FUNCTIONALIZED POLYURETHANES FOR BLOOD CONTACTING APPLICATIONS

STUDIES OF FUNCTIONALIZED POLYURETHANES FOR BLOOD CONTACTING APPLICATIONS

By

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ABSTRACT

The synthesis of novel polyurethanes for use in blood contacting applications was investigated. A series of polyurethanes containing biologically active groups in the hard segment was synthesized. Sulphonate-containing polyurethanes were produced using a sulphonated chain extender (biphenyl diamino disulphonic acid) and these polymers were subsequently reacted to incorporate arginine methyl ester groups via sulphonamide bonds.

The novel polyurethanes were initially evaluated using elemental sulphur analysis and nitrogen analysis, gel permeation chromatography and Fourier transform infrared spectroscopy to verify reaction of the sulphonated chain extender and addition of arginine methyl ester.

Three sulphonated polyurethanes covering a range of sulphonate content were produced by varying the prepolymer and hard segment lengths. These three polymers and the three corresponding arginine methyl ester containing polymers were characterized to relate both bulk and surface structure to physical and biological properties.

Elemental analysis, gel permeation chromatography and Fourier transform infrared spectroscopy were used to characterize the molecular structure. These techniques

provided results consistent with the expected reaction mechanisms. Electron spectroscopy for chemical analysis and contact angle measurements were used to analyse the surface structure. Preliminary contact angle data indicate that the sulphonated polyurethane surfaces are hydrophilic, but further investigation is required before a firm conclusion can be reached. ESCA results indicate that while soft segment surface enrichment is taking place there are significant numbers of sulphonate groups present at the polyurethane surface.

Mechanically these polymers have similar ultimate tensile strength but lower elongation than polyurethanes produced using aliphatic diamines, like ethylene diamine. This is probably due to increased microphase segregation arising partly from incompatibility of the aromatic chain extender with the aliphatic soft segment and possibly partly to ion cluster formation. The incorporation of arginine methyl ester groups increases both the elongation and the tensile strength, probably due to reduced phase segregation.

Fibrinogen adsorption experiments in plasma were conducted to evaluate the blood response of these polymers. The plasma adsorption "isotherms" for the novel polyurethanes do not show transient fibrinogen adsorption (Vroman effect) which has been observed on a number of other surfaces including a wide range of polyurethanes. Instead,

the initially adsorbed fibrinogen remains adsorbed. Also the levels of fibrinogen adsorption from plasma are three to four times greater than observed on previously studied surfaces. Conversion of some of the sulphonate groups to sulphonamide-bonded arginine methyl ester restores the Vroman effect and results in lower levels of retained fibrinogen. These data suggest the possibility of a specific interaction between sulphonate groups and fibrinogen.

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1. INTRODUCTION AND OBJECTIVES

1.1 Introduction

In 1954 the first kidney transplant was attempted. This was followed nine years later by the first liver transplant, and on December 3rd 1967 the first heart transplant was performed. In the following decades organ transplant technology advanced rapidly. As Dr. Calvin Stiller, chief of transplantation at University Hospital in London, recently commented: "it (organ transplantation) has moved from experiment to being a therapeutic procedure that people expect to have done" [1]. The limiting factor is no longer the surgical or clinical techniques, but the availability of donor organs. As a result, much research has recently gone into the development of artificial organs which can function in the place of diseased or damaged organs. The most publicized work has been on artificial heart development. Currently the artificial heart can be used to sustain a patient until an appropriate donor organ can be found. Ultimately, it is hoped that it can function as a long term replacement.

Organ replacement is only one potential area, albeit the most complex, for the use of artificial implants. In the cardiovascular system, for example, synthetic vascular

grafts can replace damaged sections of arteries or veins, and left ventricular assist devices can help a weakened left ventricle to pump blood at adequate flowrates [2,3]. Polyurethanes, because of their excellent and versatile mechanical properties, are well suited to use in this type of application.

There are, however, important limitations in the use of these technologies, the foremost being the body's sensitivity to the presence of foreign objects. In cardiovascular implants the major problem is the activation of clotting at the blood-artificial surface interface, resulting in a thrombus. This can reduce blood flow past the thrombus, or, more dangerously, the thrombus can break off to form an embolus which can result in blockage downstream (e.g. heart attack or stroke). Systemic anticoagulants must be used with these implants and, in the case of larger implants, like the artificial heart, even short-term implantation involves considerable risk. The tendency to generate thrombus is a major restriction of synthetic cardiovascular implants.

The specific interactions of blood with polyurethane surfaces are not well understood. It is known that the first event following blood contact with a foreign surface is the adsorption of plasma proteins [4]. This protein layer becomes the surface with which the blood is in contact. If

the contents of this layer could be to some degree controlled by the nature of the polyurethane surface beneath it, valuable information on the relationship between the protein layer and the tendency for thrombogenesis could be obtained. This could in turn be used to improve the antithrombogenic character of implants through the use of materials which adsorb proteins that enhance antithrombogenicity.

1.2 Objectives

In order to be suitable for use in a blood contacting environment an implant must exhibit biocompatibility and be able to withstand mechanical stress. Many polyurethanes have been synthesized which have excellent mechanical properties but only fair thromboresistance. The objectives of the present work were to synthesize and characterize a series of novel polyurethanes of improved thromboresistance which retain mechanical properties similar to "classical" polyurethanes. It was hoped that this could be done by incorporating functional groups which possess known biologic activity and have the potential to inhibit the blood's coagulation pathway.

1.2.1 Synthesis and Characterization

A major objective of this work was to synthesize and characterize novel segmented polyurethanes (SPUs) to which groups of potential anticoagulant activity are covalently attached. The goals of synthesis were to produce polyurethanes with pendant sulphonic acid groups to which amino acids could subsequently be covalently bound through sulphonamide bonds. Both sulphonic acid groups and certain amino acids appear to be important in the function of anticoagulants and have the potential to retain some of this ability when covalently attached to polymeric resins [5]. The influence of incorporating these biologically active groups into SPUs can be determined by comparison of the character of both the sulphonic acid and amino acid containing polymers with traditional SPUs.

Polymer characterization involved a number of techniques such as gel permeation chromatography, intrinsic viscosity determination, elemental analysis, electron spectroscopy for chemical analysis and contact angle measurement. These techniques can be used to verify theoretical reaction mechanisms and to gain information about the bulk and surface structures and properties of the SPU. This information can potentially be correlated with the mechanical and biological behaviour of the polymers.

1.2.2 Mechanical and Biomedical Evaluation

The mechanical properties of the SPUs synthesized can be evaluated by study of the stress-strain behaviour of cast films. Segmented polyurethanes derive their name from the tendency of their "segmented" polymer chains to phase separate and aggregate into microdomains known as hard and soft segment domains. The hard domains act as multifunctional physical crosslinks and reinforcing filler between which the amorphous soft segments can stretch and recoil. The mechanical properties of SPUs are strongly influenced by the extent of this phase separation. Other factors are hard and soft segment molecular weights and relative hard and soft segment content [6]. Phase separation of the hard and soft segments was not studied in detail in the present work; however, the known relative hard and soft segment content, along with the molecular weight data can be related to the mechanical behaviour. It was hoped that the novel SPUs would show similar mechanical behaviour to "classical" SPUs which have "tailorable" mechanical properties suitable for use in blood contact applications.

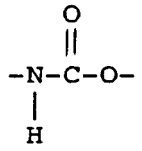
The blood response of the SPUs can be evaluated by studying the adsorption of fibrinogen (a key plasma protein in coagulation and thrombosis) from plasma onto the SPU surface. The adsorption of fibrinogen is believed to be an important factor in determining blood compatibility, even

though its exact role in this regard is not yet fully understood [7]. The adsorption of fibrinogen onto the surface of the novel SPUs can be compared to the adsorption of fibrinogen onto traditional SPUs which do not contain any functional groups. This should provide valuable information about the influence of the incorporated functional groups on fibrinogen adsorption and the blood response of polyurethanes.

2. LITERATURE REVIEW

2.1 Segmented Polyurethanes

Segmented polyurethanes (SPUs) belong to the polyurethane family of polymers. This family includes all polymers containing the characteristic urethane linkage:



Polyurethanes can have a wide variety of groups in their polymer chains. This variety leads to a diversity of mechanical properties, and polyurethanes can be used to produce anything from gums to foams to elastomers to rigid plastics.

Segmented polyurethanes have acquired their name because they are essentially copolymers of two thermodynamically incompatible chain segments, called the hard and soft segments, which tend to phase separate into microdomains. The hard segment domains act as multifunctional crosslinks and reinforcing filler particles for the soft segment domains, thus giving an SPU its elastomeric character.

SPUs have found widespread application in the field of biomaterials. Their excellent elastomeric properties and

reasonable biocompatibility are particularily suited to blood contact applications. Some examples of blood contacting devices containing SPUs are: vascular grafts [8], intraaortic balloon pumps [9], left ventricular assist devices [10], artificial kidneys [11], pacemaker wire insulation [12], synthetic heart valves [13] and the artificial heart [14].

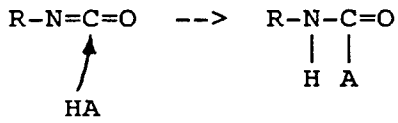
2.1.1 Polyurethane Chemistry

The versatility of polyurethanes can be attributed in part to the high reactivity of the isocyanate group. Isocyanates will react with any group having an active hydrogen [15]. Thereby allowing potential reaction with a wide variety of compounds. The reason for the reactivity of the isocyanate group lies in its electronic structure which can be written in the following resonance forms [15]:

$$R\text{-}N{=}C{=}O \;\longleftrightarrow\; \underset{\text{(major)}}{R\text{-}N{=}C\text{-}O^{+\,-}} \;\longleftrightarrow\; \underset{\text{(minor)}}{R\text{-}N^{-}\text{-}C{=}O^{+}}$$

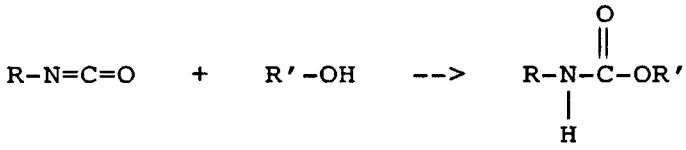
These resonance structures imply a net negative charge on the oxygen and the nitrogen and a net positive charge on the carbon. The reactions with active hydrogen-containing compounds proceed by attack of the nucleophilic centre in the active hydrogen-containing compound on the electrophilic

carbon in the isocyanate:



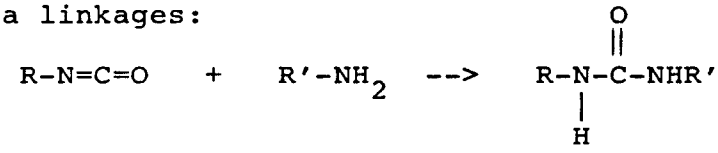
The most important of the active hydrogen containing compounds for polyurethane synthesis are alcohols, amines and water.

The reaction of an isocyanate with the -OH group in an alcohol results in the formation of a urethane linkage as follows:



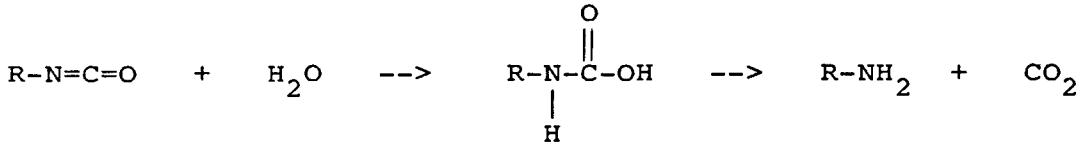
Primary alcohols are the most reactive, reacting readily at 25-50 °C, while secondary and tertiary alcohols are much less reactive due to steric hindrance [16].

Amines react with isocyanates in a similar manner giving urea linkages:



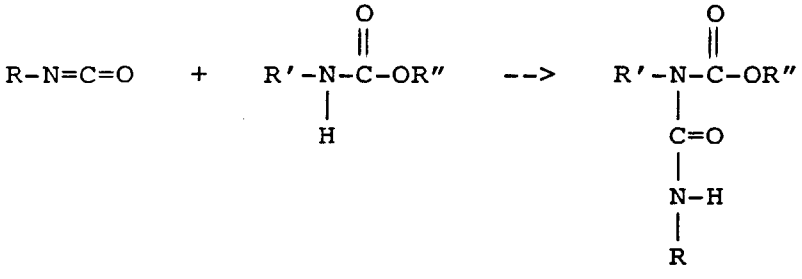
Primary aliphatic amines are the most reactive, secondary aliphatic amines are less reactive, due to steric hindrance, and primary aromatic amines are even less reactive, due to delocalization of the nitrogen's lone electron pair [17].

Water reacts with an isocyanate to give an unstable carbamic acid derivative which in turn forms an amine and carbon dioxide:

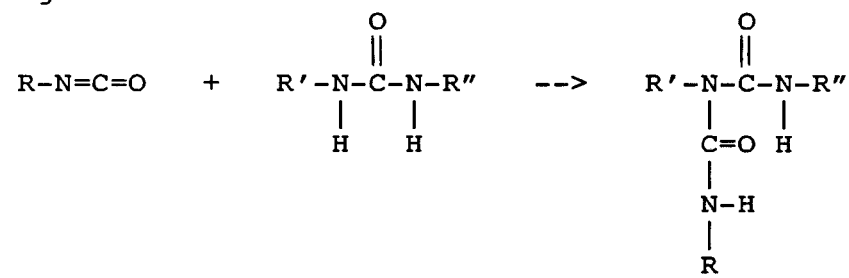


This reaction is often used in the production of urethane foams as the carbon dioxide bubbles produced become trapped in a crosslinked polymer matrix [18].

When all the components used in a synthesis are difunctional, linear polyurethane chains result. A branched or crosslinked polyurethane network can be achieved in two ways: either by the addition of a multifunctional component, which can be either a multifunctional isocyanate, amine or alcohol, or by the addition of excess isocyanate which, under appropriate conditions, can react with urethane and urea groups producing allophanate and biuret linkages. An allophanate occurs when the isocyanate reacts with a urethane linkage:



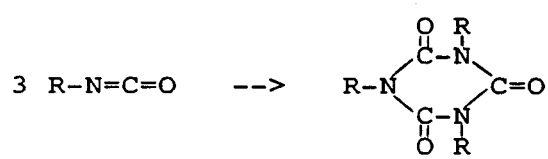
A biuret linkage occurs when the isocyanate reacts with a urea linkage:



These reactions normally require temperatures greater than 110 °C to proceed at a significant rate [15].

In addition, isocyanates can react with each other to form dimers:

or trimers:



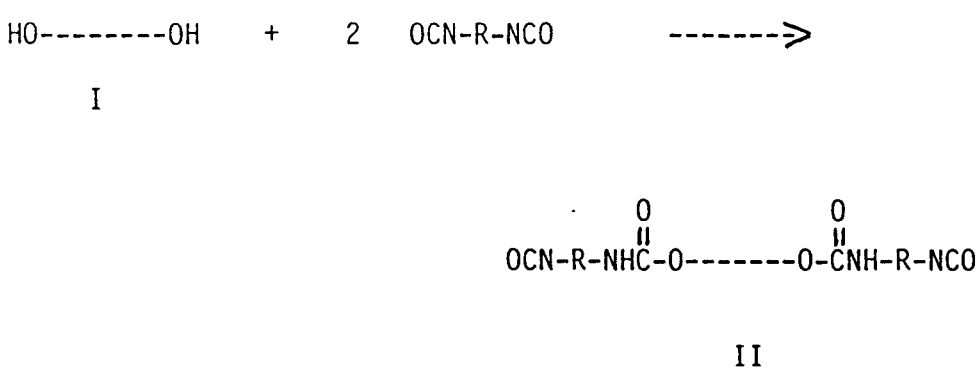
4,4′-diphenylmethane diisocyanate (MDI) will dimerize slowly on storage thereby reducing its effective isocyanate content [19]. Long storage times should, therefore, be avoided.

2.1.2 Segmented Polyurethane Synthesis

Segmented polyurethanes can be synthesized using either a one-step or a two-step procedure [19]. The one-step procedure is less common and involves the simultaneous addition of the three components: the macromolecular polyol (usually a hydroxyl-terminated polyether or polyester), the diisocyanate and the chain extender (usually a low molecular weight diamine or diol). The major drawback in this method is the differing reactivities of the components [20]. Amine groups are typically much more reactive towards the isocyanate than are hydroxyls and, in order to give a uniform polymer, approximately equivalent reactivities are desired. In cases where both amines and hydroxyls are present this can be achieved either by inhibiting the amine-isocyanate reaction or catalysing the isocyanate-hydroxyl reaction.

An alternative, more common method, is the two-step or "prepolymer" synthesis presented schematically in Figure 2.1. In the first step of this procedure a prepolymer is formed by reacting a macromolecular polyol with a diisocyanate to give an isocyanate terminated chain of molecular weight between 1,000 and 5,000. This prepolymer is then chain extended in the second step to a high molecular weight polymer using either a low molecular weight diol or

1. Prepolymer Formation

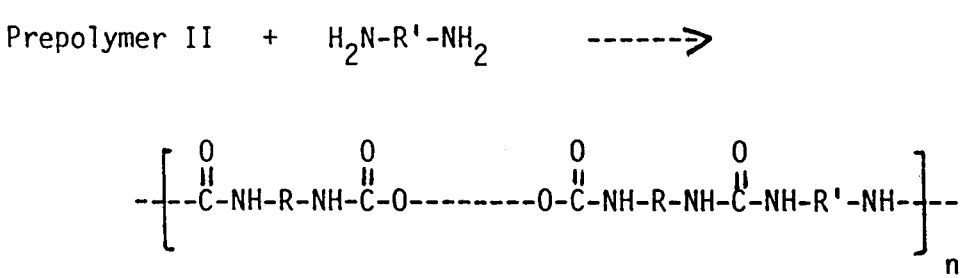


I

II

(The macromolecular diol I is usually a polyether or polyester diol.)

2. Chain Extension (illustrated with a diamine)



(Polyurethane urea)

Figure 2.1: Two Step Synthesis Of Segmented Polyurethanes

diamine as a chain extender.

Laboratory synthesis of SPUs is usually conducted in solution, and highly polar solvents are required, typically dimethylsulphoxide (DMSO), dimethylformamide (DMF) or dimethylacetamide (DMAC). Care must be taken in all SPU syntheses to avoid side reactions of the isocyanate groups. Such reactions can potentially disturb the stoichiometric equivalence of isocyanate groups with amine and hyroxyl groups, which is required to give the desired high molecular weight linear polyurethane. The reaction must be conducted under dry conditions since water reacts rapidly with isocyanates. Also the reaction temperature should be kept below 100 °C to avoid allophanate and biuret linkage formation resulting in branching and/or crosslinking.

2.1.3 Structure-Property Relationships

Segmented polyurethanes are copolymers consisting of hard and soft segments. The soft segments are the long polyether or polyester chains. The hard segment comprises the diisocyanate and chain extender moieties, and the urethane and urea linkages formed by reaction of an isocyanate group with a hydroxyl or amine group respectively. These two segments are thermodynamically

incompatible, i.e. their free energy of mixing is positive, and they tend to phase separate into microdomains. The soft segment domains are rubbery and amorphous, while the hard segment domains are rigid. The hard segment domains act as crosslinks and reinforcing filler between which the soft segment domains can stretch and recoil, giving an SPU its elastomeric character.

The mechanical and thermal properties of SPUs are determined to a great extent by the molecular weight of the polymer, the relative amounts of hard and soft segments, the molecular weight of the hard and soft segments, the degree of phase separation and the domain morphology. The type of hard and soft segments used are important factors in determining microphase structure and domain morphology.

2.1.3.1 Microphase Separation

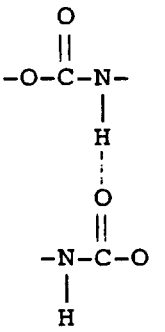
Microphase separation of hard and soft segment domains in SPUs was first suggested in 1966 by the work of Cooper and Tobolsky [21], who compared the mechanical properties of SPUs and styrene-butadiene-styrene block copolymers which were known to undergo microphase separation. Subsequently, proof of the existence of microdomains, in the form of electron micrographs was

obtained [22].

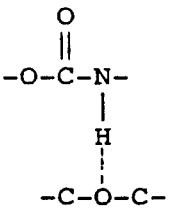
The driving force for microphase separation and domain formation is the existence of a positive interfacial free energy between the hard and soft segments on the SPU chains. Thermodynamically, this positive free energy arises from a balance of enthalpy and configurational entropy terms. There is a positive heat of mixing associated with the interface between hard and soft segment domains [23] which will motivate domain growth as a way of reducing interfacial surface area. But, as a result of domain formation, the number of possible conformations which the SPU chains can assume is restricted and entropy is decreased. This takes place in two ways: first, the joints between hard and soft segment units in the same SPU chain are confined to the interface between the hard and soft segment domains; and second, the SPU maintains an approximately constant overall density by suppressing a large number of potential polymer conformations [24]. The balance of these entropic and enthalpy terms determines the equilibrium domain size and shape.

Another important factor in domain formation is hydrogen bonding. Hydrogen bonding in polyurethanes involves the -N-H group as the donor and the urethane carbonyl, the ester carbonyl or the ether oxygen as acceptor. The hydrogen bonds which form in each of these cases are as follows [19]:

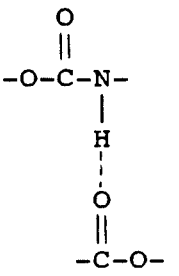
urethane-urethane:



urethane-ether:



urethane-ester:



Hydrogen bonding is especially important in hard segment domain formation and a typical polyurethane is extensively hydrogen bonded [19].

2.1.3.2 Influence of SPU Components on Microphase Separation

The interfacial free energy terms which dictate domain formation are themselves governed by the selection of components used in SPU synthesis. The type and molecular weight of soft segment, of chain extender and of diisocyanate can all have considerable influence on phase separation.

Polyether soft segment SPUs have been found to have greater phase separation than polyester soft segment SPUs, probably due to greater inter-urethane hydrogen bonding in the former [24]. Within the polyether class of soft segments, polytetramethylene oxide (PTMO) based SPUs show more phase separation than polypropylene oxide (PPO)-containing SPUs, which in turn show more phase separation than polyethylene oxide (PEO) soft segment SPUs [19]. This trend is believed to be partly due to differences in hydrogen bonding tendency [25]. It has also been found that, within a specific soft segment type, increasing the molecular weight of the soft segment results in less microphase mixing and more crystallinity [26].

The influence of the type of chain extender on microphase separation depends primarily on symmetry, ability to hydrogen bond and tendency to phase mix. For instance,

aliphatic chain extenders are more compatible with soft segment components and have more tendency to phase mix than aromatic chain extenders. As a result, aliphatically chain extended SPUs exhibit considerably less phase separation than aromatically chain extended SPUs [26]. Chang and Wilkes have also shown, using ortho- and para-phenylenediamine, that asymmetric chain extenders give less phase separation than the corresponding symmetric chain extenders [25]. In addition, SPUs prepared with a diamine chain extender have greater phase separation than those extended with a diol, possibly due to more extensive hydrogen bonding [27]. Finally, recent studies have shown that SPUs prepared with chain extenders containing an odd number of carbon atoms have less tendency to phase separate than those based on chain extenders with an even number of carbon atoms [28].

A recent study on polyurethane ionomers [29] has shown that polyurethanes which possess no hard segment, and thus exhibit no phase segregation, will display phase separation when ionic groups are placed on the polymer chains. This "domain formation" is believed to be a result of ion aggregation, also known as ion cluster formation. Several possible mechanisms for ion aggregate formation are shown in Figure 2.2. In most of the techniques used for incorporating ions into SPUs the ions are attached to the hard segment and it is unclear what the relative

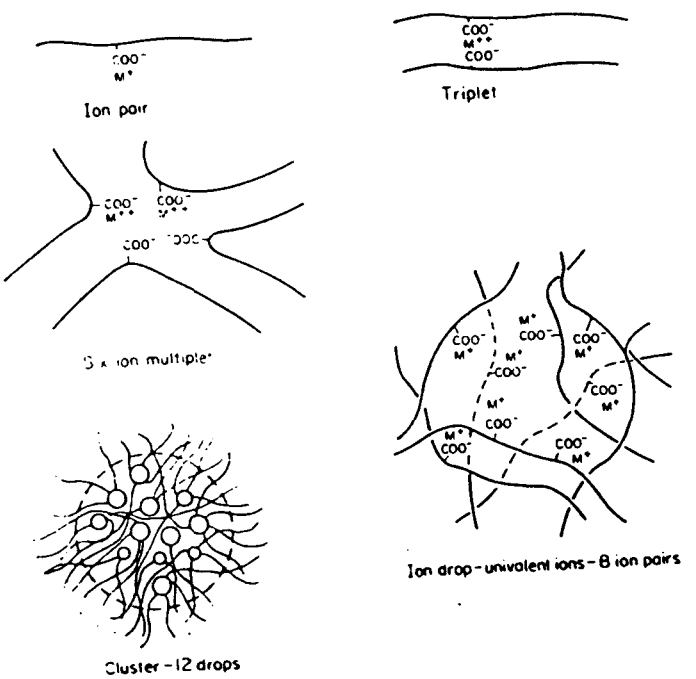


Figure 2.2: Possible mechanisms for ion aggregate formation in ion-containing polymers. (From reference 148)

contribution of ion aggregation is to domain formation. However, it would seem reasonable that, in these cases, ion aggregation will provide an increased motivation for domain segregation.

2.1.3.3 Influence of Microphase Separation on Physical Properties

There are several ways to observe the influence of microphase separation on a polymer's physical properties, the most important of which are dynamic mechanical experiments and stress/strain measurements.

In the most common dynamic mechanical experiments the storage modulus is determined as a function of temperature. This experiment provides information on the phase separation and thermal transitions of the polymer. Figure 2.3 presents typical results for several polymers of different structures; curves D and E represent segmented polyurethanes. Below Tg (the glass transition temperature) the polyurethanes are in the very high modulus glassy state, but as the temperature increases they show two regions of very sharp modulus decline. The first of these transitions is related to the Tg of the soft segment phase, while the second is associated with the hard segment phase Tg [23]. Between these two transitions, where the soft segment is

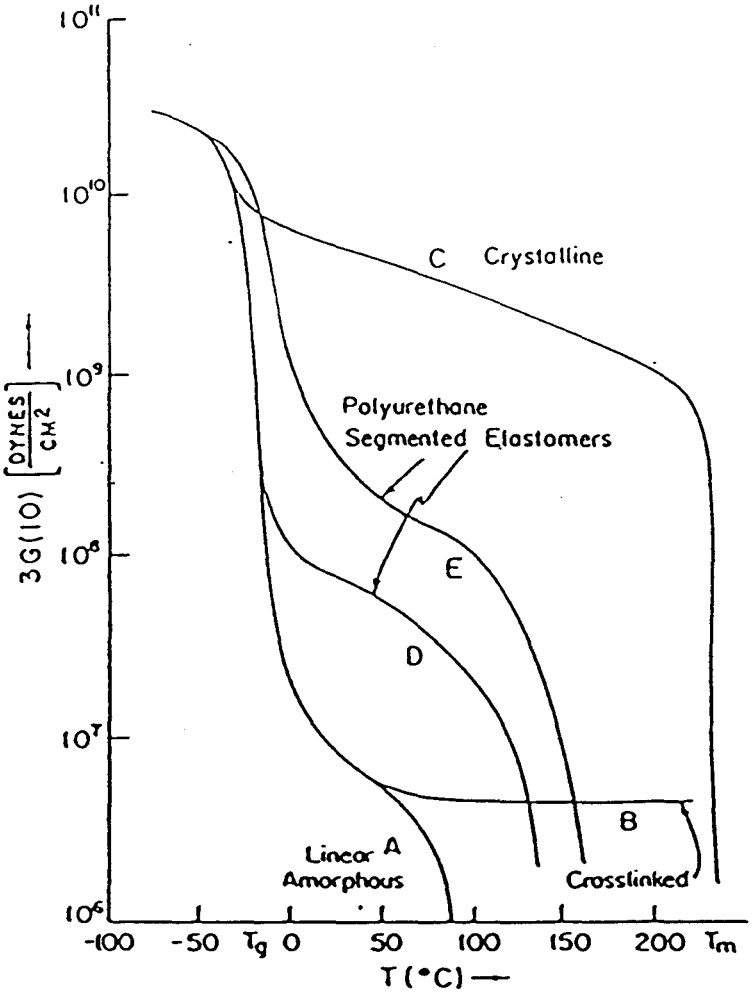


Figure 2.3: Storage Modulus vs Temperature Curves for:

A) - linear amorphous polymer
B) - cross-linked polymer
C) - semicrystalline polymer
D) - segmented polyurethane (MDI/BD/PTMA, 32% MDI by wt)
E) - segmented polyurethane (MDI/BD/PTMA, 38% MDI by wt)

(From reference 21)

above its Tg and flexible, and the hard segment is below its Tg and rigid, is the region in which the polyurethane will exhibit elastomeric character.

Sharp transitions, as shown in Figure 2.3, indicate good phase separation, while sloped or more gradual transitions indicate some degree of phase mixing.

Stress/strain testing is more useful than dynamic mechanical testing from a purely practical viewpoint. It provides a measure of the force which an elastomer is exerting under varying degrees of elongation. This is a direct indication of the mechanical performance of an elastomer under service conditions.

A comparison of several polymer stress/strain curves is shown in Figure 2.4. The initial portion of these curves, in which stress is proportional to strain, reflects the rigidity of the material. The usual measure of this rigidity is the "modulus of elasticity" or "Young's modulus", which is the ratio of stress to strain in this region. In curve c), for a tough plastic, the stress passes through a maximum value known as the yield stress. All extensions prior to this point are recoverable and the polymer is said to have elastically deformed. At extensions greater than this point the polymer cannot completely recover and is said to have plastically deformed. This phenomenon of plastic deformation gives an important distinction between a tough plastic like

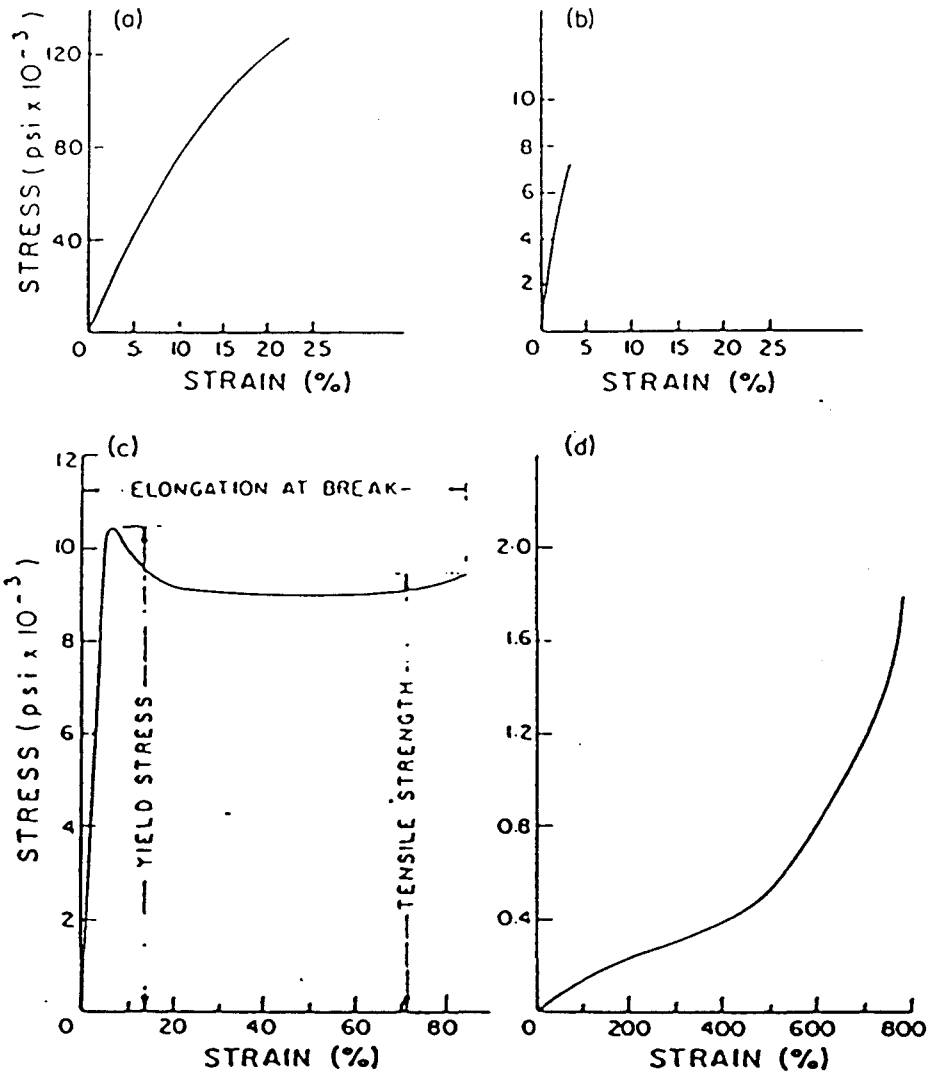


Figure 2.4: Comparison of Stress/Strain Behaviour For:
a) Synthetic fibre b) Rigid, brittle plastic
c) Tough plastic d) Elastomer
(From Reference 131)

polyethylene and an elastomer like vulcanized rubber: elastomers can recover completely from comparatively high degrees of elongation.

While segmented polyurethanes are classified as elastomers they do not exhibit exactly the same mechanical tendencies as chemically crosslinked elastomers, like vulcanized rubber. Unlike a typical crosslinked elastomer SPUs can undergo plastic deformation as a result of disruption of the hard segment domains. A similar phenomenon can occur in the recoverable or elastic region if the polymer is subjected to repeated extensions [30]. This is referred to as tensile stress hysteresis and leads to limitations in SPU elastomer applications.

In the stress-strain curves shown in Figure 2.4 the stress at the breaking point is known as the ultimate tensile strength, or failure strength, while the strain at this point is known as the ultimate elongation. These values along with the Young's modulus are the most commonly used measures of the mechanical properties of polymers.

As was previously discussed, it is the hard-soft segment microphase separation that gives an SPU its elastomeric properties. The way in which an SPU behaves under strain depends not only on the relative amounts of these two segments, although this is a major factor, but also on the relative domain sizes, the strength of the hard

segment interactions, the ability of the segments to orient in the direction of strain and the ability of the soft segment to crystallize under strain [31]. An increase in the content of hard segment domains leads to an increase in Young's modulus and ultimate tensile strength and a lower ultimate elongation [32].

Studies on the influence of soft segment molecular weight on mechanical properties have shown that as the soft segment molecular weight is increased the Young's modulus decreases and the ultimate elongation increases, as expected [33]. In addition, SPUs chain extended with aromatic chain extenders show higher initial modulus and ultimate tensile strength than those based on aliphatic chain extenders because of increased phase separation due to incompatibility of the aromatic chain extender with the aliphatic soft segment [25].

It is clear from this discussion that many factors are involved in SPU structure-property relations. This is therefore a complex problem and is for the moment only qualitatively understood at best.

2.2 Blood-Material Interactions

The application and use of all blood contacting devices is currently limited by the phenomenon of thrombosis at the blood material interface. Thrombosis, in the case of blood-material interactions, refers to the formation of a thrombus on the surface of the material at the blood-material interface. Thrombus formation occurs because the presence of a foreign body in the blood stream causes the blood's clotting mechanism to be activated. These thrombi, which are initially localized at the surface are potentially dangerous since they can either reduce blood flow through a vessel or, more seriously, break off to form emboli which can lodge in smaller vessels and block blood flow. If this blockage stops flow to a section of the heart, a heart attack results; if the affected area is the brain a stroke occurs.

There are a number of factors which can influence blood-material interactions and subsequent thrombus formation. These factors can be divided into three main classes [34]: the nature of the biomaterial; the nature of the blood flow; and the biologic environment. As far as in vivo implantation and biocompatibility are concerned, the nature of the biomaterial refers mainly to its surface properties, as it is the implant surface which is involved

in the initial blood-material interface. The nature of the blood flow (determined by parameters such as shear rate, stasis and turbulence) is a function primarily of the location and the spatial configuration of the implant. The biologic environment is determined mainly by the use of systemic anticoagulants or other drugs which could influence the blood-material interactions. The influence of each of these three classes of factors on thrombosis has been the subject of much study. Which factors play the major role in particular applications is a matter of considerable dispute, but it is reasonable to believe that each class is important and that any successful implant will originate from a consideration of all three.

The present study deals specifically with the nature of the biomaterial and its surface, since it is the surface which, through its involvement in the initial blood-material interface, influences the events which lead to thrombosis.

2.2.1 Vascular Haemostasis and Thrombosis

To understand how artificial surfaces interact with blood, a basic knowledge of the circulation's normal haemostatic and thrombotic mechanisms is helpful. Haemostasis literally means stoppage of blood flow. It

usually refers to the arrest of vessel bleeding following vessel injury, in order to maintain normal circulation.

Blood vessel leakage is stopped by the formation of a haemostatic plug composed of platelets and fibrin, in which red blood cells and granulocytes are trapped. In small blood vessels platelets are the most important component in plug formation, while in large vessels coagulation plays the crucial role [35]. In all vessels, however, the actions of platelets and coagulation are closely related.

Platelets have a dual role in haemostasis; they physically plug the disrupted vessel wall and they provide components essential to the process of coagulation. When platelets encounter damaged endothelium they adhere to the surface. In order for this adhesion to take place a protein known as von Willebrand's factor must be present [36]. More platelets can aggregate on the initial layer of adherent platelets and, in a secondary response, they secrete a number of compounds. Among these secreted metabolites are ADP and thromboxane A2 which can stimulate further platelet aggregation [36]. This process results in a growing platelet plug. In addition, when platelets undergo secretion, they release a membrane bound factor known as platelet factor 3, which acts as a catalytic surface for several of the reactions involved in coagulation [37].

Coagulation occurs in a fairly complex series of

steps known as a "cascade" illustrated in Figure 2.5. This cascade amplifies the blood's initial response to a coagulation stimulus and is subject to both positive and negative feedback [38].

There are two major known coagulation pathways: extrinsic and intrinsic. The extrinsic pathway is activated by exposure of the blood to damaged endothelium or extravascular tissue [39]. The intrinsic pathway responds when blood contacts a foreign surface, like a synthetic implant or damaged vessel wall [39]. The two pathways eventually merge to a common final path in which prothrombin is converted to thrombin. Thrombin is the "major player" in the coagulation cascade: it acts as an activator for some of the "earlier" coagulation factors; it can induce platelet aggregation and release; and it catalyzes the polymerization of fibrinogen to fibrin. Fibrin forms a crosslinked polymeric network which surrounds and binds the platelet aggregates, stabilizing them as the plug grows.

