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SHORT COMMUNICATIONS

Effects of Microphase-Separated Structure on Blood Compatibility of Grafted Poly(vinyl butyral)

Jiamin XU, Hanking GU,* Kangde YAO, Jingsheg HU,
Shaogang ZHOU, Tunyu FAN,** Qinming ZHANG,**
Zhiqiang LI, and Tengxiang MA*

Department of Applied Chemistry, Tianjin University, China
**Tianjin Institute of Urologic Surgery, China*
***Nankai University, China*

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Hydrogel of poly(acrylamide) (PAM) exhibits good blood compatibility but its wet strength is low; poly(vinyl butyral) (PVB) has good cell compatibility,[1] but its antithrombogenicity is not so good.[2] In order to develop a new antithrombogenic, permeable membranous material, a graft copolymer (VBA) was synthesized by copolymerizing acrylamide (AM) on PVB. Using PVB films as starting material, we found that the grafted layer of PAM was gradually formed on the surface, and penetrated into the matrix of PVB with increasing graft percent. Microphase-separated structures were found at a graft percent above 60%. The *in vitro* experimental results showed that the copolymers VBA having microphase-separated structure exhibit good antithrombogenicity. Adsorption of albumin and γ-globulin on the graft copolymer surface was examined in regard to antithrombogenicity of the material.

EXPERIMENTAL

Materials

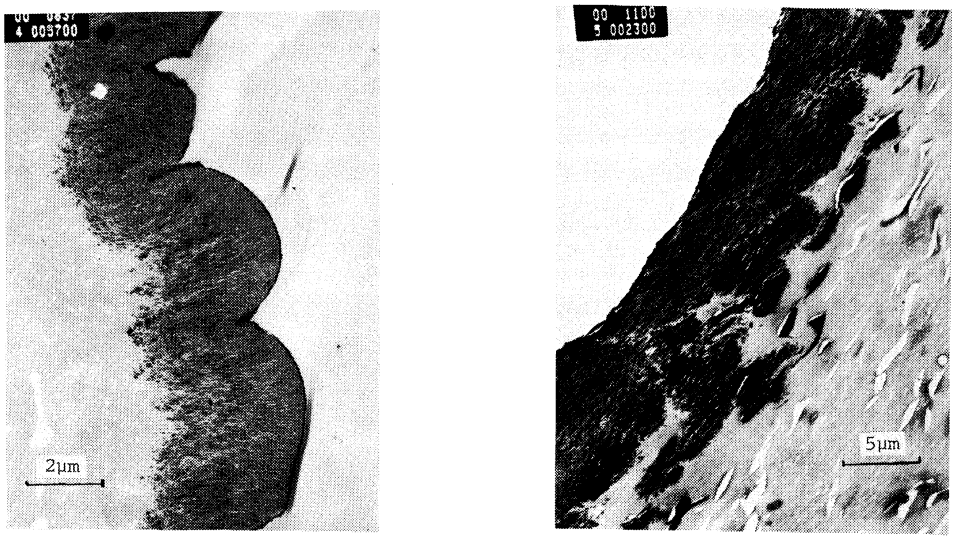
Poly(vinyl butyral) was manufactured by Tianjin Organic Chemical Plant. Its average degree of polymerization is *ca.* 490, and the degree of acetalization is 60—64%. The PVB films were prepared from ethanol solution. Acrylamide was obtained from Sigma Chemical Co., USA. Ceric ammonium nitrate of analytical grade was purchased from Beijing Chemical Engineering Plant.

To 200 mg PVB film suspended in 200 ml water, a 1 ml solution of $(NH_4)_2Ce(NO_3)_6$ in nitric acid (concentrations of Ca^{2+} and HNO_3 are 0.8 mol l^{-1} and 1 mol l^{-1}, respectively) was added, and then 120 ml 1 M acrylamide aqueous solution were incorporated. The system was bubbled with a slow stream of argon and the temperature was adjusted to 37 ± 0.5°C. The reaction was allowed to proceed for 0.5 to 8 h, and the films obtained with different percent grafting were taken out and washed with water to remove the homopolymer of acrylamide and unreacted monomer. The grafted films were dried at 40°C under vacuum. From the weight up-take, the percent grafting was calculated by the equation,

$$\% \text{ Grafting} = \frac{W_2 - W_1}{W_1} \times 100$$

where W_1 and W_2 indicate the weights of PVB and grafted PVB, respectively.

