

Graft Copolymerization of Methoxypoly(ethylene Glycohol) Methacrylate onto Polyacrylonitrile and Evaluation of Nonthrombogenicity of the Copolymer

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Synopsis

Methoxypoly(ethylene glycohol) methacrylate was grafted onto polyacrylonitrile in dimethylsulfoxide solution via thioamide formation, where ammonium peroxydisulfate was used as an initiator. Optimum conditions for the graft copolymerization, such as degree of thioamidation of the trunk polymer, feeding concentration of the acrylate and the trunk polymer, and temperature were examined. Also the rate of graft polymerization was found to be proportional to concentrations of the acrylate and the trunk polymer. An increase of the degree of the grafting increased water content of the graft copolymer and decreased interfacial free energy between the copolymer and water. *In vivo* tests showed that the graft copolymer obtained was highly nonthrombogenic.

INTRODUCTION

Previously, polyacrylonitrile (PAN) containing poly(ethyleneoxide) (PEO) side chains was found to be highly nonthrombogenic according to *in vitro*¹ and *in vivo* tests.² Here, the photografting method^{2,3} had to be used because PAN was very inert to conventional methods of thermal grafting. However, from the viewpoint of the simplicity of the synthetic process, it is desirable to develop a method of the thermal grafting.

Grafting of vinyl monomers onto polyacrylonitrile can be achieved via thioamide formation.⁴ The preparation of thioamide polyacrylonitrile (TPAN) is easily carried out by passing H₂S gas through a solution of polyacrylonitrile in dimethylformamide (DMF) at the elevated temperature.⁵ Styrene was grafted onto TPAN in DMF by using ferric laurate as redox initiator,⁴ methacrylic acid was grafted in aqueous solution by using H₂O₂ as an initiator,⁶ and methylmethacrylate was grafted in DMF by using Br₂ as an initiator.⁷ As a preliminary experiment, the grafting of methoxypoly(ethylene glycohol) methacrylate (MG) having the following formula onto TPAN was tried by using the initiators described above, in dimethylsulfoxide (DMSO) solution.



However, the graft copolymer was not obtained. On the other hand, Yamamoto et al.⁸ found that methyl methacrylate was grafted onto soy bean protein by using ammonium peroxydisulfate (APS) as an initiator which formed a redox compound with thiol group of the protein.

We could graft MG to TPAN by using the APS–thiol redox initiator. The details of the synthesis of the graft copolymers, kinetics of the graft polymerization, and the evaluation of physical properties and nonthrombogenicity for the copolymer will be described.

EXPERIMENTAL

Materials

Acrylonitrile of reagent grade was washed twice with aqueous solution of sodium hydroxide and that of phosphoric acid, washed thoroughly with distilled water, dehydrated with anhydrous potassium carbonate and stored in dark. This was distilled in Ar atmosphere before the use. MG supplied by Shin Nakamura Kagaku Co., Ltd. was used without further purification, the molecular weight distribution (M_w/M_n) of which was 1.12. Solvents, such as methyl alcohol, DMSO, and DMF of reagent grade were used after the distillation. Other reagents of reagent grade, and Ar and H₂ gases of high purity from commercial cylinders were used without further purification.

Synthesis of Graft Copolymers

Preparation of Polyacrylonitrile. Into 350 mL of DMSO, 130 mL of AN was dissolved and mixed at 60°C in Ar atmosphere. To the mixture, 28 mL of DMSO containing 1.2 g of α , α' -azobisisobutyronitrile was added. The polymerization was carried out at 60°C for 90 min and stopped by adding hydroquinone monomethylether. The reacted mixture was poured into a large quantity of aqueous methyl alcohol solution to precipitate the polymer. The polymer was washed thoroughly with methyl alcohol and dried at 40°C for 20 h in vacuum. The molecular weight M_w and its distribution M_w/M_n determined by GPC with polystyrene as a reference were 3.54×10^5 and 1.64, respectively.

