Anticlotting Membrane Based on Polypropylene Grafted by Biocompatible Monomers Under UV Irradiation

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ABSTRACT: Biocompatible membranes were successfully prepared by biaxially oriented polypropylene (BOPP) cografted with hydroxyethyl acrylate (HEA) and vinyltriethoxysilicane (VES), in which nonvapor and nonliquid photo-grafting technology was used under the irradiation of UV lamp. HEA and VES cografted BOPP (HV-g-PP) was then used to fabricate anticlotting membrane by immobilizing heparin on it. The grafting degree of BOPP host and the chemical structure of grafting layer were studied by grafting degree test, Fourier transform infrared spectrometer (FTIR), atomic force microscope (AFM), and X-ray photoelectron spectroscopy (XPS) measurements, respectively. Anticoagulation capability of the heparin immobilized HV-g-PP membrane (HHV-*g*-PP) was also investigated by hemolytic test. The results show that HEA and VES can be effectively grafted onto BOPP substrates via nonvapor and nonliquid photo-grafting method, and that the grafting degree reaches 144.8 wt % when the reaction time approaches 20 min. The heparin content of HHV-*g*-PP membrane increases from 0 to 4 wt % as the reaction time alters from 0 to 20 min. The hemolytic degree rapidly reduces from 5.1 wt % to 2.7 wt % with increasing the grafting degree from 0 to 144.8 wt %, implying a significant anticoagulation improvement of the HHV-*g*-PP membrane. Such functional membranes have large potential applications in blood-related packaging areas. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 124: E161–E168, 2012

Key words: biocompatible; biaxially oriented polypropylene (BOPP); grafting polymerization; heparinization; anticoagulation

INTRODUCTION

Packaging and transportation of blood are the most common phenomena in clinical applications. Unfortunately, almost all conventional packaging materials commercially available, like polyethylene (PE), polypropylene (PP) etc., are incompatible with blood, which directly leads to the reduction of the storage time of blood. Therefore, ample efforts have been done to improve the anticoagulation capability of packaging materials,[1–5] which mainly include the addition of anticoagulants into blood and the surface modification of polymeric substrates. In the case of the former, injection of heparin, citrate or other anticoagulants into blood is the most commonly used method in clinic.[6–8] However, many clinic researches have revealed that injection of heparin will probably result in several potential dreadful side effects such as the rheological change of blood, hypersensitivity and shock,[9–11] due to the existence of free heparin molecules. Compared with the injection of anticoagulants, surface modifications of polymeric substrates have great advantages in physiological environment related to blood and possess broad application prospects. But the processability of these materials was relatively poor, and couldn't satisfy the requirement of practical applications.[12,13] Consequently, it is apparently implied that a novel anticlotting technology, in which the injection of anticoagulants is unnecessary and the compatibility of packaging materials with blood can be substantially improved, is promising and indispensable for clinical application. Moreover, good processability and mechanical properties of the selected packaging materials should also be especially considered during the design of the material. Therefore, special modification of polymeric substrates, e.g., immobilization of anticoagulants on biocompatible polymer membrane, is feasible to solve the earlier problems. Leininger has successfully combined heparin onto several surfaces of polymers by chemically bonding method to render materials nonthrombogenic.[14] Mori et al. have developed a heparinized hydrophilic polymer through ionically bounding heparin onto polymer matrix.[15] Many similar researches have also been done to further improve the biocompatibility and anticlotting performance of polymeric materials, such as polyethylene terephthalate (PET) and polycaprolactone (PCL).[16–18] However, the high

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price and the low yield of certain polymeric membranes still preclude the development of such materials.

