Methods for the Covalent Attachment of Potentially Bioactive Moieties to Sulfonated Polyurethanes

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ABSTRACT: Methods have been developed for the attachment of amino acids and other organic molecules to sulfonated poly(ether urethane)–ureas containing sulfonate groups in the hard segment. The compounds were covalently attached to the polymers after converting the sulfonate groups to sulfonyl chloride or sulfonylimidazole. Possible reaction pathways are discussed. The degree of conversion of sulfonate to the derivatized forms was determined by elemental analysis, by titration of sulfonate and carboxylate groups, and through the use of ^{14}C-labeled amino acids. Confirmatory evidence of the structure of the derivatized polymers was obtained from infrared analysis. Molecular weight data showed that no chain degradation occurred during conversion of sulfonate groups to their derivatized forms. ESCA analysis of the original sulfonated polymer solids showed an enrichment of the soft segment and a corresponding depletion of the hard segment in the outermost surface. Derivatization was found to diminish these surface compositional gradients for reasons that are not yet understood.

Introduction

Polyurethane-based biomaterials are used in the fabrication of vascular grafts, left ventricular assist devices, total artificial hearts, intravascular catheters, and other devices where blood–material contact occurs.[1-3] Conventional polyurethanes used in these devices have been characterized for biocompatibility and material strength properties.[3,4] Data collected clearly indicate that there are still major problems associated with the use of these and other synthetic biomaterials for blood contact. One problem that has been the focus of much research is the tendency of artificial surfaces to stimulate thrombus formation when in contact with blood.[5-7] Attempts to overcome this problem by biologically modifying polymers include the incorporation of heparin,[8-10] albumin, and prostaglandins.[3,8] Many of these biologically modified materials suffer from a common drawback, namely, that the effective lifetime of the incorporated component, in contact with blood, is often short due to physical loss from the material.

More recent work has focused on permanently binding biologically active moieties to polymer chains or polymer surfaces. Larm and co-workers[11] activated polyethylene tubing with poly(ethylenimine) and then covalently bonded heparin to these surfaces. Vulic et al.[12] attached heparin covalently to a polystyrene–poly(ethylene oxide) copolymer. Both of these groups have suggested that the heparin may retain some of its anticoagulant activity in the bound state. Chapman and co-workers[13-15] have investigated the attachment of phosphatidylcholine, a phospholipid found on the outer surface of red blood cell membranes, to polymeric substrates. They showed these surfaces to be relatively inert toward coagulation.[16] Another novel approach to the development of materials that are compatible with blood is that of Jozefowicz and Jozefonvicz.[17] They have modified polystyrene resins and dextrans by covalently attaching amino acids. The resulting materials contain not only amino acids but also sulfonamide and free sulfonate groups. These latter functional groups have been shown to enhance blood compatibility.[18,19]

In the present work, synthetic pathways have been developed to attach potentially bioactive molecules to segmented polyurethanes. It is anticipated that the "substituents" will remain biologically active and that the mechanical properties that are characteristic of "classical" polyurethane materials will remain intact. It is expected that the chemical attachment methods developed will be generally useful in the design of polyurethanes having specific biological activity.

Experimental Section

Preparation of Sulfonated Poly(ether urethane)–Ureas. Sulfonated polyurethane–ureas, used as the starting point for the attachment reactions, were synthesized by a conventional two-step procedure.[3] The first step involved the reaction of excess methylenebis(4-phenyl isocyanate) (MDI) with poly(propylene glycol) (PPO) of molecular weight 1000, to yield an isocyanate-terminated prepolymer. This reaction was typically carried out for 3 h at 90 °C. The second, or chain extension, step was carried out for about 16 h at 40 °C by using a sulfonated diamine, namely 4,4′-diamino-2,2′-biphenyldisulfonic acid disodium or dipotassium salt (BDDS). The reaction solvent was dimethyl sulfoxide, and the reactions were carried out in a dry-nitrogen atmosphere. The sulfur content of the final polymers was determined by elemental analysis. The presence of free sulfonate groups was verified by titration of the acidified polymers with triethylamine. A typical reaction scheme for the synthesis of a sulfonated polyurethane is shown in Figure 1. Sulfonate content in these polymers can be varied via the stoichiometry as reported elsewhere.[20] In the present work, most of the derivatized polymers were based on polymer BDDS-1.4 containing 1.4 wt % sulfur. Polymers synthesized by using ^{14}C-labeled amino acids were derived from polymer BDDS-3.1, containing 3.1 wt % sulfur.

A nonsulfonated polyurethane based on MDI, PPO, and ethylenediamine as chain extender was synthesized for comparison purposes. The stoichiometry and reaction conditions were the same as for polymer BDDS-1.4.