Preparation of Thioamide Polyacrylonitrile. Into a three-necked flask of 1 L capacity, a definite quantities of PAN and DMF were introduced and stirred for 30 min during Ar bubbling. Into the solution, H₂ gas was bubbled at a flow rate of 50 mL/min for 60 min. The solution was poured into methyl alcohol to precipitate TPAN. The polymer was washed with methyl alcohol, reprecipitated in DMF, washed with methyl alcohol and dried in vacuum. The identification of TPAN was carried out by IR absorption (Hitachi IR 260-10) and qualitative analysis according to Ellman's method.⁹ The degree of the thioamidation was determined by element analysis and X-ray microanalysis (JEOL JXA-733).

Grafting of MG onto TPAN. In the three-necked flask of 1 L capacity, 100 mol of DMSO, a definite quantity of MG and a definite quantity of APS were completely mixed and reacted. After adding hydroquinone monomethylether to stop the polymerization, the solution was concentrated in vacuum to one half of the original volume. The solution was poured into aqueous solution of

methyl alcohol to precipitate the graft copolymer. The copolymer was reprecipitated in DMSO, and washed and dried in vacuum. The grafting was confirmed by IR absorption and NMR spectrum (JEOL GX-270).

Chemical Kinetic Measurements. At definite time intervals in the above-described graft polymerization, 10 mL of the solution was taken out and used to determine the amount of unreacted MG via thin-layer chromatography¹⁰ (Iatrosan, TH-10 TLC analyzer of Iatron Co., Ltd.) and to determine the amount of grafted MG via NMR spectroscopy where 10 mg of the precipitated and purified copolymer was dissolved into 0.5 mL DMSO- d_6 in a NMR tube of 5 mm outside diameter.

Evaluation of the Graft Copolymer

Water Absorption and Interfacial Free Energy. The water content of the film was determined from the difference between the weight of the graft polymer film soaked in distilled water for more than 24 h and that dried in vacuum at 50°C for 10 h, and expressed by the ratio (%) of the water content to the weight of the wet film. Interfacial free energy γ_{sw} between the graft copolymer film and water was calculated according to the equation of Andrade et al.¹¹ from the contact angle between an air bubble and the film and that between an octane bubble and the film. Values of the contact angles were obtained in water kept at 25°C by using a contact angle goniometer (CA-A type, Kyowa Kagaku Co., Ltd.)

Mechanical Properties. Breakdown strength and elongation were measured by an autograph (Shimazu, Type IM-100) at 25°C.

In Vivo Test. Details of the method were described previously.^{12,13} A pliable and soft suture of about 10 cm in length was coated with the test

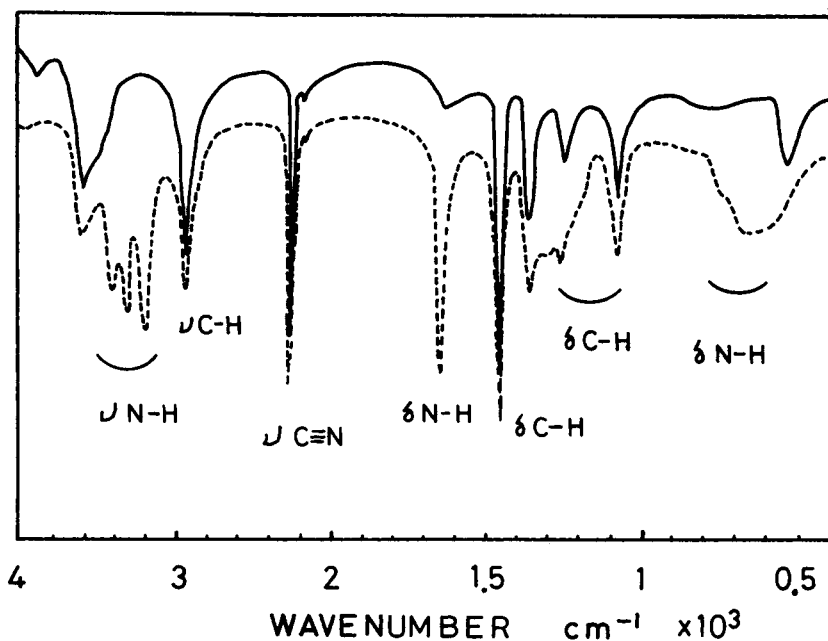


Fig. 1. IR spectrum of PAN and TPAN.