Polypropylene (PP), one of the most commonly commercial polymers, has been widely used in food, medicine, automobile, and domestic devices areas, due to its low price, excellent chemical stability, heat resistance and processability. However, nonpolar molecular chain and low surface energy of PP result in its relatively poor biocompatibility, hydrophilicity, dyeability, and adhesion. We have previously developed a nonvapor and nonliquid photo-grafting polymerization method to modify the surface energy of PP.[19–21] Relevant researches have indicated that polar monomers like acrylate acid (AA) can be effectively grafted onto inert polymeric chains of PP, and that the grafting degree of such technology is much higher than common chemical grafting reactions under same reaction conditions. In this article, two biocompatible monomers, hydroxyethyl acrylate (HEA) and vinyltriethoxysilicane (VES),[22–27] have been especially selected to graft on the surface of biaxially oriented polypropylene (BOPP) membrane by nonvapor and nonliquid photo-grafting polymerization. HEA and VES cografted BOPP (HV-g-PP) membranes have been further immobilized with heparin to obtain functional membranes (HHV-*g*-PP) with excellent anticoagulation capability. Chemical structures and anticoagulation of HHV-*g*-PP membranes have also been thoroughly investigated.

EXPERIMENTAL

Materials

BOPP membrane, treated with corona discharge, was purchased from Baoting Packing Material (Shanghai, China). Surface energy of the membrane is 38 dym/cm. Hydroxyethyl acrylate (HEA) (95%) was supplied by Aldrich (America). Vinyltriethoxysilicane (VES) was analytically pure and purchased from Kelong Chemical Reagent (Chengdu, China). Heparin was biochemical reagent (BR) and supplied by Kelong Chemical Reagent (Chengdu, China). All the other chemicals used were analytically pure, and used as received.

Preparation of the HHV-*g*-PP membranes

VES (0.26 mol/L) was dissolved in deionized water. After hydrolysis, HEA (0.43 mol/L) monomer was added and mixed. The monomer solution was prepared and preserved in a desiccator before use. BOPP membrane with the thickness of 30 μm was cut into 30 × 50 mm^2 specimens, and soaked in the appropriate amount of acetone for 24 h to remove surface stains, and then placed in xylene solution of benzophenone (BP) in a water bath at 90°C for 13 h.

The treated BOPP membrane was put into a quartz container, where the above prepared monomer solution was poured in. The UV lamp was taken on and the grafting polymerization was started. The irradiation intensity was set at 6.2 W/cm^2. After completing the reaction, the membrane was cleaned with acetone several times. Then the membrane was again extracted with acetone in a Soxhlet extractor apparatus for 24 h to remove the unreacted monomer and homopolymer. At last, the extracted membrane was put into a saturated heparin solution and maintained for 48 h. Afterward, the membrane was washed with a large amount of deionized water and dried in a vacuum oven at 60°C for 24 h.

Characterization

Grafting degree test

In this experiment, weighing method was adopted to test the grafting degree. The calculation formula is as follow[28,29]:

$$G(\%) = (G_1 - G_0)/G_0 \times 100\%$$

where G, G_0, and G_1 are the grafting degree, the weight of the membrane specimen before grafting and after grafting, respectively.

Fourier transform infrared spectrometer analysis

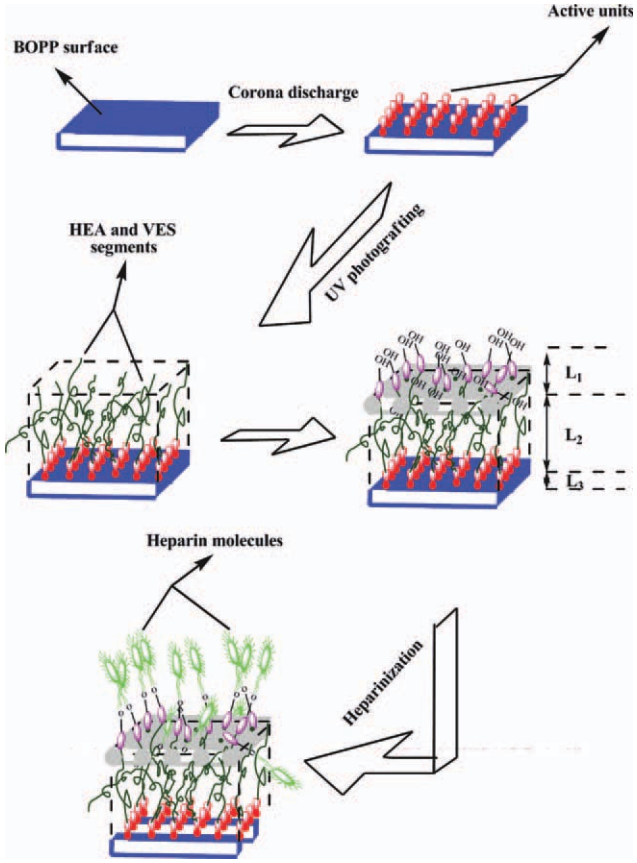
Heparin, HEA, VES, and HHV-*g*-PP membranes were characterized by using a Nicolet Fourier transform infrared spectrometer (FTIR) spectrometer (Nicolet560, Thermo Nicolet, USA) with a resolution setting of 4 cm^{-1}. Heparin, HEA, and VES were directly coated on a slice of potassium bromide for scanning, while HHV-*g*-PP membranes were characterized by using an attenuated total reflectance (ATR)-FTIR accessory. The scanning range was altered from 400 to 4000 cm^{-1}.

