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# PHYSICAL AND BIODEGRADATION PROPERTIES OF A–B–A TYPE BLOCK COPOLYMER MEMBRANES CONSISTING OF POLY(*N*-HYDROXYALKYL-L-GLUTAMINE) AS THE A COMPONENT AND POLYBUTADIENE AS THE B COMPONENT

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**Abstract**—A–B–A type block copolymer [GBG(A)] membranes consisting of poly(*N*-hydroxyalkyl-L-glutamine) (PHAG) as the A component and polybutadiene (PB) as the B component were prepared by carrying out aminoalcoholysis reaction with 2-amino-1-ethanol or 5-amino-1-pentanol (Pe) together with a crosslinking reaction with 1,8-octamethylenediamine (OMDA) on membranes of the starting block copolymer membranes consisting of poly(γ-benzyl L-glutamate) and PB. It was shown that the effective crosslink density was proportional to the molar % of OMDA in the reaction mixture. The relation between their bulk structure and membrane properties was investigated, such as the swelling ratio, *q*, in a pseudo-extracellular fluid (PECF), tensile properties, and enzymatic degradation behavior of the membranes in PECF. Tensile properties of the hydrophilic membranes were highly dependent on *q* in PECF, and on the hydrophobic portions in molecular chains, whose behavior was typical of an elastomer. Biodegradation of samples *in vitro* by papain indicated that the degradation was a bulk rather than a surface phenomenon, and that the rate of degradation was also highly dependent on *q* of membranes in PECF. Copyright © 1996 Elsevier Science Ltd

## INTRODUCTION

Poly(α-amino acid)s and their copolymers which carry hydrophilic components offer good potential for enzymatical biodegradable medical applications, such as temporary artificial skin substrates in burn therapy, temporary barriers to prevent adhesion between natural tissue planes damaged by either accidents or surgery, polymer carriers for conjugates coupled to proteins for therapeutic use and drug delivery systems [1]. For this purpose, we have prepared many types of hydrophilic poly(α-amino acid) membranes which are composed of hydrophilic and hydrophobic amino acid components. Among them, hybrid block copolymers composed of naturally occurring biopolymer blocks and synthetic polypeptide blocks are of interest due to their microheterophase structure.

In previous studies, one of the authors (T.H.) synthesized A–B–A type block copolymers (GBG) composed of poly(γ-benzyl-L-glutamate) (PBLG) and polybutadiene (PB) of high molecular weight and characterized the solid-state properties [2–5].

In this paper, we prepared an A–B–A type block copolymer [GBG(A)] consisting of *N*-hydroxyalkyl-L-glutamine [G(A)] as the A component and PB as the B component, as well as the corresponding homopolymer, poly(*N*-hydroxyalkyl-L-glutamine) (PHAG), and investigated the relationship between the bulk structures and membrane properties, such as the swelling ratio (*q*) of a pseudo-extracellular fluid (PECF), the tensile properties in PECF and the enzymatic degradation behavior *in vitro* of the membranes in PECF solution from an application point of view for the biomedical materials. PECF [6] includes 145 m-equiv/l of $Na^+$, 5 m-equiv/l of $K^+$, 118 m-equiv/l of $Cl^-$, 30 m-equiv/l of $HCO_3^-$, and 2 m-equiv/l of $HPO_4^-$, which is similar to that of physiological fluid. Papain was selected as a model protease in this study, which is a well characterized plant thiol endopeptidase [7, 8] with a broad range of specificity. It is closely related to cathepsin B, a thiol endo-peptidase that has been isolated from mammalian spleen, liver, kidney, or lung, and is released by the cells in response to inflammation.

## EXPERIMENTAL PROCEDURES

### *Materials*

*Synthesis of amine-terminated polybutadine.* Organic solvents and chemicals were purchased from Wako Pure Chem. Ind., Ltd (Tokyo, Japan). Butadiene was purified by the usual procedures and distilled over lithium aluminium hydride. Tetrahydrofurane (THF) and cyclohexane were distilled from sodium naphthalide and butyl-lithium

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solutions, respectively. Difunctional anionic initiator was prepared from 1,3-diisopropylbenzene with *sec*-butyl lithium [9]. Anionic polymerizations of butadiene, initiated by the difunctional initiator in cyclohexane at 25°C, were performed under high vacuum conditions in sealed glass reactors. The telechelic PB with amino end groups was generated by the reaction of anionic-living PB with the aminating reagent, 2,2,5,5-tetramethyl-1-(3-bromopropyl)-1-aza-2,5-disilacyclopentane which was prepared according to the method previously reported in cyclohexane and THF.