polymer. For the experiment, a dog under general anesthesia was used. An 18 gauge needle was inserted into the peripheral vein and the polymer-coated suture was inserted through the needle into the lumen of the vessel. After the needle was withdrawn, the end of the suture was fixed near the area of the vessel puncture. After a given period of time, the animal was sacrificed by acute exsanguination under general anesthesia and heparin administration. The vein in which the suture was inserted was opened and observation was

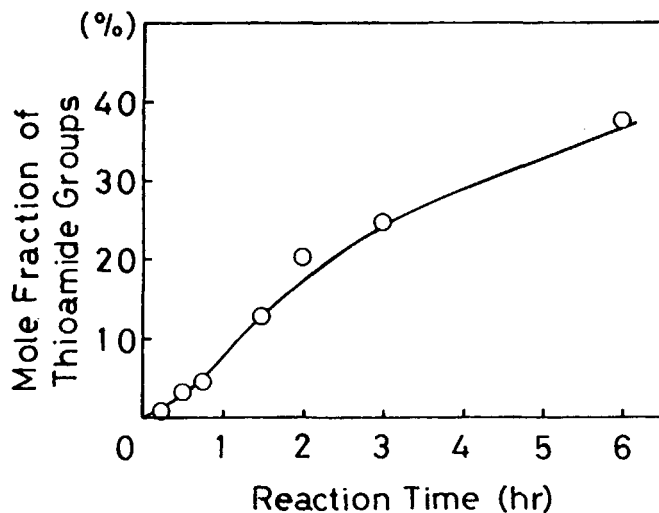


Fig. 2. Change of the degree of thioamidation with time.

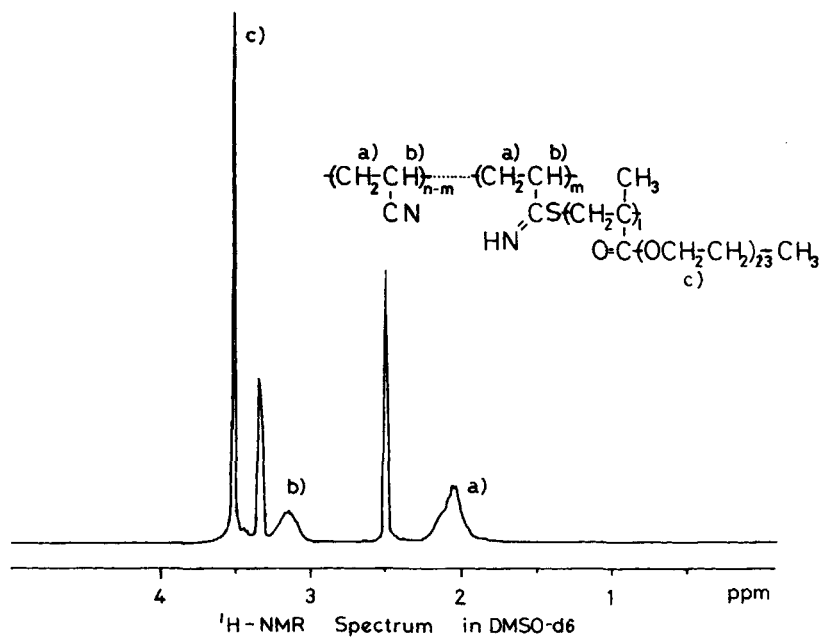


Fig. 3. NMR spectrum of graft copolymer.

carried out macroscopically and by means of light microscopy, scanning, and transmission electron microscopy.

