

# Polyurethane Membrane Prepared via a Dry/Wet Phase Inversion Method for Protein Adsorption

SHIH-LIANG HUANG,<sup>1</sup> CHENG-FANG OU,<sup>1</sup> JUIN-YIH LAI<sup>2</sup>

<sup>1</sup> Chemical Engineering Department, National Chin-Yi Institute of Technology, Taichung, Taiwan 41111, Republic of China

<sup>2</sup> Chemical Engineering Department, Chung Yuan University, Chung Li, Taiwan 32023, Republic of China

Received 7 April 1998; accepted 24 February 1999

**ABSTRACT:** Hydroxyl-terminated polybutadiene (HTPB)- and 4,4'-dicyclohexyl-methane (H<sub>12</sub>MDI)-based polyurethanes (PUs) were synthesized by solution polymerization. PU membranes were prepared by a dry/wet phase inversion method. Protein adsorption ratio of fibrinogen to albumin (F/A molar ratio) was measured. Low F/A molar ratio was found on these PUs. It was found that surface composition of these PUs has a subtle effect on F/A adsorption molar ratio. The F/A molar ratio was increased as the increase of hard segment content distributed on the surface. The variation of surface composition of these membranes and the effect on the F/A molar ratio were investigated by the difference in surface energy between nonpolar HTPB soft segment and polar hard segment, concentration, and temperature of coagulation medium, polymer content, and alcohol type. The C=O/C=C ratio, frequency shift, and difference ( $\Delta\nu$ ) as a measure of polymer homogeneity and the average strength of interpolymer hydrogen bonds were utilized to study the surface composition. © 1999 John Wiley & Sons, Inc. *J Appl Polym Sci* 74: 1334–1340, 1999

**Key words:** protein adsorption; polyurethane; dry/wet; phase inversion

## INTRODUCTION

Polyurethanes are widely used as biomaterial due to their good biocompatibility and mechanical properties. Previous studies of fibrinogen on the material's surface increases the platelet adhesion while that of albumin adsorption only has a little platelet adhesion.<sup>1,2</sup> The protein adsorption on a polymer has been thought to be important for the adhesion of platelet.<sup>3</sup> The study of surface composition in order to clarify the factors influencing the complicated mechanism of the platelet adsorption on the polymeric surface is then necessary.

Hoffmann et al.<sup>4</sup> reported that there is a strong interaction between blood and the material's surface of biomaterial with more hydrogen bonding or polar groups, and this causes higher coagulation of thrombocytes. Moreover, it has been reported that PPG-based polyurethanes (PUs) with more soft segment on the surface possesses more albumin adsorption, while there is less fibrinogen deposited and low platelet adsorption.<sup>5</sup> PUs containing alkyl chains (or C—H bond) on the surface are able to adsorb albumin and has been shown to reduce platelet deposition.<sup>6–9</sup> Others such as Costa,<sup>10</sup> Lelah,<sup>11</sup> and Ratner et al.<sup>12</sup> have reported that PUs with more C—H-bond or soft segment dispersed on the surface and possess better blood compatibility. And it is believed the ability of a polymer surface to promote platelet adhesion and activation is correlated to the adsorbed or depos-

Correspondence to: S.-L. Huang.

*Journal of Applied Polymer Science*, Vol. 74, 1334–1340 (1999)  
© 1999 John Wiley & Sons, Inc. CCC 0021-8995/99/061334-07

ited concentration ratio of fibrinogen to albumin.<sup>13,14</sup> The hydroxyl-terminated polybutadiene (HTPB) used in this study as soft segment is due to its nonpolar property and lower surface energy, and easily migrates toward the air-polymer interface of these PUs. Electron spectroscopy for chemical analysis (ESCA) indicates that the surface composition of poly(urethane urea)s with polybutadiene as soft segment has the major parts of butadiene soft segment content.<sup>15</sup>

The purpose of this study attempted to prepare HTPB-based PUs by dry/wet phase inversion method with different hard segment content of PU composition, concentration, and temperature of coagulation medium, polymer content, and alcohol type. The surface composition of these PUs were investigated by the change of C=O/C=C ratio and measured by Fourier transform IR-attenuated total reflection (FTIR-ATR). The C=O/C=C ratio, the hydrogen-bonding index (HBI) values, the IR frequency difference and shift, were used for the identification of surface composition and phase homogeneity, and for the effect on the fibrinogen to albumin (F/A) adsorption molar ratio.