Finally, when the endothelium has had an opportunity to repair itself, the clot is no longer necessary and must be destroyed to prevent embolus formation. This is accomplished through activation of the fibrinolytic pathway, which involves the formation of plasmin. At the same time as coagulation factors are activated during coagulation, plasminogen is converted to plasmin. Plasmin can cleave the

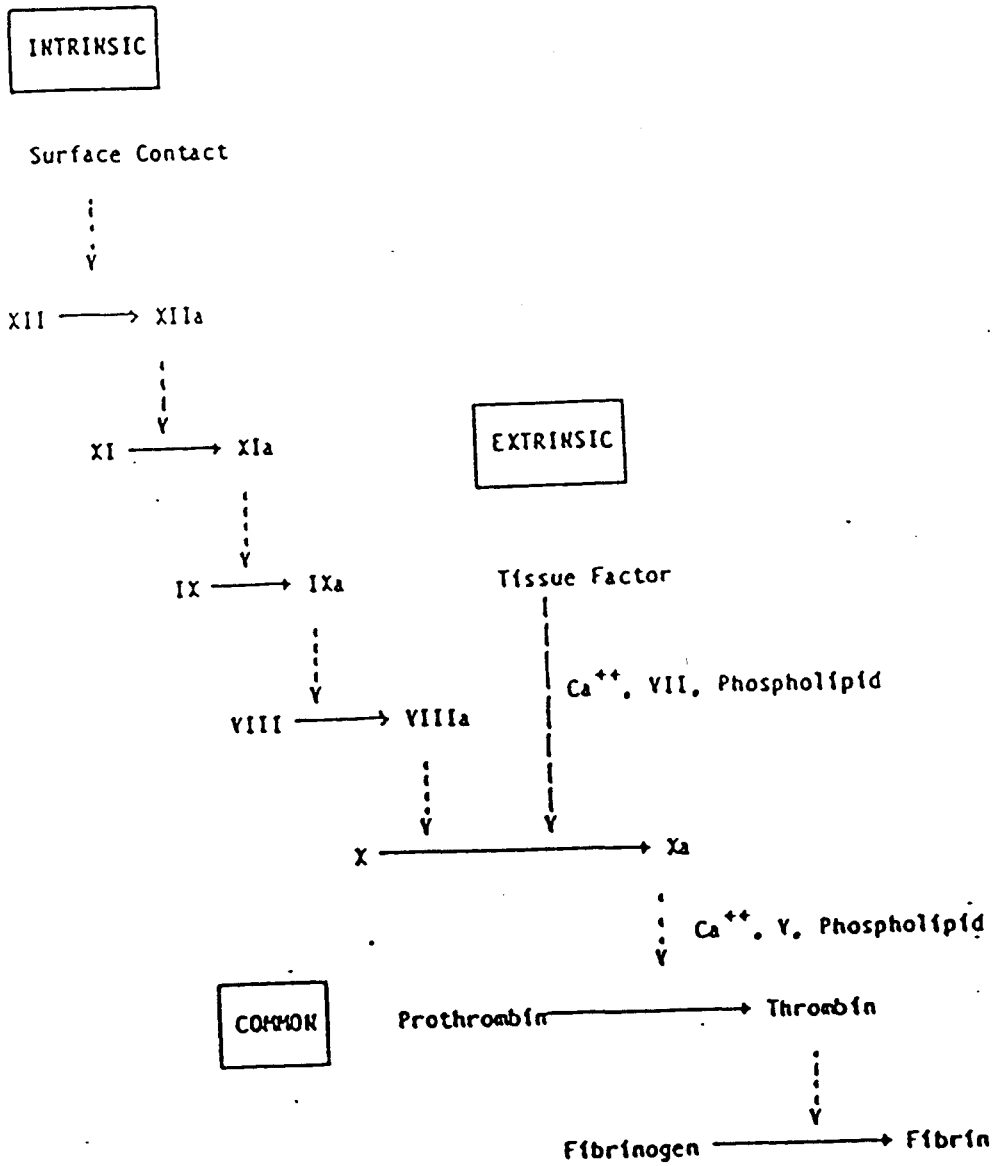


Figure 2.5: Intrinsic, extrinsic and common pathways of coagulation (From reference 39)

fibrin strands of the clot, thus degrading it into small fragments [40].

In addition to fibrinolysis, coagulation is subject to a number of other control systems for preventing undesired or excessive clot formation. The first of these is simply blood flow. The physical movement of blood prevents the local accumulation of activated factors and transports them to the liver where they are removed. Coagulation is also subject to feedback control. In addition to its many roles in the formation of the haemostatic plug, thrombin can also catalyze the production of enzymes which have an inhibitory effect on coagulation. The most important reaction of this sort is the conversion of the vitamin K-dependent protein C to protein Ca [41]. Protein Ca acts as an inhibitor of coagulation by cleaving factors Va and VIIIa [42]. A third controlling mechanism is the presence in the blood of components which inhibit certain activated factors. The most important of these inhibitors is antithrombin III, also known as heparin cofactor. Antithrombin III is the main plasma inhibitor of thrombin and can also inhibit activated factors X, IX and XII [43]. Its neutralizing action is greatly accelerated by the presence of heparin; however, heparin is not normally found in the bloodstream [44].

The normal steps in the haemostatic mechanism, then, are response to vessel wall damage, controlled clot

formation and, once the vessel has been repaired, clot degradation. When this normal haemostatic balance breaks down the formation of a thrombus in the lumen of a blood vessel may result. Thrombus formation can be initiated either by damage to the vessel wall, activation of platelets, activation of coagulation, or a combination of all three. The way in which a thrombus forms depends primarily on its location. Arterial thrombi tend to form following damage to the endothelium which causes platelet adhesion and subsequent coagulation [35]. In veins, where the blood flow and pressure are low, thrombosis is aggravated by stasis, leading to the accumulation of activated coagulation factors [35]. These activated factors generate thrombin which, in turn, induces platelet aggregation and fibrin formation.

2.2.2 Thrombogenesis on Artificial Surfaces

Thrombogenesis on artificial surfaces has much in common with thrombogenesis on vascular surfaces and is believed to involve essentially three steps: 1) plasma protein adsorption, 2) platelet adhesion and activation, and 3) activation of the intrinsic pathway of coagulation. The overall process is presented schematically in Figure 2.6.

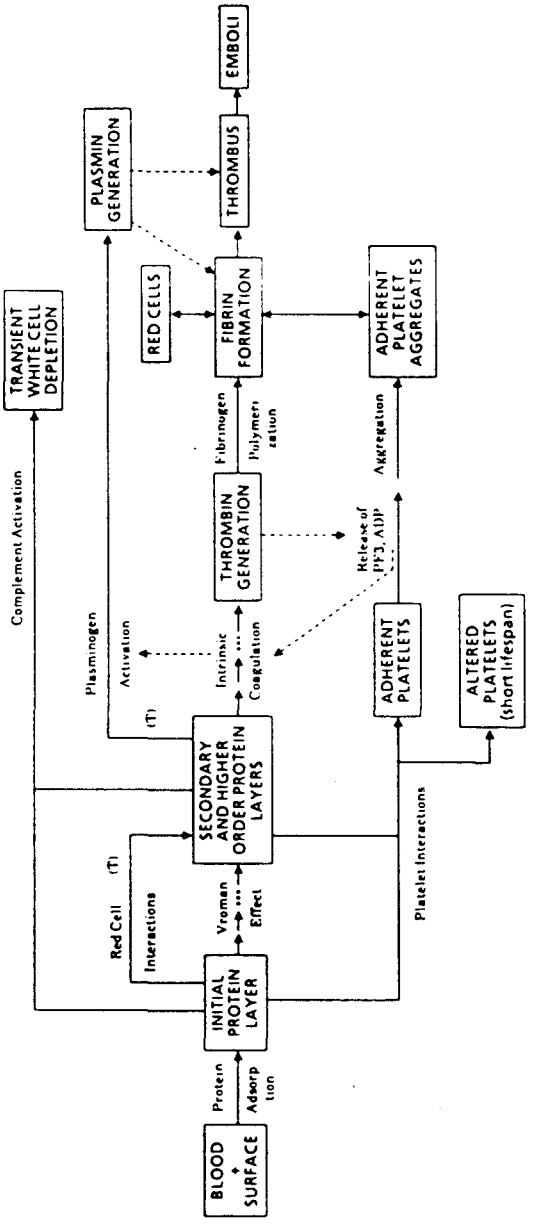


Figure 2.6: Sequence of events during blood-material interactions (T=tentative pathway) (From reference 146)

The first event following the contact of an artificial surface with blood is the adsorption of plasma proteins to the surface [45]. This protein layer rapidly becomes the effective interface with the blood and subsequent blood interactions will be determined by the layer's contents.

Protein adsorption to a surface can occur as a result of the combination of any of four basic interactions: hydrophobic, electrostatic, dipole-dipole and van der Waals [46]. When proteins adsorb to a surface it is possible for them to assume different conformations, for example a compact, highly rigid protein may maintain the same conformation it has in solution, while a loosely structured protein may become completely denatured. The conformation of the protein is important because it may determine how the protein interacts with other components of the blood.

The blood contains many plasma proteins all of which have the potential to adsorb, so protein adsorption is competitive. The initial adsorption of proteins occurs within seconds of bringing the material in contact with the blood, and it is likely that the proteins initially adsorbed will be those that are most abundant in the blood, specifically fibrinogen, immunoglobulin G and albumin. Competitive adsorption experiments with these three proteins have shown that fibrinogen adsorbs preferentially [47, 48].

However it is also known that surface-solution exchange of proteins may take place and initially adsorbed proteins may be replaced by others [49, 50]. In plasma this exchange results in the phenomenon of transient adsorption and is known as the Vroman effect [45, 50, 57]. The Vroman effect has been shown particularly by fibrinogen which is initially adsorbed and subsequently replaced on the surface by high molecular weight kininogen (HMWK) and possibly other factors of the initial or contact phase of coagulation [45, 49, 50].

The components of the protein layer formed will influence the subsequent steps of platelet adhesion and activation, and coagulation. Platelet adhesion has been found to be promoted on layers of adsorbed fibrinogen and reduced on layers of albumin [51-53]. Platelet adhesion is believed to take place about one minute after initial surface contact with the blood, when the protein layer is approximately 100 to 200 A thick [4]. After the platelets have adhered they may undergo morphological changes, including spreading, pseudopod formation and release, which in turn stimulates further platelet adhesion and surface-localized aggregation [19].

The platelets can also activate the intrinsic coagulation cascade by providing a negatively charged surface which acts catalytically in several of the cascade reactions. This negatively charged surface initiates

coagulation by binding factor XII (also known as Hageman factor) [55].

The mechanism by which factor XII is initially activated is not well understood; however, once activated it can convert prekallikrein to kallikrein which in turn can activate additional factor XII (producing activated factor XII, represented by XIIa) in a reciprocal activation step [56]. Factor XIIa can also activate factor XI. The activation of both factor XI and kallikrein by factor XIIa proceeds much more quickly when the reactants are bound to a surface. The surface binding of factor XI and kallikrein is greatly facilitated in the presence of HMWK [38], which is considered to be a cofactor of the contact phase of coagulation. Thus, although it facilitates the initial steps, it is not itself converted to an enzymatically active form. The activation of factor XII and the subsequent production of kallikrein and factor XIa constitute the contact phase of coagulation which initiates the intrinsic coagulation cascade. The contact activation mechanism is illustrated in Figure 2.7.

HMWK plays a crucial role in the contact activation sequence by promoting the adsorption of both prekallikrein and factor XI to the surface thus enabling their activation by surface bound XIIa. It has been found that the rate at which adsorbed fibrinogen is replaced via the Vroman effect

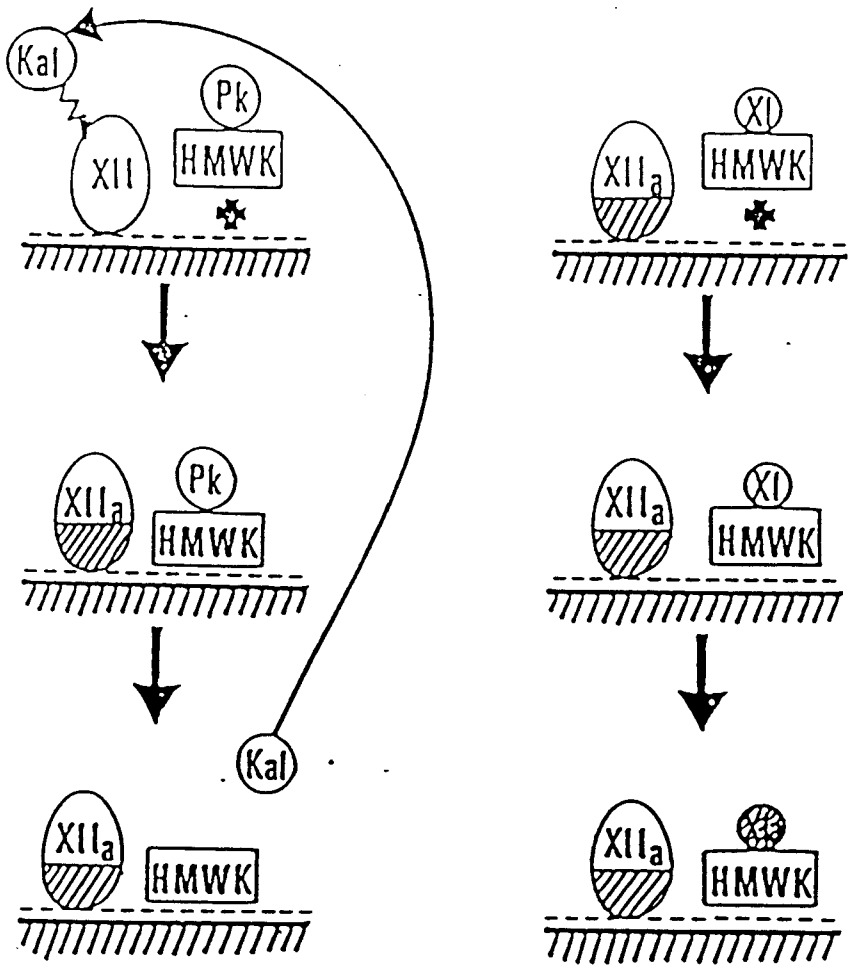


Figure 2.7: Contact activation of intrinsic coagulation.
(From reference 108)

is faster for more thrombogenically active surfaces [57]. It is thus possible that if the Vroman effect reflects fibrinogen replacement by HMWK and factor XII then the rate of fibrinogen replacement may give an indication of the ability of a surface to cause contact activation of coagulation. This possibility provides the major motivation for studying fibrinogen adsorption from plasma to segmented polyurethane surfaces in the present work.

The intrinsic coagulation pathway initiated by this contact activation mechanism involves a cascade sequence of factor activations (see Figure 2.8) which ultimately results in the catalytic conversion of prothrombin to thrombin through the cleavage of two peptide bonds [38]. Thrombin can then cleave specific arginyl-glycine bonds in fibrinogen to release fibrinopeptides A and B and form fibrin monomer. Fibrin monomer spontaneously polymerizes and is then crosslinked by factor XIIIa to provide a supporting network for the platelet plug. The activation of factors XII, XI, IX, X and prothrombin occurs by cleavage at arginine amino acid residues, producing the proteases XIIa, XIa, IXa, Xa and thrombin all of which have serine amino acid residues as their catalytically active sites [38].

In summary, immediately upon bringing a synthetic surface into contact with the blood, plasma proteins are adsorbed and provide the surface on which subsequent

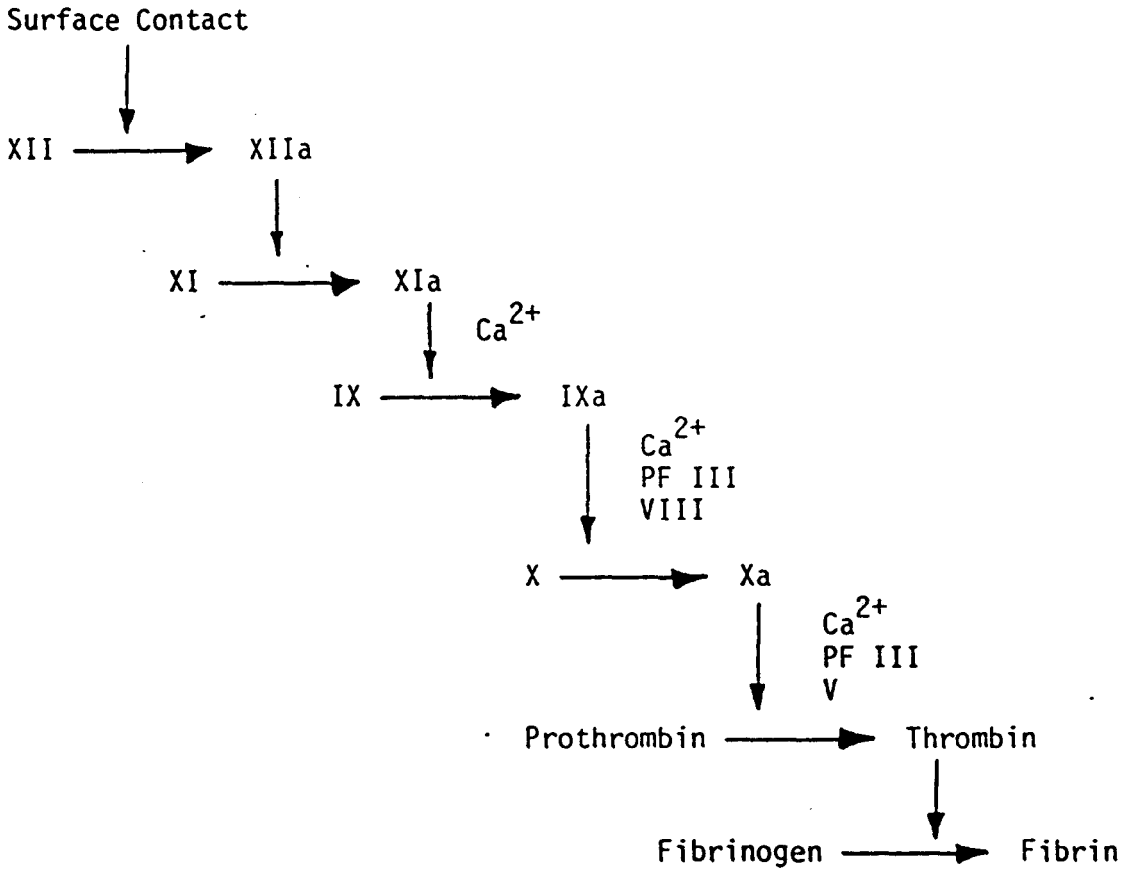


Figure 2.8: Intrinsic Pathway of Coagulation

interactions take place. Proteins in this layer may undergo surface-solution exchange involving contact phase activators, in which case, contact phase activation of the coagulation cascade can take place. Also, platelets can adhere to the adsorbed protein layer and become activated, resulting in the formation of platelet aggregates. These aggregates present a negatively charged surface which can catalyze the intrinsic pathway of the coagulation cascade, ultimately resulting in the production of fibrin which constitutes the material of clot and supports the platelet aggregates.

2.2.3 Influence of Synthetic Surface Properties on Thrombogenesis

As mentioned previously, the sequence of events leading to thrombus formation on an artificial surface begins with the adsorption of a plasma protein layer to the implant surface. The underlying material surface will influence not only the composition of this protein layer but also the conformation in which the component proteins adsorb. This combination of composition and conformation of the protein layer will govern its subsequent interactions with platelets and clotting factors and, thus, determine the potential of the surface for thrombus formation.

As the nature of the initial interaction between the plasma proteins and the material surface influences all the subsequent interactions, the factors which control this initial interaction are of considerable importance. In this regard the physical and chemical properties of the material surface are crucial. Among these are: surface chemical composition, surface roughness and porosity, crystalline-amorphous structure of the surface, distribution of chemical domains and overall hydrophobic-hydrophilic balance [34].

Water sorption is also considered important because living tissue contains a large amount of water and a material which can sorb water may, at least in this one respect, mimic the action of living tissue. Materials which can absorb significant quantities of water without dissolving are termed hydrogels. Hydrogels have been observed to possess considerable biocompatibility and it is speculated that this is due to the low interfacial free energy at the hydrogel-biological interface [34].

In fact, low interfacial free energy is one of the properties widely believed to be necessary for blood compatibility [58]. The interfacial free energy may be related to the critical surface tension of a material [59], which can be evaluated by measuring the contact angle of a number of hydrocarbon liquids, covering a range of surface tensions, on the material surface [60]. Lyman et al have

shown that the clotting time of blood following contact with a material is inversely related to the critical surface tension [61]. Subsequently, Brash and Lyman showed, consistent with this, that the degree of platelet adhesion is directly related to the critical surface tension of a material [62]. Baier has hypothesized that a critical surface tension in the range of 20-30 dyne/cm may be desirable for biocompatibility [58].

Several factors complicate the use of critical surface tension as a predictor of biocompatibility. The first is that critical surface tension is directly related to biocompatibility only for materials in which exclusively hydrophobic interactions are involved [58]; in other words for materials that are hydrophobic and possess neither surface charge nor surface chemical functionality nor surface microdomain structure. Perhaps more relevant to biomedical applications than critical surface tension is the water contact angle which provides information on the response of a surface to a fluid which is of biological importance.

The second complicating factor is the adsorption of the plasma protein layer. Since the plasma protein layer adsorbs almost instantaneously, one may question how the material itself can influence subsequent interactions when it is no longer in contact with the blood. The material

surface may do this through its influence on both the composition and the conformation of the adsorbed plasma protein layer. Since these factors will determine how the layer behaves in its subsequent interactions with platelets and clotting factors, the surface properties are, in effect, transmitted through the layer and expressed in these protein interactions.

Baier has proposed a theory related to this idea of influence that explains the desirability of low critical surface tension [58]. He has speculated that low critical surface tension may allow proteins to adsorb in a more native form, in which they are more likely to be in equilibrium with the blood. Thus, they may have a shorter period of contact with the material surface than do denatured proteins. The platelets which adhere to the native protein layer do not have time to form large aggregates before they are detached along with the layer. It should again be emphasized that critical surface tension as determined by hydrocarbon liquids probably ignores the contributions of hydrophilicity, charge, chemical functionality or microdomain structure to surface properties.

The single property which influences all of these complicating interactions and, thus, the factor most likely to determine thrombogenicity is the surface chemical

composition. Various studies have investigated the influence of the presence of polar or hydrophilic groups at the surface. It has been shown that apolar groups favour strong protein adsorption, while polar groups tend to result in a weakly adherent protein layer [63]. Consistent with this it has been found that the presence of hydrophobic groups at the surface results in protein adsorption that is both greater and less reversible than on surfaces with hydrophilic groups [64].

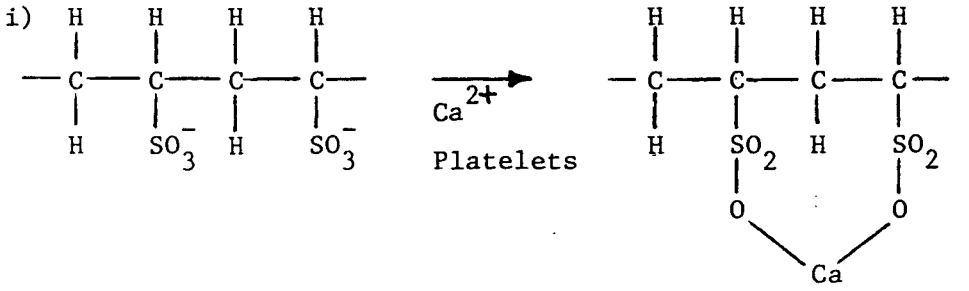
For polymers such as segmented polyurethanes, which undergo phase separation into microdomains it is possible to have both hydrophobic and hydrophilic domains in a single material. In one such case, albumin is believed to selectively adsorb to the hydrophilic domains and fibrinogen and immunoglobulin G to the hydrophobic domains [65]. In this investigation a mixture of fibrinogen, albumin and IgG, not plasma or blood, was used and it is not clear whether protein adsorption from blood would follow this pattern. It is possible, however, that manipulating the domain types, sizes and morphologies may allow some control of the composition and conformation of the adsorbed protein layer. Complicating the situation is the question of whether one domain or the other has a tendency to preferentially locate at and, thus, dominate the interface. For certain polyurethanes, it has been shown that the soft segment

domains are present to a greater degree at the surface than in the bulk [66, 67]. In addition to this, Merrill et al. have found that while soft segment analog homopolymers tend to be thromboresistant hard segment analogs are thrombogenic [68, 69]. They suggest that this is a result of surface mobility effects. The crystalline, highly hydrogen bonded hard segment polymer will not be able to rearrange or accommodate to the blood environment while the amorphous soft segment polymer should be able to do so to a much higher degree.

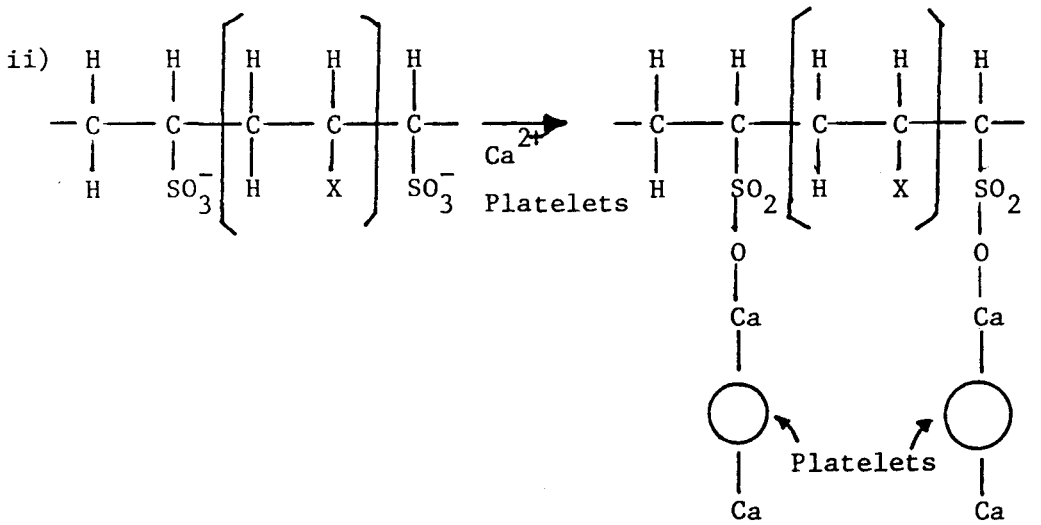
Another surface characteristic considered important is the presence of charged groups. It is believed that since vascular endothelium, platelets and plasma proteins all carry a net negative charge at blood pH [70, 71] the presence of negatively charged groups at the surface of a material implant would be beneficial as they should repel both proteins and platelets. Indeed the incorporation of sulphonate, carboxylate and glutamic acid groups into polymers has been found to give antithrombogenic properties [72-74, 83]. However, for some materials, the most notable being glass, a negatively charged surface can actually promote thrombus formation [72, 76]. Leonard [77] has developed a theory which attempts to reconcile this inability to correlate surface charge and thrombogenicity. He has suggested that the important characteristic is not

simply the presence of negatively charged groups at the surface, but rather the spatial distribution of these groups. According to this theory the spatial distribution of charge at the surface can either promote or inhibit platelet adhesion depending on the interaction with calcium ions. Calcium ions are attracted to the surface by the negative charge and if they bind they would then present a positively charged surface to which the platelet could adhere. However, if pairs of the negatively charged groups are spatially arranged in such a way that a calcium ion can bind to both and form a ring then no positive charge is presented to the platelet and adhesion is unlikely. This is illustrated in Figure 2.9. It should be noted that this theory ignores protein adsorption to the material surface and has never been subjected to experimental test.

It should be made clear that the presence of negatively charged groups will not necessarily prevent plasma proteins which possess a net negative charge, from adsorbing to the surface. While these proteins have a net negative charge, they also have specific local cationic sites which can form electrostatic bonds with the negatively charged groups on the surface and allow adsorption [78]. In addition, divalent cations, like calcium, can act as bridges between the negative charges on the surface and on the protein. Thus, the charged groups could still influence the



Platelet adhesion does not occur.



Platelet adhesion can occur.

Figure 2.9: Adhesion of platelets to a surface containing negatively charged groups, according to Leonard. (From reference 77)

type and conformation of proteins that adsorb.

An alternative theory to Leonard's calcium ion binding hypothesis for thrombogenicity on negatively charged surfaces involves the adsorption of factor XII. This theory again proposes that it is the spacing of the negative charges that is important. Nossel et al. [79] postulated that a rigid spacing of negatively charged sites may cause conformational changes in factor XII which result in the initiation of the intrinsic pathway of coagulation and subsequent thrombosis.

2.3 Polyurethanes for Blood Contact Applications

Polyurethanes were originally selected for blood contact applications because of their mechanical properties and the polymers currently used in device fabrication were designed primarily to optimize mechanical properties. It is only relatively recently that development has concentrated on improving blood compatibility. A number of promising approaches have been initiated, but because of the complex nature of blood-material interactions thorough evaluation is necessary and the time lag between research and commercialization is considerable. As a result, the polymers currently available commercially are still those which

exhibit excellent mechanical properties and adequate, but not outstanding, blood compatibility. A summary of the major commercial biomedical polyurethanes is given in Table 2.1.

Table 2.1: Commercial Biomedical Polyurethanes

Polyurethane (Developer)	Application	Composition
Biomer (duPont)	-catheters -heart assist pumps -artificial heart chambers	-based on PTMO 2000, MDI and a mix of diamines, primarily ethylene diamine
Cardiothane (Avco-Everett)	-artificial heart -intra-aortic balloons -catheters -blood conduits	-90% polyetherurethane -10% triacetoxy terminated polydimethylsiloxane
Pellethane (Upjohn)	-pacemaker connections -pacemaker lead tubing -blood bags	-based on PTMO, MDI and 1, 4 butanediol
Tecoflex (Thermo Electron)	-development and evaluation stage	-based on PTMO, hydrogenated MDI and 1,4 butanediol
Toyobo (Toyobo)	-under development	-polyetherurethane containing different soft segments
Vialon (Deseret)	-under development	- polyetherurethane

2.3.1 General Approaches to Blood Compatibility

Since most of the commercially available polyurethanes, and biomedical polymers in general, still only exhibit "adequate" blood compatibility, a lot of recent research has focussed on designing materials of improved thromboresistance. A review of the strategies which have been used and their applications to polyurethanes will be presented in this section.

Initial attempts at improving blood compatibility were based largely on laboratory observations of modifications that promoted or inhibited thrombogenesis. For instance, the observation that albumin on a surface resulted in reduced platelet adhesion [51-53] led to the development of various albumin-coated polymers as biomaterials [80-82]. Unfortunately, while these polymers did exhibit reduced platelet adhesion, it was difficult to retain the albumin coating and as a result the tendency for thrombosis was still significant enough to discourage any such polymers being produced commercially [83].

The observation that interfacial free energy appeared to be important in determining thrombogenesis led to the production of polymers which exhibit reduced interfacial tension [59, 60]. The best known polymers of this type are the water sorbing hydrogel polymers. Some

limited success has been achieved in this area with polyurethanes. The production of the most succesful polyurethane hydrogels involves grafting 2-hydroxyethyl methacrylate to a base polyurethane [84].

In recent years two approaches to blood compatibility have been pursued, both of which attempt in some way to mimic the natural vascular surface. The reason for this is quite simply that under normal conditions there is an absence of thrombosis within the vasculature. This absence can be attributed to two factors: 1) the thrombogenic inertness of cells, both endothelial cells lining the vasculature and cells contained in the blood stream; and 2) the normal coagulation control mechanisms discussed previously (section 2.2.1). These vascular simulation approaches to blood compatibility seek to endow synthetic polymers with the characteristics which inhibit thrombogenesis in the vasculature. The first approach is to develop polymer surfaces which imitate the surface of the cellular components of the vascular wall or the bloodstream, while the second involves attempts to incorporate some of the blood's natural anticoagulant activity into the biomaterial.

The first attempts at mimicking the cellular components of the vasculature focussed on the vascular wall. It is known that the vascular wall possesses a net negative

charge [70], so the incorporation of anionic groups into biomaterials has been widely studied [72-75]. This idea has been applied to polyurethanes by Lelah et al [85] and Ito et al [86] who have produced polyurethanes containing sulphonate and carboxylate groups respectively. Lelah et al were able to show improved thromboresistance using an ex vivo shunt, while Ito et al have shown moderately prolonged blood clotting times.

The most ambitious efforts to simulate the vessel wall involve lining implant surfaces with endothelial cells. Two approaches, referred to respectively as "seeding" and "sodding" have been taken.

Seeding involves placing a relatively small number of endothelial cells on the surface prior to implantation and then relies on subsequent growth and migration for monolayer formation [87]. One of the difficulties with this procedure is in getting initially seeded cells to remain adherent following implantation. Attempts to improve the adhesion have involved precoating the polymer surface with proteins important in cellular adhesion, like fibronectin, collagen and extracellular growth factors. Polymer substrates tried have included Teflon, Dacron and Biomer [87].

In sodding, on the other hand, the surface is initially seeded with endothelial cells and an intact monolayer is cultured in vitro prior to implantation [88].

The difficulties again lie in promoting the growth and adhesion of the endothelial cells. To improve this situation polymer surfaces have been coated with fibrin, collagen and human amnion (as a substitute for basement membrane). To date the best endothelialization results have been achieved with Dacron and a human amnion coating [88].

A number of studies of endothelial cell deposition on polyurethanes, using primarily Biomer as the polymeric substrate, have also shown promising results [89-92], but more work on adhesion, growth and orientation of these seeded cells would appear to be necessary. In addition, performance in vivo remains to be evaluated, and the question of whether endothelial cells retain their complex biological functions when bound to a polymeric substrate remains unanswered.

The approach of simulating the surfaces of circulating blood cells has been pursued by Hayward and Chapman among others [93-95]. In an attempt to approximate the outer surfaces of the plasma membranes of red blood cells and platelets, they have produced polymers to which phosphorylcholine, a hydrophilic group in the phospholipid bilayer which constitutes the membrane, is attached [93-95]. No data on the blood compatibility of these materials have been reported.

Another major approach to nonthrombogenicity has

been the development of surfaces which can inhibit or reverse thrombosis. Fougnot et al have postulated that "this can be achieved either by covering the given material with an antithrombogenic coating or by synthesizing materials which intrinsically possess anticoagulant properties" [83].

Antithrombogenic coating involves binding to the biomaterial surface a substance which can either inhibit thrombus formation or lyse an incipient thrombus. Three major types of substances have been explored: 1) platelet aggregation inhibitors like dipyridamole [96]; 2) plasminogen activators like urokinase or streptokinase [97]; and 3) coagulation inhibitors like heparin [98-100]. The most widely studied of these coatings is heparin. While heparinized materials have been used successfully in short term applications [83] the gradual loss of heparin from the surface limits their effectiveness in the longer term. This problem is common to all materials based on bioactive coatings. The loss of the active component can simply limit the useful lifetime of a material or, if the presence of the coating material in the bloodstream is undesired, as in the case of plasminogen activators, it can be potentially hazardous [83].

A variety of so-called "heparinized" polyurethanes have been produced. Initial work concentrated on placing cationic charges on the polymer surface to which heparin

could ionically bind [101, 102]. Heparin bound in this way is gradually released from the surface, as was discussed above, and it may be that this gradual release is responsible for the anticoagulant activity exhibited by these polyurethanes. However heparin covalently bound to a polymeric substrate has been found in some cases to retain a measure of its anticoagulant activity [103-105]. This has led to attempts to synthesize polyurethanes to which heparin is covalently bound [106, 107, 147] and preliminary results indicate that some anticoagulant activity is present in these materials.

The synthesis of materials which possess inherent anticoagulant properties generally involves attempts to incorporate into the material functional groups that are important in the action of coagulation inhibitors. As has been previously discussed, there are a number of important blood-borne inhibitors of coagulation including C1 esterase inhibitor, protein C and antithrombin III. Unfortunately, while the actions of C1 esterase inhibitor and protein C are beginning to be understood their structure and mechanisms of action are still not well known. It is known that C1 esterase inhibitor inhibits factor XIa, XIIa, plasmin and kallikrein [108] and that protein C degrades factor Va, inactivates factor VIIIa and neutralizes an inhibitor of plasminogen activation [42].

The mechanisms of action of antithrombin III and plasminogen, on the other hand, are better understood. Antithrombin III binds directly to thrombin and its anticoagulant activity is greatly accelerated by the presence of heparin [44]. The action of heparin is known to be to some extent due to the presence of sulphate and aminosulphate groups [109, 110]. As early as 1951 the incorporation of sulphonate groups onto polystyrene was found to give a marked inhibition of coagulation relative to unmodified polystyrene [75]. More recently, Fougnot et al [111] have also obtained promising results with sulphonated polystyrenes. The incorporation of sulphonate groups into polyurethanes was first accomplished in the 1960's [112], but has only recently been the subject of investigation for biomedical applications. Thus Lelah et al have found improved thromboresistance in sulphonated polyurethanes used as ex vivo shunts in dogs [85].

More recently, research on attaching amino acids to sulphonated polystyrenes via sulphonamide linkages has been conducted by Fougnot et al [83]. The rationale for this approach is not only to provide the aminosulphate linkages which are believed to be of importance in the action of heparin, but also to introduce specific amino acids which are present in the active sites of coagulation inhibitors and have the potential to inhibit coagulation directly. The

results which have been obtained with amino acid substituted polystyrenes have led to the designation of some of these materials as "heparin-like".

Specific amino acids of interest are arginine and lysine. Arginine is of interest because when antithrombin III binds to the active site of thrombin it does so via an arginine residue as illustrated in Figure 2.10 [5]. Polystyrene with arginine methyl ester groups attached via sulphonamide linkages has shown a high specific affinity for thrombin in affinity chromatography studies [113].

The attachment of lysine to a blood contacting material has the potential to influence the action of plasminogen. The role of plasminogen, as already discussed, is in clot lysis. Plasminogen is activated to form plasmin which can enzymatically cleave the fibrin strands that hold a clot together [40]. Plasminogen is known to bind specifically to lysine residues and Brash et al [114] have hypothesized that binding plasminogen to a surface may facilitate its conversion to plasmin or to a form which is plasmin-like.

The major question with regard to the attachment of single amino acids to synthetic polymers is whether or not the covalently bound amino acid retains the same activity which it has in the protein, since in many cases the action of a specific amino acid within a protein is highly

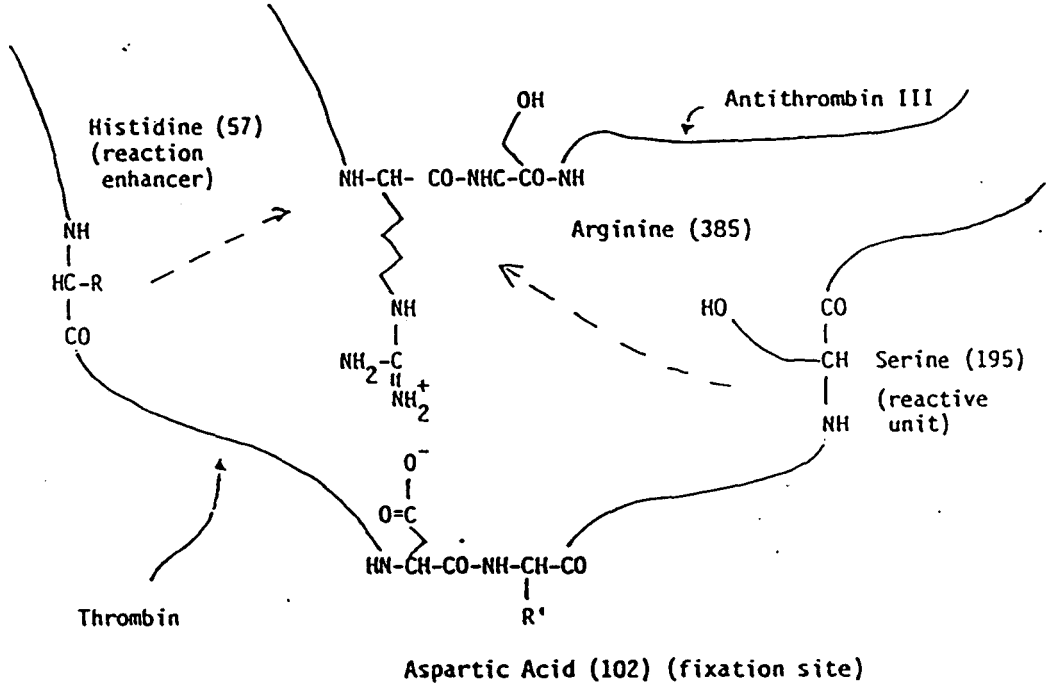


Figure 2.10: Mechanism of inhibition of thrombin by antithrombin III. (From reference 5)

dependent upon neighbouring amino acids and on protein conformation.

The attachment of single amino acids to polymers has additional potential as the initial step in forming surface-attached bioactive polypeptides.

The objective of the work described in this thesis was to incorporate potentially antithrombogenic groups into segmented polyurethanes. Specifically, sulphonate groups and aminosulphate-linked arginine methyl ester groups, which have shown considerable promise when attached to polystyrene resins, were selected for study.