RESULTS AND DISCUSSION

Existence of SH groups in the TPAN obtained was confirmed by the coloring (495 nm in DMSO) due to the SH-SS exchange reaction with 5,5'-dithiobis-(2-nitrobenzoic acid).⁹ Also, IR absorption spectrum is shown in Figure 1, which shows the N-H stretching at $3150\text{--}3450\text{ cm}^{-1}$, N-H bending at 1650 cm^{-1} , and N-H out-of-plane bending at 650 cm^{-1} . Moreover, C=N stretching of PAN at 2240 cm^{-1} decreased, and N-H at 1650 and 650 cm^{-1} increased as the degree of the thioamidation increased. These results show

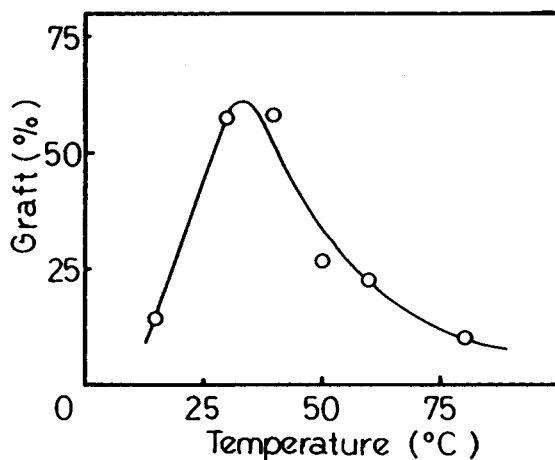


Fig. 4. Dependence of the grafted MG on the polymerization temperature.

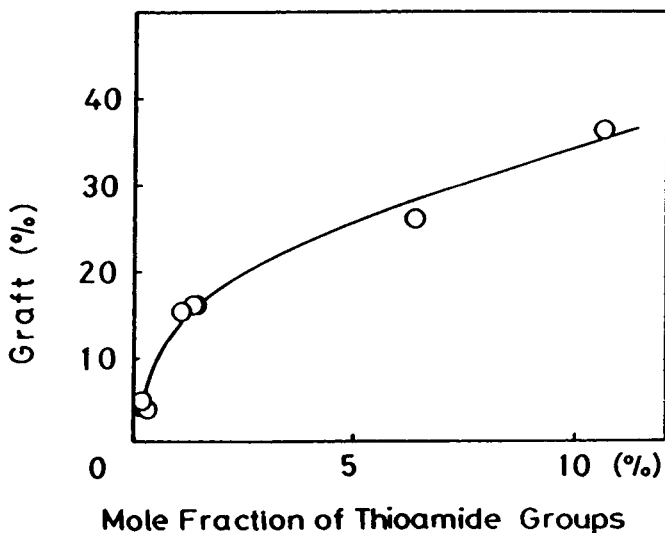
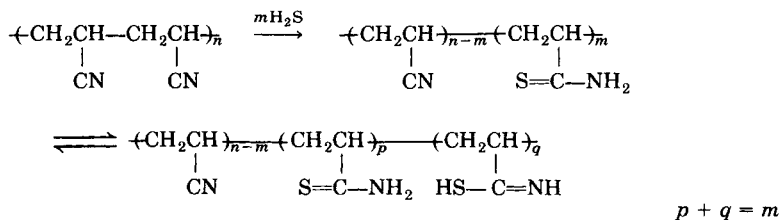


Fig. 5. Dependence of the grafted MG on the degree of thioamidation.

that the following reaction occurred as described in the literature⁵:



Since the degree of the thioamidation obtained by element analysis was proportional to the intensity of inherent X-ray of S obtained by X-ray microanalysis, the latter method was used to determine the degree of thioamidation in most of the runs. The change of the degree of the thioamidation with time is shown in Figure 2. As the degree of thioamidation increased, the TPAN obtained became a rubberlike polymer which was soluble into acetone.

Chemical structure and NMR spectrum of the graft polymer is shown in Figure 3. A signal at 3.6 ppm shows an existence of ethyleneoxide groups. Ratio of the add on weight to the weight of the trunk polymer (graft %) was calculated from the area of the signal due to methyl group (a) at 2.1 ppm and that of ethyleneoxide signal (c) at 3.6 ppm. Also, IR absorption peaks at 288, 1830, and 1110 cm^{-1} showed the existence of OCH_3 , $\text{C}=\text{O}$, and $\text{C}-\text{O}-\text{C}$. From the results, the chemical structure of the graft polymer was confirmed.

