BIOMATERIALS SCIENCE

STUDIES ON BIODURABLE POLYURETHANE POLYMERS FOR APPLICATION IN CARDIOVASCULAR DEVICES

A Thesis Presented

By

VINOY THOMAS

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of

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FOR
MEDICAL SCIENCES AND TECHNOLOGY
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DECLARATION

I, Vinoy Thomas hereby declare that I had personally carried out the work depicted in the thesis entitled 'BIOMATERIALS SCIENCE- STUDIES ON BIODURABLE POLYURETHANE POLYMERS FOR APPLICATION IN CARDIOVASCULAR DEVICES' except where external help sought is acknowledged.

Date : 19 - 10 - 2001

VINOY THOMAS

Dr. M. Jayabalan
Scientist E and Head

Polymer Division
Biomedical Technology Wing
Sree Chitra Tirunal Institute for
Medical Sciences and Technology
Thiruvananthapuram-695 012

CERTIFICATE

This is to certify that Mr. VINOY THOMAS in the Polymer Division, Biomedical Technology Wing 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 Sree Chitra Tirunal Institute for Medical sciences and Technology, Trivandrum. The work relating to his thesis entitled 'BIOMATERIALS SCIENCE-STUDIES ON BIODURABLE POLYURETHANE POLYMERS FOR APPLICATION IN CARDIOVASCULAR DEVICES' was carried under my direct supervision.

Dr. M. Jayabalan
(Guide)

The thesis
entitled

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Submitted

by

VINOY THOMAS

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TRIVANDRUM

evaluated and approved by

Dr. M. Jayabalan
(Guide)

Dr. R. Ramaswamy
(Name of Thesis examiners)

"A small body of determined spirits
fired by an unquenchable faith in their mission
can alter the course of history"

Mohandas Karamchand Gandhi, Circa 1938

To my parents...

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SYNOPSIS

SYNOPSIS

Polymers are widely used in variety of biomedical applications. However, applications, which require long term performance, warrant biocompatibility and biodurability. None of the existing polymers satisfy these requirements especially in long-term biomechanical environment. Polyurethanes have been developed from aromatic diisocyanate *viz* diphenyl methane diisocyanate (MDI); however these polyurethanes undergo thermal and thermohydrolytic degradation producing methylene dianiline (MDA), a suspected carcinogen. Aromatic polyurethanes have proved sufficient biocompatibility in a variety of blood contact applications such as experimental artificial hearts, left ventricular assist devices, blood pumps and cardiac pacemaker; however these polyurethanes could survive only for a short term. The mammary prostheses developed from toluene diisocyanate (M/S Surgitech, Meme®) have met court litigation for the clinical failure. The non-existence of these clinical devices for patient care is mainly due to catastrophic failure of the polyurethane material used in these devices.

Polyurethanes used as vital components in biomechanically sensitive blood contact devices such as pump membrane of blood pumps and flexible leaflet of artificial hearts and left ventricular assist devices have to undergo repeated cyclic flexion and flex more freely without producing adverse changes in blood flow. Therefore, it is essential to develop low elastic modulus polyurethane with increased

fatigue life. In addition to the requirement of low elastic modulus, biodurability and long-term biocompatibility in biomechanical environment is a mandatory requirement. The currently available polyurethanes undergo failure in such highly flexing environment due to bond scission at ether linkages. Biodegradation induced by hydrolytic, oxidative and environmental stress corrosion mechanism has to be prevented to avoid catastrophic failure of medical devices. The future development of heart valve and left ventricular assist device as commercially viable devices depends on performance of valve leaflet and pump membrane component, which undergo repeated cyclic flexion. Therefore, the main objective of the investigation is the development and evaluation of biodurable polyurethane polymers for application in cardiovascular devices.

The thesis consists of five chapters. Chapter I deals with the review on polymeric biomaterials, the requirements for long term implantation, polyurethane as cardiac-biomaterial and biocompatibility and biodurability of polyurethanes. The problems and risks of available polyurethanes and the need for the development of a novel high flex-life poly(urethane urea) for use in cardiovascular applications were briefed.

Chapter II describes the detailed objectives of the present investigation. They are 1. Synthesis and process optimisation of novel polyurethanes. 2. Physical, chemical and thermal characterisation and mechanical evaluation of polyurethanes. 3. Studies on biochemical and biomechanical factors on *in vitro* biostability of newly prepared polyurethanes. 4. Studies on *in vitro* calcification. 5. Studies on biocompatibility and (*in vitro* and i*n vivo)* of newly prepared polyurethanes. 6. Studies on functional performance and long-term *in vivo* biostability of polyurethanes for the

use in cardiovascular applications. The Chapter II also deals with the scope of the present investigation.

Chapter III deals with the experimental procedures used for the synthesis, characterisation and evaluation of the polyurethane. The materials used consists of hydroxy terminated polybutadiene (HTPBD), methylene bis(p-cyclohexyl isocyanate) (H_{12}MDI), 1,6-hexamethylene diamine (HDA) . Four new poly(urethane urea)s having urethane and urea linkages were synthesised using these chemicals. Two poly(ether urethane urea)s having ether, urethane and urea linkages were also synthesised using polytetramethylene glycol (PMTG-1000) along with the above materials. Two polyurethanes having only urethane functional group were also synthesised using HTPBD, H_{12}MDI and chain extender 1,4-butane diol (BD).

All the polymers were characterised for physico-chemical properties like density, crosslink density and molecular weight between crosslinks. FT-IR and wide angle X- ray diffraction studies were carried out. Mechanical properties such as tensile properties, hardness were measured using ASTM standards. Surface properties of the polymer were evaluated by water contact angle measurement using sessile drop. The thermal properties and viscoelastic properties of the materials were determined using TGA and dynamic thermal mechanical analysis (DMA) respectively.

The *in vitro* biostability of the polymers were studied by aging the polymers in different media for 30 days at 37 ^{0}C. Phosphate buffered saline (pH=7.4), Ringers solution, papain enzyme and its buffer solution, silver nitrate - sodium lactate solution, Hydrogen peroxide solution and lipid were used. The aged polymers were tested for change in weight, mechanical and surface properties. Synergistic effect of

hydrolytic enzyme and mechanical strain on the stability materials was also tested *in vitro*. Studies on environmental stress cracking-resistance and accelerated chemical degradation were carried out. Studies on the *in vitro* calcification of the materials were carried using metastable calcium phosphate solution.

The biocompatibility was evaluated by *in vitro* methods *viz* cytotoxicity and cell viability, haemolysis as per ASTM standard. The bloodcompatibility was evaluated by *in vitro* methods using whole blood by haematology. Systemic toxicity and intracutaneous irritation test were also carried out using extract of the materials as per international standards. The *in vivo* biocompatibility was evaluated by intramuscular implantation of the materials and histopathological analysis of harvested tissues as per USP standards.

The functional performance of the candidate polyurethane for the use in cardiovascular applications was also evaluated. The flex-life of candidate poly(urethane urea) was determined using an accelerated flexural fatigue tester as per ASTM standard. The endothelial cell response to the candidate material was assessed. Long-term *in vivo* biodurability was studied by subcutaneous implantation of the candidate polymer in rat animal model for 6 months. The materials were retrieved and tested for the changes in surface and bulk properties using FT-IR, scanning electron microscopic analysis, mechanical tests and surface contact angle measurements.

Chapter IV deals with the results and discussion, which is divided into 6 major sections. Section IV-1 deals with the synthesis of the new polyurethanes. All the polymers were synthesised by two step solution polymerisation technique. By optimizing the process variables *viz* nature and concentration of reactants, three types of polymers *viz* polyurethanes, poly(ether urethane urea)s and poly(urethane urea)s

were synthesised. Polyurethanes contain only urethane linkages. Poly(ether urethane urea)s contain degradation-resistant urea linkages with lesser percentages of urethane and ether linkages. Poly(urethane urea)s contain degradation-resistant urea linkages and lesser percentages of urethane linkages. All the polymers are segmented block copolymers having polyol soft segment and diisocyanate-chain extender hard segment. The isocyanate index used in the syntheses of most of the polymers is very low (1.08). Therefore, the structure of all the polymers is invariably linear structure.

Section IV-2 deals with the physico-chemical, surface and mechanical and thermal characterisation of the newly developed polyurethanes. The FTIR- ATR spectra of polyurethane-ureas reveal responses for bonded urethane carbonyls and bonded urea carbonyls (ordered) suggesting hydrogen-bonding interaction between urea-urea and urethane-urea groups resulting to a greater degree of microphase separation in these polymers. The extensive hydrogen-bonding interaction enables three-dimensional network of hydrogen bonds. The three dimensional network of hydrogen bonds leads to greater micro phase separation of hard and soft segments. The three dimensional network in hard segments appears as hard domain. WAXD results also favor the formation of ordered crystallites in these poly(urethane urea)s. The FT-IR spectral analyses of poly(ether urethane urea) reveals hydrogen-bonding interaction between the hard and soft segments (urethane-ether, urethane-urea, urea-ether and urea-urea groups) resulting to a lesser degree of microphase separation in these polymers. The thermal analysis of poly(urethane urea)s does not exhibit any softening characteristics. Poly(urethane urea)s and poly(ether urethane urea)s undergo swelling in solvents. These findings indicate that these polymers are virtually cross-linked through hydrogen bonds. The relatively higher surface water contact angle

(>70) of these polymers suggests hydrophobicity on the surface. Tensile property measurements indicated that all the polymers are low elastic modulus. The elastic modulus lies in the range 4-8 MPa, which is in the acceptable limit of elastic modulus for cardiac applications (5-8 M Pa). Shore hardness also indicated values of range 60-90 A.

Section IV-3 deals with the results and discussion on the studies on biostability of polymers. Polyurethane having only urethane linkages undergoes change in properties drastically in simulated physiological conditions. However the virtually crosslinked polyether urethane urea and poly(urethane urea) undergo segmental rearrangement leading to slight change in mechanical properties in simulated physiological conditions. There is no evidence for bond breaking at the degradation susceptible urethane, urea or ether groups in the IR spectra of aged poly(ether urethane urea) and poly(urethane urea) polymers. The change in mechanical properties of aged polymers in lipid medium is due to the plastisizing effect of the lipid absorbed and the rearrangement of crosslinks resulted from different hydrogen bonding interactions. The studies on the aging of polymer under induced-strain in hydrolytic enzyme medium reveal interesting information. The polymer strained (tension) for long-term undergoes unidirectional reorganisation of polymer chains leading to increase of elastic modulus. The spectral studies of the aged polymer indicate that unsaturated double bonds of hydro carbon polyol units in the present polymers are not only resistant to significant oxidation but also protect ether soft segment of PTMG units. The studies with accelerated chemical degradation reveal degradation of poly(ether urethane urea)s. However no degradation (weight loss) or dimensional change was observed with poly(urethane urea)s.

Section IV-4 deals with the results and discussion on the studies on calcification. The virtual crosslinking of poly(urethane urea)s plays a role on calcification. For poly(ether urethane urea)s the combined effect of complexation of ether soft segment and virtual crosslinking determines the calcification.

Section IV-5 deals with the results of the studies on biocompatibility and blood compatibility. The cytocompatibility to mouse fibroblast cells and haemolytic potential. The multiplication of viable fibroblasts in both direct and indirect (extract) close contact of polymers and preservation of their cellular morphology suggested that the materials were non-cytotoxic. The percentage haemolysis of all the polymers lies within the acceptable limit (5%). The studies on blood -material interaction *in vitro* also reveal that the present polymers are more blood compatible than polystyrene (control). Section IV-5 also reports the *in vivo* toxicological screening data. The systemic toxicity studies of the polymer do not elicit adverse systemic response. The intracutaneous irritation tests also indicate favourable response. The studies on intramuscular implantation of polymer in rabbit and gross investigation of harvested tissue along with implant reveal no adverse tissue reaction. The studies on *in vivo* biocompatibility and toxicological screening reveal biocompatibility.

Section IV-6 deals with the studies on the functional performance of the candidate poly(urethane urea) in terms of *in vivo* biostability, endothelial cell response and flex life of the polymer. The candidate poly(urethane urea) implanted subcutaneously in rat has shown no sign of change in colour or weight. The mechanical properties of the implanted sample have revealed no drastic change in elastic modulus; the elastic modulus lies within the required range of elastic modulus, 5-8 MPa intended for the polyurethane heart valves. The water contact angle of the

implanted sample reveals no drastic change in hydrophobicity. The FT-IR studies of the implanted sample showed no significant indication for the degradation at urethane, urea, and unsaturated double bonds of polyol soft segment. The scanning electron microscopic pictures reveal absence of surface degradation either in the form of pitting or micro-cracks. The studies on endothelial interaction with the solid candidate poly(urethane urea) reveal the candidate poly(urethane urea) favours appreciable endothelial growth on the surface. The studies on the flex life of the candidate poly(urethane urea) reveal flexural endurance up to 721 million cycles of flexing. However the poly(ether urethane urea) survived up to 351 million cycles of flexing. Considering that the human heart valve leaflets undergoes flexing for 40 million cycles per year, it is estimated that the new candidate poly(urethane urea) would survive flexing for 18 years. The excellent performance of high flex life of this polymer is due to the virtual crosslinking through hydrogen bonding between urea-urea, urea- urethane, and urethane-urethane linkages, hydrophobicity and phase-separated structure

Finally, the Chapter V deals with summary, conclusion and future prospects of the investigation. The potential application of the candidate poly(urethane urea) as polymeric heart valve and the relative merits of such a device over the existing commercial devices is emphasized in this section.

CHAPTER I

INTRODUCTION AND BACKGROUND

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1.1 Biomaterials

Biomaterials are materials of synthetic or natural in origin used alone or in combination with drugs as part of a device in the treatment, augmentation, or replacement of tissues or organs without causing acute or chronic harm to the host, while maintaining their intended biological and physical effectiveness during the useful service life *in vivo* (Bruck,1997). According to another definition, a biomaterial is a nonviable material used in a medical device intended to interact with biological systems (Williams, 1987). There are three fundamental properties that a biomaterial should possess; mechanical strength, functional characteristic and biocompatibility (Courtney *et al,* 1995). Mechanical strength is required to retain adequate level of performance. The functional characteristic is required so that the material has the specific property to perform the required task. What distinguishes biomaterials from other class of materials is their ability to remain in a biological environment without damaging the surroundings and without itself getting damaged. The selection of biomaterial for a given end-use must be based on several criteria such as physico-chemical properties, biomechanical properties, function desired, physiological environment, expected durability etc.

Injury, illness or degeneration of a tissue function and its assembly to ultimately an organ often represents life-limiting situations. Surgical interventions designed to correct this matter or eventual transplantation of a graft or a whole organ from a suitable donor are successful, but availability of a 'replacement organ', its compatibility with the host and the healing which must follow the implantation often pose serious questions. Currently, in the United States, over 70,000 patients are waiting for all kinds of donor organs. With ~4,000 donor organs available annually (Jennings, 1998), many patients will have no chance to become recipients. According to the estimates of the Commerce Department of USA, the global market for medical devices amounted to US$ 130 billion in 1996 and the figure might have doubled by AD 2001. Trade literature estimated the Indian market at US$ 680 million in 1995 with a projected growth rate of 15-20% annually (Kader and Preiestly, 1997). It has been claimed that biomaterials are being used to produce 2700 different medical devices, 2000 diagnostic products and 1500 disposables around the world (Valiathan *et al,* 1999). Biomaterials applications cover the entire field of medicine and every aspects of patient care.

The first implanted material was gold plate for cleft palate in 1588. Other metallic materials such as silver, platinum, stainless steel and cobalt alloys were mainly used in biomedical applications till polymer industry was developed. The first synthetic polymer to be used as biomaterial was polymethyl methacrylate (PMMA) for dentures. However, currently biomaterials include metals, natural and synthetic polymers, ceramics, composites, pyrolytic carbon materials and biological tissues. Of these biomaterials, polymers represent the largest class (Szycher, 1991). The success of a biomaterial *in vivo* depends on material properties, functional performance,

biodurability and biocompatibility and hence these aspects should be rigorously satisfied.

Biocompatibility is a *sine qua non* for all biomaterials. Clearly, there is no unique definition of biocompatibility that would fit all types of medical implants and devices. According to Williams (1987), biocompatibility is the ability of a material to perform with an appropriate host response in a specified application. Biocompatibility includes both blood-compatibility and tissue-compatibility. Blood compatibility is less well defined than the term biocompatibility, and there is no widely accepted definition also (Ratner, 1994; Ratner, 2000). It is often defined as what should not occur including thrombosis, destruction of formed elements and complement activation. Tissue- compatibility is the favourable tissue responses to biomaterials. Otherwise, biocompatibility involves the chemical interactions, not yet fully under stood, that takes place between material and the body fluids and the physiological tissue-response to these interactions. Biocompatible materials should produce responses that can be acceptable to the living physiological environment. In short, biocompatibility can be viewed as the ability of a material to exist within the living body without adversely and significantly affecting the body and without the material itself suffering any adverse and significant effect.

A new material should be evaluated for toxicity at the early stage of its consideration for biomedical application. The final device-form should again be subjected to toxicological evaluation to ensure that the procedures involved in the fabrication, processing, sterilization etc. have not introduced substances that alter its biocompatibility. The United States Pharmacopoeia (USP, 1995) includes a group of biological tests for plastics. The American Society for Testing and Materials (ASTM)

has published specifications and guidelines related to medical materials and devices, covering physical, chemical and mechanical properties and also biological evaluation (ASTM, 1998). Thus, biocompatibility evaluation of biomaterials is an extremely important part of their development for biomedical applications (VonRecum, 1999).

1.2 Polymeric Biomaterials

Polymers claim the major share of biomaterials and their medical applications already occupy the fourth-largest place among the industrial use of polymers. Polymers have been widely used for biomedical applications due to their favourable structure-property relationships (Tanazawa *et al,* 1993). Initially, the polymeric biomaterials available were limited to natural polymers and biopolymers. Progress in polymer science and technology opened up variety of synthetic materials that are available for medical use. Since the density and mechanical properties of many of the synthetic polymers resemble those of biological tissues, more closely than for metals, they were widely accepted as biomaterials. Although hundreds of synthetic polymers are available, only ten or twenty polymers are mainly used in medical device fabrication. They are mainly silicon rubber, polyvinyl chloride (PVC), nylon, polytetrafluroethylene (PTFE), polyethylene terephthalate (PET) and of course polyurethanes. Apart from favourable mechanical, thermal, electrical and optical properties polymers possess other important characteristics such as light weight, flexibility, resistance to impact and breakage, processability etc.

