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Preparation and Properties of A-B-A Tri-Block Copolymer Membranes Consisting of *N*-Hydroxyalkyl L-glutamine as the A Component and L-Alanine as the B Component

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ABSTRACT: A-B-A type tri-block copolymer consisting of *N*-hydroxyalkyl L-glutamine as the A component and L-alanine as the B component as well as the corresponding random copolymers and homopolymers were prepared by carrying out aminoalcoholysis reaction with 2-amino-1-ethanol, 3-amino-1-propanol, or 5-amino-1-pentanol, together with crosslinking reaction with octamethylenediamine on membranes of the starting polymer membranes including γ-benzyl-L-glutamate residue. The relation between their bulk structure and membrane properties was investigated, such as the swelling in water, aqueous vapor permeability, tensile properties, and enzymatic degradation behaviors of the membranes in PECF solution. The tensile properties of the hydrophilic membranes were highly dependent on the degree of swelling of PECF, and on the hydrophobicity of the side chains, whose behavior was typical of an elastomer. It was shown that a common relation was obtained between the rate of water vapor permeability and the degree of swelling of PECF of membranes in spite of the difference of the nature of the side chains. Biodegradation of samples *in vitro* by protease indicated that the degradation was a bulk rather than a surface phenomenon, and that the rate of degradation was also highly dependent on the degree of swelling of PECF of samples as well as on the hydrophobicity of the side chains of samples.

KEY WORDS A-B-A Tri-Block Copolymer / *N*-Hydroxyalkyl L-glutamine / L-Alanine / Bulk Structure / Degree of Swelling / Water Vapor Permeability / Tensile Property / Enzymatic Degradation Property / Microheterophase Structure / Hydrophobicity /

It was pointed out that the materials exhibiting the microheterophase structures of some specified dimensions are prominent in antithrombogenicity.[1] In practice, normal vascular endotherium, which is the ideal nonthrombogenic, is considered to have a microheterophase structure composed of hydrophilic and hydrophobic microdomains.[2] In previous studies,[3-5] we have synthesized A-B-A type tri-block copolypeptides consisting of γ-benzyl L-glutamate (G) and L-leucine or L-valine of high molecular weight and characterized their solid-state properties. The choice of solvent produced a significant effect on the solid-state structure of cast films. As would be expected, if such block copolymers are partly substituted by hydrophilic components, the products obtained are to be antithrombogenic and tissue compatible.[6]

On the other hand, poly(α-amino acid)s and their copolymers are typical biodegradable polymers. They have been considered very useful for biomedical applications such as a temporary artificial skin substitute in burn therapy, and as a temporary barrer to prevent adhesion between natural tissue planes that are damaged either by accident or as a result of surgery. For this purpose, we have prepared many types of hydrophilic poly(α-amino acid) membranes which are composed of hydro-

philic and hydrophobic amino acid components.

In this paper, we prepared A-B-A type tri-block copolymer (GAG) consisting of γ-benzyl L-glutamate (G) as the A component and L-alanine (A) as the B component, as well as the corresponding random copolymer (GA), homopolymers PBLG (G) and PLA1a (A). Hydrophilic polymers were obtained by carrying out the succeeding aminoalcoholysis reaction with 2-amino-1-ethanol, 3-amino-1-propanol, or 5-amino-1-pentanol, together with crosslinking reaction with octamethylenediamine (OMDA) on the G residue of the starting polymer membranes, and investigated the relation between their bulk structures and membrane properties, such as the degree of swelling of the pseudo-extracellular fluid (PECF),[7] tensile properties in PECF, water vapor permeability, and enzymatic degradation behavior *in vitro* of the membranes in PECF solution from an application point of view for the biomedical materials.

EXPERIMENTAL

Materials

N-Carboxyanhydride (NCA) of γ-Benyl L-Glutamate (G) and L-Alanine (A)

The monomers, N-carboxyanhydride of γ-benzyl L-glutamate (G-NCA) and L-alanine (A-NCA), were prepared according to the method proposed by Blout and Karlson,[8] and purified by recrystallization from an ethyl acetate solution with the addition of petroleum ether. Recrystallization was repeated from three to five times.