MDI and BDDS were obtained from Eastman Kodak Chemicals (Rochester, NY). MDI had a specified purity of 99%, and no further workup was carried out. BDDS was in the acid form and of technical grade (80% pure). This material was washed with hot water and then filtered. The filtrate was titrated with sodium or potassium hydroxide and filtered again. The BDDS salt was recrystallized from the filtered solution. Purity was verified by both titration and elemental analysis. Ethylenediamine was obtained from Aldrich Chemicals (Milwaukee, WI) and used as received. Poly(propylene glycol) (MW 1025) was obtained from BDH Chemicals (Toronto, Ontario) and was de-

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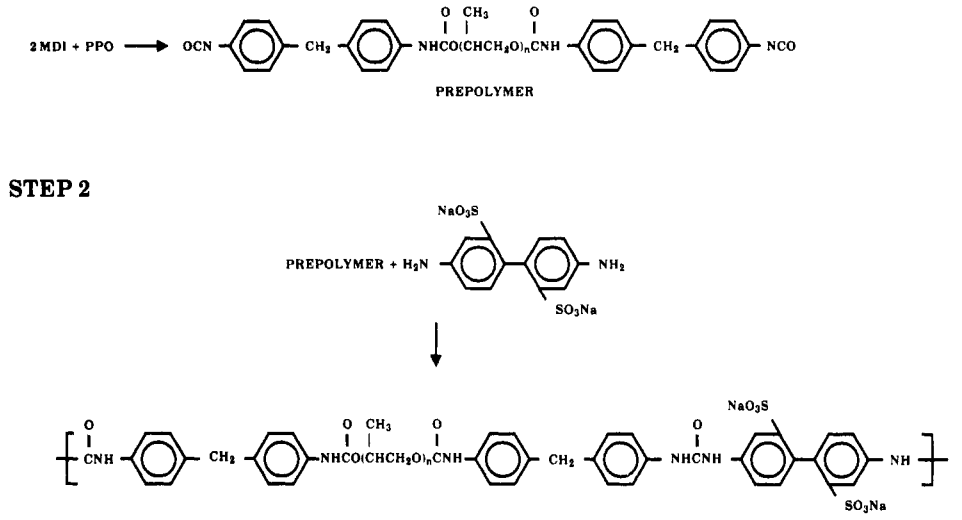
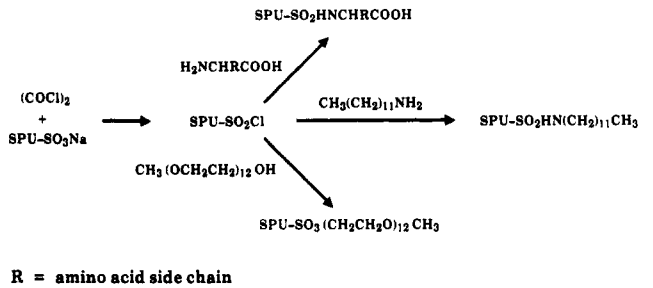
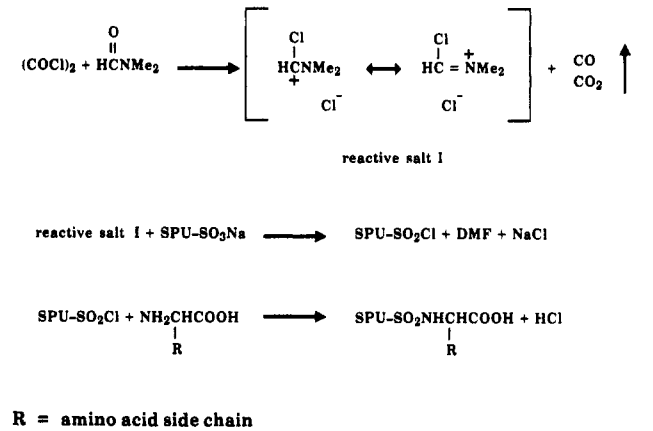


Figure 1. Sulfonated polyurethane synthesis.



R = amino acid side chain

Figure 2. Covalent attachment of hydroxyl- and amine-containing molecules to sulfonated polyurethanes.



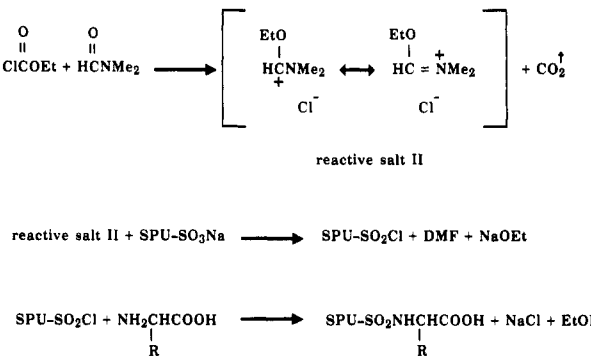
R = amino acid side chain

Figure 3. Proposed mechanism for amino acid addition to sulfonated polyurethane using oxalyl chloride. SPU-SO_3Na represents sulfonated polyurethane as described in the text.

gassed and dried at 50 °C (1 mmHg) for 24 h before use. Dimethyl sulfoxide from Caledon Laboratories (Georgetown, Ontario) was distilled under vacuum prior to use to reduce water contamination.

Derivatization of Sulfonated Poly(ether urethane)–Ureas. Covalent attachment of amino acids and other molecules (referred to as "substituents") to sulfonated polyurethanes was achieved by first converting the sulfonate groups to sulfonyl chloride and then reacting the sulfonyl chloride with the substituent. This reaction is illustrated in Figures 2–4 for several substituents.

