

# Synthesis and Property of Hydrogel Membranes Consisting of Fumaramate with Phosphorylcholine Group

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**ABSTRACT:** Fumaramate bearing a phosphoryl choline group, isopropyl-2-[2'-(trimethylammonium) ethyl phosphoryl] ethyl fumaramate (IPTPFA), was radically copolymerized with 2-hydroxyethylmethacrylate (HEMA) in the presence of various crosslinking agents, water, and 2,2'-azobis(isobutyronitrile) to obtain hydrogel membranes. The obtained hydrogel membranes adsorbed bovine serum albumin (BSA) much less than those of poly(HEMA), and the

values of water content ( $H$ ) were higher than those of poly(HEMA). The values of tensile strength and tensile elongation of the hydrogel were 68.4 g/mm<sup>2</sup> and 239%, respectively. © 2004 Wiley Periodicals, Inc. *J Appl Polym Sci* 92: 2552–2557, 2004

**Key words:** biomaterials; hydrogels; gelation

## INTRODUCTION

Biomembranes mainly consist of phospholipids and lecithin containing a phosphoryl choline group. Synthetic polymers obtained from 2-methacryloyloxyethyl phosphoryl choline (MPC) as a phosphoryl choline moiety have been extensively studied from several viewpoints of polymer chemistry, and biological, medical, and life science. The corresponding polymers exhibited biocompatibility with excellent protein adsorption–resistance properties.<sup>1–13</sup> The protein solidification onto contact lens widely used as medical polymer materials arises from adhesion of dirt and bacteria. Therefore, antiprotein adsorption is the most important property for contact lens. Fumaramate, a fumaric acid derivative, has a 1,2-disubstituted ethylene-type structure and differs from MPC with a 1,1-disubstituted ethylene structure. We have already reported the synthesis and emulsifier-free emulsion copolymerization of methyl methacrylate (MMA) with fumaramate bearing a phosphoryl choline group, isopropyl-2-[2'-(trimethylammonium) ethyl phosphoryl] ethyl fumaramate (IPTPFA), and the obtained poly(IPTPFA-co-MMA) microspheres could depress the amount of adsorbed bovine serum albumin (BSA) onto them as well as poly(MPC-co-MMA).<sup>6</sup> The hydrogel membrane prepared from IPTPFA is expected to improve durability, strength, and glass transition

temperature as a biocompatible material because of its ridged structure, as compared with that obtained from vinyl compounds.

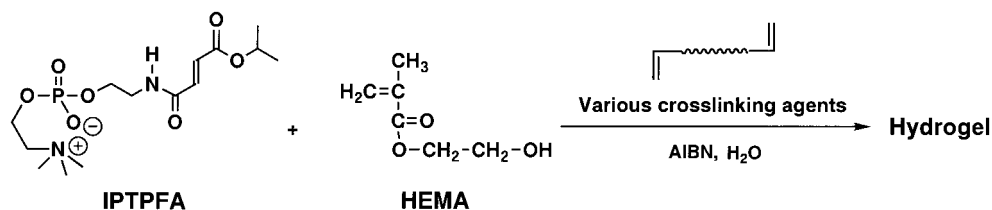
In this study, IPTPFA was radically copolymerized with 2-hydroxyethylmethacrylate (HEMA) in the presence of various crosslinking agents and water to obtain hydrogel membranes [poly(IPTPFA-co-HEMA)] (Scheme 1). Seven kinds of crosslinking agents were used, such as diallyl maleate (A), ethylene glycol dimethacrylate (B), allyl methacrylate (C), adipic acid divinyl ester (D), diethylene glycol dimethacrylate (E), triethylene glycol dimethacrylate (F), and nonaethylene glycol dimethacrylate (G), as shown in Scheme 2. The obtained hydrogel membranes were characterized by water fraction ( $H$ ), gel conversion ( $G$ ), and mechanical property. Adsorption behavior of BSA onto poly(IPTPFA-co-HEMA) hydrogel membrane was investigated, as compared with poly(HEMA).

## EXPERIMENTAL

### Materials

Various crosslinking agents and HEMA were purified by distillation *in vacuo*, except nonaethylene glycol dimethacrylate was used without further purification. 2,2'-Azobisisobutyronitrile (AIBN) was purified from methanol. BSA was purchased from Sigma Chemical Co. Ltd. (St. Louis, MO) and used without further purification. Other reagents were used without further purification. Distilled water after ion exchange was used throughout the experiment.

