Effect of polyol type on the physical properties and thrombogenicity of sulfonate-containing polyurethanes

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Polyetherurethanes (PEUs) based on polytetramethylene oxide (PTMO) as the polyol, and derivatized with propyl sulfonate functionality, have previously been shown to possess antithrombotic properties. In this article, the bulk physical properties of sulfonated and nonsulfonated polyurethanes containing either polyethylene oxide (PEO) or PTMO as the soft segment are studied. The in vitro shape-change of platelets in contact with these surfaces, and their ex vivo blood-contacting response are also investigated. It was found that PEO-base was physically weaker than PTMO-base, which is attributed to a lower degree or phase separation in the former. In the dry state, sulfonation enhanced the physical properties for PTMO-containing polyurethane (PTMO-SO3-0.20), but weakened the PEO-containing polyurethane (PEO-SO3-0.15). In vitro platelet spreading studies showed the lowest degree of platelet spreading and also the lowest platelet density on PEO-base, while platelet spreading and density on the other three materials and polyethylene (PE) was greater. The thromboreistance of these materials was evaluated using a canine arteriovenous series shunt ex vivo. It was determined that PTMO-SO3-0.20 was the least thrombogenic, followed by both PEO-base and PEO-SO3-0.15, and that PTMO-base was the most thrombogenic. © 1993 John Wiley & Sons, Inc.

INTRODUCTION

A number of investigators have shown that polymers incorporating sulfate or sulfonate groups have rather remarkable blood-contacting properties. These materials may act like heparin, a sulfated mucopolysaccharide that is commonly used as an anticoagulant. Sulfate and aminosulfate groups have been shown to be essential to the activity of heparin. Jozefowicz and Jozefonvicz have modified polystyrenes and dextrans to incorporate these ionic groups, and have shown that these materials possess anticoagulant, heparin-like activity. Ito et al. have shown that poly(vinyl sulfonate) also possesses heparin-like activity. In experiments with polyurethanes which are sufficiently sulfonated to be water soluble, it has been shown that these polymers prolong clotting times, inhibit the process of fibrin assembly, and inhibit thrombin activity.

When used as hemodialysis membranes, polysulfones and polycrylonitrile sodium methallylsulfonate (Hospal AN-69) show minimal complement activation and cause minimal granulocytopenia. Polyurethanes grafted with propyl sulfonate groups and containing PTMO as the macroglycol show strong antithrombotic behavior, with low levels of platelet deposition or spreading. Similar results were also obtained when Biomer, a commercial polyurethaneurea containing PTMO as the macroglycol, was sulfonated in the same manner. Polyurethanes surface-grafted with poly(ethylene oxide) chains that are end-terminated with sulfonate groups significantly prolong occlusion times in a rabbit arterio-arterial (AA) shunt model. Santerre et al. have shown that sulfonated polyurethanes interact strongly with fibrinogen, and that fibrinogen is not displaced from these surfaces in Vroman-effect type experiments. Since fibrinogen is typically displaced from surfaces by high molecular weight kininogen (HMWK), a protein that is involved in the contact activation of blood coagulation, it was suggested that HMWK was unable to reach the surface, and that therefore coagulation would be inhibited. Thus, polymers containing sulfate or sulfonate groups have very interesting behavior in contact with blood. Not all reports are positive, however. For example, Lai studied the response of column-washed platelets to sulfonated polyurethanes in vitro under static conditions, and found that...
platelet membranes become disrupted. This result is consistent with the observation that under certain conditions, heparin can cause platelet activation. It is possible however, that protein-surface interactions may modulate this response in vivo.

Are there any requirements for polymers to be blood compatible besides having sulfate or sulfonate groups? Okkema et al. synthesized a series of polyurethanes containing a sulfonate functionality incorporated via the chain extender and containing either PTMO (MW 1000) or polyethylene oxide (PEO) (MW 1000) polyols. Those materials, which were based on PTMO as the polyol, behaved similarly to propyl sulfonate grafted polyurethanes, but those sulfonate chain-extended polyurethanes based on PEO behaved poorly. This result was somewhat surprising, since PEO-containing polymers typically show improved resistance to thrombus accumulation, and a synergistic effect was expected. Takahara et al. synthesized a series of polyurethaneureas that contained long, hydrophilic side-chains in the soft segment. Sulfonate groups were incorporated at the end of the side chains. At the lowest levels of sulfonate incorporation there was a slight improvement in blood compatibility, but at higher levels of sulfonation the materials were actually more thrombogenic than controls. It is apparent, then, that the chemistry of a material is an important determinant of its blood compatibility.

The effect of polyl type on the physical and blood-contacting properties of sulfonate-grafted polyurethanes is investigated in this paper. A series of polyurethanes similar to those described previously, but containing either PEO or PTMO as the soft segment were synthesized, and sulfonate-grafted using the method of Hwang et al. The surface characterization of these materials using static, underwater captive bubble contact angle measurements, dynamic contact angle measurements, scanning electron microscopy, variable angle ESCA depth profiling, and cold-stage ESCA analysis of freeze-dried samples, has been reported. Polyurethanes based on PTMO showed enrichment of the hard segment, and hence the sulfonate groups at the water-polymer interface, compared to the air-polymer interface. The PEO-based polyurethanes showed no such rearrangement. It was also observed that the order of hydrophilicity was PTMO-base < PEO-base, which is the same as PEO SO3-0.15 < PTMO-SO3-0.20, as determined from dynamic contact angle measurements.