The main draw back with polymeric biomaterials for long-term use is their susceptibility to degrade or depolymerise under *in vivo* environment and undergo change of properties. The degradation products or the monomers of these polymers

may be toxic to the tissues. These changes in properties of polymeric implants under long-term are attributed to the change in molecular weight and structure. In body environment the biochemical agents such as ions (-OH, metallic ions), dissolved oxygen, free radicals, lipids and hydrolytic and oxidative enzymes are responsible for the degradation. Effect of biomechanical factors like residual stress and strain is also contributed to the degradation.

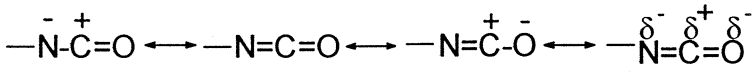
1.2.1 Polyurethanes

Otto Bayer took the initial step that led to the discovery of polyurethane in 1937. Polyurethanes, having extensive structure-property diversity, are one of the most useful industrial and engineering materials known today. They are employed in wide range of applications, including machinery, transport, furnishing, textiles, papermaking, adhesives and sealant and medicine. It was Boretos and Pierce (1967) who first time suggested for employing polyurethane elastomers as biomaterials. Since then this family of polymers has found a wide range of biomedical applications. The outstanding mechanical properties, biocompatibilitiy performance and versatility in terms of processing options and formulation design of polyurethanes makes them one of the most promising synthetic biomaterials. Polyurethane materials have been used in the development of many experimental medical devices ranging from catheters to total artificial heart (Lamba *et al* 1998; Zdrahala and Zdrahala, 1999; Wheatley *et al,* 2000)

1.2.1.1 Chemistry of Polyurethane Synthesis

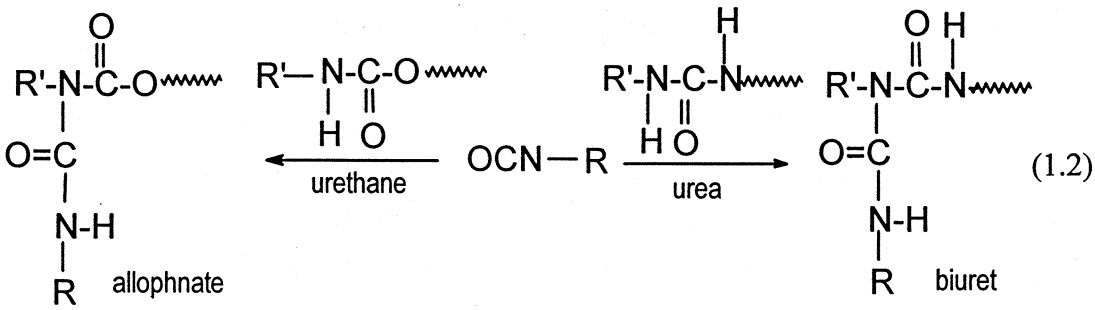
The chemistry involved in the synthesis of polyurethane centers around the basic chemical reactions of isocyanate group. The isocyanate group is very reactive

and its electronic structure consists of several resonance structures as given in equation 1.1



(1.1)

Because of the existence of multiple structures, several classes of reactions involving the isocyanate group are possible. Most reactions such as adduct formation, oligomerisation, cycloaddition and insertion reactions involve addition at N=C double bond. The undesirable side reactions during the synthesis of polyurethanes are insertion reaction and oligomer formation reactions. Dusek *et al* (1990) reported the possible network formation of polyurethanes due to side reactions. If excess of isocyanate is present, the newly formed urethane groups react with excess of isocyanate to form allophanate linkage. Like wise, urea groups react with isocyanate to yield biuret linkages as given in equation 1.2. Both the allophanate and biuret linkages are thermally labile and dissociate at elevated temperatures.



(1.2)

The second type of side reaction involving isocyanate group is the formation of oligomeric species, under special conditions. Dimerisation of isocyanate groups (uretidione) can occur in the case of aromatic diisocyanates. Formation of thermally

stable trimers (isocyanurates) can be possible from aliphatic and aromatic diisocyanate if excess of isocyanate in the reaction mixture.

1.2.1.2 Synthesis of Segmented Polyurethanes

Polurethanes are a class of heterogeneous polymers containing the urethane linkage analogous to the carbamate group in organic chemistry in the polymer chain. Usually they are prepared via the reaction between diisocynate and polyols (Lamba *et al,* 1998, Lelah *et al,* 1986a). Incorporation of other functional groups into the polyurethane polymer produces properties ranging from rigid hard thermosetting materials to softer elastomers. Synthesis of materials with high molecular weight glycols and aromatic diisocynates yielded the first polyurethane elastomer. Polyurethane copolymers are thermoplastic elastomers composed of short, alternating polydisperse blocks of soft and hard segments The soft segment is generally a low transition temperature polyether, polyester or polyalkyl diol having molecular weight 400-5000. The commonly used polyols for the synthesis of polyurethanes are given in Table 1.1.

Table 1.1 Common polyols for polyurethane synthesis

1. Polyethylene glycol	PEG
2. Polypropylene glycol	PPG
3. Poly(oxytetramethylene) glycol	PTMG
4. Hydroxy terminated poly 1,4- butadiene	HTPBD
5. Hydroxybutyl terminated polydimethyl siloxane	PDMS
6. Polyethylene adipate	PEA
7. Polycaprolactone	PCL
8. Polytetramethylene adipate	PTMA
9. Hydroxy terminated polyisobutylene	HTPIB
10. Polyhexamethylene carbonate glycol	PHMCG
11. Hydrogenated polybutadiene	HPBD

Hard segment is usually a high transition temperature aliphatic or aromatic diisocyanate linked with a low molecular weight chain extender. Most of the commercially available polyurethanes are based on aromatic diisocynates MDI or TDI. The generally used aliphatic and aromatic diisocyanates for polyurethane synthesis are given in Table 1.2.

Table 1.2 Some commonly used diisocyanates

1. 4,4'-Methylene bis (p- phenyl isocyanate)	MDI
2. 2,4 and 2,6-Toluene diisocyanate	TDI
3. 1,4- Cyclohexyl diisocyanate	CHDI
4. 4,4'- Methylene bis (p-cyclohexyl isocyanate)	H_{12}MDI
5. 3,3'-Bitoluene diisocyanate	TODI
6. 3-isocyanatomethyl-3,5,5-trimethyl cyclohexyl isocyanate (Isophorone diisocyanate)	IPDI
7. 1,5-Naphthalene diisocyanate	NDI
8. 1,6-Hexamethylene diisocyanate	HDI

The chain extenders are either diols or amines, which produce additional urethane or urea segments respectively. Polyurethanes exhibit broad range of mechano-physical properties, due to the variation in the nature, molecular weight and composition of components.

Polyurethane polymerisation reactions contain features of both addition and condensation mechanisms. Although no small molecule is eliminated during polymerisation, the reaction between the diol and diisocyanate is a condensation reaction as the kinetics of the polymerisation reaction more closely resemble the condensation polymerisation than addition polymerisation. This type condensation polymerisation, where no condensation product is formed is called polyaddition or rearrangement polymerization (Brydson, 1995). Most common method for synthesis,

particularly of biomedical polyurethanes, is the prepolymer method consisting of two steps. The first step involves the reaction of the diol and excess of diisocyanate to produce an isocyanate terminated prepolymer molecule. The prepolymer generally has low molecular weight. Subsequent reaction of this prepolymer with diol or diamine chain extender constitutes the second step, which produce the block copolymer called segmented polyurethanes.

1.2.1.3 Linear and Crosslinked Polyurethanes

Linear polyurethanes are synthesised from bifunctional ractants such as glyols and diisocyanates. A low molecular weight chain extender is employed in order to prevent crosslink formation. The polymer synthesis may be performed at 80 ^{0}C to prevent allophanate and biuret formation. The lack of crosslinks in the polymer means they are melt or solvent processable and thermoplastic. In order to prepare crosslinked polyurethane, excess isocyanate is used in chain extension step so that side reactions such as allophanate and biuret formation occur. Use of crosslinking agents and multifunctional reactants will also effect in the formation of crosslinked polyurethanes (Dusek, 1990). Crosslinked polyurethanes contain a significant number of covalent bonds that bind the polymer chain intermolecularly. Crosslinks through covalent bond are extremely strong and thermally stable. The extent of crosslinking within polyurethane will affect its mechanical properties. Crosslinking affects the mobility of polymer chains. As the degree of crosslinking increases, the modulus increases, as does the hardness; the materials no longer behave as elastomers, but start to resemble rigid thermosetting plastics.

Chemical crosslinking can be introduced either in the hard segment using a short polyol (functionality >2) as the chain extender or in the soft segment using long

triols or higher functionality polyols. The short triols used as chain extenders such as trimethylolpropane (TMP), combined with MDI, lower the crystallinity of the hard segment in comparison with linear diols and yield lower strength elastomers. Crosslink density plays role on the properties of chemically crosslinked polyurethanes. Petrovic *et al* (1991) have reported that tensile properties increased with increasing degree of crosslinking.

The crosslinked polyurethanes have received more attention for long term surgical implants also. Adiprene LW500® polymer is aliphatic polyurethane with a small amount of TMP crosslinker. Therefore, polymer has low flex life. Similarly Avcothane 51® (crosslinked silicone urethane copolymer) is also has low flex life (Nelson *et al,* 1972). Avcothane 51® has no change in molecular weight after six months subcutaneous implantation in rats (Nyilas, 1972) and is more hydrolytically stable than its linear counterpart. Crosslinked polyurethanes prepared by step growth polymerization of PEG precursor and aliphatic or aromatic polyisocyanate can be used as potential biomaterials (Gnanous *et al*, 1983). Studies on aliphatic crosslinked polyurethanes for various biomedical applications have been carried out in our laboratory. A series of biostable aliphatic crosslinked polyurethanes based on polydiols such as PEG-1000, PPG-2000 and PTMG-2000 and diisocyanates such as saturated diphenyl methane diisocyanate (SMDI), isophorone diisocyanate (IPDI) and 1,6-hexamethylene diisocyanate (HDI) have been prepared via crosslinking through trimethylol propane (TMP) and evaluated the tissue interactions for long-term implant applications (Shunmugakumar, 1991). Effect isocyanate or polyol crosslinker on the stability castor oil based potting compound were studied for potting of hollow fiber bundles in a dialyser housing component for the development of a haemodialyser

(Jayabalan, 1997). Crosslinked plyurethanes based on HDI-TMP adduct with polyols (PPG) can be used as a potential adhesive for potting of haemodialyser and oxygenator (Thomas *et al,* 2001).

1.2.2 Biomedical Polyurethanes of the Past Years

All the polyurethanes produced in the past years were mostly aromatic polyether urethane. Aromatic polyurethanes, Biomer [(Ethicon, Sommerville, USA), a poly(ether urethane urea) based on 4,4' methylene bis(phenyl isocyanate) (MDI), polytetramethylene glycol (PTMG) and ethylene diamine (EDA)], Pellethane [(Dow Chemical), a poly(ether urethane) based on MDI, PTMG and butane diol(BD)], Lycra [(Du Pont, Hemel Hempstead, UK), a poly(ether urethane urea) based on MDI, toluene diisocyanate (TDI), PTMG and EDA] etc were used for the development of biomedical devices. Biomer® can not be processed by melt extrusion or injection molding as this polymer undergoes thermal degradation during these processes. Extruded grade Biomer based on MDI:PTMG and water is no longer available now. Cardiothane 51® or Avcothane® is a block copolymer of 90% poly(ether urethane) and 10% polydimethyl siloxane. Cadiothane® possesses a reasonable degree of blood compatibility, but it has a lower flex life and creep resistance compared to Biomer®. Pellethane 2363® offer the advantage over solution grade Biomer® and Avcothane® that can be fabricated by injection moulding, compression moulding or solvent casting. However, pellethane® is inferior with respect to blood compatibility than Biomer® and Avcothane® (Nyilas, 1972). However due to catastrophic failure of pellethane® coated pacemaker lead wire insulation, the use of pellethane® has been restricted to implantation periods up to 29 days only.

In 1979 Tecoflex® (Thermedics Inc., Woburn, MA), a so-called 'second generation biomedical grade' thermoplastic polyurethane elastomer was developed by Szycher *et al* (1977). Tecoflex® is a cycloaliphatic polyether urethane produced from hydrogenated MDI, PTMG and BD. Three melt processable grades are available- Tecoflex EG-80A,EG-60D and EG-70D. Tecoflex® is claimed to be haemocompatible, thrombo-rsistant, nontoxic and noncarcinogenic. However, Stokes (1988) found that Tecoflex® showed severe cracking in 19 % of the test samples compared to Pellethane® under accelerated *in vitro* experimental conditions under accelerated *in vitro* experimental conditions.

Mitrathane® is polyether urethane urea with chemical structure similar to that of Biomer®. Vascular prostheses produced from Mitrathane® and implanted in dogs were occluded by thrombosis after six months, and were degraded on the external surface (Paynter et al 1987). Toyobo TM5® segmented polyurethane urea is based on MDI, PTMG and propylene diamine. This polyurethane has similar tensile strength and ultimate elongation to Biomer®, although its flexibility is inferior (Hayashi et al 1984). This material has been tested in LVAD pump diaphragms implanted in goats. The results indicated a significant drop in tensile strength due to the effect of blood contact. Mechanical properties of some commercial polyurethanes are listed in Table 1.3.

Table 1.3 Mechanical properties of commercial polyurethanes

Property	Biomer	Cardiothane	Cardiomat	Pellethane	Tecoflex
Ultimate tensile strength (psi)	4000-6000	6350	4070	5000-7000	6100
Ultimate Elongation (%)	600-800	525	500	350-600	580
Tensile modulus at 100% stain (psi)	400-800	--	1190	500-2000	410
Shore hardness	75A	72A	80A	80A,90A, 55D,75D	80A

1.2.3 Biocompatibility of 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 relation ship of polyurethane for biomedical applications.

Adsorption of plasma proteins on to the artificial surface is the first event to occur when blood contacts, a biomaterial surface. The adsorbed protein layer influences blood compatibility (Szycher, 1983; Brash, 1991). Since albumin passivates while fibrinogen activates surfaces, some investigators have studied the competitive adsorption between these two. Albumin was adsorbed more rapidly on a hydrophilic PEG-based-urethane than on a more hydrophobic PPG-based-urethane (Brash *et al,* 1974). It was also reported that protein adsorption on to polyurethanes containing polyethylene oxide soft segments is lower than on polyurethanes containing polytetramethylene oxide (PTMO) soft segments (Silver *et al,* 1994).

Polyol soft segment chemistry has much influence on the biocompatibility of polyurethane. Lelah *et al* (1986) found that PEG based polyurethane is more thrombogenic than the corresponding PPG and polytetramethylene (PTMG)-based-materials. Takahara *et al* (1985 b) 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 sereis. Lyman *et al* (1972) synthesized aromatic polyurethane based on MDI with PPG of different molecular masses (Mn = 425,710,1025 and 2025) and amine chain extenders, ethylene diamine / hexamethyne diamine (Lyman *et al* 1972). Tissue culture experiments using fibroblast cells indicated the normal cell growth on the surface of their PPG (Mn =710)-based-polyurethane when compared to others. Brash et al found that the fibroblast cell growth on polyester urethanes (Estane) is slightly lesser and platelet adhesion is slightly higher than on PPG or PTMG polyol-based-polyetherurethanes (Brash *et al* 1985)

Microphase separation also greatly influences the biocompatibility of polyurethanes. It is governed by the molecular nature of the groups present in the matrix. Surface morphology is often controlled by the microphase separation. Takahara et al found that the microphase sepatation in polyurethaneurea is strongly determined by the number of methylene units in the diamine chain extender (Takahara *et al,* 1985 b). The domain size of hard segment of this poly(urethane urea) increases slightly with the increase in diamine length. The polymer chain extended with ethylene diamine is more thromboresistant when compared with that extended with butane diol. This is attributed to the higher phase separation of the amine extended polyurethneureas (Takahara *et al,* 1985 b). Grasel and Cooper et al synthesised a

series of polyurethane with different types of soft segment and hard/soft segment ratio to study the effect of microphase separation on biocompatibility (Grasel and Cooper, 1986). They found that the hard segment to soft segment ratio in PEG and polydimethyl siloxane (PDMS) based polyurethanes did not influence the biocompatibility. With PTMG and PPG-based-polyurethaness, 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. Marchant *et al* (1984) have studied the in vivo biocompatibility of Biomer and other materials by cage implantation technique.

Tissue compatibility of implant is determined by the interactions at the tissue-implant surface. Subsequent to the protein adsorption and conformation, an acute inflammatory response is characterized by the presence of polymorphonuclear leucocytes (PMNs). Polyurethanes, like any other biomaterial elicit tissue reactions. This is followed by the chronic inflammation, which includes wound healing mechanisms and foreign body reactions. Anderson hypothesized that multinuclear giant cells are formed via macrophage activation (Anderson, 1988). Polyurethane like many other materials become encapsulated in a fibrous capsule. Studies on explanted breast implant coated with polyurethane foam revealed a foreign body reaction, fibrous capsule formation and degradation. It can be concluded that implant factors such as surface chemistry, size, shape, pore size, surface texture and the tissue surrounding the implant can affect the polyurethane-tissue responses (Mohanty *et al,* 1992).

1.2.4 Biodegradation of Polyurethanes

Polyurethanes are used in many biomedical applications where the long-term stability of the material is mandatory. But biodegradation of polyurethane implants leads to the catastrophic failure of these medical devices. Biodegradation can occur by many different routes. Agents responsible for the degradation of polymers include water, salts, peroxides and enzymes. Polymer degradation in chemically active media (plasma & tissue) generally includes the following processes. 1. Adsorption of medium on the polymer surface, 2. Diffusion and absorption of the medium in to the bulk. 3. Chemical interaction with unstable bounds in the polymer 4. Transport of the degradation products from the polymer matrix and surface.

1.2.4.1 Biodegradation Mechanisms

Aromatic MDI-based-polyurethanes undergo thermo-hydrolytic degradation during autoclaving and radiation sterilisation producing methylene dianiline (MDA), a suspected carcinogen (Luu and Hutter, 2000; Shintani, 1995). The implants of these polyurethanes also suffer from the oxidative degradation at the ether linkages and hydrolytic degradation at the urethane linkages during the long-term use.