Both oxalyl chloride and ethyl chloroformate react with sulfonates to give sulfonyl chlorides, which react rapidly with amine or hydroxyl groups. *N,N'*-Carbonyldiimidazole converts sulfonate or sulfonyl chloride to sulfonylimidazole groups, which also react with amine groups. All three of these compounds are common peptide-forming reagents, and their use in modifying



R = amino acid side chain

Figure 4. Proposed mechanism for amino acid addition to sulfonated polyurethane using ethyl chloroformate. SPU-SO_3Na represents sulfonated polyurethane as described in the text.

carboxylate groups is well documented.[21,22] However, there is no previous record of their use with sulfonated polyurethanes. In this respect the methods presented here appear to be novel. These reagents react rapidly with water, and therefore the reaction solvent, dimethylformamide (DMF), must be dried. In the present work it was distilled within 24 h of use and kept dry. The sulfonated polymer and the substituent were also dried prior to use.

A typical attachment reaction was carried out as follows. Sulfonated polyurethane (~10 g) was dissolved in distilled DMF at a concentration of 5 g/100 mL. Sulfonate conversion reagent (oxalyl chloride, *N,N'*-carbonyldiimidazole or ethyl chloroformate, all from Aldrich Chemicals), in stoichiometric amount relative to sulfonate groups, was added to the polymer solution, which had been cooled previously to −5 °C. The system was stirred under a dry-nitrogen atmosphere for 4 h at −5 °C. This temperature was maintained by using an ice/water/NaCl bath. The reaction evolves large amounts of CO and/or CO_2, and therefore precautions must be taken to release the pressure in the reactor upon addition of the reagent.

During the conversion of sulfonate to sulfonyl chloride, the amino acid or other substituent was dissolved/suspended in DMF (~1% w/v) in a second vessel and stirred under dry nitrogen at room temperature. The substituent was in 20% stoichiometric excess relative to the sulfonate group content of the sulfonated polymer. After 4 h the contents of the first vessel were transferred to the second vessel, with maintenance of the nitrogen atmosphere. The reaction between the amine or hydroxyl groups of the substituent and the sulfonyl chloride groups of the polyurethane was allowed to proceed under dry nitrogen for 15 h at 20 °C. The resulting polymer was precipitated in distilled water.

Table I
Polyurethane Nomenclature

E.g., BDDS-1.4-Arg
BDDS: polymer chain extended with biphenyldiaminodisulfonic acid
(ED: polymer chain extended with ethylenediamine)
1.4: polymer contains 1.4 wt % sulfur
Arg: polymer derivatized with arginine
abbreviations for substituents
Arg = arginine
ArgMe = arginine methyl ester
Asp = aspartic acid
DDA = dodecylamine
Lys = lysine
(PEO)Me = poly(ethylene oxide) methyl ether
Tau = taurine (2-aminoethanesulfonic acid)
Met = methionine

In some cases, the polymer remained partially dissolved in the water–DMF mixture, and the polymer remaining in solution was precipitated by the addition of KCl. The polymers were then washed several times in either methanol or distilled water and dried at 60 °C in a forced-air oven for 48 h.

The following amino acids and amino acid esters, used as substituents, were obtained from Sigma Chemical Co. (St. Louis, MO): arginine, arginine methyl ester, aspartic acid, lysine, methionine, and taurine (2-aminoethanesulfonic acid). Dodecylamine and poly(ethylene oxide) methyl ether (MW 550), obtained from Aldrich Chemicals, were also used as substituents. The nomenclature used to describe the derivatized polyurethanes is shown in Table I.

Fourier Transform Infrared Spectroscopy. The amino acid derivatization reactions were verified by FTIR spectroscopy. Polymer films were prepared by casting on a Teflon sheet, using a 0.5 wt % solution of polymer in dimethylformamide. The films were dried under vacuum at room temperature for 24 h and at 50 °C for an additional 24 h. Spectra were obtained on a Nicolet Model 320 FTIR instrument at a resolution of 2 cm^{-1} and were based on 64 scans. The spectra provided evidence of changes occurring in the microphase structure of the polymer due to the physical presence of the amino acid, as well as new bands assignable to the amino acid. The bands of interest were in the carbonyl region from 1600 to 1750 cm^{-1}.

Elemental Analysis. Elemental analysis was carried out by two independent methods. Analyses for sulfur and potassium were performed by scanning electron microscopy with energy-dispersive analysis of X-rays (EDAX). Analyses for nitrogen, potassium, and sulfur were also carried out using conventional methods by Guelph Chemical Labs, Guelph, Ontario. When a sulfonated polyurethane was used as the EDAX standard, agreement between the EDAX and traditional methods was good as indicated elsewhere.[20]

Acid–Base Titration. Acid–base titration was used to verify the presence of free sulfonate and carboxyl groups and to quantify amino acid substitution in some of the sulfonated polyurethanes by measuring residual sulfonate. In all cases, acidification of the polymer was required prior to titration. This was done by treating a solution of polymer in DMF with excess 1 M HCl. The solution was placed in an ice bath in order to avoid polymer degradation by the acid. After stirring overnight, the polymer was precipitated, washed, and dried. Molecular weight data showed that the polymers were not degraded by this treatment.

Titration of the polymers was carried out in DMF containing 0.1 M LiBr. The titrant was 0.025 M triethylamine (TEA; from Aldrich Chemicals) in DMF and was standardized by titration with 0.1 M HCl in DMF. The polymer titration end point was monitored by a potentiometer. Some polymers had very long equilibrium times and required up to 24 h to complete an analysis.