The mechanical properties of these materials may ultimately determine their suitability for use in biomedical devices, such as catheters, or small diameter vascular grafts. Materials without sufficient mechanical integrity would need to be used as coatings on more suitable substrates, or blended with nonionized polymers. Incorporation of ionic groups can affect the mechanical properties of polymers. Polymers containing low levels of ionic groups, called ionomers, generally show a higher modulus and tensile strength than nonionized polymers, due to the cross-linking effect of the ionic aggregates. However, for use in vivo, these materials would be hydrated, and since water acts as a plasticizer, the mechanical properties would be weakened. Evaluation of the mechanical properties is also important, however, in that it relates to the polymer's bulk and surface morphology, which in turn is related to the thrombogenicity of the materials. A thorough characterization of these parameters was therefore performed. It was a concern that these materials, which have been previously reported to absorb large amounts of water, might be partially soluble in water, and that this soluble fraction might account for their antithrombogenic behavior. Therefore, the solubility of these polymers in water was investigated.

The thrombogenicity of these materials was evaluated in an ex vivo canine arteriovenous series shunt model with modifications. The term "thrombo-resistant" is used in this paper to refer to materials that show low platelet or complex thrombus deposition on their surfaces. Goodman et al. have argued that the platelet spreading response on a bare surface correlates well with the blood compatibility determined in the ex vivo model. Therefore, platelet adhesion and spreading on these materials was evaluated in vitro.

**EXPERIMENTAL**

**Materials**

Polyurethanes were synthesized according to the method of Hwang et al. Briefly, oligomers of either polytetramethylene oxide (PTMO) (MW 1000) or polyethylene oxide (PEO) (MW 1000) in N,N'-dimethylacetamide (DMAc) were reacted with methylene-diphenylene-diisocyanate (MDI), and then chain extended with 1,4-butanediol (BD). The molar ratio of these polymers was 3:2:1 MDI:BD: (PTMO or PEO), giving a product with 47 wt.% hard segment. These materials were then derivatized by a bimolecular nucleophilic displacement of the urethane nitrogen using sodium hydride, followed by a ring-opening reaction with γ-1,3-propane sulfone. The polymers were extracted in toluene for 48 h in a Soxhlet extractor to remove low molecular weight material.

The underivatized samples are designated PTMO-base and PEO-base, to indicate which soft segment was incorporated. The sulfonated samples are designated PTMO-SO3-0.20 and PEO-SO3-0.15 to indicate
the fraction of urethane hydrogens which were substituted with propyl sulfonate groups.

Elemental analysis was performed by Galbraith Laboratories (Knoxville, TN), and the results, which are presented elsewhere, are consistent with stoichiometric predictions.

Physical characterization

Gel permeation chromatography

Gel permeation chromatography was performed using a multidetector GPC system described by Lee et al., with the exception that the low-angle light scattering detector was removed. The system consists of an ERMA ERC-3310 solvent degasser, a Beckman 114M pump, Altex μ-Spherogel columns, a Beckman 165 variable wavelength UV detector, an Altex 156 refractive index detector, and a Linear 500 multichannel chart recorder. The columns and Rhodyne 7125 sample injector were kept at 50°C in a Modular LC-750 column oven. DMAc containing 2.0 g/L of LiNO₃ was used as the carrier solvent at a flow rate of 0.5 mL/min. The UV detector was set to 285 nm and was therefore sensitive to the hard segment of the polyurethanes.

Fourier transform infrared spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR) was performed on films cast onto NaCl plates from a 1% (w/v) solution of polyurethane in DMAc. The films were thoroughly dried under vacuum at 50°C for at least 48 h before transmission spectra were collected. A Nicolet 170SX FTIR spectrometer was used, with a resolution of 2 cm⁻¹.

Stress-strain

Uniaxial stress-strain analysis was performed using a table model Instron testing machine. Films were spin cast from 10% solutions in DMAc with heat, and the cast films were vacuum dried for at least 4 days at 50°C. The films were 0.1–0.3 mm thick, and samples were cut using an ASTM 1708 standard die. Prior to testing, samples were stored in a desiccator. The samples were tested at room temperature at a crosshead speed of 0.5 in/min (57%/min).

Dynamic mechanical analysis

Dynamic mechanical thermal analysis was performed using a Rheometrics Solids Analyzer (RSAII). Spin-cast films of each polymer, with typical dimensions of 23 mm × 6.3 mm × 0.2 mm were tested in the film/fiber tension mode. The samples were tested at a dynamic strain frequency of 16 Hz over a temperature range from −150°C to failure (approximately 180°C) in 3°C temperature steps.

Differential scanning calorimetry

Thermal analysis was performed using a Perkin-Elmer Differential Scanning Calorimeter (DSC-2) over the temperature range from −150°–300°C at a heating rate of 20°C/min. The unit is equipped with a data processing module that allows subtraction of the background and normalization for sample weight. Samples weighing between 6–10 mg were prepared from films spin-cast from a DMAc solution.

Solubility in water

Because of concerns that these materials might be partially soluble in water, the two sulfonated polyurethanes were tested for leaching by flowing 6 mL of double-distilled deionized water through 45 cm segments of 0.125 in. i.d. polyurethane coated polyethylene tubing at a flow rate of around 35 mL/min. A roller pump (Masterflex model 7016.20, Cole-Parmer) was used, and the water was allowed to circulate for about 24 h. The amount eluted was determined by UV spectroscopy, comparing the absorbance at 280 nm with water-soluble solutions of similar polyurethanes of known concentration.