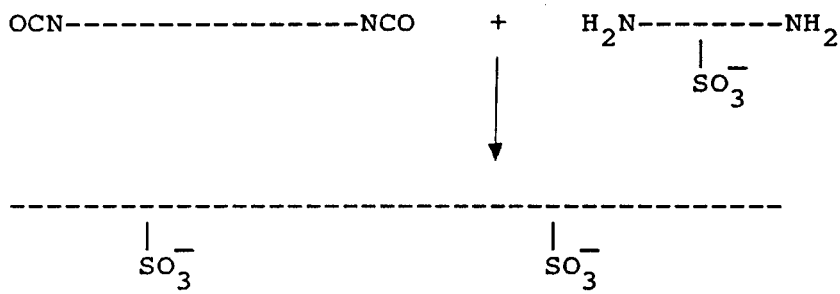
2.3.2 Approaches to Polymer Synthesis

The two major requirements of polymer synthesis in the present work were first to incorporate sulphonate groups into the polyurethane chains and, second to attach amino acids via sulphonamide bonds to the sulphonate groups.

The preparation of sulphonate containing polyurethanes was first investigated by Farbenfabriken Bayer in the 1960's in the course of a general investigation of polyurethane ionomers [112]. They found a number of interesting methods for producing anionic ionomers. The following is a summary of the methods which can be used to

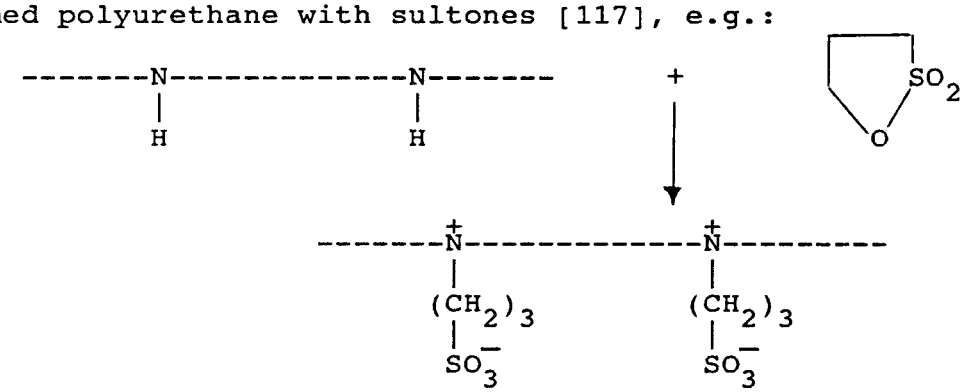
produce sulphonate containing polyurethanes:

1) Using sulphonate-containing chain extenders [115,116]:

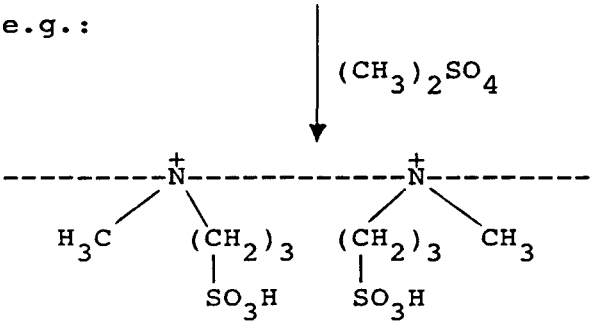


This method has the advantages that it places the sulphonate group unequivocally in the hard segment and that several appropriate chain extenders are commercially available. Having the sulphonate group in the hard segment can be considered advantageous as the hard segment is believed to be more thrombogenic than the soft segment [68]. On the other hand it is known that the hard segment domains are depleted in the surface, so the effectiveness of hard segment sulphonate groups may thus be diminished.

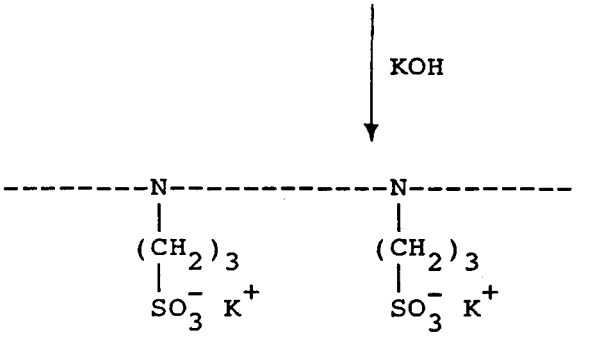
2) Reaction of secondary amines in urea segments of a preformed polyurethane with sultones [117], e.g.:



This polymer can be converted into a cationic ionomer by quaternization, e.g.:



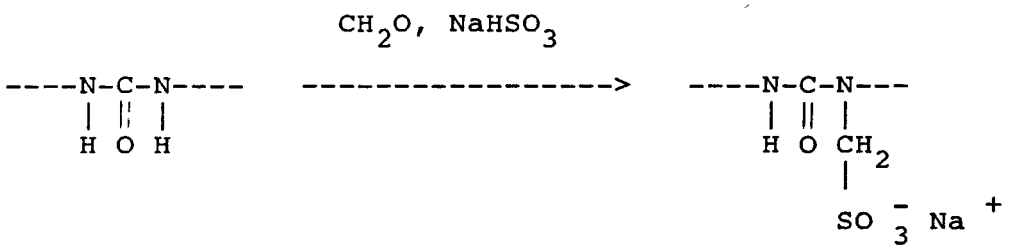
or into an anionic ionomer by reaction with a base, e.g.:



This method has been under investigation by Cooper et al for the past several years [85,118-120]. The advantages of this method are that the sulphonate group is again located in the hard segment and that it is separated from the polyurethane chain by three carbon atoms so it should have some mobility to participate in reactions with blood components. The disadvantages are the harsh nature of sultone reagents which makes reactions hazardous to carry out and the possibility of residual sultone in a polymer to be used in a biomedical

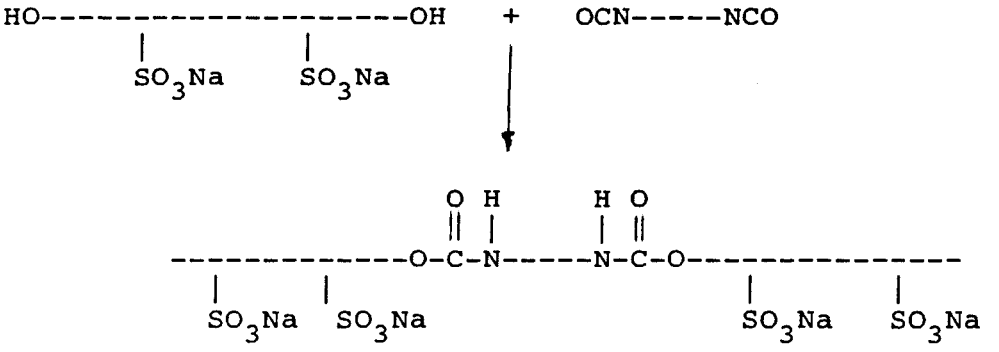
application which is highly undesirable.

3) Sulphomethylation of secondary amine in urea segments using formaldehyde and sodium bisulphite [121]:



With this method the potential again exists for placing sulphonate groups in the hard segment. However, the use of formaldehyde which has the potential to crosslink polyurethanes could complicate the reaction.

4) Using a soft segment polyester containing sulphonate groups [122]:

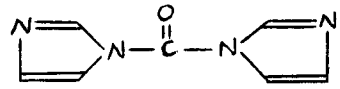


This method has two drawbacks, first the sulphonate group will be present only in the soft segment, which is less prone to thrombogenesis than the hard segment, and second,

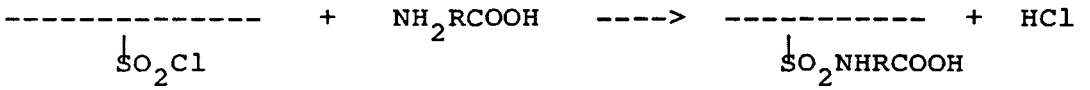
in a search of potential suppliers no commercially available polyesters of this sort could be found.

For the reasons discussed above it was decided that the use of sulphonate-containing chain extenders would be the approach used in the present work. A comprehensive study of the incorporation of sulphonate into polyurethanes should, however, encompass all of the methods available.

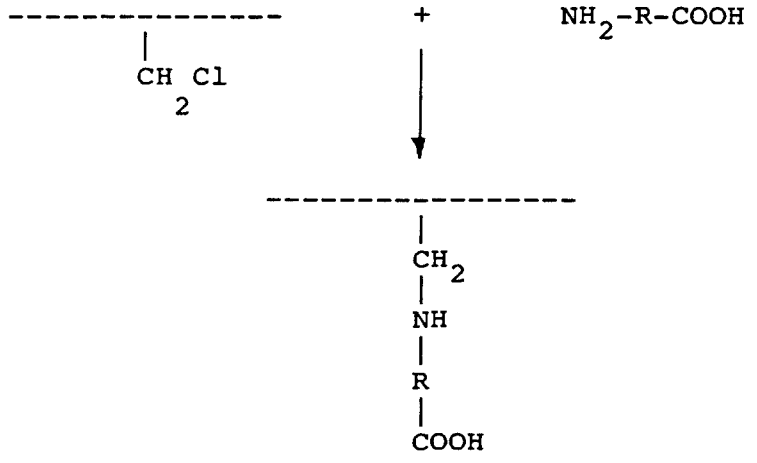
The second step in polymer synthesis is to attach amino acids to the sulphonate groups. The method of attachment used by Fougnot et al for sulphonate-containing polystyrenes was to form the highly reactive sulphonyl chloride (SO_2Cl) group which can react with the amine group of the amino acid to produce a sulphonamide bond. The method used by Fougnot et al to introduce the sulphonyl chloride groups into polystyrenes was to treat polystyrene with chlorosulphonic acid [5]. Unfortunately, chlorosulphonic acid is a harsh reagent and would be likely to degrade polyurethanes at either the ether linkages of the soft segment or the -C-O- linkages between soft and hard segments.

In the present work the existing sulphonate groups in the polyurethane were converted to sulphonyl chloride groups. Several reagents have potential in this respect: e.g. oxalyl chloride $(COCl)_2$, ethyl chloroformate ($ClCO_2Et$) and N,N'-carbonyldiimidazole 

The reaction of amino acids with sulphonyl chlorides is rapid and occurs readily at room temperature:



For the purpose of completeness an alternative method of amino acid attachment which does not involve sulphonamide bond formation should also be mentioned: the Merrifield synthesis. The Merrifield approach is widely used for the production of polypeptides [123]. The method involves attaching an amino acid to a solid polymeric substrate, then filtering to isolate the amino acid-containing substrate. The process is repeated with successive amino acids until the appropriate polypeptide is built up. The first attachment of amino acid to the polymeric substrate uses the reaction of an alkyl chloride on the polymer surface with the amine group of the amino acid:



The polymer bound amino acid can then be separated from the reaction medium by filtration, and successive amino acids may be linked together in this fashion. When the desired polypeptide has been built up it can be cleaved from the polymeric substrate to give the final product. The usual polymeric substrate for these syntheses is polystyrene [123].

3. EXPERIMENTAL METHODS

3.1 Polymer Synthesis

3.1.1 Materials

The materials used in polymer synthesis are listed in Table 3.1.

3.1.2 Solvent Distillation

Dimethyl sulphoxide (DMSO) and dimethylformamide (DMF) were both distilled to remove water prior to use in polymer synthesis. A standard vacuum distillation apparatus was used. About 700 mL of solvent was placed in the distillation flask. The first 50 mL distilled, which would contain any water, was discarded, and about 400 mL was collected for use in polymer synthesis. The distillation conditions for DMSO were: 1 to 1.5 mm Hg, 40 to 50°C; and for DMF: 1 to 1.5 mm Hg, 20 to 23 °C. The distilled solvent was used within 24 hours of distillation and was kept in a sealed flask under nitrogen until then.

Table 3.1: List of Materials Used in Polyurethane Synthesis

Material	Supplier
Dimethyl sulphoxide	Caledon Labs, BDH Chemical
Dimethyl formamide	Caledon Labs, BDH Chemical
4,4'-Diphenylmethane diisocyanate	Mobay
Polypropylene glycol 1025	BDH Chemical
Biphenyl diamino disulphonic acid	Eastman Kodak
Stilbene diamino disulphonic acid	Eastman Kodak
Diamino benzene sulphonic acid	Aldrich
Ethylene diamine	Aldrich
Methylene dianiline	Sargent Welch
Oxalyl chloride	Aldrich
Arginine methyl ester	Sigma
Methanol	Mallinckrodt
Nitrogen	Canadian Liquid Air

3.1.3 Polyether Degassing

Prior to use in synthesis, polypropylene glycol of molecular weight 1025 (PPG 1025) was degassed for 24 hours at 50 °C and 1 mm Hg.

3.1.4 4,4'-Diphenylmethane Diisocyanate Distillation

4,4'-diphenylmethane diisocyanate (MDI) was vacuum distilled at 170 °C and 1 mm Hg. Before the vacuum was applied the system was purged with high purity nitrogen to avoid contact of the MDI with water and the MDI was melted under nitrogen. Heating was done with care to avoid direct contact between the heating mantle and the distillation flask, since high temperature can initiate polymerization of MDI. The first 25 mL of MDI collected was discarded. As a result of the high distillation temperature it was necessary to wrap the vertical condenser with glass wool and the horizontal condenser with heating tape. The distilled MDI was stored under nitrogen in a sealed container at 4 °C.

3.1.5 Chain Extender Preparation and Purification

Ethylene diamine and methylene dianiline were used as received, but the sulphonic acid-containing chain extenders required additional purification. Acidic sulphonic acid-containing chain extenders were first purified by removing water soluble impurities. This was done by three repetitions of heating in distilled water at 60 °C and then hot vacuum filtering. The sodium salts of the purified sulphonated chain extenders were then prepared by addition of stoichiometric quantities of 1 M NaOH. The sodium salts of all the sulphonic acid-containing chain extenders used in this work are water soluble and any remaining insoluble impurities were removed by vacuum filtration.

The sodium salts of the chain extenders were precipitated with acetone and then dried in a 90°C air oven for 48 hours. They were stored in sealed containers at room temperature and dried for 24 hours at 90°C immediately before use.

3.1.6 Polyurethane Synthesis

The segmented polyurethanes were prepared by the two step procedure described in section 2.1.2. The apparatus is

shown in Figure 3.1. The first step in synthesis is the preparation of the prepolymer. Normally a 2:1 mole ratio MDI:PPG 1025 was used. Two prepolymer synthetic techniques were followed in this work. In the first, a 20% wt/wt solution of MDI in freshly distilled DMSO or DMF was added dropwise to the reactor containing a 40% solution of PPG held at 90-100°C under dry nitrogen. In the second, a 40% wt/wt solution of PPG was added dropwise to a 20% wt/wt solution of MDI held at 25 °C under dry nitrogen; when all the PPG had been added the temperature was raised to 90-100 °C. In both cases the solutions were allowed to react at 90-100°C under dry nitrogen for 3 hours.

After reaction the prepolymer solutions were cooled to the desired temperature, usually 40 °C, for the chain extension step. The chain extenders used were: ethylene diamine (ED), methylene dianiline (MDA), biphenyl diamino disulphonic acid (BDDS), stilbene diamino disulphonic acid (SDDS) and diamino benzene sulphonic acid (DABS). They were added either dropwise from solution (7.5% wt/wt in DMF or DMSO) or as successive small quantities of the powdered material, after an equivalent amount of solvent had been added to the prepolymer solution. Normally equimolar amounts of prepolymer and chain extender were used.

Addition of solid material in the case of the sulphonated chain extenders was necessary because of their

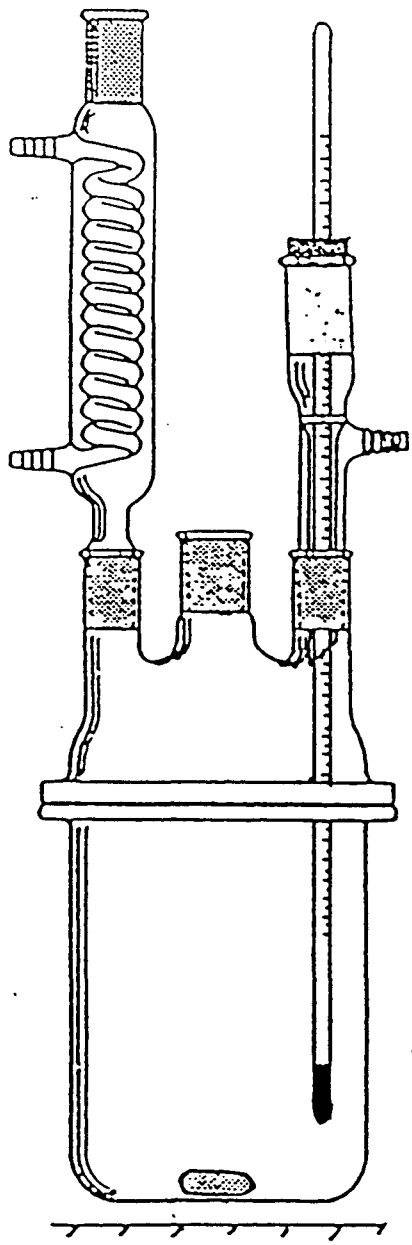


Figure 3.1: Polyurethane Synthesis Apparatus

poor solubility in the reaction solvents. This was especially true for BDDS. The sulphonated chain extender was found to dissolve as it reacted with the isocyanate and, as chain extension continued, a viscous solution resulted. To ensure completion of chain extension after addition of all the solid chain extender, the solution was maintained at the reaction temperature under nitrogen for at least twelve hours.

Following the chain extension step the polymer solution was cooled to room temperature in preparation for precipitation. The polymers prepared using ethylene diamine or methylene dianiline were precipitated in distilled water. The sulphonated polymers could not be precipitated in distilled water and had to be precipitated either in salt solutions (prepared with distilled water) or, preferably, in methanol. The precipitated polymers were then washed at least three times, with distilled water in the case of the polymers precipitated in salt solution, or with fresh methanol for those precipitated in methanol. The polymers were finally dried in a 60 ^{o}C oven to constant weight (approximately 72 hours).

3.1.7 Arginine Methyl Ester Attachment to Sulphonated Polyurethanes

The arginine methyl ester addition was a two step procedure using the apparatus shown in Figure 3.2. The first step was conversion of the sulphonate groups on the polymer to the highly reactive sulphonyl chloride groups. The polymer was dissolved in DMF (3% wt/wt solution) in vessel A under a dry nitrogen atmosphere, then cooled to -4°C, using an ice/water/salt bath. Liquid $(COCl)_2$, in an amount equimolar to the sulphonate content of the polymer was then added using a graduated pipet and the resulting reaction allowed to continue for two hours. A "drierite" packed tube was used to vent the carbon dioxide produced during the reaction.

While the conversion of sulphonate to sulphonyl chloride was proceeding in vessel A, arginine methyl ester was added to DMF (1% wt/wt) in vessel B and stirred under dry nitrogen at room temperature. Arginine methyl ester is only moderately soluble in DMF and does not dissolve completely under these conditions.

At the end of the two hour reaction time the polymer solution in vessel A was warmed to 20 °C and transferred, under vacuum, to vessel B. There it was allowed to react with the arginine methyl ester at room temperature under a

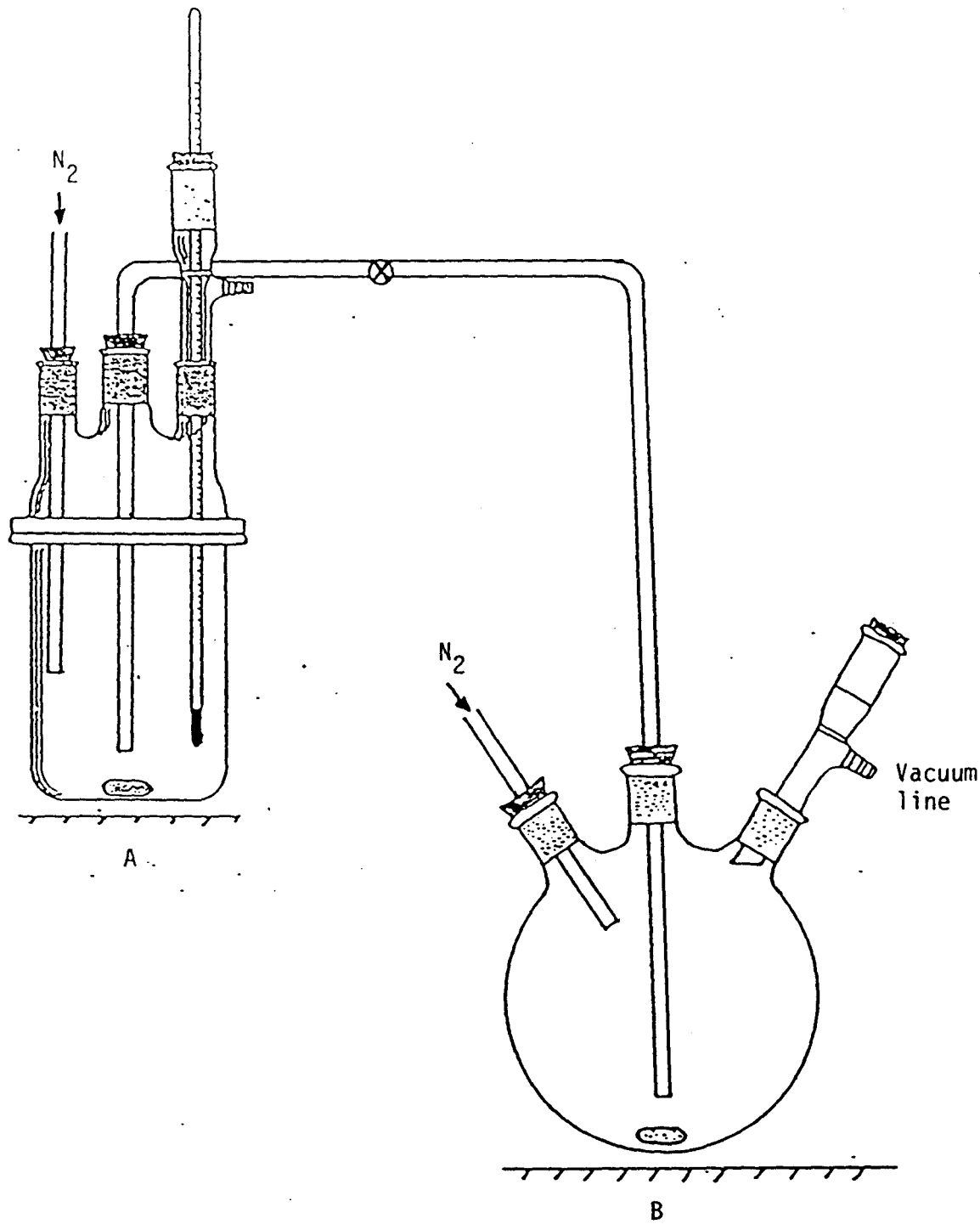


Figure 3.2: Apparatus for reactions to attach arginine methyl ester to sulphonated polyurethanes.

dry nitrogen atmosphere for at least twelve hours. Over the course of the first hour of reaction the arginine methyl ester, which is initially present as a suspension in DMF, goes into solution.

The final polymer was precipitated in distilled water, washed with distilled water at least three times and dried in a 60°C air oven to constant weight (approximately 72 hours).

3.2 Polymer Characterization

3.2.1 Gel Permeation Chromatography

Gel permeation chromatography (GPC) was used to determine the molecular weight distribution of the polyurethanes. A GPC system utilizes a mobile phase, in which the polymer samples are dissolved, and a stationary phase which separates the dissolved macromolecules on the basis of the size they assume in the mobile phase. The mobile phase consists of a suitable solvent or mixture of solvents for the polymer to be analysed. The stationary phase consists of columns packed with porous beads and the pore sizes of the beads will determine the degree of separation of the macromolecules. The concentration of the

separated macromolecules is determined as they emerge from the GPC columns and converted into a molecular weight distribution.

A schematic diagram of the gel permeation chromatography (GPC) system used is shown in Figure 3.3. The system consists of a solvent reservoir, a Model M6000A pump (Waters), a Model 7125 sample injector (Rheodyne), a series of four ultrastyragel columns (pore sizes 10^3 A, 10^4 A, 10^4 A, 10^5 A) placed in a column oven with a Model TM thermostat controller (Waters), a Model R401 differential refractive index detector (Waters), a Model DT707 screw terminal (Data Translation), a Model DT2805 data acquisition board for analog to digital conversion (Data Translation) and a Model 158 computer (Zenith) with high resolution graphics. Data were analyzed using custom developed ASYST software (MacMillan Software).

The mobile phase was a solution of 0.1 M LiBr (Fischer) in HPLC grade DMF (Caledon) flowing at 1 mL/min. The column temperature was held constant at 80°C.

All polymers were dissolved in 0.1 M LiBr in DMF. Solutions of approximately 0.2 wt% were prepared gravimetrically. All solutions were filtered through a 2 μm pore diameter precolumn filter (Millipore) to a 200 μL sample injection loop. The system was calibrated using narrow molecular weight distribution polystyrene standards (TSK).

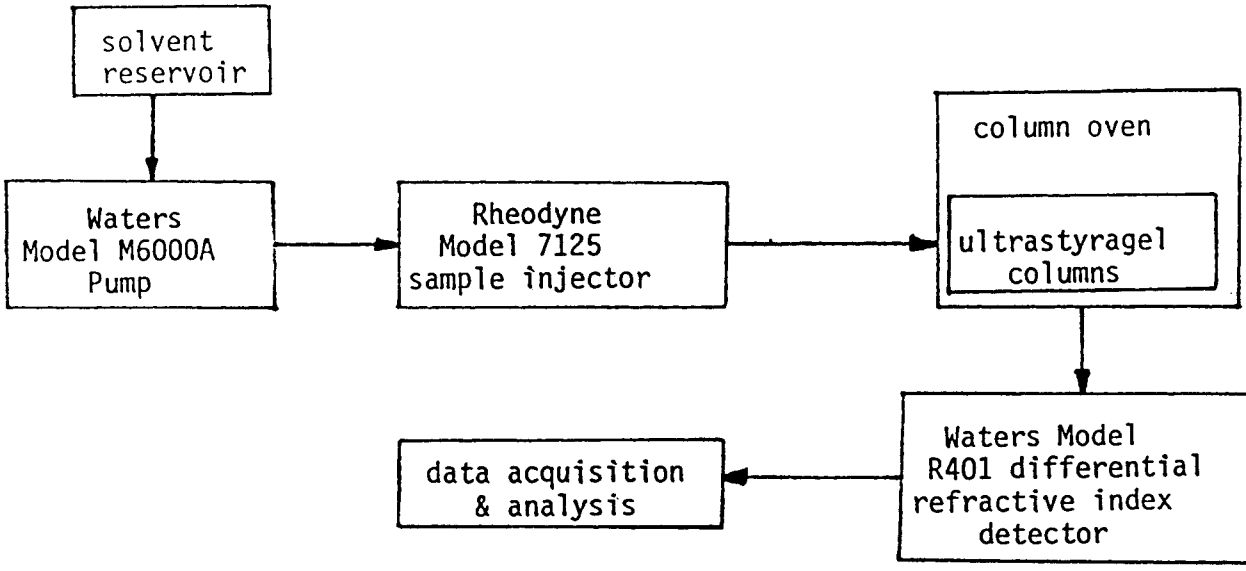


Figure 3.3: Schematic of GPC System

3.2.2 Intrinsic Viscosity Measurement

The intrinsic viscosities of the polymers were measured using an Ubbelohde suspended level viscometer. Polymer solutions in 0.1 M LiBr in DMF (the GPC mobile phase) were prepared gravimetrically. Solution concentrations of 1 g/100 mL, 0.6 g/100 mL and 0.2 g/100 mL were used. Measurements were made at the GPC operating temperature of 80°C.

3.2.3 Contact Angle Measurement

Contact angle measurements were done in the laboratory of Professor A.W. Neumann at the University of Toronto. Contact angles were measured on polymer films using a technique previously developed by Absolom et al. for measuring contact angles on hydrogels [124]. The polymer films were prepared by coating glass slides with polyurethane solutions of approximately 3% w/v in DMF which had been filtered through a 0.5 μm Millipore filter. Cleaned glass slides were placed vertically in the polymer solution and withdrawn at a constant rate, then dried in a vertical position in a vacuum oven at 60°C for at least 24 hours.

The polymer films were equilibrated in deionized,

distilled water for 24 hours and contact angles were then measured on the hydrated films as a function of time using a goniometer telescope [125]. Goniometer measurements were made using deionized, double distilled water. Droplets of water of about 10 μL volume were delivered to the polymer surface with a micropipette. The contact angle was measured by placing the cross hairs of the telescope at the point where the drop, polymer surface and air meet, known as the triple point, and reading the angle from the goniometer [125]. This is illustrated in Figure 3.4.

Time dependent contact angles were measured at 10 minute intervals in the following manner. Five drops of water were applied to the film surface at approximately the same time and advancing contact angles were measured twice on both sides of the drop. At each interval fresh drops of water were applied to new locations on the film surface. Measurements were made over a period of about 120 min.

3.2.4 Electron Spectroscopy for Chemical Analysis (ESCA)

ESCA can be used to determine the concentrations of all elements except hydrogen and helium in approximately the top 50 A of a solid specimen [126]. In ESCA an X-ray beam is used to induce photoelectron emission from the sample. The

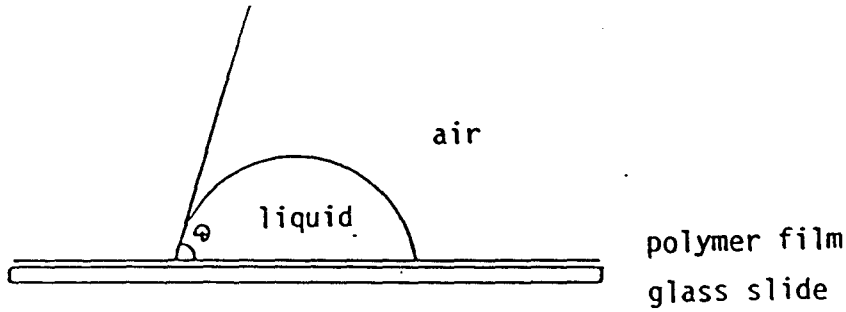


Figure 3.4: Definition of Contact Angle

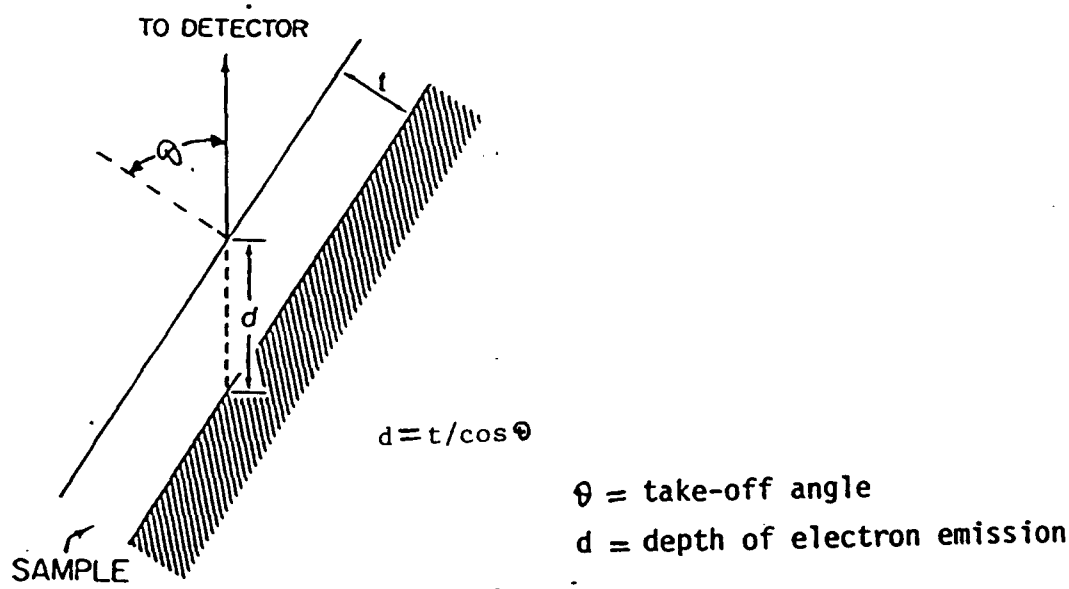


Figure 3.5: Take-off angle dependence of depth of electron emission in ESCA experiments. (From reference 126)

energy of the photoelectrons emitted identifies the chemical elements and their bonding environment while the intensity of the emission gives the concentration of the species. The atomic concentrations can be determined with a relative accuracy of about 10%.

ESCA was performed at the University of Washington, Seattle by Castner and Ratner using a Surface Science Instruments X-probe spectrometer. This spectrometer has a monochromatic aluminum X-ray source with a variable spot size (150 - 1000 μm diameter).

ESCA measurements were performed at a series of takeoff angles to determine if a compositional gradient existed near the surface of the segmented polyurethanes. The takeoff angle controls the depth from which photoelectrons are detected as they emerge from the sample. Moving from low to high takeoff angles reduces the depth of electron emission, thereby increasing the relative surface content of the elemental information. This is illustrated in Figure 3.5.

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

Polymer films coated on glass microscope slides were

used as samples. 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 at least 24 hours.

3.2.5 Elemental Analysis

Conventional elemental analyses for sulphur, sodium and nitrogen were performed at Guelph Chemical Laboratories. Sulphur was analyzed by a combustion method, in which the gases produced by combustion are absorbed in hydrogen peroxide solution and then titrated with barium perchlorate. Nitrogen was analyzed by a Perkin-Elmer C-H-N elemental analyzer with a thermal conductivity detector. Samples for sodium analysis were first digested in an acid mixture and then analysed by atomic absorption.

SEM-EDAX can be used to determine the concentration of all elements of atomic mass greater than sodium. In the present work it was used for determination of the sulphur content of polyurethanes. The basis of the technique is that electron beam excitation of a sample causes x-ray emission. The energy of the x-rays identifies the elements, while the intensity of the radiation is proportional to the relative concentration. For quantitative elemental analysis the

system must be calibrated with a sample of known elemental composition (a standard). In the present work calibration was based on samples analysed by combustion analysis at Guelph Chemical Laboratories.

Samples were prepared as thin films cast from approximately 5% w/v solutions in DMF onto cleaned microscope slide fragments. The films were dried in a vacuum oven at 60°C for 24 hours prior to analysis. The fragments were then mounted, using silver paint, on electron microscope stubs and carbon coated by vacuum evaporation. The samples were kept in a desiccator until the time of analysis (not more than 24 hours).

An ISI-DS130 model SEM was used for these measurements. It was operated under the following conditions: large spot size, 15° take-off angle, sample height of 30 (arbitrary scale) and an electron beam voltage of 15 kV.

3.2.6 FT-IR Spectroscopy

FT-IR spectroscopy can provide useful information on the chemical composition and bonding within polyurethanes; which can in turn give qualitative verification of postulated reaction mechanisms. The major purpose in

obtaining infrared spectra in the present work was to assess the extent of incorporation of arginine methyl ester into substituted polyurethanes. Such information could, in principle, be obtained by spectral subtraction methods involving subtraction of the spectra of corresponding polymers before and after reaction with the amino acid.

For these measurements a Nicolet model 320 FT-IR was used. Thin self-supporting films of the polyurethanes were used as samples in order to provide enough transmittance. They were prepared by casting 1% w/v solutions in DMF on teflon sheets. The films were dried at room temperature for 24 hours and then in a 60°C air oven for 48 hours.

The spectra for the sulphonate-containing polymers were subtracted from the spectra for the arginine methyl ester containing polymers by matching the absorbance of the $-CH_3$ peak from the soft segment at 1370 cm^{-1}.

3.2.7 Mechanical Testing

Tensile stress versus strain behaviour of the SPU's was measured using an Instron Tensile Testing System , Model TT-D (Department of Mechanical Engineering, McMaster University). Films were prepared by casting a 5% w/v solution in DMF onto a teflon plate. Multiple layers had to

be cast to provide films of adequate thickness. The initial cast layer was allowed to dry for 24 hours in a fumehood at room temperature before a second or subsequent layer was added. The finished films of about 1 mm thickness were dried in a vacuum oven at 60°C for 24 hours. An ASTM dumbbell die D was used to cut out the samples and samples were tested in accordance with ASTM D412-80 using a 10 kg load cell and a crosshead speed of 1 in/min.

3.3 Fibrinogen Adsorption

Fibrinogen adsorption was used as a means, albeit a limited one, of assessing the response of blood to the novel polyurethanes synthesized in this work. As discussed previously (section 2.2.2) the adsorption of fibrinogen from plasma to a material may give information on activation of the contact phase of coagulation. Therefore adsorption from plasma was determined. In addition it appeared from initial work that there might be a specific interaction between fibrinogen and sulphonate groups in the polyurethanes. To investigate this possibility, fibrinogen adsorption isotherms were determined from single protein solutions in buffer.

3.3.1 Tube Preparation

Glass tubing segments of 2.5 mm I.D. and 20 cm length 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 (approximately 5% w/v in DMF) for dip coating. The clean, dry tubes were dipped in the polymer solution, inverted and dipped again, then placed vertically in a drying rack and dried for 1 h in a fumehood, 1 h in a 60°C air oven and 3 h in a 60 ° C vacuum oven. The tubes were then dipped, inverted and dipped again in the polymer solution. This second coat was dried for 1 h in the fumehood, 1 h in a 60°C air oven and 24 h in a vacuum oven at 60°C.

Using this two-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 because the sulphonated polymers are hydrophilic 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 sulphonated polyurethanes was good.

3.3.2 Fibrinogen Adsorption

The materials used for the fibrinogen adsorption study were pooled, human, platelet poor plasma (with citrate anticoagulant, 6 vol. blood:1 vol. citrate, final concentration 3.7 g/l sodium citrate) from the Canadian Red Cross, Ottawa and Grade L human fibrinogen from Kabi, Stockholm. The human fibrinogen was radiolabelled with ^{125}I using the iodine monochloride method [54] and added to the plasma as a tracer in amounts not exceeding 10% of normal fibrinogen concentration (about 3 mg/mL).

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 the labelled plasma. Adsorption was studied as a function of plasma dilution in the range 0.5% to 30% of normal plasma concentration. The plasma was diluted with isotonic Tris buffer, pH 7.4. These data provide a so-called "5-minute isotherm", i.e. 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 3.6. All experiments were performed under static conditions at a constant temperature of 23°C. The tubes (20 cm long x 2.5 mm I.D.) were equilibrated overnight with isotonic Tris buffer which was then displaced by the labelled, diluted plasma using the 3-way valve system shown in the Figure, 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. 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 contact this buffer was drained from the tubes.