The molecular weight of the amine-terminated PB (ATPB; which is the abbreviation adopted here for amine-terminated polybutadiene) was calculated using gel-permeation chromatography (GPC) in THF using a TOSO HLC-8020 model, calibrated with polystyrene standards. The yield of amination was determined by titration with perchloric acid in glacial acetic acid, using Cresyl Violet as an indicator. The ratio of the weight-average molecular weight $M_w$ to the number-average molecular weight $M_n$ of the ATPB was estimated to be 1.10 by gel-permeation chromatography. The ATPB was purified before use with benzene, methanol, and water. The amine equivalent weight of the ATPB was found to be 2500 by potentiometric titration with $HClO_4$ in acetic acid. Thus, the number-average molecular weight $M_n$ of the ATPB was 5000.

*Synthesis of GBG block copolymers.* The monomer, *N*-carboxy-γ-benzyl-L-glutamate anhydride (γ-BLG-NCA) was prepared according to the method previously reported [2] and purified by repeated recrystallization from an ethyl acetate solution with the addition of petroleum ether. The respective amount of γ-BLG-NCA and ATPB was calculated to obtain the desired degree of polymerization of the polypeptide block. The polymerization was carried out in the absence of moisture, at room temperature, in a dioxane–methylene dichloride (1:1, v/v) mixture at a total concentration of γ-BLG-NCA and ATPB, 3%. The polymerization was followed by i.r. spectroscopy (disappearance of the bands at 1860 and 1790 $cm^{-1}$ was characteristic of NCA). After 48 hr, the polymerization was terminated, and the block copolymers, PBLG-PB-PBLG (GBG), were precipitated by five volumes of pure cold methanol. This method of precipitation allowed the elimination of traces of γ-BLG-NCA still present and of the oligopeptide formed by autopolymerization. Then samples were dried *in vacuo*. The purification of the block copolymers started with a selective extraction of the homopolymers included. The polydiene fragment, which was isolated, and a low molecular weight of PBLG was extracted with n-hexane. The block copolymer was then fractionated in a mixture of chloroform, n-hexane, and ethanol at 25°C. Each of the block copolymer samples was separated into four to five fractions, and the central portions of them were used for the succeeding experiments.

*Molecular characterization*

*Molecular weight of samples.* Molecular weights of samples, PBLG homopolymer and GBG block copolymers, were estimated from the intrinsic viscosity ($\eta$) measurement in dichloroacetic acid (DCA) solutions of the polymers by applying the $[\eta]$-$M_w$ relationship obtained for the GBG block polymer as well as PBLG previously [10].

Table 1. Molecular characterization of original samples

| | γ-BLG | | | $[\eta]$(dl/g) |
|---|---|---|---|---|
| Sample code | mol%* | mol%† | $M_w$* | (DCA, 25°C) |
| PBLG-1 | 100.0 | | 220,800 | 1.24 |
| GBG-1 | 91.2 | 90.9 | 208,600 | 1.18 |
| GBG-2 | 82.7 | 82.3 | 99,500 | 0.62 |

*From elemental analysis.
†From the $[\eta]$-$M_w$ relationship obtained for PBLG previously [10] in DCA, 25°C; $[\eta] = 2.78 \times 10^{-5} M_w^{0.87}$.

The preparative data of these samples are summarized in Table 1.

*Composition of block copolymers.* The composition of γ-BLG in each of block copolymer samples was determined from the data of the molecular weight of block copolymers, because the molecular weight of the middle block ATPB was obtained as 5000. Further, the composition was also determined from elemental analyses of N, C, and H atoms by using CHN-O-RAPID Type of Helaus Co. The resulting data were also listed in Table 1.

*Preparation of hydrophilic membranes*

After a polypeptide membrane (4.0 × 2.0 cm) of 0.05–0.1 mm in thickness was cast from a chloroform solution, the aminolysis reaction of γ-BLG residue was carried out by applying 2-amino-1-ethanol (E) or 5-amino-1-pentanol (Pe). The sample membrane was immersed in the mixture of 2-amino-1-ethanol and a crosslinker, 1,8-octamethylenediamine (OMDA), as well as the mixture of 1-pentanol, and OMDA with appropriate composition at 58–62°C for 48 hr. After that, the hydrophilic membrane was washed with methanol, pure water, ethyl ether, and stored in ethanol. Figure 1 illustrates a schematic diagram of the preparation of hydrophilic membranes.

