SULFONATED AND DERIVATIZED SULFONATED POLYURETHANES FOR BLOOD CONTACTING APPLICATIONS

By

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Abstract

Polyurethanes have been a material of choice for the fabrication of many conventional blood-contact devices such as vascular grafts, cardiac assist devices and total artificial hearts. Although these applications have had some success for short term applications there is a major limitation in their use for long term applications. The deficiency of the current devices and their associated materials arises from the foreign nature of these prostheses with respect to the body. Most often these devices initiate a natural physiological response that defends the body by attacking the foreign surfaces. When the materials contact with blood, the response is a series of events which lead to the eventual formation of a thrombus. In turn this often results in the dislodging of microscopic thrombi which can embolize and cause strokes.

There is therefore a need to better understand the interactions of blood with polyurethanes and to develop polyurethanes of improved blood compatibility. In this thesis the proposition is explored that improved materials may be achieved by incorporating chemical functional groups that are known to be important in the interactions of vascular and other components tending to prevent thrombogenesis. These components are the plasma proteins, the blood cells and the endothelium. Thus functional groups have been incorporated covalently into "conventional" polyurethanes as follows: (1) sulfonate groups which are an important component of the vascular wall and of the anticoagulant heparin, (2) certain amino acid moieties which are involved in the neutralization of clotting enzymes particularly serine proteases, (3) hydrocarbon chains which might be expected to bind albumin, a passivating protein, from blood, and (4) polyethylene oxide chains which might be expected to simulate the hydrogel-like nature of quiescent blood cell/plasma interfaces and which have been

shown by others to minimize interactions with proteins.

The novel polyurethane materials were extensively characterized for several aspects of bulk and surface properties and response to plasma proteins. The functionalized materials were found to have greatly modified properties relative to conventional non-functionalized polyurethanes. Using differential scanning calorimetry and Fourier transform infra-red spectroscopy it was possible to associate this modified behaviour with the formation of ionic clusters within the microdomain structures of the polyurethanes.

The different functional groups of the sulfonated and derivatized polymers allow the materials to achieve very high levels of water uptake. These elevated water levels give a hydrogel-like character to the polymers which in turn yields very low contact angle values. Furthermore, it would appear from ESCA (electron spectroscopy for chemical analysis) surface analysis data and the time dependence of sessile drop contact angle data that the material surface in air is substantially different than when the surface is contacted with an aqueous medium.

Measurements of the adsorption of fibrinogen from plasma and buffer were carried out using radiolabelling methods. The specific functionality of the different materials combine with their hydrogel-like nature to yield fibrinogen adsorption patterns in plasma that are unique and different from previously studied polyurethanes. Some of the materials were evaluated for their ability to delay plasma clotting times using standard thrombin time measurements. The results would suggest that sulfonated and some derivatized polyurethanes may be capable of deactivating thrombin, which is a key enzyme in the formation of fibrin, the primary constituent of blood clots.

The work of this thesis makes the following contributions: (i) a general method of introducing chemical function into the hard segment of polyurethanes involving reaction of amine or hydroxyl groups with sulfonyl chloride, (ii) improved understanding of polyurethane

ionomer structure, (iii) methods of controlling protein interactions and water uptake of polyurethanes by incorporation of specific functional groups.

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Chapter 1

INTRODUCTION

Section 1.1 Polyurethanes for Biomedical Applications

Segmented polyurethanes are materials of choice for biomedical applications because of their relatively good biocompatibility and appropriate mechanical behaviour. The variety of applications for these materials covers the spectrum from surgical and treatment devices to permanent implants.

Section 1.2 Blood-Contact Devices

Polyurethanes have been used in a wide range of blood contact devices including blood filters, catheters, valves, cardiac assist devices and blood oxygenators [1,2]. Vascular catheters are now in everyday use and applications of particular interest are intravenous fluid delivery and compression of plaques in coronary arteries [2]. Since catheters are usually implanted for short intervals, the blood compatibility requirements may be considered less stringent than those of permanently implanted devices [3]. Nonetheless, most vascular catheters do activate the blood coagulation sequence and attract platelets and leukocytes to form thrombi. Therefore the use of antiplatelet or anticoagulant agents can be required [4].

Artificial heart valves were among the earliest prosthetic devices to be used successfully in the vascular system of humans [5]. They are used to replace natural valves that have been damaged due to disease or congenital malformation. Between 1960 and 1980, over 600,000 valves of at least 50 different types were implanted in humans [2]. Although the principal

materials used in the fabrication of commercial artificial valves are not polyurethanes, some devices have used commercial polyurethanes such as Biomer^{r} as a component of valve cusps [6].

Cardiac assist devices such as the left ventricular assist device (LVAD) are used when the pumping function of the heart is inadequate. Polyurethane materials have been used as the bladder component in several designs of these implants [7].

Venovenous oxygenation is a process which involves withdrawing blood from the veins, oxygenating and returning it to the veins [2]. Like other blood contact devices, membrane oxygenators cause the activation of the coagulation pathway and subsequent formation of thrombi remains a problem with silicone materials used in the membrane fabrication. Polyurethanes, though not used as membranes, have been used as filters for the purpose of removing microemboli when using these oxygenators.

An organ replacement device that involves the use of polyurethanes is the artificial heart. The artificial heart program in the United States began in the sixties with the eventual development of the Jarvik-5 and Jarvik-7 pneumatically driven total artificial hearts at the University of Utah [8,9]. The general requirements for a blood-contact material in this application are minimization of thrombus formation at the blood-material interface and maximization of mechanical durability [10]. The two ventricles of the Jarvik-7 heart are made of polyurethane, supported on aluminum bases. A four layer Biomer^{r} diaphragm has graphite dispersed between the layers to act as a lubricant. The resulting flexibility permits normal blood inflow pressures to depress the diaphragm and fill the ventricle during diastole. The diaphragm can withstand pump pressures of more than 200 mm Hg and is designed so that stress points that can lead to failure are eliminated.

Synthetic vascular grafts are used for replacement of diseased arteries and veins [2]. Available synthetic polymer grafts are made from relatively thrombogenic materials such as porous Teflon^{r} and woven or knitted Dacron^{r}. These devices show a reduction of the lumen

and can completely occlude for diameters smaller than six mm. Thus, there is a need for improved materials for small-diameter vascular prostheses. Polymers such as polyurethanes, which have good mechanical properties and show a relative degree of thromboresistance, may be good candidates for this purpose.

The major difficulty in the use of artificial vascular grafts in human subjects is the failure of endothelium to grow on and cover the surface of the grafts. Thus a valid approach to the development of biomaterials for use in the fabrication of small diameter vascular grafts is to enhance the ability of its surface to support endothelialization [11]. An example of research of this type is the development of a biodegradable compliant and microporous prosthesis using poly-L-lactide-polyurethane mixtures [12]. At the present time, a number of research groups are doing extensive research in this area. However, no clinical study of a polyurethane vascular prosthesis has yet been reported [2].

Section 1.3 Biomedical Polyurethanes

Aside from the blood contact applications described in the previous section, polyurethanes have been developed for replacement of tubular organs (endotracheal tubes [13]), artificial skin [14], ligaments [15], adhesives and orthopedic casting tape, and for controlled drug delivery systems [16]. Because the applications are so varied and specific, a large number of polyurethanes have been developed. Tables 1.1 and 1.2 list some of the more common materials and indicate their reported applications.

The polyurethanes in Tables 1.1 and 1.2 may be considered to be classical polyurethanes. At the present time, research for improving the biocompatibility of polyurethanes is focussed on surface modified polyurethanes. Research groups over the past few years have reported work that includes both chemically and biologically modified polyurethanes. Chemical modifications include impregnation with carbon or cationic

surfactants, blending and grafting of copolymers and solvent polishing [2]. Biological modifications include incorporation of heparin, albumin, prostaglandin or urokinase, and the attachment of cultured endothelial cells [2]. Care must be taken to ensure that surface modification does not alter the material to the extent that it becomes susceptible to biodegradation which can be a serious problem in biomaterials [17-24].

Table 1.1: Some Commercial Biomedical Polyurethanes [2]

Polyurethane	Comments
Biomer	-supplied by Ethicon Inc. -developed by E.I. duPont deNemoours (made in sixties) -commonly used in Research and Medical Applications -used in catheters, heart-assist pumps, chambers for artificial hearts
Cardiothane	-supplied by Kontron Inc. -introduced by Avco-Everett in 1967 -used in artificial hearts, intra-aortic balloons, catheters and blood conduits
Pellethane	-supplied by Dow Chemical -developed in the seventies by Upjohn Co. -blood compatibility is not as good as for Biomer and Cardiothane -used in heart pacemaker connectors and lead tubing, and blood bags
Tecoflex	-developed in the seventies by Thermo Electron Corp. -used primarily in research
Toyobo	-developed recently by Toyobo Co. -present applications are in medical research

Table 1.2: Some Developmental Biomedical Polyurethanes [17]

Research Group	Comments
Univ. of Utah	-copolyether-urethane-ureas -used for vascular grafts
Stanford Research Institute	-Synthesized from polypropylene glycol or polytetramethylene ether glycol with a diisocyanate and primary amine. -applications are for intra-aortic balloons, heart massage cups, LVAD bladders
Southern Research Institute	-synthesized from aliphatic diisocyanates, butanediol and polytetramethylene ether glycol -applications are for facial reconstruction and soft denture lining
Ontario Research Foundation	-Pioneered research in hydrophilic polyurethanes -polyurea-urethanes based on polyoxyethylene a diamine, a diisocyanate and diethylene glycol -applied as lubricants for percutaneous and subcutaneous implants

The major drawback with the biologically modified polyurethanes is that the lifetime of the incorporated component, in constant contact with blood, is short due to leaching or diffusion from the surface. The suggestion for future development of polyurethanes is to permanently bind a biological specie to the surface of a polyurethane without destroying its biological function.

Section 1.4 Research Objectives

From this brief introduction it is clear that polyurethanes are a promising class of biomaterials. At the same time the currently available materials fall considerably short of

the ideal with respect to biocompatibility. It is clear that better materials will require an improvement in our fundamental understanding of the mechanisms by which these materials interact with biological milieux such as blood.

One of the main objectives of this research is to contribute to knowledge in this area by investigating the effects of incorporating various chemical entities into segmented polyurethanes on their interactions with plasma. These substituents have been chosen on the basis that they are similar to key constituents of the endothelial lining, anticoagulants such as heparin and inhibitors such as antithrombin III. As such they may promote the interaction of specific proteins in blood with the polymer surface as discussed in Chapter 2.

The pursuit of this objective has entailed the development of polymer synthesis and modification procedures, physical and chemical characterization methods both for the bulk material and the surface, and methods to investigate the blood interactions of the newly developed materials.

Chapter 2

Review of Literature

Section 2.1 Polyurethane Chemistry

A polyurethane is a polymer in which the repeating unit contains the urethane linkage [25]. Over the years, urethane chemistry has been used in a wide variety of polymers which, although they contain the basic urethane linkage, can differ in specific properties because of the presence of other structurally important groups in the chain. Other, more descriptive names are sometimes used such as polyesterurethane, polyetherurethane or polyurea-urethane. These names identify the chemical composition of the polymer in more detail [26].

Section 2.1.1 History

The basic chemistry of polyurethanes was developed in Germany by I.G. Farbenindustrie around 1937, and later at ICI in the United Kingdom and Dupont in the United States. Although many variations of polyurethanes exist, the synthesis of segmented polyurethanes, of interest to the present work, involves three major components: (a) a long chain polyol, usually a polyester or polyether, (b) a diisocyanate and (c) a chain extender in the form of either a short chain glycol, water or a diamine. Figure 2.1 represents the structure of a typical segmented polyurethane (SPU). These polymers are formed via the reactions of isocyanate with hydroxyl and amine groups. The chemistry of polyurethanes thus depends heavily on the chemistry of the isocyanate group.

Figure 2.1: Segmented Polyurethane Structure [27]

HO——UGUGU——UGU——UGUGUGU——UGUGU——OH

U = diisocyanate

G = chain extender

----- = polyol

OH = end hydroxy groups

Section 2.1.2 Isocyanate Chemistry

Common diisocyanates used in polyurethane synthesis are cyclohexamethylene diisocyanate (HDI), toluene diisocyanates (TDI) and diphenylmethane-4,4'-diisocyanate (MDI). The structures of these compounds are shown in Figure 2.2.

The reactivity of isocyanates with compounds containing a reactive hydrogen constitutes the basis of polyurethane synthesis. The reactivity of the isocyanate group can be explained by its resonance stabilization:

$$\underset{a}{R\text{-}N{=}C{=}O} \longleftrightarrow \underset{b}{R\text{-}N{=}\overset{+}{C}\text{-}\overset{-}{O}} \longleftrightarrow \underset{c}{R\text{-}\overset{-}{N}\text{-}\overset{+}{C}{=}O}$$

The resonance effect places a net positive charge on the carbon atom making it susceptible to nucleophilic attack. Rates of reaction will depend on the type of nucleophile. The presence of an electrophilic group adjacent to the active nucleophilic atom will hinder the reactivity of the latter. The relative reactivity of a few nucleophiles is shown in Figure 2.3.

In general, the more basic the nucleophile, the more effective is the nucleophilic

attack [29]. For example, the following order exists for reactivity of compounds with isocyanates: $CH_3NH_2 > CH_3OH > CH_3SH$.

Figure 2.2: Classical Diisocyanates [2].

1,4- cyclohexane diisocyanate

2,4- toluene diisocyanate

diphenylmethane-4,4'-diisocyanate

Figure 2.3: Reactivity of nucleophiles with isocyanates [28]

$CH_3NH_2 > C_6H_5NH_2 >$

decreasing reactivity

Likewise, the presence of electrophilic groups adjacent to the isocyanate group has an influence on reactivity. However, in this case the effect is the opposite of that in the nucleophile because the electron withdrawing group will increase the positive charge on the isocyanate carbon atom and make it even more susceptible to nucleophiles.

In addition to the considerations discussed above, both steric hindrance and aromatic ring substituents can have important effects on the reactivity of active hydrogen compounds containing aromatic rings which in turn affects the reactivity of isocyanates with these compounds. Table 2.1 provides a guide to the prediction of ring activity with respect to the isocyanates or substituted rings carrying active hydrogen groups.

Table 2.1: Effect of Substituents on Aromatic Ring Activation [29]

increasing activation	$-\ddot{N}H_2$, $-\ddot{N}HR$, $-\ddot{N}R_2$	$-\overset{O}{\overset{\|\|}{C}}R$	increasing deactivation
	$-\ddot{\underset{..}{O}}H$	$-CO_2R$	
	$-\ddot{\underset{..}{O}}R$	$-SO_3H$	
	$-\ddot{N}H\overset{O}{\overset{\|\|}{C}}R$	$-CHO$	
	$-C_6H_5$ (aryl)	$-CO_2H$	
	$-R$ (alkyl)	$-CN$	
	$-\ddot{\underset{..}{X}}$: (*deactivating*)	$-NO_2$	
		$-NR_3^+$	

Section 2.1.3: Reactions of Functional Groups with Isocyanates

Two of the most important reactions in the formation of urethane polymers are those of isocyanates with hydroxyl-containing compounds and those of isocyanate with water. Generally, all hydroxyl containing compounds will react with isocyanates to form a urethane group:

$$R\text{-}N{=}C{=}O \;+\; R'OH \longrightarrow R\text{-}\underset{H}{N}\text{-}\underset{O}{C}OR'$$

The reaction is strongly catalyzed by mild and strong bases and by metals. It is weakly catalyzed by acids [26]. As previously mentioned, steric hindrance effects can also modify the reaction rate, i.e. the reactivity of secondary and tertiary alcohols is much less than that of primary alcohols at 25-50°C.

Water reacts with isocyanates to yield an addition product which is not stable and dissociates to form an amine and releases carbon dioxide. This reaction is shown in the following equation:

$$RNCO + H_2O \longrightarrow [RNHCOOH] \longrightarrow RNH_2 + CO_2$$

The amine may then react with an isocyanate to form a substituted urea. This reaction proceeds much faster than does the formation of a urethane. For the case of amines, it has been observed that the more basic the amine the faster is the reaction. Primary aliphatic amines are very reactive, even at 25°C.

$$R\text{-}N{=}C{=}O \quad + \quad RNH_2 \quad \longrightarrow \quad R\text{-}\underset{H}{N}\text{-}\underset{O}{C}\text{-}\underset{H}{N}\text{-}R$$

Ureas and urethanes formed via the reaction of hydroxyls and amines with isocyanate can themselves react with isocyanates. These reactions can lead to extensive crosslinking and must be minimized. The urea group reacts with isocyanate to form a biuret, generally requiring temperatures in excess of 100°C [30]:

$$R\text{-}N{=}C{=}O \;+\; R\underset{H}{N}\text{-}\underset{O}{\underset{\|}{C}}\text{-}\underset{H}{N}\text{-}R' \;\longrightarrow\; RNH\overset{O}{\overset{\|}{C}}\underset{R}{N}\overset{O}{\overset{\|}{C}}NHR'$$

The urethane group is less reactive than the urea group and will react with isocyanate to form an allophanate. This reaction usually requires temperatures in excess of 120°C to proceed at significant rates.

$$R\text{-}N{=}C{=}O \;+\; R\text{-}\underset{H}{N}\text{-}\underset{O}{\underset{\|}{C}}\text{-}OR' \;\longrightarrow\; RNH\overset{O}{\overset{\|}{C}}\underset{R}{N}\overset{O}{\overset{\|}{C}}OR'$$

In addition to hydroxyl and amine groups, carboxylic and sulfonic acid functional groups are also reactive with isocyanates, carboxylic acids usually somewhat less than primary alcohols and water. They initially react to form an anhydride which then decomposes to form a variety of complexes, depending on the acid and isocyanate type:

$$RNCO + R'COOH \longrightarrow [RNH\overset{O}{\overset{\|}{C}}\text{-}O\text{-}\overset{O}{\overset{\|}{C}}R'] \longrightarrow RNHCOR' + CO_2$$

Alkyl sulfonic acids, such as methyl sulfonic acid, will react under mild conditions (< 65°C) with phenyl isocyanate to yield an anhydride [30].

$$C_6H_5NCO + CH_3SO_3H \longrightarrow C_6H_5NHCOOSO_2CH_3$$

Section 2.1.4. Chemistry of Polyurethane-Urea Elastomers

The initial development of polyurethane elastomers followed quickly after the first applications of isocyanate reactions in the formation of polymers [28]. The first step in the manufacture of a polyurethane elastomer involves the reaction of an excess of diisocyanate with a polyol to form an "extender diisocyanate" also referred to as a prepolymer. This prepolymer can then be converted into a long chain polymer by means of a "chain extender", usually a low molecular weight diamine or diol. The final polymer is a segmented polyurethane (SPU) composed of alternating blocks of soft and hard segments (see Figure 2.1). The soft segment usually consists of a polyether or polyester. Molecular weights of these materials usually range between 500 and 5000. Polyesters are quite often synthesized by condensation reactions between diols and dicarboxylic acids. The use of excess diol yields predominantly hydroxyl end groups. The polyethers most frequently used are polytetramethylene oxide, polypropylene oxide and polyethylene oxide. Some structures for typical soft segments are listed in Table 2.2.

Classical polyurethane elastomers are chain extended with simple diamines and diols such as listed in Table 2.3. In recent work by Hudson [31], functionalized chain extenders have been tried. The functional groups, provided they remain intact in the final polymer, can be used to achieve modifications of the polyurethane hard segment. Table 2.4 lists a few of these novel chain extenders.

Table 2.2: Typical Soft Segments in Polyurethane Elastomers

$HO-(-CH_2-CH_2-O-)_n-H$	polyethylene oxide (PEO)
$HO-(-CH_2\underset{\displaystyle CH_3}{\underset{\vert}{C}}H-O-)_n-H$	polypropylene oxide (PPO)
$HO-(-CH_2CH_2CH_2CH_2-O-)_n-H$	polytetramethylene oxide (PTMO)
$HO-(-(CH_2)_4-O-\overset{\displaystyle O}{\overset{\Vert}{C}}-(CH_2)_4-\overset{\displaystyle O}{\overset{\Vert}{C}}-O-)_n-H$	polytetramethylene adipate
$HO-(-(CH_2)_5-\overset{\displaystyle O}{\overset{\Vert}{C}}-O-)_n-H$	polycaprolactone
$HO-(-CH_2)_4-(Si(CH_3)_2-O-)_x-Si(CH_3)_2-(CH_2)_4-OH$	polydimethylsiloxane (PDMS) (hydroxybutyl terminated)

Table 2.3: Classical Chain Extenders

$HO-CH_2CH_2CH_2CH_2-OH$	1,4-butanediol
$H_2N-CH_2CH_2-NH_2$	ethylene diamine
	4,4' methylene bis (2-chloroaniline) (MOCA)
$HO-CH_2CH_2-OH$	ethylene glycol
$HO-(CH_2)_6-OH$	hexanediol

Table 2.4: Novel Chain Extenders

2,5 Diaminobenzenesulfonic acid (DABS)

4,4'-Diamino 2,2'- biphenyl disulfonic acid (BDDS)

4,4'-Diaminostilbene, 2,2'-disulfonic acid (SDDS)

3,5-Diaminobenzoic acid (DAB)

"Tiron"

2,5 Dihydroxy, 1,4-benzenedisulfonic acid Sodium salt (DBSNa)

1,3-Diamino 2-hydroxypropane (DHP)

Section 2.1.5. Reaction Control

The control of the isocyanate reactions for the synthesis of polyurethane elastomers is important because the possibility of multiple reactions occurring simultaneously can affect product quality and reproducibility. The polyurethane-urea synthesis involves the reaction of amines and hydroxyls with isocyanates. Isocyanates can also react with urea and urethane functions in the polymer itself and with contaminants such as water or oligomeric polyols. The relative extent to which these various reactions occur can be controlled by manipulating reaction temperature, by the use of catalysts, inhibitors, structurally hindered isocyanate groups and solvents. The sequence of monomer addition can also be used as a control variable.

The most common addition technique for the formation of polyurethane elastomers involves the prepolymer method, discussed in section 2.1.4. This reaction procedure has the advantage that the polyol-diisocyanate reaction is complete prior to the reaction with the chain-extender. An alternate procedure involves a one step method, in which the polyol, diisocyanate and chain extender are reacted simultaneously. In this case, the reaction can be controlled by the presence of specific catalyst.

Solvent types are also important in the control of isocyanate reactions. The choice of solvent may affect both the rate of the uncatalyzed reaction and the effectiveness of the catalyst [27]. Strong association of solvents with the active hydrogen reactant or catalyst may act to reduce the rate of reaction via hydrogen bonding interactions [30]. Common solvents used in the synthesis of polyurethanes are listed in Table 2.5. The optimum choice of solvent will depend upon the nature of the reactants and the desired kinetics.

Table 2.5: Common Solvents for Polyurethanes [27]

Solvent	Boiling point (°C)	Specific gravity at 20°C	Viscosity at 20°C (cp)
N,N-dimethylacetamide (DMA)	165	0.937	--
N,N-dimethylformamide (DMF)	153	0.9487	--
dimethylsulfoxide (DMSO)	189	1.1014	--
tetrahydrofuran (THF)	64-65	0.8892	0.486
m-cresol	202.6	1.0336	20.8
methyl ethyl ketone (MEK)	79.6	0.8054	0.423
toluene	110.6	0.8669	0.590

The control of impurities is also an important concern. It is quite common to find oligomeric material in commercial polyols. These materials can substantially change the nature of the product. Water is another contaminant which is to be avoided because of its reactivity with isocyanates. Water can be eliminated by solvent distillation, polyol degassing and by conducting reactions under dry, inert gases.

Another common variable used for controlling isocyanate reactions is temperature [32-34]. From room temperature up to about 50°C, reactions of isocyanates with hydroxyl groups yield predominantly urethanes; reactions with amines yield predominantely ureas; and reactions with water yield ureas and carbon dioxide. At temperatures from 80°C up to 150°C, reactions of isocyanates with urethanes yield allophanates; and reactions with ureas yield biurets. In addition dimers and trimers of isocyanates themselves may be formed. Therefore it is desirable, if one is preparing a linear polyurethane, to employ low temperatures. It is also important to note that temperatures in excess of 150°C can begin to degrade some of the less stable polymer backbone links.

Section 2.2 Polyurethane Structure

The structure and morphology of linear polyurethanes in the solid phase are governed by the microphase separation of the hard and soft segments. This phase separation will vary with molecular weight of the soft segment, prepolymer and hard segment, chemical properties of the hard segment and soft segment type, and the casting environment during formation of the solid. Phase separation has an influence on many solid state properties such as tensile strength, elongation and thermal transitions. As well it can influence the nature of the polymer solid surface, which is of particular importance if the material is to be used in contact with blood [42].

As well as undergoing microphase separation, polyurethanes are extensively hydrogen bonded. Hydrogen bonding involves the N-H group as the donor, and the urethane carbonyl, the ester carbonyl (in polyesterurethanes) or the ether oxygen (in polyetherurethanes) as the acceptor.

Section 2.2.1 Microphase Separation

Microphase separation is the result of thermodynamic incompatibility between the hard and soft segments of the polyurethane [35]. The chemical properties, concentration, size and size distribution of the domains have been shown to be influential on final polymer properties. This domain microstructure provides properties equivalent to those of a crosslinked polymer and reinforces the polymer structure.

In a typical elastomeric A/B/A block copolymer, e.g. styrene-butadiene-styrene, the A blocks aggregate to form domains which are hard (glassy or crystalline) and serve as physical crosslinks in the system. The B blocks, on the other hand, form domains which are

usually soft (rubbery or waxy) and provide the polymer with extensibility. The structures of segmented polyurethanes show similar characteristics to the domain structure of A-B-A block copolymers but are somewhat more complex because segments are shorter and the number of soft-hard sequences in a molecule is large. This results in more intersegmental interactions [36].

Thermodynamic models of these systems have been developed by several workers [37-39]. Thermodynamically, phase separation occurs because of a positive surface free energy associated with the interface between the hard and soft segment domains which tends to encourage the growth of hard segment microdomains to reduce the interfacial surface area. As a result of domain formation, the number of possible conformations which the polymer chains can assume is restricted and entropy is decreased [35]. The balance between these driving forces (entropy and tendency to reduce surface free energy) determines the equilibrium domain size and shape.

Figure 2.4 from the work of Lelah and Cooper [35] shows a typical morphological structure of a polyurethane. Estes [40] proposed a model that assumed phase separation was not complete, so that some hard segment domains are dispersed in the soft segment matrix. The domain size was estimated to be approximately 50 A in the direction of the chain axis. This agrees with the calculated contour length for an average hard segment in this work.

Figure 2.4: Morphological Structure of a Segmented Polyurethane [35]

Small angle X-ray scattering is an appropriate technique for characterizing domain size and morphology because of the size range of the domains. Using X-ray techniques, Wilkes [41] found that domains for some polyesterurethanes were lamellar in shape with an average separation of 100 to 250 Å. Larger spacings were obtained in samples with longer soft segments and higher urethane contents than the original material.

Section 2.2.2 Thermal Transitions of Polyurethanes

The microphase structure of polyurethanes is responsible for some interesting transitions observed in thermal analysis studies. These transitions have been used to assess the degree of phase separation in polyurethanes. Lelah and Cooper [35] have reported differential scanning calorimetry data that suggest phase segregation can be enhanced by thermal treatment. Two polymer systems were studied: 1) polymer ET was based on polytetramethylene oxide (PTMO), butanediol (BD) and methylene diphenylisocyanate (MDI), and 2)polymer ES was based on polytetramethylene adipate (PTMA), BD and MDI. Glass transition temperatures for the soft segments of the various polymers are shown in Table 2.6.

The data in Table 2.6 reveal two important aspects of phase separation, i.e. its dependence on hard segment solubility in the soft segment phase and on hard segment content. First there is more phase mixing in the polyester based material than in the polyether based material. This is shown by the stronger dependence of soft segment Tg on hard segment content for the polyester compared to the polyether based polyurethane. The second observation is that both systems exhibit a threshold concentration of hard segment, at which point phase mixing begins to occur and the Tg begins to increase. For the polyester material this occurs at about 31 wt% hard segment content whereas for the polyether based material the threshold is 42 wt%.

Similar studies have been carried out on the effect of chain extender length on phase separation. Blackwell and Lee [43] have demonstrated that multiple DSC melting endotherms seen for the hard domains can be assigned to the different polymorphs (crystalline forms of a polymorphic substance) within the structure.

Table 2.6: Glass Transition Temperatures (Tg) of ET and ES Polymers [35]

Polymer	Hard Segment Content (wt %)	Soft Segment Tg (°C)
ET-38-1	46	-39
ET-35-1	42	-43
ET-31-1	37	-44
ET-28-1	31	-44
ET-24-1	25	-43
ES-38-1	48	-10
ES-35-1	41	-19
ES-31-1	37	-25
ES-28-1	31	-32
ES-24-1	26	-30

Some of the major factors influencing phase separation and hence the observed thermal transitions of polyurethanes are the following: (1) crystallization of either component, (2) the steric hindrance of the hard segment units with respect to hydrogen bonding and (3) the inherent miscibility of the hard and soft segments.

A typical DSC curve for a segmented polyurethane shows several transitions depending on the degree of order within the polymer matrix (see Figure 2.5). The polymer in Figure 2.5 is a polytetramethylene oxide (PTMO) based material with a hard segment content of 38 %. The low temperature transition at about -40°C is the glass transition temperature of the soft segment. The three remaining transitions are associated with the disruption of hard segment microphase domains existing in three different structural orders. The first of these transitions occurs between 50°C and 120°C, and is associated with short range order [73]. The second of these transitions is located between 120°C and 160°C and represents the dissociation of intermolecular networks containing long range order [73]. The short and long range order transitions are not first order. The final transition, occurring near 200°C, is a crystalline melting transition [73].

Figure 2.5: DSC Curve for Polyurethane [73]

Section 2.2.3 Mechanical Behaviour of Polyurethanes

Microphase separation provides polyurethanes with excellent elastomeric behaviour because the hard segment domains act as a reinforcing filler and multifunctional crosslinks within a matrix of soft segment. Table 2.7 taken from reference [35] shows typical tensile

stress-strain data for a polymer synthesized using MDI, PTMO and ethylene diamine (ED). The data show that an increase of hard segment content with constant soft segment molecular weight leads to a higher initial modulus and lower elongation at failure.

Table 2.7: Tensile Properties of Polyurethanes [35]

Hard Segment (wt %)	Soft Segment (mol wt)	Young's Modulus (10^4 Pa)	Elongation at failure (%)	Ultimate Tensile Strength (10^4 Pa)
46	1000	69.2	600	43.0
46	2000	188.5	570	38.8
36	1000	9.3	850	34.6
36	2000	42.6	770	40.1
25	1000	3.3	1550	7.1
25	2000	4.3	1160	18.6

Polyurethanes show considerable stress hysteresis representing a serious limitation on their use. This phenomenon occurs on repeated extension or strain cycling and is attributed to disruption of hard segment domains, leading to a loss in the ability of the hard segments to reinforce the soft segment matrix [45,46].

Table 2.8: Stress-strain Properties of Functionalized Polyurethanes [47]

Polyurethane chain extender	Chain extender structure	Ultimate Tensile Strength (MPa)	Ultimate Elongation (%)
Maltose		7.35	1710
Saccharose		5.15	1720
Glucose		1.40	160
Dulcitol		0.70	180
Xylose		1.45	40
Arabinose		1.12	43

The introduction of chemical functionality into the polyurethane hard segment can cause dramatic changes in mechanical properties. Lipatova and co-workers [47] synthesized PTMO and hexamethylenediisocyanate (HDI) based polyurethanes with a variety of sugar derivatives acting as chain extenders. They demonstrated that the microphase separation process was strongly dependent on the nature of the sugar derivatives. Some stress-strain d are shown in Table 2.8. The high strength and elongation of the maltose and saccharose cl extended polymers are a result of high molecular organization at the microstructural level. The increase in ultimate tensile strength relative to the other chain extenders in Table 2.8 can be attributed to a larger and more rigid hard segment. According to Lelah and Cooper [35] an increase in hard segment size would also result in a lower ultimate elongation along with increased tensile strength. Therefore the authors concluded that the increased elongation with increase tensile strength (for the maltose and saccharose extended polymers) was a result of "supermolecular organization" between the functional groups of the hard segment.

Section 2.2.4 Hydrogen Bonding in Polyurethanes

One of the most common interactions in polymers and one that is important in the phase separation behaviour of polyurethane materials, is hydrogen bonding. Infrared spectroscopy may be used to study hydrogen bonding and several investigations [48-50] have used this technique to measure quantitatively the fraction of hydrogen bonded carbonyl groups in polyurethanes. Figure 2.6 shows some hydrogen bonding interactions involving the urethane group in a polyetherurethane.

The major infrared regions that are of concern for the study of hydrogen bonding in polyurethanes are the N-H stretching region around 3400 cm^{-1} and the carbonyl stretching region around 1700 cm^{-1}. The occurrence of hydrogen bonding in urethane models is illustrated in Figures 2.7 and 2.8 [50]. Figure 2.7 compares the N-H stretching region for a

pure ethylurethane (model compound) sample with the same regions when the sample is dissolved in a polar solvent, tetrahydrofuran (THF), and an organic nonpolar solvent, benzene. It is seen that in the pure material and in THF solution the N-H groups are strongly hydrogen bonded whereas in the benzene solution the N-H groups are "free" or non-bonded. In most polyurethanes at room temperature, less than 20 % of the N-H groups are free [35].

Figure 2.6: Hydrogen Bonding Interactions involving the Urethane Group [42]

Figure 2.7: The N-H Infrared Stretching Region of Ethylurethane [50]

Figure 2.8 compares the carbonyl infrared region of pure ethylurethane with various solutions in THF. The free, non-hydrogen bonded carbonyl peak increases with increasing dilution. In the pure state, there are many more carbonyl groups involved in hydrogen bonding than there are free groups. Changes in the hard segment type will also influence the degree of hydrogen bonding of the carbonyl groups [35].

Figure 2.8: The Infrared Carbonyl Stretching Region for Ethylurethane [50]

Section 2.3 Ionomers

The concept of "functionalizing" biomedical polyurethanes, as previously discussed in section 1.4, may be considered to include the incorporation of ions. Several research groups [51-53] have investigated the influence of these ionic segments of the polymer backbone on surface properties and blood response. Polymers containing low concentrations of ionic groups are termed ionomers. The effect of the incorporation of ions into polyurethanes as was done in the present work is important with respect to both surface and bulk properties. The background literature on these effects is now discussed.

Section 2.3.1 Covalent Incorporation of Ionic Groups in Polyurethanes

Like non-ionic polyurethanes, the first polyurethane ionomers originated from German research groups [54,55]. From 1940 through the 1960's, several techniques for ion incorporation into polyurethanes were introduced as described by Dieterick et al. [56]. The first polyurethane ionomers were synthesized using dihydroxy tertiary amines as chain extenders to incorporate quarternary ammonium ion salts into the hard segment of the polymer chain.

Schlack [54] reported the synthesis of anionic polyurethanes using diamino benzene sulfonates or diaminobenzoates to form water soluble polyurea polysulfonates and polycarboxylates. Ionic polyurethanes have also been synthesized using secondary amines followed by reaction of the amine with sultones or acid anhydrides in the presence of strong bases [58]. The use of aliphatic diamino acids such as N-(2-aminoethyl)-3-aminopropane sulfonate, N-(2-aminoethyl)-beta-aminopropionate and lysinate has also been reported [56]. These ionic aliphatic chain extenders react much faster than aromatic diaminoacids and yield polymers with high tensile strength [56]. It is important to note that the ionic group should be in the salt form, in order to prevent reaction with isocyanate groups.

Most research in this area has focussed on incorporating ions in the hard segment of the polyurethane. Very little work has yet been reported on polyurethane ionomers with the ions located within the soft segment [210]. Furthermore, no new development in polyurethane ionomer synthesis has occurred over the last 20 years.

Section 2.3.2 Ionomer Structure in Solid State

Although polyurethane ionomers have been known for nearly 50 years, there is very little detailed knowledge of their structure. Most research has focussed on property

characterization. Since in most polyurethane ionomers the ions are located in the hard segment it is difficult to uncouple ionic effects from hard segment domain effects. Research has just begun to focus on this problem. In recent work by Lee and co-workers [59,60] a structural model was proposed for polyurethane ionomers synthesized with MDI and PTMO with no chain extension. Sulfonate groups were incorporated into the polymer by reaction of the hard segment secondary amine groups with gamma-propane sultone and sodium hydride. Since no chain extender is used, the molecular weights of these polymers are low, the polymers are amorphous and microphase formation, due to hard/soft segment separation, no longer occurs. Using small angle X-ray data, Lee [59] confirmed ordering of ionic aggregates and determined their radius to be approximately 1-2 nm.

Recent theories of polyurethane ionomer structure [60] stem from research on simpler ionomers such as sulfonated polystyrenes and sulfonated fluorocarbon polymers [60-69]. Eisenberg [61] has postulated the existence of two types of ionic aggregates, termed multiplets and clusters. A multiplet is a group of a few tightly bound ion pairs, with dimensions of the order of 0.6 nm. A cluster is defined as a domain containing several multiplets as well as a significant amount of backbone chain. Cluster diameters are reported to be of the order of 2-10 nm [59] and are thus on the order of the size of hard segment domains for "classical" polyurethanes which are around 5-10 nm [35]. The existence of multiplets and clusters in a non-aqueous medium is believed to be stabilized by electrostatic interactions. Figure 2.9 depicts multiplet and cluster structures.

Section 2.3.3 Polyurethane Ionomer Properties

Although the structure of polyurethane ionomers is not well understood, several studies have been performed to evaluate key physical properties of these polymers [70,71] and these are now summarized.

Figure 2.9: Some Structural Models of Ionomers [62]

Section 2.3.3.1 Water Solubility and Swelling

Almost all polyurethane ionomers have one common feature, namely their ability to form stable emulsions [56]. Polyurethane ionomers in polar organic solvents will spontaneously form stable aqueous dispersions on addition of water, with the ionomer as the disperse phase [54]. The particle size is of the order of nanometers [54]. The stability of these dispersions is derived from the fact that hydrophobic soft segments are tightly packed in the interior of particles while the strongly hydrated urethane segments associated with the ionic groups are situated on the particle surface [71].

Al-Salah et al. [70] studied the water absorption capacity of polyurethane ionomers synthesized using MDI, polycaprolactone (PCL) and dimethylolpropionic acid (DMPA). In this work they varied the counterion and correlated ion potential with water uptake (% w/w). Water absorption for the anionic polymers studied followed the order Mg> Ba > Ni > Mn > Cd > Zn > Hg > Pb > Cu. Data are not available for polymers with Li^+, Na^+ and K^+ counterions because these materials were water soluble.

This study concluded that water absorption is greatest for the group Ia metals which tend to make the polymers water soluble, followed by the group IIa metals which showed the highest water swelling levels without dissolving, followed by the heavy metals.

Section 2.3.3.2 Mechanical Behaviour of Polyurethane Ionomers

Several studies have explained the stress-strain behaviour of polyurethane ionomers in relation to DSC data (containing information on phase separation) and FT-IR data (containing information on hydrogen bonding). These studies include comparisons of polymers with various counterions [71], soft segment types [72], hard segment concentrations [73] and ion types [74].

Data from Al-Salah's study [71] on counterion type are shown in Table 2.9. He found that the ultimate tensile strength of an MDI/PCL/DMPA polymer increased with ionic potential within a periodic group, for non-transition monovalent and divalent cations. There was no correlation with the ultimate elongation.

The increase in tensile strength with ionic potential for these materials likely indicates a stronger association between ion-dipoles rather than an increase in phase separation. This conclusion is supported by the glass transition data in Table 2.9. One would expect the Tg to decrease with higher ionic potential if an increase in ionic potential improves phase separation. As can be seen, however, the Tg values increase with increasing ionic

potential. It is possible that the stronger dipoles are attracted more strongly to the polar polyester segments thus enhancing phase mixing while strengthening Coulombic type crosslinks in the material. Increased dipole interactions based on changes in counterion type with carboxylate ionomers was also found by Hara and Wu [75]. They showed that monovalent counterion binding with COO- groups decreased in the order Li>Na>K>Cs.

Table 2.9: Physical Properties for MDI/PCL/DMPA with Different Counter Ions [70]

Metal Counter ion	Ionic Potential (charge/radius)	Ultimate Tensile Strength (MPa)	Ultimate Elongation (%)	Tg (°C)
Li^+	1.67	54.17	392	8
Na^+	1.05	48.67	395	-4
K^+	0.75	44.44	426	-10
Mg^{++}	2.29	56.06	292	18
Pb^{++}	1.65	46.60	393	13
Ba^{++}	1.48	47.53	410	5

Hwang, Speckhard and Cooper [73] studied a series of simple non-chain extended polyurethanes based on MDI/PTMO. Subsequent conversion to ionomers was carried out via a bimolecular nucleophilic displacement reaction with sodium hydride, followed by a ring-opening reaction with gamma-propane sultone. Polyurethane ionomers containing 20 wt % MDI (PTMO-20) and 38 wt % MDI (PTMO-38) were subjected to DSC, FT-IR and stress-strain measurements. The data are presented in Table 2.10.