In Figure 4, the change of the amount of the grafted MG (wt %) with temperature is shown. At about 30°C, the grafted amount becomes maximum.

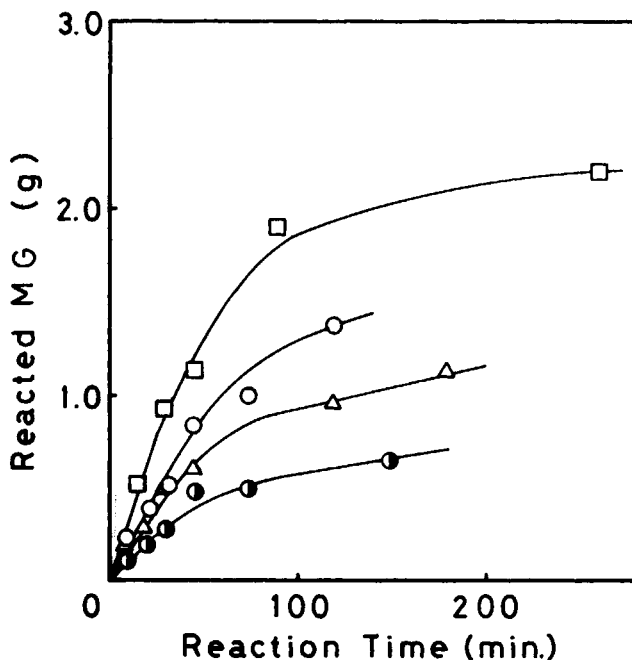


Fig. 6. Change of the reacted MG with time: temperature 30°C; degree of thioamidation 4%; MG concentration; (□) 3.0; (○) 2.0, (△) 1.5; (●) 1.0 g/100 mL, respectively.

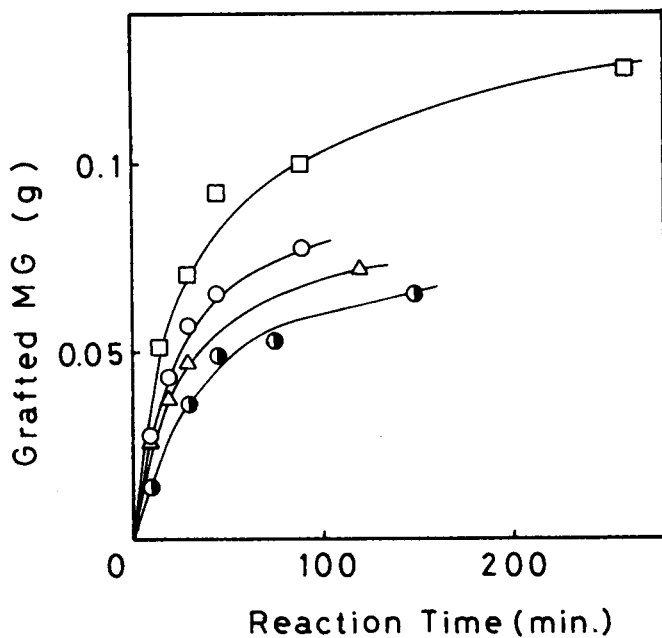


Fig. 7. Change of the grafted MG with time, Experimental conditions are the same as in Figure 6.

