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Microphase separated structure and protein adsorption of polyurethanes with butadiene soft segment

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Abstract

Membranes of hydroxyl-terminated polybutadiene-based polyurethanes (PUs) were prepared with different types of diisocyanates, hard segment contents and thickness. Three effects on the molar adsorption ratio of fibrinogen to albumin (F/A molar ratio) of polymer surface were under investigation: the deviation of surface from overall composition, the aggregation of hard segments (i.e. phase separation) and membrane thickness. The former two effects were contributed by different membrane thickness. Surface composition was quantified by the absorbance ratio of carbonyl group to butadiene group (C═O/C═C ratio) on FTIR–ATR spectra. While phase separation is expressed by hydrogen bonding index, which is the relative absorbance of the hydrogen bonded carbonyl peak to that of free hydrogen bonded carbonyl peak. We found that a suitable phase separation or surface composition on these PU polymer surface possess the lowest F/A molar ratio.

1. Introduction

Polyurethanes are widely used as biomaterial due to their lower adsorption of biomolecules and good mechanical property. While the protein adsorption on a polymer has been thought to be important for the adhesion of platelet and blood compatibility [1]. Albumin has thromboresistant ability, whereas fibrinogen promotes platelet adhesion on the polymer surfaces [2,3]. The passivation of a surface because of the presence of albumin on it in contrast to the increased adhesion of biological cells (platelets, protein etc.) in the presence of fibrinogen [4,5]. Hence, lower F/A molar adsorption ratio on polymer surface was used in this study to investigate that polymer possesses good blood compatibility and can be used as biomaterials [5,6].

The platelet adhesion and protein deposition of segmented PUs have been extensively studied and it has been shown that these depend on several factors such as the composition ratio of hard and soft segments [7], surface composition [8–12], glass transition temperature [13], type and size of the individual segment [14–16], additives [17], ionic containing [16,18–20], phase separation [13,14,21,22] and hydrophilicity [23–25]. Takahara et al. [13,14] reported that the phase separated morphology of the polyurethane-urea surface, i.e. the pattern of soft polyol domains and hard urethane, would have influence on the protein-surface interactions or protein adsorption. Then this protein adsorption in turn would effect the extent of platelet deposition, activation and thrombogenesis.

Hydroxyl-terminated polybutadiene (HTPB) is a nonpolar material due to it's composition with approximate 60 wt% of *trans*-1,4, 20 wt% of *cis*-1,4 butadiene segment and 20 wt% of vinyl-1,2 segment. Superior hydrolytic stability and high mechanical property performances of HTPB show surprising uti-

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Table 1
Designation of the PUs

Designation	Disocyanate	HTPB/diisocyanate/chain extender	Hard segment content (wt%)
H11615	H12MDI	1/16/15	67.52
H11211	H12MDI	1/12/11	60.79
H187	H12MDI	1/8/7	50.56
H143	H12MDI	1/4/3	33.08
M11211	MDI	1/12/11	59.95
M187	MDI	1/8/7	49.66
M143	MDI	1/4/3	32.27

lity in many field [26–28]. HTPB-based PUs have been intended it's uses in gas and liquid separation due to it's high phase separation between hard segments of urethane and soft segments of HTPB [29–31]. Other application to the biomedical field of lower surface energy polyol-based PUs was studied in the previous reports [15]. Protein adsorption of these HTPB-based PUs were studied in this paper due to it's nonpolar property and subsequent change of composition and phase separation. Because polymer chains of these HTPB-based PUs at the air–polymer interface are in an unsymmetrical environment in comparison with that within the membrane [32].

In this article, we try to study the effect of membrane thickness on the surface composition and phase separation of these HTPB-based PUs. These PUs were prepared with different hard segment content and types of diisocyanate. The surface composition were