The important mechanisms for the degradation of polyurethane implants are believed to be hydrolysis, oxidation and environmental stress cracking. Hydrolysis is one of the dominant mechanisms of polyurethane degradation in aqueous environment. It is the reversal of condensation reaction of polyurethane formation (Smith *et al,* 1987). Hydrolytically labile bonds (ester and urethane linkages) of polyurethane are prone to hydrolytic degradation. Rate of hydrolysis varies over wide range and is dependent on the chemical structure of the polyurethane. Schollenberger and Stewart (1971) have extensively investigated the hydrolytic stability of polyester,

polycaprolactone and polyether urethanes and found that the hydrolytic stability can be ranked in the order: polyether >polycaprolcatone> polyester. The reaction is acid catalysed. Since an acidic group is generated, the reaction is also autocatalytic. Hydrophilic polyurethanes will be more susceptible to hydrolytic degradation than hydrophobic polymers. Failures of Meme® breast implant coated with Microthane®, a polyester soft segment-based-aromatic polyurethane has been reported by many investigators (Slade and Peterson, 1982; Sinclair *et al,* 1993; Steinbach *et al,* 1993). Presence of toluene diamine (TDA) was detected in the extract of polyester foam-shells-covered breast implants (Batich *et al,* 1989). Later Szycher confirmed the presence of TDA in the first four days of exposure of polyesterurethane foam to papain solution (Szycher and Siciliano, 1991). Hydrolytic instability of polyester urethanes is due to the highly strained molecular configuration. Wang *et al* (1997) identified the principal biodegradation products of an experimental polyester urethane-urea by cholesterol esterase. Various degradable polyester polyurethanes are synthesized and evaluated for possible biomedical applications (Skarja and Woodhouse, 1998; Saad *et al,* 1997). Because of hydrolysis, physical and mechanical properties of the material can be affected through reduction in molecular weight. Though polyester based materials have not been used as permanent implants they have been used in biomaterials for temporary uses like wound dressing and gastric bubbles. Polyether-based polyurethanes and polycarbonate-based polyurethanes are resistant to hydrolysis than polyester based polyurethane due to greater phase separation in them. However, poly(ether polyurethane) and polycarbonate urethane are subject to enzyme-induced hydrolytic degradation (Wang *et al,* 1997; Tang *et al,* 2001).

Degradation of polyether polyurethane is more associated with oxidative process than with hydrolysis. Catastrophic failure of polyether urethane (Pellethane®) coated cardiac pacing lead was the first manifestation of clinical failure of polyurethane device (Stokes, 1985). Recently Wiggins (2001) also reported the clinical-biodegradation of polyether polyurethane inner insulation in bipolar pacemaker leads. The different mechanisms of oxidation include autooxidation, oxidation by peroxides and free radicals, by enzymes and metal induced oxidation (MIO). In polyether polyurethanes, cleavage occurs at α-methylene position of soft segment, by abstracting a hydrogen atom from methylene carbon as shown in equation 1.3.

$$R\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}O\text{-}R' + X^{\cdot} \longrightarrow R\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH\text{-}O\text{-}R' + HX \qquad (1.3)$$

Polyether urethanes are sensitive to metal induced oxidative degradation (MIO). The phenomenon of MIO (Stokes *et al,* 1985) and environmental stress cracking (ESC) were the two failure mechanisms led to degradation of polyether part of pellethane® (Stokes and Davis, 1985). Metal ions produced from the corrosion products of devices (reaction of metallic part with peroxide, superoxide from macrophage & other phaocytic cells) can complex with polyether soft segment of polyurethane resulting in a conformational change and subsequent embrittlement of the material (Philips and Thoma, 1989). Cobalt and molybdenum have been implicated as the promoters of MIO. Metal-ion-induced oxidation can occur by anaerobic process as well as by auto oxidation. In anaerobic mechanism, transition metals having oxidation potential greater than 7 volt initiate polymer oxidation

through the abstraction of hydrogen (Stokes *et al,* 1995). Dunkel and Bhar (1977) evaluated the *in vitro* stability of Biomer®, Pellethane 80 A®, PUX® and Avcothane 51® in bovine blood. All the polymers showed some degradation after 52 weeks immersion in blood. Lemm *et al* (1980; 1981) have found that the average molecular weight of Biomer® decreased to 50% of its original value after enzymatic exposure for 6 months implantation. Schubert *et al* (1997) established the role of oxygen in the biodegradation of polyether urethane urea elastomers by *in vitro* aging of polymers in $H_2O_2/CoCl_2$ medium and also suggested the use of antioxidant, vitamin-E to improve biostability (Schubert *et al,* 1996).

1.2.4.2 Biological Factors of Degradation

Enzymes and other biological catalysts released by the cells form the major factors of *in vivo* degradation of implants. Cells release many biochemically active compounds that can affect the degradation of polyurethane implant *in vivo* (Labow *et al,* 1995). Both macrophages and neutrophils will attempt to engulf foreign material and digest it through either on oxygen-dependent or oxygen-independent mechanisms. Kaplan *et al* (1994) found that biomaterial activates neutrophils resulting in superoxide release. These activated cells release superoxide anions, hydrogen peroxide, hydroxyl radicals and a host of halogenating substances, which are the main agents of degradation of polyurethanes (Zhao *et al,* 1991; Stokes *et al,* 1990; Thoma and Philips, 1987). The oxygen-independent mechanisms appear to involve hydrolytic enzymes. The enzymes released by the neutrophils appeared to interact preferentially with the hard segment but the soft segment also was being hydrolyzed (Labow, 1995). Papain (a protease, tripsin (a hydrolase), chymotripsin (a hydrolase), esterase, etc. degraded polyurethane *in vitro*. Smith et al investigated the

degradation of polymers including polyurethanes using enzymes like papain, trypsin and chymotrypsin (Smith *et al,* 1987). Santerre *et al* (1993) also compared the degradation of poly(ester urethane urea), poly(ether urethane urea) using radiolabelled tracers in the presence of cholesterol esterase collagenase and xanthine oxidase and found that the poly(ester urethane urea) was sensitive to cholesterol esterase. Takahara *et al* (1992) studied biodegradation of aromatic poly(urethane urea) having different soft segment chemistry including PEG (1000), PBD(2000),HPBD(2100)and PDMS(1350) with MDI/ED hard segment and 'biostable' polyurethane. They found that all linear polyurethanes except ' Biostable' polyurethane were susceptible to enzymatic attack and undergo appreciable change in tensile (Takahara *et al* 1992). Pellethane 2663-80A® is sensitive to degrade in papain, chymotripsin (Ratner et al 1988) and trypsin (Bouvier *et al* 1991). Phua *et al* (1987) found that Biomer®, also prone to degrade in vitro due to papain enzyme. To date, there has been not much report regarding the effect of enzymes on aliphatic polyurethnes or poly(urethane urea)s. Jayabalan *et al* (1994) studied interactions of cell free enzymes and fungi with experimental crosslinked polyurethanes based on hexamethylene diisocyanate (HDI) and bicyclohexyl methane diisocyanate (H_{12}MDI) containing PEG, PPG and PTMG soft segments. They found that PEG-based-polyurethanes showed marginal loss of tensile properties. They also reported that HDI-based-polyurethanes under went higher loss of tensile strength compared to H_{12}MDI-based-polyurethanes.

The enzymes released by neutrophils interact preferentially with hard segment of polyurethane. As human monocytes differentiate into macrophages cholesterol-esterase enzyme activity is present in increasing levels (Labow, 1998). Wang *et al* (1997) reported the cholesterol esterase-induced enzymatic degradation of poly(ether

urethane). Studies by Tang *et al* (1999) have shown that polycarbonate-based-polyurethanes synthesised with BD and any one of the diisocyanates, MDI, HMDI or HDI were susceptible to enzymatic degradation by cholesterol esterase. Labow *et al* (2000) have reported that though polycarbonate-based-polyurethanes might resist the reactive oxygen species provided by monocyte derived macrophages (MDM) responsible for oxidation, they were not resistant to the great degradative potential shown by the hydrolytic enzymes released from the activated MDM.

Cells appeared to be involved in calcification, surface cracking and environmental stress cracking, the major pathways of degradation of polyurethanes. Mechanisms of biomaterial activation by neutrophils are material dependent; higher the porosity of the polyurethane, the greater is the degradation rate (Merchant *et al*, 1987). Auto oxidation and the resultant surface pitting is due to the foreign body responses associated with biomaterials *in vivo* (Marchant *et al,* 1984; Anderson, 1988, Zhao *et al,* 1992). Recently, Tang *et al* (2001) reported the effect of hard segment concentration towards the enzyme-induced biodegradation of polycarbonate-polyurethanes and Labow *et al* (2001) reported the hydrolytic degradation of polycarbonate urethanes by monocyte derived enzymes.

1.2.4.3 Biomechanical Factors of Degradation

Environmental stress cracking (ESC) is one of the important causes for implant failure. In the case of polyurethane it was first observed in the failure of Pellethane made pacemaker lead wire insulation, the first clinical manifestation of polyurethane degradation (Szycher, 1991 b). ESC occurs under conditions that provide on active chemical agent and tensile stress (Stokes *et al,* 1987). The environment encountered in vivo implantation is a hostile one and thus provides a

number of suitable agents. Stress may be inherent due to the phase separated nature of polyurethanes or may be applied to a device during manufacture, implantation, or through intracorporeal movement. Environmental stress cracking is characterized by deep, ragged fracture within polyurethane occurring perpendicular to the direction of stress. Tissue surroundings the implant that has ESC is often well integrated into the material due to the tissue in-growth into cracks. ESC is not just the result of stress only, but it requires cellular interaction and other chemical agents. Macrophages and foreign body giant cell formation have vital role in ESC. Two different theories have been proposed to explain ESC. The first has been proposed by Sutherland *et al* and it involves hypochlorous acid (HOCl) and nitric oxide (NO). Polymorphonuclear leukocytes (PMN) are the major player in this phenomenon through their activities and subsequent secretion of NO and anti oxidants (Sutherland *et al*, 1993). The second theory gives greater role for the foreign body response and the oxidants secreted by macrophages and foreign body giant cells (Stokes *et al,* 1995).

1.2.4.4 Calcification of Polyurethanes

Calcification restricts the use of polyurethane as a biomaterial for cardiovascular devices. Calcification of cardiac biomaterial is associated with stiffening, failure of flexure and performance (Dostal *et al,* 1990; Glasmacher *et al,* 1987). Calcification is the deposition of calcium phosphate (mainly hydroxy apatite) mineral on material or tissue (Joshi *et al,* 1994; Schoen *et al,* 1988). It has been associated both biological materials, chemically modified for implantation and with synthetic materials. Calcification appears to occur both in material itself and in surrounding tissue associated with the biomaterial. The process of calcification is usually divided into two different types or stages. In the first type nuclear sites are

formed through the adsorption of calcium on to the material surfaces in association with proteins lipids or phospholipids, which are thought to bind calcium (Dostal *et al,* 1990; Hilbert *et al,* 1987; Schoen *et al,* 1988). In the second type, the calcium is associated with cell membranes that appear to form from the injured, degenerated or dead cells. Schoen *et al* (1988) classified calcification based on the site of mineral nucleation as intrinsic and extrinsic. In intrinsic case, the nucleation site is within the biomaterial and in extrinsic case, the site is associated with tissue, thrombus, blood elements, or cellular debris. Both *in vitro* and *in vivo* calcification of polyurethane biomaterial have been reported.

Poly(ether urethane)s, Biomer®, Mitrathane® and Pellethane® have been reported to calcify both *in vitro* and *in vivo* (Joshi *et al* 1994; Hilber *et al,* 1987). Polycarbonate based polyurethanes also higher propensity for calcification (Yang *et al*, 1999). Calcification occurs in prostheic heart valves and blood pumps. Mineral deposition on heart valves causes the material to stiffen and this renders the valve stenotic and regurgitant, compromising the haemodynamic performance and durability. Wiseman et al reported the calcification of polyurethane trileaflet valve implanted in calves (Wiseman *et al,* 1982). Calcification of Biomer heart valve implanted in the mitral position in sheep for up to 21 weeks was also reported (Hilbert *et al* 1987). They observed calcification at the interface between the polyurethane leaflet and microthrombi and fibrous sheath that were on the surface. Lo et al tested heart valves designed with Cardiomat 610®, Mitrathane M 2007® in calves. The maximum duration of survival of animal was less than one year and the problem associated with this failure was calcification, thrombosis and leaflet perforation. An addition mechanism for polyurethane calcification has been proposed based on the

removal and complexation of calcium ion from body fluid on the surface of the polymer (Philips and Thoma, 1989). Polyether soft segment of polyurethane can form complex with Ca^{2+} to form crown ether like structure, leading to increased surface concentration and ultimately surface calcification.

Other factors, which appear to influence calcification, include age of the patient, methods of preparation of biomaterial and mechanical stress and deformation, in addition to cellular adhesion. Stress-strain distribution within material has been correlated to the location of calcification. In blood pump calcification was found with the diaphragm and pump housing. In heart valves, calcification also appears to be associated with areas of mechanical stress. High mechanical stress may damage the structural integrity of the leaflet tissue, creating sites for initiation of mineral deposition at point of stress concentration (Hilbert *et al,* 1987). However it is not clear if repeated flexing causes cell deposition on the surface of the material followed by calcification or if the mechanical changes are causing damage to adjacent tissue resulting in calcification. However, reports showed that polyurethane heart valve exhibits lower degree of both in vitro and in vivo calcification than heart valves prepared from bovine pericardium (Fisher *et al,* 1992). Removal of low molecular weight component has been shown to reduce the degree of calcification (Bernacca *et al*, 1995). Though many investigators studied the modification of polyurethanes to achieve calcification resistance (Han *et al,* 1993; Joshi *et al* 1994; Bernacca and Wheatly, 1998, Alferiev, 2001), there has been no clinical success with these polymers.

1.2.5 Attempts to Improve Biocompatibility and Biostability of Polyurethanes

In order to improve the biostability and biocompatibility of polyurethanes, several efforts have been made by various investigators. Techniques such as bulk and surface modification of polyurethanes provides the major routes of modification. Bulk modification is achieved through the employment of different reagents from which the polymer is synthesized. Chemical modification is achieved for the whole polymer after polymerisation from constituent monomers. Such modifications include altering the chemical structure of diisoyanate, soft segment or chain extender. Other possible routes include derivatization of polyurethane to incorporate specific chemical groups. Cooper and coworkers incorporated sulphonate, carboxyl and tertiary amino groups on to polyurethane surface for the improvement of biocompatibility (Okkema *et al* 1991; Goddard and Cooper, 1995). These modifications were achieved through alterations in hard segment of soft segment chemistry. Santerre and Brash (1991) have altered the biological properties of polyurethanes by incorporating a biphenyl disulphonic acid chain extender in polyurethane in order to incorporate suphonate groups to get the desired biological properties. Addition of additives and stabilizers such as antioxidants has been utilized to improve the oxidative biostability of poly(ether urethane)s elastomers for long term in vivo use. Use of biological antioxidant, Vitamin E was reported to be useful to improve biostability of polyurethane urea by inhibiting oxidation and crosslinking of the polyether soft segments (Schubert *et al,* 1996; Schubert *et al,* 1997). Though use of natural antioxidant enhanced the biocompatibility over synthetic antioxidant, the UV light sensitivity of vitamin-E compromises its use. Blending of the 2-metharyloyloxyethyl phosphorylcholine (MPC) in the segmented poluyrethane was also reported as

effective method to impart nonthrombogenicity by reducing cell adhesion (Ishihara *et al* 1996).

Considerable efforts nave been focused on phospholipid-like diols and their polyurethane copolymers (Nakaya *et al* , Jpn Patent,1986; Chem Abstract, 1987) to wards the improvement of biocompatibility of polyurethanes. Studies on phospholipid polyurethanes containing phosphatidylcholine analogue in side chains (Li *et al* 1995; Yamada *et al*, 1995), in the polymer backbone (Chen *et al,* 1996) and in the main chain (Li and Nakaya, 1997) of polyurethane ureas were also reported. Since phospholipids together with proteins are principal components of biomembranes, these modified polymers exhibited good blood compatibility (Li *et al,* 1996a; 1996b). It was observed that no platelet adhesion was observed for all the new phospholipid polyurethanes. However, weak mechanical properties (ultimate strength 3.92 MPa and elongation at break 1.2%) may be the shortcoming of some of these polymers (Li and Nakaya, 1997)

The end capping of labile polyether urethanes with polydimethyl siloxane PDMS promotes a silicone rich surface due to high mobility and low surface free energy of the PDMS (Mathur *et al,* 1997). Because biodegradation begins at the biomaterial surface, a surface layer of PDMS is desirable to prevent initiation. Its believed that this may be due to a combination of providing a protective barrier and reduction in amount of total cell coverage (macrophage and foreign body giant cells) due to hydrophobicity(Capone, 1992). Mathur *et al* (1997) showed that a polyether urethane end capped with PDMS provided enhanced in vivo degradation resistance than uncapped formulation. FT-IR analysis showed that PDMS end caps did not degrade, but PTMO segments were revealed pitting and cracking in the areas of

specimens under highest strain. It is probable that under strain (400%) the oxidatively suceptible bulk of the end capped polymer could not be effectively protected by the surface layer of PDMS. Martin et al (2000) have reported a more effective way to incorporate PDMS in to a polyurethane system via. oligomeric sequence (>500 Da) in the soft segment of the polymer. They synthesized a series of thermoplastic polyurethanes with varying proportions of PDMS amd polyhexamethylene oxide (PHMO) macrodiols (Martin *et al*, 2000; Meijs, Australian Patent, 1996). Historically, efforts to achieve this has resulted in materials with poor mechanical properties and clarity due to macro-phase separation resulting from poor solubility of the very hydrophobic PDMS sequences (Shibayama *et al,* 1991).

Surface modification of biomaterials is an important method for the improvement of biomaterial performance. Surface modification include the incorporation of other polymers, ionic groups, and grafting of biological molecules such as anti-thrombotic agents, anti-platelet agents, peptides, proteins and enzymes. Many of these modifications have been applied to polyurethane elastomers. Investigators like Ko *et al* (1993) and Lee *et al* (1998) modified polyurethane to contain carboxyl groups, which were subsequently reacted with peptides to promote cell attachment. Surface grafting of PEG and immobilization of heparin on to polyurethane surface has been found to improve haemocompatibility (Han *et al,* 1989; Han *et al*, 1992; Ito *et al,* 1986). Surface modification polyurethane with biologically active substances such as anticoagulants (Ito *et al,* 1986), fibrinolytic enzymes (Ryu *et al*,1995), or by incorporating ionic functionality into the polymer (Santerre *et al,* 1992; Okkema *et a,l* 1991; Chen *et al,* 2000) can also improve blood compatibility. Bernacca et al have also studied the blood comaptibility (Bernacca *et al*, 1997) fatigue

life and calcification (Bernacca and Wheatley, 1998) of polyurethanes surface modified via covalent attachment of heparin, PEG, glucose etc. Studies by Chen *et al* (1999) on grafted polyurethane (based on polybutadiene diol and hydrogenated polybutadiene glycol with MDI bearing glucose groups revealed that glucose groups on the surface were effective for the improvement of hydrophilicity as well as haemocompatibility. A more detailed review on the heparinisation of polyurethane is available by Eloy *et al* (1988).