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Scheme 1

### Syntheses of IPTPFA

An IPTPF monomer was synthesized from maleic anhydride, isopropyl alcohol, mono-ethanolamine, 2-chloro-2-oxo-1,3,2-dioxaphospholane, and trimethylamine, as reported in the previous article.<sup>6</sup>

Yield: 40.5%, melting point (mp): 241–243°C. <sup>1</sup>H-NMR [ $\delta$  in ppm from 3-(trimethylsilyl)-1-propane-sulfonic acid, sodium salt (TMSPS) in D<sub>2</sub>O]: 6.80(d, J = 15.84 Hz, 2H, CH=CH), 5.08–4.99 (m, 1H, —COO—CH—), 4.20 (bs, 2H, (CH<sub>3</sub>)<sub>3</sub>N<sup>+</sup>—CH<sub>2</sub>—CH<sub>2</sub>—), 3.92 (t, J = 5.28 Hz, 2H, POO—CH<sub>2</sub>—CH<sub>2</sub>—NHCO), 3.56 (m, 2H, —CONH—CH<sub>2</sub>—), 3.48 (t, J = 5.28 Hz, 2H, (CH<sub>3</sub>)<sub>3</sub>N<sup>+</sup>—CH<sub>2</sub>—), 3.14 (s, 9H, (CH<sub>3</sub>)<sub>3</sub>N<sup>+</sup>), 1.23 (d, J = 6.27 Hz, 6H, —CH(CH<sub>3</sub>)<sub>2</sub>); <sup>13</sup>C-NMR ( $\delta$  in ppm from TMSPS in D<sub>2</sub>O): 167.79 and 167.31 (—C=O), 136.74 and 131.58 (CH=CH), 71.62 (—COO—CH—), 66.90 (POO—CH<sub>2</sub>—CH<sub>2</sub>—NHCO), 64.97 ((CH<sub>3</sub>)<sub>3</sub>N<sup>+</sup>—CH<sub>2</sub>—CH<sub>2</sub>—), 60.41 ((CH<sub>3</sub>)<sub>3</sub>N<sup>+</sup>CH<sub>2</sub>), 54.87 ((CH<sub>3</sub>)<sub>3</sub>N<sup>+</sup>), 41.15 (—CH<sub>2</sub>—NHCO—), 21.74 (—CH—(CH<sub>3</sub>)<sub>2</sub>). Elemental analysis: Found: H = 8.22%, C = 46.09%, N = 7.89%. Calc. for C<sub>14</sub>H<sub>27</sub>O<sub>7</sub>N<sub>2</sub>P: H = 7.43%, C = 45.90%, N = 7.65%.

### Preparation of hydrogel membrane

The reaction solutions consisting of the fixed amount of monomers (IPTPFA and HEMA), various crosslinking agents, distilled water, and AIBN were prepared (Scheme 1). The reaction solution was injected by using

a 5-mL microsyringe into the slit of glass plate with the 0.5 mm width. The slit of glass plate was put in an oven and kept at 50°C for 5 h, at 60°C for 5 h, and at 75°C for over 5 h. The chemical structure of the hydrogel membrane was confirmed by FTIR analysis with KBr method. The hydrogel membranes were used for measurements of equilibrium water content (*H*), scanning electron microscope (SEM), and amounts of adsorbed BSA.