The Tg's of the PTMO-20 polymer series decrease with increasing ion content, an effect which can be attributed to the development of phase separation. The tensile strength data support this argument. The decrease in elongation at high levels of ionization is

associated with a high degree of Coulombic interaction which may create physical crosslinks that constrain the polymer matrix. The FT-IR data in Figure 2.10 for PTMO-20 and PTMO-20-10.3 support the hypothesis of an increase in phase separation. The carbonyl peak at 1730 cm^{-1} represents non-H-bonded carbonyl groups. When phase separation increases the number of carbonyl groups in proximity to amine groups will increase. This will lead to an increase in hydrogen bonded carbonyls (with a peak at 1700 cm^{-1}) relative to free carbonyls.

Table 2.10: Physical Properties of MDI/PTMO Polymers [73]

Polymer	Tg (°C)	Ultimate Tensile Strength (MPa)	Ultimate Elongation (%)
PTMO-20-0	-55	---	---
PTMO-20-0.8	-57	0.014	300
PTMO-20-4.5	-62	0.07	1250
PTMO-20-10.3	-68	10.0	400
PTMO-20-12.1	-73	14.0	30
PTMO-38-0	-44	23.0	535
PTMO-38-1.3	-31	27.0	660
PTMO-38-3.3	-30	32.0	580
PTMO-38-6.5	-45	27.0	375

Note: The third number in the polymer name is the wt % SO_3Na content

The I.R. data for PTMO-38-0 and PTMO-38-6.5 show just the opposite effect. The PTMO-38-0 polymer already has a substantial degree of carbonyl hydrogen bonding due to the high concentration of hard segment in the material. In this case, introduction of ions interferes with the hard segment packing arrangement rather than promoting phase separation. The Tg data in Table 2.10 confirm that ionization does not improve phase separation in the same manner as observed for the PTMO-20 polymers. The ultimate tensile

strength of the PTMO-38 polymers appears to reach a plateau value, suggesting that although carbonyl-amine H-bonding interactions are being prevented, ion dipole interactions are increasing, and a balance is reached. As observed with the PTMO-20 polymers, the PTMO-38 polymer exhibits a lower elongation with high levels of ionization. Other studies [72,74] have reported similar effects.

Figure 2.10: Effect of Ionization on the Infrared Spectra of PTMO-20 and PTMO-38 [73]

Section 2.4 Pathogenesis of Thrombosis

Blood is a complex fluid composed of formed elements (red blood cells, white blood cells and platelets) suspended in a solution (plasma) consisting of proteins and electrolytes and other molecular species. Whole blood consists of about 45% formed elements and about 55% plasma by volume [76].

Under normal conditions, platelets circulate as discoid shaped, quiescent elements that do not interact with other circulating blood cells, plasma elements, or the vascular wall (endothelium) [77]. However when stimulated the platelets become involved in the pathologic process known as thrombosis. Thrombosis can be initiated in the presence of damaged vascular endothelium, agents which cause platelet aggregation or intravascular activation of blood coagulation [78]. This process forms a thrombus which is an intravascular deposit composed of fibrin and the formed elements of blood.

Section 2.4.1 Endothelium

The inner surface of the circulatory system that is in direct contact with blood is called the endothelium and is composed of endothelial cells [79]. The primary functions of endothelial cells are to supply low molecular weight nutrients to subendothelial structures, to act as a barrier to large macromolecular substances and particulate matter, and to provide a non-thrombogenic surface for circulating blood [80]. The endothelial cells also produce a portion of the factor VIII complex, known as von Willebrand factor which may be released if the vessel wall is damaged, thus providing binding sites for platelets to adhere [79].

The endothelial cell is about 0.5 to 1.2 um thick but is highly elongated and has a high surface to volume ratio. The outer surface of the cell is covered with glycocalyx [81], a complex mucopolysaccharide. The surface also contains the enzyme ADPase [81] which

degrades adenosine diphosphate (ADP), a substance released by platelets during the initial stages of platelet aggregation and adhesion to a surface. ADP release causes further platelet aggregation.

The anti-thrombogenic nature of the endothelium has a number of origins [79]:

i) Heparan sulfate, a constituent of glycocalyx, is present on the cell surface. This molecule is capable of catalyzing the reaction between antithrombin-III (AT-III) and thrombin. Thrombin is a procoagulant or clotting factor and AT-III is a naturally occurring inhibitor of thrombin.

ii) As previously stated, the surface contains ADPase, a substance that degrades ADP and hence would inhibit the aggregation of platelets on the cell surface [81].

iii) The charge repulsion effect is another important consideration. Platelets and endothelium both have a net negative electrical charge and thus could be mutually repulsive in an aqueous medium such as blood.

iv) Thrombin has been found to bind to the endothelial surface and stimulate the cells to release plasminogen activator [82]. This activator binds to fibrin and accelerates the conversion of plasminogen to plasmin. The latter is an enzyme which lyses fibrin, a major component of thrombus.

v) The blood vessel wall can generate prostacyclin (PGI_2), a potent vasodilating and anti-platelet aggregating substance [83].

vi) Current research suggests that thrombomodulin, which is an endothelial surface receptor, also enhances the anticoagulant nature of the endothelium. Thrombomodulin binds thrombin which in turn allows the activation of protein C which is regarded as a major regulatory mechanism for control of coagulation [84].

vii) The endothelium is a continuous layer with no seams and therefore acts as a barrier separating the blood cells from the vascular subendothelium which is thrombogenic in nature [78].

If a vessel is damaged, there may be endothelial loss and exposure of the relatively thrombogenic sub-endothelium to both platelets and coagulation factors. Vessel wall damage may be caused by direct injury (such as implantation of a vascular graft), immune complexes, viruses, hemodynamic stresses, tobacco products, high blood cholesterol and enzymes released from platelets and white blood cells in inflammatory states [78]. Following breakdown of the protective mechanisms provided by normal endothelium, exposed vascular subendothelium can cause platelets to adhere, aggregate and form small mural thrombi which are stabilized by fibrin. These mural thrombi can cause complications if they obstruct a major artery or if they embolize into the microcirculation.

Section 2.4.2 The Role of Proteins and Coagulation in Thrombosis

The principal coagulation proteins are listed in Table 2.11 and make up two clotting pathways, the intrinsic and extrinsic pathways.

Table 2.11: List of Coagulation Factors [79]

	Synonyms
Factor I	Fibrinogen
Factor II	Prothrombin
Factor III	Thromboplastin or tissue factor
Factor IV	Calcium
Factor V	
Factor VII	
Factor VIII	
Factor IX	Christmas factor
Factor X	Stuart-Prower factor
Factor XI	
Factor XII	Hageman factor
Factor XIII	Fibrin stabilizing factor
prekallikrein	Fletcher factor
high molecular weight kininogen	HMWK or Fitzgerald factor
Protein C	

It is common to show both the intrinsic and extrinsic clotting pathways together since in the natural vessel repair system they are both activated. The extrinsic clotting pathway is stimulated in vivo by exposure of blood to extravascular tissue which contains thromboplastin. The latter forms a complex with Factor VII which in the presence of calcium activates Factor X. As shown in Figure 2.11 the activation of Factor X eventually leads to the formation of a fibrin clot [78].

Figure 2.11: Intrinsic Coagulation Pathway [79,88]

Kal = kallikrein
PF_3 = Platelet phospholipid

The intrinsic coagulation pathway is initiated by a complex sequence of enzyme-substrate reactions in which pro-enzyme clotting factors are sequentially converted to enzymes (Figure 2.11). The final result is the formation of fibrin, the clot itself [79]. As indicated in Figure 2.11 some of the activated products feed back to accelerate the clotting process. Since it is the intrinsic pathway that likely initiates the clotting mechanism on a foreign surface, this pathway is most relevant to the study of biomaterials and is discussed in more detail.

The coagulation process may be considered to begin with contact activation (see Figure 2.12). This process involves the binding of factor XII to a surface and its immediate activation [86]. The activation of factor XII sets up the first primary feedback loop in the coagulation process. In addition to continuing the cascade of reactions by activating factor XI, factor XIIa activates prekallikrein to kallikrein, which in turn can activate factor XII. This whole process is facilitated by the presence of the cofactor HMWK [87]. The same interactions lead also to plasminogen activation and kinin formation (see Figure 2.12).

Figure 2.12: Feedback Loop of Contact Activation [87]

Following contact activation, the remaining clotting factors are activated in sequence (see Figure 2.11). The rate of activation increases down the cascade due to enzyme amplification and feedback effects. Activated factors XIIa, XIa, IXa, Xa and thrombin are termed serine proteases [80-85], meaning that the active site on these enzymes (with respect to the coagulation process) includes a serine amino acid residue [207].

A critical point in the blood clotting mechanism occurs at the stage where thrombin is formed. It is at this stage that a second feedback loop occurs which dramatically increases the rate of formation of thrombin and hence the conversion of fibrinogen to fibrin. Thrombin feeds back to accelerate clotting through the activation of factors VIII and V, and stimulates platelets to release ADP and therefore to aggregate [78]. As well thrombin is responsible in part for the activation of factor XIII which stabilizes the fibrin clot [88]. The activation of protein C also occurs via the interaction with thrombin and thrombomodulin from the endothelium [79]. Activated protein C plays an active role as an inhibitor by degrading factor V and factor VIII.

The completion of thrombus formation requires the conversion of fibrinogen to a fibrin clot. Although fibrinogen activation does not initiate feedback loops such as those discussed for factor XIIa and thrombin, it involves several reactions which lead to the polymerization of crosslinked fibrin polymers. The structure of fibrinogen will be detailed in this section because of the important role that it plays in protein adsorption to surfaces, platelet interaction and thrombus formation.

Fibrinogen has a concentration in plasma of approximately 300 mg/dL and consists of six polypeptide chains (two alpha, two beta, two gamma) that are linked by disulfide bonds to give an overall trinodular shape [89] (see Figure 2.13). The molecule has the highest molecular weight of the coagulation proteins, 340000, and is approximately 450 A in length and 90 A in diameter. The alpha, beta and gamma chains are helically wound in the regions between the nodules, while in the nodules themselves the beta and gamma chains are tightly

coiled because of their hydrophobicity [89]. The alpha chain is believed to be extended into solution because of its hydrophilic character and is subject to enzymatic degradation.

Figure 2.13 Schematic Representation of the Structure of Fibrinogen [208]

The formation of fibrin from fibrinogen occurs through a series of steps. The first step involves the cleavage of fibrinopeptide A from the alpha chain which allows the formation of polymers via end-to-end interactions of fibrin monomers. The release of fibrinopeptide B from the beta chain then provides active sites that allow lateral polymerization to proceed [89]. It is suspected that the release of fibrinopeptides A and B, which carry a net negative charge, reduces electrostatic repulsion and allows interaction between the monomers to proceed more readily. The final step in the process involves the crosslinking of the fibrin polymers via activated factor XIII. This latter protein catalyses the formation of peptide bonds between the gamma chains of the polymers.

Section 2.4.3 Protein Action in Thrombosis Regulation

As shown in the previous section, coagulation involves an enzyme amplification system and positive feedback cycles involving factor XIIa and thrombin. These same loops also provide negative feedback when critical concentrations of factors XIIa and thrombin are reached. The initial phases of these negative feedback loops are shown in Figures 2.11 and 2.12. Activated protein C and plasmin are both active in interrupting the coagulation cascade and in breaking down the fibrin matrix itself.

Four inhibitors of the coagulation cascade, each of which acts by a different mechanism, are shown in Table 2.12. C1 esterase inhibitor is a multi-specific serum enzyme inhibitor [90]. It is a primary inhibitor of complement factor C1, coagulation factors XIa and XIIa and the enzymes plasmin and kallikrein [86]. This inhibitor is an alpha 2-globulin and is unstable in the presence of heat and acid [91]. According to Triplett [79], the rate of inhibition of the coagulation factors by C1 esterase inhibitor is slow but the rate of complement factor C1 inhibition is rapid [92-95]. Although it has been stated that the inhibitory effect of C1 inactivator against factor XIa and factor XIIa has been observed only in vitro, it is possible that it may be effective in vivo, in the presence of artificial implant materials [96].

Protein C is a glycoprotein with a molecular weight of approximately 62000. As previously mentioned, activated protein C degrades factor V, inactivates factor VIII and also facilitates fibrinolysis by neutralizing an inactivator of plasminogen activator [97].

Table 2.12: Inhibitors of Coagulation [79]

Inhibitor	Molecular Weight	Plasma Concentration (mg/dL)
C1 esterase inhibitor	140000	14-30
Protein C	62000	0.5
Antithrombin-III	58000	23-40
Plasminogen	90000	10-15

Antithrombin-iII (AT-III) is an inhibitor of several of the serine proteinases produced during coagulation but its principal action is on thrombin. Some of the amino-acid residues thought to be involved in the inactivation of thrombin by AT-III are shown in Figure 2.14, constructed using reference [212]. This reaction is strongly catalyzed by heparin, a negatively charged, highly sulfated mucopolysaccharide with a molecular weight ranging between 6000 and 25000 [99]. A portion of the active unit of heparin is shown in Figure 2.15 [135].

Plasminogen can be activated and converted to plasmin via several pathways. Plasmin is a proteolytic enzyme involved in the lysis of fibrin clots after they are no longer required. When fibrin is formed, its intermolecular contacts are limited due to the nodular nature of the fibrin monomers and this leaves open channels through which plasmin and other proteins can diffuse [89]. The plasmin molecule "degrades" the crosslinked fibrin structures by cleaving a relatively small number of bonds, thus forming soluble degradation products.

Figure 2.14: Mechanism for the Inhibition of Thrombin by AT-III [212]

Figure 2.15: Structure of Pentasaccharide Unit for Heparin [135]

"Structure of the antithrombin-binding region in heparin. Structural variants are indicated by R' (-H or $-SO_3-$) and R" ($-SO_3-$ or $COCH_3$). The 3-O-sulfate group marked by an asterisk is unique to the antithrombin-binding region of the heparin molecule. In addition, each of the three sulfate groups indicated by (e) has been proven essential to the high-affinity binding of antithrombin. The functional roles of the O-sulfate groups on the two monosaccharide residues toward the reducing end (to the right in the figure) are unknown [135]."

In this section the principal blood and vascular components which contribute to the pathogenesis of a thrombus have been discussed. This discussion is pertinent to the understanding of the blood trauma which takes place in the presence of an implant device. The following section discusses blood-material interactions in detail with particular emphasis on effects leading to thrombus formation.

Section 2.5 Interactions of Blood with "foreign" Surfaces

Section 2.5.1 General Knowledge

It is generally believed that plasma protein adsorption is the first significant event to occur following blood-surface contact and that subsequent phenomena are, to a large extent, determined by interactions of the blood with the adsorbed protein layer. The normal "end point" of blood-foreign surface contact consists of coagulation, thrombus formation and

embolization [100]. An outline of the mechanisms of thrombogenesis at foreign surfaces is shown in Figure 2.16. This Figure shows several processes that occur during blood-material contact but concentrates on the initial adsorption of proteins, initiation and propagation of the plasma coagulation system. The following discussion will also emphasize the central pathway of the diagram and only briefly touch on complement activation and platelet interactions.

Figure 2.16: Sequence of Events during Blood-Material Interactions
(T = tentative pathway) [100]

Binding interactions in protein adsorption can occur via several mechanisms: i.e. hydrophobic interactions, electrostatic interactions, dipole-dipole interactions and van der Waals interactions [101]. Proteins vary considerably in structure, in particular with respect to molecular rigidity and susceptibility to conformational change. For example, a protein with a very rigid structure may be adsorbed without any appreciable conformational change, even when strong interactions are involved, whereas a loosely structured protein may be easily denatured [102,103].

The composition of the protein layer is known to affect subsequent cellular interactions with the artificial surface. Depending on the type of protein adsorbed, platelet adhesion may be either reduced or increased [103]. For instance, when albumin is adsorbed on a surface, platelet adhesion is diminished, while adsorbed fibrinogen and gamma-globulin promote platelet adhesion [104]. Once platelets adhere to the surface they often undergo release of granule contents thus activating adjacent platelets and the blood coagulation process. The process of protein adsorption is competitive and all plasma proteins have the potential to be involved. The rates of adsorption of proteins to surfaces are unknown but almost certainly differ from one protein to another.

Albumin, fibrinogen and IgG are among the most abundant proteins in plasma and competitive adsorption experiments in mixtures of the three have shown that fibrinogen most often dominates the absorbed layer relative to gamma-globulin and albumin [105]. The high surface activity of fibrinogen is probably a significant factor in relation to blood-material interactions as discussed further below.

Although these abundant proteins may be important in the initial phases of blood-material interactions, it has been shown that surface-solution exchange occurs and in a complex fluid such as plasma or blood, initially adsorbed proteins can later be exchanged with others due to differences in concentrations and in the kinetic and thermodynamic parameters for adsorption of the various components [106]. Vroman [107] demonstrated that initially

adsorbed fibrinogen was replaced by high molecular weight kininogen (HMWK), a protein involved in the initial contact phase of the intrinsic coagulation pathway as discussed above. This exchange is known as the Vroman effect, and recent work has shown that it occurs for IgG as well as for fibrinogen, and may well be a general effect in which initially adsorbed high concentration proteins are displaced by low concentration proteins of higher binding affinity [107].

As previously mentioned, the presence of fibrinogen on a surface provides favorable conditions for the uptake of platelets. Adherent platelets may then provide a surface which catalyzes the activation of the coagulation pathway. Figure 2.16 also relates the initial protein adsorption to complement activation on surfaces. Although this thesis is not concerned with the complement system, it is important to point out that artificial materials in contact with blood can activate this process. Complement activation is implicated in transient leukocyte depletion in patients undergoing hemodialysis [209]. The complement system consists of at least 20 plasma proteins capable of interacting to produce enzyme complexes which attack cell membranes [90]. It has been observed that these proteins also play an important role in the selective and competitive adsorption processes taking place on a biomaterial surface [108].

Section 2.5.2 Polyurethane-Blood Interactions

The common themes in blood-material interactions discussed in the previous section also apply to the contact of polyurethanes with blood. Many studies [see review, ref. 35] have been carried out to evaluate protein adsorption, platelet deposition, platelet activation and aggregation, thrombus formation and embolization associated with polyurethane surfaces. These studies have tried to correlate blood interactions with polyurethane structure.

Early studies of the adsorption of proteins to polyurethanes involved several single protein studies of albumin, fibrinogen and gamma-globulins [35]. This focus was rationalized on the basis of the large amount of data on the "passivating" effects of albumin and the "activating" effects of gamma-globulins and fibrinogen, as discussed in the previous section. However it soon became apparent that adsorption behaviour in single protein solutions could not be extrapolated to plasma. Brash et.al. [109] suggested that in the presence of blood or plasma, an adsorbed protein layer could be exchanging material with the fluid phase.

Lee et al. [110] studied adsorption from mixtures of albumin, fibrinogen and gamma-globulin on polyurethanes. Changes in layer composition with time were studied, and fibrinogen was found to be preferentially adsorbed in relation to the other two proteins. Although in this work adsorption from plasma was not studied, it did highlight the importance of competitive adsorption on polyurethanes which has also been noted by Brash [112]. Studies by Hudson and Brash [112] confirmed the occurrence of the Vroman effect for a variety of polyurethanes having hydrophobic (PUE1), hydrophilic (PUO1) or ionic character (PUT1) as shown in Figure 2.17.

The presence of fibrinogen on the surface of polyurethanes has been associated with platelet adhesion and aggregation [103]. Brash and Whicher [113] showed that hydrophobic polypropylene oxide based polyurethanes support a moderate amount of platelet adhesion while hydrophilic polyethylene oxide based polyurethanes showed significantly lower levels of adhesion. These studies were based on in vitro experiments. Cooper's group [114-116] studied the adhesion of fibrinogen and platelets in an ex-vivo canine shunt experiment and demonstrated the importance of adsorbed proteins in platelet-surface interactions. These studies indicated that both the surface concentration and type of hard segment were of importance in determining blood response. Both the surface properties and thrombogenicity of a particular polyurethane were changed by casting from different solvents, indicating the need to optimize and control material fabrication conditions [116].

Figure 2.17: Fibrinogen Adsorption from Plasma to Polyurethanes Based on Polypropylene Glycol [112]

Typical fibrinogen deposition profiles from Cooper's work show the presence of two peaks [116]. The first peak observed at approximately 30 seconds is likely a manifestation of transient fibrinogen adsorption (Vroman effect) [117]. The fibrinogen level then peaks again near 17 minutes. This second peak corresponds to the embolization of platelet-fibrin surface localized thrombi. Studies investigating thrombus formation on commercial

polyurethanes have also been performed [118,119], and have shown that similar mechanisms occur.

The studies of blood-polyurethane interactions carried out thus far have provided some understanding of the mechanisms governing thrombus formation on these surfaces. In so doing they have motivated research on approaches to alter polyurethane surface in such a manner as to avoid the initiation of these mechanisms [210]. The present thesis represents a contribution to this research effort.

Section 2.6 Functionalized Polymers for Improved Blood Compatibility

Chemical modification of synthetic polymers to achieve specific properties is a widely-practiced art. Graft polymers, ion exchange resins, solid phase peptide or nucleotide synthesis supports are all examples of polymers that have been chemically modified or "functionalized" with specific purposes in mind. Thus improving polymer biocompatibility by the incorporation of functional groups can be regarded simply as a new application for an old technology.

Almost all of the techniques applied to the covalent attachment of biochemically active moieties to improve the blood compatibility of polymers involve some chemical modification which either directly or indirectly interferes with the adhesion or aggregation of platelets or the formation of a fibrin network [120].

Section 2.6.1 Pharmacologically Active Polymers

A common approach to biocompatibility pursued by several groups is the incorporation of antithrombogenic drugs. Heparin continues to be one of the most popular drugs used for this purpose [120]. Incorporation of heparin is expected to give the surface

anticoagulant activity, since this molecule is known to catalyze the reaction between thrombin and antithrombin-III [98]. This activity directly interferes with the conversion of fibrinogen to fibrin and so inhibits thrombus formation.

Yoshihiro et al. [121] synthesized carboxylated polyester polyurethanes using ethyl dihydroxy-methyl propionate and L-lysine methyl esters as chain extenders. These polymers were then treated with 4 N NaOH to obtain carboxylate ionomers. The latter were then acidified and heparin was attached to the acid function using carbodiimide. The heparin-bound polyurethane-urea deactivated the blood clotting system thus leading to strong antithrombogenicity [121].

Larm et.al. [122] modified polyethylene tubing to incorporate carboxylate and sulfate groups using a concentrated sulfuric acid treatment. The surface was further treated with polyethyleneimine (PEI) to incorporate functional binding sites for heparin. The PEI activated tubing was then reacted with degraded heparin at 50 °C, in the presence of $NaBH_3CN$ at pH = 3.5. In vitro studies revealed that the heparinized polyethylene tubes were highly thromboresistant in terms of reduced platelet adhesion and surface catalyzed inhibition of adsorbed thrombin.

Kim et al. have published work on heparin immobilization to various polymers containing hydrophobic and hydrophilic "spacer" groups [123-125]. Unlike the materials discussed above where heparin was attached directly to the polymer, Kim and coworkers developed an immobilization scheme that utilized "spacer arms" to enhance heparin availability and activity when attached to a polymer. In one investigation [123], polyurethanes were hydrolyzed by treating them with 3M NaOH. This process yields free amine groups which were converted to isocyanate groups with phosgene. These isocyanate groups were then reacted with diamino alkanes of various length (i.e. 4, 6 and 12 carbon atoms) to achieve free amines at the end of the spacer. The final heparin attachment was achieved using a carbodiimide reagent as in Yoshihiro's work [121]. Compared to the base

polyurethane, the heparinized material showed improved blood compatibility with respect to activated partial thromboplastin time, thrombin time, thrombin binding and inactivation, and factor Xa binding and inactivation.

The use of hydrophilic spacer arms for the attachment of heparin to Biomer has been described by reference [125]. The materials showed reduced protein adsorption and subsequent platelet adhesion to the surface. In addition the bioactivity of the immobilized heparin was found to be enhanced by increasing the length of the spacer. Nevertheless the bioactivity of the bound heparin was less than that of free heparin regardless of the type of spacer. This is a common problem with all heparin immobilization techniques and is possibly due to chemical modification of heparin or physical inaccessibility of its AT-III binding site.

As well as immobilizing heparin which is effective against the intrinsic coagulation system, there has been interest in incorporating anti-platelet agents to minimize white thrombus formation or platelet consumption processes in situations where fibrin formation is not the limiting effect [120]. Examples of such agents are ditazole and dipyridamole, apyrase and prostacyclin (PGI_2) [120]. As for heparin, the biological activity of these compounds is highly dependent on the technique of incorporation into the polymer. Another important factor is that unlike heparin these molecules are not necessarily catalytic so that their effects are "saturable" as opposed to "renewable" and would be expected to be of limited duration.

Attempts to improve the antithrombogenicity of polymers has also been achieved by immobilizing fibrinolytic agents such as the enzyme urokinase (UK) [120]. UK converts plasminogen to plasmin which initiates fibrinolysis. Like the anti-platelet drugs attached to polymers, effective antithrombogenic character is dependent on the presence of the unreacted drug at the polymer surface.

Section 2.6.2 Ionized Polymers

Ionization of polyurethanes has been previously proposed as an approach to potential biomedical materials [126]. The incorporation of anionic character into polyurethanes may allow the surface to exhibit endothelial-like character in the broad sense that the endothelium is probably globally negatively charged through sulfonate groups [127]. Such negative charge may contribute to the inhibition of interactions of the endothelium and blood components, most of which are also negatively charged [79]. Also it is thought that heparin's unique anticoagulant properties are associated with the sulfate, aminosulfate and carboxylate groups on the molecule [128], and therefore it has been hypothesized that sulfate, sulfonate, sulfonamide and carboxylate functionalities incorporated onto polymers might simulate the action of heparinized surfaces [129].

Another possible effect of introducing ions into biomaterials is the resulting change in the structure and organization of water molecules at the aqueous interface. The structure of water near a biological interface has been discussed by Drost-Hansen [130], and Garcia et.al. [131] who have hypothesized that blood compatibility is sensitive to the hydrophilic/hydrophobic balance and ionic distribution on the surface of the polymeric system.

Lelah et al. [127] synthesized a series of ionized polyurethanes using the propane sultone technique described in Section 2.3 of this thesis. The reaction pathways are shown in Figure 2.18. Five different polymers were made, U-21.5 containing 21.5 wt % hard segment, U-38 containing 38 wt % hard segment, U-38-C (a U-38 cationomer), U-38-A (a U-38 anionomer) and U-38-Z (a U-38 zwitterionomer).

Figure 2.18: Synthesis of Polyurethane Ionomers [127]

Both platelet and fibrinogen adsorption studies were carried out by ex-vivo canine shunt experiments. The data from these studies are shown in Figure 2.19 and suggest that the zwitterionomers are more antithrombogenic than the anionomeric and cationomeric materials. While U-38-Z and U-38-A showed decreased fibrinogen uptake and platelet adsorption relative to U-38, the cationomeric polymer increased platelet adhesion and fibrinogen adsorption. As well, this study showed that a decrease in hard segment content decreases the level of protein and platelet adsorption to a larger extent than does the introduction of ionic groups.

Figure 2.19: Fibrinogen and Platelet Deposition Profiles for Ionomeric Polymers [127]

A. Platelet Deposition profiles. Peak height is used as a measure of thromboembolic potential

B. Fibrinogen deposition profiles.

A more recent study by Cooper et al. [132] showed the influence on blood response of varying sulfonate content in polyurethanes. In this process a urethane hydrogen is effectively exchanged for propane sultone. Ex vivo studies showed a significant reduction in platelet adhesion for the initial 30 minutes of surface exposure to blood, while after 60 minutes the platelet surface count is similar for all materials. Fibrinogen adsorption showed a decrease for a 10 % propane sultone substituted polymer but a significant increase (relative to the non-sulfonated polymer) for the 15 % and 20 % substituted polymers. The results are counter-intuitive since high fibrinogen adsorption is usually associated with increased platelet adhesion [133]. Cooper suggests that these results can be attributed to fibrinogen molecules bind in an altered conformation such that the normal ligands for platelet adhesion are unavailable. Another possibility is that the fibrinogen is absorbed rather than only adsorbed [134]. Water absorption experiments showed that the 15 and 20 percent substituted materials absorbed up to 10 times their weight in water at equilibrium. Materials that swell to these levels probably absorb as well as adsorb proteins and if so this could explain the abnormally high fibrinogen "uptake" relative to the constant platelet levels observed at 60 minutes for these surfaces.

As well as sulfonate groups, functional groups such as carboxylates and sulfonamide linkages have been covalently attached to polymers for blood contact applications. Jozefonvicz and coworkers [138] have modified crosslinked polystyrene by functionalizing them with sulfonate groups. These are converted to sulfonyl chlorides which are then further reacted with the alpha-amine groups of various amino-acids. The resulting materials, in particular those substituted with glutamic acid and aspartic acid show heparin-like character, in the sense that they appear to be able to catalyze the deactivation of thrombin by AT-III

[129]. Figure 2.20 compares the structure of a heparin unit with that of a "heparin-like" material. Jozefowicz et al. have shown that the antithrombic activity of crosslinked sulfonate polystyrene is linearly dependent on the surface density of the sulfonate groups. They have also shown that replacing sulfonate groups by directly backbone-bonded carboxylate groups is not sufficient to endow the resulting polymer with a significant anticoagulant activity.

Figure 2.20: Heparin-like [120] and Heparin Structures [135]

Heparin

Heparin-like Polymer

Section 2.6.3 Phospholipid-Derivatized Polymers

In work by Hayward and Chapman [136] functional groups were incorporated into polymers to mimic the thromboresistant character of blood cell membranes. The extracellular surfaces of the plasma membranes of red blood cells and quiescent platelets are thromboresistant in contrast to their cytoplasmic surfaces which are thrombogenic [136]. This thromboresistant character has been attributed to the high content of electrically neutral phospholipids which contain the phosphorylcholine head group.

Table 2.13: Phosphorylcholine Derivatives [140]

I	$(CH_3)_3\overset{+}{N}-CH_2CH_2-O-\underset{Cl}{\overset{O}{P}}-Cl$
II	$(CH_3)_3\overset{+}{N}-CH_2CH_2-O-\underset{O^-}{\overset{O}{P}}-OCH_2CH_2OSi(CH_3)_2-Cl$
III	$(CH_3)_3\overset{+}{N}-CH_2CH_2-O-\underset{O^-}{\overset{O}{P}}-O-Si(CH_3)_2-Cl$
IV	$(CH_3)_3\overset{+}{N}-CH_2CH_2-O-\underset{O^-}{\overset{O}{P}}-OCH_2CH_2NH_2$

Durrani et al. [137] described methods of preparing four phosphorylcholine derivatives for surface attachment to hydroxyl containing polymers and acid chloride

containing polymers similar to those of Jozefovicz [138]. The four compounds are shown in Table 2.13. Structures I, II and III can be covalently attached to polymers such as poly(vinyl alcohol), polyhydroxyethyl methacrylate, cellulose acetate and hydroxyl containing polyurethanes [140]. The amine of structure IV reacts readily with acid chlorides [140].

Treatment of solid substrates with the phosphorylcholine derivatives is relatively simple, but moisture content must be minimized since hydrolysis of compounds I, II and III and acid chlorides used to react with compound IV occurs readily. Confirmation of phosphorylcholine derivative attachment to such surfaces as glass and silica was carried out by infrared spectroscopy and radiolabelling of the derivatives [141]. Preliminary blood clotting test results for materials that have been modified with these compounds suggest that they have improved blood compatibility [211].

Section 2.6.4 Materials with Specific Protein Affinity

Several research groups have suggested surface modification with the objective of promoting interactions with specific proteins in plasma. Work by Munro et al. [144] was based on the hypothesis that albumin, which binds to alkyl chains of free fatty acids will specifically attach to the alkyl chains of functionalized polyurethanes. Along similar lines Grasel et al. [142] attached long alkyl chains covalently to polyurethanes to promote selective adsorption of albumin. Blood interaction studies indicated that polymers modified with alkyl groups having a carbon chain length of 18 showed lower fibrinogen and platelet deposition levels than did the corresponding non-modified polyurethanes. It was suggested that the lower level of fibrinogen adsorption may be due to selective adsorption of albumin [143].

Other groups have designed polymers that can specifically adsorb and thus effectively deactivate coagulation proteins such as thrombin. Using the same technique as described in Section 2.6.2, Boisson et al. attached arginyl residues to crosslinked polystyrene

beads [145], on the basis that AT-III inhibits thrombin and other serine proteases of the coagulation cascade by the interaction of one of its arginyl residues with the serine residue at the active site of the enzyme. In order to obtain non-thrombogenic materials able to specifically bind thrombin, crosslinked polystyrenes substituted with sulfonate and arginyl sulfamide groups were prepared. Thrombin time experiments showed that these functionalized polymers inhibit blood coagulation by high affinity binding of thrombin thus effectively removing it from the fluid phase and masking its active site. This mechanism of anticoagulation is quite different from that of the heparin-like materials previously discussed. For the arginyl polymers the active site on the polymer is non-renewable in the sense that the thrombin is irreversibly bound whereas the heparin-like materials exhibit a catalytic effect on the AT-III/thrombin interaction. The sites are continuously "renewed" when the thrombin-AT-III complex is released. Also the "serine" active site of thrombin adsorbed by heparin-like materials is available for interaction with AT-III [146].

Brash and coworkers [147,148] have shown that when surfaces are incubated with either plasma or solutions of "pure" fibrinogen containing a trace of plasminogen, considerable plasmin-induced degradation of adsorbed fibrinogen occurs. They have suggested that the surfaces may have the ability to facilitate the plasminogen-plasmin conversion. These observations all agree well with evidence that factor XIIa converts plasminogen to plasmin [87]. These studies provide rationale for attaching lysine to polyurethanes since lysine has been successfully used as an affinity ligand for the specific binding of plasminogen onto chromatographic supports [149]. The presence of lysine moieties on polyurethanes could promote the adsorption of plasminogen to the surface and hence have the potential to induce fibrinolysis of incipient fibrin clots.

Section 2.7 Proposed Contribution to Research

Based on the above review of the literature there is much information that would suggest that "functionalizing" polyurethanes with amino-acids, alkyl chains, sulfonate groups or hydrophilic components such as polyethylene oxide may allow the tailoring of specific plasma protein-surface interactions. Such interactions may be useful to improve the blood compatibility of polyurethanes since, as indicated, blood-material interactions are "controlled" by protein adsorption. It is thus the approach of the present work to modify polyurethanes to promote specific protein interactions in contact with blood. It is also of interest to determine the effects of these modifications on the physical properties of the polymers, and hopefully to be able to develop methods in which the attractive properties of the original materials are preserved.

Chapter 3

EXPERIMENTAL

Section 3.1 Materials

Materials used in the synthesis of the base polyurethanes are listed in Table 3.1, and those used for the modified polymers are indicated in Table 3.2. Modification of various synthesis materials and products required the use of other chemical reagents which are listed in Table 3.3.

Section 3.2 Polyurethane Synthesis

This section outlines the experimental procedures for the synthesis of three types of segmented polyurethanes, differing primarily in chain extender type. Although all polymers were prepared by the same general procedure, reaction conditions had to be defined in each case to obtain polymers having suitable mechanical character and in the case of sulfonated chain extenders, to optimize sulfonate content and distribution on the polymer chains.

Section 3.2.1 Solvent Distillation

Due to the reactivity of isocyanates with water and the strongly hygroscopic character of DMSO, it was required to distill the solvent prior to use for the polymer synthesis (within 20 hours). Approximately 700 mL of solvent was distilled for each reaction. The wet solvent was distilled at approximately 45 °C and 1 mm Hg. The distillate was collected in a dry flask,

which was then capped and sealed until required for use. Prior to sealing, the solvent was purged with high purity (H.P.) nitrogen gas that had been dried by passing it through a calcium chloride bed maintained at -5 °C using a salt/ice/water bath.

Table 3.1: Materials used in Polyurethane Synthesis

Material	Supplier	abbreviation
Dimethyl Sulfoxide	BDH Chemical	DMSO
4,4'-Diphenylmethane diisocyanate	Eastman-Kodak	MDI
Polypropylene Glycol M.W. 1025	BDH Chemical	PPO(1000) or PPG(1000)
Polypropylene Glycol M.W. 2025	BDH Chemical	PPO(2000) or PPG(2000)
4,4'-Diamino -2,2'-Biphenyl Disulfonic Acid	Eastman-Kodak	BDDS
Ethylene Diamine	Aldrich	ED
Methylene Dianiline	Sargent Welch	MDA
Sodium Chloride	BDH Chemical	
Potassium Chloride	BDH Chemical	
Methanol	BDH Chemical	
H.P. Nitrogen Gas	Canadian Liquid Air	

Table 3.2: Materials used in Polyurethane Modification Reactions

Material	Supplier	Abbreviation
Oxalyl Chloride	Aldrich	
N,N'- Carbonyl-diimidazole	Aldrich	
Ethylchloroformate	Aldrich	
N,N'- Dimethyl-formamide	Caledon Labs	DMF
L-Arginine	Sigma	Arg
L-Arginine methyl Ester	Sigma	ArgMe
L-Aspartic Acid	Sigma	Asp
L-Leucine	Sigma	Leu
L-Lysine	Sigma	Lys
Glycine	Sigma	Gly
L-Methionine	Sigma	Met
Taurine	Sigma	Tau
Poly(ethylene oxide) methylether (MW=500)	Aldrich	PEOMe
Dodecylamine	Aldrich	DDA
Amino Benzene Sulfonic Acid	Sigma	ABS
5-Amino-2-Naphthalene Sulfonic Acid	Sigma	ANS
5-Amino(2-ethylamino)-1-Naphthalene Sulfonic Acid	Sigma	AENS

Table 3.3: List of Materials used for Analysis and Preparation of Reactants and Products

Material	Supplier	Abbreviation
Sodium Hydroxide	BDH Chemical	
Potassium Hydroxide	BDH Chemical	
Trimethyl Benzyl Ammonium Hydroxide	Aldrich	TBA
Triethylamine	Aldrich	TEA
Concentrated Hydrochloric Acid	Fisher Scientific	
Lithium Bromide	Fisher Scientific	
Optifluor	Packard	

Section 3.2.2 Polyether Degassing

Prior to use in polyurethane synthesis, the polypropylene glycols were degassed at 50 °C and 1 mm Hg pressure for 24 hours. This treatment removes trace amounts of water and undesirable low molecular weight impurities.

Section 3.2.3 Diisocyanate Distillation

MDI was purified by distilling at 170 °C and 1 mm Hg. This procedure removes any dimerized material present in the MDI as received. In preparation for distillation, the MDI was melted at 40°C and atmospheric pressure under dry nitrogen to avoid possible reaction

with ambient moisture. Heating was carried out with care to ensure an even distribution of heat, since elevated temperatures could initiate polymerization of the MDI. Once melted the MDI was placed in the distillation apparatus. Any contact of ambient air with MDI would result in polymerization and therefore the distillation system was maintained under dry N_2 during the transfer of collecting flasks. The first 25 mL of MDI collected was discarded. Because of the high distillation temperature (170°C) and to prevent solidifying in the column it was necessary to wrap the condenser with glass wool and heating tape. The distilled MDI was stored under nitrogen in a sealed bottle at 4°C.

Section 3.2.4 Prepolymer Synthesis

Segmented polyurethane (SPU) synthesis was performed using a two step procedure: (1) the prepolymer formation and (2) the chain extension. A reaction scheme using BDDS as chain extender is shown in Figure 3.1. The reactions were designed in such a manner as to produce between 20 and 50 grams of polymer depending on how much material was required for characterization studies. For the prepolymer preparation in Figure 3.1, a 2:1 mole ratio of MDI:PPO was used. A 20 % w/v solution of MDI in freshly distilled DMSO was added dropwise to a batch reaction vessel containing a 40 % w/v solution of PPG held at 90 °C under a dry nitrogen atmosphere. A diagram of the reactor vessel is shown in Figure 3.2. After reacting at 90 °C for 3 hours with vigourous stirring the prepolymer solution was cooled to 40°C. Reaction temperatures in excess of 90°C would favour undesirable side reactions such as allophanate and biuret formation. Prepolymers were also prepared using 3:2 and 4:3 molar ratios of MDI:PPO. These stoichiometries result in prepolymers of greater average chain length.

Figure 3.1: Polyurethane Synthesis (using MDI, PPO and BDDS)

STEP 1

STEP 2

Figure 3.2: Batch Reactor for Polyurethane Synthesis

Section 3.2.5 Chain Extension of Prepolymers

The second step of the polyurethane synthesis is the chain extension of the prepolymer. Chain extension using ethylene diamine (ED) and methylene dianiline (MDA) proceeded smoothly.

The amount of chain extender used was based on a 1:1 mole ratio of chain extender to prepolymer, assuming complete conversion of reactants in the first step. To the cooled reaction mixture from the prepolymer synthesis (25°C), ED or MDA solutions (7.5 w/v in freshly distilled DMSO) are added dropwise while stirring. As the reaction progresses the mixture becomes very viscous. This reaction is usually complete within 15-20 minutes. The polymers are then precipitated in distilled water and washed several times to remove any remaining DMSO. The washed polymer is then chopped in a Waring blender and dried in a conventional forced air oven at 60°C for 72 hours.