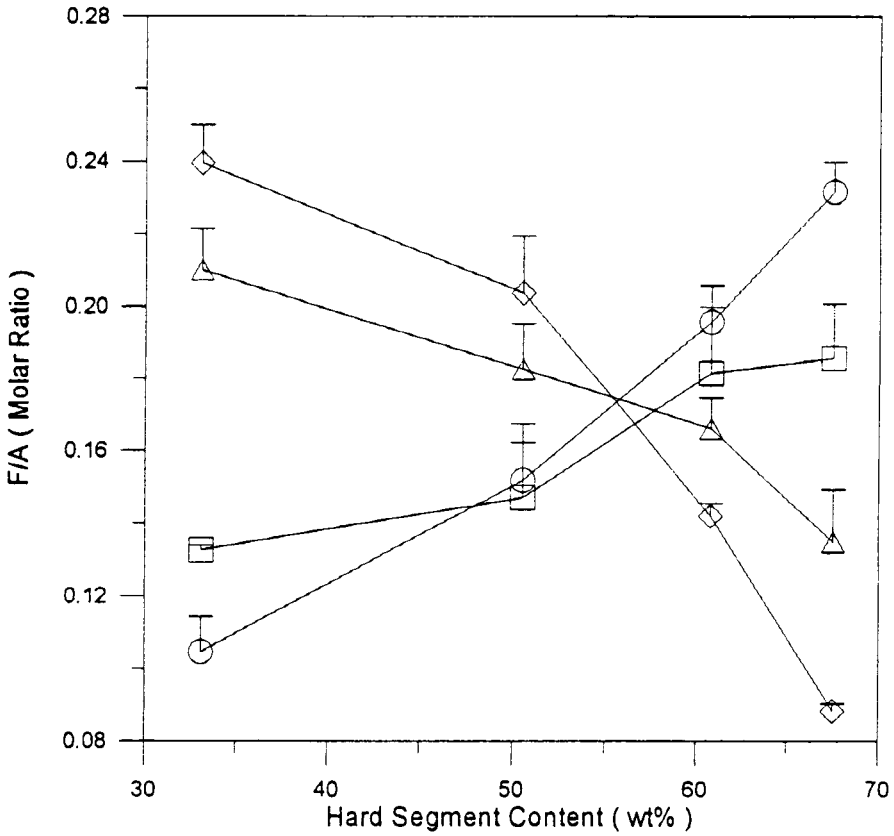

Fig. 1. Effect of hard segment content on the F/A molar ratio of H_{12}MDI-based PUs with different membrane thickness: (○) 30 μm, (□) 90 μm, (△) 120 μm, (◇) 150 μm.

investigated by the C═O/C═C ratio. While hydrogen bonding index (HBI) for the indication of phase separation was defined as the absorbance ratio of hydrogen bonded to unbonded carbonyl groups on FTIR–ATR spectra. The relationship between F/A molar ratio and membrane thickness, surface composition and phase separation were investigated.

2. Experimental

2.1. Materials

The chemicals used in this study were 4,4′-dicyclohexylmethane diisocyanate (H_{12}MDI, Desmodur W of Mobay), 4,4′-diphenylmethane diisocyanate (MDI) and hydroxyl terminated polybutadiene (equivalent weight of 1333, R-45M of ARCO). 1,4-butane diol (1,4-BD) was used as chain extender and dibutyltin dilaurate (DBTDL) was used as catalyst. Toluene and dimethylformamide (DMF) were used as solvents. Fibrinogen from human plasma of M.W. 341000 and albumin from human serum of M.W. 68000 (Sigma) were used. Sodium citrate, sodium phosphate and sodium chloride (Merck) were used for the preparation of CPBS buffer solution. Triton X-100 and dodecyl sodium sulfate and sodium hydroxide were used for the preparation of desorbed solution for protein from polymer surface.

2.2. Membrane preparation

The two-stage polyurethanes with different equivalent ratio were polymerized first by a NCO-terminated prepolymer and then chain extended with chain extender to give a 25 wt% solid content. Detailed procedures for polymerization had been reported in a previous publication [33]. All these PUs were then put in an oven under a vacuum at 70°C for 48 h for further degassing of the solvent residue. Different thickness of membrane were measured in dried state and controlled from wetted coating with same solid