## EXPERIMENTAL

### Materials

The chemicals used in this study were 4,4'-dicyclohexylmethane diisocyanate (H<sub>12</sub>MDI, Desmodur W of Mobay Co.), hydroxyl terminated polybutadiene (equivalent weight of 1333 with approximate 60 wt % of trans-1,4, 20 wt % of cis-1,4, and 20 wt % of vinyl-1,2, R-45M of ARCO Co.). The 1,4-butane diol (1,4-BD) was used as chain extender. Dibutyltin dilaurate (DBTDL) was used as catalyst. Toluene and dimethylformamide (DMF) were used as solvents for the preparation of PU solutions. Mixtures of water and alcohol were used as coagulation medium. Methanol, ethanol, and *n*-propanol were used as alcohol in the coagulation medium, respectively. Fibrinogen from human plasma of MW 341,000 and albumin from human serum of MW 68,000 (Sigma Chem. Co.) were used.

### Preparation of Polyurethane

Polyurethanes with different equivalent ratios were synthesized by two-stage method and were polymerized first by a NCO-terminated prepoly-

mer and then chain extended with chain extender to get a PU solution with 25 wt % solid content. Detailed procedures for polymerization had been reported in a previous publication.<sup>16</sup> Types of different compositions of PUs in this study are HTPB/H<sub>12</sub>MDI/1,4-BD = 1/4/3, 1/8/7, and 1/12/11, which possess 33.08, 50.56, and 60.79 wt % of hard segment content, respectively. Dry/wet membranes were prepared by pouring the PU solution onto a glass plate to a thickness of 600 μm using a Gardner knife. The casting solution was evaporated at room temperature for 5 min to degas the solvent, and then immersed in the coagulation medium with different temperature (15, 20, and 30°C) and composition (90, 70, and 50 wt % ethanol content of aqueous solution) for 10 min. Finally, the prepared membranes were dipped in distilled water for 20 min and dried in vacuum at room temperature, 48 h, for the removal of residual solvent in the membrane. Finally, the samples were kept under a vacuum at room temperature for at least 5 days prior to the test.

### IR Spectroscopy

IR spectra of PU were obtained by using a JASCO FTIR-310E spectrometer. Spectra were collected at a resolution of 2 cm<sup>-1</sup>. The peak due to hydrogen-bonded C=O stretching is centered at about 1700 cm<sup>-1</sup> and that due to free C=O stretching is centered at about 1717 cm<sup>-1</sup>, while the peak of bonded —NH stretching is at 3320 cm<sup>-1</sup> and that of free —NH stretching is at 3442 cm<sup>-1</sup>. Hydrogen-bonded carbonyl (or —NH) bands will correspond to those groups that are in the interior of hard segments, while the free bands may correspond to those groups in the hard segment domains or in the soft domains or at the interface.<sup>17</sup> In these butadiene-containing PUs, hydrogen bonding occurs only between urethane segments since the carbonyl group in the urethane linkage and the urethane alkoxy oxygen are the only proton acceptors. The extent of the carbonyl group participating in hydrogen bonding is expressed by HBI, which is the relative absorbencies of the hydrogen-bonded carbonyl peak ( $A_{\text{C=O,bonded}}$ ) to that of free hydrogen-bonded carbonyl peak ( $A_{\text{C=O,free}}$ ).<sup>18</sup> The frequency difference is defined as  $\Delta\nu = \nu_f - \nu_b$ , where  $\nu_f$  and  $\nu_b$  are the frequencies of maximum absorption for the free and hydrogen bonded —NH group, respectively. The frequency difference in the stretching frequency is considered as a measure of the strength of the hydrogen bond between molecules.