The chain extension procedure using the sulfonated chain extender BDDS was complicated by its low solubility in the reaction solvent and by the solubility properties of the resulting polyurethane. In the acid form as received from Eastman Kodak, BDDS was completely insoluble in DMSO. Conversion of BDDS from the acid form to a more soluble form such as the sodium or potassium salt was therefore required. Technical grade BDDS (80 % pure) as received was purified by first washing with hot water and then filtering to remove any water soluble contaminants. This procedure was repeated until the filtrate was clear. The filter cake was then collected and titrated with an aqueous sodium hydroxide or potassium hydroxide solution. The end point of this titration is easily detected since the acid form is insoluble in water while the Na^+ or K^+ forms are soluble in water. The resulting solution was then filtered to remove any particulate matter, and concentrated. The Na- or K-BDDS was precipitated by the addition of acetone. The final BDDS material was filtered and washed

with cold acetone, then dried. The sodium salt of BDDS is a powder and is highly soluble in DMSO. The potassium salt of BDDS is obtained as a coarse material with moderate solubility in DMSO. In addition to improving the solubility of the chain extender in DMSO this procedure reduces the chance of reaction between sulfonate and isocyanate groups during chain extension, since sulfonic acids have a higher reactivity towards isocyanates than their sodium or potassium salts. Also, a low solubility of BDDS in the solvent would limit the concentration of amine groups available for reaction with isocyanates and would therefore favour isocyanate reaction with sulfonate groups.

To obtain a polymer in which most of the sulfonate groups remain free, the chain extension was carried out using the sodium salt of BDDS at a concentration such that it dissolved completely (3 grams /100 mL). Dissolving BDDS in DMSO can take up to 12 hours and therefore must be done the day prior to the synthesis. For chain extension, the BDDS solution is added to the cooled prepolymer mixture (40°C) and allowed to stir overnight at 25°C, under nitrogen gas. Polymers containing various ratios of total sulfur to free sulfonate content were obtained by limiting BDDS concentration or adding additional MDI after the chain extension step.

Precipitation of sulfonated SPUs in the usual precipitant, water, is difficult because the polymer solution may form an emulsion rather than precipitating as do the ED and MDA-extended polymers. This problem can be overcome either by the addition of KCl or NaCl to water or by using methanol as opposed to water for precipitation. Once precipitated, the polymer is washed with either dilute aqueous NaCl or pure methanol. The polymer is then dried in a convection oven at 60°C, over a period of 48 hours. Reaction yields are usually lower for the highly sulfonated materials because of material loss in the precipitation and washing steps.

Section 3.3 Hard Segment Modification

Covalent attachment of amino-acids and other molecules to sulfonated SPUs was achieved by a two step reaction. A typical two step reaction using oxalyl chloride is:

Step 1: $(COCl)_2$ + SPU--SO_3Na ——> SPU—SO_2Cl

Step 2: SPU--SO_2Cl + $H_2NCHRCOOH$ —> SPU--$SO_2HNCHRCOOH$

R= amino-acid side chain SPU= segmented polyurethane

Other sulfonate conversion reagents are listed in Figure 3.3.

Figure 3.3: Sulfonate Conversion Reagents

Oxalyl Chloride

N,N' Carbonyldiimidazole

Ethylchloroformate

Reaction of sulfonates with oxalyl chloride and ethyl chloroformate gives sulfonyl chlorides which are highly reactive with amine groups. The reagent N,N' carbonyldiimidazole, forms a sulfonylimidazole group which is also subject to attack by amine groups. These three compounds are common peptide forming reagents and their use in modifying carboxylate groups is well documented [151-153]. However, no previous documentation on their use with sulfonates has been found. In this respect the synthetic chemistry presented here is considered novel. All three of the sulfonate conversion reagents are highly reactive with water and therefore the reaction solvent, dimethylformamide (DMF), must be distilled within 24 hours of the reaction. Also the sulfonated polymer and the compound being covalently attached to the polymer must be dried prior to use. The two step addition reaction is carried out using the apparatus shown in Figure 3.4.

A typical amino-acid attachment reaction is carried out as follows. Sulfonated SPU is dissolved in distilled DMF at a concentration of 5 g/100ml (vessel A in Figure 3.4). Sulfonate conversion reagent (in stoichiometric amount relative to sulfonate groups) is added to the polymer solution and is stirred under a very dry nitrogen atmosphere for 4 hours at -5°C. This temperature is maintained using an ice/water/NaCl bath. It is important to note that the polymer solution must be cooled prior to the addition of the conversion reagent. The reaction mixture evolves CO and/or CO_2 and therefore precautions must be taken to release the pressure in the reactor vessel upon addition of the reagent.

Figure 3.4: Apparatus for the Addition of Amine Containing Compounds to Sulfonated SPUs

During the conversion of sulfonate to sulfonyl chloride, the compound to be covalently attached is dissolved in DMF (1 % w/v) in a second reactor (vessel B in Figure 3.4) and stirred under dry nitrogen at room temperature. After 4 hours the contents of vessel A are transferred to vessel B by aspiration. The reaction between the amine groups of the molecules being attached and the sulfonyl chloride groups of the polyurethane proceeds under a dry nitrogen atmosphere for 15 hours at 20°C. After stirring overnight the resulting polymer is precipitated in distilled water. In some cases, the polymer remained partially dissolved in the water-DMF mixture. In these cases the polymer still 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 an air convection oven for 48 hours. Reaction yields between 80 and 95 % were typically achieved.

Section 3.4 Polymer Nomenclature

The nomenclature used in this thesis to identify the various polyurethanes is based on a sequence of alternating letters and numbers which identify the chain extender type, the polymer sulfur content, the fraction of sulfur in the free sulfonate form, the compound being attached to the sulfonate groups and the percent conversion of free sulfonate to sulfonamide.

All of the polymers investigated are based on MDI and PPO of molecular weight 1025. Therefore no specification of these materials is required in the polymer nomenclature. The abbreviations for the chain extenders and for the compounds that were covalently attached to the sulfonate groups are given in Tables 3.1 and 3.2 respectively. An example of polymer nomenclature is shown in Table 3.4.

Table 3.4: Polymer Nomenclature

E.g. BDDS-1.4(54)-Asp(92)

BDDS:	Polymer is chain extended with Biphenyl Diamino Disulfonic Acid
1.4 :	The polymer contains 1.4 wt % sulfur
54 :	54 % of the sulfur is in the free sulfonate form
Asp :	Sulfonate groups were converted to aspartic acid sulfonamide
92 :	92 % of the free sulfonate groups were converted to sulfonamide-aspartic acid residues

Section 3.5 Chemical Analysis

Several analytical techniques were used for chemical characterization of the polyurethanes synthesized in this work. These techniques included titration of sulfonate and carboxyl groups, Scanning Electron Microscopy (SEM) with Energy Dispersive Analysis of X-rays (EDAX) for elemental analysis, conventional elemental analysis and carbon-14 radiolabelling.

Section 3.5.1 Titration

Titration analysis was used to verify the presence of free sulfonate and carboxyl groups and to quantify amino-acid substitution in some of the sulfonated SPUs by measuring residual sulfonate.

In all cases, acidification of the polymer was required prior to titration. This was done by treating a solution of the 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.

Titration of the polymer was carried out in DMF containing 0.10 M LiBr. The titrant was 0.025 M triethylamine (TEA) in DMF and was calibrated by titration with an aliquot of a 0.1 M solution of HCl in DMF. The polymer titration endpoint was monitored using a potentiometer. Some polymers had very long equilibrium times and required up to 24 hours to complete a titration. A typical titration curve for a sulfonated SPU is shown in Figure 3.5.

Section 3.5.2 Scanning Electron Microscopy with Energy Dispersive Analysis of X-rays (SEM-EDAX)

Investigation of possible analytical techniques for the elemental analysis of sulfur and the counter ions, sodium and potassium, led to the use of SEM-EDAX. Conventional SEM has the capability of analysing polymer films for surface morphology. When the SEM is equipped with a spectrometer capable of detecting X-rays emitted by a specimen during electron beam excitation, then quantitative elemental analysis is possible because the intensity of the emitted radiation is proportional to the concentration of the element being detected. Such an analysis is termed Quantitative Energy Dispersive Spectroscopy (QEDS) [154]. The element being identified is characterized by the energy of the X-ray being detected and this energy is specific for the K,L,M shell electrons for an individual atom. Elements of atomic mass greater than sodium can be observed. Elements below this mass cannot be detected under normal laboratory conditions since the instrument cannot provide enough energy to release outer shell electrons without destroying the sample. The lower limit of detection is about 10^{-16} g [154]. Figure 3.6 shows the principle of SEM-EDAX.

Figure 3.5: Titration of BDDS-1.4(54)

Titrant: Triethylamine
Solvent: Dimethylformamide
Temperature: 25°C

Figure 3.6 Principle of SEM-EDAX [203]

For quantitative analysis the system must first be calibrated with a sample of known elemental composition. In the present work, calibration was based on "standard" polyurethane samples of known sodium, potassium and sulfur content. The SEM-EDAX samples were prepared by casting polymer films onto 5 mm square glass slides from solutions (5 % w/v) of polymer in DMF. After casting, the films were dried in a moisture-free environment for 24 hours. This casting procedure was repeated three to four times to achieve the required film thickness. The coated slides were then placed in a vacuum oven at 60°C for 24 hours. It was found that oven drying should be carried out within 48 hours of the analysis to avoid moisture uptake.

The coated slides were then mounted onto aluminum stubs using silver paint, which must touch the polymer film. This process decreases excessive heat build-up within the sample during analysis since the silver paint acts as a conducting energy "drain"; otherwise the sample would likely be destroyed. The samples were then carbon coated by vacuum evaporation to reduce charging of the surface during analysis. Samples were kept in a desiccator until the time of analysis.

The SEM-EDAX system, located in the Life Science Building of McMaster University, is an ISI-DS130 model and was operated under the following conditions: large spot size; 15° take-off angle; sample height of 30 (arbitrary scale); electron beam voltage, 15 KV. For each sample a total of 5 different "spots" were analysed. Figure 3.7 shows the EDAX spectrum of a typical sulfonated polyurethane sample. The most prominent peaks in Figure 3.7 are those of sulfur and potassium.

Section 3.5.3 Conventional Elemental Analysis

Conventional elemental analyses for sulfur, sodium, potassium and nitrogen were performed by Guelph Chemical Laboratories (GCL), Guelph, Ontario. Sulfur was analyzed by a combustion method in which the sample is burned in an oxygen flask and the resulting sulfur dioxide is absorbed in a hydrogen peroxide solution. Residual hydrogen peroxide is then titrated with barium perchlorate to determine wt % sulfur. Nitrogen was analyzed by a Perkin-Elmer C,H,N elemental analyzer with a thermal conductivity detector. Samples analyzed for sodium and potassium counter ions were first digested in an acid mixture and then analyzed by atomic absorption spectroscopy.

Figure 3.7 SEM-EDAX Analysis for polyurethane BDDS-1.0

Samples for these analyses were prepared by dissolving 2 g of polymer in 40 mL of DMF. The solutions were then centrifuged to remove insolubles and the polymers precipitated in water or methanol, followed by washing and vacuum drying. This procedure minimizes residual DMSO, unreacted chain extender and salts from the precipitating medium. DMF is a more volatile solvent than DMSO, and should be more completely removed than DMSO in the vacuum drying step.

Section 3.5.4 Carbon-14 Labelling

Carbon-14 labelling experiments were carried out to verify the composition of a few of the amino acid containing polymers. In these experiments C-14 labelled amino-acids were used for polymer synthesis using a scaled down version of the apparatus in Figure 3.5. The resulting polymers were then dissolved in DMF (1.0 g/ 100 mL) and aliquots of 100 uL, 300 uL and 500 uL of solution were mixed with a liquid scintillation cocktail (OPTI-FLUOR). The radioactivity of the solutions was determined using a Philips model PW4700 liquid scintillation counter. In order to determine the amount of amino-acid incorporated in the polymer, the solution count was compared to a series of standards made up from the labelled amino-acid.

C-14 labelled L-aspartic acid and L-lysine were purchased from Aldrich and received in small vials containing 34 ug of amino-acid with a radioactivity of approximately 50 uCi. The synthesis procedure was designed for a minimum of approximately 0.1 g of material. Also a sample count of about 6000 cpm was required for acceptable counting accuracy in relation to the background count of 500 cpm, characteristic of the scintillation counter. These conditions required that the labelled amino-acid be mixed with unlabelled amino-acid so that sufficient material would be available for the synthesis. At the same time a sufficiently high

count rate had to be obtained for the labelled polymer. The amino-acid mixture preparation for L-lysine was carried out as follows: (1) Weigh 0.5 g of unlabelled amino-acid into a test tube. (2) Add 3 mL of distilled water to the amino-acid. (3) Use the above solution to wash the contents of the labelled amino-acid vial into a crystallization dish. (4) Place the dish under vacuum (water tap) until crystallization is complete (about 1 week). (5) Place the mixture in a well sealed vial until required. For L-aspartic-acid, the same procedure was used except that 150 mL of distilled water was required to dissolve 0.5 g of amino-acid and crystallization required four weeks.

Experiments were also conducted to verify that the presence of unlabelled polymer and DMF did not cause quenching during the measurement of radioactivity. The results for this experiment are presented in Table 3.5. The data show that only a slight amount of quenching occurred and therefore the sample polymer solutions can be directly compared to standard solutions of the pure labelled mixture of amino-acid in Opti-Fluor. Labelled Lysine was used in these tests.

The standard solutions for the amino-acids were made by dissolving 0.0115 g of the labelled mixture of amino-acid in 20 mL of water and adding ethanol to complete the volume of a 100 mL volumetric flask. Appropriate aliquots of these solutions were counted to construct the calibration curve. The aliquots were placed in a scintillation vial and the solvent allowed to evaporate prior to adding the Opti-Fluor and counting.

Section 3.6 Physical Characterization of Polyurethanes

Physical characterization of the polyurethanes synthesized in this work was carried out using gel permeation chromatography (GPC) and intrinsic viscosity measurements, conventional tensile testing, differential scanning calorimetry (DSC) and measurement of water uptake.

Table 3.5: Evaluation of DMF/Polymer Quenching Effect

Sample	Sulfonated SPU Concentration (gmL^{-1})	Volume of SPU solution (uL)	Solution Count (CPM)	Counting Efficiency
Amino-acid	.1	10	6054	84.1
+ SPU in DMF	.02	50	6005	83.5
	.01	100	6059	83.2
	.002	500	4823	81.4
	.001	1000	6212	77.6
Amino-acid	---	---	6191	83.8
	---	---	5674	83.9
	---	---	5841	84.1

Note: All samples had the same amount of amino-acid
Counting efficiency is a measure of quenching effect

The presence of sulfonates or amino-acid sulfonamide groups in polyurethanes is expected to disrupt the microphase separated structure characteristic of conventional polyurethane-urea materials. The objective of the studies described in this section was to provide information leading to an understanding of the molecular chains and the internal molecular arrangement within the modified polymers.

Section 3.6.1 Gel Permeation Chromatography (GPC)

Gel permeation chromatography is the term for size exclusion chromatography (SEC) which deals with synthetic polymers. SEC is a liquid column chromatographic technique which sorts molecules according to their size. The sample solution is introduced onto the column, which consists of a rigid, porous particle packing, and is carried through the column

by a solvent (the mobile phase). Size sorting takes place by repeated exchange of the solute molecules between the bulk solvent of the mobile phase and the stagnant liquid phase within the pores of the packing. The pore size of the particles determines the molecular size range within which separation occurs [155]. Data are obtained in the form of a chromatogram which is a recording of polymer concentration versus eluent volume. The polymer molecules elute from the column in order of decreasing size.

GPC was used in the present work as an analytical procedure for obtaining number average molecular weights ($\overline{M}_n$) and weight average molecular weights ($\overline{M}_w$) of polyurethanes, defined as follows:

3.1 $$\overline{M}_n = \frac{\sum N_i M_i}{\sum N_i} = \frac{\sum h_i}{\sum (h_i/M_i)}$$

3.2 $$\overline{M}_w = \frac{\sum W_i M_i}{\sum N_i} = \frac{\sum h_i M_i}{\sum h_i}$$

3.3 Polydispersity = $\overline{M}_w/\overline{M}_n$

W_i and N_i are the weight and number of molecules of molecular weight M_i, respectively, and "i" is an incrementing index over all molecular weights present. "h_i" is the GPC chromatogram height at the ith volume increment or retention time of the GPC chromatogram. The polydispersity index gives an indication of the width of the distribution of polymer molecular weight in the sample. Using these relations, molecular weight data can be obtained from a chromatogram using standard samples to correlate molecular weight increment with retention volume increment.

A description of the GPC system used in this work is given in Figure 3.8. The system consists of: (1) a reservoir to contain the mobile phase (0.1 M LiBr in filtered DMF), (2) a

high pressure solvent delivery system (Waters M6000 A pump, delivering 1 mL/min), (3) a Rheodyne 7125 sample injector and a precolumn filter (2 um pore size), (4) a thermostatted oven for the columns, (5) four ultrastyragel columns with three different pore sizes: 10^3 Å, 10^4 Å (2 columns) and 10^5 Å, (6) a Waters temperature controller (set at 80.0°C), (7) a differential refractive index detector (Water R-401), (8) a Zenith 158 computer with high-resolution graphics, a data acquisition board for analog to digital conversion and an Epson LX-80 printer for chromatogram output, and (9) a solvent waste tank.

The operating conditions are very specific for the type of polymer being analyzed. The system developed for polyurethanes used a mobile phase of 0.1 M LiBr in DMF (HPLC grade) and an operating temperature of 80.0°C. The sample loop size was 200 uL and the sample concentration was approximately 0.2 g/100 mL. The flowrate was set at 1 mL/min and a typical chromatogram had a completed retention volume of 40 mL. In order to reduce maintenance and extend the column life, GPC solvent was filtered through Millipore FHUP 04700 0.5 um teflon filters prior to use. The teflon filters are DMF resistant but require prewetting with acetone prior to use. All polymer samples were filtered using Millex-SR 0.5 um filter units prior to injection.

DMF was chosen as the GPC solvent on the basis of its generally good solvent capability with respect to polyurethanes. These materials are completely soluble only in organic-polar solvents. Among the possibilities of DMF, DMSO and dimethylacetamide (DMAC), DMF was chosen as the most economical solvent available in high purity form.

With DMF as solvent, it was found that lithium bromide was required as an additive [156,157]. In the absence of LiBr the polyurethanes tended to form microgel particles which eluted at short retention times, thus yielding erroneously high polydispersity values. LiBr disperses the microgel particles and returns the polymer to its linear molecularly dispersed form. While several papers have been published on the effect of LiBr on various polymers [158,159], the actual mechanism of its effect is still disputed. For polyurethanes it is believed

that the salt reduces the degree of hydrogen bonding between the polymer chains and thus changes the hydrodynamic volume of the polymer.

Figure 3.8: Schematic of GPC System (see text for explanation of numbered items)

The column bank is kept at 80.0°C to reduce the viscosity of the solvent. This allows the pump to operate at a flowrate of 1 mL/min using a reasonable operating pressure (1000 psi).

The GPC calibration curve was constructed using polystyrene standards and was plotted in the form of a universal calibration curve. The universal calibration method utilizes the concept of the hydrodynamic volume of polymer molecules. The hydrodynamic volume can be expressed in terms of the product of the molecular weight, M, and the intrinsic viscosity, [η], of the polymer. In general, GPC calibration curves for polymers of different types merge

into a single plot when the calibration data are plotted as log [η]M versus retention volume, as is illustrated in Figure 3.9.

Figure 3.9: Universal Calibration Curve for GPC of Synthetic Polymers [155]

Solvent: THF. ●, PS; ○, PS (comb.); +, PS (star); △, heterograft copolymer; x, poly(methacrylate); ○, poly(vinyl chloride); ▽, graft copolymer PS/PMMA; ■, Poly(phenyl siloxane); □, polybutadiene;

From measurements of the intrinsic viscosity along with GPC data and the universal calibration estimated values of the molecular weights of the polyurethanes synthesized in this work were obtained. This approach is valid for polymers of different structures that elute at the same retention times because they have the same hydrodynamic volume and radius of gyration. Intrinsic viscosity measurements were carried out using a standard Ubbelohde viscometer at 80°C. Five solutions of the desired polymer in GPC solvent at concentrations

of 0.2, 0.4, 0.8, 1.2 and 1.6 g/100 mL were used. Three flow measurements were taken for each solution and the values averaged. A 20 minute equilibration time was allowed for the sample to reach 80.0°C in a water bath prior to taking measurements.

GPC data acquisition and data analysis were carried out using a data station which combines the features of the Zenith computer, the data acquisition board (Data Translation DT2805 Analog to Digital System), the screw terminal (Data Translation DT707) and the ASYST 1,2,3 software for graphics, statistics, analysis and acquisition. The complete software system has the following features:

1) Data can be collected from the GPC differential refractive index detector and a hardcopy of the chromatogram provided upon demand.
2) The GPC baseline can be adjusted to zero. The baseline is monitored by graphics and by voltage readout on the screen.
3) Data can be stored in files for future use. One can also store characteristic comments on the sample printout: date of GPC run, flowrate of solvent, solvent type, temperature, attenuation, sample name, operator and concentration of the sample.
4) Analysis of data can be done for Universal molecular weight or polystyrene equivalent molecular weight. This information is also provided in the form of a hardcopy.
5) Computation of a new calibration curve for standards.
6) Option to correct for baseline drift.
7) Data smoothing capabilities and graphics for the simultaneous comparison of GPC chromatograms.
8) The entire program is interactive and the different options are entered using the F-keys of the computer keyboard.

An output for the analysis of BDDS-1.4 is shown in Figure 3.10 and the program is discussed in Appendix A. The polymer peak is on the left of the chromatogram while the two inverted peaks result from the presence of LiBr and water in the system.

Figure 3.10: GPC Chromatogram Data for BDDS-1.4(54)

Mw = 67,000
Mn = 51,000
D = 1.32

Date: May 30, 1988.
Flowrate: 1.0 ml/min
Solvent: 0.1 M LiBr in DMF
Temperature: 80.0°C
Attenuation: 4X
Sample: BDDS-1.4 (May 1988)
Operator: Paul Santerre
Concentration: 0.2 g/100ml

Section 3.6.2 Tensile Stress-Strain Measurements

Tensile stress versus strain data for SPU materials were obtained using an Instron Tensile Testing System, Model TT-D (Department of Mechanical Engineering, McMaster University). Films of polyurethane samples were prepared from a 5 % w/v solution in DMF. Multiple layers were cast into teflon molds in order to provide films of adequate thickness for testing according to the ASTM D412-80 standard. The film molds were placed in a dry glovebag during the entire casting procedure to avoid the presence of moisture which would interfere with solvent evaporation, and could compromise the formation of smooth and even films. Figure 3.11 depicts the film casting arrangement.

Figure 3.11: Dry Air Glovebag for Solution Casting of Polyurethane Films

After casting, solvent was allowed to evaporate for 48 hours before the next application of the polymer solution. Casting was continued until films of approximately 0.5 mm were obtained (8-9 applications). The final films were dried in an air oven at 60°C for 24 hours and then in a vacuum oven at 25°C for an additional 24 hours. Samples for mechanical testing were cut using an ASTM dumbbell die (size D) according to the ASTM D412-80 code. A 10 lb load cell and a strain rate of 1 inch/min were used in these measurements.

Section 3.6.3 Differential Scanning Calorimetry (DSC)

DSC studies of thermal transition behaviour were carried out for all polyurethane samples using a Dupont Instruments 910 differential scanning calorimeter. Samples for DSC experiments were obtained from the untested films prepared for the tensile stress-strain measurements, thus making a comparison between mechanical and thermal transition data more meaningful.

Sample sizes were approximately 10 ± 2 mg. The specimens were scanned from -100°C to 250°C at a rate of 5°C per minute. The samples were initially cooled to -100°C using liquid nitrogen. On reaching 250°C, the samples were rapidly cooled to room temperature, then cooled again to -100°C prior to making a second scan. This second scan allows one to observe the internal structure of the material after its structural features have been relaxed by heating. It could possibly yield information on the rate of reformation and rearrangement of the polymer microstructure upon temperature perturbations.

A typical DSC curve for a sulfonated polyurethane is shown in Figure 3.12. Several transitions can be detected. The first is the glass transition temperature for the soft segment matrix of the polymer (occurs at about -20°C in Figure 3.12), the second transition is another secondary transition and is due to specific intermolecular interactions in a localized area of

the polymer matrix. The transition is not as sharp as the glass transition and can extend over a broad temperature range (occurs between 80°C and 150°C in Figure 3.13). The last transition results from hard segment crystalline melting which is a primary transition. In Figure 3.13 this transition occurs near 211°C. Since none of the structures corresponding to these transitions is 100 % pure the transitions are not sharp and usually occur over a range of temperatures. There is no accepted convention on whether a range of temperatures or a temperature within that range is reported. In this study we report the Tg as a temperature range. The presence of the phase transitions between the glass transition temperature and the hard segment melt temperature is reported as the midpoint of the transition and the hard segment melt transition is identified by the onset temperature of the transition.

Section 3.6.4 Water Uptake Studies

Most of the polymers developed in this project contain functional groups which enhance their water solubility or water swelling capacity. The corresponding classical polypropylene oxide based polyurethanes are water insoluble and have a low water uptake capacity.

The investigation of water solubility and water uptake behaviour was carried out to provide an indication of the bulk hydrophilic character of these polymers and thus to allow inferences to be drawn about the intermolecular structure and morphology of the materials in an aqueous environment.

Figure 3.12: DSC of BDDS-1.4(54)

Water uptake experiments consisted of equilibrating strips of cast SPU films in water. The films were taken from the untested samples used for mechanical and DSC studies. Approximately 1 g of polymer film was weighed, accurately, and cut into 5 mm wide x 3 cm long strips. The samples underwent water uptake for 30 days at 25°C, after which they were removed from their vials, filtered and blotted to remove adherent water and weighed. These swollen samples were then further cut into 2 mm wide x 1 cm long strips and placed in a sample vial filled with water. The vials were placed in an oven for 2 days at 70°C. The polymers were then cooled and held at room temperature for 3 days prior to filtering and weighing again. The data were recorded as weight percent water uptake.

Section 3.7 Functional Group Characterization

Functional group characterization in polyurethanes is a very difficult task because of the wealth of functionality they contain. The incorporation of sulfonate, sulfonate anhydride, sulfonamide and amino-acid groups complicates the task enormously. In the present work Fourier Transform Infra-Red (FTIR) spectroscopy was used to provide a measure of the incorporation of various chemical functions into SPU materials.

Polymer films were cast onto a teflon plate using a 0.5 wt % solution of polymer in DMF. Single layer casting was sufficient to produce films of suitable thickness (20 um) for analysis. Following casting, the solvent was allowed to evaporate for 24 hours at room temperature. The films were then further dried in an air oven at 60°C for 24 hours, peeled off the teflon sheet and mounted into the I.R. sample cell for analysis.

The resulting spectra show absorptions corresponding to transitions occurring in the microphase structure of the polymer as well as those characteristic of the functional groups. The bands representative of the incorporated functional groups are in the carbonyl region from 1600 cm^{-1} to 1750 cm^{-1}.

Section 3.8 Surface Characterization

Since it is the surface of a biomaterial which interacts with the bioenvironment one must apply surface analysis techniques to gain knowledge on the type of chemical structures present at the surface. In particular, in the present work it was important to determine whether the functional groups incorporated into the polymers to improve their blood compatibility were present on the surface as well as in the bulk of the materials.

Section 3.8.1 Electron Spectroscopy for Chemical Analysis (ESCA)

ESCA can be used to determine the concentrations of all elements except hydrogen and helium in the surface region (approximately the top 100 Å) of a solid specimen [160]. In ESCA an X-ray beam is used to induce photoelectron emission from 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 chemical species. Atomic concentrations can be determined with a relative accuracy of about 10 %.

ESCA was performed on the polyurethanes of this work at the National ESCA and Surface Analysis Center for Biomedical Problems (NESAC/BIO) located at the University of Washington, Seattle, Washington, USA. 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 between 150 and 1000 um diameter.

ESCA measurements were performed at a series of take-off angles to determine if a compositional gradient existed near the surface of the materials. The take-off angle controls the depth in the sample from which photoelectrons reach the detector (see Figure 3.13). Moving from low to high take-off angles reduces the depth at which electrons escape from the surface, thereby increasing the surface sensitivity of the measurement. This is illustrated

in Figure 3.13.

A low resolution/high sensitivity spectrum of each element present was obtained over the range of take-off angles from 0 to 80°. Intermediate resolution spectra of the carbon 1s peak were used to detect the presence or absence of carbamate and hydroxyl/ether groups.

ESCA samples consisted of polymer films coated on glass microscope slides. The films were prepared from approximately 5 % w/v solutions in DMF by spreading the solution on the surface of the slide, drying in a fumehood for 24 hours, and then in a vacuum oven at 60°C for 24 hours.

Figure 3.13: ESCA Angular Emission Geometry [53]

d = sampling depth
d cos θ = effective sampling depth
θ = takeoff angle relative to the vertical
ϵ = sample angle relative to the vertical

Section 3.8.2 Contact Angle Measurements

Water is a key constituent of all biological environments. Therefore it seemed appropriate to evaluate the polymer surfaces in an aqueous environment. This was done by measuring water contact angles, which provide information on the surface hydrophilicity of the materials.

Contact angle measurements were carried out using sessile drop and captive bubble techniques developed by Neumann [161,163] and were done at the University of Toronto. Goniometer measurements were made using deionized, double distilled water. For the sessile drop measurement, water droplets of about 10 uL in volume were delivered to the surface of the polymer films with a micropipette. In the case of the captive bubble techniques the surfaces were exposed to water for two different times, 1 minute and 24 hours, prior to the placement of the air bubble on the surface. In both the sessile drop and captive bubble methods the contact angle was measured by placing the cross hairs of a telescope eyepiece at the point where the drop or bubble, polymer surface and air meet (known as the triple point), and reading the angle from the goniometer mounted on the telescope [162]. An average of 30 drops were measured for each surface and two surface specimens were prepared for each polymer sample.

The polyurethane surfaces were prepared by a dipcoating technique. The chromerge cleaned glass slides were first coated with polymer ED by solution coating. The films were dried in a vacuum oven at 60°C for 24 hours, and then dipcoated with the test polyurethane solution and also dried in a vacuum oven at 60°C for 24 hours. Prior to taking measurements the samples were allowed to cool to room temperature.

The coating solutions used for coating the slides were made up to a concentration of approximately 2.0 g/ 100 mL in DMF. The solutions were then filtered through a 0.5 um millipore Millex-SR filter.

Section 3.9 Blood Interactions

Two methods of assessing blood interactions were used: (1) protein adsorption experiments in which the binding of proteins to the surface was studied and (2) clotting tests which give an indication of blood coagulation response to the materials.

Section 3.9.1 Fibrinogen Adsorption from Plasma

Fibrinogen adsorption is known to be involved in the early stages of blood-material interactions and the amount of fibrinogen adsorbed from plasma may give an indication of the blood compatibility of a material. In addition to developing this information for the materials of the present study it was of interest to determine how the various functional groups incorporated into the polyurethanes would influence fibrinogen adsorption, and to try to correlate any such influences with blood compatibility as assessed by other measures. The objective of the fibrinogen adsorption studies in the present research was to determine whether fibrinogen adsorbs to functionalized polyurethanes in the same manner as to classical polyurethanes and other reference surfaces (e.g. glass, Biomer, etc.). Adsorption experiments were carried out using polymer coated glass tubes Glass tubing segments of 2.5 mm I.D. x 20 cm long were used as substrates for SPU coating. The tube segments were immersed in a chromic acid cleaning mixture (Chromerge) for 3 hours and then rinsed three times with double distilled water. The tubes were then dried overnight at 60°C in a vacuum oven.

Polymer solutions were controlled to an approximately constant viscosity (corresponding to approximately 5 % w/v in DMF) for dip coating. The clean, dry glass tubes were dipped in the polymer solution, inverted and dipped again, then placed vertically in a drying rack and allowed to stand for 1 hour in a fumehood, 1 hour in an air oven at 60°C and 3 hours in a vacuum oven at 60°C. The tubes were then coated a second time using the same process.

Using this four dip technique, the tubes were first coated with a "base" coat of a hydrophobic, ethylene diamine chain extended polyurethane. Two layers of the polymer of interest were then coated over the base polymer in the same fashion. A base coat of hydrophobic polymer was required since it adheres well to glass whereas most of the functionalized polymers are hyrophilic and tend to peel away from the glass when placed in contact with water, a buffer solution or plasma. The adhesive bond between the hydrophobic and functionalized polyurethanes was good.

The plasma used in these experiments was pooled, human, platelet poor plasma prepared from blood anticoagulated with citrate (6 volume blood: 1 volume citrate) from the Canadian Red Cross, Ottawa, Ontario. Grade L human fibrinogen was obtained from Kabi, Stockholm, radiolabelled with ^{125}I using the iodine monochloride method [164] and added to the plasma in amounts not exceeding 10 % of normal fibrinogen concentration (about 3 mg/mL). Details of radiolabelling technique are given in Appendix D. Solution counts ranged between 10^3 and 10^6 CPM/mL depending on solution concentration.

The experimental procedure is based on previous work in this laboratory and consists of determining the quantity of fibrinogen adsorbed to the polymer surface during five minutes of contact with plasma. Adsorption was studied as a function of plasma dilution in the range of 0.5 % to 30 % of normal plasma concentration. The plasma was diluted with isotonic tris buffer, pH 7.4. These data can be used to construct a plot of fibrinogen adsorbed in five minutes versus plasma concentration, and provide an indication of the plasma response to the polymer surface.

The experimental procedure is shown in Figure 3.14. All experiments were performed under static conditions at room temperature. The coated tubes were equilibrated overnight with isotonic tris buffer which was then displaced by the labelled and diluted plasma, using the 3-way valve system shown in Figure 3.14 which avoids the introduction of any air bubbles into the test segment. The buffer was displaced with at least 20 tube volumes of the

labelled plasma. The final volume of plasma then remained in the tube for a predetermined time (5, 10 or 180 minutes). Following adsorption, the labelled plasma was displaced with 20 tube volumes of buffer. The buffer was left in contact with the surface for 10 minutes and was then displaced by an additional 20 tube volumes of buffer. After 15 minutes of contact this buffer was drained from the tubes.

The rinsed tubes were then cut into segments for counting. A 2 cm segment was removed from each end of the tube and discarded. The remainder of the tube was cut into four sections, each of 4 cm in length, and counted for 10 minutes in a Beckmann Biogamma counter. At the same time, a "solution" count of the labelled, diluted plasma was done (0.1 mL diluted plasma + 0.9 mL buffer).

The surface concentration (Γ in units of ug/cm^2) of adsorbed fibrinogen was calculated using the equation: $\Gamma = (Csu \times F \times 1000)/ (A \times Cpl)$. Csu is the net surface count (CPM), F is the fibrinogen concentration in diluted plasma (mg/mL), A is the surface area (cm^2) and Cpl is Solution count (CPM/mL of diluted plasma). A single pool of plasma was used for all these experiments. The fibrinogen concentration of the plasma pool was determined by the clinical hematology lab, McMaster University Medical Center, and reported to be 2.68 g/L. The surface area was calculated from measurements of the coated tube length and I.D.. The net surface count is the total surface count minus the background count. This difference is multiplied by a geometric scaling factor for glass tubing segments (determined to be 1.06) which accounts for the gamma radiation absorbed by the glass and the difference in counting geometry for the glass tubes and the reference solution count.

Some polymer surfaces were checked for preferential adsorption of labelled versus unlabelled fibrinogen by adsorbing fibrinogen from solutions all having a concentration of 1 mg/mL containing, 90 %, 50 % and 10 % labelled fibrinogen respectively. The procedure was identical to that followed for the plasma studies, except that a 3 hour adsorption time was used to allow for equilibrium adsorption to occur.

Figure 3.14: Experimental Procedure for Fibrinogen Adsorption Measurements

Section 3.9.2 Fibrinogen Adsorption from Tris Buffer

Data on fibrinogen adsorption from plasma indicated that specific interactions between fibrinogen and sulfonate groups in the polyurethanes might be occurring. To investigate this possibility equilibrium fibrinogen adsorption data were obtained using solutions of pure fibrinogen in tris buffer. The procedure was the same as described above for the plasma studies. The protein concentration range was 0.0005 to 1 mg/mL and an adsorption time of three hours was used to allow equilibrium adsorption to be approached.

Section 3.9.3 Thrombin Time Measurements

The standard thrombin time test consists of measuring the time for a clot to form in plasma after the addition of a specific concentration of thrombin. The clotting time of normal plasma in the presence of a standard concentration of thrombin is constant and any increase in thrombin time can be attributed to abnormal fibrinogen function or to anticoagulant activity in the plasma. This experiment is particularly sensitive to the fibrinogen to fibrin step of the clotting pathway.

Thrombin time experiments can also be adapted to measure the changes in the rate of clotting when plasma is incubated with polyurethane particles. For this purpose a large surface area of the material being tested is required to cause significant changes in the thrombin time, if any. For this reason, procedures were developed to produce small particles of polyurethanes (2 um in diameter) capable of giving stable suspensions in isotonic tris buffer solution (0.168 M).

The particle suspensions were prepared via a sequence of solvent manipulations. Approximately 30 mL of a 2 % w/v solution of polymer in DMF is slowly added dropwise to 300 mL of pure methanol. The resulting precipitate is stirred during this procedure to

ensure the break-up of particles. This is particularly important with materials of low sulfonate content and for most of the substituted polymers. After precipitation is complete, the suspension is stirred 24 hours. The methanol washing is repeated three times to maximize the removal of DMF. The same washing procedure is then repeated three times with distilled water and then another three times with tris buffer. The quantity of washing solution is 300 mL throughout the procedure. After washing, the suspension is stirred continuously until used for particle size analysis and thrombin time measurements. The stirring rate required depends on the tendency of the particles to aggregate.

Particle concentrations were measured in electrolyte using a Model ZB Coulter Counter at the Ceramic Engineering Laboratory, McMaster University, Hamilton, Ontario. The final concentration of particles was approximately 2 x 10^6/100uL. Particle size measurements were also obtained on the Coulter Counter and verified using a light microscope technique and a dynamic light scattering particle size analyzer (DLS). Both the Coulter Counter and light microscope provided reproducible concordant data, but the data obtained from DLS were unreliable and were not used in subsequent calculations. The Coulter Counter data can be found in Appendix B. A knowledge of particle size and concentration allows for the normalization of thrombin times to unit surface area.

Thrombin times were measured using 200 uL of pooled citrated human plasma which was incubated at 37°C with 50 uL of polymer suspension in tris buffer, for a period of 15 minutes. Thrombin time was measured from the point at which a 50 uL sample of a solution of bovine thrombin (12.5 Units/mL) in tris buffer was added. The clotting end point was detected using a fibrometer. Several thrombin time measurements were taken for each sample at 37°C. These thrombin times were then compared to "blank" samples in which 50 uL of tris buffer was used in place of the polymer suspension. Figure 3.15 depicts the experimental procedure [98].

Figure 3.15: Thrombin Time Experiment [98]

A series of different thrombin concentrations [Th] were used in the blank experiment to produce a calibration curve that would permit the calculation of the equivalent amount of deactivated thrombin corresponding to a given thrombin time prolongation. A sample calibration curve is found in Figure 3.16.

Figure 3.16: Thrombin Time Calibration Curve

Chapter 4

RESULTS AND DISCUSSION

Section 4.1 Sulfonated Polyurethane Synthesis

The incorporation of sulfonate groups in polyurethanes was achieved via a functionalized chain extender having sulfonate groups in aromatic rings. Using this approach the sulfonate groups are located necessarily in the hard segment of the polyurethane chains. The choice of sulfonate groups in aromatic rings for improving the biocompatibility of polyurethanes is justified by analogy with the work of Fougnot et.al. [138] who attached sulfonate groups to the aromatic ring of crosslinked polystyrene resins. These modified resins showed evidence of anticoagulant activity. It was decided to modify the polyurethane hard segment since there is considerable evidence correlating the surface hard segment domains with a high incidence of thrombotic events [116,165,166].

Section 4.1.1 Optimization of Synthesis Procedures

Development of the synthesis of polyurethanes chain extended with 4,4' diamino-2,2'biphenyl disulfonic acid (BDDS), required experimentation with several methods for controlling the amount of chain extender incorporated into the polymer. Methods of maximizing the reaction of isocyanate with amine and minimizing the reaction of isocyanate with sulfonate groups and optimizing the final yield of the material had to be developed.

Section 4.1.1.1 Choosing the Sulfonated Chain Extender

The choice of BDDS as the sulfonated chain extender was justified by its relatively high sulfonate content. Since polyurethanes having elastomeric mechanical properties were desirable, the reaction scheme would require that the soft segment (and therefore the prepolymer) be much larger than the hard segment (and therefore the chain extender). This constraint thus limits the level of sulfonate content obtainable in the polymer chain. In order to achieve reasonably high sulfonate content along with elastomeric behaviour a disulfonated chain extender was clearly favored over any of the mono-sulfonated chain extenders available commercially. Other disulfonated diamine chain extenders are also available, but their sulfonate groups are either: (1) both on the same ring, which would possibly leave them sterically hindered or result in decreasing the activity of the amines with respect to isocyanates, or (2) the sulfonates were located on highly conjugated ring systems which could also decrease the reactivity of the amine groups with isocyanates. BDDS has its two sulfonates on different rings and although the aromatic rings decrease the amine's reactivity with respect to the isocyanates the effect is much less than with other conjugated systems. A comparison of the structure of BDDS to those of other sulfonated chain extenders can be seen in Table 2.4.