Synthesis of Hydrophobic Block Copolymers

To make a symmetrical tri-block copolypeptide (GAG), the center block was initially prepared with the use of hexamethylenediamine initiator, followed by simultaneous polymerization of the end blocks. A typical example of the synthesis of GAG is as follows: A-NCA, 0.03 mol, was dissolved in 100 ml of dioxane–methylene dichloride (1 : 1), and 1,6-hexamethylenediamine, 2×10^{-4} mol, in dioxane was added to initiate polymerization. The polymerization reaction was monitored by the carbon dioxide evolved. The time required depended on the ratio of anhydride to initiator. On completion of the A polymerization, G-NCA in the same solvent mixture as the polymerization was added through a filter. The toral polymerization time was from 4 to 7 days.

Table I. Preparative data of original samples

Sample code	μ-BLG mol%	[η] dl g^{-1}	M_w
PBLG-1 (G-1)	100.0	1.94	371,000
PLAla-1 (A-1)	0.0	0.80	
GA-1	75.0	1.81	348,000
GA-2	49.8	1.95	370,200
GAG-1	74.3	1.95	370,600
GAG-2	43.0	2.60	517,500

Related random copolymers (GA) and the corresponding homopolymers (G and A) were also synthesized by the NCA method. The polymerization was initiated with triethylamine at an NCA-to-initiator molar ratio of 50. All starting polymers were purified and fractionated as described in the previous paper.[9] The results of all the polymerization are summarized in Table I.

Preparation of Hydrophilic Polymer Samples

The partial aminoalcoholysis of G-portion of these samples by reacting with 2-amino-1-ethanol, 3-amino-1-propanol, or 5-amino-1-pentanol, was performed as follows: after a sample membrane of *ca.* 100 μm in thickness cast from chloroform solution was immersed in the mixture of aminoalcohol and 2.0 mol% of the crosslinker, 1,8-octamethylenediamine (OMDA), at 58°C for 48 h, the membrane was washed with water and stored in ethanol. The hydrophilic polymers prepared with 2-amino-1-ethanol are denoted by G(E), GA(E), and GAG(E), those with 3-amino-1-propanol by G(P), GA(P), and GAG(P), and those with 5-

amino-1-pentanol by G(Pe), GA(Pe), and GAG(Pe), respectively.

Measurements

Composition of Block Copolymers and Random Copolymers

The molar composition of GAG and GA samples was determined by amino acid analysis. These analyses were carried out in The Peptide Institute, Inc. (Minoh-shi, Osaka). The results were summarized in Table I.

Molecular Weight Determination of Starting Polymers

The molecular weight of the starting polymers was determined in *N,N'*-dimethylformamide (DMF) at 25°C by sedimentation equilibrium using a MOM Type-3170-b ultracentrifuge equipped with a Reyleigh interference optical system and a 12 mm double sector cell.[10] The viscosity numbers were also obtained in a dichloroacetic acid (DCA) solution, using an Ubbelohde type viscometer at 25°C. These data were listed in Table I.

Conformation and Structure of Starting Polymers in Solid State

The chain conformation of starting polymers in membranes was estimated from infrared (IR) spectra measured with a Hitachi 260-30 IR spectrophotometer. Wide-angle X-ray diffraction (WAXD) profiles of membranes were obtained with a Rigaku Geigerflex Type DF-3 X-ray generator equipped with an automatic diffractometer by using Ni-filtered CuK_α radiation, setting a flat surface of the membrane normal to the beam.

Membrane Properties

The degree of swelling Q_w (%) of PECF solution of membranes were measured with a microbalance by weighing method.[11]

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

Water vapor permeation through the membranes was measured with a cylindical glass cell[12] at 37.0°C. The exposed membrane area was 12.57 cm^2.

Biodegradation of Membranes in Vitro

Enzymatic degradation studies *in vitro* were carried out by using papain. The enzyme papain was purchased from Nakarai Chem. Co. enzyme solutions were prepared by standard techniques[13] at 37.0°C. A series of the cross-linked membranes were exposed to 0.1 mg ml^{-1} of the solution of PECF at pH = 7.4 with 0.01 M cystein and 0.04 M EDTA at 37.0°C. Samples were removed from the papain solution at the appropriate time intervals, weighed, and then vacuum dried at 60°C to constant weight.