Other techniques for surface modification of polyurethanes include covalent bonding of long alkyl groups to enhance albumin adsorption (Pitt *et al,* 1988), blending of polymers having tertiary amino groups into polyurethane (Brunstedt *et al* ,1993) and plasma glow discharge to deposit functional groups that can immobilize albumin (Joseph *et al,* 1986), heparin (Kang *et al* ,1996; Kang et al 1997), enzymes (Danilich *et al,* 1992) etc on polyurethane surfaces. Covalent attachment of specific antithrombogenic agent, recombinant hirudin to polyurethane with protein binding sites was also tried to achieve antithrombogenicity (Phaneuf *et al,* 1998) It was surprising to say that none of the polyurethane modifications satisfy both biostability and biocompatibility for long-term applications.

Therefore, to date the success with the synthetic polymeric heart valve has been limited, partly due to limitations in the biostability and biocompatibility and fatigue failure of the polyurethane materials.

CHAPTER II

OBJECTIVES AND SCOPE OF THE INVESTIGATION

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The commercially available mechanical heart valves are slightly thrombogenic that the patient has to take anticoagulants continuously. Bioprosthetic heart valves are less durable due to biocalcification (Schoen *et al,* 1986). Therefore the development of polymeric heart valve which can resist thrombogenesis and biodegradation is the major challenge in the biomedical materials science. Aromatic polyurethanes, Biomer, Pellethane, Lycra etc were used for the development of cardiac devices. W. J. Kolff at the University of Utah has first developed polyurethane valves from Biomer and Pellethane. Akutsu *et al* (1959) have first implanted the polyurethane valves in animals and studied the performance. Gerring *et al* (1974) have conducted long term animal trials with polyurethane heart valves fabricated from Biomer. Ghista and Reul (1977) have also developed polyurethane heart valve from Avcothane-51 and studied the design analysis. Chandran *et al* (1989) have evaluated the polyurethane valves from Biomer and Pellethane developed by the Kolff's Laboratory at the University of Utah and reported that these valves may be used for short - term use in total artificial heart devices as a bridge to transplant. Helmholtz Institute in Aachen, Germany has developed polyurethane heart valves. However in animal trials these Aachen valves failed due to thrombosis and calcification (Herold *et al,* 1987). Mackay *et al* (1996)

have developed polyurethane heart valve from Estane 58201 [(BF Goodrich, 2260 Westerlo - Oevel, Belgium) a poly(ether urethane) based on MDI, PTMG and BD] and reported occurrence of calcification. Bernacca *et al* (1997) also have used Lycra 136C for the development of heart valve. The Lycra valves survived more than 450 million cycles of flexing in fatigue testing. However calcification was observed with and without associated failure in regions of high strain (Bernacca *et al,* 1997).

Aromatic MDI-based-polyetherurethanes, however undergo thermo-hydrolytic degradation and oxidative degradation during the long-term use. Therefore the development of aliphatic polyurethanes for long-term biomedical applications gained importance in the previous decade. An experimental ether-free polyether urethane called 'Biostable PUR' was developed by Medtronic, Inc. 'Biostable PUR' was segmented aliphatic polyurethane without ether linkages in its backbone. It was synthesised from 1,4-cyclohexane diisocyanate, 1,6-hexanediol, 9-or 10-hydroxymethyloctadecanol and dimer diisocyanate. The oxidative resistance of this 'Biostable PUR' is significantly improved (Takahara *et al*, 1991). The cycloaliphatic polyether polyurethane, Tecoflex85A® also undergoes oxidative and hydrolytic degradation during the long-term use. The oxidative and hydrolytic degradation, environmental stress corrosion cracking and calcification observed in the implants of poly(ether urethane)s during the long-term use hampered the development of polyurethane-based cardiac devices ultimately. The court litigation met with the polyurethane mammary prostheses [aromatic toluene diisocyanate (TDI) - based - polyurethane prostheses, Meme® implant, M/s Surgitech Inc] has dampened the development of polyurethane medical devices in recent years. Other court litigations *viz* DuPont legal battle arising from Vitek's use of Teflon in a temporomandibular

joint implant and Dow Corning legal battle arising from the use of breast implants have forced major companies to withdraw the polymers, Silicone, Teflon®, Delrin®, Biolon®, Dacron®, Proplast®, Pellethan®e and Biomer® from the health care market.

Some researchers have developed new generation polyurethanes using alternate chemicals and synthetic procedures. Thermo Cardiosystems Inc., Woburn, MA has claimed to produce Cardioflex®, a clone of Biomer®. Carbo Medics Inc., USA has also claimed to produce a new proprietary thromboresistant polymer. Some researchers have also developed new generation polyurethanes using polycarbonate polyols. Carbothane®PC3570A (Thermedics Inc., Woburn, MA) was developed using an aliphatic diisocyanate and polycarbonate polyol. Chronoflex®AR (Cardio Tech International, Woburn, MA), Corethane® 80A and Corethane® 55D (Corvita Corp., Miami, FL) were developed using aromatic diisocyanates and polycarbonate polyols. Subsequent to this development, researchers have proved that although polycarbonate-based polyurethanes have proven to be more oxidatively stable in comparison to polyether-based polyurethanes (Tanzi *et al,* 1997; Szycher *et al,* 1994), they have been found to suffer from hydrolytic instability at the carbonate linkage. Recent reports (Tang *et al,* 2001; Labow *et al,* 2001) on the biodegradation of polycarbonate-polyurethanes rise doubts on its use for cardiac devices. Moreover the polycarbonate - based polyurethanes, Carbothane® PC 3570A Chronoflex® AR have higher propensity for calcification (Yang *et al*, 1999).

Recently Martin *et al* (2000) have produced a thermoplastic polyurethane using the mixed polyols, poly(hexmethylene oxide) and poly(dimethyl siloxane), diisocyanate, MDI and chain extender, BD and claimed that they have achieved a combination of flexibility, strength and biostability. Elastomedic Pty Ltd. in Australia

has started to produce this polyurethane with the trademark, Elast-Eon ™ commercially. However, Elast-Eon™ consisting segments of silicone and polyurethane could be compared to the polyurethane Avcothane 51 and Enka PUR 923 (ENKA AG) which also contains segments of silicone (PDMS) and polyurethane. Bernstein *et al* (1970) have demonstrated that Avcothane-coated components used in the compact centrifugal pump for the extracorporeal left ventricular bypass have failed in half of the animal trials. AorTech International, plc. has developed the heart valve using Elast-Eon™. The study of Hunt *et al* (2000) has demonstrated that the biostability (flex-life) of Elast-Eon ™ - based heart valve could be enhanced only at the expense of biocompatibilty. The report published by AorTech International on 15th December 2000 revealed that the heart valves fabricated from Elast-Eon ™ has the flexing durability to the extent of 500 million cycles.

At present there is no aliphatic polyurethane available to meet the combined stringent requirements of low elastic modulus, higher flexing durability (>700 million cycles), blood compatibility and biostability. The future development of polymeric heart valve with tri-leaflet design (closely resemble the natural heart valve in fluid dynamic characteristic) and LVAD as commercially viable devices depends on biodurability (flex-life), biocompatibility and biostability of valve leaflet and LVAD membrane in dynamic flexing environment.

The biostability and biocompatibility of polyurethane implants depends on the chemistry of segments, microphase separation, and hydrogen bonding effects. Aliphatic polyurethanes which exclude biodegradable ether linkages but contain minimal urethane linkages and maximum number of urea linkages in the macromolecular structure can offer excellent biocompatibility and biostability.

Polyols containing unsaturated double bonds in such a macromolecular structure can provide required viscoelasticity. In polyurethane containing maximum number of urea linkages, the hydrogen bonding can lead to a three-dimensional network between the hard segments resulting in the formation of microcrystallites. The secondary interactions particularly due to hydrogen bonding between the polymer chains in poly(urethane urea)s are called virtual crosslinking or physical crosslinking. We hypothesis that aliphatic poly(urethane urea) having more urea linkages than urethane linkages can form a virtually crosslinked structure and can provide biostability, biocompatibility and flex-life required for polyurethane component in the cardiovascular applications.

Accordingly it was planned to develop novel aliphatic ploy(urethane urea)s having blood compatibility, biocompatibility, biodurability, resistance to calcification and flex-life for the potential application in cardiac assist devices *viz* polymeric heart valve and left ventricular assist device (LVAD). The detailed objectives of the investigation are:

1 Synthesis and process optimisation of new generation low elastic modulus aliphatic poly(urethane urea)s containing more urea linkages than urethane linkages.

2 Synthesis of poly(ether urethane urea)s containing ether, urethane and urea linkages and polyurethane containing only urethane linkages for comparative evaluation of poly(urethane urea)s.

3 Characterisation of poly(urethane urea)s, poly(ether urethane urea)s and polyurethanes for physico-chemical properties *viz* virtual crosslink density and number average molecular weight between crosslinks, surface properties using

FTIR and XRD techniques and contact angle, thermal properties using TGA, mechanical properties *viz* tensile properties and hardness and viscoelastic properties using dynamic mechanical analysis (DMA).

4 *In vitro* studies on the biostability of these poly(urethane urea)s, poly(ether urethane urea)s and polyurethanes in simulated physiological media by ageing in hydrolytic ionic media, hydrolytic enzyme (papain and its buffer) solution, oxidative medium and lipid media and in harsh chemical conditions.

5 *In vitro* studies on the biomechanical factors on biodegradation of polymers using accelerated environmental stress cracking system and ageing of material in strained condition in papain enzyme and its buffer media

6 Studies on *in vitro* calcification of polymers using metastable calcium solution.

7 *In vitro* biological evaluation of poly(urethane urea)s for cytotoxicity, cell-viability, haemolysis and blood material interaction (haematology).

8 *In vivo* toxicological studies of candidate polyu(rethane urea) for systemic toxicity, intracutaneous irritation and intramuscular implantation for biocompatibility and histocompatibility.

9 Studies on functional performance - Evaluation of flex-life using an accelerated flexural fatigue tester, endothelial cell compatibility and long-term *in vivo* biostability of poly(urethane urea) by subcutaneous implantation in rat.

10 Selection of candidate poly(urethane urea) for heart valve and LVAD cardiac assist devices.

CHAPTER III

EXPERIMENTAL

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EXPERIMENTAL

3.1 Materials

The monomers or raw materials used for the synthesis of new polyurethane systems were cycloaliphatic diisocyanate *viz.* 4,4'-methylene bis (cyclohexyl isocyanate) ($H_{12}MDI$) from Desmodur®, Mobay Chemicals, Pittsburgh, PA, USA, polyhydrocarbon diol, hydroxy terminated polybutadiene (HTPBD), (number average molecular mass 2620), polyether polydiol polytetramethylene oxide glycol (PTMG) (number average molecular mass 1000) from (Q. O. Chemicals, USA) and chain extenders 1,6-hexamethylene diamine (HDA) (Fluka, Inc., USA), and 1,4 butane diol (BD) (E-Merck, India,Ltd). Dibutyl tin dilaurate (DBTDL) (Fluka, Inc., USA) was used as catalyst. HTPBD. PTMG was vacuum dried before use. Spectroscopic / HPLC grade toluene and N, N-dimethyl acetamide (DMA) (SD Fine chemicals India) were used as solvents for polymerization reactions. Unless and other wise mentioned all other solvents and chemicals used for the preparation of various reagents for evaluation of the polymers were of analytical grade.

3.2 Synthesis and Purification of Polyurethanes

The present polymers were synthesized by two step prepolymer method using solution polymerization technique. In a typical experiment, the diisocyanate and

polyol were dissolved in toluene (75 ml) and heated with constant stirring up to 80 ^{0}C for 15-20 minutes in the presence of catalyst DBTDL under inert atmosphere of nitrogen to get the prepolymer. In second step, the prepolymer is cooled to room temperature and chain extender dissolved in DMA (25 ml) was added slowly at high stirring condition. The heating was started and continued to 100 ^{0}C and maintained at 100 ^{0}C for 10-15 min. The final polymer was obtained as highly viscous and transparent solution. In all cases the isocyanate index (NCO / OH) was kept nearly at one (1.08). A stirred reactor (M/s Parr instrument Co, USA) was used for the synthesis of polymers where the process parameters like temperature, pressure and stirring speed can be controlled. Controlling process variables, *viz.* temperature and time of duration of reaction, the process optimisation was carried out to get a high polymer. The final polymer solution was degassed properly and cast into thin sheets in silicon oil-coated stainless steel plate and kept at 60 ^{0} C for 48h for further curing.

The cured polymers were extracted repeatedly with hot ethanol in Soxhlet extractor to remove catalyst and the leachable low molecular weight oligomers, if any.

3.3 Characterisation of Polyurethanes

3.3.1 Evaluation of Physico-chemical Properties

The polyurethanes were evaluated for density, cross-link density and molecular weight between cross-links. The density was determined by sink-float method using water-ethanol mixtures of varying specific gravity as per ASTM D 792-91. The crosslink density was determined from swelling coefficient, which was calculated after subjecting the polymer to attain equilibrium swelling in different solvents with different solubility parameters. The solvent in which the solvent showed maximum

swelling was taken as the solvent for determining the swelling coefficient (Q). In the present polymers maximum swelling was observed in tetrahydrofuran (THF) and therefore, the accurately weighed polymer materials were allowed to swell in THF for 2 days and the increase in weight was measured. The swelling coefficient is the ratio of volume of solvent in the swollen polymer to that of dry polymer and is defined by the relation:

$$\text{Swelling coefficient (Q)} = \frac{\text{Weight of the solvent in swollen polymer}}{\text{Weight of the dry polymer}} \times \frac{\text{Density of polymer}}{\text{Density of solvent}}$$

The volume fraction of the polymer in the swollen polymer (V_r) was calculated from swelling coefficient. The solubility parameter of that solvent (δ_s) which imparts maximum swelling was taken as the solubility parameter of the polymer (δ_p). The cross-link density (γ) of swollen polymers (density of virtual or pseudo crosslinks due to extensive hydrogen bonding) was determined by using modified Flory-Rehner's equation (Flory and Rehner, 1943; Shultz, 1966). The number average molecular weight between cross-links (M_c), which is the reciprocal of crosslink density, was also calculated.

$$\gamma = \frac{-(V_r + \chi V_r^2 + \ln(1-V_r))}{d_r V_o (V_r^{1/3} - V_r/2)} = \frac{1}{M_c}$$

Where

$V_r = \frac{1}{1+Q}$, the volume fraction of the polymer in the swollen polymer.

χ= Polymer-solvent interaction parameter = Lattice constant 0.34 for $\delta_s = \delta_p$

V_o= Molar volume of the solvent

dr = density of the polymer

3. 3. 2 Evaluation of Structural and Surface Properties

3.3.2.1 ATR-FTIR and WAXD Analyses

The new polymers were analyzed by the Fourier transform infrared spectrophotometry (FT-IR) using ATR accessory and wide angle X-ray diffraction studies (WAXD). The clean polymer film was sandwiched in a KRS-5 ATR crystal and the IR spectrum was recorded. A Nicolet impacts 410 FT-IR spectrophotometer was used. The wide angle X-ray diffraction studies were carried out at room temperature using a Siemens unit (Siemens, X-ray diffractometer D 5005). Nickel filtered Cu-K_α radiation was used for obtaining X-ray diffractograms. The circular sheets of polymers were used for the measurement of diffraction angle corresponding to the crystallites. WAXD spectrum is obtained as plot of intensity against angle of diffraction in 2θ scale.

3.3.2.2 Surface Water Contact Angle Studies

The surface hydrophobicity/ hydrophilicity of the polymers was estimated from water contact angle measurements. The measurement of contact angles in an aqueous environment is particularly useful for biomedical materials, as they are employed in an environment that is predominantly water. Water contact angle, an indicator of the wettability of surface was measured by sessile drop method using an optical bench type goniometer (Kernco Instruments Co. Inc., Texas). Drops of purified water (3μl) were placed carefully on to surface of clean polymer film fixed to the glass slides through a syringe. Direct microscopic measurements of the contact angles were taken on both sides of the drop within 3 min., assuming symmetry in the

surface. The results were tabulated as a mean of 12 measurements. The interfacial free energy was derived from standard tables (Neumann *et al,* 1980).

3.3.3 Evaluation of Mechanical Properties

The tensile properties of the polyurethanes were determined as per ASTM D 412 using an Instron Automated Materials Testing system (IX) 1.09. The rectangular samples (7cm x 0.5cm) were tested with gauge length 25 mm and cross head speed of 100 mm / min at room temperature. The ultimate strength, percentage elongation and elastic modulus (stress at 100 % strain) were determined.

The Shore hardness (resistance of the cured materials to the penetration of an indentor of a specific dimension) was also determined with sheets of polymers piled to a thickness of 6 mm using a calibrated shore A durometer as per ASTM D 2240.

3.3.4 Determination of Viscoelastic Characteristics

The viscoelastic properties of the materials were determined from dynamic mechanical thermal analysis (DMA). A sinusoidal mode of deformation is applied to the sample when the sample becomes too soft to test in a given apparatus. The temperature dependence of the dynamic mechanical elasticity of polyurethanes was obtained using a microprocessor controlled Rheovibron (DDV III C) instrument, Japan at amplitude of oscillation 0.1% under a dry nitrogen purge. Samples were cooled to $\approx 120\ ^{0}C$ and data were subsequently taken at a test frequency of 35 Hz and a heating rate of $1\ ^{0}C$/ minute. The dynamic storage modulus (E'), the dynamic loss modulus (E") and the mechanical loss tangent (tanδ) were measured.

3.3.5 Evaluation of Thermal Characteristics

The thermal characteristics of polyurethanes were determined using thermogravimetric analysis (TGA). The polymer samples were heated to above 500 ^{0}C at the heating rate of 10 ^{0}C /min in inert nitrogen atmosphere. A Universal VI 12E TA instrument SDT 2960 was used for the thermal analyses.

3.4 Studies on *in vitro* Biostability and Influence of Biochemical Factors on the Biodegradation

The *in vitro* biodurability of the polyurethanes was evaluated by aging the material in simulated biological environment. The polymers were aged in simulated physiological fluid, Ringers solution, phosphate buffered saline (PBS), lipid and enzyme media. Biodurability of the polyurethanes was also evaluated using oxidising agent.