Another technique used for increasing the sulfonate content of the polyurethane was to use a "dimeric" chain extender. This approach involved reacting MDI and BDDS in a 1:2 molar ratio to form a chain extender with four aromatic sulfonates as opposed to only two.

Although experiments using this technique did yield polymers having twice the sulfonate content of similar polymers synthesized with BDDS, the materials were too brittle to be formed into useful films or coatings.

Section 4.1.1.2 The Prepolymer

To produce polymers with a low sulfonate content, the recipe was altered to obtain larger prepolymers, either by increasing the ratio of PPO 1000 to MDI or by using PPO 2000. Attempts to synthesize sulfonated polyurethanes with PPO 2000 did not succeed because of problems encountered with the chain extension step. It was found that within a few minutes after the addition of BDDS to the prepolymer there was an accumulation of small white fibres in the reaction mixture. After approximately one hour these fibres began to aggregate and finally "fused" into a large foamy mass.

The "foam phase" material contained high molecular weight polymer having good elastomeric character and precipitated in water but dissolved in methanol. Elemental analysis of the foam phase polymer showed that its sulfur content was less than predicted by stoichiometry. The polymer from the solution phase had a much higher sulfur content and was of very low molecular weight. These observations possibly suggest that the foam phase results from rapid chain extension to yield highly insoluble polymers. This process would deplete the reaction medium of prepolymer and perturb the stoichiometry so that the polymer formed subsequently would be of low molecular weight and high sulfur content. This mechanism is only speculation and further work would be required to fully analyze the nature of this reaction.

It is suspected that the high molecular weight polyol based polymers are incompatible with the salt of BDDS contained in the reaction medium and for this reason do not remain

in the reaction solution after growing beyond a certain length. This could be analogous to the precipitation of polymer systems by the addition of inorganic salts. When prepolymers are prepared with a mixture of PPO 1000 and PPO 2000 it is found that the "foam phase" is gradually eliminated as the ratio of PPO 2000 to PPO 1000 is reduced. Values of this ratio less than 1:17 are required in order to completely eliminate the foam phase.

The above difficulties were not encountered in the synthesis of "oligomeric" prepolymers based on PPO 1000 using MDI to PPO 1000 ratios less than 2:1. This would perhaps suggest that the interposition of MDI between PPO units in the prepolymer plays a critical role in the compatibility of chain extended prepolymers and the ion containing reaction medium. Also, it is likely that there exists a PPO between PPO 1000 and PPO 2000 which defines the limit of polyol size that could be used in a reaction without inducing a foam phase.

Section 4.1.1.3 Defining the range of Sulfonate Content in the Polyurethanes

Using BDDS and MDI monomers, the maximum sulfur content would be obtained when no polyether is present. This means that the polymer would be a non-elastomeric polyurea with a sulfur content of 10.03 % w/w. This polymer would be of little practical use in any application requiring flexibility, such as the medical devices discussed in section 1.2. In fact, based on mechanical considerations, polymers synthesized with BDDS, MDI and PPO 1000 are found to be impractical beyond a % wt sulfur of approximately 3.4 % or perhaps even lower, depending on how much MDI is incorporated within the material. A recipe that could produce a SPU material of 3.4 wt % sulfur would be 1:2:1, BDDS:MDI:PPO1000. The practical working range of sulfur content will also depend on the solubility properties of the materials. Clearly water soluble materials would be of limited usefulness if the material required mechanical integrity.

Lowering sulfur content by increasing the size of the prepolymer aiso has limits based on practical considerations. If the prepolymer is increased to a size beyond a molecular weight of 16000, using MDI and PPO1000 the resulting polymer is gummy and sticky, and although it can be stretched to extremely long extensions it has very poor elastomeric capacity. A BDDS chain extender attached to a prepolymer of molecular weight 16000 would have an approximate sulfur content of 0.4 % wt.

A practical working range of sulfur compositions (based on mechanical properties) using a BDDS/MDI/PPO 1000 system would therefore have sulfur content in the range of 0.4 % wt and 3.4 % wt. The polymers that were ultimately chosen for detailed study had sulfur content in the range of 0.2 to 3.1 % wt.

Section 4.1.1.4 Reaction of Isocyanates with Sulfonates

In addition to varying the sulfonate content of the sulfonate containing polymers as discussed, one can change the form of sulfonate in the material. This can be done by allowing isocyanate groups to react with the sulfonate to yield sulfonic acid anhydrides.

It was found that this is a convenient approach for decreasing the amount of free sulfonate while tailoring the mechanical properties of the polymer. This is explained in the following manner. For instance, if a polymer of sulfur content 0.2 % w/w were desired, a reaction

stoichiometry of 1:1 chain extender to prepolymer would require a prepolymer of molecular weight 32000. This would result in a polymer with very low mechanical strength. However, if the stoichiometry were arranged such that the amine/isocyanate ratio is less than 1, partial reaction of the sulfonate groups with isocyanate would occur over time. Supposing the amine/isocyanate ratio were fixed such that one could react half of the sulfonate groups, as well as all of the amine groups. Using a prepolymer of molecular weight 16000, it would be possible to achieve a polymer with a sulfur content of 0.2 % w/w, while yielding a polyurethane with mechanical properties of similar nature to a polymer synthesized with a prepolymer of molecular weight 16000. The only drawback in this procedure is that the polymer would lose some free sulfonate functionality to yield branched sulfonates. Figure 4.1 illustrates the structure of the above two hypothetical polymers.

To further manipulate mechanical properties as well as other physical properties of the polymer (e.g. water uptake) it is possible to change the size of the branching unit. In these experiments the smallest branch would consist of a single MDI molecule while the largest branching unit would be defined by a prepolymer type structure or a chain extended type structure. The incorporation of MDI as a side chain would yield a polymer with minimal changes in sulfur content but dramatic changes in mechanical properties.

In summary, reaction variables include the prepolymer size, the hard segment size and the degree of side chain branching via the sulfonate group. Changes in these variables manipulate the polyurethanes characteristics which include free sulfonate concentration, sulfur concentration, molecular weight and physical character.

Figure 4.1: Hypothetical Sulfonate Polyurethane Structures

The subsequent discussion will refer frequently to "branching" and "chain extension" and it is essential for the reader to have a clear understanding of the two terms. Chain extension refers to the reaction of the BDDS amine with the prepolymer isocyanate, while branching refers to the reaction of the BDDS sulfonate with an isocyanate.

Section 4.1.2 Sulfonated Polyurethanes for Characterization Studies

Following exploration of the variables discussed in the previous section, a series of six sulfonated polyurethanes were synthesized. These polymers were used as the basis for our investigation of the effect of sulfonate groups on polymer properties and blood interactions. They are designated BDDS-0.2, BDDS-0.8(50), BDDS-1.4(54), BDDS-2.0(61), BDDS-2.6(47) and BDDS-3.1(86).

The reaction stoichiometries for all six polymers are given in Table 4.1 and the product compositions are found in Table 4.2. Comparing final compositions from Table 4.2 to the original stoichiometries of Table 4.1 it becomes apparent that the reaction mechanisms require some clarification since sulfonation levels are much lower than anticipated by the original reaction stoichiometry. The next section will address this issue. Polymers BDDS-0.2, BDDS-0.8(50) and BDDS-1.4(54) were synthesized using the potassium salt of BDDS and prepolymers of varying size. All three of these polyurethanes were precipitated using solutions of KCl and water. In this precipitation step the concentration of KCl was gradually increased as the sulfur content of the polymer increased. The yields for these reactions range between 65 and 80 percent.

Polymers BDDS-2.0(61), BDDS-2.6(47) and BDDS-3.1(86) were made with the same prepolymer as used for the BDDS-1.4(54) material. However they were chain extended with the sodium salt of BDDS which is more soluble than the potassium salt in DMSO. Sulfur and sulfonate content were controlled via the chain extension step. Both BDDS-2.0(61) and BDDS-3.1(86) were branched to varying degrees using "excess" prepolymer, while BDDS-2.6(47) was branched using "excess" MDI and the prepolymer. Note that the product stoichiometry comes much closer to matching the reactant stoichiometry for the three sodium BDDS polymers than for the three potassium BDDS polymers. This is directly associated with the solubility of the different salt types of BDDS chain extenders and will be discussed in the

subsequent sections.

Table 4.1: Reaction Stoichiometry of Selected Sulfonated Polyurethanes

Polymer	Moles of MDI	Moles of PPO 1000	Moles of BDDS	BDDS Counterion
BDDS-0.2	4.0	3.0	1.0	K
BDDS-0.8(50)	3.0	2.0	1.0	K
BDDS-1.4(54)	2.0	1.0	1.0	K
BDDS-2.0(61)	2.5	1.2	1.0	Na
BDDS-2.6(47)	2.4	1.0	1.0	Na
BDDS-3.1(86)	1.8	1.0	1.0	Na

Precipitation of the three sodium BDDS chain extended polymers was much more difficult because the elevated sulfonate content increased the solubility of the polymers in water. BDDS-2.0(61) was precipitated in a saturated aqueous solution of KCl. BDDS-2.6(47) and BDDS-3.1(86) were both precipitated in methanol saturated with KCl. The reaction yields were about 70 % for BDDS-2.0(61) and about 50 to 60 % for BDDS-2.6(47) and BDDS-3.1(86).

Table 4.2: Product Composition of Selected Sulfonated Polyurethanes

Polymer	Moles of MDI	Moles of PPO 1000	Moles of BDDS	BDDS Counterion
BDDS-0.2	28	26	1	K
BDDS-0.8(50)	8	6	1	K
BDDS-1.4(54)	5	3	1	K
BDDS-2.0(61)	3.8	2	1	Na
BDDS-2.6(47)	3	1.3	1	Na
BDDS-3.1(86)	2.4	1	1	Na

Section 4.1.3 Sulfonated SPU Chemical Structure

The chemical structures of the polymers discussed in section 4.1.2 were determined using GPC, FTIR, elemental analysis and titration analysis. The data obtained are discussed in this section.

Section 4.1.3.1 Gel Permeation Chromatography

Gel permeation chromatography data for various experimental polymers and for the commercial polyurethane Biomer[r] are shown in Table 4.3.

The molecular weight data confirm several important features of the polyurethane synthesis. In the BDDS-0.2, BDDS-0.8(50) and BDDS-1.4(54) series, a decrease in molecular weight is observed as the prepolymer size decreases. With similar conversion and the same stoichiometry in the chain extension reaction, the final molecular weight is expected to reflect the size of the prepolymer. Although it may not be apparent when first examining the GPC data, it may be deduced that the number of prepolymer units incorporated into a polymer molecule decreases with increasing prepolymer size. Using the intrinsic viscosity data, a better estimate of the molecular weight can be obtained with the Universal Calibration principle discussed in section 3.6.1. Table 4.4 gives the recalculated molecular weight values for BDDS-0.2, BDDS-0.8(50) and BDDS-1.4(54). Also included in Table 4.4 is an estimate of a prepolymer size (based on elemental analysis data) that would yield sulfur contents of 0.2, 0.8 and 1.4, and the number of prepolymer units per polymer molecule based on the data in columns 1 and 2.

Table 4.3: GPC Data (a)

Polymer	$\overline{Mw}$ (b)	$\overline{Mn}$ (b)	MWD	[n] (dL/g)
BDDS-0.2	209000	135000	1.5	0.47
BDDS-0.8(50)	87000	68000	1.3	0.32
BDDS-1.4(54)	67000	51000	1.3	0.31
BDDS-2.0(61)	44000	34000	1.3	--
BDDS-2.6(47)	48000	36000	1.3	--
BDDS-3.1(86)	44000	32000	1.4	--
ED	270000	159000	1.7	0.30
MDA	82000	66000	1.2	--
Biomer	298000	183000	1.6	0.66

(a) Molecular weight data are polystyrene equivalent molecular weights (see section 3.6.1 for details)
(b) Precision of the molecular weight calculation is approximately +/- 10 %.

The data in Table 4.2 suggest that the longer prepolymers hinder the ability of the BDDS chain extender to react with other chains after growth has exceeded a certain level. The reactivity of the chains may be limited due to a loss of mobility in the increasingly viscous solution. In addition these are multifunctional systems with functional groups of very different reactivity. Such a system does not follow the simple classical mechanism for step growth polymerization [105].

Table 4.4: Degree of Chain Extension with Different Prepolymers

Polymer	Universal Mw Estimate	Estimated soft segment size	Number of prepolymers in final polymer
BDDS-0.2	182000	32000	5.7
BDDS-0.8	68000	8000	8.5
BDDS-1.4	45000	4500	10.0

Note: Intrinsic viscosity data for polystyrene in DMF at 80.0°C were obtained from ref. [158].

The molecular weights of polymers BDDS-3.1(86), MDA and ED in Table 4.3 allow an assessment of chain extender reactivity because all three of these polymers were synthesized using similar stoichiometry. It is clear that the molecular weight of polymer ED is much greater than those of polymers MDA and BDDS-3.1(86). These differences are perhaps not surprising since ethylene diamine (the chain extender for ED) is more reactive than the aromatic diamines MDA and BDDS. The sulfonate group is an electron withdrawing group and will further reduce the activity of the amine groups in BDDS compared to MDA.

Section 4.1.3.2 Elemental Analysis

Elemental analysis data obtained using various techniques are reported in Table 4.5. Sulfur analysis permits determination of chain-extender incorporation into the polymer, while nitrogen analysis enables one to detect the presence of both MDI and BDDS. Sulfonate counter-ion analysis can be used to determine the concentration of free sulfonate anions. This last analysis is complicated by the fact that most of the sulfonated SPU materials

required the use of salt in the precipitation step during product work-up. The possibility of counter-ion contamination therefore exists and this reduces the validity of quantitative analysis of free sulfonate via potassium and sodium content.

From the sulfur analysis data in Table 4.5 it can be seen that both analytical techniques are in good agreement. It can also be seen that in general the nitrogen content increases with increasing sulfur content as expected. However on comparing BDDS-1.4(54) and BDDS-2.0(61) it is seen that the nitrogen content data show the opposite trend. Since the sulfur content of BDDS-1.4(54) is lower than that of BDDS-2.0(61), the higher nitrogen content of BDDS-1.4(54) cannot be associated with chain extender incorporation and therefore must be associated with an increased incorporation of MDI. This might occur by reaction of isocyanate with the sulfonate groups of BDDS. This hypothesis is supported by the fact that the percentage of sulfur in the form of free sulfonate in BDDS-1.4(54) is only 54 % compared to 61 % in BDDS-2.0(61).

Table 4.5: Elemental Analysis of Sulfonated SPUs

Polymer	Sulfur Content * % w/w (S.D. = 0.3)	Sulfur Content ** % w/w (S.D. = 0.2)	Nitrogen Content ** % w/w (S.D. = 0.3)
BDDS-0.2	0.2	0.07	2.87
BDDS-0.8(50)	0.75	0.86	3.70
BDDS-1.4(54)	1.40	1.40	4.50
BDDS-2.0(61)	1.96	1.97	4.17
BDDS-2.6(47)	2.50	2.66	5.28
BDDS-3.1(86)	3.20	2.98	5.65

* Determined by SEM-EDAX
** Determined by conventional elemental analysis

To verify the branching hypothesis, a nitrogen and sulfur mass balance using the GCL analytical data was done to predict free sulfonate concentration. Table 4.6 gives analytical data for BDDS-1.0(48). In order to make the calculations statistically significant, the elemental analysis was repeated on five samples each obtained from polymers synthesized with the same reactant stoichiometry. The data used in the mass balance is the average of these five samples.

Based on the average % w/w S and % w/w N values in Table 4.6 the polymer contains 7.3 molecules of MDI and 4 molecules of PPO1000 for every BDDS molecule. This implies that there is an excess of MDI molecules in the product stoichiometry since one would normally expect that the total amount of amine and hydroxyl functions would equal the amount of isocyanate functions. One possible explanation is that the presence of excess MDI results from the occurrence of branching reactions via the sulfonate groups. If this is so then one would expect to see a reduction in titratable sulfonate groups.

Table 4.6: Elemental Analysis of BDDS-1.0

Sample	Sulfur Content (% w/w)	Nitrogen Content (% w/w)	Sodium Content (% w/w)
1	0.89	4.22	0.67
2	1.20	4.29	0.74
3	1.08	3.85	1.51
4	1.07	4.15	0.70
5	0.92	3.73	0.22
Avg	1.03 ±.15	4.05 ±.3	0.77 ±.6

(errors are standard deviations)

The sodium analysis reveals that there is counterion present. However since the standard deviation in this data set is so high it would be impossible to quantify the concentration of sulfonate in the free sulfonate form and the concentration in the branched sulfonic acid anhydride form. For the purpose of this analysis free sulfonate content was determined by titrating the acidified polymer with triethylamine in DMF. Data for the BDDS-1.0(48) polymer are shown in Table 4.7.

The titration data shown in Table 4.7 along with the nitrogen mass balance, suggest that reaction of some of the isocyanate groups with sulfonate groups is occurring thus decreasing the concentration of free sulfonate in the polymers. As will be illustrated in a later discussion, certain physical properties (i.e. water uptake) of the polymers are particularly sensitive to the degree of branching introduced by the sulfonate-isocyanate reaction. Table 4.8 lists the titration data for the polymers used in the physical characterization and biological evaluation experiments. Titration equilibrium times of up to four hours were sometimes required. Data for the BDDS-0.2 polymer were not obtained because titration equilibration times were prohibitively long and the experimental error is larger than the value being measured.

Table 4.7: Titration of BDDS-1.0 with Triethylamine

Trial	Moles of titratable SO_3^- per gram of polymer x 10^4	S in free SO_3^- form (mole %)
1	1.3	41
2	0.9	29
3	1.9	58
4	1.4	44
5	1.7	54
6	2.0	63
Avg	1.54 ± 0.41	48 ± 12

(Errors are standard deviations)

Table 4.8: Titration Data for Sulfonated SPUs

Polymer	* Moles of titratable SO_3^- per gram of polymer x 10^4	S in free SO_3^- form (mole %)
BDDS-0.8	1.2	50
BDDS-1.4	2.2	54
BDDS-2.0	3.8	61
BDDS-2.6	3.8	47
BDDS-3.1	8.2	86

* Standard deviation is $\pm$ .45x10^{-4} and was estimated from Table 4.7.

Section 4.1.3.3 Fourier Transform Infra-Red (FTIR) Analysis

In addition to using elemental analysis, the composition of polyurethanes can be investigated using FT-IR to detect chemical functional groups. The infra-red spectrum of BDDS-1.4(54) is shown in Figure 4.2 and tentative assignments of some principal peaks are given in Table 4.9 and are based on reference spectra libraries [204].

As previously mentioned, the infra-red spectra of polyurethanes are complex. Nonetheless it is possible to distinguish features which are indicative of sulfonated chain extender content. Figures 4.3 and 4.4 show the spectra of three sulfonated polyurethanes ranging in sulfur content from 0.8 to 2.6 % w/w. The peaks of interest are grouped at 1730, 1710 and 1680 cm^{-1} in Figure 4.3, at 790, 730 and 641 cm^{-1}, and at 561 and 541 cm^{-1} in Figure 4.4.

Figure 4.2: FT-IR Spectrum of BDDS-1.4(54)

Table 4.9: FT-IR Peak Assignment for BDDS-1.4(54) (assignments made from ref. [204])

3310	N-H Stretch
3100	N-H Overtone
3050	C=C-H
2975	Aliphatic C-H
2935	"
2900	"
2870	"
1730	non-H-bonded N-C(=O)-O
1709	H-bonded N-C(=O)-O
1680	N-C(=O)-N Shoulder
1600	C=C, N-H
1539	N-H
1510	C=C, N-H
1470	CH_2
1458	"
1415	C=C
1375	CH_3
1310	N-H, C-N
1230	"
1215	S=O
1100	C-O
1031	S=O
1011	C=C-H
925	"
830	"
790	Ring substitution
730	"
641	S-O
561	SO_2 scissoring
541	"

The peaks located near 1700 cm^{-1} (Figure 4.3) are assigned to the urea and urethane linkages in both the "free" (1730 cm^{-1}) and hydrogen bonded (1710 cm^{-1}) states. It can be seen that with increasing chain extender content the free urethane peak gradually decreases relative to the H-bonded urethane and urea peaks. This is to be expected since an increase in chain extender content necessarily increases the number of urea groups and the number

of amine groups available for hydrogen bonding.

Figure 4.4 shows a series of peaks at wavenumbers less than 800 cm^{-1} which are assigned to a combination of aromatic ring and ring substitution vibrations, and S-O stretching and SO_2 scissoring vibrations. The peaks at 730 and 641 cm^{-1} are likely associated with S-O stretching and ring substitution as well as aromatic ring vibrations [204]. As the sulfur content of the polymers increases, the magnitude of the peaks at 730 and 641 cm^{-1} surpasses that of the 790 cm^{-1} peak, and indicates the change in functional group composition between the three polymers. Similarly one can see that the SO_2 scissoring peaks (near 561 cm^{-1}) increase in size relative to the peak at 519 cm^{-1}. There are other trends in the spectra which are also observed but the interpretation of these is more difficult because of the complex combination of chemical functional groups located at each wavenumber.

Section 4.2 Derivatization of Sulfonated Polyurethanes with Amino-acids, Dodecylamine, Polyethylene oxide methyl ether, and Amino-sulfonic-acids.

Results and data relating to the incorporation of amino-acids and other organic compounds into sulfonated SPUs are presented and discussed in this section. These moieties were chosen in the expectation of altering the hydrophilic character, physical character, chemical functionality and biological response of these materials.

Section 4.2.1 Derivatization Chemistry

Section 4.2.1.1 Sulfonate Conversion Reagents

The derivatization or substitution reaction involves two steps: (1) conversion of sulfonate to sulfonyl chloride or sulfonyl imidazole and (2) reaction of substituents with sulfonyl chloride or sulfonyl imidazole via amine or hydroxyl groups.

Figure 4.3: Effect of Increasing Chain Extender Content on FT-IR Peaks Associated with Urea and Urethane Groups: a. BDDS-0.2, b. BDDS-1.4(54), c. BDDS-2.6(47)

Figure 4.4: Effect of Increasing Chain Extender Content of Polyurethanes on FT-IR Peaks Associated with Aromatic Rings and SO_3 Groups: a. BDDS-0.2, b. BDDS-1.4(54), c. BDDS-2.6(47)

Figure 4.5 shows the probable chemical pathway for substituent incorporation using oxalyl chloride to convert sulfonate to sulfonyl chloride [205]. Upon addition of oxalyl chloride to the reactor containing a solution of polymer in DMF there is a rapid reaction between the solvent and the oxalyl chloride. Both CO_2 and CO are evolved and a white solid precipitates, but then rapidly dissolves. The solid (believed to be the salt (I) shown in Fig. 4.5) reacts with sulfonate groups in the polymer to form sulfonyl chloride groups. The reaction solvent acts as a catalyst since, after the reactive salt (I) has reacted with the sulfonate group, dimethyl formamide is formed once again. In the absence of sulfonated polymer, the white salt slowly goes into solution.

Following formation of the sulfonyl chloride in step 1, the desired substituent is added to the solution. Either the hydroxyl or amine group of the specified substituent will react with the sulfonyl chloride group, with the amine having a much higher reactivity than the hydroxyl [29]. The amine groups form sulfonamide bonds while the hydroxyl groups form sulfonate bonds. Although reactions are rapid, the procedure may take up to four hours since most of the amino-acids have a low solubility in DMF. It was found that some esters of amino-acids and quaternary ammonium salts of amino-acids have increased solubility in DMF and thus give enhanced reaction rates.

Like oxalyl chloride, ethylchloroformate is also believed to form a complex with dimethylformamide. The proposed mechanism is shown in Figure 4.6. Upon addition of ethylchloroformate to DMF a white solid is formed and CO_2 gas is released. This metastable salt reacts rapidly with sulfonate groups to form sulfonyl chlorides and ethanoate ions.

In the absence of the substituent the ethanoate ion can react with sulfonyl chloride, thus eliminating this group for subsequent substituent incorporation. In this case a two step reaction is not feasible and all reagents must be added at the same time. This is also a problem in the presence of most amino-acids since the latter are of low solubility in DMF.

Ethylchloroformate works best when the amino-acid substituent is completely in solution because the reactivity of the amine group is much greater than that of the ethanoate ion [205]. Using a two-step procedure, this reagent did not provide high conversions of sulfonate to amino-acid sulfonamide. For this reason the oxalyl chloride was the preferred reagent.

Figure 4.5: Proposed Mechanism for Substituent Incorporation into Sulfonated Polyurethanes using Oxalyl Chloride

Figure 4.6: Proposed Mechanism for Substituent Incorporation into Sulfonated Polyurethanes using Ethylchloroformate

A third reagent, N,N' carbonyldiimidazole was also investigated. This reagent does not form a salt with the solvent and only dissolved in DMF in the presence of sulfonated polymer (see Figure 4.7). The evolution of CO_2 gas is not nearly as extensive as for the other two reagents. Also, conversion to the substituent derivative was lower than for oxalyl chloride. It is important to note that the chloride ion is a much better leaving group in nucleophilic substitution reactions than is the imidazole ion and the results obtained may be rationalized

on this basis.

Of the three reagents, oxalyl chloride gave the best results and was chosen for the synthesis of all the amino-acid substituted polymers presented and discussed in this thesis.

Figure 4.7 Proposed Mechanism for Substituent Incorporation into Sulfonated Polyurethanes using N,N'-Carbonyldiimidazole

Section 4.2.1.2 Sulfonate Substituents

Several amino-acids and other compounds were chosen as substituents to the sulfonated SPU materials. Some of these compounds were selected based on previous research relating them to specific blood interactions while others were chosen to investigate the versatility of the chemical groups that could be coupled to the sulfonated polyurethanes. Most of these interactions were discussed in Section 2.6 and include heparin-like activity, adsorption and subsequent deactivation of specific plasma enzymes and the promotion of protein resistant materials.

Table 4.10 lists all of the substituents used, including their intended biological activity and a qualitative "rating" of the success of the substitution reaction. A rating of "good" means the compound was successfully attached and the substituent apparently retained its chemical and physical character. A rating of "moderate" means that greater than 50 % conversion of the sulfonate with the particular substituent was difficult to achieve but the substituent apparently retained its chemical and physical properties. A rating of "poor" means that the analytical techniques were unable to confirm that derivatization occurred even though physical changes in the polymer were sometimes noticed.

Section 4.2.2 Reaction Conditions

The reaction conditions were investigated as preliminary work to this phase of the project. These conditions included possible degradation reactions, solvent pH, solvent type and counterion type both for the amino-acids and the polymer.

Table 4.10: Compounds Reacted with Chlorosulfonated SPUs

Compound	Structure	Biological Function Intended	Reaction Rating
Arg	$H_2N\underset{CH_2CH_2CH_2NHC(=NH)NH_2}{CHCOOH}$	High affinity for thrombin [135]	moderate
ArgMe	$H_2N\underset{CH_2CH_2CH_2NHC(=NH)NH_2}{CHCOOCH_3}$	Same as Arg	good
Asp	$H_2N\underset{CH_2COOH}{CHCOOH}$	heparin-like activity [129]	good
Leu	$H_2N\underset{CH(CH_3)_2}{CHCOOH}$	Experimental	no evaluation
Lys	$H_2N\underset{CH_2CH_2CH_2CH_2NH_2}{CHCOOH}$	High affinity for plasminogen [148,149]	good
Gly	H_2NCH_2COOH	Experimental	good
Met	$H_2N\underset{CH_2CH_2SCH_3}{CHCOOH}$	Experimental	poor
Taurine	$H_2NCH_2CH_2SO_3H$	Experimenal	poor
PEOMe	$CH_3(OCH_2CH_2)_nOH$	Protein Resistant [169,170]	good
DDA	$CH_3(CH_2)_{11}NH_2$	Promote albumin adsorption [143,144]	good
ABS		Extend sulfonate groups out from the polymer backbone [127]	moderate
ANS		Same as ABS	moderate
AENS		Same as ABS	poor

Note: See Table 3.2 for abbreviation definition

Section 4.2.2.1 Stability of Sulfonated Polyurethanes

To investigate possible polymer degradation in the substitution reaction, molecular weights were determined by gel permeation chromatography. It was found that polymer degradation occurred when un-sulfonated SPUs were treated with oxalyl chloride. Table 4.11 shows molecular weight information for ED (samples #1 and #2). The molecular weight of ED following treatment with oxalyl chloride (sample #2) is lower than the original ED polymer (sample #1). In the absence of suitable functional groups (sulfonic acid or carboxylic acid) it is likely that the reactive salt formed by oxalyl chloride and DMF will attack the urea or urethane or even the aromatic rings [205]. For this reason, excess reagent was avoided in these reactions.

Samples 4 to 8 in Table 4.11 represent a variety of modified 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. Comparing sample #3 to samples #4 through #8 it is seen that although the molecular weights vary somewhat there is no significant change in either the molecular weight or polydispersity following substitution for any of the polymers.

Table 4.11: Molecular Weights of Substituted Polymers (a)

Sample #	Polymer	$\overline{Mw}$ $\times 10^{-4}$	$\overline{Mn}$ $\times 10^{-4}$	$\overline{Mw}$ / $\overline{Mn}$	[η] (dL/g)
1	ED	27	16	1.6	0.30
2	ED(treated with $(COCl)_2$)	17	11	1.7	--
3	BDDS-1.4(54)	7.7	5.9	1.3	0.31
4	BDDS-1.4-ArgMe(100)	7.4	5.7	1.3	--
5	BDDS-1.4-Lys(100)	8.6	6.1	1.4	0.26
6	BDDS-1.4-PEOMe(100)	8.0	5.7	1.4	--
7	BDDS-1.4-DDA(100)	7.2	5.1	1.4	--
8	BDDS-1.4-Asp(92)	7.5	5.7	1.3	0.30

(a) Molecular weights are reported as polystyrene equivalent values

Section 4.2.2.2 pH of Substitution Reaction

If reaction temperatures much in excess of 25°C were used it would be important to maintain the pH near 7 because the formation of a sulfonamide bond by reaction of sulfonyl chloride with the amine containing substitutents produces HCl which could degrade the polymer by hydrolysis of urea and urethane bonds in the polymer. In the present work, the reaction temperature ranged between -5°C 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 degree of sulfonyl chloride conversion to substituent linkage would be compromised since the hydroxide ions could convert the sulfonyl chloride groups back to free sulfonate ions. Bases such as triethylamine were used to control acidity in some instances but their presence appeared to hinder the reaction and therefore no further attempts to control the pH during the experiment were made. It is now thought that the TEA was contaminated with water and this led to lower degrees of conversion since the water would readily react with the sulfonyl chloride groups.

It would however be desirable to control the pH of the reaction solution in a manner that would ensure that the reacting amine is not in the cationic form, so as to enhance the reaction between amine and sulfonyl chloride.

Section 4.2.2.3 Reaction Solvents

Based on general polyurethane solubilities, solvents for sulfonated polyurethanes are limited to dimethylformamide (DMF), dimethylsulfoxide (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 substituent derivatization reactions. DMF was

chosen because it dissolved the sulfonated polymers quite well and because it acts as a catalyst for the covalent incorporation of substituent onto the sulfonated polyurethane, in the presence of oxalyl chloride and ethylchloroformate [151,152,205]. 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. Therefore every attempt must be made to avoid competing reactions for the sulfonyl chloride groups, between the desired substituents and highly DMF soluble materials.

Section 4.2.2.4 Substituent and Sulfonate Counterions

The use of different amino-acid salts is 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 carboxylate form of the amino-acid. However it was found that the conversion of sulfonate to sulfonamide amino-acid in the polymers was reduced when the metal salts were used. It is suspected that the removal of all the water from these salts is difficult. Residual water could have reacted with and reduced the number of active SO_2Cl groups, thus reducing the yield of the amino-acid derivatization reaction. It was found that amino-acids which were pretreated with benzyl trimethyl ammonium hydroxide were more soluble in dimethylformamide than the free acid, potassium or sodium salt forms.

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

Section 4.2.3 Quantitative Chemical Analysis of Derivatized Polymers

Quantitative chemical analysis of the derivatized polyurethanes has proven to be a difficult task. Many reagents commonly used to determine amino-acid incorporation in peptide synthesis could not be used because of possible side reactions that could occur with other functional groups on the polyurethane. Titration of carboxylate groups did not always yield quantitative information because the titration curves were not sharp enough to determine end points with sufficient precision. On the other hand the determination of free sulfonate groups was achieved without difficulty.

An alternate method used for the quantitative determination of amino-acid incorporation into polyurethanes, involves elemental analysis for nitrogen. Increases in nitrogen content are expected due to the incorporation of amino-acids of high nitrogen content such as lysine and arginine.

Section 4.2.3.1 Analytical Data

The analytical data used to calculate the conversions are given in Table 4.12. As seen in Table 4.12 the nitrogen content increases with the incorporation of amino-acid, but the differences are in general within analytical precision, which makes these data questionable for determining conversion. As well, it has previously been mentioned that the titration curves for the determination of aspartic acid did not have sharp end points thus calling into question the level of confidence in the titration data alone. A comparison of the titration curves for BDDS-1.4(54) and the BDDS-1.4(54)-Asp(92) is shown in Figure 4.8. It is clear from this figure that the end point of the sulfonic acid is sharper than that of the carboxylic acid. Note that the pH at the end point for the sulfonic acid is near pH = 6.5 whereas that of the carboxylic acid material is near pH = 8. With the incorporation of aspartic acid it is

anticipated that the number of acid groups would double and would require more titrant. This is confirmed as shown in Figure 4.8.

Table 4.12: Analytical Data for Derivatized SPU Materials

Polymer	* Nitrogen Content (% w/w)	**Sulfur Content (% w/w)	***Moles of acid titrated per gram of polymer x 10^4	
			amino acid	SO_3^-
BDDS-0.2	2.9	0.1	--	--
BDDS-0.2-Lys	3.4	0.15	--	--
BDDS-0.8(50)	3.6	0.8	--	1.20
BDDS-0.8-Lys(21)	3.9	0.8	--	--
BDDS-0.8-Asp(82)	--	--	1.16#	.258
BDDS-1.4(54)	4.3	1.4	--	2.20
BDDS-1.4-Arg(30)	5.5	1.8	--	--
BDDS-1.4-ArgMe(100)	5.1	1.2	--	--
BDDS-1.4-Lys(100)	4.9	1.4	--	--
BDDS-1.4-Asp(92)	--	--	2.34#	.246
BDDS-1.4-Gly(67)	--	--	1.47	.73
BDDS-1.4-DDA(100)	--	1.2	0	0
BDDS-1.4-PEOMe(100)	--	1.4	0	0
BDDS-1.4-Tau(0)	--	1.3	--	--
BDDS-1.4-Met(0)	--	1.2	--	--
BDDS-2.0(61)	4.2	2.0	--	3.84
BDDS-2.0-ABS	4.7	2.2 2.7	--	3.65
BDDS-2.0-ANS	5.0	2.6 2.5	--	3.80
BDDS-2.0-AENS	2.9	1.7	--	--
BDDS-3.1(86)	5.0	3.1	--	8.18
BDDS-3.1-Asp(61)	--	--	4.97#	3.21

* S.D. = ± 0.3 %
** S.D. = ± 0.2 %
*** S.D. = ± 0.45 x 10^{-4} moles
\# 1 mole of amino acid has 2 equivalents of CO_2^- acid

Note: Conversions are based on residual sulfonate groups.

Figure 4.8: Titration Curves for BDDS-1.4(54) and BDDS-1.4(54)-Asp(92)

Section 4.2.3.2 Quantitative Analysis using ^{14}C Labelled Amino-Acids

Verification of the analytical methods presented in the previous section was carried out by comparing titration and elemental analysis data with levels of amino acid incorporation obtained from C-14 labelling experiments. Data from the experiments are shown in Table 4.13 and agree with the results of elemental nitrogen analysis for the lysine derivatized polymer and of titration analysis for the aspartic acid derivatized polymers. Sulfonate conversions for the ^{14}C-labelled experiments were determined from calibration data which are presented in Appendix C.

Table 4.13: Comparison of Polymer Composition by C-14 Labelling with Elemental Analysis and Titration (data reported as mole of amino-acid per mole of SO_3 (%))

Polymer	Trial	N-analysis or Titration	C-14 labelling
BDDS-3.1-Asp	#1	3.12	2.85
BDDS-3.1-Asp	#2	5.59	5.99
BDDS-3.1-Lys	#1	27.6	28.0

The experiments carried out with ^{14}C-labelled amino-acids were designed to convert approximately 60% of the sulfonate groups to sulfonamide. The conversion achieved for the preliminary trial of the ^{14}C-lysine polymer was only 28% while that for the ^{14}C-aspartic acid polymer was only about 3%. It is believed that these low conversions are a direct result of the method of amino-acid preparation which involved a recrystallization from water. It seems likely that some water may remain in the crystals and if so, could deactivate sulfonyl chloride groups during reaction. From an examination of the data it appears that the effect

is more significant for the aspartic acid polymer than for the lysine polymer. Both substituents were reacted under the same conditions, the only difference being that the aspartic acid was recrystallized from 150 mL of water while lysine was recrystallized from 3 mL. Therefore the possibility of water contamination would be even greater for the aspartic acid than for the lysine. The problem could be corrected by longer drying times or changing the preparation of the ^{14}C-labelled materials.

To test this theory two additional experiments were carried out. In the first, ^{14}C-labelled aspartic acid was further dried in a vacuum oven prior to reaction (trial #2 in Table 4.13). One can see that the conversion for trial #2 is twice that for trial #1. A second experiment was carried out using unlabelled amino-acid which had not been recrystallized from water. The conversion for this polymer, BDDS-3.1-Asp(61) is shown in Table 4.14. The conversion achieved agrees well with the expected value of 60%. Both of these experiments support the hypothesis that water contamination of the amino acids may be responsible for low conversion. However it is clear from Table 4.13 that the radioactivity data agree well with the titration or nitrogen analysis data thus confirming the validity of the latter.

Section 4.2.3.3 % Conversion of Derivatized Polyurethanes

Derivatization reaction yields for all of the polymers synthesized were calculated from the data in Table 4.12 and are shown in Table 4.14, along with the technique used for the quantitative analysis. The reaction conversions shown in Table 4.14 vary from one substituent to another. Because of limitations due to the error in the analytical data it was impossible to determine the degree of substitution of amino-acid in the BDDS-0.2 polymer. Data for the BDDS-0.8(50) polymer are also questionable but at least one can conclude whether the amino-acid is or is not present in the polymer.

The BDDS-1.4(54) series provides much more reliable data on the derivatization reactions. Substituents such as lysine and aspartic acid were incorporated at significant levels.

Arginine was incorporated at a moderate level of 30% conversion. This value is significantly less than the 100% conversion achieved for the arginine methyl ester. The lower conversion for arginine may be due to a lower solubility of arginine relative to arginine methyl ester, in the reaction solvent. This comparison highlights a major advantage of the amino-acid ester over the corresponding amino-acid. Use of the ester provides another method for improving compound solubility in DMF along with the use of quaternary ammonium ions as discussed previously.

Dodecylamine and polyethylene oxide methyl ether are both very soluble in DMF and gave high values of sulfonate conversion. On the other hand, taurine in the acid form was completely insoluble in DMF and had to be converted to the quaternary ammonium salt by titration with benzyl trimethylammonium hydroxide. The resulting salt was recrystallized from water and dried for use in the derivatization reaction. The subsequent reaction of this salt with the chlorosulfonated polymer gave a very low yield. Two explanations appear possible for this behaviour. The first is that water contamination, resulting from the recrystallization procedure, may interfere with the reaction as discussed for other substituents. The second is based on the fact that since there is no control of pH, the reaction solution becomes acidic with conversion causing acidification of the unreacted taurine salt which then precipitates out of the reaction medium, as observed.

Table 4.14: Quantitative Chemical Analysis of Derivatized SPU Materials

Substituent type	Sulfonated SPU (base polymer)	Analytical Technique	Percent Conversion of Sulfonate Groups
Lys	BDDS-0.2	1	not determined
Asp	BDDS-0.2	2	not determined
Lys	BDDS-0.8(50)	1	21
Asp	BDDS-0.8(50)	2	82
Lys	BDDS-1.4(54)	1	100
Asp	BDDS-1.4(54)	2	92
Arg	BDDS-1.4(54)	1	30
ArgMe	BDDS-1.4(54)	1	100
Gly	BDDS-1.4(54)	2	67
DDA	BDDS-1.4(54)	3	100
PEOMe	BDDS-1.4(54)	3	100
Tau	BDDS-1.4(54)	4,5	0
Met	BDDS-1.4(54)	4,5	0
ABS	BDDS-2.0(61)	4,5	50
ANS	BDDS-2.0(61)	4,5	50
AENS	BDDS-2.0(61)	4,5	not determined
Asp	BDDS-3.1(86)	2	61

Analytical Techniques:
1. Elemental analysis of nitrogen (difference between the "base" polymer nitrogen content and the substituted polymer nitrogen content).

2. Titration of excess H^+ due to the presence of carboxylic acid groups. There is a potential for two carboxylic acid groups per original sulfonate group.