The IR absorbance of carbonyl group measured by FTIR-ATR are calculated by the addition of the respective hydrogen-bonded C=O absorbance peak height and free C=O absorbance peak height. The IR absorption band of butadiene soft segment are to be the *trans*-1,4 form at  $972\text{ cm}^{-1}$ , 1,2 form at  $912\text{ cm}^{-1}$ , and *cis*-1,4 form at  $685\text{ cm}^{-1}$ .<sup>19</sup> The C=O/C=C ratio on the surface is the ratio of total C=O absorbance peak height with *trans*-1,4 form absorbance peak height. Larger value of C=O/C=C ratio indicates that more hard segment content are dispersed on the surface.

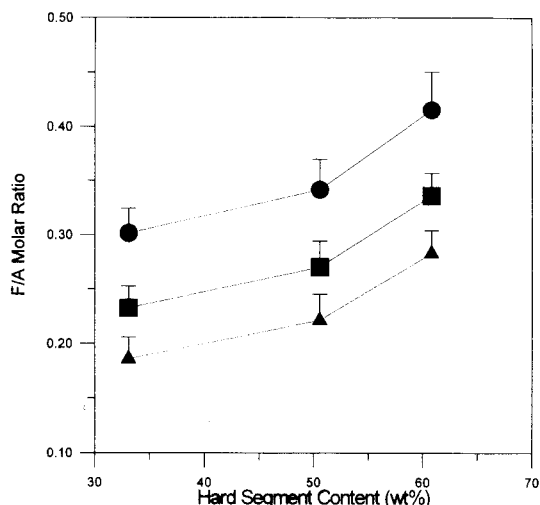
### Molar Ratio of Fibrinogen to Albumin Adsorption

The films with  $8\text{ cm}^2$  surface area were immersed into CPBS buffer solution (0.01M sodium citrate, 0.01M sodium phosphate, 0.12M sodium chloride, pH 7.40) for 12 h.<sup>20</sup> The concentrations of albumin and fibrinogen were 1.0 and 0.2 mg/mL, respectively, in CPBS buffer solution. The film surface was then quickly blotted with absorbent paper to remove surface buffer solution. The films were then filled with 2 mL of protein solution at  $30^\circ\text{C}$  for 1 h. After desorption, the films were rinsed with deionized water. The absorbed proteins were desorbed with 1% Triton X-100 and 1% dodecyl sodium sulfate in 0.01N NaOH at  $30^\circ\text{C}$  with agitating at 100 rpm for 1 h.<sup>21</sup> Afterward, the boric buffer solution was added into the desorbed proteins. 0.5 mL fluorescamine solution with a 3:2 volume ratio in acetone (20 mg/100 mL) were added to the mixture with vigorous stirring. The protein quantity was determined by a fluorescence spectrophotometer (Hitachi Co., F-2000) and the fluorescence intensity was measured at 392 nm with excitation and at 491 nm with emission.