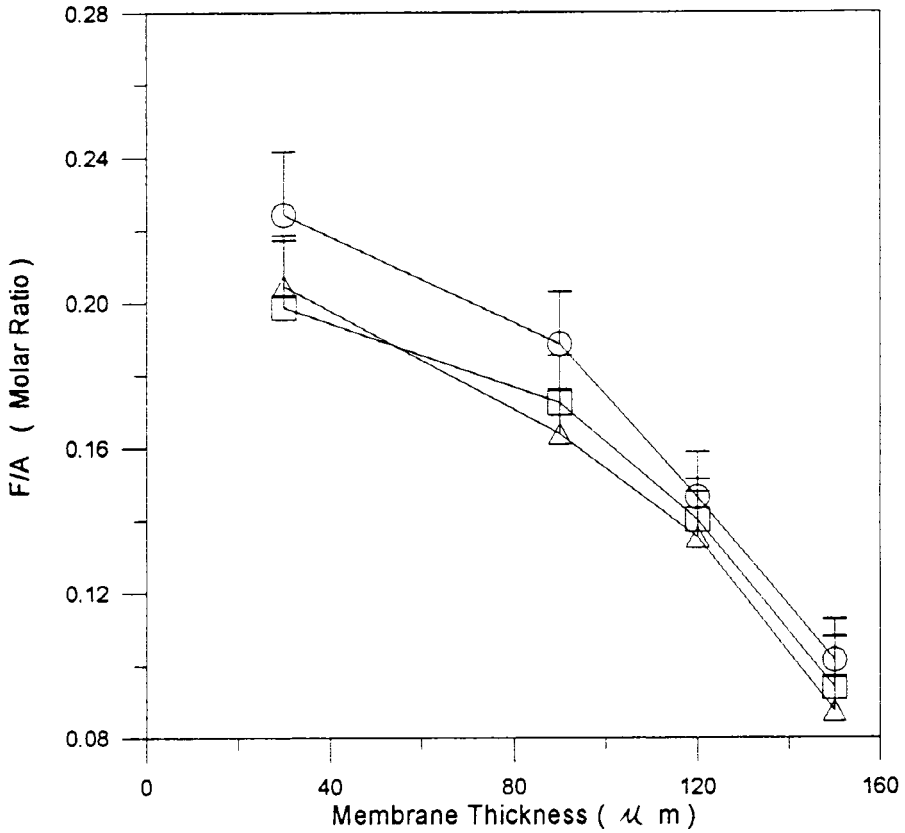

Fig. 2. Effect of membrane thickness on the F/A molar ratio of MDI-based PUs with different hard segment content: (○) M11211, (□) M187, (△) M143.

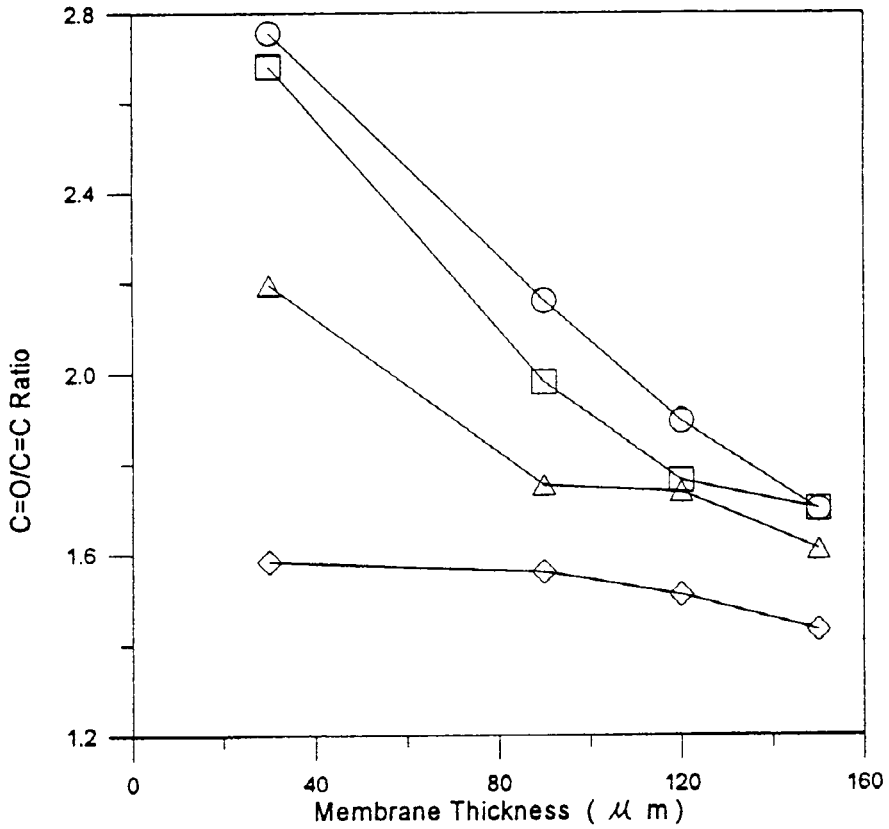

Fig. 3. Effect of membrane thickness on the C═O/C═C ratio of H12MDI-based PUs with different hard segment content: (○) H11615, (□) H11211, (△) H187, (◇) H143.

content and nearly the same viscosity. Finally, the samples were kept under a vacuum at room temperature for at least 5 days prior to the test. In this study, PU films with different compositions, for instance, the equivalent ratio of HTPB/H_{12}MDI/1,4-BD = 1/12/11 and HTPB/MDI/1,4-BD = 1/8/7 are denoted by H11211 or M187, respectively. All the PU compositions are presented in Table 1. These seven types of PUs are all prepared with four different membrane thickness of 30, 90, 120 and 150 μm, respectively.