### Measurement of water content (*H*) and gel conversion (*G*)

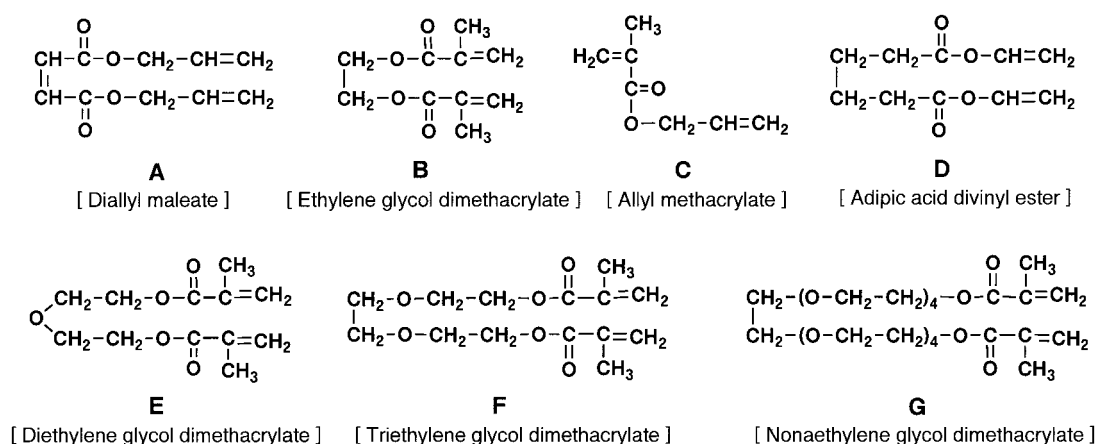
The hydrogel membrane was immersed in water to equilibrate at 30°C. The saturated hydrogel membrane that removed excess water by wiping with a dry filter paper was weighted every hour until the weight stayed constant. Water content (*H*) was calculated from the equation

$$H = \frac{[(\text{weight of hydrated membrane}) - (\text{weight of dry membrane})]}{(\text{weight of hydrated membrane})}$$

where gel conversion (*G*) was obtained by changed to ethanol from water on the above manipulation.

### Adsorption procedure of BSA

The hydrogel membrane (10 × 10 mm) was immersed in water at 30°C for 3 h. After removing excess water



Scheme 2

TABLE I  
Synthetic Results of Hydrogel Membranes<sup>a</sup>

Run	IPTPFA		HEMA		Crosslinking agent <sup>b</sup>			G <sup>c</sup> %	H <sup>d</sup> %
	wt/wt	g	wt/wt	g	Type	wt/wt	g		
1	50	1.0	50	1.0	D	3	0.06	46.1	55.2
2	40	1.5	60	2.3	A	3	0.11	86.9	50.6
3	40	1.5	60	2.3	B	3	0.11	76.0	45.8
4	40	1.5	60	2.3	C	3	0.11	90.3	45.6
5	40	2.0	60	3.0	D	3	0.15	84.0	54.1
6	40	0.8	60	1.2	E	3	0.06	73.1	44.7
7	40	0.8	60	1.2	F	3	0.06	73.9	43.2
8	40	0.8	60	1.2	G	3	0.06	55.1	51.4
9	40	0.8	60	1.2	D	0.5	0.01	60.0	45.7
10	30	0.4	70	0.9	D	3	0.04	54.3	45.8
11	0	0	100	5.0	B	3	0.05	82.7	33.9
12	0	0	100	5.0	C	3	0.05	90.2	35.7

<sup>a</sup> Initiator: 2,2'-azobis(isobutyronitrile) (AIBN); [AIBN] / [IPTPFA + HEMA] = 1.0/100 (wt/wt); [water]/[IPTPFA + HEMA] = 20/100 (wt/wt).

<sup>b</sup> Crosslinking agent: A, diallyl maleate; B, ethylene glycol dimethacrylate; C, allyl methacrylate; D, adipic acid divinyl ester; E, diethylene glycol dimethacrylate; F, triethylene glycol dimethacrylate; G, nonaethylene glycol dimethacrylate.

<sup>c</sup> Gel conversion.

<sup>d</sup> Equilibrium water fraction.

by wiping with a dry filter paper, the hydrogel membrane was immersed in 4.5 g dL<sup>-1</sup> of BSA solution (800 μL) at room temperature (RT) for 6 h. The amount of BSA adsorbed on hydrogel membrane was determined by the micro-BCA method.<sup>14</sup> The amount of adsorbed BSA was calculated from the content of BSA solution eluted from the hydrogel membrane in sodium dodecyl sulfate (SDS) aq. solution. To calculate the amount of BSA in the SDS solution, a protein assay kit (Micro-BCA Protein Assay Reagent Kit, no. 23235, Pierce, Rockford, IL, USA) was used. The amount of adsorption of BSA onto hydrogel membrane was calcu-

lated by measuring absorbance at 562 nm based on BAS by UV-Vis measurement with a Shimadzu UV-2200 spectrophotometer. The calibration curve was made from the adsorption value and the BSA concentration.