The occurrence of graft copolymerization



(a) (b)

Figure 1

Table I. Grafting of AM onto PVB surface at 37°C

Sample code	wt% Grafted
PVB	0
VBA-8	8
VBA-20	19.45
VBA-60	60
VBA-80	80

was identified by means of the elemental analysis of nitrogen, FT-IR spectra (1630 and bonyl) and ^{13}C NMR spectra (182 ppm, attributed to the carbonyl group). Table I shows the graft percents of the copolymers obtained.

Plasma proteins used were bovine serum albumin obtained from Chang Yang Pharmaceutical Manufactory, Shanghai, and human serum γ-globulin purchased from West Asia Trade Co., Hongkong.

Methods

The morphology of the surface of specimens of VBA after being stained with a saturated palladium chloride aqueous solution was observed with a Hitachi X-650 scanning electron microscope. All the samples were treated with evaporated gold before SEM examination. The morphology of cross sections of the stained membranes embedded in epoxy resin and microtomed was observed under a Philips EM-400ST transmission electron microscope.

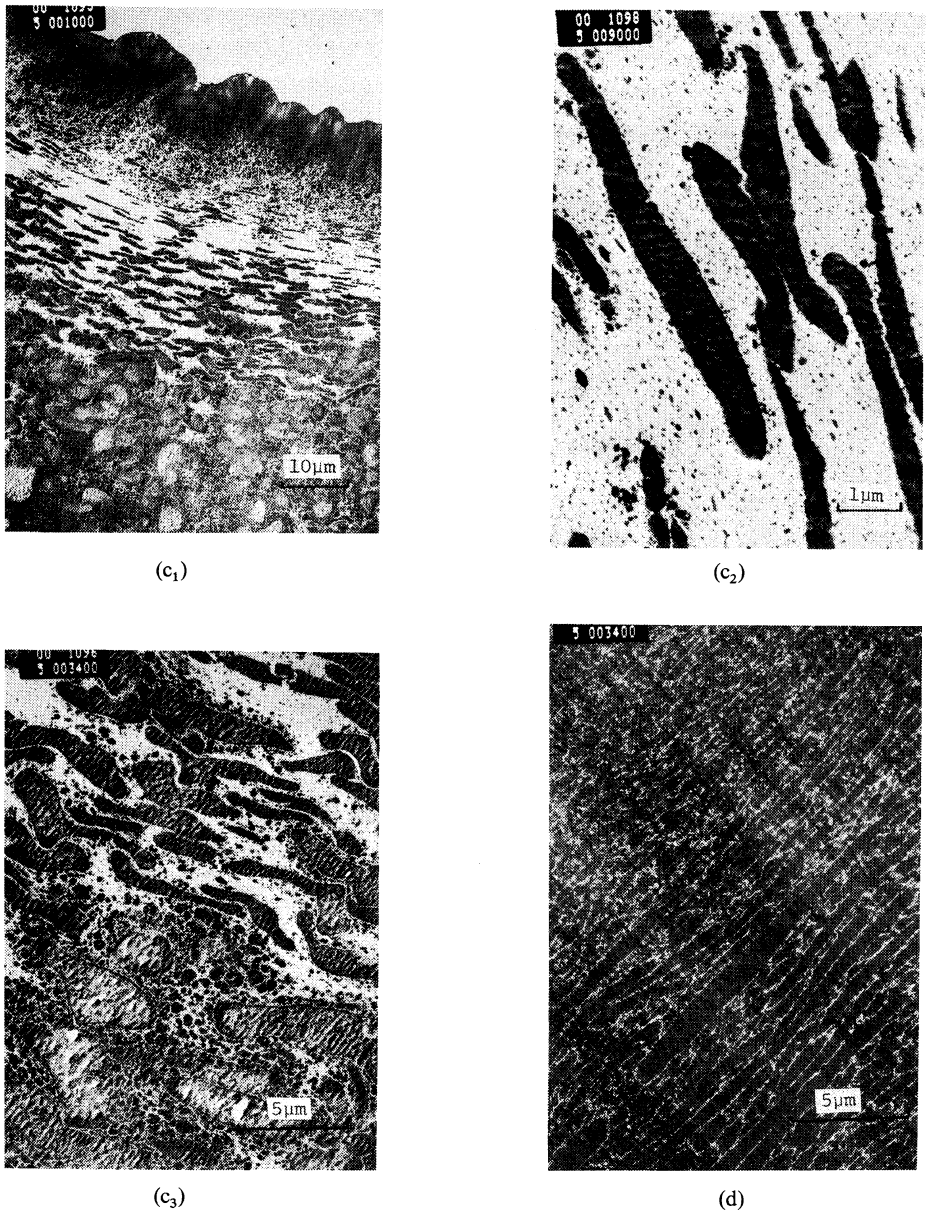
The water absorption of membranes after being immersed in water for 24 h and dried centrifugally at 1000 rpm for 4 min was evaluated by the following equation.