2.3. Molar ratio of fibrinogen to albumin adsorption

The PU films with 8 cm^2 surface area were immersed into CPBS buffer solution (0.01 M sodium citrate, 0.01 M sodium phosphate, 0.12 M sodium chloride, pH 7.40) for 12 h [34]. The film surface was then quickly blotted with absorbent paper to remove buffer solution from surface. Protein concentrations of albumin and fibrinogen were 1.0 and 0.2 mg/ml, respectively, in CPBS buffer solution. The films were then filled with 2

Table 2
Solubility parameter of raw materials[a]

Materials	HTPB	MDI	H12MDI	1,4-BD
Solubility parameter (δ, $cal^{1/2}/cm^{3/2}$)	8.67	13.95	8.98	11.00

[a] Values obtained by estimation method.

ml of protein solution at 30°C for 1 h. After desorption, the films were then rinsed with deionized water. The absorbed proteins were desorbed with solution of 1% Triton X-100 and 1% dodecyl sodium sulfate in 0.01 N NaOH at 30°C with agitating at 100 rpm for 1 h by shaker [35]. Afterwards buffer solution with pH 9.30, which composes of both 0.05 M boric acid and KCl, was added into the desorbed protein solutions. 0.5 ml of 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, F-2000) and the fluorescence intensity was measured at 392 nm with excitation and at 491 nm with emission.

2.4. Infrared spectroscopy

Infrared spectra of PU were obtained by using a JASCO FTIR-310E spectrometer. The films were pressed against a 45° Germanium crystal. Spectra were collected at a resolution of 2 cm^{-1}. C═O/C═C absorbance ratio is defined as the absorbance ratio of carbonyl (hydrogen bonded plus unbonded) with 1,4-*trans* C═C group of butadiene segment.

The carbonyl absorption band between 1800 and 1600 cm^{-1} splits into two peaks. The peak due to hydrogen-bonded C═O stretching is centered at about 1700 cm^{-1} and that due to free C═O stretching is centered at about 1717 cm^{-1} (H_{12}MDI) and 1734 cm^{-1} (MDI). Hydrogen-bonded carbonyl 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 [36].

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 absorbances of the hydrogen bonded carbonyl group to free hydrogen bonded carbonyl group [37]. The purpose of this study only need the change of

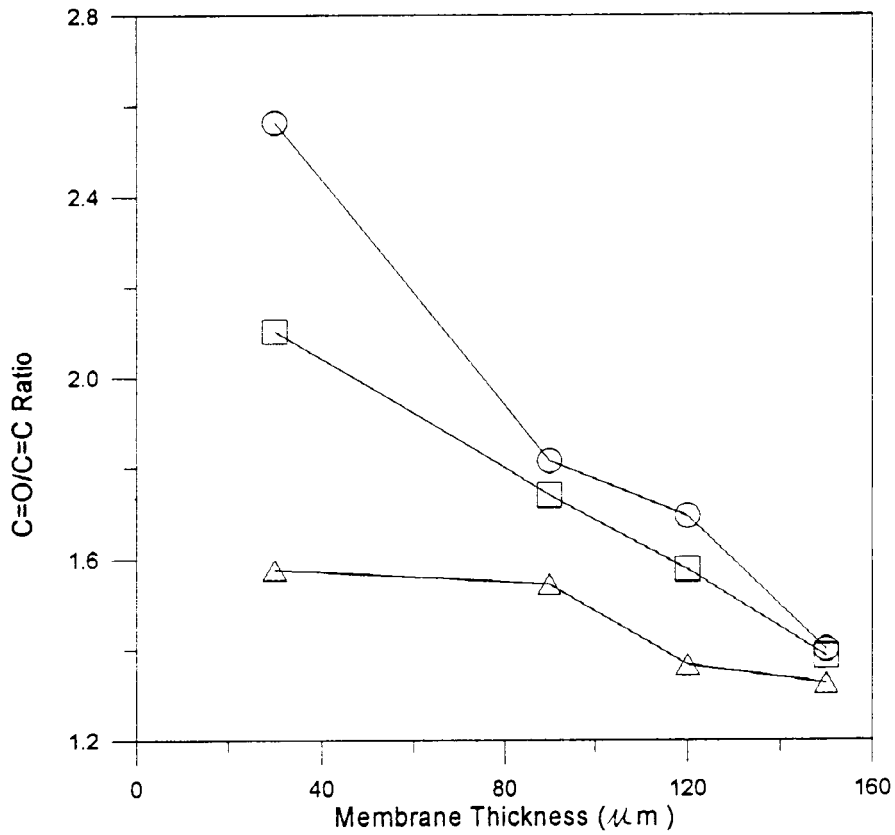

Fig. 4. Effect of membrane thickness on the C═O/C═C ratio of MDI-based PUs with different hard segment content: (○) M11211, (□) M187, (△) M143.