3.4.1 Evaluation of *in vitro* Hydrolytic Stability

The *in vitro* hydrolytic stability of the polymers was evaluated by aging the polymers in Ringers solution, PBS (Appendix A). PBS was prepared as per the standard procedures (Jayabalan *et al*, 2000; Thomas *et al,* 2000). Solutions of 0.2 M disodium hydrogen phosphate and 0.2M sodium dihydrogen phosphate in 0.9% saline were prepared separately and mixed in the ratio 77:23 (v/v). The pH was adjusted to 7.4 by adding dilute solution in distilled water (1:2) of disodium hydrogen phosphate (if acidic) or sodium dihydrogen phosphate (if alkaline). Ringer's solution (Appendix B) was prepared as per the procedure by Ritchie et al (1990).

Accurately weighed rectangular thin strips (7cm x 0.5cm) of polymers (n=6) were immersed in the hydrolytic media in screw capped bottles and aged for 30 days

at 37 °C. To ensure the activity of the media, the media were changed with fresh solution for every 5 days interval. The aged samples were vacuum-dried and tested for any weight loss. The hydrolytic degradation was assessed from FT-IR spectral analysis, mechanical and surface properties. Aging studies of polymers were carried out in PBS medium 37 °C for 6 months also.

3.4.2 Evaluation of Degradation in Hydrolytic Enzyme

In order to investigate the biostability in hydrolytic enzyme environment, the polymers were aged *in vitro* papain medium and its buffer (control). 10 mg of papain enzyme (2-8 units / mg solid and 1 unit can hydrolyze 1.0 mole -NH-CO- group) was used in 10 ml HEPES buffer of pH 6.8 (Appendix C). The accurately weighed polymer films (n=6) of rectangular shape (7cm x 0.5 cm) were immersed in enzyme and also in buffer (control) media and aged for 30 days at 37 ^{0}C. The activity of enzyme was maintained by adding fresh enzyme (10 mg) for every 3 days. The aged samples were rinsed with distilled water, vacuum-dried at 60 ^{o}C and characterized by FT-IR spectral analysis .The aged polyurethanes were evaluated for change in weight, mechanical and surface properties also.

3.4.3 Evaluation of *in vitro* Lipid Sorption

Lipid adsorption/absorption behavior of the polyurethanes were studied by aging the polymers in palm oil (commercial food grade), a lipid rich medium Palm oil (Koeneman *et al*, 1995) and also in Dulbecco's modified eagle medium (DMEM) (Lykissa *et al*, 1997) with L-glutamine (Appendix D). DMEM contains, additionally, glucose (1g), sodium bicarbonate (3.7 g) per liter) and 1.6 g cholesterol per liter

(supplied by Himedia Labs, Bombay, India). Accurately weighed specimens of polymers were kept in screw capped bottle containing the lipid medium and aged at 37 ^{0}C for 30 days. The samples from palm oil medium were rinsed gently with hexane to remove the excess oil adsorbed on the surface. The aged polymers were analyzed for weight change and tensile properties after drying at 60 ^{0} C.

3.4.4 Evaluation of *in vitro* Oxidative Stability

In vitro oxidation medium was prepared by mixing equal volume of 0.1M silver nitrate and 0.1M sodium lactate solutions as per the procedure published elsewhere (Takahara *et al*, 1991). Accurately weighed rectangular polymer films were immersed in the medium in screw-capped bottles and aged at 37 ^{0} C for 30 days. Since silver ions (standard reduction potential, E^0 = 0.7991 V) have tendency to under go reduction even in the normal condition, the medium was changed for every 5 days interval to maintain the ionic strength (Mueller, 2001; Niemi, 1985). The aged polymers were tested as described in the previous section. The polymers were aged in another oxidation medium, 20% H_2O_2 / $CoCl_2$ solution at 37 ^{0} C (Wu *et al,* 1994) for 6 months also.

3.4.5 Studies on Accelerated Chemical Degradation of Polyurethanes

The stability of polymers under accelerated chemical degradation was evaluated using harsh chemical treatment. Aging of polymers in boiling water for 100 h and also in boiling alcoholic potassium hydroxide (0.5N) for 4h were carried out (Lemm, 1984). Weight loss and visible changes of samples were noted.

3.5 Studies on the Influence of Biomechanical Factors on the Degradation of Polyurethanes

3.5.1 Studies on the Synergistic Effect of Strain and Enzyme on Biostability

In order to investigate the biostability polyurethanes *in vitro* under the combined effect of strain and hydrolytic medium, the polymers strained with 20 % longitudinal strain were aged in hydrolytic enzyme, papain. The hydrolytic enzyme solution comprises 500 mg of papain enzyme (2-8 units / mg solid and 1 unit can hydrolyze 1.0 mole) in 1000 ml HEPES buffer of pH 6.8. The rectangular polyurethane coupons (5 x 0.5 cm) were stretched for the maximum 20 % tensile strain and fixed at their ends over PMMA support by two clamps using stainless steel screws as reported by Fare *et al* (1999). These strained samples were aged in papain enzyme solution at 37^0C for 30 days. The activity of enzyme was maintained by using fresh enzyme medium for every 3 days. Aging of strained-polyurethane was also carried out in enzyme-free buffer solution as a control. The aged samples were washed with distilled water, dried and tested for change in weight, IR and tensile properties.

3.5.2 Studies on Environmental Stress Corrosion Cracking

Studies on biodegradation under environmental stress were carried out *in vitro* using stress-induced polymers. An environmental- stress- corrosion cracking resistance test apparatus was used for aging. The aging was carried out in Ringers solution and PBS media at 50 ^{o}C for 2 days as per the ASTM standard D 1695. The percentage of failure of sample was noted.

3.6 Studies on *in vitro* Calcification of Polyurethanes

It has been reported that mechanical stress can accelerate polyurethane calcification (Hilbert *et al*, 1987). Experiments on calcification were performed using metastable calcium phosphate solution as reported elsewhere (Golomb, 1991; Chandy *et al*, 1994; Vasudev *et al*, 1997). The metastable calcium phosphate solution was prepared in 0.05M tris-buffer of pH 7.4. The concentration product of calcium ($CaCl_2.2H_2O$) and phosphate (K_2HPO_4) in the incubation solution was kept at $3.741mM^2$ (2.499mM calcium and 1.497mM phosphate). The concentration of calcium in the incubation solution was similar to that of total serum levels (10 mg / 100 ml) and the ratio of Ca / PO_4 1.67 was similar to that in hydroxyapatite. Ultrasonically cleaned coupons of polyurethane polymers (5x 0.5 cm size, n=5) were stressed equally by bending the specimens in screw capped test tubes containing the calcification solution. After the incubation for 30 days at 37 ^{0}C, the polymer strips were taken out from the solution and dried at 110 ^{0}C for 2 h. The deposits on the surface of polymer strips were hydrolysed by exposing the aged polymer strips in 2 ml HCl (2N) solution at 60 ^{0}C for 48 h as reported elsewhere (Golomb,1991; Vasudev *et al*, 1997). The calcium and phosphate concentrations were determined from HCl hydrolysate by spectrophotometric method using standard kits (Dr. Reddy's Diagnostics Division, Hyderabad, India).

Diagnostic calcium kit contains a buffer solution, a colour reagent and a standard calcium solution (10-mg%). The concentration of calcium has been determined quantitatively by converting it into red coloured cresolphthalein complex and measuring at 570 nm. Diagnostic Phosphorous kit contains a catalyst solution,

molybdate reagent, metol reagent and a standard phosphorus solution (5mg%). The concentration of phosphorous has been determined quantitatively by converting phosphate ions into phosphomolybdate complex, which is then reduced to blue coloured complex by metol. The absorbance of colour measured spectrophotometrically at 680 nm is proportional to the inorganic phosphorus concentration.

3.7 Studies on *in vitro* Biocompatibility of Polyurethanes

In vitro biocompatibility of the newly synthesized polymers was assessed by *in vitro* haemolysis test, whole blood haematology analyses, *in vitro* cell culture tests for cytotoxicity and cell viability. Clean and sterile polymers (quantity as per the standard used) were used for the studies.

3.7.1 Cleaning and Sterilisation of Polymers

Since ultrasonic cleaning can improve the surface quality, polyurethane test samples were cleaned ultrasonically. Polymeric samples (getting after purification with ethanol extraction) with required dimension and quantity (as per each standard) were sonicated 20 min in 0.5% liquid soap solution (Extran®, 02 MA from E-merck India Ltd) and 5 min each twice in distilled water. Samples were rinsed three times after each sonication using distilled water. Cleaned wet samples were dried at 50 ^{0}C for 48 h in clean room facility. The samples were packed, sealed (separately for each analysis) and radiation-sterilized using a Panoramic Batch Irradiator (PANBIT) having Cobalt-60 gamma source for 2.5 Mrad at a dose rate of 0.4-0.49 Mrad/h. Commercial poly(ether urethane), Tecoflex 85A® (Thermedics, Inc., Mississippi,

USA) was also cleaned, sterilized and used in all tests for comparison of test data.

3.7.2 Evaluation of *in vitro* Heamolysis

The haemolytic potential of materials was evaluated by *in vitro* rabbit blood haemolysis test using a procedure published elsewhere (O'Leary and Guess, 1968; Austian, 1977). Short-term acute haemolytic activity is significant with respect to potential acute toxic liability of biomaterials. The detection of overt haemolysis (lysis of red blood corpuscles) is therefore of immediate significance with respect to toxicity. 1 g of biomaterial cut into small pieces was placed in test tubes (n =3), containing 25 ml PBS and 0.4 ml rabbit blood and incubated at 37 ^{0}C for 60 minutes. Similarly a positive control (0.4-ml blood and 25 ml 0.1 % sodium carbonate solution) and a negative control (0.4 ml blood diluted with 25 ml PBS) were set for incubation. After incubation all the test tubes were centrifuged at 3000 rpm for 5 minutes and supernatant was carefully separated. The percentage of haemolysis was calculated by measuring the optical density (O.D) of the supernatant at 545 nm using an UV-Visible spectrophotometer (Shimadzu UV-1601). The test was performed for each sample in duplicate.

$$\text{Haemolysis (\%)} = \frac{\text{O.D (test sample)} - \text{O.D (negative cotrol)}}{\text{O.D (positive control)} - \text{O.D (negative control)}} \times 100$$

3.7.3 Evaluation of *in vitro* Blood Compatibility- Haematology

The blood compatibility of the polymers was investigated by measuring blood - material interaction parameters *viz.* the reduction in platelet and WBC count and haemoglobin content after exposure to human blood. Human blood was collected from voluntary blood donors in test tubes containing acid-citrate-dextrose (from

blood bank of Hospital complex, SCTIMST). Two ml blood was incubated with polymer material [uniform circular samples of 7.75 mm radius and 0.5 mm thickness with total surface area of 400 mm^2] in multi-well tissue culture plate for 1h at room temperature with occasional shaking (n =3). Platelet consumed / mm^2 on the surface of material, count reduction in WBC, RBC and concentration of haemoglobin due to interaction with the whole blood were determined using an automated Hematology analyzer (Cobas-Minos Vet 704, Roche Diagnostics, France). Blood taken in the tissue culture plate (without material) was used as the control.

3.7.4 Evaluation of Cytocompatibility

The cytocompatibility of the polymers was evaluated by cell culture tests. This assay is mainly used to determine the toxicity potential in the cellular level. Cytotoxicity assay was carried out with the polyurethane samples (0.5 cm x 0.5 cm size, n=3) by direct contact of the polymers with a monolayer of mouse fibroblast cells (L 929) as per ASTM standard F 813-83. The fibroblast cells were subcultured from stock culture (supplied by NCCS, Pune, India.) by trypsinisation and seeded on to multi well tissue culture plate. Cells were fed with Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% bovine serum and incubated at 37 ^{o}C in humidified 5% carbon dioxide atmosphere. When the cells attained confluent monolayer, the material was kept in contact with the cells for 24 h. The morphology of the cells was assessed by an inverted phase contrast microscope, MIL 090-131.002 (Leitz DMIL). The morphology of the cells was compared with negative control (cells on polystyrene culture plate without material) and positive control (Zinc diethyl dithiocarbamate, a known cytotoxic mecapto-compound with cells). The experiment was also carried out for Tecoflex 85A®.

3.7.5 Evaluation of Cell Viability -MTT assay

Cell viability studies were carried out by indirect contact method (MTT assay) using extract of the polymers as reported elsewhere (Dekker *et al*, 1994). The MTT test is based on the conversion of the tetrazolium salt [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT)] to an insoluble blue MTT formazan product by mitochondrial succinate dehydrogenase. In this method, the polymer was extracted in cell culture medium of DMEM supplemented with glutamine, antibiotics and 10 % calf serum for 24 h at 37^{0}C as per ISO 10993-5 (1999) protocol. The ratio between the surface area of polymer and volume of medium was approximately 1 square cm / ml. A confluent monolayer of fibroblast cell was grown in a multi-well tissue culture plate as narrated in cytocompatibility test. When a confluent monolayer of cells had been formed in the wells of culture plate, the medium was aspirated and the extract (100%) or a medium containing 50% dilution of extract. After 24h incubation at 37 °C, cell viability was determined. For that MTT solution (20 µl, final conc. 0.44 mg / ml) was added to each well of the tissue culture-plate. Plates were shaken briefly and incubated further for 4 h at 37 ^{0}C. After careful aspiration of the supernatant, 100µl of 2-propanol was added to each well to dissolve the formazan product and absorbance was measured spectrophotometrically at 590 nm against blank wells (without cells but incubated with MTT solution) using a microtiter plate reader (Biotek, USA). The relative percentage of formazan product was calculated with respect to the control cells without any extract. The relative percentage of formation of formazan product is a measure of the percentage of metabolically active cells or the cell viability. Three replicate of each concentration were used and the results were presented as the average absorbance of three replicate wells.

3.8 *In vivo* Toxicological Studies for Biocompatibility

3.8.1 Evaluation of *In vivo* Systemic Toxicity

The systemic response of the mice to the extract of the polyurethane was evaluated for candidate HPL18-PU. The samples were cleaned and sterilized by gamma radiation with dosage of 2.5 Mrad. The United States Pharmacopoeia protocol (1995) was adopted for this study. The extract of the polyurethane was prepared using 0.9% sodium chloride (polar medium) and cottonseed oil (non-polar medium) medium. Extract of the polyurethane was injected in Swiss albino mice through intravenous and intra-peritoneal route with the dosage of 50-ml/ Kg body weight. The observation period was 4, 24, 48 and 72 h and up to 7 days. Toxic symptoms, feed and water consumption, body weight and mortality were observed during this period.

3.8.2 *In vivo* Intracutaneous Irritation Test

In order to evaluate the local tissue response to the extracts of the candidate polyurethane HFL18-PU in rabbit, intracutaneous irritation test was carried out. Albino Rabbit animal model was used. The ISO 10993-10 (1995) protocol, (meeting requirements of USP and ASTM F749-98 standards) was followed. The extracts of polyurethane in 0.9% sodium chloride and cottonseed oil were prepared as mentioned in previous section. Four rabbits were clipped closely without any mechanical irritation and trauma and the extract was injected through intracutaneous route at 10 sites with the dosage of 0.2ml /site. The animals were observed for immediate tissue reaction, 24, 48, and 72 hours. The animals were observed for any toxic symptoms (erythema, edema etc.) feed and water consumption and mortality.

3.8.3 *In vivo* Biocompatibility by Intramuscular Implantation

The *in vivo* biocompatibility of candidate poly(urethane urea) HFL18-PU implant was evaluated by intra muscular implantation as per the USP protocol 1995 (meeting ASTM requirements). Polymer strips (cut into size 10 x 1 x 1mm) with smooth edges were cleaned sterilized by gamma radiation. Clean and sterile ultra high molecular weight polyethylene (UHMWPE) was used as control. The healthy adult New Zealand White rabbits (from small animal facility of SCTIMST) were anaesthetized using sodium pentabarbitone (dose of 45mg/ kg). The polymeric material was implanted intra muscularly in the dorsal paravertebral muscle of rabbits with body weight more than 2.5 kg, whose fur can be clipped closely on both sides of spinal column. 4 strips of the test sample (HFL18-PU) were implanted on one side of spinal column and 2 strips of control samples (UHMWPE) on the other side of spine. Implantation was done under clean and aseptic condition using sterile needle. The animals were sacrificed after the post- implantation periods of 7, 30, 90 days, the sites of implantation were macroscopically examined for edema, erythema, haemorrhage, necrosis, discoloration and infections and the implants were retrieved. The test and control samples with tissue surrounding the material were harvested, fixed in 10 % buffered formalin. The tissues were processed for histopathological investigation. For this the tissues were dehydrated in ethanol-water solutions of successively higher concentrations and then cleaned in xylene. Then these samples were embedded in paraffin wax and after adequate infiltration of paraffin wax, the samples were sectioned using a macrotome cryostat and observed through an optical microscope.

3.9 Studies on Functional Performance of the Poly(urethane urea)s

3.9.1 Studies on Accelerated Flexural Fatigue Test

The accelerated flexural fatigue test was carried out using elastomer strips (5 x 0.5 x 0.1cm, n=3) of polyurethane urea (HFL18-PU) and poly(ether urethane urea) (HFL17-PU) as per ASTM standard D 671. A schematic diagram of the accelerated flexural fatigue machine is given in Figure 2.1. The flexural fatigue tester consists of adjustable base on which a spring steel was fixed which acts as dynamometer. A dial gauge was used to measure the deflection of the spring on either side. Specimen was fixed to the spring at one side and at holder on the other side. The holder was connected to a variable eccentric through a connecting rod. The eccentric was driven by a motor and automatically stop when the holder touches the electrical contact when the specimen breaks due to fatigue failure. Initially the tester was calibrated. The variable eccentric was set to zero load and a dead weight (995.11g) was attached to the holder. The unit was run manually and the amplitude of vibration (test span) was noted using the dial gauge on the spring. The spring constant (dead weight/amplitude of vibration) was found as 1.7157 Kg/mm. Then the specimen was fixed on the unit. The variable eccentric was varied slightly and the amplitude of vibration on the specimen was found with the dial gauge. The load applied on the specimen by the variable eccentric was calculated by multiplying the amplitude of vibration. The load applied on the sample and flexural fatigue stress applied on the test specimen was calculated (3.8432 Kg and 1.3975 Kg/cm^2). The test specimen was subjected to flexing with flexing rate of 1425 cycles per min. The number of cycle before the failure was noted. The flex life was calculated.