Atomic force microscope

BOPP membrane and HHV-*g*-PP membranes were characterized by using an AFM spectrometer (NanoScope MultiMode, Veeco, USA) with both lateral and topographic force contact modes. The scanning size and rate were ~ 3.5 μm and 2 Hz, respectively.

X-ray photoelectron spectroscopy

The element content of BOPP membrane and HHV-*g*-PP membranes were characterized by using a XPS



Scheme 1 Mechanism of HHV-*g*-PP membranes. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

spectrometer (XSAM800, Kratos, Japan) with a monochromatic MgK_{α} phone source.

Hemolytic test

The heparinized sample was immersed in 10-mL saline, and placed in a water bath at 37°C for 30 min. Then 0.2-mL anticoagulant of rabbit blood[30] was added, and the temperature was maintained at 37°C for 60 min. To obtain the hemolysis degree, both the positive responding specimen and the negative responding specimen were prepared. The positive responding specimen is prepared by mixing 10 mL distilled water and 0.2 mL anticoagulant of rabbit blood, and the negative responding specimen is obtained by mixing 10 mL saline and 0.2 mL anticoagulant of rabbit blood. After a centrifugation treatment, the earlier liquids were drawn in cuvettes, and the corresponding absorbance was measured through an ultraviolet-visible spectrophotometer (UV-2100, Shimadzu, Japan) with a wavelength of 545 nm. The hemolysis degree was calculated by the following formula[31,32]:

$$\mathrm{HD}(\%) = (D_s - D_{nr})/(D_{pr} - D_{nr}) \times 100\%$$

where HD, D_s, D_{nr}, and D_{pr} are the hemolysis degree, the absorbance of sample and the absorbance of negative and positive responding specimen, respectively. Hemolysis degree is an average value, and is obtained after measuring five specimens.

RESULTS AND DISCUSSION

Grafting polymerization of HV-*g*-PP membranes

It is not easy for VES to be homo-polymerized in a free radical polymerization because of the steric hindrance of side groups. Therefore, monomers composed of HEA and VES are selected to copolymerize. Scheme 1 briefly shows the grafting polymerization of HV-*g*-PP membranes. As can be seen from it, -OH group is introduced onto the surface of the membrane through the cografting polymerization of HEA and VES, which has an antioxidant ability.[33,34] Then the introduced Si—OH groups can react with -OH groups of heparin, and further form Si—O—C covalent bonds by dehydration reaction.[35] Consequently, heparin is indirectly anchored onto the surface of the membrane and the heparinization is completed.