PLATELET ADHESION STUDIES

Platelets were obtained by drawing 10 mL of blood from a normal, healthy adult male volunteer who had taken no medication for the previous 2 weeks. The blood was drawn into a polypropylene syringe containing acid citrate dextrose (ACD) (1.4 mL ACD for 10 mL blood), mixed gently by inversion, and then centrifuged for 15 min at 160 g. The platelet-rich plasma (PRP) was drawn off with a polystyrene pipette and applied to a Sepharose 2B-300 column (Sigma Chemical Co., St. Louis, MO) (bed volume 40–50 mL) pre-equilibrated with HEPES-Tyrodes buffer. The HEPES-Tyrodes buffer was then used to elute the platelet suspension into polystyrene test tubes.

The test polymers were coated onto 0.125 in. i.d. polyethylene tubing and cut onto U-shaped segments 0.5 cm in length. The tubing was washed in distilled water and equilibrated in HEPES-Tyrodes buffer at
room temperature for about 1 h. Then 100 μL aliquots
of the platelet suspension, at 37°C, were pipetted
onto the test materials with a micropipette. To verify
the activity of these platelets, parallel experiments
were performed in which the platelets were allowed
to adhere onto polyethylene substrates, and typical
spreading behavior was confirmed.

Platelets were allowed to adhere and activate on the
tubing sections for time periods of 5, 20, and 45 min.
Incubation took place at 37°C in an air atmosphere
controlled at 100% relative humidity. Platelet acti-
vation was arrested by placing freshly prepared 2%
glutaraldehyde in 0.1 M phosphate buffer (pH 7.35)
on each segment after appropriate incubation times.

The tubing specimens were dehydrated using a
graded ethanol series, followed by critical point dry-
ning with CO2 as the transitional fluid. Specimens were
mounted on stubs and sputter coated with 4-6 nm
of gold prior to viewing with a JEOL JSM-35C scan-
ing electron microscope at 15 kV. Micrographs were
recorded at 1000× and 5000× magnification for sub-
sequent analysis.

The level of platelet deposition was below mono-
layer coverage for all materials and all time points
studied. Platelets were categorized according to the
extent of shape change, as previously described.23,24
Each platelet was assigned to one of five activation
levels based on morphological features.

Blood contacting response

The blood-contacting properties of these surfaces
were evaluated using an ex vivo canine series shunt
experiment,22 with modifications.7

Adult mongrel dogs, which were selected after
hematological screening, were injected with autolo-
gous 111In-labeled platelets and 125I-labeled fibrino-
gen. No anticoagulants were used in this procedure.
The shunts contained two replicates of each material,
and were filled with degassed phosphate buffered
saline (pH 7.35) containing no azide, and hydrated
overnight. The blood-contacting times for the shunts
were 1, 2, 5, 10, 15, 20, 25, 30, 45, and 60 min. A total
of three surgeries were performed. The blood flow
was continuously monitored using an electromagnetic
flow probe. The initial flow rate was controlled at
280 ± 20 mL/min using a clamp distal to the test
specimens. Blood samples were collected hourly to
determine bulk radioactivity, platelet and fibrinogen
centration, hematocrit, blood gas analysis, and
hematological function.

Blood flow was stopped after appropriate intervals,
and the shunts were flushed with Tyrodes buffer
at a rate of approximately 60 mL/min. Immediately
following flushing and detachment from the animal,
the shunts were fixed with 2% glutaraldehyde. The
test sections were subdivided into sections for gamma
scintillation counting and scanning electron micro-
scopic (SEM) examination. Samples were examined in
a JEOL-JSM 35C scanning electron microscope using
an accelerating voltage of 15 kV, at magnifications of
390× and 2000×.

RESULTS

Gel permeation chromatography

The number average (Mn) and weight average
molecular weights (Mw) along with the polydispersity
for each sample are shown in Table I. The values are
based on comparisons with polystyrene standards.
The number average and weight average molecular
weights are comparable for all four materials.
PTMO-SO3-0.20 showed slightly higher values than
PTMO-base, although the difference is probably not
significant. This could be due to either the 7% increase
in molecular weight by addition of propyl sulfonate
groups, or to a slight increase in the interaction of
the mobile phase with the stationary phase in the
column. It is also possible that the toluene extraction
process could vary in its effectiveness in the base
and sulfonated polymers, causing a slight difference
in molecular weight distributions. PEO-SO3-0.15 was
prepared from a different batch of base polyurethane,
and so has a somewhat broader molecular weight
distribution than the other samples, and a slightly
higher Mw than PEO-base. Sulfonation is calculated to
add an additional 5% to the weight of the PEO-base
polyurethane.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number-Average Molecular Weight (Mn)</th>
<th>Weight-Average Molecular Weight (Mw)</th>
<th>Polydispersity Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTMO-base</td>
<td>2.74 × 10^4</td>
<td>6.49 × 10^4</td>
<td>2.37</td>
</tr>
<tr>
<td>PTMO-SO3-0.20</td>
<td>3.23 × 10^4</td>
<td>6.98 × 10^4</td>
<td>2.16</td>
</tr>
<tr>
<td>PEO-base</td>
<td>2.69 × 10^4</td>
<td>5.85 × 10^4</td>
<td>2.17</td>
</tr>
<tr>
<td>PEO-SO3-0.15</td>
<td>2.40 × 10^4</td>
<td>8.74 × 10^4</td>
<td>3.64</td>
</tr>
</tbody>
</table>
FTIR

