New-Type Sulphonated Polymer Surfaces for Improving Blood Compatibility

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ABSTRACT: A new-type sulphonated polymer surface obtained by the solution technique is reported. The surface properties of the modified polymer surfaces are characterized by SEM and ESCA, and their *in vitro* blood compatibility is evaluated in terms of TT, PRT, PTT, and platelet adhesion. The modified polymer surface is found to be the least thrombogenic when the modifying polymer molecule contains hydrophilic PEO chains. The PEO chains can serve as "spacers" inserted into the film surface and SO_3^- group. This kind of polymer surface is easily mobile for reconstructing to carry out the function of the SO_3^- group and PEO chains. © 1999 John Wiley & Sons, Inc. J Appl Polym Sci 74: 2826–2831, 1999

Key words: solution technique; polymer surface modification; PEO chain; SO_3^- group; blood compatibility

INTRODUCTION

Surface-induced thrombogenicity of blood-contacting biomaterials is a key problem in clinical practice. Much work has focused on understanding the relationship between polymer surfaces and thrombogenicity.[1,2] Hydrophilic polymer surfaces appeared to be less thrombogenic. Polyethylene oxide (PEO) is widely appilied in improving polymer surface hydrophilicity.[3] On the other hand, bioactive substances such as heparin are introduced into the polymer to endow polymer surface nonthrombogenic to some extent. Moreover, Jozefonicz and colleagues reported that a sulfonate group on the polystyrene showed a similar antithrombogenic property to that of heparin, which provided a possibility to develop biofunctional polymer.[4]

The surface grafting method is usually used for surface modification of polymers. This method has its own shortcoming. The candidate polymer should have a reactionable group on the surface, which confines this method to a few polymer types such as polyurethane. In our study, new-type sulphonated polymer surfaces for improving blood compatibility are prepared by a solution technique. Polystyrene is chosen as a model for the polymer surfaces. Sulphonated steary polyethylene oxide (SSPEO) serves as the modifying polymer. The surface property of the thus obtained sulphonated surfaces of PS films are characterized, and their *in vitro* blood compatibility are investigated to determine the role of the sulphonate group and PEO chains in blood compatibility of the modified surfaces.

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EXPERIMENTS

Synthesis of Sulphonated SPEO Derivatives

The synthesis of sulphonated SPEO derivatives is carried according to the reference with modification.[5] SPEO (M_n 1700) solves in isopropanol, and sodium carbonate solves in a little water. Then SPEO and sodium carbonate solution are mixed and kept stirring for 20 h in 40–45°C water bath and under nitrogen atmosphere. Then 1,3-propane sulfone/DMSO solution is added into this reaction mixture, and the reaction continues for another 20 h, again under the same condition. Finally, the reaction mixture is concentrated by vacuum distillation. The concentrated solution is poured into a great amount of anhydrous ethylene ether to obtain a yellowish solid product. This product (described as SSPEO) is characterized by IR and NMR.

Preparation of Sulphonated PS Films

Sulphonated PS films are prepared by the solution technique (detailed report in previous article).[6] Briefly, sulfonated SPEO derivatives solve in THF/H_2O (80/20 v/v) mixture solvents. Then SSPEO solution of a little amount keeps contacting with the PS film (1 × 1 cm) surface for 30 min (R.T.). Thus, treated PS film is immediately rinsed in deionized water for 7 days (with exchanging water every day). After water rinsing, the modified PS film is dried in air. The modified films are dried in vacuum for 10 h before surface characterization.

Surface Characterization of the Modified PS Films

The surface properties of the modified PS films are characterized by scanning electron microscopy (SEM; JEOL JEM type) and energy spectroscopy for chemical analysis (ESCA; ESCALAB MARK II type). PS films are mounted to sample platforms by conductive silver adhesive. Before SEM observation each film surface is sprayed gold. For the ESCA observation the measurement condition is described as follows: X-ray source AlK^{α}, analysis room pressure $< 10^{-8}$ Torr, energy analysis passage 50 ev, scanning times 3, whole spectroscopy path length 0.8 ev, remaining time 50 ms, narrow spectroscopy path length 0.1 ev, remaining time 200 ms.