#### Other measurement

FTIR spectra were obtained by using a Shimadzu FTIR 8100A spectrophotometer. The surface of the membrane was observed by SEM with a Hitachi S-2300 instrument after Pt sputtering on the membrane surface. Transparency of several hydrogel membranes was measured

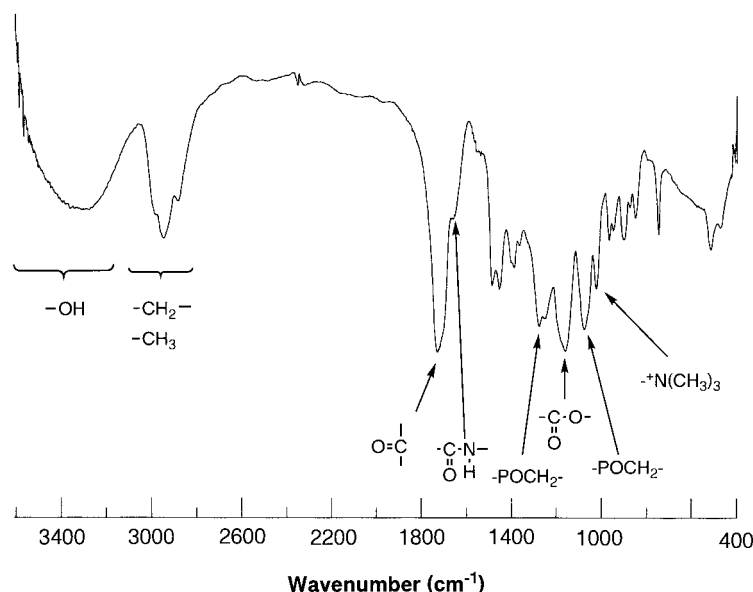
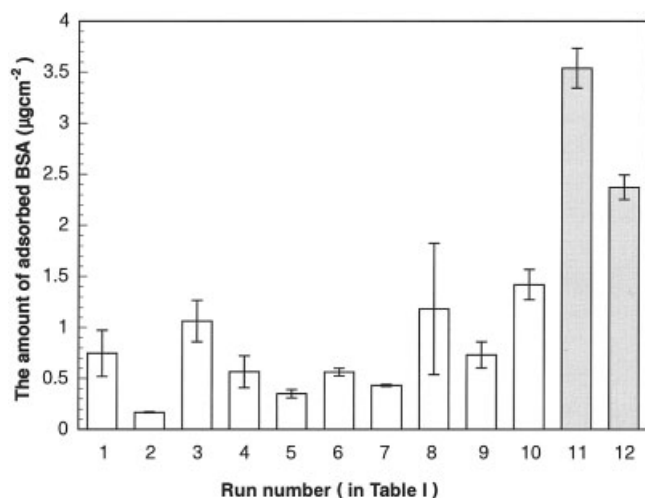


Figure 1 IR spectrum of hydrogel having fumaramate with phosphoryl choline group (run 5).



**Figure 2** Amount of BSA adsorbed onto hydrogel membranes after contact for 60 min.

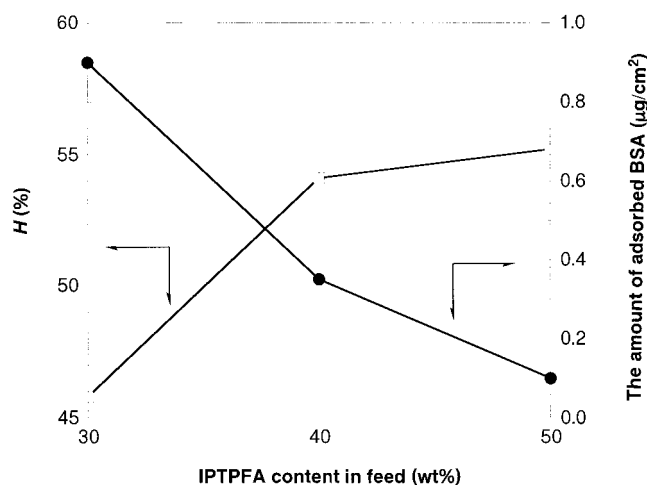
with the eye. Tensile tests for hydrogel membrane (10 × 5 × 0.5 mm) were carried out by using an Insuteko testing machine 2005 with a tensile speed of 2 mm/min at 23°C.