Use of Radiolabeled Amino Acids. In a few cases ^{14}C-labeled amino acids were used to verify the amino acid content of the derivatized polymers. ^{14}C-labeled amino acid was incorporated into the sulfonated polyurethane using the same procedure as described above. The derivatized polymer was then dissolved in DMF at a concentration of 1.0 g/100 mL, and 0.1 mL of the solution was added to a liquid scintillation cocktail (Opti-Fluor, from Canberra Packard, Mississauga, Ontario) for determination of radioactivity. In order to estimate the amount of amino acid incorporated, the solution count was compared to a series of standards made from the labeled amino acid. ^{14}C-labeled aspartic acid and lysine used in these experiments were obtained from Sigma Chemicals. They were recrystallized from water.

Molecular Weight Determination. Molecular weights were determined by size-exclusion chromatography (SEC). Four Waters Ultrastyragel columns with pore sizes of 10^3, 10^4, 10^4, and 10^5 Å and a differential refractive index detector were used. The operating temperature was 80 °C, and the mobile phase was dimethylformamide containing 0.1 M LiBr. The sample size was 200 μL, and the polymer concentration was approximately 0.2 g/100 mL. With a flow rate of 1 mL/min, a typical chromatogram showed a retention volume of 40 mL. The system was calibrated with TSK polystyrene standards obtained from Toyo Soda Manufacturing Co., Ltd., Tokyo. Polystyrene equivalent molecular weights of the polyurethanes are reported.

Electron Spectroscopy for Chemical Analysis (ESCA). In ESCA an X-ray beam induces photoelectron emission from a solid sample. It is a surface-sensitive technique and can be used to determine the concentrations of all elements, except hydrogen and helium, in approximately the outermost 100 Å of the sample. The energy of the photoelectrons emitted identifies the chemical elements and their bonding environment, while the intensity of the emission gives the concentration of the species. Atomic concentrations can be determined with a relative accuracy of about 10%.

ESCA measurements were performed at the National ESCA and Surface Analysis Center for Biomedical Problems (NESAC/BIO), University of Washington, Seattle, WA. The data were collected on a Surface Science Instruments X-probe spectrometer, which has a monochromatic aluminum X-ray source with a variable spot size (150–1000 μm). Data were taken at a series of take-off angles to determine if a compositional gradient existed near the surface of these segmented polyurethanes as has been found by others.[23] The take-off angle controls the depth from which photoelectrons are detected as they emerge from the sample. The greater the take-off angle (measured from the normal to the surface), the greater the surface contribution to the compositional information. A low-resolution/high-sensitivity spectrum of each element present was obtained at several angles over the range from 0 to 80°. Intermediate resolution spectra of the carbon 1s peak were used to determine the presence or absence of carbamate, hydroxyl, and ether groups.

ESCA samples consisted of polymer films coated on glass microscope slides. The films were prepared from 5% w/v solutions in DMF by spreading on the slide and drying in a dust-free enclosure at room temperature for 24 h and then in vacuo at 60 °C for 24 h.

Results and Discussion

Derivatization Chemistry. General Considerations. The derivatization reaction involves two steps: (1) conversion of sulfonate groups in the polyurethane to sulfonyl chloride or sulfonylimidazole and (2) reaction of amine or hydroxyl groups of the substituents with sulfonyl chloride or sulfonylimidazole.

Figure 3 shows the probable mechanism of substituent attachment using oxalyl chloride to convert sulfonate to sulfonyl chloride. Upon addition of oxalyl chloride to a solution of polymer in DMF, gases (CO and CO_2) are evolved and a white solid precipitates. The solid, believed to be the salt (I) shown in Figure 3, rapidly dissolves and converts sulfonate groups in the polymer to sulfonyl chloride. Analogous mechanisms have been proposed by Contreras and Jones[24] for the formation of carboxylic and sulfonic acid chlorides using phosgene or thionyl chloride in the presence of DMF.

DMF acts as a catalyst in these reactions, since, after the salt (I) reacts with sulfonate, dimethylformamide is regenerated. When oxalyl chloride is added to DMF in the absence of sulfonated polymer, the salt forms and slowly goes into solution.

Like oxalyl chloride, ethyl chloroformate is also believed to form a complex with dimethylformamide. Upon addition of ethyl chloroformate to DMF, a white solid is formed and CO_2 is released. A possible mechanism is shown in Figure 4. Reaction with sulfonate groups leads to the formation of sulfonyl chlorides and ethoxide ion. These can then react with each other, thus eliminating the sulfonyl chloride groups for subsequent substituent attachment. In this case, a two-step reaction is therefore not feasible and all reagents must be added at the same time. An excess of amine groups must be present at all times since otherwise the sulfonyl chloride groups will react with ethoxide ions as these are generated. Since most amino acids are only slightly soluble in DMF, it is difficult to maintain these conditions. For this reason, oxalyl chloride was preferred over ethyl chloroformate as a chlorination agent.

A third reagent, *N,N'*-carbonyldiimidazole, was also investigated. This reagent does not form a salt with DMF and was found to dissolve only in the presence of sulfonated polymer. Gas evolution was not as evident as for the other reagents. Also, conversion to the derivatized polymers was much lower than that for oxalyl chloride.

Of the three reagents, oxalyl chloride was the most convenient to use and gave the highest yields. It was used in the synthesis of all the derivatized sulfonated polyurethanes discussed in this paper.