HBI value to indicate the trend of the degree of separation of the same diisocyanate series. The greater HBI values indicate increased participation of the carbonyl group in hydrogen bonding between intermolecular hard segment and hence the lower degree of separation between hard and soft segments [33] of these butadiene-containing PUs.

The infrared 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 total absorbances of carbonyl group can be used as an indication of hard segment content on surface. The infrared absorption band of butadiene soft segment are to be *trans*-1,4 form at 972 cm^{-1}, 1,2 form at 912 cm^{-1} and *cis*-1,4 form at 685 cm^{-1} [38]. 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 of C═C group. The C═O/C═C absorbance ratio can then be used as the ratio of hard segment content to soft segment content. Larger value of C═O/C═C ratio of membrane indicates that more hard segment content dispersed on the surface than the other membrane with lower C═O/C═C ratio.

3. Results and discussion

3.1. Effect of membrane thickness

To confirm the effect of membrane thickness on F/A adsorption ratio, HTPB-based PU solutions of various hard segment contents were synthesized and membranes with different thickness were then prepared. Fig. 1 shows the effect of hard segment content on F/A adsorption ratio of H_{12}MDI-based PU membranes with different thickness. The F/A molar ratio increased with increasing hard segment content of membranes with 30 and 90 μm thickness. However, F/A molar ratio decreased with increasing hard segment content of membranes with 120 and 150 μm thickness. Fig. 1

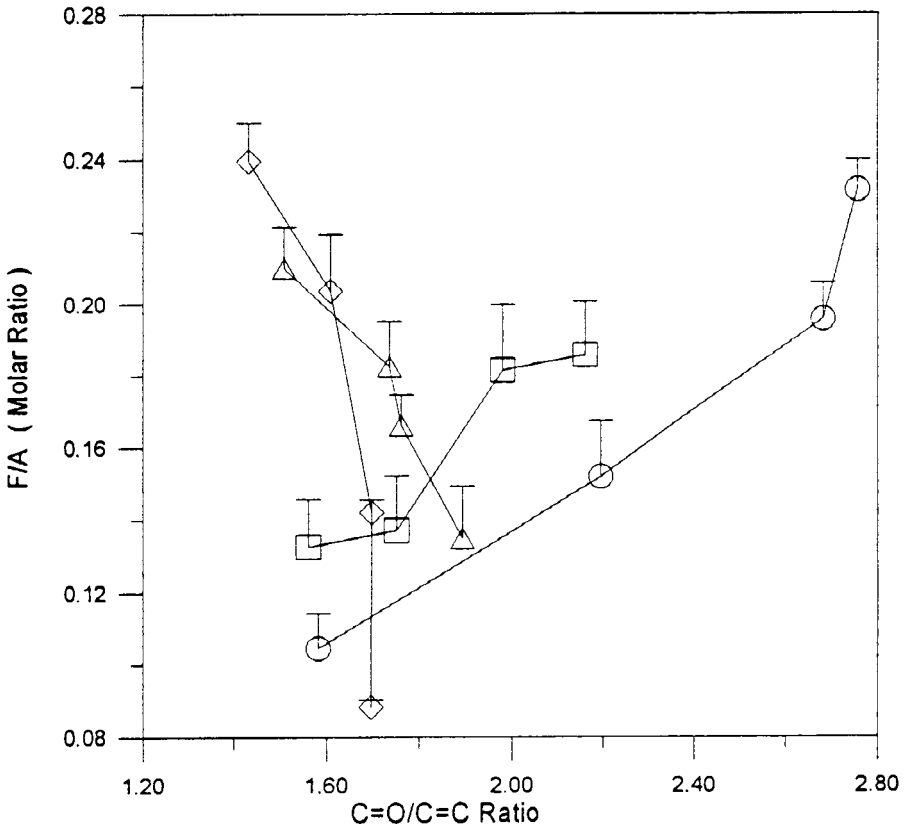

Fig. 5. Relationship between C═O/C═C ratio and F/A molar ratio of H_{12}MDI-based PUs with different membrane thickness and hard segment content: (○) 30 μm, (□) 90 μm, (△) 120 μm , (◇) 150 μm.

also shows the two types of membrane with lowest F/A ratio: (1) membranes containing least hard segment content and possessing thinnest membrane thickness are present on the underside of the left of the figure and (2) membranes with highest hard segment content and thickness are present on the underside of the right of the figure.

