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Synthesis and Blood-Compatibility of Poly(ethylene-*g*-γ-benzyl-L-glutamate)†

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ABSTRACT: A novel graft copolymer consisting of commercial chlorosulfonated polyethylene and γ-benzyl-L-glutamate was synthesized. The graft yield was over 70% at a mild reaction condition while we did not observe any crosslinking reaction. Polypeptide units were grafted into sulfonyl groups of the polyethylene. They formed an α-helical conformation as evidence by FT-IR, circular dichroism spectra, and wide angle X-ray diffraction patterns. The mechanical properties of copolymers showed that the Young's modulus and the yield stress increased with peptide content in the polyethylene. The dynamic mechanical property showed that the side chain mobility was increasing due to incoporation of the peptide units.These affected the blood compatibility performance. The clotting time of the GP-4 and GP-5 samples was about 51 minutes. The platelet adhesion performance for the GP-4 and GP-5 samples was less than 5% as investigated by Coulter counter. Fibrin networks were not observed on the surface of GP-4 and GP-5 examined by SEM pictures.

KEY WORDS Chlorosulfonated Polyethylene (CSM) / γ-Benzyl-L-glutamate / Blood-Compatibility / Side Chain Effect / Graft Copolymer /

In many applications, polymeric materials have been used for medical devices and their roles as biomedical materials are increasing. One of the most essential properties for biomedical materials is biocompatibility. Biocompatibility is necessary for all materials that are brought into contact with living systems. Biocompatibility also represents the properties of materials not recognized by the living body as a foreign material and does not induce various biological refusal reactions. Biocompatibility includes blood-compatibility and tissue-compatibility, which can be understood as interfacial phenomena between a living body and foreign materials.[1]

Synthetic poly(amino acid) has been known to be promising as biomedical material because the modified protein components of natural tissue or organs have been widely used in reconstructive surgery and also known to have a good biocompatibility. For these resons, the preparation of graft[2,3] and block[4–7] copolymers containing polypeptide units have been conducted by our research group to obtain biomaterials with improved anti-thrombogenic properties. We investigated the commercial polyurethane, poly(propylene oxide), poly(ethylene oxide) and silicone as the main trunk polymer. The peptide units in the copolymer affected the mechanical properties and blood-compatibility.[2,3,6,7] We also observed that the blood-compatibility of graft copolymer was

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affected by the side chain mobility and conformation of the grafted peptide.[2,3]

The main objectives of the present study are the synthesis of the graft copolymer consisting of polyethylene and poly(γ-benzyl-L-glutamate)[γ-BLG] and investigation of the mechanical properties and blood compatibility of the graft copolymer. We chose chlorosulfonated polyethylene (CSM) as a trunk polymer. CSM is a rubbery material and shows various physical properties depending upon chlorination degree in polyethylene units. The chlorine in CSM can be substituted to an amine unit which will act as an initiator for γ-GLG *N*-carboxy anhydride (NCA).

EXPERIMENTAL

Materials

Chlorosulfonated polyethylene (CSM, Hypalon 40 MW = 34000) was obtained from Du Pont Co. It was dissolved in toluene, precipitated in methanol and then dried in vacuum at 40°C, for a later use. γ-Benzyl-L-glutamate (γ-BLG) from Sigma Chemical Co. and trichloromethyl chloroformat (TCF, phosgene dimer) from Hodogawa Co. were used as received. 1,2-Dichloroethane, chloroform, tetrahydrofuran, ethylacetate, *n*-hexane, toluene, methanol, and benzene were dried and purified with a usual solvent purification process.

Synthesis of Graft Copolymers

A schematic diagram of the synthetic route of graft copolymers is given in Figure 1. After CSM was dissolved in 1,2-dichloroethane, an excess of alkali solution (30% NH_3) was added to the polymer solution and reacted for 15 hours at room temperature. We chose a mild reaction condition to prevent any possible crosslinking reaction.[8] Amine substituted polyethylene (CSM-*s*-NH_2) was prepared after washing with methanol. The graft copolymers were synthesized by reaction of CSM-*s*-NH_2 and γ-BLG NCA which was prepared by the phosgene method.[9] In this reaction, the substituted amine groups on polyethylene act as an initiator for NCA.[2] The graft copolymers were washed with benzene several times to remove a homopolypeptide (PBLG).