Figure 1(a) shows the infrared spectra for PTMO-base and PTMO-S03-0.20. These spectra compare closely with those previously published. The N-H absorption band at 3340 cm\(^{-1}\) was reduced in intensity following sulfonation (data not shown). The most striking change after derivatization is the relative decrease in the peak intensity at 1702 cm\(^{-1}\) as compared to the peak intensity at 1732 cm\(^{-1}\). The peak at 1702 cm\(^{-1}\) has been assigned to carbonyl groups that are hydrogen bonded (presumably to the urethane hydrogens) and the peak at 1732 cm\(^{-1}\) has been assigned to carbonyl groups that are not hydrogen-bonded. The ratio of these two peaks is indicative of the degree of phase separation found in these polymers. A more highly phase-separated material will show a large degree of hydrogen bonding, while a more phase-mixed material will show less hydrogen bonding. For PTMO-S03-0.20, the decrease in hydrogen bonding could be due to the interaction of the urethane hydrogens with the sulfonyl groups instead of the carbonyl groups, or it could be a result of disruption of the ordered hard segment packing upon sulfonation. Shifts in the sulfonyl O=O stretching region (1050 cm\(^{-1}\)) due to possible hydrogen bonding are difficult to confirm, due to the small peak area.

Comparing PEO-base with PTMO-base (Figs. 1[a] and 1[b], respectively), the former shows comparatively less hydrogen bonding, which indicates less phase separation for PEO-base. The lower bonded-to-free carbonyl ratio for PEO-base may be attributed to an increase in the ether oxygen content; ether oxygen may hydrogen bond with urethane N-H groups. The greater polarity of the PEO polyol, as compared with PTMO, may result in a greater degree of phase mixing between the hard and soft segments.

PEO-S03-0.15 shows a further reduction in the bonded-to-free carbonyl ratio compared with PEO-base, as seen in Figure 1(b), suggesting a further decrease in phase separation. As with PTMO-S03-0.20, however, the decrease in hydrogen-bonded carbonyl content could also be due to hydrogen-bonding with the sulfonyl groups.

**Tensile properties**

The tensile properties of the polymers are summarized in Table II. The stress-strain curves for PTMO-base and PTMO-S03-0.20 are shown in Figure 2(a), and those for PEO-base and PEO-S03-0.15 are shown in Figure 2(b). For the PTMO-containing polyurethanes, the Young's modulus and ultimate stress of the material was dramatically increased by incorporation of sulfonylate groups. This is expected, since the ionic groups may act as reinforcing cross-links. There was also an expected decrease in the ultimate elongation of the sulfonated polymer as compared to the nonsulfonated base. The properties of these materials are similar to those synthesized previously. For the PEO-containing polyurethanes, however, the incorporation of sulfonylate groups resulted in a very weak, viscoelastic material, with an ultimate elongation of >1000%. The decrease in the Young’s modulus and ultimate stress for PEO-S03-0.15 may be due to disruption of hard segment packing by sulfonylate groups, which is in agreement with the IR data. It could also be due to the hygroscopic nature of the soft segment. Water absorption studies showed that films of PEO-base absorbed 40% of its...
TABLE II

Tensile Properties

<table>
<thead>
<tr>
<th>Sample</th>
<th>Young's Modulus (MPa)</th>
<th>100% Tensile Modulus (MPa)</th>
<th>Ultimate Stress (MPa)</th>
<th>% Elongation at Break</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTMO-base</td>
<td>21 ± 6</td>
<td>5.8 ± 1.2</td>
<td>20 ± 6</td>
<td>477 ± 52</td>
</tr>
<tr>
<td>PTMO-S03-0.20</td>
<td>128 ± 18</td>
<td>14.5 ± 1.7</td>
<td>38 ± 4</td>
<td>327 ± 23</td>
</tr>
<tr>
<td>PEO-base</td>
<td>23 ± 4</td>
<td>3.3 ± 0.4</td>
<td>3.6 ± 0.6</td>
<td>399 ± 69</td>
</tr>
<tr>
<td>PEO-S03-0.15</td>
<td>2.1 ± 0.3</td>
<td>0.40 ± 0.09</td>
<td>0.26 ± 0.06</td>
<td>1157 ± 76</td>
</tr>
</tbody>
</table>

Dry weight in water when immersed in water for 24 h, as compared with 0.5% for PTMO-base. Any water absorbed by PEO-S03-0.15 would result in a decreased cohesion of the ionic aggregates, and would act as a plasticizer, thus weakening the material. The modulus determined for PEO-S03-0.15 at room temperature in tensile testing is an order of magnitude lower than that determined using dynamic-mechanical analysis, suggesting that this sample was water-plasticized. Although the samples were stored in a desiccator, the sample may have absorbed water for several hours prior to testing, as well as during testing due to humidity. Samples tested in the RSAII were subjected to a nitrogen purge in the sample chamber, thereby minimizing the effect of humidity. This difference in modulus may also be due to the fact that cyclic testing was used in dynamic mechanical analysis, as compared to the noncyclic stress-strain testing. The Young's modulus of PEO-Base is comparable to that found previously for a PEO-containing polyurethane, although the ultimate properties are lower.