The effect of membrane thickness also existed for HTPB-based PU membranes using MDI as the hard segment component. Fig. 2 shows the effect of membrane thickness on F/A molar ratios of MDI-based membranes containing various amount of hard segment contents. Surprisingly, the membrane thickness had strong effect on F/A molar ratio. The F/A molar ratio decreased with increasing membrane thickness but had little variation among membranes of the same thickness. These two phenomena were studied in the following section that a suitable phase separation between hard segment and soft segment or surface composition in the above PU membranes with lower F/A molar adsorption ratio.

3.2. Surface composition

Surface composition and phase separation may be the two possible factors, which attributed to the effect of membrane thickness. Surface composition was measured by FTIR–ATR and represented by the C═O/C═C ratio. It was suspected that two effects may be introduced by the variation of surface composition from the bulk. One effect is the compatibility between H_{12}MDI and HTPB and the other was the aggregation of hard segment. As is shown in Table 2, the solubility parameter of H_{12}MDI and HTPB are nearly the same. The same solubility parameter indicates that H_{12}MDI and HTPB are compatible. The former compatibility effect induces that less variation of surface composition from the bulk or the increase of C═O/C═C ratio on the surface. While the latter aggregation effect of hard segment intends the lower surface energy polyol of HTPB soft segment migrating toward the air–polymer surface and has the reverse effect on the decrease of C═O/C═C ratio on the sur-

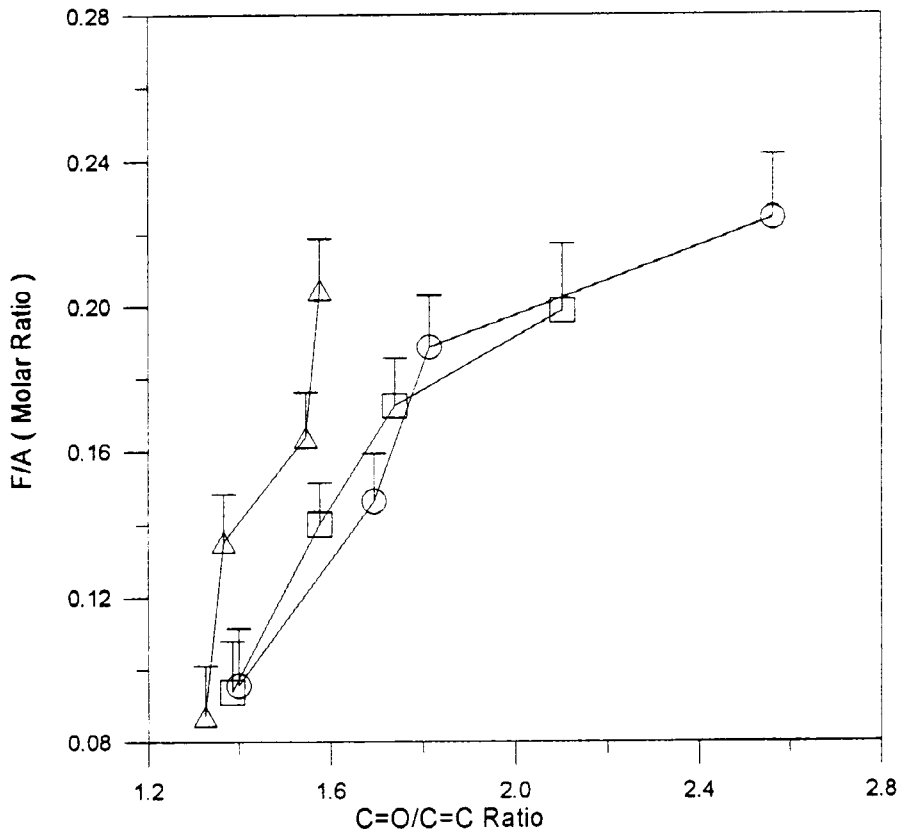

Fig. 6. Relationship between C═O/C═C ratio and F/A molar ratio of MDI-based PUs with four different membrane thickness and hard segment content: (○) M11211, (□) M187, (△) M143.

face. PUs with high hard segment content contain longer aggregation of hard segment compared with PUs of low hard segment content [39]. The variation of surface composition with respect to bulk composition of H_{12}MDI-based PUs at different membrane thickness is shown in Fig. 3. Fig. 3 indicates that the contents of carbonyl group increased along with the increase of hard segment content for membranes of all thickness, while the surface composition of thicker membranes did not strongly depend on the bulk composition. In other words, the surface of thicker membranes contained lesser carbonyl groups than that of the thinner ones. It is also shown in Fig. 3, as the membrane thickness increased up to 150 μm, the surface compositions were nearly the same among membranes of different bulk hard segment contents.