Following formation of the sulfonyl choride, the desired substituent is added to the solution. The hydroxyl or amine group of the substituent reacts with the sulfonyl chloride group to give the corresponding sulfonate or sulfonamide. Amines are much more reactive than hydroxyls.[25] Although these reactions are rapid, an entire procedure using 10 g of polymer usually took several hours due to the low solubility of the substituents, particularly the amino acids, in DMF. The amino acids generally dissolved slowly as the reaction proceeded. It was found that some esters and some quaternary ammonium salts of the amino acids are more soluble than the amino acids themselves, and thus more rapid procedures for attachment to the polyurethanes are possible in these cases.

Effect of pH. The formation of sulfonamides and sulfonates by reaction of sulfonyl chlorides with amine and hydroxyl groups produces HCl, which could degrade the polymer by hydrolysis of urea and urethane bonds. This hydrolysis can be rapid at temperatures greater than about 25 °C. In the present work, the reaction temperature ranged between −5 and +25 °C, thus minimizing possible acid-catalyzed polymer hydrolysis. It must also be noted that if pH were controlled by the addition of hydroxide ions the conversion of sulfonyl chloride by substituent could be compromised since the hydroxide ions could convert the sulfonyl chloride groups back to sulfonate. Triethylamine was used in an attempt to control acidity in some instances but appeared to hinder the reaction, and therefore no further attempts to control pH were made. It is possible that the triethylamine contained traces of water and that this led to lower conversion since water readily reacts with sulfonyl chloride groups. It is, of course, desirable that the pH of the reaction be high enough that the amine groups of substituents are not in the less reactive cationic form.

Reaction Solvent. The most common polyurethane solvents are dimethylformamide (DMF), dimethyl sulfoxide (DMSO), and dimethylacetamide (DMAC). DMSO and oxalyl chloride cannot be used together because they react violently. No attempt was made to use DMAC for the derivatization reactions. DMF was chosen because it is a reasonably good solvent for the sulfonated polymers and because it acts as a catalyst for the reaction of the substituent with the sulfonated polyurethanes in the presence of oxalyl chloride and ethyl chloroformate as discussed. Also it can be vacuum distilled at low temperatures, and this is convenient for water removal. A major disadvantage is the fact that the solubility of most amino acids in DMF is low, as discussed above. This is true of most nonaqueous solvents.

Table II
Yield of Derivatization Reactions

polymer	analytical technique	conv of sulfonate to derivative, %
BDDS-1.4-Arg	increase in nitrogen content	30
BDDS-1.4-ArgMe	increase in nitrogen content	100
BDDS-1.4-Asp	titration of carboxyl groups	92
BDDS-1.4-DDA	titration of unreacted sulfonate	100
BDDS-1.4-Lys	increase in nitrogen content	100
BDDS-1.4-(PEO)Me	titration of unreacted sulfonate	100
BDDS-1.4-Tau	sulfur content	0
BDDS-1.4-Met	sulfur content	0

Counterions. A brief investigation of different sulfonate counterions was carried out in order to ascertain whether the reaction was influenced by the counterion type. The potassium and sodium salts of sulfonated polymers showed about equal reactivity with oxalyl chloride.

The use of different salts of the amino acid carboxyl group provides, in principle, a method of controlling pH and enhancing the solubility of the amino acids in DMF. Titration of the amino acids with potassium hydroxide or sodium hydroxide yields the corresponding metal salts. However, it was found that the conversion of sulfonate groups in the polymers to the amino acid derivatives decreased when the metal salts were used. It may be significant that removal of all water from these salts is difficult, since residual water could react with and reduce the number of active sulfonyl chloride groups, thus reducing the yield of the amino acid derivatization reaction.

Characterization of Polymers. Chemical Composition of Polymers. Determination of amino acid incorporation into polyurethanes proved to be a difficult task. Common reagents for the determination of amino acid addition in peptide synthesis[25,26] could not be used because of interferences from other chemical functions present in the sulfonated polyurethanes. Titration of polymers derivatized with aspartic acid did not always yield reliable data because the carboxylate end point was not sharp enough. However, the determination of residual free sulfonate groups by titration was achieved without difficulty. Quantitative analysis of lysine- and arginine-containing polymers was also attempted by determination of the difference in the nitrogen content of the precursor and derivatized polymers. Molar conversions for selected polymers determined by these various methods are shown in Table II.

Table II shows that conversion of sulfonate depends on the nature of the substituent. Compounds such as arginine methyl ester, poly(ethylene oxide), and dodecylamine gave conversion levels of 100% and may be considered a "good" substituents. Arginine showed a maximum level of sulfonate conversion of about 30% and is considered a "fair" substituent. The relatively low level of conversion in this case may be due to the much lower solubility of arginine relative to arginine methyl ester in DMF. Finally, taurine and methionine are clearly "poor" substituents since no incorporation was achieved. Taurine in its acid form is insoluble in DMF, but the quaternary ammonium

Table III
Derivatization of Sulfonated Polyurethane BDDS-3.1 with ^{14}C-Labeled Amino Acids: Estimates of Conversion by Titration, Nitrogen Content, and ^{14}C Labeling

		conv of sulfonate to derivative, %	
expt	amino acid	titration or N content	^{14}C
1	lysine	27.6	28.0
2	aspartic acid	3.12	2.85
3	aspartic acid	5.59	5.99

salt is soluble. Therefore, taurine was titrated with benzyltrimethylammonium hydroxide, and the resulting salt was crystallized and dried for use in the polymer derivatization reaction. However, the reaction of the quaternary ammonium salt with the chlorosulfonated polymer also gave a very low yield, due to the fact that as the reaction proceeds the taurine salt rapidly becomes acidified and precipitates. Water contamination from the recrystallization procedure may also have limited the yield in this reaction.