Dynamic mechanical properties

The dynamic mechanical data showing plots of the storage modulus $E'$, the loss modulus $E''$, and tan $\delta$ are shown for PTMO-base and PTMO-S03-0.20 in Figure 3(a), and for PEO-base and PEO-S03-0.15 in Figure 3(b). The thermal transitions are summarized in Table III.

PTMO-S03-0.20 shows a $T_g$ comparable to PTMO-base. The rubbery plateau occurs at a higher modulus for PTMO-S03-0.20 as compared to PTMO-base. A higher temperature transition peak, labeled $\beta'$, is seen at 103°C for PTMO-S03-0.20. This transition has been observed previously between 95 and 100°C, and may be due to disruption of ionic aggregates. The gamma peaks, which are indicative of short range chain motions, occur at about $-130^\circ$C for PTMO-base and PTMO-S03-0.20, and are somewhat lower than the transition for PEO-base and PEO-S03-0.15, at about $-121^\circ$C. The properties of PTMO-base and PTMO-S03-0.20 agree well with those found by others.

Comparing PEO-base with PEO-S03-0.15, it can be seen that the glass transition temperature is higher for the sulfonated polymer. The rubbery plateau occurs at a lower modulus and extends over a narrower temperature range for the ionic polymer than for the base. This result is somewhat surprising, since ionomers typically show a rubbery plateau at a higher modulus which extends to higher temperatures than nonionic polymers.

Figure 2. Uniaxial stress-strain curves for a) PTMO-base and PTMO-S03-0.20 and b) PEO-base and PEO-S03-0.15.
Thermal analysis

The DSC thermograms for the initial heating of each material are shown in Figure 4. The soft segment $T_g$ and other thermal transitions for the polyurethanes are summarized in Table IV.

For the PTMO-containing polyurethanes the $T_g$ midpoint occurs substantially above the $T_g$ for the pure PTMO homopolymer\(^{29}\) of $-89^\circ$C. This indicates that some phase mixing of hard and soft segments occurs in these polymers.\(^{31}\) PTMO-base and PTMO-SO3-0.20 have the same $T_g$ and therefore show about the same degree of phase separation. For a similar series of polyurethanes,\(^{18}\) low levels of sulfonation resulted in disruption of hard segment domains, but as the level of ionization increased, the degree of phase mixing returned to that of the underivatized sample. This was attributed to the increase in segmental polarity difference offsetting the disruption of hard segment packing.

For PEO-base, a soft segment phase $T_g$ of $-23^\circ$ was observed. The polarity difference between hard and soft segments is less for PEO-base than PTMO-base, and therefore a higher degree of phase-mixing is seen in the former.\(^{30,32}\) For PEO-SO3-0.15, the soft segment $T_g$ is even higher, which may be due to disruption of hard segment domains, as ionization interferes with hard segment packing.

Transitions are observed for these materials from 55°C up to 135°C, which may be attributed to annealing or other events related to thermal or mechanical history.\(^{33}\) Transitions related to crystallinity in the hard segment phase\(^{34}\) are seen for the underivatized samples at temperatures ranging from 165–200°C.
### Water absorption and leaching

Water absorption data for these materials have been previously presented. PTMO-base absorbs <1%, while PEO-base absorbs roughly 40%, of its dry weight in water. Sulfonation dramatically increases water absorption by these materials, with PTMO-S03-0.20 and PEO-S03-0.15 absorbing roughly 100 and 300%, respectively, of their dry weights in water. Since at higher levels of sulfonation (30% or greater), these materials become water soluble, the average release rate of these polymers into aqueous solution was measured. PEO-S03-0.15 and PTMO-S03-0.20 were released into solution at an average rate of $2.51 \times 10^{-3}$ and $5.29 \times 10^{-3} \, \mu g/cm^2 \, \text{min}$, over a period of 24 h. Instantaneous release kinetics were not measured. These values are an order of magnitude less than the minimum release rate of $4.00 \times 10^{-2} \, \mu g/cm^2 \, \text{min}$ for ionically bound heparin to be pharmacologically active, as determined by Basmadjian et al. This means that the antithrombotic effects seen for these polyurethanes are probably not due to release of the polymers into the bloodstream.

### In vitro platelet adhesion

When column-washed platelets come in contact with an artificial surface, they undergo a process of adhesion and spreading. This process has been categorized by Goodman into five stages, identifiable by morphological changes which typically occur in this process. Inactivated platelets, such as those found in circulating blood, are round or discoid in shape (R), with a diameter of about 2 µm. As the platelets become more activated, they extend pseudopodia (D), and then the membrane between the pseudopods extends as it is pushed out by the hyaloplasm (SD or S), until the platelet reaches its fully spread state (FS). In the final state, the platelet typically has a diameter of 7–10 µm. It has been observed that there is a correlation between the degree of platelet adhesion and spreading on an artificial surface in vitro, and the thrombogenicity of that material evaluated using an ex vivo canine series shunt experiment.

