STUDIES ON THE STABILITY OF POLYURETHANE MATERIALS AND THEIR INTERACTION WITH TISSUE

A THESIS PRESENTED

BY

N. SHUNMUGA KUMAR

TO

THE DIVISION OF TECHNICAL EVALUATION OF BIOMATERIALS
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN THE SUBJECT OF
BIOMATERIAL SCIENCE AND TECHNOLOGY

SREE CHITRA TIRUNAL INSTITUTE FOR
MEDICAL SCIENCES AND TECHNOLOGY
THIRUVANANTHAPURAM

NOVEMBER 1991

The thesis

entitiled

Studies on the stability of polyurethane materials and their interaction with tissue

submitted by

N.Shunmuga Kumar

For

Doctor of Philosophy

in

Biomaterial Science and Technology

of

SREE CHITRA TIRUNAL

INSTITUTE FOR MEDICAL SCIENCES & TECHNOLOGY

THIRUVANANTHAPURAM

evaluated and approved

by

DR.M.JAYABALAN
(Guide)

Viva - Voce Board

1.
2.
3.

CERTIFICATE

I N.Shunmuga Kumar hereby certify that I had personally carried out the work depicted in the thesis entitiled " Studies on the stability of polyurethane materials and their interaction with tissues " except where external help sought are acknowledged.

N.SHUNMUGA KUMAR
Date : 23-11-91.

DR.M.JAYABALAN

(Division of Technical Evaluation of Biomaterials, Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences & Technology, Poojapura, Thiruvananthapuram-695012)

(Declaration)

This is to certify that Sri.N.Shunmuga Kumar in the division of Technical Evaluation of Biomaterials of this institute, has fulfilled the requirements of the regulations relating to the nature and prescribed period of research for the Ph.D degree of the Sree Chitra Tirunal Institute for Medical Sciences & Technology, Thiruvananthapuram. The work relating to his thesis entitiled ' Studies on the stability of polyurethane materials and their interaction with tissue ' was carried out under my direct supervision.

DR.M.JAYABALAN
(Guide)

CONTENT

ACKNOWLEDGEMENT

I wish to place on record my indebtness to my Ph.D guide and Doctoral advisory committee Chairman Dr.M.Jayabalan, for his guidance ,critical analysis of my work,encouragement and constant support throughout my work. I wish to express my gratitude to my doctoral advisory committee members, Dr.C.P.Sharma and Dr.A.Jayakrishnan of this institute for teaching courses and for their useful suggestions.

My sincere gratitutde is also extended to Prof.M.S.Valiathan, Director, Mr.A.V.Ramani, former Head, BMT wing and Dr.G.A.Nair, Registrar of this institute for providing facilities, adminstrative helps and encourgements for fulfilling this task.

I thank Mr.K.Rathinam, Dr.C.C.Kartha, Dr.Mira, Dr.T.V.Kumari, Dr.A.V.Lal, Dr.J.Shanmugam, Mr.Ravindranath of this institute and Dr.Prabha,Regional Cancer Centre, Trivandrum, and Dr.Prasanna Kumar, Medical College, Trivandrum for their assistance in biological experiments.

I thank Dr.K.Sreenivasan and Dr.P.D.Nair of this department for their cooperation throughout this work.I thank Mrs.S.Vasanthi Artist, for her help.

My sincere gratitude to Dr.P.C.Deb , Director, NCML, Defence R & D Organ., Bombay., for the encouragement during the preparation of thesis.

Finally, I extend my sincere appreciation to all the members of my family for their patience, tolerence and continuous support.

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES

SYNOPSIS

Among a large number of polymers available for biomedical applications, polyurethanes are an interesting family of materials. The widely varying physical and chemical properties, superior mechanical properties and biocompatibility made them so appealing for biomedical applications. Generally polymers intended for long term application should be totally inert. Unfortunately most of the polyurethanes available us on today for biomedical uses undergo delayed degradation and leaching affecting both biocompatibility and biofunctionality of the devices inspite of their excellent performance in short term use. This undesirable property also results in adverse tissue interaction. Such failures are either due to biological molecules attack or biomechanical stress. Failures of soft tissue implants, indwelling polyurethane catheters, polyurethane heart valves etc. are still a challenge to biomaterial scientists. Therefore it was our frontic effort to prepare a new crosslinked polyurethanes, study the various parameters which influence the stability and to understand the nature of tissue interaction with crosslinked polyurethanes.

The introductory chapter begins with the brief historical background of biomaterials. This chapter also deals with the general requirements of polymers used in biomedical application. The relation between physico chemical and surface properties and biocompatibility and biofuntionality of the biomedical

device/materials is highlighted. This chapter also highlights the special qualities of polyurethanes used in the biomedical application.

Chapter 2 deals with the background of the investigation with special reference to biocompatibility and stability of polyurethanes used in biomedical applications. The various material failures associated with the polyurethanes used in blood and tissue contact applications are reviewed. The degradation mediated by the attack of biological molecules such as enzymes, lipids and also by the biomechanical fatigue is presented in this chapter. The failures due to problems associated with the material-tissue interaction are also presented in this chapter.

Chapter 3 deals with the aims and objectives of the investigations. The importance of synthesis of new aliphatic crosslinked polyurethane and the need for the study of stability and interactions of crosslinked polyurethanes with tissue are narrated.

Chapter 4 deals with the materials and methods used and analytical facilities employed for the studies. Synthesis of various aliphatic crosslinked polyurethanes are presented. Polyurethanes were synthesized using diisocyanates, such as IPDI,SMDI and HDI and polyols such as poly(tetramethylene oxide) glycol, poly(propylene oxide)glycol,poly(ethylene oxide) glycol. The crosslinker used for the above synthesis was

trimethylol propane. The technique for preparing porous polymers for the studies on material-tissue interface are also mentioned. The methods of preparation of the implants and various biological tests for biocompatibility are also given. Experiments on sterilizability and stability of polyurethane under various chemical and biochemical environment are detailed. The fungal susceptibility tests and in vivo stability of the polymers in rat are also narrated. A detailed procedure for the in vitro studies on material - cell interaction as a part of material-tissue interaction is also presented. The studies on the tissue response of polyurethane implanted in biomechanically different implant sites in pig are also narrated.

Chapter 5 deals with results and discussion. This chapter contains five sections. First section deals with the chemistry of polyurethane synthesis. The rationate behind the formation of biuret links and its quantitative estimation by amine decomposition method are discussed.

The second section deals with the physico - chemical characterization. The change in density and MC of the polyurethanes with respect to the formulations are discussed. The tensile strength, percentage elongation, modulus and hardness are discussed in terms of their variation in the composition (hard segment content) and MC. The variation of surface parameters such as surface energy, polar and dispersion components of surface free energy with respect to composition of

the polymers are also discussed. The water absorption studies highlights the degree of hydrophilicity/hydrophobicity of the polymers. The infrared spectra of polyurethanes are discussed. The formation of hydrogen bonding in polyurethanes is discussed. The change in thermal properties of the polyurethanes with respect to their composition is also discussed .

The third section deals with the biocompatibility studies. The results of various biological tests such as hemolysis test, recalcification time test, systemic toxicity test, intracutaneous irritation test are discussed. The histotathological results of intramuscular implantation test in rabbit are discussed in detail.

The fourth section presents the studies on the stability of synthesized materials. The first part of this section deals with sterilizability of polyurethanes as evaluated by autoclaving and γ-radiation. The change in mechanical properties and swelling properties are correlated with the variation in hard segment content and molecular weight between crosslink.

The second part this section deals with the stability in different chemical and biochemical environment. The hydrolytic stability of the polymers are determined in water and in different ionic environment such as phosphate buffered saline, peseudoextracelluar fluid. The catalytic hydrolysis of the synthesized polyurethane also discussed. The result of catalytic transamination and catalytic transesterification are presented to

ascertain the esterifying and aminification potential of the material. The stability of the polyurethane in methanol, which is used in extracting low molecular weight components is also discussed. The oxidizing potential of the polyurethane material is also evaluated using a mild oxidizing agent(sodium oxychlorite). The stability of polyurethane in enzymes and lipids is also discussed.

The third part of this section is concerned with the results of the studies on calcification. The calcifying potential of the polyurethane is discussed. The effect of stress on the calcification is found to be higher in stressed condition in comparing to the unstressed condition.

The fourth part of this section is concerned with the results and discussion of in vivo stability of polyurethane after implantation in rats 4 months. The change in mechanical properties of the retrieved implants is discussed. The SEM studies on a implanted sample are also discussed. This studies revealed the appreciable stability in the SMDI and HDI based polyurethanes in comparison with the IPDI based polyurethanes.

The fifth section of the results and discussion deals with the studies on material-tissue interaction. The first part of this section deals with the results of the in vitro studies on material -cell interaction and material extract-cell interaction using L-929 fibroblastic cell lines. The studies indicate appreciable cell adhesion and growth with test polyurethanes.

The second part of this section is concerned with the studies on tissue interaction of synthesized materials implanted in biomechanically different tissue sites. The results of the studies on the effect of tissue reaction in maximal motion site and minimal motion site are discussed. The effect of material biostability and implantation site on material-tissue interaction is discussed.

The last chapter summarizes the results and discussion. The conclusion and future area of investigation are presented.

INTRODUCTION

I N T R O D U C T I O N

Biomaterials are as old as medicine itself. Materials have been implanted in the human body for the surgical purposes for many centuries. As early as the 17th century Hieronymous and others used gold, bronze, iron wires for sutures . Nearly 4000 years ago the Egyptians used linen, a natural polymeric material, for suturing wounds. About 600 B.C the Indians used leather in repairing wounds. Undoubtably there were earlier examples for prosthetic application. However not until recently the techniques of the modern surgery have allowed more or less routine replacement of many fault and diseased part of the body.

Polymer as Biomaterial :-

Many of the remarkable medical achievements made in recent years are due to the modern materials like ceramics, metals, composites and polymers. Among which polymers are widely used for biomedical application due to its favorable mechanical, thermal, electrical and optical properties. The other important characteristics of polymers are light weight, flexibility, resistance to impact and breakage, and lower unit cost.

All living matters consist of primarily polymers. The biological polymers have been used for healing purposes. Although nature provides a huge selection of different polymers, it is sometime difficult to select a natural macromolecule which would fulfill all demands of pharmacy and medicine.Progress in macromolecular chemistry had led to the hope that synthetic

polymers could be tailored to fit in desired functions in the body better than natural polymers. However, synthesis of a new polymer for a special purpose requires complete theoretical understanding of the role of the synthetic polymer that would play in the body.

Biocompatibility of polymers:-

The main requirement of any polymeric implant is biocompatibility. The physical and chemical properties of polymers are governed by the structure, molecular weight and orientation of polymer chain[1-3]. All the factors should be considered for biomedical application [4,5]. The surface properties which govern biocompatibility include critical surface tension, surface composition, roughness and the presence of impurities on the surface. There are many controversies regarding the value of polar and dispersive components of surface energy of polymers required for biocompatibility[6-12]. Now it has been accepted that the value of polar and dispersive components should be around 3.0 $[dyn/c.m]^{1/2}$ and 4.7 $[dyn/c.m]^{1/2}$ respectively for the blood contact application [13]. Zeta potential value approaching extremely low value has ambiguous relevance to biocompatipility[14]. The groups which easily form hydrogen bonds present on the surface have strong tendency to interact with biological components resulting in high thrombogenicity[15]. Surface roughness is one of the important factors which also influences biocompatibility. The cells are very sensitive to surface roughness. Surface texture is also an important property

which affects biocompatibility of the devices[16].

The important mechanical properties which influence the biocompatibility are hardness, elastic modulus, dilation modulus and flex life. Hard surfaces are not good for tissue contacting application particularly at maximal motion site . Similarly elastic modulus, compliance and dilation modulus are important parameters for vascular graft [17,18]. The elastic modulus of the implant is important in the modeling phase of the wound healing, and the mismatch of the elastic modulus of implant and tissue creates stresses and movement at the interface. Soft tissue materials with higher moduli produce a thicker capsule. The presence of a higher modulus implant in bone also affect ,the surrounding tissue[19].

There is no evidence that the thermal properties are directly related to biocompatibility. The important thermal properties of polymer by which one can try to link with biocompatibility are T_g (glass transition temperature) and thermal activation energy. At T_g there is no chain mobility other than vibration about a fixed position. If the T_g lies at around body temperature then the properties of the material are not as expected and the interaction with biological system is of course different[20]. Many attempts were made to relate the activation energy of the polymer to carcinogenesis. However, there are lot of exceptions in this correlation[21].

The electrical properties and their relationship with biocompatibility are not fully understood. Generally intrinsic semiconducting materials have low thrombogenicity[22-23].

Size,shape and porosity are also important parameters for determining the biocompatibility. As the size of the implant increases the foreign body reaction also increases. So minimum surface area of contact is advisable [20]. The shape is equally important like size. The implant with minimum pointed corners have minimum foreign body reaction. It is well known that triangular implant has more foreign body reaction than the rectangular implant, and circular implant has least foreign body reaction[24,25]. Porosity is another important parameter determining blood compatibility and tissue compatibility. Material porosity is considered both as bulk as well as surface property. The size of the pores in polymers for biomedical applications ranges from about 1 to 200 μm. For vascular graft the optimal pore size has been found to be in the range of 20 to 50 μm and for the soft tissue ingrowth pore sizes in the range from 100 to 200 μm have been found to be suitable for mechanical interlocking[26-32].

Absorption and leaching are the important parameters influencing biocompatibility. Water sorption may results in expansion or shrinkage. It causes interfacial problems like bacterial penetration etc. Some biological molecules are easily absorbed by the polymer through diffusion resulting in

degradation of the polymer matrix . Similarly, degraded products inside the polymer matrix may easily leach out and cause cytotoxic problem[33].

The biomaterials should be sterlizable, hydrolytically stable, processable and corrosion and wear resistant. For biomedical applications the polymers should be either totally inert or totally biodegradable. Unfortunately, most of the polymers fall between these two extremes affecting both biocompatibility and biofunctionality of the medical devices.

Polymer degradation in the living body may be interpreted as an interaction between the organism tissue components and a material acting as foreign body. Theoretically, the degradation process could be catalyzed by free amino acids, hormones, vitamins and low molecular weight compounds. However, their content in the tissue is extremely small and their catalytic activity might be negligible. Thus the information available on long term degradation of implant materials in the presence of these biological components is scarce.

Polyurethane as biomaterials:-

Biological application of polyurethanes go back to the early 1960 s [34-39]. Since the initial unpromising results might be due to the presence of impurities, additives, catalyst, residues and polymer structural irregularities in the commercial polymers used, they gave rise to a common clinician's opinion that polyurethanes have doubtful qualities as implant materials in

surgery [38]. However extensive research on polyurethanes has led to the development of more promising biocompatible polymers.

Among polymers for biomedical applications, polyurethanes are an interesting family of materials with broad possibility for processibility, superior physical and mechanical properties and biocompatibility which made them so appealing for biomedical applications. The polyurethanes used in various biomedical applications are given in table 1.1. Polyurethane elastomers have also been successfully applied in heart assist devices, in the fabrication of tubing for hemodialysis units, intravenous feeding kit and in the construction of blood bags and solution containers.

Polyurethanes used in biomedical device should fulfill the following requirements[40].

1. It should be reproducibly produced as pure material.
2. It should be fabricated into the desired form without being degraded or adversely changed.
3. It should be sterilized without change in properties or form
4. It should have the required chemical, physical and mechanical properties for performing their functions.
5. It should not induce inflammatory and foreign body reaction.
6. It should not be carcinogenic, mutagenic, teratogenic or toxic.
7. It should be stable throughout their residence in the physiological environment.

Table 1.1 Various Appilcation of Polyurethane

Total artificial hearts	Heart valves
Vascular prostheses	Pericardial patchs
Vascular stents	Intraaortic ballons
Mammary implants	Ureteral prostheses
Fallopian tubings	Adhesives
Orthopedic casting tapes	Dialysis membranes
Filters in Oxygenators	Meniscus reconstruction membranes
Endovascular embolization	Pacing leads insulation
Angioplasy baloons	Gastric baloons and feeding tubings
Catheters and cannulas	Sutures ,ligaments
Wound dressings and drape	Blood bags
Peripheral nerve repair device	
Shock Absorbing elements for root	
Liners in dentistry and paradental membranes	
Implants for craniofacial and maxillofacial application	
Oseophagal and tracheal prostheses	
Roller pump tubings in artificial heart or blood pumps	
Enveloping membranes for soft organ fixation	
Endotracheal tubing	

At present there are no polymers available which meet all these specifications. It is also believed that some extent of interaction between the materials and it's environment is required if the material is to be performed effectively[41]. Required properties of polyurethanes are usually obtained by using additives (antioxidants, stabilizers, plasticizer, opacifier) by polymer orientation, crystallization, introducing cross links and pendent groups, and by blending materials with high molecular weight and copolymerization etc. All these additives, if not removed in biomedical polyurethane, not only affect biocompatibility but also induce tumor [21]. Other structural morphology affect to a greater or lesser extent the biocompatibility[42].

Polyurethanes are quite stable for the short term applications. However in the long run polyurethane components are deteriorating under the influence of physiological environment affecting both biocompatibility and biofunctionality.

Material - Tissue interface :-

The events occuring at the material tissue interface are prime importance for the incorporation and durability of polymer implants [19,43]. The problems associated at the material- tissue interface are mediated primarily through the chemical and physical characteristic of the surface. Although specific tissue response is dependent on bulk mechanical properties, size and shape are known to be important. The ability of the wide variety

of materials to produce a wide range of host response must depend on critical events at the interface[44].

Most materials in contact with soft or hard tissue elicit a response consisting of inflammation and subsequent fibrosis[45 -51]. It may be desirable to control this response for the better functioning of the device or for the comfort of the patient. Primary factors, which can be used to determine this reaction, have been identified and examined systematically. These are the content of water at the implant surface, the charge distribution, surface free energy, and the relative movement of implant and tissue[44]. These parameters determine the specific characteristics of what appears to be nonspecific response in the tissues. The reaction is mediated through protein deposition which is the immediate interfacial response in tissue fluid. Subsequent cellular reactions are due to the changes in this protein layer. It may be desirable in some case to promote or prevent direct adhesion of the tissue to the implant. Examples of characteristics which will promote interfacial adhesion in soft tissues are increasing surface water and certain counter ions in hydrophilic polymers. The direct attachment of bone is facilitated by controlled surface active glasses and ceramics which mimic the structure and composition at the bonding interface. Although adhesion may be promoted by manipulation of surface characteristics, it will not be effected unless the relative movement of implant and tissue is prevented[52].

The potentially most catastrophic consequence of a long term biomaterial-tissue interaction would be a malignant tumor. Such potential has been demonstrated in experimental rodent models. However, Homer Lawerence has summarized some material associated tumors in human, although a cause and effect relationship has not been demonstrated in humans[52a]. It is clear that induction of a malignant tumor in rodents depends on the size and shape of the foreign body, whether the foreign material is fibrous or solid, whether it is in contact with susceptible tissues, and also on the chemical nature of the material[52a]. It is likely that a common mechanism exists for fibrous tumor induction by the solid materials which depends on the interaction at the interface.

The factors governing the stability of the polymer - tissue interface are

1. Nature of the material
2. Stability of the material
3. Cell / biological components interaction with the material and
4. Biomechanical factors.

The problem associated with the stability of polyurethane and it's interaction with tissue attract the attention of biomaterial scientist due to the need for the development of more reliable biomedical devices for long term use.

BACKGROUND LITERATURE

BACKGROUND LITERATURE

This chapter highlights background of the present investigation. The factors which affect the biocompatibility and biostability of polyurethanes, the problems associated with the use of polyurethanes and failures of polyurethane implants in medicine and surgery are precisely reviewed.

Structure - Property (biocompatibility) relationship in polyurethanes

Bulk and surface properties of polyurethanes are governed by the molecular architecture, which influences the biocompatibility. Extensive research works have been carried out to explore the structure-property (biocompatibility) relationship of polyurethane for biomedical applications.

The polyol present in the polyurethane plays an important role in determining biocompatibility. Lemm found that polypropylene glycol (PPG) based polyurethanes are more thrombogenic than the polyethylene glycol (PEG) counterpart[53]. Interesting studies were carried out by Lelah et al who found that the PEG based polyurethane is more thrombogenic than the corresponding PPG and polytetramethylene glycol (PTMG) based materials[54]. Takahara et al have indicated that PPG (Mn 1000),PTMG (Mn 2000) and PEG (Mn 600) based polyurethanes are more blood compatible than the other polyurethanes of their corresponding polyol series[55]. Lyman et al synthesized polyurethane based on diphenylmethane diisocyanate (MDI), PPG (Mn

= 425, 710, 1025 and 2025) and ethylene diamine / hexamethylene diamine[56]. Tissue culture experiments using fibroblastic cells indicated the normal growth on the surface of their PPG (Mn 710) based polyurethane when compared with others. They also made similar studies on segmented copolyurethane with bony hamster kidney cell culture in vitro[57].

Brash et al found that the fibroblastic cell growth on the polyester urethanes(estane) is slightly lesser and the platelet adhesion is higher than on the PPG or PTMG based polyetherurethanes[58].

Phase separation also influences the biocompatibility of polyurethanes. It is governed by the molecular nature of the groups present in the matrix. The surface morphology is often controlled by the microphase separation. Takahara et al found that the microphase separation in polyurethane urea is strongly influenced by the number of methylene units in the diamine(chain extender)[59]. The domain size of the hard segment of this polyurethane increases slightly with increase in diamine length. The ethylene diamine chain - extended polymer is more thromboresistence when compared with the butanediol chain - extended polyurethane. It is due to the higher phase segregation of the former[59].

Cooper et al synthesized a series of polyurethane with varying different types of soft segment and/or hard/soft segment ratio to study the effect of phase separation on

biocompatibility[60]. They found that the hard to soft segment ratio in PEG and polydimethyl siloxane (PDMS) based polyurethanes does not influence the blood compatibility. With PTMG and PPG based polyurethanes , material containing relatively large amount of soft segment and possessing large levels of phase separation, causes the formation of relatively large thrombus during blood contact.

The effect of alkyl groups in the polyurethane structure on the microphase separation and biocompatibility was studied by some researchers in recent years. Pitt et al grafted alkyl chains containing 2,10 and 18 carbon atoms to 10% of the urethane nitrogen in a polyether urethane[61]. They found that as the alkyl chain length increases upto C18 the polymer surface becomes less polar and the total amount of human serum albumin adsorbed in the first four hours decreased with the increase in chain length.

Like flexible polymers polyurethanes also reorient in certain circumstances[62]. The dynamic nature of the surface plays an important role in influencing the blood compatibility. Vacuum spectroscopy has been used to study the surface of biomer and avocothane[63,64]. The role of these polymer surfaces on the coagulation of blood has been studied by Lyman et al[65-67].

The surface morphology of polymer is often varied with the type of casting technique. Sung et al[68,69] and Ratner et al[70] studied the effect of different casting techniques on the surface chemical composition of two types of segmented polyurethanes

using FTIR spectroscopic and ESCA studies. The casting solvent also has some effect on biocompatibility[54]. It would be expected that solvent polarity, volatility and viscosity would be among the factors that influence surface structure and composition on casting. Sevastinor et al studied the effect of molding characteristics on the surface structure of polyurethanes and their relation with blood compatibility[71]. The cleaning and extraction of polyurethane also determine the biocompatibility of polyurethane. Grasel et al studied the effect of extraction on bulk and surface properties of polyurethanes[72]. It is found that the methanol extraction improved the blood compatibility[54]. We have found that, for some linear Isophorone diisocyanate based polyurethanes, methanol extraction leads to degradation of the macromolecular chain[73].

Beahan et al[74], Kohjiya et al[75] and Fialta et al[76] synthesized polyurethanes using MDI. In vitro and in vivo studies showed that they are biocompatible for short term application. Tanzi et al synthesized a heparinized blood compatible polyurethane[77]. Boretos et al evaluated the in vivo biocompatibility of polyurethane (Lycra) [78]. Pollock et al[79], White et al[80] studied the effect of pore size on the biocompatibility of porous polyurethane in rat for 12 weeks.

Sharp et al studied the effect of concentration of carbon in bioelectric polyurethane based on polyesterurethane on blood compatibility [81]. They found that 10% carbon containing

polyurethane is better than 5% carbon containing polyurethane for blood contacting application. Hull et al implanted crosslinked polyurethane in dorsum of canines, goats and pigs for 12 months[82]. They found a mild tissue reaction in the absence of infection. The blood compatibility of a biomaterial mainly governed by the nature of the prosthetic surface[83]. The electrical properties of some polyurethanes have been studied for blood contacting application[84-86].

Protein adsorption is an important initial event taking place at material-blood/tissue interface. Several mechanisms were suggested for the protein adsorption on the polyurethane surface[87-89]. Anderson et al studied the in vivo biocompatibility of biomer and other materials by cage implantation technique[90-93].

Failures and problems associated with polyurethane used for biomedical application.

Problems associated with processibility of polyurethane:-

Aromatic polyurethanes are very sensitive to the processing condition[94]. During extrusion if the temperature is slightly increased above the processing temperature, the polymers easily undergo degradation. It is well known that aromatic polyurethanes are susceptible to UV and Visible light. The exposure of aromatic polyurethane to sunlight results in a concurrent loss of mechanical properties and discoloration. Brauman et el evaluated the light stability of various polyurethanes and

polyurethaneureas[95]. The order of stability from their findings is : Aliphatic polyurethane > Aliphatic amine extended polyurethane urea > Aromatic amine extended aliphatic polyurethane urea > aromatic polyurethane > Aliphatic amine extended aromatic polyurethane urea > Aromatic amine extended aromatic polyurethane urea.

Chu et al evaluated the effect of additives on the sunlight stability of polyurethane elastomer for maxillofacial application[96,97]. In 1978 Darby et al found the presence of methylene diamine (MDA) in the aqueous extract of pellathane. MDA is an aromatic amine analog of MDI[98]. MDA is a carcinogen, Mutagen and is very toxic[98,99]. There are two ways for the amine formation. They are

$$R\text{-}OOC\text{-}NH\text{-}R\text{-}NH\,COO\text{-}R \xrightarrow{150\text{-}200^{\circ}C} H_2N\text{-}R\text{-}NH_2 + CO_2 + CH_2{=}CHR$$

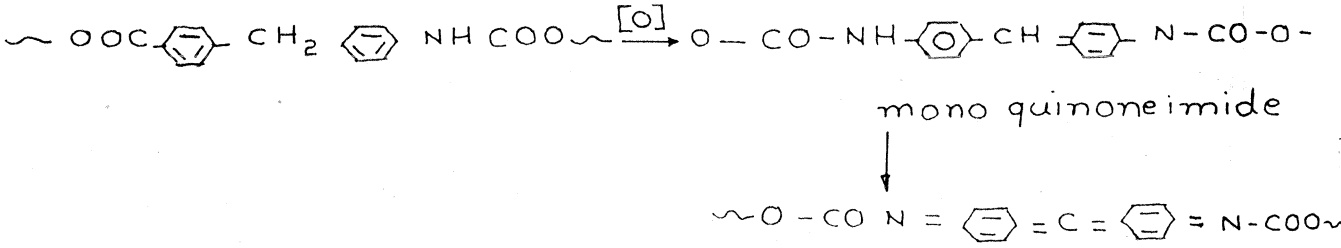
$$R\text{-}OO\text{-}NH\text{-}R\text{-}NH\text{-}COOR \xrightarrow{150\text{-}200^{\circ}C} OCN\text{-}R\text{-}NCO + HOR$$

$$OCN\text{-}R\text{-}NCO + 2H_2O \xrightarrow{150\text{-}200^{\circ}C} N_2\,N\text{-}R\text{-}NH_2 + 2CO_2\uparrow$$

The first mechanism involved is thermal degradation. The second mechanism is thermohydrolytic decomposition which can only occur in the presence of moisture[100]. MDA has been found in trace amounts in aromatic polyurethane when these polyurethane are subjected to a single steam sterilization cycle at 120C°[101]. Mazzu and Smith found 3 to 5 ppb MDA in aqueous extracts of polyurethanes under prolonged steam autoclaving[101]. Works of

O. Mara et al also confirmed the presence of MDA in the aqueous MDI based polyurethanes[102].

Upon exposure to UV radiation or heat MDI based polyurethanes first develop a deepening of colour which change from a very yellow to amber and to a dark brown on extensive exposures [103]. The colour change is due to the production of diurethane bridges formed from aromatic diisocyanates The diurethane bridges are capable of autooxidation, resulting in a quinone-imide structure.



The best way to improve UV stability of aromatic polyurethane is to incorporate antioxidants or UV stabilizers. However in the long run these additives cause cytotoxic problems.

Degradation in the physiological environment:-

Polyurethanes are inherently stable in the body environment. However polyurethanes components are deteriorating under the influence of minerlization, environmental stress cracking oxidation and hydrolysis[104-105].

Generally polyester urethanes are susceptible to hydrolysis

and because of this property it's use in the biomedical field is very limited[106]. The hydrolytic instability of polyesterurethanes are due to the highly strained molecular configuration[107]. The polyether urethanes are relatively insensitive to moisture[107]. While thermoplastic segmented polyurethanes have been valuable in producing medical devices mainly for blood contacting application the crosslinked polyurethanes have received more attention for long term surgical implants. Kolff et al compared the stability of polyurethane VC (linear polyurethanes) with polyurethane sponge (crosslinked polyurethane) after 6 months implantation in dogs[108]. They found no loss of mechanical strength with the polyurethane sponge when polyurethane VC lost 50% of its tensile strength.

Dunkel et al evaluated the in vitro stability of polyurethanes (Biomer, Pellathane 80A, PUX and Avocothane 51) in bovine blood[109]. All the polymers showed some degradation after 52 weeks immersion in blood; the degree of degradation is less in polyetherurethanes. Lemm et al have found that the average molecular weight of biomer decreased to 50% of its original value after enzymatic exposure for 6 months and approximately 20% after 6 months implantation[110-111]. In vitro experiments of Chawla et al found that polyether insulation used in cardiac pacing leads is susceptible to oxidative degradation[112].

A number of hypotheses have been developed to explain the nature of biodegradation. They are enzymatic chain cleavage,

simple oxidative chain cleavage, autooxidative processes involving metal catalyzed peroxide decomposition, metal-catalyzed and metal ion - catalyzed polymeric break down, stress cracking, auto oxidative process etc. Differences in the degree of these degradation are reported. Lemm and Bucheral considered changes in surface properties or loss of mechanical strength as biodegradation[113]. Potts et al defined biodegradable material as those which, because of their chemical structure, are susceptible to be assimilated by microorganisms such as fungi and bacteria[114].

Sufficient mechanical strength is required for polymers used in biomedical application. It is supposed that sorption of body fluid components may have direct effect on the mechanical properties of biomedical elastomers. It has been reported that silicone rubber heart puppets used in prosthetic heart valves degrade in vivo due to the absorption of lipid from blood[115-118]. Variety of enzymes such as hydrolytic , proteolytic, oxidative enzymes present in the body are also responsible for degradation of polyurethane . Huang et al[116] defined biodegradation as enzyme induced degradation. Marchant et al[91] presented biodegradation as "degradation occurs on many different structural levels ie molecular, macromolecular, microscopic and macroscopic depending on the mechanism ''. Works of Ratner et al indicate that all the macromolecules slightly undergo degradation in the presence enzyme[118]. Williams and his

workers studied the degradation of polyurethanes using a variety of enzymes[119-122].

Leinmain and workers studied the changes of properties in various polymers during implantation[123]. They found that polyurethane film is disintegrated in dogs within 16 months. Boretos et al implanted polyurethane (Lycra) in mice, dogs and rabbits for studies on tissue reaction[124]. The specimen which have been implanted in dog for 18 months exhibited stability. They used estane(polyester urethane) as control. After 4 months the colour of the estane material has turned to a deep yellow, deteriorating progressively throughout the 18 months test. The material become badly fragmented that less than 20 % of any specimen could be found. Bichnon et al evaluated the biostability of polyester based polyurethane coated catgut sutures by subcutaneous implantation in rats[125].

Calcification or minerlization is an important route of polyurethane degradation leading to mechanical failure[126,127]. Hennics et al investigated the calcification with Avocothane-51 and Pellathane used in heart valves and blood pumps for left heart assist devices in Jersy Calves[128]. They observed calcification on moving parts of all pump bladder and valve and hair line fissures as early as 10 days after implantation of pellathane implant. There are reports regarding problems due to minerlization of circulatory assist devices[129-138]. Philips and Thoma proposed a mechanism for calcification, involving metal ion

complexation through cyclic ether[139].

Artificial heart and heart valve replacement are considered as a culmination point in Biomedical science and Technology. Kolf et al made a strong attempt to develop artificial heart valves using linear polyurethane[108]. The valve failed within 5 weeks and the problems associated with this device is mechanical failure and thrombus formation. Experimental heart valves with some components made of polyurethanes have been designed and tested by various research groups[140-144]. Lo et al tested heart valves designed with cardiomat 610, Mitrathane M2007 in calves[145]. The maximum survival time of the animal was less than one year and the problem associated with this failure was calcification, thrombosis and leaflet perforation.

Boretos et al tested Lycra-126(polyether urethane) for left ventricular heart assist pump in calves for a period upto 35 weeks [146,147]. They found cracks resulted during continuous flexing. Solution grade Biomer, Avocothane-51,PU-1025, Pellathane 2363-80A and Mitrathane have been used/tested for heart chamber fabrication[148-157].

An interesting study by Hunter et al showed an increase in molecular weight with the retrieved polyurethane heart implant implanted in canines upto 7 months[158]. Hayashi et al studied the effect of implantation on mechanical properties of polyurethanes diaphram of left ventricular assist devices[159]. Byrd et al evaluated the degradation of polyurethane endocardial pacing

leads made up of pellathane 80A and 50D[160]. They found that all the polymers exhibit surface frosting to some degree. Skalasky et al performed testing of polyurethane(pellathane-90A) in cardiac pacing leads in animal models[161].

Polyurethane used in vascular prosthesis:

Synthetic materials are widely used for the replacement or bypass of obstructed arteries in man. Majority of prosthesis having an internal diameter of 6 mm or less will occlude because of thrombus formation within them or the late development of thrombus formation within them or the late development of pseudo-intimal hyperplasia[162,163]. The importance of surface roughness have seen studied by Sharp and his workers [164,165]. The importance of electrical properties in vascular prosthesis was studied by several groups[166-168]. Annis et al used elastrostatically spun fibrous tube of polyurethane as vascular grafts [169]. Many research groups stressed the importance of complaiance on the patency of polyurethane vascular graft[170-173]. Many works in the development of arterial prosthesis were found elsewhere[174-182].

Problems associated with polyurethanes used in soft tissue application-

The interaction of polyurethanes with soft tissue leads to the formation of thin fibrous collagenous tissue around the implant. Salvatore et al used commercially available polyurethane(Ostoma) for osseous lesions [183] and found only

microscopic traces of samples at the site of implantation in mongrel dogs. Schwope et al have evaluated the viability of the epigard foam dressing in experimental animals and clinically[184]. Several polyurethane materials such as Lyofoam[185], and mixture of polyacetide and estane[186] are investigated as burn coverings.

Polyurethane used in other medical devices:-

Human trachea has no regenerative power if it is destroyed. Several research groups tried /developed the microporous tracheal prosthesis[187-190]. Intermittent dialysis of blood by an artificial kidney is a substitute for kidney function. Hemodialyser does the function of kidney. Polyurethane materials are used as blood filter which efficiently remove the aggregates[191]. Several experiments were carried out to study the effectiveness of polyurethane materials in the dialyser membrane[192-194].

Aliphatic polyurethane for medical use :-

In general aliphatic polyurethanes are less rigid than their corresponding aromatic counterpart. The rigidity of the aromatic polyurethane is due to the presence of aromatic ring in the polyurethane backbone. The flexibility of a polyurethane made up of cycloaliphatic diisocyanate is due to the presence of several isomers in the diisocyanate which reduce the tendency of the hard segment to crystallize. In general aliphatic polyurethanes are more difficult to synthesize due to the lower reactivity of

aliphatic diisocyanate. The commonly used aliphatic polyurethanes for biomedical applications are Adiprene LW 500 and Tecoflex[195].

Aliphatic polyurethanes also produce a diamine, MDA analogue, during thermal degradation but are less toxic than the aromatic counterpart. The higher U.V and light stability of aliphatic polyurethane is attributed to the resistance to autooxidation reaction[196].

Szycher et al evaluated aliphatic polyurethane material in the development and fabrication of ventricular assist pump[196]. Accelerated flexural testing of various commercially available polyurethane indicated that adiprene LW 500 and Tecoflex HR have higher flex life. Merrill et al developed platelet compatible hydrophilic segmented polyurethanes from PEG and cylohexane diisocyanate[197]. These polyurethanes are elastic and flexible during the contact with blood in the range of 0-37°C. Experiments of Merrill et al also showed that polyurethane made with aromatic diisocyanates like TDI and MDI with PEG 1500 showed much lower p ' (platelet index value) than polyurethane made from the same PEG-1500 with aliphatic IPDI.[198].

Wilker et al developed a series of chemically well defined segmented polyurethane and their mixture with predictable and controlled morphology for biomedical application[199].

Lipatova et al synthesized polyurethanes based on HMDI/MDI PTMG and various aminoacids and dipeptides[200]. They found that all HMDI based polyurethane showed an incomplete phase

separation. Their studies concluded that phase separation was influenced by the nature of diisocyanate. The type of extender has little influence on the phase separation. However, the increase of the extender size improve the phase separation of the components in the polyurethanes. Schauwecker et al used microporous Tecoflex 80 A as the inner layer of an artificial skin[201].

Crosslinked polyurethanes have received more attention for long term surgical implants. The adiprene LW 500 polymer is aliphatic polyurethane with a small amount of trimethylol propane (TMP) crosslink.So as expected this polymer has low flex life. Similarly, Avcothane 51 is crosslinked silicone urethane copolymer which has low flex life[202]. The avcothne 51 has no change in weight average molecular weight after six months subcutaneous implantation in rats[203]. It has more hydrolytic stability than their linear counterpart[204]. Gnanuos et al synthesized crosslinked polyurethane by step growth polymerization using polyethylene oxide glycol precursor polymer and aliphatic or aromatic pluroisocyanate[205]. These polymer are optically transparent highly hydrophilic with satisfactory mechanical properties. This polymer can be used as potential biomaterials.

AIMS AND OBJECTIVES

AIMS AND OBJECTIVES OF THE INVESTIGATIONS

Generally polymers declared as long term biomedical material should be totally inert and stable throughout the implantation period. Unfortunately most of the polymers undergo delayed degradations and leaching of polymer fragments affecting biocompatibility and biofunctionality of the device intended for specific task. The different class of polyurethane available in the market are associated with the degradative failure during the long term use in spite of their excellent performance in short term use. Failures of the soft tissue implants , indewlling polyurethane catheters , polyurethane heart valves etc are still a challenge to biomaterial scientist. The international conference on Materials Technology and Profit held in Melborne, Australia 16 - 18 November 1987 has also recommended for the research and development of biostable polyurethanes for long term biomedical applications. Therefore it was decided to prepare a new class of aliphatic crosslinked polyurethane for biomedical applications since aliphatic polyurethanes possess appreciable light stability which may be a required for implants of soft tissue reconstruction.

The physical and chemical parameters of polyurethane influences the biocompatibility and biostability of the implantable polymers. Conventional characterization techniques using polymer solution can not be adopted for crosslinked

polyurethanes . It was aimed therefore to evaluate the influence of the molecular weight between two cross links (Mc) and hard segment content on the variation of surface properties. The required surface properties of biocompatible linear segmented polyurethanes are normally reflected as hydrophilic/hydrophobic balance. It was therefore necessary to explore weather such possibility excist in new crosslinked polyurethanes . It was aimed to explore the degree of hydrophobic \hydrophilic character with reference to the variation of polyols and diisocyanate.

The biocompatibility of a new polymer has to be evaluated for a possible attempt to use that polymer as an implantable material. The biocompatibility is not only governed by the surface characteristics but also by the integrity of the material free from leaching. The impurities and low molecular weight fragments from crosslinked polymer can leach out and cause incompatibility. Therefore it was planned to study the biocompatibility of the crosslinked polyurethanes both by in vitro and in vivo tests.

Biostability is an important parameter for long-term applications. Degradation of implants not only cause device failure but also immunological injury to tissue. Degradation of polymers in vivo biological environment is complex event influenced by biochemical factors, environmental stress cracking and biomechanics of the tissue at the material - tissue

interface. It is very difficult to attribute the real cause for the degradation of polymeric implant during long term use. Therefore it was aimed to study the influence of various chemical biological fluids and blood components on the degradation of the present polyurethane. It was also planned to carry out the studies on the biostability of polyurethane by in vivo implantations in rat and surface analysis of explanted polymers.

The tissue response to long term polymeric implants depend on material tissue interaction(linkage). Generally this interaction is mediated by primary event of solid material fibroblast interaction and material extract - fibroblast interactions. Therefore it was planned to study material - fibroblast cell interaction using solid polyurethane materials and its extract . The studies on influence of surface characteristics of the polyurethanes on the cellular interaction was aimed. The material - tissue interaction is also influence by the chemical (biostability) factors and biomechanical factors. Therefore it was planned to use the porous sample of present polyurethane and to study the effect of biomechanically different implant sites as well as biodegradation on the tissue response of the long term implants. The study was aimed to be carried out in pig using experimental prosthetic material which can stimulate clinical prosthesis . The present investigation also aimed at possible identification of polyurethane materials for long term tissue contact applications.

EXPERIMENTAL

EXPERIMENTAL

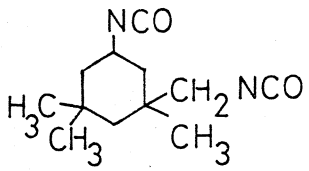
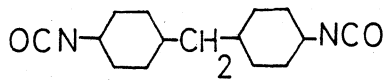
4.1 SYNTHESIS

The chemicals used for the synthesis their structure and sources are given in table 4.1. The purity of the diisocyanates was estimated as per ASTM D 1648 standard. The polyols (Polytetramethylene oxide glycol(PTMG), Polypropylene oxide glycol (PPG) and poly ethylene oxide glycol (PEG) used for the synthesis were vaccum dried before use. The crosslinker, trimethylol propane and catalyst,dibutyl tin dilaurate were used as such. The catalyst,triethyl amine was vaccum distilled before use. HPLC grade dimethyl acetamide was used as solvent.

The polyurethanes were synthesized as prepolymer method. The flow chart was given in figure 4.1. The synthesis of the present polyurethanes involved initially the synthesis of biuret of isocyanate.

Biurets of isophorone diisocyanate(IPDI) and bicyclohexylmethane diisocyanate(SMDI) were synthesized by reacting the known quantity of diisocyanate with water in the presence of catalyst at 50-60°C in the nitrogen atmosphere for 20 -30 minutes in a reaction kettle, which was fitted with nitrogen inlet, dropping funnel,mechanical stirrer and condenser equipped with $CaCl_2$ desiccant tube.The amount of catalyst used was 0.1% of the diisocyanate by weight. Biuret of hexamethylene diisocyanate (HDI) was synthesized by reacting the diisocyanate and water in the presence of triethylamine catalyst(0.25 % by

Table 4.1 Chemicals used for Polyurethane synthesis

Chemical	Structure	Source
(a) Diisocyante		
Isophorone diisocyanate (IPDI) 99.1 %		Chemik He Werre Holls West Germany.
Hexamethylene diisocyanate (HDI) 99.5 %	$OCN-(CH_2)_6-NCO$	Fluka Chemie A.G
Bicyclohexylmethyl diisocyanate(SMDI) 99 %		Bayers A.G,W.Germany.
b, Polyols		
POly(tetra methylene oxide) glycol(PTMG) $\bar{M}n$: 2000	$HO-\left[CH_2-CH_2-CH_2-CH_2-O\right]_n-H$	Q.O Chemicals, USA.
Poly(propylene oxide) glycol(PPG) $\bar{M}n$: 2000	$HO-\left[CH_2-CH-(CH_3)-O\right]_n-H$	Aldrich Chemicals USA.
Poly(ethylene oxide) glycol(PEG) $\bar{M}n$: 2000	$HO-\left[CH_2-CH_2-O\right]_n-H$	Polysciences, USA.
c.Crosslinker/ Chain extender		
Trimethylol Propane (TMP)	$CH_3CH_2C(CH_2OH)_3$	Merck, W.Germany.
d.Catalyst		
Dibutyl tin dilaurate (DBTDL)	$\left[C_4H_9\right]_2SN\left[OCO-(CH_2)_{10}-CH_3\right]_2$	Fluka Chemie A.G.
Triethyl amine (TEA)	$(C_2H_5)_3N:$	Merck, W.Germany.
e.Solvent		
Dimethyl acetamide (DMA)	$CH_3CO-N(CH_3)(CH_3)$	Sd Fine Chemicals

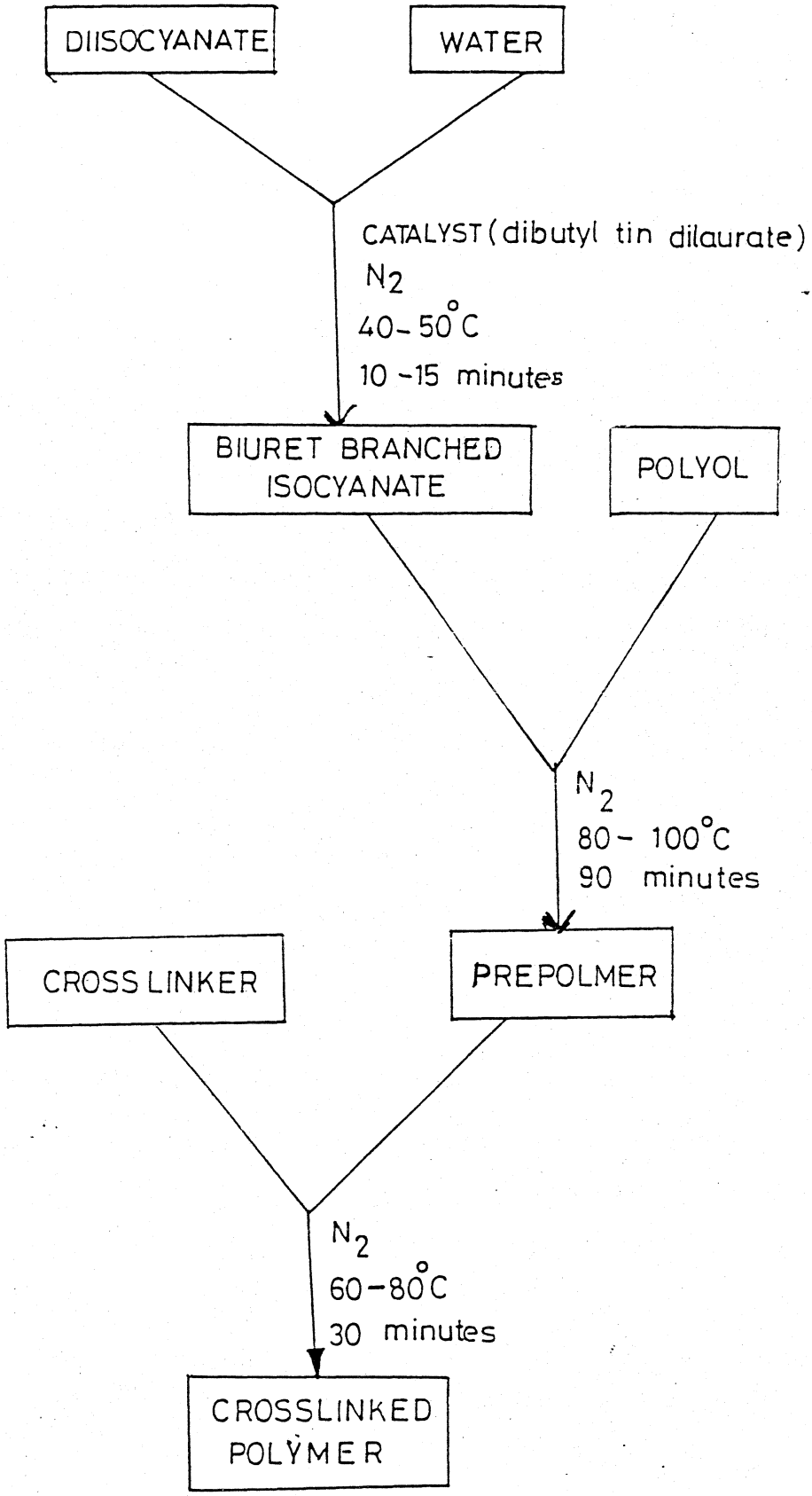
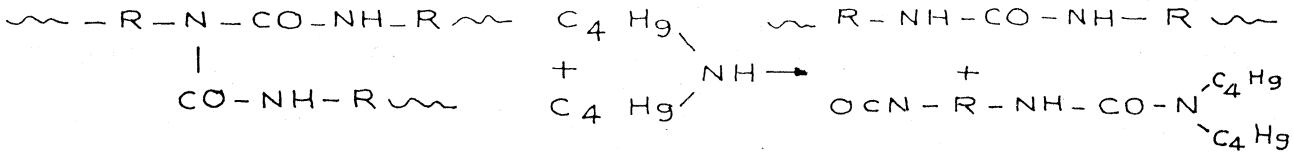


Figure 4.1 Synthesis of crosslinked polyurethane.

weight) at 40 -50°C for 10 minutes in the nitrogen atmosphere. Solvent(DMA) was used to get the homogeneous solution.

Quantitative analysis of biuret estimation was carried out. A known quantity of diisocyanate and water was taken in a reaction vessel. The reaction was carried out at 50-60° C in nitrogen atmosphere for 30 minutes and capped the unreacted -NCO groups by n-butanol. This was made into a solution using dimethyl acetamide. An excess amount of dibutylamine was added to this solution. The reaction mixture was heated at 60°C in a closed container for 16 hours. The excess dibutylamine was titrated against the standard hydrochloric acid using bromocresol green indicator. The reaction of dibutylamine and the biuret of diisocyanate is as follows.,



The effect of increase in diisocyanate and water content on biuret formation was tested.

To the biuret reaction mixture known quantity of polyol was added through separating funnel. The reaction was carried out at 70 to 80°C for 90 to 120 minutes. In the case of HDI based polyurethane the reaction was carried out for about 2 hours. In the case of PTMG and PPG polyols, the duration of prepolymer formation was one and half hour. In the case of PEG polyols the

duration was two and half hours. After cooling the reaction mixture, known quantity of 10-15% of DMA solution of trimethylol propane was added. The reaction mixture was heated at 60°C for 30 minutes. Then the reaction mixture was cooled, degassed and poured on a glass plate. The polymer was cured for 60°C for 12 hours and 80°C for 6 hours. In the case of HDI system an additional quantity of diisocyanate was added as crosslinking agent before trimethylol propane was added.

The porous polyurethane was prepared by salt replication method . Sodium carbonate has been found to be a catalyst for the condensation reaction in polyurethane synthesis. Sodium carbonate has been used for final curing and void formation. Sodium carbonate granules (M/S Sd. fine chemicals, Bombay) were sieved to get uniform granules of particle size 250 to 355 μ. During the polyurethane preparation, these sodium carbonate granules were added to the polymer melt with 40 pbw for the 60 pbw of the polymer. The semi solid was mixed throughly and cast in the hot glass plate and cured at 60°C for 12 hours and at 80°C for 6 hours. The cured polymer was repeatedly immersed in fresh water until all the sodium carbonate dissolves. The complete removal of sodium carbonate was confirmed by qualitative test with aqueous extract using PH paper. After that, the traces of sodium carbonate if any , was removed ultrasonically using deionised water. Then the porous polyurethane was dried and kept ready for implantation. The cross and longitudinal section of the porous

polyurethane was photographed. The porous polymer was found to have open and closed cellular structure. The air side and mold side were covered with thin polymer film. Only the cut surface has been open to cellular structure. A representstive photomicrographs of the polyurethane(I1) is given in the figure 4.1a

The cured polyurethane was cooled and cleaned with 10% rectified spirit for one hour and distilled water for overnight. The polymers were conditioned at 50°C till get the reproduceable weight. Various polyurethanes were synthesized by varying hard segment and soft segment ratio.(Table 4.2 - 4.4)

4.2 PHYSICO-CHEMICAL CHARACTERIZATION

The density of the polymers was determined as per ASTM D standard. The crosslink density of the polymers was determined using modified Flory- Rehner equation[206].

$$r = \frac{-Vr + X\,Vr^2 + \ln(1-Vr)}{drVo(Vr^{1/3} - Vr/2)} = \frac{1}{Mc}$$

where

r is the effective number of moles of crosslinked units per gram of rubber

Mc is the molecular weight between two crosslinks

Vr is the volume fraction of rubber in the swollen vulcanized.

X is the polymer solvent interaction parameter

dr is the density of polymer

Vo is the molar volume of the solvent

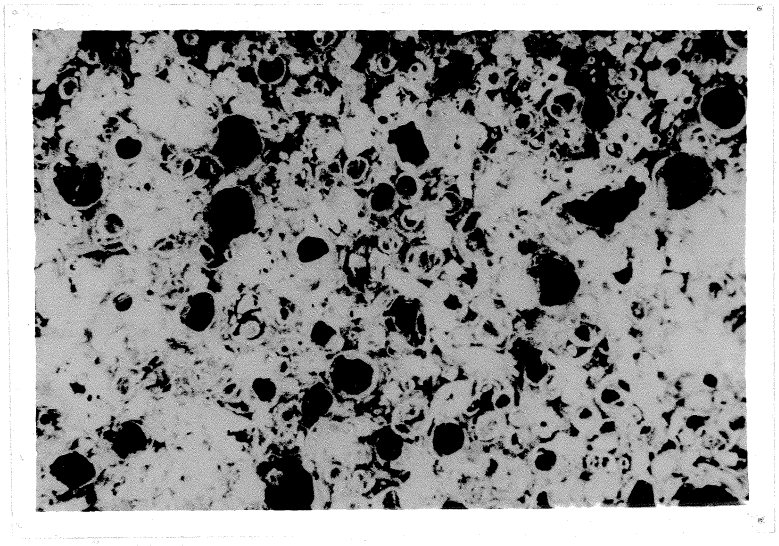


Figure . 4 . Photomicro graph of porus polyurethane

Table 4.2 The Formulation of IPDI based Polyurethanes

PU	DI (mole)	Water (mole)	Polyol (mole)	TMP (mole)	% Hard segment by weight
I1	9.0	3.0	1.5 (PT)	1.5	41.47
I2	9.0	3.0	3.0 (PT)	1.0	25.53
I3	9.0	3.0	4.0 (PT)	0.33	19.74
I4	9.0	3.0	1.5 (PP)	1.5	41.47
I5	9.0	3.0	3.0 (PP)	1.0	25.53
I6	9.0	3.0	4.0 (PP)	0.33	19.74
I7	9.0	3.0	1.5 (PE)	1.5	59.74

DI = Isophorone Diisocyanate

PT = Poly(tetra methylene oxide) glycol

PP = Poly (propylene oxide) glycol

PE = Poly (ethylene oxide) glycol

TMP =Trimethylol propane

Table 4.3 The Formulation of SMDI based Polyurethanes

PU	DI (mole)	Water (mole)	Polyol (mole)	TMP (mole)	% Hard segment by weight
S1	8.0	2.0	2.0(PT)	0.67	33.36
S2	7.0	2.0	2.0(PT)	0.67	30.38
S3	6.0	2.0	2.0(PT)	0.67	28.42
S4	15.0	5.0	6.0(PT)	1.00	24.60
S5	8.0	2.0	2.0(PP)	0.67	33.36
S6	7.0	2.0	2.0(PP)	0.67	30.38
S7	6.0	2.0	2.0(PP)	0.67	28.42
S8	15.0	5.0	6.0(PP)	1.00	24.60
S9	9.0	1.5	2.0(PE)	0.67	44.42

DI = Bicyclohexylmethane Diisocyanate

PT = Poly(tetra methylene oxide) glycol

PP = Poly (propylene oxide) glycol

PE = Poly (ethylene oxide) glycol

TMP =Trimethylol propane

Table 4.4 The Formulation of HDI based Polyurethanes

PU	DI (mole)	Water (mole)	polyol (mole)	TMP (mole)	% hard segment by weight
H1	10+2	5.0	3.0(PT)	1.0	26.62
H2	10+3	5.0	5.0(PT)	1.0	18.70
H3	10+1.5	2.0	6.0(PT)	1.0	15.13
H4	10+1.5	2.0	6.5(PT)	0.33	13.18
H5	10+1.5	2.0	7.5(PT)	0.33	12.08
H6	10+2	5.0	3.0(PT)	1.0	26.62
H7	10+1.5	2.0	7.5(PT)	0.33	12.08
H8	10+1.5	2.0	4.0(PE)	0.33	50.33

DI = Hexamethylene Diisocyanate

PT = Poly(tetra methylene oxide) glycol

PP = Poly (propylene oxide) glycol

PE = Poly (ethylene oxide) glycol

TMP =Trimethylol propane

$$Vr = 1/1+Q$$

where

$$Q = \frac{\text{weight of solvent in gel} \times ds}{\text{weight of gel} \times dr}$$

where

dr is the density of the polymer

ds is the density of the solvent

The polymer solvent interaction parameter was determined using Bristor and Watson semiempirical equation[207]

$$X = \beta + (Vs/RT)\,(\delta s - \delta p)^2$$

where

β is the lattice constant usally about 0.34

Vs is the molar volume of solvent

R is the gas constant

T is the absolute temperature.

and δp and δs is the solubility parameter of the polymer and swelling agent.