*Physical measurements of hydrophilic membranes*

The swelling ratio $q$ in PECF was determined by equilibrating the membrane in PECF solution at 37°C. The membrane was removed, blotted to remove surface PECF, and weighed until a constant weight was achieved. The membrane was then dried in a vacuum oven. $q$ was defined as the ratio of the weight of PECF swelling hydrophilic membrane to that of the dried one. Table 2 summarizes experimental data of the membranes.

The tensile properties of hydrophilic membranes were measured by a Tensilon UTM-II-20 (Toyo-Boldwin Co., Tokyo, Japan) using the standard techniques in PECF at 25°C. All the samples were tested at an elongation rate of 40% per min.

*Biodegradation of hydrophilic membranes* in vitro

Enzymatic degradation studies *in vitro* were carried out using papain. Papain (3.5 m Anson μg/mg, No. 7144, Sigma) was purchased from Nacalai Tesque (Kyoto, Japan), and used without further purification. Unless stated, all measurements were made in a system containing 10 mM cystein and 40 mM ethylene diaminotetraacetate (EDTA) in the PECF solution. A series of the crosslinked hydrophilic membranes were exposed to PECF solution at pH 7.4 and 37°C. These membranes were removed from the enzyme solution at appropriate time intervals, weighed, and then vacuum-dried at 60°C to constant weights.

## RESULTS AND DISCUSSION

### *The swelling ratio of hydrophilic membranes in PECF*

The swelling ratio $q$ in a solvent is determined by the interaction energy between the solvent molecules and polymer segments as well as the elastic energy (crosslink density) for a solvent-swollen polymer. On the other hand, the precise crosslink density has not been determined because of the uncertainty in the relative reactivities of 2-amino-1-ethanol and OMDA, and also because estimation of the fraction of the reacted diamine molecules which form effective crosslinks is difficult. The effect of the crosslinker, OMDA, concentration in the reaction on $q$ of the crosslinked membranes in PECF is shown in Fig. 2. $q$ in PECF decreases with increasing OMDA molar concentration in the reaction mixture.