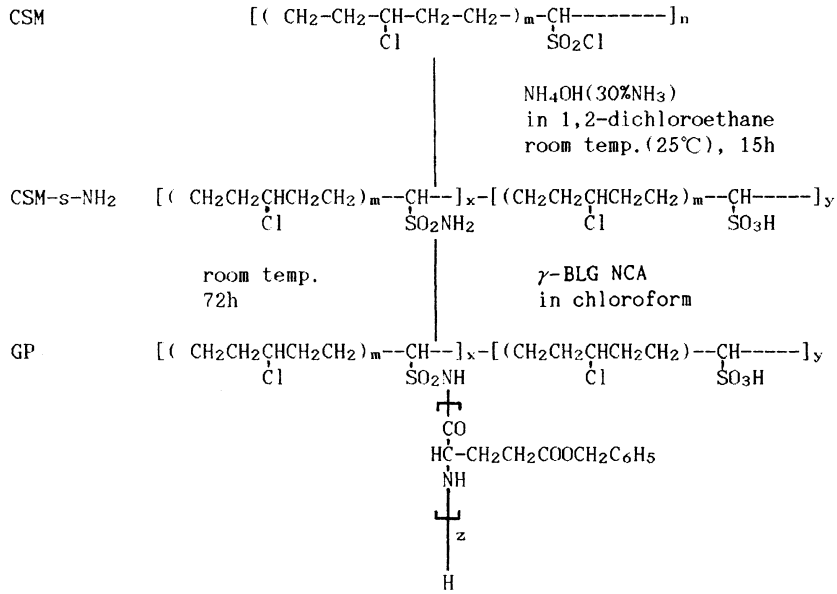

Figure 1. Synthesis of the graft copolymer consisting of γ-benzyl-L-glutamate and chlorosulfonated polyethylene.

Characterization

The composition of copolymers was measured by an elemental analyser (Perkin Elmer, Model 2400). The conformation of poly(γ-benzyl-L-glutamate) was measured by circular dichroism (CD, JASCO Model J-500A), FT-IR (NICOLET 5DX) and wide-angle X-ray diffractometer (RIGAKU, Model DMAX-III B, Ni filter). The mechanical properties of graft copolymers were determined by Instron (INSTRON, Model No. 420) and Rheovibron (ORIENTEC, Model No. D.D.V-2/3, 110 Hz). Scanning electron microscope (SEM, JEOL, Model JSM-35CF) was used to investigate the morphology of adhering platelets on the polymer surface.

Lee–White Method[10]

Polymer samples were coated onto the inside wall of a test tube 15 mm × 125 mm in size (Corning Ltd.). Two ml of fresh human blood collected from a healthy male donor of 24 years old were brought into a test tube to contact the coated polymer. The clotting time was measured and the test was terminated after the blood started to clot.

Column Method[11]

We used a column method to count the number of platelets and investigate the morphology of platelet adhering on the polymer surfaces. Glass beads (40—60 mesh) precoated with 1 (w/v)% polymer solution are packed into a glass tube (10 cm in length, 3 mm in inner diameter). Fresh human blood was collected from a healthy male donor 24 years old, and was passed through the tube packed with precoated glass beads for 3 min at a flow rate of 1 ml min^{-1}. Passed blood was mixed with anticoagulant (ethylenediamine tetraacetic acid [EDTA]) in the sampling bottle to determine the number of platelets using the Coulter counter (Model S-plus). Glass beads precoated with polymer were in good contact with blood for 20 minutes, and the morphology of platelets adhering on the polymer surfaces was investigated using SEM after the platelets on the polymer surface were fixed with 1.25% glutaraldehyde solution.[12]