In Vitro Blood Compatibility of the Modified PS Films

PRT TT and PTT Tests

Plasma recalcification time (PRT), thrombin time (TT), and prothrombin time (PTT) of the modified PS films are detected according to ref. 7. In short, fresh plasma (containing anticoagulant) incubate on the films surfaces in 37°C water bath for 60 s. Then the corresponding reagent [$CaCl_2$, thrombin, or plasminogen activator containing calcium (II)] is added in the plasma. Meanwhile, the start time and the time at which the fibrous substance appears are recorded and the clotting time is calculated.

Platelet Adhesion Experiments

Platelet-rich plasma (PRP) is obtained by centrifuging fresh whole blood (within 2 h after blood collection from a healthy human in the Red Cross Hospital, Hangzhou) for 10 min at 1200 rpm. PRP contacts the modified PS film surfaces for 30 min (R.T., static). Then the films are washed in phosphate-buffered saline (PBS, PH 7.2) for several times and dried in air. The adhered platelets on the modified PS films are investigated by SEM.

For comparison study octadecanol ($C_{18}H_{37}OH$, ODO) has also been chosen to carry out the above same reaction and tests as SPEO.

RESULTS AND DISCUSSION

SPEO and ODO Sulfonate Derivatives

The IR spectroscopes of sulphonated SPEO product are shown in Figure 1. Compared with SPEO itself, SSPEO exhibits new IR absorbance peaks at 1035 and 1200 cm^{-1}, which are attributed to —SO_2— vibration. In the ^{1}H-NMR spectroscopy of SSPEO (shown in Fig. 2) new peaks also appear in the range of 3.1–3.4 ppm, attributed to the hydrogen connected with SO_3 (i.e., $C\underline{H}_2SO_3$). These results show that the reaction of SPEO and 1,3-propane sulfone under this condition is successful. The schematic reaction formula is shown as the following:

$$\text{SPEO—CH}_2\text{—OH} + \text{1,3-propane sulfone} \xrightarrow[\text{isopropanol}]{\text{Na}_2\text{CO}_3/\text{H}_2\text{O}} \text{SPEO—CH}_2\text{—O—CH}_2\text{CH}_2\text{CH}_2\text{—SO}_3^-$$

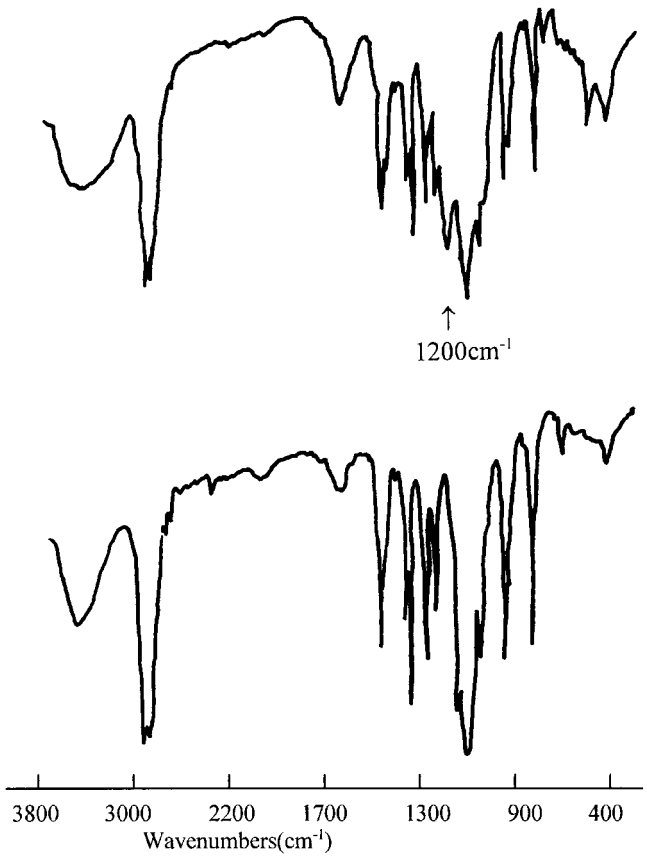


Figure 1 IR spectroscopes of sulphonated product of SPEO (KBr) (up: product; down: SPEO).