RESULTS AND DISCUSSION

Conformation and Structure of Copolymer Membranes

IR spectra were measured with sample membranes cast from solution. In each case, bands of amide I, II, and V of polypeptide chains appeared at 1650, 1550, and 610 cm^{-1}, just at the same wave numbers for the corresponding homopolymers. It is concluded from the experimental results that the polypeptide chains in sample membranes exist in α-helical conformation, and moreover, it is shown that the characteristic bands of ester bond at 1730 cm^{-1} as well as those of phenyl group at 700 and 750 cm^{-1} decrease with increasing content of *N*-hydroxyalkyl L-glutamine (OH).

The WAXD patterns for GAG-1 block copolymer, the corresponding homopolymers, and GA-1 random copolymer are shown in Figure 1. The first main reflextion corresponds to an intermolecular spacing of the α-helix chains. GA-1 random copolymer shows a single reflection at an intermediate spacing. The actual interhelical spacing is known to depend on the composition of the copolymer and increases as the amount of G increases.[14] It appears that these two comonomer units co-

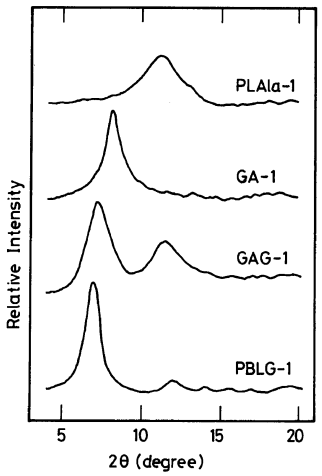

Figure 1. Wide-angle X-ray diffraction profiles of unoriented films for PBLG-1, GAG-1, and GA-1 cast from chloroform (CF), and for PLAla-1 cast from CF-trifluoro acetic acid solution.

crystallize in random copolymers and isomorphic structures are obtained.

Diffraction patterns for GAG-1 block copolymer show two main reflections, basically similar to the reflections of the two homopolymers. It appears that the phase separation has occurred, and that the G and A domains assume the same structural modification as the corresponding homopolymers qualitatively. The interfacial zone, G/A, is made up of the coiled peptide residues near the ends of both polypeptide chains G and A, owing to the difference of the equivalent radius of cross-section of the G and A α-helix rods. Thus, the relative amount of the crystalline portion in the GAG block copolymer membrane should be lower than those of the corresponding homopolymer membranes.

Degree of Swelling of PECF of Membranes

The relation between the degree of swelling Q_w (%) and the molar percent of the OH group in each case of the membranes is illustrated in Figure 2, in which Q_w of membranes was measured after dipping membranes in PECF for 48 h at 37.0°C. It is seen that the Q_w values obtained with GAG block copolymer membranes at a given OH (mol%) are remarkably higher than those obtained with PBLG and GA random copolymer membranes in low OH (mol%) region. Another remarkable characteristic of the GAG block copolymer membranes compared with the cases of PBLG or GA random copolymer is that the depression in the slope of Q_w *vs.* OH (mol%) curves is observed in high OH (mol%) region, as shown in Figure 2. Such characteristics for the GAG membranes should be attributed mainly to the specific feature of the microheterophase structure of the block copolymers.

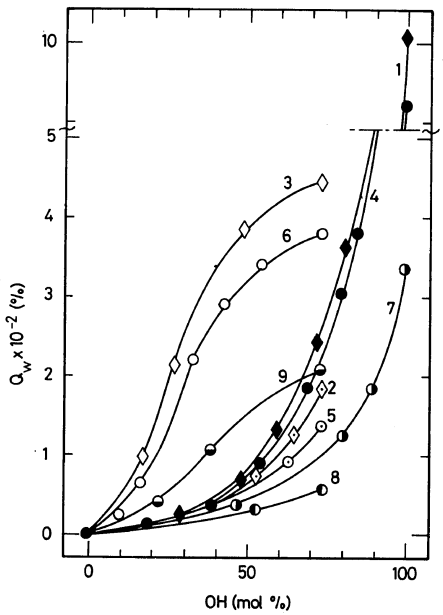

Figure 2. The degree of swelling Q_w (%) of PECF plotted against OH (mol%) for membranes: (1) G(E)-1, (2) GA(E)-1, (3) GAG (E)-1, (4) G(P)-1, (5) GA(P)-1, (6) GAG(P)-1, (7) G(Pe)-1, (8) GA(Pe)-1, and (9) GAG(Pe)-1 series at 37.0°C.