The derivatization reaction with methionine was also unsuccessful, and no increase in the sulfur content of the polymer was observed. However, a reaction did occur, since when methionine was added to the polymer solution in DMF, it was taken up instantly, whereas in the absence of sulfonated polyurethane it dissolved very slowly in DMF. It is possible that a secondary reaction occurring at the sulfide bond removes the methionine sulfur from the amino acid side chain. Further work will be required to understand this system.

In the case of lysine and aspartic acid, verification of amino acid incorporation into polymer BDDS-3.1 was carried out by using the ^{14}C-labeled amino acids. Radioactivity data, as indicated at Table III show good agreement with the results of nitrogen analysis for the incorporation of lysine and of titration for the incorporation of aspartic acid. The conversion levels are lower in Table III than in Table II partly because the experiments were designed to convert 60% of free sulfonate groups rather than 100%. In addition, the efficiency of the reactions using labeled amino acids was influenced by the presence of water in the amino acid crystals. The labeled amino acids were recrystallized from water, and drying of the crystals was more difficult for aspartic acid than for lysine. As can be seen from Table III, the lysine conversion was greater than the aspartic acid conversion for the same stoichiometry. Experiment 3 using aspartic acid was carried out by using material that had undergone additional vacuum drying and gave a higher yield. In general, these experiments with ^{14}C-labeled amino acids provide validation of the other analytical techniques used for estimating incorporation.

Infrared Spectroscopy. Useful information on the structure of the derivatized polyurethanes was obtained by FTIR. For example, FTIR data indicated the presence of specific amino acid moieties in the polymers, the manner in which substitution occurred, and changes in the polymer solid structure due to the presence of the substituents.

Figure 5 shows the spectra of a model compound, *N*-tosylarginine, containing no urethane functionality,[27] polymer BDDS-1.4, and polymer BDDS-1.4–Arg. The spectrum of the model compound indicates that a significant peak at about 1700 cm^{-1} should appear upon incorporation of arginine into the sulfonated polyurethane. Comparison of the peak near 1700 cm^{-1} for the two polymers indicates an increase in peak height for the arginine-derivatized polymer. This can be attributed in part to the incorporation of a carboxyl group. Another feature is a prominent shoulder at about 1625 cm^{-1}, which appears on the

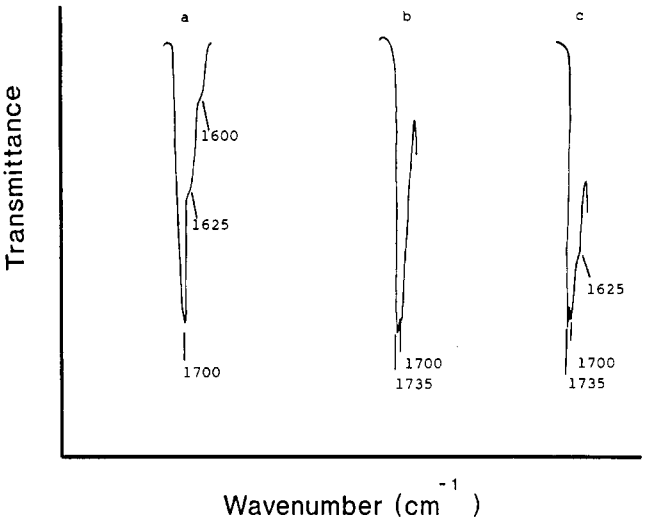


Figure 5. Infrared spectra of (a) *N*-tosylarginine (taken from ref 27, with permission, copyright Sadtler Research Laboratories, Division of Biorad Laboratories, Inc., 1977), (b) polymer BDDS-1.4, and (c) polymer BDDS-1.4–Arg.

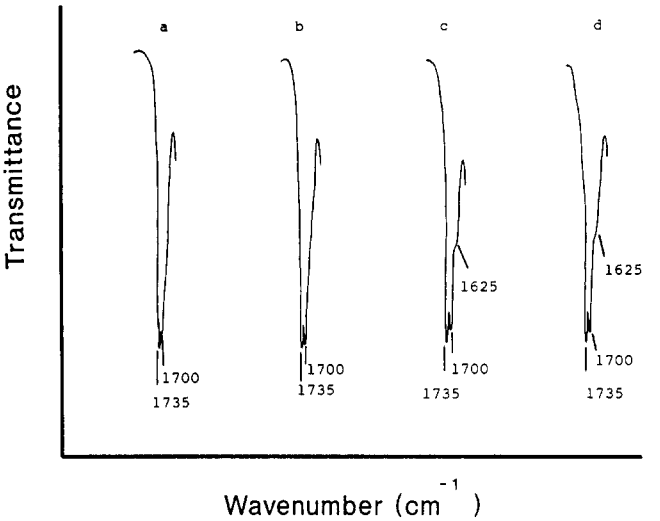


Figure 6. Infrared spectra of (a) polymer BDDS-1.4, (b) polymer BDDS-1.4–Asp, (c) polymer BDDS-1.4–ArgMe, and (d) polymer BDDS-1.4–Lys.