Figure 1 reveals the influence of reaction time on the grafting degree of HV-*g*-PP membranes. It can be seen that the grafting degree sharply increases when reaction time changes from 0 to 5 min. The increment of the grafting degree becomes slowly with reaction time. Afterward when the reaction time exceeds 15 min, the grafting degree rapidly increases and reaches 144.8 wt % at a reaction time of 20 min, indicating that a high grafting degree can be obtained by such a nonvapor and nonliquid photografting method in a short reaction time. It can be accounted for the fact that the copolymerization

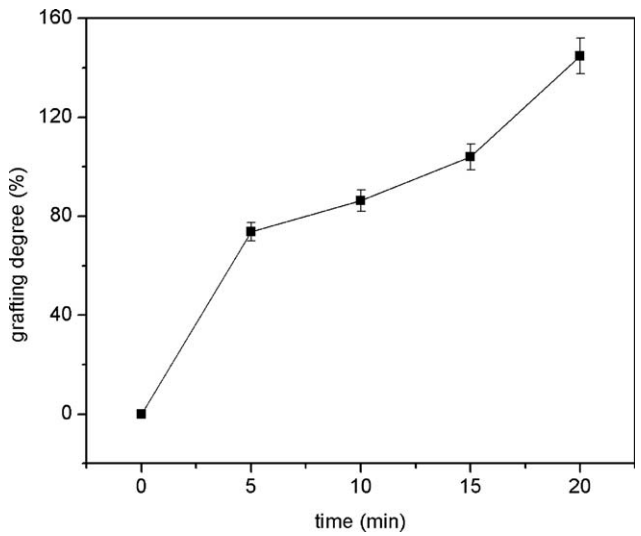


Figure 1 Influence of reaction time on the grafting degree of HV-*g*-PP membranes.

between HEA and VES is a typical free radical polymerization, and the rate of photoinitiated polymerization can be described by the following equation.[36–38]

$$R_p = k_p[M]\left(\frac{2k_d[I]}{k_t}\right)^{\frac{1}{2}}$$

where R_p, k_p, $[M]$, k_d, $[I]$, and k_t are the rate of photoinitiated polymerization, propagation rate coefficient, monomer concentration, initiator decomposition rate coefficient, initiator concentration, and chain termination rate coefficient, respectively. At the beginning of the reaction, the grafting degree raises with reaction time resulting from a high concentration of both $[M]$ and $[I]$. With decreasing the content of $[M]$ and $[I]$, the grafting degree changes slightly. After 15 min, the grafting degree again significantly increases due to gel effects.[39]

FTIR analysis

FTIR is commonly used to qualitatively and quantitatively characterize the grafting degree of polyolefin membranes,[40,41] especially to investigate the vibration of polar groups grafted on inert substrates. FTIR spectra of heparin, HEA and VES are illustrated in Figure 2. The characteristic absorption band of heparin at 969 cm^{-1} is observed in Figure 2(a). While the characteristic absorption bands of —OH and —CO— of HEA are detected at 3443 and 1724 cm^{-1} in Figure 2(b). In Figure 2(c), the characteristic