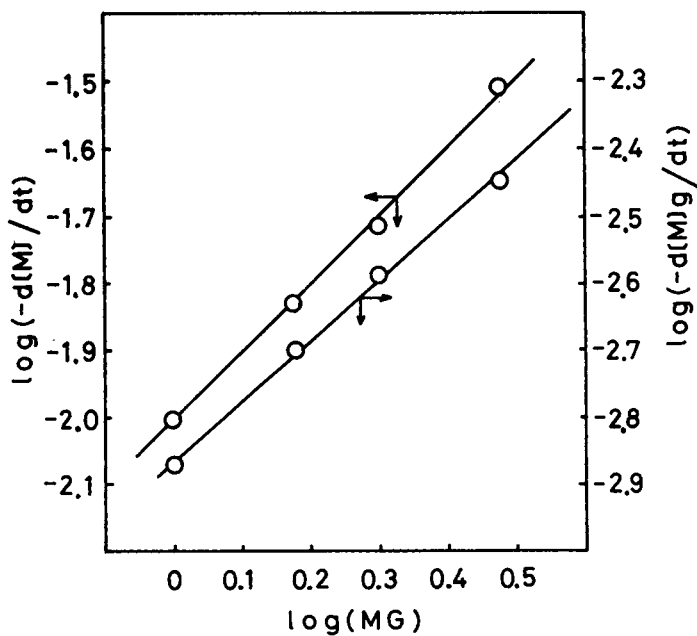


Fig. 8. Dependence of the overall polymerization rate and the grafting rate on the MG concentration. Experimental conditions are the same as Figures 6 and 7.

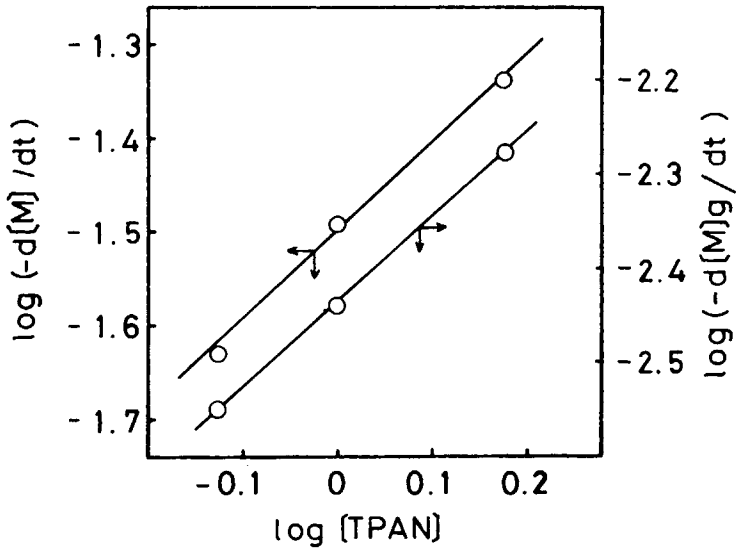


Fig. 9. Dependence of the overall polymerization rate and the grafting rate on the TPAN concentration: temperature 30°C; degree of thioamidation 4%; TPAN concentration, 1.50, 1.00, and 0.75 g/100 mL, respectively.

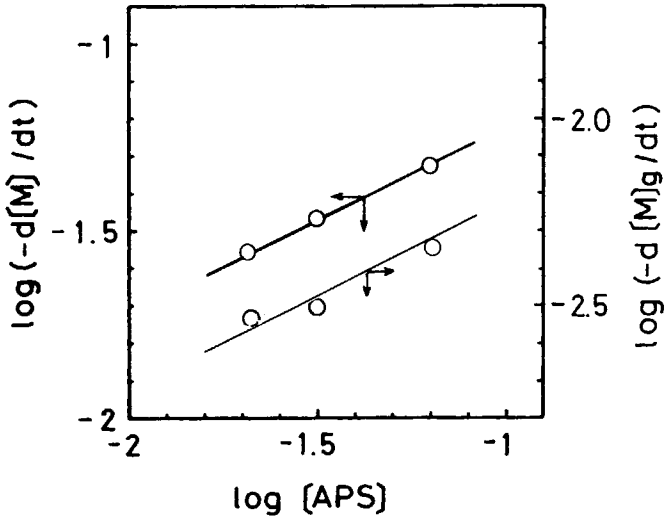


Fig. 10. Dependence of the overall polymerization rate and the grafting rate on the APS concentration: temperature 30°C; degree of thioamidation 4%; APS concentration 0.0619, 0.0315, and 0.0210 g/100 mL, respectively.