1700-cm^{-1} peak in the derivatized polymer. This shoulder is probably associated with the guanidyl group on the side chain of arginine as suggested by the model compound spectrum.

Figure 6 shows the spectra of the arginine methyl ester derivatized, the aspartic acid derivatized, and the lysine derivatized polymers. Again the arginine methyl ester containing polymer shows the presence of the guanidyl group as a shoulder on the peak at 1700 cm^{-1}. The spectrum of BDDS-1.4–ArgMe differs from that of BDDS-1.4–Arg (Figure 5) in that the carbonyl peak appears at a higher wavenumber. Also the ratio of the peaks at 1735 and 1700 cm^{-1} is greater for the arginine methyl ester derivatized polymer than for the arginine derivatized polymer.

The aspartic acid derivatized polymer would be expected to show a more substantial change in the peak at 1700 cm^{-1} because of the incorporation of two carboxyl functions per amino acid as opposed to one for the lysine and arginine polymers. As can be seen in Figure 6, the ratio of the 1700-cm^{-1} peak to the 1730-cm^{-1} peak for the aspartic acid containing polymer is indeed higher than those for the other two amino acid derivatized polymers. The lysine-

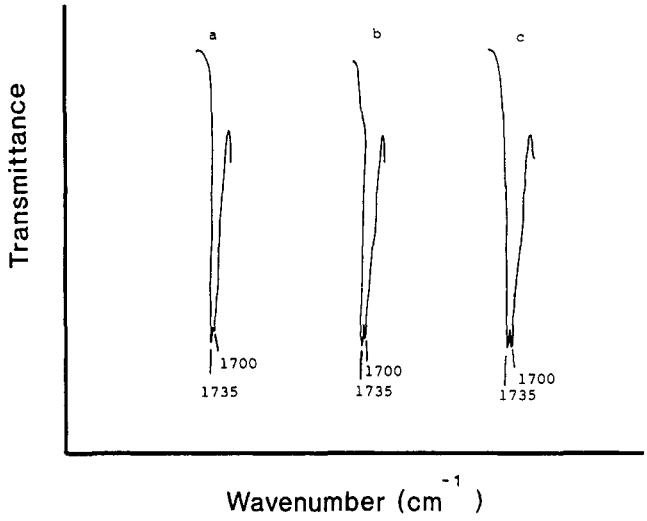


Figure 7. Infrared spectra of (a) polymer BDDS-1.4, (b) polymer BDDS-1.4-DDA, and (c) polymer BDDS-1.4-(PEO)Me.

containing polymer also shows a peak at 1700 cm^{-1} of increased intensity relative to BDDS-1.4 and a shoulder at about 1625 cm^{-1}, which is probably due to the amine group on the side chain of lysine.

Although FTIR spectroscopy is a convenient qualitative tool for confirming the derivatization reactions, it does not provide a quantitative measure of substituent content in the modified polymers. Changes in the polymer solid, caused by the addition of small concentrations of substituent, probably disrupt its microphase structure. These changes may be related to changes in the infrared bands associated with the urea and urethane functionalities. The latter bands happen to overlap the characteristic amino acid infrared bands, so that any changes in the infrared spectra of the modified polymers are associated not only with the substituent directly but also with the environment created by the substituent, i.e., the microphase structure of the hard and soft segments.

For example, if microstructural changes influence hydrogen bonding, there should be significant shifts in the carbonyl and amine peaks. Coleman and co-workers[28] showed that for certain polyurethanes the urethane N–H stretching frequency varies from 3450 to 3350 cm^{-1}, depending on whether the group is involved in hydrogen bonding. Likewise, the frequency of the carbonyl stretching band occurs at higher wavenumbers if the carbonyl group is free rather than hydrogen bonded.

Evidence of structural changes in the polymers associated with the covalent attachment of other molecules to the sulfonate groups in the hard segment is provided in Figure 7, which shows the infrared spectra of the DDA- and (PEO)Me-derivatized polymers. These are relatively long molecules compared to the amino acids discussed earlier. Both spectra show changes in the group of peaks around 1700 cm^{-1}, suggesting that modifications in microstructure are occurring in the hard-segment phase, since the substituent molecules themselves do not possess any functional groups that are expected to show significant absorption in the 1700-cm^{-1} region. There appears to be an increase in the proportion of H-bonded carbonyl in the derivatized compared to the underivatized polymers, and the effect is more pronounced for poly(ethylene oxide) than for dodecylamine. These effects suggest increased hard-segment association in the hard domains for the derivatized polymers. It might be expected that the DDA and (PEO)Me chains would decrease rather than increase hard-segment association. We have no explanation for this apparent anomaly but are currently investigating these aspects using differential scanning calorimetry.

Table IV
Molecular Weight of Precursor Versus Derivatized Polyurethanes[a]

polymer	$M_w \times 10^{-4}$	$M_n \times 10^{-4}$	M_w/M_n
ED	27.0	16.0	1.6
ED, $(COCl)_2$-treated	17.0	11.0	1.7
BDDS-1.4	7.7	5.9	1.3
BDDS-1.4-Arg	7.4	5.7	1.3
BDDS-1.4-Lys	8.6	6.1	1.4
BDDS-1.4-(PEO)Me	8.0	5.7	1.4
BDDS-1.4-DDA	7.2	5.1	1.4
BDDS-1.4-Asp	7.5	5.7	1.3

[a] Data are polystyrene equivalent molecular weights.

