Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Membrane Science 245 (2004) 137–146
journal of MEMBRANE SCIENCE
www.elsevier.com/locate/memsci

Immobilization of heparin on PVDF membranes with microporous structures

Dar-Jong Lin[a], Dong-Tsamn Lin[b], Tai-Horng Young[c], Fang-Ming Huang[a], Ching-Chung Chen[a], Liao-Ping Cheng[a,*]

[a] *Department of Chemical and Materials Engineering, Tamkang University, Taipei 25137, Taiwan, ROC*
[b] *Department of Laboratory Medicine, College of Medicine, National Taiwan University, Taipei 10016, Taiwan, ROC*
[c] *Institute of Biomedical Engineering, College of Medicine and College of Engineering, National Taiwan University, Taipei 10016, Taiwan, ROC*

Received 30 October 2003; received in revised form 1 July 2004; accepted 15 July 2004
Available online 7 October 2004

Abstract

Heparin (an anti-thrombin) was immobilized onto microporous poly (vinylidene fluoride) (PVDF) membranes that were prepared either by dry or immersion precipitation method. Immobilization of heparin was carried out by a procedure consisted of two steps. First, poly (acrylic acid) (PAA) was grafted on PVDF membranes with various surface porosities by plasma-induced polymerization. Then, heparin was covalently bonded to PAA with the aid of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDAC). The highest attainable grafting yield of PAA was 0.68 mg/cm^2. For heparin immobilization, it was found that the preparation parameters such as, EDAC activation time, concentration of reactants, and pH affected significantly the amount of immobilized heparin. The maximal value was 9.68 μg/cm^2. Furthermore, blood compatibility tests were carried out on membranes with and without heparin by platelet adhesion method. The results indicated that immobilized-heparin could effectively inhibit platelet adhesion on the membranes.

Keywords: Composite membranes; Plasma polymerized membranes; Immobilization; Heparin

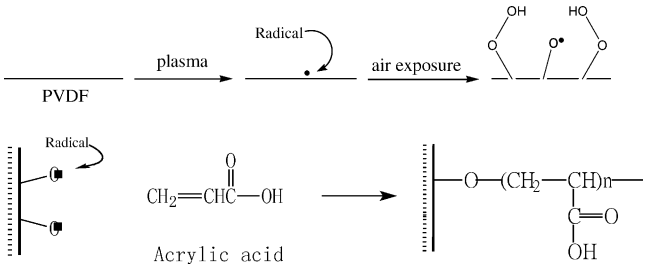
1. Introduction

Poly (vinylidene fluoride) (PVDF) is an acid resistant, chemically inert, and mechanically strong polymer that has been used in various biomedical applications [1,2]. PVDF can be processed by phase inversion technique into microporous membranes that encompass a wide spectrum of surface morphologies. For example, it can be a layer of PVDF crystallites that close-packed into a nonporous dense skin; by contrast it can also be totally open for being composed of interlinked globules [3,4]. With such a variety in surface morphology, it is of great interest to investigate how much the surface morphology affects the efficiency of chemical modification on these membranes.

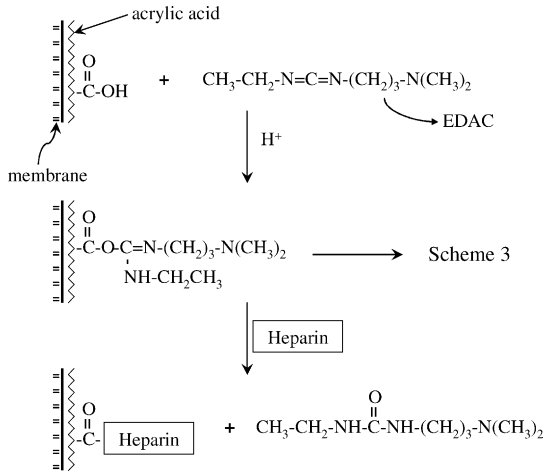
Plasma-induced polymerization has been known as a useful method for grafting polymer chains on the surface of a polymeric material; the purpose of which is to provide the surface with a specific property, e.g., hydrophilic, hydrophobic, biocompatible, and so forth, that serves a particular need [5–8]. PVDF membranes can be used as substrates subjected to various treatments to prepare composite membranes [9–11]. In the current work, attempts were made to immobilize heparin on them. This was made possible by introduction of poly (acrylic acid) (PAA) as an inter-linkage between PVDF and heparin. PAA was first grafted on PVDF substrate by plasma-induced polymerization. Subsequently, the carboxylic acid group of PAA was chemically reacted with heparin by the action of a carbodi-

* Corresponding author. Tel.: +886 2 26215656x2725; fax: +886 2 26209887.
E-mail address: lpcheng@mail.tku.edu.tw (L.-P. Cheng).

0376-7388/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.memsci.2004.07.028



Scheme 1. Plasma-induced grafting of PAA on PVDF.



Scheme 2. Immobilization of heparin to form heparin/PAA/PVDF membrane.

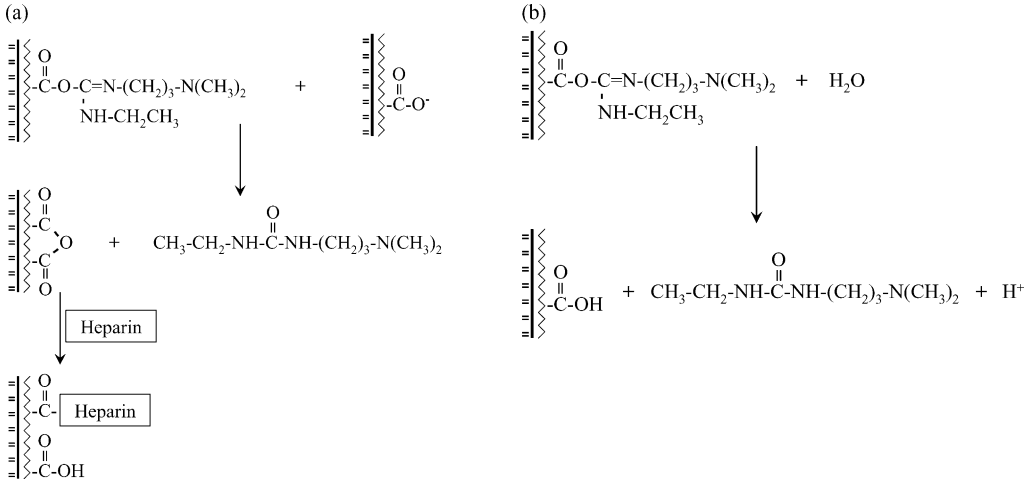
imide (1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDAC)) to form heparin/PAA/PVDF composite membranes. The synthesis routes for these composite membranes are shown in Schemes 1–3 below [12–17]. In Scheme 1 for grafting PAA, PVDF was plasma treated and then exposed to air to form peroxides, which decomposed into free radical $-CO^{\bullet}$ at elevated temperatures or in the presence of Fe^{2+} (e.g., Mohr salt). These radicals served to initiate the free radical polymerization of AA monomers to produce a layer of PAA on the surface of the PVDF membrane. To immobilize heparin on PAA, Kang's method was adopted [14,15]. As shown in Scheme 2, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride was first reacted with carboxylic acid to form *o*-acrylisourea derivative, which was then reacted with amine group (nucleophilic) of heparin to yield amide bond between PAA and heparin, and leave the isourea derivative as a byproduct. Scheme 3 shows the possible side reactions, in which route (a) also results in heparin immobilization via reaction of amine with anhydride. The detailed mechanism can be found in the literature [14–17].