Furthermore, it is pointed out that the degree of swelling Q_w of PECF for membranes containing *N*-hydroxypentyl L-glutamine (Pe) becomes much lower than those for membranes containing *N*-hydroxyethyl L-glutamine (E) and *N*-hydroxypropyl L-glutamine (P). While the degree of swelling of water is determined by the interaction energy

between water and polymer segment, and the elastic energy for a water swollen sample, it is also related to conformation and configuration of polymer segment. The hydrophobic nature of glutamine side chain increases from E to Pe. Lotan, *et al.* have shown that the helical content increases with increasing length of side chain in aqueous solution, which can be explained by hydrophobic side chain interactions.[15] Poly(*N*-hydroxyethyl L-glutamine) (PHEG) is completely in the random coil conformation, while poly(*N*-hydroxypentyl L-glutamine) (PHPeG) is almost completely in the α-helix conformation. Poly(*N*-hydroxypropyl L-glutamine) (PHPG) shows the intermediate behavior[15] between the above two.

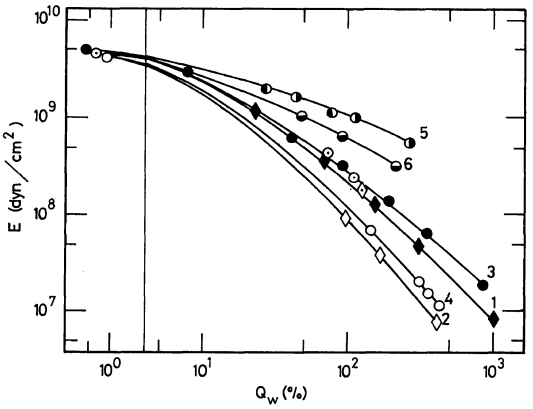

Figure 3. Young's modulus E (dyn cm^{-2}) as a function of the degree of swelling Q_w (%) of PECF for membranes: (1) G(E)-1 and GA(E)-1, (2) GAG(E)-1, (3) G(P)-1 and GA(P)-1, (4) GAG(P)-1, (5) G(Pe)-1 and GA(Pe)-1, and (6) GAG(Pe)-1 series.

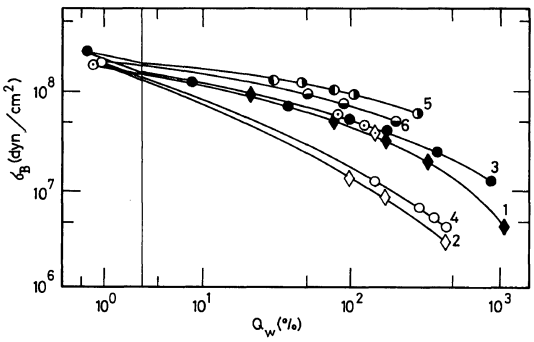

Figure 4. Tensile strength at breakage δ_B (dyn cm^{-2}) as a function of the degree of swelling Q_w (%) of PECF for membranes: (1) G(E)-1 and GA(E)-1, (2) GAG(E)-1, (3) G(P)-1 and GA (P)-1, (4) GAG(P)-1, (5) G(Pe)-1 and GA(Pe)-1, and (6) GAG(Pe)-1 series.

Tensile Properties of Membranes in PECF

The tensile properties of hydrophilic membranes are highly dependent on the degree of swelling Q_w (%) value in PECF. Further, elastomeric block copolymers are highly suited to biomedical applications, which include lipid control by hydrophobic–hydrophilic block copolymers, artificial organs, and reconstructive prothesis and cosmesis. For example, the mechanical properties and appearance of human skin can be simulated by pigmented low-modulus high-strength block copolymer thermoplastic elastomers.[16]

Figures 3 and 4 show the experimental values of Young's modulus E at an elongation of 1%, and the strength at the breakage point σ_B as a function of the degree of swelling Q_W(%) for membranes in PECF at 25°C, respectively. In Figures 3 and 4, it is shown that the PECF-swelling hydrophilic GAG block copolymer membranes tend to maintain attractive mechanical properties for artificial skin substitute application in much broader region compared with that of PBLG or GA random copolymer membranes, *i.e.*, the hydrophilic GAG block copolymers give softer membranes than do PBLG or GA random copolymers at the same order of the degree of swelling of PECF. Further, it is pointed out that the hydrophobic nature of glutamine side chain affect the mechanical properties of membranes, and the longer of side chain, the higher values of tensile parameters (E and σ_B) are obtained for each case of membranes.