The rinsed tubes were then cut into segments for counting. Two 2 cm segments were cut from either end of the tube and discarded. The remainder of the tube was cut into 4 sections of 4 cm each. These sections were 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 of adsorbed fibrinogen was calculated using the following equation:

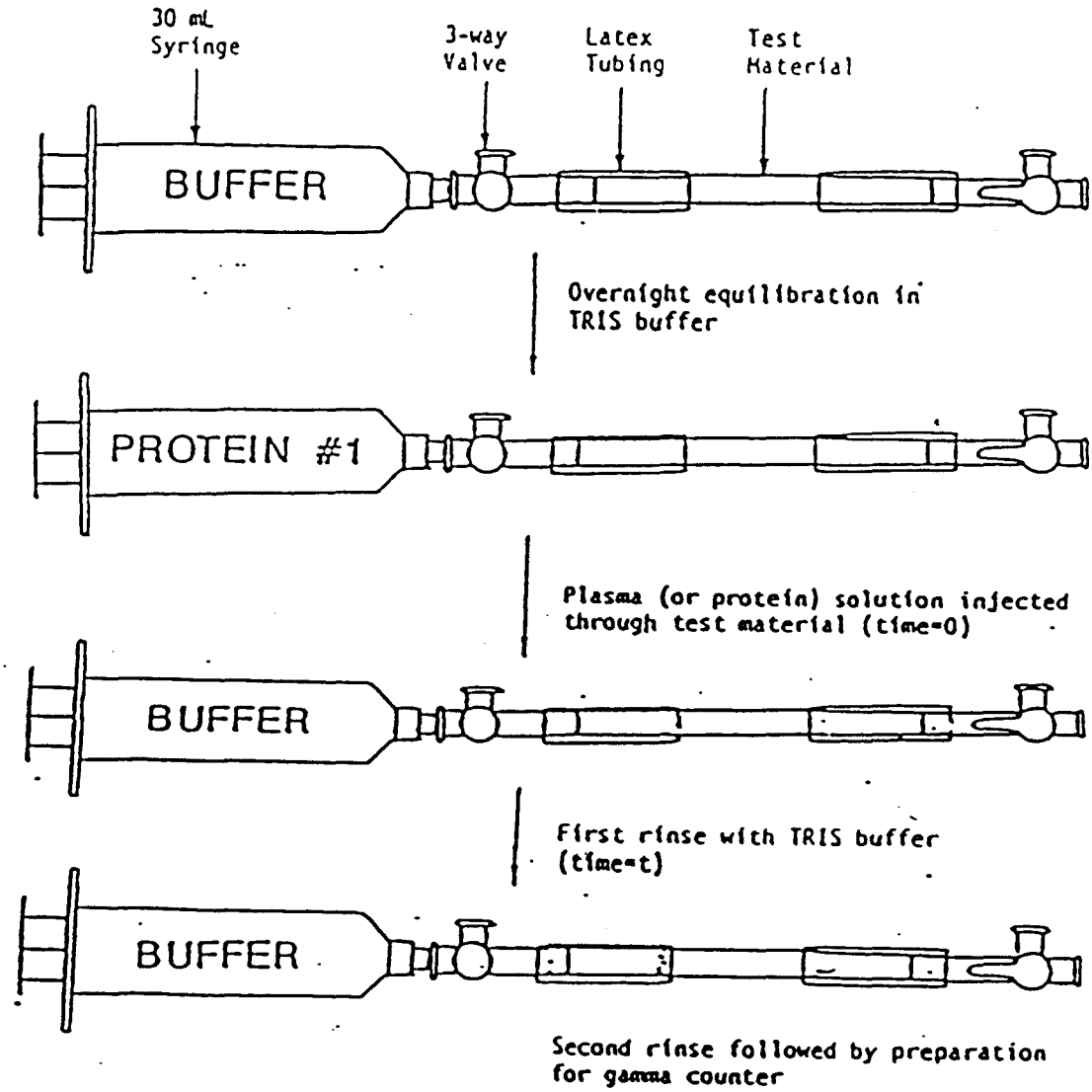


Figure 3.6: Experimental procedure for fibrinogen adsorption measurements.

$$\text{Surface Conc } (\mu g/cm^2) = \frac{\text{Net surface count (CPM)}}{\text{Surface area (sq cm)}} \times \frac{\text{Fibrinogen conc in diluted plasma (mg/mL)}}{\text{Solution count (CPM/mL diluted plasma)}} \times 1000$$

The fibrinogen concentration of the plasma pool was determined by the clinical hematology lab, McMaster University Medical Centre and reported to be 2.68 g/L . The surface area was calculated from measurements of the 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 the glass tubing segments (determined to be 1.06) which accounts for the gamma radiation absorbed by the glass.

Each polymer surface was checked for preferential adsorption of labelled versus unlabelled fibrinogen by preparing 3 solutions of identical fibrinogen concentration (1 mg/mL in isotonic tris buffer) containing 90%, 50% and 10% labelled fibrinogen respectively. The procedure was identical to that followed for the plasma "isotherms", except that a 3 hour adsorption time was used to allow for equilibrium adsorption to occur.

Fibrinogen adsorption isotherms from isotonic Tris buffer were also determined. In these experiments the range of concentrations examined was 0.0005 to 1 mg/mL. An adsorption time of three hours was used to allow equilibrium adsorption to occur.

4. RESULTS AND DISCUSSION

The initial phase of this work involved development and optimization of the sulphonated polyurethane synthesis for both sulphonate content and molecular weight. In the next phase three polymers which covered an appropriate range of sulphonate content were selected for derivatization with arginine methyl ester. The three sulphonated polymers and the three corresponding arginine methyl ester-containing polymers were then extensively characterized and their interactions with fibrinogen both in plasma and buffer were studied.

4.1 Optimization of SPU Synthesis Using Sulphonated Diamines

For optimization of the sulphonated polyurethane synthesis two goals were of particular importance: 1) to produce polymers with a high sulphonate content so that any influence the sulphonate groups and the subsequently attached arginine methyl ester might have on physical and biomedical properties would be evident; and 2) to produce polymers of acceptably high molecular weight which would be likely to have good mechanical properties. To optimize these

properties both sulphur content (by SEM-EDAX) and molecular weight (by GPC) were determined.

Data for both sulphur content and molecular weight were verified independently in other laboratories. SEM-EDAX results were verified by combustion analysis (Table 4.1).

Table 4.1: Comparison of SEM-EDAX and Combustion Analysis Data For Elemental Composition of Polymers

Polymer Sample	Wt% S SEM-EDAX**	Combustion*
1	0.7	0.7
2	0.9	1.0
3	1.0	0.9

* - precision ± 0.3%
** - calibrated with standard determined by combustion analysis.

The two sets of data are in good agreement. In order to check the reliability of both the instrument and the data handling software of our GPC system, a chosen polymer sample was analysed at the Waters Co. laboratories in Boston using different columns and software with the same mobile phase conditions. Again agreement between the two data sets was good (Table 4.2).

Table 4.2: Verification of GPC System*

System	Mw	Mn	D
McMaster	87,000	67,500	1.3
Waters	89,000	63,000	1.4

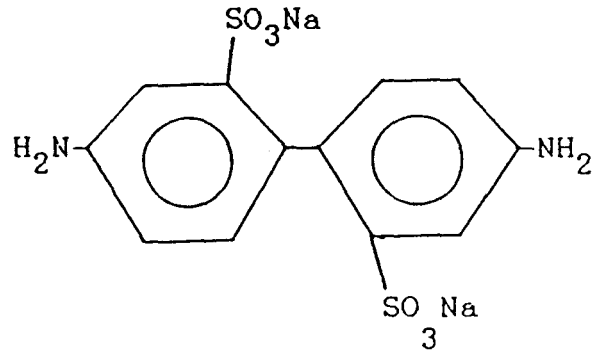
* The polymer analysed was chain extended with BDDS at 40°C in DMSO. The GPC was operated using a mobile phase of 0.1 M LiBr (McMaster) and 0.05 M LiBr (Waters) in DMF at a flowrate of 1.0 mL/min and a column temperature of 80.0°C. Molecular weight values are polystyrene equivalents.

GPC and SEM-EDAX were then used routinely as analytical tools in the optimization of reaction conditions. The formation of the prepolymer by reaction of PPG 1000 and MDI has been thoroughly studied in previous work [7] and the optimum reaction conditions established. However, little work has been done with sulphonated chain extenders and it was necessary to optimize the conditions of the chain extension step. The variables investigated were: type of chain extender, type of solvent, chain extender addition technique and temperature of the chain extension step.

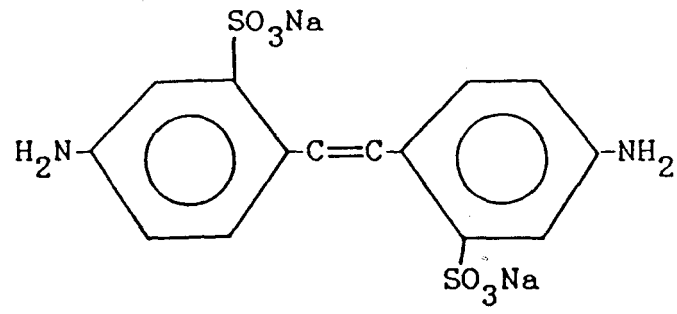
Three different chain extenders were evaluated (Table 4.3a): biphenyl diamino disulphonic acid (BDDS), stilbene diamino disulphonic acid (SDDS) and diamino benzene sulphonic acid (DABS) (see Figure 4.1 for structures). Initial experiments with DABS gave polymers of lower molecular weight than the other two chain extenders, which gave very similar molecular weights. However BDDS gave a slightly higher sulphur content polymer than SDDS and therefore became the chain extender of choice.

Since the nature of the solvent can affect the reactivity of all of the components [19], the use of two different reaction solvents (DMF and DMSO) was studied (Table 4.3b). The use of DMSO resulted in polymers of higher molecular weight, sulphur content and yield. This solvent dependence can be partly attributed to differing

Biphenyl diamino disulphonic acid disodium salt
(BDDS)



Stilbene diamino disulphonic acid disodium salt
(SDDS)



Diamino benzene sulphonic acid sodium salt
(DABS)

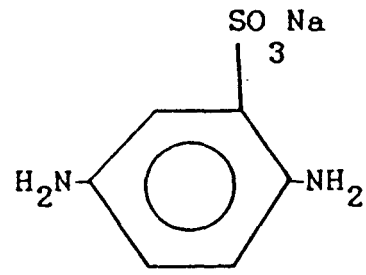


Figure 4.1: Sulphonated Chain Extender Structures

Table 4.3: Optimization of Chain Extension Step in Polyurethane Synthesis Using Sulphonated Diamine Chain Extenders

a) Influence of Chain Extender Type

Chain Extender	Mw	Mn	D	Wt% S
BDDS	87,160	67,740	1.3	0.98
SDDS	87,460	60,610	1.4	0.43
DABS	66,950	52,180	1.3	0.74

Chain extension conducted at 40°C in DMSO; stoichiometry: 1:2:1, PPG:MDI:Extender.

b) Influence of Solvent Type: Extender - BDDS

Solvent	C-X Temp	Mw	Mn	D	Wt% S
DMF	30	54,370	43,470	1.3	n/a
DMSO	30	75,470	50,990	1.5	0.72
DMF	40	42,000	31,040	1.4	0.86
DMSO	40	87,160	67,740	1.3	0.98

Stoichiometry: 1:2:1, PPG:MDI:BDDS.

c) Influence of Chain Extender Addition Technique: Extender - SDDS

Addition Technique	Mw	Mn	D	Wt% S
powder, small increments	87,460	50,990	1.5	0.43
solution, small increments	82,970	59,370	1.4	0.51

Chain extension conducted at 40°C in DMSO; stoichiometry: 1:2:1, PPG:MDI:SDDS.

d) Influence of Chain Extension Temperature: Extender - BDDS

Temperature	Mw	Mn	D	Wt% S
30	75,470	50,990	1.5	0.72
40	87,160	67,740	1.3	0.98
70	70,790	47,560	1.5	0.83

Chain extension conducted in DMSO; stoichiometry: 1:2:1, PPG:MDI:BDDS.

e) Influence of Catalyst on Chain Extension: Extender - BDDS

Catalyst	Mw	Mn	D	Wt% S
none	87,160	67,740	1.3	0.98
stannous octoate	79,310	59,480	1.3	0.60
benzoic acid	91,950	64,510	1.4	0.53

Chain extension conducted at 40°C in DMSO;
stoichiometry: 1:2:1, PPG:MDI:BDDS;
catalyst concentration 1% wt/wt of reactants.

f) Influence of Prepolymer, Hard Segment Length: Extender - BDDS

Prepolymer	Reaction Stoichiometry (PPG:MDI:BDDS)	Mw	Mn	D	Wt% S
long	1:2:1	70,100	53,900	1.3	1.00
short	1:2:1	55,060	40,620	1.4	1.56
short	1:4:2*	43,740	28,550	1.5	2.50

Chain extension conducted at 40°C in DMSO;
short prepolymer prepared by adding PPG dropwise to excess MDI, long prepolymer prepared by adding MDI dropwise to excess PPG.
* Chain extension stoichiometry: 1:2:2, Prepolymer:MDI:BDDS, a longer hard segment is expected in this reaction.

reactivities, but probably also results from the fact that DMF may decompose to give small quantities of dimethylamine which could remove isocyanate functionality [127]. DMSO was selected as the reaction solvent for the remainder of the experiments.

BDDS is only moderately soluble in DMSO and has to be added to the prepolymer solution as small quantities of solid (powder). It is assumed that the BDDS which is in solution reacts rapidly with the isocyanate groups of the prepolymer, allowing more of the BDDS powder to dissolve and react until eventually all the BDDS has dissolved and reacted. The net effect of this mechanism should be similar to a dropwise solution addition technique in which small increments of a chain extender solution are added. The polymers produced should also be similar. This was checked by comparing polymers produced using both methods with SDDS which has a relatively high solubility in DMSO. As shown in Table 4.3c there is no significant difference between polymers prepared using these two methods, so it would appear that the powder addition technique, while unorthodox, gives satisfactory and logical results.

The final variable screened was the temperature at which chain extension was conducted (Table 4.3d). In the range of 30 to 70°C a temperature of 40°C gives the highest molecular weight and chain extender incorporation although

no dramatic temperature dependence of the BDDS reactivity is apparent over the practically possible temperature range. Temperatures higher than 70 °C were not studied because of the increased chance of allophanate and biuret formation.

With these variables selected, the task of increasing the molecular weight and sulphur content could be addressed. Since the molecular weight is determined by the conversion of the chain extension step, the most obvious way to increase the molecular weight is to increase the reactivity of the amine groups on the chain extender. There are two ways to accomplish this: 1) temperature variation and 2) catalysis. The data in Table 4.3d indicate that the amine reactivity is not significantly temperature dependent. In addition, catalysis of the amine-isocyanate reaction has not been extensively studied because the aliphatic amines generally used in these reactions are highly reactive and not in need of catalysis [15]. Two catalysts, both of which are known to catalyse the isocyanate-hydroxyl reaction, namely stannous octoate [15] and benzoic acid [128], have been tried with varying degrees of success with aliphatic amine chain extenders.

Attempts in this work to catalyse the amine-isocyanate reaction (Table 4.3e) were disappointing. Only slight differences in molecular weight are apparent and the degree of sulphonation (and therefore conversion of the

chain extension step) is less in the "catalyzed" than in the "uncatalyzed" reactions. The latter effect may be a result of increasing the reactivity of any remaining hydroxyl functionality in the prepolymer and, thereby, increasing the prepolymer molecular weight. Then in order for the sulphur content to remain the same a longer hard segment would have to be generated. The likelihood of reactions giving longer hard segments may be less than those producing free hard segment units.

Changing the sulphonate content of the BDDS-extended SPUs is a different matter. Sulphonate content can be manipulated only by changing the relative size of the hard and soft segments. An increase in sulphonate content can be achieved by either decreasing the prepolymer (soft segment) molecular weight or increasing the hard segment molecular weight.

The prepolymer molecular weight can be adjusted using the stoichiometry of the prepolymer forming reaction. The greater the molar excess of MDI over PPG the lower the prepolymer MW. Stoichiometry can be controlled by the manner in which the MDI and PPG are mixed. Adding the PPG in small increments to an excess of MDI should result in a small, largely "monomeric" prepolymer, MDI-PPG-MDI, while adding the MDI in small increments to an excess of PPG should increase the probability of forming longer prepolymer chains

of the type -MDI-(PPG-MDI)$_{\overline{n}}$. As the results indicate (Table 4.3f) this appears to happen. A decrease in the prepolymer molecular weight reduces the final SPU molecular weight and increases the sulphonate content.

It should be possible to increase the hard segment molecular weight by adding additional MDI at the chain extension step thus increasing the probability of hard segments of structure -(BDDS-MDI)$_{\overline{n}}$ with n > 1. A reaction was conducted (Table 4.3f) using the "short" prepolymer produced by adding PPG to MDI, in which the stoichiometry of the chain extension step was 1:2:2, prepolymer:MDI:BDDS, compared to standard chain extension stoichiometry of 1:1, prepolymer:BDDS. The difficulty with adjusting the stoichiometry of the chain extension step in this way is that in addition to increasing the hard segment lengths in the final SPU, "free" hard segment homopolymers -(BDDS-MDI)$_{\overline{n}}$ can be produced which would have low molecular weight and high sulphonate content. As shown by the data in Table 4.3f this may have taken place, since the molecular weight of the polymer has decreased slightly and the relative sulphonate content has increased dramatically. An alternative method is to preform the hard segment units by reacting MDI with BDDS in stoichiometric amounts to produce amine terminated hard segment units, then reacting these hard segment units with the prepolymer. These variations in prepolymer and hard

segment length give a wide available range of sulphonate content suitable for further study.

The overall molecular weights of these BDDS-extended polymers are low, relative to commercially available polyurethanes, like Biomer, and other polymers which are chain extended using aliphatic diamines (see below, Table 4.8) and this may be a problem with respect to mechanical properties. Future work should consider potential ways to increase the MW of BDDS-extended segmented polyurethanes.

Based on the findings presented in this section of the thesis and discussed above, three polymers which cover a suitable range of sulphonate content were selected for attachment of arginine methyl ester. The chemistry involved in the reaction of amino acids with sulphonate groups has been studied in detail in other work in this laboratory [129] and the reaction scheme is shown in Figure 4.2. The reaction of arginine methyl ester with sulphonated polyurethanes in the present work was done stoichiometrically on the basis of the sulphonate content. Unfortunately, the sulphonate content data used in calculating the stoichiometry were erroneously low for the samples used (this problem will be discussed in more detail in the next section) and a maximum of only about one half of the sulphonate groups were in fact derivatized. The other half would necessarily remain as free sulphonate groups.

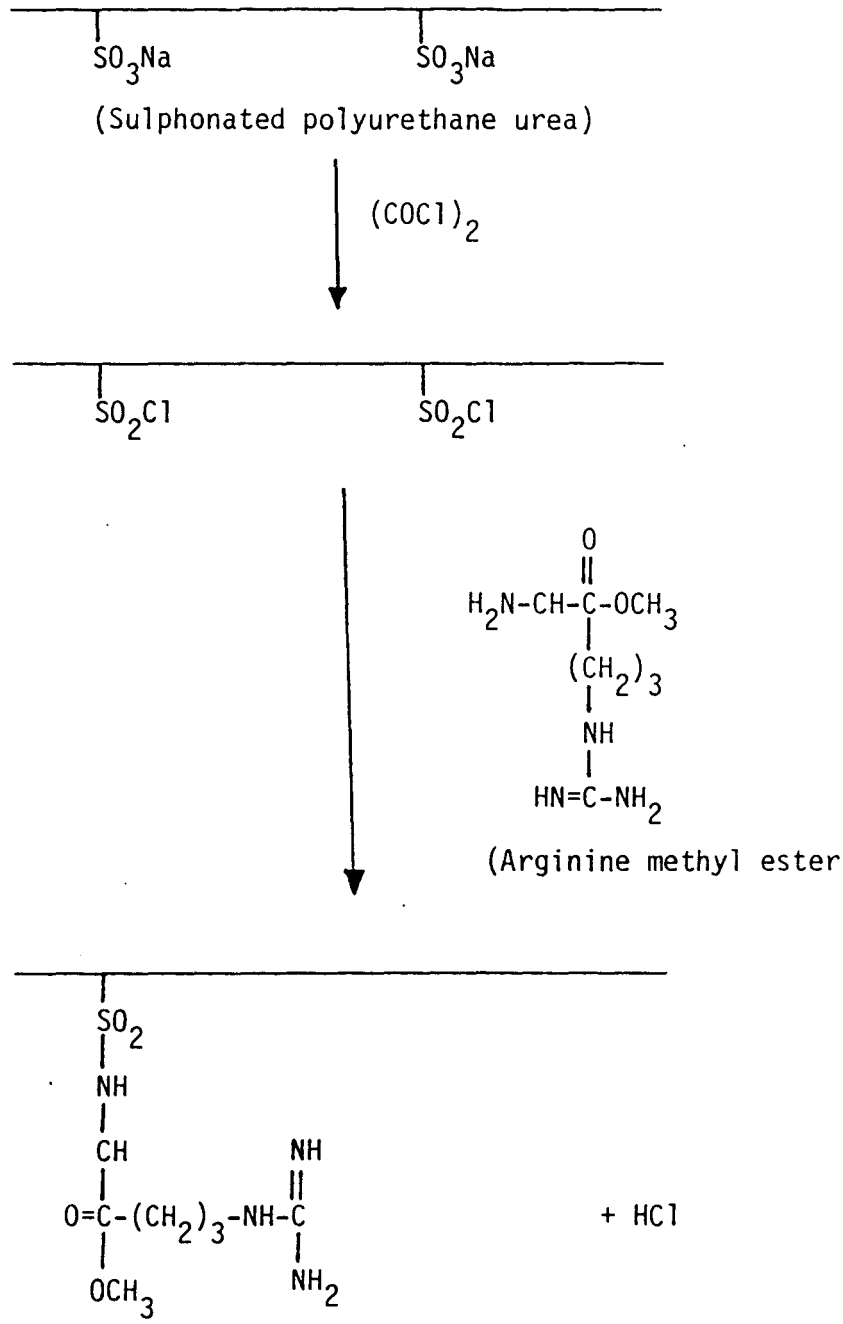


Figure 4.2: Reaction scheme for addition of arginine methyl ester to sulphonated polyurethanes.

The three sulphonated and corresponding three arginine methyl ester containing polymers were extensively characterized and their interactions with fibrinogen in plasma and buffer were studied in detail as discussed in the following sections.

4.2 Polymer Characterization

The purpose of physical characterization was to provide information on the molecular and bulk polymer structure, which could be used to verify the reaction mechanisms and to understand the polymer mechanical properties. In addition surface characterization was required to provide information which could be related to fibrinogen adsorption and possibly other blood interaction behaviour. Elemental analysis, Fourier transform infrared spectroscopy, gel permeation chromatography and intrinsic viscosity were used as physical characterization methods. Contact angle measurements and ESCA provided information on the surface structure of the polymers.

In the discussion that follows the three sulphonated polymers are identified by the chain extender abbreviation followed by the expected sulphur content (for simplicity, approximate values are used):

BDDS-3 (synthesized using the "large" prepolymer)

BDDS-4 (synthesized using the "small" prepolymer)

BDDS-5 (synthesized using the "small" prepolymer and a larger hard segment)

The corresponding arginine methyl ester modified polymers are identified by the designation AM, e.g. BDDS-3-AM.

4.2.1 Elemental Analysis

Elemental analysis was conducted to confirm the expected structure of both the sulphonated polymers and the arginine methyl ester derivatized polymers. Elemental analysis both by combustion and by SEM-EDAX (which requires calibration using standards determined by combustion analysis) was done.

Elemental analysis for sulphur and sodium (the counterion to the sulphonate groups) provides a quantitative measure of the content of sulphonated chain extender. The sodium elemental analysis also allows a check that the sulphonate group remains pendant and unreacted, as expected since sulphonates are much less reactive than amines. Also, any reaction of sulphonate with isocyanate would probably result in crosslinking which is experimentally observable as insolubility in the reaction solvent. This was not the case.

Elemental analysis for nitrogen provides a quantitative measure of both the hard segment content of the sulphonated polymers and the degree of arginine methyl ester (AM) incorporation (see Figure 4.2 for the structure of AM) in the modified polymers.

Elemental analysis was also used to verify the reproducibility of polymer synthesis. When samples from two independent syntheses were analysed by SEM-EDAX the results were similar (Table 4.4), although the sulphur contents were consistently higher in the second trial.

Table 4.4: Comparison of Sulphur Incorporation (SEM-EDAX) and GPC Data for Replicate Syntheses of Sulphonate-Containing Polyurethanes

Polymer	Trial	Mw	Mn	D	Wt% S
BDDS-3	1	70,100	53,900	1.3	1.0
BDDS-3	2	61,300	46,700	1.3	1.6
BDDS-4	1	55,060	40,620	1.4	1.6
BDDS-4	2	58,200	46,300	1.3	1.9
BDDS-5	1	43,740	28,550	1.5	2.5
BDDS-5	2	42,000	33,200	1.3	2.8

SEM-EDAX was calibrated using a standard determined by combustion elemental analysis, the standard used was BDDS-3 with a sulphur content of 1.0%;
GPC results were obtained using a mobile phase of 0.1 M LiBr in DMF at a flowrate of 1.0 ml/min and a column temperature of 80.0°C. Molecular weight values listed are polystyrene equivalents.

When the data from combustion elemental analysis are compared with SEM-EDAX results a systematic and significant difference is evident (Table 4.5). Also combustion analysis

data for the two synthesis trials of polymer BDDS-3 gave sulphur contents of 1.0 and 3.4% (data not shown in Table 4.4). In this connection it should be noted that different precipitation methods were used in the two trials. In the first trial the polymers were precipitated in saline solution (prepared with distilled water), while in the second, the polymers were precipitated in methanol. A subsequent third synthesis trial was conducted in which the polymer was divided into two parts, one of which was precipitated in methanol, the other in saline. Combustion elemental analysis of these samples showed the sulphur content to be the same for both precipitation methods (Table 4.6) and consistent with the lower value (1%) which had been previously found for this polymer. The reason for the difference in the elemental composition between the two initial trials is not understood at the present time, but may be related to differences in prepolymer length, since the higher sulphur content BDDS-3 polymer was also found to be slightly lower in molecular weight than the lower sulphur content BDDS-3 polymer (Table 4.4).

Table 4.5: Comparison of SEM-EDAX and Combustion Results for Elemental Analysis

Polymer	Wt% S SEM-EDAX	Combustion	ESCA (max. depth data)
BDDS-3	1.6	3.4	1.4
BDDS-4	1.9	4.4	3.3
BDDS-5	2.8	5.4	-
BDDS-3-AM	1.6	3.0	2.6
BDDS-4-AM	1.9	3.2	2.9
BDDS-5-AM	1.8	4.4	4.9

Combustion analysis - precision $\pm$ 0.3%
SEM-EDAX was calibrated using a standard determined by combustion. The standard was BDDS-3 with a sulphur content of 1.0%.

Table 4.6: Influence of Precipitation Method on Elemental Composition and Molecular Weight of BDDS-3

Precipitant	Wt% Sulphur	Mw	Mn	D
Methanol	1.08	69,400	52,300	1.3
Saline	1.07	68,900	51,900	1.3

Wt% sulphur was determined by combustion analysis.

At the time of selecting samples for further characterization and study the difficulty associated with reproducing the BDDS-3 polymer having the higher sulphur content of about 3% was not known. However, the elemental composition of this polymer has been verified in subsequent combustion analyses and is believed to be representative of the BDDS-3 polymer which was characterized and studied.

The apparently systematic difference between the combustion and SEM-EDAX results is difficult to explain.

While the SEM-EDAX data are in agreement with the combustion data as far as qualitative trends are concerned they may not be quantitatively accurate. It is possible that the relationship between sulphur content and SEM-EDAX response is not linear for this system. The calibration standard used had a sulphur content of 1% whereas the materials in Table 4.5 are in the range 3 to 5%. It would appear that while SEM-EDAX may be adequate for quantitative analysis over a limited range of values close to the calibration standard, significant deviations result outside this range. It is therefore believed that, until the problems associated with SEM-EDAX are understood and resolved, SEM-EDAX should be used only as a qualitative indicator of relative sulphur content, and that combustion analysis data should be used for quantitative analysis.

The combustion analysis data are confirmed by the maximum depth ESCA data, also shown in Table 4.5 and discussed subsequently in section 4.2.6. The ESCA data are slightly lower than those found by combustion, as ESCA is a surface sensitive technique and the surface exhibits hard segment depletion and soft segment enrichment. BDDS-5 shows no sulphur content in ESCA analysis, but this may be due to sample preparation, as the film casting required for ESCA analysis was difficult with this polymer.

The discrepancy between SEM-EDAX and combustion

analysis was not known when the stoichiometry for the arginine methyl ester-sulphonate reaction was calculated. Since the SEM-EDAX values for sulphonate content, which are only about one half of the actual values, were used, a maximum of only about 50% of the sulphonate groups could have reacted, leaving a minimum of 50% as free sulphonate.

A comparison of the expected elemental composition for each of the polymers selected for detailed study (Table 4.5) and the experimentally observed results, from combustion analysis, is shown in Table 4.7. The expected values are based on the repeat unit structures predicted by reaction stoichiometry. BDDS-3 and -4 were prepared with the same overall stoichiometry of 2:1:1, MDI:BDDS:PPG but different mixing techniques, presumably resulting in prepolymers of different molecular weight distribution. For BDDS-3, the MDI was added in small increments to the PPG 1025, so the potential exists for the MDI to react with more than one PPG unit resulting in a relatively long prepolymer. For BDDS-4 the PPG 1025 was added in small increments to the MDI, so that each PPG chain should be endcapped with MDI, giving a short "monomeric" prepolymer. However, the expected repeat unit, based uniquely on stoichiometry and assuming complete conversion, is the same for both these polymers, i.e. $[-MDI-PPG-MDI-BDDS-]_n$. The expected and experimental results are in good agreement in both cases. BDDS-3 shows

somewhat lower %S and %Na compared to BDDS-4 which is consistent with a slightly increased prepolymer length. For BDDS-4 a shorter prepolymer would mean that the potential for MDI to react with BDDS to form longer hard segments is increased thus increasing the sulphur and sodium content and to a lesser extent the nitrogen content. This assumes that conversion of the amine-isocyanate reaction is not complete, as appears likely since the molecular weight of the polymers is relatively low.

Table 4.7: Comparison of Theoretical and Observed Elemental Composition of Segmented Polyurethanes

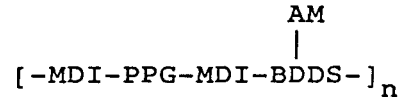
Polymer	Theoretical			Experimental		
	%S	%Na	%N	%S	%Na	%N
BDDS-3	3.4	2.4	4.4	3.4	1.6	4.3
BDDS-4	3.4	2.4	4.4	4.4	2.8	4.2
BDDS-5	5.0	3.6	5.5	5.4	4.4	4.8
BDDS-3-AM	3.1	0	6.8	3.0	0.1	5.9
BDDS-4-AM	3.1	0	6.8	3.2	0.1	6.1
BDDS-5-AM	4.5	0	8.9	4.4	0.2	9.5

Data given as weight percent.

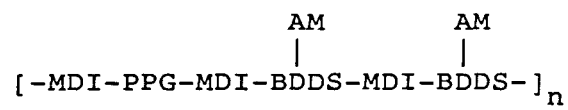
For BDDS-5 the expected repeat unit is $[-MDI-PPG-MDI-BDDS-MDI-BDDS-]_n$. The experimentally observed results for both %S and %Na are slightly higher than expected. This increase can be attributed to the presence of hard segment homopolymers $-[BDDS-MDI]_n-$ which would have a very high sulphur and sodium content. In general the hard segment units of polyurethanes have poor solubility in both

DMF and DMSO [19] and it was observed that the BDDS-5 polymers were not completely soluble. The insoluble portion is believed to be due to the presence of "free" hard segment homopolymer and not to crosslinking. Crosslinked polymers tend to swell in solvent, but in this case the insoluble material was unswollen precipitate as expected for a hard segment homopolymer. To explore this possibility further a synthesis was conducted in which the hard segment homopolymer was prepared by reacting equimolar amounts of MDI and BDDS. The resulting polymer was not soluble and did not swell in DMSO.

As was previously discussed, when these sulphonated polymers were modified to incorporate arginine methyl ester, erroneous elemental analysis data were used to estimate the required stoichiometric quantities of arginine methyl ester, resulting in a maximum conversion of only about one half of the sulphonate groups. Assuming that on average one of the two sulphonate groups per BDDS has reacted, then the repeat unit for BDDS-3-AM and -4-AM would be:



and the repeat unit for BDDS-5-AM would be:



On this basis the expected and observed values for these

three polymers are all in good agreement. Only slight differences are evident and these can be attributed to incomplete conversion of sulphonate to arginine methyl ester sulphonamide.

The observed and expected values for BDDS-5-AM are in better agreement than those for BDDS-5 (which had higher sulphur and nitrogen values than expected, probably due to hard segment homopolymer) because of the procedure used in the addition of AM. This procedure involves transferring the polymer solution from one reaction vessel to another through a U tube. During the transfer procedure any of the polymer which has not dissolved (i.e. probably any hard segment homopolymer) would be left in the bottom of the original vessel, where the transfer tube cannot reach, and would not be involved in the formation of BDDS-5-AM. On the other hand, these hard segment units would have necessarily contributed to the deviations observed for BDDS-5.

For all this elemental analysis data it must be remembered that the repeat units used to calculate the expected compositions are the ideal cases based solely on stoichiometry and complete conversion. The polymer produced in any given synthesis will have a distribution of chain lengths and compositions as determined by the statistical nature of step growth polymerisation. The chain length distribution in these syntheses is determined mainly by

stoichiometry and functional group (i.e. isocyanate, hydroxyl and amine) conversion. Nonetheless, the values confirm approximately the reaction mechanisms involved in polymer formation and are consistent with the assumed mechanism of arginine methyl ester-sulphonate reaction. However, it is recommended that in future work combustion sulphur analysis rather than SEM-EDAX data be used to calculate the stoichiometric amounts of amino acids because of the complications, as yet not understood, in the use of the latter technique.

4.2.2 Gel Permeation Chromatography and Intrinsic Viscosity

While elemental analysis can verify the expected repeat units of the polymers, it provides no information on the number of repeat units in a chain, or on whether degradation of these chains occurs during the subsequent reactions involved in conversion of sulphonate to arginine methyl ester sulphonamide. To obtain information on the molecular weight of the polymers, at various stages in their preparation, gel permeation chromatography (GPC) and intrinsic viscosity measurements were used.

The basis of the GPC method is the relationship between the elution volume of a polymer and its molecular

weight. GPC columns can be calibrated to relate the elution volume to the molecular weight using polystyrene standards. Using a technique known as universal calibration this polystyrene calibration curve can be converted to a calibration curve for any other polymer system for which the Mark-Houwink-Sakurada (MHS) constants are known. The MHS constants relate the intrinsic viscosity, $[\eta]$, of a polymer to its viscosity average molecular weight, M_v, by the following equation:

$$[\eta] = KM_v^a$$

Where K and a are constants which depend on the polymer type, solvent type and temperature. The MHS constants can be used to relate the molecular weight of another polymer which appears at the same elution volume as the polystyrene of known molecular weight, using the following equation:

$$[\eta]_1 M_1 = K_1 M_1^{a_1+1} = [\eta]_2 M_2 = K_2 M_2^{a_2+1}$$

where the product $[\eta]M$ is known as the hydrodynamic volume of the polymer. This equation allows conversion of the calibration curve, so that the elution volume data for the new polymer system can be converted directly into molecular weight data. If the MHS constants are not known the polystyrene equivalent molecular weights are often used. A polystyrene equivalent molecular weight is simply the molecular weight of a polystyrene sample which would have eluted at the same elution volume as the polymer of

interest. It is not the true or absolute molecular weight of the polymer and is normally only used for comparative purposes.

Difficulties arise in the GPC analysis of polyurethanes because of their tendency to form aggregates in solution and to interact with many of the chromatographic supports used in GPC work. To brea up aggregates and to minimize the potential for polar interactions between the polymers and the GPC support a mobile phase of 0.1 M LiBr in DMF was used in this work. This solvent has been used successfully used by Hann for GPC of SPUs [130].

In addition, comparative GPC analysis of polymers, such as the polyurethanes developed here, which vary in composition as well as molecular weight is more difficult than for homopolymers because of the limitations of current detection methods. The most commonly used detector in GPC work is the differential refractive index detector. This detection method, along with others which use light absorption to detect changes in concentration, assumes that the refractive index of the polymer solution emerging from the column depends only on the polymer concentration. This is true for homopolymers but is unlikely to be true for systems where the polymer chains have variable chemical composition. In chemically complex polymers it is probable that the refractive index varies with composition. The

refractive index difference therefore depends on the polymer composition as well as its concentration.

If the polymer has a constant average composition, for example if the ratio of monomers in a copolymer is relatively constant and independent of molecular weight, then the refractive index difference arising from composition is unimportant and the copolymer can be treated as a homopolymer. To determine whether the average composition is constant requires a method of detecting the concentration of one of the monomers in the copolymer as well as a method of detecting the copolymer concentration. If this is the case the Mark-Houwink-Sakurada (MHS) constants can be determined by several methods [131].

If the copolymer average composition varies with molecular weight the only available technique involves using the relative monomer content to correct the copolymer detector reading for compositional change [132]. This corrected reading can then be used to calculate the MHS constants.

To date the only in-depth investigation of the analytical problems involved in the use of GPC with polyurethanes has been conducted by Cooper et al [133]. They found that the best results are achieved using a multidetector method which allows correction for compositional change. However, they also found that the

polystyrene equivalent molecular weights give a good approximation to these results. For reasons of expediency and simplicity the data presented in this thesis are polystyrene equivalent molecular weights. While these values give a good approximation they should not be considered as absolute molecular weights for the polyurethanes.

A GPC chromatogram typical of the polyurethanes studied in the present investigation is shown in Figure 4.3 for the sulphonated polyurethane BDDS-3. In addition to the broad polymer peak there is a sharp positive and a sharp negative peak. These peaks are believed to be the result of small gradients in LiBr concentration which result upon sample injection, since they also appear upon injection of polystyrene standards and the pure mobile phase itself. Similar chromatograms have been observed by Andrade et al for SPUs in a LiBr/DMAC mobile phase [151].

The calibration curve of polystyrene molecular weight vs retention time is shown in Figure 4.4.

GPC analysis was used in addition to elemental analysis as a means of monitoring the reproducibility of polymer preparative procedures. As seen in Table 4.4 the molecular weights of polymers BDDS-4 and -5 compare well between the two trials, but BDDS-3 is slightly lower in molecular weight in the second trial. In subsequent experiments it was found that the higher of the two values

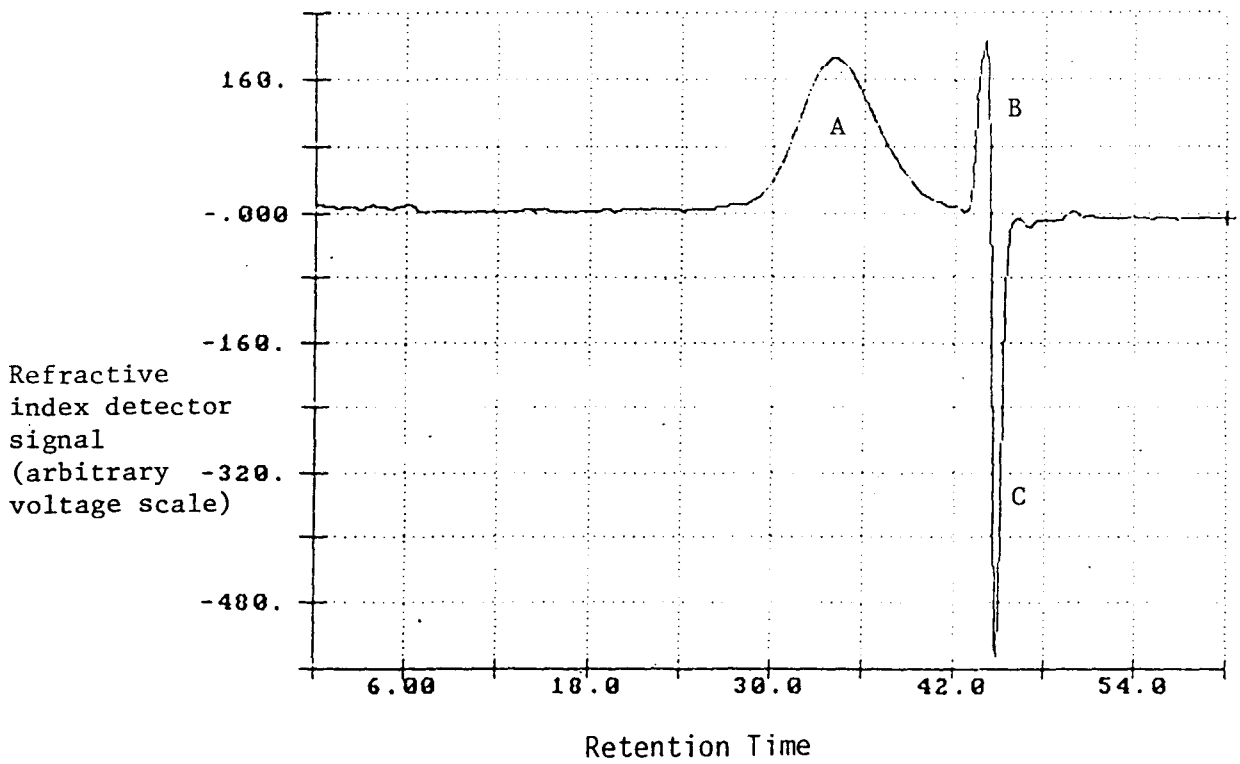


Figure 4.3: Typical GPC chromatogram for polyurethane BDDS-3
Conditions: mobile phase - 0.1 M LiBr in DMF, 80 C, flowrate=1.0 mL/min
A:polymer peak; B,C:LiBr concentration gradient peaks?