Immobilization of heparin on polymeric materials has been investigated by Kang et al. [14,15] and Shiomi and coworkers [18,19] using EVAL and PU dense films, respectively, as the substrate materials. However, because these films were nonporous, the immobilization yields were limited to a relatively low level. To increase the amount of immobilization and to understand the effects of substrate's surface morphology on the immobilization yields, a series of PVDF membranes with very different surface porosity were prepared and employed as the substrates for immobilization. Also, blood compatibility test was performed on heparin-immobilized membranes to see their anticoagulation capabilities with respect to platelet rich plasma (PRP).

2. Experimental

2.1. Material

PVDF (Elf Ato Chem, Kynar 740, $M_\eta = 245\,000$) was a semicrystalline polymer. *N*-Methyl-2-pyrrolidone (NMP, Acros, 99%, $d = 1.028$ g/ml) and *N*,*N*-dimethyl formamide (DMF, Across, 99%, $d = 0.945$ g/cm^3) were used as the solvent whereas distilled and deionized water was used as the nonsolvent for PVDF membrane preparation. Acrylic acid



Scheme 3. Reactions of *o*-acrylisourea.

(Acros, 99.5%, $d = 1.0510$ g/ml) was vacuum distillated to removed trace of inhibitor MEHQ prior to grafting reaction. Heparin sodium salt aqueous solution (anticoagulant activity = 100 unit/mg) was purchased from Aldrich. EDAC was purchased from TCI.

2.2. PVDF membrane formation

PVDF membranes were prepared both by the dry (solvent evaporation) and the immersion–precipitation method. For the dry method, a dope solution composed of 20 wt.% PVDF in DMF was cast on a glass plate. Then solvent was allowed to evaporate under ambient condition for 1 day. The formed film was put in vacuum at an elevated temperature to remove trace of residual solvent. The membrane thus obtained was relatively transparent and nonporous. For the immersion–precipitation method, the dope (20 wt.% PVDF in DMF or NMP) was cast on a glass plate and then immersed in a coagulation bath to induce precipitation. The nascent membrane was washed in a series of nonsolvents and then dried at 45 °C. The conditions for membrane preparation are summarized in Table 1. The morphology of the membrane was observed using SEM (Hitachi, S800). Dried samples were sputtered with a layer of Au–Pd and imaged at 20 kV. Some structures of the membranes prepared according to the conditions shown in Table 1 (cases P1, P2, and P3) have been published previously [3,20–22]. They are similar to those shown in Fig. 1, except for a little variation in P1 membrane (compared with Fig. 4 in reference [3]). However, it can be certain that the porosity of P1 is between P0 and P2 in both cases.

Table 1
Preparation conditions for PVDF membranes with various structures

Code	Components	Dope	Bath	Method
P0	Water–DMF–PVDF	A	AIR	Dry
P1	Water–DMF–PVDF	A	Water	Wet
P2	Water–NMP–PVDF	B	Water	Wet
P3	Water–NMP–PVDF	B	70% NMP/water	Wet

Dope A: 20% PVDF in DMF; Dope B: 20% PVDF in NMP.

2.3. PAA/PVDF composite membrane formation

PAA was covalently grafted onto the surface of a PVDF membrane via plasma-induced free radical polymerization. The membrane was plasma-irradiated (50 W, Argon at 52.33 Pa (0.4 Torr)) for 1 min and then exposed to the air for 10 min to form peroxide on the membrane surface, after which it was immersed directly in a deaerated PAA/water solution containing 0.0015 M Mohr's salt at 80 °C for a specific

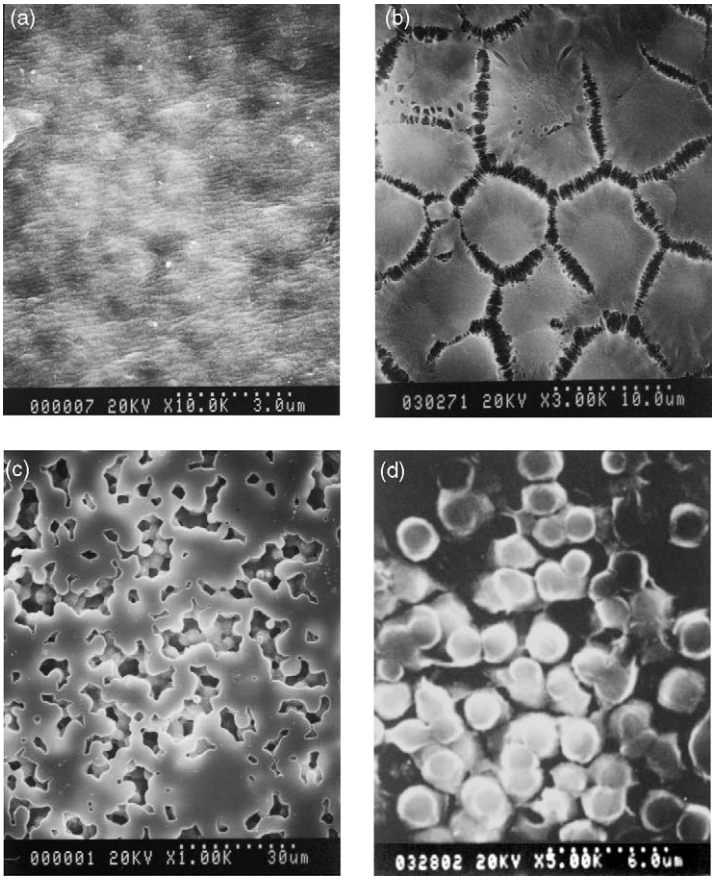


Fig. 1. SEM photomicrographs of the bottom surfaces of membranes prepared by the conditions shown in Table 1. (a) P0; (b) P1; (c) P2; and (d) P3.