RESULTS AND DISCUSSION

Composition

Table I lists the composition of CSM-*s*-NH_2 samples. Chlorosulfonated polyethylene (CSM) contains about 1 wt% sulfur as determined by the elemental analysis. We can estimate the mole of sulfur and mole of amine group from the wt% of sulfur and nitrogen in the base polymer. The net result was about 10.6 mol of sulfur/mol of trunk polymer. In a similar manner, the number of nitrogens in the CSM-*s*-NH_2 samples was determined to be about 5.6 mol mol^{-1}. This amount of nitrogen contributed to the incoporation of amine groups into the polymer. Amine groups will act as an initiator for NCA. We can also estimate the content of sulfonic acid groups formed as a side product present in the graft copolymer.

Table II lists the degree of polymerization and the graft % of peptide in the copolymer. In graft copolymers, the graft percent varies from 10 to 222% and the degree of polymerization of peptide units varies from 3.0 to 64.0. Also, the graft yield is over 70% for all the samples. Because the reaction was carried out at room temperature, cross-linking of $-SO_2-$ type may not occur.[13]

Table I. Composition of CSM-*s*-NH_2

Samples	Wt % of S[a]	Wt % of N[a]	No. of S[b]	No. of N[b]	No. of sulfonic acid[c]
CSM	1.0	—	10.6	—	—
CSM-*s*-NH_2	1.0	0.23	10.6	5.6	5.0

[a] Determined by elemental analysis.
[b] Calculated from the wt% of S and N, respectively.
[c] [number of S]−[number of N].

Table II. Molecular characterization of graft copolymer

Samples	[A]/[I][a]	Nitrogen %[b] in copolymer	Graft % of peptide[c] in copolymer	Graft[d] yield %	Degree of[e] polymerization of peptide
GP-1	4.0	0.79	10	83	3.0
GP-2	14.0	1.34	26	76	7.0
GP-3	26.0	2.28	59	73	17.0
GP-4	36.0	2.88	87	72	24.0
GP-5	44.0	3.27	115	77	32.0
GP-6	76.0	4.32	222	82	64.0

[a] Ratio of amino acid content to initiator ($-NH_2$) content.
[b] Determined by elemental analysis.
[c] {[wt of copolymer − wt of trunk polymer]/wt of trunk polymer} × 100 (%).
[d] {[wt of copolymer − wt of trunk polymer]/wt of init. amino acid} × 100 (%).
[e] [increased wt of copolymer]/[unit wt of animo acid]. Increased wt of copolymer can be calculated from b or c.

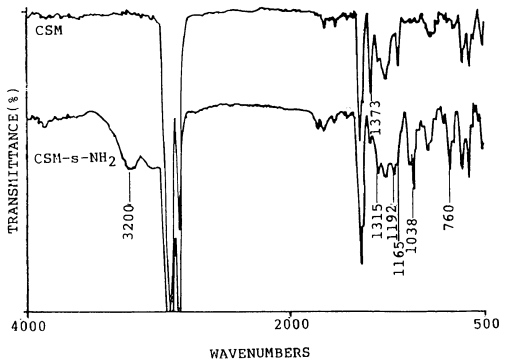

Figure 2. Infrared spectra of CSM and CSM-*s*-NH_2 films cast from chloroform solution.

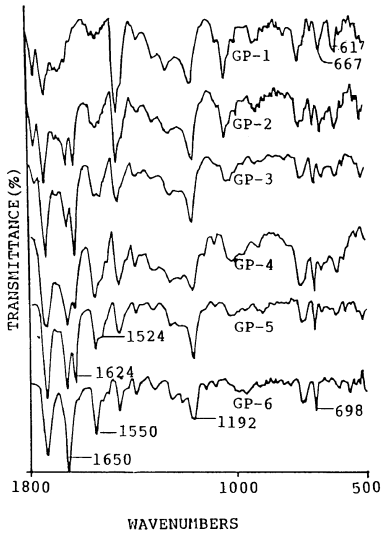

Figure 3. Infrared spectra of graft copolymer films cast from chloroform solution.