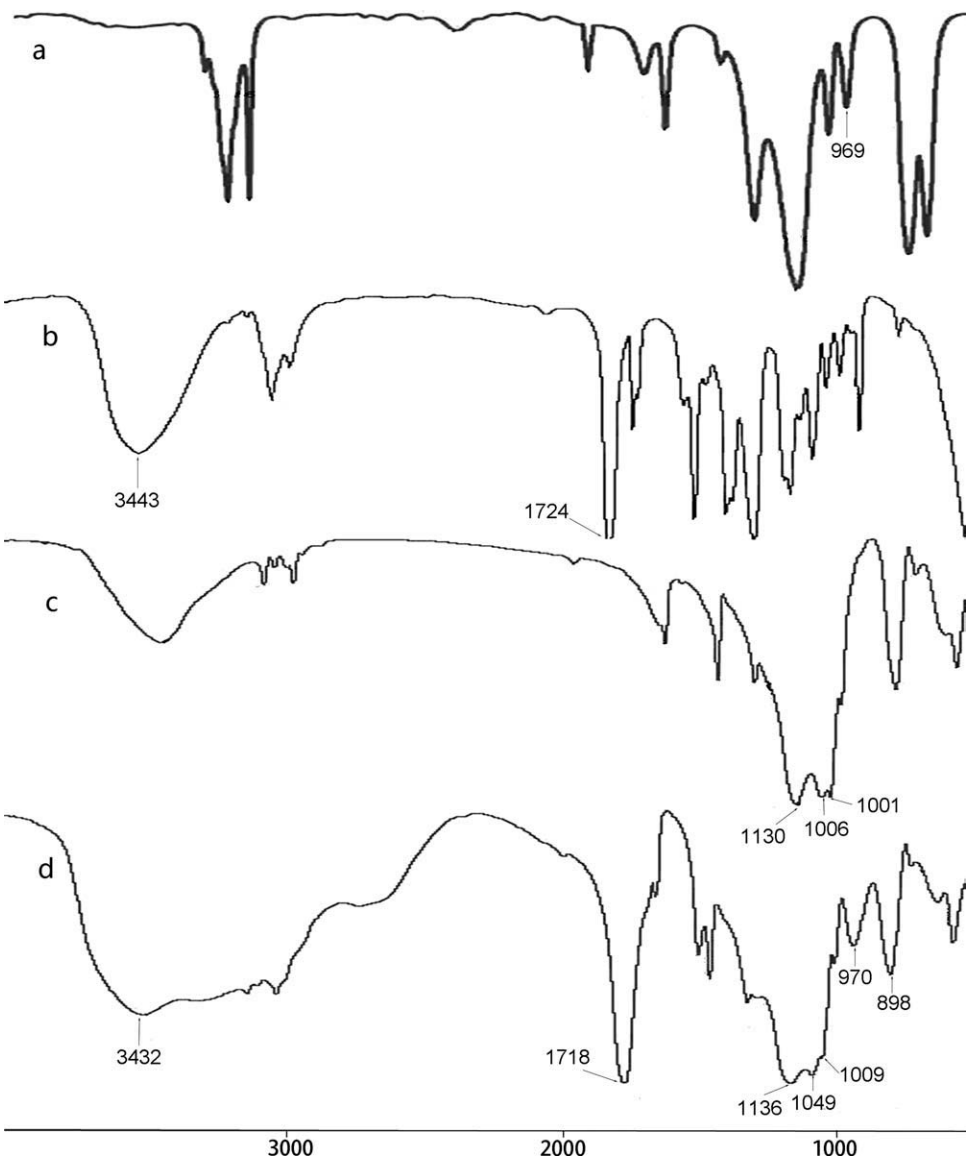


Figure 2 FTIR spectra of heparin, HEA, VES, and HHV-*g*-PP membrane (a, heparin; b, HEA; c, VES; d, HHV-*g*-PP membrane).