Water Vapor Permeability of Membranes

A large variety of synthetic polymeric membranes has been investigated in the treatment of burns.[17–19] Among them, for example, formulation of a crosslinked polymer in the

form of hydrogel appears to provide added capability for encouraging cellular migration into the graft and vascularization.[17] In designing an effective wound closure or an artificial skin, at least two of the functions of skin are urgently essential to survival. The first is the ability of skin to keep most bacteria out. The second is its ability to keep tissue and organ water from evaporating away. Massive infection and severe fluid loss are recognized as major threats to patient's survival. Thus, for the first time, effective wound closure requires maintenance of an appropriate rate of water vapor permeability through the membranes. If the rate of water vapor permeability V_f (g m^{-2} day^{-1}) through the membrane is excessively low, water accumulates at the interface between woundbed and impermeable graft, and edema results. The graft-woundbed interfacial contact is thereby undermined. Consequently, optimization of the rate of water vapor permeability, probably close to the human physiological level of about $V_f = 200$ to 500 (g m^{-2} day^{-1}),[20] appears to be a necessary component of an appropriate design. As a result, to maintain the ability to wet the woundbed and thereby maintain an air-free interface, an inner skin substitute membrane should exhibit higher V_f value than 500 (g m^{-2} day^{-1}).

Figure 5 illustrates the relation between the rate of water vapor permeability V_f (g m^{-2} day^{-1}) of PECF and the degree of swelling Q_w (%) for membranes at 37.0°C. It may be also shown that the V_f value is highly dependent on the Q_w value in PECF. It may be judged in Figure 5 that GAG block copolymer membranes are more effective than PBLG and GA random copolymer membranes in an appropriate Q_w region for an application such as artificial skin substitutes, while a common relation is obtained between V_f and Q_w of membranes in spite of the difference of the architecture of polymer chains in higher Q_w region.

Biodegradation of Membranes in Vitro

Williams[21] has shown that numerous proteases may be present at the wound site. Enzymes of the inflammatory response that likely to degrade poly(α-amino acid)s include the endopeptidase cathepsin B and the exopeptidases carboxypeptidase and leucine

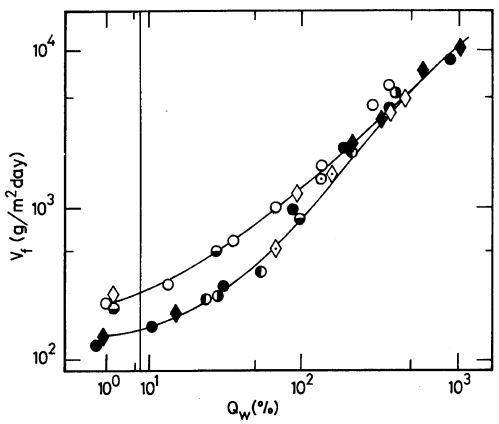

Figure 5. The rate of water vapor permeability V_f (g m^{-2} day^{-1}) as a function of the degree of swelling Q_w (%) of PECF at 37.0°C for membranes of G(E)-1 (◆), GA(E)-1 (◇), GAG(E)-1 (◇), G(P)-1 (●), GA(P)-1 (⊙), GAG(P)-1 (○), G(Pe)-1 (◑), GA(Pe)-1 (◐), and GAG(Pe)-1 (◒) series.

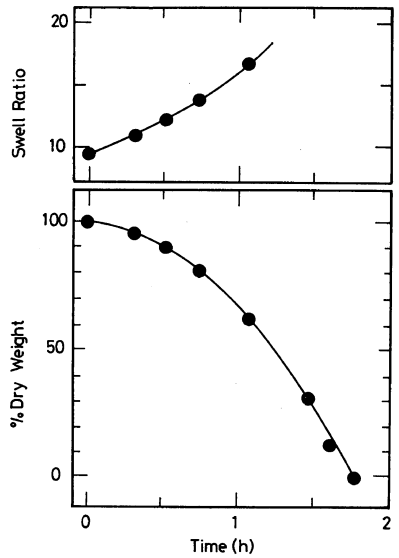

Figure 6. Dry weight ratio (%) and the swelling ratio of PECF for G(P)-1 (100 mol% of OH) membrane as a function of papain digestion time (h) at 37.0°C and pH = 7.4.