time. The grafted composite membrane was washed in a ultrasound water bath and then rinsed in water to remove residual AA and un-grafted PAA. Presence of PAA on PVDF membrane was identified by FTIR/ATR at the absorption peak of the carbonyl group (1710 cm^{-1}). The amount of grafted PAA was determined by titration method. The grafted membrane was put in a 0.01N NaOH solution, which was shaken for 24 h and then the solution was titrated against 0.001N HCl.

2.4. Immobilization of heparin

PAA/PVDF membrane (3 cm × 4 cm) was put in a 30 ml buffer solution (pH 5, molar ratio of sodium hydroxide/citric acid = 1/1, 0.1 M in water) containing a known quantity of EDAC. Reaction was allowed to proceed at 4 °C for a period of 1–24 h to activate the carboxylic acid group of PAA (cf. Scheme 2). A known amount of heparin solution (0.1–0.3 ml) was subsequently added to the solution wherein immobilization reaction was carried out for 24 h. The formed heparin/PAA/PVDF composite membrane was washed in a buffer solution containing 0.1% Triton X-100 for 1 h to remove residual un-reacted heparin and byproduct, isourea. The composite membrane was then rinsed in water and freeze dried. The amount of heparin immobilized on the PAA/PVDF composite membrane was determined by UV colorimetry using toluidine blue (631 nm) as the reagent, as described in the literature [23].

2.5. Platelet adhesion test

Platelet adhesion test was performed by observation under SEM of adsorption of human PRP [24] on the prepared membranes [15,25]. Membrane was rinsed with phosphate buffer solution (PBS, pH 7.4) and then immersed in PRP for 60 min to allow interaction between PRP and membrane. Subsequently, the membrane was washed repeatedly with PBS and then immersed in PBS solution containing 2.5% glutaraldehyde for 1 h to fix the adhered platelets. After thorough washing with PBS, the platelets were dehydrated by graded ethanol changes and then critical point dried. Sample was then gold sputtered in vacuum and examined by SEM.

3. Results and discussion

3.1. Grafting of PAA on PVDF membranes

The surface morphologies of the PVDF membranes employed as the substrates for preparing PAA/PVDF composite membranes are shown in Fig. 1(a)–(d). The preparation conditions for these membranes can be found in Table 1. Membrane ‘P0’, prepared by dry method, appears to be dense and nonporous while the other membranes have a porous bottom surface. For membrane ‘P1’, the bottom surface is composed of large polygonal clusters whose boundaries broke into linear cavities. These clusters have been shown previously to be PVDF impinging spherulites. Membranes ‘P2’ and ‘P3’ were prepared by precipitation from baths of different strengths. Their bottom surfaces are composed of globular particles (commonly called nodules). For the case of precipitation in a water bath, liquid–liquid demixing dominate the precipitation process to yield a primarily cellular structure. PVDF crystallization takes place at a late stage on the cell walls or close to the bottom surface of the membrane. By contrast, in a soft bath (e.g., 70 NMP/water for membrane ‘P3’), mass transfer is slow and thus crystallization process competes with liquid–liquid demixing to form a mixed structure in the cross section. At the bottom surface, however, crystallization dominates and nearly full spherulites are produced. The flatten effect of the particles as a result of growth against glass support is obviously stronger in membrane ‘P2’. For the membranes shown in Fig. 1, it appears that the porosity increases in the following order: ‘P3’ > ‘P2’ > ‘P1’ > ‘P0’. It is therefore anticipated that the grafting yield follows a reversed trend.

PAA was grafted by plasma-induced polymerization on the bottom surfaces of the membranes ‘P0’–‘P3’. The presence of PAA on the formed PAA/PVDF composite membrane was verified qualitatively by FTIR/ATR. Fig. 2 shows the spectra of two typical composite membranes with different grafting yields together with that of the pure membrane substrate ‘P3’. The absorption peak at 1710 cm^{-1}, being characteristic of C=O stretching vibration of PAA, is evident for the PAA/PVDF composite membranes, yet it is not observed for the pure PVDF membrane. This clearly confirms that PAA has been grafted on the membrane substrate. The grafting yield for common plasma-induced free radical polymerization, as discussed in the literature, depends upon factors such as plasma treatment time, concentrations of reactants, reaction temperature, reaction time, etc. [26–30]. In the present work, attention has been focused on the effects of the monomer concentration, the reaction time, and the morphology of the grafting surface. The results are demonstrated in Figs. 3 and 4.

In Fig. 3, the amounts of grafted PAA versus reaction time are demonstrated for different membranes. The initial concentration of AA monomer was 30 wt.% for all cases. It can be seen that the grafting yields increase with increasing grafting time throughout the reaction. Given the fact that only a definite quantity of active sites were initially generated on the surface of the PVDF membrane by plasma bombardment, the substantial increase of PAA with time was thought to be growth of PAA chain rather than creation of new polymer chains. As the bulk solution also underwent free radical polymerization, the reaction mixture gelled or solidified after a certain period of time, depending upon the initial monomer concentration. For an already gelled sample, characterization of the PAA/PVDF composite membrane was difficult, and the data were not reported here. Fig. 3 also indicates the effect of the surface porosity of the PVDF substrate. Obviously, more porous surface exhibits higher graft-