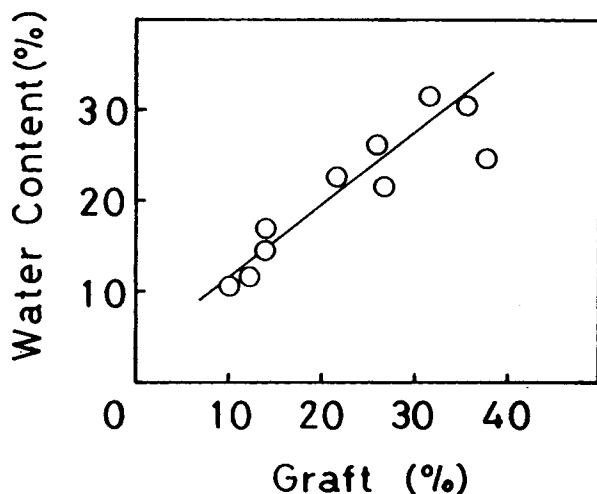


Fig. 11. Change of water content with grafted MG %.

This may be due to a balance between an increase of the polymerization rate and an increase of the session of the side chains.⁶

In Figure 5, it is shown that the grafted MG increases with an increase of the degree of the thioamidation. Also, the gelation occurred at the high degree of the thioamidation. Therefore, mole fractions of thioamide groups were kept below 7% in the most of runs.

Figures 6 and 7 show that the reacted MG and grafted MG increase with time. From the initial slope of the plots, the initial rates of the overall polymerization and the grafting polymerization were obtained. The dependence of these rates on the concentration of MG, $[M]$, are shown in Figure 8. The plots show that the both rates are approximately proportional to $[M]$. Similarly, the dependence of the rates on the $[TPAN]$ and $[APS]$ are shown in

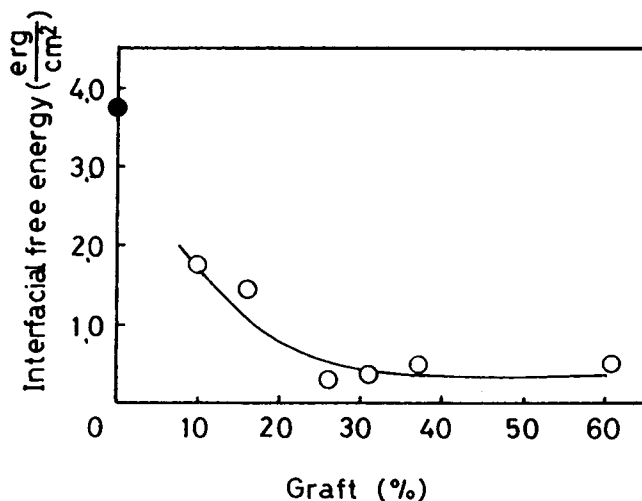


Fig. 12. Change of interfacial free energy with grafted MG %: (○) graft copolymer; (●) PAN.