The results of octadecanol (ODO) and 1,3-propane sulfone reaction are the same as that of SPEO. The obtained sulfonated product is described as SODO.

Surface Properties of the Modified PS Films

PS films surfaces are modified by SSPEO or SODO solution. SEM observation results of the modified PS surfaces show the morphology of the modified films are basically smooth, and there are no apparent holes on the surfaces. The C_{1s} peak of the different electron escape angles from the normal line of the modified PS film surfaces can be recorded by the ESCA technique. Considering the possible existing elements and carbon bond on the surfaces, the C_{1s} peak might be separated into three peaks by software analysis. In this case, the C_{1s} peak may belong to the contribution of carbon atoms in C—C single bond (inner electron binding energy 285.0 ev), carbon atoms in C—O single bond (binding energy 286.5 ev), and carbon atoms in C═O double bonds or C—S single bond (binding energy 288.0–288.5 ev). As the C═O double-bond component does not exist in the SSPEO or SODO molecular, the third peak (binding energy 288.0–288.5 ev) is attributed to the C—S single bond. The processed data of the angle-dependent

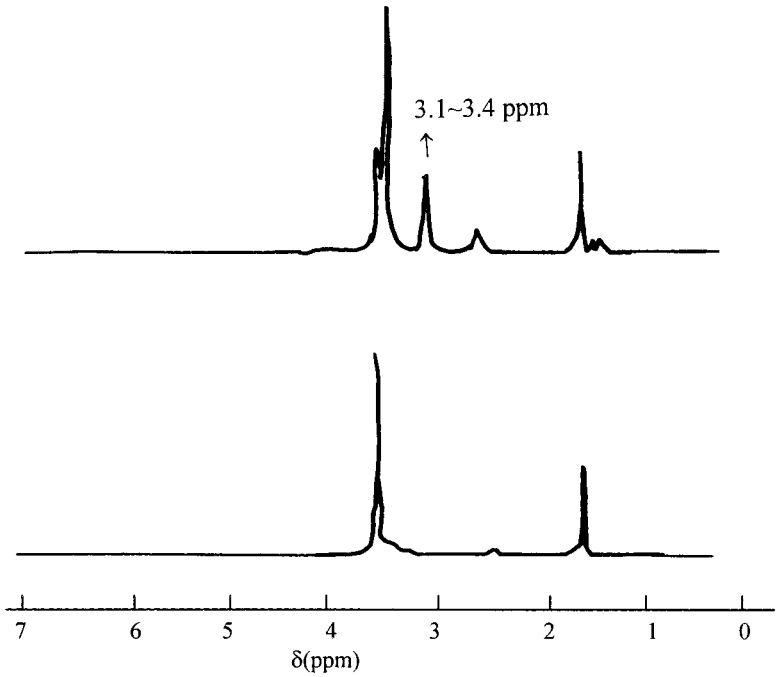


Figure 2 NMR spectroscopes of sulphonated product of SPEO up: product (*DMSO-d_6 solvent peak); down: SPEO ($CDCl_3$).

Table I Data of Surface Characterization of the Modified PS Films

	ESCA (θ)		
Sample Code	Escape Angle (°)	C—C : C—O : C—S	Contact Angle (°)
96-12-12SS	20	1 : 0.77 : 0.65	
	40	1 : 3.52 : 1.61	56.8
	60	1 : 4.22 : 1.77	
96-12-12DS	20	1 : 2.48 : 1.68	
	40	1 : 1.24 : 0.81	78.6
	60	1 : 0.49 : 0.056	

SS stands for SSPEO-modified surface, DS stands for SODS-modified surface. Escape angle is defined as the angle formed by the normal line of sample surface and the route of escape electrons from sample surface region to energy analyzer. The contact angle is carried out in R.T. and by the sessile drop method. The data is detected five to six points every film.

ESCA (θ) of the modified PS films are listed in Table I.

From Table I, the presence of the C—S component on the modified surfaces indicates that sulphonated SPEO (SSPEO) or SODO has been introduced into the PS film, and can be immobilized in the surface region of several hundreds of angstroms by this method. This result shows that the PS film surface modification by the solution technique is workable for building a sulfonated surface. This method provides a possible new attempt to immobilize an expensive bioactive substance into the polymer surfaces.