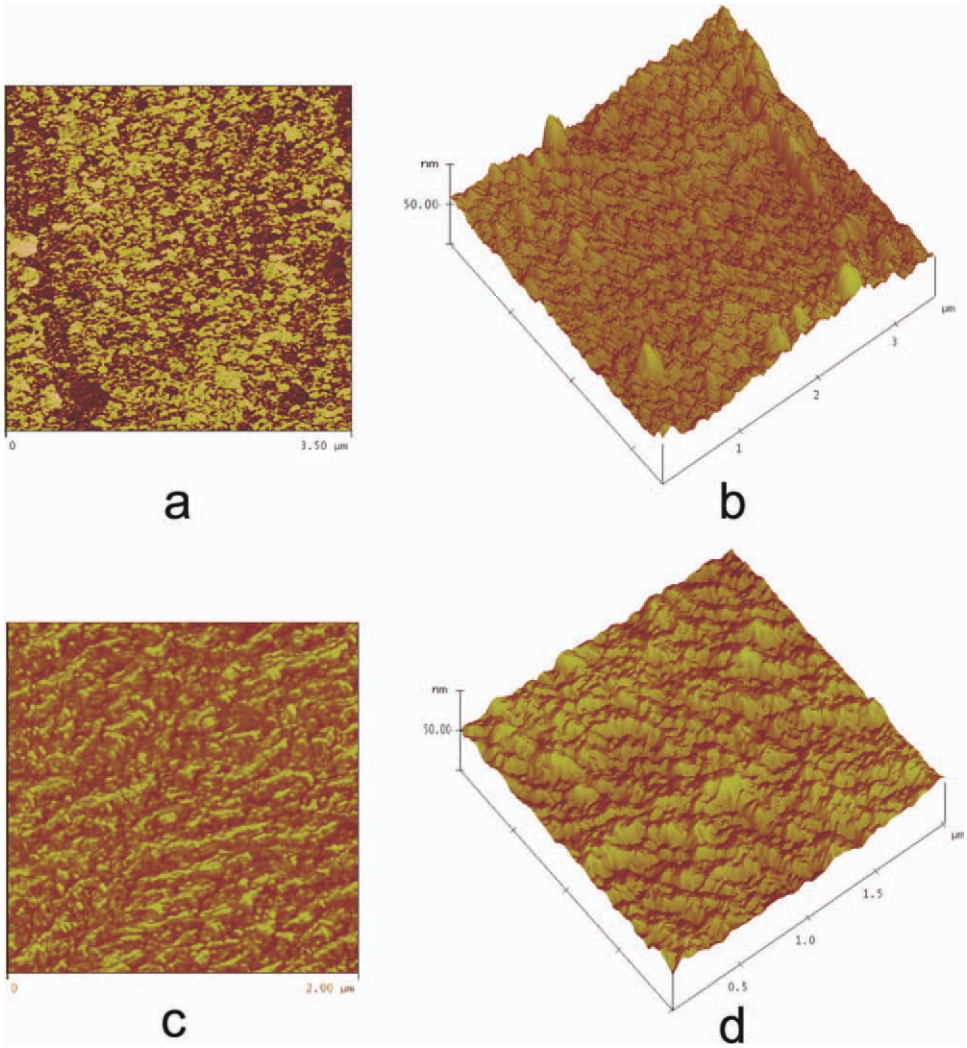


Figure 3 AFM micrographs of the surface of HHV-*g*-PP membrane and BOPP membrane (a, b, HHV-*g*-PP membrane; c, d, BOPP membrane). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

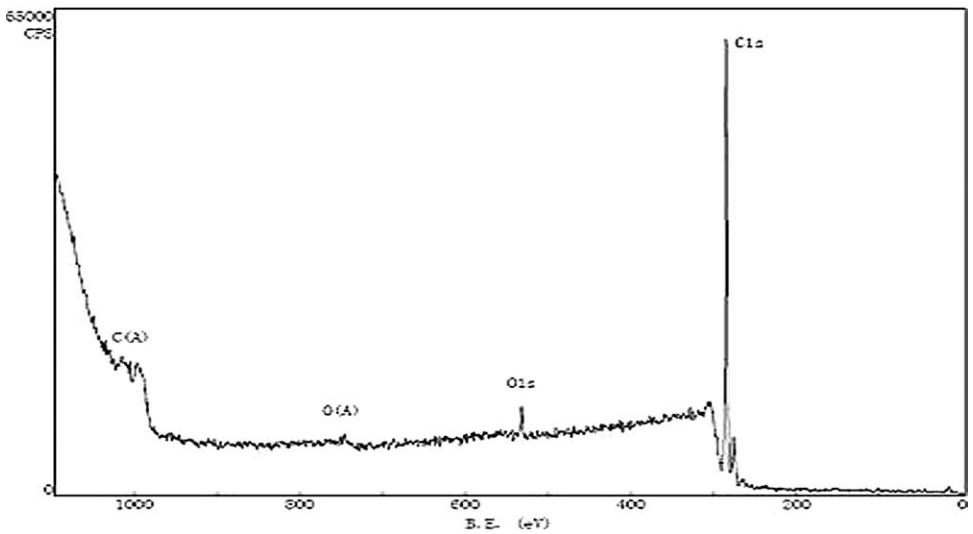


Figure 4 XPS spectrum of the BOPP membrane.