3. Titration of unreacted sulfonate groups.

4. Elemental analysis for sulfur by combustion analysis (difference between the "base" polymer sulfur content and the substituted polymer sulfur content).

5. Elemental analysis for sulfur by SEM-EDAX.

The derivatization reaction with methionine was unsuccessful based on sulfur analysis. However a reaction did occur, since when methionine was added to the polymer solution in DMF it was taken up into solution instantly and indeed much faster than any other

substituent used with the BDDS-1.4(54) polymer. In nonreacting conditions methionine dissolves very slowly in pure DMF. It is believed that a secondary reaction at the sulfide bond could be taking place which removes the methionine sulfur from the amino acid side chain functionality. There is no data or references to support this speculation and further work will be required to fully understand these observations. This example stresses the importance of protecting groups for specific functionality on the amino acids and future work should incorporate this consideration.

Derivatization reactions attempted on the BDDS-2.0(61) polymer included the substituents ABS, ANS and AENS. In these materials it was expected that the sulfonate groups would be "extended" from the backbone of the polymer.

The derivatization of BDDS-2.0(61) with AENS was complicated by the precipitation of a material during the reaction. Elemental analysis revealed this material to be of similar nature to the AENS substituent. It is likely that the unreacted AENS salt precipitated in the acid medium of the reaction solution. No further work was attempted on this polymer. The polymer from reaction with ABS showed a moderate degree of substitution but the results of different sulfur analysis techniques were conflicting and therefore it was difficult to assess the true degree of substitution. ANS gave conversions of sulfonate groups of 50%. Titration of the ANS- and ABS-substituted materials confirmed as required by the reaction stoichiometry that the amount of free sulfonate groups was unchanged after the substitution reaction.

Section 4.2.4 Qualitative Features of the Derivatized Polymers

The addition of amino acid moieties imparts interesting qualitative features to the sulfonated polyurethanes. These observations are discussed in this section.

A property of sulfonated SPUs is enhanced solubility in water relative to the ED or MDA polyurethanes. This behaviour was noted in trying to precipitate the polymers in water and was expected to be due to the presence of sulfonate groups. Acidification experiments showed that these polymers will precipitate from water much easier in the acid form than in the potassium or sodium salt form. This was also apparent in all the reactions in which substituents were incorporated into the polyurethanes following conversion of sulfonate to sulfonyl chloride with oxalyl chloride.

As well, the substituted polymers tend to adopt some of the physical characteristics of the substituents. For instance, most of the amino-acids used in the substitution reactions have a sweet smell which is also found in the corresponding derivatized polymers. Colour was also sometimes transmitted from substituent to substituted polymer. The base sulfonated polyurethanes all have a yellow-brown colour, but after reaction with ANS, which is purple, polymer BDDS-2.0(61) itself turned purple.

Qualitative analysis of the substituted materials proved feasible by Fourier transform infra-red spectroscopy (FT-IR). For example, FT-IR data indicated the presence of specific amino acid moieties in the polymers, the manner in which substitution had occurred, and changes in polymer structure due to the presence of the substituents.

Figure 4.9 shows the spectra of polymers BDDS-1.4(54), BDDS-1.4-Arg and a model compound, N-tosyl arginine, containing no urethane functionality [202]. The spectrum of the model compound indicated that one should find a significant IR peak between 1650 cm^{-1} and 1700 cm^{-1} upon substitution of arginine into the base polymer. Comparing the IR peaks near 1700 cm^{-1} for the arginine derivatized and corresponding non-derivatized polymers indicates that the peak height for the derivatized polymer has increased. This can be attributed in part to the incorporation of a carboxyl group. Another feature is a prominent shoulder located on the 1700 cm^{-1} peak in the derivatized polymer. This shoulder is associated with the guanidyl group on the side chain of arginine.

Figure 4.9: FT-IR Spectra for: a. N-tosyl Arginine (taken from Sadtler I.R. spectra [202]) b. BDDS-1.4(54) and c. BDDS-1.4-Arg

Figure 4.10 shows the IR spectra for the arginine methyl ester , the aspartic-acid and the lysine derivatized polymers. Again the arginine methyl ester containing polymer shows the distinct appearance of the guanidyl peak as a shoulder to the peak at 1700 cm^{-1}. The guanidyl peak is unique to the arginine amino-acid and is not observed for the other two polymers shown in Figure 4.10. The spectra for BDDS-1.4-ArgMe differs from that of BDDS-1.4-Arg since the ester carbonyl appears at a higher wavenumber. The ratio of the peaks at 1735 cm^{-1} and 1700 cm^{-1} is larger for the arginine methyl ester polymer (Figure 4.10) than for the arginine polymer (Figure 4.9).

One would expect that the aspartic-acid derivatized polymer would show a more substantial change in the peak at 1700 cm^{-1} because of the incorporation of two carboxyl functions as opposed to one for the other amino-acid derivatized polymers. As can be seen in Figure 4.10 this is in fact the case: the ratio of the 1700 cm^{-1} peak to the 1730 cm^{-1} peak for the aspartic acid containing polymer is higher than for the other two amino-acid derivatized polymers shown in Figure 4.10. With the exception of the carboxyl peak near 1700 cm^{-1} the lysine-containing polymer does not exhibit significant IR spectral features that can be distinguished from those of the BDDS-1.4(54) base polymer (Figure 4.9). If one examines the spectra for BDDS-1.4-Lys it is possible to notice a shoulder to the right of the 1700 cm^{-1} peak which could be due to the amine group on the side chain of lysine.

Although FT-IR is a convenient qualitative tool for confirming the derivatization reactions, at this time it cannot provide a quantitative measure of substituent content in the modified polymers. The probable reason is that subtle changes in the polymer caused by the addition of minute concentrations of substituent, appears to disrupt its microphase structure. These changes appear to be related to changes in the infrared peaks associated with the urea and urethane functionalities. The latter peaks happen to overlap the characteristic amino acid infra-red peaks. Therefore any changes in the infrared spectra of a modified polymer are linked not only to the substituent but also to the environment created by the substituent, i.e.

the microphase structure of the hard and soft segments. Since these effects cannot be easily uncoupled it is concluded that quantitative analysis using FT-IR, is not possible at this time.

Figure 4.10: FT-IR Spectra for: a. BDDS-1.4-Asp, b. BDDS-1.4-ArgMe and c. BDDS-1.4-Lys

For example, if microstructural changes influence hydrogen bonding there will be significant shifts in the carbonyl and amine peaks. Coleman et.al. [46] showed that for urethane polymers, the urethane N-H stretching frequency varies from 3450 cm^{-1} to 3350 cm^{-1} depending on whether the hydrogen is involved in hydrogen bonding or not. Likewise, the carbonyl stretching band can shift to higher wavenumbers if the carbonyl group is not involved in hydrogen bonding.

Evidence of structural changes in the polymer, associated with the covalent attachment of molecules to the sulfonate groups in the hard segment, is shown in Figure 4.11. In this figure the IR spectra for DDA-and PEOMe-substituted polymers are shown. These substituents are relatively long molecules compared to the amino-acids discussed earlier. Both spectra show changes in the group of peaks around 1700 cm^{-1}. This may suggest that changes in structure are occurring in the hard segment phase, since the substituent molecules themselves do not possess any functional groups that could show significant absorption at a wavenumber of 1700 cm^{-1}. The effect is more pronounced with polyethylene oxide than with dodecylamine. This may be due to the fact that the PEOMe chain (MW = 500) is more than twice as long as the DDA chain and contains ether linkages which could potentially be involved in hydrogen bonding. Further to this, the discussions on mechanical and thermal analysis studies in the subsequent sections will support the above observations.

Section 4.3 Physical Characterization

It was anticipated that functional group incorporation to moderate levels within polyurethanes would improve the biocompatibility of these polymers without seriously compromising the physical properties which have made them attractive for biomedical applications.

Figure 4.11: FT-IR Spectra for: a. BDDS-1.4-DDA and b. BDDS-1.4-PEOMe

The physical properties of the functionalized materials were assessed by tensile stress-strain testing, differential scanning calorimetry (DSC) and water uptake measurements. In the following discussions, the conversion number will sometimes be omitted from the polymer name for the sake of convenience. For example BDDS-0.8(50)-Lys(27) is the same as BDDS-0.8-Lys. This should cause no confusion since only one polymer of a given sulfonate content with a given substituent was subjected to characterization.

Section 4.3.1 Tensile Testing

Tensile stress-strain data for all polymers studied are listed in Table 4.15. These include tensile strength, initial modulus and ultimate elongation.

Section 4.3.1.1 Tensile Stress-Strain Testing of Sulfonated SPU Materials

Stress-strain curves for some sulfonated SPU materials are shown in Figure 4.12 and are compared to the non-functionalized polymers ED and MDA. The curves reflect a variety of structural features which include polyurethane chain extender type and degree of branching via reaction of isocyanate with sulfonate. All of the polymers shown in Figure 4.12 were made with the same prepolymer.

The use of stiff chain extenders such as BDDS and MDA dramatically increases the initial modulus and tensile strength of the materials relative to those chain extended with ethylene diamine. On the other hand the more flexible ED polymer has a much higher ultimate elongation.

Table 4.15: Tensile Stress-Strain Evaluation of SPU Materials

Polymer	Tensile Strength MPa (S.D. = ± 10%)	Initial Modulus at 100 % E MPa (S.D. = ± 15%)	Elongation at Break % E (S.D. = ± 12%)
ED	12.8	3.0	1340
MDA	10.1	33.6	365
BDDS-0.2	>0.8	6.0	>3900
BDDS-0.2-Asp	>0.9	2.0	>1300
BDDS-0.2-Lys	>0.8	0.9	>1300
BDDS-0.8(50)	6.0	8.0	750
BDDS-0.8-Asp	12.0	4.8	800
BDDS-0.8-Lys	21.8	4.0	1200
BDDS-1.4(54)	28	60	380
BDDS-1.4-ArgMe	26	18	450
BDDS-1.4-Arg	31	24	400
BDDS-1.4-Asp	36	140	390
BDDS-1.4-Gly	34	100	400
BDDS-1.4-Lys	37	32	380
BDDS-1.4-DDA	15	120	210
BDDS-1.4-PEOMe	21	264	250
BDDS-1.4-BDDS	35	48	420
BDDS-2.0(61)	11	100	240
BDDS-2.0-ABS	19	232	250
BDDS-2.0-ANS	19	380	150
BDDS-2.6(47)	Too brittle to test		

Note: relative standard deviations were calculated based on five tests for each material.

Figure 4.12: Tensile Stress-Strain Behaviour of SPU Materials

Both sulfonated aromatic amine chain-extended polymers show a higher initial modulus than the non-functionalized aromatic amine chain extended, MDA. This could be due, first, to the presence of branching on the BDDS polymer chains and, second, to the possible formation of ion pair aggregates which could enhance polymer strength in a similar fashion to hydrogen bonding.

Comparing the BDDS-1.4(54) and BDDS-2.0(61) materials it is seen that the initial modulus is greater for the latter polymer, suggesting that intermolecular interactions in the hard segment are increasing with sulfonated chain extender content. On the other hand, a higher degree of branching gives BDDS-1.4(54) a greater tensile strength than BDDS-2.0(61). Comparing polymer BDDS-1.4(54) to polymer MDA it can be seen that both materials have a similar ultimate elongation. This result correlates well with the fact that both materials have somewhat similar structures. On the other hand these two materials have very different tensile strengths. Again the degree of chain branching and functional group interactions are suspected to be the key elements in the enhanced strength of BDDS-1.4(54).

Figure 4.13 compares three sulfonated polyurethanes having similar hard segments but varying in prepolymer size. Increasing the prepolymer size increases the polymer molecular weight (see Table 4.3) and polymer extensibility but reduces hard segment concentration which in turn reduces both the material stiffness and functional group interactions. As previously discussed for Figure 4.12 these characteristics will cause a decrease in both the initial modulus and tensile strength and an increase in elongation as the prepolymer size is increased. Figure 4.13 confirms this interpretation. Polymer BDDS-0.2 displays much higher elongations than BDDS-0.8(50) and BDDS-1.4(54). On the other hand BDDS-1.4(54) has a relatively high initial modulus along with a very high tensile strength. While the latter two polymers are moderately good elastomers, BDDS-0.2 is not a good elastomer.

Figure 4.13: Stress-Strain Behaviour Dependence on Prepolymer Size

A summary of the trends observed in Figures 4.12 and 4.13 is given in Table 4.16. Prepolymer extensibility can be increased by increasing the length of the prepolymer. Intermolecular interactions via functional groups include hydrogen bonding, ion pair aggregate formation and van der Waals interactions and tend to create coherent structures within the material. Chain branching as a result of the reaction of isocyanate with sulfonate yields polymers with increased tensile strength. The arrangement of microdomain structure for the sulfonated polyurethanes will be discussed in more detail when differential scanning calorimetry data are presented in Section 4.3.2, however it is important to recall that microdomain structure is a direct result of the tendency for polyurethanes to undergo phase separation. In general, increased phase separation leads to improved tensile strength and reduced elongation [171]. These concepts were discussed in Section 2.3.

Table 4.16: Stress-Strain Behaviour Trends for SPU Materials

Polymer Property	Tensile Strength	Young's Modulus	Ultimate Elongation
prepolymer flexibility	v	v	^
chain extender stiffness	^	^	v
functional group interaction	^	^	N
branching between chains	^	^	N
microdomain formation	^	^	v
molecular weight	^	^	^

^ increasing with increasing property
v decreasing with increasing property
N no distinct trend observed

Section 4.3.1.2 Tensile Stress-Strain Testing of Derivatized Materials

Figures 4.14 and 4.16 to 4.19 show tensile stress-strain curves for the derivatized sulfonated SPUs. Figure 4.14 shows data for the derivatized BDDS-0.2 polymers. Lysine incorporation appears to decrease the initial modulus while increasing the strength (at 1000 % E) of the material. The elongations are greater than 1000%. This behaviour is interesting since a decrease in initial modulus usually is associated with a decrease in strength, as seen in Table 4.16.

Likewise, aspartic acid incorporation decreases the initial modulus, although to a lesser extent than lysine. Also the strength at 1000 % elongation increases relative to that of BDDS-0.2 and BDDS-0.2-Lys. Since these materials were all based on a relatively large prepolymer they all have very high elongation and their tensile strength could not be evaluated due to limitations on the elongations that could be obtained on the tensile testing apparatus.

The behaviour of the derivatized BDDS-0.2 polymers can be explained by considering possible ion interactions and other functional interactions occurring in the polymer matrix [173]. Both aspartic acid and lysine have the capacity to increase the strength of the material by increasing ionic interactions between the polymer chains. For every sulfonate group converted, two carboxylate ions or a carboxylate and an ammonium ion are formed (see Figure 4.15). Although an increase in the concentration of ionic groups probably enhances strength, it has reduced the initial modulus of the material. This suggests for the case of BDDS-0.2, where ionic concentration is very low, that only ion pair multiplet formation is taking place and that ion clustering does not play an important role in the ionic interactions. If ionic clustering were prominent one would expect to see an increased modulus for the aspartic-acid derivatized material based on data shown in Table 4.15 comparing BDDS-1.4 to MDA. It is perhaps not surprising to see the decrease in stiffness associated with the lysine substituted polymer since one could expect that the long lysine side chain offers a means for more mobility of the ion pairs, as opposed to the rigidity of aromatic ring sulfonates.

Figure 4.14: Stress-Strain Behaviour of BDDS-0.2 Derivatized Materials

The principal features of the data presented in Figure 4.14 are summarized in Table 4.17 and tensile properties for the materials are given in Table 4.15.

Table 4.17: Trends in Stress-Strain Properties for Derivatized BDDS-0.2 Polymers

- There appears to be no ion pair clustering.
- Initial modulus decreases with increasing ion carrier chain mobility.
- Material strength at 1000 % elongation increases with increasing ionic interactions.
- Ultimate elongations and tensile strength cannot be measured.

Figure 4.16 shows stress-strain curves for the derivatized BDDS-0.8(50) polymers and their tensile properties are given in Table 4.15. Again it can be seen that an increase in ion pair content increases the tensile strength of the materials. The presence of flexible side chains carrying the ions is likely responsible for the decrease in initial modulus of the derivatized BDDS-0 8(50) polymers. The relatively low ionic concentration, as for the BDDS-0.2 series, is believed to preclude the formation of ion clusters. It is difficult to make a fair comparison between the two derivatized polymers since their degrees of conversion vary by a significant amount. The ultimate elongations of both the derivatized BDDS-0.8(50) polymers are much lower than those of the derivatized BDDS-0.2 polymers, probably reflecting the strong dependence of elongation on the size of the prepolymer.

Figure 4.15: Ionic Functionality of BDDS and Derivatized Polymers

BDDS Segment

Lysine Substituted BDDS Segment

Aspartic Acid Substituted BDDS Segment

Figure 4.16: Stress-Strain Behaviour of Derivatized BDDS-0.8 Materials

The BDDS-1.4(54) polymer was used as a base polymer for the incorporation of a wide variety of amino-acids. The stress-strain curves for these polymers are shown in Figure 4.17 and their tensile properties can be found in Table 4.15. Several interesting trends are associated with the functional character of each amino-acid. All of the polymers which were derivatized with carboxylic acid-containing moieties show higher tensile strength than the precursor BDDS-1.4(54) polymer, while the amino-acid ester derivatized material shows a tensile strength similar to that of the base polymer. This observation yields information on possible ionic group interactions occurring in the BDDS segment of these polymers.

The pendant carboxylate groups appear to produce stronger ion pair interactions than do the sulfonate groups attached directly to the polymer chain backbone. In the base polymers the sulfonate groups are presumably restricted by the mobility of the macromolecular backbone, while in the derivatized polymers anions have the potential to extend away from the chain, making them more available for interactions such as ion aggregation as proposed by Eisenberg [61]. The elevated ion concentration in the BDDS-1.4(54) series makes this situation particularly favorable.

Since the arginine methyl ester substituted polymer has no carboxylate ions its tensile strength is relatively low. However, the quaternary ammonium-chloride ion pair shows a similar capability to the sodium sulfonate ion pair to enhance material strength.

Ultimate elongation is again determined primarily by the size of the prepolymer, and does not show significant change from one polymer to another. On the other hand there is a wider range of initial modulus data. It appears that ion concentration in the BDDS-1.4(54) series is high enough to allow for ion pair aggregation to form clusters which would tend to increase the modulus values. Both the aspartic-acid and glycine derivatized polymers show increased initial modulus compared to the precursor BDDS-1.4(54) polymer, while the lysine, arginine and arginine methyl ester derivatized polymers show decreased initial modulus. All of the latter three substituents have long side chains bearing quaternary ammonium ions. As

of the latter three substituents have long side chains bearing quaternary ammonium ions. As previously explained, these side chains enhance flexibility in the ion interactions which would reduce polymer stiffness. As could be expected the arginine methyl ester had the lowest initial modulus of the three materials.

In addition to studying the mechanical behaviour of amino-acid derivatized polymers, studies were also done on three additional polymers, containing respectively a hydrophilic, a hydrophobic and an ionic substituent. BDDS-1.4(54) was used as the base polymer. As can be seen in Figure 4.18 and Table 4.15 derivatization with BDDS increases the strength of the polymer but has very little effect on the initial modulus and elongation. The increased strength is likely due in part to an increase in ionic interactions and to the presence of a higher concentration of aromatic rings which could enhance the strength of the hard segment domains.

The DDA and PEO derivatized materials show substantial changes in initial modulus, ultimate elongation and tensile strength relative to the precursor polymer and other derivatized BDDS-1.4(54) materials. The initial modulus of the dodecylamine derivatized material is relatively high and of the same order as the aspartic-acid derivatized polymer. It is quite apparent that this is not a result of ionic interaction or the presence of rigid side chains since the substituent is a 12-carbon alkyl chain. The enhanced initial modulus may be a result of van der Waals interactions and entanglements between the alkyl side chains themselves. Such an analysis implies the formation of a third phase within the polymer and the differential scanning calorimetry data shown in Section 4.3.2 confirm this implication. This would account for the high observed value of Young's modulus. One may also expect that the material would have a weaker tensile strength because the hydrocarbon chains would interfere with the packing of hard segments in the micro-domains. In addition to this, the

Figure 4.17: Stress-Strain Behaviour of Amino-Acid Derivatized BDDS-1.4(54) Materials

Figure 4.18: Stress-Strain Behaviour of Derivatized BDDS-1.4(54) SPU Materials

hydrophobic interactions would be much weaker than the ionic and hydrogen-bonding forces discussed previously. Without proper cohesion within the hard segments, the materials' ultimate elongation is also expected to decrease. Figure 4.18 confirms this analysis, since both tensile strength and ultimate elongation have been lowered relative to the BDDS-1.4(54) polymer. These interpretations are also supported by the FT-IR data presented in Section 4.2.4. IR spectra showed that the DDA derivatized polymer had peaks in the carbonyl region which were different from those of BDDS-1.4(54) and suggested that there was disruption of the hard segment domain structure. This would clearly affect the mechanical properties of the material.

The PEOMe substituted material also shows increased initial modulus combined with a reduced tensile strength and ultimate elongation compared to the precursor polymer. Polymer BDDS-1.4-PEOMe has the highest initial modulus of all the BDDS-1.4(54) derivatized polymers and this may reflect the capacity of the PEO chain to form hydrogen bonds with the hard segment domains. The molecular weight of the PEOMe residue is 550, corresponding to about 12 ethylene oxide units. Such chains provide many hydrogen bonding possibilities which would account for the substantial changes observed in the FT-IR spectra shown in Figure 4.11. Although this material shows an elevated initial modulus, its tensile strength is decreased and again DSC studies (discussed in Section 4.3.2) suggest a disruption in the micro-domain structure.

It is important to note that although the presence of substituents appears to be modifying micro-domain structure, it is not implied that microphase separation is reduced. Table 4.18 list some tentative conclusions on mechanical testing data for the BDDS-1.4(54) series.

A final data set showing mechanical property variations resulting from derivatization of BDDS polymers is shown in Figure 4.19, for the BDDS-2.0(61) series. These data illustrate

the effect of incorporating rigid amino-sulfonic-acid moieties into the polymer hard segment. Data for the substituents ABS and ANS are shown in Table 4.15.

Table 4.18: Conclusions Regarding Mechanical Properties for Derivatized BDDS-1.4(54) Polymers

- High ionic concentration enhances ion pair interactions.
- Extension of ionic groups away from the polymer backbone enhances ion pair interactions
- Tensile strength is increased with increasing ionic interactions in the hard segment domains
- Substitution of ionic groups for non-ionic hydrophilic and hydrophobic chains decreases tensile strength and ultimate elongation.
- Initial modulus is dependent on the mobility of the substituent carrying the ion.
- Young's modulus increases with van der Waals, hydrogen bonding and entanglement interactions.

Derivatization with both ABS and ANS causes an increase in the tensile strength of the BDDS-2.0(61) polymer, in line with the behaviour of amino-acid substituents previously discussed. The initial modulus of both of these derivatized polymers is substantially increased over that of the precursor polymer. This higher value is no doubt directly related to increased ionic interactions and to the incorporation of rigid substituents. Based on the latter comment it is not surprising that the initial modulus of the ANS material is much greater than that of the ABS polymer. Incorporation of ABS did not affect the ultimate elongation of the BDDS-2.0(61) polymer. On the other hand, the ultimate elongation was substantially decreased with the incorporation of the rigid naphthalene based amino-sulfonic acid.

Figure 4.19: Stress-Strain Behaviour of Derivatized BDDS-2.0(61) SPU Materials

Section 4.3.2 Differential Scanning Calorimetry (DSC)

As suggested by the mechanical property studies discussed in the previous section, polyurethane micro-domain structure may be modified by the incorporation of chemical functionality into the hard segments. A useful technique for detecting changes in the micro-domain structure is DSC. As well, this technique gives a measure of the degree of phase separation occurring in the material.

The following DSC data presented here includes thermograms for the "original" materials as prepared for mechanical testing. These materials were also subsequently cooled and subjected to a second DSC test. The data sets for these tests are labelled "reheat". In addition to the detailed thermograms presented throughout this section, thermal data are summarized in Table 4.19.

Section 4.3.2.1 DSC Thermal Analysis of Non-Derivatized Polyurethanes

Figure 4.20 shows DSC data for the non-functionalized ED and MDA polymers and compares them with a sulfonated polymer, BDDS-1.4(54). All three polymers were synthesized with prepolymers of similar molecular weight and composition.

The degree of phase separation in a segmented polyurethane material may be assessed by the sharpness and temperature of the glass transition. In particular changes in glass transition temperature (Tg) of the soft segment phase relative to that of the PPO used in the synthesis of the prepolymer, are of interest. In this case PPO of molecular weight 1000 was used. The glass transition temperature for this material is approximately -70 °C and its DSC thermogram is shown in Figure 4.21. The PPO is relatively pure and shows a sharp transition occurring over a range of about 4°C.

Table 4.19: DSC Data for Non-Derivatized SPU Materials
(Data are °C)

Polymer	Soft Segment Glass Transition (Tg)	Short Range Order Transition	Long Range Order Transition	Hard Segment Melt Temp. (T_{HSM})
PPO1000	-70 to -66 ES	--	--	--
ED	-27 to -16 VS	107 M	172 S	210 VS
ED R	-33 to -23 VS	--	--	--
MDA	-22 to -8 VS	118 VW	169 W	225 M
MDA R	-42 to -29 VS	--	172 VW	214 VW
MDA R2	-45 to -33 VS	88 S	192 W	213 S
BDDS-0.2	-27 to -20 VS	--	189 VW	213 W
BDDS-0.2 R	-38 to -22 VS	--	--	207 VW
BDDS-0.8(50)	-29 to -14 S	--	142 W	190 M
BDDS-0.8(50) R	-35 to -21 VS	--	--	213 VW
BDDS-1.4(54)	-20 to -7 S	115 M	--	213 S
BDDS-1.4(54) R	-35 to -20 S	--	140 VW	205 VW
BDDS-1.4(54) R2	-41 to -25 S	35 M	131 S	210 S
BDDS-2.0(61)	-30 to -10 S	85 VW	188 VW	228 VW
BDDS-2.0(61) R	-39 to -13 VS	136 W	--	212 W
BDDS-2.6(47)	30 to 78 VW	133 W	187 VS	--
BDDS-2.6(47) R	-46 to -23 M	145 VW	--	225 M

ES = extremely strong
VS = very strong
S = strong
M = medium
W = weak
VW = very weak
R = Reheat experiment
R2 = Second Reheat experiment

Figure 4.20: DSC Thermograms for SPU Materials
(y axis division = 0.1 W/g)

Figure 4.21: DSC Thermogram of PPO1000

The degree of crystallinity within the hard segment can be assessed by the sharpness of the hard segment crystal melt temperature (T_{HSM}). This transition gives a measure of how well packed and ordered the hard segments are in the micro-domains.

Examining the thermogram transitions for the original samples (solid line) in Figure 4.20 one can see several differences among the SPU materials. Trends in both microphase separation and micro-domain structure are quite evident. The "classical" non-functionalized polyurethane, ED, shows the highest degree of phase separation. Both Tg and T_{HSM} are sharp and the presence of a long range order transition at 172°C suggests a high degree of order in the hard segment micro-domains, a result of both tight packing and extensive hydrogen bonding.

Incorporation of the rigid chain extenders MDA and BDDS increases the Tg, which

may indicate increased hard segment mixing into the soft segment phase [35]. As well, the magnitude of the T_{HSM} is reduced for both materials and the long range transitions are not as prominent as for the ED polymer. While polymer MDA shows hardly any short range order transition, polymer BDDS-1.4(54) exhibits a noticeable transition at about 115°C, probably representing sulfonate group interactions.

Reheat experiments show phase separation behaviour in the materials after heat treatment. Rapid cooling after initial heating "locks in" the "melted" state of the material. Reheat thermograms (dashed lines) in Figure 4.20 show that phase separation, as indicated by the glass transition, is only slightly altered for the simple alkyl amine chain-extended polymer (ED) while both of the more rigid polymers MDA and BDDS show decreased Tg values indicating an improved phase separation exceeding even that of the ED material. These results imply that the phase separation process for polymers MDA and BDDS-1.4(54) is highly dependent on temperature, and that perhaps the mechanical properties of these materials could be improved by annealing processes to match those of ED. Such considerations also open the possibility of studying phase separation kinetics for both the MDA and BDDS-1.4(54) polymers. The difference in Tg values (comparing original to reheat) for the BDDS-1.4(54) material is less than that for the MDA material and likely reflects the fact that the BDDS-1.4(54) polymer has side branches which would extend into the soft segment phase and thus constitute phase mixing.

Heating of all three materials dramatically reduces ordering between the hard segments since the short range order, long range order and T_{HSM} transitions are all weak. This does not mean that such ordered structures cannot reform, it simply suggests that their rate of formation (involving ionic, hydrogen bonding, crystallization, etc.) is much slower than the rate of quench-cooling.

If the BDDS and MDA polymers were heated to high temperatures, then cooled to room temperature and maintained at 25°C for an extended period, it would be expected that the

hard segment domain structure would reform and exhibit both short and long range order transitions and possibly a T_{HSM}. This experiment was carried out for both the BDDS-1.4(54) and MDA polymers. The second reheat analyses were performed several months after the first reheat analysis (six months for BDDS-1.4(54) and four months for MDA). Comparisons of the original, first reheat and second reheat analyses for MDA are shown in Figure 4.22 and for BDDS-1.4(54) in Figure 4.23.

Both sets of data show that Tg decreases with each reheat. For MDA, Tg changes from -22°C (original sample) down to -45°C (second reheat) while the width of the transition decreases slightly with each additional cycle. The same trends are observed for polymer BDDS-1.4(54) which shows a Tg of -20°C for the "original sample" and -41°C for the "second reheat" analysis.

It can also be seen from the short and long range transitions that after one heat cycle the samples have lost hard segment domain structure. However, if the material is allowed to stand at room temperature in an unstressed state for an extended period, the hard segment structures appear to be reformed. For both the MDA and BDDS-1.4(54) materials the second reheat curves show short and long range order secondary transitions and a melt transition.

Figure 4.24 shows data for three polymers of varying hard segment structure: BDDS-1.4(54), BDDS-2.0(61) and BDDS-2.6(47). All three materials have very different morphological features. BDDS-1.4(54) and BDDS-2.0(61) both have a similar concentration of branched sulfonates although BDDS-2.0(61) has about twice the concentration of free sulfonates of BDDS-1.4(54). The thermograms of the "original" samples show that the glass transition for BDDS-2.0(61) begins at about -30°C while that of BDDS-1.4(54) begins at -20°C. This difference would normally suggest better phase separation for the BDDS-2.0(61) polymer. However the width of the latter material's glass transition is almost twice that for the BDDS-1.4(54) polymer.

Figure 4.22: Effect of Heating Cycles on Thermal Transitions in MDA
(y axis division = 0.1 W/g)

Figure 4.23: Effect of Heating Cycles on Thermal Transitions of BDDS-1.4(54) (y axis division = 0.1 W/g)

A comparison of T_{HSM} of the BDDS-2.0(61) and BDDS-1.4(54) polymers suggests that the latter has more structured order in the hard segment domains than does the former. In studies of sulfonated hard segment polyurethanes Grasel and Cooper [132] have associated this loss of order in the hard segment domains to structural interferences resulting from ion pair interactions. Such interactions could also explain the large width of the glass transition in these materials, a break-up of the hard segment would encourage phase mixing.

The BDDS-2.6(47) polymer has the same concentration of free sulfonates as the BDDS-2.0(61) but has a greater concentration of branched sulfonates along with a relatively high hard segment content. These differences appear to cause a dramatic alteration in the degree of phase mixing. The glass transition for polymer BDDS-2.6(47) is very high, beginning at 30°C and extending to 78°C. It is evident that hard segment domains mix with soft segment regions of the molecular matrix since T_{HSM} as shown in Table 4.19 occurs at 187°C compared to 213°C for BDDS-1.4(54). Heating of all three polymers, as indicated by the Tgs shown for the dashed lines, causes enhanced phase separation. The most dramatic change occurs for the highest hard segment content material, BDDS-2.6(47). Again, these data highlight the temperature dependence of phase separation for SPU materials chain extended with rigid molecules.

Previous work by Cooper and co-workers [171] shows that this dependence is either not observed at all or the trends are reversed for polyurethanes chain extended with butanediol. As previously noted, hard segment micro-domain interactions do not have time to redevelop when the sample is rapidly quench-cooled to -120°C and therefore the reheat curves exhibit neither second order transitions above the Tg nor a T_{HSM}. Thermal analysis data from the curves in Figure 4.24 can be found in Table 4.19.

Figure 4.24: DSC Thermograms of Sulfonated SPU Materials of Varying Hard Segment Structure (y axis division = 0.1 W/g)

Figure 4.25 shows the effect of increasing prepolymer size on the degree of phase separation and hard segment microdomain structure in sulfonated SPUs. Thermal analysis data for these materials are given in Table 4.19. In this particular series, the prepolymer size increases as the sulfur content decreases. The Tg values for the original samples show that phase separation initially increases (i.e. Tg decreases) with increasing prepolymer size but appears to level off for prepolymers with molecular weights exceeding 10000. This observation may reflect the fact that, at very low hard segment concentration the driving force for phase separation is lost and the modification of Tg relative to pure PPO 1000 increasingly reflects the concentration of MDI distributed throughout the prepolymer matrix. A plot of Tg versus prepolymer size is shown in Figure 4.26.

The hard segment melting transitions, near 200°C in Figure 4.25, for these three polymers support the previous discussion. As prepolymer size increases and microphase separation is diminished there is a lowered concentration of microdomains reflected by the decrease in the size of the transition at the T_{HSM}.

Reheat thermograms show that all three polymers have very similar Tg values following heating and rapid quenching. After reheat and subsequent quenching, no ordered hard segment structure is formed in the micro-domains. This behaviour is identical to the other SPU materials. Conclusions from the thermal analysis of Figures 4.22 to 4.26 are summarized in Table 4.20.

Figure 4.25: DSC Thermograms for Sulfonated SPU Materials of Varying Prepolymer Size (y axis division = 0.1 W/g)

Figure 4.26: Tg Dependence on Prepolymer Size for BDDS-0.2, BDDS-0.8(50) and BDDS-1.4(54) (Onset of the glass transition is shown)

Table 4.20: Conclusions from Thermal Analysis Data for Non-Derivatized Polyurethanes

- SPU materials synthesized with ethylene diamine chain extender show better phase separation than aromatic diamine chain extended materials.
- Phase separation of aromatic diamine chain-extended polymers is greater than that of alkyl amine chain extended polymers after annealing processes.
- Using BDDS as chain extender and increasing free sulfonate content while keeping sulfonate branch content constant, causes an increased phase separation but decreased hard segment crystallization.
- Using BDDS as chain extender and increasing sulfonate branch content while keeping free sulfonate content constant, contributes to increased phase mixing.

Section 4.3.2.2 DSC Thermal Analysis of Derivatized Polyurethanes

Thermal analysis data for derivatized sulfonated polyurethanes are given in Table 4.21. DSC thermograms for derivatized BDDS-0.2 polymers are shown in Figure 4.27. The degree of phase separation for BDDS-0.2-Asp and BDDS-0.2-Lys polymers is slightly greater than that of the BDDS-0.2 material as indicated by the lower value of Tg. It should be noted that the strength at 1000 % elongation for the two derivatized polymers was also slightly greater than that of the base polymer. There appears to be no short or long range order transitions. T_{HSM} could not be observed since the sample leaked out of the cell above 200°C. Rather than using a solid sample cell, a liquid sample cell should have been used. It appears that incorporation of amino-acids into the BDDS-0.2 material has very little effect on the polymer's microstructure. This behaviour was expected since, in this particular case, the microstructure is predominantly controlled by the prepolymer size.

Figure 4.28 shows thermograms for BDDS-0.8(50) SPU materials derivatized with aspartic-acid and lysine. From the curves for the "original" samples one can see that the order of Tg is BDDS-0.8-Asp < BDDS-0.8 < BDDS-0.8-Lys. However the spread of the values is small and it seems likely that substitution has no effect on degree of phase separation in this system. Again, the prepolymer size appears to play a more dominant role in influencing phase separation than does the incorporation of functional groups.

The BDDS-0.8(50) polymer shows transitions at 142°C and 190°C (Table 4.19) which suggest that there are interactions creating order in the hard segment domains. However there does not appear to be hard segment packing to the extent required to create crystalline structures since a melt transition is not apparent. On the other hand the BDDS-0.8-Lys polymer shows a weak transition at 174°C and a melt transition near 213°C, suggesting that interactions occur between polyurethane hard segments and could account for the increased tensile strength and ultimate elongation observed for this material. In addition to the transitions already discussed a new endothermic transition appears at about 240°C for the BDDS-0.8-Lys polymer. This peak may be due to reaction between free amine groups of lysine and other functional groups in the polymer.

Reheat runs show a lowering of the Tg values for all three materials in Figure 4.28. Of particular interest is the reheat run for the BDDS-0.8-Lys polymer in which the sharp transition which occurred at 240°C in the original heating cycle is no longer observed, suggesting that most of the lysine amine groups have reacted during the original heating cycle.

The most extensive study of derivatization reactions was carried out for the BDDS-1.4(54) derivatized polymer series. DSC data for this series are presented in Figures 4.29 to 4.31 and the corresponding data can be found in Table 4.21.

Figure 4.27: DSC Thermograms for Derivatized BDDS-0.2 SPUs
(y axis division = 0.1 W/g)

Table 4.21 DSC Data for Derivatized SPU Materials
(Data are given as °C)

Polymer	Soft Segment Glass Transition	Short Range Order Transition	Long Range Order Transition	Hard Segment Melt Temperature
BDDS-0.2-Asp	-32 to -22 VS	--	--	--
BDDS-0.2-Lys	-32 to -21 VS	--	--	--
BDDS-0.8-Asp	-31 to -20 VS	122 VW	--	220 M
BDDS-0.8-Asp R	-32 to -20 VS	--	--	--
BDDS-0.8-Lys *	-27 to -15 VS	--	174 W	213 M
BDDS-0.8-Lys R	-36 to -25 VS	--	--	--
BDDS-1.4-ArgMe *	-26 to -11 S	104 M	193 S	212 S
BDDS-1.4-ArgMe R	-37 to -20 S	--	172 M	212 M
BDDS-1.4-Arg *	-22 to -12 S	128 M	--	215 M
BDDS-1.4-Arg R	-36 to -20 VS	--	186 M	225 M
BDDS-1.4-Lys **	-27 to -10 S	117 M	194 VS	--
BDDS-1.4-Lys R	-36 to -10 S	133 W	202 S	--
BDDS-1.4-Asp	-25 to -10 S	120 S	161 VS	200 M
BDDS-1.4-Asp R	-29 to -20 VS	--	--	--
BDDS-1.4-Gly	-26 to -10 S	122 W	--	226 M
BDDS-1.4-Gly R	-34 to -18 S	--	171 W	215 M
BDDS-1.4-DDA	-25 to -7 M	64 W	--	--
BDDS-1.4-DDA R	-36 to -8 VS	--	--	211 M
BDDS-1.4-PEOMe	-25 to -10 S	100 VS	190 S	--
BDDS-1.4-PEOMe R	-30 to -17 S	--	--	216 M
BDDS-1.4-BDDS	-22 to -15 M	85 W	--	210 S
BDDS-1.4-BDDS R	-29 to -10 S	--	--	--
BDDS-2.0-ABS	-34 to -8 M	--	180 M	--
BDDS-2.0-ABS R	-40 to -20 S	107 M	--	--
BDDS-2.0-ANS	-29 to -8 W	93 M	140 VW	205 M
BDDS-2.0-ANS R	-45 to -20 M	--	176 M	--

** several endothermic peaks, * 1 endothermic peak
R = reheat, VW = very weak, W = weak, M = medium, S = strong, VS = very strong,

Figure 4.28: DSC Thermograms for Derivatized BDDS-0.8 SPUs
(y axis division = 0.1 W/g)

Figure 4.29 compares the sulfonated base polymer, BDDS-1.4(54) with the glycine and aspartic acid derivatized polymers. Glycine substitution effectively replaces one sulfonate with one carboxylate group while aspartic acid derivatization replaces one sulfonate with two carboxylate groups. It can be seen that in the original samples, both carboxylated materials show increased phase separation relative to BDDS-1.4(54). The Tg range of the derivatized polymers begins at -26°C while that of the non-substituted material starts at -20°C. All three polymers show a similar width for the glass transition. The increased phase separation may be in part responsible for the increased tensile strength of the two carboxylated polymers relative to the sulfonated SPU.

Neither of the polymers containing carboxylates exhibits the same value of T_{HSM} shown for BDDS-1.4(54) but they do show hard domain long range interactions. Of the two derivatized polymers the BDDS-1.4-Asp material has the most pronounced transition for long range interactions (at about 160°C) and this may be due to the increased concentration of ion pairs present in the hard segment domains. It should be recalled that aspartic acid derivatized polymer exhibited a high initial modulus relative to the other two polymers, which could have resulted from these long range interactions.

Reheat experiments showed an increase in phase separation for all three polymers and substantially reduced thermal transitions associated with the hard segment domain. Of the three polymers, BDDS-1.4-Asp showed the smallest increase in phase separation. This may indicate the importance of ionic interactions in governing phase separation processes since the aspartic acid material has twice the ion pair concentration of the other two polymers.