The solubility parameter of the polymer was determined by conducting swelling experiments using small rectangular specimens in nine different solvents ranging in solubility parameter 7.3 (cal c.m $^{-3}$) $^{1/2}$ δ (n-hexane) to 14.4 (cal c.m $^{-3}$) $^{1/2}$ δ (methanol). The swelling coefficient, ' Q ' was evaluated as mentioned above. A graph between ' Q ' verses δs was plotted. The peak of the curve gives the solubility parameter of the polymer sample.

The water absorption studies were carried out as per ASTM standard D 570. Thin square pieces of polyurethane were placed in

deionised water at 25° C for 96 hours. After that the surface was cleaned with tissue paper and the change in weight was noted. The mechanical properties of the polyurethane rectangular sheets were determined as per ASTM standard D 812 using Instron Universal Testing machine(model 1193) The chart speed and crosshead speed were 100 mm/min. The gauge length was 3 c.m in each test. The tensile strength, % elongation and modulus at break were calculated . The hardness of the polymers were determined using a shore A hardness tester(Durometer). The infrared surface spectral analyses were carried out on ATR accessory with KRS 5 crystal (Perkin Elmer IR spectrophotometer). The dynamic mechanical analyses (thermal analyses) of the polymers were carried out using DuPont thermal analyser unit. The temperature range was ambiant to 300°C. The testing rate was 5°C/min. at 10 Hz. The thermogravimetric analysis was carried out for the polymers using Dupont thermal analysis under nitrogen atmosphere. The temperature range was ambiant to 800°C. The heating rate was 10°C/min.

The surface properties of the polymer were determined using a Goniometer. The contact angles for captive air and octane with polymer surface were noted for the polyurethanes after conditioning in water for 24 hours. The adhesion tension of the polymer - water was determined using Youngs equation as per method of Andrade et al[208].

$$(\gamma sv - \gamma sw) = \gamma wv \cos 0$$

where

γ is the interfacial free energy for the hydrated polymer water vapour(γsv) and hydrated gel water(γsw). γwv is the interfacial tension of the water. 0 is the contact angle of the air with hydrated polyurethane surface. The solid - water, non dispersive force (polar) was calculated using the following equation[208].

$$Isw = 50.5\ (1 - \cos 0)$$

where 0 is the contact angle of the octane - polyurethane surface.

The dispersive componants of the surface energy of the polyurethane was calculated using the following equation

$$\gamma^{d}_{sv} = \frac{[(\gamma sv - \gamma sw) - Isw + 72.1)]^{2}}{9.3}$$

The polar components of the surface energy of the polyurethane was calculated using the following equation

$$\gamma^{p}_{sv} = Isw^{2} / (4\ \gamma^{p}_{wv})$$

where

γ^{p}_{wv} = 50.5 dyn c.m

γ^{d}_{sv}, γ^{p}_{sv}, γ^{d}_{sv}, γ^{p}_{sv}, polar/dispersion ratio and γsw of the polymers were calculated.

4.3 BIOCOMPATIBILITY TESTS

4.3.1 Preparation of implants

The newly prepared polymers were cleaned under clean room conditions with neutral soap solution(Labolein) to avoid contamination. They were then washed in an ultrasonic cleaner for

15 minutes and then washed in hot water for 5 minutes and finally with soap solution to remove lint and skin oil. The cleaning was repeated again. After this the following procedure was adopted.

1. 5 minutes hot water rinse containing 0.1% benzalkonium chloride
2. 5 minutes distilled water rinse
3. 15 minutes in the ultrasonic cleaner with hot water.
4. 5 minutes distilled water rinse.
5. 15 minutes in the ultrasonic cleaner with distilled water. The benzalkonium chloride was preferred due to its ability to kill pathogens[208a]

The implants were dried at 40°C and sealed in polyethylene bags and sterilized by γ-radiation with dosage of 2.5 Mrad using Co^{60} source. 4.3.2.1 Hemolysis test

This test is used to asses the hemolytic potential of the material. Red blood cell (RBC) lysis in the presence of material was tested by treating 10-20 mg of ultrasonically cleaned and powdered material with 2 ml of anticoagulated calf blood at 37°C for 1 hour. The blood was centrifuged twice at 150g for 15 minutes for the complete removal of RBCs. 0.4 ml of above plasma was mixed with 3.6 ml of distilled water and the absorbance was measured at 429nm, 414nm and 398 nm. The hemoglobin release was analysed as per the method of Raphael[209].

The number of mgs of plasma hemoglophin release per 100 ml of plasma $= [A_{414} - (A_{429} + A_{398})/2] \times 266$

The control was untreated blood. This test was carried out for all the polymer samples

4.3.2.2 Plasma recalcification time

Plasma recalcification time denotes the intrinsic clotting potential of plasma. In this experiment, 0.2 ml of ultrasonically cleaned and powdered polymer sample and 0.4 ml of plasma were incubated for 2 minutes at 37°C. To this 0.1ml of 0.025M $CaCl_2$ solution was added under warm condition . The time for the formation of fibrin clot was noted as described elsewhere [210]. This experiment was conducted for all the polymer samples.

4.3.2.3 Systemic toxicity test

This test is used to detect the systemic leachable toxicants present in the material. Saline and cotton seed oil extracts of materials are commonly used in systemic toxicity test. Extracts were prepared as per USP standard using cotton seed oil and saline as per the method described elsewhere[211]. 4 g of the material was extracted in 20 ml of saline/cotton seed oil at 70 ± 2°C for 24 hours. The saline and cotton seed oil extracts were used. Albino mice of either sex weighing between 17 to 23 g were used in this test. Two groups of five mice were injected intravenously with saline extract and intraperitonially with cotton seed oil extract with dosage of 50 ml/kg. Extracting medium alone was injected to 5 mice to serve as control. The test animals were observed for two weeks for notable change.

4.3.2.4 Intracutaneous irritation test

This test can reveal the irritant response elicited by the extract. The extract used in the systamic toxicity tests were

used here.

In this test 0.2 ml of extract prepared with saline and cotton seed oil was injected intracutaneously at 10 sites on the one side of the dorsal surface of each of two rabbits previously clipped of hair. The other side of the dorsal surface was injected with negative control (extracting medium alone) at 10 sites of the animal. Medium alone acted as negative control whereas ethylalcohol acted as positive control. The animals were observed for erythema ,odema and necrosis for 7 days. The scoring system ranges from 0 to 5 ; the zero being the response to the negative control).

4.3.2.5 Intramuscular Implantation studies

Implantation techniques primarily are used for assesment of biocompatibility of materials or devices that will be in contact with tissues.

In this study rabbit was anaesthetised using sodium pentabarbitone with a dose of 45 mg/kg and placed in ventral recumbancy. The skin over the lumber and pelvic regions was clipped and swabbed with an antiseptic. Implants of size 1 x 1 x 10 mm were implanted in gluteal muscle using bone marrow puncher. Six samples (5 test materials + 1 control material) were implanted in a animal. The animals were sacrificed after the post implantation period of 7,30 and 90 days and the implants were retrieved. Histopathological evaluation was performed on the excecised tissue surrounding the implant. For this the samples with the surrounding tissues were transfered in a container

containing formalin. Then these samples were dehydrated in ethanol - water solutions of successively higher ethanol concentration and then cleared in xylene. Then this samples were embedded in paraffin wax and after adequate infiltration of paraffin wax, the samples were sectioned using a microtome. The sections were wetted with a few drops of 50% ethanol and then floated on a heated water bath. After that the sections were stained with hemotoxylin and eosin.

Twelve histopathological criteria were examined for tissue response. They include

1. Necrosis
2. Inflammation
3. Polymorphonuclear lekocytes
4. Macrophages
5. Lymphocytes
6. Plasma cells
7. Giant cells
8. Foreign body debris
9. Fibroplasia
10. Fibrosis
11. Fatty infiltration
12. Relative size of involved area.

Each of these criteria was then scored as mentioned below.

0 = item not present

1 = item occasionally present

2 = item present to a mild degree

3 = item present to a moderate degree

4 = item present to a marked degree

Each one of representative sample of test polyurethane material was implanted for each series of polyurethanes.

4.4. STUDIES ON THE STABILITY OF POLYURETHANES

Polymer strips of 0.5 x 5.0 cms were used to study the change in mechanical properties and weight. For the studies on change in equilibrium swelling ratio (%), polymer strips of 0.5 x 0.5 cm were used. The solvent used for these studies was toluene.

4.4.1 Sterilizability

The stability to radiation sterilization of the polymers was evaluated. The samples were exposed to γ-radiation for a dosage 2.5 Mrad using Co^{60} source. The stability to steam sterilization was investigated under 15 psi pressure at 121°C for 15 minutes. The change in swelling coefficient, weight, and mechanical properties was determined.

4.4.2 Hydrolytic stability

The hydrolytic stability of the polymer was evaluated by immersing the polymer strips in deionised water at 60°C for 96 hours. The polymers were dried heating at 50°C in vacuum until to get the constant weight. After drying, the change in swelling coefficient , weight, and mechanical properties was determined.

4.4.3 Stability in ionic environment

The polymer was immersed in pseudoextracellular fluid and phosphate buffered saline at 80 ±2°C for 6 hours The composition of these biological solutions is given in table 4.5 . After drying the polymer as mentioned above the change in swelling coefficient, weight and mechanical properties was evaluated. The change in P_H of the solution was noted before and after experiment.

4.4.4 Stability in catalytically reactive environment

The stability of the polymers in reactive environment such as catalytic hydrolysis, transamination and transesterifications was also tested. The composition of reactants, duration of the treatment and temperature are given in table 4.6. The change in properties was investigated after drying the polymers.

4.4.5 Stability in the environment of oxidative degradation

The stability of the polyurethanes in a mild oxidizing agent, sodium oxychloride (NaOCl) was tested. 0.1 N solution of NaOCl solution was used for this study. The NaOCl solution was standardized by iodometric method[212]. The polymer samples were immersed in NaOCl solution for 36 hours at 37 ±2°C. The change in properties of the polymers was evaluated after drying the polymers.

4.4.6 Stability in lipid solution

A standard lipid solution was prepared as per the formulation of Takahara et al[213]. The polymers were immersed in

Table 4.5. The Composition of Physiological Solutions

(a) Pseudoextracellular fluid (PECF)

Ion	Concentration (m.eq./lit)	
	Physiological	PECF
Na^+	145	145
K^+	5	5
Cl^-	113	118
HCO_3	30	30
HPO_4	2	2

(b) Phosphate buffered saline (PBS)
(for one liter)

$Na_2HPO_4.2H_2O$	0.120 M
$NaH_2PO_4.2H_2O$	0.030 M
NaCl	0.147 M

Table 4.6. The Composition and Conditions of Polyurethane Catalytic Degradation.

Degradation Reaction	Composition of Reactants	Temperature (°C)	Duration (Hours)
Catalytic hydrolysis	3 % polymer 5 % oxalic acid 92 % water	80	12
Catalytic transamination	1 % polymer 1 % ZnCl2 98 % water	70	12
Catalytic transesterification	4 % polymer 4 % Oxalic acid 92 % Ethonol	80	12

lipid solution for 5 weeks at 37±2°C. The change in properties was determined after drying the incubated polymers.

4.4.7 Stability of polymers in the environment of enzymatic degradation

The stability of polyurethanes in enzymes was studied using hydrolytic and oxidative enzymes (Papain, lactic dehydrogenase, Trypsin and Bromealin) The composition of buffers are given in the table 4.7. Enzyme solutions were prepared with activity needed to break all susceptible bonds. The enzyme activity was determined as per standard procedures. The enzyme was changed every 2 days of incubation. The experiment was carried out at 37°C for two weeks. After two weeks the polymers were removed, cleaned and dried. The change in properties was determined.

4.4.8 Stability of polymers in blood

The stability in blood was evaluated using calf blood at 8°C for 5 weeks. The blood was preserved with antibiotics such as penicillin hydrochloride (10,000 units/litre) and 0.2% sodium azide (NaN_3) preservatives. The blood was changed weekly with fresh blood before the bacterial contamination might occur with old blood. After 5 weeks the polymers were taken out cleaned and dried. The change in properties was determined. In the same experiment the amount of lipid absorbed was evaluated using phosphovannilin reagent after extracting it in chloroform-methanol(2:1 v/v) mixture [214].

Table 4.7. The Composition and Activity of Enzyme Solution

Enzyme	Buffer	Activity after 24 hours
Papain	0.1 M HEPES 0.2% NaN_3 0.005 M Cystene 0.01 M EDTA 0.06 M Mercaptoethanol (pH 6.8)	70 %
Lactic dehydragenase	0.1 M Phosphate buffer with 0.2% NaN_3	48 %
Trypsin	0.46 M Tris-HCl with 0.0115 M $CaCl_2$ and 0.2% NaN_3	61 %
Bromealin	0.1 M Citrate with 0.2% NaN_3	52.5 %

4.4.9 In vivo stability test

In this study rats weighing 200-300 g were chosen as animal model. The animals were anesthetized using sodium pentabarbitone with dosage 25 mg/kg. The anesthetized animal was placed under ventral recumbence and the dorsal trunk region was clipped , shaved and swabbed with antiseptic agent. Four transverse skin incisions of 2.0 cm in length was made for subcutaneous implantation. One ultrasonically cleaned and sterile polyurethane sample of size.1 x 0.5 x 5.0 cm was placed in each pocket. One sample prepared from Tecoflex 85 A was negative control. The skin was closed with sterile cotton thread. The animals were kept under standard laboratory post operative care. After the post implantation period of 120 days the animals were sacrificed and samples were retrieved. The polymers were cleaned with water and 0.1% papain(proteolytic enzyme). Then the polymers were ultrasonicated in water for 15 minutes and dried at 50°C in vaccum. After conditioning the polymers the change in mechanical properties was investigated. The surface analysis of polymer was investigated by SEM analysis for the possible development of microcracks at the surface. The SEM analysis studies were carried out using Jeol 30 scanning electron microscope at the electron gun voltage of15 KV.

4.4.10 Studies on calcium deposition

Studies on calcium deposition were carried out using calf blood plasma at 37°C in a special indigenous experimental set up

as shown in the figure 4.2. Six rectangular strip of polyurethane (0.5x 0.5 x 5 cm) was immersed in calf blood plasma containing antibiotics in six test tubes each containing one sample. The test tubes were kept in a hard bottle containing water as shown in the figure. The water was kept in the bottle in such a way that no water can enter into the test tube. The static pressure inside the bottle was generated by passing nitrogen gas from a cylinder which was monitored with a pressure gauge. 400 mm Hg of pressure was maintained through out the experiment. The calcium ion content of the calf blood plasma was determined by potassium permanganate method before the incubation with polyurethanes [215]. The experiment was conducted for four weeks. At the end of each week the plasma was changed with new plasma before bacterial contamination might start with old blood plasma. At the end of the experiment the polymers were taken out and the surface was cleaned gently with double distilled water containing 0.01 % papain. The calcium deposited on the surface was extracted and analysed. The calcium was extracted with known quantity of 0.15 M M HCl at 60°C for 16 hours. The extracted solutions were analysed for calcium ions using atomic absorption spectrophotometer(Instrumentation Lab, USA) The experiment was repeated for samples without applying pressure. A biomedical grade polyurethane (Tecoflex 85A) was used as the control. Polyurethane based on IPDI and SMDI were chosen for this study.

4.4.12 Susceptibility of polyurethanes to fungal attack

The polyurethane strips of 0.1 x 0.5 x 5 cm kept in a paper cover for one year at 20-25°C and relative humidity of 60% were taken for this study. The fungal attack on the polyurethane was analysed by the following method. A small piece of polyurethane was cleaned with sterile saline and kept in salmons froth and salmons slope for 7 days. Polyvinyl butryl rubber was used in this experiment as positive control[216]. At the end of seventh day the samples were tested for fungal growth by visual observation. salmons solution was used as control.

4.4.13 Study on inhibition of fungal growth by polyurethanes using fungus spore

The fungi used for this study were Aspergillus niger and Penicillium species which are common contaminants of plastic devices. Salt agar medium was prepared as per the composition given below for one litre.

$NaNO_3$	3.00 gm
K_2HPO_4	1.00 gm
$MgSO_4.7H_2O$	0.25 gm
Yeast extract	0.20 gm
Agar	10.00 gm

The yeast extract was added to the composition to ensure germination of fungus spores. The yeast extract normally supports a very small amount of growth and ss frutility. Therefore it provides a basis for judging zones of inhibition around the

material under test. The newly prepared polyurethane samples were sterilized by γ radiation with a dose of 2.5 Mrad. The polymer strips of 0.1x 0.5 x 5.0 cm were placed in the middle of the salt agar slope. The spores of above mentioned fungus were spread around and over the material. The growth of fungus was checked by visual observation at the end of 1 , 2, 3, 4 and 5 weeks. Cotton seed oil socked PVC was used as the positive control.

4.5 STUDIES ON MATERIAL - TISSUE INTERACTION

These studies were planned to understand the material cell interaction and to study the material-tissue interaction under the influence of biomechanically different tissue sites.

4.5.1 Studies on material - cell interaction

A continuous cell line of fibroplast L-929 cell was used in the present experiment. This cell line was procured from the National Institute of Virology(NIV),ICMR,Poona and passaged at the microbiology laboratory of our institute as per the general laboratory procedure. Minimum Essential medium(MEM)-95% with 5% sterile goat serum was used for growing the monolayer of the fibroblast. The composition of culture medium was as follows

Minimum Essential medium	93 ml
$NaHCO_3$ (3.5%)	5.5 ml
Glutamine(3%)	1.0 ml
Penicillin and Streptomycin	0.2 ml

Phenol red was used to check the PH of the medium. MEM

(completed), 95 ml and sterile calf serum, 5 ml was used as growth medium.

Tissue culture grade polystyrene bottles were used for the present study. One percent cell culture grade Agarose solution was prepared using deionised water,sterilized and used for sticking the polymer on the bases of polystyrene bottle . The cytotoxic effect of agarose solution was tested by placing agarose drop in the tissue culture bottles and culturing it for 3 days. In this study MEM containing $1x10^{-5}$ cells/ml was used.

Sterile polyurethane strips of 0.2 x 4 x 20 mm were sticked inside the tissue culture grade bottle . 10 ml of the growth medium was added. PVC strips of same size soaked in 0.1% aqueous dibutyltin dilaurate was used as positive control, whereas the biomedical grade polyurethane Tecoflex 85A was used as negative control. The morphological change of the fibroblast was investigated for the material-cell interaction.

4.5.2 Study of the interaction between fibroblastic cells and polymer extract

Polyurethane weighing 0.2 gram was put in 20 ml of the pseudoextracellular fluid (PECF) and autoclaved at 121°C and 10 lbs for 10 minutes. The sterile extract was cooled and used for the experiment. 0.1 % phenol aqueous solution was used as the positive control. The negative control was biomedical grade polyurethane, Tecoflex 85 A. The cells from the culture bottles were dispersed by Trypsin-Phosphate-Versene-Glucose(TPVG- PH 7.4)

solution. The dispersed cells were seeded into Leighton's tubes having coverslips (10 mm x22mm size) at it's bottom. One ml of MEM containing 1.5 million cells were added into each Leightons tube. The seeded tubes were incubated at 37°C for four or 5 days, until a complete monolayer of fibroblast cells was formed. The fully grown monolayer was washed with maintenance medium(without goat serum) after the removal of the growth medium and then 0.2 ml of the polymer extract was added into the two tubes, spread gently over the fibroblastic cell monolayer and incubated for adsorption at 37°C for 60 minutes. Later 0.8 ml of the maintenance medium was added into each tube and incubated at 37°C for 72 hours. Four tubes of fibroblast cells were also incubated with PECF alone for the comparison .

After 72 hours of incubation at 37°C the medium containing polymer extract was removed from the tubes and the cover slip having monolayer was washed gently with sterile PECF. Then the cover slips were air dried and than stained with dilute carbol fuchsin for one minute inside the jar and fixed with cold acetone at +4°C for 10 minutes. The coverslips were washed well with distilled water and air dried by keeping over a microslide. After drying they were mounted upside down on a clean and grease free micrislaide and labelled. Then they were examined for the evidence of morphological changes under a light microscope and compared with PECF control cells.

STUDIES ON TISSUE RESPONSE TO POLYURETHANES IMPLANTED IN BIOMECHANICALLY DIFFERENT TISSUE SITES

The studies on tissue response on implants were carried out to explore mainly the biomechanical factors which affect the soft tissue response to implants. The pig was chosen as experimental model for this study. Three porous crosslinked polyurethanes , (one from each series) were implanted. The dimension of the implant was 4 cm length 1.0 cm width and 0.1 cm thick. The polymers were cleaned, packed and sterilized as mentioned in section 4.3.1. The biomedical grade polyurethane, Tecoflex 85 A of same size was served as the negative control. The animals were anesthetized by the administration of atropine sulphate (0.05 mg/kg) (intramuscular) and triflurobromazine hydrochloride (1 mg/kg) (intramuscular). After 15 - 20 minutes latter general anesthesia was induced by administering thiopentanone with standard cuffed tube and automatic ventilation with 70 % nitrous oxide and 30% oxygen. Flaxedil was used for muscle relaxation and repeated when necessary. A single incision was made on the dorsal aspect of the paravertebral region. The blunt scissor dissection was made to make a pocket in the muscles. The candidate material was implanted intramuscularly in the posterior side and the control Tecoflex 85 A was implanted in the anterior side. The paravertebral region of the animal constitutes the minimal motion site. For the comparison of the effect of tissue

responses in the minimal motion site to that of the maximal motion site, a similar intramuscular implantation was carried out in hind legs of the same pig. The candidate material was implanted in the left back leg and the control was implanted in the right leg. The animals were sacrified at the post implantation period of 6,9 and 12 months. The implants and tissue surrounding the implant were excised and histopathological analysis was carried out as described in section 4.3.2. The tissue section was stained with hemotoxylin and eosin.

RESULTS AND DISCUSSION

5.1. S Y N T H E S I S

Biuret formation:-

The first step of polyurethane synthesis involves the formation of biuret as per the following routes. The model reaction on the biuret formation clearly indicates the feasibility of synthesis of crosslinked polyurethane with biuret links.

$OCN-R-NCO + H_2O \longrightarrow OCN-R-NHCOOH \longrightarrow OCN-R-NH_2$

Diisocyanate(I) Carbamic Acid(II) Amine(III)

$I + III \longrightarrow OCN-R-NH-CO-NH-R-NCO$

Substituted Urea (IV)

$IV + I \longrightarrow OCN-R-N(-CO-NH-R-NCO)-CO-NH-R-NCO$

Biuret (Triisocyanate) (V)

As represented in the in the scheme of biuret formation, the reaction is not necessarily stochiometric. Usually the amine decomposition method is used to determine the allophonate and /or biuret content of the linear polyurethane[216,217]. This method is not applicable in the present investigation because crosslinked polymers do not dissolve in the titration medium. So after biuret formation the isocyanate group was blocked with n-butanol prior to the estimation of biuret. (vide experimental section 4.1) Since the reaction between the dibutyl amine and biuret molecule is stoichiometric, estimation of biuret was possible quantitatively.

The results of the biuret estimation is given in Table 5.1. Statistically significant differences are not observed in the biuret content from the individual diisocyanate with different mole ratio of diisocyanate and water. Among the three biuret formation the amount of biuret formed is relatively more with HDI monomer followed by IPDI and SMDI monomers. This is due to the higher reactivity of HDI followed by IPDI and SMDI, with water and substituted urea. In the biuret formation, corresponding amines of diisocyanates are involved as a second step. The reactivity of the amines of the diisocyanates are therefore be in the same order.

The reactivity of the amines is related to the basicity of the amines. As the basicity of these amines increases the reactivity also increases. The less reactivity of amine of IPDI and SMDI is due to the nonplanarity of the molecule.

It was found earlier that in the presence of organotin catalyst and the absence of water the reaction between the hydroxyl and isocyanate groups gives urethane at temperature below 100°C[218-221]. Theoretical study of Dusek also proved that formation of urethane is the fastest reaction. But in the presence of water the reaction leads to biuret formation which is

Table 5.1 Estimation of Biuret Content by Amine Decomposition Method

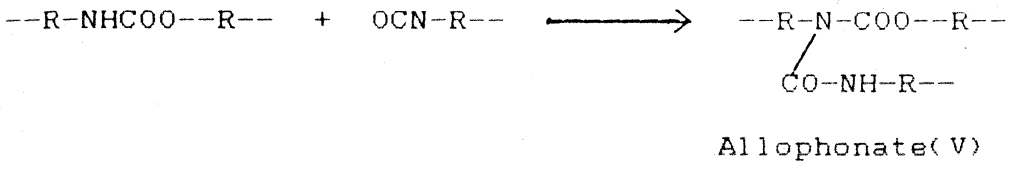
DI	Concentration (mole)		Biuret content (mole)	
	Diisocyanate	Water	calculated	estimated
HDI	3.0	1.0	1	0.8892
	3.5	1.0	>1	0.8913
	4.0	1.0	>1	0.9123
	3.0	1.1	<1	0.8801
	3.0	1.2	<1	0.8741
IPDI	3.0	1.0	1	0.8102
	3.5	1.0	>1	0.8231
	4.0	1.0	>1	0.8401
	3.0	1.1	<1	0.8001
	3.0	1.2	<1	0.7891
SMDI	3.0	1.0	1	0.7113
	3.5	1.0	>1	0.7314
	4.0	1.0	>1	0.7412
	3.0	1.1	<1	0.6992
	3.0	1.2	<1	0.6642

DI- Diisocyanate

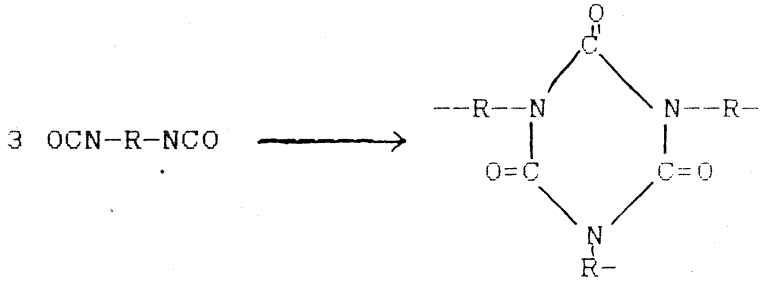
governed by an equilibrium of two reactants, the free isocyanate and substituted urea. The concentration of free isocyanate(R), substituted urea(T) and biuret(U) are governed by the following equilibrium[222].

$$K_B = [U]/[T][R]$$

Under this condition the formation of allophonate(V) and isocyanurate(vi) linkage is not possible[222].



Allophonate(V)



Isocyanurate (VI)

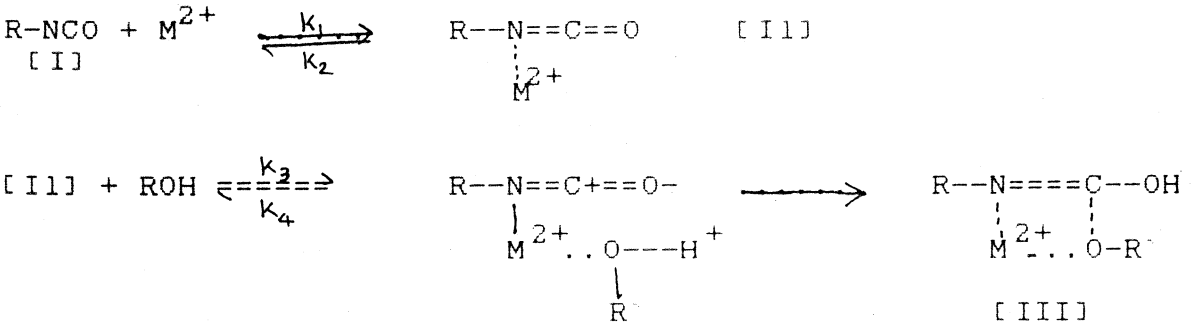
More over the formation of biuret from the substituted urea is much faster than allophonate forming from urethane as the equilibrium constant of biuret formation is comparatively higher than that of the allophonate[223]. Therefore it can be inferred that the polyurethanes synthesized newly consist biuret structures in the backbone.

Reactivity of diisocyanate in polyurethane formation

As the reaction proceeds the chances of gelation is higher.

Traces of moisture can affect the reaction by acting like a non reactive diluents , or as a monofuntional blocking agent and an effective chain extender linking branch points. The total behaviour is a combination of all the three aspects and is determined by the relative speeds of reaction of water and hydroxyl groups[224]. But it is known that the reaction of diisocyanate with hydroxyl group is faster when compared with water[224] . Usually structural asymmetry of diisocyanate retards chain extension[225,226]. Formation of the other trifuntional group promotes gelation and increases the crosslink density of the network[227]. However the difference in reactivity due to asymmetry of diisocyanate is very less[225,226].

Second order kinetics for the reaction between isocyanate and hydroxyl group has been suggested by some groups[228,229]. Pseudo first order kinetics was also suggested for the reaction of aliphatic isocyanate with excess alcohol[230]. The most acceptable mechanism for the polyurethane formation and the factors affecting the rate of the reaction was suggested by Robins[231].

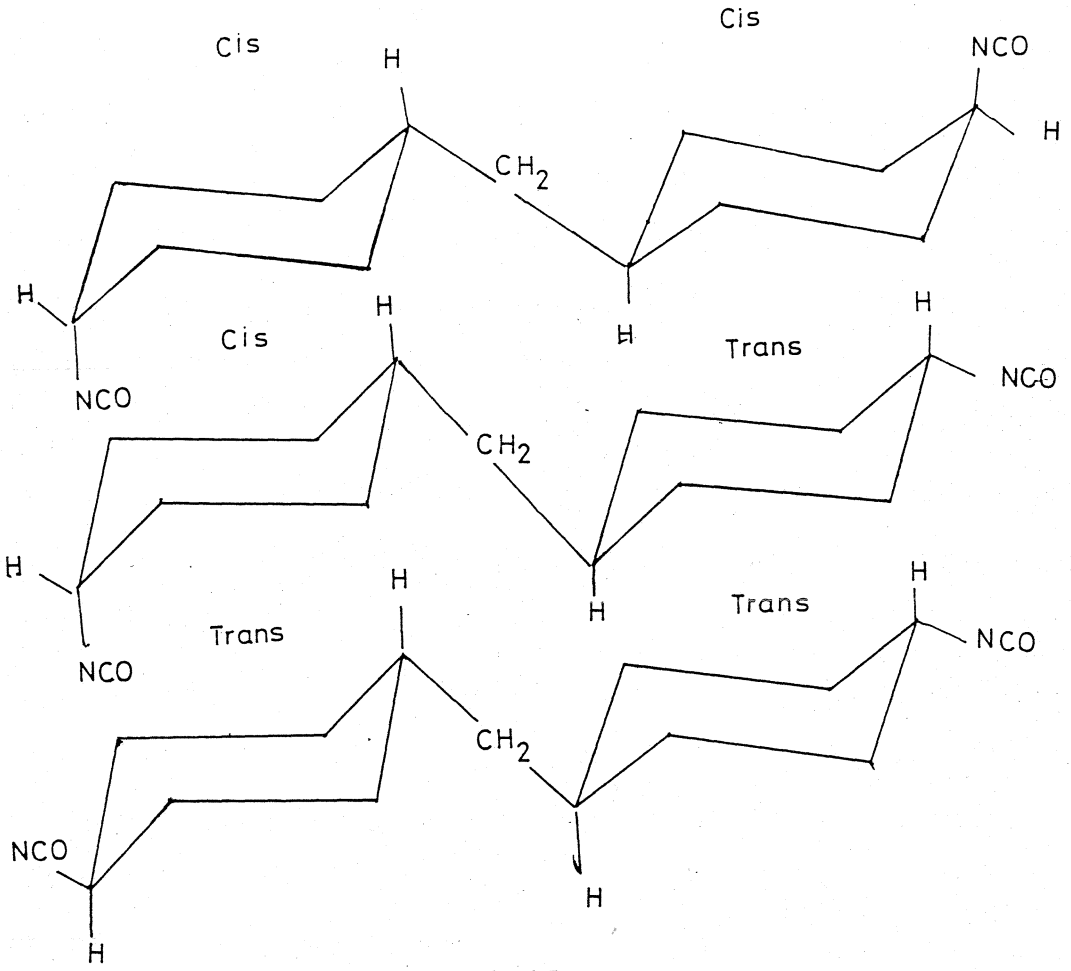


[III] ------- RNH COOR^{-} + M^{2+}

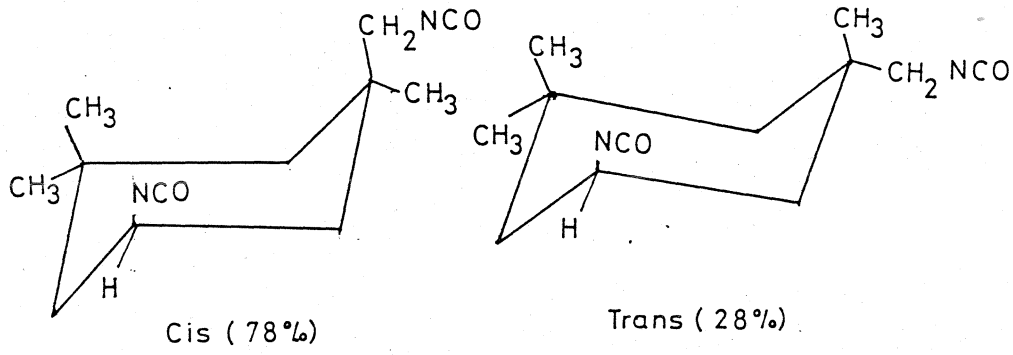
$d[NCO]/dt = -kC\,[NCO]\,[OH]\,[C]$

The reaction rate also depends on the nature of diisocyanate. In HMDI the reactivity of the two isocyanates is same . In the case of SMDI and IPDI the reactivity of the isocyanate groups is not same. The unequal reactivity of the isocyanates of SMDI is due to their existence as geometrical isomers. [trans-trans, trans-cis and cis-cis] (Figure 5.1) In this case the isomer having axial -NCO group is more reactive than the isomer having the equitorial one[232]. However the difference in reactivity among themselves is very less [232]. The IPDI exists as two isomers [233]. They are cis and trans isomers. Measurement of the isomer ratio using Proton and C^{13} NMR and chromatography analysis indicates about 72 % as cis isomer and 28 % as trans isomer[234]. The reactivity of the isocyanate group of IPDI can also be differentiated by the presence of primary and secondary NCO. The primary -NCO group is more reactive than the secondary -NCO group[234]. This also adds the difference in the reactivity among isocyanate groups of IPDI[73,234]. In general linear diisocyanate is more reactive than cycloaliphatic one[235]. Thus HDI is more reactive than other diisocyanate in the present synthesis of various polyurethanes. The relative reactivity of the diisocyanates is in the following order[236].

HDI>IPDI>SMDI



SMDI



Cis (78%)

Trans (28%)

IPDI

Figure 5.1 The isomers of diisocyanate

The commonly used catalyst for polyurethane synthesis are metal and amine catalysts. The metal catalysts are more effective than amine catalyst. In the present synthesis of polyurethanes based on SMDI and IPDI, dibutyl tin dilaurate was used in order to increase the reaction rate. The tin compounds with shorter alkyls groups have higher reactivity than those with longer groups. Diethyltin is the most effective catalyst , but toxicity considerations, alkyl groups shorter than butyl are rarely used commercially. Here also highly substituted tin compounds have been used. The mechanism of catalytic behavior of dibutyltin dilaurate(DBTDL) in DMF was published elsewhere[237-239]. The catalyst employed for the HDI based polyurethane synthesis was triethyl amine(TEA). Due to the high reactivity of HDI the employment of DBTDL catalyst resulted in gelation. Because of the lower catalytic activity of TEA the duration of the synthesis of polyurethane was increased.

Preparation of polyurethane

The second stage polyurethane synthesis involves prepolymer formation using the modified diisocyanate (biuret). After the biuret formation the reaction was carried with polyol at 80° C to give urethane .

$$-R-NCO + HO---R---OH \longrightarrow --R-[NHCOO]-R--OH$$

Urethane(V)

The duration for the prepolymer formation in the case of PEG based polyurethane is very long in compared with that of PPG and

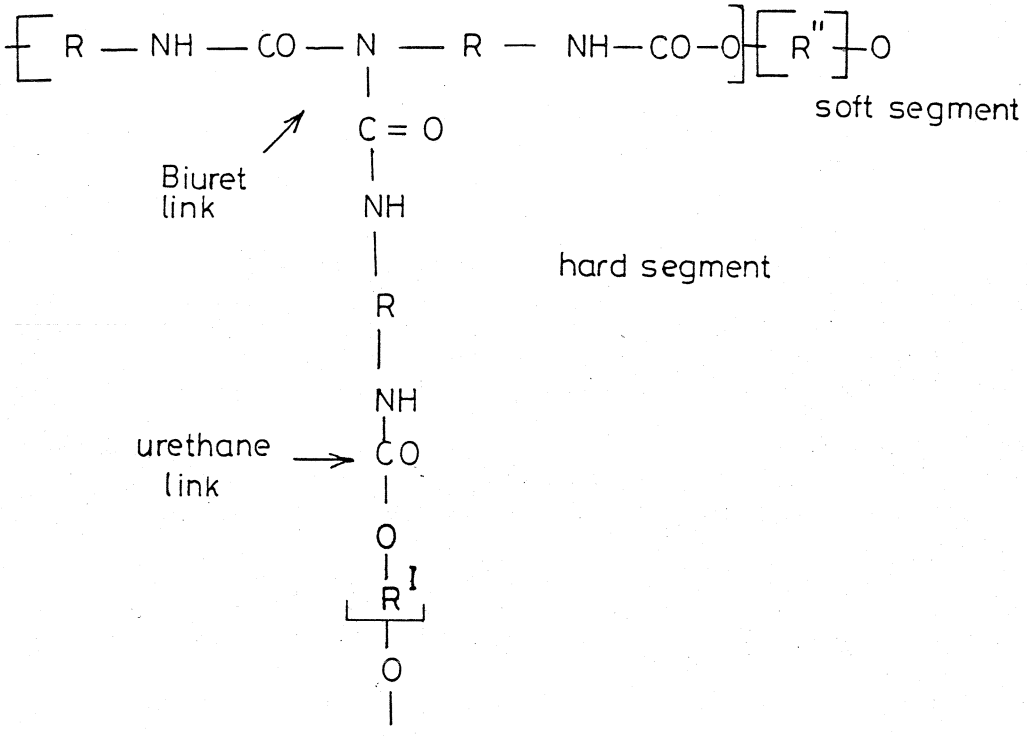
PTMG based polyurethanes. It may be due to the highly flexible nature of PEG which prevent the easy formation of cyclic intermediate. In the case of PPG one end has secondary hydroxyl group and the other end has primary hydroxyl group. The primary hydroxyl group is more reactive than the secondary hydroxyl group which makes the PPG relatively lesser reactive than PTMG. However the difference in reactivity between PTMG and PPG is less pronounced . The order of reactivity of polyols is given below[240].

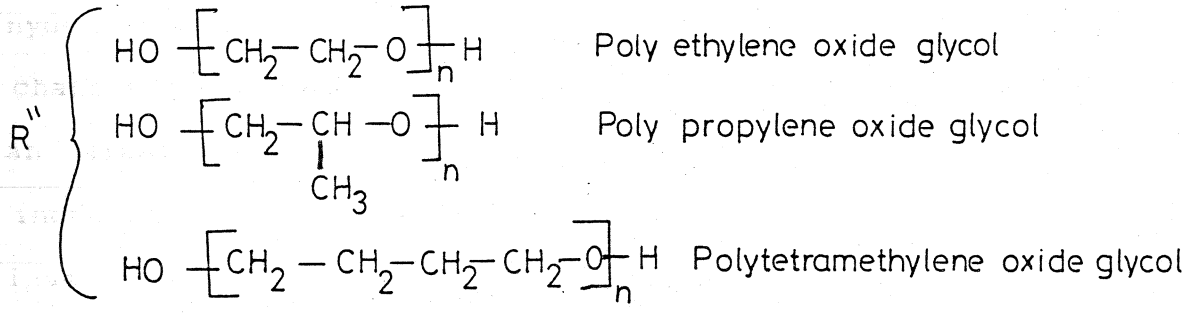
PTMG>PPG>PEG

The final addition to the reaction mixture with the crosslinker (trimethylol propane) enables further crosslinking. Curing is very important part of polyurethane synthesis which determines final properties of the polymer. The curing reactions of polyurethanes can be accelerated by electron donars (e.g hydroxyl groups) and electron accepting groups(e.g Carboxyl group and DBTDL)[241]. Generally original monomer or oligomer disappears very early in the reaction. After the gel point most of the reactive functional groups are attached to the three dimensional crosslinked network. In the present polyurethane systems the duration of prepolymer formation was long due to the decreased mobility of trifuntional network. When the mobilities of these reactive functional groups becomes seriously restricted, The curing reaction becomes diffusion controlled. The curing rate decreases with increasing extent of reaction[241].

The hard segment consist of diisocyanate and crosslinker units whereas the soft segment consist of polyol units.(Fig 5.2) A series of polyurethanes with different hard segments yield varying properties.

In conclusion the candidate polyurethanes are associated with biuret and urethane linkages in the hard segment and soft segment in the macromolecular architecture. The biuret and trifuntional hydroxyl extender forms the junction for the crosslinked network whereas the soft segment forms a linear segment in the final network.





R^1 { Crosslinker

R { Diisocyanate

Figure 5.2 The Hard and Soft segments of PU

PHISICO - CHEMICAL CHARACTERIZATION OF POLYURETHANES

The structure - property relationship of linear polyurethanes has been studied by some investigators[242-246]. There are very few studies on the effect of crosslinking on the properties of polyurethane elastomers [247-250]. In general changes in the degree of crosslinking do not affect the properties in all urethane polymers in the same way. In those noncrystalline linear polymers having high degree of effective intermolecular forces, increase in crosslinking first serve to reduce the effective intermolecular forces by reducing the fit. As the molecular weight between two crosslinks (Mc) decreases those forces eventually become relatively week so that further changes in Mc values results in changes which might be predicted for hydrocarbon elastomers. As crosslinking increases the polymer changes progressively from a linear thermoplastic soluble form to an elastomer with moderate crosslinking and finally with increase to a thermoset, rigid structure. In the present investigation, an attempt was made to correlate the effect of hard segment, which is composed of biuret,urethane and crosslinker , on the properties of polyurethanes.

5.2.1 Density

Density is an important bulk property. which can influence the material- tissue interactions. The density of the present polyurethanes are given in table 5.2. No difference could be observed significantly between the polyurethanes of each

PHISICO - CHEMICAL CHARACTERIZATION OF POLYURETHANES

The structure - property relationship of linear polyurethanes has been studied by some investigators[242-246]. There are very few studies on the effect of crosslinking on the properties of polyurethane elastomers [247-250]. In general changes in the degree of crosslinking do not affect the properties in all urethane polymers in the same way. In those noncrystalline linear polymers having high degree of effective intermolecular forces, increase in crosslinking first serve to reduce the effective intermolecular forces by reducing the fit. As the molecular weight between two crosslinks (Mc) decreases those forces eventually become relatively week so that further changes in Mc values results in changes which might be predicted for hydrocarbon elastomers. As crosslinking increases the polymer changes progressively from a linear thermoplastic soluble form to an elastomer with moderate crosslinking and finally with increase to a thermoset, rigid structure. In the present investigation, an attempt was made to correlate the effect of hard segment, which is composed of biuret,urethane and crosslinker , on the properties of polyurethanes.

5.2.1 Density

Density is an important bulk property. which can influence the material- tissue interactions. The density of the present polyurethanes are given in table 5.2. No difference could be observed significantly between the polyurethanes of each

Table 5.2 Physical properties of Polyurethanes

DI	PU	% Hard segment	Density	Q	$\bar{M}c$
IPDI	I1(PT)	41.47	1.024	1.6	802
	I2 ,,	25.53	1.019	4.2	4302
	I3 ,,	19.47	1.014	4.3	4396
	I4(PP)	41.47	1.023	2.6	1503
	I5 ,,	25.53	1.018	5.2	4901
	I6 ,,	19.47	1.012	6.1	5275
	I7(PE)	59.54	1.161	1.9	1476
SMDI	S1(PT)	33.36	1.081	1.7	1902
	S2 ,,	30.39	1.072	1.9	2509
	S3 ,,	28.42	1.064	3.3	3466
	S4 ,,	24.60	1.021	3.9	4100
	S5(PP)	33.36	1.063	2.9	2092
	S6 ,,	30.39	1.052	3.4	3806
	S7 ,,	28.42	1.043	4.2	4266
	S8 ,,	24.60	1.032	2.7	4654
	S9(PE)	44.42	1.147	2.4	2167
HDI	H1(PT)	26.62	1.147	4.6	4566
	H2 ,,	18.70	1.146	5.0	4776
	H3 ,,	15.13	1.146	5.3	4864
	H4 ,,	13.18	1.144	5.7	5066
	H5 ,,	12.08	1.142	6.8	5480
	H7(PP)	26.62	1.146	5.0	4796
	H8 ,,	12.08	1.147	5.8	5032
	H_8 (PE)	33.50	1.171		

Q = Equilibrium swelling ratio, $\bar{M}c$ = Molecular weight between crosslinks.

diisocyanate. However in the case of PEG based IPDI polyurethane(I7) the density is found to be slightly high, which may be due to higher amount of hard segment in comparison with the other polymer and easily crystallizing nature of PEG. Linear decrease of density with increase of soft segment concentration was observed with linear polyurethanes [251]. In general, at high soft segment concentration density is a measure of the soft segment packing and phase separation[252]. The PPG molecule has pendent methyl group which can renders more free volume in comparison with the PTMG and PEG based polyols in a linear polyurethane. The density of the present crosslinked polyurethanes based on PPG polyol does not differ much from that of PTMG based polyurethanes. HDI based polymers are more denser than the other polymers based on IPDI and SMDI due to more packing of the molecule with the HDI . The denser implant is not advisable as soft tissue implant, because it causes stress permanently to the tissue causing inflammation or irritation to the tissues. This is more pronounced when the implant is in motion.

5.2.2 Molecular weight between crosslink (Mc)

Molecular weight is an important parameter for the polymer concerned. In general the properties of any polymer may decrease or increase with the increase of molecular weight. However the molecular weight of a linear polymer has no effect on the properties after a particular level[242]. However in the

crosslinked polymer the properties vary with crosslink density[242]. The molecular weight between the two crosslink gives an idea about the degree of crosslinking. Higher the Mc value lower the crosslink density.

The molecular weight between two crosslinks(Mc) of the polymers are given in table 5.2. In the IPDI based polyurethanes, the Mc decreases with increase in % hard segment by weight. Very low Mc value of I1,I4 and I7 is due to the presence of large amount of biuret crosslinks when compared with that of other polymers of this series. When we compare the PTMG and PPG based polyurethane, the PPG based polyurethane shows higher Mc values. This is due to the fact that the pendent methyl group in the PPG molecule can give some degree of steric hindrance to the growing macromolecular chain.

According to Kontou et al the effective crosslink density of any polymer is sum of physical and chemical crosslinks[253]. In such case as the % of hard segment increases the effective crosslink density decreases due to decrease in the physical crosslink density. In the present polyurethanes, chances for the formation of physical crosslinks are limited since the crosslinking was carried out with the crosslinker(TMP) during the synthesis itself. Increase in Mc with increase in soft segment content(dilution) was observed as reported by Allen et al and Shen et al [254,255]. This leads to a decrease in the physical entanglement at higher dilution[254-255].

In SMDI based polyurethane also, Mc of the polymer decreases as the amount of hard segment by weight increased. Both in PTMG and PPG based polyurethanes the amount of crosslinker (trimethylol propane) is same except S4 and S8 (Table 4.3). But the amount of diisocyanate decreased from S1 to S3 and S5 to S7. Higher the diisocyanate, higher is the formation of biuret linkages.

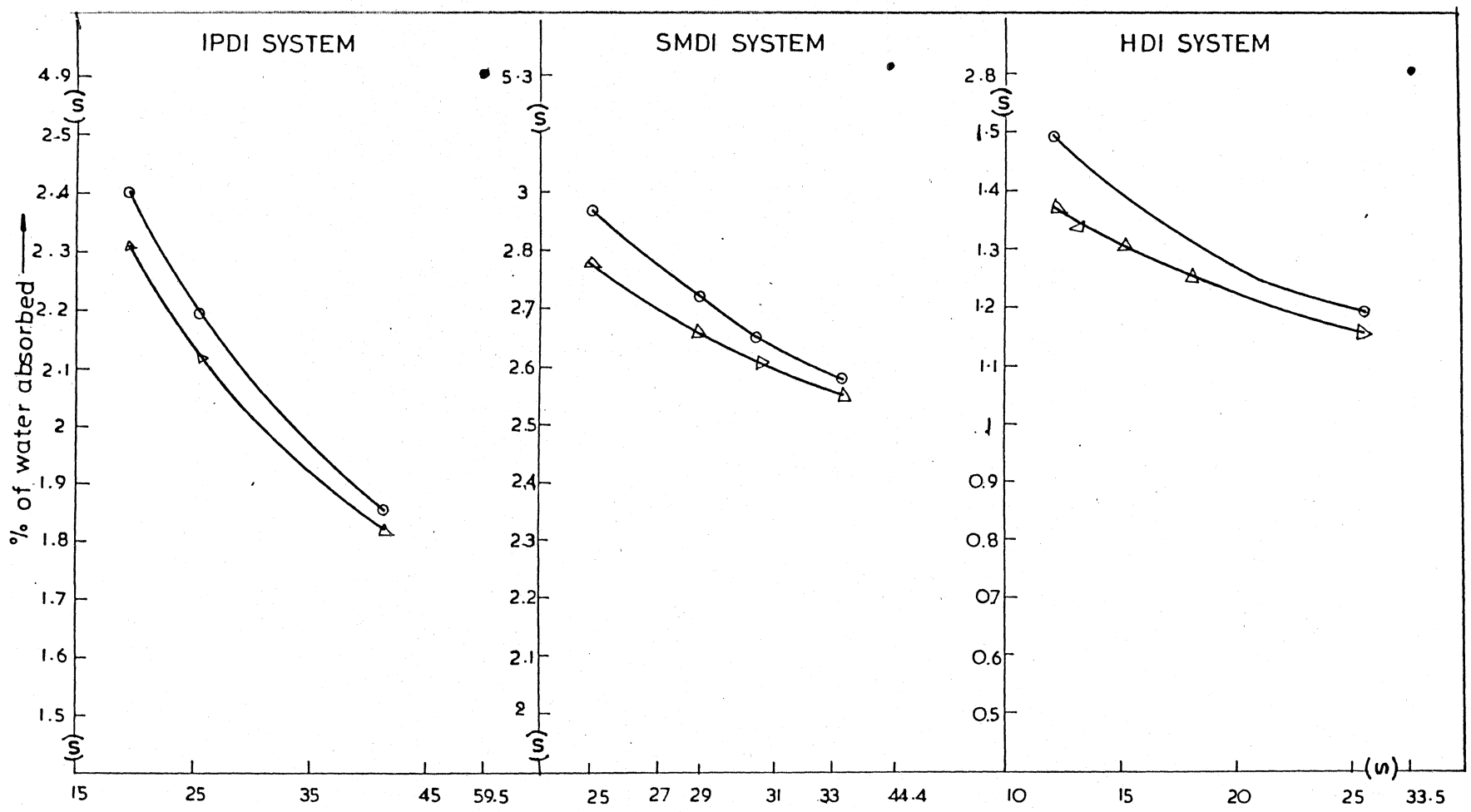
In HDI based polyurethanes also, Mc value increases with decrease in hard segment content.

5.2.4 Water absorption studies

The water absorption(%) of the three series of polymers are given in figure 5.3. Among the polyurethanes, PEG based polyurethanes absorbs more water in comparison with PPG and PTMG based polyurethanes. This is due to the fact that PEG is more hydrophilic than PTMG and PPG. The solubility parameter values of the urethane and polyols is given below[256,257].

Urethane	10.0	$\mu[cal\ c.m^{-3}]^{1/2}$
PTMG	8.2	$\mu[cal\ c.m^{-3}]^{1/2}$
PPG	8.7	$\mu[cal\ c.m^{-3}]^{1/2}$
PEG	11.1	$\mu[cal\ c.m^{-3}]^{1/2}$
Water	23.0	$\mu[cal\ c.m^{-3}]^{1/2}$

It is known that when the solubility parameter of the polymer approaches to that of the solvent, the polymer dissolves in the case of linear polymer ; but in the case of crosslinked polymer it swells. In the present polyurethanes the the solubility


IPDI SYSTEM
SMDI SYSTEM
HDI SYSTEM
% of water absorbed
% Hard segment
% Hard segment
% Hard segment
⊙PPG based Polyurethane
△ PTMG based Polyurethane
●PEG based Polyurethane

5.1

parameter of PEG is relatively close to the solubility parameter of water when compared to the other polyols.

However in the crosslinked polymer system the crosslinking plays a major role in the water absorption. As the Mc decreases, the water absorption decreases in all the polyurethanes based on HDI, SMDI and IPDI. However among PPG and PTMG based polyurethanes , PPG based polyurethanes absorb relatively more water which may be due to several factors such as loosely packed macromolecular chains and relatively higher solubility parameter.

5.3 Spectral analysis

I.R spectrum has been shown to be an effective technique to study the morphology of polyurethanes. Hydrogen bonding in polyurethanes results from the presence of the -NH group which is the proton donor and the carbonyl or ether oxygen groups which are hydrogen bond acceptors. The hydrogen bond acceptor may be either the hard segment (the carbonyl of the urethane group) or the soft segment (ether oxygen). Relative amounts of two types of hydrogen bonds determine the morphology of the polyurethane[258-260].