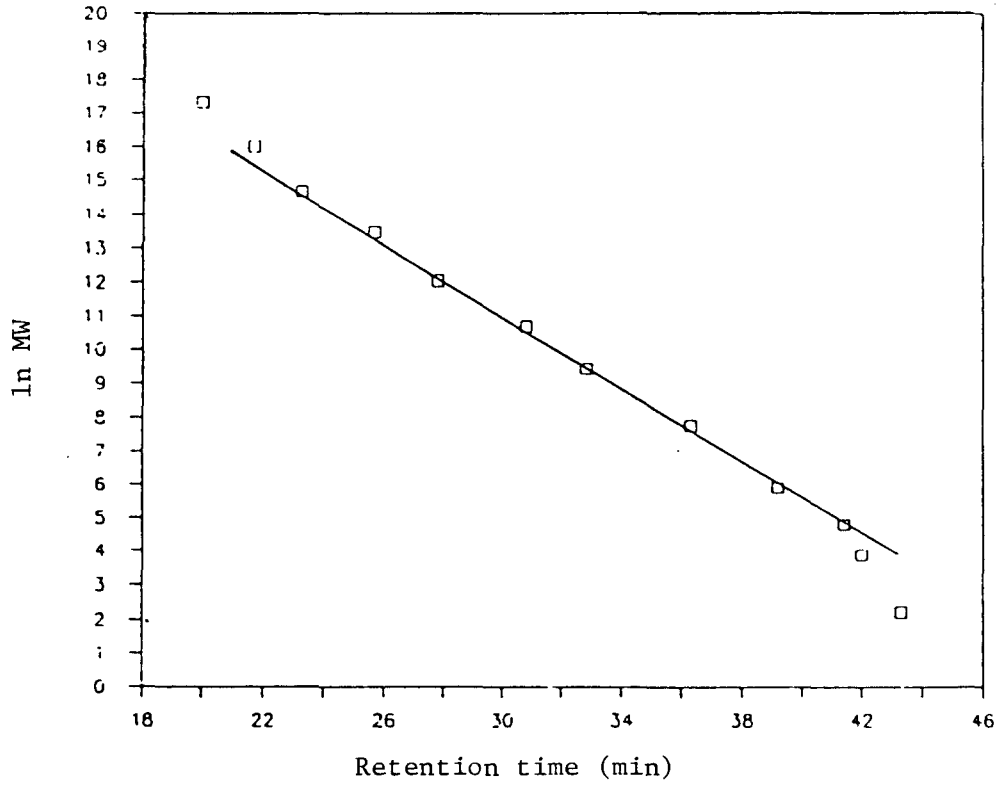


Figure 4.4: GPC Calibration curve (Calibrated using polystyrene standards)
Conditions: mobile phase - 0.1 M LiBr in DMF, 80 C, flowrate - 1.0 mL/min

is consistently obtained (Table 4.6) suggesting that there was a difference in the prepolymer size in the two initial BDDS-3 trials which may have contributed to the difference found in the elemental composition, as previously discussed.

The GPC and intrinsic viscosity results for all six polymers studied in this investigation are presented, along with data for a typical ethylene diamine chain extended polymer (ED) and Biomer, in Table 4.8. The molecular weights for the BDDS chain extended polymers are considerably lower than those for the other polymers. This is probably due to the lower reactivity of the aromatic diamines resulting in a lower conversion in chain extension as previously discussed. Again, the molecular weight decreases as the prepolymer length decreases and as the hard segment size increases. The AM modified polymers have about the same molecular weights and intrinsic viscosities as the corresponding precursor sulphonated polymers. Thus, it may be concluded that no significant chain breaking occurs during conversion of sulphonate to arginine methyl ester sulphonamide.

Table 4.8: GPC Molecular Weights of Segmented Polyurethanes

Polymer	Intrinsic Viscosity $[\eta]$	PS Equivalent Molecular Weights Mw	Mn	Polydispersity (D)
BDDS-3	0.20	61,300	46,700	1.3
BDDS-4	0.16	58,200	46,300	1.3
BDDS-5	0.10	42,000	33,200	1.3
BDDS-3-AM	0.21	63,000	47,000	1.3
BDDS-4-AM	0.16	60,800	46,500	1.3
BDDS-5-AM	0.14	43,500	35,200	1.2
ED	0.30	277,000	159,000	1.7
Biomer	0.66	298,000	183,000	1.6

GPC results obtained using a mobile phase of 0.1 M LiBr in DMF at a flowrate of 1.0 ml/min and a column temperature of 80.0°C. Intrinsic viscosity data determined under the same conditions.

4.2.3 Tensile Stress-Strain Properties

The results of mechanical testing are presented in Table 4.9 and Figure 4.5. For comparison with the functionalized polymers developed in this project, a typical ethylene diamine (ED) chain extended polymer is also shown. Introduction of sulphonate groups on an aromatic chain extender appears to increase the tensile strength and initial modulus and decrease the elongation at break. Conversion of sulphonate to arginine methyl ester sulphonamide increases the tensile strength and elongation at break and decreases the initial modulus. In general the introduction of sulphonate groups greatly compromises the

elastomeric nature of these polyurethanes and with only 5% by weight of sulphur a glassy material which was too brittle to allow testing was obtained.

Table 4.9: Tensile Stress-Strain Properties of Segmented Polyurethanes

Polymer	Tensile Strength (MPa)	(PSI)	Initial Modulus (MPa)	Elongation at Break (%)
BDDS-3	18.3 (±1.0)	2650	140 (±23)	75 (±10)
BDDS-4	17.9 (±1.9)	2600	150 (±18)	10 (±2)
BDDS-5	n/a - too brittle to test			
BDDS-3-AM	26.2 (±2.4)	3800	15 (±3)	400 (±50)
BDDS-4-AM	26.9 (±1.5)	3900	110 (±17)	160 (±30)
BDDS-5-AM	n/a - too brittle to test			
ED	12.4	1800	3	1340

Values shown are the average of three determinations.
Standard deviations are shown in brackets.

As discussed previously, there are many factors, a number of which are interrelated, which influence the mechanical properties of polyurethanes. The primary influences are total molecular weight, soft segment molecular weight, stiffness of chain segments, effective intermolecular forces and degree of hard/soft segment domain segregation. It is difficult to specifically relate mechanical property changes to differences in the polyurethane chain structure without a detailed knowledge of the polymer solid microstructure which was not studied in this work.

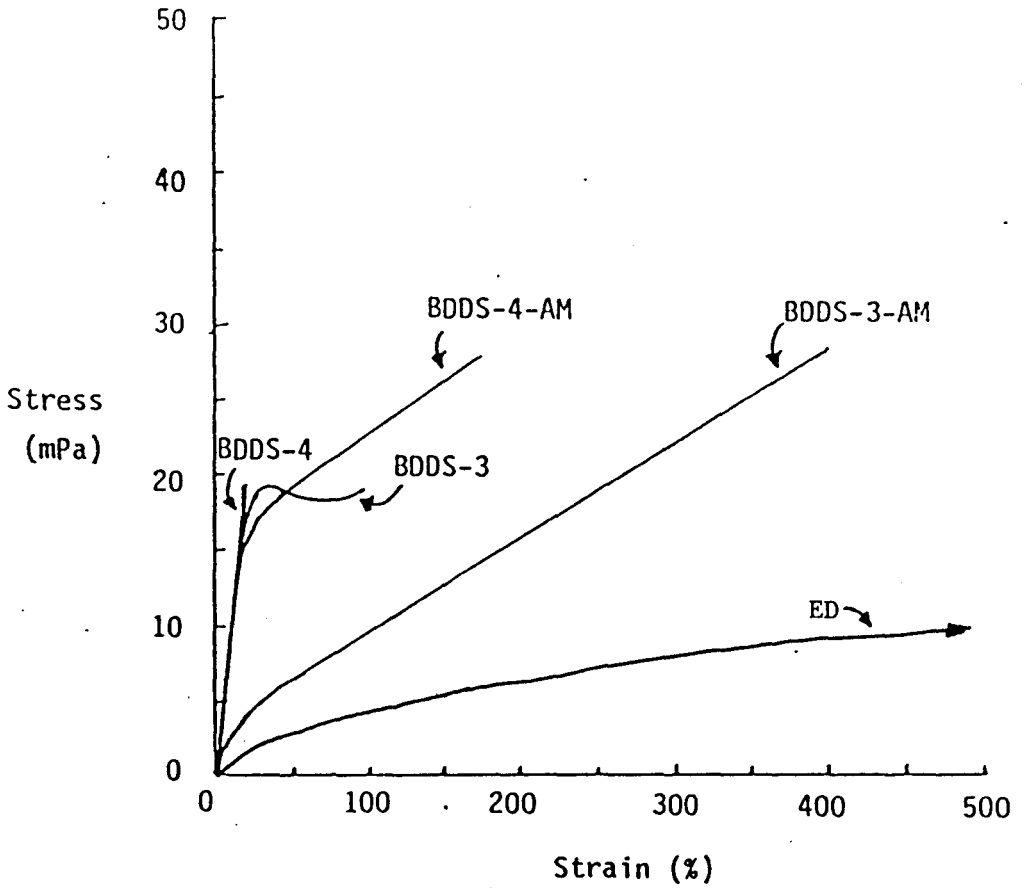


Figure 4.5: Stress-Strain Behaviour of Polyurethanes

The influence of the overall molecular weight in polyurethanes is similar to that for other polymer types. The tensile strength, modulus and ultimate elongation reach plateau values at a particular molecular weight [15]. As the functionalized polyurethanes studied in this work cover a relatively limited range of molecular weight values it is difficult to determine the influence of the overall molecular weight of the polymer on the mechanical properties observed. It would be necessary to produce some higher molecular weight polymers in order to determine whether or not the plateau values of mechanical properties as a function of molecular weight have been reached.

The influence of soft segment molecular weight is different from that of overall molecular weight. If the relative content of hard and soft segments remains the same, the elongation increases with soft segment molecular weight [23]. In opposition to this, elongation is known to decrease as the degree of phase separation increases. The degree of phase separation also contributes to the polyurethane's ultimate tensile strength. As the degree of phase separation increases the potential for hard segment domain formation increases causing a relative increase in hard segment domain content. Increasing the hard segment domain content is equivalent to increasing the crosslink density in a crosslinked elastomer and results in a polyurethane of

increased ultimate tensile strength [23].

In addition, ion containing polymers have a tendency to form ion aggregates which act in a manner similar to the hard segment domains of polyurethanes with respect to mechanical properties [148]. The formation of ion aggregates has recently been found to occur in polyurethanes which contain sulphonate ions but no hard segments [29]. Thus, for the sulphonated polyurethanes studied here, it is likely that both ion aggregate formation and hard/soft segment domain separation contribute to the microphase structure and mechanical properties.

The data in Table 4.9 and the stress-strain curves in Figure 4.5 can be interpreted along the lines indicated above. Thus, the low elongation, high initial modulus and high tensile strength of the BDDS polymers relative to the ED polymer are to be expected on the basis of the relatively low molecular weight of the BDDS polymers and the increased tendency for phase separation which arises from the aromatic nature of the chain extender [26] and the tendency for sulphonate ions to form ion aggregates.

Within the group of BDDS polymers, increasing the prepolymer length (BDDS-3 vs BDDS-4) produces a polymer with higher elongation since the soft segment molecular weight is increased. Increasing the hard segment length produces a polymer (BDDS-5) which is too brittle even to be tested

presumably because of an increase in hard segment domain size. The increased sulphonate content of BDDS-5 may also be responsible for its mechanical brittleness.

The BDDS-AM polymers show an increased elongation and tensile strength but a reduced initial modulus relative to the corresponding BDDS precursor polymers, probably because the arginine methyl ester groups are added to the chain extender section of the hard segment. The presence of this aliphatic side group will reduce the ability of the hard segment chains to pack tightly, thus reducing the tendency of the hard segment to phase separate. These factors will reduce the relative hard segment domain content, thus giving the higher elongation observed. Further, the addition of arginine methyl ester groups converts charged sulphonate groups to uncharged sulphonamide bound AM groups, thereby reducing the relative ion content of the polymers and the tendency to form ion aggregates.

Further study is necessary in order to better understand the microphase structure of these polymers and the influence of the sulphonate and arginine methyl ester groups. It is recommended that in future work differential scanning calorimetry and dynamic mechanical testing be used to provide information on the degree and nature of microphase separation in these polyurethanes.

4.2.4 Fourier Transform-Infrared Spectroscopy

Fourier transform infrared (FT-IR) spectroscopy analysis was used to verify the incorporation of the arginine methyl ester and to provide information on the changes in the microstructure which result from arginine methyl ester addition.

A typical spectrum for the polyurethane BDDS-3 is shown in Figure 4.6. The major peaks along with probable assignments as determined by reference to previous FT-IR work on polyurethane block copolymers [134-137] are listed in Table 4.10. The peak assignments are consistent with those found by Hwang et al for sulphonated polyurethanes [137].

As a model compound for structures with an aromatically bound sulphonamide of arginine methyl ester, tosyl-L-arginine methyl ester was used. Its FT-IR spectrum is shown in Figure 4.7. The highlighted peaks at 1720 and 1660 cm^{-1} are, respectively, contributions of the methyl ester and the guanidyl groups of arginine methyl ester. These peaks can be used to verify arginine methyl ester attachment to the sulphonated polyurethanes because they occur at wavenumbers at which the precursor sulphonated polyurethanes do not show significant infrared absorption.

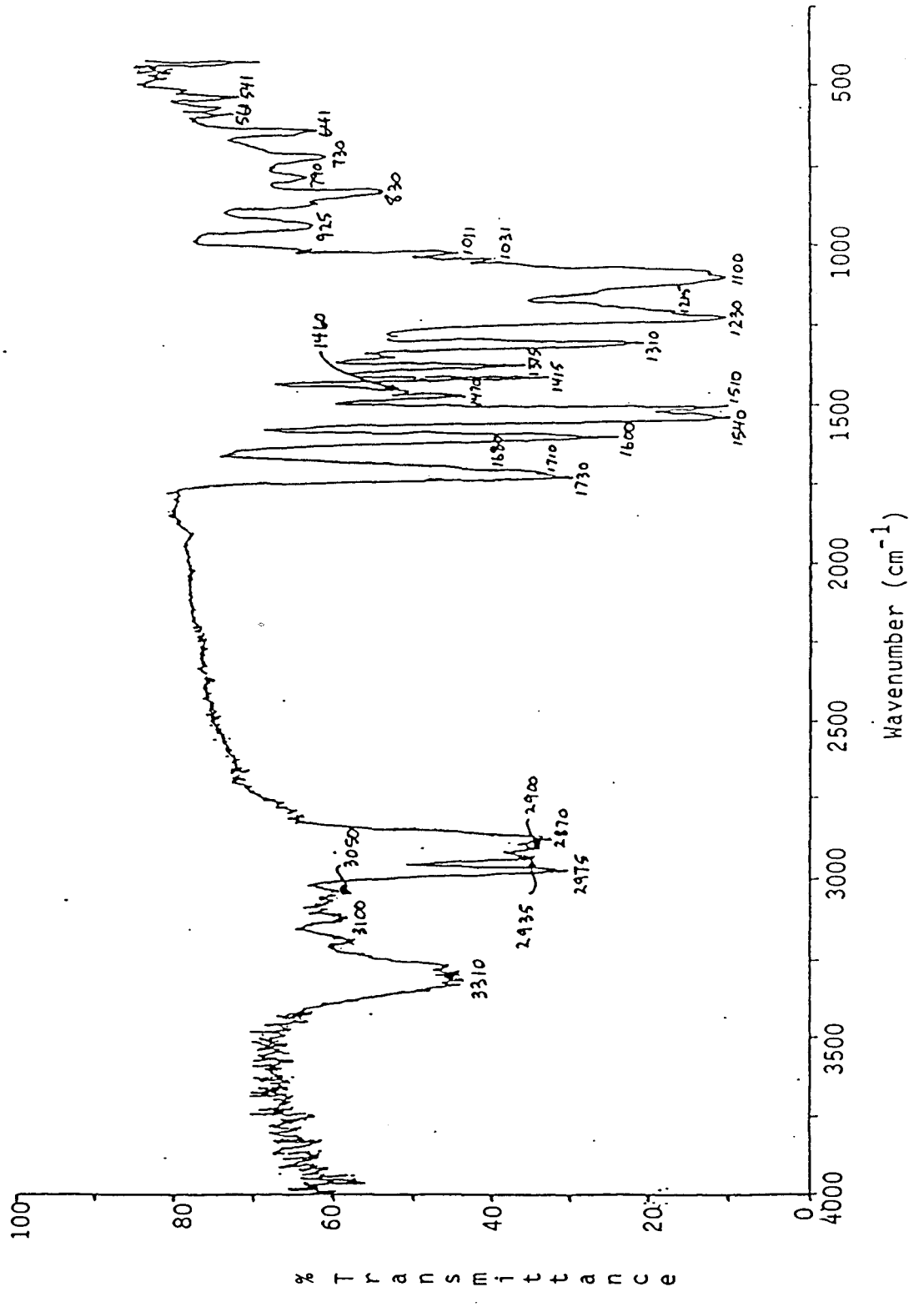


Figure 4.6: FT-IR Spectrum of polyurethane BDDS-3

Table 4.10: Assignment of FT-IR Peaks in the Spectrum of Polyurethane BDDS-3

Frequency (/cm)	Identity
3310	N-H Stretch
3100	N-H Overtone
3050	C=C-H
2975	Aliphatic C-H
2935	"
2900	"
2870	"
1730	Free $N-\overset{O}{\overset{\|}{C}}-O$
1709	H-bonded $N-\overset{O}{\overset{\|}{C}}-O$
1680	$N-\overset{O}{\overset{\|}{C}}-N$ 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	"

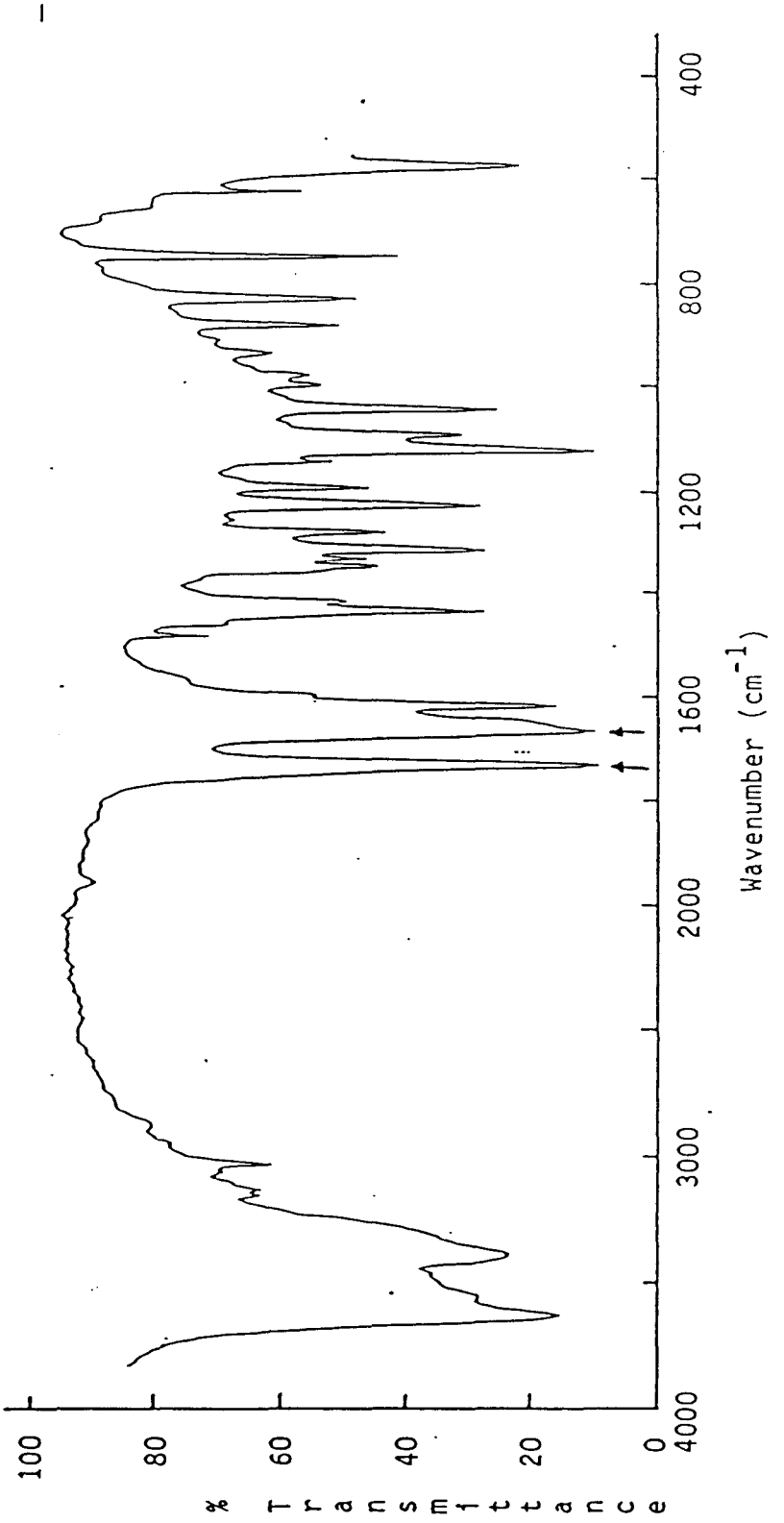


Figure 4.7: FT-IR Spectrum of tosyl-l-arginine methyl ester

As a possible means to estimate the incorporation of arginine methyl ester the difference spectra for the precursor and derivatized polymers were investigated. These spectra were obtained by equalizing the absorbance of the non H-bonded $-CH_3$ peak from the PPO groups at 1370 cm^{-1} for the BDDS polymer and the corresponding BDDS-AM polymer and then subtracting the spectra. The difference spectra then show the changes which have taken place in the polyurethane as a result of AM incorporation.

To substantiate the subtraction procedure two spectra for different samples of polymer BDDS-3 were subtracted. The difference spectrum (Figure 4.8) consists only of noise lines which result from slight differences between peaks which were off scale in the original spectra. No absorption peaks are visible, as expected for the subtraction of identical polymer spectra. A similar result was obtained for polymer BDDS-4 (Figure 4.9).

The difference spectra for BDDS-3-AM versus BDDS-3 and BDDS-4-AM versus BDDS-4 are shown in Figures 4.10 and 4.11 respectively. Again, a number of noise lines are visible but the methyl ester peak at 1720 cm^{-1} and guanidyl peak at 1660 cm^{-1} characteristic of the sulphonamide of arginine methyl ester are also clearly evident. Also evident is a considerable change in the N-H stretch and overtone region (3100-3300 and 1230 cm^{-1}) resulting from the four new

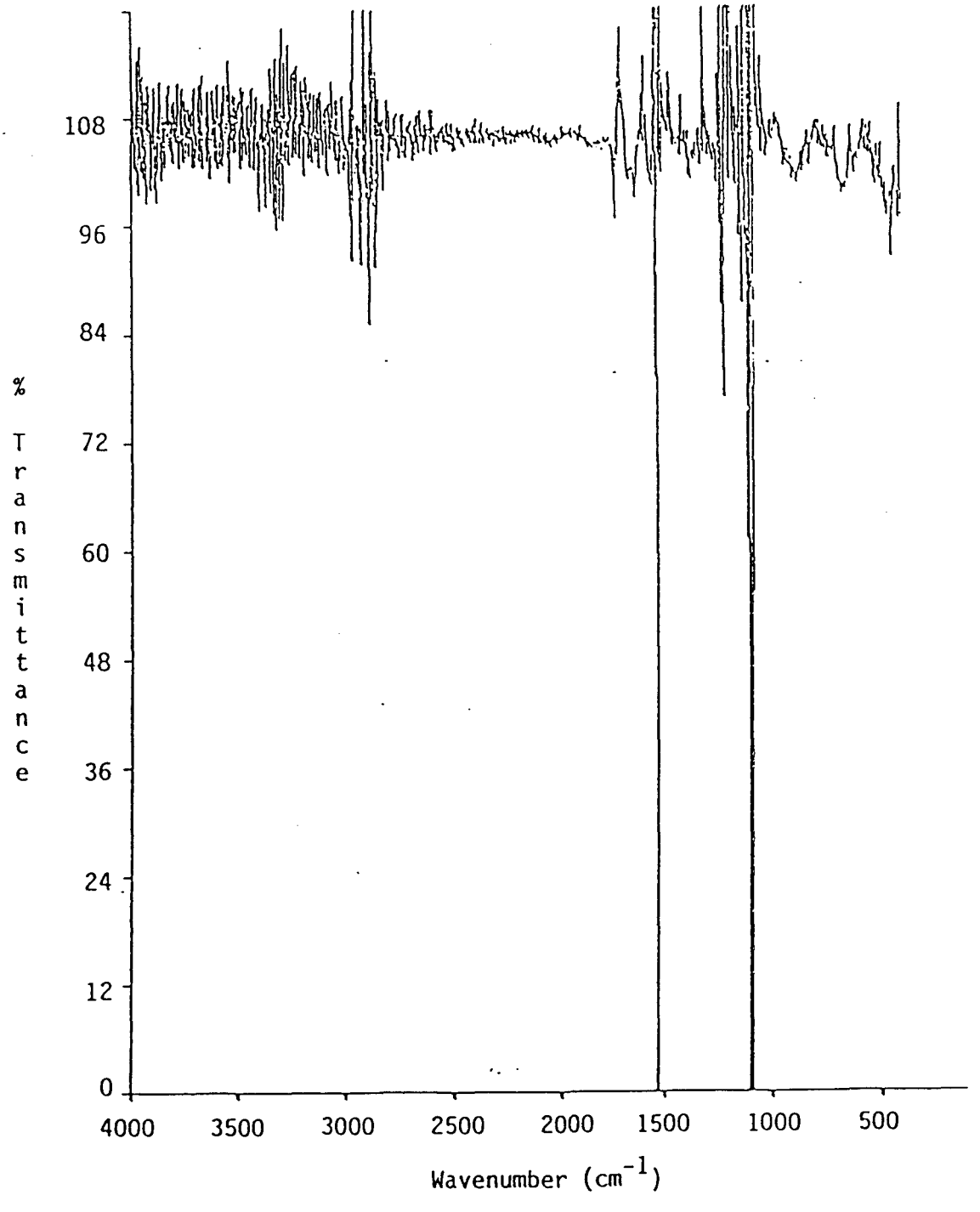


Figure 4.8: FT-IR Difference spectrum for two BDDS-3 samples

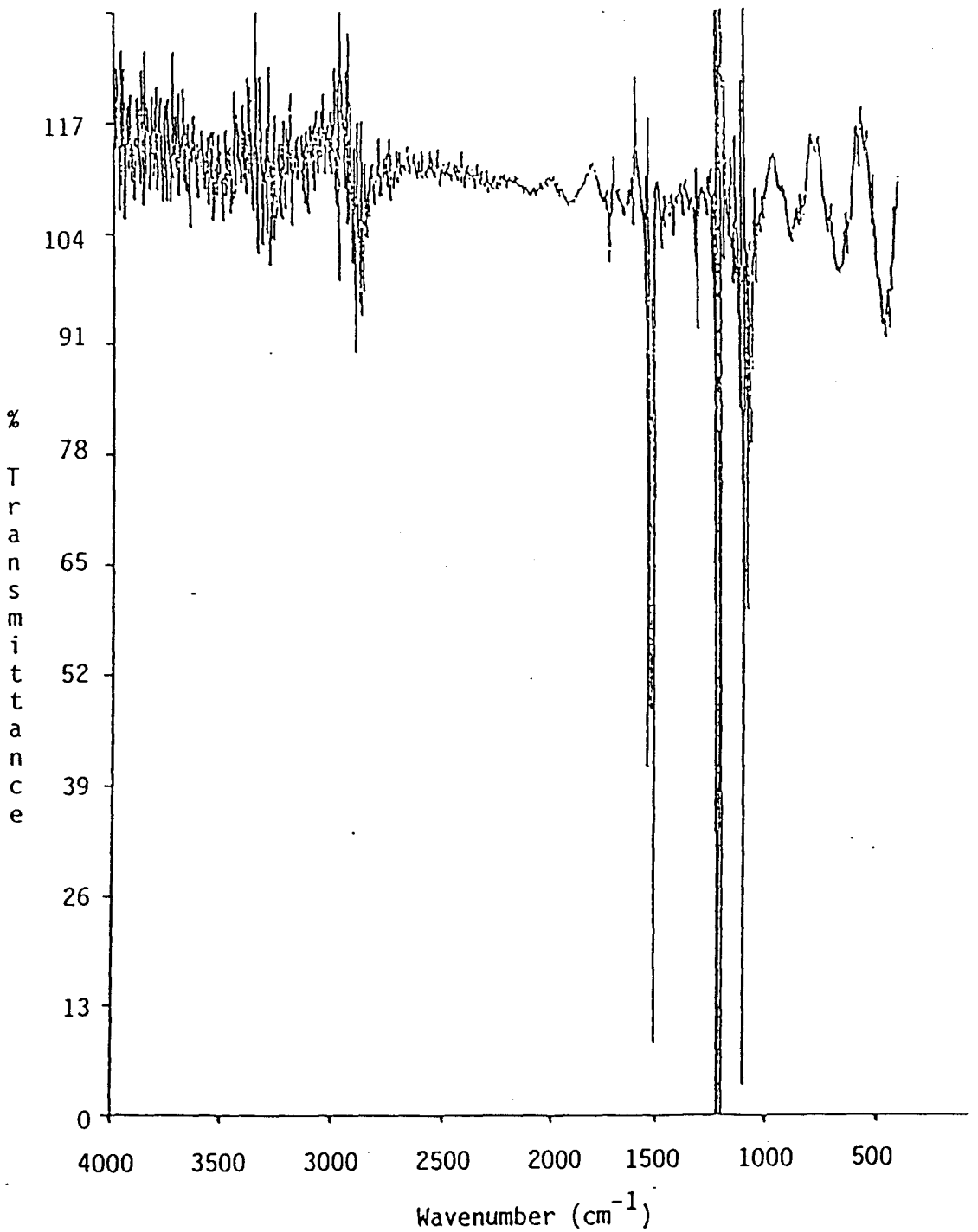


Figure 4.9: FT-IR Difference spectrum for two BDDS-4 samples

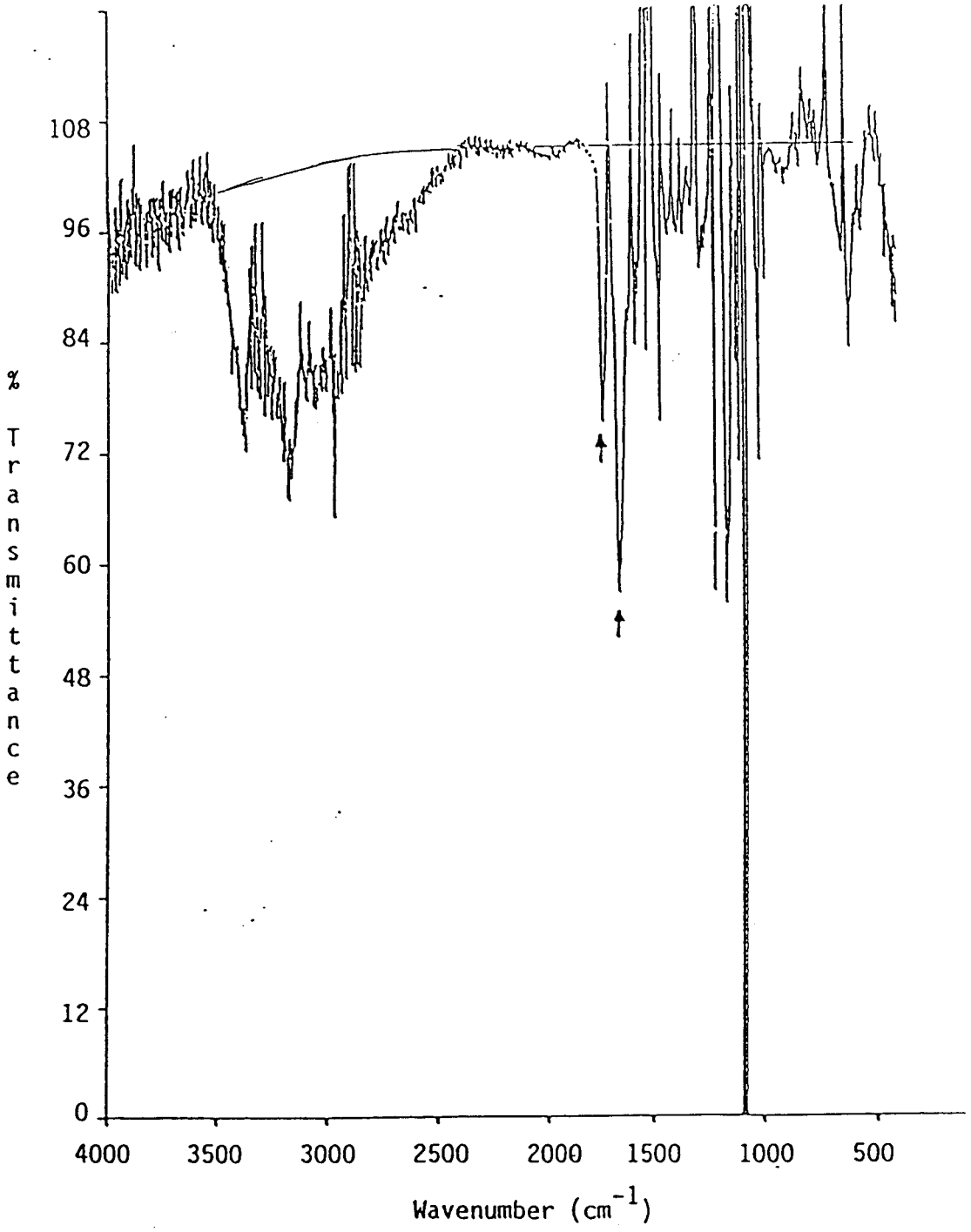


Figure 4.10: FT-IR Difference spectrum for BDDS-3-AM and BDDS-3

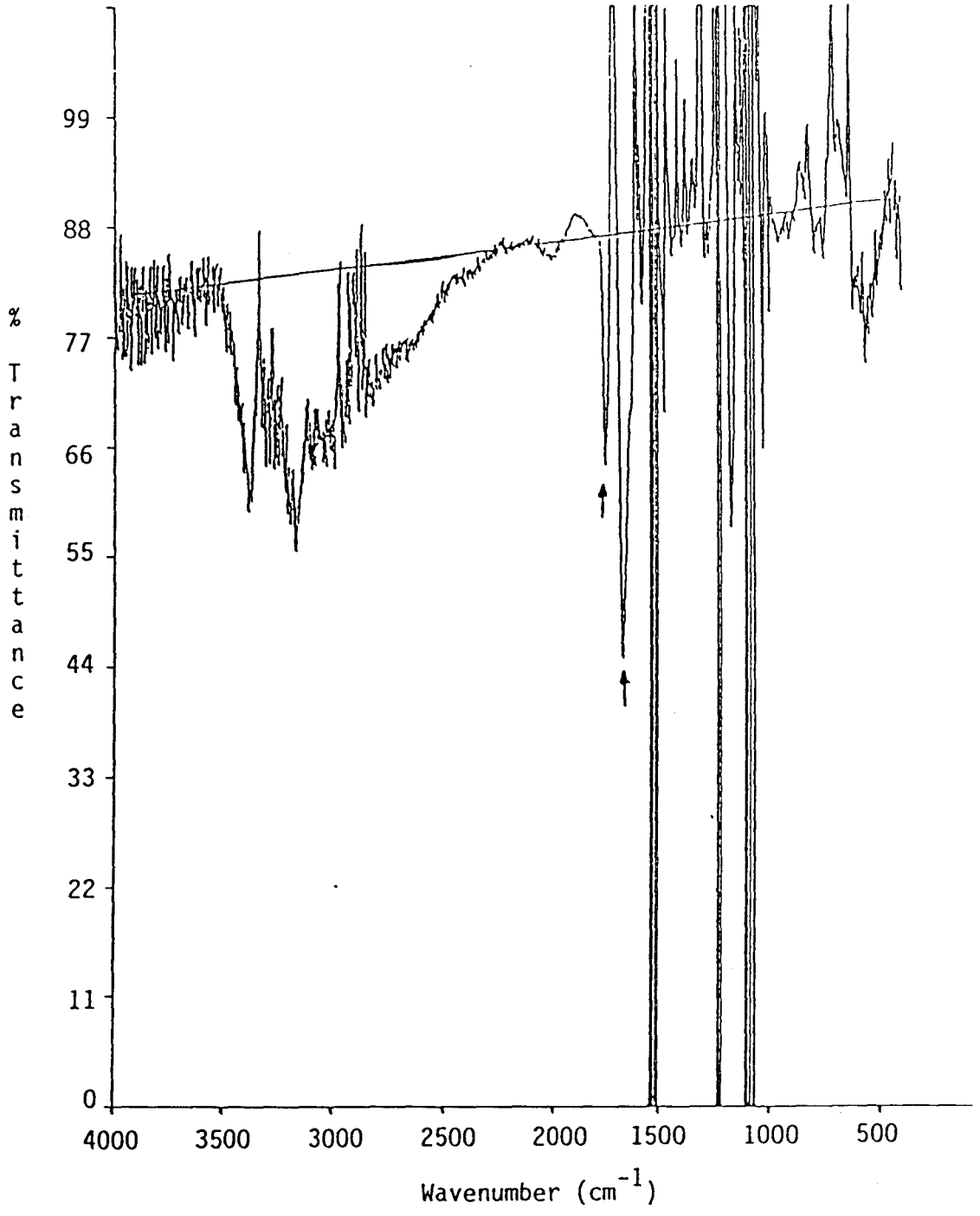


Figure 4.11: FT-IR Difference spectrum for BDDS-4-AM and BDDS-4

N-H bonds generated by incorporation of AM. Figure 4.12 shows the FT-IR spectrum of polymer BDDS-5-AM. A difference spectrum versus its precursor BDDS-5 could not be obtained because BDDS-5 is too brittle to be formed into a film for use in FT-IR. However, the spectrum of BDDS-5-AM does show the new methyl ester and guanidyl peaks as expected at 1720 and 1660 cm^{-1} respectively.

These infrared spectra, then, provide verification that arginine methyl ester is incorporated into the polyurethane. The combination of these data and the nitrogen elemental analysis data provide considerable support for the proposed mechanism of addition of AM to the polyurethanes via the sulphonate groups on the chain extender moieties.

4.2.5 Contact Angle Studies

Contact angle measurements are a simple and convenient way of assessing the physical nature of the surfaces of biomaterials. Knowledge of contact angles allows, for example, the determination of the solid surface tension which in turn is a direct indication of the hydrophilicity or hydrophobicity of a polymer surface. A low surface tension indicates a hydrophobic surface, while a high surface tension indicates a hydrophilic surface [125].