Figure 4.29: DSC Thermograms for Derivatized BDDS-1.4(54) SPUs
(Carboxylated substituents, y axis division = 0.1 W/g)

Figure 4.30 shows DSC curves for the three materials containing pendant amine groups. The most striking feature of these three curves is the presence of endothermic reaction peaks above 200°C, as was previously observed for polymer BDDS-0.8-Lys. The presence of these peaks provides strong evidence that free amine groups are present in these polymers which on heating are able to react with other functional groups. The nature of the reactions occurring is unknown, but it is clear that it is dependent on polymer internal structure since the peaks occur over a range of temperatures from 210°C to 240°C. Thermal transitions in the T_{HSM} range are most pronounced for the lysine material suggesting that extensive interactions occur between lysine functional groups and the hard segment.

Both the lysine- and arginine methyl ester-derivatized materials show a more pronounced degree of phase separation, relative to the BDDS-1.4(54) polymer (see Figure 4.29), than does the arginine derivatized polymer shown in Figure 4.30. This is not surprising since in the latter only 30 % conversion of sulfonate was achieved while in the other two polymers conversion of free sulfonate to amino-acid sulfonamide was close to 100 %.

Reheat runs for these polymers show enhanced phase separation for all three polymers. Onset Tg values change from -27 to -36°C for BDDS-1.4-Lys, from -26 to -37°C for BDDS-1.4-ArgMe and from -22 to -36°C for BDDS-1.4-Arg. In the reheat runs, hard segment domain ordering appears to be reduced for all three polymers, with lysine retaining the highest degree of structure following heat treatment as shown in the thermogram near 200°C. This structure may be locked in by the hypothesized amine group reaction which occurs in the first heating cycle. There is no evidence of reaction endotherms in the reheat runs.

Perhaps the most interesting microdomain structures of any of the substituted polymers are shown by the dodecylamine- and polyethylene oxide-derivatized polymers (see Figure 4.31). A thermogram of polymer BDDS-1.4-BDDS, is also shown in Figure 4.31 and is almost identical to that of the base polymer prior to derivatization.

Figure 4.30: DSC Thermograms for Derivatized BDDS-1.4(54) SPUs
(Amine substituents, y axis division = 0.1 W/g)

The "original" sample run for BDDS-1.4-DDA implies an increase in phase separation since the onset of the glass transition occurs at -25°C compared to -20°C for BDDS-1.4(54). However the magnitude of the transition is smaller and the width is greater (18°C compared to 13°C for BDDS-1.4(54). These data suggest that the presence of the long alkyl chains is enhancing the separation of hard and soft segments thereby decreasing Tg. However at the same time, the long C_{12} chains may be mixing with the soft segment matrix thus broadening the Tg range. The existence of a unique transition at about 64°C in this material supports the occurrence of van der Waals interactions between the alkyl chains, as was suspected from the tensile data. This transition is not observed in any other polymer in the present study. The presence of the long alkyl chains in the hard segment appears to disrupt ordering in the hard segment micro-domain since there is no observable transition in the T_{HSM} range. This behaviour is not observed for any of the other thermograms in this series. The relatively low tensile strength and ultimate elongation observed for this particular polymer presumably also reflect disruption of hard segment domain structure.

The reheat thermogram for the BDDS-1.4-DDA polymer shows completely different patterns from those observed previously. The onset of the glass transition is reduced to -36°C from -25°C which would suggest increased phase separation. However the width of the Tg range increases to 28°C from 18°C and the the transition increases considerably in "strength". These observations indicate increased separation of hard and soft segments. At the same time there appears to be "contamination" of the soft segment phase, causing an increase in the width of the Tg range. Figure 4.32 shows a schematic diagram of a model that could explain these conflicting data with respect to phase mixing.

Figure 4.31: DSC Thermograms for Derivatized BDDS-1.4(54) SPU Materials (Hydrophobic-Hydrophilic Functions, y axis division = 0.1 W/g)

The driving force for the proposed mechanism shown in Figure 4.32 is identical to that for classical polyurethane microphase separation. The hard segment of the polyurethane is highly polar and is incompatible with the long alkyl chains, even more than with the polyether chains of the soft segments. Thus the incorporation of these long alkyl chains should increase the polar/non-polar incompatibility which is the driving force for hard segment domain formation. Under the film casting conditions used in this work, the ability of the system to respond to this driving force is limited. However as has been shown for all of the polymers based on rigid chain extenders, specifically BDDS, the degree of phase separation is increased with annealing processes. The BDDS-1.4-DDA polymer is no exception. In fact for this material, phase separation and micro-domain structuring apparently occurred during the first heating and quenching (note the appearance of a melting transition in the reheat thermogram at 211°C). The BDDS-1.4-DDA polymer is the only material which showed an increase in microdomain ordering upon heating.

Additional support for the microphase structuring depicted in Figure 4.32 is provided by the transition observed at -5°C which could be associated with the break-up of alkyl chains. It is possible that after annealing the polymer, the hydrocarbon chains had formed a third microdomain structure of their own which is indicated by the appearance of the transition at -5°C. The normal melting point of dodecylamine is 28°C which makes it quite possible to find a transition for the DDA substituents near -5°C particularly since they are interdispersed with other polyurethane components.

Figure 4.32: Hypothesized Mechanism for Phase Mixing in BDDS-1.4-DDA

Some of the above ideas developed for the BDDS-1.4-DDA polymer are also applicable to polymer BDDS-1.4-PEOMe. The "original" thermogram for BDDS-1.4-PEOMe reveals the presence of very strong transitions occurring at 100°C and 190°C, yet no melting transition is observed. The onset of the soft segment Tg occurs at -25°C compared to -20°C for the base polymer while the width of the glass transition remains similar to that of BDDS-1.4(54). Therefore the incorporation of long PEOMe chains increases phase separation but does not "contaminate" the soft segment phase. Yet even with improved phase separation there is no evidence of hard segment crystallization as in the BDDS-1.4(54) polymer. This observation confirms that the long PEOMe chains are interfering with hard segment packing as reflected also by a lower tensile strength and ultimate elongation for this material relative to its parent polymer.

The pronounced interactions between the PEOMe chains and polyurethane hard segments may also account for the strong transitions at 100°C and 190°C which may be the result of extensive hydrogen bonding networks within the hard segment micro-domains. An increase in hydrogen bonding is suggested by FT-IR data shown in Figure 4.11. The interactions are clearly much stronger than the van der Waals interactions discussed for the BDDS-1.4-DDA as reflected by the fact that the tensile strength, initial modulus and ultimate elongation for the PEOMe derivatized polymer are greater than those of the DDA derivatized polymer.

A summary of the observations for the thermal transitions of the BDDS-1.4(54) polymer series is given in Table 4.22. The summary outlines the modifications in polyurethane structure upon incorporation of different functional groups into the hard segment.

Table 4.22: Summary of Thermal Transitions for the BDDS-1.4(54) Series

- Phase separation of the SPU hard and soft segment domains increases with amino-acid incorporation into the hard segment.
- Quench cooling of amino-acid derivatized polymers immediately after heating to 250°C shows that the annealing process increases phase separation and decreases interactions in the hard segment domains.
- At temperatures in excess of 200°C free amine groups, in amino-acid derivatized polymers may react with SPU functional groups such as urea linkages.
- Long chain, non-ionic, hydrophilic and hydrophobic substituents can dramatically enhance hard segment phase separation.
- Long chain, non-ionic, hydrophobic substituents may form a third phase upon annealing of the polymer film.

Figure 4.33 contains thermograms for the derivatized BDDS-2.0(61) series. It will be recalled that BDDS-2.0(61) has a fairly high hard segment content relative to BDDS-1.4(54). The incorporation of ABS and ANS should increase the size of the hard segment which would affect both the Tg and T_{HSM}. Increases in the rigidity of the BDDS-2.0-ANS and BDDS-2.0-ABS polymers as shown by tensile data clearly indicate the elevated size of the hard segments. If the hard segment microdomains are too large they tend to increase the amount of phase mixing in the polymer [35]. This is shown by the decrease of intensity in the Tg, going from BDDS-2.0(61) to BDDS-2.0-ANS and the lack of a well defined T_{HSM} in the latter. The thermogram for BDDS-2.0-ANS in Figure 4.33 is truly indicative of a phase mixed material. Upon annealing, the ABS and ANS derivatized materials show improved phase separation.

Figure 4.33: DSC Thermograms for Derivatized BDDS-2.0(61) SPUs
(y axis division = 0.1 W/g)

Section 4.3.3 Water Uptake Studies

The interaction of materials with water is an important aspect of "biocompatibility" and there is continuing debate regarding the relative merits of hydrophobic versus hydrophilic materials [175]. The materials developed in this work are essentially hydrophilic due to the presence of sulfonate and certain amino acid residues. Two relatively easily available measures of hydrophilicity are water uptake, which gives an indication of "internal" hydrophilicity, and contact angle which relates to surface or interfacial hydrophilicity. This section will deal with the former of the two techniques while contact angle measurements will be discussed in section 4.4.

The importance of water interactions with both the implant-blood interphase and the material itself cannot be overemphasized since, in physiological conditions, water is an essential component of both blood and intracellular structures. Blood plasma is a solution which contains 90 % water [175], while intracellular water comprises approximately 80 % of the mass in most living cells [176].

The water uptake experiments carried out in the present work were intended to measure the capacity of the polyurethane ionomers to absorb and retain water. As will be described in discussions to follow, the water uptake data have important implications for protein sorption to these materials.

Experiments were carried out to measure water uptake at room temperature (i.e. under conditions corresponding to those used in blood interaction studies) and over a period of 30 days. It was also desired to measure the maximum capacity of these materials for water uptake and this was done by incubating the polymers (previously pre-swollen at 25°C for 30 days) in distilled water at 70°C for a period of two days. Uptake at longer times was also evaluated. However the materials did not show further uptake and therefore data are reported only for two day incubations at 70°C.

The water uptake data are summarized in Table 4.23. Also included in Table 4.23 is a relative oualitative ranking of the strength of the materials after uptake was complete. These rankings were obtained by observing the materials' resistance to tear upon pulling on both ends of a polymer strip. All samples underwent water uptake during the same period of time and were manipulated in the same manner in order to allow for comparison. The samples were taken from the same films that were prepared for mechanical testing. Different sections of the film for a selected polymer showed reproducible mechanical properties which tends to give confidence in the consistency of the bulk physical structure of the films.

Section 4.3.3.1 Water Uptake for Non-Derivatized SPUs

The data are actually quite surprising for the first experiment, carried out at room temperature. It was expected that the polymers would show increasing water uptake with increasing sulfonate content, however this is not the case. The room temperature water uptake is not proportional to total sulfonate content (branched and free) or even to free sulfonate content. This is emphasized in Figure 4.34.

The fact that polymer ED in Table 4.23 shows a higher room temperature water uptake than either MDA or BDDS-1.4(54) highlights the dependence of water uptake on chain extender type and perhaps more importantly on the type of bulk and surface structure.

The water uptake data for the BDDS series correlate well with the microstructure revealed by DSC experiments. Water uptake for BDDS-0.2 and BDDS-0.8(50) appears to be dependent more on the prepolymer structure than on sulfonate content. Previous physical property studies emphasized that the ion concentration in these two polymers did not substantially influence microphase separation or initial modulus. On the other hand, in BDDS-1.4(54) sulfonate groups are present in high enough concentration to strongly affect the material's initial strength and microphase structure. In this case it appears that most of

Table 4.23: Water Uptake Data for SPU Materials

Polymer	Percent (w/w) Water Uptake after 30 days at 25°C	* Relative Strength after 30 days at 25°C	Percent (w/w) Water Uptake after 2 days at 70°C	* Relative Strength after 2 days at 70°C
ED	40	ES	63.5	ES
MDA	5	ES	9.5	ES
BDDS-0.2	37	S	205	M
BDDS-0.2-Asp	34	S	383	M
BDDS-0.2-Lys	51	S	359	M
BDDS-0.8(54)	38	S	374	M
BDDS-0.8-Asp	30	S	1018	M
BDDS-0.8-Lys	22	S	610	M
BDDS-1.4(54)	18	S	237	S
BDDS-1.4-ArgMe	18	S	503	S
BDDS-1.4-Arg	19	S	298	M
BDDS-1.4-Asp	20	S	718	M
BDDS-1.4-Gly	22	S	707	S
BDDS-1.4-Lys	22	S	705	W
BDDS-1.4-DDA	77	M	801	VW
BDDS-1.4-PEOMe	69	S	1457	S
BDDS-1.4-BDDS	22	S	294	S
BDDS-2.0(61)	410	W	4600	VW
BDDS-2.0-ABS	440	W	6100	W
BDDS-2.0-ANS	490	M	6200	M
BDDS-2.6(47)	180	M	no noticeable change in uptake, but polymer is partially dissolved	W
BDDS-3.1(86)	dissolved after 2 days (uptake > 1000 after 1 day)	--	--	--

* Qualitative Assessment
ES = extremely strong, VS = very strong, S = strong
M = moderate , W = weak , VW = very weak

Figure 4.34: Water Uptake for Non-Derivatized Polyurethanes

the sulfonates are internalized in the polymer's micro-domain structure and are not immediately available for hydration. Water uptake must actually breakup ion pair aggregates which appear to be highly structured according to DSC thermograms. Because of this state the hydration of the sulfonates appears to be inhibited which then causes the lower room temperature water uptake observed relative to the BDDS-0.8(50) and BDDS-0.2 polymers.

The DSC data in Figure 4.33 showed that increasing the sulfonate content by BDDS incorporation, eventually resulted in phase mixing. This makes the sulfonate groups much more available for hydration since the ability of water to access sulfonate groups is enhanced. Electron spectroscopy for chemical analysis data to be presented in section 4.4 also confirm phase mixing of the high BDDS content materials. Thus room temperature water uptake is

increased for BDDS-2.0(61) and BDDS-2.6(47) relative to BDDS-1.4(54). The lower water uptake of BDDS-2.6(47) relative to BDDS-2.0(61) is attributed to the higher level of branched sulfonates in the former. The magnitude of the difference is, however, surprisingly great and there may well be other phenomena occurring which for the moment remain obscure.

DSC studies were not carried out on the BDDS-3.1(86) polymer but it is clear that a low level of branching combined with a very high sulfonate content contribute to an extremely high degree of water uptake followed by eventual dissolution of the material. Dissolution of BDDS-3.1(86) was complete after four days of water exposure at 25°C (1 g/100mL).

As was shown via DSC measurements the hard segment structure of BDDS polyurethanes can be relaxed by heating the materials. It was reasoned, therefore, that if the swelling experiment were continued at an elevated temperature one should expect to see the BDDS polymers swell to a much higher degree than the two unsulfonated materials ED and MDA. Examining the two-day, 70°C water uptake data in Table 4.23 it can clearly be seen that all of the BDDS polymers swell to a higher degree than either ED or MDA, and that of all the polymers MDA takes up the least amount of water. This supports the idea that the hydration of sulfonates is facilitated at higher temperature.

The higher temperature studies also show again that within the non-derivatized BDDS series water uptake does not necessarily increase with sulfonate content but is strongly dependent on the concentration of branched sulfonates within the structure and their ratio relative to free sulfonate. The 70°C water uptake data for these materials are shown in Table 4.24 along with concentration of free sulfonates per gram of polymer and the ratio of branched sulfonates relative to free sulfonates.

Table 4.24: Water Uptake for Non-Derivatized BDDS Polymers at 70°C

Polymer	Percent (w/w) water uptake after 2 days at 70°C	Moles of Free sulfonate per gram of polymer x10^4	Ratio of (branched sulfonates/ free SO_3)
BDDS-0.2	205	--	--
BDDS-0.8(50)	374	1.2	1.00
BDDS-1.4(54)	237	2.2	0.85
BDDS-2.0(61)	4600	3.8	0.64
BDDS-2.6(47)	dissolving	3.8	1.12
BDDS-3.1(86)	dissolved	8.2	0.16

Another interesting aspect of the water uptake experiments within the BDDS series is the apparent hydration potential of the PPO soft segments. In the BDDS-0.2 polymer the sulfonate concentration is very low and although no titration was carried out on this polymer it may be assumed that a fraction of the sulfonate groups are in the branched form. Therefore it is unlikely that all of the water uptake capacity of these polymers is related to the sulfonates and that a significant portion is attributable to the ether linkage of the PPO chains. PPO is much less soluble in water than PEO, yet solubility studies have shown that PPO of molecular weight less than 2000 is soluble in water [177].

Polymer BDDS-2.6(47) exhibits unique water uptake behaviour and merits further discussion. This polymer swells quite rapidly in the first five days at room temperature. However subsequent water uptake is very small, presumably because of branching within the polymer. At the same time the polymer continues to "hydrate" at the water-polymer interface since it begins to dissolve. This behaviour was particularly evident in the experiment at 70°C since the final weight of the polymer was less than the weight following room temperature

swelling and the water took on the colour of the polymer. This emphasizes the fact that the ultimate degree of hydration is dependent on the total sulfonate content, independent of the number of free or branched sulfonates, since both BDDS-2.0(61) and BDDS-2.6(47) had the same concentration of free sulfonates yet BDDS-2.0(61) did not dissolve. On the other hand the rate of water uptake will be strongly dependent on the concentration of free sulfonate and the ratio of branched sulfonates to free sulfonates, since it is seen that BDDS-2.0(61) is taking up water at higher rates relative to BDDS-2.6(47).

In order to check that the incubation at 70°C in water did not degrade the polymers, GPC molecular weight evaluations were carried out on several of the polymers. No significant difference was observed in molecular weights between the original samples and the water-swollen polymers.

Section 4.3.3.2 Water Uptake Studies for Derivatized Polyurethanes

Water uptake experiments were also carried out for the derivatized polymers and the data are shown in Table 4.23 along with data for the corresponding base polymers. One can see that in the room temperature water uptake experiment, the swelling for all of the derivatized polymers, with two exceptions, is similar to that of the corresponding BDDS polymer.

The most convincing evidence in relation to this point is for the BDDS-1.4-PEOMe and BDDS-1.4-DDA polymers. According to DSC studies these are the only two derivatized polymers which exhibit a gross distortion of hard segment micro-domain structure, suggesting that "virtual" cross-links that are normally associated with the hard segment micro-domains for a polymer of the BDDS-1.4(54) type were no longer operative. If this interpretation is correct, one would expect to see a dramatic enhancement of room temperature water swelling in these two polymers as is in fact observed.

As was seen for the non-derivatized BDDS polymers heating at 70°C relaxes the hard segment interactions in the derivatized polymers and enhances the exposure of ionic groups to the water medium. In all cases water uptake is greater than for the "parent" BDDS polymers. The capacity of "pendant" ionic groups to enhance the water uptake of the BDDS polymers is enormous and suggests that the proximity of the BDDS sulfonate groups to the rigid hard segment domains greatly limits the potential for ionic hydration and polymer swelling.

It should be pointed out that the enhancement of water uptake is in proportion to the degree of substitution. For example BDDS-1.4-Arg and BDDS-1.4-BDDS had substitution levels of 30 % and showed much less water uptake than the other derivatized BDDS-1.4(54) materials, which had higher levels of substitution.

The importance of chain branching and PPO content is again reflected in the "ultimate" water uptake capacity of the substituted polymers. Comparing BDDS-1.4-Asp to BDDS-0.8-Asp it can be seen that the latter shows greater water uptake than the former. Based on free ionic content alone the reverse would be expected.

A closer examination of the BDDS-1.4(54) series allows one to evaluate the effect of different substituents on water uptake. The arginine methyl ester polymer shows lower water uptake than do the aspartic acid, lysine and glycine derivatized polymers. This may reflect the fact that polymer BDDS-1.4-ArgMe has no anionic group on the polymer. What actually differentiates the "hydrophilicity" of the three other amino-acid derivatized polymers is not their water uptake but their relative strength after swelling. Both the glycine and aspartic acid derivatized polymers retained the water taken up and showed a reasonable material strength when removed from water contact. The lysine derivatized polymer was weak and lost water within a few minutes of removal from the aqueous medium. This may suggest that there is a difference between the "type" of water in the glycine and aspartic acid derivatized polymers and the lysine derivatized material. For example polymers BDDS-1.4-Gly and

BDDS-1.4-Asp may contain more "structured" bound water than does the BDDS-1.4-Lys material.

From the data for the BDDS-1.4-DDA and BDDS-1.4-PEOMe polymers it can be seen that these materials also have high water uptake. The level of water uptake observed for BDDS-1.4-DDA in the two day experiment at 70°C is perhaps surprisingly high since it could be argued that the presence of C_{12} hydrocarbon chains would reduce the ultimate hydration of the polymer. As can be seen it showed water uptake similar to the amino-acid containing materials. It is clear that the mechanism of water uptake must be different from that of the amino-acid derivatized polymers, as suggested by the strength of the swollen polymer. The wet polymer is very weak and upon removal from water the "hydration" water seeps out at a rapid rate. In the swollen state, when the material is in the water, the sample loses its shape, while all of the amino-acid derivatized materials retain a somewhat dry-state shape with well defined edges. As opposed to being "structured" or bound water it is suggested the water within the swollen BDDS-1.4-DDA polymer is almost completely made up of bulk water.

The BDDS-1.4-PEOMe polymer shows the highest level of swelling of any of the derivatized BDDS-1.4(54) polymers. This is not surprising since PEO is itself water soluble. In addition, the water swollen polymer has a material "strength" comparable to the BDDS-1.4-Asp and BDDS-1.4-Gly derivatized polymers and retains its water after being removed from the aqueous environment. These observations suggest that unlike polymer BDDS-1.4-DDA, the PEOMe containing material promotes a certain degree of water structuring in the swollen state.

The polymers which showed the greatest resemblance to classical hydrogels were the BDDS-2.0(61) series. The ion concentration in these materials is too low to allow the polymers to dissolve in water. However the level of sulfonate branching is low enough to allow "swelling" up to 4600 percent in the underivatized polymer and up to 6200 percent for the derivatized materials. These data correspond to polymer concentrations in the swollen state of 2.2 g/100 g of water for the underivatized polymer and 1.6 g/100 g for the derivatized polymer. Removal of the polymers from water results in immediate loss of the absorbed water suggesting that the better part of this water is in the "bulk" state. Samples of all three polymers retained defined edges but were somewhat distorted from their pre-swollen shape.

Section 4.3.3.3 General Conclusions on Water Uptake Data

The actual physical state of the functionalized polymers (sulfonated or derivatized) in the water-swollen state is almost certainly similar in nature to hydrogels. In a sense they can be regarded as concentrated solutions of polymers in water, since in the final swollen state the polymer concentrations range from 1.6 g/100 g for BDDS-2.0-ABS to 52.9 g/100g of water for BDDS-0.2. Due to a combination of effective crosslinks and limited solubility on the part of specific segments within the different polymer matrices, they are not infinitely dilutable, but reach an equilibrium state of swelling, in which they possess shape and elasticity.

Roorda et al. [182] described the internal structure of such hydrogels in the following manner. The internal structure should not be regarded as that of a rigid polymer framework through which water filled pores or channels run, but rather as that of a highly mobile mixture of water and polymer chains. The average mobility of water in these structures is expected to be significantly reduced by the presence of polymer.

Although an extensive study of water structuring was not carried out for the polymers of the present study, a few DSC thermograms were obtained for polymer films that were cast in an environment of high moisture content. These showed different transitions from the films that were cast in a controlled moisture-free environment. Recent work by Roorda et al. [182] showed that DSC can be used to study hydrogel structure. Figure 4.35 shows the DSC thermograms for BDDS-1.4-DDA and BDDS-1.4-ArgMe cast in a moist environment. One can see that there are transitions near 0°C and 125°C for both materials. Neither of these transitions is apparent in the DSC thermograms for the corresponding dry-cast polymers shown in Figure 4.36. It is believed that these transitions are the result of structural alterations created by the presence of water in the film making process. These transitions are likely not associated with bulk water since the shape of the transition is not characteristic of a free water transition (see Figure 4.37).

Figure 4.37 shows the DSC thermograms for two BDDS-1.4-Lys films. Both films were cast in a dry environment, however, one was exposed to moist air prior to performing the DSC experiment. The wet film shows two very sharp peaks at approximately 0°C and 100°C while the dry film shows no transition of this nature. These peaks are clearly associated with the bulk water absorbed in the polymer film and are not at all similar to the peaks observed for the materials in which water was incorporated within the polymer film during the casting procedure. These simple DSC studies serve to illustrate the different character that water can exhibit when interacting with polymer films.

Figure 4.35: DSC Thermograms of SPU Materials Cast in a Moist Environment

Figure 4.36: DSC Thermograms of SPU Materials Cast in a Dry Environment (y axis division = 0.1 W/g)

Figure 4.37: Comparison of DSC Thermograms for "Dry" and "Wet" SPU Material (y axis division = 0.1 W/g)

In concluding this section it is important to note the potential role that a hydrogel-like character can play in the biocompatibility of the polyurethanes. Several research groups have postulated and given evidence for the existence of a carbohydrate-rich coat covalently attached to the surface of the plasma contacting cells [178-181]. This carbohydrate-rich layer is highly hydrated and gelatinous and has pronounced effects on the interfacial energy of the blood-cell interface [175]. Such a surface is in part responsible for the blood compatibility at the endothelial cell-plasma interface and the different structures of water within this surface are most important in the biological phenomena occurring at the cardiovascular wall [178]. Therefore the hydrogel-like polyurethanes presented here may be able to mimic to some degree the carbohydrate-like structures of the endothelial wall.

Section 4.4 Surface Characterization

Surface characterization was performed to provide information on the nature of chemical functionality at the polyurethane surfaces. In particular, the relationship between bulk and surface chemical composition is of interest. Surface chemical data are also useful in the interpretation of the blood interactions of these materials (section 4.5).

Two methods were used for surface characterization: water contact angle measurements using both the sessile drop and captive bubble techniques, and electron spectroscopy for chemical analysis (ESCA). Contact angles provide a measure of the interfacial tension between water and the material, while ESCA gives a mapping of the elemental composition in approximately the top 100 A layer at the material-vacuum interface.

Section 4.4.1 Electron Spectroscopy for Chemical Analysis

Over the past decade ESCA has become an important technique for surface chemical analysis of biomaterials because it is simple, nondestructive and gives chemical compositions in a region closer to the surface than other available methods [160].

In the present experiments a survey scan (0 to 1000 eV binding energy) of each sample was done at a takeoff angle of 0° (i.e. normal to the surface) to determine which elements were present in the surface region. Spectra of elements detected in the survey scans and high resolution C_{1s} spectra were acquired at takeoff angles of 0, 39, 55,68 and 80 degrees (the takeoff angle is defined as the angle between the normal to the sample surface and the analyzer lens (see Figure 3.14). The greatest sampling depth (approximately 100 Å) occurs at a takeoff angle of 0° and the smallest sampling depth (about 20 Å) at a takeoff angle of 80° [160].

A listing of ESCA peak positions for various carbon atom types found in polyurethanes is given in Table 4.25 taken from reference [53]. The C_{1s} spectra of the polymers developed in this work show three peaks which are assigned to hydrocarbon (285.0 eV), ether (286.6 eV) and urea-urethane (288.8-289.5 eV) species in the sample. The C_{1s} spectra, acquired at 0° takeoff angle, for BDDS-1.4(54), BDDS-0.8(50) and BDDS-0.2 are shown in Figure 4.38 [183]. In this Figure one can see a gradual change in chemical composition as the prepolymer size increases. The small peak in the 289 eV region is much broader for BDDS-1.4(54) than for BDDS-0.2, indicating that in the latter this peak is due to urethane carbons while in the BDDS-1.4(54) the 289 eV peak is due to both urea and urethane carbons. The ratio of the ether carbon peak at 286.6 eV to the hydrocarbon peak at 285.0 eV is greater for BDDS-1.4(54) than for BDDS-0.2. This reflects the fact that in the latter the hard segment:soft segment ratio is relatively low. Similar observations can be made in comparing BDDS-0.8(50) to either BDDS-0.2 or BDDS-1.4(54).

Table 4.25: C1s ESCA Peak Positions for Some Carbon Atoms Found in SPU Materials [53]

GROUP	PEAK POSITION (eV)	DESIGNATION
CH Hydrocarbon	285.0	Peak A
-CN Amine	285.7 - 286.3	
-C-O Ether, Alcohol	286.5	Peak B
$-\overset{O}{\overset{\|\|}{C}}-$ Ketone	288.0	
$-\overset{O}{\overset{\|\|}{C}}-\overset{H}{\overset{\|}{N}}-$ Amide	288.2	
$-\overset{O}{\overset{\|\|}{C}}-O-$ Acid, Ester	288.8	
$-\overset{H}{\overset{\|}{N}}-\overset{O}{\overset{\|\|}{C}}-\overset{H}{\overset{\|}{N}}-$ Urea	288.8	
$-\overset{H}{\overset{\|}{N}}-\overset{O}{\overset{\|\|}{C}}-O$ Urethane	289.3 - 289.7	Peak C

Complete listings of all the high resolution C1s spectra are shown in Table 4.26 for the "base" SPU materials and in 4.27 for derivatized SPU materials. A general trend seen in Table 4.26 and 4.27 is the depletion of urethane/urea carbon in the surface relative to the bulk. Previous ESCA studies on polyurethane films have also noted this phenomenon which may be interpreted as showing that the surface is rich in soft segment [35, 53, 184]. The C1s high resolution data also give an indication of the favored orientation of the surface localized soft segment. While urethane carbon decreases as the surface is approached, the hydrocarbon content generally increases relative to the ether carbon content. This would suggest that for most of the BDDS polymers, the preferred orientation of the soft segment at the polymer-vacuum interface is such that the methyl groups are at the interface as opposed to being in

the bulk polymer. This concept is depicted in Figure 4.39.

Figure 4.38: C_{1s} Spectra for SPU Materials [183]

Table 4.26: ESCA Analysis of SPU Materials: High Resolution C1s Peak Composition at Various Takeoff Angles (relative accuracy = 10%)

Polymer	Takeoff Angle (degrees)	Atomic Percent Carbon Hydrocarbon	Ether	Urethane/Urea
ED *	0	45.6	49.5	2.4
	80	42.2	54.2	2.2
BDDS-0.2	0	47.3	50.3	2.3
	39	50.6	47.6	1.8
	55	50.8	47.4	1.8
	68	53.3	45.3	1.5
	80	53.8	45.0	1.2
BDDS-0.8(50)	0	48.7	49.0	2.3
	39	47.5	50.6	1.9
	55	50.2	47.5	2.3
	68	49.8	48.7	1.6
	80	50.7	47.6	1.7
BDDS-1.4(54)	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-3.1 **	0	53.8	43.7	2.5
	39	54.6	43.1	2.3
	55	51.8	45.6	2.6
	68	55.0	42.8	2.3
	80	54.4	43.9	1.5

* Taken from ref. [53]
** Taken from ref. [52]

Table 4.27: ESCA Analysis of Substituted SPU Materials: High Resolution C1s Peak Composition at Various Takeoff Angles (relative accuracy = 10 %)

Polymer	Takeoff Angle (degrees)	Atomic Percent Carbon Hydrocarbon	Ether	Urethane/Urea
BDDS-0.2-Lys	0	42.4	55.6	2.0
	39	46.0	52.3	1.8
	55	43.8	53.7	2.4
	68	45.5	52.7	1.8
	80	47.6	51.0	1.4
BDDS-0.2-Asp	0	47.3	50.9	1.8
	39	49.5	48.5	2.0
	55	48.5	49.6	1.9
	68	49.6	48.8	1.5
	80	49.7	48.6	1.6
BDDS-0.8-Lys	0	47.0	51.0	2.0
	39	47.2	50.7	2.1
	55	47.8	50.6	1.7
	68	45.7	52.2	2.1
	80	46.3	52.0	1.8
BDDS-0.8-Asp	0	46.9	51.3	1.8
	39	48.4	49.7	1.9
	55	48.7	49.5	1.8
	68	49.6	49.0	1.4
	80	47.6	50.8	1.6

Table 4.27 cont'd: ESCA Analysis of Substituted SPU Materials: High Resolution C1s Peak Comparison at Various Takeoff Angles (relative accuracy = 10%)

Polymer	Takeoff Angle (degrees)	Atomic Percent Carbon Hydrocarbon	Ether	Urethane\Urea
BDDS-1.4-Arg	0	49.7	48.4	1.9
	39	49.1	48.7	2.1
	55	51.6	46.9	1.5
	68	49.7	48.4	2.0
	80	51.7	46.0	2.4
BDDS-1.4-ArgMe	0	49.3	48.5	2.2
	39	50.7	46.8	2.5
	55	50.0	47.9	2.1
	68	49.4	48.4	2.2
	80	49.6	48.6	1.9
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-Lys	0	51.2	46.1	2.7
	39	50.8	47.1	2.1
	55	55.1	43.2	1.6
	68	51.7	46.4	1.9
	80	54.0	44.4	1.6
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
BDDS-1.4-BDDS	0	51.2	46.1	2.7
	39	50.8	47.1	2.1
	55	55.1	43.2	1.6
	68	51.7	46.4	1.9
	80	54.0	44.4	1.6

Section 4.4.1.1 ESCA Data for Non-Derivatized BDDS and ED Polyurethanes

Table 4.28 lists the atomic composition data at various takeoff angles for the BDDS base polymers and ED. As can be seen there is silicon contamination in these materials. From the angular dependence, the silicon appears to be enriched at the surface. The silicon binding energy is consistent with the presence of a siloxane such as poly(dimethylsiloxane) (PDMS) [183].

To test the PDMS contamination hypothesis, calculations for the surface content of BDDS, MDI, PPG and PDMS were carried out on three random sets of data taken from Table 4.28. From atomic balances one can confirm that these four compounds, which includes the contaminant PDMS, account for the observed surface composition. The proportions of BDDS, MDI, PPG and PDMS are determined from the C, N, S and Si contents. This provides enough information to calculate oxygen content for comparison to the value of oxygen content obtained from the ESCA data. Sample data showing the atomic distribution for each compound are listed in Table 4.29. For all three random data sets the calculated atomic oxygen content, based on oxygen contributed from BDDS, MDI, PPG and PDMS, is in good agreement with the oxygen content obtained from the ESCA analysis. Thus atomic balances are consistent with the conclusion that these four species make up the surface of the materials.

Figure 4.39: Orientation of Soft Segment Methyl Groups on Sulfonate SPU Materials

a) Favored Methyl Group Orientation

b) Un-favored Methyl Group Orientation

Table 4.28: ESCA Analysis of Base BDDS and ED SPU Materials: Elemental Composition in Atom Percent at Various Takeoff Angles (relative accuracy = 10%)

Polymer	Takeoff Angle (degrees)	Atom Percent C	O	S	N	Si
ED *	0	73.4	22.7	--	3.8	--
	20	73.2	22.7	--	4.2	--
	40	74.7	21.8	--	3.5	--
	60	74.6	22.5	--	2.9	--
	80	74.8	22.2	--	3.0	--
BDDS-0.2	0	72.6	22.2	--	2.2	3.0
	39	72.5	21.6	--	2.1	3.9
	55	70.6	22.8	--	2.0	4.7
	68	70.1	22.0	--	1.8	6.0
	80	67.9	22.9	--	1.4	7.8
BDDS-0.8(50)	0	75.0	20.5	0.3	2.6	1.7
	39	75.8	19.8	0.2	2.4	1.9
	55	72.8	22.4	0.1	2.3	2.3
	68	72.4	21.9	0.1	2.1	3.5
	80	71.2	22.8	0.1	1.6	4.3
BDDS-1.4(54)	0	73.7	20.9	0.6	3.6	1.2
	39	73.8	21.3	0.6	2.9	1.6
	55	72.8	22.2	0.4	3.0	1.6
	68	73.8	20.9	0.3	2.4	2.6
	80	72.7	22.1	0.3	2.2	2.6
BDDS-3.1 **	0	70.9	20.4	1.4	3.8	1.7
	39	71.8	19.3	1.3	3.7	2.1
	55	71.1	19.9	1.2	3.5	2.2
	68	69.9	20.8	1.3	3.3	2.9
	80	70.4	20.6	0.9	2.9	3.8

* Taken from ref. [53]
** Taken from ref. [52]

Table 4.29: Atom Balance Calculation Based on C, S, N, Si Content

Sample	Takeoff	element	Atom Percent of C,S,N,Si and O contributed by MDI	BDDS	PPG Unit	PDMS	Total
BDDS-0.8(50)	0°	C	17.25	1.8	52.55	3.4	75.0
		O	2.3	0.9	17.25	1.7	22.4
		N	2.3	0.3	--	--	2.6
		S	--	0.3	--	--	0.3
		Si	--	--	--	1.7	1.7
ESCA Oxygen Content: 20.5 atom %							
BDDS-1.4(54)	80°	C	14.25	1.8	51.4	5.2	72.7
		O	1.9	0.9	17.1	2.6	22.5
		N	1.9	0.3	--	--	2.2
		S	--	0.3	--	--	0.3
		Si	--	--	--	2.6	2.6
ESCA Oxygen Content: 22.1 atom %							
BDDS-3.1	80°	C	15.00	3.6	44.2	7.6	70.4
		O	2.0	2.7	14.7	3.8	23.2
		N	2.0	0.9	--	--	2.9
		S	--	0.9	--	--	0.9
		Si	--	--	--	3.8	3.8
ESCA Oxygen Content: 20.6 atom %							

The silicon concentration increases with increasing takeoff angle, indicating that silicon contaminant is located on the surface of the films. Other research groups have found silicon surface contamination as revealed by ESCA data [53, 132], and it has been suggested that the silicon is an artifact of sample preparation for ESCA analysis and is likely linked to the presence of PDMS based vacuum grease.

The presence of this surface contamination could make interpretation of the ESCA data more difficult. However, if one assumes that the silicon contamination is present only at the surface then elemental composition can be "corrected" by giving atomic percentages on a PDMS free basis. The corrected ESCA composition profiles for the base BDDS and ED polyurethanes are presented in Table 4.30. Comparing the data in Tables 4.28 and 4.30 one can see that the correction does not change any of the trends in the data. Its only effect is to cause slight changes in the atom percent composition.

Examining the data in Table 4.30 it can be seen that there is a decrease in nitrogen content as the surface is approached. This implies surface depletion of hard segment since nitrogen is not found in the polyether chains which make up the bulk of the soft segment in polyurethanes. This depletion is observed in all materials and confirms the soft segment surface enrichment predicted by the C1s spectra discussed previously. Since the BDDS molecule is located in the hard segment it is understood that the sulfur content will also decrease as the surface is approached. No sulfur is detected for the BDDS-0.2 material since the lower limit of detection is about 0.2 atom %. The data suggest that the surface of BDDS-0.2 contains very little sulfonate and that the material's surface is primarily composed of soft segment. Based on the elemental composition for the upper surface layer (take-off angle = 80°), the BDDS-0.8(50) material contains 1 BDDS molecule for every 18 MDI molecules and 22 PPO1000 molecules, compared to bulk values of 1 BDDS molecule for every 8 MDI molecules and 6 PPO1000 molecules. This gives a clear idea of how few sulfonate groups are actually on the surface.

Table 4.30: Corrected ESCA Analysis of Base BDDS and ED SPU Materials: Elemental Composition in Atom Percent at Various Takeoff Angles (relative accuracy = 10%)

Polymer	Takeoff Angle (degrees)	Atom Percent C	O	S	N
ED *	0	73.5	22.7	--	3.8
	20	73.2	22.7	--	4.2
	40	74.7	21.8	--	3.5
	60	74.6	22.5	--	2.9
	80	74.8	22.2	--	3.0
BDDS-0.2	0	75.7	21.8	--	2.5
	39	76.6	20.9	--	2.5
	55	75.3	22.3	--	2.4
	68	76.5	21.1	--	2.4
	80	76.1	21.9	--	2.0
BDDS-0.8(50)	0	76.7	20.2	0.3	2.8
	39	77.9	19.4	0.2	2.6
	55	75.2	22.2	0.1	2.5
	68	76.0	21.5	0.1	2.4
	80	75.6	22.4	0.1	1.9
BDDS-1.4(54)	0	74.9	20.7	0.6	3.8
	39	75.3	21.0	0.6	3.1
	55	74.4	22.0	0.4	3.2
	68	76.6	20.4	0.3	2.7
	80	76.1	21.2	0.3	2.4
BDDS-3.1 **	0	73.9	20.4	1.5	4.2
	39	75.3	19.2	1.4	4.1
	55	74.9	19.9	1.3	3.9
	68	74.0	20.7	1.5	3.8
	80	75.3	20.2	1.1	3.5

* Taken from ref. [53]
** Taken from ref. [52]

The ratio of surface sulfur content to bulk sulfur content improves as BDDS content in the polymer increases. Thus for BDDS-1.4 the content of BDDS at the surface is 1 BDDS molecule for every 8 MDI molecules and 6 PPO1000 molecules while in the bulk there is 1 BDDS molecule for every 5 MDI and 3 PPO1000. For BDDS-3.1 there is 1 BDDS molecule per 2 MDI and 2 PPO1000 molecules. For this particular MDI/PPO1000/BDDS system, the BDDS-3.1 has a surface sulfonate concentration similar to that of the bulk which is 2.4 MDI and 1 PPO1000 for every BDDS molecule.