$$\text{Water absorption} = \frac{W - W_0}{W_0} \times 100\%$$

where W and W_0 indicate the weights of wet and dry membranes, respectively.

The isotherms of protein adsorption on VBA and PVB films were investigated in a vibrator at initial concentrations 4 and 1 mg l^{-1}, respectively, for albumin and γ-globulin in physiological saline at 37°C. The changes of the plasma protein concentrations were followed by absorbances at 260 and 280 nm, using a Shimadzu UV-360 spectrophotometer.

The antithrombogenicity of VBA was evaluated by *in vitro* test; for this purpose, the blood taken from a dog femoral artery was passed into a polyvinyl chloride tube covered



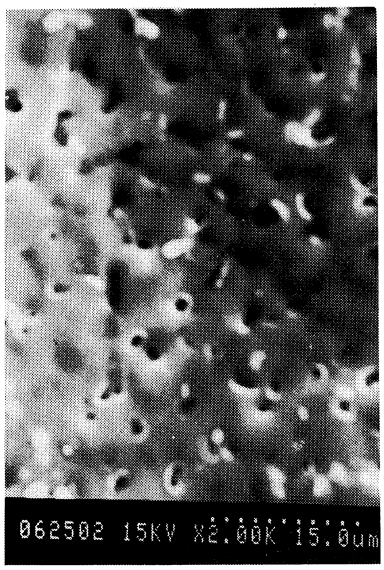
(c_1) (c_2) (c_3) (d)

Figure 1. Typical morphological patterns observed in transmission electron micrographs. The dark portions indicate polyacrylamide domains. (a) VBA-8 (scale 2 μm), (b) VBA-20 (scale 5 μm), (c) VBA-60 (c_1, scale 10 μm; c_2, scale 1 μm; c_3, scale 5 μm), and (d) VBA-80 (scale 5 μm).

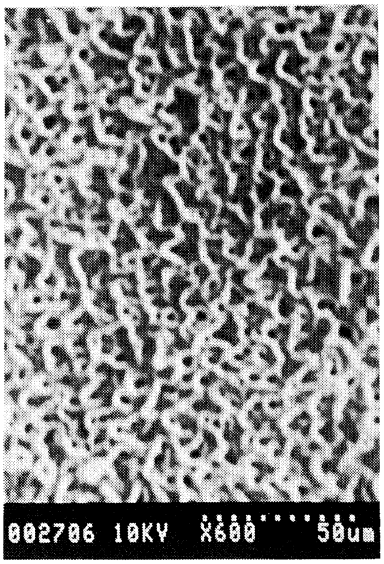
with VBA at a flow velocity of 10 ml/min for 10 minutes. Then, the tube was washed with physiological saline containing paraformaldehyde for about 5 min, and fixed in a 2.5% glutaraldehyde solution. The specimens were examined for antithrombogenicity with a Hitachi X-650 scanning electron microscope.