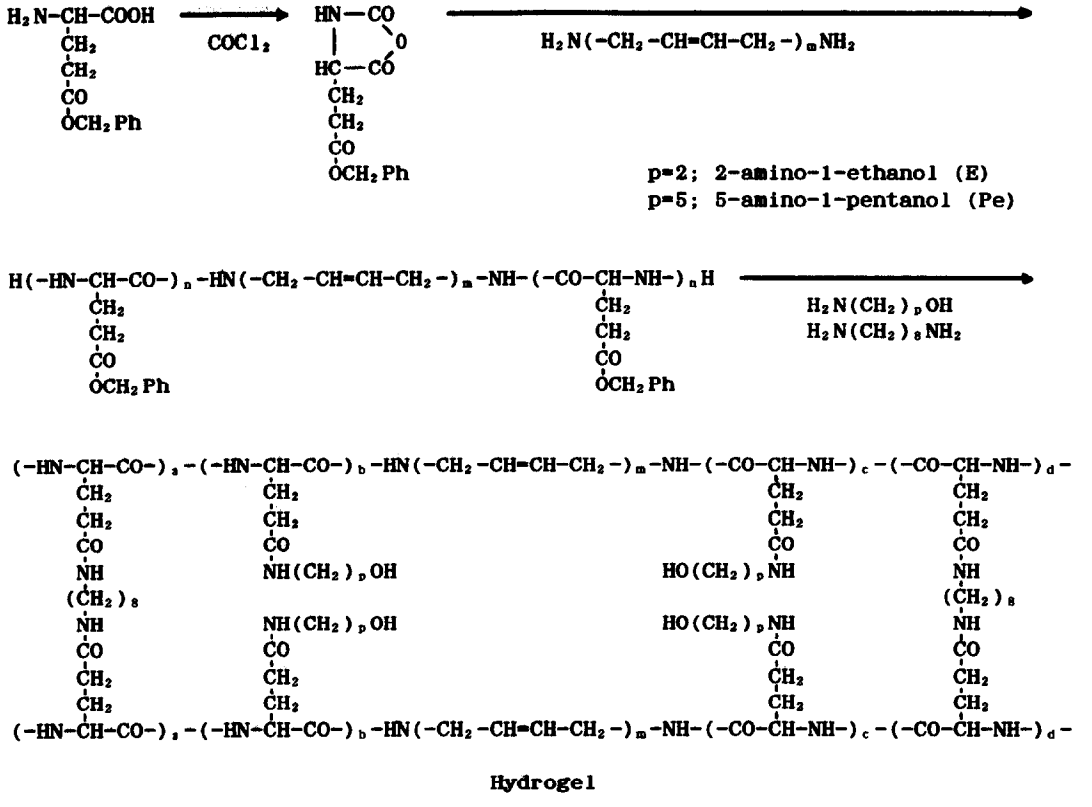


Fig. 1. Schematic diagrams of the preparation of hydrophilic membranes.

When $q$ is quite large, it is given by the following equation according to the rubber elasticity theory [11]:

$$q^{5/3} = (vM_c)\,(1 - 2M_c/M)^{-1}(1/2 - \chi_1)/V_1 \quad (1)$$

where: $M_c$ is the molecular weight per crosslinked unit; $M$ the primary molecular weight; $v$ the specific volume of polymer; $V_1$ the molar volume of solvent; and $\chi_1$ the interaction parameter. The factor $(1 - 2M_c/M)$ expresses the correction for network imperfections resulting from chain ends. For a quite high molecular weight polymer chain, it reduces to unity. As the effective crosslink density, $f_c$ is proportional to the value of $M_o/M_c$, where $M_o$ is the molecular weight of the repeat unit (monomeric unit), equation (1) may be simplified as

$$q^{5/3} = k(vM_o)\,(1/2 - \chi_1)V_1 f_c. \quad (2)$$

Thus, log $q$ is in linear relation with log $f_c$ with the slope as $-\frac{3}{5}$.

Table 2. Preparative data of hydrogels

| Sample code | Starting polymer | OMDA (mol%) | $q$ ($W_w/W_d$) |
|---|---|---|---|
| PHEG-1-1 | PBLG-1 | 1.0 | 15.2 |
| PHEG-1-2 | | 2.0 | 9.9 |
| PHEG-1-3 | | 3.0 | 7.8 |
| PHEG-1-4 | | 4.0 | 6.7 |
| GBG(E)-1-1 | GBG-1 | 1.0 | 11.5 |
| GBG(E)-1-2 | | 2.0 | 8.2 |
| GBG(E)-1-3 | | 3.0 | 6.4 |
| GBG(E)-1-4 | | 4.0 | 5.8 |
| GBG(E)-2-1 | GBG-2 | 1.0 | 6.8 |
| GBG(E)-2-2 | | 2.0 | 5.4 |
| GBG(E)-2-3 | | 3.0 | 4.9 |
| PHPeG-1-1 | PBLG-1 | 1.0 | 4.2 |
| PHPeG-1-2 | | 2.0 | 3.5 |
| PHPeG-1-3 | | 3.0 | 3.2 |
| GBG(Pe)-1-1 | GBG-1 | 1.0 | 3.1 |
| GBG(Pe)-1-2 | | 2.0 | 2.9 |
| GBG(Pe)-1-3 | | 3.0 | 2.6 |

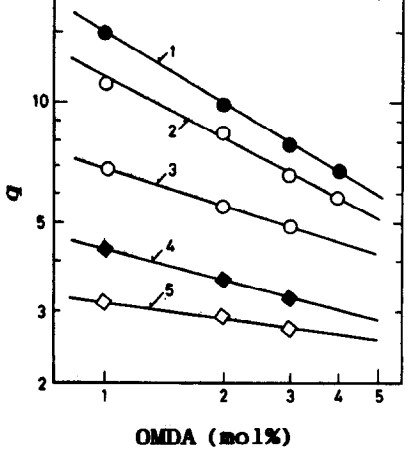


Fig. 2. The swelling ratio $q$ ($= W_w/W_d$) of PECF vs mol% of OMDA for: (1) PHEG-1; (2) GBG(E)-1; (3) GBG(E)-2; (4) PHPeG-1; and (5) GBG(Pe)-1.