Table V
Sulfur Content of Derivatized Polyurethanes by ESCA[a]

	take-off angle, deg				
polymer	0	39	55	69	80
BDDS-1.4	0.6	0.6	0.4	0.3	0.3
BDDS-1.4-ArgMe	0.5	0.5	0.4	0.4	0.3
BDDS-1.4-Asp	0.6	0.5	0.8	0.6	0.5
BDDS-1.4-Lys	0.8	0.5	0.7	0.4	0.5
BDDS-1.4-DDA	0.5	0.6	0.5	0.5	0.5

[a] Data given as atom percent sulfur.

Molecular Weight of Derivatized Polymers. To investigate possible polymer degradation occurring during the derivatization reactions, molecular weights were determined by gel permeation chromatography. The data are shown in Table IV. It was found that chain degradation occurs when nonsulfonated polyurethanes are treated with oxalyl chloride as shown by the data for polymer ED, which was synthesized by using MDI, PPO, and ethylenediamine as chain extender. In the absence of functional groups such as sulfonic acid or carboxylic acid, it is possible that the reactive salt formed by oxalyl chloride and DMF attacks the urea or urethane linkages or even the aromatic rings of the polymers. For this reason, excess reagent was avoided in these reactions.

Table IV also shows data for several derivatized polymers, synthesized using a two-step reaction procedure in which stoichiometric amounts of oxalyl chloride relative to sulfonate and a 20% excess of substituent were used. Upon comparison of the precursor polymer BDDS-1.4 to the derivatized polymers, it is seen that there is no significant change in either the apparent molecular weight or polydispersity following derivatization for any of the polymers.

It is clear also from Table IV that the molecular weight of polymer ED is significantly higher than those of the BDDS series. This difference is believed to be due to the higher reactivity of ethylenediamine compared to BDDS. A polymer synthesized using the same stoichiometry with methylenedianiline gave a molecular weight similar to the BDDS polymers,[20] so its seems unlikely that the lower molecular weights of the sulfonated polymers are due to interactions of the sulfonate groups with the column packing.

Chemical Composition of Surfaces. The ESCA data shown in Table V were collected at various take-off angles and give the sulfur content as a function of depth into the solid. An angle of 0° gives information from a depth of about 100 Å, while an angle of 80° probes the sample to about 20 Å.

The data for polymer BDDS-1.4 clearly show a decrease in sulfur content toward the surface, probably reflecting soft-segment enrichment in the surface as observed

Table VI
ESCA Analysis of Derivatized Polyurethanes: High-Resolution C_{1s} Peak

polymer	take-off angle, deg	carbon atom, % hydrocarbon (285 eV)	ether (286.6 eV)	urethane–urea (288.8–289.5 eV)
BDDS-1.4	0	50.5	47.4	2.2
	39	52.2	45.4	2.3
	55	48.5	50.1	1.4
	68	51.4	47.1	1.5
	80	48.7	50.1	1.2
BDDS-1.4–Asp	0	49.5	49.0	1.5
	39	52.5	45.1	2.3
	55	51.2	46.6	2.2
	68	51.8	46.7	1.5
	80	52.9	45.1	2.0
BDDS-1.4–DDA	0	52.2	45.6	2.2
	39	53.3	44.7	2.0
	55	54.4	43.6	2.0
	68	52.8	45.0	2.2
	80	53.2	44.6	1.9

previously for other types of segmented polyurethanes.[3,23] Another possibility is that hydrocarbon contamination at the surface causes the "apparent" decrease in sulfur content in the surface layers. Data for the C_{1s} peak, shown in Table VI, do not support this conclusion. This peak can be resolved into three main components at 285, 286.6, and 288.8–289.5 eV, corresponding to hydrocarbon, ether, and urethane–urea carbons, respectively.[29,30] Whereas there is no clear trend in the hydrocarbon component, the urethane–urea component, indicative of hard segment, clearly decreases strongly toward the surface for BDDS-1.4. In the derivatized polymers the decrease in sulfur content at the surface is less pronounced (Table V), suggesting the substituent incorporation alters the microphase structure in a manner that decreases soft-phase enrichment at the surface. Again, C_{1s} data for the derivatized materials (shown for BDDS-1.4–DDA and BDDS-1.4–Asp in Table VI) confirm the fact that less surface depletion of the hard segment occurs than for BDDS-1.4. At present we have no convincing explanation for this behavior. Increased phase mixing is one possibility, but the infrared data for BDDS-1.4–DDA suggesting an increase in H-bonding relative to BDDS-1.4 argue against such a possibility. The microstructure of these materials appears to be unique, and further study will be required to fully understand it.

In conclusion, it is appropriate to point out that the derivatization reactions described in this paper have great potential versatility. They could in principle be used to attach any molecule containing amine or hydroxyl functions to any polymer containing sulfonate or carboxyl groups. Among the more interesting possibilities is the attachment of bioactive peptides via their N terminus. The polymers discussed in the present paper are currently being investigated with respect to biological properties such as protein binding and their effect on blood coagulation.

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References and Notes

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Registry No. (MDI)(PPO)(BDDS) (copolymer), 134736-38-2; Arg, 74-79-3; ArgMe, 2577-94-8; Asp, 56-84-8; DDA, 124-22-1; Lys, 56-87-1; (PEO)Me, 9004-74-4.