RESULTS AND DISCUSSION

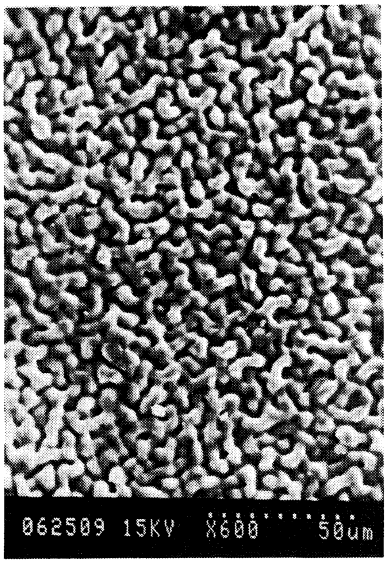
The $PdCl_2$-stained cross sections of the membranes were observed with a transmission



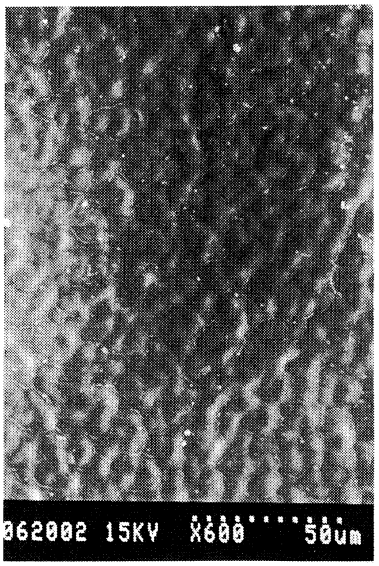
(a)



(b_1)



(b_2)

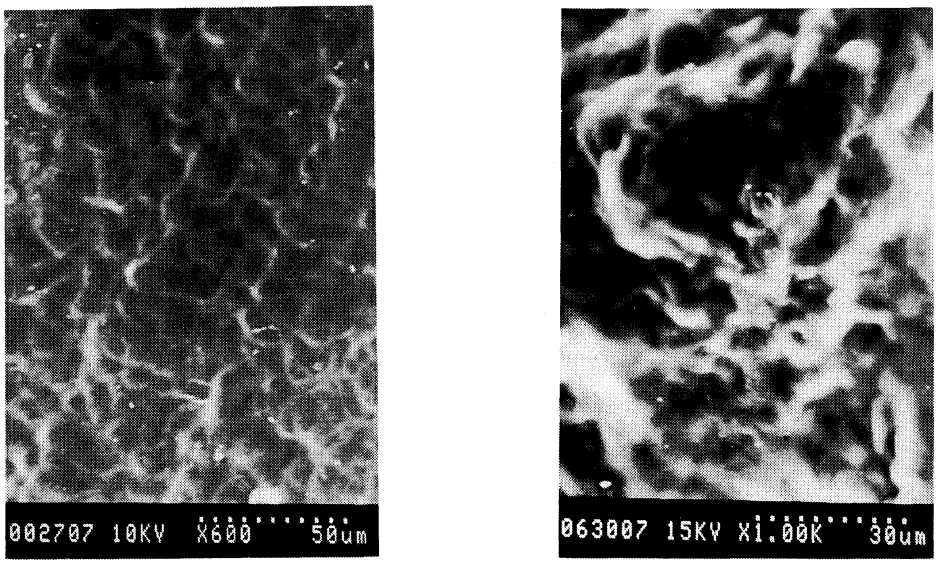


(b_3)

Figure 2

electron microscope (Figure 1). As can be seen in Figure 1, the grafted layer of PAM is gradually formed (a), and penetrates into the matrix of PVB (b) with increasing graft percent. At graft percents above 60% ((c) and (d)), microphase-separated structures appear, and the volume of the grafted membranes increases.

The corresponding surface structures of VBA were observed by a scanning electron microscope (Figure 2). Figure 2(a) indicates the surface structure of Ce(IV)-treated PVB, in which a lot of activated centers due to the effect of Ce(IV) was observed. The graft re-



(c) (d)

Figure 2. Scanning electron micrographs of VBA copolymers. The light portions are polyacrylamide domains. (a) Ce(IV)-treated PVB, (b) VBA samples with increasing graft percent. b_1, b_2, and b_3 indicate VBA-8, VBA-20, and VBA-less than 60, respectively, (c) VBA-60, and (d) VBA-80.