Figure 5 and Table V show the results of these experiments. Platelet coverage was submonolayer on all surfaces and at all time points studied. On polyethylene (PE), which was used as a control surface, the platelets appear to be evenly distributed throughout the four more activated categories of platelet morphology, with no discernible trends towards activation over time. On PTMO-base, after 5 min, most of the platelets were found in the spread (S) state. After 20 min on this material, most of the platelets were found in the fully spread (FS) state, or in the spreading dendritic (SD) state. This is because platelets are continually deposited on the surface from the suspension. Thus, the total number of platelets seen after 20 min increased from 29 to 60. After 45 min, most of the platelets were found in the spreading-dendritic (SD) stage, indicating that many new platelets had been recruited to the surface (Table V). For PTMO-S03-0.20, as for PTMO-base, most platelets were found in the spread state (S) after 5 min. At 20 min, most of the platelets were still in the spread (S) stage, or had advanced to the fully spread (FS) state. After 45 min, platelets were equally distributed between the four most activated morphologies. On PEO-base, few platelets were observed to achieve the spread (S) or fully spread (FS) morphologies at any time, and fewer total platelets were observed on this material than on any other at the 20 and 45 min time points. PEO-base is the only surface on which some platelets remained inactivated (R) after both 5 and 45 min. On PEO-S03-0.15, however, more platelets were seen than on PEO-base, and they were more activated.

### Blood contacting response

Platelet and fibrinogen deposition profiles for all four polyurethanes are shown in Figure 6. The data shown are an arithmetic mean for three surgeries. A maximum in platelet deposition is seen on all of the materials between 15 and 45 min of blood-contacting time. The peak signifies the occurrence of a thromboembolitic event, with platelet aggregates or more complex thrombi increasing in size, and then being removed from the surface. In general, the lower the platelet maximum, the smaller the emboli. It can be seen that PTMO-base is the most thrombogenic, PEO-base and PEO-S03-0.15 are of equal and intermediate thrombogenicity, and PTMO-S03-0.20 is the least thrombogenic material.

The fibrinogen deposition profiles show similar trends to the platelet deposition profiles, as expected,
since platelets and fibrinogen are the primary components of a thrombus. However, the relative amount of fibrinogen seen on PTMO-S03-0.20 is about as great as on PEO-base and PEO-S03-0.15, which differs from the results seen for platelets. This reinforces the previous observations\textsuperscript{7,11} that PTMO-S03-0.20 interacts strongly with fibrinogen.

Scanning electron microscopic observation showed that submonolayer to monolayer coverage by pseudopodial platelets was present on all surfaces after 5 min of blood-contacting time (Fig. 7). The response to each of these materials was roughly equivalent at this early time. For PTMO-base and PTMO-S03-0.20, the platelets are seen to be pseudopodial at higher magnification (Fig. 9a,b).

PTMO-base is the most thrombogenic, exhibiting maximal platelet deposition of about 1100 platelets/1000 \(\mu m^2\) at 20 min of blood-contacting time (Fig. 6[a]). Small platelet aggregates (20 \(\mu m\)) developed after 15 min. Macroscopic platelet thrombi were observed on this material at 30 min (Fig. 8[a]), along with large numbers of adherent, spread leukocytes, which can be seen more clearly at higher magnification (Fig. 9[c]). The type of leukocytes present was not determined. At 60 min, macroscopic thrombi remained on some of the samples, with many adherent, spread leukocytes.

The lowest amount of thrombus formation was observed on PTMO-S03-0.20. Figure 6(a) shows a maximum of about 100 platelets/1000 \(\mu m^2\) at 25 min. At 15 min, very small platelet aggregates (5 \(\mu m\)) were seen on the surface of this material. The platelet aggregates did not increase significantly, in size at either 30 min (Figs. 8[b] and 9[d]) or 60 min. However,

\begin{table}[h]
\centering
\caption{Platelet Morphology Results}
\begin{tabular}{|c|c|c|c|c|c|c|c|c|c|}
\hline
\textbf{Material} & \textbf{Time (min)} & \textbf{Sample size} & \textbf{\%R} & \textbf{\%D} & \textbf{\%SD} & \textbf{\%S} & \textbf{\%FS} \\
\hline
PE & 5 & 28 & 3.57 & 17.84 & 32.16 & 28.59 & 17.84 \\
& 20 & 70 & 2.85 & 12.86 & 34.27 & 20.00 & 30.02 \\
& 45 & 100 & 1.00 & 22.00 & 33.00 & 15.00 & 29.00 \\
PTMO-base & 5 & 29 & 3.45 & 31.03 & 17.24 & 37.93 & 10.34 \\
& 20 & 60 & 1.67 & 20.00 & 36.67 & 6.67 & 35.00 \\
& 45 & 212 & 3.28 & 31.58 & 47.19 & 8.96 & 8.99 \\
PEO-base & 5 & 22 & 13.65 & 22.73 & 59.08 & 4.55 & 0.00 \\
& 20 & 29 & 3.47 & 5.16 & 31.08 & 6.89 & 6.89 \\
& 45 & 105 & 10.48 & 53.36 & 34.26 & 0.00 & 1.90 \\
PTMO-S03-0.20 & 5 & 52 & 1.92 & 19.24 & 26.91 & 38.47 & 13.46 \\
& 20 & 86 & 0.00 & 11.63 & 13.93 & 39.54 & 34.90 \\
& 45 & 162 & 1.90 & 28.40 & 25.30 & 18.50 & 25.90 \\
PEO-S03-0.15 & 5 & 27 & 22.23 & 14.81 & 44.46 & 14.81 & 3.69 \\
& 20 & 109 & 3.67 & 11.93 & 22.02 & 15.60 & 46.79 \\
& 45 & 204 & 3.45 & 37.22 & 47.05 & 5.89 & 6.39 \\
\hline
\end{tabular}
\end{table}
some of the samples showed submonolayer regions of adherent, spreading leukocytes at 30 min. Another sample had a fibrin meshwork on part of the surface at 60 min, although very few cells of any type were entrapped in this meshwork.

