conformational change in the adsorbed BSA (Fig. 10), caused by the diminution on their molecular mobility, plus associated interactions with water molecules on hydrogel [28].

The SEM photographs of DMAA-G-PTFE, DMAA-G-PETFE, and DMAA-G-PE films are showed in Fig. 11. As can be seen in the pictures, the number of adhered platelets or the fibrin formation was decreased compared to that PTFE, PETFE, and PE ungrafted films.

CONCLUSION

It was shown that the nature of polymer surface controls protein adsorption. However the grafting degree of poly(*N,N'*-dimethylacrylamide) onto *PTFE, PETFE, and PE surfaces* was an important factor for the adsorption process of plasma proteins such as albumin, α-globulin, and fibrinogen. The diminution on the fibrinogen adsorption and increased adsorption of albumin for copolymerized samples as well as the existence of a certain range of degrees of grafting for an improved hemocompatibility, hints that it must due to the surface composition, molecular mobility of the grafted chains, and interaction of the plasma proteins with water in polyDMAA hydrogels. However, the precise reason for such an enhancement in the hemocompatibility of a certain range of degree of grafting is still

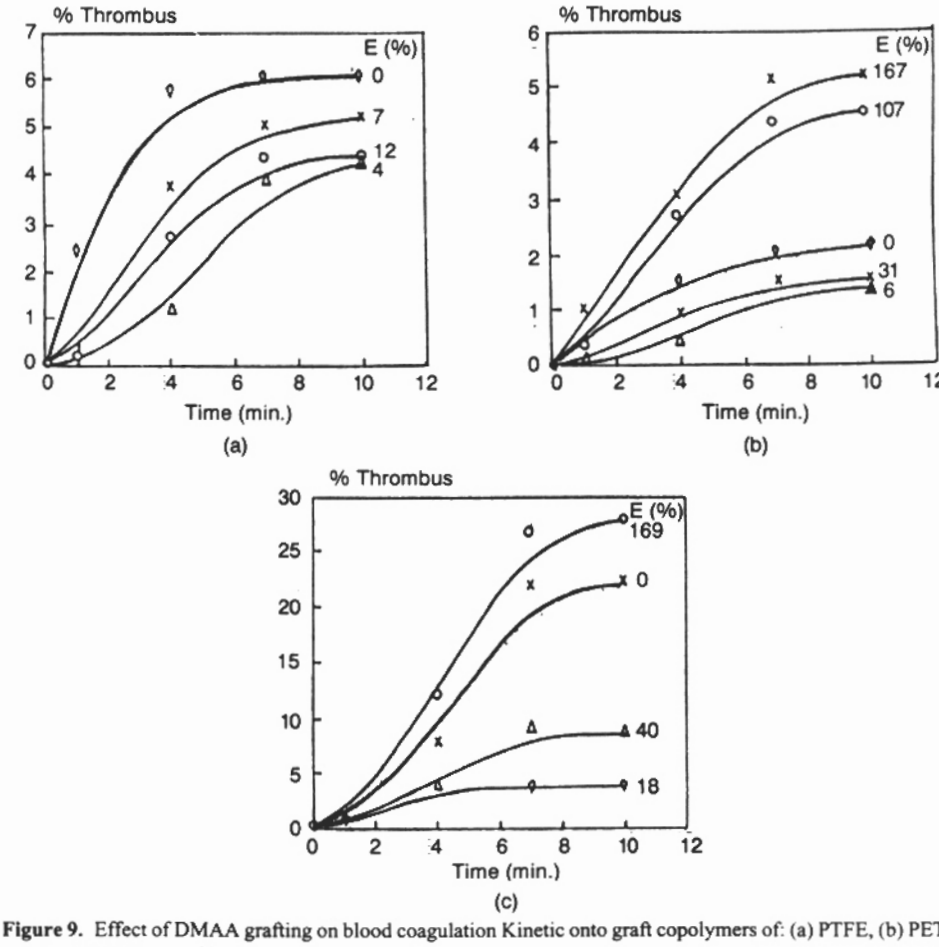


Figure 9. Effect of DMAA grafting on blood coagulation Kinetic onto graft copolymers of: (a) PTFE, (b) PETFE and (c) PE. E(%): grafting degree.