The ATR-I.R spectrum of the representative cross linked polyurethane is given in figure 5.4. The infrared band assignments for polyurethanes are given in Table 5.3. The spectral assignment clearly shows the polyurethane formation with the disappearance of the characteristic infrared peak for the the isocyanate group at 2265 $c.m^{-1}$., and no residual isocyanate

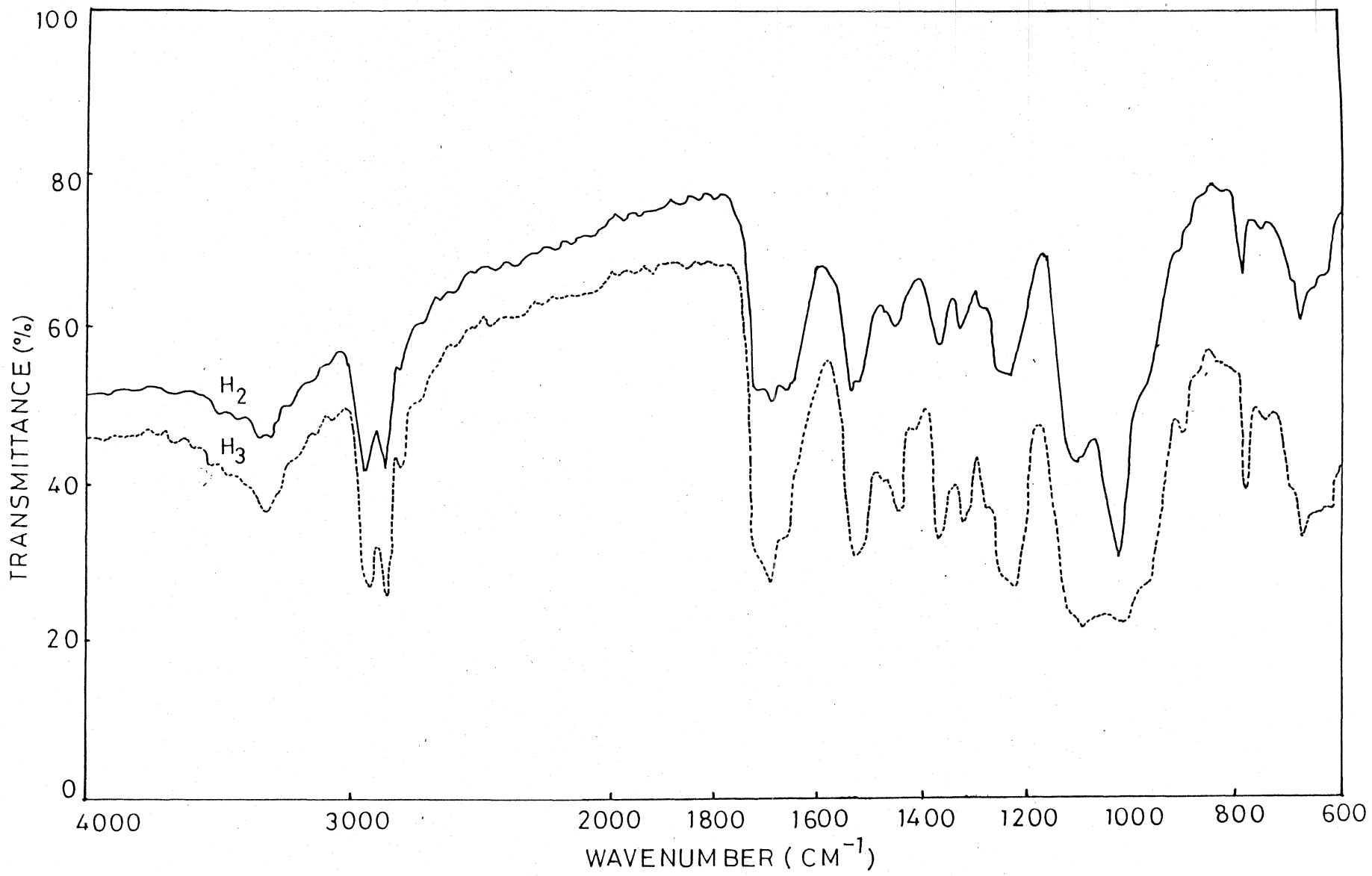


Fig. 5.5 ATR Infrared spectra of crosslinked polyurethanes

Table 5.3 Infra red band Assignments of Polyurethane

Wave Number CM^{-1}	Main assignment
3420 - 3445	ϑ(N-H) free N-H
3305 - 3330	ϑ(N-H) bonded N-H
2950 - 3020	ϑ- CH2- streching
2850 - 2900	ϑ- CH2- streching
1721 - 1735	C=0 free
1635 - 1645	C=O bonded
1700 - 1720	C=0 stretching, biuret
1080 - 1100	- 0 - C - 0 - ether

groups were detected in any of the materials tested. It has been observed that the frequency shift in the NH groups which is defined as

$$\Delta = vf - vb$$

where vf and vb are the frequencies of maximum absorption for the free and hydrogen bonded NH groups respectively. The shift Δv is considered as the measure of strength of hydrogen bond. As the hydrogen bond strengthens the (NH....O=C) distance, measured in the solid state by X ray crystallography, decreases and this decrease is usually accompanied by an increase in the difference between the associated NH stretching frequency and the non-associated NH stretching frequency[261].

In the present polyurethanes also there is frequency shift which is approximately $115cm^{-1}$ due to crosslinked nature of polymer matrix . Relatively higher value(80 - 120 $c.m^{-1}$) was observed for the linear polyurethanes [253,261,262].

5.2.4 Mechanical properties

One of the most important properties of a biomaterial, is it's ability to withstand the various mechanical forces placed upon it . In most clinical situations several forces are likely to act simultaneously. But for purposes of property evaluation they are usually measured separately . The important mechanical forces acting on a implant is tension, compression,shear, torsion and bending. These properties influence biofuntionality of soft tissue implants.

In the linear polyurethanes , the polar groups are free to align themselves and form very strong physical bonds. The introduction of crosslinks tend to prevent the polar groups aligning themselves to the full extent. Thus the mechanical properties of crosslinked polymers are different from that of the linear polyurethanes.

In IPDI polymers the polymers I1 and I4 are found to have higher tensile strength in comparison to their respective polyurethanes due to higher content of hard segment content and lower Mc. The % elongation increases with increase in soft segment concentration as reported elsewhere [263,264]. With PEG based polyurethanes, the tensile strength is found to be less due to the presence of highly flexible ethylene oxide group in the molecular chain. The stress - strain diagrams of IPDI polymers indicate the elastomeric nature in all polymers except I1 and I4. (figure 5.5). The stress-strain diagram of all the other polyurethanes are given in figures 5.6, and 5.7. With all the polymers except S4 and S8 , the modulus decreases with increase in Mc in their respective polyurethane series. (Table 5.4) For polymers S4 and S8 the modulus is very high due to the relatively higher crosslinker(TMP) content than in other polyurthanes. The PPG polymers show lesser tensile strength when compared with PTMG based polyurethane due to the pendent methyl group present in the PPG which prevent the cohesiveness of the molecule.

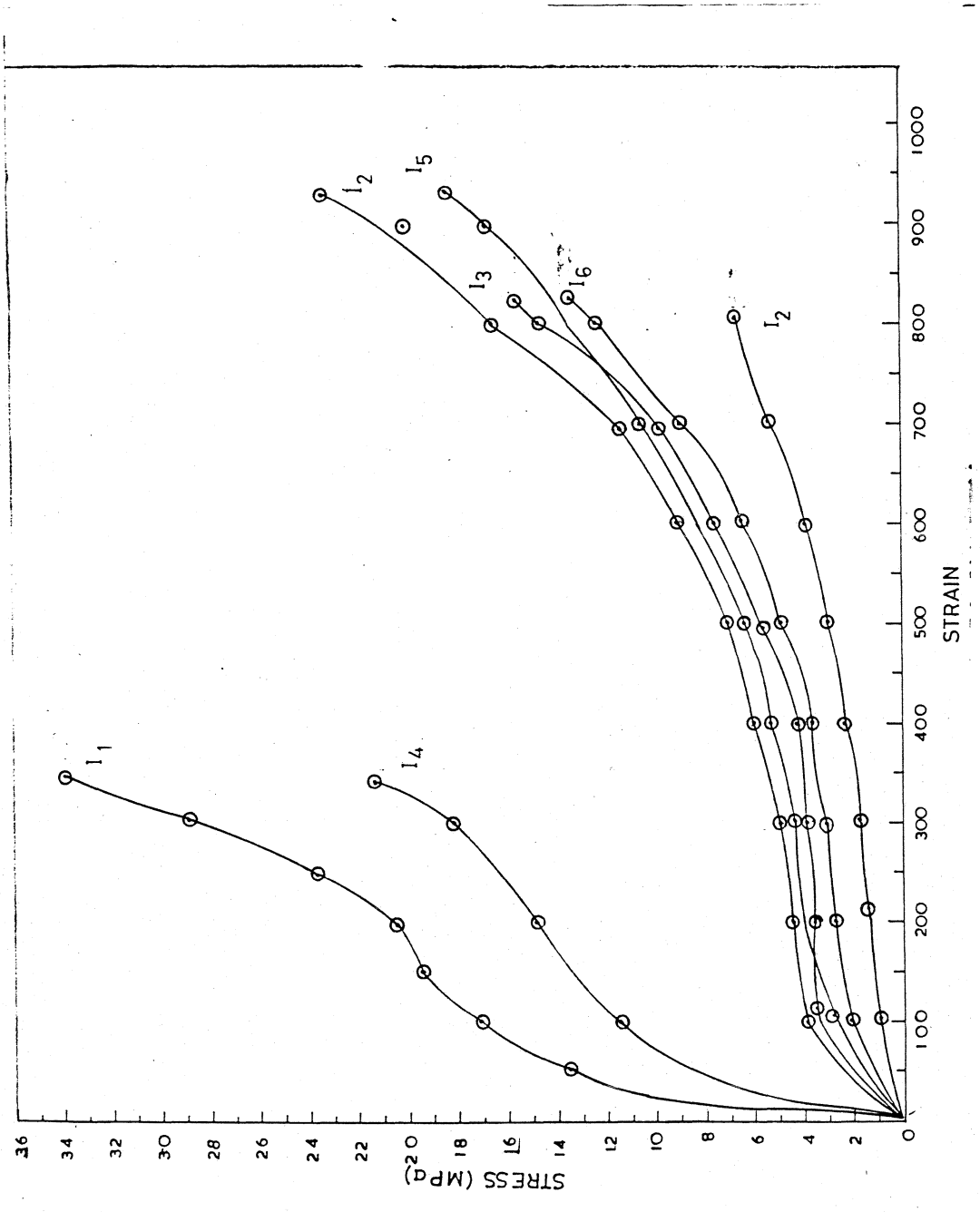


Figure 5.5 Stress Strain diagram of IPDI based PU

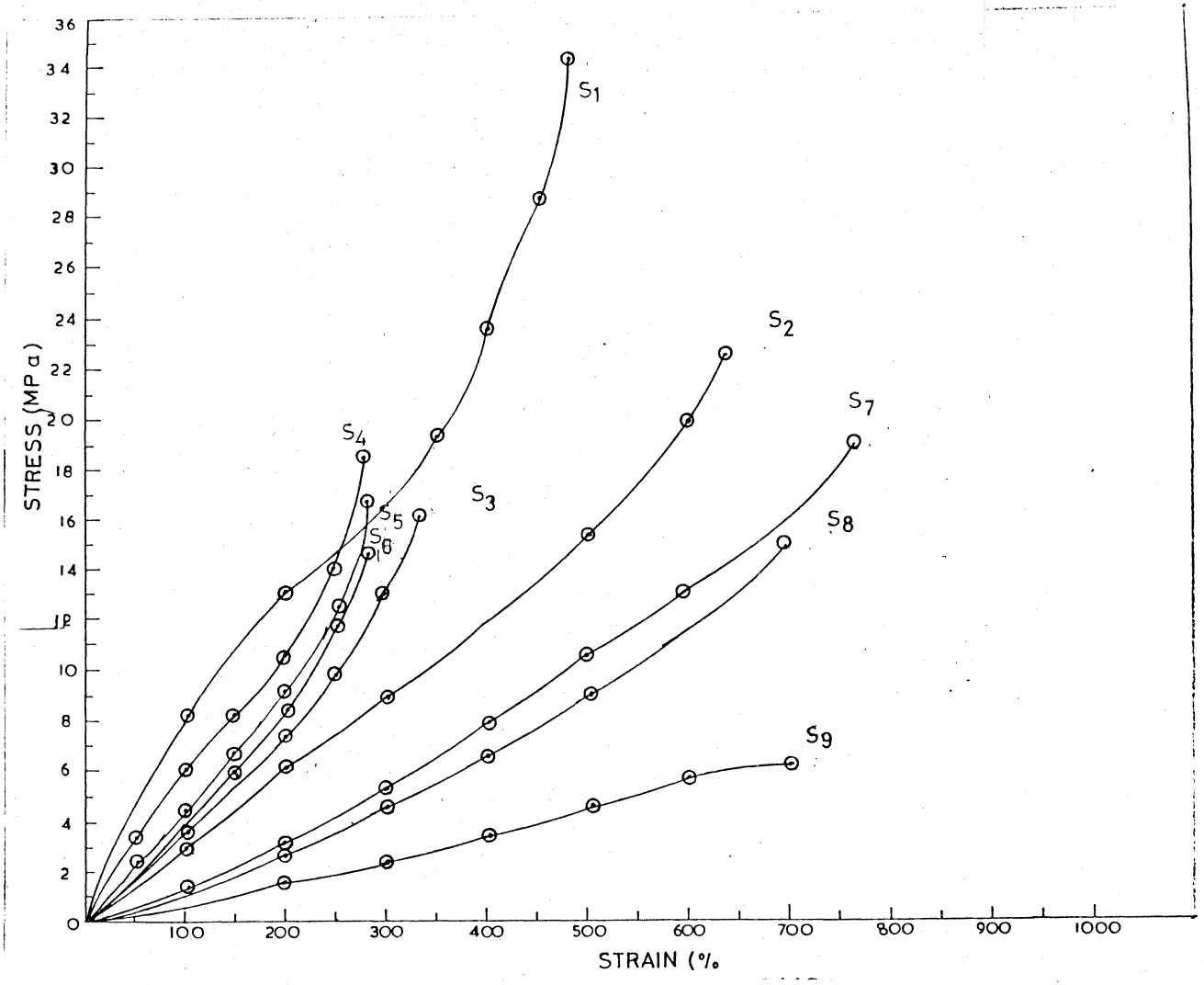


Figure 5.5 Stress strain diagram of SMDI based PU

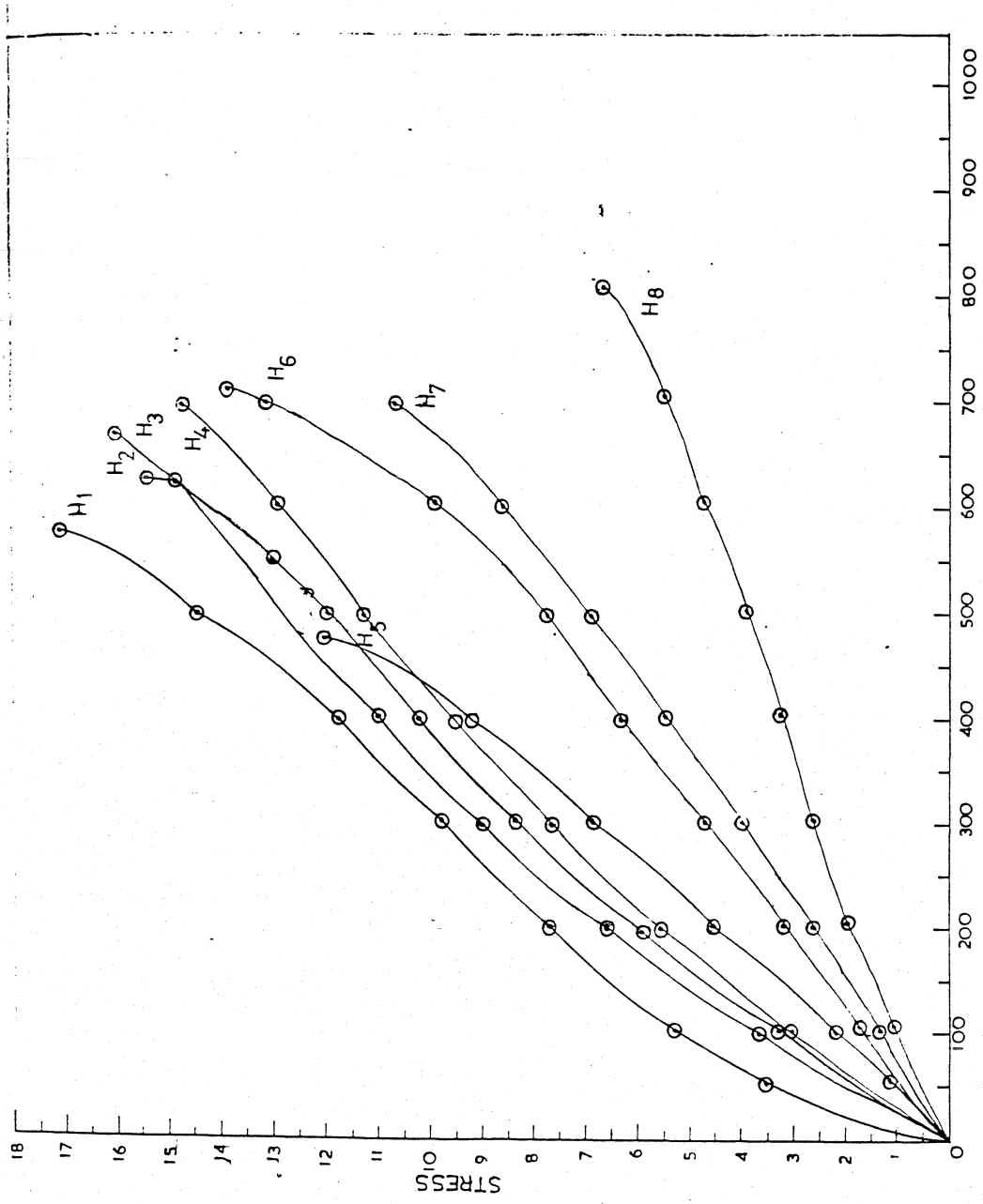


Figure 5.7 Stress Strain diagram of HDI based PU

Table 5. The Mechanical properties of Polyurethane

DI	PU	% Hard segment	Tensile[a] strength(Mpa)	% Elongb ation	Modulus	Hardness shore A
IPDI	I1(PT)	41.47	33.98	321.20	10.58	82
	I2 ,,	25.53	23.28	934.32	2.49	79
	I3 ,,	19.47	15.69	833.33	1.88	78
	I4(PP)	41.47	21.39	344.42	6.22	80
	I5 ,,	25.53	18.20	933.33	1.95	77
	I6 ,,	19.47	13.59	898.46	1.51	75
	I7(PE)	59.54	6.81	816.32	0.83	55
SMDI	S1(PT)	33.36	34.54	475.00	7.27	88
	S2 ,,	30.39	22.54	636.67	3.54	82
	S3 ,,	28.42	19.10	767.06	1.96	81
	S4 ,,	24.60	16.90	275.00	6.15	79
	S5(PP)	33.36	18.62	278.88	6.70	85
	S6 ,,	30.39	16.06	335.00	4.77	82
	S7 ,,	28.42	14.80	753.00	2.26	81
	S8 ,,	24.60	14.60	269.23	5.42	77
	S9(PE)	44.42	6.30	699.32	0.90	58
HDI	H1(PT)	26.62	17.13	580.20	2.95	79
	H2 ,,	18.70	16.10	670.00	2.40	78
	H3 ,,	15.13	14.73	700.2	2.10	77
	H4 ,,	13.18	15.10	620.7	2.43	76
	H5 ,,	12.08	13.92	712.80	1.96	75
	H6(PP)	26.62	10.69	470.20	2.27	78
	H7 ,,	12.08	12.16	696.20	1.75	71
	H8(PE)	33.50	6.67	812.00	0.82	51

Standard deviation a = < 5% , b = < 8%

Hardness has definitely an impact on biocompatibility of the material. If the material is harder than the surrounding tissues or biological environment, certainly the material leads to irritation and adverse inflammation. It certainly causes damage to the surrounding tissues. This effect is acute if the implant is in maximal motion site. Hardness is a measure of stiffness of the molecular chain . Chain units having very limited rotational or configurational possibilities tend to stiffen the polymer chains. Crystallization of the polymer units restrict the rotation resulting in higher value of hardness. Similarly increased crosslinking both physical and chemical crosslinking results in higher value of hardness [265].

The values of hardness of the present polyurethane systems are given in table 5.4. In IPDI polyurethanes the shore A hardness decreased as the soft segment concentration increased. This is true for PPG as well as PTMG based polyurethane. In PEG based polyurethane the shore A hardness value is very less due to the presence of more flexible PEG molecule. When we compare the PTMG and PPG based polyurethanes, the PPG molecule have restricted rotational or configurational possibilities to rotate polymer chains resulting in low hardness value. Thus the hardness of the polyurethane is directly proportional to the amount of hard segment[265]. This trend is same in SMDI and HDI based polyurethanes.

5.2.5 Surface properties

The first event after contact of a implant with the body fluid is the adsorption of proteins from the fluid at the polymer -liquid interface. All the process like cell adhesion, platelet adhesion and blood coagulation depend on this protein layer. The adsorption of protein is depend on the surface nature of the polymer. In general polymers adsorbing albumin is more compatible than adsorping other proteins. Commercial biomedical grade Polyurethanes having surface energy 30 to 70 dyns/cm and critical surface tension in the range of 27-29 dyns/cm is within the " zone biocompatibility (ie the range 20 - 30 dynes/cm critical surface tension) "[266]. Several authors have indicated that the importance of polar and dispersion components of the surface free energy for clear understanding of the biomaterial surfaces[267-269].

The various surface energy parameters of the polyurethanes are given in tables 5.5 to 5.7. In all the polyurethanes the surface energy are within the surface energy range of biomedical grade polyurethanes as mentioned above. Variation of polar and dispersion components of surface energy is not marked in PPG and PTMG based polyurethanes except in S5. The contact angle studies indicated that the present polyurethanes are highly hydrophilic in character. Higher polar - dispersive component ratio and low solid-water interfacial tension, $\gamma sp/\gamma sd$ are found in PEG based polyurethanes. Thus in the set of above three

Table 5.5 Contact angle measurements of IPDI based PU

PU	air	octane	tsp	$\sqrt{tsp}$	tsd	$\sqrt{tsd}$	$\sqrt{tsp}/\sqrt{tsd}$	SW
I1	39.75	133.10	35.77	5.98	20.91	4.57	1.21	1.24
I2	38.75	133.10	35.77	5.98	21.70	4.66	1.28	1.24
I3	38.75	132.10	35.22	5.94	22.35	4.73	1.26	1.35
I4	43.00	133.27	35.86	5.99	18.24	4.27	1.40	1.37
I5	39.00	130.40	34.29	5.86	23.21	4.82	1.18	1.57
I6	36.25	136.00	37.32	6.11	21.80	4.67	1.31	0.97
I7	31.00	146.00	42.23	6.50	19.95	4.47	1.45	0.38

rsp = Polar components of surface energy.

rsd = Dispersion components of surface energy.

SW = Interfacial surface energy between solid and water.

Standard deviation of all the measurements are less than 5 %

Table 5.6 Contact angle measurements of SMDI based Polyurethanes

PU	air	octane	tsp	$\sqrt{tsp}$	tsd	$\sqrt{tsd}$	$\sqrt{tsp}/\sqrt{tsd}$	SW
S1	45.00	133.50	35.86	5.99	16.61	4.08	1.47	1.55
S2	45.57	128.37	32.57	5.71	19.18	4.38	1.30	1.27
S3	50.10	120.29	28.57	5.35	20.76	4.56	1.23	3.09
S4	48.00	123.34	30.32	5.50	20.48	4.53	1.21	2.55
S5	40.50	120.75	28.84	5.37	29.21	5.40	0.99	3.62
S6	44.20	126.32	30.35	5.51	23.85	4.88	1.13	2.14
S7	51.20	123.40	30.35	5.51	17.51	4.18	1.33	2.80
S8	49.00	126.21	31.95	5.65	17.65	4.20	1.35	3.16
S9	33.00	136.23	37.44	6.12	24.04	4.90	1.25	1.01

rsp = Polar components of surface energy.

rsd = Dispersion components of surface energy.

SW = Interfacial surface energy between solid and water.

Standard deviation of all the measurements are less than 5 %

Table 5.7 Contact angle measurements of HDI based Polyurethane

PU	air	octane	tsp	$\sqrt{tsp}$	tsd	$\sqrt{tsd}$	$\sqrt{tsp}/\sqrt{tsd}$	SW
H1	46.10	126.20	31.94	5.65	20.17	4.49	1.26	2.65
H2	45.20	128.10	33.01	5.74	19.66	4.44	1.29	1.68
H3	45.10	129.30	33.68	5.80	18.97	4.36	1.33	1.78
Q H4	45.40	127.10	32.44	5.70	20.16	4.49	1.27	1.99
H5	45.20	128.10	33.01	5.74	19.67	4.44	1.29	1.68
H6	46.80	124.80	31.15	5.58	20.53	4.53	1.23	2.31
H7	46.20	125.90	31.77	5.64	20.29	4.50	1.25	2.10
H8	36.10	146.20	42.47	6.51	16.60	4.07	1.60	2.55

rsp = Polar components of surface energy.

rsd = Dispersion components of surface energy.

SW = Interfacial surface energy between solid and water.

Standard deveation of all the measurements are less than 5 %

polyurethanes, the polyurethanes based on PEG shows more hydrophilic in comparision with other polyol based polyurethanes. The polymers based on HDI has more captive air contact angle which indicate the less hydrophilic nature compared with the other polyurethanes.

5.2.6.Thermal properties :-

The important thermal property which affect the processability conditions are thermal degradation. The TGA analysis indicates that the candidate polyurethanes are stable upto 200°C. The representative TGA thermograms are given in figure 5.8. The relative thermal thermal stability of the candidate polyurethanes for 10% weight loss is in the following order:

HDI > SMDI > IPDI

The thermal property which can affect biocompatibility is glass transition temperature . For all the polyurethanes there is no significant glass transition temperature at around 37°C The DMA analysis indicate that the glass transition temperature of the most of the polyurethanes predominately appears around -30°C which may be due to soft segment (polyol) of the candidate polyurethanes. Representative DMA thermograms are shown in figure 5.9.

The standard differential thermal analysis of the present polyurethanes indicate the cross linked nature. All the polyurethanes except that of IPDI exhibit no softening endothermic peaks(figure 5.10) The IPDI based polyurethane shows

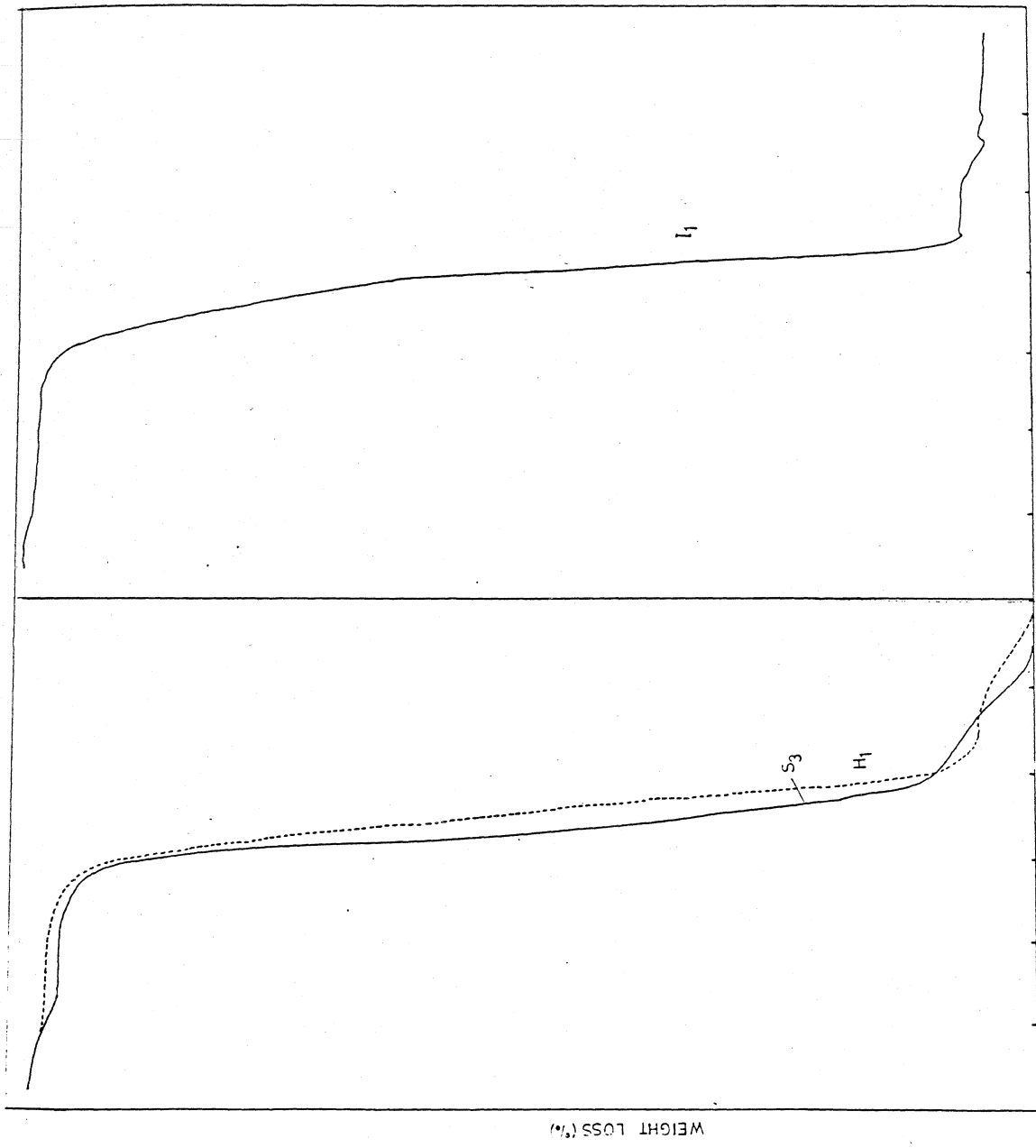


Figure 5.8 TG Analysis of Polyurethane

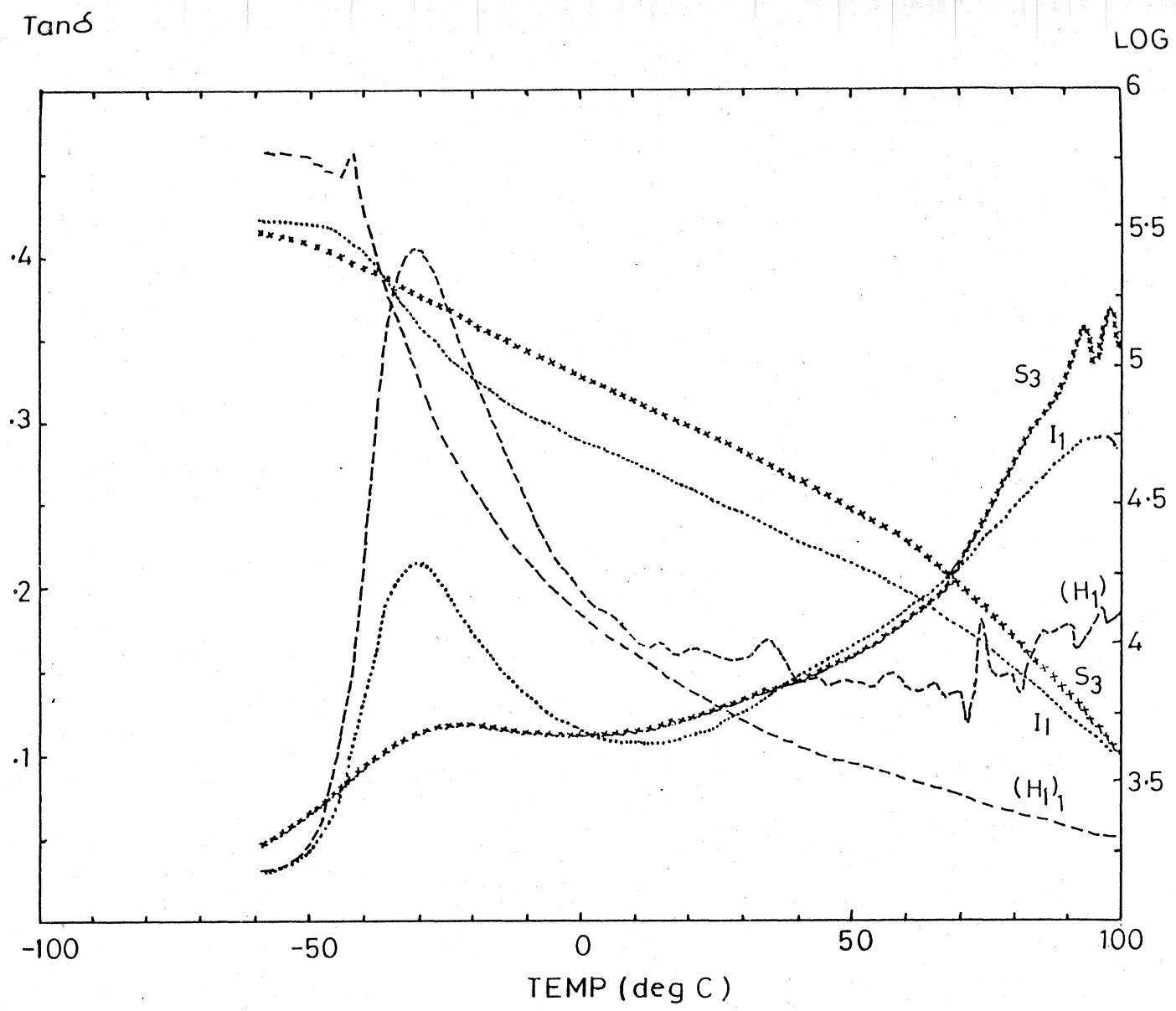


Figure 5.9 THERMO MECHANICAL SPECTRA OF PU

a relatively weak endothermic peak at around 150°C followed by elevation of the curve indicating higher thermal conductivity of the mass. Such trend is associated with the thermal degradation as observed in the TGA curves of this polymer (figure 5.8) All other curves based on HDI and SMDI does not elicit significant changes upto the initiation of degradation. Though the IR spectral studies showed the presence of hydrogen bonding. The DTA analysis do not reveal any significant changes which may be attributed to the effect of hydrogen bonding.

In conclusion the present crosslinked polyurethanes are found to possess the following general characteristics.

1. HDI based polyurethanes are denser in comparison with IPDI and SMDI based polyurethanes.
2. As the hard segment content increases the Mc decreases in all the polyurethanes.
3. Water absorption studies shows that the PEG based polymer absorbs more water following the PPG and PCTMG based polyurethanes.
4. I.R spectral analyses showed that the present polyurethanes also possess hydrogen bondings in the network
5. Mechanical properties studies showed that the present polyurethanes are elastomeric in nature. Higher the Mc higher will be the tensile modulus.
6. All the polyurethanes are relatively more hydrophilic.

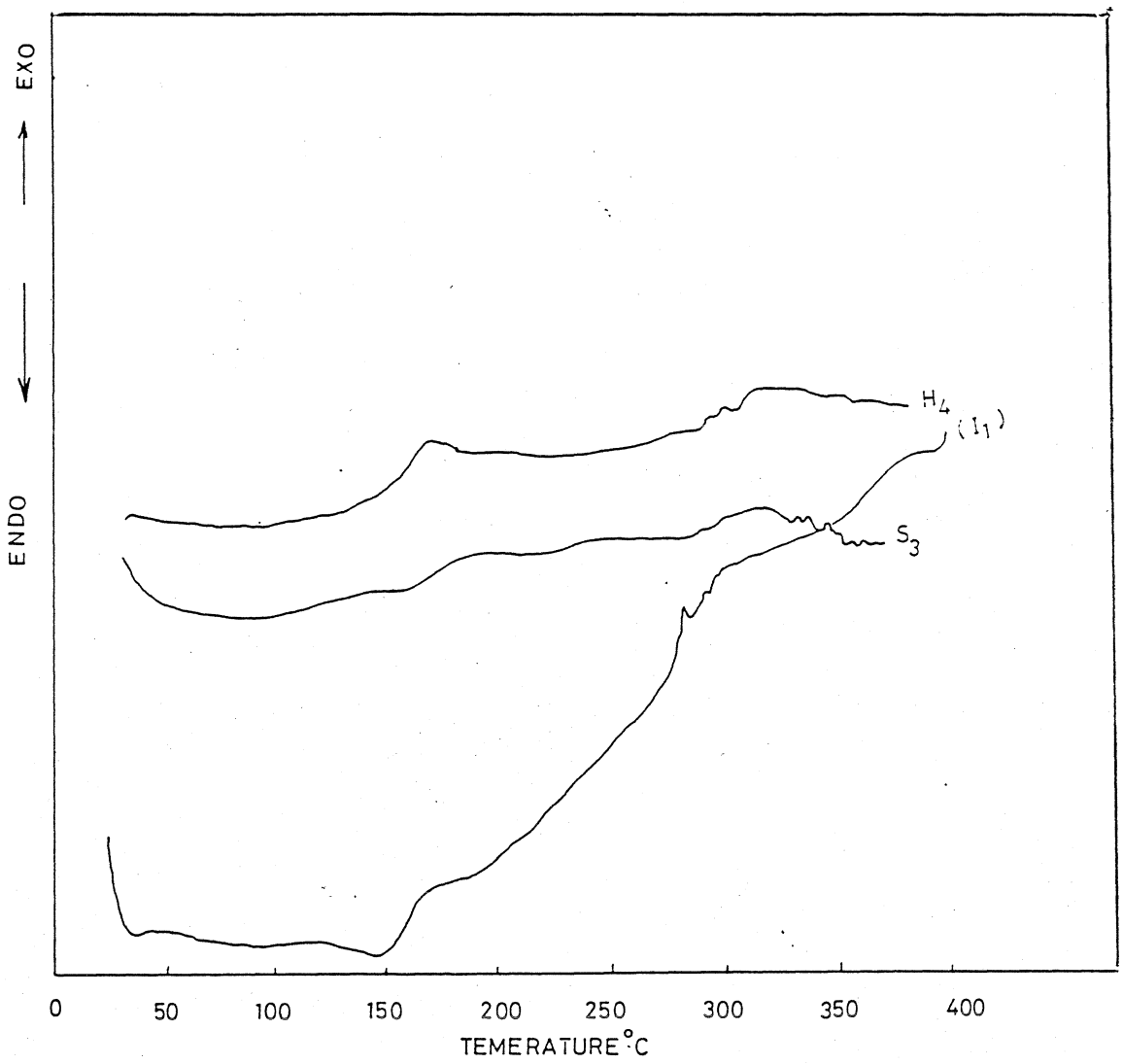


Figure 5.10 DTA Thermogram of PU

7. All the polymers are stable upto 200°C with a T_g at around -30°C and does not show any softening with the exception of IPDI based polyurethanes which shows a weak softening point.

STUDIES ON BIOCOMPATIBILITY

A safe medical implant should perform as intended and present no significant harm to the patient. One important aspect of biocompatibility is the potential toxicity of a biomedical implant. The toxicity and /or unfavorable host response of the implant may arise by material - biological component interactions. The following are the different types of undesirable responses.

(a) Sorption of cellular constituents from tissue leading to change of physical ,chemical and mechanical properties of materials.

(b) Leaching of ingredients from the implant.

(c) Sorption of vital cellular constituents such as antigen, antibodies,hormones and various circulating drugs.

(d) Material - blood contact leading to thrombus formation.

(e) Material - tissue contact leading to scar tissue and immobilization of the implant.

(f) Physical or solid state carcinogenesis.

The results of following in vitro and in vivo biological tests asses the toxic potential of the candidate materials.

Biological tests

Hemolysis test is important biological test used to asses the hemolytic potential of the material. The results of hemolysis test are given in table 5.8. All the candidate materials shows less than the permitted limit ie 20 mg /100ml of blood, and also

Table 5.8 In vitro Biocompatibility test results

SMDI	Wt	H.lysis(%)	R.C.T	IPDI	Wt	H.lysis	R.C.T	HDI	Wt	H.lysis	R.C.T
S1	a,10.2 b,19.2	9.2 13.3	46	I1	a,11.1 b,18.3	8.1 11.3	39	H1	a, 9.1 b,11.9	6.2 11.3	37
S2	a, 9.8 b,16.4	8.4 9.9	47	I2	a, 9.1 b,16.2	7.2 13.4	41	H2	a, 7.1 b,12.4	8.2 10.9	42
S3	a, 8.9 b,16.9	9.3 12.2	42	I3	a, 8.9 b,15.9	8.1 12.5	38	H3	a, 8.9 b,10.3	8.6 10.0	44
S4	a,10.1 b,17.9	8.2 13.2	41	I4	a, 9.1 b,16.4	7.6 12.9	41	H4	a, 6.9 b,17.3	5.4 16.2	42
S5	a, 8.7 b,17.5	9.0 12.2	39	I5	a, 8.7 b,17.1	6.2 13.3	41	H5	a, 8.2 b,12.6	7.1 10.1	39
S6	a, 9.6 b,16.9	8.6 11.9	38	I6	a, 9.0 b,15.9	8.1 12.7	37	H6	a, 9.1 b,14.2	8.1 11.2	37
S7	a, 8.7 b,16.7	9.2 10.9	42	I7	a, 9.1 b,18.1	9.8 18.6	39	H7	a, 8.3 b,16.1	7.2 12.1	41
S8	a, 9.8 b,18.3	6.4 13.4	41					H8	a, 7.9 b,19.3	9.1 18.2	39
S9	a,10.1 b,18.1	8.2 19.8	44								
T-85	a, 9.2 b,16.2	9.1 12.9	37								

H.lysis = Haemolysis

R.C.T = Recalcification time(sec)

comparable with the biomedical grade polyurethane. However the polyurethane based on polyethylene glycol I7,S9 and H8 showed relatively higher hemolysis value. The recalcification time of the synthesized polymers are also given in table 5.8. This test measures the clotting potential of the blood on the material. This test clearly shows that the candidate materials behaves like biomedical grade polyurethanes.

Systemic toxicity of the polymers indicates the polar and nonpolar toxicants. The animals injected with saline and cotton seed oil extract were in sound health during the observations. It shows that the synthesized polymers do not leach any systemic toxicants. The intracutaneous irritation tests elicited no edema, erythema or necrosis with cotton seed oil and saline extract of the test polyurethanes. This test also indicate the candidate polymers do not leach any irritant.

Studies on intramuscular implantation

The testing of a new biomaterial in man regardless of its proposed specialized use, usually follows the gross and microscopic examination of the response to the material implanted in test animals. The extent and duration of acute and chronic inflammatory response evoked by intramuscular implants anticipates the response to them[270-271]. Further more the extent and duration of the inflammatory response found in rodents or dogs are similar to those found in primates allowing accurate inferences to be obtained from responses in test animals as to the anticipated responses in man[272].

Seven days post implantation period

The first observable changes of inflammation are ' color ' (heat), 'rubor' (redness),' tumor' (swelling) and 'dolor' (pain). In addition to cells and proteins, lymph vessels are also hold widely open for the flow of fluid containing excess of protein. The first cell involved in the inflammatory process is the polymorphonuclear neutrophils (PMN) and it takes 2-9 minutes to penetrate the wall.They are the first fight against bacteria being armed with peroxides, lysozyme and other proteolytic and hydrolytic enzymes contained within the granules[273,274]. Several hours latter the monocytes predominates and in the tissues these large cells become phagocytic and are called macrophages, their emigration continued after the emigration of polymorphs has stopped. In the present investigation the lecocytes concentrated at 7 days post implantation period for all most all the polymer implants.(table 5.9 to 5.11). A similar observation was made by Rigdon et al [273,274]. The degree of necrosis varies from +1 to +4 for all the samples (table 5.9 to 5.11) The neutrophils accumulation are more in all the samples. Lymphocytes and plasma cells were also evidenced. Usually it is observed at the site of chronic inflammation, the number of plasma cells and lymphocytes is related to the underlying cause of the inflammation and in conditions of chronic immunological injury, these cells predominate over other inflammatory cells[275]. In the absence of immunological injury the presence of these cells is not fully

Table 5.9 The Histopathological Response to IPDI based Polyurethanes (7 days)

	C	I3	I6	I1	I4	I7
Degree of necrosis	2+	4+	4+	3+	2+	4+
Neutrophils	1+	4+	3+	3+	3+	2+
Macrophages	4+	4+	4+	4+	3+	4+
Lymphocytes	3+	4+	3+	3+	2+	3+
Plasma cells	2+	4+	2+	2+	2+	2+
Giant cells	2+	2+	3+	3+	3+	3+
Eosinophils	1+	4+	2+	2+	1+	1+
Foreign body debri	KS	-	K	-	K	-
Fibroplasia	2+	2+	1+	2+	2+	2+
Fibrocytes	3+	2+	2+	3+	2+	2+
Fatty infiltration	0	1+	0	-	0	0
Calcification	0	0	0	-	0	0
Haemoraghe	1+	1+	1+	1+	1+	3+
Oedema	0	0	0	-	1+	2+
Any other	-	0	-	C	-	-

KS = Keratin, Squamous epithelim and few hair follicles.

K = Keratinised epithelium.

C = Cartilege,bone marrow tissue and adipose tissue are seen in one section.

Table 5.10 The Histopathological Evaluation of Tissue Response to SMDI based Polyurethanes (7 Days)

	C	S4	S1	S8	S5	S9
Degree of necrosis	2+	3+	3+	3+	1+	3+
Neutrophils	3+	3+	3+	3+	3+	3+
Macrophages	3+	3+	2+	2+	3+	3+
Lymphocytes	3+	3+	2+	2+	3+	3+
Plasma cells	1+	1+	1+	1+	1+	2+
Giant cells	2+	3+	3+	2+	1+	3+
Eosinophils	3+	0	0	3+	1+	1+
Foreign body debri	0	0	S	0	0	0
Fibroplasia	2+	1+	1+	2+	2+	2+
Fibrocytes	1+	1+	1+	1+	2+	2+
Fatty infiltration	0	0	0	0	1+	0
Calcification	0	0	0	0	0	0
Haemoraghe	1+	1+	1+	2+	0	2+
Oedema	0	0	0	0	0	0
Any other	0	0	0	0	0	0

S =Squamous epithelium dermal collagen and keratin.

Table 5.11 Histopathological Evaluation of Tissue Response to HDI based Polyurethanes (7 Days)

	C	H1	H5	H6	H7	H8
Degree of necrosis	1+	4+	3+	3+	1+	0
Neutrophils	1+	4+	2+	1+	1+	0
Macrophages	1+	4+	3+	2+	1+	0
Lymphocytes	1+	4+	3+	3+	1+	1+
Plasma cells	1+	3+	2+	1+	0	0
Giant cells	2+	4+	2+	2+	1+	M
Eosinophils	1+	2+	1+	1+	0	0
Foreign body debri	0	S	-	H	0	0
Fibroplasia	2+	2+	2+	3+	1+	1+
Fibrocytes	2+	3+	2+	4+	1+	1+
Fatty infiltration	0	0	0	0	0	0
Calcification	0	0	0	0	0	0
Haemoraghe	0	1+	1+	0	1+	0
Oedema	0	0	0	0	0	0
Any other	0	0	0	0	0	0

S = Skin

H = Hair

M =Muscle giant cells

understood. The muscle giant cells were observed at the 7th day post operative period. We could not observe any foreign body giant cells at this period. The formation of foreign body giant cells by the fusion of macrophages on the polymer surface is also different for different polymers. For most polymers foreign body giant cells were noted on the surface even at 4 days following implantation. Anderson et al speculated that the foreign body giant cell formation is a function of polymer chain mobility at the surface which facilitate the mobility and subsequent fusion to form foreign body giant cells[276].

In the present investigations few eosinophils were noted in almost all the samples(Table 5.9 to 5.11). The function of the eosinophils are somewhat elusive, but present indications are that this cell is concerned mainly with endogeneous substances such as mast cell and lympocyte products, kinins and complement. Foreign body debri in general not present but keratin, squamous epithelium and hair follicle are present with the investigative tissues in some sections due to experimental error.

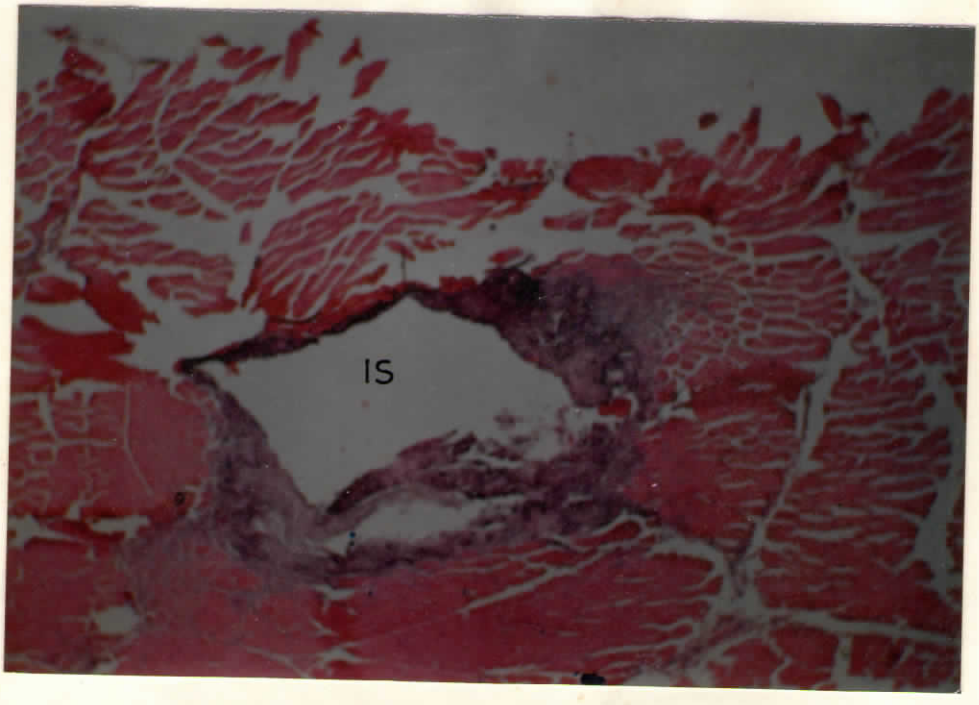
The presence of fibroplasia and fibrocytes indicate by the beginning of repair of wounds. Fatty infiltration was not observed in almost all sections except for the sample I3. Calcification was not found in any of the sections . However haemoraghe was present in many sections due to experimental reason. Oedema was not observed in all the samples except for the I4 and I7 samples.

The implantation site of the control polymer(Tecoflex 85 A) is shown in the microphotograph 5.11(a). The accumulation of inflammatory cells are also shown in the figure 5.11(b) for the control polymer. The inflammatory cells are shown in higher magnification for the polymers S4 and H5 in figures 5.12(a) and 5.12(b) respectively.

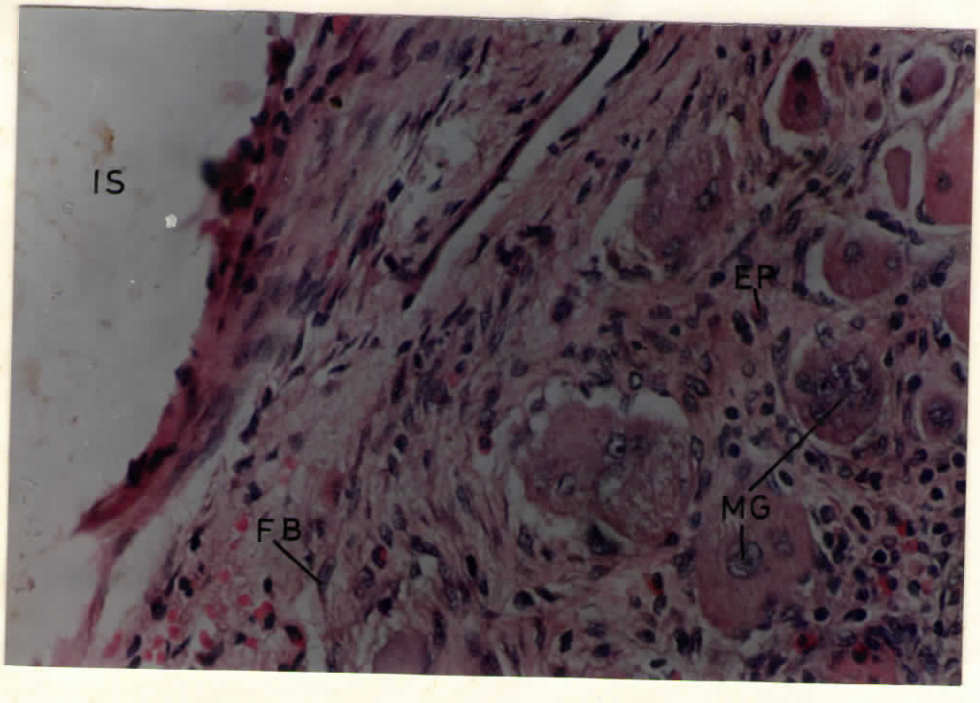
The overall 7 days post implantation results showed that tissue response of the candidate materials are similar to that of control biomedical grade polyurethane.