Conformation

Conformation of the graft copolymers was measured by FT-IR (Figure 2). In the case of CSM-*s*-NH_2, N–H stretching vibration appeared at 3200 cm^{-1} and S=O asymmetric and symmetric stretching peaks were shown at 1315 cm^{-1} and 1192 cm^{-1}, respectively. Characteristic asymmetric stretching peak of S=O for sulfonyl chloride in CSM appeared at 1373 cm^{-1}. As the CSM is aminated or grafted, the peak intensity at 1373 cm^{-1} is reduced.

Figure 3 shows infrared spectra of the grafted peptide in copolymers. Broat *et al.*[13] already reported that characteristic peaks representing an α-helical conformation of peptide appear at 1650 cm^{-1} and at 1550 cm^{-1} for amide I and II, respectively. Also the characteristic peaks for β-sheet form appeared at 1630 cm^{-1} and at 1530 cm^{-1} for amide I and II, respectively.[13] For graft copolymers studied here we confirmed that the peptide units in the graft copolymer have an α-helical conformation and that an α-helical content increased with the grafted peptide content.

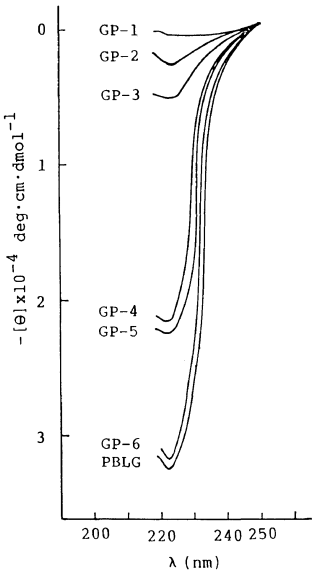

Figure 4. Circular dichroism spectra of graft copolymers and PBLG homopolymer in THF solution measured at 25°C.

Table III. $[\theta]_{222}$ values in THF (25°C)

Sample	Graft % of peptide in copolymer	$-[\theta]_{222}\times10^{-4}$	$[\theta]^c_{222}/[\theta]^o_{222}$
GP-1	10	0.0237	0.0073
GP-2	26	0.2542	0.0785
GP-3	59	0.5058	0.1561
GP-4	87	2.1739	0.6710
GP-5	115	2.2270	0.6873
GP-6	222	3.1852	0.9831
PBLG	0	3.2400	1.0000

Figure 4 shows circular dichroism spectra of graft copolymers and PBLG homopolymer which were dissolved in tetrahydrofuran solution at 25°C. The negative peak appeared at around 222 nm indicating that the grafted polypeptide has an α-helical conformation.[14] The negative peak intensity increases with polypeptide content in the graft copolymer. GP-6 shows a similar CD pattern with PBLG. The helical content in the graft copolymer can be calculated as the ratio of ellipticity of graft copolymer to that of polypeptide (PBLG) itself

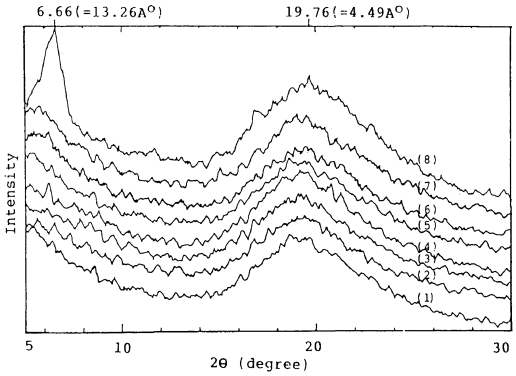

Figure 5. WAXD profiles of graft copolymers: (1) CSM; (2) CSM-*s*-NH_2; (3) GP-1; (4) GP-2; (5) GP-3; (6) GP-4; (7) GP-5; (8) GP-6.