PEO-base was less thrombogenic than PTMO-base, but more thrombogenic than PTMO-S03-0.20. From Figure 6(a), a maximum of about 300 platelets/1000 μm² was deposited at 25 min. At 15 min, the surface showed a monolayer of pseudopodial platelets. At 30 min, platelet aggregates (10 μm) developed on some samples, while on other samples, large numbers of adherent, spread leukocytes were observed, and macroscopic clots were seen (Fig. 8[c]).

At 60 min, the surface looked similar to that observed after 30 min.

PEO-S03-0.15 was very comparable to PEO-base. From Figure 6(a), the maximum in platelet deposition was also approximately 300 platelets/1000 μm², but was deposited after 30 min instead of 25 min. At 15 min, leukocytes began to adhere to the surface, although the number was still significantly below monolayer coverage. At 30 min, some samples showed small (20 μm) platelet aggregates, while others showed macroscopic white thrombi (100 μm), and lots of adherent, spread leukocytes (Fig. 8[d]). At 60 min, macroscopic thrombi were visible, along with a near monolayer of adherent, spread leukocytes.

**Figure 6.** a) Platelet deposition profiles and b) fibrinogen deposition profiles for PTMO-base, PTMO-S03-0.20, PEO-base, and PEO-S03-0.15.
DISCUSSION

Altering the polyol from PTMO to PEO resulted in dramatic alterations of the physical properties of the sulfonated polyurethanes. For the PTMO-containing polyurethane, sulfonation resulted in a sixfold increase in Young’s modulus, and roughly a doubling of the ultimate strength, although the ultimate elongation was somewhat reduced. The improvement in tensile properties upon sulfonation is also supported by the dynamic-mechanical data, which showed that sulfonation resulted in an increase of the rubbery plateau modulus. No difference in molecular weight or molecular weight distribution was seen using GPC for these polymers. However, IR spectra show that sulfonation causes a decrease in the fraction of hydrogen-bonded carbonyl groups, which signifies a possible decrease in hard segment packing. Alternatively, this could be due to hydrogen bonding between the urethane hydrogens and the sulfonyl groups, instead of the carbonyl groups. However, the DSC data show that both PTMO-base and PTMO-S03-0.20 have the same $T_g$, indicating that both polymers have about the same degree of phase separation. This conclusion is in agreement with that seen previously.$^{18}$

For the PEO-containing polyurethanes, however, sulfonation had the opposite effect on physical properties. In this case, Young’s modulus and the ultimate tensile strength were reduced by an order of magnitude, although the ultimate elongation exceeded 1000%. PEO-S03-0.15 had a lower modulus in tensile testing than in dynamic-mechanical testing, possibly due to plasticization by water, or to the cyclic vs. noncyclic testing modes. Dynamic-mechanical data show a lowering of the rubbery plateau modulus, an elevated $T_g$, and a rubbery plateau extending over a narrower temperature range for PEO-S03-0.15 compared to PEO-base. The rubbery plateau region is so narrow, in fact, that the polymer behaves almost like a linear, amorphous material. The molecular weights of these materials were comparable, as determined by GPC, although the sulfonated polymer was synthesized from a different base batch of polymer. The IR data for these materials show that there is a decrease in hydrogen bonding of the carbonyl carbon in PEO-S03-0.15. This decrease in phase separation seen for PEO-S03-0.15 using IR is supported by an increase in the soft segment phase glass transition temperature seen for this material using both dynamic-mechanical analysis and DSC.

Comparing PEO-base with PTMO-base, the former shows about a five-fold lower ultimate tensile strength. Since the molecular weights and molecular weight distributions are roughly comparable, the weakness of PEO-base is attributable to its lower degree of phase separation. The IR spectra, which show that PEO-base has fewer hydrogen bonded carbonyl groups than PTMO-base, support this conclusion. This
is likely due to the smaller polarity difference between hard and soft segments for PEO-base as compared to PTMO-base. The fact that the PEO-base has a higher glass transition temperature than the PTMO-base as determined both by DMTA and DSC also support this conclusion.

**Blood compatibility**

*In vitro* platelet spreading assays conducted on these polyetherurethanes showed that platelet attachment and spreading was most inhibited on PEO-base. This result is in good agreement with Goodman,\(^6\) who found that a PEO-based polyurethaneurea produced very little shape change or activation of platelets. PTMO-base caused most of the platelets to reach the spreading (S) stage within 5 min, and continued to promote spreading throughout the assay. This is also in agreement with Goodman, et al.,\(^6\) who observed significant platelet spreading on a similar polyurethaneurea.

The *ex vivo* blood compatibility for the nonionized PEUs compares well with the ability of platelets to spread on these surfaces. PTMO-base was found to be significantly more thrombogenic than PEO-base, and also showed a greater degree of platelet spreading.