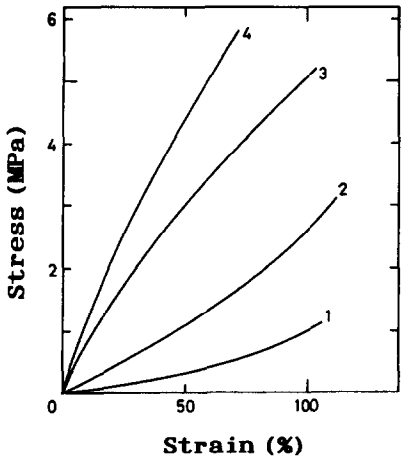


Fig. 3. Stress–strain curves of membranes at 25°C in PECF for: (1) PHEG-1-2; (2) GBG(E)-1-1; (3) GBG(Pe)-1-1; and (4) PHPeG-1-1.

Figure 2 illustrates the effect of OMDA concentration in the reaction mixture on $q$ of the crosslinked hydrophilic membranes. The effective crosslink density $f_c$ may be proportional to the crosslinker OMDA concentration (mol%) in the reaction mixture. As shown in Fig. 2, $q$ decreases with the increasing OMDA molar concentration in the reaction mixture. The slope of the log–log plots for PHEG-1 has the value of $-\frac{3}{5}$ as predicted in equation (2). On the other hand, the slope for GBG(E)-1 block copolymer membranes slightly decrease from $-\frac{3}{5}$. The order of the decrease becomes higher with decreasing $q$ values of the membrane which may attribute to the content of the hydrophilic portion, PB, in block copolymer chains.

Further, PHPeG-1 and GBG(Pe)-1 (Fig. 2) with lower $q$ cannot be dealt with in terms of the rubber elasticity theory, showing much less than $-\frac{3}{5}$.

*Tensile properties of hydrophilic membranes in PECF*

The tensile properties of hydrophilic membranes are highly dependent on $q$ in PECF. Further, elastomeric membranes are highly suited to biomedical applications, such as membranes for artificial organs, reconstructive prosthesis, and cosmesis.

Figure 3 illustrates the stress–strain curves of some hydrophilic membranes in PECF at 25°C. Table 3 lists the experimental findings of Young's modulus $E$ at an elongation of 1%, the tensile strength of $\sigma_B$ and elongation $\epsilon_B$ at the breakage point with $q$ values for membranes in PECF. It is shown in Fig. 3 that tensile behavior of the hydrophilic membranes was classified in two types, namely, the skin-type and elastomer-type by the shape of the stress–strain curve [12]. The skin-type membranes such as PHEG-1-2 or GBG(E)-1-1 membrane show a concave behavior with low Young's modulus as a typical case of human skin, while the elastomer-type hydrogels such as PHPeG-1-1 or GBG(Pe)-1-1 show sigmoidal behavior with the transition in the low strain region. The skin-type behavior has been accounted for by the entropy elasticity without stress relaxation, creeping and hysteresis, while the elastomer-type behavior has been considered to include the contribution of energy elasticity with stress relaxation, creeping and hysteresis. The difference in the shape of stress–strain curves between these two cases was discussed in detail previously [12]. Lotan *et al.* have shown that the helical content increases with the increasing length of the hydrocarbon of the side-chain in aqueous solution [13], and that the PHPeG and/or GBG(Pe) series exists almost completely in α-helix conformation, while the PHEG and/or GBG(E) series exists in random coil conformation. The hydrophilic copolypeptide membranes which exist in α-helix or interrupted α-helix conformation show elastomer-type behavior, while those which contain random coil conformation show skin-type behavior.

Table 3. Tensile properties of hydrogels at 25°C in PECF

| Sample code | $q$ ($W_w/W_d$) | $E$ (MPa) | $\sigma_B$ (MPa) | $\epsilon_B$ (%) |
|---|---|---|---|---|
| PHEG-1-1 | 15.2 | 0.21 | 0.50 | 105 |
| PHEG-1-2 | 9.9 | 0.42 | 1.05 | 108 |
| GBG(E)-1-1 | 11.5 | 2.24 | 3.12 | 112 |
| GBG(E)-2-1 | 6.8 | 2.88 | 3.95 | 118 |
| PHPeG-1-1 | 4.2 | 11.82 | 5.80 | 72 |
| GBG(Pe)-1-1 | 3.1 | 8.50 | 5.15 | 104 |

*Biodegradation of membranes* in vitro

Williams [14] has shown that numerous proteases may be present at the wound site. Although papain is a general plant thiol endopeptidase, it attacks preferentially peptide bonds where the amino acid residue of the carbonyl group is arginine, lysine, or glutamine and where this amino acid is joined on either side by amino acids with hydrophobic side chains. Preweighed sample membranes were exposed to 0.10 mg of papain in 1 ml of the appropriate activator solution at 37°C, and results obtained with PHEG-1-2 and GBG(E)-1-1 membranes are illustrated in Fig. 4.