Fig. 4 shows that the surface composition of another MDI-based PU membranes decreased as the hard segment content and membrane thickness increased also. The difference between surface and bulk composition was even more obvious. It was suspected that the tendency of low-surface energy HTPB migrating to the top surface of membranes might attribute to composition difference between the surface and bulk polymer. Since the solubility parameter of MDI is higher than that of H_{12}MDI, which is shown in Table 2, it was expected to see more severe deviation between bulk and surface composition of MDI-based PUs.

3.3. Effect of surface composition on F/A adsorption ratio

The variation of F/A molar ratio of H_{12}MDI-based PU membranes with surface composition is shown in Fig. 5. Minimum F/A molar ratio can be obtained at the region of C═O/C═C ratio starting from 1.6 to 2.0. These results are in agreement with that reported by Kajiyama et al. [40]. They reported that PUs with both the soft or hard segment rich surfaces are thrombogenic and the appearance of minimum platelet adhesion corresponds to PUs with

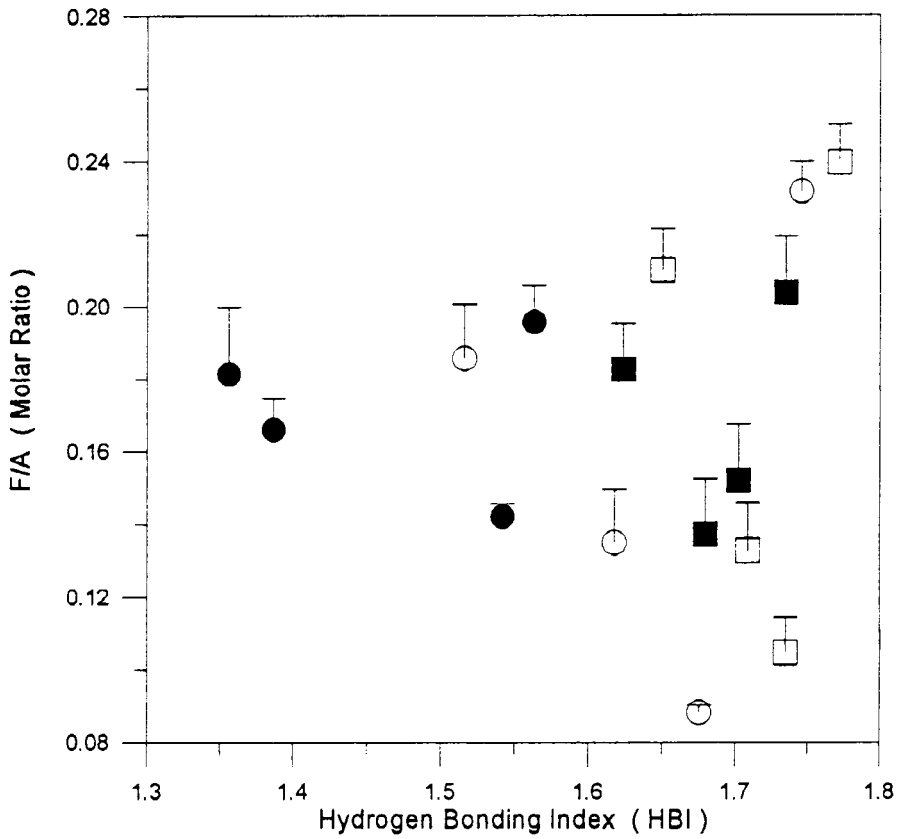

Fig. 7. Relationship between F/A molar ratio and hydrogen bonding index (HBI) of H_{12}MDI-based PUs for all studied membrane thickness: (○) H11615, (●) H11211, (■) H187, (□) H143.

suitable amount of hard segment content on the surface. However, the F/A molar ratio of these PUs still depend on membrane thickness. The F/A molar ratios on membranes of similar surface composition varied from one membrane thickness to another. This result indicated that there existed other factors affecting fibrinogen and albumin adsorption in addition to the chemical composition of membrane surface.

As mentioned previously, the overall composition of MDI-based PU membranes had little effect on F/A molar ratio. However, the F/A ratio did not vary with surface composition, which is shown in Fig. 6. When the membrane thickness reached 150 μm, it was also found that membranes of different overall compositions had similar surface composition as shown in Fig. 4. The lowest F/A ratio is 0.085 at surface C═O/C═C ratio around 1.35 as shown in Fig. 6. It was obvious that the F/A molar ratio on MDI-based PUs, like that on H_{12}MDI ones, was strongly affected by membrane thickness. The surface composition alone would not be able to explain the membrane thickness effect.