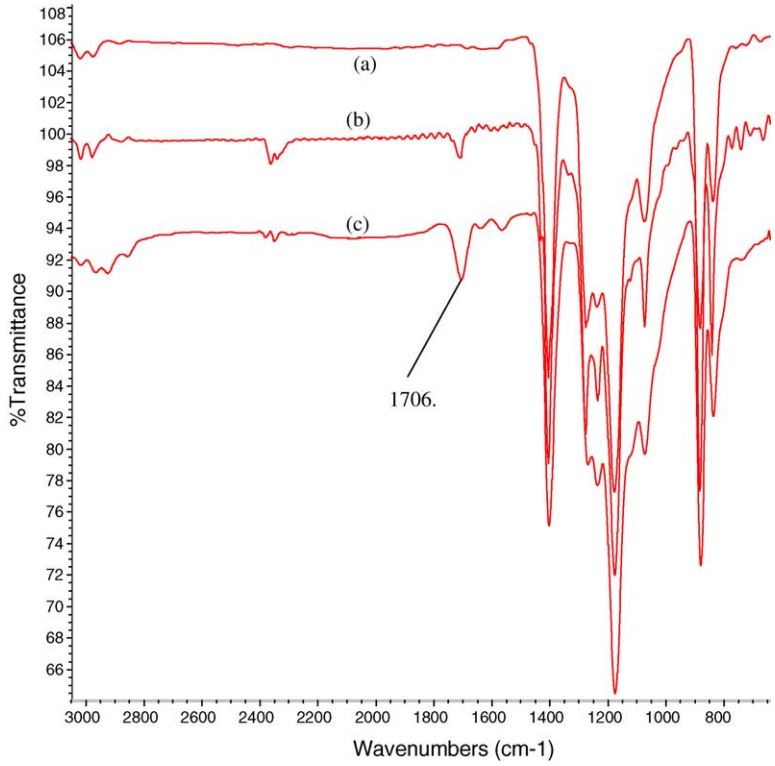


Fig. 2. FTIR/ATR spectra of pure PVDF membrane and PAA/PVDF composite membranes with different amounts of grafted PAA. (a) Membrane P3; (b) graft yield = 0.34 mg/cm^2; and (c) graft yield = 0.65 mg/cm^2.

ing yield. This is associated with the much higher surface area available for more porous membranes both in terms of radical generation by plasma radiation and pore filling during chain growth. The highest grafting yield for membrane 'P3' reached 0.65 mg/cm^2 whereas that for nonporous film 'P0' was only 0.27 mg/cm^2.

Fig. 4 shows the amount of grafted PAA versus initial monomer concentration for different membranes. The grafting time was 3 h for all cases. The grafting yield, as is expected, increases with increasing monomer concentration over a wide concentration range (10–40 wt.% AA). Similar trends have been reported in the literature for various systems [5–7,26–30]. The fact that high surface porosity favors PAA grafting is also evident in Fig. 4; the grafting yield of 'P3' was ca. three times that of 'P0'. Fig. 5 shows the SEM images of the grafted surface of a typical composite membrane. The PVDF substrate was 'P3' and the grafting yield was 0.68 mg/cm^2. Compared with Fig. 1(d), the original 'P3', it is clear that the pores on membrane 'P3' surface were covered with a nonporous and rough PAA layer. This layer func-

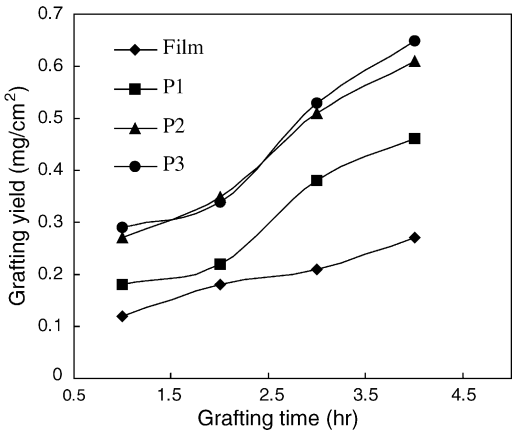


Fig. 3. The effect of reaction time on the grafting yield of PAA for various membranes. Initial AA concentration = 30 wt.%.

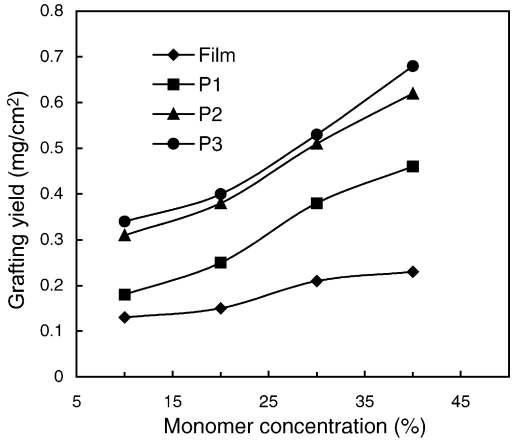


Fig. 4. The effect of monomer concentration on the grafting yield of PAA for various membranes. Grafting time = 3 h.

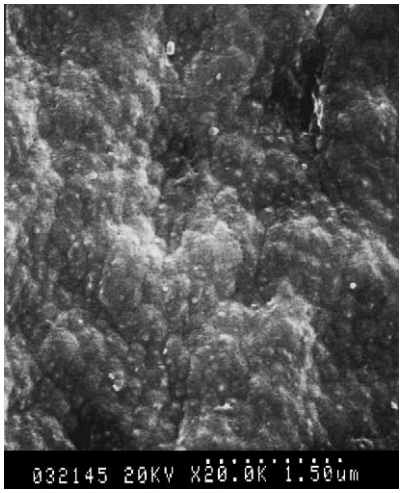


Fig. 5. An SEM photomicrograph of a PAA-grafted surface. The substrate was membrane P3 and the grafting yield was 0.68 mg/cm^2.

tioned as an effective bridge for heparin immobilization via chemical linkage.

3.2. Immobilization of heparin on PAA/PVDF composite membrane

Immobilization was achieved by forming amide bond between amine groups of heparin and carboxylic acid groups of PAA with the aide of EDAC. These reactions were carried out on various PAA/PVDF composites membranes having different amounts of grafted PAA. The results are summarized in Fig. 6 in terms of immobilization yield of heparin versus amount of PAA grated on PVDF. For all cases, 30 mg of EDAC and 0.1 ml of heparin were added to the initial buffer solution (30 ml); the pH was 5 and the activation time for PAA was 2 h (cf. Scheme 2). It can be seen that the amount of heparin immobilized on the membrane increases with increasing grafted amount of PAA. This is because membranes with more PAA offer more carboxylic acid for amidization with amine of heparin. However, it is worth to notice that as the grafted PAA exceeded ca. 0.5 mg/cm^2, the amount of immobilized heparin leveled off and reached a constant value, 6.43 μg/cm^2. We think that there are three possible reasons for this uncommon behavior: (1) the highest amount of carboxylic acid that could be activated was reached for the employed EDAC concentration; (2) the activation time (2 h in this case) was not long enough such that there were still free carboxylic acids remaining to be activated; (3) the concentration of heparin in the buffer solution was too low. These points will be discussed further in following sections. Fig. 6 also indicates that the immobilized heparin of membrane 'P0' is considerably lower than the other membranes because it has a nonporous surface and thus a small amount of grafted PAA.