## RESULTS AND DISCUSSION

### Effect of Concentration of Coagulation Medium

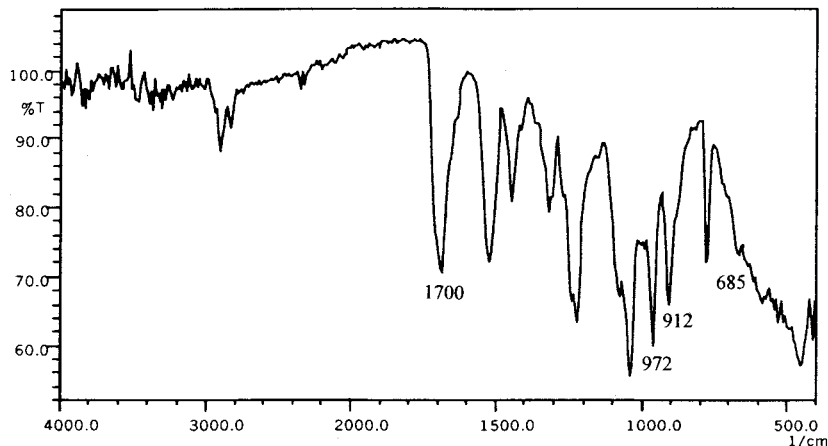
Different mixtures of ethanol and water were used as coagulation medium in this study except for the final section of discussing the effect of alcohol type. The F/A molar adsorption results of membranes prepared by using different coagulation media are shown in Figure 1. It shows that the F/A molar ratios increase in the following sequence of ethanol content of coagulation medium: 90 wt % < 70 wt % < 50 wt %. This phe-



**Figure 1** Effect of hard segment content on the F/A molar ratio with different ethanol content (wt %) in coagulation medium. (●) 50 wt %, (■) 70 wt %, and (▲) 90 wt %.

nomenon might be explained by the change of C=O/C=C ratio, which is an indication of hard segment content on the surface. In order to determine the polymer surface composition, ESCA analysis and FTIR-ATR spectra were carried out respectively to calculate the ratio of oxygen to carbon element and C=O/C=C ratio in our previous report.<sup>22</sup> It shows that the O/C ratio at the surface increases with the increase of the C=O/C=C ratio. Hence the C=O/C=C ratio calculated by FTIR-ATR spectra was used in this article to present the change of surface composition. Figure 2 shows the FTIR-ATR spectrum of polyurethane. The carbonyl group absorbance band appears at about  $1700\text{ cm}^{-1}$ , while that of butadiene segment are to be the *trans*-1,4 form at  $972\text{ cm}^{-1}$ , 1,2 form at  $912\text{ cm}^{-1}$ , and *cis*-1,4 form at  $685\text{ cm}^{-1}$ . On the other hand, the variation of surface composition with respect to the concentration of coagulation medium is shown in Fig. 3. The C=O/C=C ratio of surface of these dry/wet membranes are increased with the decrease of ethanol content of coagulation medium and has the same sequence as F/A molar ratio. It is really possible to obtain the result that the F/A molar ratio are increased as the increase of C=O/C=C ratio. This phenomenon may explain that PUs containing alkyl chain are preferentially to adsorb albumin as studied in the previous report.<sup>6</sup>

Frequency difference ( $\Delta\nu$ ) and shift were utilized in this study to discuss the hard segment distributing on the surface (i.e., C=O/C=C ra-