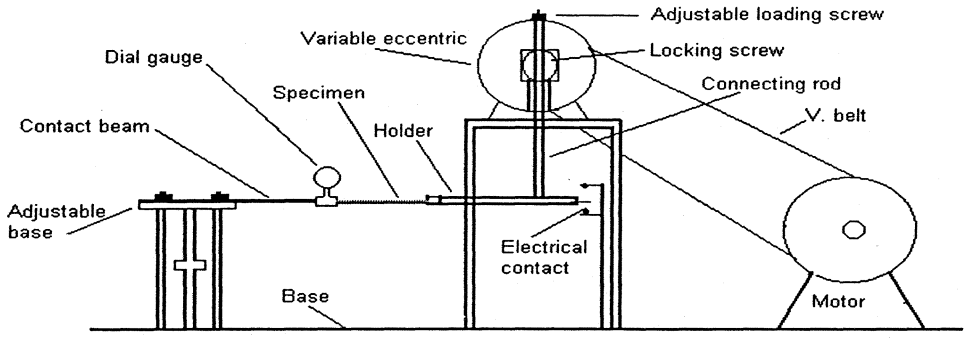

Figure 2.1 Flexural Fatigue Tester

3.9.2 Studies on Endothelial Cell Response to Candidate Poly(urethane urea)

Endothelial cells have been frequently used in cytotoxicity testing of materials, especially polymers, used in blood contacting implants as well as for investigating seeding technologies for vascular prostheses. In order to understand the endothelial cell response for the blood compatibility, the cell response was evaluated using cell culture technique by direct contact method. A cell line HUV-EC-C (human umbilical cord), obtained from NCCS Pune India, was used. The candidate material HFL18-PU was exposed the endothelial cells grown in Isocoves 's medium enriched with 20 % fetal calf serum and incubated. The endothelial cell-lysis or proliferation on the poly(urethane urea) was evaluated by assessing the morphology of cells in contact with the surface of polymer using optical microscopy. The growth of endothelial cells on the surface of polyurethane was also assessed by cell seeding with endothelial cells and followed by scanning electron microscopic study using SEM, Hitachi S2400.

3.9.3 Studies on Long-term *in vivo* Biodurability of Candidate Poly(urethane uea) by Subcutaneous Implantation in Rat

The long-term *in vivo* biodurability was evaluated for candidate polyurethane HFL18-PU. The clean and sterile polymer samples (7 x 0.5 cm, n=6) were implanted subcutaneously in rat animal model as per ASTM F 763-87. The animals were sacrificed after the period of 6 months. The adhered tissues were removed from the polymeric samples using 0.1% sodium hydroxide solution. The polymeric samples were vacuum- dried. The changes in weight, mechanical properties and surface properties were determined as described earlier. The samples for SEM were prepared by critical point drying (CPD) and gold sputtering. The surface pitting, if any occurred, was investigated using scanning electron microscope (SEM,) after. The chemical changes in the bulk structure of the polymer were investigated using infrared spectroscopy (FTIR-ATR).

CHAPTER IV

RESULTS AND DISCUSSION

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4.1 Synthesis of Polyurethanes

Commercial aliphatic poly(ether urethane)s based on polytetramethylene oxide glycol (PTMG), viz. Tecoflex® or polyurethanes based on polydimethyl siloxane (PDMS), viz. Avcothane® failed to satisfy the requirements of long-term biomedical uses. Recent reports on enzyme-induced biodegradation (Tang *et al,* 2001) and hydrolytic degradation by monocyte-derived macrophages (Labow *et al,* 2001) of polycarbonate polyurethanes pose doubt on their use in long-term blood contact applications. Therefore, development of aliphatic poly(urethane urea)s having minimal degradable linkages, but having more virtual or physical crosslinking and phase-separated structure was planed. Hydroxy terminated polybutadiene (HTPBD) is a versatile polyol that polyurethanes based on which have been widely used as high performance composite propellants for space applications (Manjari *et al,* 1993). But the application of HTPBD in biomedical uses for the fabrication of long-term devices is not yet explored. This may be due to poor biostability of polyurethane containing HTPBD as reported by Takahara *et al* (1991).

The high flex-life, the finite requirement of materials for applications such as leaflets of heart valve, membrane of blood pump etc could be achieved by using long alkenyl chain molecule. Therefore, it was planned to synthesis aliphatic poly(urethane

urea)s using $H_{12}MDI$ as diisocyanate, HTPBD as polyol and 1,6-hexamethylene diamine (HDA) as chain extender and compare with poly(ether urethane urea)s and polyurethanes. The chemical structures and molecular weights of the reactant monomers used as the raw materials for the synth esis of present polymers are given in Figure 4.1.

4. 1.1 Synthesis and process optimisation of polyurethanes, poly(ether urethane urea)s and poly(urethane urea)s

The conventional two step prepolymer preparation method was used to synthesis the present polyurethanes due to certain advantages. Solution polymerisation by prepolymer method can give materials with better properties than one shot process. The schematic representation of synthesis of polyurethanes and poly(urethane urea)s is given in Figure 4.2. Since the reactions of aliphatic diisocyanates and the polyols are very slow, DBTDL, a tin catalyst was used in the first step itself. In presence of the catalyst, the diisocyanate $H_{12}MDI$ reacts with polyol to form reactive prepolymer with -NCO end groups. In the second step the prepolymer reacts with chain extender HDA or BD in presence of the catalyst to form cured Poly(urethane urea) or polyurethane respectively as the final product. Poly(ether urethane urea)s were obtained by using mixture of polyols, HTPBD and PTMG as per the method adopted for poly(urethane urea)s. The completion of the reaction was assessed using IR analysis. A representative FT-IR spectrum of poly(urethane urea), HFL18-PU is given in Figure 4.3. Absence of characteristic peak for free isocyanate group around 2260 cm^{-1} suggested the completion of the polymerization reaction.

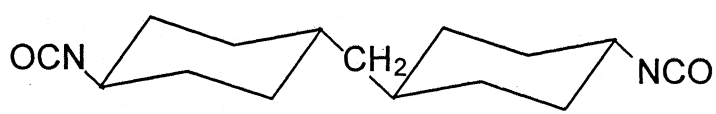
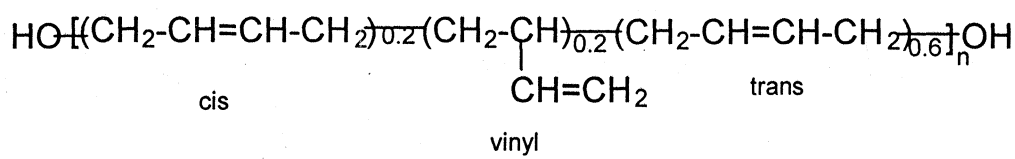
Monomer	Mol. wt	Chemical structure
BD	90	$HO\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}OH$
HDA	116	$H_2N\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}NH_2$
$H_{12}MDI$	250	
HTPBD	2620	
PTMG	1000	$HO\text{-}[CH2\text{-}CH2\text{-}CH2\text{-}CH2\text{-}O]_n\text{-}H$

Figure 4.1. Chemical structures of the monomers used for the synthesis of the various polymers

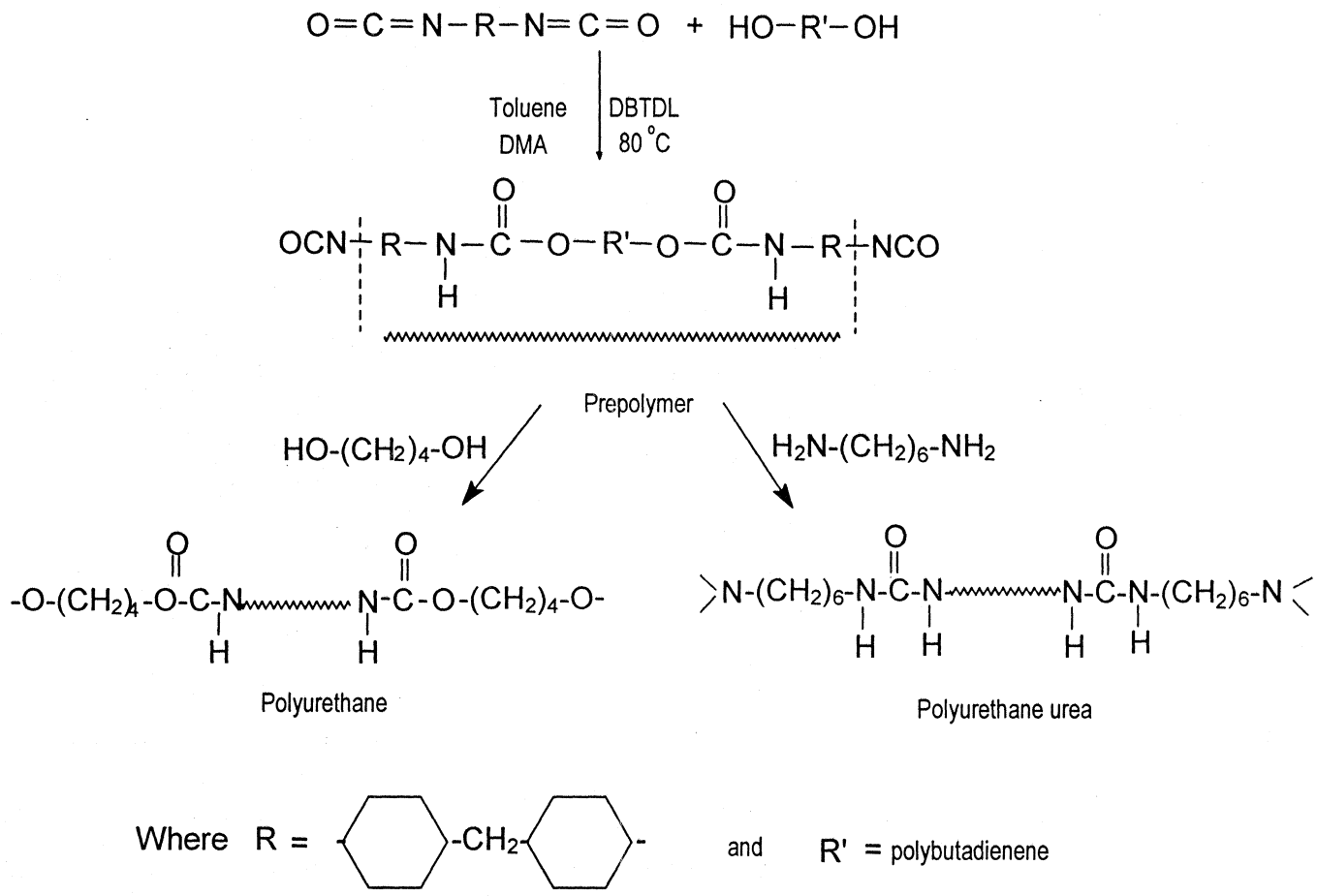


Figure 4.2. Synthesis of polyurethane and polyurethane ureas

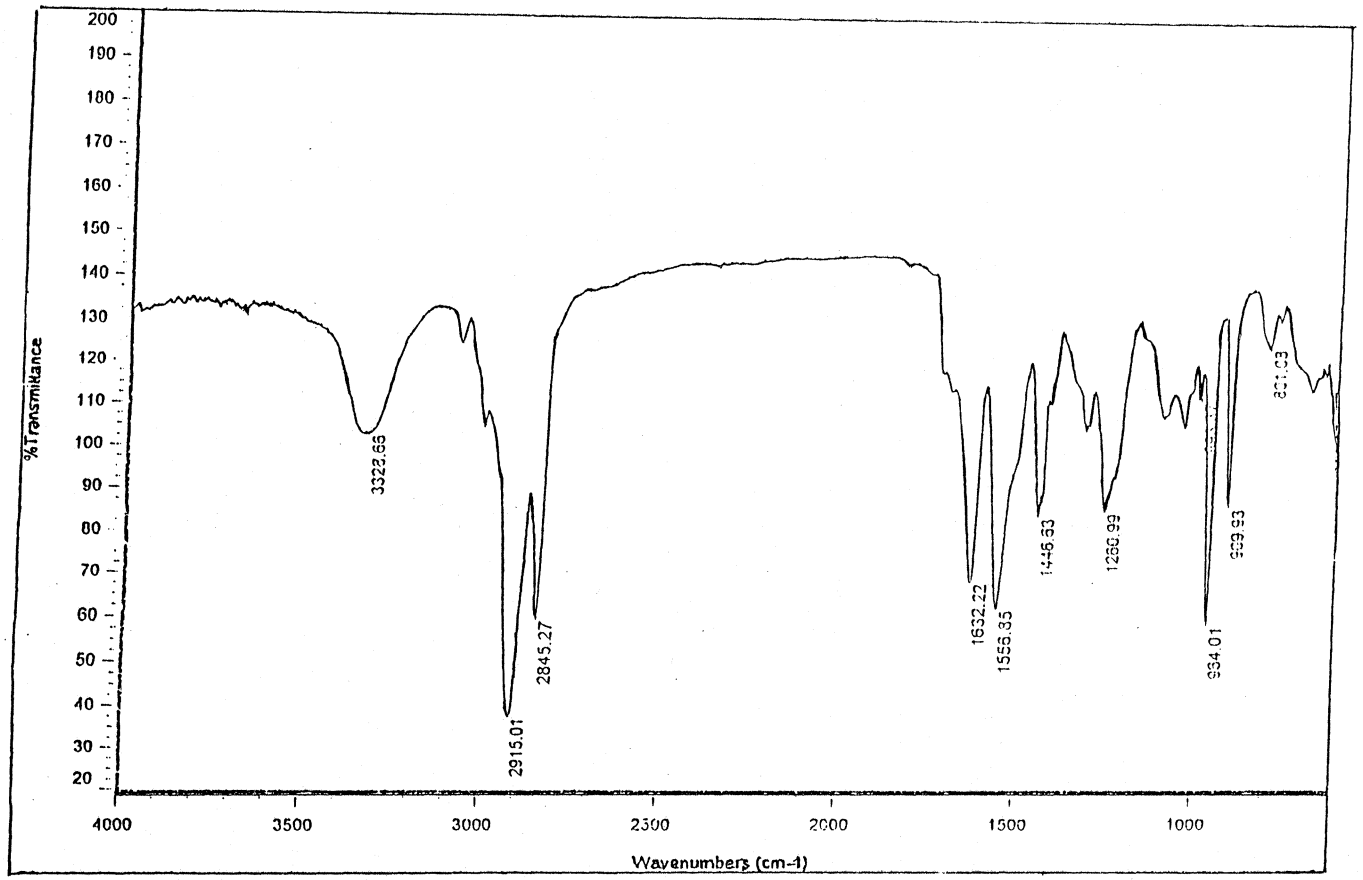


Figure 4.3 Infrared spectrum of poly(urethane urea) HFL18-PU

The polymerisation reaction for the formation of polyurethane has features of both addition and condensation polymerisation and hence referred to as polyaddition or rearrangement polymerisation (Brydson, 1995). Process optimisation is important for the tailor making of polyurethanes because process variables will affect the required properties of the polymer. The process variables *viz.* concentration of reactants, temperature and duration of reaction, are optimised during synthesis

The cured polymers were extracted continuously with hot ethanol in Soxhlet extractor to remove catalyst and the leachable low molecular weight oligomers, if any Alcoholic extraction using soxhlet method is found to be more effective in the case of polyurethanes to ensure complete purity. The thorough extraction of polyurethans is important as the presence of the catalyst dibutyl tin dilaurate and leachables from the implant can cause chronic inflammations *in vivo* physiological environment.

Three types of polymers were prepared. Two polyurethanes containing urethane linkages were prepared and coded as HFL1-PU and HFL3-PU. Two poly(ether urethane urea)s containing ether, urethane and urea groups were prepared and coded as HFL16-PU and HFL17-PU. Four poly(urethane urea)s containing urethane and urea linkages were prepared and coded as HFL9-PU, HFL13-PU, HFL15-PU and HFL18-PU. Polyurethanes and poly(ether urethane urea)s were synthesised to compare the properties and performance of poly(urethane urea)s. The formulation variables for the synthesis of various polymers are given in Table 4.1. Polyurethanes, HFL1-PU and HFL3-PU chain extended with 1,4-butane diol contain only urethane linkages in the hard and hard-soft segment interface. Poly (urethane urea)s HFL9-PU, HFL13-PU, HFL15-PU and HFL18-PU are ether-free polymers.

Table 4.1 Formulation variables of polymers

Polymer	Reactant concentration (mol)				
	Diisocyanate	Polyol		Chain extender	
	H_{12}MDI	HTPBD	PTMG	HDA	BD
Polyurethane					
HFL1-PU	3.5647	1.2152	-	-	2.0254
HFL3-PU	3.5647	1.1828	-	-	2.1186
Poly(urethane urea)					
HFL9-PU	3.5647	1.4000	-	1.9000	-
HFL13-PU	3.5647	1.0607	-	2.2400	-
HFL18-PU	3.5647	0.8260	-	2.4760	--
HFL15-PU	3.5647	0.6600	-	2.6405	-
Poly(ether urethane urea)					
HFL16-PU	3.5647	0.7000	0.7000	1.9000	-
HFL17-PU	3.5647	0.5305	0.5303	2.2397	-

Since they are chain-extended with 1,6-hexamethylene diamine, they contain degradation-resistant urea linkages in hard segment and lesser percentages of urethane linkages in soft segments. Easily degradable ether or ester linkages were avoided and urethane linkages were minimised in the preparation of poly(urethane urea)s. Poly(ether urethane urea)s, HFL16-PU and HFL17-PU contain urea linkages in hard segment and lesser percentage of urethane and ether linkages in soft segment. The mole concentration of diisocyanate (3.5746) and the isocyanate index (1.08) were kept constant for all the polymers. The composition of individual polymers was formulated by varying the chain extender or polyol content. Therefore, the chain extender content directly gives the hard segment composition. The percentage composition of soft segment and hard segment of the polymers is given in Table 4.2.