In fact, element analysis cannot detect the existence of sulfur element in the DS and SS films. These results indicate that the amount of the sulfur element introduced is very little, and the sulfur element exists only on the surface region of the polymer films. Therefore, this method has a distinctive feature. It can modify the surface region of polymer films while no apparent effect is caused in the bulk.

On DS or SS film surfaces, the C—C component might come from steary in SPEO or SODS molecular or surface PS chains. The ESCA analysis software cannot distinguish the resource difference of the C—C bonds. Therefore, the quantitative value of the sulfonate group concentration cannot be obtained by ESCA determination. The relative ratio of the C—C, C—O, and C—S component is considered into discussion.

From the ESCA (θ) data in Table I, the ratio of the C—O and C—S components to the C—C components on the air—SS film interface increases as the detection depth increases (i.e., the escape angle increases). In contrast, the ratio decrease as the detection depth increases on the DS surface. The constituted difference of the air–film interface between SS and DS films results mainly from their different free energies of the PS chain, PEO chain, steary chain, and SO_3^- group, which has the following order from large to small: SO_3^- group $\gg$ PEO chain $>$ steary chain $\approx$ PS chain. The free energy of the PEO chain is relatively near to that of the steary and PS chains.

When the modifying solution contacts the PS films surface, the solvent containing THF can solve the film surface region and induce the modifying polymer to fuse into the film surface. As the solution used is very little, the solvent vapors fast, and the mobility of the chains may be frozen. During this process of forming a new surface, there may occur film surface molecular solvation and chain–chain or chain–group interaction on the surface region. Finally, the modified films are dried in air and will be put into a vacuum atmosphere to carry out the ESCA measurement. According to the least free energy principle, the chain of the lowest free energy will occupy the upper layer of the surface, and the PEO chain or the SO_3^- group of the higher free energy will be buried under the deeper layer. This case is suitable for the SS film surface. But for DS film surface, the case has some difference due to the relatively very high free energy of the SO_3^- group, which makes the SO_3^- group and steary, the SO_3^- group, and PS chain incompatible in the view of thermodynamics. And the nearness of the steary and PS chain free energy makes them interact with each other by a hydrophobic interaction. Thus, the DS surface is formed with the SO_3^-

Table II Results of PRT TT and PTT of the Modified Surfaces

Sample Code	TT(S)	PRT(S)	PTT(S)
96-12-12SS	*	282(316)	49(48)
DS	*	177(190)	43(43)
96-12-12SH	*	410(400)	79(97)
DH	*	261(246)	54(56)
PS	19	194(190)	25

SS stands for SSPEO-modified surface, DS stands for SODS-modified surface. SH and DH are the modified PS films by SPEO–heparin and ODO–heparin solution.[8] The values in the parentheses are obtained by detecting the modified surfaces for the second time after being rinsed in PBS buffer for 7 days.

group occupying the upper layer of air–films interface.

Blood Compatibility of Modified PS Films

The PRT, TT, and PTT data of modified PS films are listed in Table II. The PRT, TT, and PTT all become longer in comparison with the values of the unmodified PS films. The results indicate that sulfonated polymer surfaces exhibit antithrombogenic. It is worth noticing that the TT values of modified sulfonated surfaces are undetectable in the range of the experimental period (6 h), similar to the results of heparinized surfaces. The sulfonate group incorporated into the PS surface is considered to be the main reason. Jocefonivz and colleagues[4] reported the antithrombogenic property of sulfonated polystyrene. The SO_3^- group could interact with the antithrombogenic factor AT III, and prompt the reaction of ATIII and thrombin in the blood. Moreover, the sulfonated surfaces retain their antithrombogenicity after soaking in water. This result indicates that the immobilized SSPEO or SODO molecules on the PS surfaces are stable in buffer.

The PRT and PTT values of SS surfaces are bigger than that of DS surfaces. This result indicates that SS surfaces are less thrombogenic than the DS surfaces. The thrombogenic difference between the two kinds of sulfonated PS surfaces might arise from the different modifying polymer, while other conditions are similar.