Thirty days post implantation period

With the repairing process the degree of necrosis was decreased continuously and neutrophil accumulation was less and in some cases there were no neutrophil at 30 days post operative period.The macrophage concentration also reduced continuously (figure 5.13(a) & (b). Salthouse has shown that macrophages adhere to the many implanted polymers such as Dacron, Teflon etc[277]. The behaviour of the macrophages depends upon the surface characteristics of the polymer. Roughened surfaces confined considerable number of macrophages while on smooth surfaces, there were few cells even after one month of implantation[277]. Behling and Spector also observed macrophages adhered to the polymers such as polyethylene and polysulphone[278]. Lymphocytes and plasma cells were reduced considerably. The surface characteristics play a major role on the giant cell formation. Anderson et al found that hydrophobic surface evoke



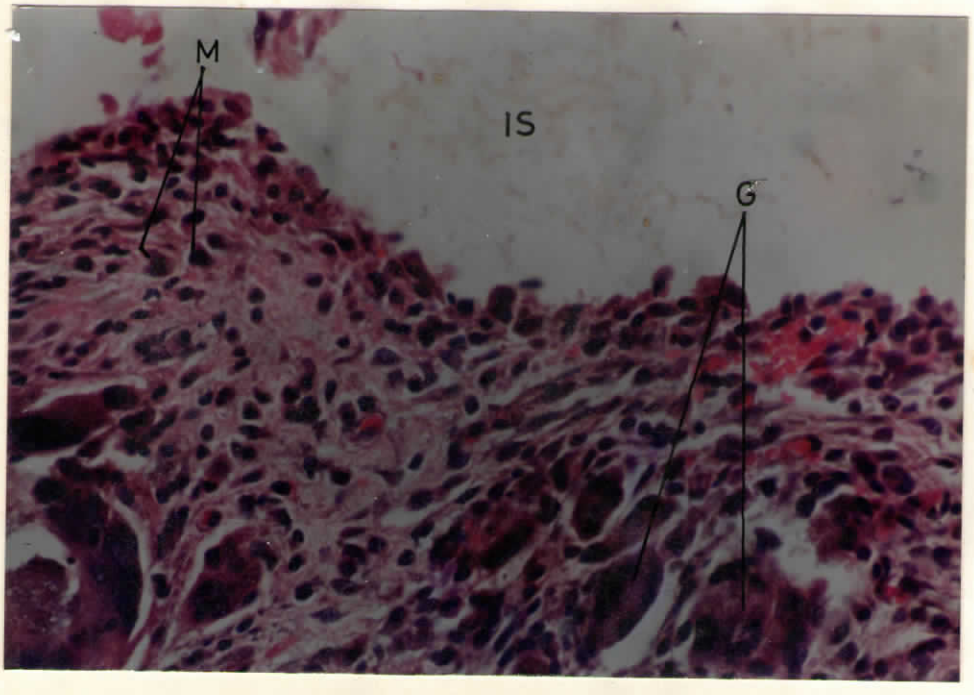
(a)



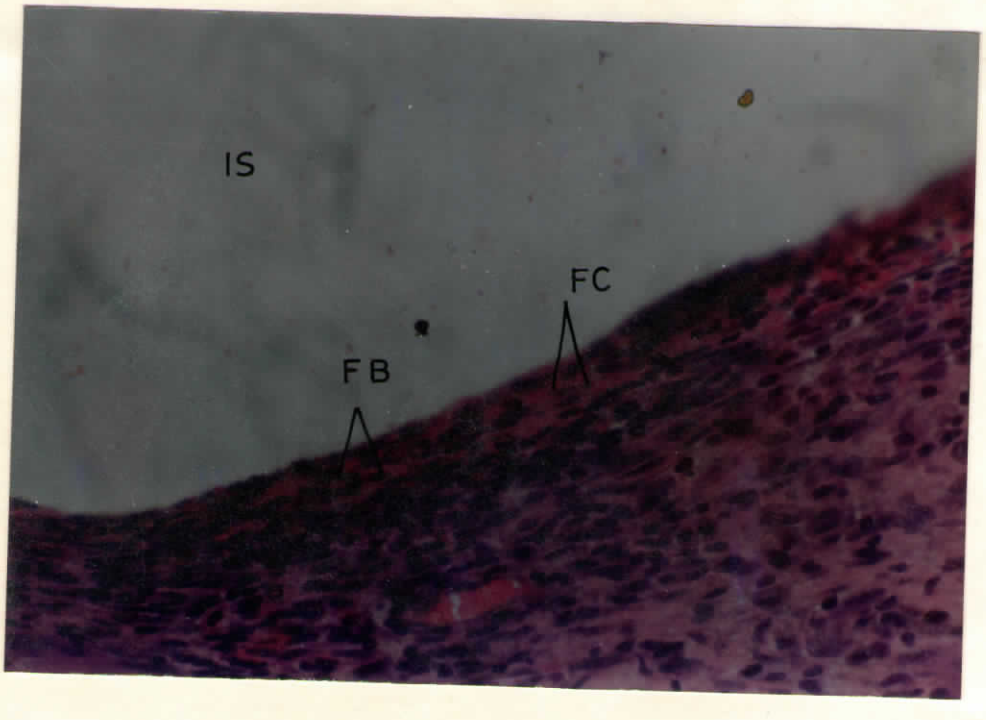
(b)

Figure 5.11(a) The implantation site of control polyurethane Eosin & Hematoxylene X 40 - 7 days

Figure 5.11(b) indicats the enlarged accumulation of neutrophils and other cells X 400 -Control polyurethane - 7 days.
Is - Implantation site,Fb - Fibroblast , MG Muscle Giant cell, Ep - Eosinophils.



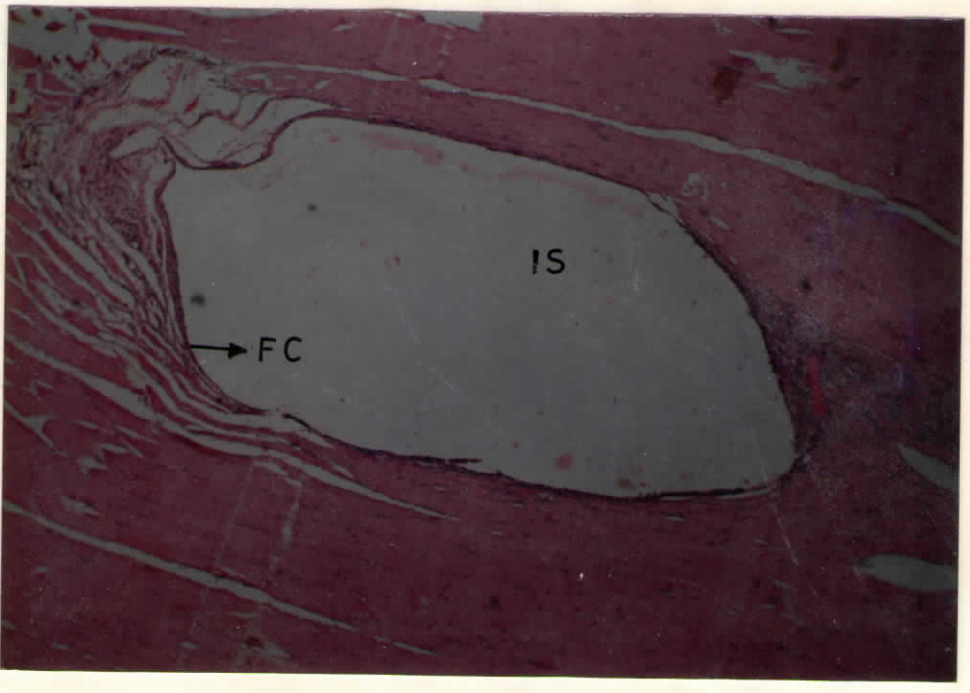
(a)



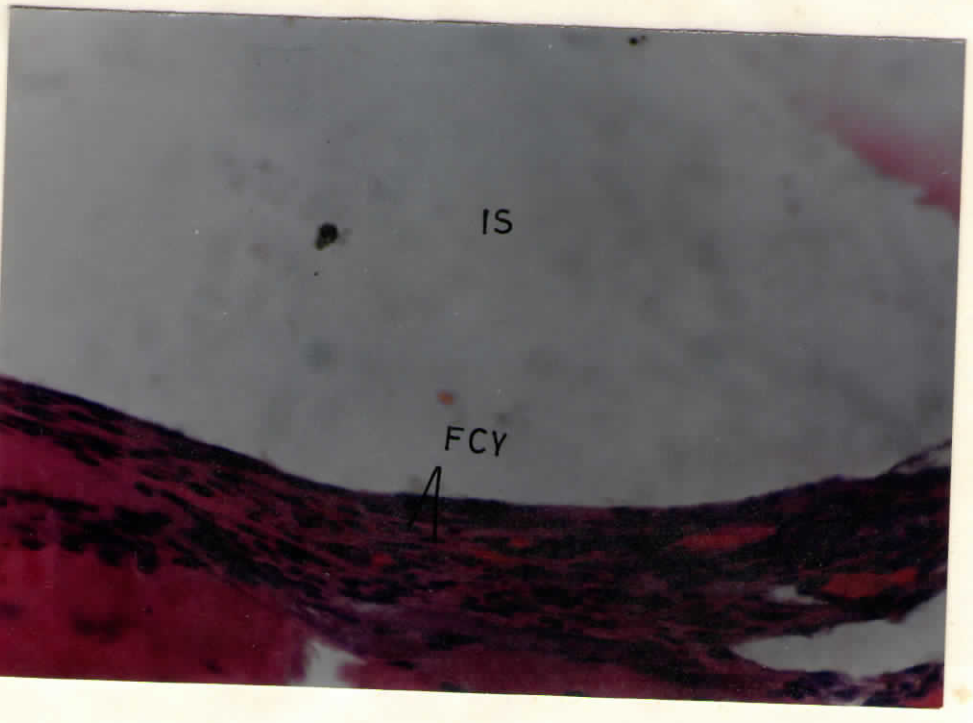
(b)

ure 5.12(a) shows the predominance of neutrophils . Eosin & Hematoxylene stain, 7 days post implantation period- S1 polyurethane X 400

ure 5.12(b) indicates the initiation of fibrisis. Eosin & Hematoxylene stain 7 days post implantation period - H5 Polyurethane X 400
M - Macrophage , G - Giant cell , Is - implantation site, Fb -Fibroblasia, Fc - Fibrocyte



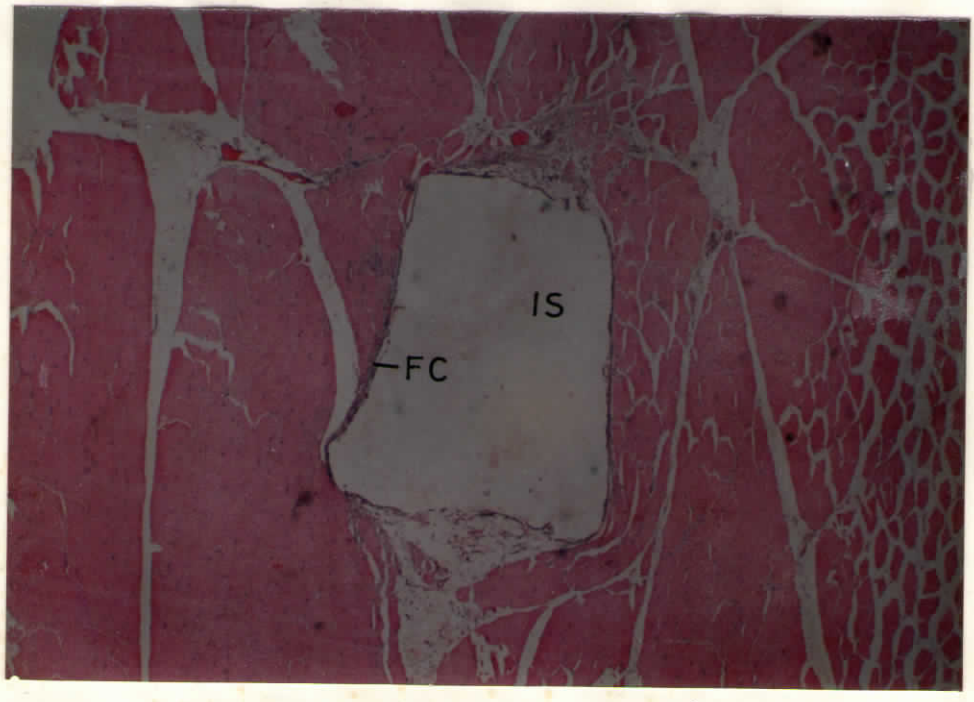
(a)



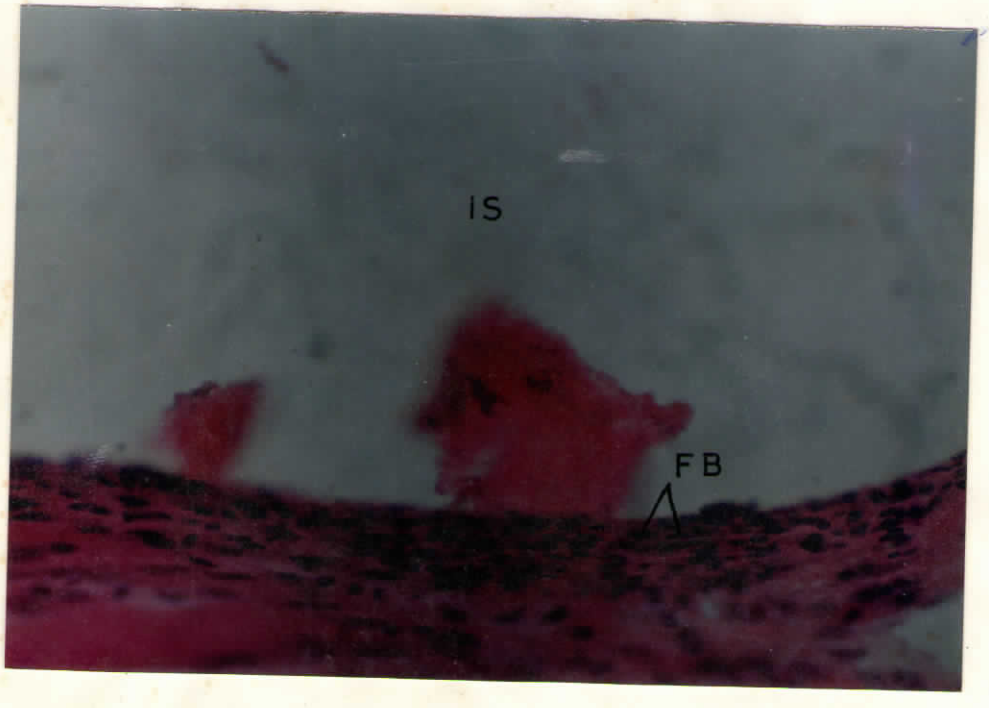
(b)

.13(a) shows the immplantation site. Eosin & Hematoxylene stain, 30 days post implantation period, Control polymer, X 40

.13(b) shows the fibrosis. Eosin & Hematoxylene stain, I3 polyurethane, X400



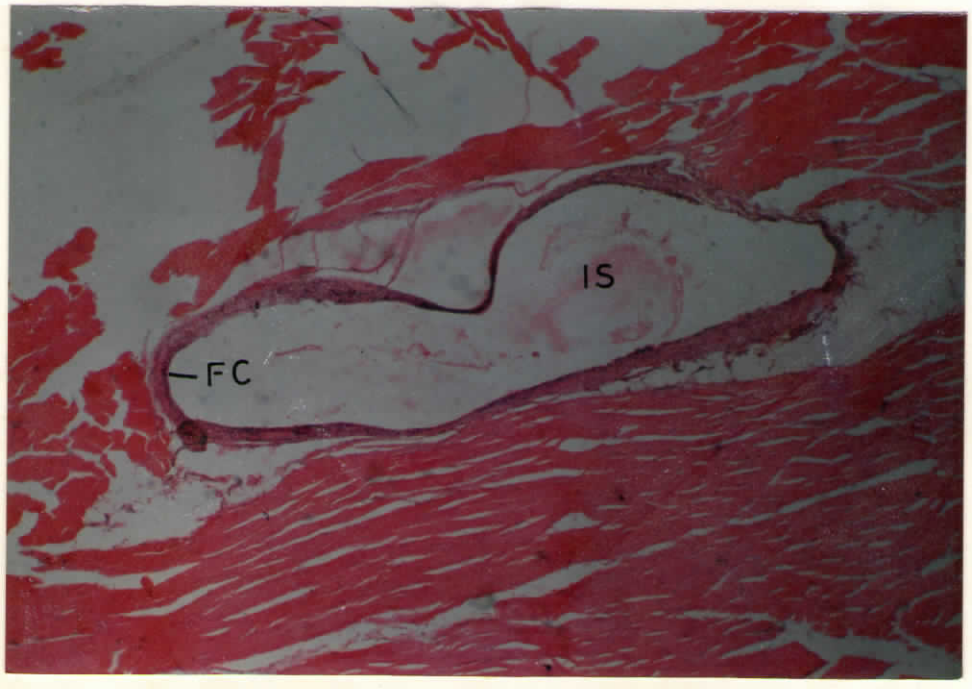
(a)



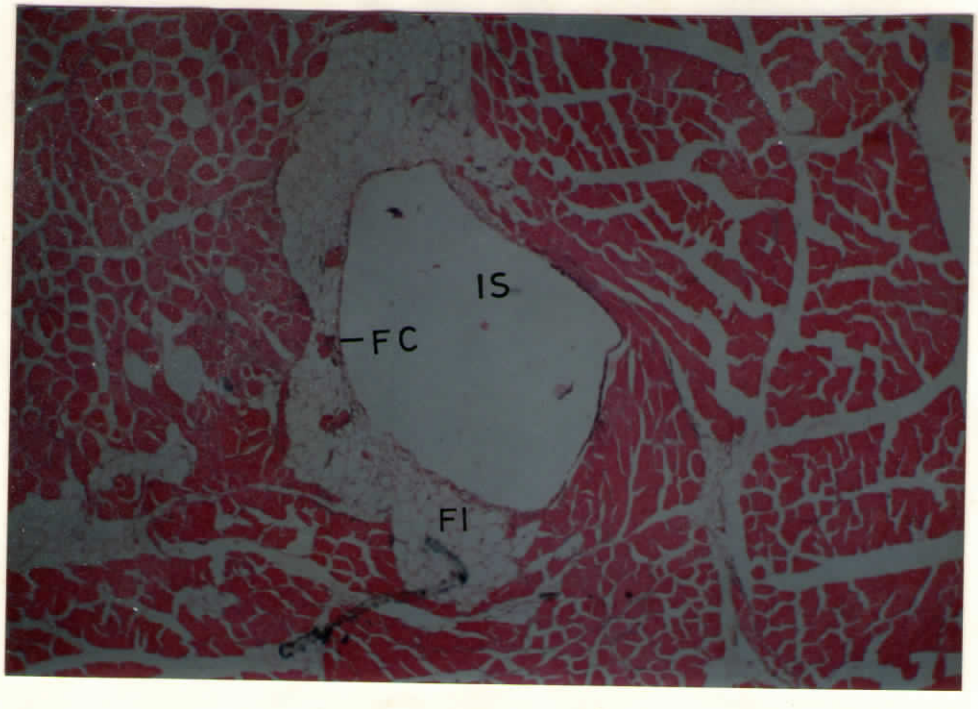
(b)

5.14(a) shows the immplantation site. Eosin & Hematoxylene stain, 30 days post implantation period, H5 polyurethane, X 40

5.14(b) shows the fibrosis. Eosin & Hematoxylene stain, S1 polyurethane, X400



(a)

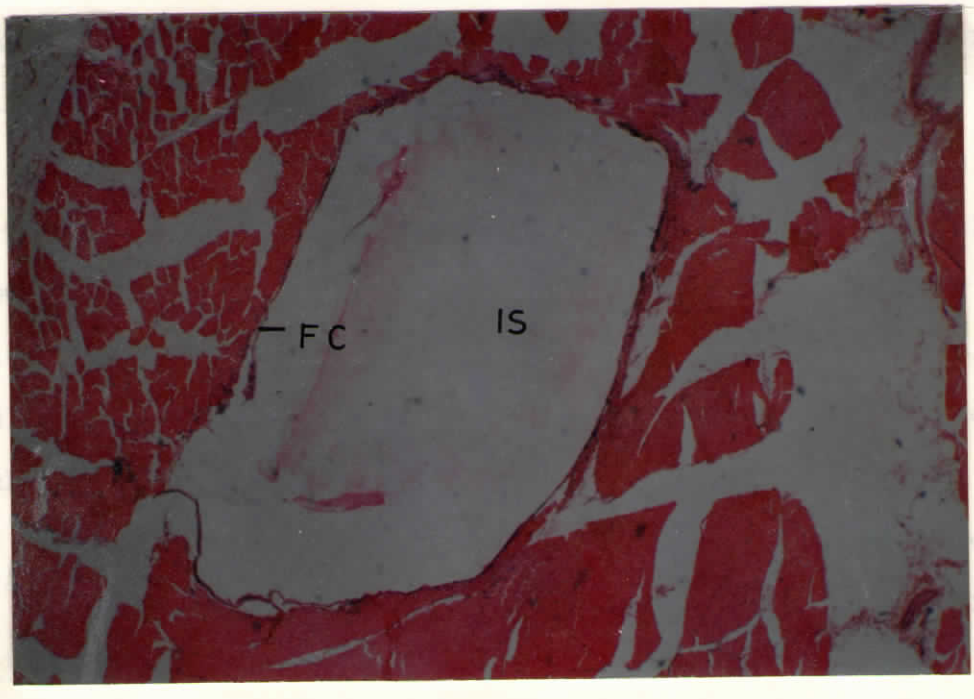


(b)

5-15(a) shows the implantation site. Eosin & Hematoxylene stain, 90 days post implantation period, Control polymer, X 40

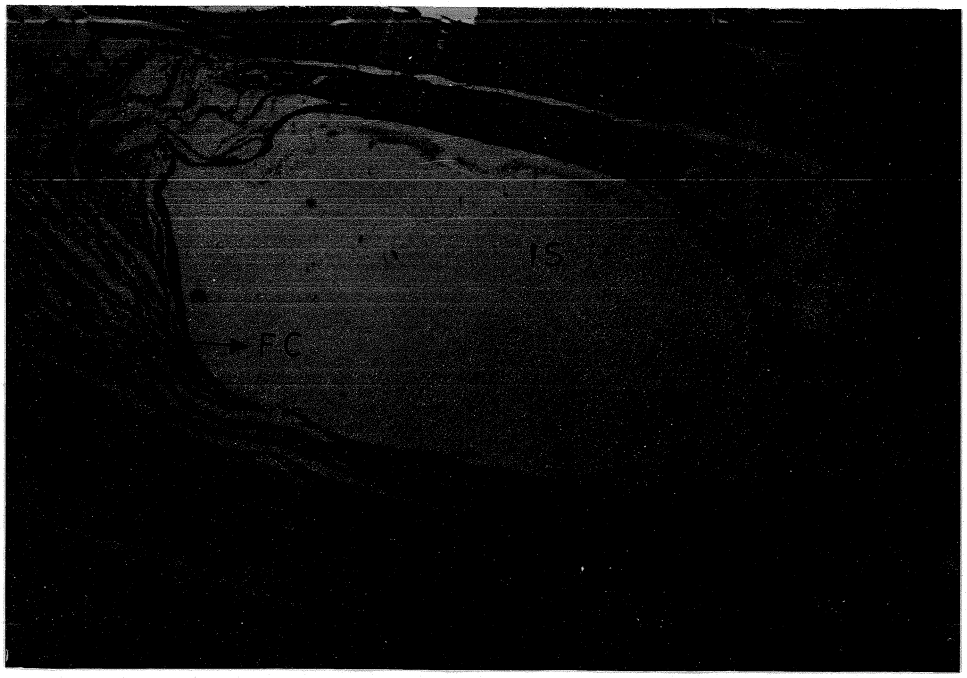
5-15(b) shows the immplantation site. Eosin & Hematoxylene stain, 90 days post implantation period, S1 polyurethane , X 40

(a)

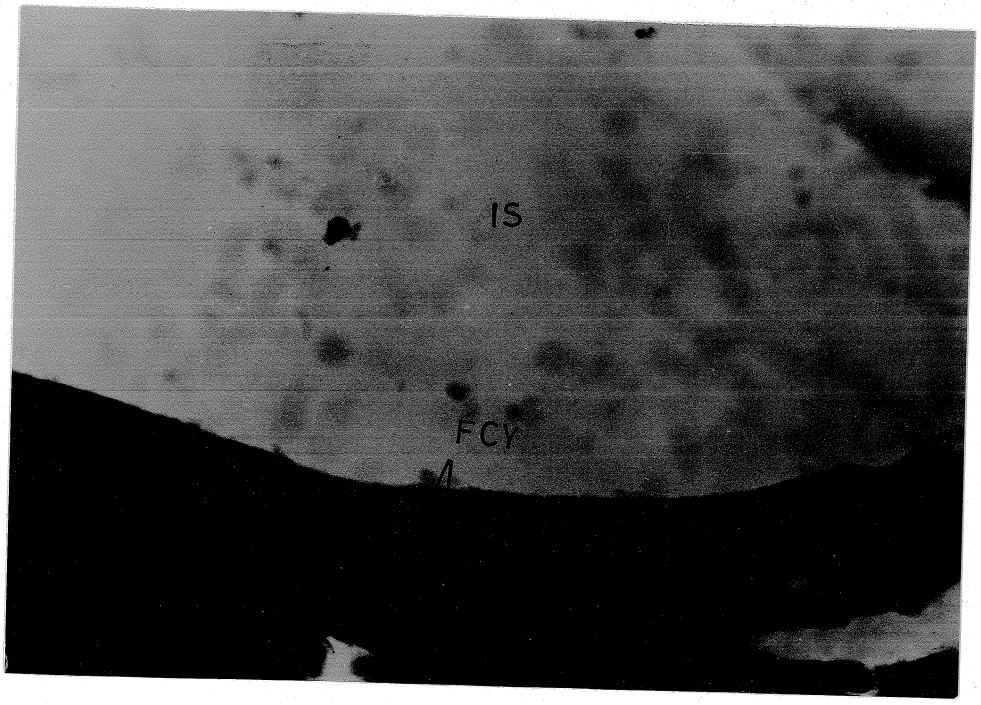


(b)

. 5-16 shows the immplantation site. Eosin & Hematoxylene stain, 90 days post implantation period, H5 polyurethane, X 40



(a)



(b)

ig. 5.13(a) shows the immplantation site. Eosin & Hematoxylene stain, 30 days post implantation period, Control polymer, X 40

ig. 5.13(b) shows the fibrosis. Eosin & Hematoxylene stain, I3 polyurethane, X400

preferential giant cell formation[279]. Eosinophils were rarely found. Fibroplasia and fibrocytes were also seen in many sections which are required for the collagen synthesis (5.13 and 5.14). Fatty infiltration was found in some sections. Calcification, haemoraghe and Oedema were not seen in any of the sections.

The histopathological report for 3Ø days post implantation period is given in tables 5.12 to 5.14. The tissue response of the the candidate materials are similar to that of biomedical grade polyurethanes at 3Ø days post implantation period.

Ninty days post implantation period

The 9Ø days post implantation report indicates the resolution of inflammatory response with the disappearance of inflammatory cells, PMN, macrophages and lymphocytes in most of the polymers based on HDI (Tables 5.15 to 5.17) The HDI polyurethanes based on PPG (H7P and PTMG (H1) are found to be highly biocompatible. Though H5, a PTMG based polyurethane, elicits a mild degree of giant cell presence due to the foreign body debri. The connective tissue response indicates it's biocompatibility. The PEG based polymer (H8) shows the presence of lymphocytes due to the fragmentation of polymer . However the polymer also elicits favorable connective tissue response. Considering the tissue response , it seems that polyurethanes is not influenced with type of polyol present in polymer. Lymphocytes are observed to a certain degree in SMDI based polyurethanes through the fibrous tissue encapsulation and

Table 5.12 The histopathological response to IPDI based polyurethanes (30 days)

	C	I3	I6	I1	I4	I7
Degree of necrosis	1+	0	1+	3+	1+	1+
Neutrophils	3+	0	0	3+	1+	1+
Macrophages	4+	0	2+	4+	3+	2+
Lymphocytes	4+	0	3+	4+	2+	3+
Plasma cells	3+	0	2+	3+	2+	2+
Giant cells	4+	0	2+	3+	3+	3+
Eosinophils	3+	0	1+	2+	1+	2+
Foreign body debri	F	-	-	K	F	-
Fibroplasia	3+	1+	2+	2+	2+	2+
Fibrocytes	4+	1+	2+	2+	2+	2+
Fatty infiltration	1+	0	1+	0	2+	3+
Calcification	0	0	0	0	0	0
Haemoraghe	1+	0	0	1+	1+	0
Oedema	1+	0	0	1+	0	0
Any other	0	-	-	-	0	O

F = Fragments of refratile material.

K = Keratin and hair follicles.

Table 5.13 The Histopathological Evaluation of Tissue Response to SMDI based Polyurethanes (30 Days)

	C	S4	S1	S8	S5	S9*
Degree of necrosis	0	1+	1+	0	1+	
Neutrophils	0	0	0	0	1+	
Macrophages	1+	1+	2+	2+	3+	
Lymphocytes	2+	1+	3+	3+	3+	
Plasma cells	0	1+	1+	0	1+	
Giant cells	M	1+	1+	0	1+	
Eosinophils	0	0	0	0	0	
Foreign body debri	0	0	F	0	0	
Fibroplasia	1+	1+	2+	1+	2+	
Fibrocytes	1+	1+	2+	2+	2+	
Fatty infiltration	1+	0	0	1+	0	
Calcification	0	0	0	0	1+	
Haemoraghe	0	0	0	2+	0	
Oedema	0	0	0	0	0	
Any other	0	0	0	0	0	

M =Muscle giant cells.

F =Fragments of polymer.

* Sample not traceable

Table 5.14 Histopathological Evaluation of Tissue Response to HDI based Polyurethanes (30 Days)

	C	H1	H5	H6	H7	H8
Degree of necrosis	0	0	0	1+	0	0
Neutrophils	0	0	0	0	0	0
Macrophages	0	2+	2+	2+	0	3+
Lymphocytes	1+	2+	1+	3+	1+	3+
Plasma cells	0	0	0	0	0	1+
Giant cells	0	2+	1+	2+	0	2+
Eosinophils	0	0	0	1+	0	1+
Foreign body debri	0	F	0	F	0	K,P
Fibroplasia	1+	2+	1+	3+	1+	3+
Fibrocytes	1+	3+	1+	2+	0	2+
Fatty infiltration	1+	2+	1+	1+	0	0
Calcification	0	0	0	0	0	0
Haemoraghe	0	0	1+	0	0	0
Oedema	0	0	0	0	0	0
Any other	0	0	0	0	0	0

K = Polymer fragments

P = Keratin with squamous epithelium._

Table 5.15 The histopathological response to IPDI based polyurethanes (90 days)

	C*	I3	I6	I1	I4*	I7
Degree of necrosis		0	0	0		0
Neutrophils		0	1+	0		0
Macrophages		0	3+	1+		0
Lymphocytes		0	3+	3+		1+
Plasma cells		0	2+	0		0
Giant cells		0	2+	0		0
Eosinophils		0	1+	0		0
Foreign body debri		0	2+	0		0
Fibroplasia		1+	2+	2+		1+
Fibrocytes		0	2+	2+		1+
Fatty infiltration		0	1+	1+		1+
Calcification		0	0	0		0
Haemoraghe		0	0	0		0
Oedema		0	0	0		0
Any other		0	0	0		0

* Sample not traceable

Table 5.16 The Histopatological Evaluation of Tissue Response to SMDI based Polyurethanes (90 Days)

	C	S4*	S1	S8	S5	S9
Degree of necrosis	0		0	0	0	0
Neutrophils	0		0	0	0	0
Macrophages	0		1+	0	0	0
Lymphocytes	0		2+	2+	1+	1+
Plasma cells	0		0	0	0	0
Giant cells	0		1+	0	0	0
Eosinophils	0		0	0	0	0
Foreign body debri	0		0	F	0	F
Fibroplasia	1+		2+	2+	1+	2+
Fibrocytes	1+		2+	2+	1+	2+
Fatty infiltration	1+		2+	2+	1+	1+
Calcifiacation	0		0	0	0	0
Haemoraghe	0		0	0	0	0
Oedema	0		0	0	0	0
Any other	0		0	0	0	0

F = Fragments-polymer

* Sample not traceable

Table 5.17 Histopathological Evaluation of Tissue Response to HDI based Polyurethanes (90 Days)

	C	H1	H5	H7	H8
Degree of necrosis	0	0	0	0	0
Neutrophils	0	0	0	0	0
Macrophages	1+	0	0	0	0
Lymphocytes	2+	0	0	0	2+
Plasma cells	0	0	0	0	0
Giant cells	1+	0	1+	0	0
Eosinophils	0	0	0	0	0
Foreign body debri	0	0	1+	0	F
Fibroplasia	2+	1+	1+	1+	1+
Fibrocytes	1+	1+	1+	1+	1+
Fatty infiltration	1+	1+	1+	1+	1+
Calcification	0	0	0	0	0
Haemoraghe	0	0	0	0	0
Oedema	0	0	0	0	0
Any other	0	0	0	0	0

F = Fragments-implant material

fibrocytes are seen remarkably well. Interestingly the degree of necrosis and plasma cell found to nil indicates further that the presence of lymphocytes may only be due to lesser sensitivity of the polymers. The fragmentation was observed in the polymers S8 and S9 (SMDI based polymers) However these polymers did not show any adverse inflammatory reaction. The polymer S5 based on PPG shows a minimum concentration of lymphocytes with connective tissue response. The polymer based on PTMG (S1) shows inflammatory response. Figure 5.15 (b) indicates the fatty infiltration surrounded indicating the influence of polyol in the SMDI based polyurethane on biocompatibility.

Polyurethanes based on IPDI show interesting results on biocompatibility with sample I3 eliciting zero inflammatory reaction and thin fibrous tissue encapsulation in comparison with all the polyurethanes. Relatively the polymer I7 also excells equally with minimum level of fibrosis and fibrocytes and with little concentration of lymphocytes.

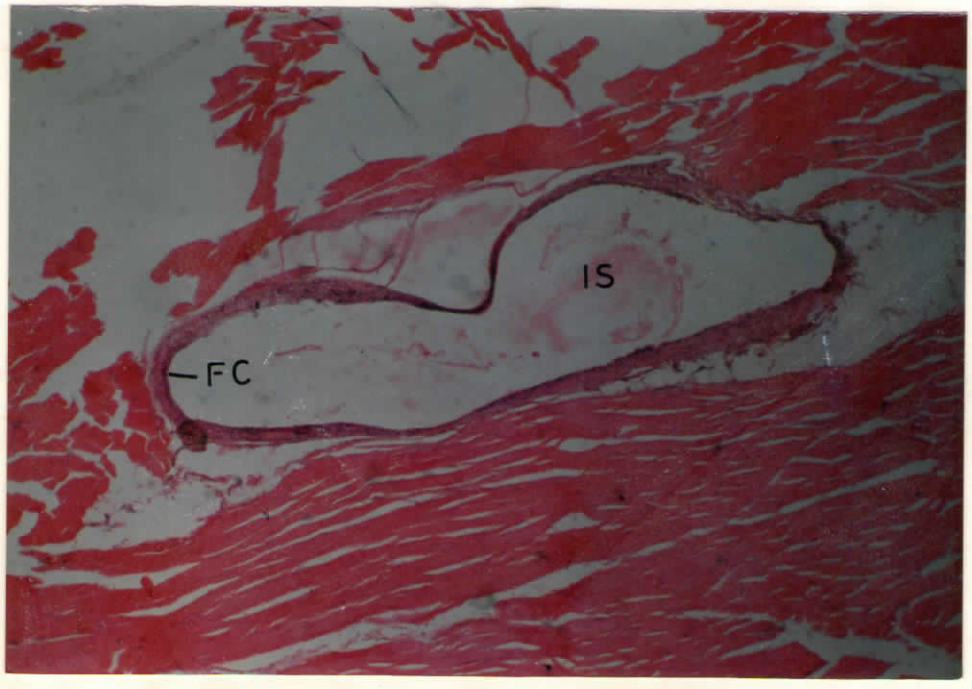
Considering the connective tissue response at the post implantation period of 90 days, the following candidate polymers are considered as biocompatible.

HDI based polyurethane H1,H5,H7 & H8

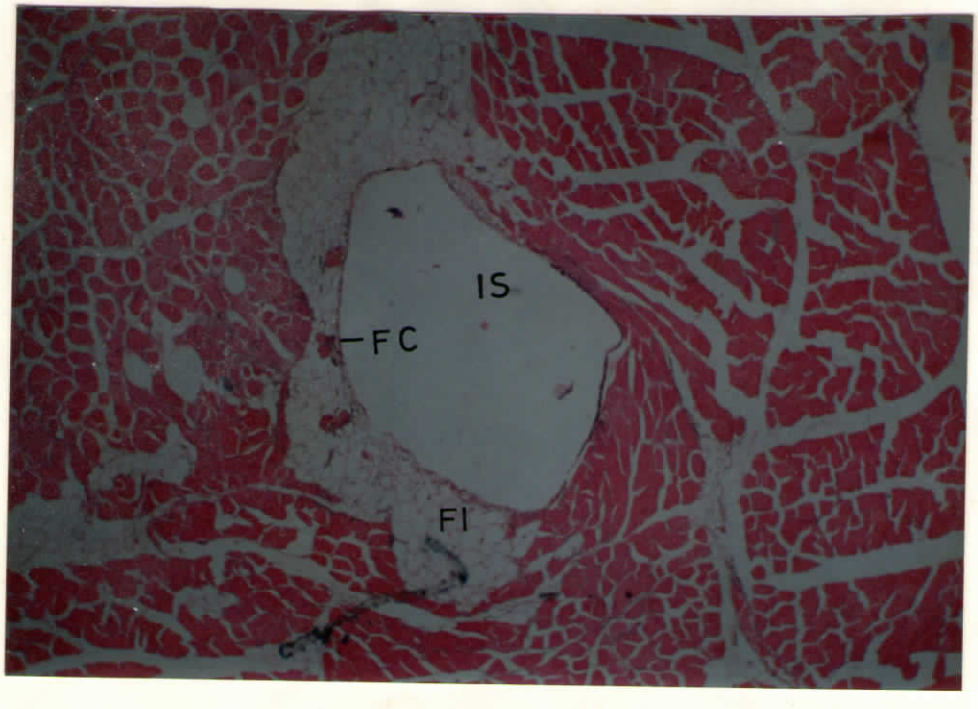
SMDI based polyurethane S5,S8 & S9

IPDI based polyurethane I3 and I7

The degree of fibroplasia associated with the above implants at the post implantation period of 90 days indicates the 1+



(a)

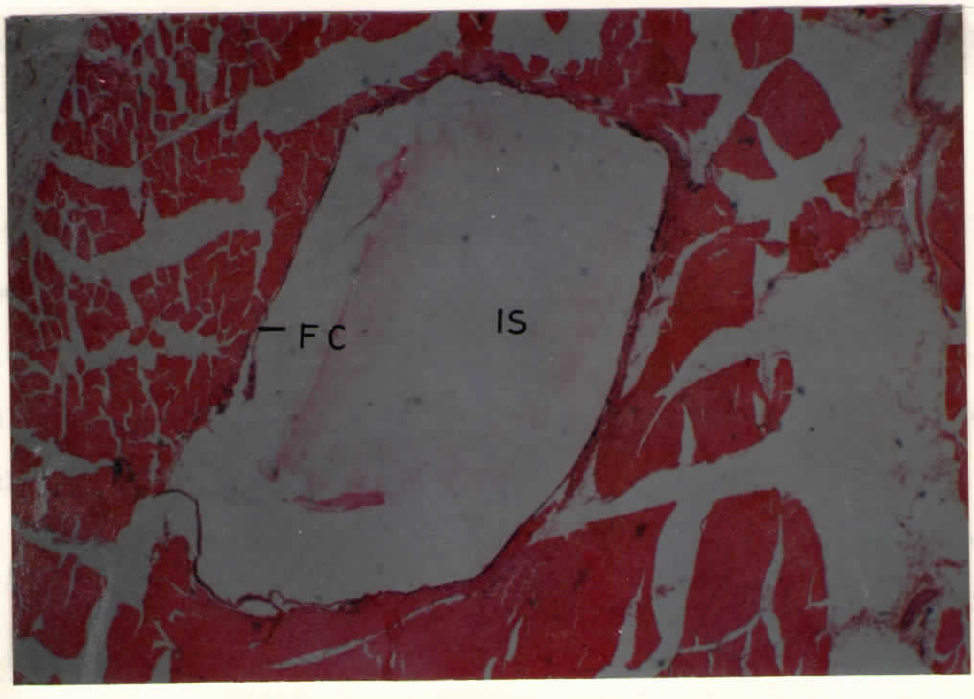


(b)

5-15(a) shows the implantation site. Eosin & Hematoxylene stain, 90 days post implantation period, Control polymer, X 40

5-15(b) shows the immplantation site. Eosin & Hematoxylene stain, 90 days post implantation period, S1 polyurethane , X 40

(a)



(b)

. 5-16 shows the immplantation site. Eosin & Hematoxylene stain, 90 days post implantation period, H5 polyurethane, X 40

STUDIES ON STABILITY

Considering the extensive use of polyurethanes in biomedical and general applications , it is important to assess the stability of polyurethanes. In biomedical applications , environmental stability tends to be particular important in three areas - processing, sterilization and long term implantation[280-285]. Degradation during processing is primarily chemical in nature, especially if melt processing techniques are used[94,286-289] . Sterilization effects can be thermal, radiative or hydrolytic, depending on the sterilization technique. Long term effects primarily involve chemical and hydrolytic degradation. The physiological environment is complex and synergistic interactions between components of tissue or blood may enhance biodegradation of polyurethane implants.

5.4.1 Effect of sterilization

Sterilization is defined as the use of physical or chemical procedure to destroy all microibial life, including highly resistant bacterial endospores. Sterility of most biomedical devices is of prime importance but the choice of sterilization methods are limited by characteristics of the materials. The materials should withstand the three phases of steam sterilization cycle. The three phases are heating phase, sterilizing phase and venting phase. Steam sterilization is not recommended for polyurethanes[290]. Air removal and moisture penetration are essential for effective autoclaving[291].

The effect of autoclaving on physical and mechanical properties is given in table 5.18 The present polyurethanes are stable. The PEG based polyurethanes show slight decrease in tensile strength in comparison with others. The change in swelling ratio also supported with it.

Another mode of sterilizing biomedical devices is τ-radiation. In τ-radiation, the lower energy electrons produce chemical changes in the medium and lead to the destruction of microorganisms. The commonly used radiation sources are Co^{60} and Ce^{137}. The general radiation level used to sterilize biomedical device is 2.5 Mrad. In general bacteria requires 0.5 Mrad, spores require 2.0 Mrad and some resistant spores require 2- 6 Mrad. The spores of Bacillus and Micrococus radioduran and Bacterial virus requires more than 6 Mrad[292].

For the present studies radiation dose used was 2.5 Mrad. which is an internationally acceptable dose level. The effect of radiation sterilization could be understood with change in properties. The properties of the present polymers are not changed appreciably with this dosage.(Table 5.19) Many polymers like polycarbonate, styrene, acrylics do not lose their tensile strength even after 100 Mrad radiation[293]. In polyurethanes higher the amount of urethane better the will be the resistant to radiation doses[294].

5.4.2 Hydrolytic stability

Nearly 50.4 % total weight of human is water. The urethane group in polyurethane is susceptible to hydrolysis. The

Table 5.18.Change in properties after autoclaving

SMDI-PU	T.S[a]	Wt[b]	Q[c]	IPDI-PU	T.S[a]	Wt[b]	Q[c]	HDI-PU	T.S[a]	Wt[b]	Q[c]
S1	0.1	0.1	102.6	I1	0.1	0.1	100.1	H1	0.2	0.1	102.3
S2	0.3	0.0	103.4	I2	1.1	0.2	101.5	H2	1.0	0.2	104.2
S3	0.5	0.1	102.9	I3	1.3	0.3	107.2	H3	0.2	0.4	105.1
S4	0.9	0.1	104.1	I4	0.3	0.1	102.2	H4	1.8	0.5	105.0
S5	0.2	0.0	100.2	I5	1.2	0.2	105.7	H5	2.0	0.5	104.9
S6	0.4	0.1	100.9	I6	1.8	0.3	106.3	H6	0.8	0.4	104.1
S7	0.8	0.1	101.1	I7	2.1	0.6	109.5	H7	2.8	0.6	104.7
S8	1.3	0.1	101.3					H8	3.8	0.8	105.1
S9	1.8	0.2	106.1								

a = loss of Tensile stregnth(%) std.deviation < 5.5 %
b = loss of weight (%) std.deviation < 1.1 %
c = change in Q value(%) std.deviation < 8.2 %

S1 to S4, I1 to I3 & H1 to H5 PTMG based Pus in the decreasing order of hard segment content.
S5 to S8, I4 to I6 & H6 to H7 PPG based Pus in the decreasing order of hard segment content.
S9,H8 & I7 ,PEG based polyurethanes.

Table 5.19. Change in properties after r-radiation

SMDI-PU	a T.S	b Wt	c Q	IPDI-PU	a T.S	b Wt	c Q	HDI-PU	a T.S	b Wt	c Q
S1	0.9	0.0	100.4	I1	0.2	0.0	100.9	H1	0.1	0.0	100.2
S2	0.0	0.1	101.1	I2	0.1	0.0	99.8	H2	0.4	0.1	100.1
S3	0.1	0.1	100.3	I3	0.0	0.0	99.5	H3	0.2	0.1	100.0
S4	0.0	0.1	100.9	I4	0.1	0.0	99.9	H4	0.0	0.0	99.9
S5	0.4	0.1	101.4	I5	0.8	0.0	99.8	H5	0.6	0.1	99.9
S6	0.2	0.1	100.2	I6	0.7	0.0	101.0	H6	0.1	0.1	99.8
S7	0.3	0.1	101.3	I7	0.8	0.1	101.0	H7	0.0	0.0	100.0
S8	0.2	0.0	100.2					H8	0.0	0.1	100.1
S9	0.5	0.0	101.1								

a = lose of Tensile stregnth(%) std.deviation < 6.5 %
b = lose of weight (%) std.deviation < 1.2 %
c = change in Q value(%) std.deviation < 8.0 %

S1 to S4, I1 to I3 & H1 to H5 PTMG based Pus in the decreasing order of hard segment content.
S5 to S8, I4 to I6 & H6 to H7 PPG based Pus in the decrasing order of hard segment content.
S9,H8 & I7 ,PEG based polyurethanes.

hydrolysis resulted in two shorter chain compounds, one having hydroxy terminated and other amine terminated, which resulted in lose in tensile strength.

The results of hydrolytic stability studies are given in table 5.2Ø. This study clearly indicates that PEG based polyurethanes are more susceptible to hydrolysis than PPG and PTMG based polyurethanes. This is due to the fact that PEG is more hydrophilic than PTMG and PPG. So the polyurethanes of PEG polyol sorps water which results in appreciable change of properties. In the SMDI system as the % of hard segment content decreases the lose in properties (Tensile strength and weight) increases. Eventhough hydrolysis mainly occurs at the urethane linkage for the polymers having higher hardsegment content absorbs lesser amount of water when compared with the others. So the change in properties is less. The same trend is observed in PPG based polyurethanes. The loss in weight clearly indicate, there is leaching. The amount of leachants was determined from the weight loss for unit area of the sample. The amount of leachants vary from Ø.Ø25 to Ø.275 mg/cm2 for SMDI polyurethane, Ø.Ø25 to Ø.325 mg/cm2 for IPDI polyurethanes and Ø.Ø4Ø to Ø.72 mg/cm2 for HDI polyurethanes. The variation of properties of IPDI and HDI based polyurethanes are like that of

Table 5.20.Change in properties after hydrolytic treatment

SMDI-PU	T.S[a]	Wt[b]	Q[c]	IPDI-PU	T.S[a]	Wt[b]	Q[c]	HDI-PU	T.S[a]	Wt[b]	Q[c]
S1	6.6	0.1	102.7	I1	4.0	0.1	102.8	H1	3.8	0.2	104.3
S2	6.9	0.2	101.9	I2	8.2	0.9	103.2	H2	8.1	0.4	106.3
S3	7.1	0.4	103.1	I3	9.8	1.0	114.3	H3	9.8	0.6	107.1
S4	7.9	0.7	103.9	I4	5.6	0.8	105.5	H4	9.9	0.8	107.1
S5	6.6	0.3	103.2	I5	9.6	0.9	117.1	H5	10.9	1.0	107.1
S6	7.9	0.3	104.1	I6	10.1	1.0	119.3	H6	6.8	0.5	107.0
S7	8.0	0.5	105.1	I7	11.8	1.3	126.2	H7	9.8	1.2	108.2
S8	8.2	0.8	106.3					H8	13.9	1.4	109.1
S9	9.8	1.1	111.1								

a = loss of Tensile stregnth(%) std.deviation < 5.5 %
b = loss of weight (%) std.deviation < 0.8 %
c = change in Q value(%) std.deviation < 7.0 %

S1 to S4, I1 to I3 & H1 to H5 PTMG based Pus in the decreasing order of hard segment content.
S5 to S8, I4 to I6 & H6 to H7 PPG based Pus in the decreasing order of hard segment content.
S9,H8 & I7 ,PEG based polyurethanes.

SMDI based polyurethanes. In all the cases as the Mc increases the hydrolytic stability decreased as observed by Masar et al[295].

5.4.3 Stability in biological fluids (Aqueous salt solution)

Phosphate buffered saline(PBS) is commonly used biological fluid. The change in properties after PBS treatment is given in table 5.21. The change in properties are similar to that found with hydrolytic stability studies. The ionic permeation inside the polyurethane matrix is very less, so the effect of inorganic ions on the degradation of polymer is less[296]. For SMDI based polyurethane the leaching vary from Ø.Ø5 to Ø.25 mg/cm2, for IPDI based polyurethanes the amount of leachants vary from Ø.Ø5 to Ø.325 mg/cm2 and for HDI based polyurethanes the leachants vary from Ø.Ø75 to Ø.725 mg/cm2.

The studies on the stability of polyurethanes in PECF is very important since this solution just mimics the concentration of ions present in the biological extracellular fluid. The results of PECF is given in table 5.22. The change in properties are similar to that found in hydrolytic stability studies. The amount of leachants vary from Ø.Ø25 to Ø.225 mg/cm2 for SMDI systems, Ø.Ø25 to Ø.275 mg/cm2 for IPDI systems and Ø.Ø25 to Ø.Ø325 mg/cm2 for HDI based polyurethanes.

5.4.5 Stability in catalytic reactive environment

Polyurethane can also undergoes other type of catalytically induced reactions such as trans esterification, transamination in addition to hydrolysis in vivo biological environment.

Table 5.21 Change in properties after PBS treatment

SMDI-PU	a T.S	b Wt	c Q	IPDI-PU	a T.S	b Wt	c Q	HDI-PU	a T.S	b Wt	c Q
S1	6.2	0.2	103.9	I1	4.2	0.2	104.7	H1	6.3	0.3	103.2
S2	6.9	0.4	104.2	I2	8.9	0.2	108.5	H2	8.4	0.4	104.1
S3	6.8	0.6	105.1	I3	10.2	0.9	116.5	H3	10.6	0.7	104.0
S4	7.1	0.8	105.9	I4	6.0	0.6	107.0	H4	11.9	0.9	104.1
S5	6.4	0.3	104.2	I5	9.9	1.2	109.7	H5	12.9	0.8	104.1
S6	8.0	0.6	105.3	I6	W	W	W	H6	7.7	0.4	104.0
S7	9.2	0.9	106.2	I7	11.9	1.3	130.8	H7	11.3	2.9	109.2
S8	9.1	1.0	107.9					H8			
S9	9.8	0.7	112.1								

a = lose of Tensile stregnth(%) std.deviation < 6.5 %
b = lose of weight (%) std.deviation < 1.2 %
c = change in Q value(%) std.deviation < 8.0 %

S1 to S4, I1 to I3 & H1 to H5 PTMG based Pus in the decreasing order of hard segment content.
S5 to S8, I4 to I6 & H6 to H7 PPG based Pus in the decreasing order of hard segment content.
S9,H8 & I7 ,PEG based polyurethanes.

Table 5.22. Change in properties after PECF treatment

SMDI-PU	T.S[a]	Wt[b]	Q[c]	IPDI-PU	T.S[a]	Wt[b]	Q[c]	HDI-PU	T.S[a]	Wt[b]	Q[c]
S1	5.9	0.1	102.7	I1	4.2	0.1	102.9	H1	4.1	0.1	104.3
S2	7.1	0.2	102.9	I2	8.6	0.3	104.1	H2	8.3	0.3	106.3
S3	7.2	0.5	103.4	I3	9.6	0.4	114.3	H3	9.7	0.4	1 7.1
S4	8.1	0.7	103.9	I4	5.4	0.4	104.5	H4	9.9	0.8	107.1
S5	6.4	0.4	103.1	I5	9.8	0.5	108.1	H5	10.8	1.1	108.2
S6	7.9	0.6	104.9	I6	10.2	0.7	118.2	H6	6.1	0.6	106.2
S7	8.2	0.7	104.9	I7	13.8	1.1	120.3	H7	9.9	1.0	1 7.9
S8	8.4	0.8	105.6					H8	14.3	1.3	111.1
S9	10.1	0.9	113.2								

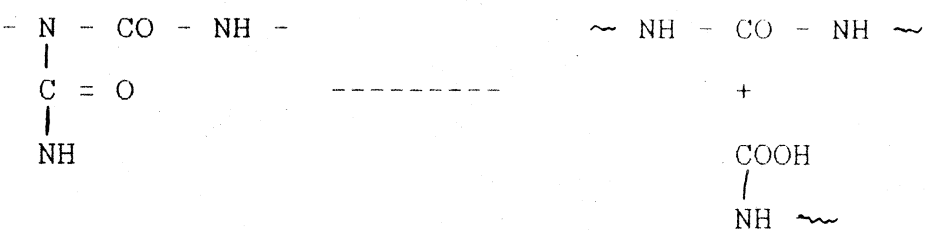
a = loss of Tensile stregnth(%) std.deviation < 4.5 %
b = loss of weight (%) std.deviation < 0.2 %
c = change in Q value(%) std.deviation < 4.0 %

S1 to S4, I1 to I3 & H1 to H5 PTMG based Pus in the decreasing order of hard segment content.
S5 to S8, I4 to I6 & H6 to H7 PPG based Pus in the decreasing order of hard segment content.
S9,H8 & I7 ,PEG based polyurethanes.

Therefore it is relevant to study the stability in in vitro conditions. The change in properties after catalytic hydrolytic studies are given in table 5.23. The change in properties are similar to that found in simple hydrolytic environment. The acidic catalyst has some effect on the degradation of some polyurethanes. There is marked change in properties in comparison with the simple hydrolytic environment. The amount of leachants vary from Ø.Ø25 to Ø.225 mg/cm2 for SMDI based polyurethane, Ø.Ø25 to o.475 mg/cm2 for IPDI based polyurethane and Ø.Ø5 to Ø.65 mg /cm2 for HDI based polyurethane. The change in swelling ratio also supported with above trend. The polyurethanes I5 and I6 are warped due to significant effect on the polymer. The degradation of urethane bonds in the catalytic hydrolytic environment is given below.

$$-NH - COO\sim + H_2O \longrightarrow -NH_2 + CO_2$$

In the case of biuret link, the degradation occurs with resulting in urea and amine.



The change in properties after catalytic transamination studies are given in table 5.24. The change in tensile strength is relatively low in SMDI based polyurethanes in comparison with that found in hydrolytic stability studies.

Table 5.23.Change in properties after catalytic hydrolysis studies

SMDI-PU	T.S[a]	Wt[b]	Q[c]	IPDI-PU	T.S[a]	Wt[b]	Q[c]	HDI-PU	T.S[a]	Wt[b]	Q[c]
S1	6.1	0.1	100.2	I1	3.9	0.1	100.3	H1	6.1	0.2	104.6
S2	7.2	0.3	103.2	I2	8.8	0.9	104.5	H2	6.8	0.4	106.8
S3	7.9	0.4	105.3	I3	9.4	1.2	121.2	H3	7.9	0.6	107.1
S4	8.6	0.6	107.1	I4	7.0	1.4	111.6	H4	7.9	0.9	107.3
S5	6.4	0.2	105.9	I5	W	W	W	H5	6.9	0.9	107.2
S6	7.7	0.3	108.2	I6	W	W	W	H6	5.4	1.1	108.0
S7	8.1	0.5	109.1	I7	9.9	1.9	133.4	H7	6.6	1.4	108.9
S8	9.9	0.8	111.2					H8	8.9	2.6	110.7
S9	10.9	0.9	113.1								

a = loss of Tensile stregnth(%) std.deviation < 6.3 %
b = loss of weight (%) std.deviation < 1.1 %
c = change in Q value(%) std.deviation < 7.8 %

S1 to S4, I1 to I3 & H1 to H5 PTMG based Pus in the decreasing order of hard segment content.
S5 to S8, I4 to I6 & H6 to H7 PPG based Pus in the decreasing order of hard segment content.
S9,H8 & I7 ,PEG based polyurethanes.