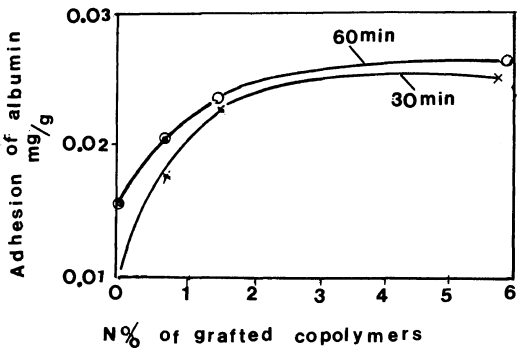


Figure 3. Adsorption of bovine serum albumin on the surface of VBA plotted against N% of grafted copolymers.

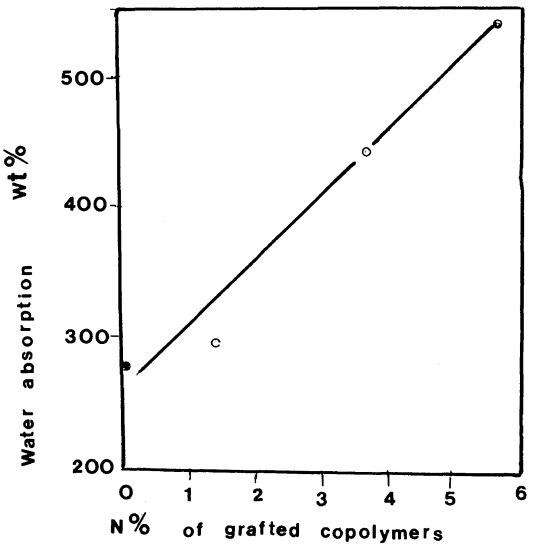


Figure 4. Water absorption of VBA plotted against N% of grafted copolymers.

action is considered to start from these places. Figures 2(b_1) and (b_2) show the surfaces of films of 8 and 20% graft percent, respectively. Polyacrylonitrile grafted displayed embossed surface structures. In these cases, films changed from transparent to opaque. With increasing graft percent, the grafted layer thickened. As the graft percent further increased, the grafted layer penetrated the mother film, and the embossed pattern on the surface flattened. Above graft percents of 60% (Figures 2(c) and (d)), the grafted layer extended into the mother film completely. Thus, microphase-