## RESULTS AND DISCUSSION

### Synthesis and property of hydrogel membrane

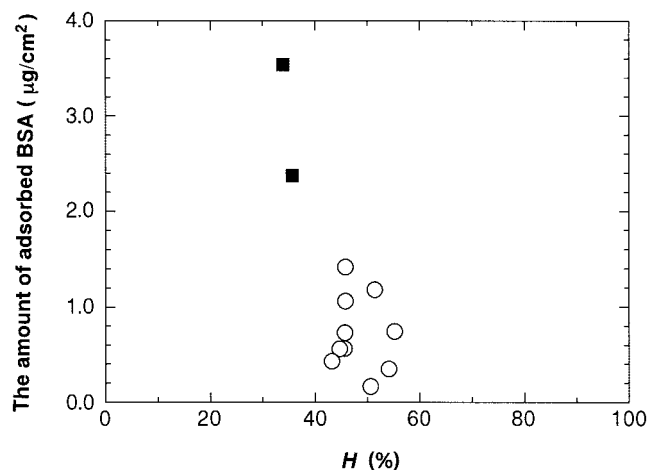
The hydrogel membranes [poly(IPTPFA-co-HEMA)] having phospholipid polar groups were synthesized from IPTPFA, HEMA, various crosslinking agents, and water. When the ratio of IPTPFA in the feed was more than 50 (wt/wt), the solution became heterogeneous. Thus, IPTPFA less than 50 wt % was used. The weight ratios of IPTPFA in feed were 30, 40, or 50 (wt/wt) in the case crosslinked with adipic acid divinyl ester (D). In other cases, the weight ratio of IPTPFA/HEMA/(crosslinking agent) was 50/60/3 (wt/wt/wt). Table I summarizes the results of the synthesis of poly(IPTPFA-co-HEMA) hydrogels (runs 1–10) and poly(HEMA) hydrogels (runs 11 and 12). In all compositions performed in this experiment, hydrogels were performed. In the IR chart of hydrogel obtained in run 5, the hydrogel membrane exhibited characteristic peaks due to both HEMA and IPTPFA units, as shown in Figure 1. The IR peaks assigned to —NHCO— (1660 cm<sup>-1</sup>), —POCH<sub>2</sub>— (1240 and 1080 cm<sup>-1</sup>), and —N<sup>+</sup>(CH<sub>3</sub>)<sub>3</sub> (970 cm<sup>-1</sup>) for IPTPFA were observed, which suggested that the phosphoryl choline unit existed in the hydrogel membrane.

The introduction of IPTPFA in hydrogel membrane effectively enhanced the water content (*H*). That is, the value of water content (*H*) of hydrogel membrane introduced IPTPFA was higher than that of a nonintroduced one (runs 11 and 12). In the case of crosslinked adipic acid divinyl ester (D) (runs 1, 5, and 10), the water content (*H*) increased with increasing the concentration