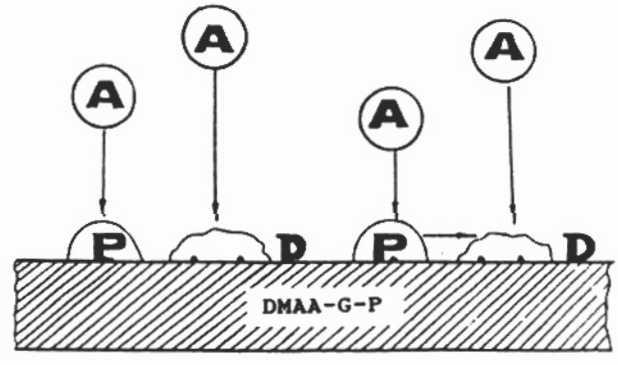


Figure 10. Conformational change on plasma proteins by interaction with polymeric surface. A: free BSA, P: Adsorbed BSA, D: denaturated BSA. P-G-DMAA: Graft copolymers (DMAA-G-PTFE, DMAA-G-PETFE and DMAA-G-PE).

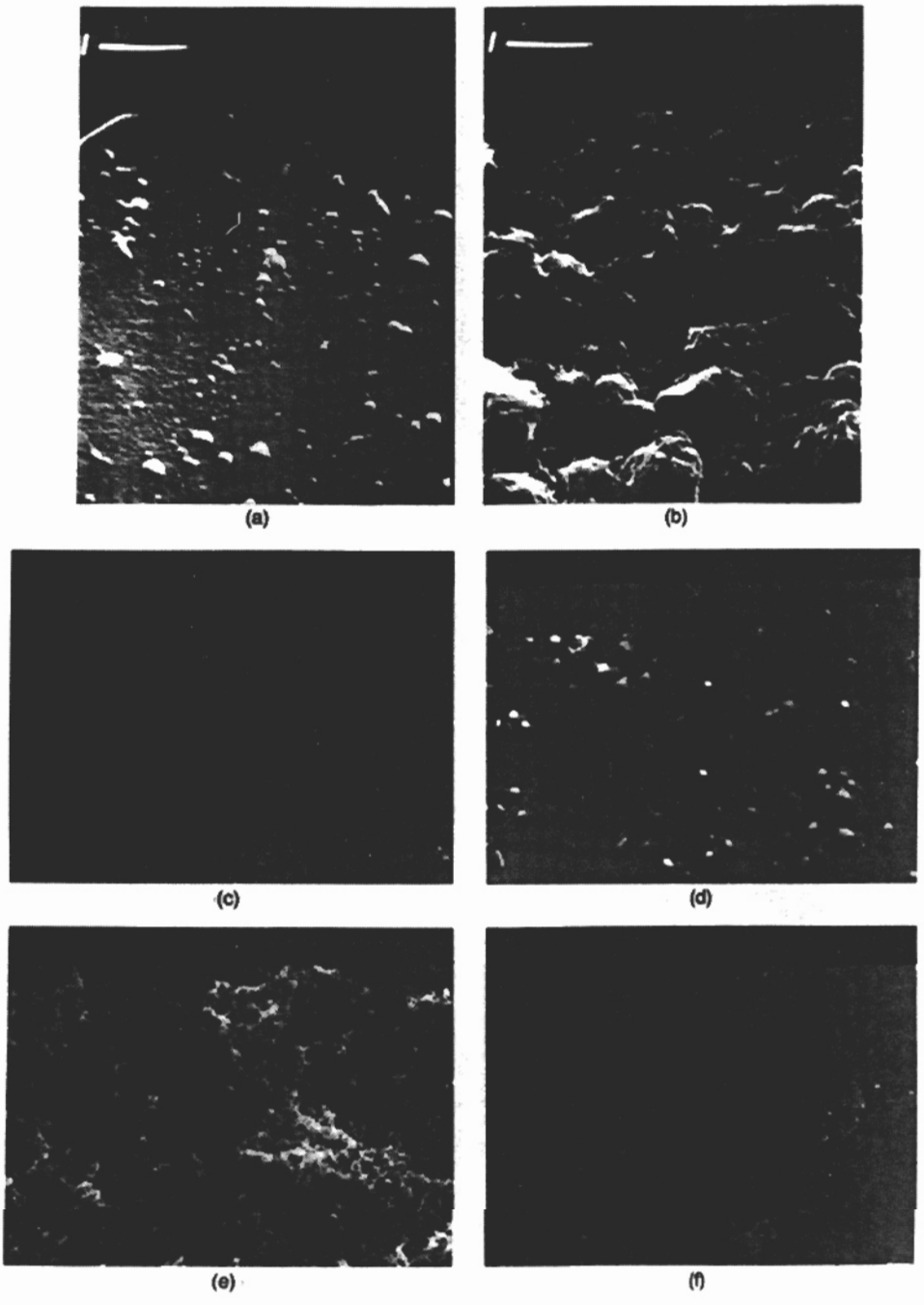


Figure 11. Scanning electron micrographs of polymer surfaces after incubation with human blood at 37°C for 3 min. (a) Virgin PTFE, (b) DMAA-G-PTFE (grafting degree: 9%), (c) Virgin PETFE, (d) DMAA-G-PETFE (grafting degree: 9%), (e) Virgin PE, (f) DMAA-G-PE (grafting degree: 19%). Original magnifications of Figures × 1000.