Table 5.24 Change in properties after catalytic transamination

SMDI-PU	a T.S	b Wt	c Q	IPDI-PU	a T.S	b Wt	c Q	HDI-PU	a T.S	b Wt	c Q
S1	4.8	0.0	103.7	I1	4.1	0.3	103.1	H1	3.9	0.2	104.6
S2	4.9	0.1	104.1	I2	7.8	0.7	104.2	H2	8.3	0.4	106.2
S3	5.1	0.3	104.7	I3	9.9	0.9	112.2	H3	9.9	0.6	108.1
S4	5.9	0.4	105.1	I4	6.1	0.7	105.4	H4	10.2	0.9	107.3
S5	4.6	0.1	102.9	I5	9.7	0.8	116.1	H5	10.7	1.1	109.1
S6	5.9	0.3	103.1	I6	W	W	W	H6	6.0	0.6	109.0
S7	6.0	0.4	106.2	I7	11.9	1.1	120.3	H7	9.9	1.3	110.2
S8	6.2	0.6	108.1					H8	14.1	1.6	111.2
S9	7.8	0.8	112.1								

a = loss of Tensile stregnth(%) std.deviation < 5.5 %
b = loss of weight (%) std.deviation < 0.5 %
c = change in Q value(%) std.deviation < 6.2 %

S1 to S4, I1 to I3 & H1 to H5 PTMG based Pus in the decreasing order of hard segment content.
S5 to S8, I4 to I6 & H6 to H7 PPG based Pus in the decreasing order of hard segment content.
S9,H8 & I7 ,PEG based polyurethanes.

The leaching vary from Ø.Ø25 to Ø.2Ø mg/cm2 for SMDI based polyurethanes, Ø.Ø75 to Ø.225 mg/cm2 for IPDI based polyurethanes and Ø.Ø5 to Ø.4Ø mg/cm2 for HDI based polyurethanes.The other trend is same as in hydrolytic stability studies.

The change in properties after catalytic transesterification studies are given in table 5.25. The transesterification reaction is given below.

$$R - NH - COO - R' + R'' - OH \longrightarrow R - NH - COOR'' + R' - OH$$

The leaching vary from Ø.Ø25 gm/cm2 to o.375 gm/cm2 for SMDI systems, Ø.Ø25 to Ø.425 mg/cm2 for IPDI system and Ø.Ø25 to o.425 mg/cm2 for HDI systems. The trend in change of properties is same as hydrolytic stability studies.

5.4.8 Stability in oxidative degradation environment

The polyurethanes are susceptible to oxidative degradation also. The oxidative environment in physiological condition is by the phagocytic attempt by macrophages and giant cells and also by the peroxide radical formation during the normal biological function. The results of oxidative (mild oxidant) treatment are given in table 5.26. The oxidation takes place at the soft segment. It has been found that the methylene group next to ether oxygen of polyol is susceptible oxidation [296]. So the degree of oxidative stability of the polyurethanes are directly proportional to the amount of soft segments present in the

Table 5.25.Change in properties after catalytic transesterification

SMDI-PU	T.S[a]	Wt[b]	Q[c]	IPDI-PU	T.S[a]	Wt[b]	Q[c]	HDI-PU	T.S[a]	Wt[b]	Q[c]
S1	5.9	0.1	103.1	I1	4.3	0.1	103.1	H1	3.9	0.1	104.3
S2	6.9	0.2	103.9	I2	8.6	0.8	103.6	H2	8.9	0.3	106.3
S3	6.9	0.4	104.1	I3	10.1	0.9	115.3	H3	10.2	0.6	107.1
S4	8.1	0.8	104.6	I4	5.7	0.3	106.8	H4	10.7	0.8	107.3
S5	6.4	0.4	104.3	I5	9.9	0.9	119.1	H5	11.2	1.1	107.1
S6	7.9	0.5	104.9	I6	10.7	1.5	119.9	H6	4.8	0.6	107.0
S7	8.3	0.6	106.3	I7	12.9	1.7	129.2	H7	10.1	1.4	108.2
S8	8.7	0.7	107.3					H8	14.1	1.7	109.1
S9	10.8	0.9	119.1								

a = loss of Tensile stregnth(%) std.deviation < 5.5 %
b = loss of weight (%) std.deviation < 1.1 %
c = change in Q value(%) std.deviation < 7.0 %

S1 to S4, I1 to I3 & H1 to H5 PTMG based Pus in the decreasing order of hard segment content.
S5 to S8, I4 to I6 & H6 to H7 PPG based Pus in the decreasing order of hard segment content.
S9,H8 & I7 ,PEG based polyurethanes.

Table 5.26. Change in properties after oxidative degradation

SMDI-PU	T.S[a]	Wt[b]	Q[c]	IPDI-PU	T.S[a]	Wt[b]	Q[c]	HDI-PU	T.S[a]	Wt[b]	Q[c]
S1	4.6	0.9	103.4	I1	2.0	0.8	110.4	H1	1.9	0.8	105.2
S2	6.6	1.1	106.9	I2	9.6	1.0	116.4	H2	2.4	0.9	105.8
S3	8.8	1.3	107.2	I3	10.8	1.4	121.0	H3	2.9	1.0	105.9
S4	9.8	1.4	108.3	I4	3.3	1.1	109.9	H4	3.3	1.1	106.0
S5	9.3	1.0	104.3	I5	9.9	1.3	118.2	H5	3.5	1.1	106.1
S6	14.0	1.3	108.1	I6	11.2	1.4	123.4	H6	3.6	1.0	107.5
S7	16.0	1.4	109.2	I7	13.8	1.3	108.4	H7	5.3	1.4	107.9
S8	16.8	1.5	111.2					H8	7.8	1.3	108.2
S9	10.9	0.9	108.2								

a = loss of Tensile stregnth(%) std.deviation < 6.8 %
b = loss of weight (%) std.deviation < 0.4 %
c = change in Q value(%) std.deviation < 8.0 %

S1 to S4, I1 to I3 & H1 to H5 PTMG based Pus in the decreasing order of hard segment content.
S5 to S8, I4 to I6 & H6 to H7 PPG based Pus in the decreasing order of hard segment content.
S9,H8 & I7 ,PEG based polyurethanes.

polymer backbone. In the present polyurethanes also as the % of soft segment by weight increases the change in properties also increases. These observations are similar to that found in hydrolytic stability studies. The amount of leachants vary from Ø.Ø225 to Ø.4Ø mg/cm2 for SMDI systems , Ø.ØØ2Ø to Ø.Ø.325 mg/cm2 for IPDI system and Ø.ØØ2Ø to Ø.325 mg/cm2 for HDI system.

5.4.7 Stability of polymers in enzymes

Two types of enzymes commonly present in the inflammatory reactions with biomaterials. They are hydrolytic and oxidative enzymes. The results of stability studies with hydrolytic enzymes(Papain,Bromealin and Trypsin) are given in table 5.27 to 5.29 and result of stability study with oxidative enzyme is given in table 5.3Ø. The change in properties such as loss in tensile strength, weight and change in equilibrium swelling ratio is found to be relatively low in comparison with that found in all other treatments. Though the PTMG and PPG polyol based polyurethanes are found to be stable relatively, polymers based on PEG undergo relatively more degradation in enzymes. A similar type of observation was made by Smith et al for linear polyurethanes[297].

5.4.8 Stability of polymers in lipids

The change properties after treatment in lipid solution treatment are given in table 5.31. The PEG based polyurethanes show more change in properties when compared with the others. Here the permeability of water play a major role for lipid molecules mobility. As PEG based polymers absorbs relatively more

Table 5.27. Change in properties after the enzyme (Trypsin) attack

SMDI-PU	T.S[a]	Wt[b]	Q[c]	IPDI-PU	T.S[a]	Wt[b]	Q[c]	HDI-PU	T.S[a]	Wt[b]	Q[c]
S1	0.8	0.0	100.5	I1	0.3	0.1	100.3	H1	0.1	0.0	100.2
S2	0.9	0.1	101.9	I2	0.1	0.0	99.9	H2	0.4	0.1	100.1
S3	0.9	0.1	100.8	I3	0.8	0.0	99.6	H3	0.2	0.1	100.0
S4	0.8	0.2	100.9	I4	1.1	0.0	100.9	H4	0.0	0.0	99.9
S5	0.9	0.1	101.6	I5	0.9	0.1	99.9	H5	0.6	0.1	99.9
S6	1.2	0.1	100.4	I6	1.3	0.0	101.3	H6	0.1	0.1	99.8
S7	1.3	0.1	101.9	I7	1.8	0.1	101.4	H7	0.0	0.0	100.0
S8	1.2	0.1	100.9					H8	0.0	0.1	100.1
S9	1.5	0.0	101.2								

a = loss of Tensile stregnth(%) std.deviation < 6.5 %
b = loss of weight (%) std.deviation < 1.0 %
c = change in Q value(%) std.deviation < 8.3 %

S1 to S4, I1 to I3 & H1 to H5 PTMG based Pus in the decreasing order of hard segment content.
S5 to S8, I4 to I6 & H6 to H7 PPG based Pus in the decreasing order of hard segment content.
S9,H8 & I7 ,PEG based polyurethanes.

Table 5.28. Change in properties after the enzyme (Bromealin) attack

SMDI-PU	T.S[a]	Wt[b]	Q[c]	IPDI-PU	T.S[a]	Wt[b]	Q[c]	HDI-PU	T.S[a]	Wt[b]	Q[c]
S1	0.9	0.0	100.4	I1	0.2	0.0	100.9	H1	0.1	0.0	100.2
S2	0.0	0.1	101.1	I2	0.1	0.0	99.8	H2	0.4	0.1	100.1
S3	0.1	0.1	100.3	I3	0.0	0.0	99.5	H3	0.2	0.1	100.0
S4	0.0	0.1	100.9	I4	0.1	0.0	99.9	H4	0.0	0.0	99.9
S5	0.4	0.1	101.4	I5	0.8	0.0	99.8	H5	0.6	0.1	99.9
S6	0.2	0.1	100.2	I6	0.7	0.0	101.0	H6	0.1	0.1	99.8
S7	0.3	0.1	101.3	I7	0.8	0.1	101.0	H7	0.0	0.0	100.0
S8	0.2	0.0	100.2					H8	0.0	0.1	100.1
S9	0.5	0.0	101.1								

a = loss of Tensile stregnth(%) std.deviation < 6.5 %
b = loss of weight (%) std.deviation < 1.2 %
c = change in Q value(%) std.deviation < 8.0 %

S1 to S4, I1 to I3 & H1 to H5 PTMG based Pus in the decreasing order of hard segment content.
S5 to S8, I4 to I6 & H6 to H7 PPG based Pus in the decreasing order of hard segment content.
S9,H8 & I7 ,PEG based polyurethanes.

Table 5.29. Change in properties after the enzyme (Papain) attack

SMDI-PU	T.S[a]	Wt[b]	Q[c]	IPDI-PU	T.S[a]	Wt[b]	Q[c]	HDI-PU	T.S[a]	Wt[b]	Q[c]
S1	1.9	0.0	100.5	I1	0.4	0.0	100.9	H1	0.7	0.0	100.3
S2	2.2	0.1	101.4	I2	0.6	0.0	99.9	H2	0.3	0.1	100.2
S3	2.1	0.1	100.3	I3	1.0	0.0	99.4	H3	1.1	0.1	101.0
S4	2.8	0.1	100.8	I4	1.4	0.0	99.9	H4	1.9	0.0	99.9
S5	3.4	0.0	101.4	I5	1.8	0.0	99.8	H5	0.6	0.1	99.9
S6	3.2	0.1	100.2	I6	0.7	0.0	101.0	H6	0.1	0.1	99.8
S7	3.3	0.1	101.3	I7	0.8	0.1	101.0	H7	3.2	0.0	100.0
S8	3.8	p.0	100.2					H8	0.0	0.1	100.1
S9	4.5	0.0	101.1								

a = loss of Tensile stregnth(%) std.deviation < 4.5 %
b = loss of weight (%) std.deviation < 1.1 %
c = change in Q value(%) std.deviation < 7.0 %

S1 to S4, I1 to I3 & H1 to H5 PTMG based Pus in the decreasing order of hard segment content.
S5 to S8, I4 to I6 & H6 to H7 PPG based Pus in the decreasing order of hard segment content.
S9,H8 & I7 ,PEG based polyurethanes.

Table 5.30. Change in properties after the enzyme (Lactic dehydragenase)

SMDI-PU	T.S[a]	Wt[b]	Q[c]	IPDI-PU	T.S[a]	Wt[b]	Q[c]	HDI-PU	T.S[a]	Wt[b]	Q[c]
S1	0.7	0.0	100.1	I1	1.2	0.1	100.8	H1	0.1	0.0	100.2
S2	0.6	0.1	101.2	I2	0.6	0.0	99.9	H2	0.4	0.1	100.1
S3	0.9	0.1	100.9	I3	0.7	0.0	99.5	H3	0.2	0.1	100.0
S4	0.9	0.1	101.2	I4	0.9	0.0	99.9	H4	0.0	0.0	99.9
S5	0.8	0.2	101.9	I5	1.8	0.0	99.8	H5	0.6	0.1	99.9
S6	0.9	0.1	100.9	I6	1.7	0.0	101.0	H6	0.1	0.1	99.8
S7	0.6	0.1	101.3	I7	2.8	0.1	101.0	H7	0.0	0.0	100.0
S8	0.9	0.1	100.9					H8	3.0	0.1	100.1
S9	2.5	0.0	101.1								

a = loss of Tensile stregnth(%) std.deviation < 6.8 %
b = loss of weight (%) std.deviation < 1.0 %
c = change in Q value(%) std.deviation < 8.0 %

S1 to S4, I1 to I3 & H1 to H5 PTMG based Pus in the decreasing order of hard segment content.
S5 to S8, I4 to I6 & H6 to H7 PPG based Pus in the decrasing order of hard segment content.
S9,H8 & I7 ,PEG based polyurethanes.

Table 5.31 Change in properties after lipid solution treatment

SMDI-PU	T.S[a]	IPDI-PU	T.S[a]	HDI-PU	T.S[a]
S1	5.1	I1	7.2	H1	8.1
S2	5.9	I2	7.9	H2	8.4
S3	6.3	I3	8.3	H3	9.8
S4	7.1	I4	7.9	H4	11.4
S5	5.3	I5	8.3	H5	13.9
S6	6.3	I6	9n1	H6	10.1
S7	6.9	I7	10.9	H7	14.1
S8	7.5			H8	19.2
S9	9.9				

a = loss of Tensile stregnth(%) std.deviation < 5.5 %

S1 to S4, I1 to I3 & H1 to H5 PTMG based Pus in the decreasing order of hard segment content.
S5 to S8, I4 to I6 & H6 to H7 PPG based Pus in the decreasing order of hard segment content.
S9,H8 & I7 ,PEG based polyurethanes.

water than the other polyol based polyurethanes there is more lipid molecule permeation which causes more degradation with in loss in tensile strength as observed by Zartnack et al[298]. However the mechanism of degradation is not fully understood. Takahara et al have investigated that the effect of lipid absorption on fatigue strength and other mechanical properties[213]. In the present investigations, among PTMG and PPG based polyurethanes, as the % of hard segment content increases the loss in properties also decreases.

5.4.9 Stability of polymers in blood

The results of studies on the stability of polyurethanes in blood are given in table 5.32. The exposure of polymers to blood was carried out at 8°C in order to avoid the damage of blood components. The PEG based polyurethanes are less stable when compared with the PTMG and PPG based polyurethanes. Among PTMG and PPG based polyurethanes, as the % hard segment content decreases the loss in tensile strength also increases. Here the degradation is attributed to the absorption of blood components, particularly lipids.

The amount of absorption of lipids of the representative polymers are given in table 5.33. Among various polymers ,PEG based polymers absorbs more lipids when compared with the others. This process is governed by the permeability of lipid molecules. Thus it is understood that the absorption of lipid is not only governed by the solubility parameter but also the

Table 5.32 Change in tensile strength after 4 months immersion in blood

SMDI-PU	T.S	IPDI-PU	T.S	HDI-PU	T.S
S1	6.1	I1	7.2	H1	9.1
S2	7.9	I2	7.9	H2	9.4
S3	9.3	I3	9.3	H3	9.8
S4	9.9	I4	7.9	H4	13.4
S5	6.3	I5	8.3	H5	17.9
S6	8.3	I6	10.1	H6	10.1
S7	10.9	I7	20.8	H7	19.1
S8	12.5			H8	20.2
S9	19.9				

a = loss of Tensile stregnth(%) std.deviation < 5.5 %

S1 to S4, I1 to I3 & H1 to H5 PTMG based Pus in the decreasing order of hard segment content.
S5 to S8, I4 to I6 & H6 to H7 PPG based Pus in the decrasing order of hard segment content.
S9,H8 & I7 ,PEG based polyurethanes.

Table 5.33 The amount of lipid absorbed by the polyurethanes

Polyurethane	Solubility parameter	Amount of lipid absorbed(%)
I1	10.1	1.12
I3	10.0	1.21
I4	10.4	1.23
I7	10.3	1.38
I8	11.2	1.98
S1	10.2	1.12
S4	10.2	1.22
S5	10.3	1.31
S8	10.3	1.30
S9	11.3	2.23
H1	10.2	1.21
H5	10.3	1.22
H6	10.3	1.31
H7	10.4	1.42
H8	11.5	2.41

Table 5.33 The amount of lipid absorbed by the polyurethanes

Polyurethane	Solubility parameter	Amount of lipid absorbed(%)
I1	10.1	1.12
I3	10.0	1.21
I4	10.4	1.23
I7	10.3	1.38
I8	11.2	1.98
S1	10.2	1.12
S4	10.2	1.22
S5	10.3	1.31
S8	10.3	1.30
S9	11.3	2.23
H1	10.2	1.21
H5	10.3	1.22
H6	10.3	1.31
H7	10.4	1.42
H8	11.5	2.41

permeation.

5.4.1Ø Fungus susceptibity of polyurethanes

The fungus susceptibility test does not indicate any no trace of fungus in any of the aged polymer. The test does not show any growth of fungus after keeping the polymers in the slope and froth of fungus culture medium.

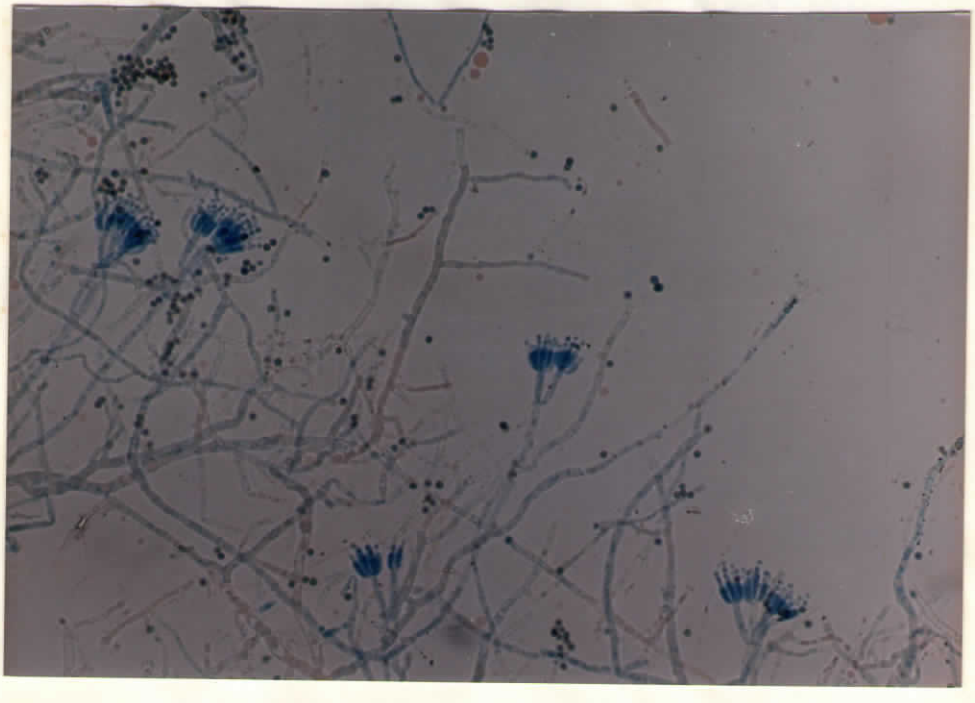
5.4.11. Fungus inhibition test

Aspergilus niger and Pencilium sp. are commonly occurring fungus damaging medical devices. (figures 5.17). The fungus inhibition test clearly indicates that PEG base polyurethanes are slightly susceptible for fungus growth.(figure 5.18 & 19). All other test polyurethanes do not show any fungal growth either the Aspergilus niger or to pencillium sp. Darby and Kaplan demonstrated that some fungus (Aspergillus Sp. and Pencillium Sp.) attack only those polymers with three or more methylene groups between urethane linkages. But in the present case the attack of polymers are not only based on the molecular structure but also on the nature of the polyurethane. The PEG based polyurethanes are more hydrophilic and absorbs more moisture compared with the others, which favours the fungus growth.

4.14 Calcification on polyurethanes

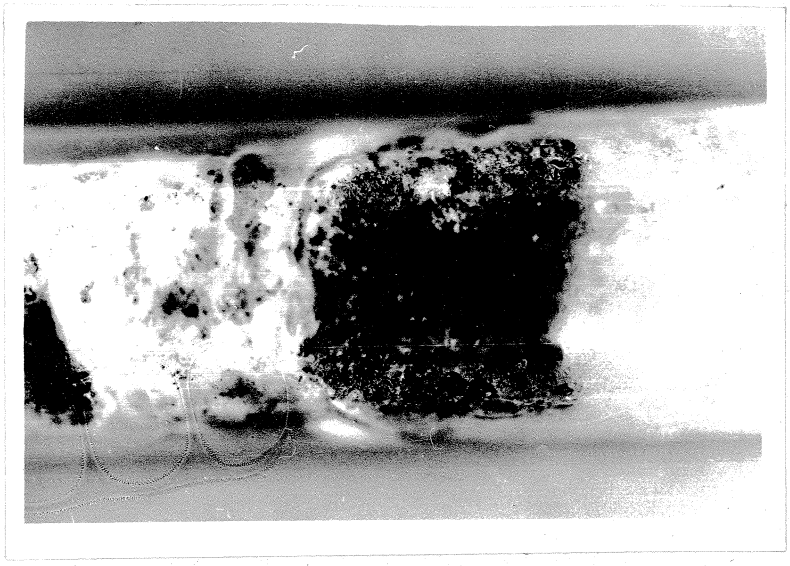
Calcification of biomedical polymers leads to catastrophic failure. The amount of calcium deposited on the polyurethane surface is given in table 5.33. The interaction of Ca^{2+} ion with polyol ether results in the formation of a crown ether is shown

(a)

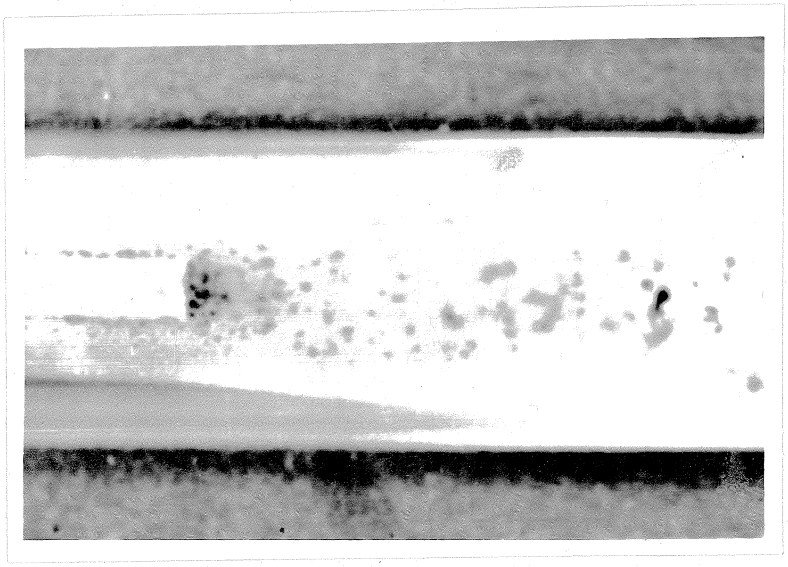


(b)

re 5.17(a) Aspergilus Niger (b) Pencilium Sp.



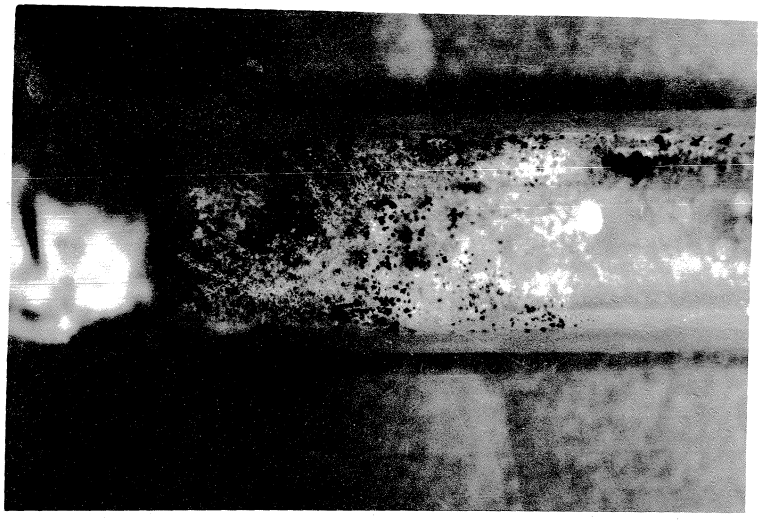
Positive Control



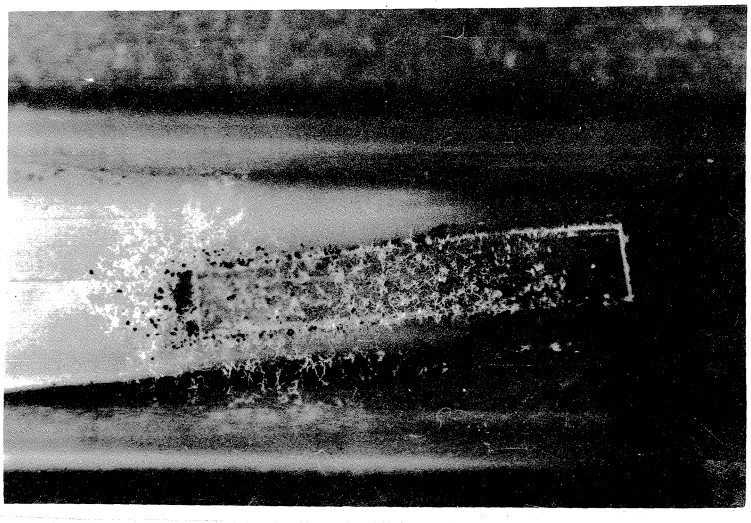
S_9

H_8

Figure 5.18 Photograph showing fungus (Aspergilus niger) attack of positive control and test material



POSITIVE CONTROL



S_9

Figure 5.19 Photograph showing fungus (Pencilium Sp.) attack of positive control and test material

Table 5.34 Calcification of plyurethanes

PUs	Amount of Ca++ deposited μ.gm/cm2		Ratio =stressed/unstressed
	stressed	unstressed	
I1	12.67	7.34	1.45
I2	13.00	-	
I3	17.19	-	
I4	10.40	-	
S1	29.11	20.07	1.44
S2	19.37	-	
S3	14.83	-	
S5	17.48	-	
Teco flex 85A	4.99	3.16	1.58

in the figure.5.2Ø The crown ether formation is more feasible with symmetrical polyols like PTMG. In the case of present IPDI based polyurethane as the % of PTMG by weight increased the amount of calcium ion deposited on the polymer decreases. In PPG based polyurethane(I4) the calcium ion deposition is less , this is due to pendent methyl group present in the molecule which gives steric hindrance to the ether formation with calcium. In the case of SMDI based polyurethanes the reverse trend was observed. The decreased calcium ion deposition in S3 polymer (with increase in soft segment content) may be due to the higher interfacial tension between polymer - water and some other factors related to surface morphology. When we compared the stressed and unstressed polymers the stressed polymers has more amount of calcium ion deposition. From the table 5.33, it is understood that the crosslinked polyurethanes are less susceptible to accelerated calcification.

5.4.1Ø In vivo stability of polyurethanes

The loss in tensile strength of polyurethanes are given in figures 5.21 to 5.23. The histograms clearly indicate that the PEG based polyurethanes are less stable when compared with the other polyurethanes. For PTMG and PPG based polyurethanes as the % of hard segment decreases the loss in tensile strength also increases. When comparing the biostability of the three types of the polyurethanes containing the hard segments content around 25 %.. The loss in tensile strength is in the order

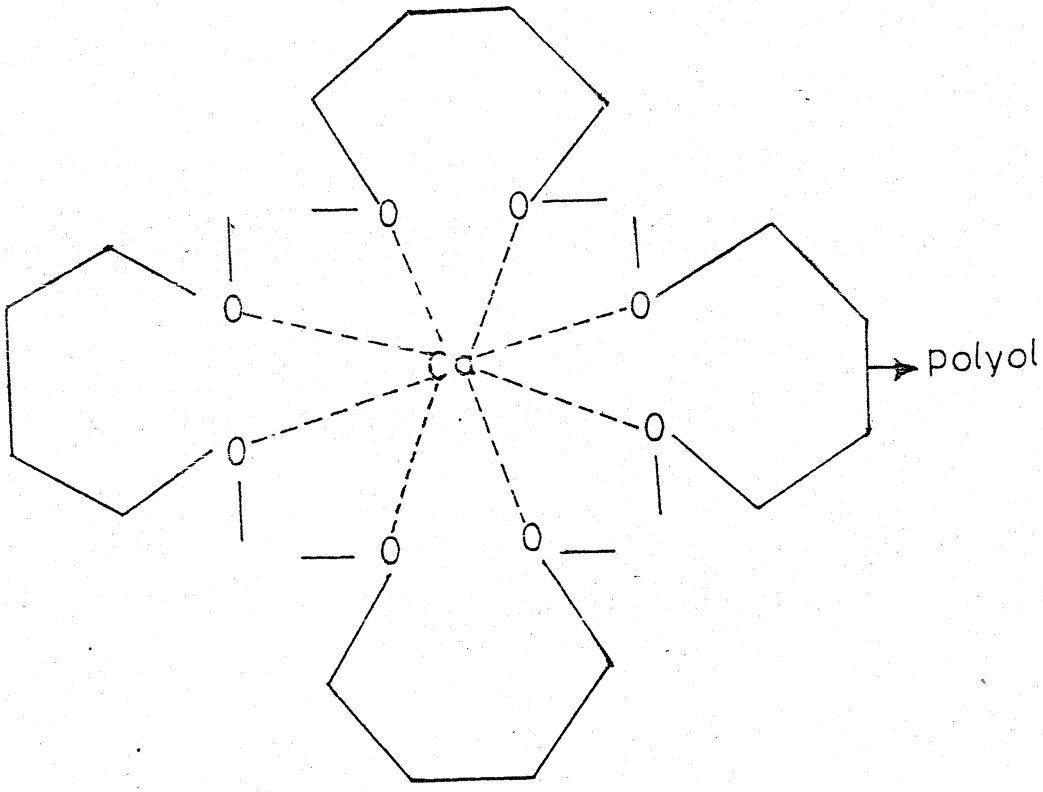


Fig.5.20.Crown ether formation of PTMG molecule with calcium ion

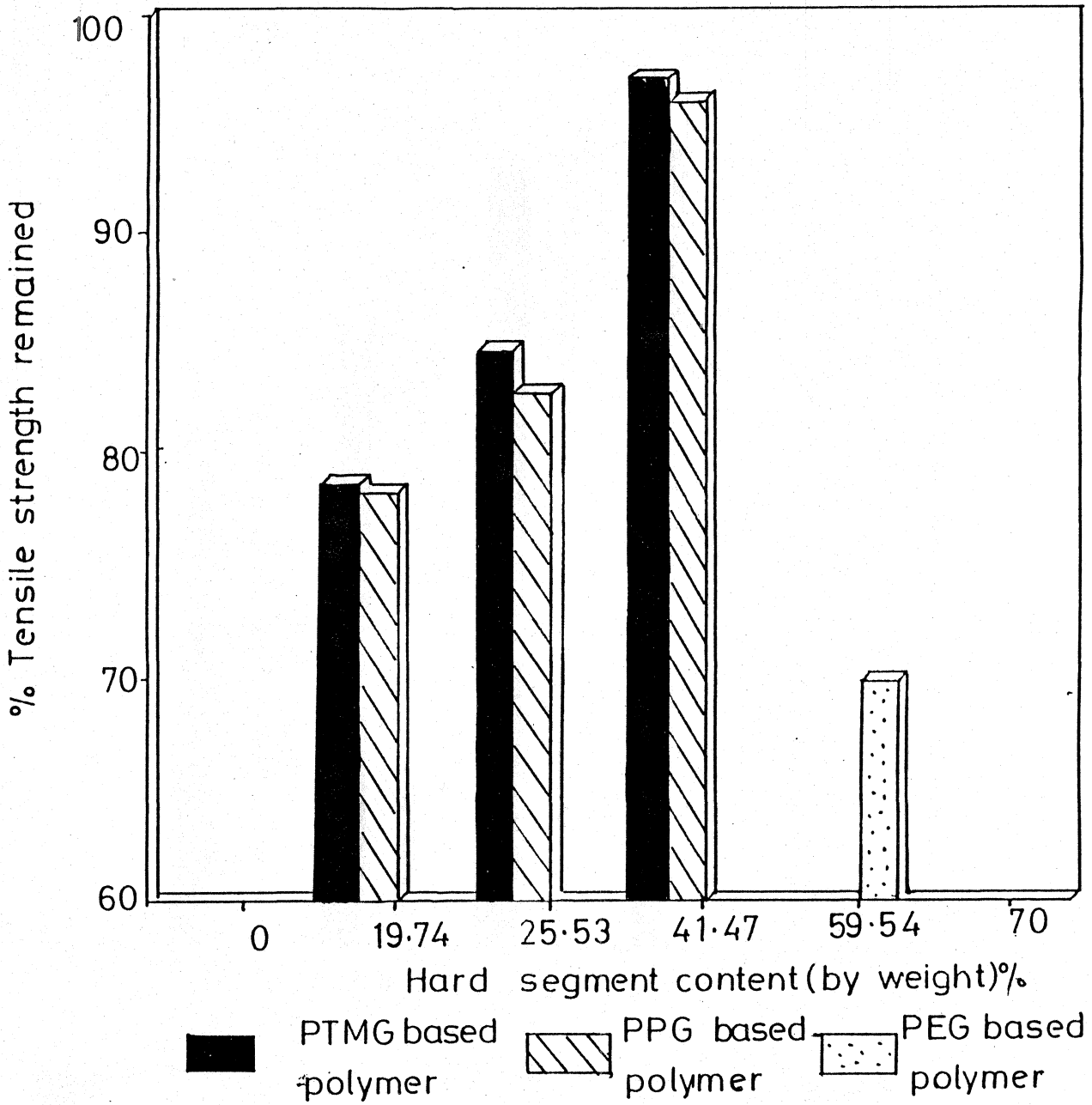


Fig . 5-21. Change in tensile properties of IPDI polymers after subcutaneous implantation for 4 months.

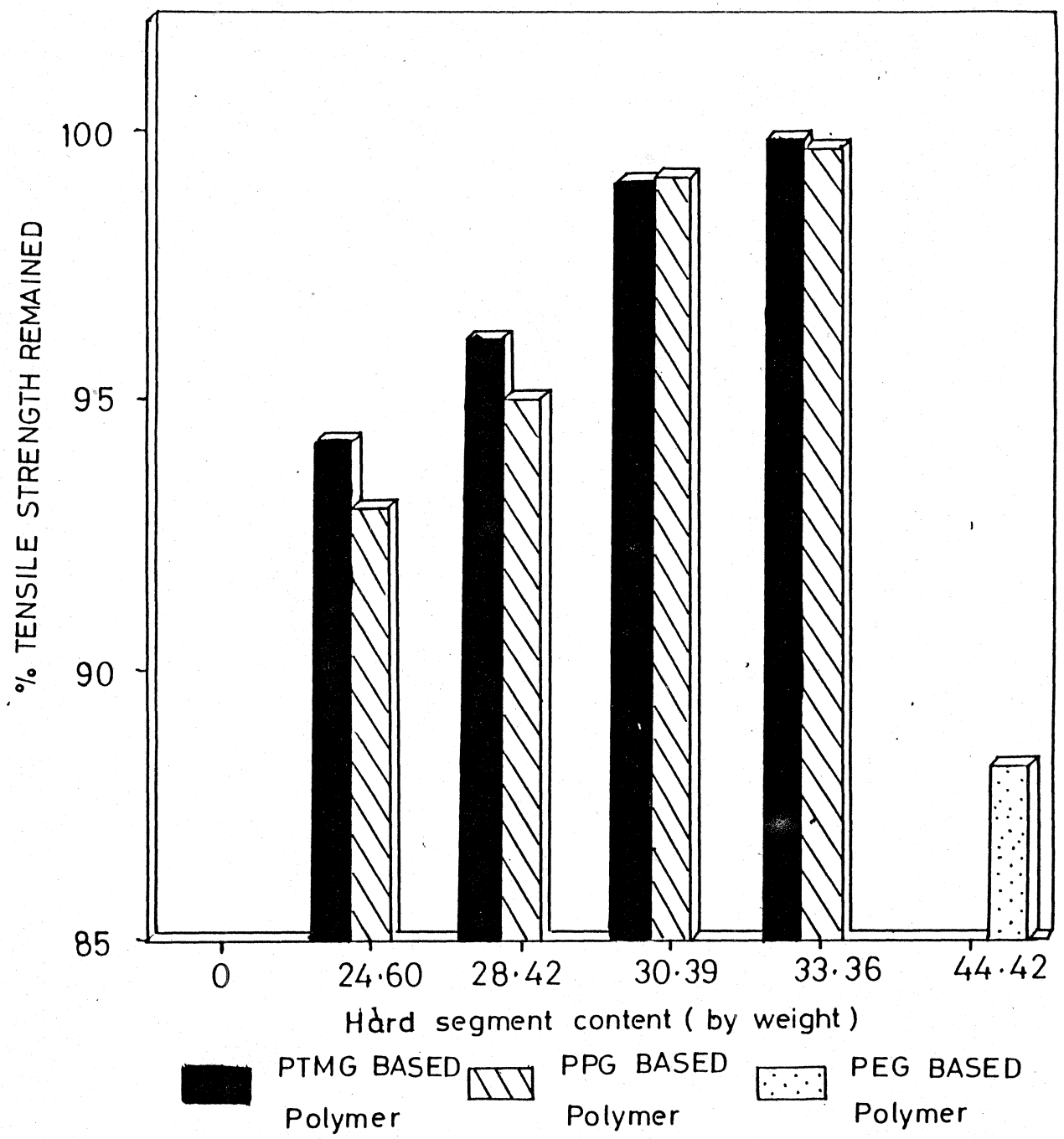


Figure 5.22 Change in tensile properties of SMDI plymer after subcutaneous implantation for 4 months

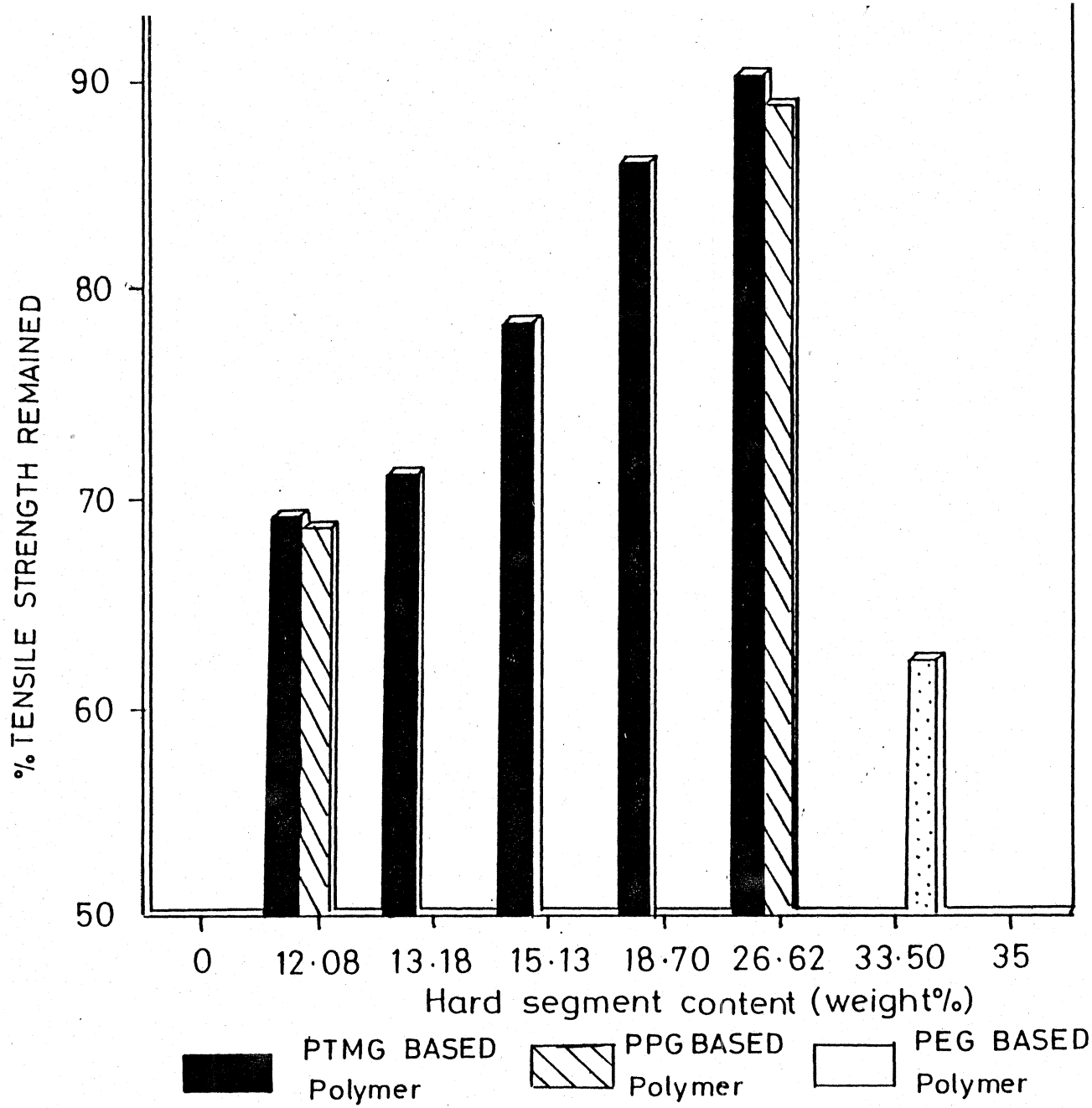
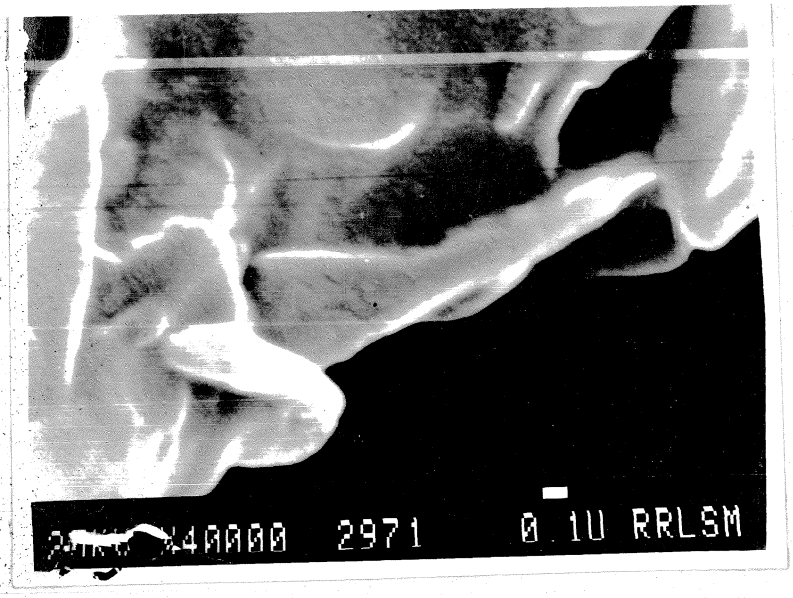


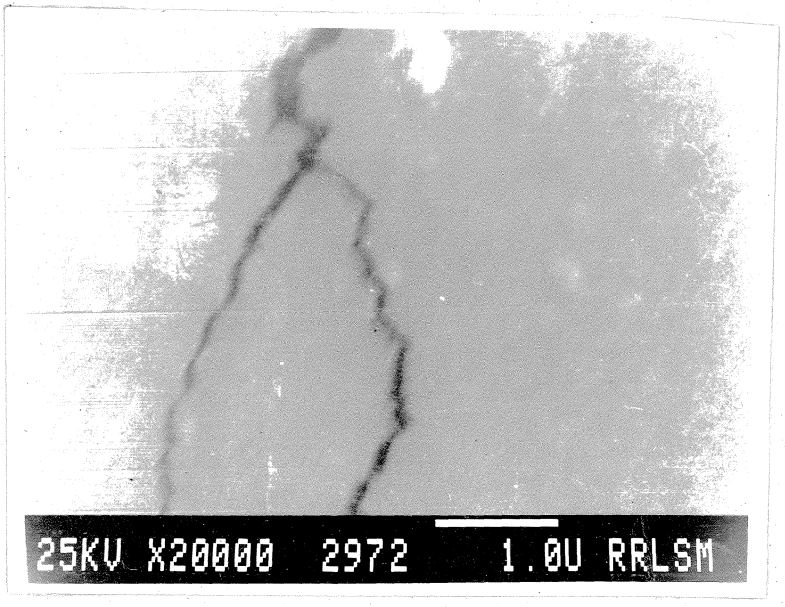
Figure 5.23 Change in tensile properties of HDI Polymer after subcutaneous implantation for 4 months

SMDI > HDI > IPDI

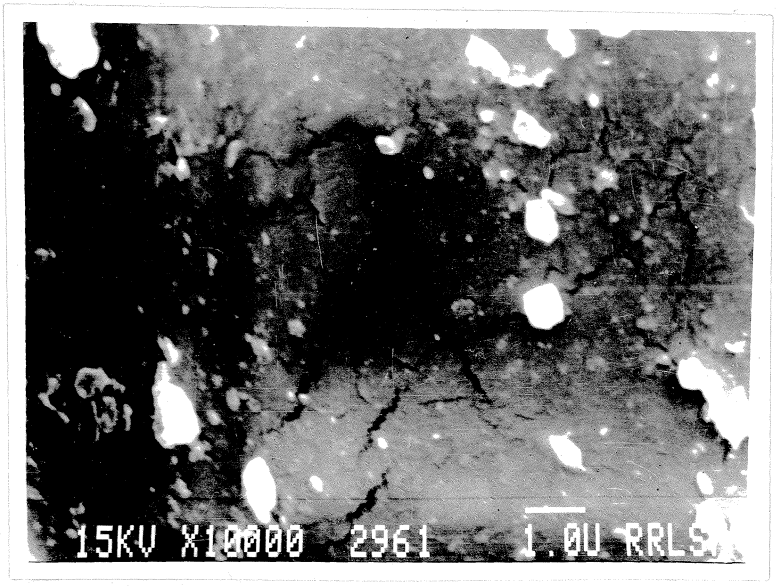
The above trend is observed for both PTMG and PPG based polyurethanes. While comparing the loss in tensile strength due to the effect of polyol PTMG and PPG all the three classes of polyurethanes, a insignificant excess decrease of around 2 % loss of tensile strength is noticed with PPG based polyurethanes in class of polyurethane. This indicates that the biostability is not appreciably influenced by polyols but only by the hard segments and Mc. The tensile studies can reveal the change in bulk properties. But in order to study the change in surface integrity, SEM photomicrographs are used. Figures 5.24-5.26 indicates the appearance of microcracks on the surface of the present polyurethanes for postimplantation period of 4 months. The degree of microcracks formation in all the three classes of polyurethanes is in the order, PEG based PU > PPG based PU > PTMG based PU. These microcracks are associated with environmental stress cracking in which the influence of the present elastomers polyurethane, biological attacking medium and formation of tensile stresses are involved. The formation of tensile stress is possible due to the leaching of some components (of course leaching is significantly small) from the polymer as observed in the in vitro stability studies. The tensile stress can also arise due to the mechanical surface deformation by the absorption of biological components at the surface. This can lead to the



I_1(a)

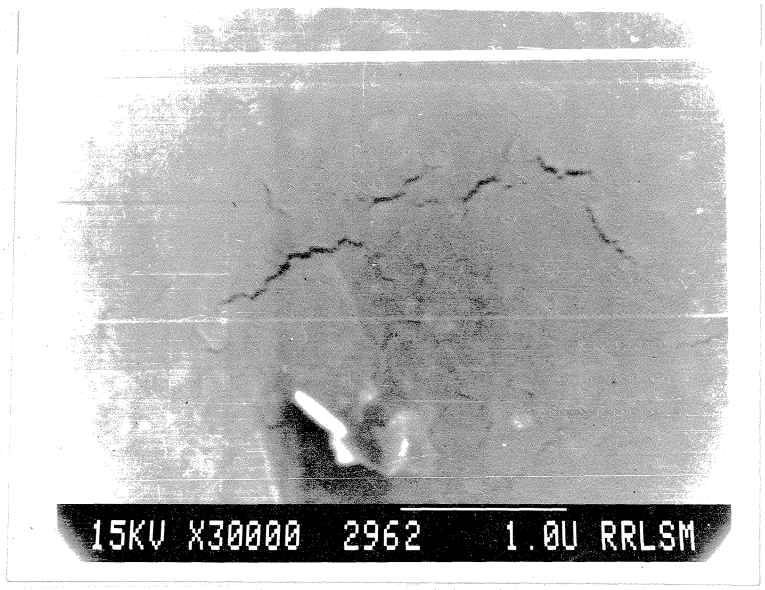


I_4(b)

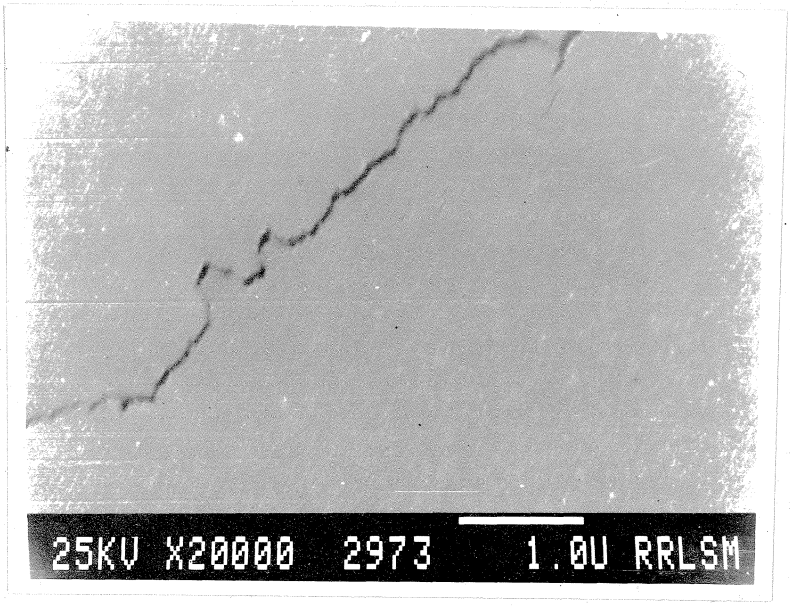


I_7(C)

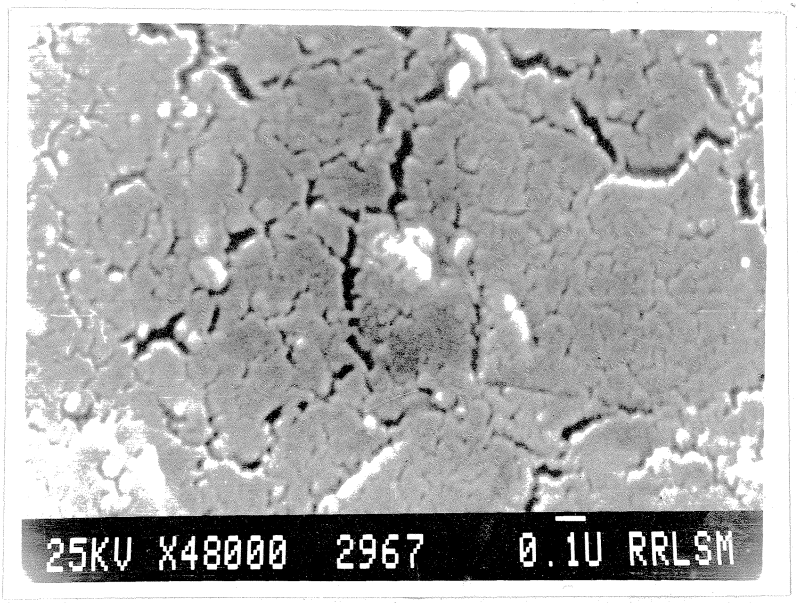
Figure.5.24.SEM microphotograph showing microcracks of IPDI based Polyurethane
(a = PTMG based PU , b = PPG based PU, c = PEG based PU



S_2 (a)

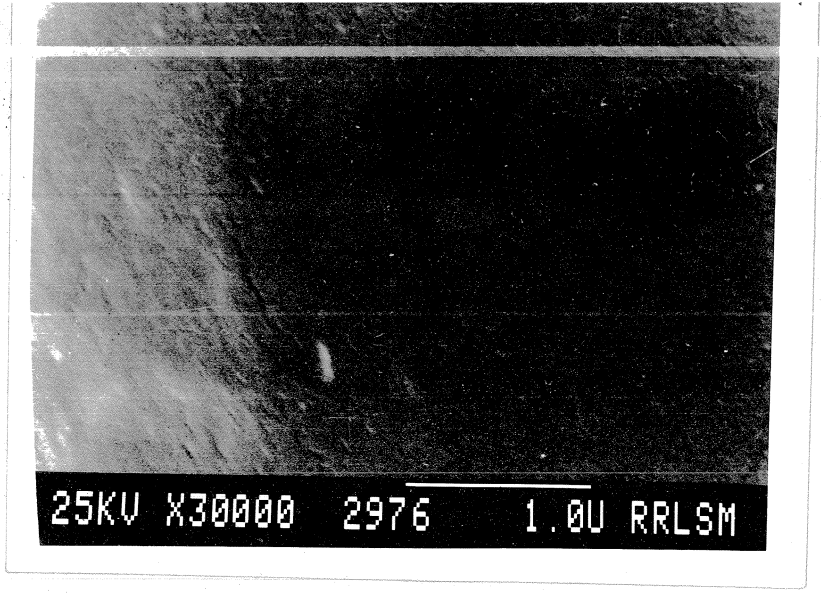


S_8 (b)

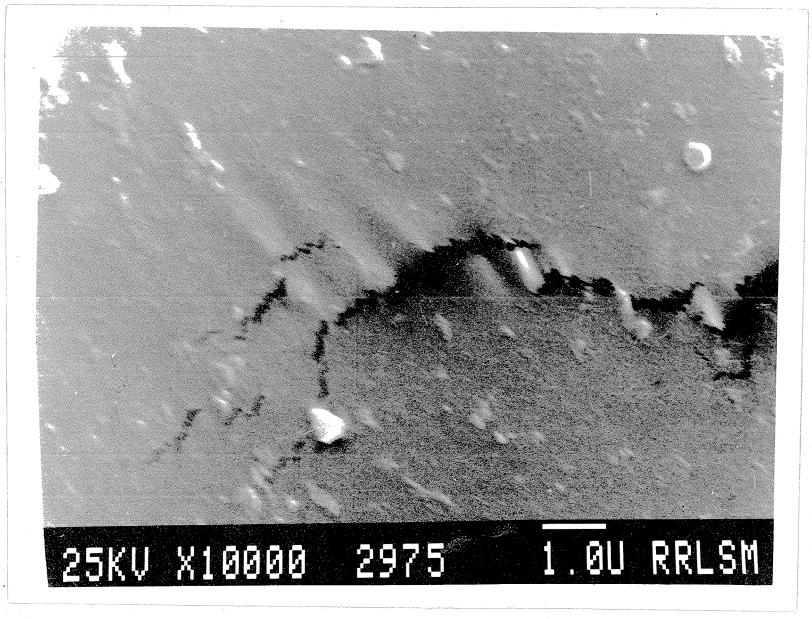


S_9 (C)

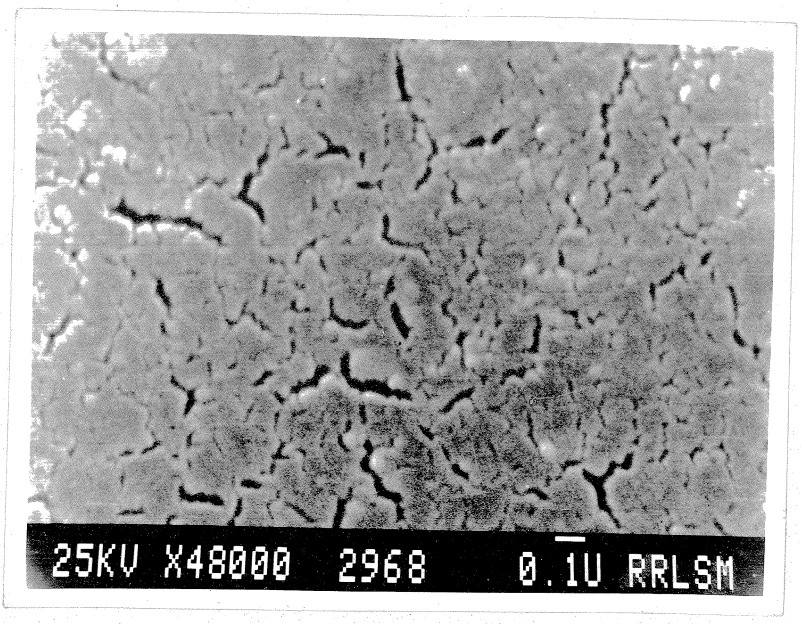
Figure 5.25. SEM microphotograph showing microcracks of SMDI based Polyurethane
(a = PTMG based PU , b = PPG based PU, c = PEG based PU



$H_1(a)$



$H_7(b)$



$H_8(C)$

ure.5.26. SEM microphotograph showing microcracks of HDI based Polyurethane
(a = PTMG based PU , b = PPG based PU, c = PEG based PU

formation of surface crazes on the surface under the tensile stress. These surface crazes able the attacking biological components at the surface. This can lead to the formation of surface crazes on the surface under the tensile stress. These surface crazes unable the attacking biological medium to penetrate into the polymer. This favours the propagation of such microcracks in the polymers. The polymers based on PPG polyol show propagation of such microcracks under higher magnification. The relatively high modulus PTMG based polyurethanes show relatively lesser degree of microcracks on the surface. The IPDI based PTMG polyurethane showed fracture surface with feathery patterns (figure 5.24 c) The PEG based polyurethanes show higher degree of microcracks interconnected through the surface. The degree of leaching from this polymer, low tensile modulus and high water and lipid absorption are the characteristics responsible for the higher degree of microcracks formation at the surface.