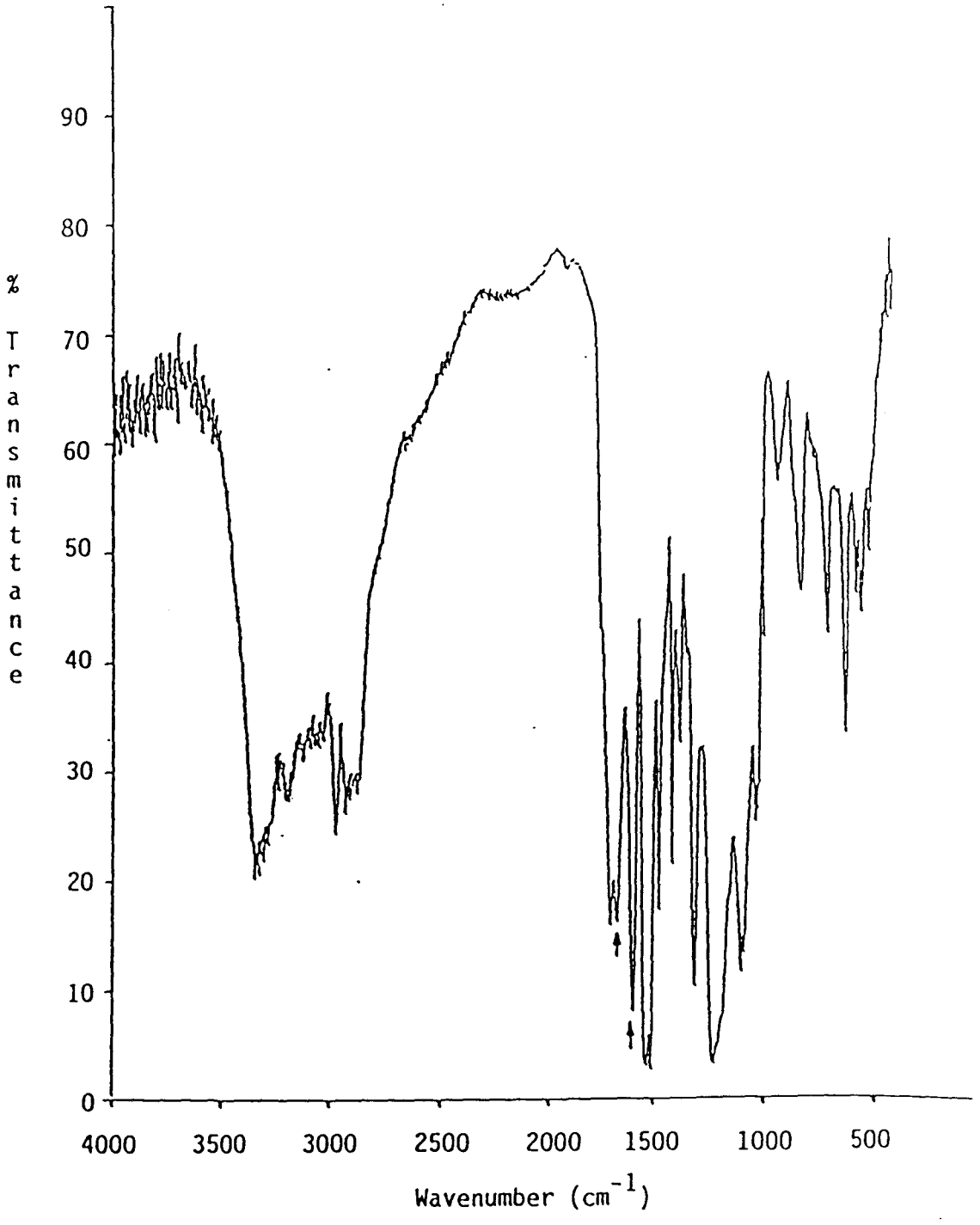


Figure 4.12: FT-IR spectrum of polyurethane BDDS-5-AM

The measurement of contact angles on hydrophilic surfaces such as the present sulphonated polyurethanes involves a number of difficulties since the water droplets used for measuring the contact angles can be absorbed by the surface and can cause rearrangement of the surface over time, both of which can change the angle being measured. Indeed, the relevance of contact angles measured on a dry surface as a means of characterizing surfaces which are to be used in contact with blood can be questioned. As a result, an attempt has been made to apply a method previously developed for measuring contact angles on hydrogels [124] to the polyurethane films produced in this work.

This method involves equilibrating the polymer films in distilled water for twenty-four hours, then measuring the time dependent contact angles of the hydrated surface at ten minute intervals. This results in a plot of contact angle versus time which consists of three basic regions. In the first region the contact angle gradually increases to a plateau value as a result of evaporation of excess water from the surface. The second, or plateau region, is considered the true contact angle for the hydrated surface of the substrate. In the third region the contact angle either increases or decreases with time as a result of physical and/or chemical changes within the film and on its surface [124].

The application of the method used in this work to hydrophilic polymer films instead of hydrogels is still in its early stages [149] and only preliminary results are available at this time. The results, given as the contact angle observed in the plateau region, are presented in Table 4.11 for BDDS-3 and BDDS-5. The values are slightly higher than those which have been previously found for polyacrylamide hydrogels (which range between 8 and 14 degrees) but indicate a considerable degree of hydrophilicity, as expected for polymers with a significant content of sulphonate groups. There appears to be little difference in surface hydrophilic character between polymers BDDS-3 and BDDS-5.

Table 4.11: Water Contact Angles Of Hydrated Sulphonated Polyurethane Films

Polymer	Contact Angle (degrees)
BDDS-3	18
BDDS-5	16

The contact angles listed are the so-called "equilibrium" angles which are found at the plateau of the contact angle versus time curve.

From these preliminary results it is evident that this method is worth pursuing both for the characterization of the remainder of the polymers synthesized in this work and for future work involving hydrophilic polymers. It would be interesting to compare results obtained using this method

to results obtained using the underwater captive bubble technique [125], which allows the measurement of a contact angle for a surface which is in equilibrium with a fluid. It is recommended that future work also encompass a comparative study of the use of these two techniques for contact angle measurement.

4.2.6 Surface Characterization by ESCA

In recent years ESCA has become the dominant method for surface chemical analysis of biomaterials because it is simple, nondestructive and gives information in a region closer to the surface than other available methods [126]. ESCA analysis was used in this work to provide information on the surface chemical composition and to determine if the hard/soft segment ratio was the same in the surface as in the bulk, or if surface enrichment of one of the domains occurs.

The results of ESCA analyses conducted at the University of Washington by Ratner and Castner are presented in Tables 4.12 and 4.13 and in Figures 4.13 - 4.18. The results in Tables 4.12 and 4.13 are presented in the form of content of various chemical elements versus takeoff angle. As the takeoff angle increases, so does the contribution of

the surface to the elemental composition. Table 4.12 presents the results for the high resolution carbon 1s spectra. Three peaks were identified in the carbon 1s spectra consistent with the presence of hydrocarbon (285 eV), ether (286.6 eV) and urethane (289.5 eV) carbons in the polyurethane samples [138]. In Table 4.13 the elemental composition at 5 takeoff angles is presented. Figures 4.13-4.18 show the compositional depth profiles (CDP) for each sample; these are generated from the variable takeoff angle data [150].

In a number of ESCA studies of polyurethane films soft segment surface enrichment has been found [7,19,27]. The results for the polyurethanes analysed here indicate that their surfaces are also soft segment enriched as the outer 10 A of the surfaces show a depletion of nitrogen, sulphur and urethane carbon all of which are associated with the hard segment. However, the data indicate that there are significant numbers of sulphonate groups present at the surface.

The results for BDDS-5 are curious since they indicate the absence of any sulphur in the sample. This may be a result of the sample preparation. BDDS-5 is a brittle polymer and the films produced from it had a tendency to crack and form spotty coatings. It may be that during ESCA analysis the region on which the X-ray beam was focused was

Table 4.12: ESCA Analysis of Sulphonated Polyurethanes: High Resolution C1s Peak Comparison At Various Takeoff Angles

Sample	Takeoff Angle	Atomic Percent Carbon		
		Hydrocarbon	Ether/Alcohol	Urethane
BDDS-3	0	48.0	50.1	1.8
	39	53.2	45.5	1.4
	55	51.8	46.5	1.7
	68	54.0	44.2	1.8
	80	59.7	40.3	-
BDDS-4	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	54.6	43.9	1.5
BDDS-5	0	44.8	53.8	1.4
	39	45.3	53.0	1.7
	55	44.7	54.0	1.3
	68	46.1	52.4	1.5
	80	43.4	56.6	-
BDDS-3-AM	0	50.8	46.2	3.0
	39	49.3	47.6	3.2
	55	50.5	46.6	2.8
	68	48.8	48.2	3.0
	80	49.2	47.7	3.1
BDDS-4-AM	0	51.6	44.7	3.7
	39	49.3	47.9	2.9
	55	52.0	45.4	2.7
	68	48.6	49.8	1.6
	80	48.0	51.3	0.8
BDDS-5-AM	0	58.6	37.0	4.4
	39	59.5	35.6	4.9
	55	60.5	33.6	5.9
	68	60.9	35.4	4.4
	80	59.7	38.2	2.1

Table 4.13: ESCA Analysis of Sulphonated Polyurethanes: Elemental Composition in Weight % At Various Takeoff Angles

Sample	Takeoff Angle	Weight Percent					
		C	O	S	N	Si	Na
BDDS-3	0	65.5	24.7	1.4	3.1	4.0	1.2
	39	65.2	24.2	1.4	2.9	5.2	1.0
	55	63.8	23.8	1.2	2.9	7.4	1.0
	68	62.4	23.9	1.2	2.0	10.0	0.5
	80	59.1	24.5	0.7	1.7	13.7	0.3
BDDS-4	0	62.3	23.9	3.3	3.9	3.5	3.0
	39	63.1	22.6	3.0	3.8	4.3	3.0
	55	62.3	23.3	2.8	3.6	4.5	3.5
	68	60.6	24.0	3.0	3.3	5.9	3.2
	80	61.1	23.8	2.1	2.9	7.7	2.3
BDDS-5	0	68.4	27.9	-	3.8	-	-
	39	71.1	25.2	-	3.7	-	-
	55	71.9	24.9	-	3.3	-	-
	68	72.1	25.3	-	2.6	-	-
	80	72.5	25.4	-	2.1	-	-
BDDS-3-AM	0	63.3	25.7	2.6	5.1	3.3	-
	39	63.5	25.7	2.1	4.6	4.2	-
	55	62.6	25.9	2.1	4.1	5.2	-
	68	61.9	26.3	1.6	3.6	6.6	-
	80	60.5	26.5	0.9	2.2	9.9	-
BDDS-4-AM	0	64.3	25.8	2.9	5.4	1.7	-
	39	63.9	25.8	2.9	5.1	2.3	-
	55	64.6	25.1	2.6	4.7	2.9	-
	68	65.2	25.0	1.9	4.3	3.6	-
	80	63.1	26.3	1.2	3.0	6.4	-
BDDS-5-AM	0	59.9	23.3	4.9	7.6	4.2	-
	39	60.2	23.4	4.9	7.6	3.9	-
	55	60.7	22.7	4.2	7.5	4.9	-
	68	58.9	23.9	3.7	6.2	7.3	-
	80	59.3	23.6	2.5	4.6	9.9	-

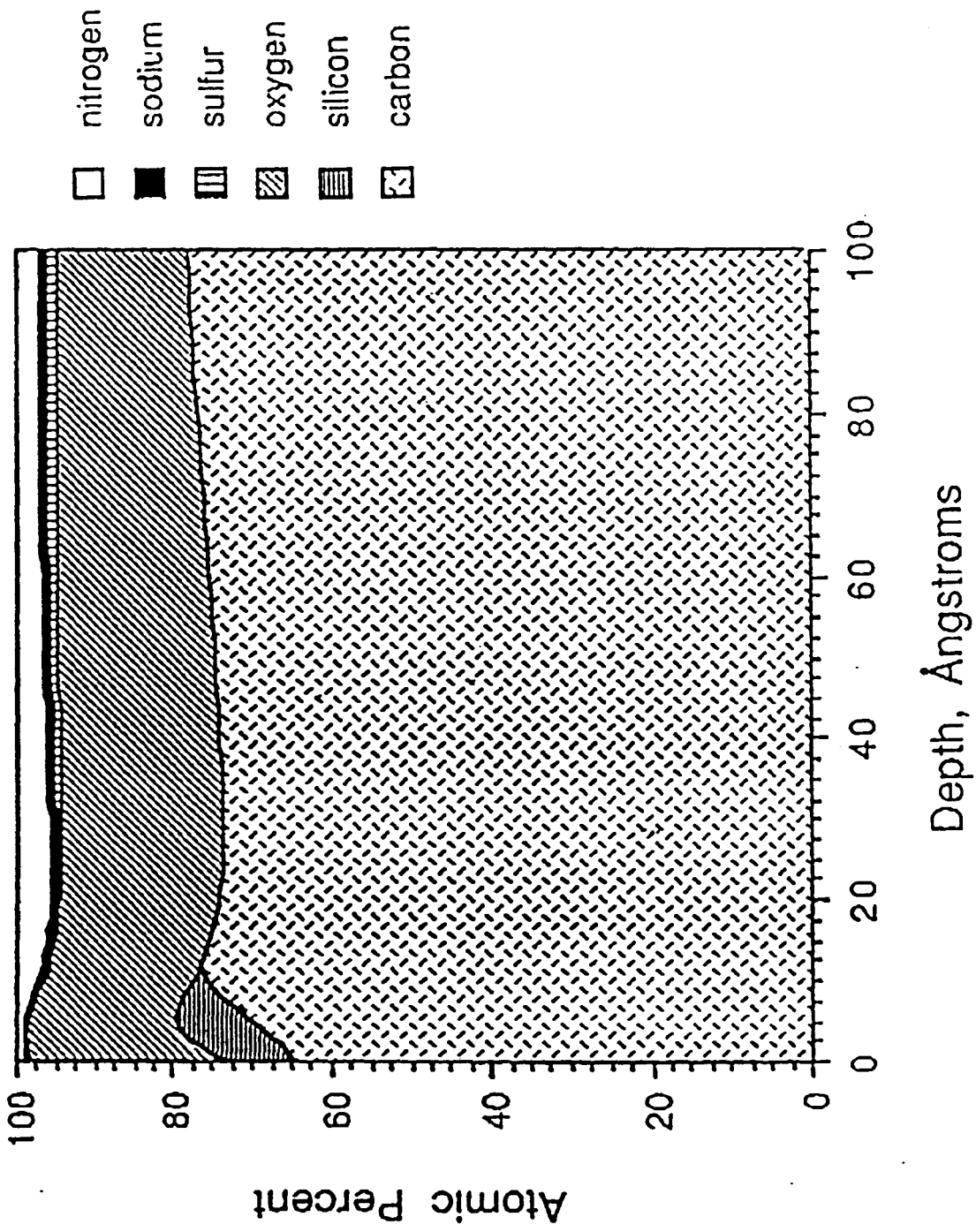


Figure 4.13: ESCA Composition Depth Profile for Polyurethane BDDS-3

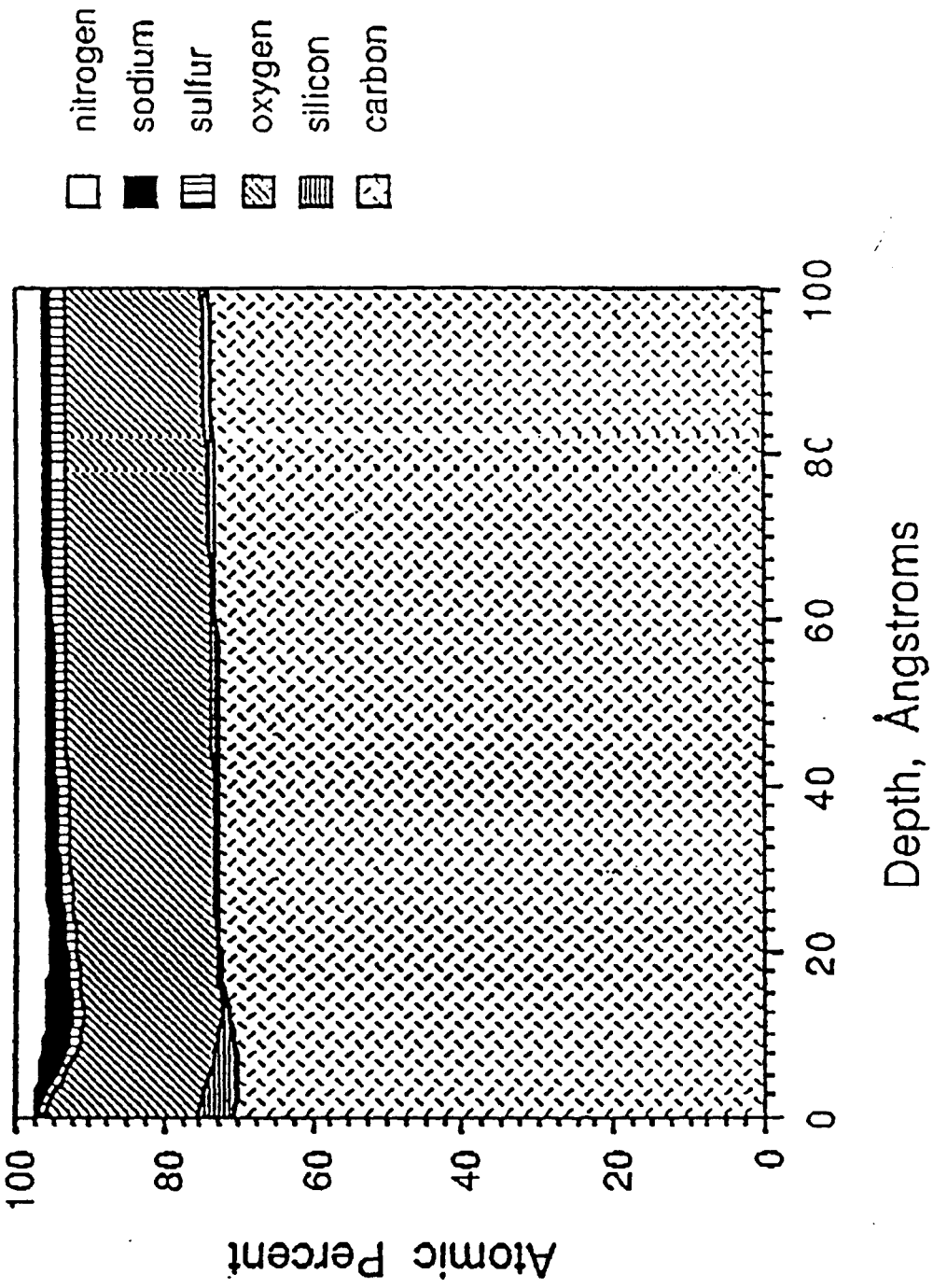


Figure 4.14: ESCA Composition Depth Profile for Polyurethane BDDS-4

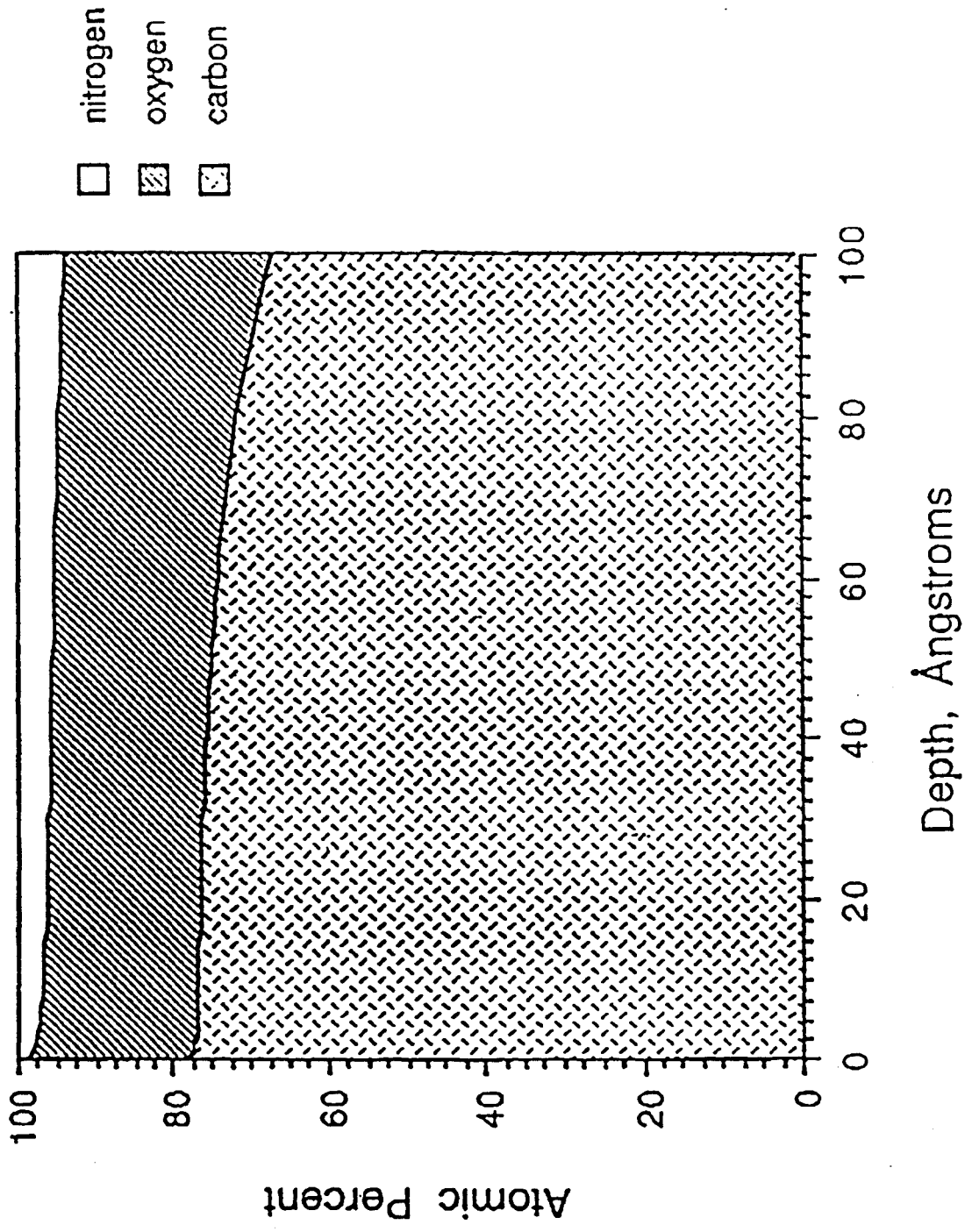


Figure 4.15: ESCA Composition Depth Profile for Polyurethane BDDS-5

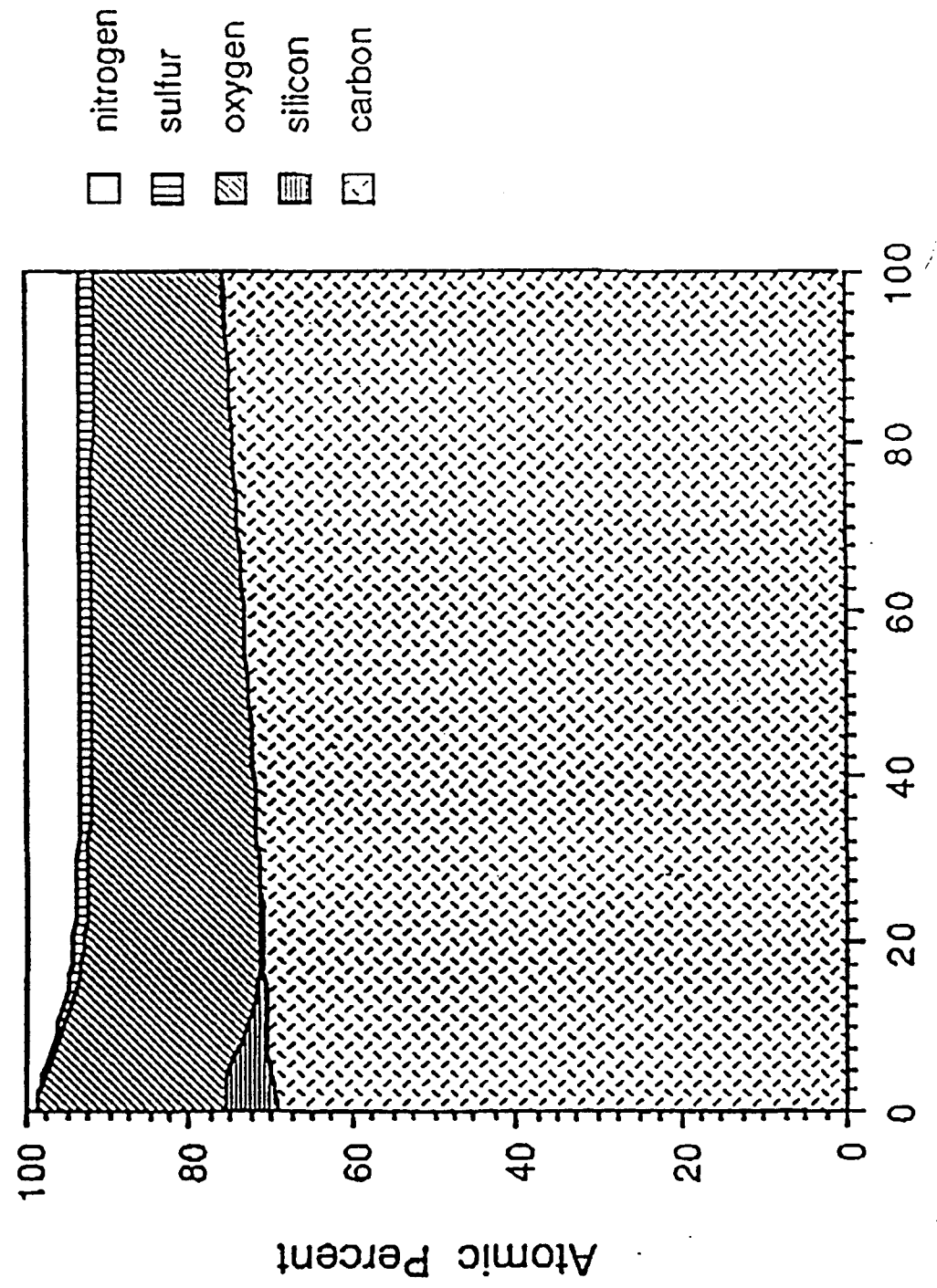


Figure 4.16: ESCA Composition Depth Profile for Polyurethane BDDS-3-AM

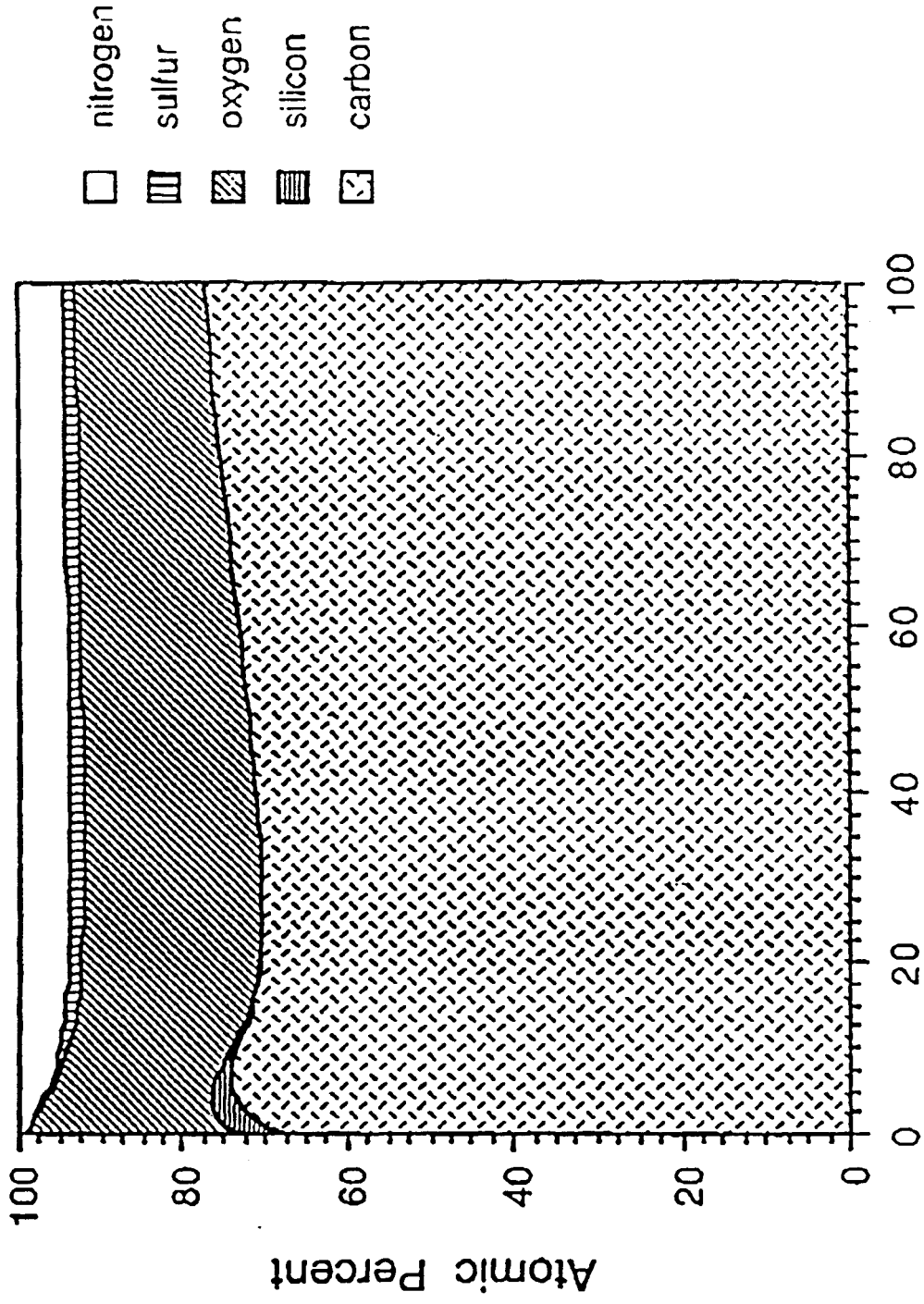


Figure 4.17: ESCA Composition Depth Profile for Polyurethane BDDS-4-AM

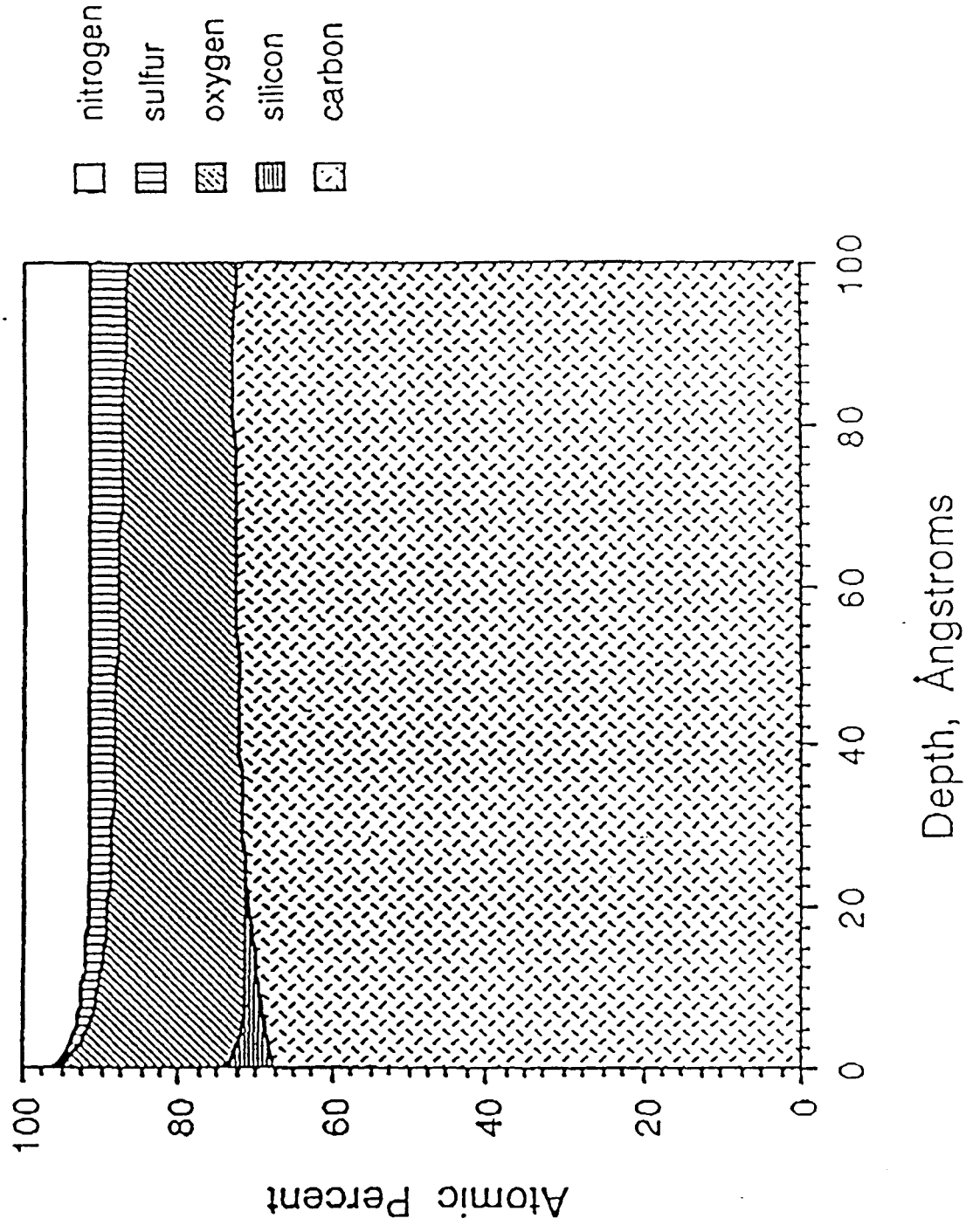


Figure 4.18: ESCA Composition depth profile for polyurethane BDDS-5-AM

one in which the BDDS-5 film had cracked revealing the base coat of ethylene diamine chain extended polyurethane. This would explain the fact that the data are very similar to those observed for the ethylene diamine chain extended polymer [7].

BDDS-3 shows a lower sulphur content in the ESCA analysis than in the combustion analysis, possibly due to soft segment surface enrichment. For all the other polymers the ESCA values of sulphur content are in good agreement with the combustion analysis results.

Elemental silicon, probably associated with a siloxane compound such as polydimethylsiloxane [138], is found on the surface of all the polyurethane films, an observation which has also been made for other polyurethanes [7]. The origin of the silicon is not known, but it may have been introduced at some point in the preparation of the films for ESCA analysis. This surface contamination makes interpretation of the ESCA data more difficult as it will complicate the angular dependence of elemental composition. However, if the silicon is assumed to be a surface contaminant and the elemental composition values are calculated on a silicon free basis, the values show only a slight change in magnitude and soft segment surface enrichment is still evident.

The addition of arginine methyl ester to the

polyurethanes results in a significant increase in elemental nitrogen content, evident both in the bulk and in the surface. The ESCA values closely parallel those for bulk elemental nitrogen analysis in magnitude and in trends. Thus the increase in nitrogen content increases with the sulphonate content of the precursor polyurethane. As the surface is approached (i.e. as the takeoff angle increases) the concentration of nitrogen decreases due to soft segment enrichment of the surface, confirming that the arginine methyl ester is associated with the hard segment of the polyurethanes.

In the interpretation of ESCA data it must be remembered that the results are obtained under conditions very different from blood contact. ESCA measurements are performed in vacuum, not in contact with plasma, and this method of elemental analysis cannot reflect the possible surface rearrangement which may occur in contact with blood. Nonetheless, these ESCA results are consistent with the bulk elemental composition data obtained by combustion analysis, and with the expected reaction pathways.

4.3 Fibrinogen Adsorption Studies

Fibrinogen adsorption experiments were conducted in order to assess the response of plasma to the materials synthesized. It was hoped that comparison of the fibrinogen adsorption characteristics of the novel SPUs synthesized in this work, to those of traditional SPUs would provide a direct indication of the influence of the sulphonate and arginine methyl ester groups on plasma response. The fibrinogen adsorption characteristics of the BDDS and BDDS-AM polymers were therefore compared with those for a methylene dianiline (MDA) chain extended polymer, an ethylene diamine (ED) chain extended polymer and Biomer. Methylene dianiline is an aromatic nonsulphonated diamine chain extender having the same structure as BDDS but without the sulphonate groups. Comparison of the fibrinogen adsorption characteristics of the BDDS chain extended polymers with the MDA chain extended polymer should therefore give a direct indication of the influence of the sulphonate and sulphonamide bonded arginine methyl ester groups on fibrinogen adsorption.

4.3.1 Influence of Radiolabelling on the Adsorption of Fibrinogen From Buffer Solution

Before fibrinogen adsorption from plasma can be assessed using radiolabelled protein, the influence, if any, of radiolabelling on the adsorption of fibrinogen to these surfaces must be determined. Measurements of the adsorption of fibrinogen from buffer solutions provides a way of verifying that radiolabelling does not significantly alter the behaviour of the protein, at least in terms of adsorption. This was done by comparing fibrinogen adsorption from three solutions having different ratios of labelled to unlabelled fibrinogen but the same total fibrinogen concentration. If the labelling procedure does not change the adsorption characteristics then the adsorption measured, by counting the surface bound radioactivity, should not vary with the percentage of labelled protein in the solution.

Experiments were conducted using solutions containing 10%, 50% and 90% labelled and 90%, 50% and 10% unlabelled fibrinogen, respectively, at a total fibrinogen concentration of 1.0 mg/mL and an adsorption time of 2 h. Previous measurements performed in this laboratory have shown the standard deviation in such measurements to be about $\pm$ 0.05 μg/sq cm. The data (Figures 4.19 and 4.20) show that in most cases there is either no dependence or only a relatively slight dependence of adsorption on fibrinogen

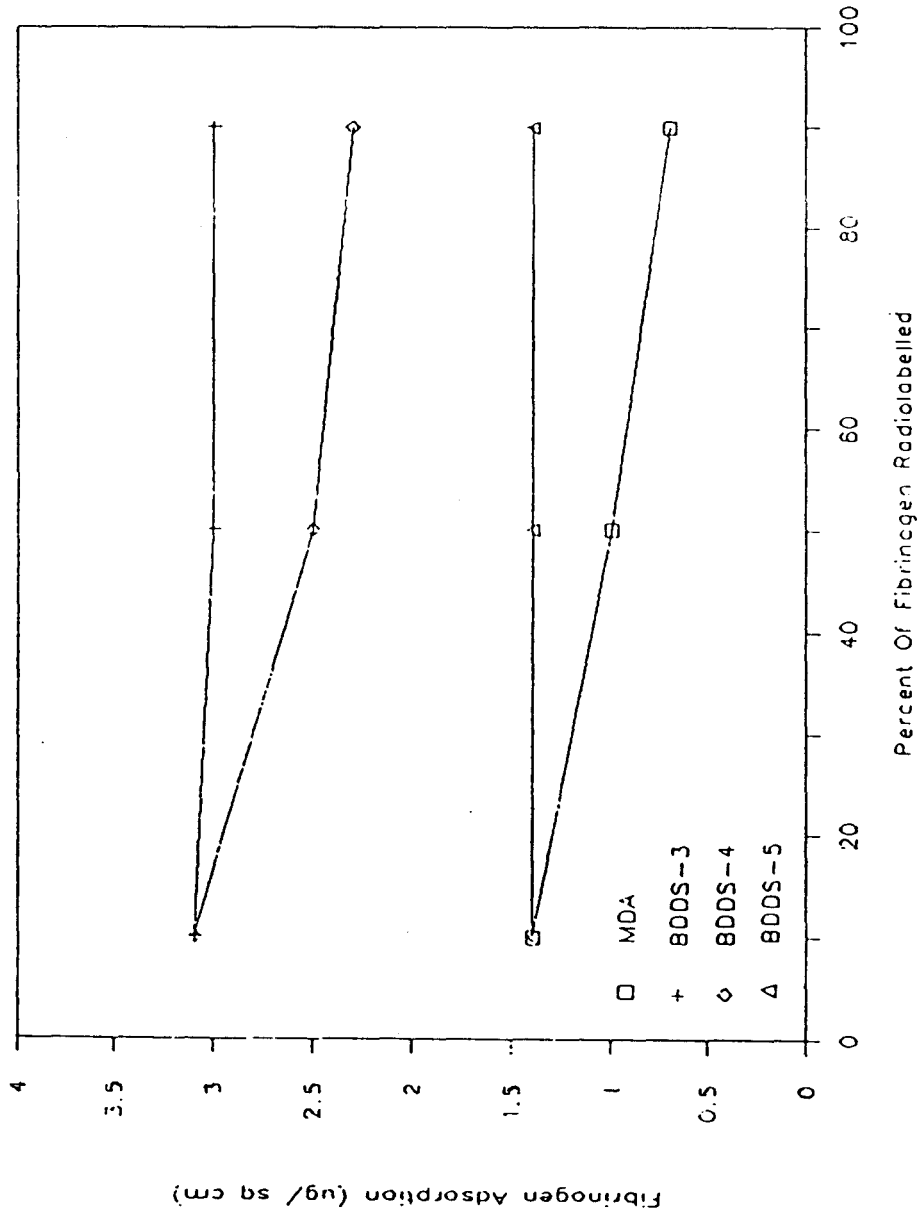


Figure 4.19: Fibrinogen adsorption from Tris buffer to sulphonated, segmented polyurethanes at labelled fibrinogen concentrations of 90%, 50% and 10%. Total fibrinogen concentration, 1 mg/mL; adsorption time, 2 hours.