Table IV. Mechanical properties of CSM CSM-*s*-NH_2, and the graft copolymer

Sample	Graft % of peptide in copolymer	Young's modulus	Yield stress
		kg cm^{-2}	kg cm^{-2}
CSM	—	28.0	13.0
CSM-*s*-NH_2	—	31.0	12.0
GP-1	10	30.0	13.0
GP-2	26	59.0	22.0
GP-3	59	157.0	36.0
GP-4	87	259.0	47.0
GP-5	115	260.0	46.0
GP-6	222	694.0	103.0

and is listed in Table III. Note that the helical content increases rapidly when the graft % of peptide in graft copolymer is more than 50%. The GP-6 sample has about the same helical content as the PBLG.

Wide-angle X-ray diffraction patterns of graft copolymers and CSM are presented in Figure 5. In these profiles, a typical peak of amorphous region in polyethylene is shown at $2\theta=19.76$ and the crystalline peak of peptide appeared at $2\theta=6.66$.

Mechanical Properties

Static mechanical properties of graft copolymers and CSM were measured by Instron. CSM behaves like an uncrosslinked rubber.

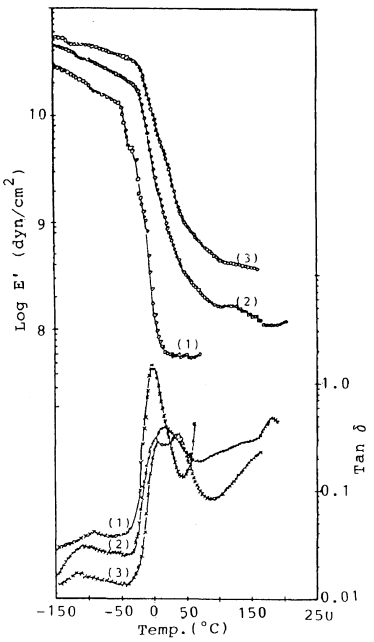

Figure 6. Dynamic mechanical properties of (1) CSM, (2) GP-2, and (3) GP-6.

However the graft copolymers containing the peptide behave like cross-linked rubber. Their toughness increased. Young's modulus and yield stress of graft copolymers are listed in Table IV. The incoporation of peptide, in the polyethylene unit changed the rubber-like CSM into a rigid graft copolymer. Young's modulus increased rapidly when the graft % of peptide in the copolymer exceeds about 50%.

Figure 6 shows the dynamic viscoelastic properties of the graft copolymer. From the tangent delta to temperature profile of GP-2 and GP-6, the chain mobility of graft copolymers increased with side chain length. We speculate that CSM has only micro-Brownian motion of the main chain, while the graft copolymers has not only micro-Brownian motion of the main chain but also side chain mobility due to incoporation of a rigid side chain. We expect that this side chain mobility should affect the blood-compatibility of graft copolymers.

From the above results, CSM, GP-1, GP-2, and GP-3 are relatively strong and rubbery materials, while GP-4, GP-5, and GP-6 samples are similar to the cross-linked rubber. Note that the mobility of graft copolymers is increasing with the side chain length.

Table V. Platelets adhesion performance and degree of polymerization

Samples	Platelets adhesion performance[a]	Degree of polymerization[b] of peptide
	%	
CSM	14.9	—
GP-1	8.3	3.0
GP-2	6.9	7.0
GP-3	4.7	17.0
GP-4	4.7	24.0
GP-5	4.7	32.0
GP-6	12.3	62.0

[a] [number of platelets in the column]/[number of platelets in the fresh blood]
[b] See d in Table II.

Table VI. Clotting time and ratio of CSM and the graft copolymer measured at 37°C

Samples	Clotting time	Clotting time ratio[a]
	min	
CSM	9	1.1
GP-1	25	3.1
GP-2	24	3.0
GP-3	26	3.2
GP-4	51	6.4
GP-5	51	6.4
GP-6	22	2.7
PBLG	12	1.5
Glass	8	1.0

[a] [clotting time of polymer samples]/[clotting time of glass].