open to discussion. A more detailed study is now in progress and the results will be reported in the future.

REFERENCES

1. C. T. Lynch (Ed.), *Nonmetalic Materials and Applications*. CRC Press, New York (1975).
2. R. W. Tess and G. W. Poehlein (Eds), *Applied Polymer Science*. ACS, New York (1985).
3. E. Piskin and A. S. Hoffman (Eds), *Polymeric Biomaterials*. Martinus Nijhoff, Netherlands (1986).
4. O. Witcherle and D. Lim, *Nature* **9,** 117 (1960).
5. A. S. Hoffman, A. T. Horbett and B. D. Ratner, *Ann. N.Y. Acad. Sci.* **283,** 372 (1977).
6. D. F. Williams (Ed.), *Biocompatibility of Clinical Implant Materials*. CRC Press, New York (1981).
7. A. S. Hoffman, *Radiat. Phys. Chem.* **18,** 323 (1981).
8. T. Okano, S. Nishiyama, I. Shinohara, T. Akaike and Y. Sakurai, *Polym. J.* **10,** 223 (1978).
9. T. Okano, N. Yui, J. Tanaka, K. Sanvi, N. Ogata and K. Kataoka, *Polym. J.* **16,** 119 (1984).
10. V. P. Wang, *Radiat. Phys. Chem.* **25,** 491 (1985).
11. B. D. Ratner, A. S. Hoffman, S. R. Hanson, L. A. Harker and J. D. Whiffen, *Polym. Sci. Polym. Symp.* **66,** 363 (1979).
12. S. D. Bruck (Ed.); *Blood Compatible Synthetic Polymers,* Charles C. Thomas, New York (1974).
13. N. D. Skuba, J. Kala, I. Vacik and O. S. Voronocya, *Nakromol. Chem. Suppl.* **9,** 145, (1985).
14. K. Otsuhata, M. T. Razzak, R. L. Castanares, Y. Tabata, F. Ohashi and A. Takeuchi, *Radiat. Phys. Chem.* **25,** 537 (1985).
15. T. Beugeling, *J. Polym. Sci. Polym. Symp.* **66,** 419 (1979).
16. M. Josenfowicz and J. Josenfowicz, *Pure Appl. Chem.* **56,** 1335 (1984).
17. W. Norde and J. Lyklema, *J. Biomater. Sci. Polym. Edn.* **2,** 183 (1991).
18. P. L. Biscayart, A. C. Palladini, N. Vita and L. P. Roguin, *J. Immunoassay* **10,** 37 (1989).
19. Y. Imai and Y. Nose, *J. Biomed. Mater. Res.* **6,** 165 (1972).
20. J. E. Wilson (Ed.), *Radiation Chemistry of Monomers, Polymers and Plastics*. Marcel Dekker, New York (1974).
21. M. T. Razzak, (PhD Thesis), Radiation grafting studies on the modification of fluoropolymers and natural rubber for biomaterials, Tokyo University, Tokyo (1988).
22. B. D. Ratner, A. B. Johnston and T. J. Lenk, *J. Biomed. Mater. Res. Apply Biomater.* **21,** 59 (1990).
23. A. F. Recum (Ed.), *Handbook of Biomaterials Evaluation*. MacMillan, New York (1986).
24. C. G. Gebelein and F. Koblitz (Eds), *Biomedical and Dental Applications of Polymers*. Plenum, New York (1981).
25. A. S. Hoffman, *J. Appl. Polym. Sci. Appl. Poly. Symp.* **46,** (1990).
26. S. W. Kim and E. S. Lee, *J. Polym. Sci. Polym. Symp.* **66,** 429 (1979).
27. J. L. Brash and S. Uniyal, *J. Polym. Sci. Polym. Symp.* **66,** 377 (1979).
28. M. S. Jhon and J. D. Andrade, *J. Biomed. Mater. Res.* **7,** 509 (1973).