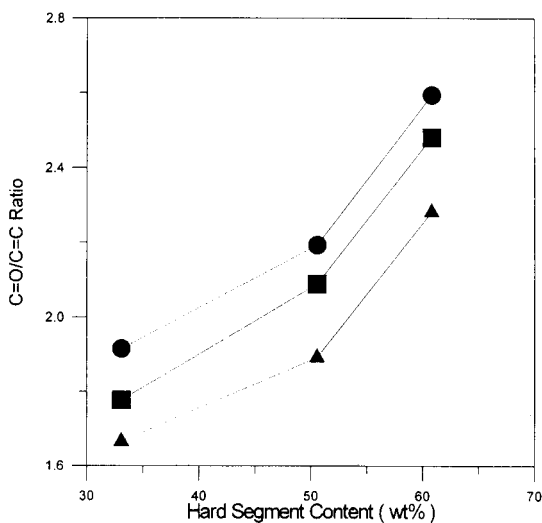


**Figure 2** FTIR-ATR spectrum of HTPB-based polyurethane.

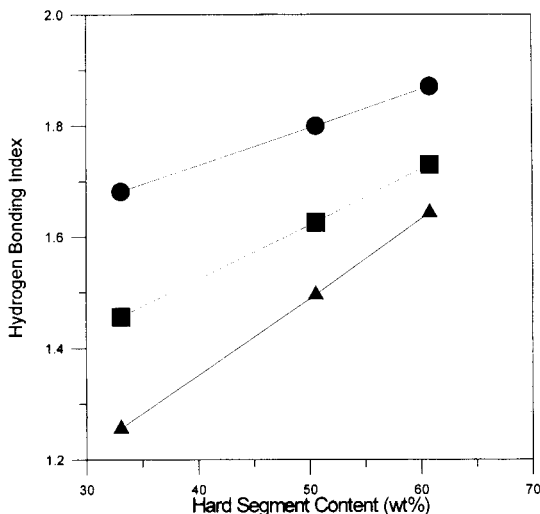
tio), while this ratio is affected by the interaction between water (or ethanol) and N—H (or C=O) groups of these membranes and can be evidenced by frequency shift and difference. The absorption frequency of bonded N—H groups shift from 3331 to 3320  $\text{cm}^{-1}$  as the membranes dipped in ethanol or water. Upon the association of membrane with coagulation medium, the energy and force constant of the N—H group absorption decrease and the absorption band shifts to a lower frequency, while that of frequency difference changes from 122 (nonsolvent treated) to 134  $\text{cm}^{-1}$  (dipped in ethanol or water). These may indicate that there is a strong

interaction between ethanol (or water) and N—H groups of hard segment as the membranes dipped in ethanol or water.

Coleman and co-workers<sup>23</sup> used the frequency difference ( $\Delta\nu$ ) between hydroxyl groups and those of the hydrogen-bonded hydroxyl groups as a measure of the average strength of intermolecular interactions. The frequency difference or the average strength of the hydrogen bonds between coagulation medium and membranes is in the following order: dipped in ethanol > dipped in water > nonsolvent treated. This indicates that the interaction between membranes and ethanol is higher than that with water, while that of the frequency difference decreases in the following order: dipped in 50 wt % > dipped in 70 wt % > dipped in 90 wt %. The reason may be that when the membranes are immersed in water-ethanol mixture, the ethanol molecules are adsorbed on the polymer chains of HTPB soft segment, which is easily swelled by ethanol. The polymer interacts with the hydrophobic moiety of the ethanol and the OH groups of the ethanol remain exposed on the surface.<sup>24</sup> This hydrophilization of the somewhat hydrophobic membrane by the ethanol is probably responsible for the effect on the demixing rate between casting solution and coagulation medium. Then the increase of water content in coagulation medium will increase the demixing rate with casting solution, which contains polar solvent (i.e., DMF). The result is that the C=O/C=C ratio increases as the water content in coagulation medium increases. This may explain that membranes prepared by dipping in 50 wt % ethanol content of coagulation



**Figure 3** Effect of hard segment content on the C=O/C=C ratio with different ethanol content (wt %) in coagulation medium. (●) 50 wt %, (■) 70 wt %, and (△) 90 wt %.



**Figure 4** Effect of hard segment content on the HBI values with different ethanol content (wt %) in coagulation medium. (●) 50 wt %, (■) 70 wt %, and (▲) 90 wt %.

medium have the largest hard segment content on the surface.