3.2.1. Activation of PAA by EDAC

The action of EDAC to activate PAA on PVDF membrane was affected by the activation time and the initial concentration of EDAC. These two parameters were investigated and the results are demonstrated in Fig. 7, in which the amounts of heparin immobilized on membrane 'P2' are plotted versus the activation time for various EDAC concentrations. For all cases, 0.3 ml heparin solution was added to a 30 ml buffer solution and the pH was adjusted to 5. Fig. 7 indicates that the amount of immobilized heparin increases both with increasing immobilization time and the quantity of initially added EDAC. It follows that the amount of activated carboxylic acid of PAA depends similarly on these two factors. The largest immobilization yield, 9.68 μg/cm^2, was several times higher than those reported in the literature [15,18,19,31]. However, when insufficient quantity of EDAC was used (e.g., EDAC = 30 mg), the amount of activated PAA could reach its saturated value very rapidly within 2 h. As a result, even with a prolonged activation time the amount of immobilized heparin remained at a constant value of 6.1 μg/cm^2. This is related to the leveling-off situation shown previously in Fig. 6; i.e., the amount of immobilized heparin became a constant as the grafted PAA exceeded 0.5 mg/cm^2. Now, it is clear that because the initial concentration of EDAC was too low, the

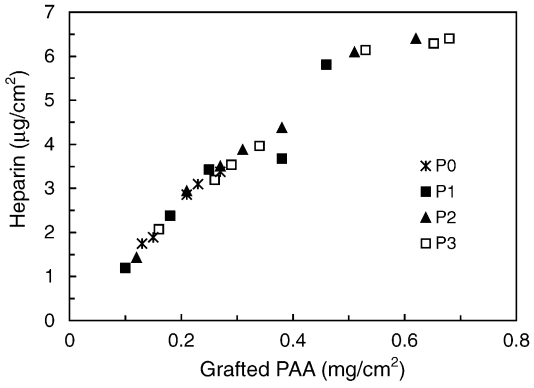


Fig. 6. The effects of PAA grafting yield on the immobilization yield of heparin for various membranes.

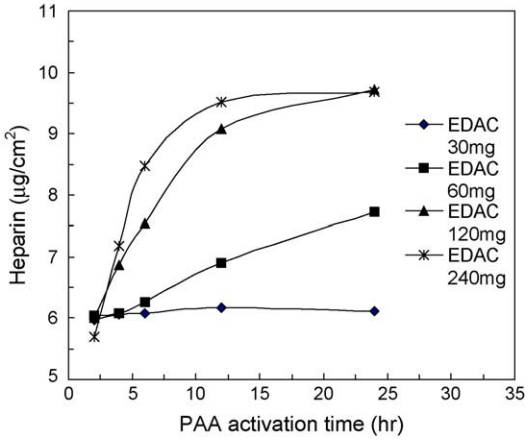


Fig. 7. The effects of PAA activation time and EDAC concentration on the immobilization yields of heparin for membrane 'P2'.

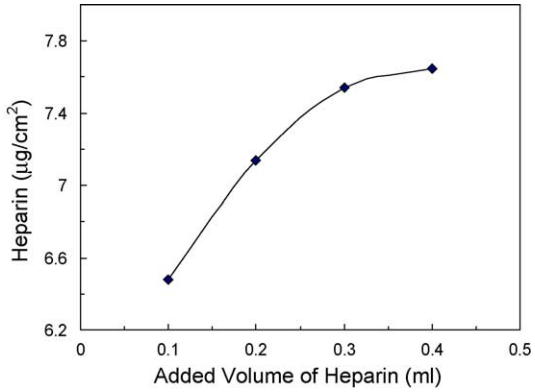


Fig. 8. The effects of initial heparin concentration on the immobilization yields of heparin for membrane 'P2'.

activated carboxylic acid soon reached a saturated level even with an increased amount of PAA.

3.2.2. The effect of heparin concentration

The amount of heparin immobilized on the PVDF membrane depends on the initial concentration of heparin in the reaction mixture. This effect is shown in Fig. 8. The immobilization reaction was carried out on membrane 'P2' with the preparation parameters: 120 mg EDAC was added to a 30 ml buffer solution at pH 5, and the activation time was 6 h. Fig. 8 indicates that the immobilization yield increases as the volume of heparin solution initially added increases. Obviously, this is because more heparin provided more opportunity to react with PAA. However, possibly due to the steric effect, as the volume of heparin solution exceeded ca. 0.3 ml, the increase in the immobilization yields slowed down.

3.2.3. The effect of reaction pH

Immobilization was conducted in both acidic and basic environments to see their effects on the immobilization yields. Three different pHs were studied, namely pH 5, 7, and 11, and the immobilization yields for these cases were 6.09, 5.46, and 3.97 μg/cm^2, respectively. Membrane 'P2' with 0.51 mg/cm^2 grafted PAA was used as the substrate. The other preparation parameters included: 30 mg EDAC and 0.1 ml of heparin were added to a 30 ml buffer solution, and the activation time was 2 h. It appears that higher pH results in lower heparin immobilization; in other words, the reaction favors acidic conditions. This is also associated with the activation of PAA by EDAC, which is known to be effective in acidic conditions, and only under which *o*-acrylisouurea derivatives [14–17] can be formed for subsequent condensation reactions between heparin and PAA. It follows that heparin immobilized in basic solutions was attached to the membrane largely by physical absorption rather than covalent bonding, which is confirmed by the immobilization stability test shown below.

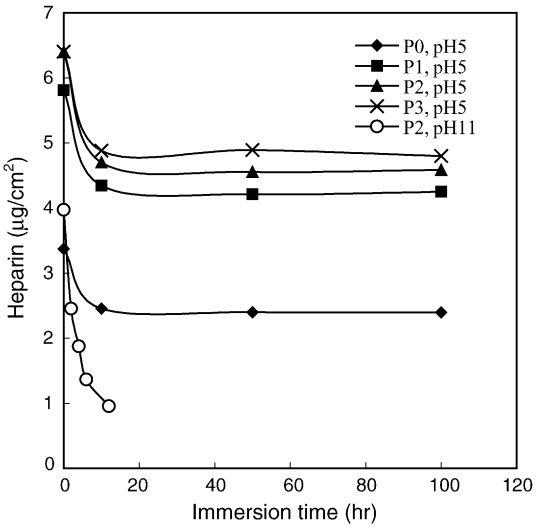


Fig. 9. Stability of heparin immobilized at pH 5 and 11 on various membranes.