Degradation of these membranes was measured by changes in the dry weight ratio $W_r/W_o$ as well as $q$ (Fig. 4). As shown in Fig. 4, with papain digestion an immediate increase in the swelling ratio of hydrophilic membranes was observed, while weight loss occurred slightly and more slowly in the beginning step in both cases. An endopeptidase must make two incisions in a chain segment to produce a soluble

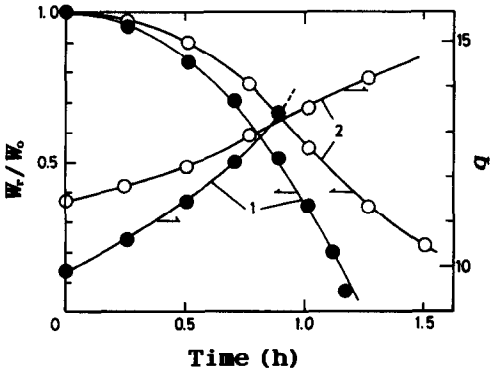


Fig. 4. Dry weight ratio ($W_r/W_o$) and $q$ of membranes vs papain digestion time $T$ (hr) at pH 7.4 and 37°C in PECF for: (1) PHEG-1-2; and (2) GBG(E)-1-1.

fragment, but a single cleavage will decrease the effective crosslink density.

In comparison between GBG(E)-1-1 and PHEG-1-2 membranes with papain digestion, it was pointed out that the former could maintain the shape of membrane in the region of $W_r/Wo > 30\%$, while the latter broke down its shape at 50% of $W_r/W_o$. It may suggest that the block copolymer membrane is more tough than the corresponding homopolymer membrane due to the existance of microheterophase structure of hydrophobic block component.

Next, it is also important to know exactly the change of the remaining tensile strength of membranes under enzymatic digestion from the applicational point of view. Figure 5 illustrates the relative tensile strength ($\sigma_{B,r}/\sigma_{B,o}$) and the $W_r/W_o$ for GBG(E)-1-1 and PHEG-1-2 membranes as a function of papain digestion time at 37°C and pH 7.4 in PECF. It was commonly shown in both samples that the order of the decreasing of the tensile strength compared to that of mass for papain digestion is rather high, suggesting that the cleavage of crosslink occurs practically under enzymatic digestion. In Fig. 5, it was shown that the remaining tensile strength of GBG(E)-1-1 membrane became zero at 40% of $W_r/W_o$. On the other hand, that of the PHEG-1-2 membrane became zero at 65% of $W_r/W_o$. The fact suggests again that the block copolymer membrane is more tough than the corresponding homopolymer membrane.

Figure 6 summarizes the rate of papain digestion $V_{\frac{1}{2}}$ ($hr^{-1}$) as a function of $q$ in PECF for these membranes. $V_{\frac{1}{2}}$ ($hr^{-1}$) is defined as the reciprocal of the time required for the sample weight to be reduced to one-half its initial value. It is clearly shown that the order of $V_{\frac{1}{2}}$ among these hydrophilic membranes is as follows:

PHPeG-1 > GBG(Pe)-1 > PHEG-1 > GBG(E)-1

which is in the same order of $q$ for each membrane.