TABLE I
Element Analysis of HHV-*g*-PP Membrane

Element	Area	Q factor	A/Q	At%
C1s	79198.1	0.493	160645.2	75.8
O1s	40296.1	0.914	44087.6	20.8
N1s	1526.5	0.813	1877.6	0.9
S2p	1225.4	1.62	756.4	0.4
Si2s	716.5	1.08	663.4	0.3
Si2p	846.7	0.225	3763.1	1.8

absorption band of 1000–1130 cm^{-1} is observed, attributed to the Si—O vibration of VES. Figure 2(d) indicates the infrared spectra of HHV-*g*-PP membrane. It can be apparently seen that the characteristic absorption band of heparin at 898 and 970 cm^{-1}, and the absorption bands of —OH and —CO— at 3432 and 1718 cm^{-1} are observed, respectively. Moreover, three characteristic absorption bands of Si—O at 1009, 1049, and 1136 cm^{-1} are also observed. The above discussion clearly demonstrates that HEA and VES have been successfully grafted onto the surface of the membrane, and that the heparinization of the membrane is also considerable.

AFM analysis

AFM micrographs of the surface of HHV-*g*-PP membrane are shown in Figure 3. In Figure 3(a), the lateral force mode shows that most sphere-like heparin molecules regularly align in rows, and only a few heparin molecules gather in the scanning view. Figure 3(b) reveals the topographic morphology of the surface of HHV-*g*-PP membrane. As seen from that the average height of *Z* axis reaches 62.83 nm, which is significantly higher than that of BOPP [Fig. 3(d)], revealing an increase of roughness of HHV-*g*-PP membrane. The increase of roughness is evidently derived from the complicated photografting reaction of HEA and VES, and from the heparinization. All the results above indicate that most heparin molecules are evenly distributed and reacted, and small amount of cluster-type heparin molecules aggregate on the surface of modified BOPP membrane.

XPS analysis

To further clarify the successful completion of both the photografting polymerization and the heparinization, XPS measurement was used. XPS spectrum of empty BOPP membrane is displayed in Figure 4. As can be seen from it, only the elements of C and O can be observed, and the element of O is assigned to the surface oxidation.

Table I presents the element analysis result of HHV-*g*-PP membrane. In the case of the heparinized membrane, the content of the characteristic element, N and S, approaches 0.9 wt % and 0.4 wt %, respectively. While the content of element S was 10% in pure heparin. Therefore, the content of heparin on the surface of the grafted membrane is calculated to be around 4 wt %. Figure 5 demonstrates the spectrum of HHV-*g*-PP membrane. The intensity of characteristic elements of C, O, N, S, and Si are evident. The element of O is originated from HEA, while the elements of S, N, and Si are derived from heparin and VES, respectively. On the basis of what have been discussed above, it can be concluded that HEA and VES have been successfully grafted onto the surface of the membrane through the cografting polymerization, and that heparin has also been immobilized onto the surface of the membrane.

Hemolytic analysis

Hemolytic test, an important assessment for ranking anticlotting materials, is usually used to analyze the extent of both the dissolution of erythrocyte and the