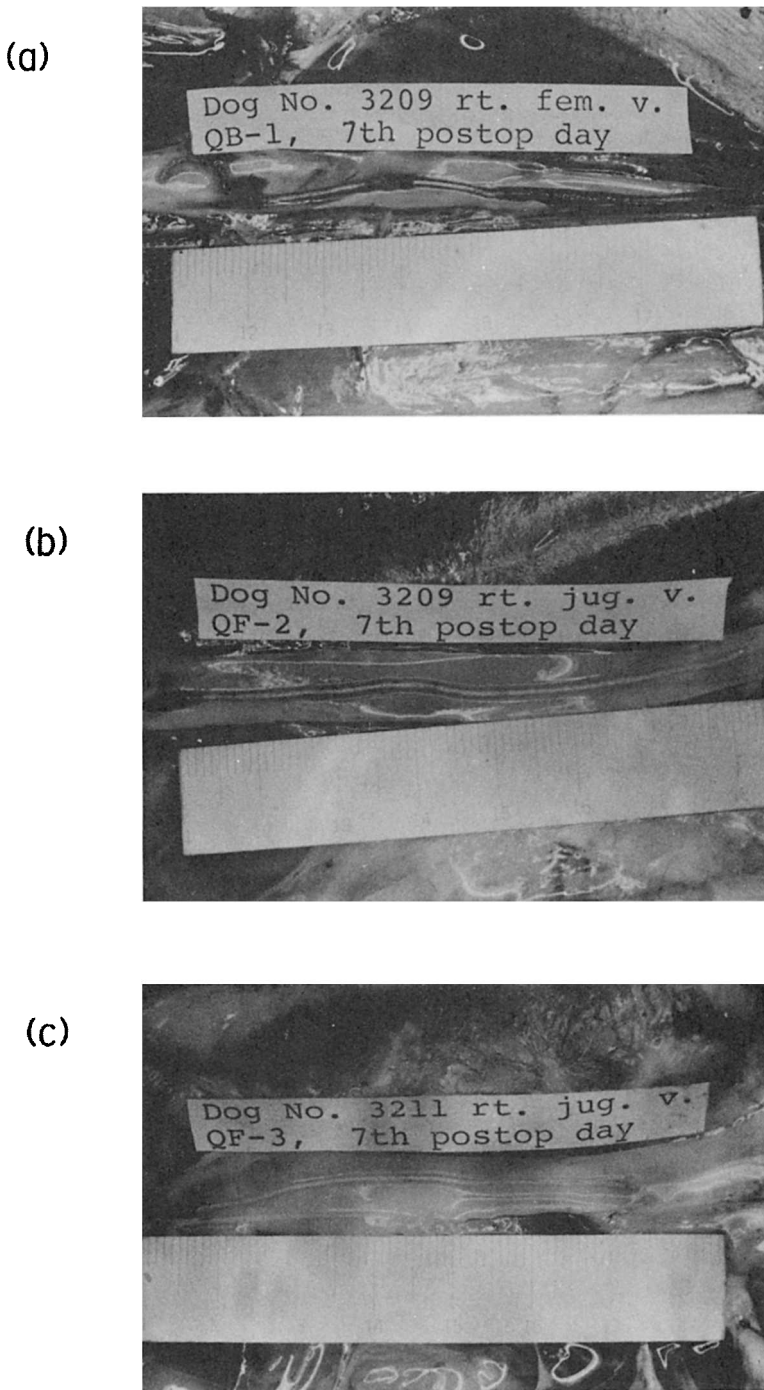


Fig. 13. Open views of peripheral vein in which a structure counted with the polymer was inserted: (a) TPAN QB-1; degree of thioamidation is 0.3%; (b) graft copolymer QF-2; grafted amount of MG and water content are 37.7 and 24.1%, respectively; (c) graft copolymer QF-3; grafted amount of MG and water content are 59.6 and 36%, respectively.

Figures 9 and 10, respectively. The plots show that the both rates are approximately proportional to [TPAN] and $[\text{APS}]^{1/2}$, although the data for the graft polymerization rate against [APS] are scattered. Therefore, the rates of the overall polymerization and the graft polymerization are considered approximately proportional to $[\text{M}] [\text{TPAN}] [\text{APS}]^{1/2}$. This is similar to the rate expression for the graft polymerization of methyl methacrylate¹⁴ onto silk with potassium peroxydiphosphate, where potassium peroxydiphosphate and thiol of the silk forms a redox catalyst and the termination occurs via the two polymer radicals recombination. As shown in Figures 11 and 12, the water content of the graft copolymer increases with an increase of the grafted MG, and the interfacial free energy between the polymer and water decreases with an increase of grafting. This behavior is very similar to that of the graft polymer obtained by the photo-grafting method.^{1,2}

Since the graft copolymer obtained by the present method had almost the same chemical structure and physical properties of that obtained by the photochemical method, it is expected that the graft copolymer has excellent nonthrombogenic property as in the previous report.^{1,2} From the *in vivo* results shown in Figure 13, it is obvious that the graft copolymer shows no thrombus formation, while the trunk polymer shows appreciable thrombus formation. The breakdown tensile strength and elongation of the sample QF-2 in wet state were 654 kg/cm² and 33%, respectively. This shows that the graft copolymer is tough enough for the practical use.

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