It is difficult to assess the exact area of the surface covered by sulfonate since there is no information available on the conformation of the polymer chains at the surface. As a rough estimate one can calculate the area based on the addition of bond lengths for the different molecules, assuming that the molecules are fully extended. Data on bond lengths were obtained from reference [29]. The results of such a calculation are shown in Table 4.31 taking the ionic radius of a sulfonate anion to be of the order of 5 A [206]. The sulfonate coverages at the immediate surface are estimated at 4 BDDS molecules per 10000 A^2 for BDDS-0.8, 19 for BDDS-1.4(54) and 57 for BDDS-3.1.

Table 4.31: Estimate of Sulfonate Surface Concentrations in Base Polyurethanes

Polymer	Surface Concentration BDDS molecules/ 10000 A^2
BDDS-0.8(50)	4
BDDS-1.4(54)	19
BDDS-3.1	57

Table 4.32: Comparison of ESCA Sulfur Content with other Elemental Analytical Techniques

Analytical Technique	Polymer Sulfur Content (Wt %)		
	BDDS-0.8	BDDS-1.4	BDDS-3.1
SEM-EDAX	0.75	1.4	3.2
Combustion	0.86	1.4	2.9
ESCA, 0°	0.73	1.4	3.3
ESCA, 39°	0.48	1.4	3.0
ESCA, 55°	0.24	1.0	2.8
ESCA, 68°	0.24	0.72	3.0
ESCA, 80°	0.23	0.71	2.1

It should also be pointed out that the ESCA elemental analysis data are in good agreement with the bulk chemical analysis data obtained using SEM-EDAX and combustion analysis. Table 4.32 presents data for all three techniques in terms of weight percent sulfur. The ESCA data at a takeoff angle of 0°, equivalent to a sampling depth of about 100 Å, are similar to those from the bulk analytical techniques. This confirms that sulfonate depletion only occurs within the top 100 angstroms of the surface. It is also observed that the depletion effect occurs at a lower depth as BDDS content increases in the bulk polymer (i.e. depletion is less for BDDS-3.1 than for BDDS-0.8). This possibly had to do with the fact that phase mixing is usually greater in high hard segment content materials and thus would promote a more even distribution of sulfur throughout the surface region [35].

Section 4.4.1.2 ESCA Data for Derivatized BDDS Polyurethanes

The ESCA data giving atomic composition for the derivatized polymers are presented in Table 4.33. Data corrected for PDMS surface contamination as described in the previous section are shown in Table 4.34.

Comparing the ESCA depth profiles of the BDDS-0.2-Asp and BDDS-0.2-Lys polymers to the original BDDS-0.2 polymer in Table 4.30 one can see no major changes in the distribution of N, C, and O compositions and the absence of sulfur is still noted.

The derivatized BDDS-0.8 polymers do show slight differences relative to their parent polymer. The data for BDDS-0.8(50) in Table 4.30 clearly show a decrease in sulfur content as the surface is approached. However in the two derivatized BDDS-0.8 polymers (Table 4.34) this effect is somewhat attenuated. The decrease in nitrogen toward the surface appears to be of the same order for all three polymers. These observations suggest that the incorporation of the substituents "enhances" the presence of the hard segment at the surface.

Reduced sulfonate depletion at the surface is also observed for the derivatized BDDS-1.4 polymers relative to BDDS-1.4 itself. This effect appears to be a general trend since a large number of substituents were examined in this series. The sulfur gradient across the top 100 Å layer is clearly reduced for almost all of the BDDS-1.4 based materials shown in Table 4.34 in comparison to the BDDS-1.4(54) polymer (Table 4.30). In addition one can also observe that the nitrogen gradient is also reduced. Since the depletion of sulfur from the surface is almost completely eliminated, one must associate the better part of nitrogen depletion with the MDI moieties in the prepolymer and not with the BDDS-MDI chain extender segments. This suggests that the interactions occurring between the derivatized BDDS-MDI segments limits their capacity to migrate away from the surface, perhaps because of extensive phase mixing or increased surface activity of the substituent molecules.

Table 4.33 ESCA Analysis of Derivatized SPU Materials: Elemental Composition in Atom Percent at Various Takeoff Angles (relative accuracy = 10 %)

Polymer	Takeoff angle	Atom Percent C	O	S	N	Si
BDDS-0.2-Lys	0	74.5	22.1	--	2.5	1.0
	39	75.0	21.0	--	2.7	1.4
	55	73.8	21.9	--	2.3	2.0
	68	74.4	21.0	--	2.1	2.4
	80	72.8	21.8	--	1.8	3.5
BDDS-0.2-Asp	0	74.8	20.8	--	2.4	2.1
	39	74.6	20.5	--	2.2	2.7
	55	73.7	20.7	--	2.0	3.7
	68	73.0	20.4	--	1.7	4.9
	80	71.6	20.6	--	1.6	6.2
BDDS-0.8-Lys	0	75.4	20.4	0.3	2.9	1.0
	39	75.5	20.2	0.3	2.6	1.4
	55	75.2	20.1	0.3	2.4	2.1
	68	74.6	19.9	0.2	2.0	3.3
	80	73.7	20.3	0.2	2.0	3.9
BDDS-0.8-Asp	0	72.7	22.7	0.3	3.1	1.3
	39	73.3	21.8	0.4	2.7	1.8
	55	73.7	21.7	0.3	2.0	2.4
	68	72.2	22.1	0.2	2.2	2.8
	80	72.4	22.2	0.2	2.1	3.2

Table 4.33 cont'd: ESCA Analysis of Derivatized SPU Materials: Elemental Composition in Atom Percent at Various Takeoff Angles (relative accuracy = 10%)

Polymer	Takeoff Angle	Atom Percent C	O	S	N	Si
BDDS-1.4-Arg	0	72.9	21.9	0.3	3.0	1.9
	39	73.7	20.4	0.4	3.1	2.4
	55	73.7	20.0	0.3	2.8	3.2
	68	73.2	20.4	0.2	2.4	3.7
	80	68.5	22.1	0.3	1.6	7.5
BDDS-1.4-ArgMe	0	74.5	20.1	0.5	3.9	1.1
	39	73.5	20.9	0.5	3.6	1.6
	55	72.9	21.3	0.4	3.2	2.1
	68	73.3	21.1	0.4	2.8	2.5
	80	72.9	20.7	0.3	2.5	3.6
BDDS-1.4-Asp	0	72.3	22.0	0.6	3.8	1.3
	39	72.1	21.6	0.5	3.9	1.9
	55	71.9	21.7	0.7	3.6	2.3
	68	71.9	21.6	0.5	3.2	2.8
	80	71.0	21.6	0.4	2.9	4.0
BDDS-1.4-Lys	0	73.3	20.7	0.7	3.5	1.8
	39	73.9	19.0	0.5	3.7	2.8
	55	73.6	19.3	0.6	3.6	3.0
	68	72.6	20.1	0.4	2.7	4.2
	80	71.0	21.8	0.5	2.3	4.4
BDDS-1.4-DDA	0	72.8	21.0	0.5	3.8	1.8
	39	72.7	21.0	0.5	3.3	2.6
	55	72.7	20.9	0.4	2.8	3.2
	68	71.4	21.2	0.4	2.6	4.5
	80	69.5	22.4	0.4	2.6	5.2
BDDS-1.4-BDDS	0	75.8	19.3	0.6	3.6	0.8
	39	75.5	19.5	0.6	3.2	1.2
	55	75.5	19.5	0.5	2.8	1.8
	68	74.4	19.8	0.4	2.4	3.0
	80	74.1	20.0	0.5	2.2	3.3

Table 4.34: Corrected ESCA Analysis of Derivatized SPU Materials: Elemental composition in Atom Percent at Various Takeoff Angles (relative accuracy = 10%)

Polymer	Takeoff Angle	Atom Percent C	O	S	N
BDDS-0.2-Lys	0	75.4	22.0	--	2.6
	39	76.4	20.7	--	2.9
	55	75.9	21.6	--	2.5
	68	77.0	20.6	--	2.3
	80	76.6	21.3	--	2.1
BDDS-0.2-Asp	0	77.0	20.4	--	2.6
	39	77.6	20.0	--	2.4
	55	77.8	19.9	--	2.3
	68	78.6	19.3	--	2.1
	80	78.7	19.1	--	2.1
BDDS-0.8-Lys	0	76.5	20.2	0.3	3.0
	39	77.0	19.9	0.3	2.8
	55	77.5	19.6	0.3	2.6
	68	78.3	19.2	0.2	2.3
	80	78.0	19.4	0.2	2.4
BDDS-0.8-Asp	0	73.9	22.5	0.3	3.3
	39	75.1	21.6	0.4	2.9
	55	76.2	21.3	0.3	2.2
	68	75.6	21.7	0.2	2.5
	80	76.6	21.8	0.2	2.4

Table 4.34 cont'd: Corrected ESCA Analysis of Substituted SPU Materials: Elemental Composition in Atom Percent at Various Takeoff Angles (relative accuracy = 10%)

Polymer	Takeoff Angle	Atom Percent C	O	S	N
BDDS-1.4-Arg	0	74.8	21.6	0.3	3.3
	39	76.2	20.0	0.4	3.4
	55	77.2	19.3	0.3	3.2
	68	77.4	19.6	0.2	2.8
	80	76.5	20.9	0.3	2.3
BDDS-1.4-ArgMe	0	75.8	19.7	0.5	4.0
	39	75.1	20.6	0.5	3.8
	55	75.1	21.0	0.4	3.5
	68	75.8	20.6	0.4	3.1
	80	76.8	20.0	0.3	2.9
BDDS-1.4-Asp	0	73.6	21.8	0.6	4.0
	39	74.0	21.3	0.5	4.2
	55	74.0	21.1	0.8	4.1
	68	74.6	21.2	0.6	3.6
	80	75.1	21.0	0.5	3.4
BDDS-1.4-Lys	0	75.0	20.4	0.8	3.8
	39	77.0	18.3	0.5	4.2
	55	76.7	17.5	0.7	4.1
	68	79.6	17.1	0.4	2.9
	80	78.2	18.8	0.5	2.5
BDDS-1.4-DDA	0	74.7	20.7	0.5	4.1
	39	75.2	20.5	0.6	3.7
	55	76.0	20.3	0.5	3.2
	68	76.0	20.3	0.5	3.2
	80	74.5	21.7	0.5	3.3
BDDS-1.4-BDDS	0	76.6	19.1	0.6	3.7
	39	76.8	19.2	0.6	3.4
	55	77.4	19.1	0.5	3.0
	68	77.7	19.1	0.5	2.7
	80	77.7	19.2	0.6	2.5

It is interesting to note that associated with these changes between the derivatized and non-derivatized materials, the balance of carbon and oxygen at the surface shows readjustments, thus confirming the mobility of material segments to rearrange and minimize their interfacial free energy in response to their environment [175]. In this regard it must be kept in mind that the ESCA data are obtained in a vacuum which constitutes an environment quite different from blood plasma. Therefore, ESCA analysis cannot possibly reflect the surface rearrangements which may occur when the polymer material contacts plasma. Nonetheless these ESCA results are consistent with the bulk elemental composition data and some structural features of the materials which were previously detected by DSC studies. In the absence of surface chemical analytical methods that can operate in water ESCA is the best currently available technique to acquire this kind of data. Finally, the ESCA data may represent reasonably accurately the surface chemistry at an air interface. This information is relevant in the interpretation of contact angle data at the air-material interface.

Section 4.4.2 Contact Angle Measurements

Water contact angle measurements on polyurethane films were carried out in Dr. A.W. Neumann's laboratory at the University of Toronto using the sessile drop and captive bubble techniques [163]. Angles were measured on a number of areas of the coated glass slides to obtain statistically significant data. Data were obtained from both "underwater" (captive air bubble) and "droplet in air" (sessile drop) experiments.

As the water uptake studies showed, these polymers are able to absorb water due to their hydrophilic nature. Upon placing a water droplet for contact angle measurement on a dry surface, the water droplet may slowly change shape as water is absorbed.

Table 4.35: Water Contact Angle Data (Sessile Drop Method)

Polymer	Number of Drops Measured	In-air Contact angle (degrees ± S.D.)
BDDS-0.2	66	83 ± 2
BDDS-0.8(50)	79	82 ± 2
BDDS-1.4(54)	84	75 ± 2
BDDS-2.0(61)	72	74 ± 4
BDDS-2.6(47)	67	73 ± 1
BDDS-3.1(86)	54	55 ± 5
BDDS-0.2-Lys	63	84 ± 2
BDDS-0.2-Asp	48	74 ± 6
BDDS-1.4-Lys	61	77 ± 2
BDDS-1.4-Asp	50	69 ± 2
BDDS-1.4-PEOMe	69	74 ± 1

For the non-derivatized polymers, the droplet contact angles (Table 4.35) show a definite decrease as sulfonate content increases, reflecting the more hydrophilic nature for polymers of higher sulfonate content. Although the trend is clear the effect is not as pronounced as might have been expected. Previous work by Cooper et al. [132] showed that the incorporation of sulfonate groups into MDI/PTMO/BD based polyurethanes at a level corresponding to 1.6 % w/w sulfur caused a change in contact angles from 78° to 37°. A possible explanation for the small changes observed in the droplet contact angles in this work relates to the prepolymer. In particular PTMO does not contain pendant methyl groups which could potentially mask the oxide portion of the soft segment. As discussed in relation to the ESCA data the methyl groups appeared to be oriented outwards from the surface. Also it will be recalled that the estimated surface coverage of sulfonate groups was only 5.7 % for BDDS-3.1. Thus the surface is dominated by soft segment with the methyl groups preferentially

oriented outwards from the surface. The relative hydrophobicity of PPO versus PTMO could account for the higher contact angles in these polymers compared to those of Cooper et al. [132].

Droplet contact angle data for some of the derivatized BDDS polymers are also shown in Table 4.35. Aspartic-acid derivatization causes a decrease in the contact angle while lysine derivatization has no effect. This can be seen for both the derivatized BDDS-0.2 and BDDS-1.4(54) polymer series. ESCA data showed that the concentration of sulfur-containing groups was increased at the surface after the addition of substituents. Thus it is likely that the substituents themselves will manifest some effect at the surface. Aspartic acid would be expected to increase hydrophilicity since it has two carboxylate groups. On the other hand the single carboxylate group of lysine may be masked by its long alkyl amine side chain, thus possibly altering the hydrophilicity of the surface in a manner that causes no significant change in contact angle.

The presence of PEOMe as a substituent did not change the contact angle to any significant degree (see Table 4.35). The highly hydrophilic sulfonate groups are replaced by long hydrophilic chains and the net effect appears to be a surface of similar hydrophilicity to the parent BDDS-1.4(54) polymer. It is interesting to note that the changes in the droplet contact angles of these materials do not run parallel to the water uptake data. As reported in section 4.3, BDDS-1.4(54) and BDDS-1.4-PEOMe absorb .18 and .69 grams of water per gram of polymer respectively, for the room temperature experiment. One might expect that the BDDS-1.4-PEOMe material would have a much lower contact angle than BDDS-1.4(54). The data show that the contact angles are not greatly different, suggesting that water interaction at the surface may be substantially different from in the bulk.

In order to determine contact angles for the polymers in the wet state, underwater captive bubble contact angles were measured for surfaces exposed to water for 1 minute (Table 4.36) and 24 hours (Table 4.37). The most striking observation from the 1 minute

water exposure data is that all of the contact angles are now the same for the underivatized series, except for BDDS-3.1. A possible explanation for this observation is that in air the methyl groups have a preferred orientation outwards from the surface, while in an aqueous environment the methyl groups are oriented toward the bulk and the more hydrophilic oxide groups are oriented towards the water-polymer interface. This concept was proposed by Yasuda and Sharma [185] to explain similar behaviour for swollen hydrogels. They suggested that since polymer molecules have many degrees of freedom for rearrangement, in the bulk or at the surface, the occurrence of such orientations would have a high probability.

Table 4.36: Contact Angle Data for Underwater Captive Bubble Method (1 minute water exposure)

Polymer	Number of Bubbles Measured	Underwater Contact Angle (degrees ± S.D.)
BDDS-0.2	31	75 ± 1
BDDS-0.8(50)	16	74 ± 2
BDDS-1.4(54)	27	72 ± 2
BDDS-2.0(61)	28	74 ± 2
BDDS-2.6(47)	28	72 ± 1
BDDS-3.1(86)	26	41 ± 1
BDDS-0.2-Lys	42	77 ± 3
BDDS-0.2-Asp	32	80 ± 4
BDDS-1.4-Lys	26	72 ± 2
BDDS-1.4-Asp	30	66 ± 1
BDDS-1.4-PEOMe	22	72 ± 1

If an exchange of methyl groups for oxide groups is indeed taking place at the surface then one would expect the change in contact angle measured by the two methods to be most dramatic for polymers containing large amounts of soft segment. This is indeed the case: BDDS-0.2 and BDDS-0.8(50) show changes in contact angle of 8° (significant relative to the standard deviations shown in Tables 4.35 and 4.36) while BDDS-1.4(54) shows a change of 3° and BDDS-2.0(61) and BDDS-2.6(47) shows no significant change. Similar differences were also observed for the derivatized BDDS polymers with the exception of BDDS-0.2-Asp which actually showed an increase in contact angle, although the difference may not be significant for this polymer since the standard deviation was high.

A one minute exposure to water is probably not sufficient for the surface layer to reach equilibrium water uptake and presumably to expose further ionic functionality. Thus the contact angles for the more highly sulfonated materials do not show the low values expected of highly hydrophilic materials.

Data on captive bubble contact angle measurements for surfaces exposed to water for 24 hours are shown in Table 4.37. Comparing the standard deviations for Table 4.35 with those found in Table 4.37 it can be seen that the standard deviation is greater for the water treated materials. As the materials adsorb water it can be expected that the surfaces will distort thus resulting in non uniform surfaces which could cause a greater variation in contact angle data. These data show that contact angle values are in the expected low range when the samples are exposed to water for a prolonged period. Within the non-derivatized BDDS polymer series the trend in contact angle values is similar to that seen for the droplet data, namely that the contact angle decreases with increasing sulfonate content. The low contact angle of 11°C for BDDS-3.1(86) suggests that this material is highly hydrophilic as was previously observed in water uptake studies.

Table 4.37: Contact Angle Data for Underwater Captive Bubble Method (24 hour water exposure)

Polymer	Number of Bubbles Measured	Underwater Contact Contact Angle (degrees ± S.D.)
BDDS-0.2	28	25 ± 5
BDDS-0.8(50)	32	24 ± 3
BDDS-1.4(54)	20	23 ± 4
BDDS-2.0(61)	36	21 ± 3
BDDS-2.6(47)	32	17 ± 3
BDDS-3.1(86)	14	11 ± 1
BDDS-0.2-Lys	34	29 ± 8
BDDS-0.2-Asp	29	29 ± 6
BDDS-1.4-Lys	36	27 ± 3
BDDS-1.4-Asp	32	25 ± 8
BDDS-1.4-PEOMe	36	22 ± 3
BDDS-3.1-Asp	32	21 ± 3

Comparing these data to the measurements taken after one minute exposure to water, it can be concluded that the surfaces "evolve" gradually upon contact with water. To illustrate this behaviour, kinetic data for contact angles on the BDDS-3.1(86) surface are shown in Figure 4.40 (sessile drop technique). Similar data for BDDS-1.4-PEOMe are compared to BDDS-3.1(86) data in Figure 4.41. As can be seen from Figure 4.41, the rate at which the contact angle decreases for BDDS-3.1 is twice that for BDDS-1.4-PEOMe. This gives an indication as to how different chemical functionalities adapt to the aqueous environment.

In general all of the BDDS and derivatized BDDS materials are very hydrophilic and this nature is highly dependent on the number of sulfonate and sulfonamide groups in the materials. For instance, the BDDS-3.1 materials are more hydrophilic than the BDDS-1.4 polymers. The nature of functional groups incorporated into the derivatized sulfonated polyurethanes has a relatively small effect on surface compared to bulk properties.

Figure 4.40: Sessile Drop Contact Angle Kinetics for the BDDS-3.1(86) Surface

Figure 4.41: Sessile Drop Contact Angle Kinetics for the BDDS-1.4-PEOMe and BDDS-3.1 Surfaces

Section 4.5 Interactions of Polyurethanes with Blood

Blood interaction studies were carried out to assess both the early stages of plasma protein interactions and the coagulation response to the materials. These studies consisted of fibrinogen adsorption measurements from both plasma and pure fibrinogen solutions, and thrombin time measurements.

Section 4.5.1 Fibrinogen Adsorption from Plasma

Section 4.5.1.1 Vroman Effect

It was hoped that fibrinogen adsorption studies from plasma would provide insight into the initial response of plasma to these surfaces since fibrinogen adsorption is one of the first events to take place when a foreign surface is exposed to blood [100]. To date most artificial surfaces examined with respect to fibrinogen adsorption from plasma exhibit the Vroman effect. The Vroman effect is a maximum in fibrinogen adsorption, occurring within seconds of plasma-surface contact, resulting from the displacement of initially adsorbed fibrinogen by low concentration proteins of high adsorption affinity, in particular the contact phase clotting factors HMWK and factor XII [106]. The Vroman effect can be illustrated in two different ways, either in a time domain or in a concentration domain. In the time domain the classical Vroman effect is illustrated for a fixed concentration of plasma and one observes the amount of fibrinogen adsorbed to the surface as time changes. Since fibrinogen surface concentrations change very fast, most adsorption experiments cannot record the changes occurring at the surface. Therefore another method of simulating the Vroman behaviour is to keep time fixed and vary the concentration of plasma. In this manner at very low concentrations of plasma there is not enough HMWK or other low concentration proteins to

displace all of the fibrinogen protein from the surface and therefore fibrinogen is retained. As the plasma concentration increases beyond a critical value (classically near 1 % normal plasma concentration) HMWK and other proteins are in high enough concentration to begin displacing fibrinogen. As normal plasma concentration is approached virtually all of the fibrinogen is displaced.

Figure 4.42: Fibrinogen Surface Concentration versus Bulk Concentration from Tris and from Plasma for Five Minutes (Adsorption onto Glass, ref. [167])

The experimental design used in this work requires the use of the concentration domain to simulate the Vroman effect. Typical data are presented in Figure 4.42 for a glass surface. In this Figure adsorption from plasma is compared to pure fibrinogen solution at comparable concentrations, onto glass. The quantity of fibrinogen adsorbed is plotted against

bulk concentration for 5 minute adsorption times. Bulk concentration is expressed as percent of normal plasma concentration. For the single protein data 100 % plasma corresponds to 2.5 mg/mL fibrinogen. In the pure fibrinogen experiment, fibrinogen adsorption increases up to a plateau level corresponding to monolayer coverage. Fibrinogen adsorption both from plasma and pure fibrinogen at concentrations lower than 0.5 % is linear with concentration and the slopes are similar. In this regime the surface is presumed to be filling with protein and little interaction takes place among the molecules on the surface. At a bulk concentration greater than 0.5 %, interactions between molecules and competitive effects begin to occur. The adsorption and removal of fibrinogen on glass from 5 % normal plasma is essentially complete within five minutes.

Section 4.5.1.2 Experimental Reproducibility of Adsorption Data

With the large number of functionalized and derivatized materials to evaluate it was impractical to run repeat experiments for all the data sets. Therefore polymers were chosen at random for repeat experiments. These experiments (Figures 4.43 to 4.45) showed that the variation in data and curve form was in general small but could be quite high for highly swelling polymers such as BDDS-2.0(64). Figures 4.43 and 4.44 for BDDS-1.4-PEOMe and BDDS-2.6(47) show very little scatter in repeat runs. Room temperature water uptake for these two polymers were 69 w/w % and 182 w/w % respectively. On the other hand there is more scatter for fibrinogen adsorption from plasma for polymer BDDS-2.0(61) which had a room temperature water uptake value of 410 w/w %, during a three hour adsorption period (see Figure 4.45). These data represent the two extremes of scatter for the experimental work presented in this section. It is important to note that even for the high scatter case shown in Figure 4.45 the adsorption patterns are quite reproducible.

Figure 4.43 Reproducibility for Fibrinogen Adsorption from Plasma onto BDDS-1.4-PEOMe: Five Minute Exposure Time

Figure 4.44: Reproducibility for Fibrinogen Adsorption from Plasma onto BDDS-2.6(47): Five Minute Exposure Time

Figure 4.45: Reproducibility for Fibrinogen Adsorption from Plasma onto BDDS-2.0(61): Three Hour Exposure Time

Section 4.5.1.3 Fibrinogen Adsorption from Plasma for Non-Derivatized Polymers

Figure 4.46 shows data on fibrinogen adsorption from plasma for four different polymer surfaces, including Biomer^r, ED, MDA and BDDS-1.4(54). The latter three polymers vary only in the chain extender used, while Biomer is a MDI/PTMO/ED based polyurethane. The fibrinogen adsorption curve for the sulfonated polymer is significantly different from those of the other three. For the non-sulfonated polymers, complete desorption of fibrinogen has occurred within five minutes at plasma concentrations greater than about 10 %. On the sulfonated SPU, fibrinogen adsorption is still significant at 30 % plasma concentration. In addition, the peak fibrinogen surface concentration is almost double that for Biomer and is a factor of twelve greater than that of ED and MDA. The fibrinogen adsorption peak for the BDDS-1.4(54) material occurs at a higher plasma concentration relative to the other three polymers.

A satisfactory mechanistic explanation for these differences cannot be given at this time. However it is clear that fibrinogen adsorption is dependent on surface hydrophilicity. Polymers ED and MDA are essentially hydrophobic. Biomer is also hydrophobic although as suggested in the section on contact angle data, PTMO is possibly less hydrophobic than PPO. The presence of sulfonate groups in the BDDS-1.4(54) polymer makes it hydrophilic as shown by water uptake and contact angle data.

High fibrinogen adsorption on the sulfonated polymer may be in part the result of a gel-like surface layer consisting of plasma swollen polymer. Such a layer would parallel the concept of a hydrogel carbohydrate layer which is postulated to make up the vascular endothelial layer [175], and was described as a solution of various endothelial polymer components in plasma. It seems likely that the surface region of the dry BDDS-1.4(54) could swell during exposure to Tris buffer, and in the swollen state would certainly have a high

capacity for protein uptake since protein could effectively be dissolved in the gel phase. Fibrinogen displacement by HMWK and other proteins could also be less rapid since protein mobility within this layer may be reduced in relation to bulk solution. It should be noted that such a layer would not necessarily be limited in protein uptake because of protein size if it is hypothesized as a solution rather than a microporous material. This is an important distinction with respect to the ability of the gel surface to absorb large molecules like fibrinogen (M.W.=340000).

An alternative explanation for the high fibrinogen adsorption is that there are specific interactions with sulfonate groups. If these interactions are relatively strong then displacement would be more difficult, the adsorption peak would tend to be higher and the "desorption" phase more gradual, exactly as is the case for polymer BDDS-1.4.

Figure 4.47 shows fibrinogen adsorption from plasma for all of the underivatized BDDS polymers studied. Fibrinogen adsorption is seen to increase with sulfonate content. Polymer BDDS-0.2 behaves much like Biomer and exhibits a Vroman peak at 1 % normal plasma with an approximate surface concentration of 0.1 ug/cm^2. For the BDDS polymers, peak height and plasma concentration at which the peak occurs both increase with increasing sulfonate content for polymers BDDS-0.2, 0.8, 1.4 and 2.0. The adsorption curve for the BDDS-2.6(47) polymer shows no Vroman effect and resembles a Langmuir adsorption isotherm. In addition, the peak adsorption for BDDS-2.0 is greater than any level of adsorption achieved for the BDDS-2.6(47) polymer. However adsorption at 30 % normal plasma is considerably higher for BDDS-2.6(47) than for BDDS-2.0(61). The BDDS-3.1(86) polymer shows still higher adsorption at the peak.

Figure 4.46: Fibrinogen Adsorption from Plasma onto SPU Materials: Five Minute Exposure

Figure 4.47: Fibrinogen Adsorption from Plasma onto Sulfonated SPU Materials: Five Minute Exposure

This series of adsorption curves is important in demonstrating the effect of sulfonation on the adsorption of fibrinogen to polyurethane surfaces. The enhancement of fibrinogen adsorption onto the sulfonated SPU surface BDDS-1.4(54) in Figure 4.46 was rationalized on the basis of two independent hypotheses. From the data in Figure 4.47 it appears that neither of these two hypotheses alone can explain both the high uptake and apparent binding affinity of fibrinogen on the sulfonated SPU polymers.

If fibrinogen uptake were determined solely by absorption into a polymer-plasma gel layer, then it would be expected to increase with increasing thickness of the gel layer. Assuming that the top 100 A of a dry surface is subject to swelling and that the density of plasma in the gel is 1 g/ml, one can estimate the swollen layer thickness and the polymer concentration in the layer, using water uptake data presented in Table 4.23. These estimates are shown in Table 4.38 along with the surface concentration of adsorbed fibrinogen at 30 % normal plasma and at the peak (or plateau level), for all the sulfonated polymers. The amount of fibrinogen remaining on the polymer at 30 % normal plasma is a crude indication of how well stabilized is the fibrinogen-surface interaction. The peak or plateau (when peaks were not observed) level is a crude indication (not a measure) of the capacity that the material has for the protein in plasma.

Table 4.38: Estimated Polymer-Plasma Swollen Layer Properties and Five Minute Fibrinogen Adsorption from Plasma Solution

Polymer	Polymer/ plasma layer Thickness (A)	Adsorbed fibrinogen at 30 % Normal Plasma (ug/cm^2)	Peak adsorbed Fibrinogen (ug/cm^2)
BDDS-0.2	300	0.008	0.084
BDDS-0.8	470	0.012	0.159
BDDS-1.4	340	0.064	0.191
BDDS-2.0	4700	0.271	0.552
BDDS-2.6	280	0.441	0.442
BDDS-3.1	>1100	0.412	0.574

As can be seen in Table 4.38 the surface concentration of fibrinogen at 30 % normal plasma does not increase monotonically with polymer plasma layer thickness but it does increase with polyurethane sulfonate content (with the exception of BDDS-3.1). On the other hand the peak adsorption levels show no correlation with increasing thickness of the polymer-plasma gel layer but do increase with sulfonate content (with the exception of BDDS-2.6). These observations suggest that the hydrogel-layer is not as predominant a factor in the uptake of fibrinogen as is the presence of the sulfonate group itself. Based on this one may favor a mechanism that would imply specific interactions between sulfonate and fibrinogen and would not implicate the hydrogel-layer.

Evidence against only specific fibrinogen-sulfonate interactions and no absorption effect comes from the actual magnitude of the BDDS-3.1(86) and BDDS-2.0(61) peak fibrinogen levels. Such a specific binding mechanism implies that adsorption is taking place at the immediate surface. If this were true then the surfaces of the above two polymers could be completely covered with fibrinogen since monolayer coverage is between 0.2 ug/cm^2 and 1.57 ug/cm^2 [186]. Although this is possible, it is difficult to believe that the protein layer in contact with plasma consists only of fibrinogen, when there are over two hundred other proteins present in plasma. It is the author's belief that both mechanisms are taking place: hydrogel effect and specific interaction effect. As to which of the effects is more important, no comment can be made until further investigations are done.

A longer experiment would be expected to show higher fibrinogen adsorption in the region where displacement is not important and lower adsorption where displacement is the main effect, i.e. the peaks should move to lower plasma concentration [167]. Data were obtained for BDDS-2.0, BDDS-2.6 and BDDS-3.1 for three hours plasma contact and are shown in Figures 4.48, 4.49 and 4.50 along with five minute data for comparison.

In Figure 4.48, BDDS-2.0 shows much higher fibrinogen adsorption at three hours than at five minutes. Moreover the maximum value in the three hour experiment, 3.3 ug/cm^2 is

well beyond the maximum value for a monolayer. If multilayer adsorption is highly unlikely, then the hypothesis of a polymer-plasma layer becomes more attractive. As can be seen also the Vroman effect is now more pronounced but displacement is more gradual than expected at higher plasma concentration, also in agreement with a surface gel layer in which protein diffuses slowly.

Adsorption for both five minute and three hour plasma exposure times to the BDDS-2.6 polymer are shown in Figure 4.49. Fibrinogen adsorption at three hours is greater than at 5 minutes and the Vroman effect is readily apparent. An interesting feature is that fibrinogen displacement stops after 10 % normal plasma, at an adsorption level of 0.6 ug/cm^2. It should be emphasized that BDDS-2.6 is a relatively low-swelling polymer compared to BDDS-2.0. As was previously noted in Figure 4.47 it retains a significant amount of fibrinogen at high plasma concentrations. The questions to be answered here are: (1) why is there an abrupt end to fibrinogen displacement; (2) what is the nature of the surface protein interaction; and (3) what role do the sulfonate groups play in this interaction? Kinetic adsorption data shown in Figure 4.51 for BDDS-2.6 at 5 % plasma, confirm that at three hours the adsorption is close to being at steady state. This suggests that the fibrinogen plateau shown in Figure 4.49 represents irreversibly bound fibrinogen.

The BDDS-3.1 data shown in Figure 4.50 again indicate that longer plasma exposure times are required in order for the Vroman effect to be evident. For the BDDS-3.1 polymer it seems possible that the swollen state probably evolves rapidly to become a dilute polymer solution in plasma. Even though the polymer has a higher sulfonate content and therefore more of the fibrinogen may be specifically bound, the displacement of fibrinogen is greater for BDDS-3.1 than for BDDS-2.0 since the peak level of fibrinogen is greater for BDDS-2.0 than for BDDS-3.1. This would argue in favor of the absorption rather than the specific interaction mechanism. Similar to BDDS-2.0, the displacement of fibrinogen from BDDS-3.1 continues as plasma concentration increases. There is no indication of limited fibrinogen

displacement as was seen for BDDS-2.6.

Figure 4.48 Fibrinogen Adsorption from Plasma at Different Exposure Times for BDDS-2.0(61)

Figure 4.49: Fibrinogen Adsorption from Plasma at Different Exposure Times for BDDS-2.6(47)

Figure 4.50: Fibrinogen Adsorption from Plasma at Different Exposure Times for BDDS-3.1(86)

Figure 4.51: Fibrinogen Adsorption from Plasma: BDDS-2.6 Kinetic Data at 5 % Normal Plasma

Three hour adsorption data for BDDS-0.2 are shown in Figure 4.52. The Vroman peak for the three hour experiment shifts to the left as expected. Perhaps more interesting is the portion of the three hour adsorption curve, at higher plasma concentrations where adsorption appears to be increasing after the Vroman peak. This effect was not apparent for the higher sulfonate content materials and is for the moment difficult to rationalize. There is no apparent reason for this behaviour and the system will have to be examined in more detail.

Grasel and Cooper [132] have given explanations similar to those presented here for the adsorption of fibrinogen to other sulfonated polyurethanes. They refer to the idea of fibrinogen absorption to explain the apparent multilayer adsorption levels of fibrinogen observed on their polymers. They also discuss the possibility of specific interactions between the fibrinogen and sulfonate surfaces but lack information on the nature of such a mechanism.

Further evidence to support enhanced fibrinogen uptake on sulfonated SPUs due to the formation of a polymer-plasma gel layer and to specific sulfonate-fibrinogen interactions, comes from the adsorption data shown in Figures 4.53 and 4.54. In these experiments BDDS-2.0 and BDDS-2.6 surfaces were equilibrated for different times with isotonic Tris buffer, pH 7.4, prior to exposing them to plasma. If a polymer-plasma gel layer is in fact formed then increasing the equilibration time should increase the layer thickness and the levels of fibrinogen adsorption. Figure 4.53 shows five minute fibrinogen adsorption data for BDDS-2.0 equilibrated for ten minutes, five hours and 20 hours. As can be seen, there is an increase in adsorption with increased buffer equilibration time. However, even at the lowest swelling time, fibrinogen uptake is still quite high, suggesting that the specific interaction mechanism could be implicated. It may also be that swelling is very rapid and does not change much after ten minutes as could well be the case for the thin layers of polymer used in the adsorption experiment (< .1 mm thick).

Figure 4.52: Fibrinogen Adsorption from Plasma at Different Exposure Times for BDDS-0.2

Figure 4.53: Effect of Pre-Equilibration in Tris Buffer on Fibrinogen Adsorption from Plasma onto BDDS-2.0(61): five minute exposure

Figure 4.54 shows similar data for polymer BDDS-2.6. For this polymer, swelling is not as great and one would not expect to see a strong influence of pre-equilibration time on adsorption. The data confirm this expectation. Although there is a change in fibrinogen adsorption from ten minutes to five hours, there is no change between five hours and 20 hours and the shapes of the curves are identical.

Section 4.5.1.4 Fibrinogen Adsorption from Plasma onto Derivatized SPUs

Fibrinogen adsorption measurements from plasma were also carried out for a large number of the derivatized sulfonated polyurethanes. Figure 4.55 compares BDDS-0.2 with the corresponding polymers derivatized with lysine and aspartic-acid. Amino-acid incorporation is seen to decrease the peak value of fibrinogen adsorption. Both of the amino-acid containing materials show similar curves although the aspartic-acid polymer retained more fibrinogen at higher plasma concentrations than the BDDS-0.2 and BDDS-0.2-Lys polymers. These differences are relatively small and perhaps not significant. The behaviour of these polymers is expected to be dominated by the soft segment; the hard segment whether derivatized or not, is expected to have little influence.

Figure 4.56 shows fibrinogen adsorption data for the BDDS-0.8(50) series. In this case there are noticeable differences among all three polymers. The non-derivatized polymer shows the highest peak adsorption followed by BDDS-0.8-Lys and then BDDS-0.8-Asp. This order is the inverse of water uptake data (see Table 4.24). Fibrinogen adsorption appears to be dependent more on binding and thus on surface chemistry rather than on the thickness of the polymer-plasma gel at the interface. This does not imply that fibrinogen uptake into the polymer-plasma gel does not occur. Rather the amino-acid moieties appear to have a lower fibrinogen binding affinity than do free sulfonate groups.

Figure 4.54: Effect of Pre-Equilibration in Tris Buffer on Fibrinogen Adsorption from Plasma onto BDDS-2.6(47): Five Minute Exposure

Figure 4.55 Fibrinogen Adsorption from Plasma onto Derivatized BDDS-0.2 SPU Materials: Five Minute Exposure

Figure 4.56: Fibrinogen Adsorption from Plasma onto Derivatized BDDS-0.8(50) SPU Materials: Five Minute Exposure

Fibrinogen adsorption data for the derivatized BDDS-1.4(54) polymers are shown in Figures 4.57 and 4.58. Of the derivatized polymers, BDDS-1.4-Asp polymer shows the lowest peak level of adsorbed fibrinogen while BDDS-1.4-Lys shows the highest. The peak adsorption for the lysine derivatized polymer is almost identical to that of the non-derivatized BDDS precursor which has the highest value of this series. However, fibrinogen displacement occurs at a lower plasma concentration for the lysine derivatized polymer than for BDDS-1.4(54), suggesting stronger binding to free sulfonate groups.

Beyond the adsorption peak, all of the polymers in Figure 4.57 show a continuous decrease in fibrinogen adsorption as the plasma concentration increases until all or most of the fibrinogen is displaced. This is contrary to the three non-anionic derivatized polymers shown in Figure 4.58 which show a levelling off at a value of 0.04 ug/cm^2. The significance of this is unknown but it may be noted that arginine methyl ester is the only amino substituent not bearing an anionic group. In addition, the two polymers derivatized with DDA and PEOMe display almost identical behaviour to the ArgMe derivatized polymer. Like the other derivatized polymers, BDDS-1.4-DDA and BDDS-1.4-PEOMe have lower adsorption peaks than does BDDS-1.4(54).

Adsorption data for a group of derivatized BDDS-2.0 polymers are shown in Figure 4.59. All of these materials have substituents containing free sulfonate groups. It can be seen that both the amino benzene sulfonate derivatized polymer (BDDS-2.0-ABS) and the naphthalene sulfonate derivatized polymer (BDDS-2.0-ANS) have markedly reduced levels of fibrinogen adsorption in the region of the peak and beyond. Since all of these materials have high water uptake and about the same free sulfonate group content they would have been expected to show similar adsorption. A possible explanation for the lower adsorption on the ANS and ABS derivatized materials is that for steric reasons, the sulfonate groups are less readily available.

Figure 4.57 Fibrinogen Adsorption from Plasma onto Derivatized BDDS-1.4(54) SPUs: Materials: Five Minute Exposure

Figure 4.58: Fibrinogen Adsorption from Plasma onto Derivatized BDDS-1.4(54) SPU Materials: Five Minute Exposure

Figure 4.59: Fibrinogen Adsorption from Plasma onto Substituted BDDS-2.0 SPU Materials: Five Minute Exposure

Section 4.5.1.5 General Conclusions

The conclusions of the above discussions on fibrinogen adsorption from plasma are summarized in Table 4.39. It is generally accepted that surfaces which adsorb high levels of fibrinogen tend to promote platelet adhesion and activation [189] and one may conclude from the data presented in the previous sections that the sulfonated materials and their derivatized analogs would be highly platelet-reactive. However, recent work by Grasel and Cooper [132] has shown that platelet adhesion and activation is actually reduced on sulfonated polyurethanes even though these surfaces adsorb relatively large quantities of fibrinogen. These apparently conflicting observations are for the moment without an explanation.

Fibrinogen adsorption from plasma onto the functionalized surfaces developed in this research appears to be dependent on the thickness and nature of the polymer plasma-gel layer which we hypothesize exists at the plasma-SPU interface, the nature of functionality and the conformational orientation state (i.e availability) of the functional groups. From the data obtained it is difficult to uncouple these three effects.