Blood Compatibility

In this study, the Lee–White method and microsphere column method were utilized to evaluate the blood-compatibility of graft copolymers *in vitro*. The results are shown in Table V. The platelets adhesion performance of polymers is calculated as the ratio of the

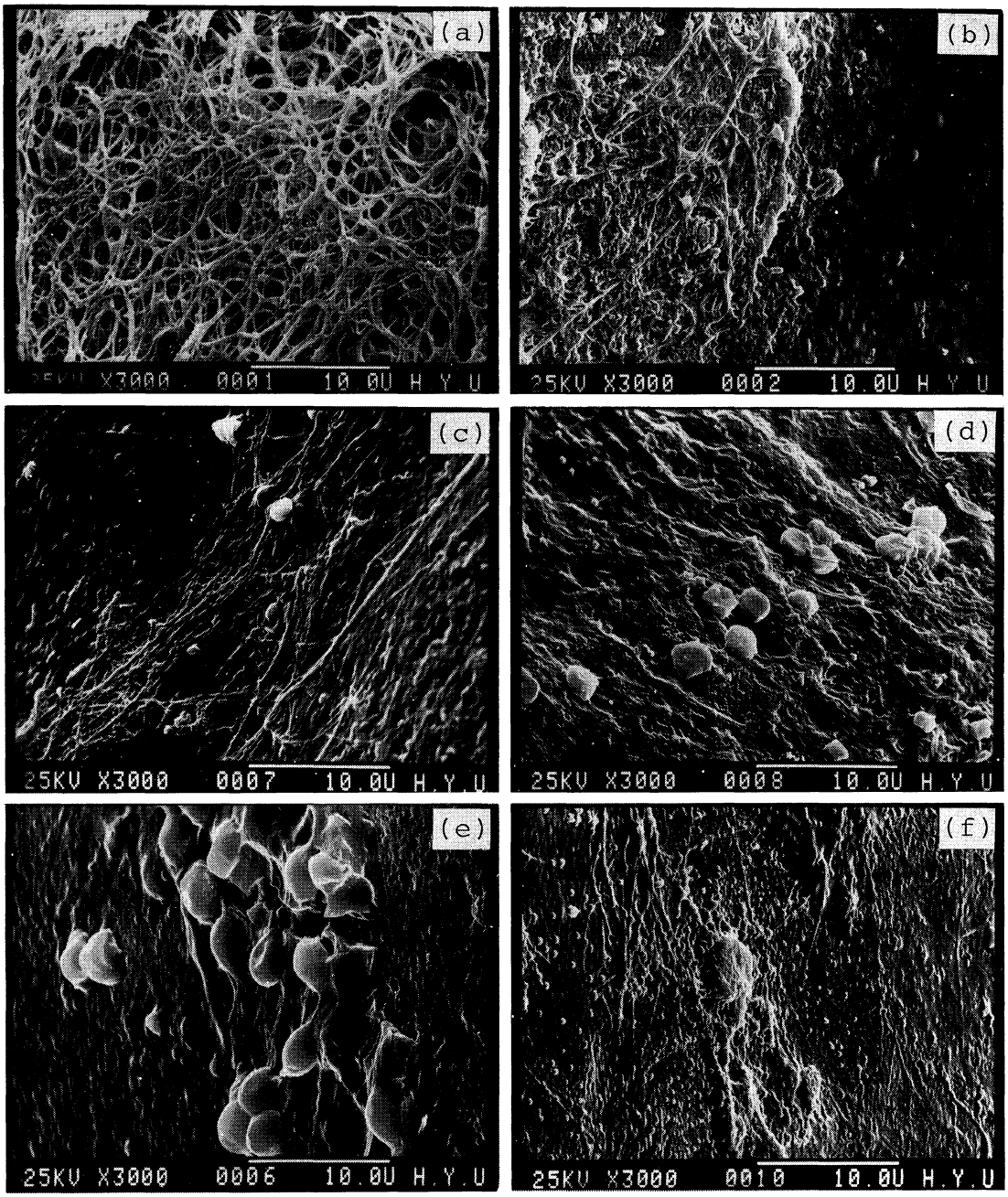

Figure 7. Scanning electron microscope pictures of platelets adhering onto sample surfaces: (a) CSM; (b) PBLC; (c) GP-2; (d) GP-4; (e) GP-5; (f) GP-6.

number of platelets adhered into the glass column to the number of platelets in the fresh whole blood. GP-4 and GP-5 samples show less than 5% platelets adhesion performance.