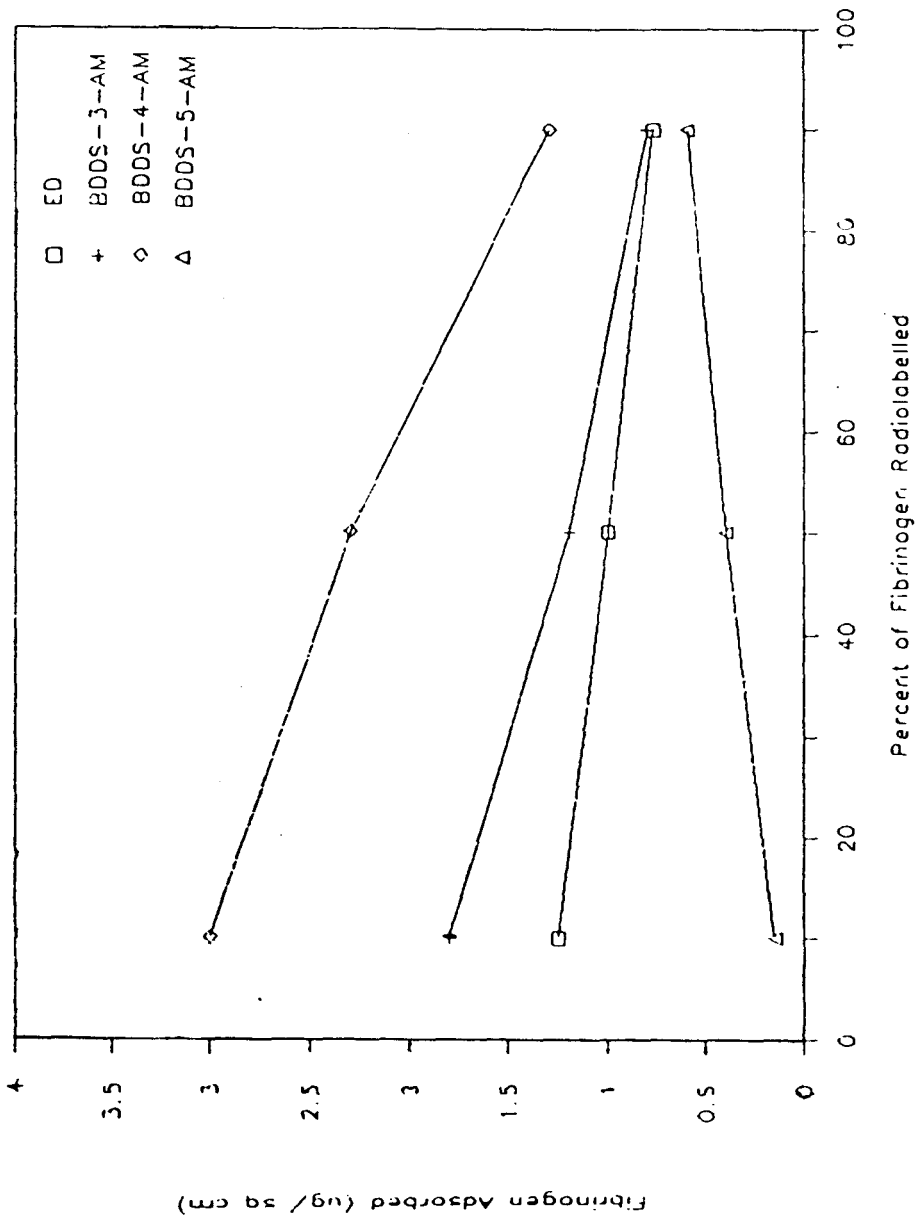


Figure 4.20: Fibrinogen adsorption from Tris buffer to arginine methyl ester-containing polyurethanes at labelled fibrinogen concentrations of 90%, 50% and 10%. Total fibrinogen concentration, 1 mg/mL; adsorption time, 2 hours.

labelling. The BDDS-AM polymers and particularily BDDS-4-AM show a stronger labelling dependence than the BDDS polymers. For most of the polymers that do show a dependence of adsorption on labelling the trend is for decreasing adsorption with increasing percent labelled protein, suggesting that fibrinogen labelling inhibits its adsorption on these surfaces. Accordingly it may be appropriate in these cases to work at the minimum possible percent labelled protein and to consider the adsorption data as minimum values. In the adsorption studies reported below, the percent fibrinogen present as labelled fibrinogen in either plasma or solutions in buffer was never more than 10%.

Interestingly, the BDDS polymers all show considerably higher fibrinogen adsorption than the corresponding BDDS-AM polymers or the ethylene diamine chain extended polymer. This trend and other aspects of these data will be discussed further in the following sections.

4.3.2 Adsorption of Fibrinogen From Plasma

Previous work has shown that fibrinogen adsorption from plasma to a wide variety of surfaces, including a range of different polyurethanes, polyethylene and glass, is transient [57,139]. This phenomenon is known as the Vroman

effect. In the first few seconds of contact between the surface and plasma, fibrinogen is extensively adsorbed. After this time, fibrinogen is competitively displaced by a number of other plasma proteins, including high molecular weight kininogen (HMWK), in a process which may be related to the contact activation of coagulation. The rate of fibrinogen adsorption and displacement is dependent upon the surface type and may be an indicator of the procoagulant activity of the surface [45].

The critical time for fibrinogen adsorption and displacement appears to be on the order of one to two seconds following contact, but current experimental procedures cannot measure adsorption at such short times. As an equivalent way of observing this short-time behaviour Brash and ten Hove [57] have conducted adsorption experiments at longer times, usually 5 minutes, but using diluted plasma. Dilution of the plasma decreases the concentration of the displacing proteins, which are believed to be present at low concentration, so that displacement of adsorbed fibrinogen is slowed or eliminated. However the fibrinogen concentration is still sufficient to give significant adsorption. Thus if fibrinogen adsorption is measured as a function of plasma dilution, the data go through a maximum. The magnitude of the plasma dilution at which fibrinogen adsorption reaches a maximum, for a given

time of adsorption, increases with the time required, at normal plasma concentrations, for displacement to begin.

A typical experiment, showing adsorption after five minutes as a function of plasma concentration for fibrinogen adsorption to glass, is shown in Figure 4.21 [139]. Curves for adsorption of fibrinogen from both plasma and a single protein solution at equivalent concentration are shown. The initial portion of both curves is linear as the surface is not covered with protein and little interaction between proteins is taking place. For the single protein solution the adsorption increases and reaches a plateau value. Adsorption from plasma, on the other hand, illustrates the Vroman effect. The fibrinogen surface concentration passes through a maximum value and then rapidly decreases.

Fibrinogen adsorption from plasma as a function of plasma concentration for the BDDS and BDDS-AM polymers as well as for the methylene dianiline (MDA) and ethylene diamine (ED) chain extended polymers and Biomer were determined. The adsorption time in these experiments was five minutes and the curves generated are referred to as "isotherms".

The first set of experiments conducted for these polymers covered a range of plasma concentrations from 0.25% to 14% normal plasma. The results for the BDDS polymers are shown in relation to the MDA-based polymer in Figure 4.22;

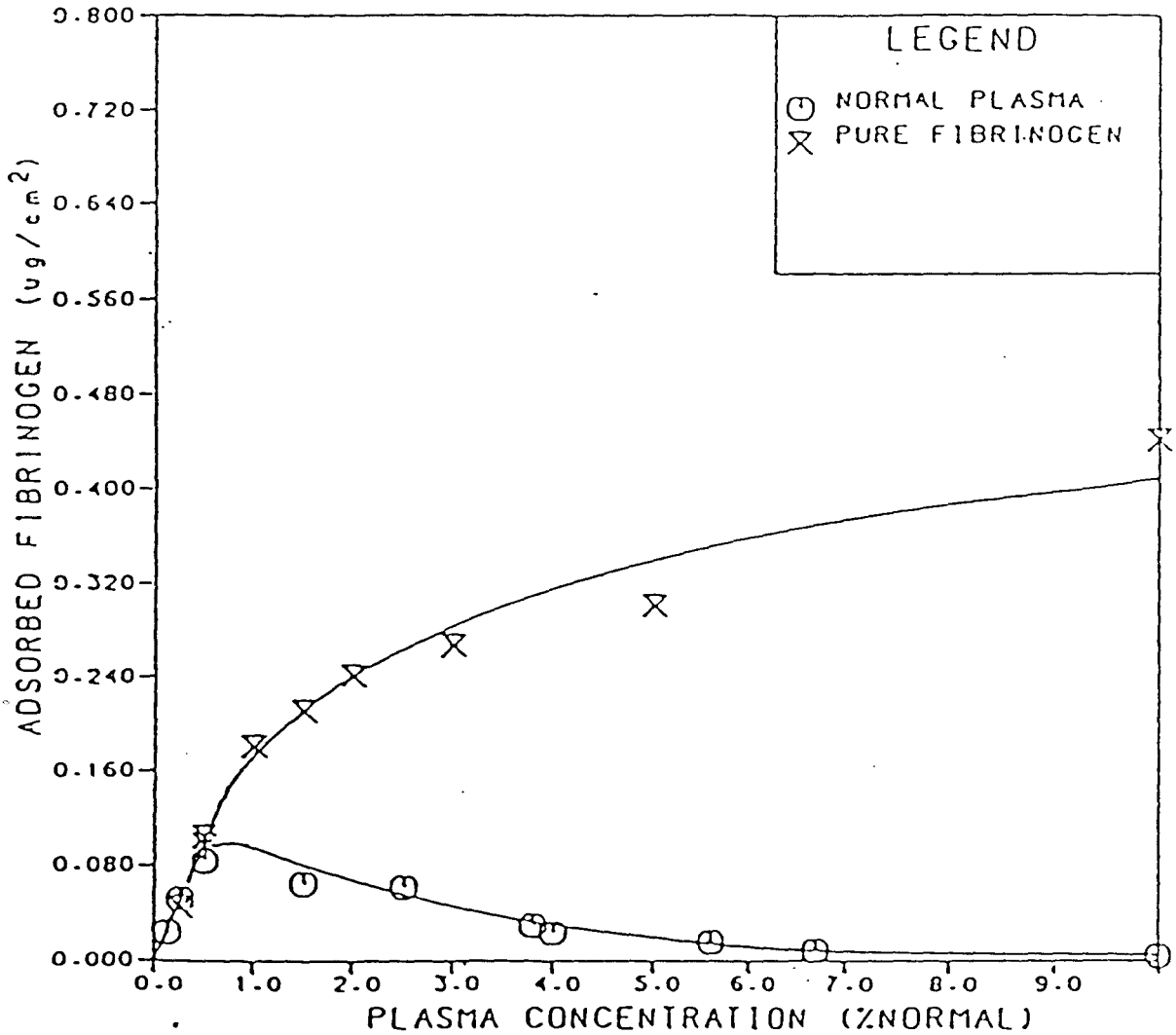


Figure 4.21: Fibrinogen surface concentration versus bulk concentration from Tris and from plasma at 5 minutes. (From reference 139)

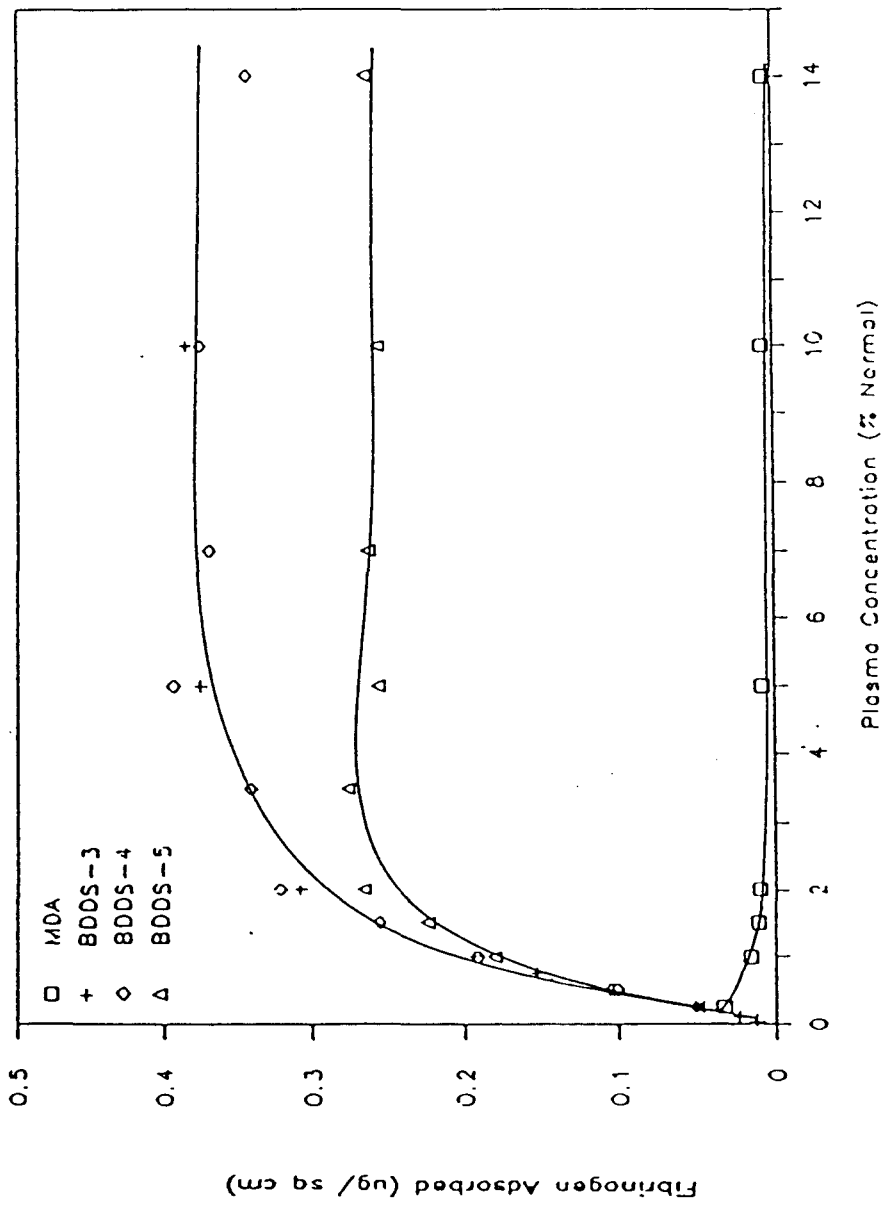


Figure 4.22: 5-minute fibrinogen adsorption from plasma to polyurethanes: effect of sulphonation. Run 1.

and BDDS-4 is shown in relation to the MDA based polymer, Biomer and an ethylene diamine-based polymer in Figure 4.23. The MDA based polymer, Biomer and the ED based polymer show the typical transient adsorption associated with the Vroman effect, but none of the BDDS polymers exhibit this behaviour. On these polymers the initially adsorbed fibrinogen is not displaced, but remains bound to the surface either irreversibly or in dynamic equilibrium with solution fibrinogen.

To verify this behaviour and to observe whether fibrinogen displacement occurs at higher plasma concentrations on the sulphonated polymers, a second set of experiments which covered a range of plasma concentrations from 0.25% to 30% normal plasma was done (Figure 4.24). Average values for those plasma dilutions covered in both runs are shown in Figure 4.25 for the BDDS polymers and comparisons of the two runs for each polymer are shown in Figures 4.26-4.28. The second experiments confirm the trends seen in the initial data and extend the data to higher plasma concentrations. Some variation in the plateau surface concentrations for the repeat runs is evident, possibly due to the difficulty in reproducing the surface on both a microscopic and a macroscopic scale, but the general behaviour remains the same: the Vroman effect is absent.

The quantity of fibrinogen adsorbed to the surfaces

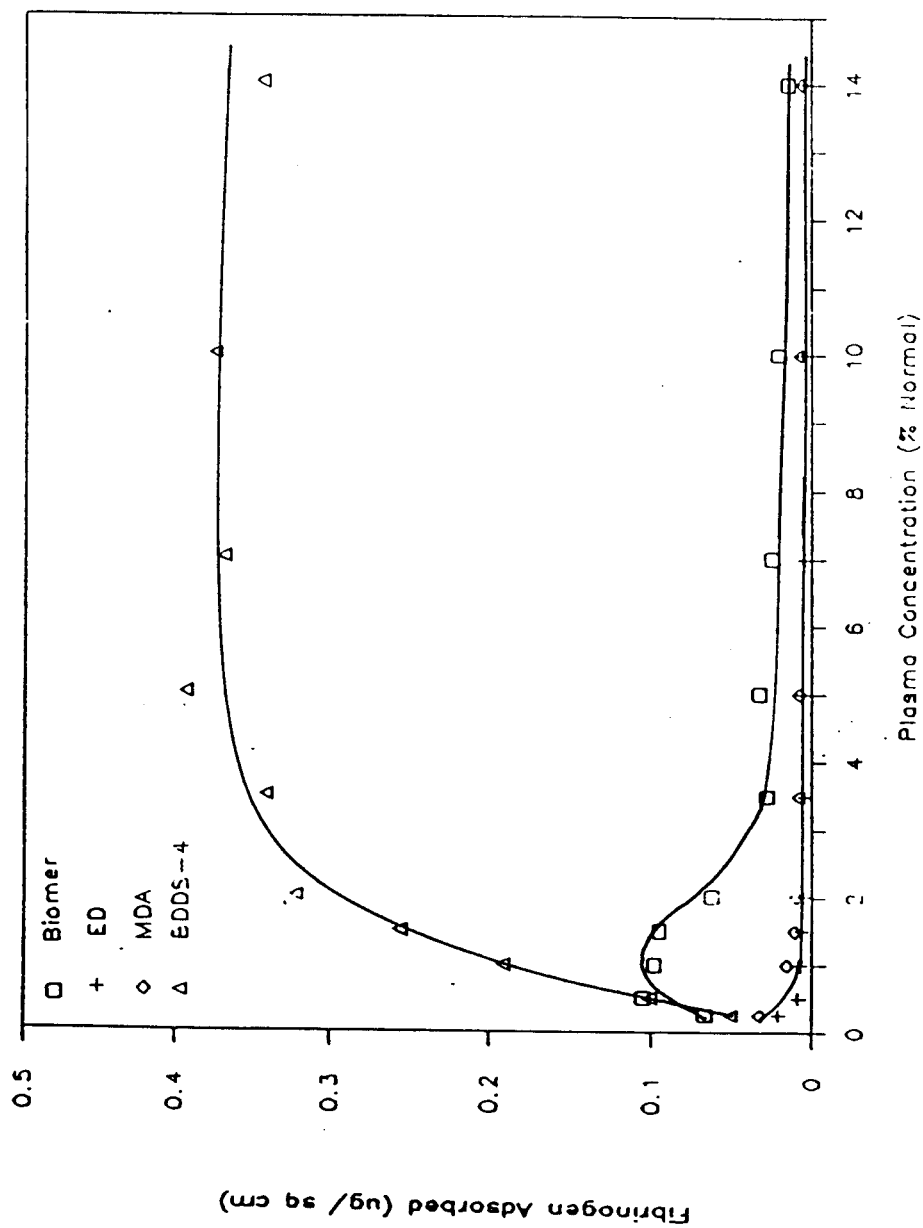


Figure 4.23: 5-minute fibrinogen adsorption from plasma to polyurethanes: effect of sulphonation. Run 1.

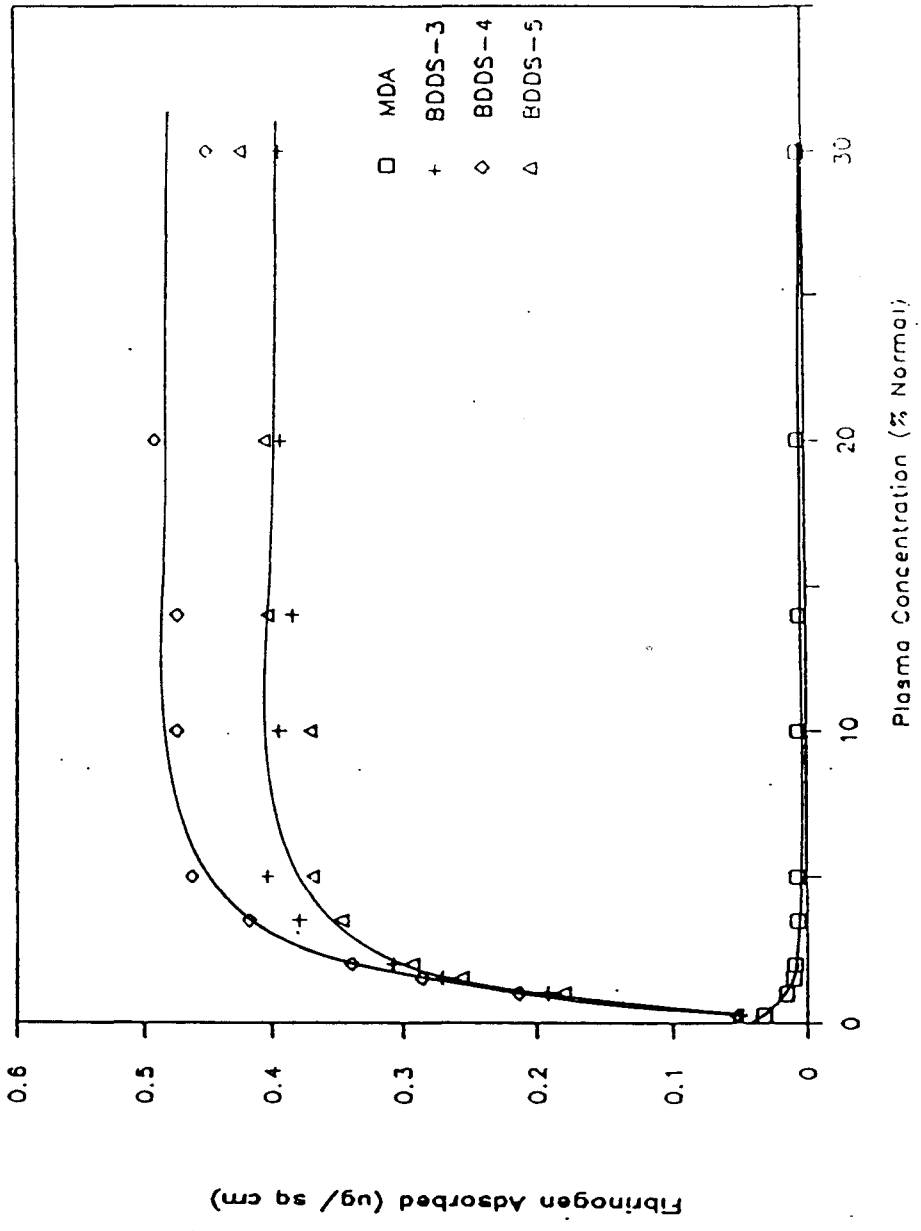


Figure 4.24: 5-minute fibrinogen adsorption from plasma to polyurethanes: effect of sulphonation. Run 2.

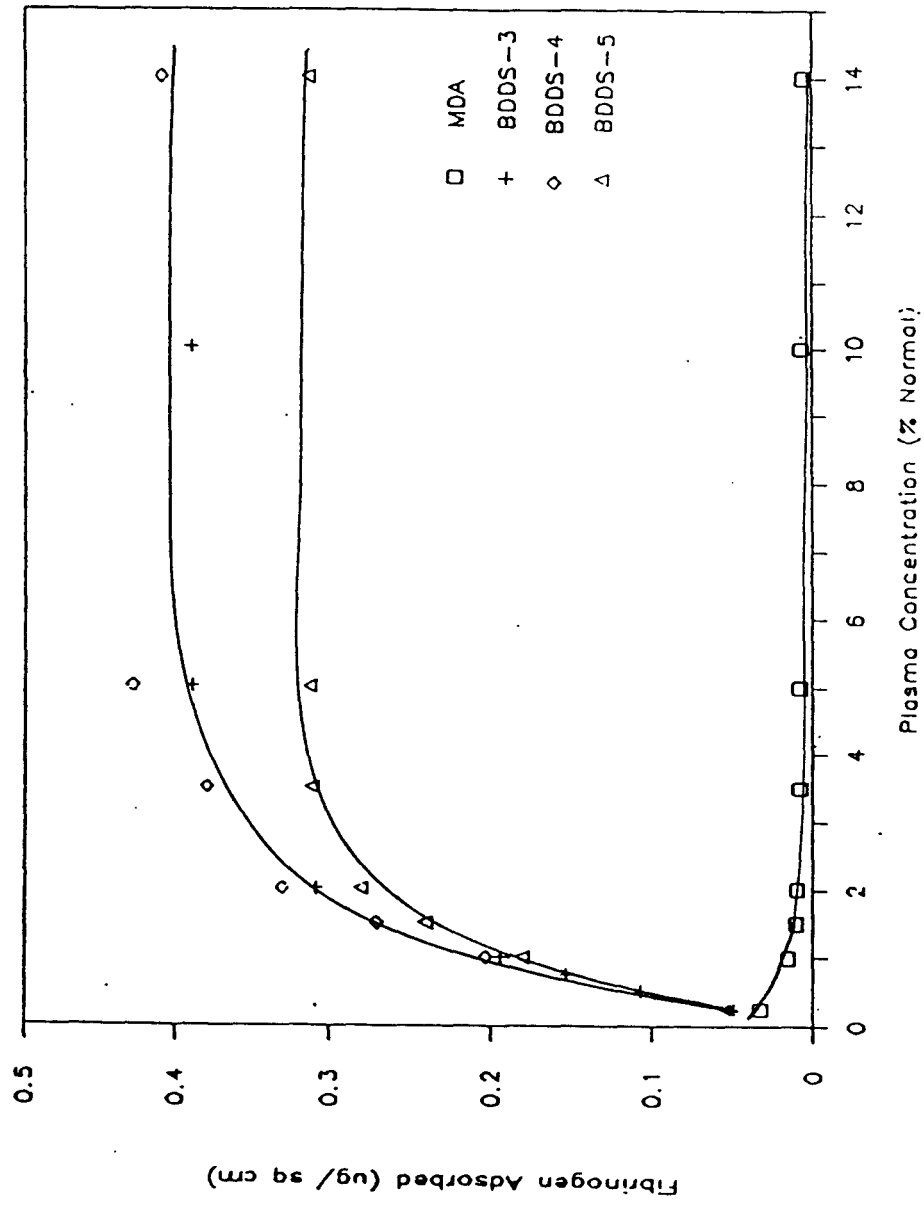


Figure 4.25: 5-minute fibrinogen adsorption from plasma to polyurethanes: effect of sulphonation. Average of runs 1 and 2.

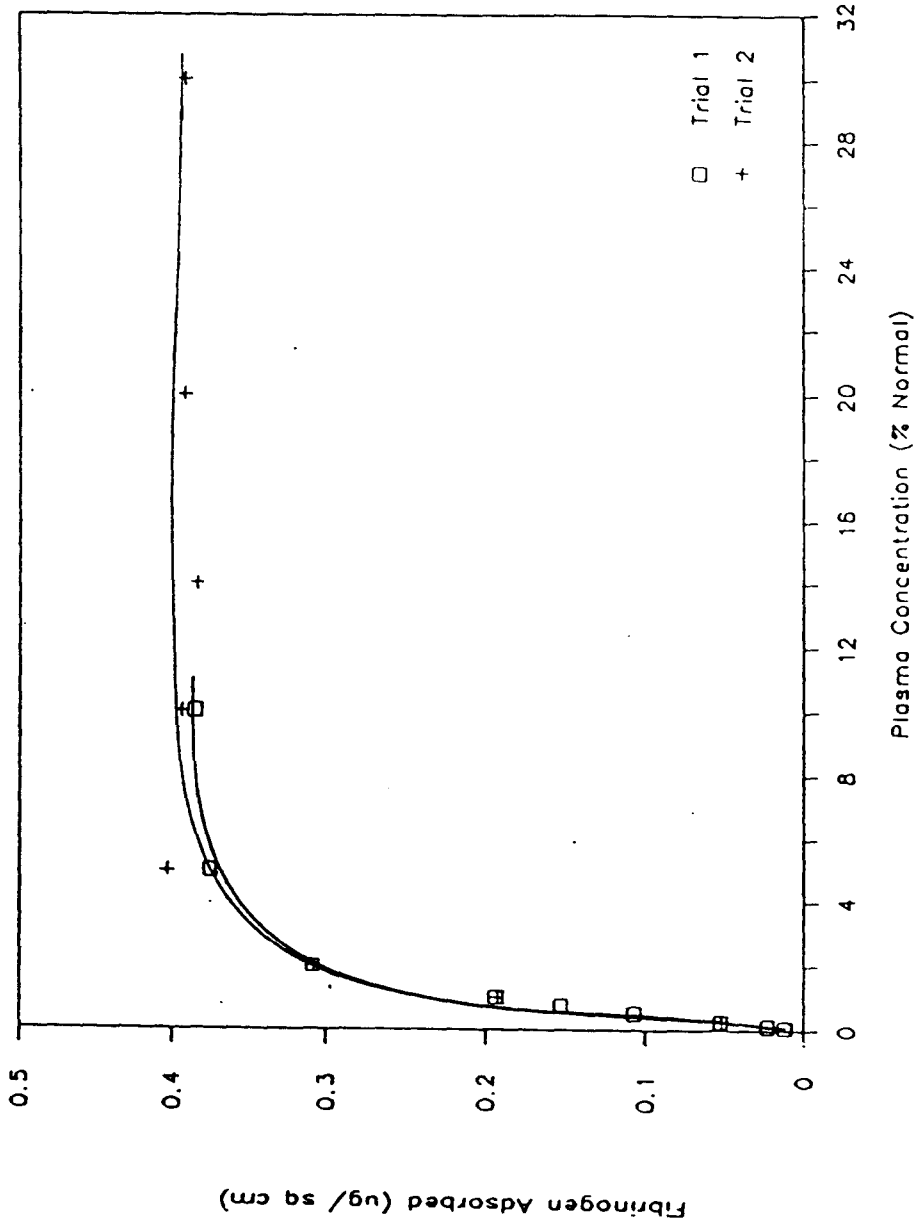


Figure 4.26: 5-minute fibrinogen adsorption from plasma to polyurethanes: comparison of runs 1 and 2 for BDDS-3.

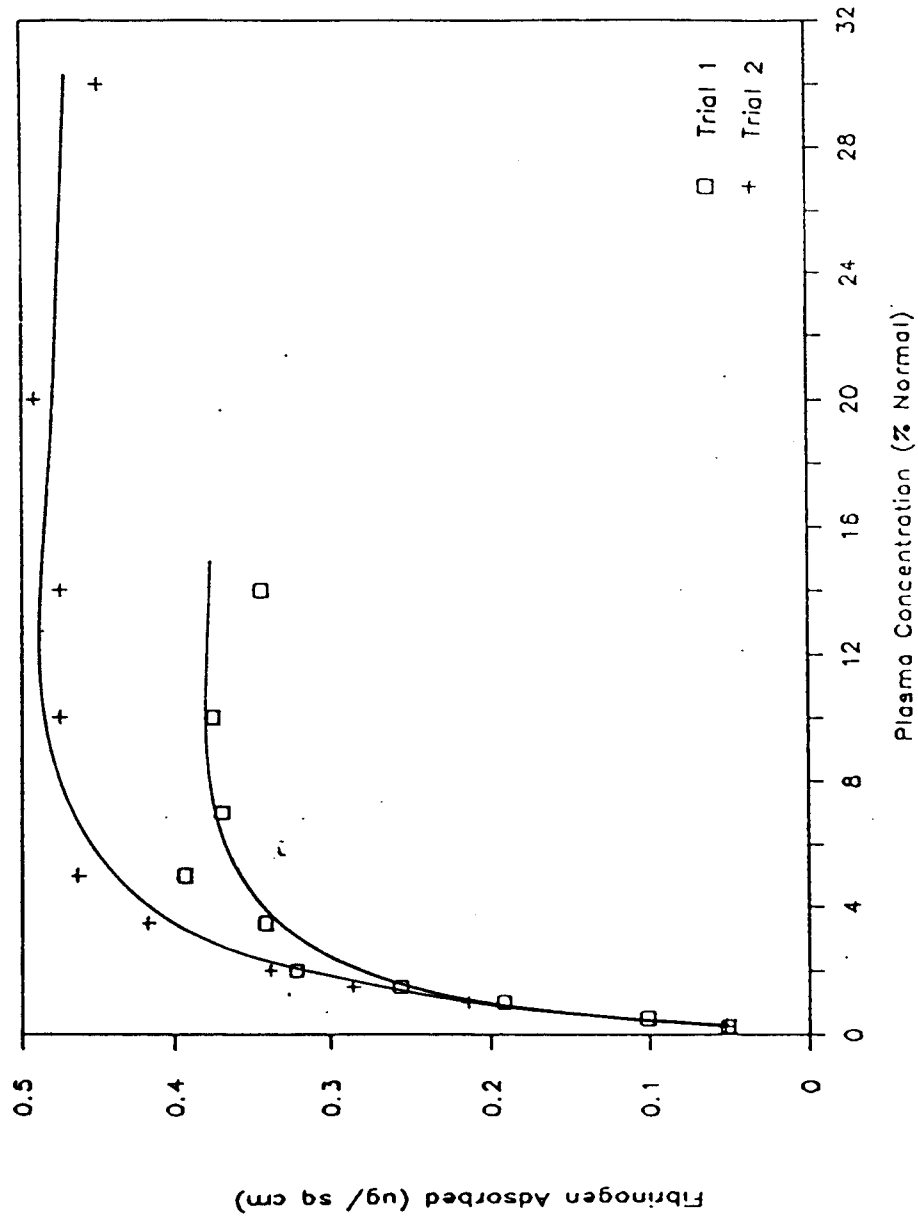


Figure 4.27: 5-minute fibrinogen adsorption from plasma to polyurethanes: comparison of runs 1 and 2 for BDDS-4.

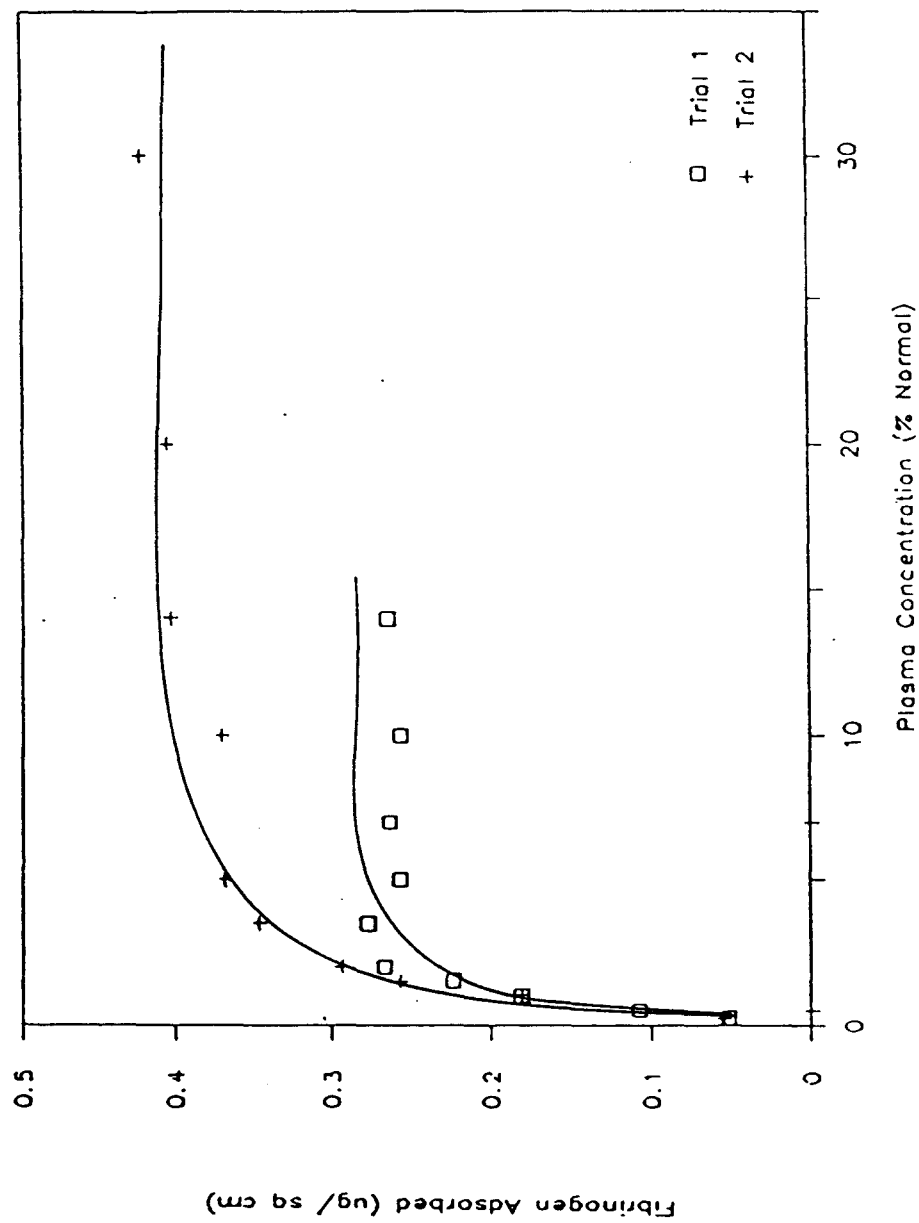


Figure 4.28: 5-minute fibrinogen adsorption from plasma to polyurethanes: comparison of runs 1 and 2 for BDDS-5.

of all the BDDS polymers is very high in comparison with other surfaces which have been tested. The levels are between those expected for a close-packed, end-on oriented monolayer (1.8 μg/sq cm) and a side-on oriented monolayer (0.2 μg/sq cm) [139], suggesting that the adsorbed protein layer is largely composed of fibrinogen.

The fact that the adsorbed fibrinogen is not displaced on the sulphonated polyurethane surfaces suggests that there may be a specific interaction between the sulphonate group and fibrinogen. This specific interaction could result in either reversible or irreversible binding of fibrinogen via the sulphonate groups. In the latter case the plateau in the adsorption isotherms would represent saturation of the sulphonate-related surface binding sites.

The decrease in fibrinogen adsorption on BDDS-5 (which has the greatest sulphonate content) relative to BDDS-3 and -4, would appear to contradict the surface binding site saturation hypothesis. However, lower adsorption may occur on this material if, as seems likely, the surface of these SPUs is dynamic and able to undergo rearrangement in response to a change in contacting fluid (e.g. water vs air). When the SPU is equilibrated with the buffer solution, surface rearrangement could take place and is likely to involve surface enrichment of the hydrophilic sulphonate groups. The mobility of the sulphonate groups is

dependent on the mobility of the hard segment: the greater the degree of phase separation of the domains, the lower the mobility is likely to be. Thus, the high hard segment content of BDDS-5 may have reduced mobility to such an extent that surface rearrangement is limited. Even though the total sulphonate content is higher in BDDS-5 than in BDDS-3 and -4, the surface content may be lower. Alternatively, the adsorption of fibrinogen may pass through a maximum as a function of sulphonate content. Such a maximum could be dependent on a number of factors, including surface distribution of sulphonates, or of hard and soft segment domains.