#### Effect of Hard Segment Content

The higher the hard segment content was, the higher was the F/A adsorption molar ratio regardless of any type of coagulation medium, as shown in Figure 1. The variation of surface composition, which will affect the protein adsorption and the platelet adhesion, from the bulk depends on the interaction between hard and hard segment and the compatibility between hard and soft composition segment. In general, that on the air-polymer interface will possess more nonpolar HTPB soft segment, while that on the glass face has a more polar hard segment.<sup>25</sup> The increase of hard segment content will restrict the movement of soft segment toward the surface. Hence the C=O/C=C ratios on the surface, which is shown in Figure 3, are increased as the hard segment content increases. On the other hand, Figure 4 shows that the HBI values increase as the hard segment content increases. Membranes prepared by dipping in 50 wt % ethanol content of coagulation medium have the largest HBI values than the other two types of membranes. The reason may be that more hard segment distributes on the surface and hence, more N-H (or C=O) groups are hydrogen bonded on the surface as the HBI value increases. Hence, films with high hard segment content will have a low affinity for albumin ad-

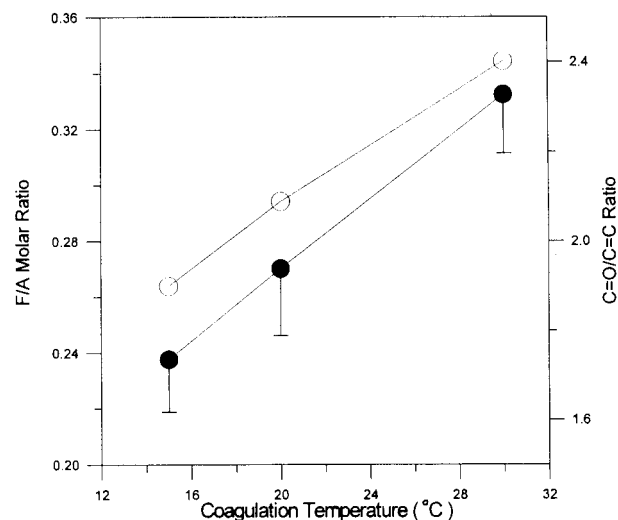
sorption (i.e., high F/A molar ratio) due to the increase of C=O/C=C ratio on the surface.<sup>5</sup>

#### Effect of Coagulation Temperature

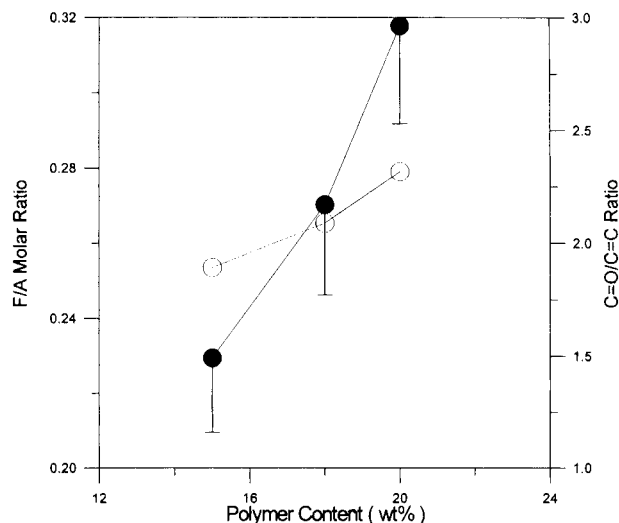
In this and the following section, dry/wet membranes were prepared by dipping in 70 wt % ethanol content of coagulation medium and with equivalent ratio of 1/8/7, due to the medium void concentration among these three types of membranes. Figure 5 shows that the F/A molar ratio increases with the increase of coagulation temperature. The increase of coagulation temperature induces that more hard segment, which possesses larger surface energy, compared with that of HTPB soft segment, which possesses lower energy, migrating toward the surface of the membranes as dipped in coagulation medium. The other effect is the increase of demixing rate between polymer solution and coagulation medium. Then the soft segment or hard segment migrating toward the surface of membranes possess nearly the same rate as the membranes prepared at higher coagulation temperatures. Hence the increase of C=O/C=C ratio on the surface, which is shown in Figure 5, as the coagulation temperature is increased. The change of C=O/C=C ratio may be the reason for the variation of F/A molar ratio with the change of coagulation temperature.