3.4. Effect of phase separation on F/A adsorption ratio

The formation of hard segment domain on the surface may be another factor related to the membrane thickness effect. The aggregation of hard segment is presented by the HBI which is defined as the ratio of the absorbance height of hydrogen bonded C═O peak to that of unbonded one. The effect of HBI value was presented by plotting F/A molar ratio versus HBI values of membranes for all thickness since membranes of different overall compositions had different surface composition. As shown in Figs. 7 and 8, the lowest F/A ratio of H_{12}MDI containing PU membranes happened at about HBI = 1.65; while that of MDI-based PUs happened at about 1.40. According to the above information, the manufactured membrane should not contain too many or too few hydrogen bonded carbonyl groups on the surface, in order to obtain a membrane with a low F/A ratio. HBI value indicates participation of the carbonyl group in hydrogen bonding between intermolecular hard segment. Hence, greater HBI value means increased participation of the

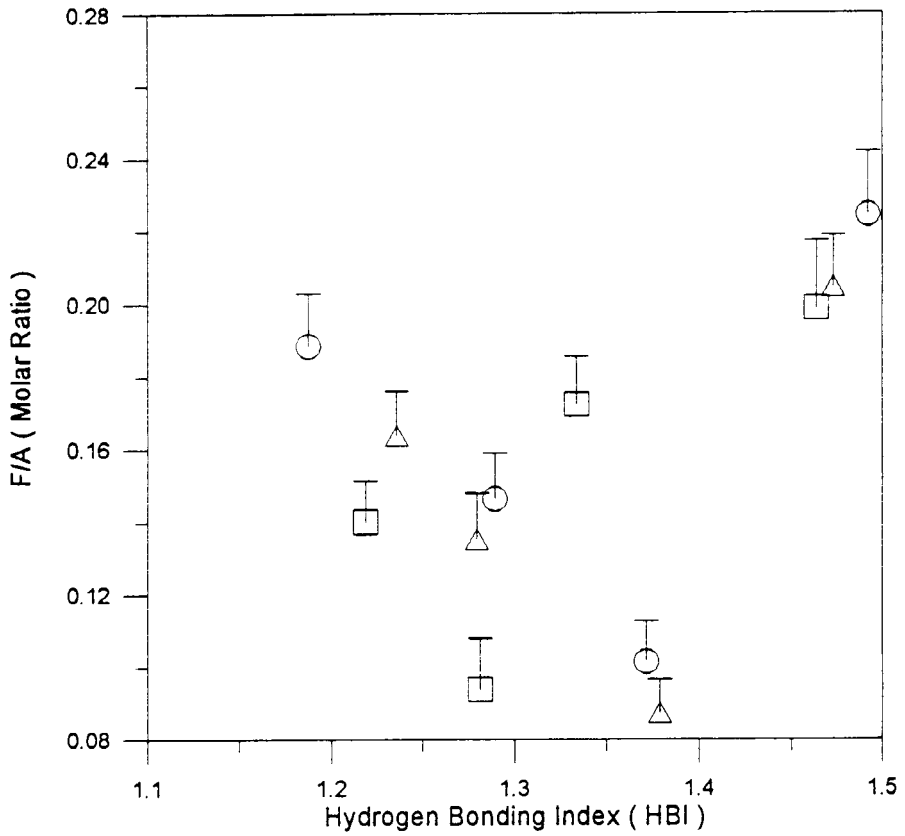

Fig. 8. Relationship between F/A molar ratio and hydrogen bonding index (HBI) of MDI-based PUs for all studied membrane thickness: (○) M11211, (□) M187, (△) M143.

carbonyl group in hydrogen bonding between intermolecular hard segment and hence the lower degree of separation between hard and soft segments of these butadiene-containing PUs [33]. In other words, membranes with an appropriate HBI value as described in the above two HBI values means a suitable degree of micro-phase separation and smallest fibrinogen (i.e. lowest F/A ratio) deposited, which is consistent with that reported by Takahara et al. [14].

4. Conclusions

Surface composition and aggregation of hard segment were contributed by different membrane thickness. In this study, phase separation was revealed by the ratio of hydrogen bonded to unbonded carbonyl groups. According to our results, there is an optimal surface composition which yields the lowest F/A molar ratio. There exists also an optimal HBI value. For H_{12}MDI-based PUs, The optimum conditions happened at surface C═O/C═C ratio around 1.6 and HBI around 1.7; for MDI-based PUs, the optimum happened when C═O/C═C ratio and HBI equal to 1.35 and 1.4, respectively. Although a universal index to describe the behavior of F/A molar ratio on any polymer has not yet been found, this study implies that any procedure taken in membrane processing that can affect its phase separation and hence its biocompatibility.

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