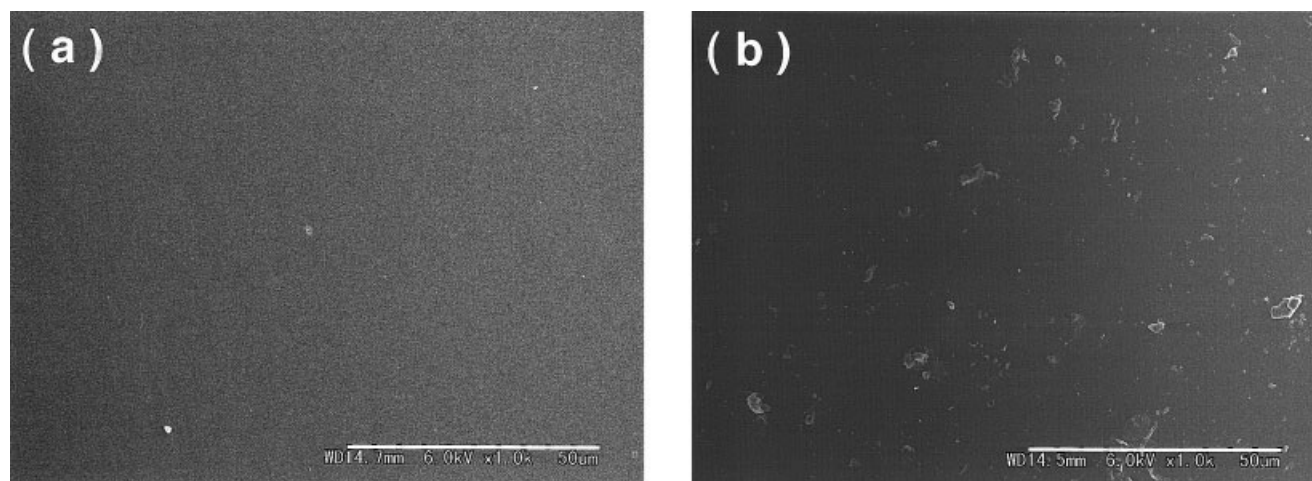


**Figure 3** Amount of BSA adsorbed onto hydrogel membranes and water content (*H*) for IPTPFA content in poly(IPTPFA-co-HEMA) series crosslinked with D: ●, BSA adsorption; □, *H*.

ratio of IPTPFA in feed, which was attributed to introduce the hydrophilic phospholipid polar group in IPTPFA. It seems that the reaction among IPTPFA, HEMA, and a crosslinking agent can form excellent hydrogel network structures. In general, oxygen permeability is improved with increasing water content (*H*); therefore, higher water content (*H*) is required for contact lens materials.<sup>15</sup> The value of water content (*H*) of the hydrogel crosslinked with adipic acid divinyl ester (D) was the highest (*H* > 0.54) (runs 1 and 5). In comparison with the weight ratio of the crosslinking agent at 0.5 and 3 (wt/wt), the values of water content (*H*) and gel conversion (*G*) for the hydrogel membrane prepared with the weight ratio of 3 (wt/wt) (run 5) were higher (run 9). This may result from the fact that hydrogel network structures proceeded with increasing the weight ratio of crosslinking agent in feed. Higher gel conversion (*G*)



**Figure 4** Relation between amount of BSA adsorbed onto hydrogel membranes and water content (*H*): ○, poly(IPTPFA-co-HEMA) series; ■, poly(HEMA) series.



**Figure 5** SEM pictures of hydrogel membrane surface after contact with BSA for 60 min: (a) poly(IPTPFA-co-HEMA) (run 2); (b), poly(HEMA) (run 12).

indicated that nonreacted monomer, noncrosslinked oligomers, and homopolymers existed very little in the hydrogel membrane. The gel conversion ( $G$ ) of the hydrogel crosslinked with allyl methacrylate (C) reached a higher value of 90%.

#### Assay of BSA absorbed amount using micro-BCA method

The amount of BSA adsorbed onto surface of the hydrogel membrane was examined. The results of BSA adsorption test are shown in Figure 2. Run numbers in the figure correspond to those in Table I. Bar graphs depict the average of the absorbed amount obtained by the measurement five times. It was noted that the amount of BSA adsorbed onto the surface of the hydrogel membrane drastically decreased by the introduction of the IPTPFA component in copolymer. The amount of BSA adsorbed in the poly(IPTPFA-co-HEMA) hydrogel was lower than that in poly(HEMA) gel. The hydrogel crosslinked with diallyl maleate (A) (run 2) indicated the most excellent antiprotein adsorption in all hydrogels.

Plots of the water content ( $H$ ) or the amount of absorbed BSA against the weight ratio of IPTPFA in feed

are shown in Figure 3 for hydrogel membranes crosslinked with adipic acid divinyl ester (D) (runs 1, 5, and 10). The water content ( $H$ ) increased with an increase of the IPTPFA content in the hydrogel membranes, and the BSA adsorption amount decreased with an increase of IPTPFA. Variations of both the water content ( $H$ ) and the BSA adsorption amount between 30 (wt/wt) and 40 (wt/wt) of the IPTPFA content were larger than those between 40 (wt/wt) and 50 (wt/wt).

The relationship between the BSA absorption amount and water content ( $H$ ) in all hydrogel membranes is shown in Figure 4. Two distinct groups were found between plots of poly(IPTPFA-co-HEMA) series with a phosphoryl choline unit and a poly(HEMA) series. The enhancement of water content ( $H$ ) significantly depressed the BSA adsorption amount.