The morphology of the adhering platelets on the polymer surfaces was investigated with

SEM and are shown in Figure 7. We found that the fibrin networks were formed on the polymer surfaces except for GP-4 and GP-5 samples, where the platelets morphology only changed to a small extent, that is, platelets only adhered on their surfaces.

The VI shows the results of the Lee–White method. From blood clotting time data using this method, we concluded that the blood-compatibility of the graft copolymers was superior to the unmodified polyethylene. Note that GP-4 and GP-5 samples have the clotting time of over fifty minutes that is similar to the blood compatibility of the commercial biomedical materials.

CONCLUSIONS

We synthesized a novel graft copolymer consisting of commercial chlorosulfonated polyethylene grafted with γ-benzyl-L-glutamate. The graft yield was over 70% at a mild reaction condition where we did not observe any crosslinking reaction. The polypeptide units were grafted into the sulfonyl groups of polyethylene and formed an α-helical conformation as evidenced by FT-IR, circular dichroism spectra and wide angle X-ray diffraction patterns. The mechanical properties of the copolymers showed that the Young's modulus and yield stress increased with peptide content in polyethylene. The dynamic mechanical property data showed that the side chain mobility increased due to incorporation of the peptide units. These affected blood compatibility performance. The clotting time of the GP-4 and GP-5 samples was about 51 minutes. The platelets adhesion performance for the GP-4 and GP-5 samples was less than 5% as investigated by Coulter counter and we did not find fibrin networks on the surfaces of GP-4 and GP-5 samples as seen in the SEM pictures.

REFERENCES

1. H. Tanzawa, S. P. Nakaba, G. H. Atsumi, N. Kondou, and M. Siba, "Biomedical Polymer," Kyoritsu Publishing Co., Tokyo, 1978, p 62.
2. K. Y. Kim, S. W. Jang, J. H. Kim, Y. K. Sung, and C. S. Cho, *Polymer (Korea)*, **12**, 455 (1988).
3. K. Y. Kim, S. W. Jang, Y. K. Sung, and C. S. Cho, *Polymer (Korea)*, **13**, 238 (1989).
4. K. Y. Kim, Y. M. Lee, Y. G. Lee, and K. J. In, *Polymer (Korea)*, **7**, 53 (1983).
5. K. Y. Kim, Y. G. Yang, Y. Kuroyanagi, and M. Seno, *Polymer (Korea)*, **9**, 572 (1985).
6. C. S. Cho, S. C. Song, S. O. Sub, K. Y. Kim, S. W. Jang, and Y. K. Sung, *Polymer (Korea)*, **13**, 134 (1989).
7. K. Y. Kim, S. W. Jang, D. S. So, C. S. Cho, and Y. K. Sung, *Polymer (Korea)*, **13**, 529 (1989).
8. K. J. Saunders, "Organic Polymer Chemistry," Chappan and Hall, London, 1976, p 58.
9. R. B. Woodward and C. H. Scharm, *J. Am. Chem. Soc.*, **69**, 1551 (1947).
10. R. I. Lee and D. D. White, *J. Am. Med. Sci.*, **145**, 495 (1913).
11. K. Kataoka, T. Tsuruta, T. Akaike, and Y. Sakurai, *Makromol. Chem.*, **179**, 1121 (1978).
12. H. H. Weetal and A. A. Filbert, "Method in Enzymology," Vol. 44, Academic Press, New York, N.Y., 1976, p 59.
13. E. R. Broat and A. Asabourian, *J. Am. Chem. Soc.*, **78**, 145 (1956).
14. T. Hayashi, A. G. Walton, and J. M. Anderson, *Macromolecules*, **10**, 346 (1977).