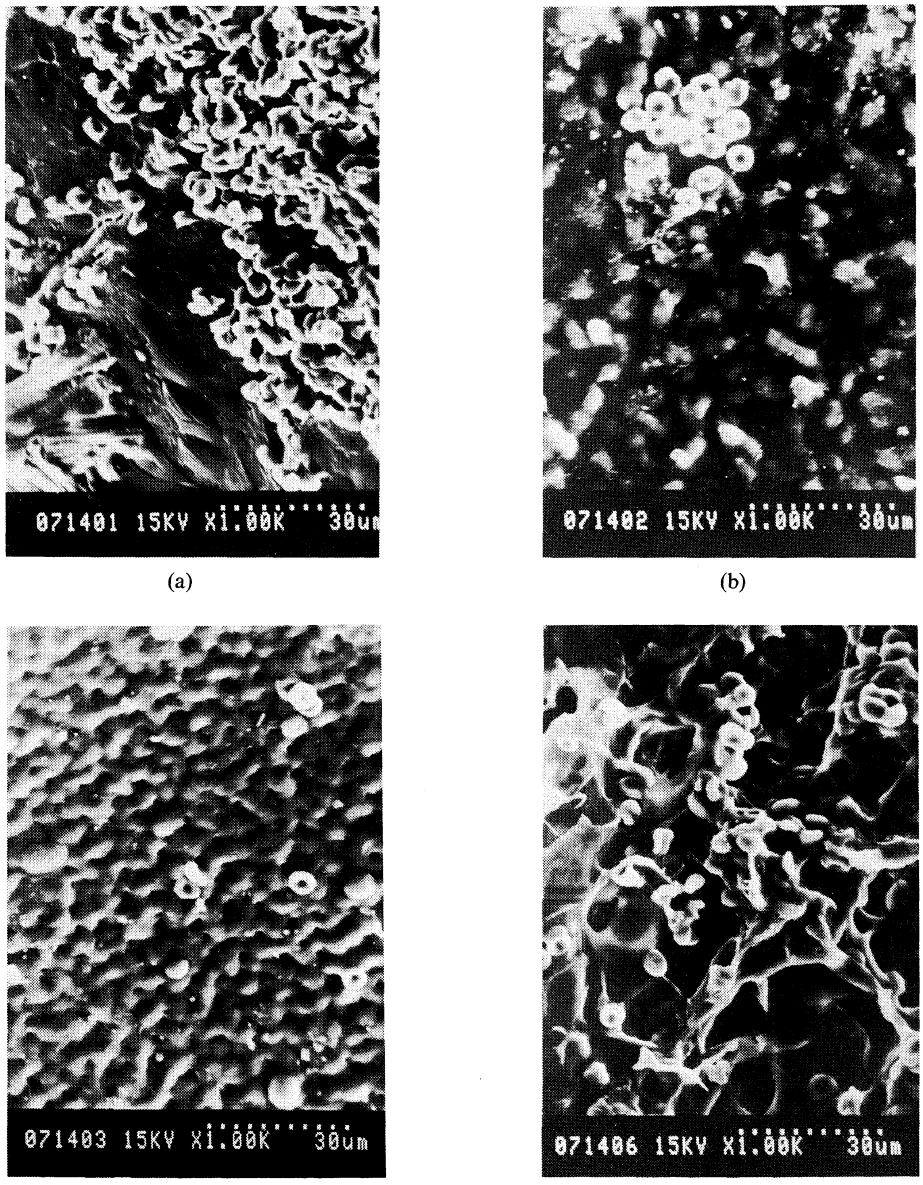


Figure 5. Scanning electron micrographs about blood components adhering to the surface of PVB and VBA. (a) PVB, erythrocytes adhering to the surface obviously. (b) VBA-20, erythrocytes adhering to the surface without denaturation. (c) VBA-60, erythrocytes slightly adhering to the surface. (d) VBA-80, erythrocytes considerably adhering to the surface.

separated structures were formed (see Figure 1(c)).

The adsorption of proteins and adhesion of cells in the blood to the grafted surface were examined. It was found that human γ-globulin did not adhere to the VBA surface, but bovine serum albumin did (see Figure 3). In Figure 4, the water absorption is plotted against the

nitrogen (N)% of the grafted polymers. The water absorption increases with increasing graft percent. Such results may be attributed to the fact that albumin preferably adheres to the hydrophilic domains of the surface, but γ-globulin adheres to the hydrophobic domains of the surface. Our results are in accord with the findings by Okano *et al.*[3,4]

It was also found from the scanning electron micrographs shown in Figure 5 that, in contrast to PVB, the erythrocytes adhered to the surface of VBA-20 without denaturation, and only slightly to the microphase-separated VBA surface, the graft percent of which was about 60%. In our SEM measurements, we did not examine the morphology of plateles. So our present discussion is based on the behavior of erythrocytes toward the polymer surface. The good antithrombogenicity of VBA-60 is thought to come from the coexistence of hydrophilic and hydrophobic microphases at an optimum balance.

Finally, by using spherical active carbon (BAC-MUAZ) encapsulated[5] with VBA (N%: 5), and with PVB as the adsorbents, changes in blood composition and biochemical index by absorption were evaluated by hemoperfusion on living animal body (dogs) through mini-column filled with the adsorbent. It was found that the decrease in the platelet number due to adhesion onto VBA (N%: 5) was only 8.1%. Such a result was compared with 28.9% for PVB, and 28.6% for poly(hydroxyethyl methacrylate).[2,6] Thus antithrombogenicity seems to be correlated closely to the microphase-separated structure of the materials.

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