3.3. Stability of heparin immobilized on PVDF membrane

To see how stable heparins immobilized at different pHs were bound to the PVDF membranes, binding stability tests were carried out. The prepared heparin/PAA/PVDF membranes were immersed in a mildly shaken PBS solution and the quantities of heparin bound to the membranes were determined at different times, which might last for as long as 100 h, after immersion. The results are manifested in Fig. 9. For the case of immobilization at pH 11, the loosely bound heparin was removed from the membrane surface very rapidly. For example, at 5 h after immersion, only ca. 30% of the heparin was left on the surface. In other words, the escaped heparins were originally attached to the surface merely by phys-

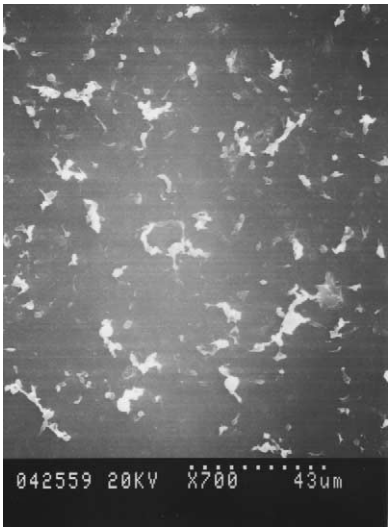


Fig. 10. An SEM photomicrograph showing platelet adhesion on pure PVDF membrane 'P0'.

ical attractions or chain entanglements. This verifies the fact that heparin cannot be effectively immobilized by means of EDAC under basic conditions. Fig. 9 also shows the binding stability of heparin on various membranes that were immobilized at pH 5. For all of the tested membranes, ca. 20–25% of the non-chemically bound heparin departed from the membrane surface within the first 10 h, and ca. 75–80% of the heparin still stayed on the surface even after 100 h of immer-

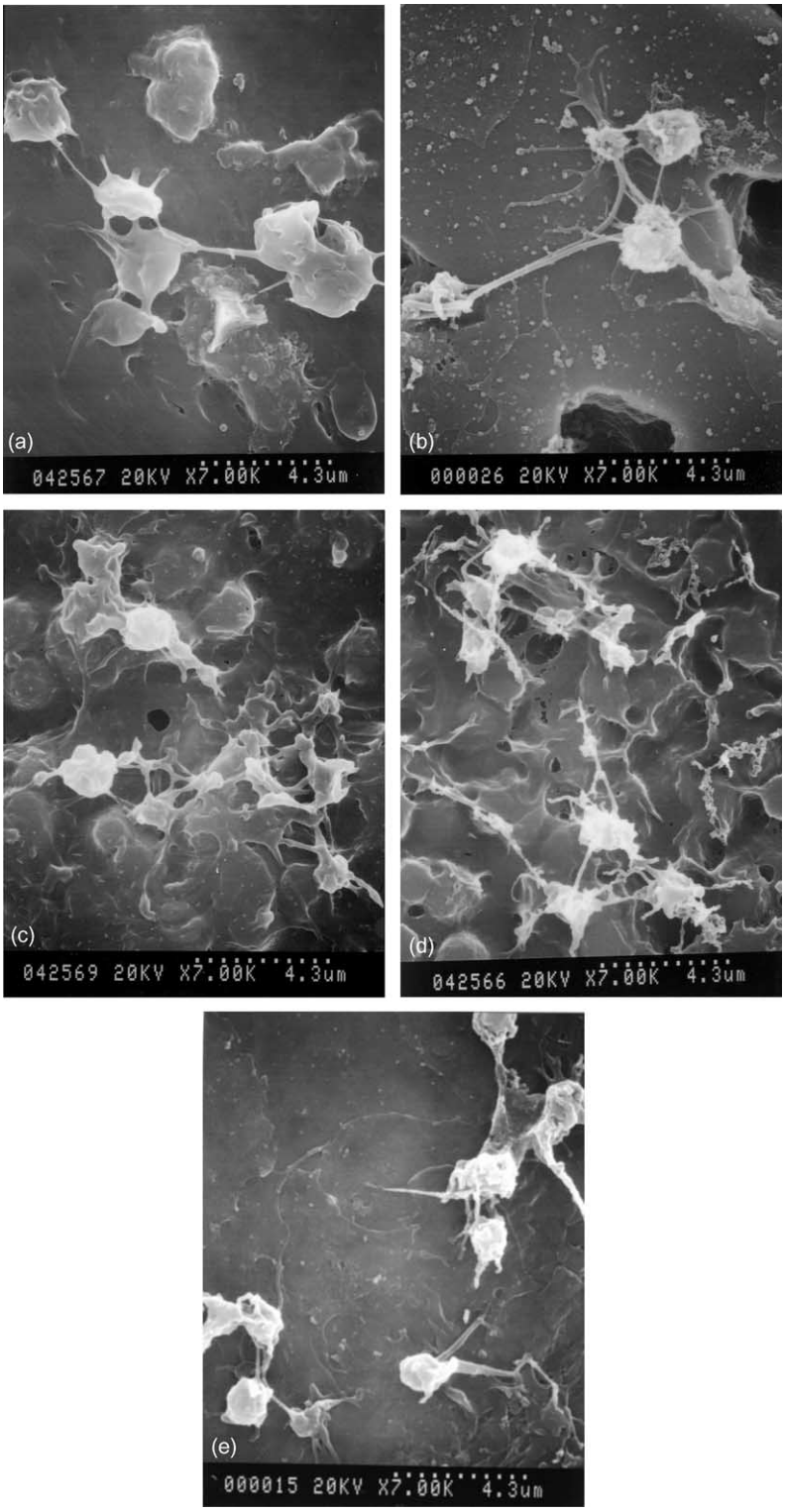


Fig. 11. SEM photomicrographs showing platelet adhesion on pure PVDF and PAA/PVDF membranes at a higher magnification. (a) P0; (b) P1; (c) P2; (d) P3; and (e) PAA/P3, grafting yield = 0.68 mg/cm^2.

sion. This suggests that the heparins were strongly bound to the membrane surface through covalent bonds.