Table 4.2. Chemical composition of polymers

Polymer	Hard segment	(mol %)	Soft segment (mol %)		Isocyanate index
Polyurethane					
HFL1-PU	H_{12}MDI-BD	(62.5)	HTPBD	(37.5)	1.08
HFL3-PU	H_{12}MDI-BD	(64.2)	HTPBD	(35.8)	1.08
Poly(urethane urea)					
HFL9-PU	H_{12}MDI-HDA	(57.5)	HTPBD	(42.5)	1.08
HFL13-PU	H_{12}MDI-HDA	(67.9)	HTPBD	(32.1)	1.08
HFL18-PU	H_{12}MDI-HDA	(75.0)	HTPBD	(25.0)	1.08
HFL15-PU	H_{12}MDI-HDA	(80.0)	HTPBD	(20.0)	1.08
Poly(ether urethane urea)					
HFL16-PU	H_{12}MDI-HDA	(57.5)	HTPBD-PTMG	(42.5)	1.08
HFL17-PU	H_{12}MDI-HDA	(67.9)	HTPBD-PTMG	(32.1)	1.08

All the newly prepared polyurethanes, poly (ether urethane urea)s and poly (urethane urea)s are segmented block copolymers having polyol soft segment and diisocyanate-chain extender unit together as hard segment. Urethane linkages connect the soft segments to the hard segments. The chemical architecture of the three types of polymers are given in Figures 4.4 - 4.6. Polyurethanes, HFL1-PU and HFL3-PU are composed of soft segment HTPBD and hard segment H_{12}MDI-BD unit. The poly(urethane urea)s, HFL9-PU, HFL13-PU, HFL18-PU and HFL15-PU are composed of soft segment of HTPBD and hard segment H_{12}MDI-HDA unit. The poly(ether urethane urea)s, HFL16-PU and HFL17-PU, are comprised of mixed soft segment of polyether polyol (PTMG) and polyhydrocarbon polyol (HTPBD) and hard segment H_{12}MDI-HDA unit. The soft segment-volume in the poly(ether urethane urea)s can be a regular array of either HTPBD or PTMG or alternate HTPBD and PTMG or irregular array of HTPBD and PTMG. H_{12}MDI, aliphatic analogue of

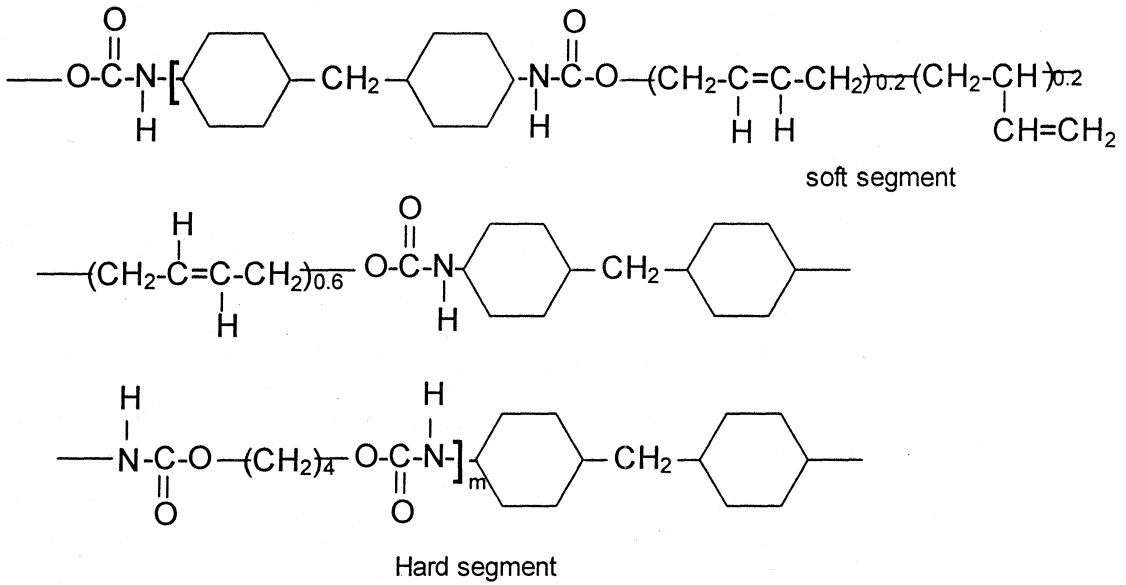


Figure 4.4. Structure of polyurethane (H_{12}MDI-HTPBD-BD)

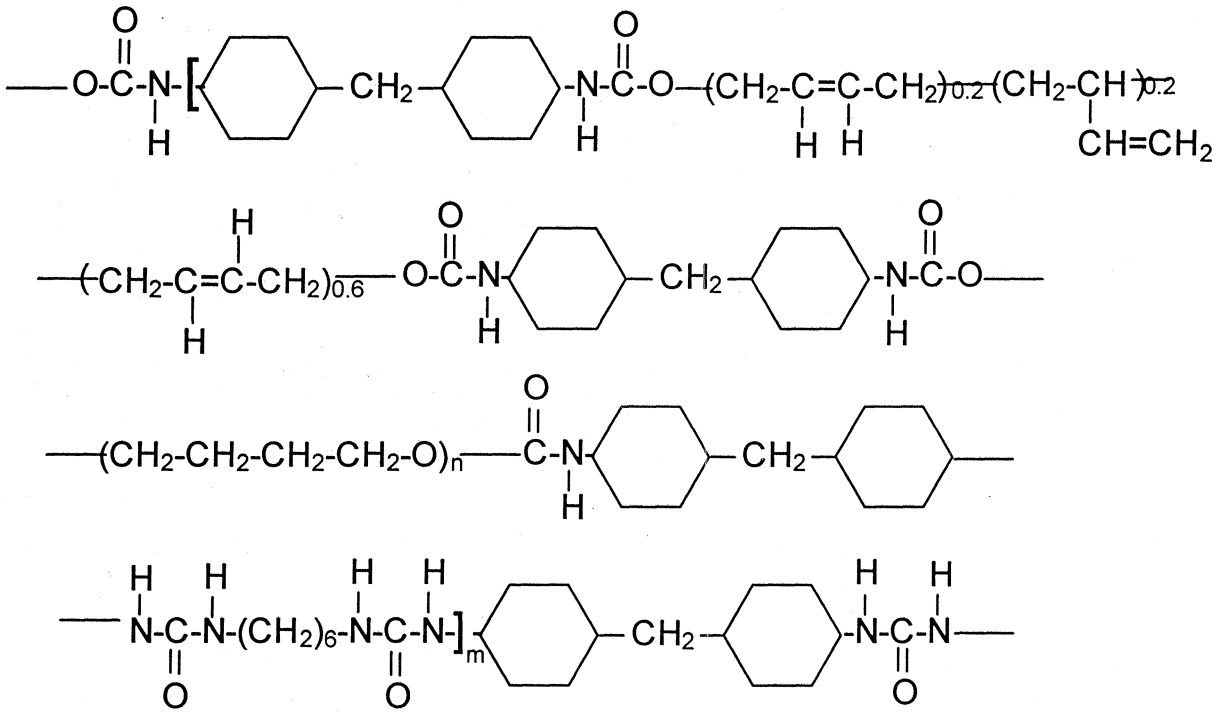


Figure 4.5. Structure of poly(ether urethane urea) (H_{12}MDI-HTPBD/PTMG-HDA)

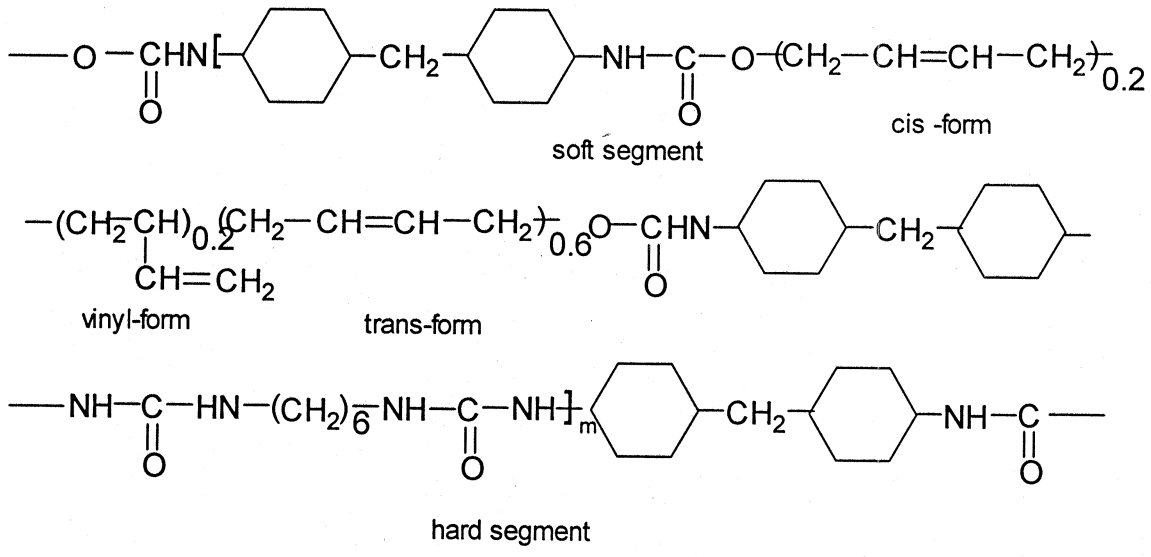


Figure 4.6. Structure of poly(urethane urea) (H_{12}MDI-HTPBD-HDA)

4,4'-methylene bis(phenyl isocyanate) (MDI) exists as isomeric mixtures of *cis-cis*, *cis-trans* and *trans-trans* as given in Figure 4.7.

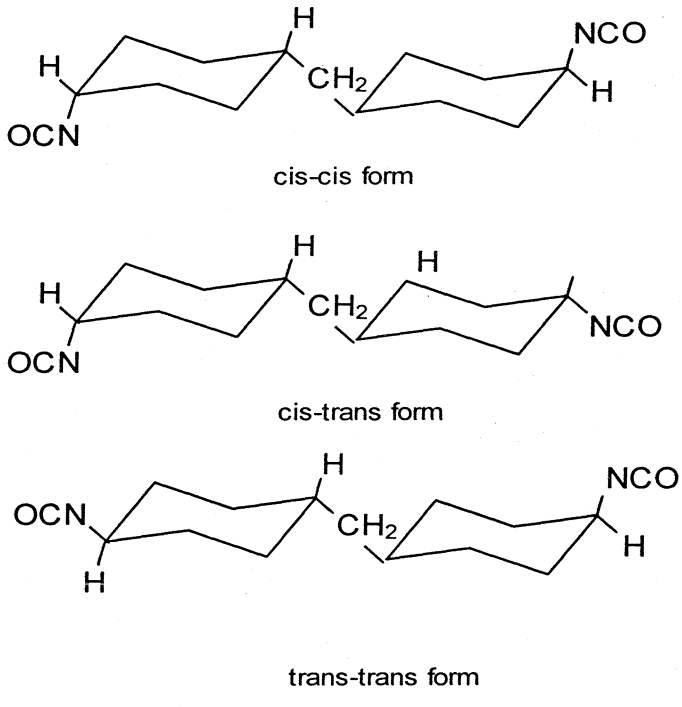


Figure 4.7. Isomeric structures of H_{12}MDI (Desmodur W=30% trans-trans; 65%trans-cis and 5%cis-cis isomer mixture)

The isocyanate index [the ratio of mole concentration of -NCO to (-OH and -NH_2) or -OH functionality] used for the synthesis of all polymers is nearly equal to one. The low value of isocyanate index restricts the occurrence of side reactions leading to the formation of allophanate or biuret linkages in the polyurethanes. Further, the DBTDL is a specific catalyst for urethane bond formation rather than allophanate or biuret linkages in the polyurethanes. Therefore, the structure of all the polymers is invariably linear (Figure 4.4 - 4.6).

All the polymers were prepared using mixture of solvents, dimethyl acetamide/ toluene having different solubility. Therefore, the premature phase-

separation of hard and soft segments occurs in the polymers due to the initial immiscibility of the reactants. However, the polyhydrocarbon polyol based polyurethanes exhibit a high level of macro-phase separation when they are prepared in bulk and a high level of micro-phase separation when they are prepared in solution under homogeneous conditions. These effects are also attributed to a large difference in their segmented structure and polarity as well as absence of inter-segmental hydrogen bonding (Cuv'e *et al,* 1991). Moreover, polyurethanes based on hydroxy terminated polybutadiene do not favour hydrogen-bonding interaction between hard and soft segment units. As a result of this, from thermodynamic point of view, there exists a positive surface free energy (ΔG) and a positive heat of mixing (ΔH). This serves as a driving force for the growth of phase-separated hard and soft segment domains. Hence the present polyhydrocarbon polyol based poly(urethane urea)s are more phase-separated than polyether polyol based poly(ether urethane urea)s and polyurethanes. In brief, all the present poly (urethane urea)s are considered to be segmented, linear and microphase-separated polymers.

4.2 Characterization of Newly Prepared Polyurethanes

4.2.1 Physico-chemical Characterization- Virtual Crosslink Density

Polyurethanes, poly(urethanes urea)s and poly(ether urethane urea)s were characterised for physico-chemical and structural properties such as density, swelling coefficient, crosslink density and molecular weight between crosslinks. Data on swelling studies are given in Table 4.3. The swelling data of the present polymers indicated that polyurethane and poly(ether urethane urea)s swell maximum in

dimethyl acetamide (DMA) whereas poly(urethane urea)s showed maximum swelling in tetrahydrofuran (THF).

Table 4.3 Physico-chemical properties of polymers

Polymer	Density (g/cm^3)	Swelling coefficient	Crosslink density (x 10^4) (mol/cm^3)	Mol. wt between crsslinks
Polyurethane				
HFL1-PU	0.9440	6.2110	1.1660	8575
HFL3-PU	0.9500	5.773	1.3670	7301
Poly(urethane urea)				
HFL9-PU	0.8916	4.1027	1.8520	5399
HFL13-PU	0.8905	2.5680	6.7810	1475
HFL18-PU	0.9483	2.2486	6.9460	1440
HFL15-PU	0.9555	1.9533	8.7375	1144
Poly(ether urethane urea)				
HFL16-PU	0.9552	4.2716	2.2600	4427
HFL17-PU	0.9174	3.6526	3.0900	3238

Swelling property of a polymer depends on its solubility parameter of the solvent and polymer. Maximum swelling can be observed in that solvent having solubility parameter close to that of polymer. The virtual or physical crosslink density, *i.e*, the effective number of virtual or physical crosslinks is of great importance because of its effect on the physical and mechanical properties of polymers. The most well known theoretical model for determining these parameters is the model of Flory and Rehner (1943) based on equilibrium swelling theory.

The physical or virtual crosslink density is defined as the sum of all the secondary level molecular interactions like hydrogen bonding, dipole interactions and other Vanderwaal's forces. In the present polymers, virtual crosslink density is the

degree of secondary level of molecular interactions, particularly hydrogen bonding, present along the polymer chain of poly (urethane urea)s. The virtual crosslink density and the number average molecular weight between crosslinks are given in Table 4.3. The crosslink density and the molecular weight between the crosslinks are inversely related to each other. As virtual crosslink density increases the polymer assumes more thermoset-like character. These characteristics indicate that poly(ether urethane urea)s and poly(urethane urea)s are invariably cross-linked due to inter chain hydrogen bonds. These virtual cross-linking in poly(urethane urea)s, HFL9-PU, HFL13-PU, HFL18-PU and HFL15-PU are through hydrogen bonds between urethane-urea, urea-urea and urethane- urethane linkages. The predominant hydrogen bonding *viz* urethane-urea and urea-urea linkages in the polymer chains of poly(urethane urea)s and urethane-urethane, urethane-urea, urea-urea, urethane-ether and urea-ether linkages in the polymer chains of poly(ether urethane urea)s which lead to virtual crosslinking are given in Figure 4.8.

As the percentage of chain extender increases the virtual crosslink density increases in the present polymers. The dependence of virtual crosslink density with hard segment content or chain extender percentage is given in Figure 4.9. Poly(urethane urea)s showed higher value of virtual crosslink density compared to polyurethanes. Even for a poly(urethane urea) having lower hard segment content (HFL9-PU) in compared with polyurethane (HFL1-PU), the virtual crosslink density was found to be higher in the former than in the latter. Higher crosslink density was observed for poly (urethane urea)s HFL13-PU, HFL18-PU and HFL15-PU in comparison with other polymers (Table 4.3).

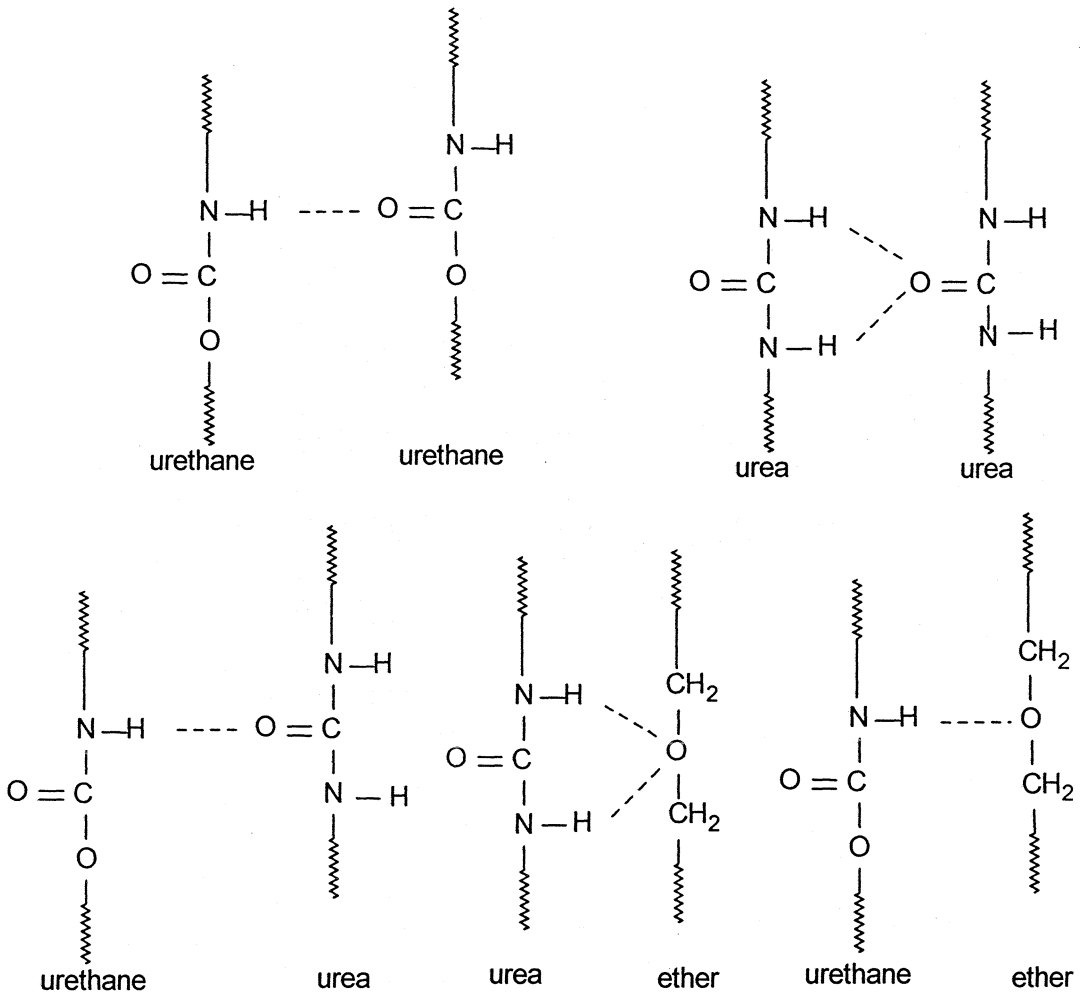


Figure 4.8 Hydrogen bonding interactions

The increased crosslink density observed in HFL15-PU and HFL18-PU is due to increased urea-urea hydrogen bonding because of the higher percentage of hard segment content. Higher the concentration of amine chain extender in a particular series of poly(urethane urea), higher the virtual crosslink density. In general As the percentage of amine content increases in poly(urethane urea)s, the sites of hydrogen bonding (urea linkage) increases and virtual crosslink density increases. This is because urea or diamide group has C=O bond flanked by two N-H groups.