#### Effect of Polymer Content

Dry/wet membranes were prepared with different polymer content of casting solution from 15, 18, to



**Figure 5** Effect of coagulation temperature on the F/A molar ratio (■) and C=O/C=C ratio (○).



**Figure 6** Effect of polymer content (wt %) on the F/A molar ratio (●) and C=O/C=C ratio (○).

20 wt %. Figure 6 shows that the F/A molar ratios of these three membranes increase as the increase of polymer content of casting solution. The increase of polymer content will lead to a much higher polymer concentration on the surface and restrict the movement of polymer chains, especial for soft HTPB segment. On the other hand, an increase in polymer concentration will decrease the demixing rate between polymer solution and coagulation medium, and hence increase the lower energy of soft segment migrating toward surface. The competition of the above two effects shows that the former has a larger influence on the surface composition distribution. The result is the increase of C=O/C=C ratio on the surface, which is shown in Figure 6, as the increase of polymer content of the casting solution. And the increase of C=O/C=C ratio on the surface may be the reason for the increase of F/A molar ratio as the increase of polymer content in casting solution.

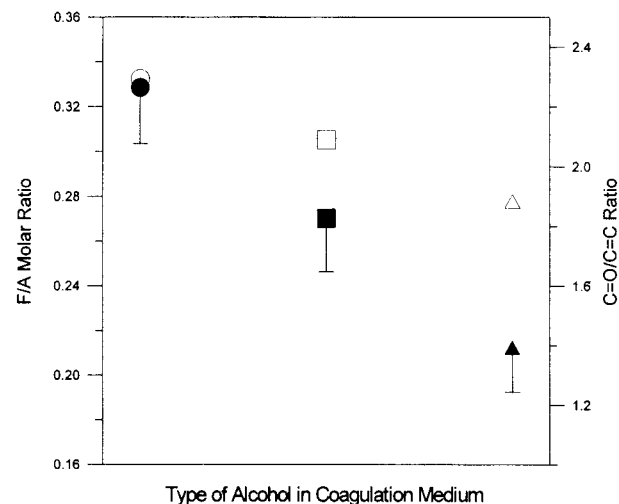
#### Effect of Alcohol Type in Coagulation Medium

In this section, dry/wet membranes were prepared by dipping in 70 wt % alcohol content of methanol, ethanol, and *n*-propanol, respectively. Figure 7 shows that the F/A and C=O/C=C ratios are all decreased in the order as the membrane dipped in different alcohol solution: methanol > ethanol > *n*-propanol. There are three effects on the surface composition of membranes prepared with different alcohol coagulation me-

dium. One is that the demixing rate of casting solution and coagulation medium is decreased due to the increase of alcohol molecular volume and hence the decrease of C=O/C=C ratio as the coagulation medium content of alcohol changed from methanol, ethanol, to *n*-propanol. Second is the decrease of interaction between C=O (or N—H) groups and coagulation medium, which can be evidenced by the changes of frequency difference from 138 (methanol), 134 (ethanol) to 130  $\text{cm}^{-1}$  (*n*-propanol), and induces the decrease of C=O/C=C ratio as discussed in the above section. Third is the increase of interaction between soft HTPB segment and coagulation medium of alcohol as the polarity of these alcohol decreases from methanol, ethanol, to *n*-propanol, and may increase the demixing rate and induce the increase of C=O/C=C ratio. The competition of the above three effects shows that the decrease of alcohol polarity has a larger influence on the decrease of C=O/C=C ratio, which may be the reason for the decrease of F/A molar ratio as the C=O/C=C ratio decreases.