3.4. Platelet adhesion test for the heparin/PAA/PVDF membrane

The PVDF membranes before and after heparinization were examined for their human blood compatibility via observation of adherence and coagulation of PRP on the membranes. An SEM photomicrograph of the unmodified 'P0' membrane surface exposed to PRP for 60 min is shown in Fig. 10. It appears that many platelets were deposited on the membrane surface during this period. Similarly, a considerable attachment of plasma platelets on the surface of other unmodified PVDF membranes ('P1'–'P3') was observed after contacting with PRP for 60 min (not shown here). The morphological change of the adherent platelets was observed using SEM at a higher magnification, as shown in Fig. 11. For all tested pure PVDF membranes, pseudopodium growth of the cells was in evidence and many of the cells underwent serious shape transformation. This implies that the platelets have activated to a relatively high level and ready for aggregation. For membranes grafted with PAA, pseudopodium growth was also observable, as shown in Fig. 11(e). The grafting yield of this membrane, 'P3', was 0.68 mg/cm^2. Membranes with lower grafting yields gave similar growth results. In other words, PAA does not show much anti-coagulation capability with the range of grafting density in the present study.

By contrast, examination of heparin/PAA/PVDF composite membranes by SEM at a low magnification showed very few platelets on their surfaces for the same contacting time (SEM not shown here). At a higher magnification, Fig. 12 indicates very little morphological change of adherent platelets on the PVDF membranes after heparinization, in comparison with those before heparinization. The shapes of the platelet cells were largely spherical; only minimal pseudopodium formation was observed. Obviously, the adherent platelets do not appear to be activated. Therefore, the heparinized mem-

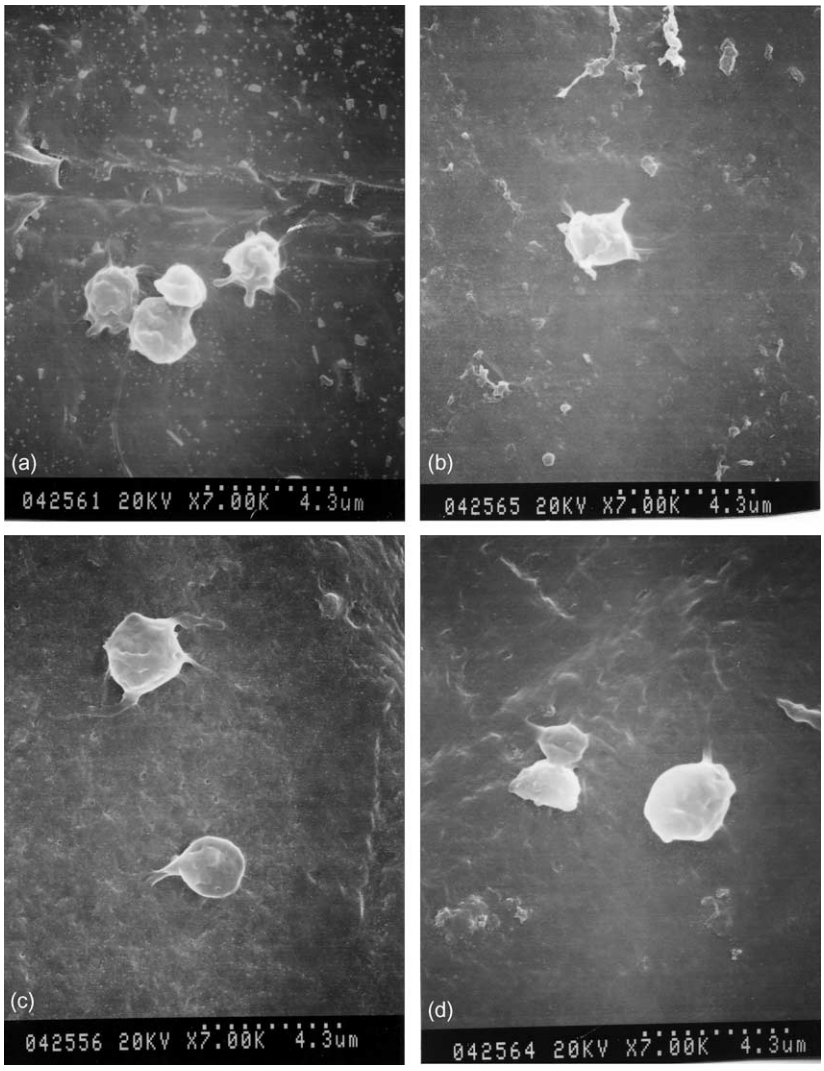


Fig. 12. SEM photomicrographs showing platelet adhesion on membranes after heparin immobilization. (a) P0; (b) P1; (c) P2; and (d) P3.

branes could effectively inhibit the coagulation activity of human PRP.

4. Conclusion

Heparin was covalently immobilized on PVDF membranes with different porous surface structure. The formed composite membranes demonstrated significant improvement on anticoagulation activities of human blood in comparison with pure PVDF membrane. The immobilization yields of heparin were found to increase as the following preparation parameters increased: (1) the quantity of PAA grafted on PVDF, (2) the activation time of PAA by EDAC, (3) the initial concentration of EDAC, (4) the initial concentration of heparin. Also, heparin could be effectively immobilized by chemical bonding on the membrane only in acidic environments.

Acknowledgement

This research was supported by the National Council of Taiwan, ROC (NSC88-CPC-E-032-014).