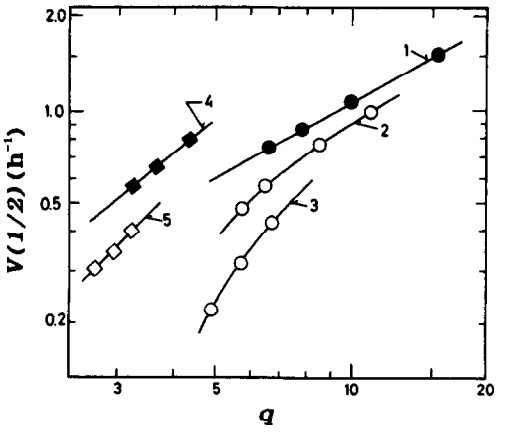


Fig. 6. The rate of papain digestion $V_{\frac{1}{2}}$ ($hr^{-1}$) vs $q$ at pH 7.4 and 37°C in PECF for: (1) PHEG-1; (2) GBG(E)-1; (3) GBG(E)-2; (4) PHPeG-1; and (5) GBG(Pe)-1.

## CONCLUSIONS

The effective crosslink density is proportional to the mol% of the crosslinker (OMDA) in the reaction mixture. It is pointed out that the swelling ratio $q$ of membranes plays an important role in the membrane properties, and that the hydrophilic block copolymer membranes are shown to maintain their toughness more than the corresponding homopolymer one in the course of the enzymatic digestion due to the existence of microheterophase structure of hydrophobic block component. The enzymatic hydrolysis of these block copolymer membranes by papain *in vitro* indicates that the degradation could be regarded as a bulk rather than a surface phenomenon. The rate of degradation of samples was also highly dependent on the $q$ value of membranes, and it increased with increasing the hydrophobicity of side-chains. Finally, block copolymer membranes, which maintain their toughness moderately in the course of the enzymatic digestion, will be more useful than the corresponding homopolymer membrane for the clinical applications and drug delivery systems.

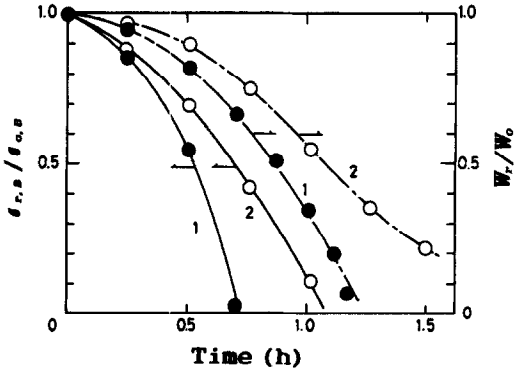


Fig. 5. Dry weight ratio ($W_r/W_o$) and relative tensile strength at break ($\sigma_{B,r}/\sigma_{B,o}$) of membranes vs papain digestion time $T$ (hr) at pH 7.4 and 37°C in PECF for: (1) PHEG-1-2; and (2) GBG(E)-1-1.

## REFERENCES

1. J. M. Anderson, K. L. Spilizewski and A. Hiltner. *Biocompatibility of Tissue Analogs* (edited by D. F. Williams), p. 68. CRC Press, Boca Raton (1985).
2. T. Hayashi, A. G. Walton and J. M. Anderson. *Macromolecules* **10,** 346 (1977).
3. T. Hayashi, J. M. Anderson and A. Hiltner. *Macromolecules* **10,** 352 (1977).
4. A. Nakajima, T. Hayashi, K. Kugo and K. Shinoda. *Macromolecules* **12,** 840 (1979).
5. A. Nakajima, K. Kugo and T. Hayashi. *Macromolecules* **12,** 844 (1979).
6. C. A. Homsey. *J. Biomed. Mater. Res.* **4,** 341 (1971).
7. T. N. Salthouse. *J. Biomed. Mater. Res.* **10,** 197 (1976).
8. J. Drenth, J. N. Jansonius, R. Koekoek and B. G. Solthers. *Papain X-ray Structure in the Enzymes*, Vol. 3, p. 485. Academic Press, New York (1971).
9. R. P. Foss, H. W. Jacobson and W. H. Sharkey. *Macromolecules* **10,** 287 (1977).
10. A. Nakajima, K. Kugo and T. Hayashi. *Polym. J.* **11,** 995 (1979).
11. P. J. Flory. *Principles of Polymer Chemistry*, p. 576. Ithaca, NY, Cornell University Press, Ithaca, NY (1953).
12. E. Nakanishi, K. Hamada, E. Sugiyama, S. Hibi and T. Hayashi. *Polym. J.* **23,** 1053 (1991).
13. N. L. Lotan, A. Yaron, A. Berger and M. Sela. *Biopolymers* **3,** 625 (1965).
14. D. F. Williams. *J. Bioengng* **1,** 279 (1977).