aminopeptidase.[22] In the present investigation, the plant thiol endopeptidase papain was selected as a commercially available analog of cathepsin B. Papain is an ellipsoidal protein molecule with the approximate dimensions $5.0 \times 3.7 \times 3.7$ nm.[23] Although papain is a general thiol endopeptidase, it has preferences for 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.[24] Papain is closely related to cathepsin B, a thiol endopeptidase that has been isolated from mammalian spleen, liver, kidney, and lung[24] and is released by the cells of the inflammatory response. Preweighed crosslinked sample membranes were exposed to 0.100 mg of papain in 1 ml of the appropriate activator solution at 37.0°C, and a result obtained with PHPG-1 membrane is illustrated in Figure 6. Although papain is a macromolecule whose molecular weight is about 21,000, it is apparent that the polymer network does not exclude papain molecule. Degradation of the PHPG-1 was measured by changes in a bulk property, the swelling ratio (Figure 6). Since an immediate increase in the swelling ratio was observed, the degradation must be a bulk rather than a surface phenomenon. Geometrical considerations are also consistent with easy access of the enzyme to the bulk of the membrane. A rough calculation of the PHPG-1 membrane pore diameter in the PECF-swollen state gives the value of about 20 nm for a 2.0% crosslinked PHPG membrane. Even assuming a hydration sphere for the enzyme, this is adequate for rapid diffusion. As shown in Figure 6, with papain digestion an immediate increase in the swelling volume of the PHPG-1 sample was observed, while weight loss occurred slightly more slowly. An endopeptidase must make two incisions in a chain segment to produce a soluble fragment, but a single cleavage will decrease the effective crosslink density.

Figure 7 summarizes the rate of papain digestion $V(1/2)(h^{-1})$ as a function of the degree of swelling Q_w (%) of PECF for hydrophilic sample membranes. $V(1/2)$ is defined as the reciprocal of the time required for the sample weight to be reduced to one-half its initial value. It is also clearly shown that a common relation is obtained between $V(1/2)$ and Q_w of sample membranes for each case of *N*-hydroxyalkyl L-glutamine groups. In Figure 7, the order of the rate of papain digestion among the copolymers was; *N*-hydroxypentyl L-glutamine (Pe) > *N*-hydroxypropyl L-glutamine (P) > *N*-hydroxyethyl L-glutamine (E) at the same Q_w value. Hydrophobic effects of the side chain portion may be important in explaining the faster digestion of the more highly hydrophobic side chains in membranes; that is, papain concentration within the membrane may increase as the hydrophobicity of the side chain increases. As a result, degradation would occur more rapidly in the more highly hydrophobic membrane.

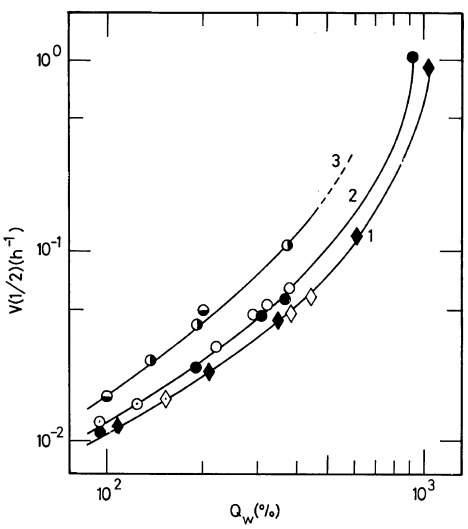

Figure 7. The rate of papain digestion $V(1/2)$ (h^{-1}) as a function of the degree of swelling Q_w (%) for hydrophilic polymer membranes: (1) G(E)-1 (◆), GA(E)-1 (◇) and GAG(E)-1 (◇), (2) G(P)-1 (●), GA(P)-1 (⊙) and GAG(P)-1 (○), and (3) G(Pe)-1 (◑), GA(Pe)-1 (◐) and GAG(Pe)-1 (◒) series at 37.0°C and pH = 7.4.

In conclusion, it is pointed out that the

microheterophase structure of hydrophilic–hydrophobic block copolymers highly contributes the bulk properties of membranes, and that the degree of swelling of water plays an important role to the membrane properties. Further, the rate of enzymatic digestion increased with increasing the carbon numbers of the end of the side chain in membranes. This phenomenon was attributed to hydrophobic effects.

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