References

[1] V. Guenard, R.F. Valentini, P. Aebischer, Influence of surface texture of polymeric sheets on peripheral nerve regeneration in a two-compartment guidance system, Biomaterials 12 (1991) 259.
[2] H. Chen, G. Soldani, P.M. Galletti, M. Goddard, Microporous small diameter PVDF-TrFE vascular grafts fabricated by a spray phase inversion technique, ASAIO J. 38 (1992) 201.
[3] L.P. Cheng, D.J. Lin, C.H. Shih, A.H. Dwan, C.C. Gryte, PVDF membrane formation by diffusion induced phase separation-morphology prediction based on phase behavior and mass transfer modeling, J. Polym. Sci., B: Polym. Phys. 37 (1999) 2079.
[4] L.P. Cheng, Effect of temperature on the formation of microporous PVDF membranes by precipitation from 1-octanol/DMF/PVDF and water/DMF/PVDF systems, Macromolecules 32 (1999) 6668.
[5] S.D. Lee, G.H. Hsiue, C.Y. Kao, Preparation and characterization of a homobifunctional silicone rubber membrane grafted with acrylic acid via plasma-induced graft copolymerization, J. Polym. Sci., A: Polym. Chem. 34 (1996) 141.
[6] K.S. Chen, N. Inagaki, K. Katsura, Adhesion of glow discharge polymers, J. Appl. Polym. Sci. 26 (1981) 2197.
[7] A.K. Sharma, F. Millich, E.W. Hellmuth, Wettability of glow discharge polymers, J. Appl. Polym. Sci. 26 (1981) 2205.
[8] T. Yamaguchi, S. Nakao, S. Kimura, Plasma-graft polymerization: preparation of a new type of pervaporation membrane for organic liquid mixtures, Macromolecules 24 (1991) 5522.
[9] Y.M. Lee, J.K. Shim, Plasma surface graft of acrylic acid onto a porous poly(vinylidene fluoride) membrane and its riboflavin permeation, J. Appl. Polym. Sci. 61 (1996) 1245.
[10] S. Hietala, A.L. Maunu, F. Sundholm, Structure of styrene grafted poly(vinylidene fluoride) membranes investigated by solid-state NMP, Macromolecules 32 (1999) 788.
[11] X. Yang, C.O. Too, L. Sparrow, J. Ramshaw, G.G. Wallace, Polypyrrole–heparin system for the separation of thrombin, React. Funct. Polym. 53 (2002) 53.
[12] K. Fujimoto, H. Tadokoro, Y. Ueda, Y. Ikada, Polyurethane surface modification by graft polymerization of acrylamide for reduced protein adsorption and platelet adhesion, Biomaterials 14 (1993) 442.
[13] J. Chen, Y.C. Nho, J.S. Park, Grafting polymerization of acrylic acid onto preirradiated polypropylene fabric, Radiat. Phys. Chem. 52 (1998) 201.
[14] I.K. Kang, O.H. Kwon, M.K. Kim, Y.M. LEE, Y.K. Sung, Preparation and surface characterization of functional group-grafted and heparin-immobilized polyurethanes by plasma glow discharge, Biomaterials 17 (1996) 841.
[15] I.K. Kang, O.H. Kwon, M.K. Kim, Y.M. LEE, Y.K. Sung, In vitro blood compatibility of functional group-grafted and heparin-immobilized polyurethanes prepared by plasma glow discharge, Biomaterials 18 (1997) 1099.
[16] D.R. Lloyd, C.M. Burns, Coupling of acrylic polymers and collagen by use of a water-soluble carbodiimide. I. Optimization of reaction conditions, J. Polym. Sci., A: Polym. Chem. 17 (1979) 3459.
[17] D.R. Lloyd, C.M. Burns, Coupling of acrylic polymers and collagen by use of a water-soluble carbodiimide. II. Investigations of the coupling mechanism, J. Polym. Sci., A: Polym. Chem. 17 (1979) 3473.
[18] J.S. Bae, E.J. Seo, I.K. Kang, Synthesis and characterization of heparinized polyurethanes using plasma glow discharge, Biomaterials 20 (1999) 529.
[19] T. Shiomi, M. Satoh, M. Miya, K. Imai, Binding of heparin onto ethylene–vinyl alcohol copolymer membrane, J. Biomed. Mater. Res.: Appl. Biomater. 22 (1988) 269.
[20] C.L. Chang, M.S. Chang, Preparation of composite membranes of functionalozed silicone polymers and PVDF for pervaporation of ethanol–water mixture, Desalination 148 (2002) 39.
[21] D.J. Lin, C.-L. Chang, T.C. Chen, L.P. Cheng, Fine structure of PVDF membranes prepared by phase inversion from water–NMP–PVDF system, J. Polym. Sci., B: Polym. Phys. 42 (2004) 830.
[22] D.J. Lin, C.L. Chang, H.Y. Shaw, Y.S. Jeng, L.P. Cheng, Formation of multilayer poly(acrylic acid)/poly(vinylidene fluoride) composite membranes for pervaporation, J. Appl. Polym. Sci. 93 (2004) 2266.
[23] P.K. Smith, A.K. Mallia, G.T. Hermanson, Colorimetric method for the assay of heparin content in immobilized heparin preparations, Anal. Biochem. 109 (1980) 466.
[24] C. Thomas, M.L. Daniel, H.R. Gundu, Platelet adhesion and spreading on protein coated surfaces: variations in behavior in washed cells, PRP, and whole blood, J. Biomater. Appl. 13 (1998) 46.
[25] N.M.K. Lamba, J.D.S. Gaylor, J.M. Courtney, Complement activation by cellulose: investigation of the effects of time, area, flow rate, shear rate and temperature on C3a generation in vitro, using a parallel plate flow cell, J. Mater. Sci.: Mater. Med. 9 (1998) 409.
[26] T. Hirotsu, Water–ethanol separation by pervaporation through plasma graft polymerized membranes, J. Appl. Polym. Sci. 34 (1987) 1159.
[27] H. Wang, K. Tanaka, H. Kita, K.-I. Okamoto, Pervaporation of aromatic/non-aromatic hydrocarbon mixtures through plasma-grafted membranes, J. Membr. Sci. 154 (1999) 221.
[28] H. Matsuyama, A. Kariya, M. Teramoto, Characteristics of plasma polymerized membrane from octamethyltrisiloxane and its application to the pervaporation of ethanol–water mixture, J. Membr. Sci. 88 (1994) 85.
[29] T. Masuoka, T. Iwatsubo, K. Mizoguchi, Pervaporation membranes for ethanol–water mixture prepared by plasma polymerization of fluorocarbons. II. Perfluoropropane membranes, J. Membr. Sci. 69 (1992) 109.
[30] K.R. Lee, S.J. Yu, S.L. Huang, D.M. Wang, J.Y. Lai, Pervaporation of water–ethanol mixtures through plasma graft polymerization of polar monomer onto crosslinked polyurethane membrane, J. Appl. Polym. Sci. 67 (1998) 1789.
[31] I.K. Kang, K.D. Baek, M.Y. Lee, K.Y. Sung, Synthesis and surface characterization of heparin-immobilized polyetherurethanes, J. Polym. Sci., A: Polym. Chem. 36 (1998) 2331.