The surface cracking of the polyurethanes is generally mediated by the giant cell attack. Zhas et all[299] have proposed that the adherent foreign body giant cell induces localized surface cracking directly under the giant cells during the implantation period. The formation conclude that the adherent macrophages and foreign body giant cells attack the invading foreign body in the facial cell - polymer contact areas[299].

5.5 MATERIAL- TISSUE INTERACTION STUDIES ON POLYURETHANES

The long term functional stability of the implant is largely governed by the material - tissue interaction. It is well known that such interaction is influenced by the surface characteristics, biostability and sample morphology of the implant and also by the biomechanics acting at the interface. Unfavorable surface properties and biodegradation not only affect the mechanical locking of the implant with tissue but also leads to chronic inflammatory reaction Therefore solid material fibroblast cell interaction can reveal the degree of tissue compatibility at the solid surface. The interaction of fibroblast cell with extract of the material can reveal the influence of leachants on the growth of the fibroblast at the material - tissue interface. The long term functional stability of the implant can be judged by the evaluation of tissue response to materials implanted in biomechanically different implant sites.

5.5.1 INTERACTION OF FIBROBLAST CELL WITH SOLID POLYURETHANE MATERIAL

Fibroblasts are a group of cells which are, present in all tissues, arise from all the three germ layers. Fibroblasts function in the deposition, maintains degradation and rearangement of extracellular matrix. Therefore this cell is specialized for establishment and maintenance of the matrix structure. They are also important in wound healing. following implantation of a biomaterial the responses that occur at the

interface of the implanted material and in the surrounding environment are important events in determining the biocompatibility of the implant. Successful incorporation of the implant in the human body depends on several factors such as biocompatibility, complete incorporation and maintenance of function. If the biocompatibility and rate of incorporation could be controlled, maintenance of function would be guaranteed.

In the case of implanted materials excessive fibroblast proliferation leads to fibrosis and tissue encapsulation, which alter the function of the implanted device. Therefore the knowledge of the behaviour of fibroblastic cells at the tissue polymer interface is essential to establish tissue compatibility as well, as functionality of implant. Normal adhesion and growth of mammalian fibroblasts depend upon the cell substrate interaction. Therefore the properties of synthetic polymer substrate which can regulate the cell behaviour are the primary parameters to be considered. Several substratum properties have been proposed as influencing cell behaviour[302]. These properties include chemical group expression(eg. substratum hydroxyl group content), hydrophilicity/ hydrophobicity(fractional polarity) physical and chemical Anisotropy and substratum contacility (equilibrium water content).

The present polyurethanes are crosslinked and insoluble. Therefore these polyurethanes do not swell in water appreciably. These polyurethanes do not contain any hydroxyl group in the

polymer chain. Interestingly these polyurethanes posses polar dispersive components of surface free energy distinctly different from each other, the polar components being higher than the dispersive components in most of the polymer.

Vnder Valk et al have shown a relationship between the polar component of surface free energy and fibroblast adhesion of verify polymer including a polyether urethane(Estanne 574 F1) using a transformed cell line of mouse lung fibroblasts. Though they have not observed variations in cell division rate with various polymer substrates, they have observed marked variation in cell spreading on the various substrates. All spreading appeared to be dependent on the polar surface free energy. Cell spreading is low when the polar surface free energy ($r^p s$ of the bare polymer surface is lower than 5 erg/cm^2, marked spreading occurs when r^p_s is higher than 15 erg/cm^2

A similar relationship was found in the protein coated polymer surfaces. Cell spreading appeared independent of the dispersion surface free energy.3Ø2 Lydon et al has proposed a correlation between the fractional polarity of the polymer substrate and fibroblast(spontaneously transformed rat dermal fibroblast) adhesion on the polymer surface[3Ø3]. Their studies were aimed to locate the polarity within which all materials so far tested support full cell adhesion and spreading. Schadenraad et al have found a relationship between cell spreading and substratum surface free energy ($r^p s$) using a primary cell culture

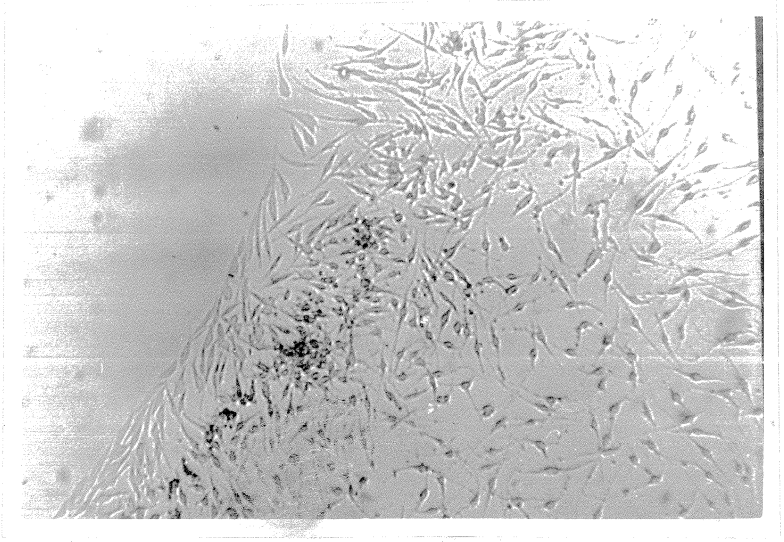
of human skin fibroblast[3Ø4]. They found that cell growth in the presence of serum protein did not differ significantly on the various polymers with reference to their rs values. However they noticed lowest relative cell growth on a polyether urethane. (Platilon u Ø1, m/s plate chemie, FRG). This polyurethane was found to have surface energy 53 erg/cm^2 with dispersive components 4Ø erg/cm^2. In the absence of serum, they could distinguish two groups of polymers with respect to cell growth. The first group showed increasing cell growth with increasing, rs, whereas the second group showed continuously low cell growth. The polyurethane, platilon u Ø1 falls in the second group. Schakenraad et al [3Ø4] also found that cell spreading and substratum surface free energy showed a characteristic sigmoid relationship both in the presence and in the absence of serum protein; good spreading only occurred when rs was higher than approximately 57 erg/cm^2. The polyurethane, platilon vo1, showed higher degree of relative cell spreading in the absence of serum than in the presence of serum. Schakenraad et al also confirmed these in vitro findings with an in vivo rat model using smooth muscle in tubular grafts made from various polymers including a pellathane 75D, polyether urethane[3Ø5].

Fibroblast cells have a notoriously high replicative activity. Analysing the proliferation of these cells, using the uptake of a radioactive tracers for the DNA of the dividing cell, it was found that fibroblasts showed significant variations

from their normal duplicating rhythm when placed in contact with toxic materials[3Ø6].

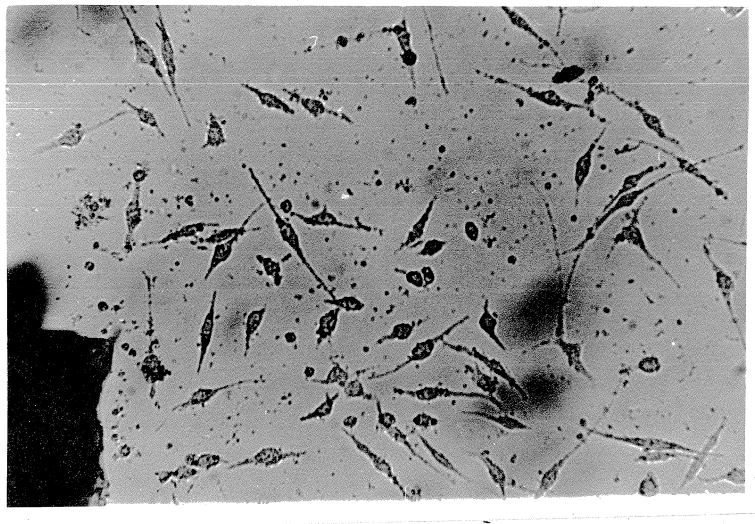
The morphology of fibroblast L-929 cells is shown in figure 5.27 a. The cells are elongated and spindle shaped. Some of the cells are also clustered together. The photomicrograph of the fibroblast cells in the presence of agarose solid sample clearly indicate viability of cells.(fig 5.27b). The interface at agarose - cellular medium indicates the uniformly adhered fibroblast cells with cytoplasmic spreading. The next adjacent cells have their cytoplasmic tags pointed towards the initially adsorbed cells on the surface of the agarose. Cell clustering is also noticed no cell lysis was noticed near the agarose sample or in the medium. These suggest that marked cellular proliferation was favored by the agarose sample. The biomedical grade polyurethane Tecoflex 85A exhibited some degree of cell lysis (fig 5.28 a) This may be due to the physical contact of the solid polyurethane materials with the cell layer which might induce bad nutrient and gas supply and lead to decreased cell growth as reported by Wieslander et al 3Ø7. The candidate polyurethanes exhibited good cell adhesion and spreading (fig 5.29-5.31) The cytoplasmic spreading was noticed appreciably, in 17,S5,H7 and H8 polyurethanes. Moreover heavy accumulation of cells on the polymer surface and near the polymer is noticed for S9 polymer (fig5-3Øb) . Significantly no cell lysis was noticed with any of the test material except with 13 where some cell lysis was noted

(a)

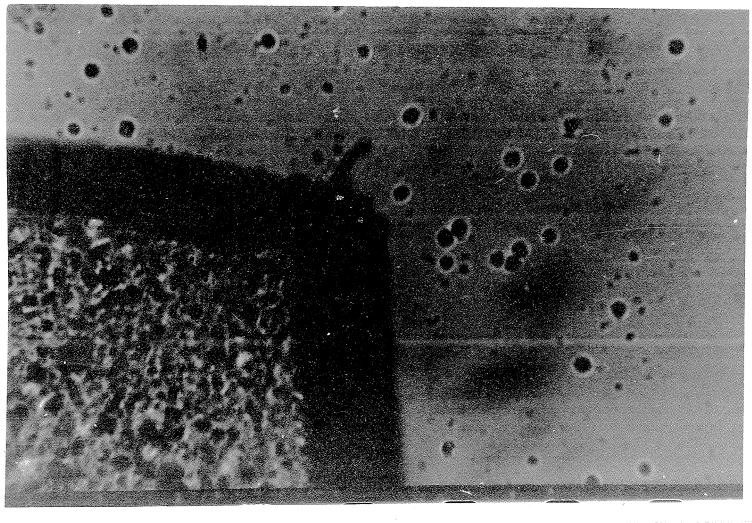


(b)

Figure 5.27 Photomicrographs of fibroblast cells.
(a: Fibroblast cell -L929 ,b: Fibroblast cell with agarose) X100

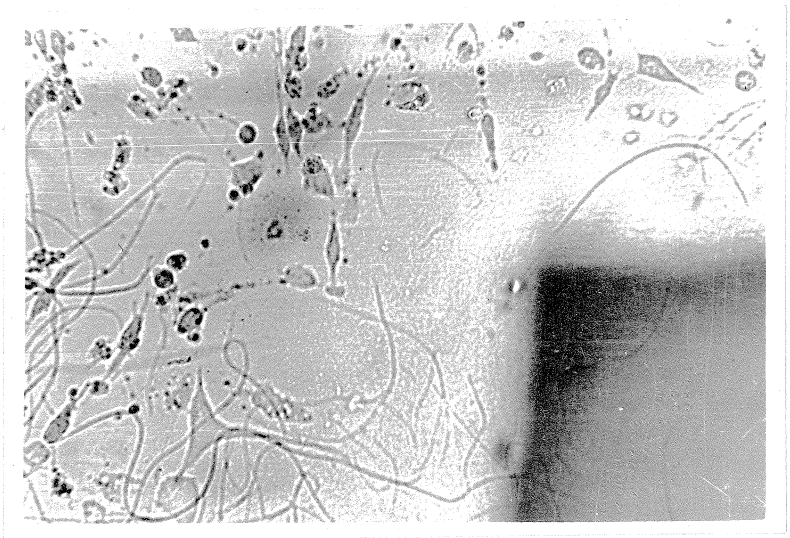


(a)



(b)

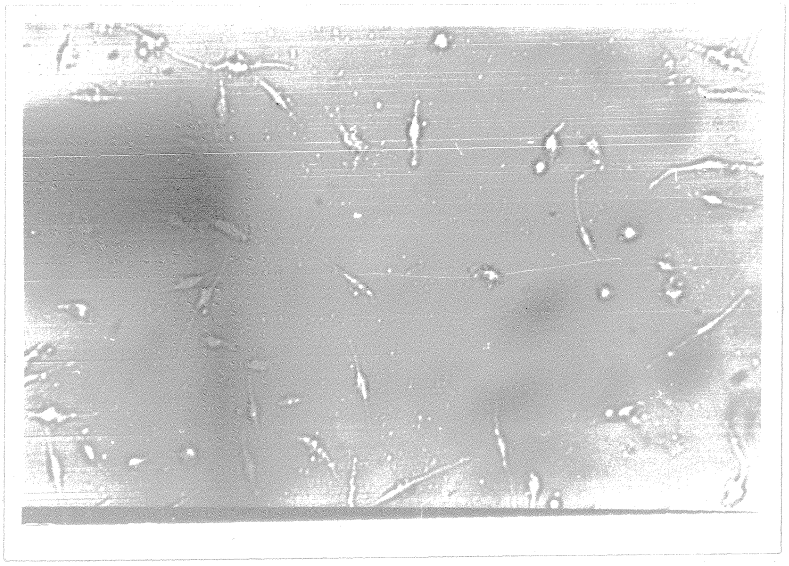
Figure 5.28 Photomicrographs of fibroblast cells with polymers. (a: Tecoflex 85 A negative control, b: Positive control) X100



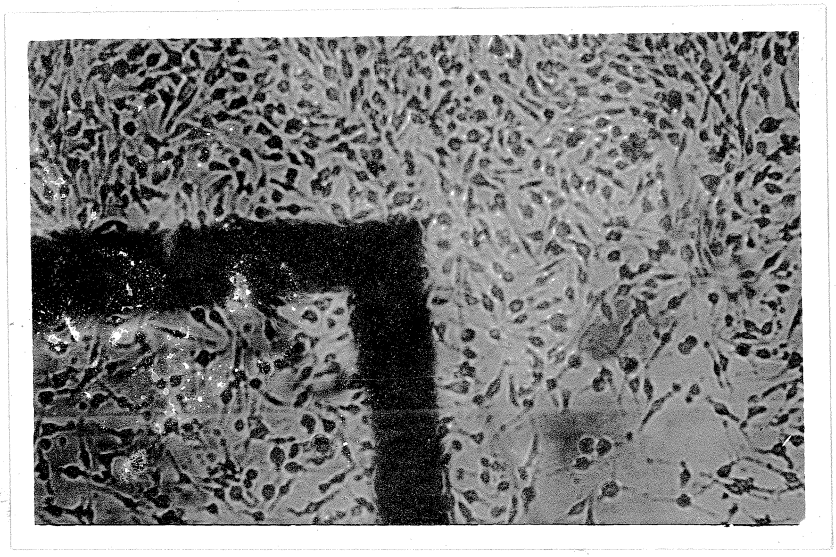
(a)

(b)

Figure 5.29 Photomicrographs of fibroblast cells with IPDI based polyurethanes
(a: I3 , b: I7) X100



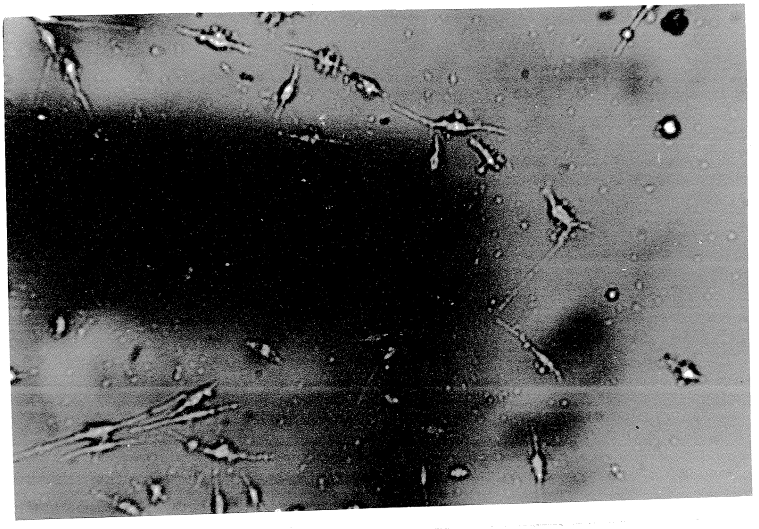
(a)



(b)

Figure 5.30 Photomicrographs of fibroblast cells with SMDI based polyurethanes
(a: S5 , b: S9) X100

(a)



(b)

Figure 5.31 Photomicrographs of fibroblast cells with HDI based polyurethanes
(a: H7 , b: H8) X100

(fig 5-29 a) This cell lysis resembles to that seen with negative control Tecoflex 85A. The cells under the polymer film also seems to be viable in most of the candidate polyurethanes. The microphotograph of the cells associated with the positive control polymer PVC , clearly indicated the cell lysis over, under and near the material(fig 5-28b). This toxic response clearly indicates the incompatibility at the PVC material-cell interface.

The present polyurethanes are found to elicit appreciable fibroblast growth (adhesion and spreading) on the material. Most of the candidate polyurethanes the polar surface free energy higher than 15 erg/cm^2 and the surface energy around 5Ø erg/cm^2 vide Tables 5.5-5.7).

Therefore the surface energy parameters of the present polyurethanes are favorable for fibroblast cell growth on and around the material. The investigation clearly indicates that there polyurethanes can elicit more favorable tissue response at the material-tissue interface.

5.5.2 INTERACTION OF FIBROBLAST CELL WITH EXTRACT OF POLYURETHANE MATERIAL

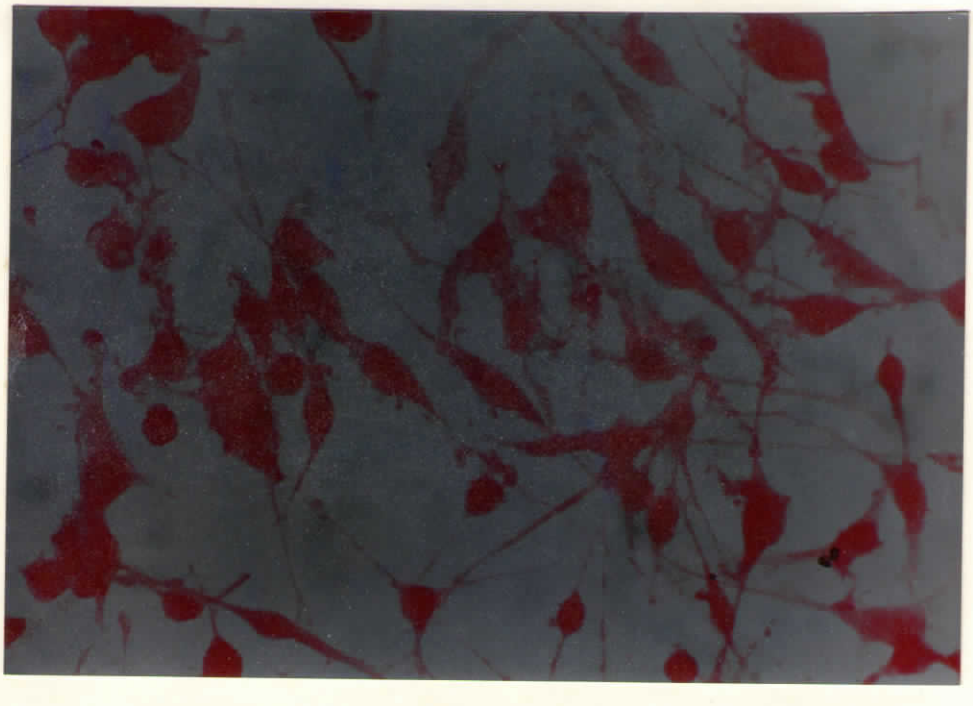
Polymer materials may contain additives such as monomers, catalysts, lubricants which have been used during polymer synthesis and processing. It may also contain oligomers. These additives have to be removed from the polymer by extensive

extraction. During the processing of the polyurethane, excessive heat can lead to degradation of polyurethane chain to give amine compounds as mentioned in chapter 2. Moreover autoclaving and sterilization by boiling water can also degrade. These additives and degraded fragments leach out from the polyurethane when it is in contact with biological fluids if they are not removed already. The additives soluble in polar fluids not only affect the cell behaviour at the material cell interface leading to unfavorable tissue response at the material-tissue interface but also influence systemically.

The intracutaneous irritation test and systemic toxicity tests of the present polyurethanes have revealed the compatibility of the polymer extract locally and systematically. However the interaction of fibroblast cell with the extract of the polyurethanes is one of the determining factor on the tissue compatibility. The fibroblast cell grown in the presence of PECF fluid is shown in the fig 5.32a. The morphology of the cell shows that the cells are viable . Appreciable cytoplasmic spreading is also observed. The fibroblast cell grown in the presence of extract of the biomedical grade Tecoflex 85A showed the viable nature of the cell with limited cytoplasmic spreading in compression to that seen with PECF fluid. The nuclei do not show only marked morphological changes.

Cytopathic effect is considered as the morphological changes in the cytoplasm. Usually it is due to the flexibility

(a)



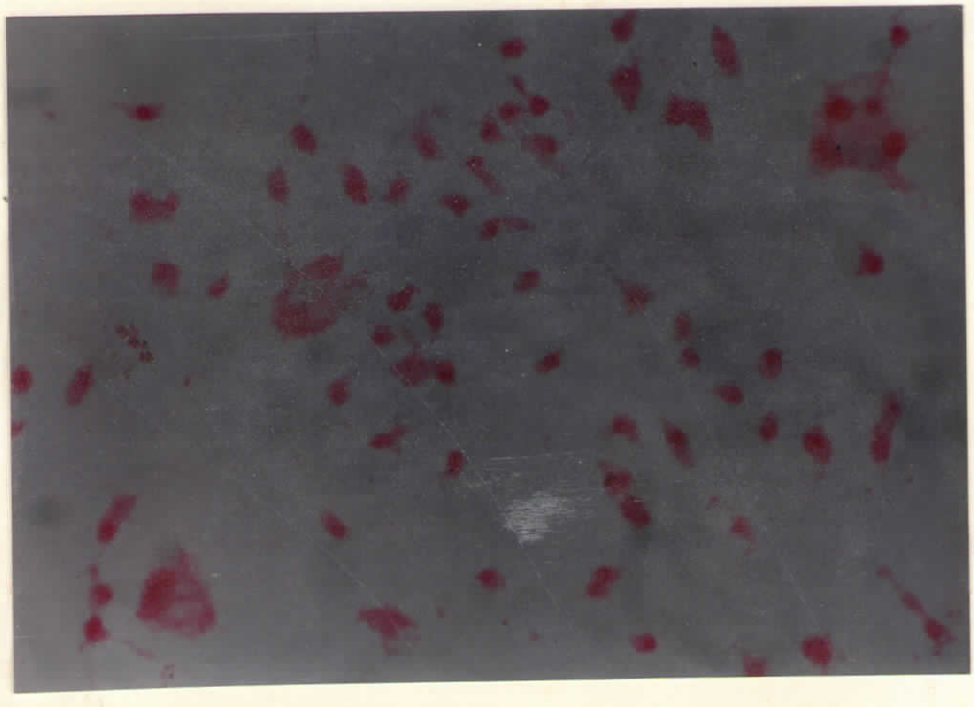
(b)

e 5.32 Photomicrograph of fibroblast cells.
(a: cell in PECF control, b: cell in extract of Tecoflex 85A negative control) Carbol fushin stain
X 250

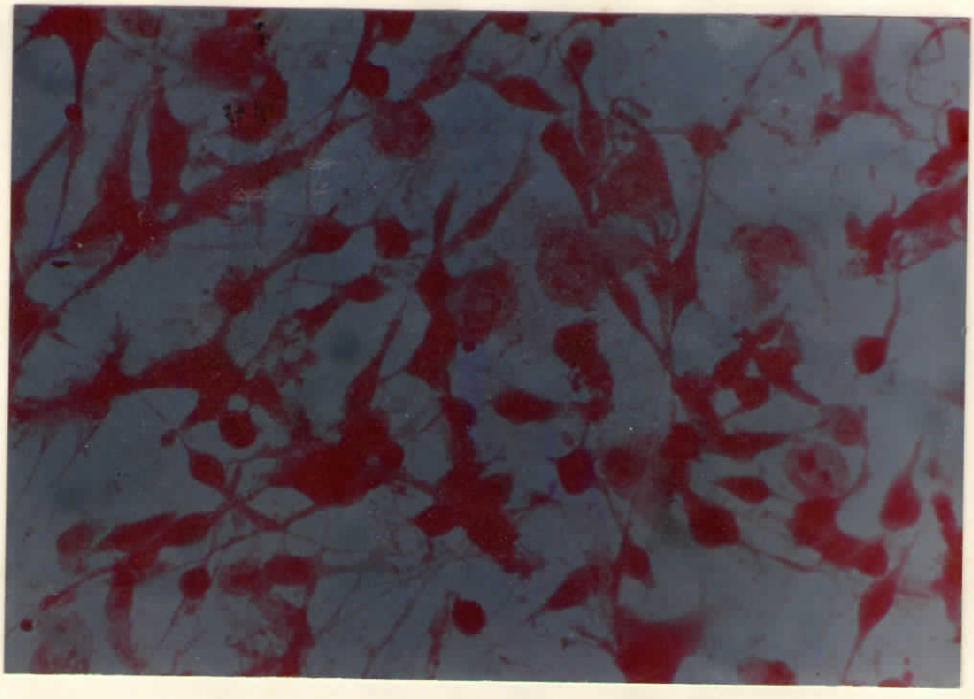
(invagination) and permeability of cell membrane. Such invagination is possible whenever the osmotic pressure difference excists across the membrane (between the cytoplasmic content and external medium). The membrane becomes permeable to lipid soluble molecule, certain small molecules (water, methyl alcohol and formamide) and ions (Na, K and Cl) sugar molecules and amino acids[13Ø8]. The whole process finally leads to the formation of vacuoles in the cytoplasm. The cytopathic effect need not affect the nucleolus of the cell and still keep the viability of the cell.

The photomicrograph for the positive control shows the cell lysis (Fig 5.33) Few of the cells also clustered. the polyurethanes I1 and S1 showed limited cytoplasmic spreading with occasional cell lysis(Fig 5.34a and 5.36a) as observed with biomedical grade , control Tecoflex 85A (Fig 5.32b). On the other r hand polyurethanes I4, H7 and S5 show more cell clustering with occasional cell lysis (fig 5.34 b, 5.35 a, fig 5.35 b) The polyurethane S9 shows more cytoplasmic spreading of fibroblast with no cell lysis (fig 5.36) in comparing to the other test polyurethanes as well as biomedical grade Tecoflex 85A A polyurethane.

The fibroblast growth in the presence of extract of the present crosslinked polyurethanes are comparable to that of the growth in the case of biomedical grade Tecoflex 85A. In comparing the response of the test materials to that of toxic PVC (positive



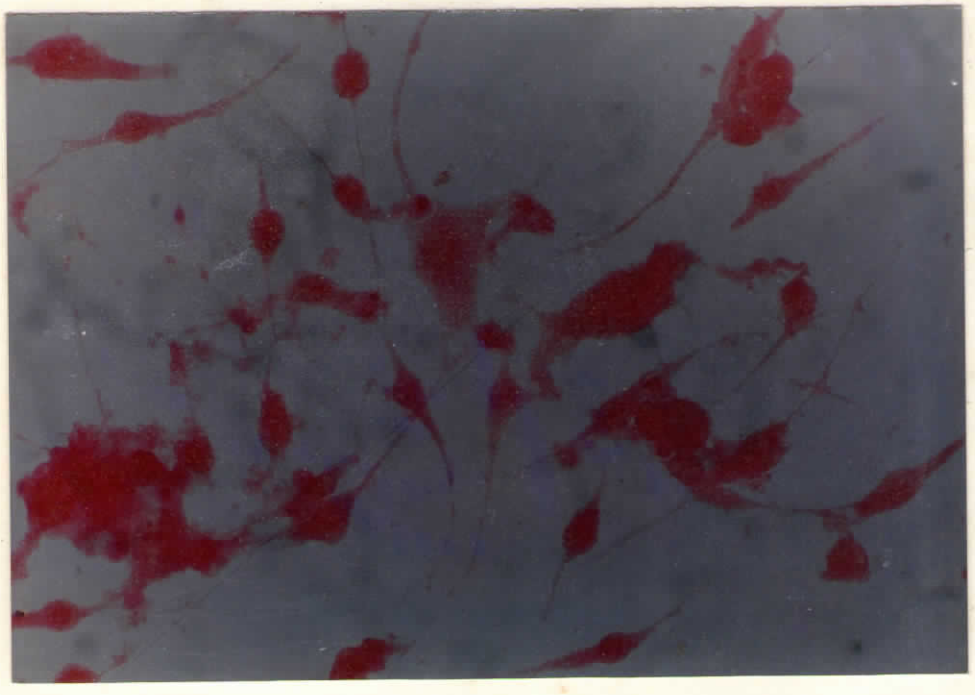
5.33 Photomicrograph of fibroblast cells in the extract of toxic PVC (Positive control) Carbol fushin stain X 250



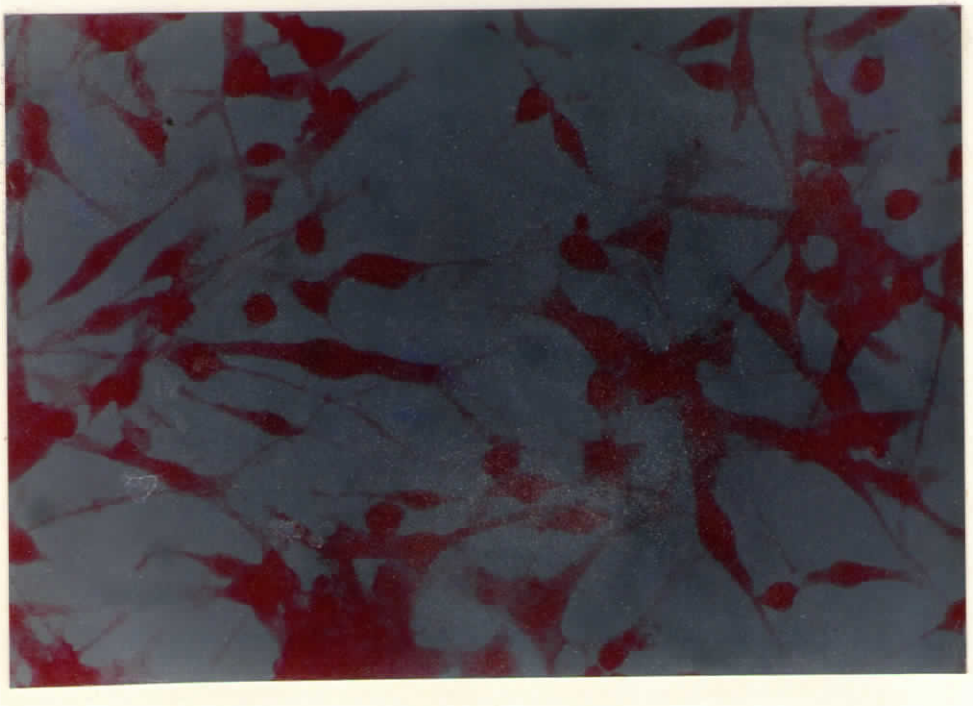
(a)

(b)

5.34 Photomicrograph of fibroblast cells in the extract of IPDI Polyurethanes
(a: cell in PECF extract of I1, b: cell in extract of I4) Carbol fushin stain X 250

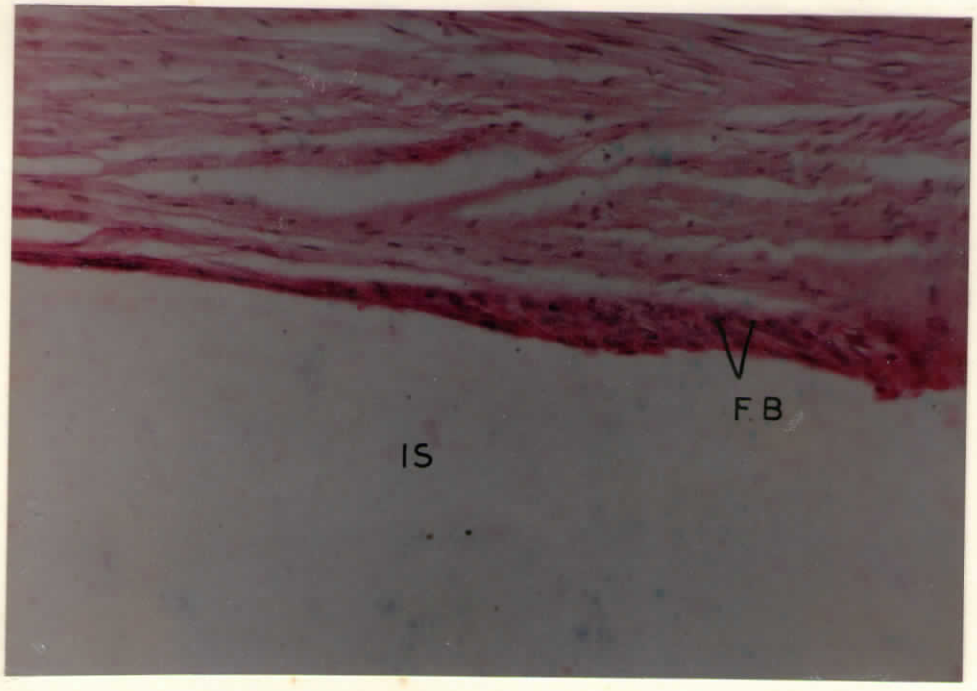


(a)

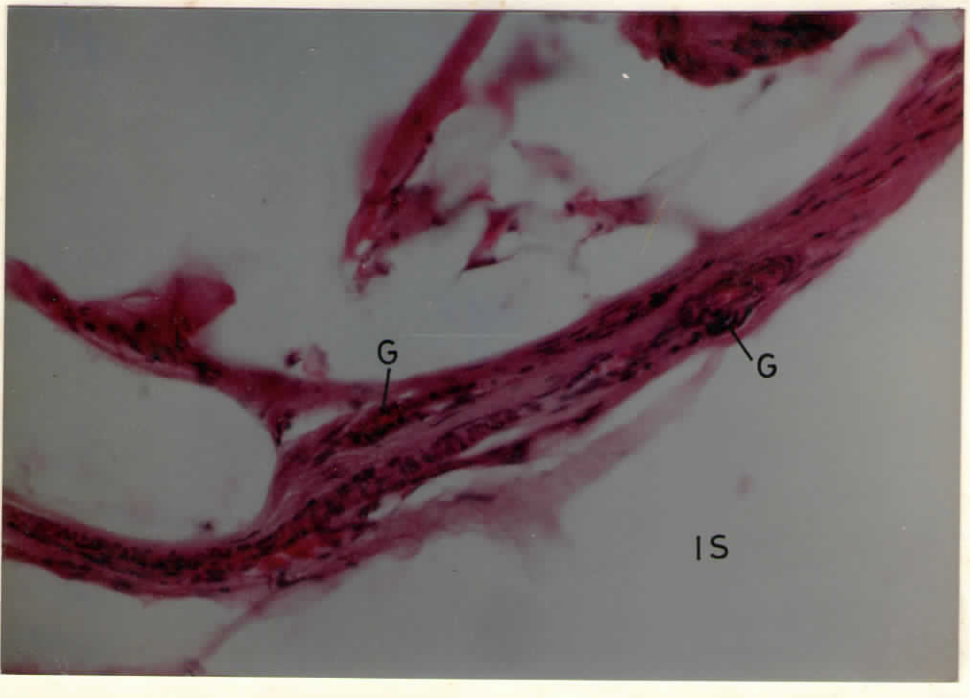


(b)

re 5.35 Photomicrograph of fibroblast cells in the extract of HDI and SMDI Polyurethanes
(a: cell in PECF extract of H7, b: cell in extract of S5) Carbol fushin stain X 250



(a)



(b)

e 5.37 Tissue response at minimal motion site. 25 weeks post implantation period, (a:Control polymer b: IPDI sample) Hematoxylene Eosin stain, X 250
Is-Implant site, G-Giant cell,FB-Fibroblast

control) it can be inferred that the extract of the present materials do not induce cell lysis at the material cell interface significantly.

5.5.3 TISSUE RESPONSE TO POLYURETHANES IMPLANTED IN BIOMECHANICALLY DIFFERENT IMPLANT SITES

The tissue response to a passive biomaterial implant is largely governed by the chemical factors of the biomaterial. When an implant is placed in tissue a host response will occur depending on the material characteristics. This host response includes acute, chronic inflammatory reaction, foreign body reaction and scar tissue encapsulation. Most implants are not truly inert, thus providing many chemical stimuli for inflammation. These stimuli include the residues of polymers by Pitting corrosion or degradation. One of the factors which affect the tissue response is geometrical factors of the implant. Samples with triangular, pentagonal and circular shape elicit different host response. The greatest reaction was found with the triangular shaped implants, both in the number of inflammatory cells present and in the amount of enzyme activity[3Ø9]. The third and usually neglected major factor affecting soft tissue reaction to implants is mechanical. Kaminski et al [31Ø] compared different implantation sites and found muscle to have the greatest reaction. They felt that this was probably the result of the extension and contraction movement invariably present in muscle.

In the present investigation the effect of motion and biostability of the candidate polyurethanes on the tissue

response is also considered to be the controlling parameters which affect the material-tissue linkage.

Anderson and his workers have observed normal foreign body reaction in human implants[311] and chronic inflammatory reaction. The normal foreign body reaction is characterized by the foreign body reaction with granulation tissue development. The chronic inflammatory reaction is characterized by the presence of mononuclear cells including lymphocytes and plasma cells. The 'normal foreign reaction' is considered to be the normal wound healing response to inert biocompatible materials[311] Lymphocytes and plasma cells are involved principally in immune reaction and are key mediators of antibody production and delayed hyper sensitivity responses. Their roles in nonimmunological injuries and inflammation remain largely unknown. Little is known regarding normal immune responses and cell mediated immunity to synthetic biomaterials.

The foreign body reaction to human implants is composed of foreign body giant cells and the components of granulation tissue (macrophages, fibroblast and capillarities in varying amounts depending upon the form and topography of the implanted material[311]). Relatively flat and smooth surfaces (ex: human breast prostheses) have foreign body reaction composed of a layer of macrophages[311]. But relatively rough surfaces (ex: human vascular prosthesis) were associated with the foreign body reaction with macrophages and foreign body giant cells[311]. In

the present investigations both test and control polyurethanes are rough and porous. Although a biomaterial may e considered to be biocompatible, the form in which it s used in an artificial organ may alter the observed tissue reaction and inflammatory response[311]. This is clearly noticed with present control biomedical grade Tecoflex - 85A polyurethane. The control material was associated with 'normal foreign body reaction.'

The 'normal foreign body reaction' appears to persist for th life time of the human implant although the cellular content and the degree of vascularization and fibrosis may vary within the implant site with time[311]. Charnely has observed multinucleate giant cell around polymethyl methacrylate implant after the 9 months implantation and declared that giant cells need not be indicative of a harmful state[312]. With the biocompatible materials the composition of the foreign body reaction in the implant site may be controlled by the surface properties of the material, the form of the implant and the volume of the implant. For example: high surface to volume implant, such as fabric vascular graft will have higher ratios of macrophages and foreign body giant cells in the implant site or interface[311]. However the subject implant site undergoes fibroconnective tissue proliferation leading to fibrous encapsulation[311]. In these cases fibrosis is the major component of the 'normal foreign body reaction[311],.

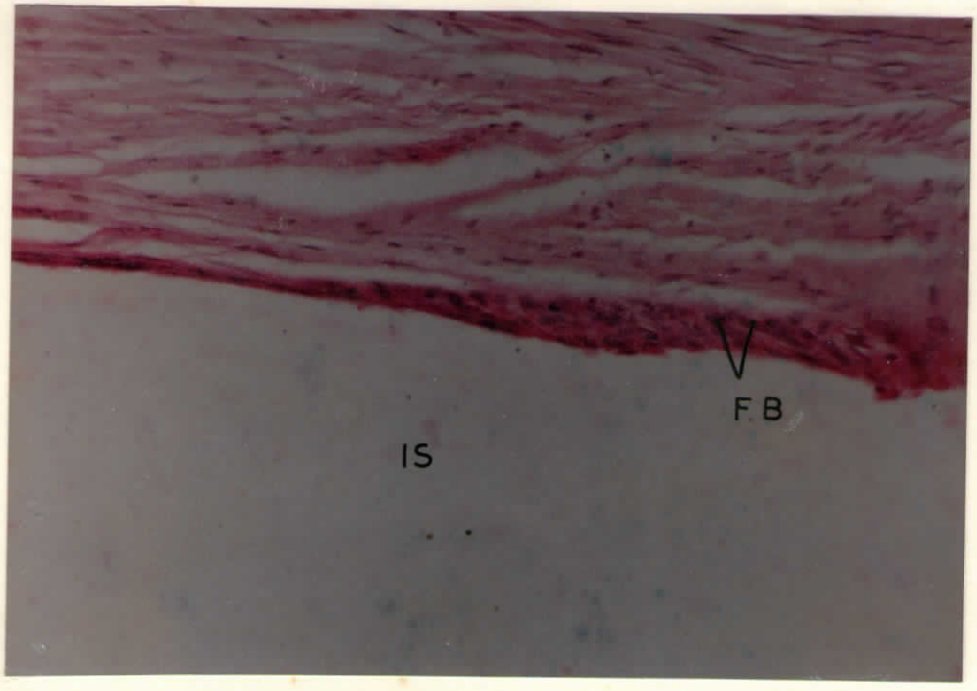
STUDIES ON TISSUE RESPONSE TO IPDI BASED POLYURETHANE [I3]

The long term tissue response to the IPDI based polyurethane was studied by comparing the histopathological parameter of the implants retrieved at the different post-implantation period. The histopathological parameters of the candidate crosslinked polyurethane(I3) are compared with that of the biomedical grade polyurethane Tecoflex^{R} 85 A.

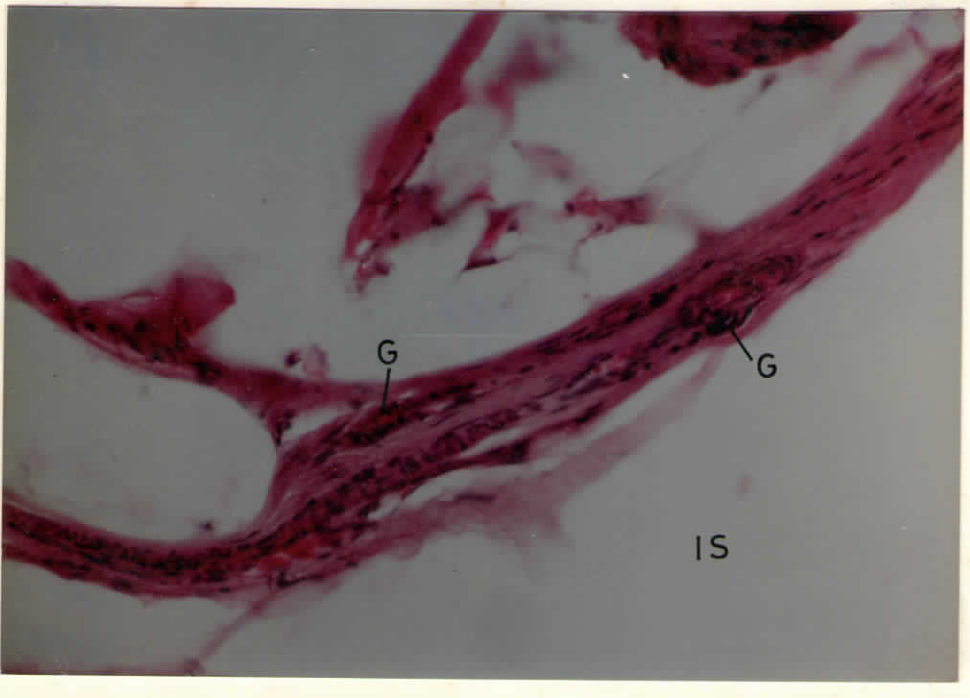
The histopathological parameters of the implant retrieved from the paravertebral muscle (minimal motion site) and hind leg (maximal motion site) are compared based on the chemical(instrinsic) and mechanical (extrinsic) factors.

1. Post - implantation period of 25 weeks.

The histopathological investigation on the crosslink polyurethane retrieved from the minimal motion site after the post implantation period of 25 weeks indicates the surrounding of the implant area with a capsule of fibrous tissue. This capsule is of varying thickness and is composed of numerous fibrocytes. Adjacent to this capsule, numerous macrophages, lymphocytes and few giant cells containing refractile material are seen (Fig.5.37 b) There is an ingrowth of the capsule in to the implant area in some places. The control material (Tecoflex 85A) retrieved from the anterior side of the paravertebral muscle is associated with the formation of extensive fibroplasia a on one aspect of the implant site and a much thinner layer of fibrous tissue along the other aspect (Fig 5.37a). Numerous clear spaces with giant cells



(a)



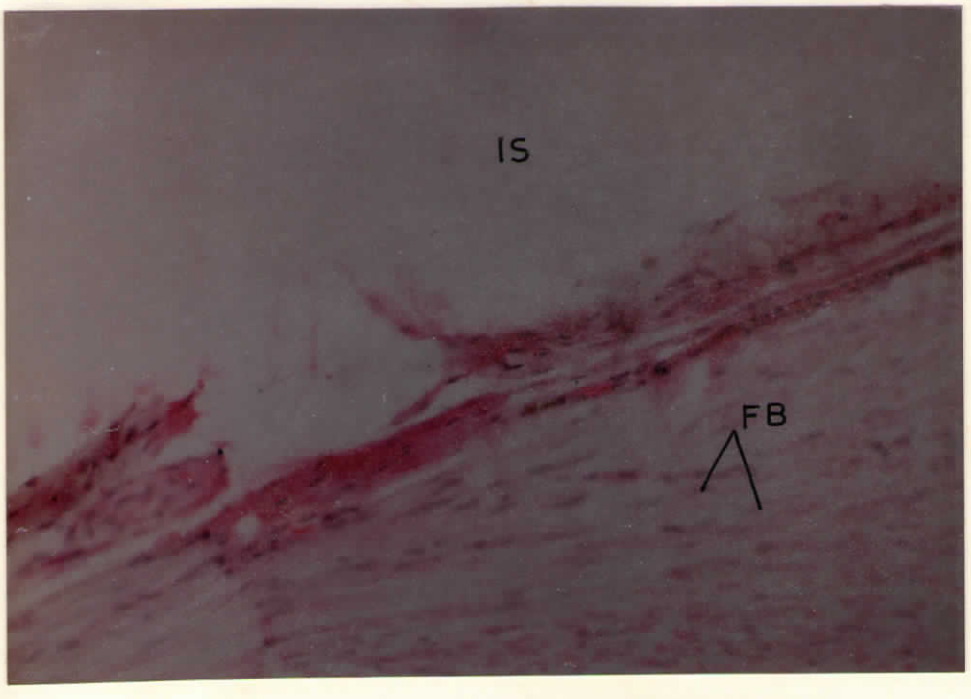
(b)

e 5.37 Tissue response at minimal motion site. 25 weeks post implantation period, (a:Control polymer b: IPDI sample) Hematoxylene Eosin stain, X 250
Is-Implant site, G-Giant cell,FB-Fibroblast

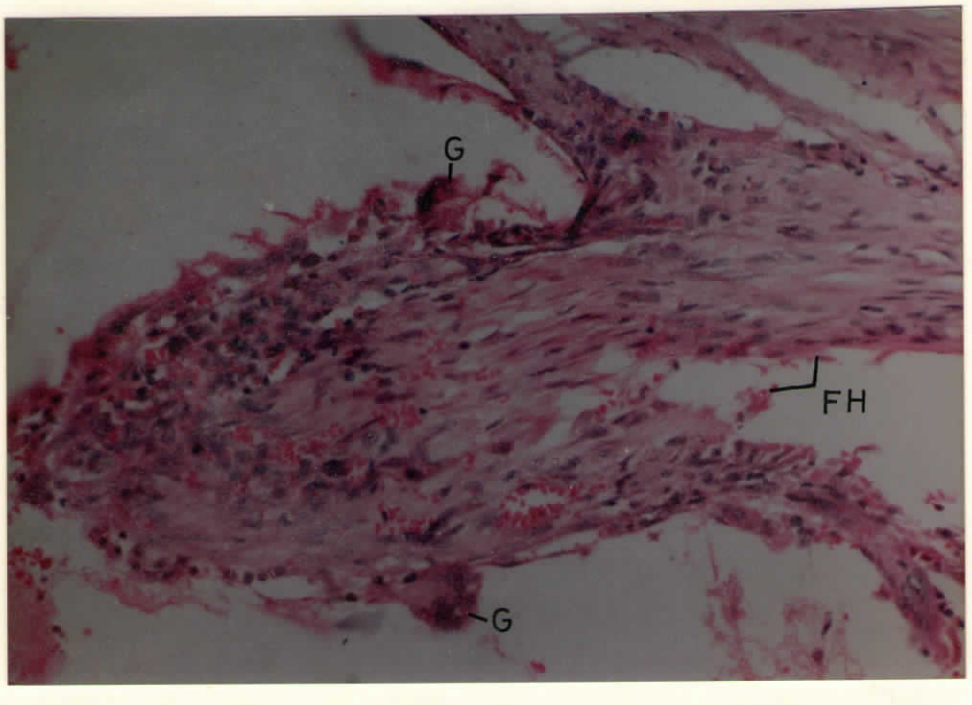
are seen on the fibroblastic area. Here also there is evidence of ingrowth of tissue from the encapsulated area into implant areas, with numerous giant cells and scant necrotic material. The candidate crosslinked polyurethane retrieved from the maximal motion site (left hind leg) also elicit the similar histopathological responses as seen with test material implanted in minimal motion site but with more strands of tissue in the implant area itself. (Fig.5.38 b) The histopathological picture of control material harvested from the maximal motion site (right hind leg) indicated the capsule surrounding the implant site with varying thickness from Ø.125 μm to 2.8 μm. It is predominately fibroblastic (Fig 5.38 a). This response extend at various places into the implant site with a marked macrophages and giant cell response. These giant cells are also seen at places in the capsule itself.

post implantation period of 4Ø weeks

The test crosslinked polyurethane harvested from the minimal motion site at the post implantation period of 4Ø weeks indicates the formation of very severe fibroplasia with inflammatory cells(Fig.5.39 b) The capsule is fund to be very thick. The control polyurethane material retrieved from the anterior side of the paravertebral muscle also showed inflammatory cells with highly cellular connective tissue. Figure 5.39a indicates the histopathological response of the tissue surrounding the implant. .