From the above surface analysis the C—S and C—O components exist on the surface region of the modified PS films. The surface constitute may experience a change when contacting with plasma. The "real time" ESCA data about the films in water is very difficult to obtain. But the contact angle measurements provide the indirect proof. The contact angles of all these modified PS films decrease compared to that of the PS film (see Table I). The hydrophilic components such as the PEO chain and SO_3^- group will usually move to a film–water interface to decrease the interface free energy. Moreover, the hydrophobic interaction between the steary and surface PS chain on the SS film forces the turnover of the surface constitute. Consequently, PEO chains on the SS surface come up to the upper surface region together with the SO_3^- group connected.

The PEO polymer has been well known for its excellent hydrophilicity, flexibility, and mobility. PEO chains on the SS film surface serve as "spacers" between the SO_3^- group and film surface. So the properties of the PEO polymer are very helpful for the SO_3^- group immobilized on the SS surfaces to interact with antithrombogenic factors in the blood.

On the other hand, steary directly connects with SO_3^- in the SODS molecular. Steary chains are relatively stiff, and steary and PS chains have strong hydrophobic interaction. Consequently, SO_3^- groups reach the DS surface more nearly, and the mobility of the SO_3^- on the DS surfaces is reduced. Figure 3 describes the surface structure of the SS and DS films in water. Kim et al. reported that the sulphonated surfaces by the grafting reaction of PEO onto polyurethane films and further with 1,3-propane sulfone to improve blood thrombogenicity.[5]

Platelet adhesion results are shown in Figure 4. Few platelets adhere on the SS surface, while a lot of platelets adhere on DS surface. This result indicates that the SSPEO-modified surface is more platelet resistant than the DS surface. Two possible reasons are considered. One is the increased hydrophilicity of the SS surface, both by PEO and SO_3^-. The other is the resistance of the PEO chain to the platelets. PEO is good at reducing cell adhesion. Considering the above reasons,

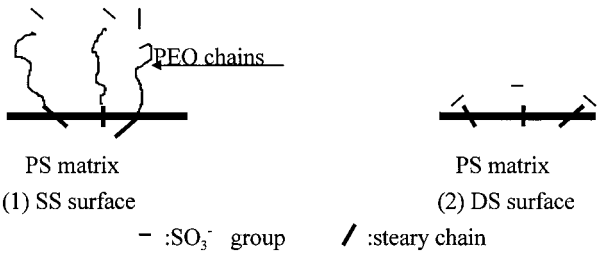
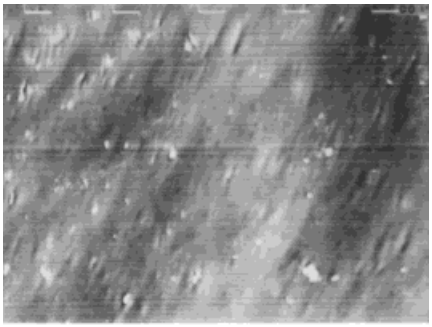
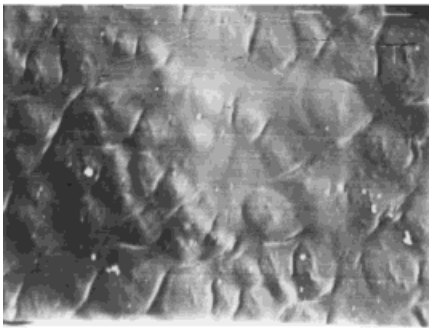


Figure 3 Schematic surface structure of modified PS films in water.



(1) on DS surfaces



(2) on SS surface

Figure 4 SEM observation of adhered platelets on the surfaces (SEM 2000×; scale length "—" in the figure means 10 μm).

the SS surfaces exhibit good platelet adhesion resistant.

From the above study, the sulfonated SPEO-modified polymer surfaces (SS) show better blood compatibility than the SODS-modified surfaces. In respect to blood coagulation and platelet adhesion of modified surfaces, the PEO chain and SO_3^- group exert a combined effect on the antithrombogenic property of the SS surfaces.

CONCLUSION

Sulfonated surface of polystyrene films has been prepared by a simple solution technique. The new-type sulfonated surfaces exhibit antithrombogenic property. A hydrophilic and flexible PEO "spacer" between the functional group and the matrix surface is helpful for the immobilized SO_3^- group to interact with antithrombogenic factors and for resistance of platelet adherence.

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