When the BDDS and BDDS-AM polymers are compared there are significant differences in the shape of the adsorption "isotherms" (Figures 4.29-4.31). Not only is the overall adsorption lower on the BDDS-AM polymers (also observed in the buffer-single protein data), but fibrinogen displacement occurs at plasma concentrations above 5% normal. This is still by no means a typical Vroman effect since the fibrinogen adsorption normally peaks at very low plasma concentrations (on the order of 0.5 to 1%) and descends rapidly to zero. For the BDDS-AM polymers the peak occurs at about 5% normal plasma concentration and is broad compared to a typical Vroman peak. Fibrinogen adsorption appears to equilibrate at significant levels and

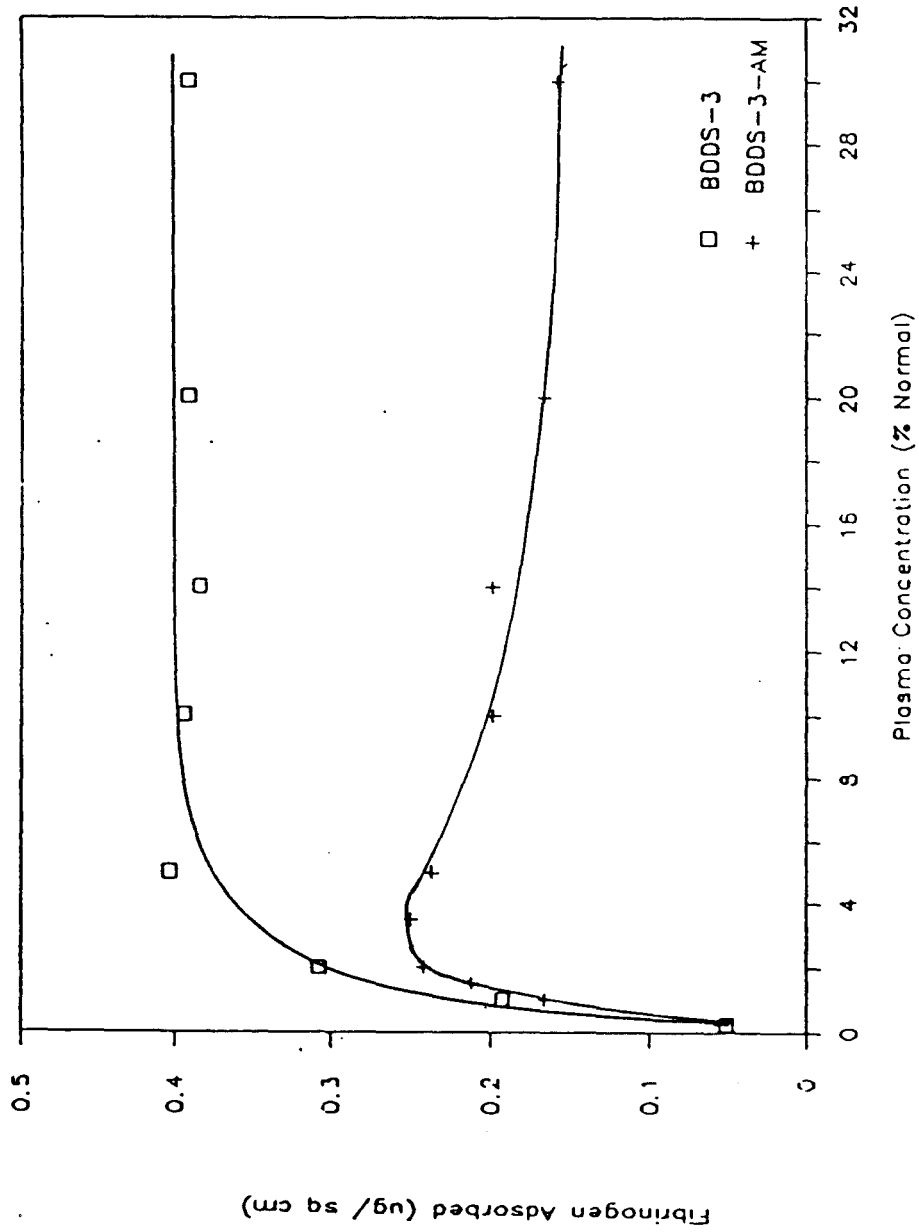


Figure 4.29: 5-minute fibrinogen adsorption from plasma to polyurethanes: effect of arginine methyl ester incorporation in BDDS-3.

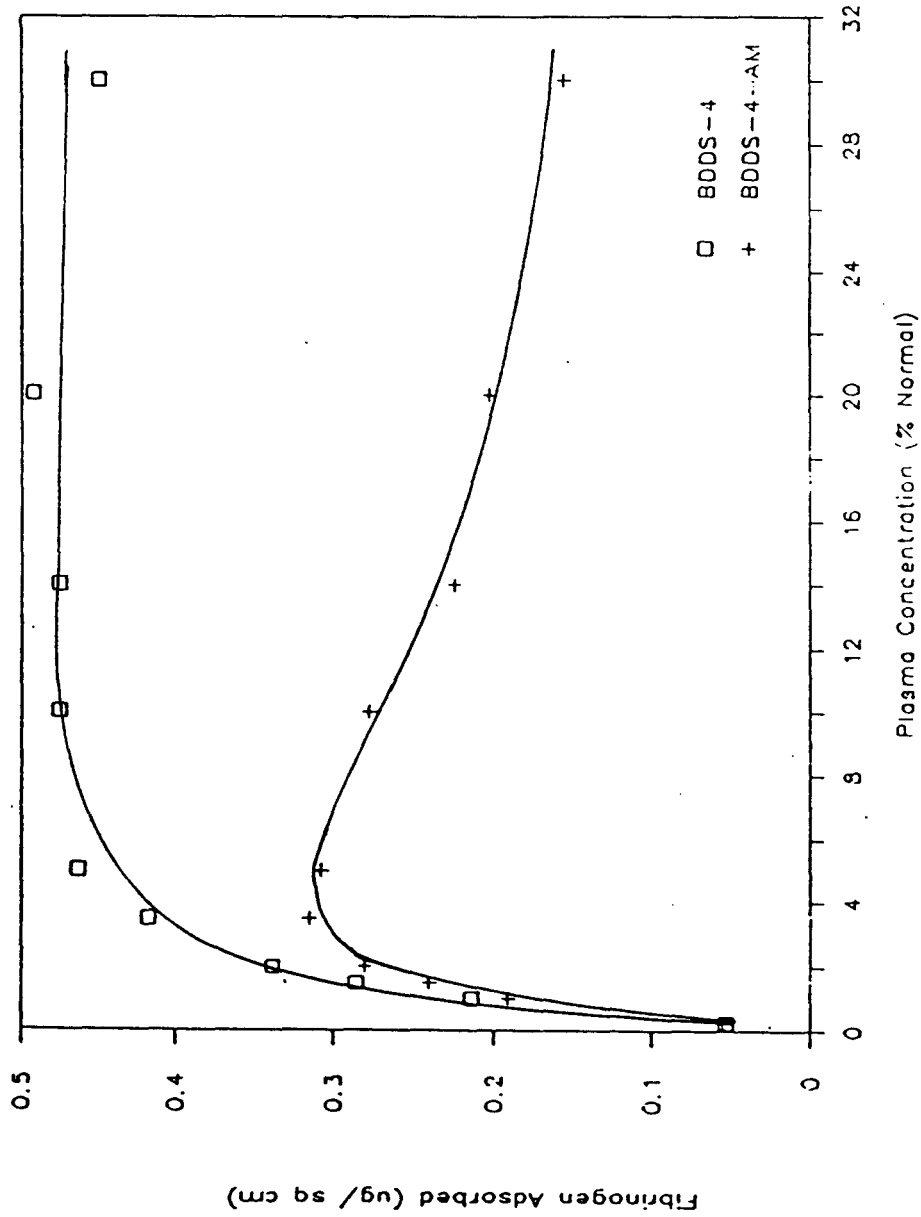


Figure 4.30: 5-minute fibrinogen adsorption from plasma to polyurethanes: effect of arginine methyl ester incorporation in BDDS-4.

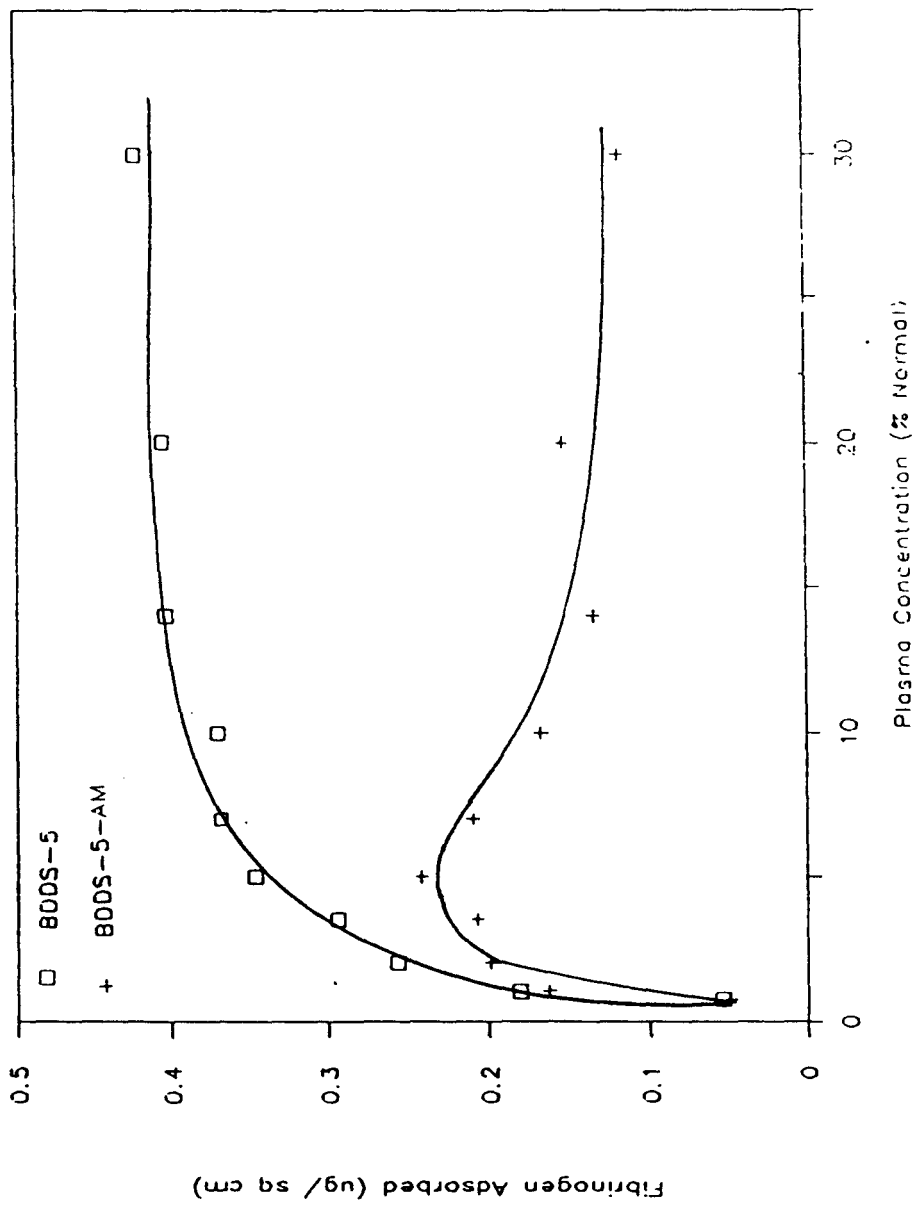


Figure 4.31: 5-minute fibrinogen adsorption from plasma to polyurethanes: effect of arginine methyl ester incorporation in BDDS-5.

displacement is never complete.

The plateau value reached with all the BDDS-AM polymers is lower than that for the corresponding precursor BDDS polymers, a fact which is in line with the hypothesis that there is a specific interaction between fibrinogen and sulphonate, since the AM polymers have reduced concentrations of sulphonate groups.

The appearance of fibrinogen displacement with arginine methyl ester substitution of sulphonate is interesting. A number of explanations are possible. For example one could postulate the existence of weak specific interactions between AM or the sulphonamide bond and fibrinogen which are overcome at high plasma concentrations. However, on the basis of these preliminary results it can only be concluded that in order to elucidate these mechanisms further study is required.

The implications of the lack of fibrinogen displacement in relation to thrombogenicity can be cast in either a positive or a negative light. On the positive side the fact that fibrinogen is not displaced, presumably by HMWK and other contact phase factors, may mean that contact activation of coagulation is inhibited. However, on the negative side, glass surfaces to which fibrinogen has been preadsorbed have been found to be platelet reactive [51]. How this finding relates to a polyurethane surface,

especially one in which a specific interaction with fibrinogen may be involved, is not clear. Cooper et al in studies of sulphonated polyurethanes (which covered a range of sulphur content between about 0.2 and 0.75 wt %) in canine ex vivo shunts, have found high levels of fibrinogen adsorption but low levels of platelet adhesion and activation [140]. This suggests that the important factor may not be the quantity of fibrinogen adsorbed, but some other factor, perhaps the conformation of the adsorbed fibrinogen.

Related to this, Lindon et al have found considerable differences in platelet adhesion to polyalkyl methacrylate surfaces which adsorb similar total amounts of fibrinogen [141]. They also found that the level of platelet adhesion paralleled the affinity of the surface bound fibrinogen for anti-fibrinogen antibodies. This led them to speculate that fibrinogen in its native form (which would bind the antibody) is more platelet reactive than fibrinogen which has undergone a conformational change, as assessed by changes in the antibody binding.

Clearly, given these data, the behaviour of the sulphonated polyurethanes towards blood should be further investigated. To this end, both the nature of the interaction between sulphonate and fibrinogen, and the reactivity of these surfaces towards platelets should be

assessed. As far as the sulphonate-fibrinogen interaction is concerned, it would be interesting to attempt to desorb the adsorbed fibrinogen from the surface by displacing the plasma with buffer solutions of increasing ionic strength. The ionic strength of the solution required for desorption should give an indication of the strength of any electrostatic interactions involved. In addition, to determine whether the surface has a high specific affinity for fibrinogen rather than a high affinity for plasma proteins in general, measurements of the adsorption of other proteins, like IgG or albumin should be conducted. The reactivity of these materials towards platelets could be assessed by measuring the extent of platelet adhesion and subsequent release of granule contents. A number of methods are available for such measurements, including the use of a flow cell, a packed column [142] or a Couette flow [143] experiment. Perhaps most importantly, the overall coagulation response of plasma to these materials should be established by measurement of a parameter like the thrombin clotting time [5].

Furthermore, since the reason for incorporating arginine methyl ester groups was to promote the binding of thrombin to the surface, it is recommended that experiments to assess the interaction between these surfaces and thrombin be undertaken [5].

4.3.3 Adsorption of Fibrinogen From Buffer Solution

As the fibrinogen adsorption from plasma data were significantly different from those previously obtained for other polyurethane surfaces and suggested a specific interaction between fibrinogen and sulphonate groups, more information on the interaction between fibrinogen and the sulphonated surfaces was desired. There are a number of factors which can influence the adsorption of proteins to surfaces. Among the factors which influence the surface activity of a protein are: size (proteins are believed to form multiple contacts with the surface when adsorbed), charge and charge distribution (most charged amino acids are believed to reside at the protein's exterior), structural factors, and chemical differences arising from different amino acid sequences [144]. The influence of the adsorbing surface is determined by its interactions with these protein surface activity factors. Horbett and Brash have stated that in cases in which the surface possesses fixed ionic charges "the role of protein surface charge is particularily important and probably dominant" [144].

It is difficult to obtain information on the role of specific protein characterisitics in relation to adsorption. However information on the overall interactions between fibrinogen and the BDDS and BDDS-AM surfaces can be obtained

by studying the adsorption of fibrinogen from buffer solution. A range of fibrinogen concentrations from 0.0005 mg/mL to 1.0 mg/mL were studied using a 3 hour adsorption time, to allow the determination of the equilibrium adsorption isotherms.

The shape of single protein true equilibrium isotherms can provide information on the free energy of adsorption between the protein and the surface [145]. In the initial part of the isotherm, where surface coverage is low the protein molecules interact with the surface only. It is from this region that protein-surface binding free energies can be derived. In the plateau region, where surface coverage is high, the adsorbed molecules will interact laterally as well as with the surface [145]. The initial portion of these isotherms has not been well studied as, due to analytical limitations, the trends tend to be uncertain [145]. The plateau value has usually been found to correspond to a close packed monolayer, either in a side-on or end-on orientation.

The fibrinogen adsorption isotherms obtained for the BDDS, BDDS-AM, MDA and ED polymers are presented in their entirety in Figures 4.32 and 4.33, and the initial portions of the isotherms are shown in Figures 4.34 and 4.35. The initial slope and the quantity of fibrinogen adsorbed at the plateau is listed for each isotherm in Table 4.14. It is

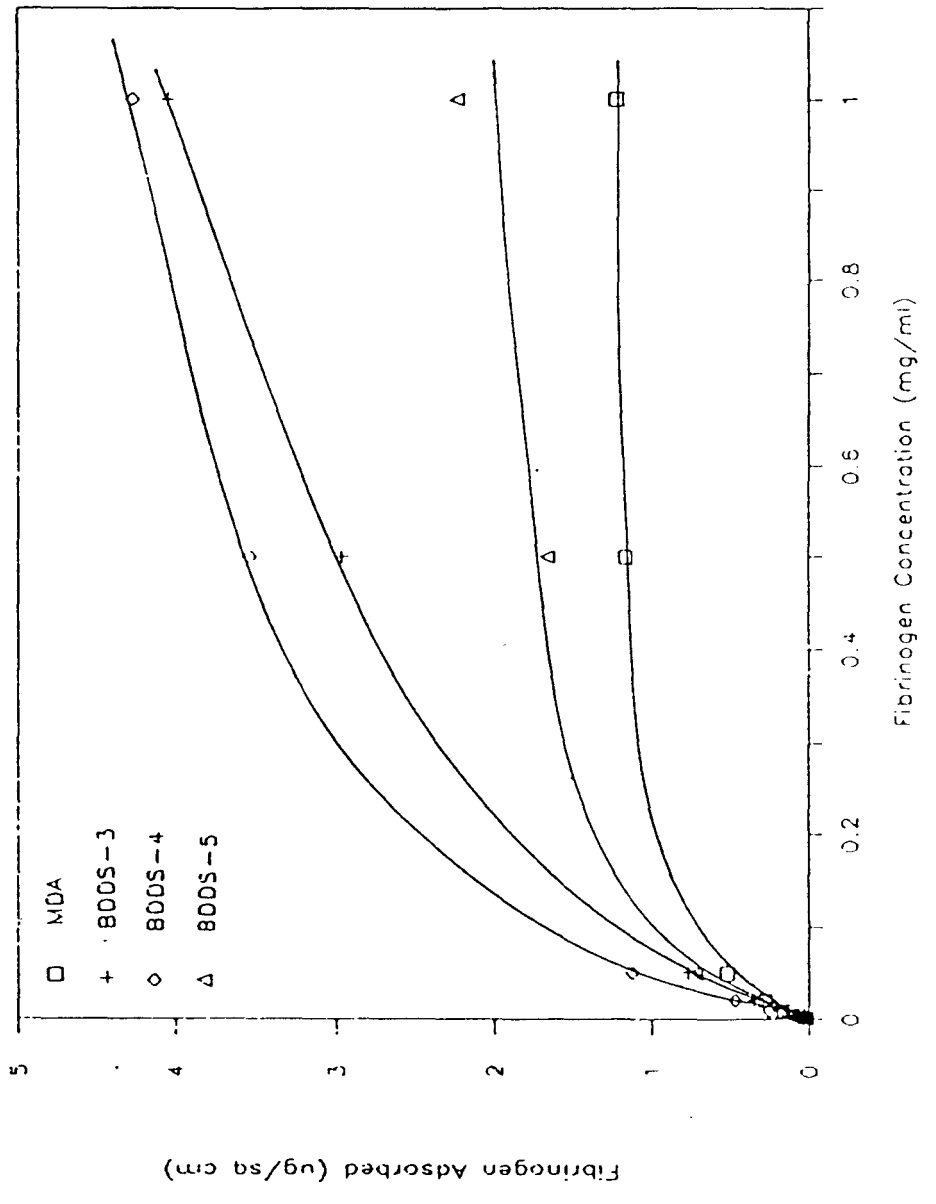


Figure 4.32: Fibrinogen adsorption isotherms from Tris buffer to polyurethanes. (Adsorption time, 3 hours).

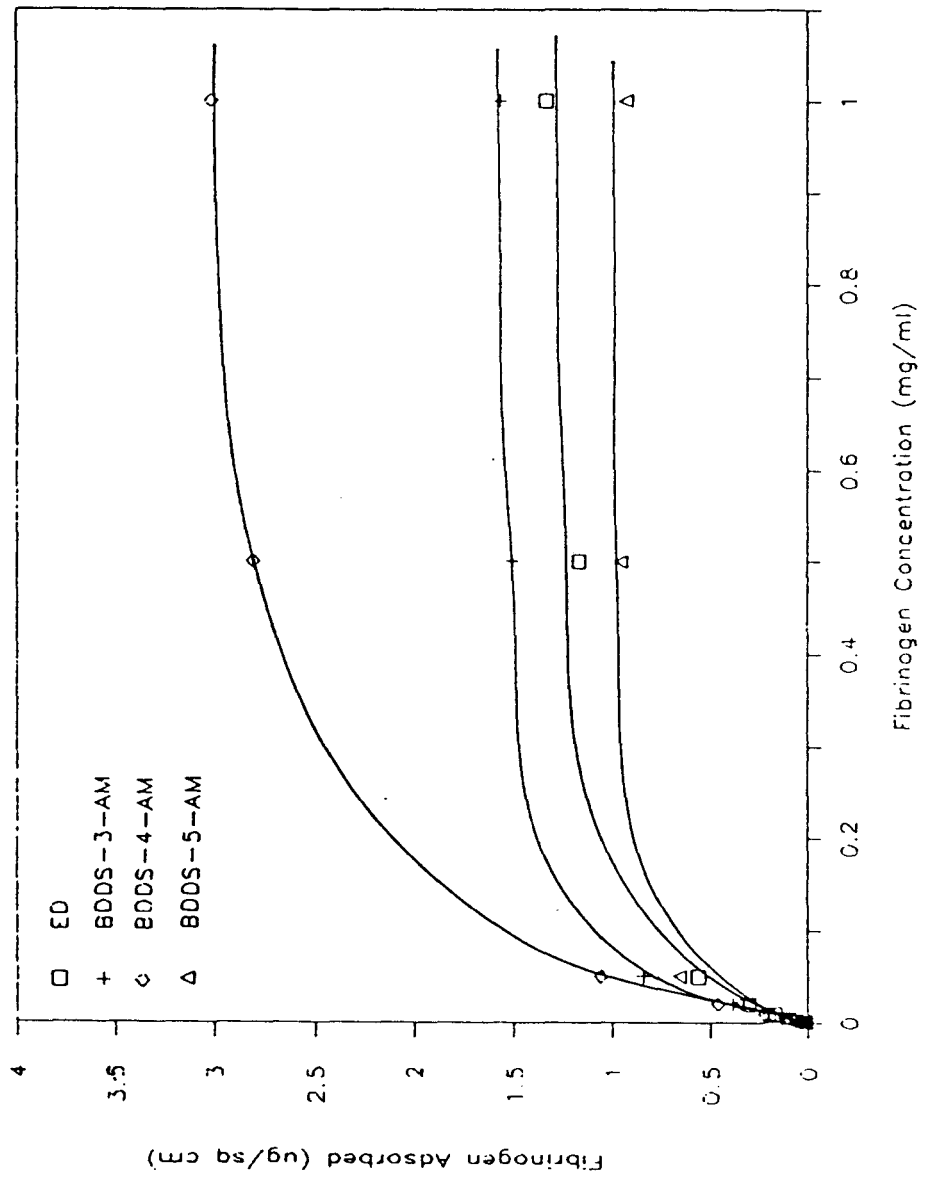


Figure 4.33: Fibrinogen adsorption isotherms from Tris buffer to polyurethanes. (Adsorption time, 3 hours).

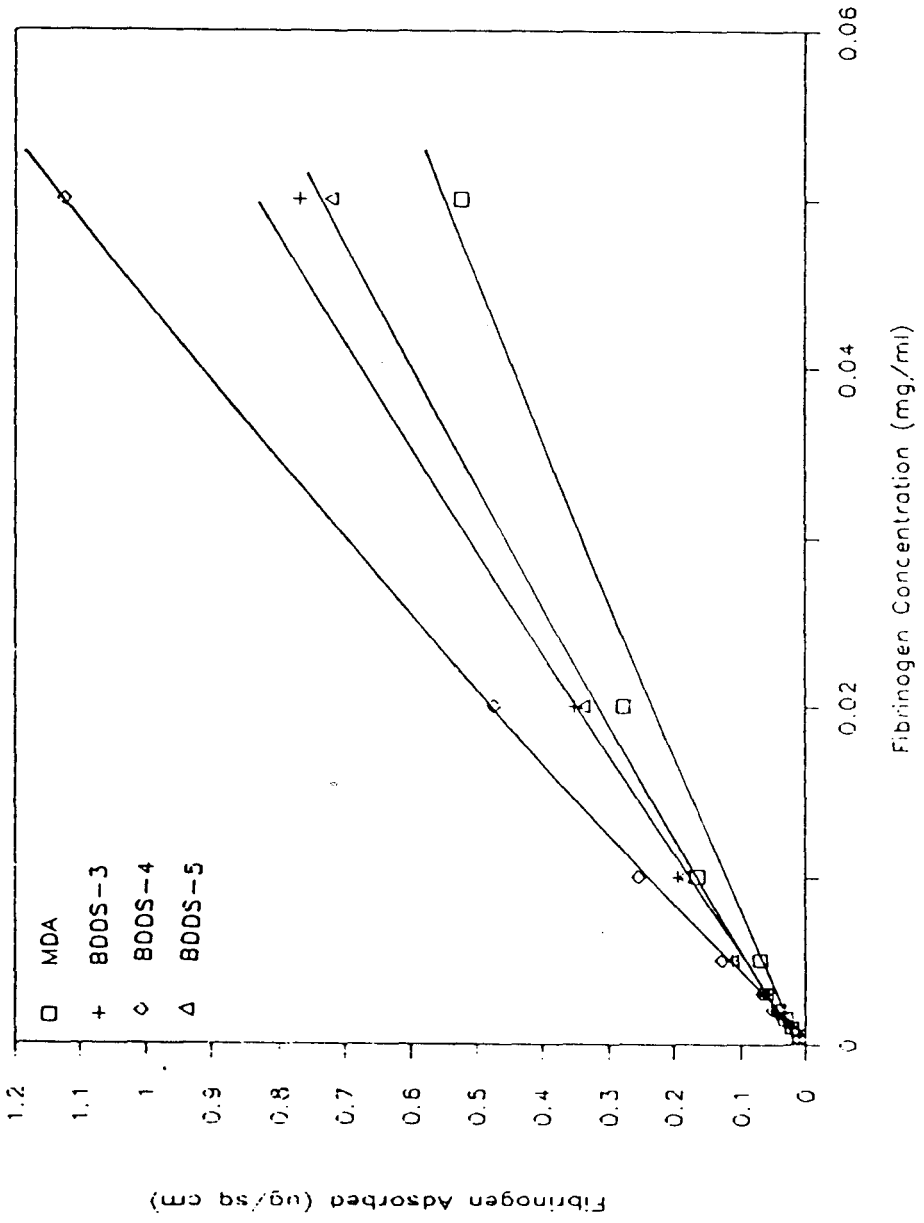


Figure 4.34: Fibrinogen adsorption isotherms from Tris buffer to polyurethanes: low concentration portion. (Adsorption time, 3 hours).

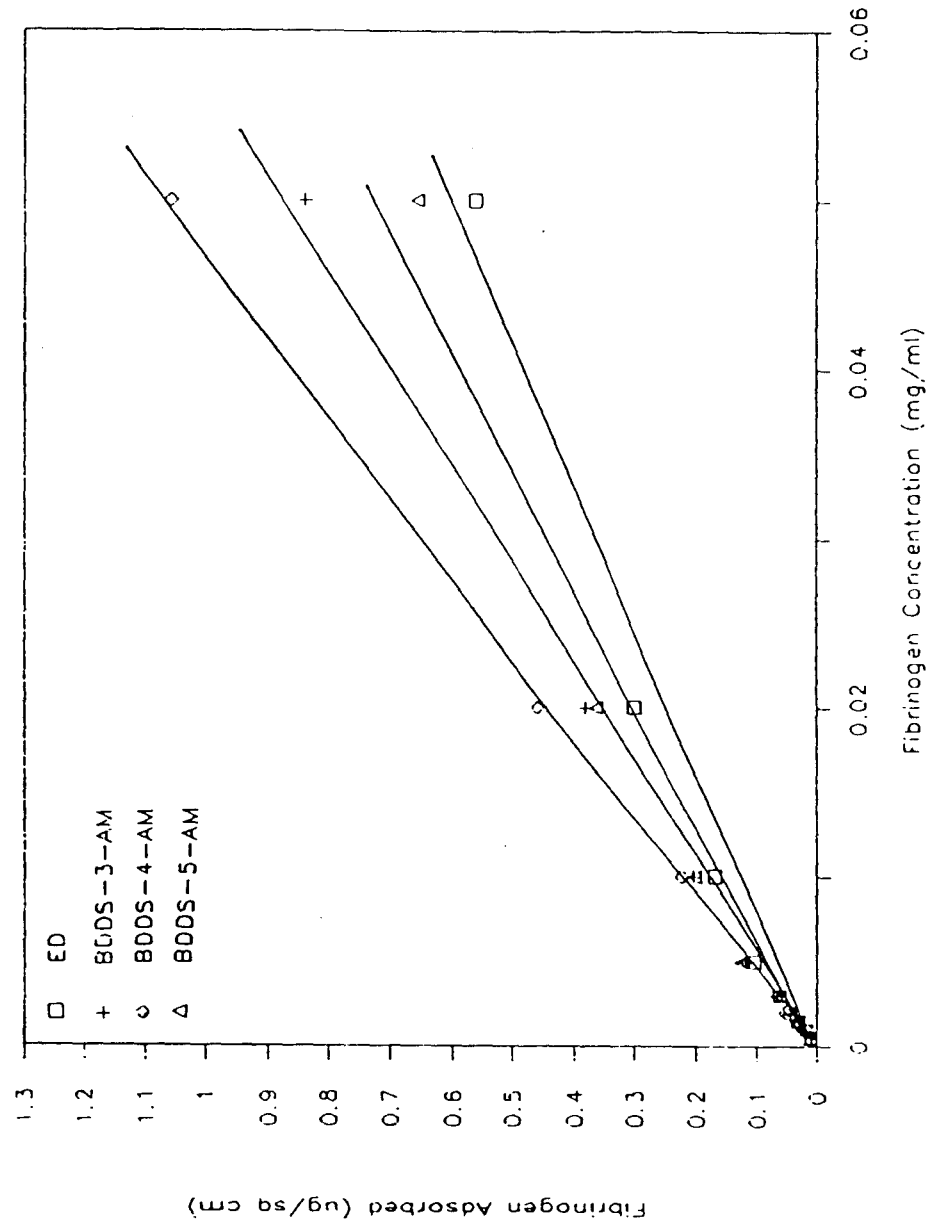


Figure 4.35: Fibrinogen adsorption isotherms from Tris buffer to polyurethanes: low concentration portion. (Adsorption time, 3 hours).

difficult to draw any definite conclusions from the initial slope data since in general the differences from polymer to polymer are not great. The slope values for the BDDS and BDDS-AM polymers, with the exception of BDDS-5-AM, are higher than those for the MDA and ED polymers. This suggests that fibrinogen has a higher affinity for the sulphonated surfaces whether AM derivatized or not, but given the limited range of data involved and the potential for dilution error at low concentrations this conclusion should be considered as tentative. The magnitude of the initial slope values parallels the levels of fibrinogen binding in the plasma experiments. The highest adsorption plateau values are shown for BDDS-4 and -3, and all the BDDS-AM polymers show lower plateau adsorption than the corresponding BDDS surfaces.

Table 4.14: Summary of Data for Adsorption of Fibrinogen From Tris Buffer to Segmented Polyurethanes. Total Adsorption Time, 3 Hours.

Polymer	Initial Slope of Isotherm ($cm \times 10^3$)	Amount of Fibrinogen Adsorbed at Plateau ($\mu g/sq\ cm$)
BDDS-3	17.4	4.1
BDDS-4	23.9	4.3
BDDS-5	16.6	2.2
BDDS-3-AM	18.9	1.6
BDDS-4-AM	22.7	3.0
BDDS-5-AM	13.3	0.9
MDA	13.7	1.3
ED	14.8	1.3

The level of fibrinogen adsorbed at the plateau is also higher for the BDDS and BDDS-AM polymers than for the MDA and ED polymers, again with the exception of BDDS-5-AM. In fact the plateau adsorptions are greater than those expected for a close packed monolayer (0.2 μg/sq cm, side on orientation; 1.8 μg/sq cm, end on orientation), suggesting either multilayer adsorption or absorption of fibrinogen into the polyurethane material structure. It is possible that the hydrophilic sulphonate groups result in a surface which is to some degree water swellable. Then the surface area which is exposed to the solution would be greater than the value used in the calculations, which is based on the nominal inner surface area of the tube. As with the initial slope data, the plateau values indicate the same trends as he plasma adsorption results, with BDDS-4 and -3 showing the highest adsorption, and all the arginine methyl ester polymers exhibiting lower levels of adsorption than their precursor sulphonated polymers.

On the basis of these results it is impossible to conclude whether or not a specific interaction between fibrinogen and sulphonate groups is taking place. Nonetheless, consistent with the plasma adsorption results, the sulphonated surfaces do exhibit a high affinity and capacity for fibrinogen. Further investigation is warranted as to the nature of these interactions.

4.4 Summary

In the initial part of this work the synthesis of segmented polyurethanes using a sulphonated diamine chain extender was studied and appropriate reaction conditions were determined. A series of three sulphonated polyurethanes covering a range of sulphonate contents, produced by varying the prepolymer and hard segment lengths, were selected for further study. These three sulphonated polyurethanes were modified to incorporate arginine methyl ester groups via sulphonamide bonds. These six polymers (three sulphonated and the corresponding three arginine methyl ester derivatized polymers) were then characterized and studied to relate both molecular and surface structures to physical and biological properties.

The polymer structure was evaluated by elemental analysis, gel permeation chromatography and Fourier transform infrared spectroscopy; attempts were then made to relate this structural information to the polymer mechanical properties. The surface structure was evaluated by ESCA and contact angle measurements. The blood response was evaluated by studying the adsorption of radiolabelled fibrinogen from both plasma and solution.

Elemental analysis confirmed the expected structure of the sulphonated polymers, showing a slight increase in

sulphonation with decreasing prepolymer length and a substantial increase in sulphonation with increasing hard segment length. Also in accord with expectations the arginine methyl ester-modified polymers exhibited a significant increase in nitrogen content compared to the precursor sulphonated polymers.

The molecular weights of the sulphonated polyurethanes were lower than those for polyurethanes prepared using the "classical" chain extender ethylene diamine. Such a decrease is expected since aromatic amines are less reactive than aliphatic amines because of electron delocalization. The molecular weight of the polymers remained the same after the arginine methyl ester attachment procedure, indicating that no degradation occurred as a result of the attachment procedure.

Fourier transform infrared spectroscopy confirmed qualitatively the presence of arginine methyl ester groups in the polyurethane, since peaks for the guanidyl and methyl ester groups of arginine methyl ester appeared in the arginine methyl ester polymers which were not observed in the precursor sulphonated polyurethanes. The combined elemental analysis, gel permeation chromatography and FT-IR data provide support for the reaction pathways expected in forming these polymers.

Mechanically these polymers have similar ultimate

tensile strength but lower elongation than classical polyurethanes. This is probably due partly to the increased microphase separation arising from incompatibility of the aromatic chain extender and the aliphatic soft segment and partly to the tendency of the ionic sulphonate groups to aggregate and form ion clusters. The incorporation of arginine methyl ester decreases the initial modulus and increases the elongation significantly and the tensile strength slightly, probably due to decreased hard-soft segment phase incompatibility resulting from the incorporation of an aliphatic side chain.

A limited amount of contact angle data indicated that the sulphonated polymers form films with hydrophilic surfaces, but further study is necessary to confirm this conclusion. The ESCA results obtained show that while the surface does exhibit soft segment enrichment and hard segment depletion there are still significant concentrations of sulphonate groups present at the surface.

The fibrinogen adsorption results for the sulphonated polyurethanes are quite different from those for "classical" polyurethanes. For the sulphonated polymers, fibrinogen adsorption "isotherms" from plasma do not show the Vroman effect, i.e. a peak in the curve of adsorption versus plasma concentration reflecting transient adsorption of fibrinogen from plasma. This effect has been reported in

the literature for a wide range of surfaces including many polyurethanes. On the sulphonated polyurethanes fibrinogen is retained on the surface at all plasma concentrations studied up to 30% normal. The arginine methyl ester modified polymers, which contain about half as many free sulphonate groups as their base polymers, show some displacement of initially adsorbed fibrinogen, but it is by no means a traditional Vroman effect since the displacement begins at a relatively high plasma concentration, continues over a wide range of plasma concentrations and is never complete. These results suggest that there could be a specific interaction between the sulphonate groups and fibrinogen.

The data for adsorption of fibrinogen from Tris buffer show very high levels of adsorption for both the sulphonated and the arginine methyl ester containing polymers, suggesting possible multilayer adsorption or absorption into the bulk polymer material. The same trends observed in the plasma adsorption data were again observed in these results, with lower levels of adsorption occurring for the arginine methyl ester polymers than for their precursor sulphonated polyurethanes.

The retention of fibrinogen on a biomaterial surface in contact with blood may be desirable as the displacement of fibrinogen involves HMWK and other coagulation factors which are important in the contact phase activation of

coagulation. However, other studies have shown that fibrinogen-coated surfaces are highly platelet reactive. Preliminary ex-vivo shunt experiments which have been performed on sulphonated polyurethanes by Cooper et al [140] indicate that while the sulphonated polyurethanes have higher levels of adsorbed fibrinogen they also exhibit a lower tendency for thrombus formation than unsulphonated polyurethanes [140]. These data and others obtained by Lindon et al [141] suggest that the most important factor in the platelet reacivity of a surface with a high level of adsorbed fibrinogen is not the quantity of fibrinogen adsorbed, but may rather be the conformation of the adsorbed fibrinogen. Thus, the specific adsorption of fibrinogen to a biomaterial surface may be desirable for blood compatibility as it may deter the contact phase activation of coagulation without causing an increase in platelet adhesion and activation.

5. SUGGESTIONS FOR FUTURE WORK

The work presented in this study has yielded some interesting results but represents only a first attempt to synthesize and characterize these functionalized polyurethanes. Further investigation in several directions is warranted, including polymer synthesis, polymer characterization and blood-polymer interaction studies. Some of these are indicated below.

Polymer Synthesis:

1. The synthesis of polyurethanes using the biphenyl diamino disulphonic acid chain extender that cover a wider range of sulphonate contents should be undertaken.
2. The molecular weight of the polymers synthesized using biphenyl diamino disulphonic acid should be increased to be sure that the molecular weight region has been reached in which mechanical properties are independent of molecular weight.
3. Alternative methods for incorporating sulphonate groups into polyurethanes should be investigated. They should include the use of other sulphonated chain extenders.
4. Incorporation of other potentially bioactive amino acids

or amino functional oligomers or polymers into the polyurethanes should be investigated.

Polymer Characterization:

1. More detailed information on the microstructure of the polyurethanes should be obtained. For example differential scanning calorimetry and dynamic mechanical testing data would give information on the extent of microphase separation.
2. Contact angle studies should be continued including further investigation of the contact angles of the hydrated surfaces and determination of underwater contact angles.
3. Other mechanical properties should be evaluated, such as creep, stress relaxation and fatigue behaviour which are important from a biomaterials standpoint.

Blood-Material Interactions

1. The interaction between fibrinogen and the sulphonated polyurethanes should be further investigated to determine if indeed a specific interaction is involved. It would be of interest to study the adsorption of other plasma proteins to observe whether the surface simply adsorbs high levels of all plasma proteins. Also elution studies with specific eluents could help to establish the fibrinogen-sulphonate binding mode.

3. The thrombotic response of blood to the sulphonated and amino acid containing polyurethanes should be evaluated directly by measurements such as thrombin clotting time and platelet adhesion.

4. Experiments should be conducted to determine whether or not the arginine methyl ester which has been incorporated in the polyurethane results in the preferential adsorption of thrombin to the surface. This behaviour has been reported for other materials derivatized with arginine methyl ester.

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