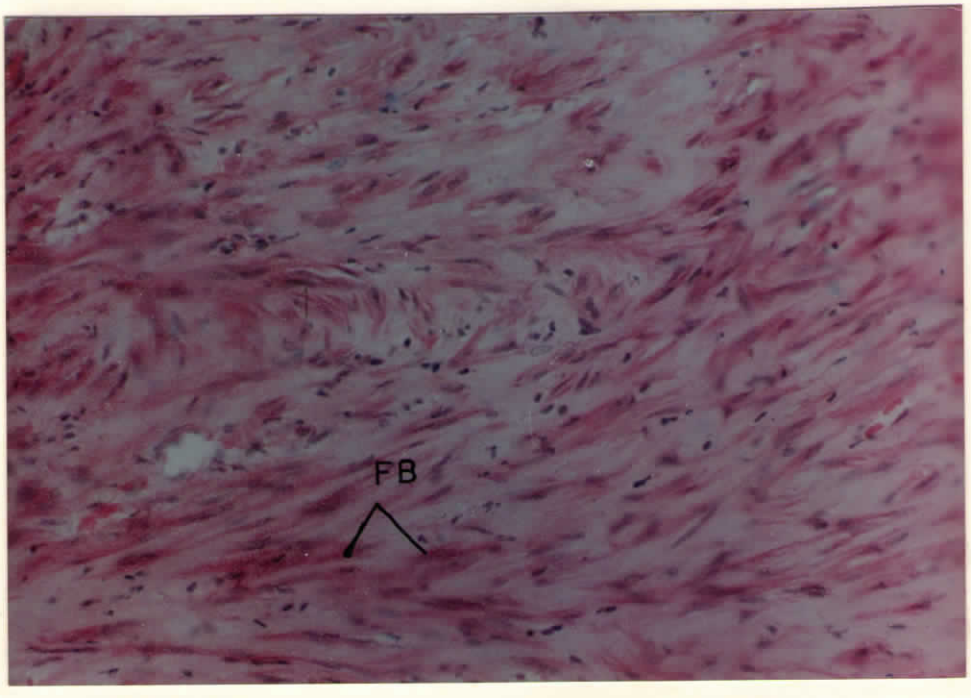


(a)

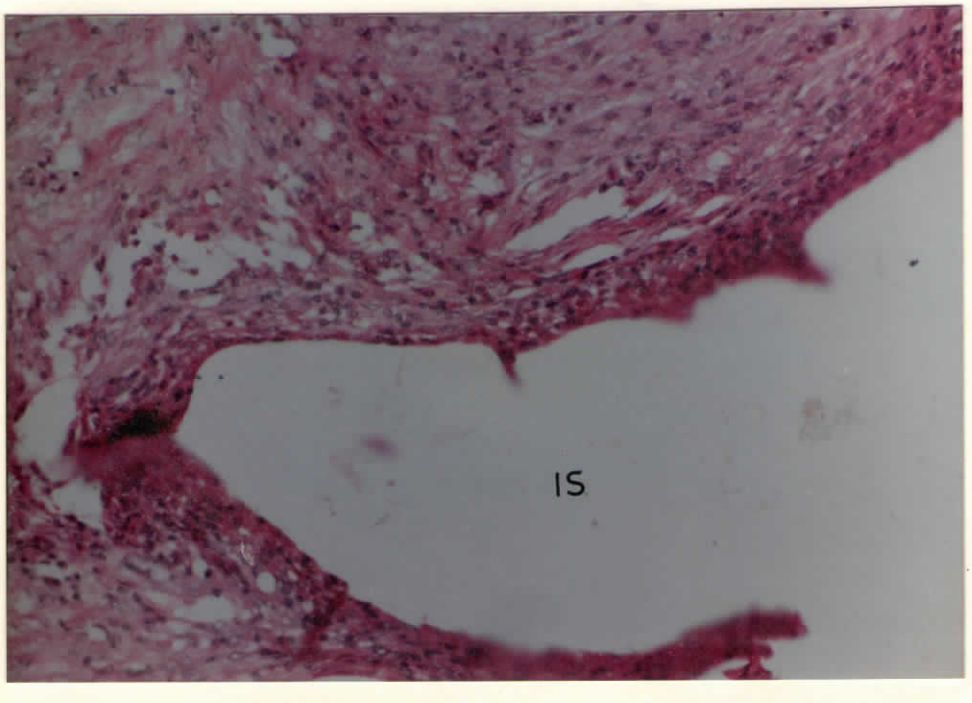


(b)

.38 Tissue response at maximal motion site. 25 weeks post implantation period, (a:Control polymer b: IPDI sample) Hematoxylene Eosin stain, X 250
Is-Implant site, G-Giant cell,FB-Fibroblast

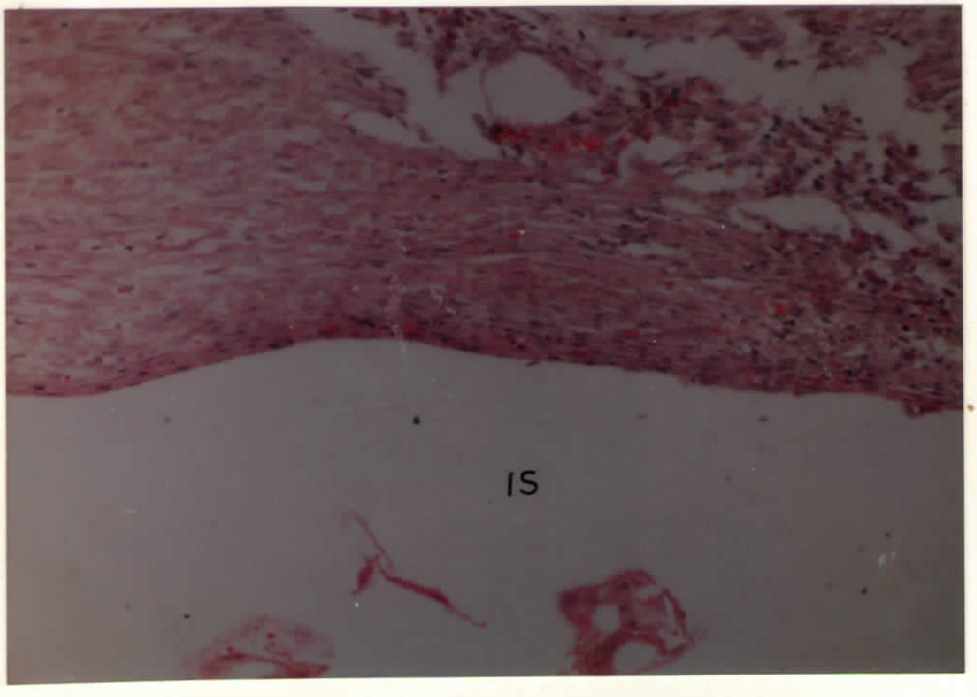


(a)

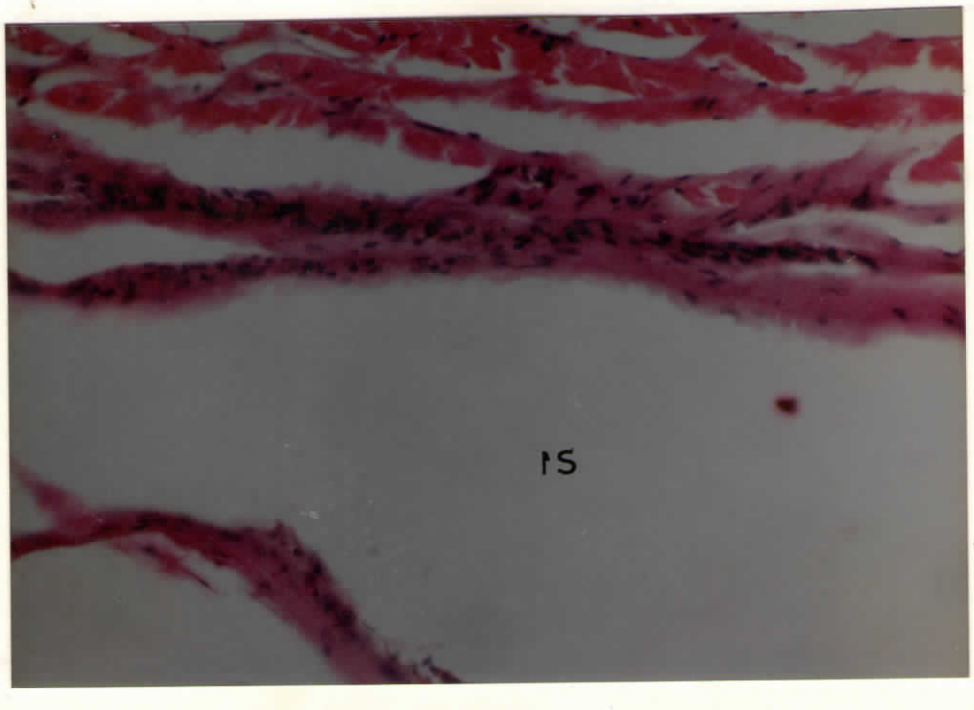


(b)

5.39 Tissue response at minimal motion site. 40 weeks post implantation period, (a:Control polymer b: IPDI sample) Hematoxylene Eosin stain, X 250
Is-Implant site, G-Giant cell,FB-Fibroblast

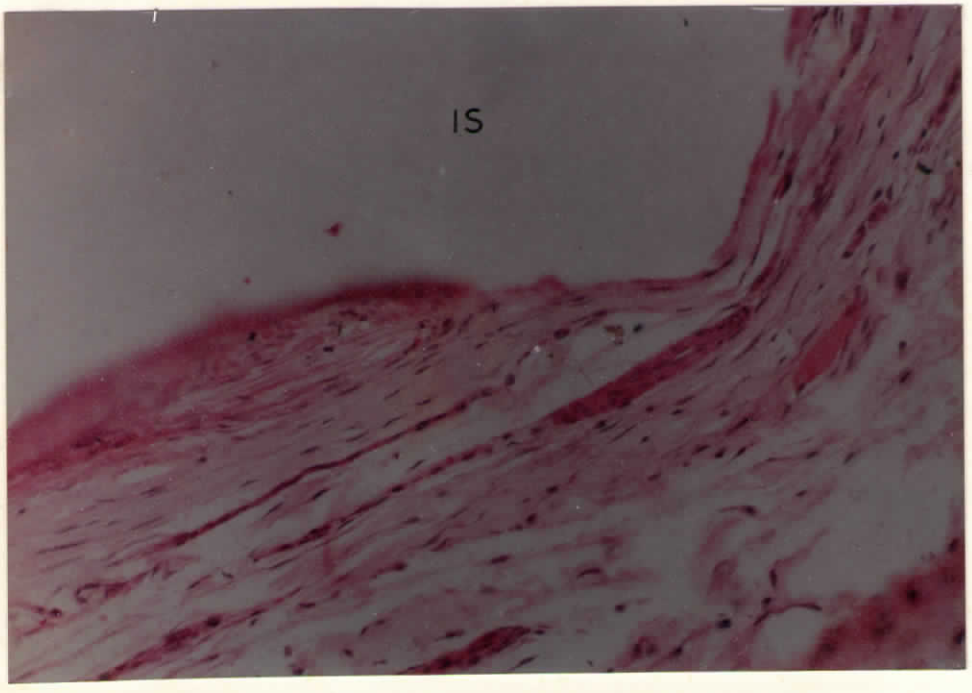


(a)

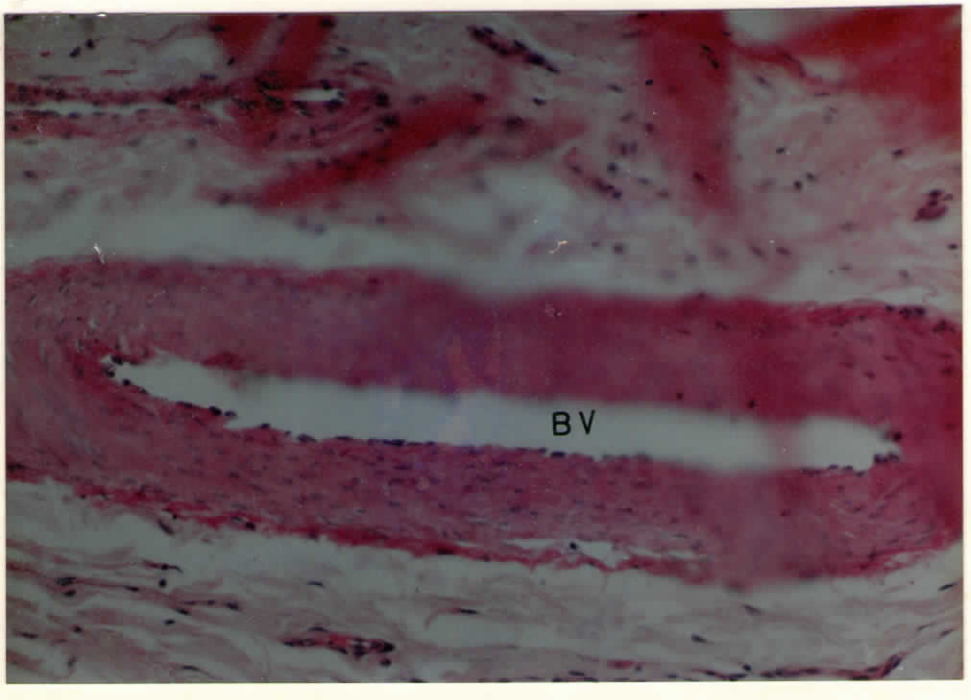


(b)

re 5.42 Tissue response at minimal motion site. 49 weeks post implantation period, (a:Control polymer b: IPDI sample) Hematoxylene Eosin stain, X 250
Is-Implant site, G-Giant cell, FB-Fibroblast

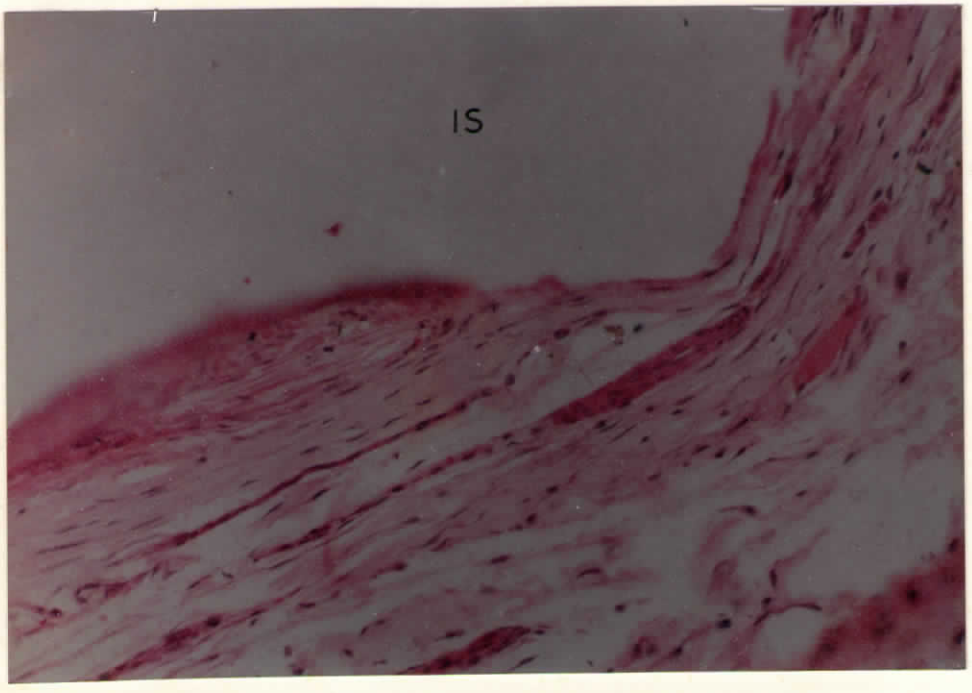


(a)

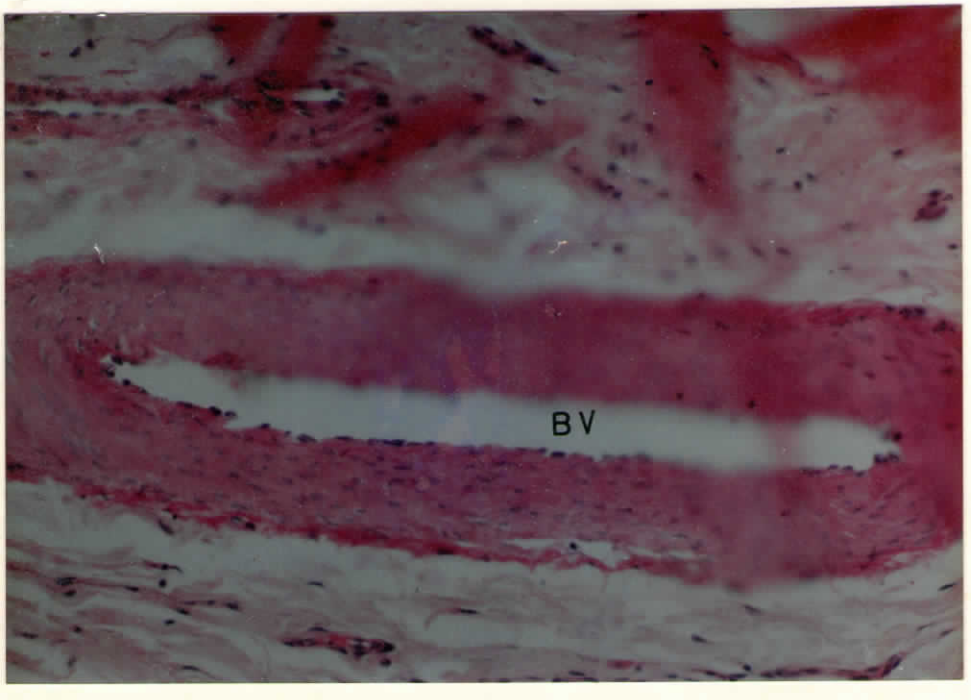


(b)

re 5.41 Tissue response at minimal motion site. 49 weeks post implantation period, (a:Control polymer b: IPDI sample) Hematoxylene Eosin stain, X 250
Is-Implant site, FB-Fibroblast, BV- Blood vessel

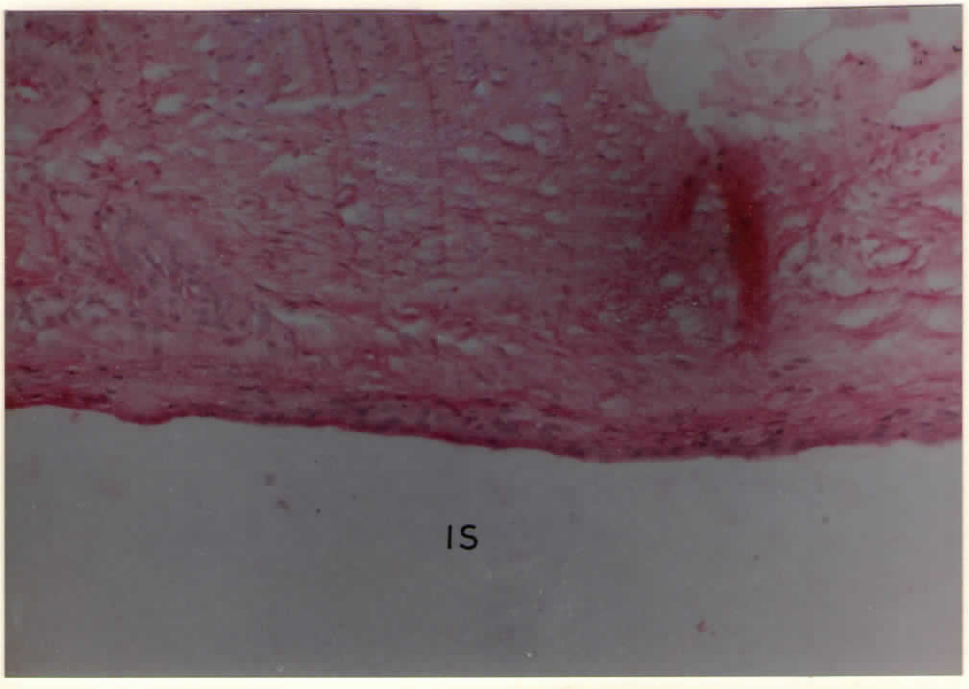


(a)

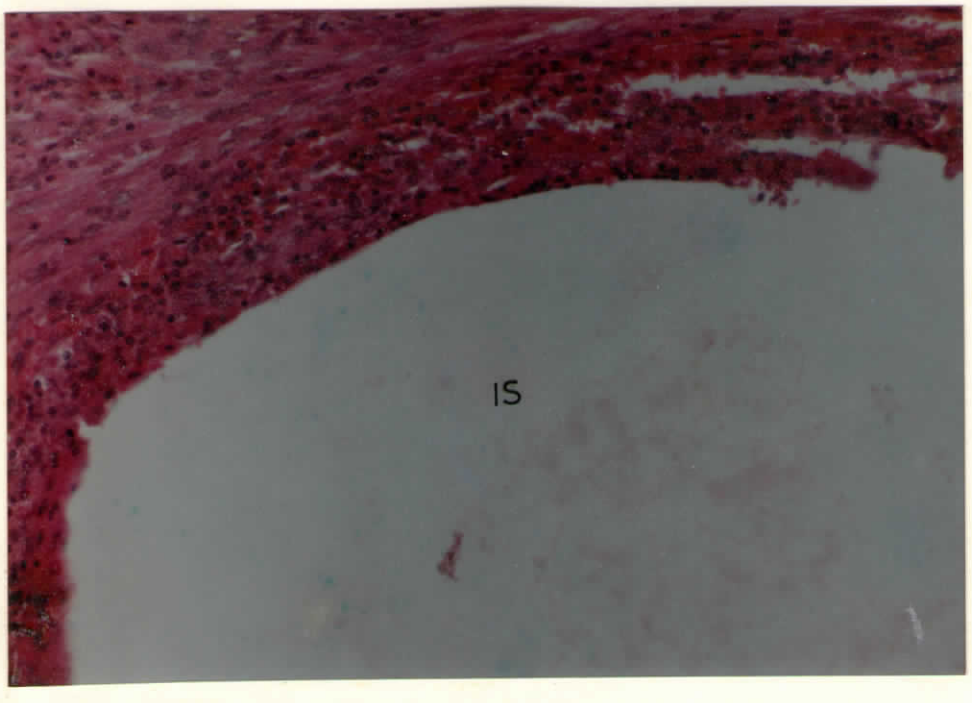


(b)

re 5.41 Tissue response at minimal motion site. 49 weeks post implantation period, (a:Control polymer b: IPDI sample) Hematoxylene Eosin stain, X 250
Is-Implant site, FB-Fibroblast, BV- Blood vessel



(a)



(b)

ure 5.40 Tissue response at maximal motion site. 40 weeks post implantation period, (a:Control polymer b: IPDI sample) Hematoxylene Eosin stain, X 250
Is-Implant site, FB-Fibroblast

are seen on the fibroblastic area. Here also there is evidence of ingrowth of tissue from the encapsulated area into implant areas, with numerous giant cells and scant necrotic material. The candidate crosslinked polyurethane retrieved from the maximal motion site (left hind leg) also elicit the similar histopathological responses as seen with test material implanted in minimal motion site but with more strands of tissue in the implant area itself. (Fig.5.38 b) The histopathological picture of control material harvested from the maximal motion site (right hind leg) indicated the capsule surrounding the implant site with varying thickness from Ø.125 µm to 2.8 µm. It is predominately fibroblastic (Fig 5.38 a). This response extend at various places into the implant site with a marked macrophages and giant cell response. These giant cells are also seen at places in the capsule itself.

post implantation period of 4Ø weeks

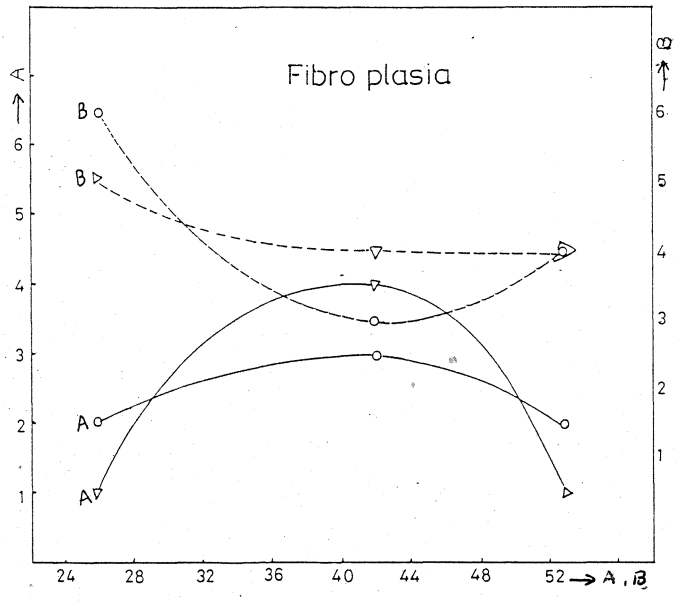
The test crosslinked polyurethane harvested from the minimal motion site at the post implantation period of 4Ø weeks indicates the formation of very severe fibroplasia with inflammatory cells(Fig.5.39 b) The capsule is fund to be very thick. The control polyurethane material retrieved from the anterior side of the paravertebral muscle also showed inflammatory cells with highly cellular connective tissue. Figure 5.39a indicates the histopathological response of the tissue surrounding the implant. .

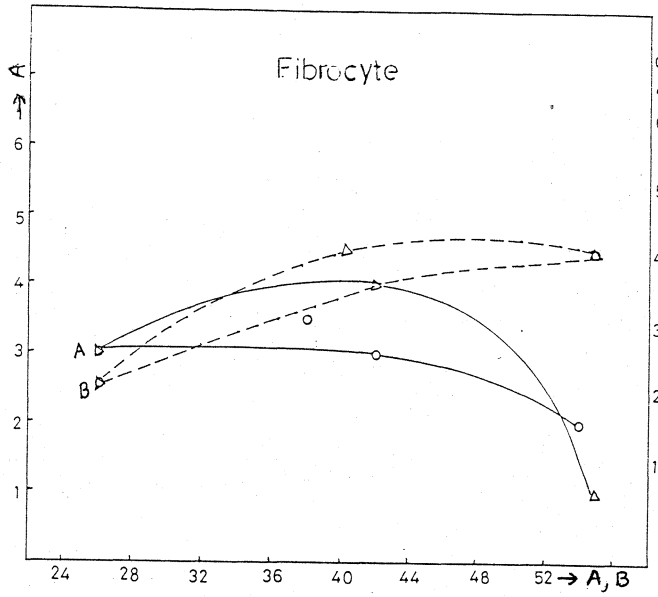
The test polyurethane retrieved from the left hind leg also showed inflammatory reaction surrounding the implant site. A granuloma is also noted. However the fibrous capsule is not very thick (Fig5.4Ø b). The control material harvested from the right hind leg indicates the formation of thick fibromuscular capsule surrounding the implant site (fig 5,4Ø a). Chronic inflammatory reaction is minimal with occasional foreign body giant cells.

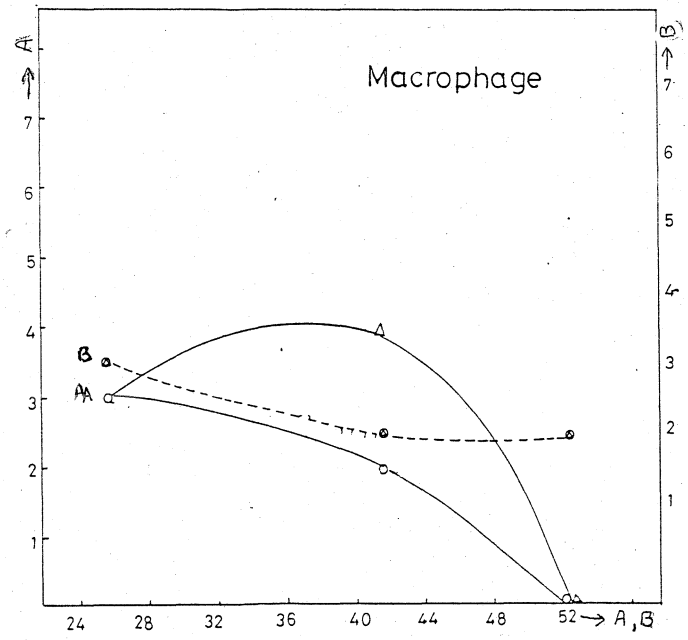
Post implantation period of 49 weeks

The histopathological investigation on the test crosslinked polyurethane harvested from the minimal motion site indicates the formation of a thinner fibrous capsule surrounding the implant with scant inflammatory infiltrates and blood vessels. Fig 5.41 a indicated the presence of blood vessel in the connective fibrous tissue surrounding implant. The control material retrieved from the anterior side of the paravertebral muscle indicates the formation of thick fibrous capsule with cellularity and thick blood vessels (Fig 5.41 a) The test material harvested from the left hind leg indicates histopathological responses different from aspect to another. In one aspect of the implant, a thick fibrous capsule with chronic inflammatoy cell is seen. On other aspect, fragment of implant material are surrounded by fibrocollagenous septae which has blood vessels, inflammatory cells (chronic) and occasional foreign body giant cells (Fig 5.42 b) The control material harvested from the right hind leg indicates the formation of thick capsule on one aspect of the implant site with fibromuscular tissue (Fig.5.42a)

By considering the histopathological responses to both candidate and control polyurethane materials, it can be inferred that, both candidate and control polyurethane materials, elicit a 'normal foreign body reaction. The degree of such reaction can be rationalized by comparing the tissue responses with respect to the post implantation period. The figures 5.43 a & b illustrate the relation between the concentration of cells and time. Interestingly the normal foreign body reaction for candidate material observed at the minimal motion site for the post implantation period of 4Ø weeks is associated with higher concentration of macrophages, giant cells, lymphocytes, plasma cells, fibroplasia and fibrocytes in comparing with that observed in maximal motion site for the candidate material with same implantation period. This indicates that the biomechanical factors are not responsible for this varying tissue response. This is further clear from the zero level concentration of foreign body debri at 4Ø weeks-implantation period with both implant site. On the other hand a reverse trend was observed for the post implantation period of 49 weeks for the test material with decreased lymphocytes, giant cells, fibroplasia and fibrocytes. Macrophages and plasma cells, however have reduced to zero level, concentration. The resolution of the 'normal foreign body reaction' to a favorable tissue response is also complimented by the appreciable biostability of the test material







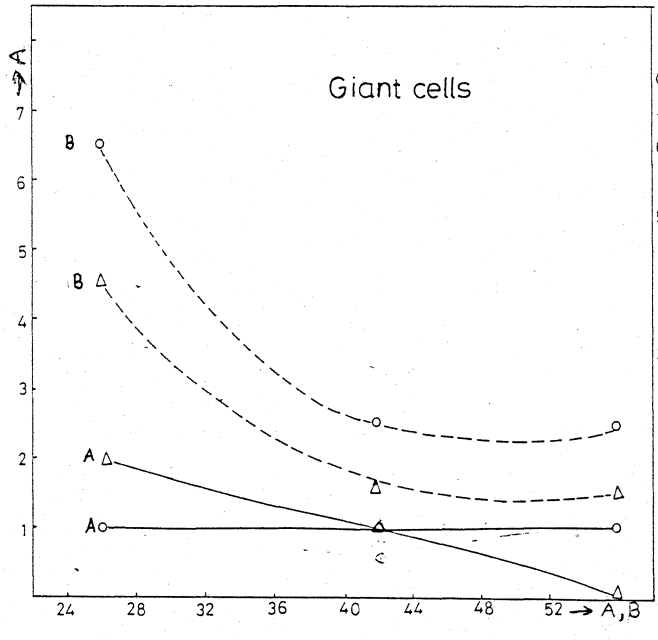
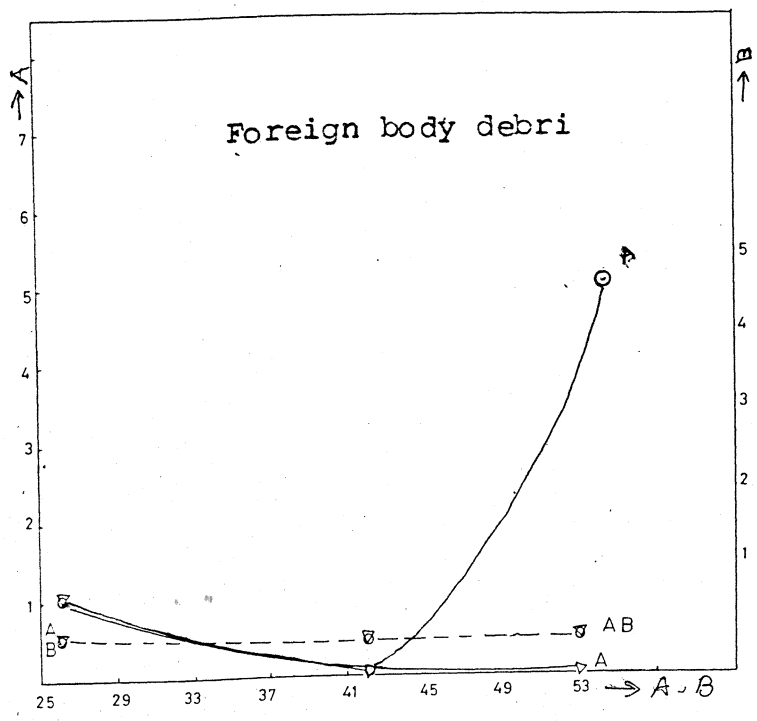
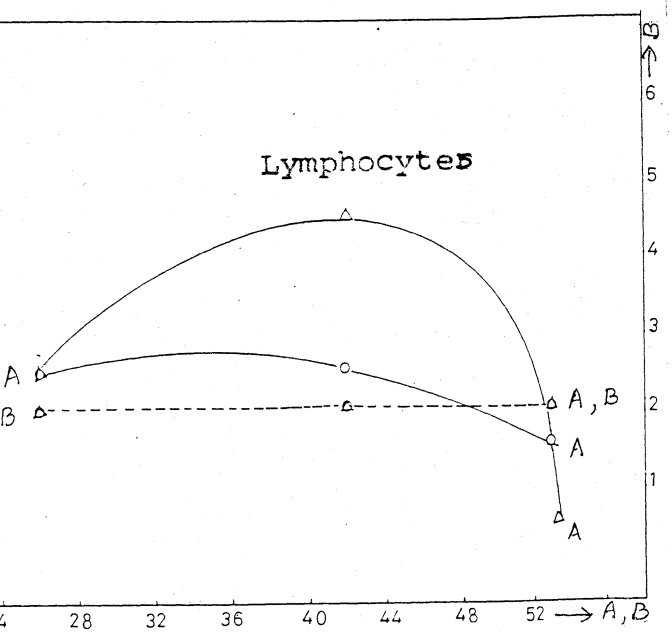


Fig. 5.43a. Variation of host response to polyurethane (I_3) with time
(– test ..... control, Δ-min, o-max) (x. axis: time y. axis: concentration).
(weeks)





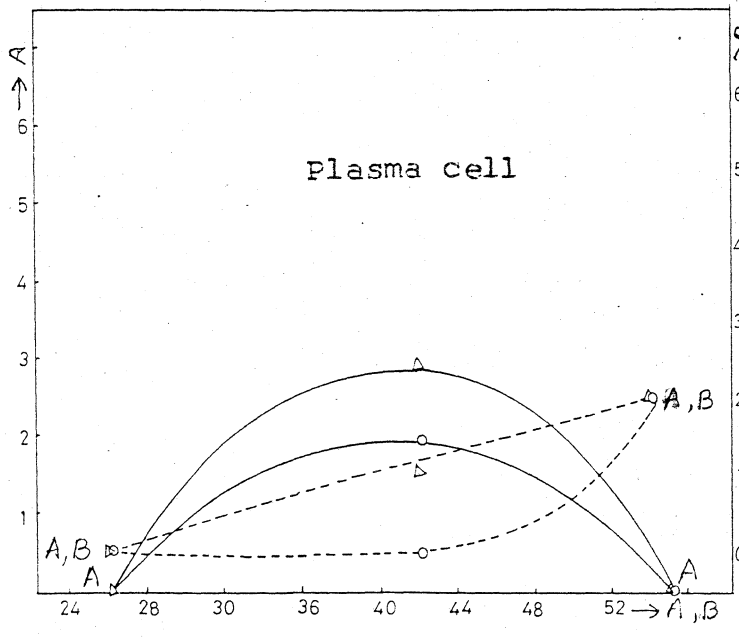


Fig.5.43b. Variation of host response to polyurethane (I3) with time (- test, ... Control, Δ-min, o-max) (x.axis; time (weeks) y, axis; concentration)

at the minimal motion site. The foreign body debri is found to be low in minimal motion site in comparison with that in maximal motion site at the 49 weeks post implantation period. (Fig.5.43 b) The higher concentration of foreign body debri at maximal motion site is also due to possible environmental stress cracking as observed in the section 5.4 stability of polyurethane.

Though the candidate polyurethanes elicit favorable tissue response in minimal motion site, the biostability of this material in maximal motion site is not realized. The biomedical grade polyurethane elicit higher concentration of macrophages, lymphocyte, plasma cell, fibrocyte and fibroplasia at both implantation sites for the 49 weeks implantation period in comparison with the test material for the same implantation period though the control material does not produce any debri. There fore at this period the biomechanical factors does not influence the tissue response of the control material since the material is appreciably stable. The higher concentration of lymphocyte and plasma cells are therefore attributed to the delayed hypersensitivity responses.

Studies on tissue response to SMDI based polyurethane (S5)

The histopathological investigation rating with variation of post implantation period also indicates the 'normal foreign body reaction' associated with the candidates SMDI based polyurethane (S5) Fig 5.44 a & b illustrate the variation of cellular concentration with time.

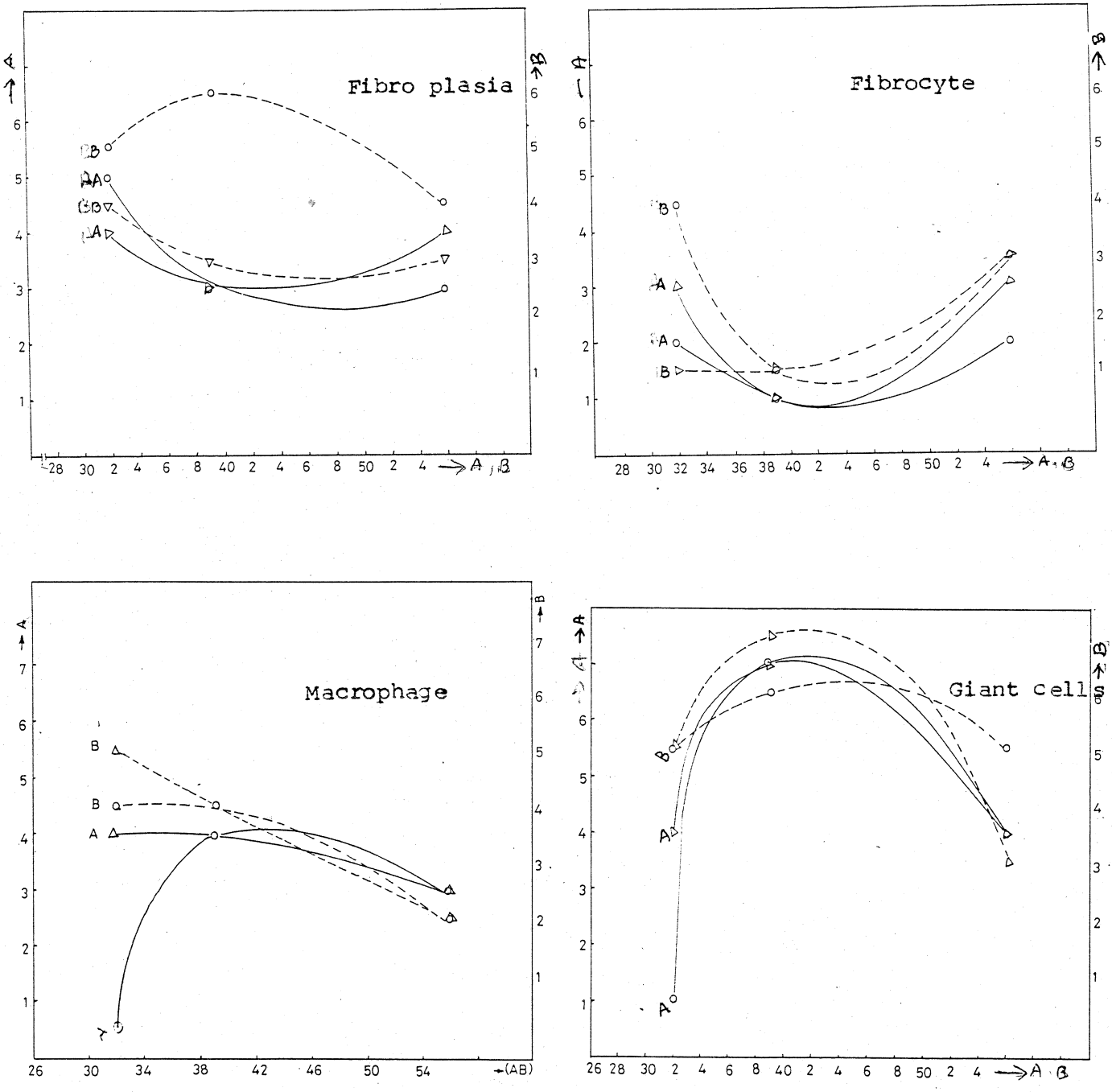
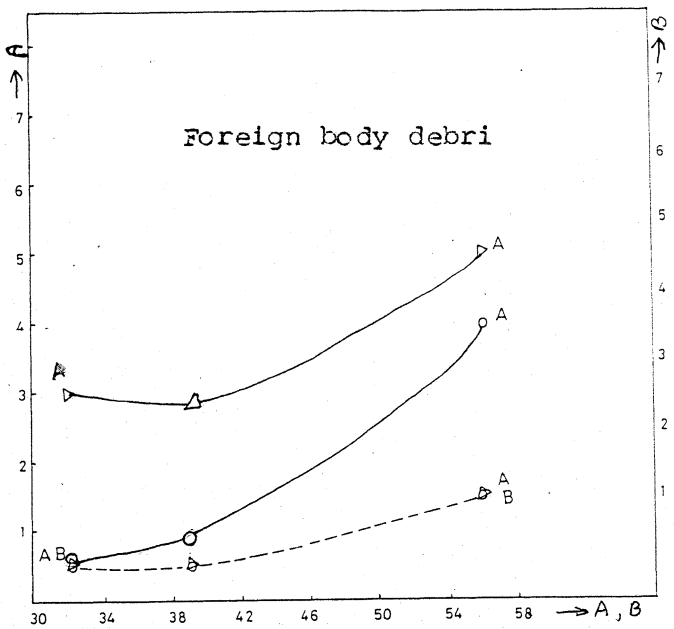
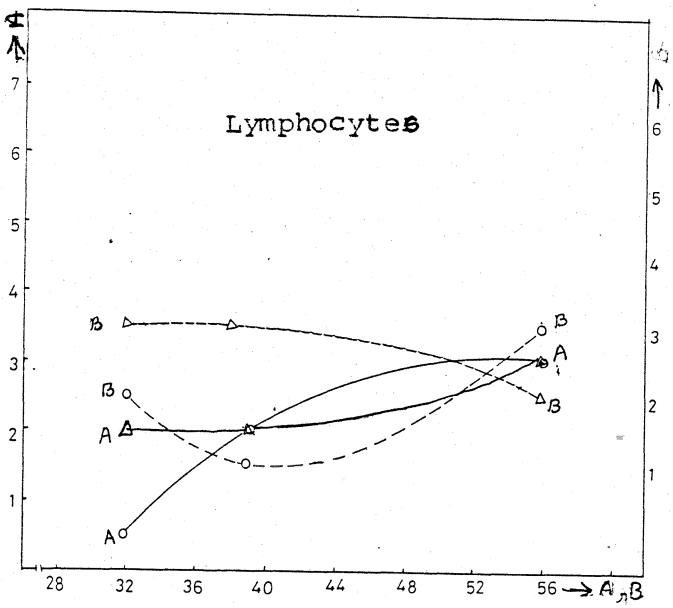


Fig.5.44a. Variation of host response to polyurethane (Sa) with time (-test.....Control, Δ-min, o-max) (x.axis;time weeks y, axis;concentration)





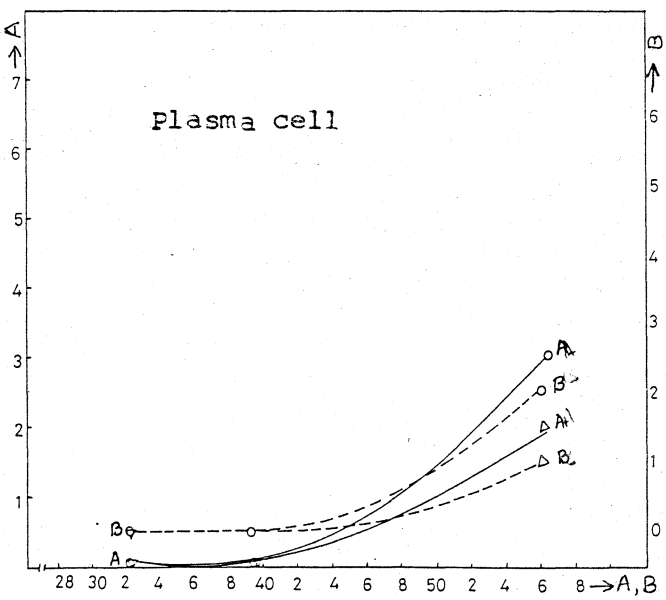


Fig. 5.44b. Variation of host response to polyurethane (Sg) with time (- test ▪▪▪.. control, △-min, o-max) (x.axis; time (weeks) y, axis; concentration)

The test material from the minimal motion site elicits higher concentration of macrophages. Fibroplasia and fibrocytes after post implantation period of 56 weeks in comparison with that found in maximal motion site for the same post implantation period. However giant cell and lymphocytes are seen with the same concentration. Interestingly the biostability of this sample is corresponds with fragmentation of the material. Such fragmentation does elevate the concentration of plasma cells and lymphocytes in comparison with that in post-implantation period of 39 weeks.

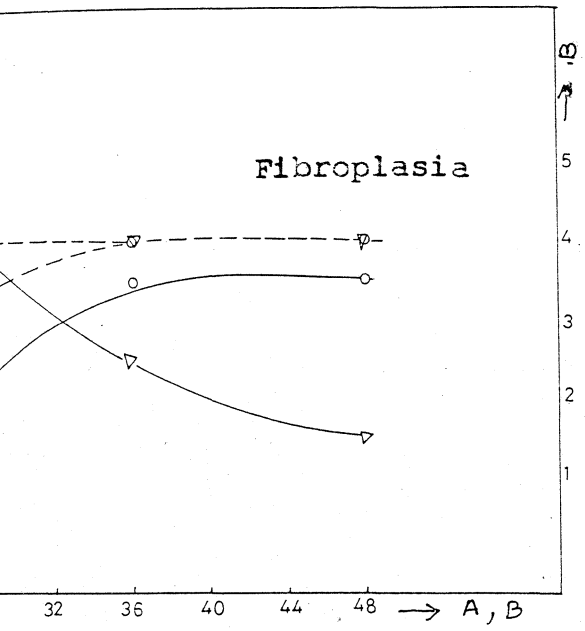
The persistent increase of the plasma cells and presence of lymphocytes in the control materials in both implantation sites at the post-implantation period of 56 weeks also indicate the delayed hypersensitivity of the control material. The behaviour of the control material in both the implantation sites are similar to that observed with the control material used in studies in the tissue response to the IPDI based polyurethane.

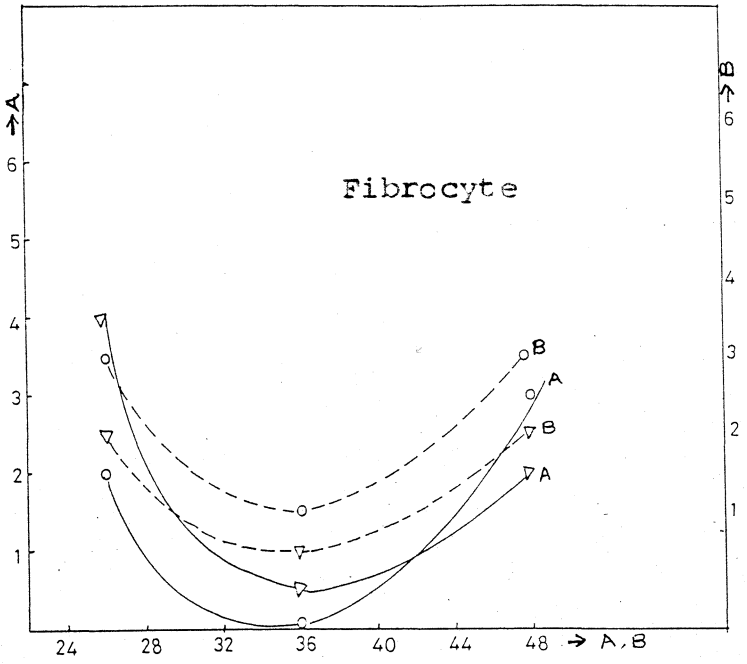
Studies on tissue response to HDI based polyurethane (HI)

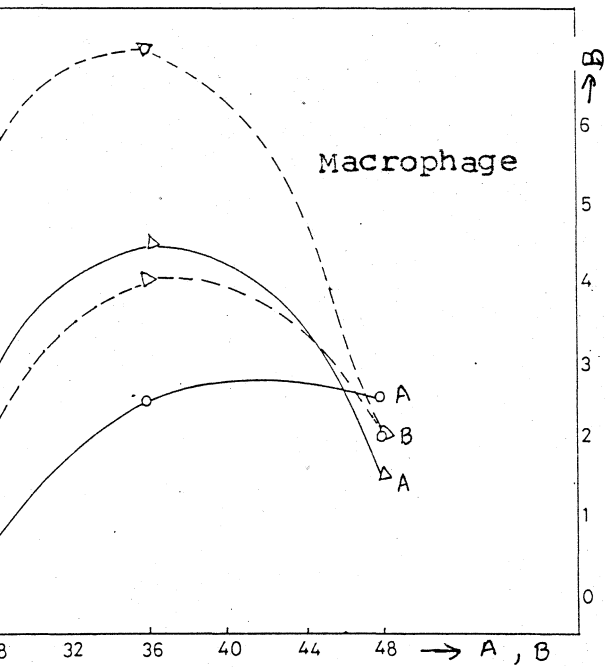
We have observed the tissue response to the test HDI based polyurethane implants (HI) as well as control implants as 'normal foreign body reaction' in both minimal and maximal motion sites. The concentration of macrophages and giant cells are found to follow a gaussian pattern with time. (Fig 5.45a) as observed with 1the SMDI based polyurethanes. The fibroplasia is found to increase with time and reach a steady state which means the fibroblast proliferation and collagen synthesis are reduced. An

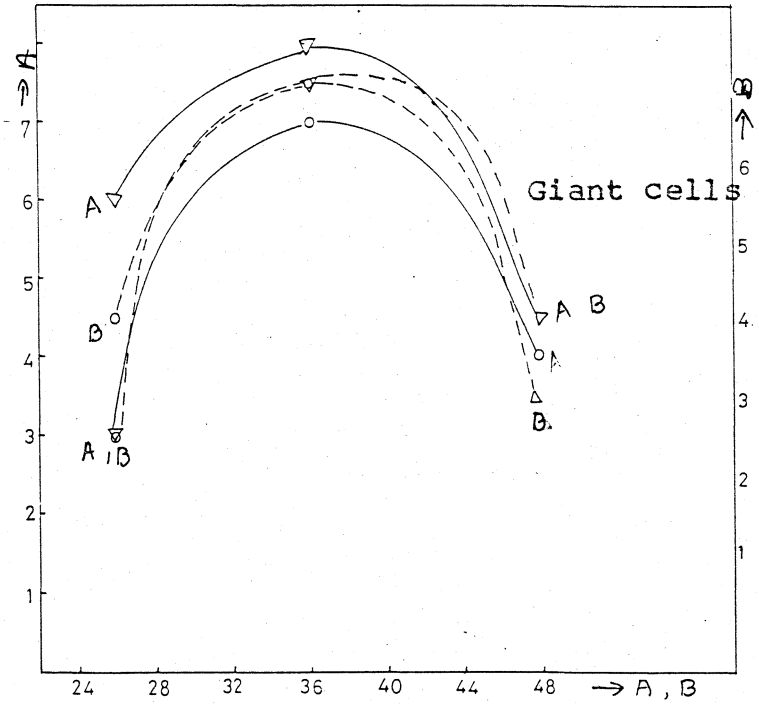
exception is seen with the test implant in minimal motion site after the post implantation period of 48 weeks. in this case the fibroplasia decreased in comparing to its previous period (fig 5.45 a)

When comparing the performance of biomedical grade polyurethane Tecoflex 85A it is concluded that chemical factors alone are not responsible for it's tissue response. As mentioned earlier the porous structural morphology of this material has elicited a normal foreign body reaction as observed in the studies with IPDI and SMDI polyurethane. Comparing the tissue response, the S9 has higher foreign body reaction than that with the control material. Interestingly materials implanted in minimal motion site are associated with higher degree of concentration in comparing with that in maximal motion site throughout the time (Fig 5.45b). The maximal motion site elicits higher degree of shear stress on the implant in comparing the tissues at the minimal motion site. The lower concentration of giant cells due to the low probability of fusion of macrophages in maximal motion site into giant cells. The concentration of lymphocytes found in maximal motion site except at the maximum period of implantation is always lower than that in minimal motion site. The concentration of lymphocytes and macrophages is found to be varied in a linear relationship. Higher the concentration of lymphocytes higher will be concentration of macrophages. The stress at the material-tissue interface can activate the macrophages to extracellular release of









5a. Variation of host response to polyurethane (H1) with time (- test .....control, Δ-min, o-max) (x, axis;time) (weeks) y, axis;concentration)

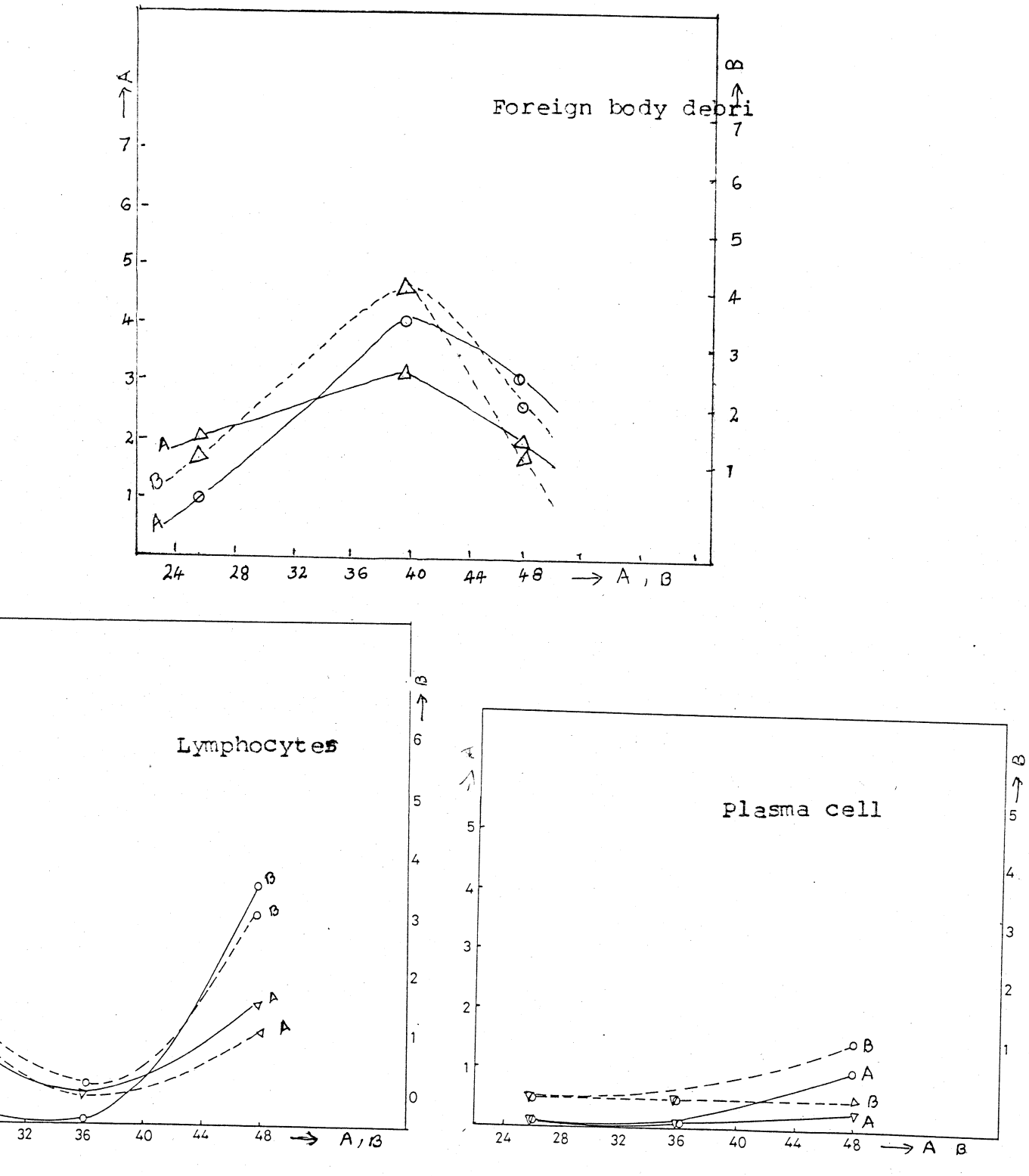


Fig.5.45b. Variation of host response to polyurethane (H1) with time (- test, ...control, Δ-min, o-max) (x.axis; time (weeks) y, axis; concentration)

interlukin 1 and mediators. Interlukin 1 was found to have multiple effects on both inflammatory and immune responses 313. In such inflammatory reactions, lymphocytes get sensitized and secret macrophages migration inhibition factors (MIF) which arrest the migration of macrophages. The high concentration of such macrophages therefore favorably leads to the fusion of macrophages to give giant cells. Therefore it is obvious that both implantation sites elicit normal foreign body reaction with test and control materials; maximal motion site elicits lesser degree of giant cell growth, lymphocyte and plasma cell concentration in comparing with that in minimal motion site.