#### BSA adsorption distribution of the hydrogel membrane surface

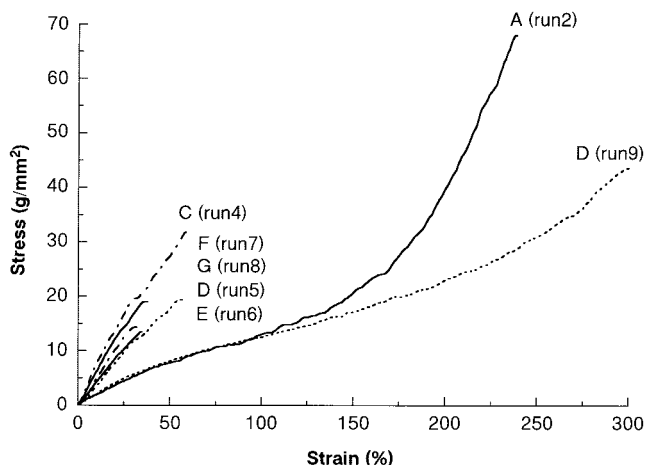
Figure 5 shows photographs of SEM for hydrogel membranes of (a) poly(IPTPFA-co-HEMA) (run 2), and (b) poly(HEMA) (run 12) after contact with BSA for 60 min. From the SEM pictures, the BSA adsorp-

**TABLE II**  
Properties of Hydrogel Membranes

Run <sup>a</sup>	Crosslinking agent types	Young's modulus (g mm <sup>-2</sup> )	Tensile elongation (%)	Tensile strength (g mm <sup>-2</sup> )	Transparency <sup>b</sup>
2	A	14.8	239	68.4	+
3	B	—	—	—	+
4	C	84.7	59	32.2	+
5	D	45.6	35	13.7	—
6	E	58.1	57	19.5	+
7	F	62.8	38	19.1	+
8	G	55.4	32	14.4	+
9	D	17.9	300	43.7	+

<sup>a</sup> in Table I.

<sup>b</sup> +, good; —, poor.



**Figure 6** Stress-strain curves of hydrogels: (A–G) corresponding to the crosslinking agents.

tion was widely observed on the poly(HEMA) hydrogel membrane. The protein adsorbed amount on poly(IPTPFA-*co*-HEMA) was clearly depressed in comparison with poly(HEMA).

#### Physical property of hydrogel membrane

Mechanical properties and transparency of hydrogel membranes are summarized in Table II. The polymer crosslinked with ethylene glycol dimethacrylate (B) (run 3) was difficult to measure stress-strain curves because the cracking was formed by swelling in the water. Figure 6 shows stress-strain curves of poly(IPTPFA-*co*-HEMA) hydrogel membranes having phosphoryl choline units. The curves for hydrogel membranes crosslinked with diallyl maleate (A) (run 2) and adipic acid divinyl ester (D) (run 9) showed lower Young's modulus, longer tensile elongation, and higher tensile strength, and they drew the ideal stress-strain curves as a hydrogel. Other hydrogel membranes exhibited higher Young's modulus, shorter tensile elongation, and poor tensile strength. They are not useful for contact lens materials because hydrogel membranes were hard and fragile. This may result from the fact that the molecular weight of hydrogels membrane was relatively low. It may be necessary for soft contact lens to be over 150% of tensile elongation and more than 70 g/mm<sup>2</sup> of tensile strength.<sup>16</sup> The hydrogel crosslinked with diallyl maleate (A) (run 2) showed the best mechanical property.

All hydrogel membranes showed good transparency except for the membrane crosslinked with adipic acid divinyl ester (D) (run 5). The membrane obtained with adipic acid divinyl ester (D) turned white on swelling in water and then its transparency decreased. When the

weight content of adipic acid divinyl ester (D) in feed decreased from 3 (wt/wt) to 0.5 (wt/wt), the transparency of the hydrogel was improved (runs 5 and 9).

#### CONCLUSION

1. Fumaramate bearing a phosphoryl choline group, IPTPFA, was synthesized and radically copolymerized with HEMA to obtain hydrogel membranes [poly(IPTPFA-*co*-HEMA)].
2. The content of water (*H*) in poly(IPTPFA-*co*-HEMA) hydrogel membrane was higher than poly(HEMA) hydrogel membrane.
3. The content of water (*H*) in poly(IPTPFA-*co*-HEMA) hydrogel membranes increased with an increase of the IPTPFA content in feed.
4. Hydrogel membranes of poly(IPTPFA-*co*-HEMA) adsorbed BSA much less than those of poly(HEMA) hydrogel membranes.
5. The values of tensile strength and tensile elongation of the hydrogel reached 68.4 g/mm<sup>2</sup> and 239%, respectively.

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