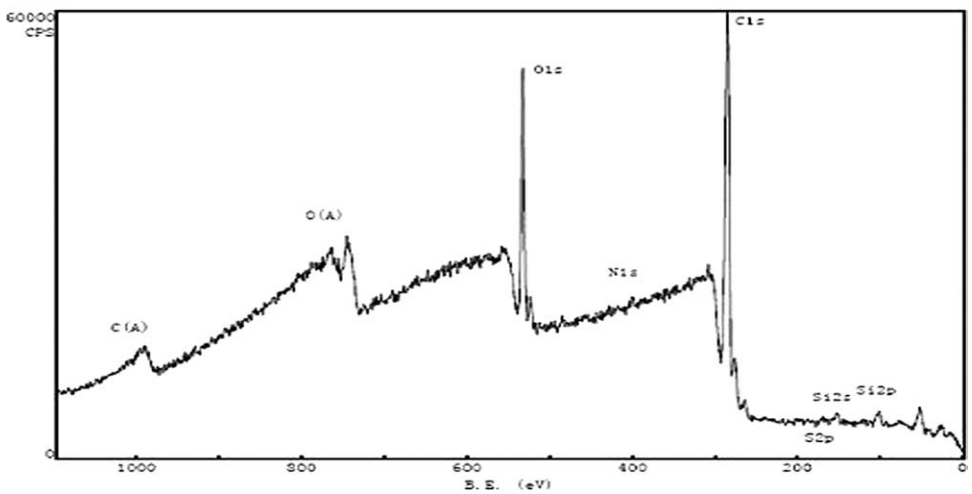


Figure 5 XPS spectrum of HHV-*g*-PP membrane.

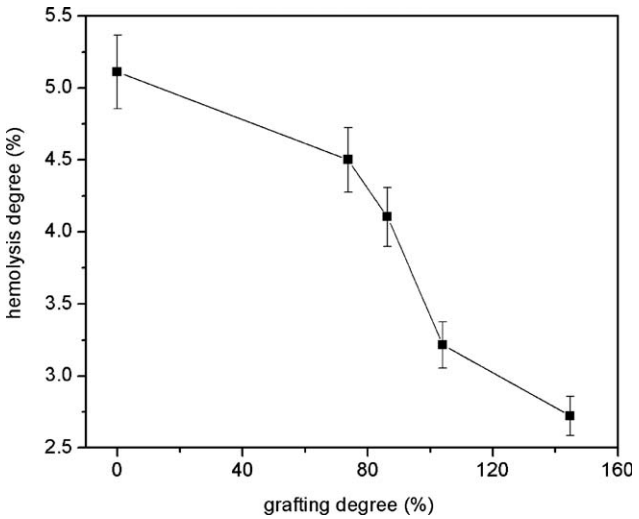


Figure 6 Effect of grafting degree on hemolysis degree of HHV-*g*-PP membrane.

dissociation of hemoglobin. It is convinced that the larger the hemolysis degree is, the poorer the biocompatibility is.

Effect of the grafting degree on hemolysis degree of HHV-*g*-PP membrane is shown in Figure 6. It evidently reveals that the hemolysis degree of HHV-*g*-PP membrane decreases with the grafting degree. When the grafting degree reaches 144.8%, the hemolysis degree sharply decreases from 5.1 wt % to 2.7 wt %, indicating about a 47 wt % decrease of the hemolysis degree.[42,43]

Furthermore, it can be seen that the hemolysis degree slowly decreases when the grafting degree varies from 0 to 73.7%, which is attributed to the little amount of the grafted chain. With increasing the grafting degree, the hemolysis degree rapidly decreases deriving from ample grafted chains. However, the hemolysis degree slightly decreases with further enhancing the grafting degree. Because the surface area of the grafted membrane is almost a constant, therefore, only a small amount of heparin molecules can penetrated into the subsurface of HEA and VES segments to react with Si—OH groups. Relevant mechanism is briefly shown in Scheme 1.

It evidently indicates that the anticoagulation of HHV-*g*-PP membrane has been dramatically improved after a compound treatment of grafting modification and heparinization, and that the evident enhancement of anticoagulation is mainly attributed to the introduction of biocompatible polymeric segments, e.g., HEA and VES segments.

CONCLUSIONS

Anticlotting membrane substrate can be successfully prepared by BOPP cografted with biocompatible monomers HEA and VES. Nonvapor and nonliquid photo-grafting technology has been used to prepare the cografted membrane under an irradiation of UV light. The cografted membranes are able to further immobilize with heparin to obtain anticlotting membranes. The heparin content of HHV-*g*-PP membrane reaches 4 wt % as the reaction time approaches 20 min. The corresponding hemolytic degree of HHV-*g*-PP membrane evidently reduces by 47 wt % with increasing the grafting degree from 0 to 144.8 wt %, implying the excellent anticoagulation of the HHV-*g*-PP membrane.

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