The foreign body debri found at the maximal motion site at 48 weeks with both test and control materials is relatively higher than that in minimal motion site. However such debri have not increased the giant cell concentration (Fig.5.45 a & b). On the other hand the lymphocytes and plasma cells are found in appreciable number in maximal motion site at this period (48 weeks). This indicates that this debri would have caused delayed hypersensitivity to the surrounding tissue.

The in vivo biostability studies indicate that all the polyurethanes undergo microlevel surface cracking. Therefore the debri would have been produced either by the environmental stress corrosion, pitting corrosion or crazing at microlevel. The polymeric debri produced during the implantation can act as micro

irritants such micro irritants can cause persistent presence of giant cells at the material-tissue interface.

In conclusion the studies on the interaction of fibroblast cell with the solid polyurethane materials and it's extract show the biocompatibility of the present polyurethanes as well as favorable material - cell response which can lead to material - tissue interaction.

When comparing the tissue response at the maximum implantation time it is observed that I3 and H1 polyurethane is comparatively more stable and elicit favorable normal foreign body reaction in minimal ,motion site in comparison with that in maximal motion site. The degree of chronic inflammatory cells in maximal motion site is relatively higher than that in minimal motion site. The SMDI based polyurethane (S9) undergo fragmentation both in minimal and maximal motion site due to the biodegradation as observed previously in section 5.4 and possess increased degree of chronic inflammatory response in comparison with that of IPDI in maximal motion site. Though the HDI polyurethane , is relatively more stable than IPDI polyurethane, the polymeric debri from the microcracks induce chronic inflammatory response in addition to the ' normal foreign body reaction . The control, tecoflex 85 A , also showed a mixed response of normal foreign body reaction and chronic inflammatory reaction at maximal motion during during the maximum period of implantation.

irritants such micro irritants can cause persistent presence of giant cells at the material-tissue interface.

In conclusion the studies on the interaction of fibroblast cell with the solid polyurethane materials and it's extract show the biocompatibility of the present polyurethanes as well as favorable material - cell response which can lead to material - tissue interaction.

When comparing the tissue response at the maximum implantation time it is observed that I3 and H1 polyurethane is comparatively more stable and elicit favorable normal foreign body reaction in minimal ,motion site in comparison with that in maximal motion site. The degree of chronic inflammatory cells in maximal motion site is relatively higher than that in minimal motion site. The SMDI based polyurethane (S9) undergo fragmentation both in minimal and maximal motion site due to the biodegradation as observed previously in section 5.4 and possess increased degree of chronic inflammatory response in comparison with that of IPDI in maximal motion site. Though the HDI polyurethane , is relatively more stable than IPDI polyurethane, the polymeric debri from the microcracks induce chronic inflammatory response in addition to the ' normal foreign body reaction . The control, tecoflex 85 A , also showed a mixed response of normal foreign body reaction and chronic inflammatory reaction at maximal motion during during the maximum period of implantation.

CONCLUSION AND FUTURE EFFORTS

6. SUMMARY AND CONCLUTIONS

With an aim to develop biostable polyurethanes for long-term biomedical application, aliphatic and crosslinked polyurethanes were synthesized using three diisocyanates, IPDI, SMDI and HDI, polyether polyols, PTMG, PPG and PEG and crosslinker, TMP by a modified bulk polymerization technique. The crosslinked macromolecular architecture was developed by the crosslinks of biuret of the diisocyanate and triol, TMP. The polymers possess polyol as soft segment. The hard segment composes of urethane linkages associated with biuret and TMP. Polyurethanes having different mechanical properties were synthesized by varying hard segment content and polyol in each series. Higher the hard segment or lower the MC, higher will be the tensile modulus and hardness. Most of the polyurethanes are elastomeric in character. All the polyurethanes show glass transition temperature below room temperature. All the polyurethanes are thermally stable upto 200°c. These polyurethanes also reveal the presence of hydrogen bonding. All the polyurethanes exhibit relatively higher surface energy. Most of them have polar to dispersive component of surface energy indicating high hydrophilicity. Some of the polyurethanes possess equal polar and dispersive component of surface energy.

In vitro toxicological tests reveal that the haemolytic potential and recalcification time are within the limit of

6. SUMMARY AND CONCLUTIONS

With an aim to develop biostable polyurethanes for long-term biomedical application, aliphatic and crosslinked polyurethanes were synthesized using three diisocyanates, IPDI, SMDI and HDI, polyether polyols, PTMG, PPG and PEG and crosslinker, TMP by a modified bulk polymerization technique. The crosslinked macromolecular architecture was developed by the crosslinks of biuret of the diisocyanate and triol, TMP. The polymers possess polyol as soft segment. The hard segment composes of urethane linkages associated with biuret and TMP. Polyurethanes having different mechanical properties were synthesized by varying hard segment content and polyol in each series. Higher the hard segment or lower the MC, higher will be the tensile modulus and hardness. Most of the polyurethanes are elastomeric in character. All the polyurethanes show glass transition temperature below room temperature. All the polyurethanes are thermally stable upto 200°c. These polyurethanes also reveal the presence of hydrogen bonding. All the polyurethanes exhibit relatively higher surface energy. Most of them have polar to dispersive component of surface energy indicating high hydrophilicity. Some of the polyurethanes possess equal polar and dispersive component of surface energy.

In vitro toxicological tests reveal that the haemolytic potential and recalcification time are within the limit of

6. SUMMARY AND CONCLUTIONS

With an aim to develop biostable polyurethanes for long-term biomedical application, aliphatic and crosslinked polyurethanes were synthesized using three diisocyanates, IPDI, SMDI and HDI, polyether polyols, PTMG, PPG and PEG and crosslinker, TMP by a modified bulk polymerization technique. The crosslinked macromolecular architecture was developed by the crosslinks of biuret of the diisocyanate and triol, TMP. The polymers possess polyol as soft segment. The hard segment composes of urethane linkages associated with biuret and TMP. Polyurethanes having different mechanical properties were synthesized by varying hard segment content and polyol in each series. Higher the hard segment or lower the MC, higher will be the tensile modulus and hardness. Most of the polyurethanes are elastomeric in character. All the polyurethanes show glass transition temperature below room temperature. All the polyurethanes are thermally stable upto 200^{o}c. These polyurethanes also reveal the presence of hydrogen bonding. All the polyurethanes exhibit relatively higher surface energy. Most of them have polar to dispersive component of surface energy indicating high hydrophilicity. Some of the polyurethanes possess equal polar and dispersive component of surface energy.

In vitro toxicological tests reveal that the haemolytic potential and recalcification time are within the limit of

biomedical grade polymeric materials. The systemic and intracutaneous irritation tests do not reveal any toxic response systemically as well as locally. The itramuscular implantation test in rabbit using smooth sample (USP Standard) indicates biocompatibility in most of the polyurethanes. The histopathological analyses of the tissue surrounding the implant harvested at 3 months post-implantation period show thin fibrous tissue encapsulation, with fibroblasts and fibrocytes. Cells indicating chronic inflammatory response are absent. In IPDI series of polyurethanes, formulations based on PTMG polyol, I3 and PEG polyol, I7 are biocompatible. In SMDI series, formulations based on PPG polyol,S5 and S8 and PEG polyol, S9 are biocompatible. In HDI series, formulations based on PTMG polyol, H1 and H5 PPG polyol, H7 and PEG polyol, H8 are biocompatible. In some of the formulations, the presence of chronic inflammatory cells, lymphocytes, plasma cells, macrophages and giant cells is noticed. This unfavorable tissue response is attributed to degradation of the polyurethane on the surface in micro level.

The in vitro and in vivo studies on biostability of the polyurethanes reveal both PTMG and PPG based polyurethanes are appreciably more stable than the PEG based polyurethanes. More over the hard segment content and MC also influence the biostability. The higher the hard segment content or lower the MC higher will be the stability. The in vivo studies on biostability indicate that the loss of tensile properties do not differ much

between the PTMG and PPG based polyurethanes containing around 25% hard segment content.

However the nature of diisocyanate has some influence on the in vivo biostability. For polyurethanes containing hard segment content around 25%, the in vivo biostability is in the following order: SMDI based polyurethanes>HDI based polyurethanes>IPDI based polyurethane.

The SEM analysis indicated the micro level cracking at the surface. Higher degree of cracks is observed in PEG based polyurethanes. The degree of surface micro cracks formulation is in the following order.

PEG based polyurethanes > PPG based polyurethanes > PTMG based polyurethane.

These micro cracks formation is attributed to the environmental stress cracking under the combined influence of elastomeric character, mechanical surface deformation due to flexibility of the polymer and the biological medium. The higher concentration of surface adhered macrophages and foreign body giant cells at the interface of materials-biological Medium enable degradation on the surface in micro level.

The tissue response of the implant intended for long-term use is influenced by the nature of fibroblast interaction with solid material and its extract. The present polyurethanes elicit favorable fibroblast cell growth on the polymer surfaces. It is due to the higher surface energy and relatively higher

hydrophilicity of the polymer. The present polyurethanes also do not leach any toxic additives which can lyse the fibroblast significantly at the interface of material-biological medium. The studies on the effect of biomechanics and biostability on the tissue response of implants, implanted in biomechanically different implant sites have revealed that those polyurethanes, (ex, H1) having appreciable stability posses'normal foreign body reaction' at minimal motion site and possess'normal foreign body reaction ' and chronic inflammatory reaction at maximal motion site. But those polyurethanes (ex.S9) having lower biostability elicit the mixed tissue response at both implant sites.

In conclusion the present investigation reveals that some selective aliphatic crosslinked polyurethanes (I3, H1, H5, H7 and S5) posses appreciable biocompatibility, biostability and elicit good tissue compatibility similar to that clinical implants.

FUTURE AREAS OF INVESTIGATION

Since the present crosslinked polyurethanes exhibit a broad spectrum of mechanical properties, biostability and tissue compatibility, proper selection of polyurethanes for a specific application largely depends on the design and evaluation of experimental and clinical prostheses. One of the soft tissue applications in which these polyurethanes can be used is the tendon graft. Tendon healing is one of the challenging area even today for biomaterial scientists. Since the regenerative capacity of tendon is relatively less and also due to the fibrous tissue in-growth associated with the wound healing, the sliding motion

of the grafted tendon is not achieved so far with any of the available tendon graft, The present porous polyurethanes for example I3 or H5 or H7 can be used for the tendon graft development. A novel composite consisting a micro porous polymeric film on the porous solid block of polyurethane (leaving the cross sectional area) can enable permeation of nutrients only for tendon healing and anastomosis through the cross sectional area of the graft. An extensive research has to be undertaken to explore this concept.

REFERENCES

1. Odian G, "Principles of polymerization", Mcgrow Hill Book Company, New York, P:1 (1971).

2. Billmeyer Jr.F.W, "Text book of polymer science", Wiley Interscience Publication, New York, P:261 (1984).

3. Boeing H.V, "Structure and properties of polymers", Stuttgart Georg Theieme Publishers, P:14Ø (1973).

4. Hastings G.W, Polymer, 26,1331 (1985).

5. Rembaun A.R and Shenm, "Biomedical polymers", Marcel Dekker Inc., New York, P:174 (1971).

6. Bair R.E, Gott V.L, and Feruse A, Trans. Amer. Soc. Artif. Intern. Organs, 16,5Ø (197Ø).

7. Schrader M.D, J.Colloid. Interface Sci.,88, 296 (1982).

8. Coleman D.L, Gregoins D.E, Andrade J.D, J. Biomed. Mater. Res., 16, 381 (1982).

9. Ratner B.D, Hoffman A.S, Hanson S.R, Harker et al., J. Polym. Sci., Polym. Symp., 66, 363 (1979).

1Ø. Hanson S.R, Ratner B.D, Hoffman A.S and Harker L.A, J. Lab. Clin. Med., 95,289 (198Ø).

11. Bruck S.D, Nature, 243,416 (1973).

12. Uroman L, Leonard E.F (eds)" The behaviour of blood and its components at interface", Ann. New York Acad. Sci., 283 (1977).

13. Sharma C.P, Biomaterials, 2, 57 (1981).

14. Bruck S.D, "Properties of bilmaterals in the physiological environment", CRC Press Inc., New York, P:9 (198Ø).

15. Fowkes F.M,"Interfacial aspects of biomedical polymers", vol 1, Andrade J.D (eds) Plenum Press, New York, P:337 (1985).

16. von Recum A.F, "Biomedical Materials" Elsevieer Science Publishers Ltd., P:1 (199Ø).

17. Lelah M.D, Grasel T.G, Pierce J.A, Lambrecht L.K, and Cooper S.L, Trans. Amaer. Soc. Artif. Intern. Organs, 3Ø, 411 (1984).

18. Lyman M.D, Fuzzio F.J, Voorhee S.H, Robinson G, and Albo P, J. Biomed. Master. Res., 12, 337 (1978).

19. Hench L.L, and Ethridge E.C, "Biomaterials: An interfacial approach", Acadamic press, P:1Ø5 (1982).

2Ø. Shunmuga kumar N, and Sharma C.P, Trends in Biomat. Art. Organs, 3, 61 (1981).

21. Autian J, Sing A.R, Turner J.E, Hung G.W, et al, Cancer Reserach, 35, 1591 (1975).

22. Bruck S.D, Polymer, 16, 25 (1975)

23. Hiratzka L.F, Goeken J.N, White R.A, and Wright C.B, Arch. Sur., 11Ø, 698 (1979).

24. Wood N.K, Kaminiski F.J, and Oglesby R.J, Biomed Mater Res, 4, 1 (197Ø).

25. Matlaga B.F, Yasenchak L.P, and Salthouse T.N, J.Biomed. Mater. Res., 1Ø, 391 (1976).

26. Sprincle J.Kopecok, and Lim.D, J. Biomed. Mater. Res., 5, 447 (1971).

27. Hulbert S.F, Morrison S.J, Klawitter J.J, J. Biomed. Mater. Res., 6, 347 (1972).

28. Taylor D.F and Smith F.B, J. Biomed. Mater. Res., 6, 467, (1972).

29. White R.A, Hirofe F.M, Sprout R.W, Lawrence R.S and Nelson R.J, Biomaterials, 3, 145 (1982).

3Ø. White R.A, Shore E, Mirand R.M, Klein S.R, Golburg L et al, Biomaterials, 2, 171 (1981).

31. Leinder J, Wang E.W.C, and Noval A, J. Biomed. Mater. Res., 17, 229 (1983).

32. Wilka G.C and Samuley S.L, J. Biomed. Mater. Res., 7,541 (1973).

33. Borchard U, "Syatemic aspects of biocompatibility", Williams D.F (ed) vol 2, CRC Press, Florida, P:1Ø7 (1981).

34. Lelah M.D and Copper S.L, "Polyurethane in Medicine", CRC press, Florida (1986).

35. Bloch B, Hastings G.W, "Plastic materials in surgery", Thomas and Publishers, Springfield, Illionis (1972).

36. Lee H, Neville K, "Handbook of Medical Plastics", Passadena Technology press, Passadena (1971).

37. Dreyer B, Akutsu T, Kolff W.J, J. Appl. Physiol., 15, 18 (196Ø).

38. Williams D.F and Roaf R, "Implants in surgery", Saunders, London (1973).

39. Kronenthal R.L, Oser Z, Martin E, "Polymers in Medicine and surgery", Plenum Press, New York (1975).

4Ø. Lyman D.J, Rev. Macromol. Chem, 1, 3559 (1966).

41. Gogolewski S, Colloid & Polymer Sci., 267, 757 (1989).

42. Gibbons D.F, British Polym. J, 1Ø, 232 (1978).

43. Hench L.L, Science, 2Ø8, 836 (198Ø).

44. Hench L.L, J. Biomed. Mater. Res., 14, 8Ø3 (1987).

45. Hanker J.S and Giammara B.L, Science, 242, 885 (1988).

46. Gristina A.G, Science, 237, 1588 (1987).

47. Bruck S.D, Biomat. Med. Dev. Artif. Organs, 5, 97 (1977).

48. Boretos J.W and Eden M, "Contempory Biomaterials", Noyes Publishers, New York (1984).

49. Coleman D.L, King R.N and Andrade J.D, J. Biomed. Mater. Res., 8, 199 (1974).

5Ø. Coleman D.L, King R.N and Andrade J.D, J. Biomed. Mater. Res., 5, 65 (1974).

51. Black R.D, Biomaterials, 5, 12 (1984).

52. Bagnal R.D, Biomaterials, 1, 51 (198Ø).

53. Lemn. W, Proc. European Society of Artificial organs, 2, 154 (1975).

54. Lelah M.D, Greasel T.G, Pierce J.A and Cooper S.L, J. Biomed. Mater. Res., 2Ø, 433 (1986).

55. Takahara A, Tashita J, Kajyama T, Takuyanoi M and Macnight W.J, Polymer, 26, 987 (1985).

56. Lyman D.J, Hill D.W, Stirk R.K, Adam C and Mooney B.R, Trans. Amer. Soc. Art. Intern. Organ, 18, 19 (1972).

57. Lyman D.J,Gett C.K, Zwart H.H.J, Bland A, Elaswood N, Kwajet al, Trans. Amer. Soc. Art. Intern. Organ, 17, 456, (1971).

58. Brash J.L, Chan B.M.C, Szota P and Thibodeau J.A, J. Biomed. Mater. Res.,19,1Ø17(1985).

59. Takahara A, Tashita J, Kajiyama T,Takayanagi M and Mcknight W.J, Polymer, 26, 978 (1985).

6Ø. Grasel T.G and Cooper S.L, Biomaterials, 7, 315 (1986).

61. Pitt W.G, Greasel T.G and Cooper S.L, Biomaterials,9, 36 (1988).

62. Lelah M.D, Pierce J.A, Lambrecht L.K, and Cooper S.L, J. Colloid, Interfac. Sci. 1Ø4,422 (1985).

63. Graham S.W and Hercules D.M, J.Biomed. Mater. Res., 15, 463 (1981).

64. Graham S.W and Hercules D.M, J.Biomed. Mater. Res., 15, 349 (1981).

65. Lyman D.J. Muir W.M. and Lee L.J, Trans. Amer. Soc. Art. Intern. Organ., 11, 3Ø1 (1965).

66. Lyman D.J, Metcalf L.C, Albo D, Richards K.F and Lamb J, Trans. Amer. Soc. Art. Intern. Organ., 2Ø, 474 (1974).

67. Lyman D.J, Khutson K, Mc Neill B and Shibatani K, Trans. Amer. Soc. Art. Intern. Organ, 21, 49 (1975).

68. Sung C.S.P, Hu C.B, Merril E.W and Sulzman E.W, J. Biomed. Mater. Res., 12, 791 (1978).

69. Sung C.S.P and Hu C.B, J. Biomed. Mater. Res., 13, 161 (1979).

7Ø. Ratner R.D. and Paynter A.E, Polyurethane in Biomedical Engineering voli(ed) Planck H, Edberg G and Syre I,Elsevier Science Publishers, Amsterdam P:41 (1984).

71. Sevastianor V.I. Eberhart R.C and Kim S.W. Trans.Amer.Soc. Art.Intern.Organ., 34, 1Ø(1988).

72. Greasel T.G. Lee D.C. Okkema A.Z. Slowinski T.J and Cooper S.L. Biomaterials, 9, 383(1988).

73. Shunmuga Kumar N and Jayabalan M. Proced. Indian Acad.Sci,Chemical sci.,1Ø1, 465 (1989)

74. Beahan P and Hull D, J.Biomed.Mater.Res., 16, 827(1982).

75. Kohjiya S.Ikeda Y and Yamshita S. Polyurethane in Biomedical Engineering vol 1(ed) Planck H,Edberg G and Syre I, Elsevier Science Publishers, Amsterdam P:197(1984).

76. Fialta V., Sotolova O., Trbsex V.,Vrbora M, Vasku A et al Biomaterials, 8, 259 (1987).

77. Tanzi M. C, Fumero. R and Tiohi.G, Polyurethane in Biomedical Engineering vol 1 (ed) Planck H., Edberg G and Syre I.Elsevier Science Publishers, Amsterdam, P:197 (1984).

78. Boretos J.W and Pierce. W,J Biomed Mater Res.,2, 121 (1968).

79. Pollock E, Andrews E.J, Lentz D and Sheikh K, Trans Amer. Soc. Art.Intern.Organ, 27, 4Ø5 (1981)

8Ø. White R. A , Biomaterials 2, 171 (1981).

81. Sharo W.V, Gardner D.C. and Anderson G.,J. Trans. Amer. Soc.Art.Intern.Organ., 12, 179 (1966).

82. Hull C.W, Adams C.M and Ghidoni J.J, Trans. Amer. Soc.Art.Intern. Organ., 21, 281 (1973)

83. Lelah M.D, Lamrech L.K, Young B.R and Cooper S.L, J.Biomed. Mater.Res., 17, 1 (1983)

84. Million. Cleminshaw W.T and Edmark K.E, Biomaterials, 7, 445 (1973).

85. Bruck S.D, Trans. Amer.Soc.Art.Intern.Organ.,18, 1 (1972)

86. Wright A. Becker R and Donath E, J.Biomed. Mater.Res.,18,317 (1984).

87. Matsude T. Takario H. Hayathi K. Takahara Y et al. Trans. Amer. Soc.Art.Intern.Organ., 3Ø, 237 (1977).

88. Pitt W.G and Cooper S.L, Biomaterials, 7, 34Ø (1986).

89. Stuoo S.I, Kauffman J.W. and Cooper S.L. J.Biomed. Mater.Res., 11,237 (1977).

9Ø. Marchant R.E, Phua K, Hiltner. A and Anderson J.M, J. Biomed.Mater.Res., 18, 3Ø9 (1984).

91. Marchant R.E, Anderson J.M, Phua K and Hiltner A. J.Biomed.Mater.Res, 18, 561 (1984)

92. Anderson J.M.Marchant R. Suzuki S. Phua S et al Polyurethane in Biomedical Engineering vol 1(ed) Planck H, Edberg G and Syre I. Elsev ier Science Publishers. Amsterdam P:134,(1984).

93. Marchant R.E, Miller K.M and Anderson J.M, J.Biomed. Mater.Res.,18, 1969 (1984).

94. Schollenberger C.S. and Stewart F.D. J.Elastoplastics, 4,294 (1972).

95. Brauman S.K. Mayorga G.D. and Heller, J.Annals of Biomed. Engineering 9,45 (1981).

96. Chu C.C. and Fisher T.E, J.Biomed.Mater.Res., 12,347 (1978).

97. Chu C.C. and Fisher T.E, J.Biomed.Mater.Res.13, 965 (1979).

98. Darby T.D, Johnson H.B. and Northup S.J., Toxicology and applied pharmacology, 46, 449 (1978)

99. Cassaret L.J, Toxicology: The basic science of poisons (ed) Casarett L.H. and paull J. Macmillan. New york P:245 (1976).

1ØØ. Szycher M, Oliver V.C and Dempsey D.J, J.Elastomer. Plast. 15, 81 (1983).

1Ø1. Massu A.C. and Smith C.P., J.Biomed.Mater.Res., 18, 961 (1984).

1Ø2. Mara M.M.O, Erner D.A. and Hanshumaker D.J., Polyurethane in Biomedical Engineering vol 1 (ed) Planck H. Edberg G and Syre I. Elsevier Science Publishers. Amsterdam P: 83 (1984).

1Ø3. Schollenberger C.S. and Stewart F.D. J. Ellasto. Plast.,3, 28 (1971).

1Ø4. Coury A.J, Stokes K.B, Cahalan P.T. and Slaikey P.C,Life suport system, 5,25 (1987).

1Ø5. Gogolewski S., Life support system, 5,41 (1987).

1Ø6. Grassie N and Zulfigar M, J.Polym.Sci.Polym. Chem.Ed,16,1653 (1978).

1Ø7. Boretos J.W,,Biocompatibility of clinical implant material (ed) Williams D.F.,CRC Press. Inc.,Florida, P:127 (1983).

1Ø8. Kolff W.J, Akutsa T, Dreyer B and Norotan H, Trans. Amer.Soc.Art.Intern.Organ., 5, 298 (1961).

1Ø9. Dunkel. W and Bhar D.W, Proc.Europ.Soc.Artif.Org.,4,2Ø3 (1977).

11Ø. Lemn W, Pirling E.S and Bucher I.S, Proc.Europ.Soc.Artif. Organs,7, 86 (198Ø)

111. Lemm.W.Krukenberg T.Regier.G. Gerlach.K and Bucherl E.S, Proc.Europ.Soc.artif.Organs. ,8,71 (1981)

112. Chawla A.S, Bleis. P, Hinberg. I , Artificial cells Biomaterials. Artificial Organs,16,785 (1988).

113. Lemm W and Sucherl E.S, Biomaterials and Biomechanics,(ed) Ducheyne P, Van der Perre G and Aubert A.E, Elsevier Science Publishers, Amsterdam, P:319 (1984).

114 Potts J.E, Clending R.A,, Ackart W.B.and Niegisch W.D. Polymers and Ecological problems, Polymer Science and Technology series vol 3 (ed) Guilet. Pienum, New York. P:61 (1973).

115. Swanson J.W. and Lebeau J.E, J. Biomed. Mater. Res., 8,357 (1974).

116. Huang S.J, Bansleben D.A. and Knox J.R, J.Appl.Polym.Sci.,23. 429 (1987).

117. Smith R. , Williams D.F. and Oliver C. J.Biomed. Mater.Res.,21,1149 (1987).

118. Ratner B.D, Gladhill K.W. and Hoobett T.A. J.Biomed.Mater.Res., 22, 5Ø9 (1988).

119. Williams D.F, Physiological and microbiological corrosion. CRC Crit Rev., Biomaterials, 1, 1 (1983).

12Ø. Williams D.F, J.Mater.Sci.,17,1233 (1982).

121. Smith R and Williams D.F, J.Mater.Sci. Lett.,4,547 (1985).

122. Smith R., Oliver C and Williams D.F. , J.Biomed. Mater.Res.,21,991 (1987).

123. Leininger R.I, Mirkovitch V, Peters. A and Hawks W.A. Amer.Soc.Art.Intern.Organ.,1Ø,32Ø (1964).

124. Boretos J.W, Petmer D.E and Donachy J.H, J. Biomed.Mater.Res., 5,373 (1971).

125. Bichnon D., Booroelez W and Zoopi A.C.C, Biomaterials, 5, 255 (1984).

126. Coleman D.C,Lim D. Kessler T and Andrade J.D, Trans. Amer.Soc.Art.Intern.Organ., 27,97 (1981).

127. Coleman D.C. Trnas. Amer.Soc.Art.Intern,Organ., 27, 7Ø8 (1981).

128. Hennics E, Keilbach H, Holde, D and Bucherl E.S, Proc. European Soc. Artif. Organ., 8, 76 (1981).

129. Hennics F and Bucherl E.S, Polyurethane in Biomedical engineering 1 (ed) Planck H. Egbers. G and Syre I, Elsevier Science Publishers, Amsterdam (1984).

13Ø. Anderson H.C. Trans. Amer.Soc.Art.Intern.Organ., 27,7Ø2 (1981).

131. Lian J.B, Levy R.J, Bernharat W and Szycher M, Trans. Amer. Soc.Art.Intern.Organ., 27, 683 (1981).

132. Long M.M and Urry D.W. Trans.Amer.Soc.Art.Intern.Organ., 7, 69Ø (1981).

133. Woodward S.C., Trans Amer.Soc.Art.Intern.Organ., 27, 697 (1981).

134. Nose Y , Harasaki H and Murray J, Trans.Amer.Soc.Art. Intern.Organ., 27,714 (1981).

135. Glasmacher B, Reul H, Rau G, Ercker and Wieland J, Polyurethane in Biomedical Engineering II (ed) Planck H. Syre I, Dauner.M and Egberg .G, Elsevier Science Publications, Amsterdom, p 151 (1987).

136. Woters L.H.G. Rouseau E.P.M. Vansteenhoken A.A. and German A.L.Polyurethane in Biomedical Engineering II (ed)Planck H, Syre I, Dauner M and Egberg G, Elsevier Science Publications, Amsterdom, p 169 (1987).

137. Colemam D.L,, Trans. Amer.Soc.Art.Intern.Organ., 27, 7Ø8 (1981).

138. Turner S.A, Milton L.J, Porer V.C and Norman J.C, Artificial Organs, 5, 18 (1981).

139. Phillips R.E. and Thoma R.J. Polyurethane in Biomedical Engineering II (ed) Planck H, Syre I. Dauner M and Egberg G. Elsevier Science Publications, Amsterdom, p. 91 (1987).

140. Gerring E.L. Bellhouse B.J. Bellhouse F.H. and Haworth W.S. Trans. Amer. Soc.Art.Intern.Organ., 20, 703 (1974).

141. Hagher S.D, Coleman D.L, Dew P.A, Burns G.L, Olsen D.P and Kolffaj, Trans.Am.Soc.Art.Intern.Organs, 30, 75 (1984).

142. Sawyer P.N, Stanczewski B, Srinivasan S, Stempack J.G. and Kammloff G.W, Trans. Amer. Soc. Art. Intern.Organ, 20, 692 (1974).

143. Wisman C.B, Pierce W.C, Donachy J.H, Pae W.E, Mayer J.I. and Prophet G.A, Trans. Amer.Soc.Art.Intern.Organ., 28, 164 (1984).

144. Sharp W.V, Uardner D.C and Anderson G.J, Trans. Amer.Soc. Art.Intern.Organ., 12, 179 (1966).

145. Herold M, Lo. H.M, Reul H.B, Mucketer H, Taguchi .H et al Polyurethane in Biomedical Engineering II (ed) Planck H.Syre I, Dauner M and Egberg G, Elsevier Science Publications. Amsterdom, p.231 (1987).

146. Boretos J.W, J.Biomed. Mater.Res., 6, 473 (1972).

147. Boretos J.W, Pierce W.S, Bair R.E, Lerous A.F and Donachg H.J, J.Biomed. Mater.Res., 9, 327 (1975).

148. Kolff W.J, Trans. Amer.Soc.Art.Intern.Organ. 29.6 (1983).

149. Lawson J.H, Olsen D.B, Hershgold.E, Kolff J, Hadgield K and Kolff W.J, Trans. Amer.Soc.Art.Intern.Organ. 21, 368 (1975).

150. Shaffer L.J, Donuchy J.H, Rosenberg G, Phillips W.M. et al Trans. Amer.Soc.Art.Intern.Organ, 25, 254 (1979).

151. Imachi K, Fujimasa I, Miyalke H, Takido N et al, Trans. Amer.Soc.Art.Intern.Organ., 27,118 (1981).

152. Joyce L.P, Devries W.C, Hastings G.W, Olsen D.B et al, Trans. Amer.Soc.Art.Intern.Organ,. 29,81 (1983).

153. Nose M,Vikamudi A.K, Kudo T, Akutstand H.C. Trans. Amer. Soc.Art.Intern.Organ, 25,268 (1979).

154. Zartnack F,Punkcel W,Affeld K and Bucherl E.S, Trans. Amer.Soc.Art.Intern.Organ., 24,6ØØ (1978).

155. Lyman D.J, Gett C.K, Zwart H.H.J, Bland A et al, Trans. Amer.Soc.Intern.Organ., 17, 456 (1971).

156 Mestres C.A, Nicnot S. and Barriuso C et al, Polyurethane in Biomedical Engineering II (ed) Planck H. Syre I. Dauner m and Egberg G. Elsevier Science Publications. Amsterdom. p. 39 (1987).

157. Mestres C.A. River A., Cutgat E. Nihot S et al, Polyurethane in Biomedical Engineering II (ed) Pland H. Syre I. Dauner M and Egberg G, Elsevier Science Publications, Amsterdom, p.45 (1987).

158. Hunter K.S, Gregoris D.E, Coleman D.C, Andrade J.D. et al Trans. Amer.Soc.Intern.Organ., 28, 473 (1982).

159. Hayashi. M, Matsuda T. Tajabi H, Umezu. M, Taenaxa Y and Nakamum T. Biomaterials, 6, 82 (1985).

16Ø. Byrd C.L, Schwartz S.J, Sivina M, Yahr W.Z. et al Polyurethane in Biomedical Engineering I (ed) Planck H. Egbers G and Syre I, Elsevier Science Publications, Amsterdom, p 65 (1984).

161. Skalasky M. Vaughen J.P. and Chapman, Polyurethane in Biomedical Engineering I (ed) Planck H. Egbers G. and Syre I. Elsevier Science Publications, Amsterdom, p 75 (1984).

162. Annis D, Life support system, 5, 47 (1987).

163. Kambic H.E, Trans. Amer.Soc.Art.Intern.Organ., 34, 1Ø47 (1988).

164. Taylor B.C, Sharp W.V, Wright J.I, Ewines K.L and Wilson C.L, Trans. Amer. Soc.Art.Intern.Organ., 17, 22 (1971).

165. Miller B.G, Dyer K.A, Taylor B.L, Wright J.I and Sharp W.V. Trans. Amer.Soc.Art.Intern.Organ, 2Ø, 91 (1974).

166. Helmus M.N, Gibbons D.F, and Jones R.D, J. Biomed.Mater.Res.. 18,165 (1984).

167. Sharp W.V. Taylor B. Wright J. Nuwayser E et al trans. Amer.Soc.Art.Intern.Organ. 18.232 (1972).

168. Sharp W.V, Gardner D.C. and Anderson J.M, Trans. Amer.Soc.Art.Intern.Organ. , 14, 73 (1968).

169. Annis D, Bornat A,. Edwards R.O, Higham A et al, Trans. Amer.Soc.Art.Intern.Organ, 24, 2Ø9 (1978).

17Ø. Lyman D.J, Faxxio F.J, Vorhes H, Robinson G and Albo D, J. Biomed. Mater. Res. , 1Ø,337 (1978).

171. Gogolewski S and Pennings A.J, Polyurethane in Biomedical Engineering I (ed) Plank H, Egbers G. and Syre I, Elsevier Science Publications. Amsterdom, p.279 (1984).

172. Martz H, Guidoin R,Beaudoin G, Pynter R and King M, Life support systems, 4, 77 (1986).

173. Lyman D.J, Albo D, Jackson R and Knutson K, Trans. Amer.Soc.Art.Intern.Organ., 23, 253 (1977).

174. Noidhiki Y, Nagaoka.S, Kuchi T.K and Mori Y, Trans. Amer. Soc.Art.Intern.Organ, 27,213 (1981).

175. Wilson G.J, Mcgregor D.C, Klement P, Lee J.M, Nido P.J. et al, Trans. Amer.Soc.Art.Intern.Organ, 29, 26Ø (1983).

176. Lommen E, Gogoewski. S, Pennings A.J, Wildeutur C.R.H. et al Trans. Amer.Soc.Art.Intern.Organ. 29,255 (1983).

177. Matsumoto H, Kira K, Miyawaki F, Hiamatsu K, Saito H, Kondo K. and Takumatsu T, Life support system, 44, 89 (1986).

178. Martz H, Guidon R, Paynter R, Forest J.I and Downs A, Life support system, 4, 85 (1986).

179. Martz H, Paynter R, Forest J.C, Downs A et al, Biomaterials, 8, 3 (1987).

18Ø. Hayashi K, Polyurethane in Biomedical Engineering I(ed)Plank H.Egbers G and Syre I, Elsevier Science Publications. Amsterdom, p.129 (1987).

181. Annis P, How T.V, and Fither A.C, Polyurethane in Biomedical Engineering I (ed) Plank H, Egbers G and Syre I. Elsevier Science Publications, Amsterdom, p. 287 (1984).

182. Hess F, Jerrsales C, Braun B and Grande P, Polyurethane in Biomedical Engineering I (ed) Planck H. Egbers G. and Syre I, Elsevier Science Publications, Amsterdom, p. 3Ø1 (1984).

183. Salvatore J.E, Moncrief W.H, Mandrino M.P and Sherman R.T. Trans. Amer. Soc.Art.Intern.Organ., 5, 325 (1964).

184. Schwope A.D, Wise D.L, Sell K.W, Skdrnik W.A and Presslar D.P, Trans. Amer.Soc.Art.Intern.Organ., 2Ø,1Ø3 (1974).

185. Salisbury R.E, Berin A.G., Dingeldein G.P., and Grishan, J. Arch.Surg.,119, 1188 (1979).

186. Gogolewski S and Pennings A.J, Macromol.Chem.Rapid Commun. 5, 675 (1983).

187. Nelson R.J, White R.A, Lawrence R.S, Walkinshow M.D. et al Trans. Amer. Soc.Art.Intern.Organ 25,8 (1979).

188. Everett R.L. and Bailey B.J, Trans. Amer. Soc.Art. Intern. Organ., 26, 418 (198Ø).

189. Schawecker H.H, Daudert A, Phipips A, Schnoy N and Plank H. Life support system, 3, 139 (1985).

19Ø. Schauwecker H.H, Gerlach J.C, Planck H, Schmaykal T and Pucherl E.S, Polyurethane in Biomedical Engineering II (ed) Planck Syre 1, Daunner M and Egbers.G Elsevier Science Publications, Amsterdom. p. 29 (1987).

191. Bischel M.D, Orell F.C, Scholer B.G, Mohler J.G. and Barbour B.H, Trans. Amer.Soc.Art.Intern.Organ., 19,192 (1973).

192. Gotch F, Sargent J, Keen M, Holmer G and Teisinger C. Trans. Amer.Soc.Art.Intern.Organ., 15, 237 (1964).

193. Lyman D.J. and Loo B.H, J.Biomed Mater.Res., 1,17 (1969).

194. Hunter S.K, Gregoins D.E, Coleman D.L, Hanover B et al, Trans. Amer.Soc.Intern.Organ. 29, 25Ø (1983).

195. Szycher M, Poirier V.L and Dempsey D, 35'th Ann. Tech.Conf. Soc. of Plastics Engineers, 35, 743 (1977).

196. Szycher M. Poirrier V and Keiser J, Trans. Amer.Soc.Art.Intern. Organ. 23, 116 (1977).

197. Merrill E.W, Sulzman E.W, Wan W, Mahmud N et al, Trans. Amer. Soc.Art.Intern.Organs, 28,482 (1982).

198. Merrill E.W, Costa V.S.D, Salzman E.W., Russel D.B. et al, Advances in chemical series 199 (ed) Cooper S.L. and Peppas N.A. Washington D.C.American chemical society,, p 95, (1982).

199. Wilker G.L, J.Biomed. Mater. Res., 7,541 (1973).

2ØØ. Lipatova T.E, Pkhakdzo G.A, Vasil D.V.C and Shilor V.V, Biomaterials, 4, 2Ø1 (1983).

2Ø1. Schauwecker H.H, Kamof W.D, Planck, Schamaylat T et al Polyurethane in biomedical Enginerring I (ed) Planck H, Egbers G, and Syre I, Elsevier Science Publications, Amsterdom, P:19 (1987).

2Ø2. Nelson R.J, White R.A, Lawrence R.S, Walkinshow M.D et al., Trans. Amer. Soc. Art. Intern. Organ, 2, 58 (1979).

2Ø3. Nyilas E, J. Biomed. Mater. Res., 3, 97 (1972).

2Ø4. Nyilas.E, 23rd Anuual Conf. Eng. in Medicine and Biol. Washington D C (197Ø)

2Ø5. Gnanous Y,Hild G and Rempp P, Macromolecules,16,775 (1983).

2Ø6. Gnanous Y,Hild G. Encyclopedia of polymer science and technology, Interscience publishers, London,4,331(1966)

2Ø7. Hourston D.J and Zia, J.Appl .Polym.Sci., 28,2139 (1983)

2Ø8. Andrade J.D, King R.N, Gregonis D.E and Coleman D.L,Jpolim. Sci, Polym. Symposium, 66,313 (1979)

2Ø9. Raphale S.S, Lynch's Medical Laboratory Technology, Igacushion, Saunder International Ed., Japan, 1983 P:265.

21Ø. Bauer J.D, Clinical laboratory Methods, The C.V Mosby Company, St. Louis, Toronto, P:292 (1982).

211. The United States Pharmacopea, 21 st edition, P:1235 (1985).

212. Vogel A.I, A Textbook of quantitative inorganic analysis, 4th ed, The ELBS society, Logman,P:37Ø (1979).

213. Thkahava A, Tathita J, Kajiyama T and Takangi M, J.Biomed.Mater .Res.I9, 8Ø (1985).

214. Bauer J.D, Clinical Laboratory Methods, The C.V Mosby Company, St. Louis, Toronto, (1982) P:552.

215. Frankel S, Reitman S, Sopnnenwinch A.C, Gradwohl's Clinical laboratory methods and diagnosis, vol.1 The C.V Mosby Company, Saint Louis, 197Ø P:143.

216. Spirkova M and Dusek K,Polymer Bulletin, 22, 191 (1989).

217. Oikawa H,Nagai T and Murakami K,J.Polym.Sci,Polym.Phy.27,795 (1989).

218. Illavsky M and Dusek, K, Polymer, 24, 1323 (1983).

219. Illavsky M and Dusek. K,Polym. Bull,8, 359 (1982).

22Ø. Dusek K, Illavsky M and Matejka L, Polym.Bull, 12, 33 (1984).

221. Illavskey. M,Bouchal K and Dusek K, Polym. Bull,14, 495 (1985).

222. Dusek. K,Polym,Bull, 17, 481 (1987).

223. Spirkova.M, Kubin.M and Dusek.K, J.Macromol. Sci-Chem A24,1151 (1987).

224. Gandhi K.S and Babu S.U, Macromolecules ,13, 791 (198Ø).

225. Gandhi K.S and Mall S, Polymer,26, 595 (1985).

226. Wright P and Cummings A.P.C,solid polyurethane elastomers, Macmillan and sons, London P 29 (1969).

227. Dusek K,Spirkova,m and Havlieck I,Macromolecules,23,1774, (199Ø).

228. Lipschitz S.D and Macoko C.W, J.Appl.Polym.Sci., 21, 2Ø24 (1977).

229. Broyer E, Macoko C.W, Critchfield F,C and Lawler , Polym. Engineering and Science, 18, 382 (1978).

23Ø. Squiller E.P and Rosthauser J.W,Polym.materi.Sci.Eng.,55,64Ø (1986).

231. Robins J,J. Appl.Polym.Sci. 9, 821 (1965).

232. White K.A, Ward R.S,Riffle J.S, Trans 7th Intern.Biomat. Sym., SanDiego, California, 8, 57 (1985).

233. Cunliffe A.V, Davis A, Farey M and Wright J Polymer,26, 3Ø1 (1985).

234. Frish K.C,Advances in Urethane Science and Technology, Technamic Publications,west port, CT,8,75 (1981).

235. Kanub P and Camberlin Y,J.Apl.Polym.Science,32,5627 (1986).

236. Dieter J.W and Byrene C.A, Polym.Eng.SC1.27,673 (1988).

237. Lipatova T.E, Bakalo L.A, Niselsky Y.N and sirtiuskukaya, J.macromol. Sc1,chem A4,743(1976)

238. Entelis S.G, Nesterov and Tiger R.P,J.Cell. Plast., 3, 360 (1967).

239. Frish K.C, Reegan S.C and Thir B J. Polym. Sci, 16,2191 (1967).

240. Lyman D.J, Step growth polymerisation, (ed) Soloman, P 99

241. Chern S.C J.Appl. Polym. Sci., 40, 2189 (1990)

242. Saunders J.H and Frisch K.C, Polyurethane Chemistry and Techenology parts I and II,Intersciences, New Yourk, 1962.

243. Kajiyana T and Macknight W.J , Macromolecules, 2, 254 (1969).

244. Harrel L.L, Macromolecules, 2, 607 (1969).

245. Seymour R.W, Estes G.M, Huh D.S and Cooper S.L, J.Polym.Sci., Part A-2, 10, 1525 (1972).

246. Aitken R.R and Jeffs, Polymer, 18, 197 (1977).

247. Russo R and Thomas E.L, J.Macromol. Sci.Phys B22, 553 (1983).

248. Bonart R, J.Macromol. Sci.Phys B2, 115 (1968).

249. Seymour R.W and Cooper S.L, J.Polym.Sci, 9,689 (1971).

250. Petrovic Z.S Illavsky M, Duesk K, Vidakovic et al,J .Appl. Polym.Sci., 42, 391 (1991).

251. Petrovic Z.S and Simendic J.B, Rubber Sci. Tech,58,685(985).

252. Petrovic Z.S and Ferguson J, Hem. Ind, 32, 531 (1978).

253. Kontou E, Spathis G, Niaounakis M and Kefalas V, Colloid Polym Sci, 268, 639 (1990).

254. Allen G, Boeden M.J, Blundell, Hutchinson F.G et al Polymer, 14, 597 (1973).

255. Shen M.C and Tobolsky A.V, J. Polym. Sci., A2, 2513 (1964).

256. Brandrup J and Immergut F.H (eds) Polymer hand book, 2nd edition IV-337,Miley,New Yourk (1975).

257. Kojima H, Nishi mura H and Funaki M, Reports Res.Lab, Ashasi Glass Co, Ltd, 31, 109 (1981).

258. Sung C.S.P, Schneider N.S, Mater. Sci, 13, 1689 (1978).

259. Bonart R, Morbitzer 1, Muller E.H et al, Macromol. Sci-Phy, B9, 447 (198Ø).

26Ø. Sung C.S.P, Smith T.W, Sung N.H, Macromolecules, 15, 71 (1982).

261. Brunette C.M, Hsu S.L, Macknight, Macromolecules, 15, 71 (1982).

262. Gardner K.H, Blackwell, Polymer, 2Ø, 13 (1979).

263. Petrovic Z.S, and Simendic J.B, Rubber Sci. Tec.,58, 7Ø1 (1985).

264. Hourston D.J and Zia y, J.Appl. Polym. Sci, 28, 139 (1983).

265. Petrovic Z.S, Ilavasky M, Dusek K, Vidakovic et al J.Appl. Polym. Sci., 42, 391 (1991).

266. Lyman D.J, Rev.Macromol.Chem, 1, 355 (1966)

267. Nyilas E, Morton W.A, Cumming R.D, Ledeman D.M et al, J.Biomed. Master.Res.,Symp.,8,51 (1977).

268. Sharma C.P, J.Sci. Ind. Res. 39.453(198Ø).

269. Biridi K.S, J. Theoret.Biol,93,1(1981)

27Ø. Dermer O.C, Curtis V.S and Leach R.F, Biochemical indicators of surface Pollution, Ann.Arbor Science, Publishers,198Ø P:6Ø.

27Øa,Sprugel.J.M Mcpherson A.W and Rogs.R, Am.J.Pathology, 129, 6Ø1 (1987)

27Øb,Delustron F and Leroy , J.Reticuloendothelial.Soc., 31,295,(1982)

27Øc,Vanbrunt J and Klausen A, Biotechnology, 6,25 (1988)

271. Robbins S.L and Cotran R.S, Pathologic basis of diseease,2nd ed, Saunders, Philadelphia, 1979 P:56

272. Anderson W.A.D , Pathology, 6th ed, Mosby, St. Louis, 1971,P:16.

273. Rigdon R.H, J.Biomed .Mater.Res.,8,97(1974)

274. Rigdon R.H, Biomed. Mater.Res.,7,79(1973)

275. Woodward S.C and Salthouse T.N, Evaluation of biocompatibility testing, P:364.

276. Anderson J.M, Trans. Am.Soc.Arti.Intern. Organs,34,1Ø1(1988)

277. Salthouse T.N, J.Biomed.Mater.Res.,18,395(1984)

278. Behling C.A and Ppector M,J. Biomed. Mater.Res., 2Ø,653(1986)

279. Spilizedwski K.L, Marchant R.E, Anderson J.M,and Hiltner A Biomaterials 8,12(1987)

28Ø. Bruck S.D, Biomaterials,2,14(1981)

281. Autian J,Fundamental aspects of biocompatibility (ed) Williams,CRC Press, Boca Raton, Florida,2,63(198Ø)

282. Ashar B and Turcotte L.R Trans.Am.Soc.Artif. Intern. Organs, 27,372(1981)

283. Chawla A.S and Blais P,12th Ann. Meeting of the socity of bio materials, Minneapolis,1986 p:192

284. Gogolewski S and Galleti G, Life Support sysyem,2,324(1984)

285. Grifin G.J.L, Pure and Applied Chemistry,52,399(198Ø)

286. Saunders J.H,Rubber Chem. Technopl. 32m337 (1959)

287. Schollenberger C.S Scott H and Moor G.R, Rubber World Technomic,Westport, CONN(1985)

288. Hepburn C,Polyurethane elastomers, Applied Sciences, London (1982)

289. Grassie N and Zulfigar M. Development of polymer stabilization (ed) Scott G, Applied Sciences,Londopn (1979)

29Ø. Gogolowski S, Colloid. Polym.Sci., 267,757 (199Ø)

291. Ernst R.R, Disinfection, Sterlization and Preservation (ed) Block H Philadeelphia, Penn: Lea and Febiger Press, (1977) P:499

292. Szcher M, Biocompatible materials and devices, (ed) Sharma C.P and Szcher M, Technomic, Lancaster, Penn(1989) :P39.

293. Szher M, Blood compatible materials and devices, (ed) Sharma C.P and Szher M, Technomic, Lancaster, Penn(199Ø) P: 188.

294. Lawandus S.N and Amera A.M,J.Appl.Polym.Sci, 37,283 (1989)

295. Masar B and Cefelin P, Lipatova T.E et al,J.Polym.Sci. Polym.Sym.66,259(1979)

296. Wells L.A Cassidy P.E, Aminabhavi T.M et al Rubber Chemistry and Technology,63,66 (199Ø)

297. Smith R, Williams D.F and Oliver C, J.Biomed.Mater.Res., 21,1149 (1987)

298. Zartnack F, Dunkel W,Alfred K and Buchari E.S, Trans.Am. Soci.Art Intern.Organ., 24,6ØØ (1978).

299. Stoker M and O'Nell'C Berryman and Waxman V, Int,J Cancer

3ØØ. Maroudas N.G J.Cell.Physiol,9Ø,511 (1977)

3Ø1, Baker D, Van Blitterwijk C.A, Hesseling S.C et al, Biomaterials, 9,14 (1988)

3Ø2. Lydon M.J, Minett T.W and Tighe B.J, Biomaterials, 6, 396 (1985)

3Ø3. Van der Valk P, Van Pelt A.W.J, Busscher H.J et al., J.Biomed.Mater.Res., 17,8Ø7 (1983)

3Ø4. Schakenraad, J.M, Busscher H.J, Wilderur C.R.H and Arends , J.Biomed.Mater.Res , 2Ø,773 (1986)

3Ø5. Schakenaad J.M, Kuit J.H, Arends J et al Biomaterials 8, 2Ø7, (1987)

3Ø6. Pizozerato A, Vespucci A, Ciapetti G and Stea S, Biomaterials, 6,346 (1985)

3Ø7. Wieslander A., Magnusson A and Kjellstrand., Biomat.Art.Cells, Art.Org., 18, 367 (199Ø)

3Ø8. Ambrose E.J and Easty D.M, Cell Biology, The ELBS and Nelson, Polymong (UK), P:258 (1975).

3Ø9. Matlaga B.F, Yasenchak L.P and Salthouse T.N, J.Biomed.Mater.Res., 1Ø, 397 (1976)

31Ø. Kaminski E.J, Oglesby R.J, Wood N.K and Sandric J, J.Biomed.Mater.Res., 2, 81, (1968)

311. Anderson J.M, Trans Am. Soc. Artif. Intern.Organs., 34,12 (1988)

312. Charnley J, Acrylic cement in orthopaedic surgery, Edinbargh, Livigstone, 197Ø, P:198.

313. Dinarello C.A.D, Rev.Infect.Dis, 6, 51 (1984).

ABBREVATIONS

ATR-IR	Attunated Total Reflection - Infrared Spectrophotometer
DMA	Dimethyl Acetamide
DMA	Dynamic Mechanical Analysis
DTA	Differential Thermal Analysis
DBTDL	Dibutyl Tin Dilaurate
HDI	Hexamethylene Diisocyanate
IPDI	Isophorone Diisocyanate
PEG	Poly ethylene Oxide glycol
PTMG	Poly tetramethylene oxide glycol
PPG	Poly propylene oxide glycol
$\bar{M}c$	Molecular weight between two crosslinks
TEA	Triethyl amine
TMP	Trimethylol Propane
SMDI	Methylene bis cyclohexyl Diisocyanate.

APPENDIX (i)

PHYSICAL PROPERTIES OF TECOFLEX-85 A REFERENCE BIOCOMPATIBLE MATERIAL

Property	Value
Ultimate tensile Strength(psi)	6420
Ultimate Elongation (%)	600
Hrdness in cast block form (shore) A	85

APPENDIX ii

PROPERTIES OF POROUS POLYURETHANE MATERIALS

Property	Value
Tecoflex -85 A	
Pore volume (%)	35-40
Pore diameter (μ)	100-120
IPDI based PU	
Pore volume (%)	35-40
Pore diameter (μ)	150-200
SMDI based PU	
Pore volume(%)	38-44
Pore diameter(μ)	150-210
HDI based Pu	
Pore volume (%)	40-42
Pore diameter (μ)	175-210

APPENDIX-iii

1 . Biocompatible Materials and Devices - A Review, in Trends in Biomaterials and Artificial Organs, 3(2), 1989 P:61-86.

2 . Studies on the effect of extraction of isophorone diisocyanate based polyurethane, in Proc. of Indian Acc.Sci., (Chemical Sci.) 101, 1989 P: 455-465.

3 . Evaluation of stability of castor oil based polyurethanes for biomedical applications in Trends in Biomaterials and Artificial Organs.,6(1) 1991 P:

4 . In Vivo Biocompatibility of an aliphatic crosslinked polyurethane in rabbit, in J.Biomedical. Mater.Res.,(in Press)

5 . The pressure induced calcium deposition on crosslinked polyurethanes in Artificial Organs (in Press)

(List of publications)