#### CONCLUSIONS

The hard segment content on the surface of HTPB-based PUs affects the adsorption of albumin and fibrinogen. The C=O/C=C ratio and F/A molar ratio are all increased with the increase of hard segment content of PU composition, coagu-



**Figure 7** Effect of alcohol type in coagulation medium on the F/A molar ratio—(●) methanol, (■), and ethanol (▲) *n*-propanol—and C=O/C=C ratio—(○) methanol, (□) ethanol, and (△) *n*-propanol.

lation temperature, and polymer content. Membranes prepared by dipping in 50 wt % ethanol content of coagulation medium have the largest hard segment content on the surface and F/A molar ratio, while that of membrane dipped in methanol aqueous solution shows the largest C=O/C=C ratio and F/A molar ratio too. All the above results can be explained by the increase of soft segment content or the decrease of C=O/C=C ratio on the surface. This study implies that the surface composition of these HTPB-based PUs affects the adsorption of albumin and fibrinogen.

## REFERENCES

- Ito, Y.; Sisko, M.; Imanishi, Y. *J Biomed Mater Res* 1994, 23, 191.
- Chuang, H. Y. K. *J. Biomed Mater Res* 1984, 18, 547.
- Sheppard, J. I.; McClung, W. G.; Feuerstein, I. A. *J Biomed Mater Res* 1994, 28, 1175.
- Hoffman, A. S. *ACS Adv Chem Ser* 1982, 3, 199.
- Okano, T.; Nishiyama, S.; Shinohara, I.; Akaike, T.; Sakurai, Y. *Polym J* 1978, 10, 223.
- Pitt, W. G.; Cooper, S. L. *J Biomed Mater Res* 1988, 22, 359.
- Rahman, R.; Ratner, B. D. *J Polym Sci Part A Polym Chem* 1989, 27, 2673.
- Marconi, W.; Galloppa, A.; Martinelli, A.; Piozzi, A. *Biomaterials* 1995, 16, 449.
- Grasel, T. G.; Pierce, J. A.; Cooper, S. L. *J Biomed Mater Res* 1987, 21, 815.
- Costa, V. S.; Russel, D. B.; Trudel, G.; Waugh, D. F.; Salzman, E. W.; Merrill, E. W. *J Colloid Inter Sci* 1980, 76, 594.
- Lelah, M. D.; Grasel, T. G.; Pierce, J. A.; Cooper, S. L. *J Biomed Mater Res* 1986, 20, 433.
- Ratner, B. D.; Yoon, S. C.; Kaul, A.; Rahman, R. *Polyurethanes in Biomedical Engineering II*; Elsevier Science Publishers BV: Amsterdam, 1987; p 317.
- Brash, J. L.; Unival, S. *J Polym Sci* 1979, 66, 377.
- Shih, C. Y.; Lai, J. Y. *J. Biomed Mater Res* 1993, 27, 983.
- Li, Y. J.; Nakamura, N.; Wang, Y. F.; Kodama, M.; Nakaya, T. *Chem Mater* 1997, 9, 1570.
- Huang, S. L.; Lai, J. Y. *J Membrane Sci* 1995, 105, 137.
- Coleman, M. M.; Skrovanek, D. J.; Hu, J.; Painter, P. C. *Macromolecules* 1988, 21, 59.
- Seymour, R. W.; Estes, G. M.; Cooper, S. L. *Macromolecules* 1970, 3, 579.
- Don, T. M.; Chiu, W. Y.; Hsieh, K. H. *J Appl Polym Sci* 1992, 43, 2193.
- Absolom, D. R.; Zingg, W.; Neumann, A. W. *J Biomed Mater Res* 1987, 21, 161.
- Kaifu, K.; Komai, T. *J Biomed Mater Res* 1987, 16, 757.
- Huang, S. L.; Yu, S. J.; Lai, J. Y. *J Adhesion Sci Technol* 1998, 12(4), 445.
- Moskala, E. J.; Varnell, D. F.; Coleman, M. M. *Polymer* 1985, 26, 228.
- Ruckenstein, E.; Sun, F. *J Membrane Sci* 1994, 95, 207.
- Tanaka, K.; Yoon, T. S.; Takahara, A.; Kajiyama, T. *Macromolecules* 1995, 28, 934.