Section 4.5.2 Fibrinogen Adsorption from Tris Buffer Solution

The plasma experiments discussed in the previous section suggest the possibility that fibrinogen interacts specifically with sulfonate groups. To investigate this possibility adsorption of fibrinogen as a single protein from buffer was studied. It seemed likely that a better measure of fibrinogen-sulfonate interactions would be obtained without competition from the other plasma proteins. In principle adsorption isotherms should provide an indication of binding affinity [167]. Adsorption isotherms for ED, MDA and BDDS-1.4 are shown in Figure 4.60. The initial slope region of the isotherms is used to derive information

on the binding affinity of the protein to the surface. During adsorption fibrinogen exchange at the surface is slow and it is thought that exchange allows fibrinogen to progressively rearrange and adsorb in a more end-on conformation/orientation permitting the monolayer to exceed 0.20 ug/cm^2 [167]. Work in this laboratory has shown that fibrinogen binding is for the better part non-reversible in these experiments [167]. The plateau values which indicate the capacity of the surface for fibrinogen have usually been found to correspond to a close packed monolayer [167].

Table 4.39 Conclusions of Fibrinogen Adsorption Studies from Plasma to Sulfonated and Derivatized SPUs

- Sulfonated polyurethanes adsorb and retain large amounts of fibrinogen
- Water uptake data suggest the presence of a polymer-plasma gel layer at the interface. This layer may cause the observed increase in the level of fibrinogen uptake.
- The adsorption data suggest that specific interactions may occur between sulfonate groups and fibrinogen.
- Adsorption to lysine derivatized polymers is greater than to aspartic acid derivatized polymers. Derivatization, in general, decreases fibrinogen adsorption.
- Arginine methyl ester, DDA and PEO chain substituents give materials with similar fibrinogen adsorption levels and tend to retain higher levels of fibrinogen, at high plasma concentration, than do amino-acid substituents.
- Surface affinity for fibrinogen adsorption is apparently dependent on functional group availability.

Since it was not an objective of this work to model protein adsorption either from an equilibrium or kinetics point of view, fibrinogen adsorption data will be discussed in a qualitative manner. Interpretation of initial isotherm slopes and adsorption capacity will be made for the purpose of comparing the different materials. Both initial slope and apparent plateau levels for fibrinogen adsorption from Tris buffer onto all of the polymers, are listed

in Table 4.40. The initial slopes were calculated using the first five data points of the curve where the isotherm was linear and the apparent fibrinogen capacity is recorded as the level of adsorbed fibrinogen for a solution concentration of 1 mg/mL.

Section 4.5.2.1 Fibrinogen Adsorption from Tris Buffer onto Non-Derivatized SPUs

The data in Table 4.40 show that the non-functionalized surfaces (ED and MDA) are different from the functionalized polymers with respect to initial fibrinogen adsorption from a single component system. This is not surprising in view of their different behaviour with respect to fibrinogen adsorption from plasma. The initial slopes of the MDA and ED isotherms are approximately half the value of those for the BDDS-1.4 polymer.

At equilibrium the initial slope of the isotherm gives a measure of adsorption affinity. However it is always possible that even at long times (in the case of three hours) the adsorption experiment is diffusion rather than adsorption limited, in which case the variation in surface concentration should follow the solution to the diffusion equation [191]:

$$\Gamma = 2C_b (Dt/\pi)^{1/2}$$

where Γ is the bulk concentration of fibrinogen, "D" is the diffusion coefficient and "t" is the time of contact with the surface. In this work Γ/C_b is the slope given in Table 4.40 and "t" is 10800 seconds (3 hours). The data at low C_b are indeed linear and could thus be diffusion limited. The values of D calculated from the initial slopes of the Γ versus C_b curves, however, are 1.2 x 10-8 cm^2/sec, 1.4 x 10-8 cm^2/sec and 5.2 x 10-8 cm^2/sec respectively for ED, MDA and BDDS-1.4. These must be compared to a literature value of D (fibrinogen) of 2 x 10^{-7} cm^2/sec [167]. It is clear therefore that the data are not diffusion limited in these experiments, and that the isotherm slopes should give at least an indication of relative binding

affinity. On this basis the affinity of the fibrinogen is higher for the BDDS-1.4 than for the unsulfonated materials.

Table 4.40: Summary of Data for Adsorption of Fibrinogen from Tris Buffer to SPU Materials

Polymer	Initial Slope ($ug\ cm^{-2}/ug\ cm^{-3}$)	Fibrinogen Adsorbed at Apparent Plateau (ug/sq cm)	Water Uptake at 25°C (wt %)
ED	0.013	1.29	41
MDA	0.014	1.22	6
BDDS-0.2	0.022	0.43	37
BDDS-0.2-Lys	0.005	0.13	34
BDDS-0.2-Asp	0.010	0.42	52
BDDS-0.8(50)	0.026	0.66	38
BDDS-0.8-Lys	0.020	0.51	30
BDDS-0.8-Asp	0.021	0.28	22
BDDS-1.4(54)	0.027	0.66	19
BDDS-1.4-Arg	0.022	0.75	20
BDDS-1.4-ArgMe	0.023	0.69	19
BDDS-1.4-Asp	0.023	0.68	20
BDDS-1.4-Lys	0.023	0.84	22
BDDS-1.4-DDA	0.025	0.72	77
BDDS-1.4-PEOMe	0.023	0.27	69
BDDS-2.0(61)	0.025	7.564	410
BDDS-2.0-ABS	0.026	4.612	439
BDDS-2.0-ANS	0.024	5.005	486
BDDS-2.6(47)	0.022	1.981	182
BDDS-3.1(86)	0.024	7.142	>1000

Figure 4.60: Fibrinogen Adsorption from Tris Buffer onto SPU Materials: Three Hour Exposure

Figure 4.61 illustrates three hour adsorption isotherms for the complete BDDS series and Table 4.40 contains the data pertaining to the initial slopes and apparent plateau values. The initial slopes for all the sulfonated polyurethanes are similar with an average value of 0.024 ± 0.002 cm (again significantly lower than required for a diffusion contol mechanism). It is clear that there is no significant difference in the initial binding affinity of fibrinogen for any of these materials and it seems likely that the initial part of the isotherm represents fibrinogen binding to sulfonate groups. In the early stages of adsorption (low coverage) the hydrogel layer does not appear to play a dominant role.

As discussed previously, gel layers may be present on the highly sulfonated materials and this can affect the uptake of fibrinogen. In agreement with this idea, the isotherm for BDDS-0.2 levels off with a much lower fibrinogen uptake than does BDDS-3.1. The existence of a possible polymer-buffer gel layer in the high sulfonate content materials could explain the observed adsorption capacities which are well beyond end-on monolayer coverage (see Figure 4.61).

Another example showing the probable importance of the polymer-buffer gel layer is provided by comparing BDDS-2.0(61) and BDDS-2.6(47). Both materials have high sulfonate content and could be expected to adsorb large amounts of fibrinogen. Based on sulfonate content alone it would be expected that BDDS-2.6(47) would adsorb more fibrinogen than BDDS-2.0(61). However as seen in Figure 4.61 this is not the case. BDDS-2.0(61) adsorbs more than three times as much fibrinogen as does BDDS-2.6(47). This is probably due to the much greater water uptake of BDDS-2.0(61), making the polymer-buffer gel layer for BDDS-2.0(61) thicker and increasing its capacity apparently to adsorb beyond monolayer levels.

Figure 4.61: Fibrinogen Adsorption from Tris Buffer onto Sulfonated SPU Materials: Three Hour Exposure

Section 4.5.2.2 Fibrinogen Adsorption from Tris Buffer onto Derivatized SPU Materials

Figure 4.62 shows fibrinogen adsorption data from Tris buffer solution for the derivatized BDDS-0.2 polymers. It is obvious at once that the initial slopes for the lysine and aspartic acid materials are lower than those of the non-derivatized material (see Table 4.40). Note that even though the initial slope of BDDS-0.2-Asp is half that for BDDS-0.2, the apparent plateau level for the aspartic-acid derivatized polymer is similar to that for BDDS-0.2. Again, this suggests the presence of a greater effective surface area, possibly due to the presence of a polymer-buffer interfacial gel phase. It is interesting to note that the BDDS-0.2-Lys polymer has a low plateau value relative to the other two polymers in Figure 4.62. Unfortunately no explanation for this behaviour is immediately obvious.

Fibrinogen adsorption isotherms for the derivatized BDDS-0.8(50) polymers are presented in Figure 4.63. Here again, the incorporation of amino-acids into the polymers has resulted in a lowering of the initial isotherm slope, however the effect is much less significant than it was for the BDDS-0.2 derivatized polymers (see Table 4.40). The apparent plateau levels for the amino-acid derivatized polymers are less than that of the non-derivatized BDDS-0.8(50).

Single protein fibrinogen adsorption data for BDDS-1.4(54) polymers derivatized with different amino-acids, are shown in Figure 4.64. There are no significant differences among the initial slopes of the adsorption isotherms for the amino-acid derivatized BDDS-1.4(54) polymers. As well, all the derivatized polymers have lower sulfonate concentration and therefore show a lower uptake of fibrinogen than the non-derivatized BDDS-1.4(54) at low fibrinogen concentrations. All of the amino-acid derivatized materials eventually reach "plateau" adsorption exceeding that of the BDDS-1.4(54) material. Again, this is probably at least partly due to the fact that polymer-buffer gel layers on these materials are thicker than on the BDDS-1.4(54) polymer, thus enabling them to expose more of their binding sites.

Figure 4.65 contains adsorption data for the BDDS-1.4(54) materials derivatized with dodecylamine, polyethylene oxide (M.W.= 550) and arginine methyl ester. These three polymers retain significant sulfonate character through the sulfonamide and sulfonate functional groups and display initial adsorption isotherm slopes similar to those of the other BDDS-1.4 polymers.

The BDDS-1.4-DDA and BDDS-1.4-ArgMe isotherms are similar to those of the amino-acid derivatized polymers in Figure 4.64. However polymer BDDS-1.4-PEOMe exhibits a plateau adsorption level lower than any of the other BDDS-1.4(54) materials. The adsorption isotherm for this material resembles more that of BDDS-0.2. The long PEOMe chain incorporates ethylene oxide groups into the BDDS-1.4(54) polymer which dramatically enhances the polymer's water uptake character and likely exposes a large number of PEOMe chains to the protein solution. Previous work by Hudson [53] showed that the incorporation of PEO in the soft segment of polyurethanes dramatically reduced the level of fibrinogen adsorption. These observations are in agreement with those of other groups that showed minimal protein adsorption to PEO surfaces [194,195].

Figure 4.66 shows fibrinogen adsorption isotherms for derivatized BDDS-2.0 materials. The initial slopes and apparent plateau levels are given in Table 4.40. The initial isotherm slopes for both the ABS and ANS derivatized polymers are similar to that of the precursor BDDS-2.0(61) polymer. Fibrinogen uptake capacities are well in excess of monolayer coverage probably reflecting both enhanced binding affinity and uptake by a polymer-buffer gel layer. Nonetheless, as was found for adsorption from plasma, it appears that the capacity of the BDDS-2.0(61) polymer for fibrinogen is greater than that of the polymers that have the sulfonate groups extended out from the polymer backbone. The availability of sulfonate groups for fibrinogen binding may be diminished due to orientation/conformational and steric arrangements of the pendant sulfonate groups.

Figure 4.62: Fibrinogen Adsorption Isotherms from Tris Buffer onto Derivatized BDDS-0.2 SPU Materials: Three Hour Exposure

Figure 4.63: Fibrinogen Adsorption Isotherms from Tris Buffer onto Derivatized BDDS-0.8(50) SPU Materials: Three Hour Exposure

Figure 4.64: Fibrinogen Adsorption Isotherms from Tris Buffer onto Derivatized BDDS-1.4(54) SPU Materials (amino-acids): Three Hour Exposure

Figure 4.65: Fibrinogen Adsorption Isotherm from Tris Buffer onto Derivatized BDDS-1.4(54) SPU Materials (non-acid): Three Hour Exposure

Figure 4.66: Fibrinogen Adsorption Isotherm from Tris Buffer onto Derivatized BDDS-2.0(61) SPU Materials: Three Hour Exposure

Table 4.41 contains a summary of the principal conclusions from the pure fibrinogen adsorption isotherms. These data support the concept of a polymer-buffer gel layer and of high protein binding affinity associated with sulfonate groups. It would be interesting to carry out additional experiments on other single proteins in order to determine if the enhanced binding affinity is specific to fibrinogen or applies to plasma proteins in general. If the gel layer concept is an important mechanism in fibrinogen uptake then it is likely that other proteins will also show elevated uptake.

Table 4.41: Conclusions on Fibrinogen Adsorption Studies

- In general the BDDS containing polymers have a higher initial isotherm slope than do the non-functionalized polymers.
- The presence of a polymer-buffer gel layer exposes additional binding sites.
- Functional group chemistry and availability affect the type of interactions and number of fibrinogen binding sites at the polymer-buffer solution interface.

Section 4.5.3 Thrombin Time Studies

The sulfonated polyurethanes and certain of their amino-acid derivatives were synthesized with the hope of obtaining polyurethanes having anticoagulant properties. Others have demonstrated that the incorporation of sulfonate and amino acid sulfonamide groups into crosslinked polystyrenes and dextrans render these materials anticoagulant [196,197].

Therefore, in addition to studying the influence of sulfonate and other chemical functional groups on the early stages of protein adsorption from plasma, it was also desired to evaluate, in some way, the coagulation response of plasma to these modified polyurethanes. Thrombin time measurements were carried out for this purpose.

The details of the experiment were previously explained in section 3.9.3. Essentially the thrombin time experiment consists of measuring the time for a clot to form in plasma in the presence of a standard concentration of thrombin. The experiment is generally done in a test tube with only the surface of the tube in contact with the plasma. Under these conditions the thrombin time of normal plasma is constant. The test can be adapted to assess the effect of surfaces by adding particles of high specific surface area to the plasma.

This experiment is particularly sensitive to the fibrinogen-to-fibrin conversion. Any delay or increase in thrombin time in the presence of the SPU particles can be attributed to abnormal fibrinogen function or to the presence of anticoagulant activity [98].

The measured thrombin times for a series of "blank" samples, using 50 uL aliquots of solutions having different thrombin concentrations, are shown in Table 4.42. One Unit of thrombin is defined as the activity expressed in National Institute of Health (NIH) units obtained by direct comparison to a NIH thrombin reference lot J. The NIH assay procedure uses 0.2 mL diluted plasma (1:1) as a substrate and 0.1 mL of thrombin solution (stabilized in a 1 % buffered albumin solution) and is based on a modification of the method of Biffs [198]. The data shown in Table 4.42 were used to construct the calibration curve in Figure 3.16.

Table 4.42: Thrombin Time Calibration Data

Thrombin Conc., Th (Unit/mL)	1/ Th (mL/Unit)	Thrombin Time (sec ± 8 %)
50	0.02	5
25	0.04	10
12.5	0.08	23
6.25	0.16	65
3.125	0.32	200

Experimental conditions: Thrombin (Bovine), 50 uL
Plasma (pooled human citrated), 200 uL at 37°C
Blank, 50 uL, 0.168 M Tris Buffer, pH 7.4

As seen in Table 4.42, decreasing the concentration of thrombin causes the thrombin time to be prolonged. Any prolongation in thrombin time resulting from the presence of polymer particles can then be related via the calibration curve to the equivalent amount of thrombin deactivated.

Table 4.43 contains data for a few of the functionalized SPU materials synthesized in this project along with two materials known to exhibit anticoagulant properties. These two materials were obtained from Prof. M. Jozefowicz (Universite de Paris-Nord, France) and are respectively a sulfonated crosslinked polystyrene ($PSSO_3$-10(100)) and a glutamic acid derivatized sulfonated crosslinked polystyrene ($PSSO_3$-10-Glu(88)). The $PSSO_3$ material has a sulfur content of 10 % w/w and the derivatized polymer has 12 % free sulfonate and 88 % sulfonamide glutamic acid residues. The particle size and surface area data for the materials listed in Table 4.43 are shown in Table 4.44. Surface area was calculated based on the assumptions that the particles were spherical and non porous (see Appendix B for data)

When interpreting the data in Table 4.43 one must consider the particle size and particle area information given in Table 4.44. Because it is impossible to precisely control the particle concentration and total particle area in the polymer suspensions there are slight variations in these parameters from one sample to another. Therefore a direct measure of thrombin time is not sufficient to compare one material to another. The deactivation of thrombin must be normalized to the surface area of the sample.

The data in Table 4.43 show that thrombin times are prolonged as the sulfonate content of the the non-derivatized BDDS materials increases, implying that the sulfonated SPU polymers have some anticoagulant activity. A prolongation of thrombin time could also be due to abnormal fibrinogen function which could be checked by comparing the reptilase times of the polymer-treated plasmas to that of normal plasma [98]. Although this was not done in the present work, it is considered unlikely that the fibrinogen was significantly affected by the polymer. Also while the sulfonated SPU materials have been shown to take up large amounts of fibrinogen from plasma the corresponding depletion would not be sufficient to influence the thrombin times. Data on fibrinogen adsorption from plasma showed that the peak amount of fibrinogen adsorbed to BDDS-3.1(86) is 1.9 ug/cm^2 for a three hour exposure. In the thrombin time experiment for this material, the polymer surface area was 0.34 cm^2 so that at most, 0.646 ug of fibrinogen could be adsorbed to the surface compared to a total content of about 600 ug of fibrinogen in 200 uL of plasma. Therefore, at most, only 0.1 % of the fibrinogen would potentially be depleted and one may conclude that the sulfonated polymers are prolonging the thrombin times via their interaction with thrombin rather than fibrinogen.

Recent studies by Grasel and Cooper have also shown prolonged thrombin times for MDI/PTMO/BD based sulfonated polyurethanes [199]. Such prolongation could be due to a direct action of the polymers on thrombin or it could be due to heparin-like properties of the materials which allow them to catalyze the antithrombin III-thrombin neutralization reaction.

Such properties have been attributed to the sulfonated polystyrene resins used as "controls" in this study [98]. Additional investigation would be required to show whether the present polyurethanes have heparin-like properties.

Table 4.43: Thrombin Times of Functionalized Polyurethanes

Polymer	Thrombin Time (sec) S.D. = ± 8 %	% Thrombin Deactivated	Thrombin deactivated/ Surface Area (Units/cm^2) S.D. = ± 1.5
(no polymer)	24	--	--
BDDS-1.4(54)	26	7.7	3.5
BDDS-1.4-Lys(100)	31	23	2.4
BDDS-1.4-Asp(88)	32	25	4.5
BDDS-2.6(47)	47	49	13.5
BDDS-3.1(86)	69	67	23.8
BDDS-3.1-Asp(50)	70	66	22.9
$PSSO_3$-10.0(100)	37	35	6.5
$PSSO_3$-10.0-Glu(88)	34	30	2.7

Experimental conditions:

Thrombin: Bovine, 50 uL of a 12.5 U/mL solution
Plasma : pooled human citrated, 200 uL at 37°C
Sample : polymer particles suspended in Tris Buffer, 50 uL, pH 7.4

Previous work by Jozefowicz and coworkers showed that the incorporation of certain amino-acids having acid side chains into sulfonated polystyrene resins gives them heparin-like character and that the biological activity of these resins is dependent upon the proximity of the amino and carboxylic acid functions [129]. Maximal anticoagulant activity is attained

with a separation consisting of three methylene groups [200]. Biological activity is reduced with spacers that are less than or greater than this critical size. This same study showed that the ester analog of the sulfonamide amino-acid moieties did not have heparin-like activity. Other studies by the same group showed that carboxylated polystyrene resins do not have any heparin-like activity [200] while the incorporation of dicarboxylic amino-acids into sulfonated polymers gave materials with heparin-like activity greater than that of the sulfonated polystyrene [201]. The general conclusion is that the chemical requirements for heparin-like activity are (1) sulfonate groups and (2) sulfonamide groups with an acid function.

Table 4.44: Particle Size and Solution Particle Concentration Corresponding to the Data in Table 4.43

Polymer	number of * Particles per 50 uL x 10^{-6}	Avg** Particle Size (um)	Avg *** Particle Area (um^2)	Total Particle Area in 50 uL (cm^2)
BDDS-1.4(54)	1.14	2.76	23.9	0.27
BDDS-1.4-Lys	6.10	2.50	19.6	0.70
BDDS-1.4-Asp	3.40	2.56	20.8	0.70
BDDS-2.6(47)	0.65	4.72	70.0	0.46
BDDS-3.1(86)	1.57	2.64	21.9	0.34
BDDS-3.1-Asp	1.66	2.63	21.7	0.36
$PSSO_3$-10.0(100)	0.0109	44	6.07×10^3	0.66
$PSSO_3$-10.0-Glu	0.0123	60	1.13×10^4	1.39

* measured using a Coulter counter
** measured using a Coulter counter and light microscope
*** calculated based on particle diameter and assuming spherical particles

A comprehensive study of thrombin times for the derivatized polyurethanes developed in this project was not possible because of limitations in micro particle preparation, which were discussed in section 3.9.3. However, thrombin time data were obtained for two aspartic-acid containing polymers and one lysine derivatized material as indicated in Table 4.43. These data show that amino-acid incorporation has no significant effect on thrombin times relative to the non-derivatized sulfonated polyurethane. However it would be unwise to draw any general conclusions from such a small data base and future work should focus on the accumulation of more data both on thrombin times and heparin-like activity.

Comparing the sulfonated SPU materials to the polystyrene resins one must conclude that the segmented polyurethanes have a greater ability to prolong thrombin times than do the polystyrene materials. Polymer BDDS-3.1(86) has a substantially lower sulfonate content than does $PSSO_3$-10.0(100) yet the BDDS-3.1(86) had an apparent thrombin deactivation value of 23.8 Units/cm^2 while the sulfonated polystyrene material has an apparent thrombin deactivation value of 6.5 Units/cm^2. The data do not show that the $PSSO_3$-10.0-Glu polymer has a greater capacity to deactivate thrombin than $PSSO_3$, as reported previously by Fougnot and Jozefonvicz [138].

No thrombin time experiments were carried out on the arginine and arginine methyl ester materials because they could not be prepared in the form of microparticles, however it would be useful to examine these materials since the polystyrene analogs of these polymers have been shown to deactivate thrombin through a mechanism that is not heparin-like [145]. Perhaps if these amino-acids were substituted in a BDDS material having a higher sulfonate content, the particle suspensions would be stable and thrombin times could be determined.

Section 4.5.4 Biocompatibility of Sulfonated and Derivatized Sulfonated Polyurethanes

It was the objective of the blood interaction studies to assess the potential blood compatibility of the sulfonated and derivatized sulfonated polyurethanes. The sulfonated surfaces adsorb high levels of fibrinogen at low plasma concentrations relative to conventional polyurethanes, and show much "delayed" displacement of the adsorbed fibrinogen. This behaviour would be expected to alter the sequence of usual events at the material-blood interface, i.e. the rapid displacement of initially adsorbed fibrinogen by the intrinsic coagulation pathway contact activation proteins (HMWK and Factor XII) and subsequent formation of thrombin. It is possible that fibrinogen is adsorbing in a passivated state and if so these materials may lead to a more acceptable blood-contact surface than the conventional materials.

No readily discernible effects of the introduction of amino acids, dodecane or polyethylene oxide on protein adsorption were evident. Indeed the major effect appeared to result from the concomitant decrease in sulfonate content. It may well be that the two effects would have to be uncoupled before any definite conclusion is possible.

Most of the sulfonated and derivatized polyurethanes show high "affinity" for water which would tend to allow them to mimic the hydrogel nature of the blood vessel wall, a property which is believed to be important for blood compatibility. Several of the sulfonated and derivatized sulfonated polyurethanes have shown the ability to delay thrombin clotting times of plasma. The inhibition of thrombin activation and the deactivation of thrombin are again principal functions of the endothelial surface inside the cardiovascular system.

In summary, both sulfonated and derivatized sulfonated polyurethanes show the potential to alter both contact activation (as indicated by fibrinogen displacement) and thrombin function. Both of these play important roles in thrombus formation which are a principal limitation in today's cardiovascular devices.

Chapter 5

CONCLUSIONS

The work reported in this thesis shows that the synthesis of sulfonated polyurethanes using 4,4'-diamino-2,2'-biphenyl disulfonic acid (BDDS) as a chain extender is feasible. By controlling reaction stoichiometry it is possible to obtain materials having a range of sulfonate content. Reactions between isocyanate and sulfonate functional groups can lead to "chain branching". This reaction also reduces the content of free sulfonate ion in the polymers.

Synthetic pathways were developed for the covalent binding of amino-acids, alkyl chains and polyether chains to sulfonated polyurethanes by first converting sulfonate groups to sulfonyl chloride. These sulfonyl chloride groups were found to react readily with amine and hydroxyl groups of the different compounds being attached to the polymers. The method provides a general scheme for the incorporation of amine- or hydroxyl- containing compounds, provided the compounds are soluble in the reaction solvent.

Conclusive evidence of substituent attachment to the sulfonated polyurethanes was provided by titration analysis, elemental analysis, ^{14}C labelling of amino-acids and Fourier transform infrared spectroscopy. However none of these analytical technique provided high precision quantitative data on substituent content.

Stress-strain measurements showed that the incorporation of BDDS into a polyurethane increases its tensile strength and initial modulus relative to similar polyurethanes incorporating ethylene diamine as the chain extender. Evidence was given to demonstrate that the change in properties is the result not only of additional aromatic rings (making the backbone stiff) in the hard segment. The probable existence of ionic aggregates most likely also plays an important role in the enhanced strength of the polymers.

Derivatized sulfonated polyurethanes exhibit a wide range of tensile properties

depending on the nature of the substituent. Ionic substituents (e.g. glycine and aspartic acid) enhance both the initial modulus and tensile strength of the polymers relative to the base polymer, but in general do not significantly affect the ultimate elongation. On the other hand, elimination of sulfonate ions by non-ionic substituents (i.e. polyethylene glycol and dodecylamine) results in a decrease of both tensile strength and ultimate elongation. Surprisingly, the initial modulus for these two polymers is significantly increased compared to that of the base polymer, particularly the polyethylene oxide derivatized polymer (BDDS-1.4-PEOMe). Differential scanning calorimetry thermograms and Fourier transform infrared data for the BDDS-1.4-PEOMe polymer showed that there is significant hydrogen bonding which probably contributes to the elevated initial modulus of the material.

Differential scanning calorimetry provided useful insights on the internal structure of these novel polymers, as follows:

- Phase separation of the segmented polyurethane hard and soft segment domains increases with amino-acid incorporation into the hard segment.
- Quench cooling of sulfonated and amino-acid derivatized polymers immediately after heating to 250°C shows that the annealing process increases phase separation and decreases interactions in the hard segment domains.
- At temperatures in excess of 200°C, free amine groups in amino-acid derivatized polymers reacts with other segmented polyurethane functional groups.
- Long chain, non-ionic, hydrophilic and hydrophobic substituents can dramatically enhance hard segment phase separation.
- Long chain, non-ionic, hydrophobic substituents may form a third phase upon annealing of the polymer film.

Water uptake data for the sulfonated and derivatized sulfonated polyurethanes show a strong dependence on the concentration and type of functionality incorporated into the polymer. This behaviour is shown to be important in protein interaction studies for these materials. Changing functionality type and concentration on the polyurethanes allows one to

control the morphology of the swollen polymer, the degree of swelling and even the level of dissolution.

Water contact angle studies using both sessile drop and captive bubble techniques allowed the author to monitor the dynamics of the material surface as the novel polymers were contacted with water. The different surfaces clearly responded to water contact in their own unique fashion. This was particularly emphasized in going from high to low sulfonate content.

Surface analysis using ESCA provided insight into the distribution of functional groups in the surface region of the polymer. The data showed that the concentration of sulfonate at the surface of the sulfonated polyurethanes depends on the process of hard segment microdomain depletion from the surface. However, the derivatized polymers appear to be much less subject to this effect, resulting in a higher than expected concentration of functional groups at the surface of the materials.

Fibrinogen adsorption studies from plasma show that the sulfonated materials have adsorption patterns very different from the more conventional polyurethanes such as Biomer. As sulfonate content increases in the polyurethane the level of adsorbed fibrinogen increases to beyond monolayer coverage. As well the displacement of adsorbed fibrinogen by other plasma proteins (the process known as the Vroman effect) occurs over a much longer time scale and in some cases does not seem to take place at all (i.e. BDDS-2.6(47)). At the present time it is believed that a combination of both specific affinity for the surfaces and the hydrogel-like nature of the materials is responsible for the observed behaviour. The implications of this behaviour are of some importance, since the adsorption of fibrinogen to a biomaterial surface is one of the first events when it is contacted with blood. The delayed displacement of fibrinogen could dramatically change the sequence of events that occur at the material surface. The substituents of the derivatized sulfonated polyurethanes appear to show a lesser influence on the overall fibrinogen adsorption curve relative to the presence of the

sulfonate and sulfonamide groups, however most derivatized materials display a unique fibrinogen displacement pattern.

Thrombin time measurements of plasma in which the polymers are suspended show that both sulfonated and some derivatized sulfonated polyurethanes have the ability to interfere with the clotting process by either deactivating thrombin or effectively removing it from the plasma and masking its active sites. A marked increase of thrombin time was seen with increasing concentration of sulfonate or sulfonamide groups in a series of polymers. Further studies will be required to determine the mechanism responsible for these effects. It appears in any event these materials are in some sense anticoagulant in nature.

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Appendix A: "GPC" Program for Data Manipulation

This program was developed using MacMillan's Asyst 123 software and a Zenith PC IBM clone computer. The program was developed by Paul Santerre and Nick Vanderkamp. It is accessed within ASYST using the key word "GPC", the acronym for gel permeation chromatography. Upon typing "GPC" followed by a carriage return, the user is prompted for a choice of data manipulation subprograms:

<F1> Acquires data from the GPC unit

<F2> Finds new GPC calibration coefficients

<F3> Stores chromatogram data

<F4> Computes molecular weights

<F5> Corrects for baseline drift

<F6> Smooths your chromatogram data

<F7> Allows you to compare chromatograms

<F8> Exits GPC and returns you to ASYST

In the program itself, the above "F" keys are associated with the following subprograms:

<F1> DATA.ACQ

<F2> CALIBRATION

<F3> DATA.STORE

<F4> UNIV.MOLWT

<F5> BASELINE.DRIFT

<F6> DATA.SMOOTH

<F7> GRAPH.COMPARE

<F8> NABORT

The subprogram "DATA.ACQ" consists of two subprograms which perform the following tasks: "INIT" which defines the channel from which data is acquired and "DT2800" which sets up the on screen display of data and stores the acquired data in a temporary working file. The working file can later be stored and manipulated using the other "F" key functions.

Appendix B: Particle Size Distribution and Coulter Counter Parameters for Particle Size Measurements and Concentration

The following data list the orifice size of the particle tube analyser, the calibration constant for the glass tube, the particle size for the calibration of the glass tube and the particle size distribution determined from the Coulter Counter measurements.

1. BDDS-1.4(54)

Orifice Size: 200 um
Calibration constant: 5.586
Particle Size Standard: 19.81 um

Particle Size Distribution:

Number of Particles	Particle Diameter (um)
87	60.7
191	48.1
178	36.2
127	30.3
89	24.0
51	21.4
67	13.5
25	10.7
112	8.5
161	5.3
606	3.8
716	3.0
3848	2.4
16665	1.1

2. BDDS-1.4-Lys(100)

Orifice Size: 140 um
Calibration Constant: 3.78
Particle Size Standard: 19.81

Particle Size Distribution:

Number of Particles	Particle Diameter (um)
3	41.0
5	32.6
34	25.8
98	20.5
235	16.3
198	14.5
188	12.9
180	11.5
348	10.2
301	7.2
865	5.7
1090	4.7
2446	3.6
864	3.2
73	2.6
2603	2.0
7138	1.6
5465	0.7
3522	0.4

3. BDDS-1.4-Asp(88)

Orifice Size: 140 um
Calibration Constant: 2.88
Particle Size Standard: 19.81

Particle Size Distribution:

Number of Particles	Particle Diameter (um)
2	62.5
4	49.6
28	39.4
105	31.2
234	24.8
324	19.7
497	15.6
572	12.4
405	11.0
354	9.8
424	8.8
404	7.8
57	6.9
1314	5.5
1630	4.4
1994	3.4
3369	2.8
4065	2.0
9509	1.5
20185	1.2

4. BDDS-2.6(47)

Orifice Size: 100 um
Calibration Constant: 2.023
Particle Size Standard: 9.79 um

Particle Size Distribution:

Number of Particles	Particle Diameter (um)
53	59.0
138	46.8
281	37.1
425	29.5
615	23.4
470	20.8
470	18.6
790	16.6
551	14.7
814	13.1
2260	10.4
2981	8.3
4496	6.5
5711	5.2
7703	3.6
11360	2.9
24341	2.3
6343	1.1

5. BDDS-3.1(86)

Orifice Size: 100 um
Calibration Constant: 2.02
Particle Size Standard: 9.79 um

Particle Size Distribution:

Number of Particles	Particle Diameter (um)
25	13.8
125	11.0
527	8.7
469	7.8
694	6.9
863	6.2
1065	5.5
1128	4.9
3266	3.9
4504	3.1
5512	2.4
6823	1.9
184	1.7
7477	1.4
13368	1.1

6. BDDS-3.1-Asp(50)

Orifice Size: 140 um
Calibration Constant: 3.78
Particle Size Standard: 19.81 um

Particle Size Distribution:

Number of Particles	Particle Diameters (um)
4	41.0
8	32.6
25	25.8
35	20.5
58	16.3
124	14.5
102	12.9
117	11.5
196	10.2
277	9.1
631	7.2
1104	5.8
1312	4.5
3804	3.6
46	2.6
3862	2.0
5118	1.6
7573	0.7

7. $PSSO_3$-10.0(100)

Orifice Size: 200 um
Calibration Constant: 5.59
Particle Size Standard: 19.81 um

Particle Size Distribution:

Number of Particles	Particle Diameter (um)
4	121.3
76	96.3
322	76.4
286	60.7
67	48.1
72	36.2
69	30.3
55	24.1
72	21.4
40	19.1
89	17.0
131	15.1
252	13.5

8. $PSSO_3$-10.0-Glu(88)

Orifice Size: 200 um
Calibration Constant: 5.61
Particle Size Standard: 19.81 um

Particle Size Distribution:

Number of Particles	Particle Diameter (um)
510	100
11	6.5
64	5.4
15	4.6
95	3.8
186	3.0

Appendix C: Calibration Data for C-14 Labelling Experiments

C-14 scintillation counting data for the calibration solutions and labelled polymers are given in Table C.1 (lysine data) and Table C.2 (aspartic-acid data). These data were used to compute the conversion values given in Table 4.13. All the calibration data show identical counting efficiencies which confirms the validity of the calibration curve. The counting efficiency is a measure of the amount of count quenching. For the two derivatized polymers there is a slight dependence of efficiency on polymer sample size which is accounted for by DMF quenching as previously discussed in section 3.5.4.

Table C.1: Scintillation Count Data for C-14 labelled BDDS-3.1-Lys

Sample	Sample Conc. (g/100 mL)	Sample Size (uL)	Counts per min.	Counting Efficiency
L-lys standard	.0115	100	1696	83.6
"	"	300	5523	83.1
"	"	500	8849	83.1
"	"	700	13149	83.4
"	"	1000	18815	83.0
BDDS-3.1-Lys	1	100	6595	82.1
"	"	300	19355	80.4
"	"	500	31605	78.7

Table C.2: Scintillation Count Data for C-14 Labelled BDDS-3.1-Asp

Sample	Sample Conc. (g/100mL)	Sample Size (uL)	Counts per min.	Counting Efficiency
L-Asp standard	0.0113	100	1975	83.3
"	"	300	5193	83.2
"	"	500	7926	83.2
"	"	700	10840	83.0
"	"	1000	16221	83.2
BDDS-3.1-Asp trial #1	1	100	776	82.4
"	"	300	2465	81.1
"	"	500	3893	79.7
BDDS-3.1-Asp trial #2	"	100	1148	83.2
"	"	300	3249	81.5
"	"	500	5255	80.1

Appendix D: Preparation of Radiolabelled Fibrinogen

The present study employed a radio-tracer technique exclusively [214]. In each experiment fibrinogen was labelled with ^{125}I and adsorbed to different materials from a Tris buffer or human blood plasma. This radiolabelling method makes it possible to monitor the adsorption of fibrinogen on the surface from multicomponent systems such as plasma. No other technique can give quantitative data for the plasma system with comparable reliability.

The possibility of preferential adsorption of labelled versus unlabelled protein was previously studied for sulfonated and derivatized polyurethanes [52]. A small but insignificant effect was found on each surface that was tested. The gamma radiation count rate on the surface could therefore be related directly to surface concentration based on the previously measured solution count rate.

A list of materials used in the experiments is as follows:

Pooled citrated human blood plasma- from Canadian Red Cross, Ottawa, courtesy of Dr. George Adams.

Human fibrinogen - from Kabi, Stockholm, Sweden.

Pyrex glass tubing (0.24 cm ID).

3 way valves (teflon) - from Pharmaseal, Toa Alta, Puerto Rico.

Latex tubing (1/8"x1/16") - from Canlab.

30 mL syringes - from Becton-Dickinson, Rutherford CA.

Gamma counter (Biogamma) - Beckman, Fullerton CA.

Tris (hydroxymethyl) amino methane (electrophoresis quality) - from Biorad, Richmond CA.

Na ^{125}I (approximately 20 mCi/mL)- from Dupont, New England Nuclear, Boston MA.

Chromerge - from Manostat, New York NY.

Preparation of Tris Buffer:

In order to make two litres of isotonic Tris buffer 48.4 g of electrophoretically pure Tris was brought up to a volume of 1000 mL in deionized water to form a stock solution. 840 mL of this stock solution was combined with 700 mL of 0.4 N HCl solution. To the resulting mixture, 460 mL of deionized water was added. The pH was adjusted to a value between 7.35 and 7.40 by the addition of a small amout of the HCl solution. The buffer was stored in a plastic bottle and refrigerated.

ICl Preparation:

The ICl used in the labelling procedure was prepared in the following manner: 150 mg of NaI was dissolved in 8 mL of 6 N HCl and 108 mg $NaIO_3.H_2O$ was dissolved in 2 mL deionized water. The resulting solutions were mixed and the volume brought up to 40 mL with deionized water. This was shaken up with 5 mL CCl_4 repeatedly until the pink colour was no longer visible in the organic phase. Residual CCl_4 was removed by aerating for one hour and the volume of the solution was made up to 45 mL with deionized water to form the stock ICl solution. For use in the labelling procedure one part stock solution was mixed with nine parts of a 2 M NaCl solution to give a 0.0033 M ICl solution in 1.8 M NaCl.

Glycine Buffer Preparation

75 g of glycine and 58.5 g NaCl were dissolved in deionized water and the volume was brought up to 500 mL to form a 2 M glycine solution in 2 M NaCl. To this was added 2.0 N NaOH until the pH reached 8.8.

^{125}I Labelling of Fibrinogen

Fibrinogen was labelled using the method of McFarlane [164] resulting in a substitution of iodine in the aromatic rings of the tyrosine residues. The biological activity of fibrinogen has been shown to be unaffected if a substitution level of about one iodine atom per molecule or less is used [215].

The amount of fibrinogen labelled for each experiment was essentially the same. 0.5 mL of fibrinogen solution (approximately 9 mg/mL in isotonic Tris) was prepared for labelling by the addition of two drops of glycine buffer (pH 8.8). One drop of glycine buffer was added to 0.1 mL ICl solution (0.0033 M). 4.0 uL of Na ^{125}I was mixed with the ICl for one minute. The fibrinogen was then carefully mixed with the ICl avoiding air bubbles as much as possible. The unreacted ^{125}I was then separated from the labelled fibrinogen by passing the "hot" solution through an anion exchange column (AG1X4) which consisted of a 3 mL disposable syringe, plugged with glass wool, filled with anion exchange resin and equilibrated with Tris buffer. The column was rinsed with approximately 10 mL of Tris buffer yielding a labelled fibrinogen solution that could be used as a trace label for 10 to 15 mL of human plasma or for a simple fibrinogen in Tris system. The percent labelled ranged between 10 and 20 % fibrinogen. The ^{125}I-fibrinogen stability was determined by measuring free iodide in solution (approximately 2.5 % free iodide).

Preparation of Dilute Plasmas

Pooled normal human plasma was used (10 mL or 15 mL) and added to the labelled fibrinogen prepared earlier. It was then brought up to a volume of 100 mL (or 75 mL) with isotonic Tris buffer in order to make 10 % (or 20 %) normal plasma. From this solution, other solutions ranging from 0.01 % to 10 % were made. 1 mL samples of each dilution were placed

in scintillation vials and counted in the Beckman Biogamma counter in order to check that they were properly made and to determine the specific radioactivity of the labelled fibrinogen. Each dilution was taken up into a 30 mL syringe to minimize the effects of the air-solution interface in the time between their preparation and their injection into the adsorption tubes.

The determination of fibrinogen concentration in the undiluted plasma was done by the Clinical Haematology Laboratory in the McMaster University Medical Centre (MUMC) using the thrombin clotting technique developed by Clauss [215].