The results of the platelet spreading assays on the sulfonated polyurethanes are more complicated. Lai et al.\(^12\) have shown that when column-washed platelets are allowed to adhere on sulfonate-grafted polyurethanes under static conditions, the platelet membranes become disrupted. These results are not observed for surfaces that are preadsorbed with plasma proteins, or for platelets in PRP. This may be due to the Vroman effect, in which cell adhesive proteins such as fibrinogen and fibronectin are displaced from the surface. However, Santerre et al.\(^11\) have shown that fibrinogen is not easily displaced from the surfaces of sulfonated polyurethanes. Therefore, since the response of platelets to PTMO-S03-0.20 appears to be strongly influenced by the intervening protein layer, the results of the *in vitro* platelet spreading assay may not be directly comparable to that seen in the canine *ex vivo* model.

It is also interesting that the thrombogenicity of PEO-base and PEO-SO3-0.15 evaluated in the *ex vivo* model is roughly comparable, while significantly less adhesion and spreading of platelets is observed *in vitro* on the nonionized polyurethanes. It may be that the sulfonate groups interact with the platelets, as with PTMO-S03-0.20. However, cold-stage ESCA studies of PEO-SO3-0.15 show that when it is hydrated, there is no tendency for the sulfonate groups to be enriched.
POLYOL TYPE POLYURETHANE PROPERTIES

Figure 9. Scanning electron micrographs after 5 min of blood exposure for PTMO-base (a); PTMO-S03-0.20 (b). Scanning electron micrographs after 30 min of blood exposure for PTMO-base (c); PTMO-S03-0.20 (d) (original magnification 2000×).

CONCLUSIONS

Sulfonation had a very positive effect on the mechanical properties of the PTMO-containing polyurethanes, although in a hydrated state, as would be the case in vivo, it is unlikely that such properties would be maintained. Due to disruption of hard segment ordering, PTMO-SO3-0.20 may be even weaker in vivo than PTMO-base. A sixfold increase in Young's modulus and a doubling of the ultimate tensile strength was seen, which may be attributed to the cross-linking effect of ionic aggregates. Further, this material was seen to have about the same degree of phase separation, in comparison with its nonionized analog, as determined from T_g measurements using DSC and DMTA. Thus, the improved strength of PTMO-SO3-0.20 could be due to the effect of increased segmental polarity exceeding the effect of disruption of hard segment packing.

For the PEO-containing polyurethanes, sulfonation had the opposite effect, resulting in a weak, viscoelastic material. The ultimate tensile strength was reduced by an order of magnitude upon sulfonation, and the rubbery plateau modulus extended only over a very narrow temperature range. The fraction of hydrogen bonded carbonyl groups was quite low, as measured by FT-IR, suggesting a decrease in the degree of hard segment ordering. This observation was supported by the elevated soft segment glass transition temperatures seen using DSC and dynamic-mechanical analysis.

PEO-base had a lower Young's modulus and ultimate tensile strength as compared to PTMO-base. This was attributed to a lower degree of phase separation, due to the reduced polarity difference between hard and soft segments for PEO-base. This conclusion is supported by IR data, as well as T_g measurements. It would be interesting to verify this observation using small angle x-ray scattering techniques.

BLOOD COMPATIBILITY

This article provides support for the idea that polymer morphology, as well as incorporation of sulfate or sulfonate groups, is an important determinant of blood compatibility. For PEO-base and PEO-SO3-0.15, no positive effect of sulfonation on blood compatibility was seen. Surface analysis of these polymers, using dynamic contact angle measurements and ESCA
of frozen, hydrated samples, showed that reorienta-
tion of the sulfonated hard segment domains toward
the water-polymer interface occurs for PTMO-S03-
0.20, but not for PEO-S03-0.15. This may occur be-
cause PTMO-S03-0.20 is more strongly phase sepa-
rated, allowing distinct orientations of the two phases,
while PEO-S03-0.15 is more phase mixed, so that
reorientation is less likely. Thus, determination of structure-property relations in this system provides
an interpretation of the surface properties, which are
related to the hemocompatibility of these materials. As
discussed in the introduction, polyurethanes which in-
corporated sulfonate functionality in the chain exten-
sion did not correlate with the water uptake
related to the hemocompatibility of these materials. As
an interpretation of the surface properties, which are
structure-property relations in this system provides
conclusion is also supported in the present study.
The thrombogenicity of these materials as measured in
an ex vivo canine model correlates with the
receding contact angle measurements. It was found
that PTMO-base was the least hydrophilic, PEO-base
and PEO-S03-0.15 were of intermediate and equal
hydrophilicity, and that PTMO-S03-0.20 was the most
hydrophilic. Interestingly, the contact angle mea-
surements did not correlate with the water uptake
measurements for these materials. Also, the most
thrombogenic material, PTMO-base, is the only one
which could not be classified as a hydrogel. Polyvinyl
alcohol hydrogels show low levels of platelet adhe-
sion, but are platelet consumptive. This observation
may be of significance in the blood compatibility
of these polyurethanes. However, comparing PEO-
base with PEO-S03-0.15, the thrombogenicity is
quite similar, while the level of water absorption is
nearly 10 times lower in the former. For PTMO-
S03-0.20, the level of water absorption is lower than for
PEO-S03-0.15, but the former is more thromboresistant. Finally, the ex vivo blood compat-
ibility of the nonsulfonated polyurethanes agrees with the in vitro platelet spreading assay. However,
for the sulfonated polyurethanes, the in vitro platelet
spreading assay does not directly compare with ex vivo thrombogenicity of these materials.

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