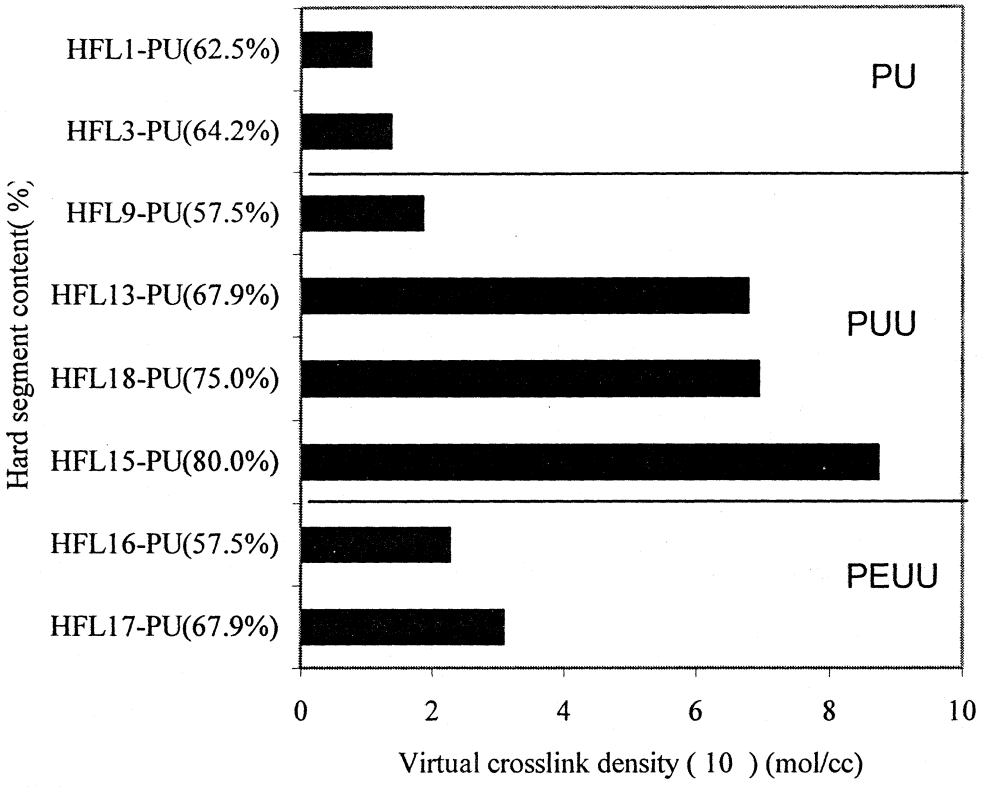


Figure 4.9 Virtual crosslink density Vs. hard segment content or chain extender percentage

The oxygen atom of the C=O group of one hard segment part can effectively undergo hydrogen bonding with hydrogen atoms of the -N-H groups of second hard segment part and so on. This will lead to short and long range of secondary interactions leading to micro-crystallites formation in poly(urethane urea)s. The crosslink density of HFL9-PU and HFL13-PU reveal appreciable difference. The chemical composition and crosslink density of these two poly(urethane urea)s indicate the transition from highly elastomeric nature in the former to the reduced elastomeric nature in the latter. This suggests that addition of PTMG polyol in these two poly(urethane urea)s alter the crosslink density differently. The addition of PTMG in

the formulation of HFL9-PU (resulting HFL16-PU) increases the crosslink density in poly(ether urethane urea), HFL16-PU. While the addition of PTMG in the formulation of HFL13-PU (resulting HFL17-PU) reduces the crosslink density in poly(ether urethane urea), HFL17-PU.

In poly(ether urethane urea)s, HFL16-PU and HFL17-PU in addition to crosslinking through urea-urea and urethane-urea linkages there are two more types of virtual crsslinking viz. ether-urea and ether-urethane (Figure 4.8). However, the crosslinks of these types provide phase-mixing between the hard and soft segments, as ether group in poly(ether urethane urea)s is present in the soft segment as reported by Hu *et al* (1982). However, the phase-mixing in HFL16-PU and HFL17-PU could be moderate due to the presence of hydrophobic polyols. In polyurethanes, HFL1-PU and HFL3-PU the virtual crosslinking is due to urethane-urethane hydrogen bonding only. In short, in poly(urethane urea) the virtual crosslinking is of three kinds, urethane-urethane, urethane-urea and urea-urea. While in polyether urethane urea, there are four types of bonding like urethane-urethane, urethane-urea, ether-urethane and ether-urea. Among these, ether-containing hydrogen bonding is responsible for the phase-mixing and the other two favours the phase-separation. In poly(urethane urea), HFL9-PU, HFL-13PU, HFL15-PU and HFL18-PU, only urethane-urea interactions may lead to phase-mixing. Since the concentration of urethane linkages in these poly(urethane urea)s is very low, the chances of phase-mixing are minimal.

4.2.2 Fourier Transform Infrared Spectral Analyses

The Fourier transform infrared-attenuated total reflectance (FTIR-ATR) spectral analyses were used to characterise polymers. The major ATR-IR peak

responses of the poly(urethane urea)s are given Table 4.4. A representative spectrum of HFL18-PU is given in Figure 4.3. ATR-IR peak responses of polyurethanes and poly(ether urethane urea)s are given Table 4.5.

The IR peak appeared at around 1702-1708 cm^{-1} for all polymers is due to the 'ν (C=O) urethane amide I hydrogen bonded' as reported by Lin *et al* (1983; 1985) and by Miller *et al* (1985). The absence of peaks at around 1735-1710 cm^{-1} is due to the absence of ν (C=O) urethane amide I 'non-hydrogen bonded' as reported by Xu *et al* (1981) and by Brunette *et al* (1981). Moreover, the presence of urethane carbonyl near 1700 cm^{-1} reveals presence of 'crystalline region' and the absence of peak near 1735 cm^{-1} gives absence of' amorphous region' in the IR spectrum as reported by Corish (1959). The presence of peaks at the spectral region 1620-1660 cm^{-1} for ν(C=O) the urea amide I (hydrogen bonded) reveals the extent of hydrogen bonding of urea groups. The absence of peak for urea amide I at 1660 cm^{-1} reveal the absence of non-hydrogen-bonded urea or free urea as reported by Sung *et al* (1978).

All the present poly(urethane urea)s and poly(ether urethane urea)s showed bonded urea carbonyl peak at around 1632 cm^{-1}. The shift of urea peak towards lower wave number region to an extent of ~30 is, therefore, an indication of the extent of hydrogen bonding between the urea carbonyl and the -N-H unit of the another or the same polymer chains as discussed earlier. The absence of peak at around 3420-3445 cm^{-1} further confirmed the absence of free -N-H units in the present poly(urethane urea)s.

Table 4.4. ATR-IR spectral responses of the virgin poly(urethane urea)s

Spectral response	HFL 9-PU1	HFL13-PU	HFL15-PU	HFL18-PU
Bonded N-H stretching	3334 cm^{-1} (s)	3334 cm^{-1}(s)	3325 cm^{-1}(s)	3329 cm^{-1} (s)
Free –N-H- stretching	Absent at 3420-3445	Absent at 3420-3445	Absent at 3420-3445	Absent at 3420-3445
Aliphatic -CH2- stretching	2913 (s),2843cm^{-1}(s)	2913(s),2844cm^{-1}(s)	2911(s),2844 cm^{-1}(s)	2915(s) ,2845(s)
Free = C=O stretching	Absent at 1720-1735	Absent at 1720-1735	Absent at 1720-1735	Absent at 1720-1735
Bonded =C=O (urethane)	1706 cm^{-1} (m)	1702 cm^{-1} (m)	1701 cm^{-1} (m)	1702 cm^{-1} (m)
Bonded =C=O (urea), ordered	1635 cm^{-1} (s)	1632 cm^{-1} (s)	1629 cm^{-1} (s)	1632 cm^{-1} (s)
-(C-N)- + (N-H) amide II	1558 cm^{-1}(s)	1559 cm^{-1}	1558 cm^{-1} (s)	1557 cm^{-1} (s)
-C-H in CH_2	1441 cm^{-1}(s)	1444 cm^{-1}(s)	1445(s)	1446 cm^{-1} (s)
-(C-N)- + (N-H) amide III + urethane ether(OC-O-C)	1248cm^{-1}(s)	1247 cm^{-1}(s)	1247 cm^{-1}(s)	1261 cm^{-1} (s)
-C=C -(trans1,4 butadiene)	964 cm^{-1} (s)	964 cm^{-1} (s)	962 cm^{-1} (s)	964 cm^{-1} (s)
-C=C- (vinyl PBD)	910 cm^{-1} (s)	910 cm^{-1} (s)	909 cm^{-1} (s)	910 cm^{-1} (s)
-C=C-(cis1,4 polybuta diene) expected at 995 cm^{-1}	Absent	Absent	Absent	Absent

Table 4.5. ATR-IR spectral responses of the virginpolyurethanes and poly(ether urethane urea)s

Spectral response	HFL1-PU	HFL3-PU	HFL16-PU	HFL17-PU
Bonded N-H stretching	3320 cm^{-1} (w)	3325 cm^{-1}(w)	3325 cm^{-1} (m)	3325 cm^{-1} (m)
Free –N-H- stretching	3425 cm^{-1}	3421 cm^{-1}	Absent at 3420-3445	Absent at 3420-3445
Aliphatic -CH2- stretching	2911 (s),2838 (s)	2911(s), 2841(s)	2915(s) 2847 cm^{-1} (s)	2911(s), 2844 cm^{-1} (s)
Free = C=O stretching	Absent at 1720-1735	Absent at 1720-1735	Absent at 1720-1735	Absent at 1720-1735
Bonded =C=O (urethane)	1706 cm^{-1} (S)	1701 cm^{-1} (S)	1705 cm^{-1} (m)	1702 cm^{-1} (s)
Bonded =C=O (urea), ordered	---	---	1632 cm^{-1} (s)	1630 cm^{-1} (s)
-(C-N)- + (N-H) amide II	1543 cm^{-1} (s)	1544 cm^{-1} (s)	1559 cm^{-1} (s)	1549 cm^{-1}(s)
-C-H in CH_2			1445 cm^{-1}(s)	1446 cm^{-1}(s)
-(C-N)- + (N-H) amide III + urethane ether	1247 cm^{-1} (s)	1247 cm^{-1} (s)	1246 cm^{-1} (s)	1241 cm^{-1}(s)
-C-O-C- (ether,PTMG)	---	----	1105 cm^{-1} (s)	1101 cm^{-1} (s)
C-H in trans1,4 polybutadiene	967 cm^{-1} (w)	959 cm^{-1} (s)	965 cm^{-1} (s)	964 cm^{-1}(s)
-C-H in (vinyl PBD)	914 cm^{-1} (s)	909 cm^{-1} (s)	910 cm^{-1} (s)	914
-C=C-(cis1,4 polybuta diene) expected at 995 cm^{-1}	Absent	Absent	Absent	Absent

The peak appeared around 1525-1560 cm^{-1} shows ν(C-N)+(δ N-H) amide II bands as reported by Marchant *et al* (1987) and by Mathews *et al* (1987). Strong peak appeared at 1247 cm^{-1} is assigned to the combined effect of amide III band for ν(C-N)+(δ N-H) and ω(C-H) in -CH_2- as reported by Ishihara *et al* (1974), Nakayama *et al* (1969) and by Knutson *et al* (1982) . In contrary, Srichatrapimuk *et al* (1978) have reported that the peak near 1250 cm^{-1} may be due to ν(C-O-C) in urethane O=C-O-C- also. All the polymers indicated well-defined peaks for polybutadiene soft segments at around 964 and 910 cm^{-1} for -C-H in *trans* and *vinyl* forms of polybutadiene diol respectively. However, the peak corresponding to *cis* form near 995 cm^{-1} (ν C-H in *cis* 1,4- polybutadiene) is absent in all the polymers indicating that the *cis* form is concentrating in the bulk of the polymer and surface is dominated by the *trans* and *vinyl* forms of polybutadiene segments.

The spectra of poly(ether urethane-urea)s, HFL16-PU and HFL17-PU exhibit an additional ether peak at 1105 cm^{-1} and 1101 cm^{-1}. HFL16-PU and HFL17-PU showed peaks at 1702 cm^{-1} for bonded urethane carbonyls and at 1630 cm^{-1} for bonded urea carbonyls (ordered). The mixed spectral response for carbonyls in poly(ether urethane urea) reveals hydrogen-bonding interaction between urethane-ether, urethane-urea, and urea-ether and urea-urea groups resulting to a lesser degree of microphase separation in these polymers.

Polyurethanes prepared with butane diol chain extender, HFL1-PU and HFL3-PU showed a peak for free N-H bond at 3425 cm^{-1}. This indicated that poly(urethane urea)s chain extended with diamine exhibited extensive hydrogen bonding interaction compared to polyurethane chain extended with butanediol.

The spectral region of carbonyl stretching vibrations from 1600 to1800 cm^{-1} is in focus because it provides useful information on the short range and long range ordering of polymer segments. Chen *et al* (1999) have observed peaks at around 1632 cm^{-1} and 1700 cm^{-1} for ordered hydrogen bonded urea and urethane carbonyl groups respectively for aliphatic poly(urethane urea)s. The absence of the peak at 1666 cm^{-1} for C=O stretching of urea carbonyl bonded (disordered) and urethane carbonyl at around 1721 cm^{-1} in the present poly(urethane urea)s confirmed the formation of short and long range ordering of urea hydrogen bonding which reflects the formation of crystallites in hard domains as reported elsewhere (Ning *et al* , 1996; Ning *et al*, 1997). Earlier, Ning *et al* (1996) have reported the presence of IR peak for disordered urea hydrogen bonding at 1666 cm^{-1} for solid segmented poly(urethane urea) samples.

The extent of hydrogen bonding interaction between urea and urea groups is higher than the extent of hydrogen bonding interaction between urethane and urea groups. This is due to the lower molecular weight of the diamine in comparison with that of the polyol as evident from peak intensities of urea and urethane. Such interaction enables three-dimensional network of hydrogen bonds. The three dimensional network of hydrogen bonds leads to greater microphase separation of hard and soft segments. The three-dimensional network in hard segments appears as hard domain.

All the poly(urethane urea)s are linear polymers. Therefore, none of the polymers has showed peaks for allophanate linkages due to true covalent crosslinking. The absence of the band at 2270 cm^{-1} suggested the absence of physisorbed free isocyanates in the polymer as unreacted form and the absence of free isocyanate end

groups as reported by Kwok *et al* (2000). Allophanates are formed due to reaction between free isocyanates and the early formed urethane bond during the prepolymer stage of synthesis of the polyurethanes, provided isocyanate index is high. Allophanate linkages are normally characterized by a triplet of intense IR band centered at 1220, 1280 and 1310 cm^{-1} as well as unique N-H bands at 3298, 3267 and 3233 cm^{-1} (Stovbun *et al,* 1996). The absence of these characteristic bands in the IR spectra of the present polymers demonstrated that undesirable side reactions had not occurred during the synthesis of present polymers.

The present IR spectral studies suggest that the presence of micro-crystallite structure in poly(urethane urea)s. The extensive hydrogen bonding between urea-urea groups in the present poly(urethane urea)s impart physical cross-linking or virtual cross-linking between polymer chains and such physical crosslinking in hard segment results in the formation of microcrystallites. The hard segments in the present poly(urethane urea)s aggregate to form microcrystallites, in spite of the isomeric forms of the present diisocyanate H_{12}MDI due to extensive hydrogen bonding interactions, which were hitherto observed only in aromatic poly(urethane urea)s. Since the number of polar groups per volume is more in the hard segment blocks compared to soft segment, physical crosslinking exists mainly in the hard segments. These extensive interactions in the hard segment region result in the ordering of hard segment in short and long range fashion leading to micro- crystallisation.

4.2.3. Wide Angle X-ray Diffraction Analyses

The wide-angle X-ray diffraction analyses (WAXD) data of poly(urethane urea)s also support the formation of micro crystallites through virtual cross-linking. The WAXD spectra for poly(urethane urea)s, poly(ether urethane urea) and

polyurethane are given in Figure 4.10. The WAXD peak of poly(urethane urea) is more intense at 19^0 of 2θ angle scale corresponding to 4-5 A^0 d-spacing. Peak intensity at 19^0 of 2θ for WAXD spectrum of poly(urethane urea)s is more than that of poly(ether urethane urea)s containing the same hard segment percentage. For example, the WAXD peak of poly(urethane urea), HFL13-PU is more intense than that of HFL17-PU. Similar is the case of HFL9-PU and HFL16-PU. The more peak intensity at 19^0 is due to the presence of microcrystallites in poly(urethane urea)s by short range ordering in hard segment domains due to urea-urea hydrogen bonding. The microcrystallites formed due to hard segment ordering is represented in Figure 4.11. Microcrystallites has been observed by earlier investigators in the case of poly(ether urethane urea)s based on toluene diisocyanate, PTMG, poly (butylene adipate) and ethylene diamine (Sung *et al,*1980). Microcrystallites has also been noticed only in aromatic poly(ether urethane urea)s such as Biomer® based on MDI.

No reports on the crystallites formation in aliphatic poly(urethane urea) have appeared so far. The present studies reveal the formation of crystallites in aliphatic poly(urethane urea)s based on H_{12}MDI. Though poly(ether urethane urea)s, HFL16-PU and HFL17-PU contain crystallizable PTMG soft segments, there is no more intense peak other than that observed in poly(urethane urea)s. This is due to the lack of greater phase-separation in them, resulting from the soft and hard segment-mixing through additional ether-urea hydrogen bonding in poly(ether urethane urea)s.

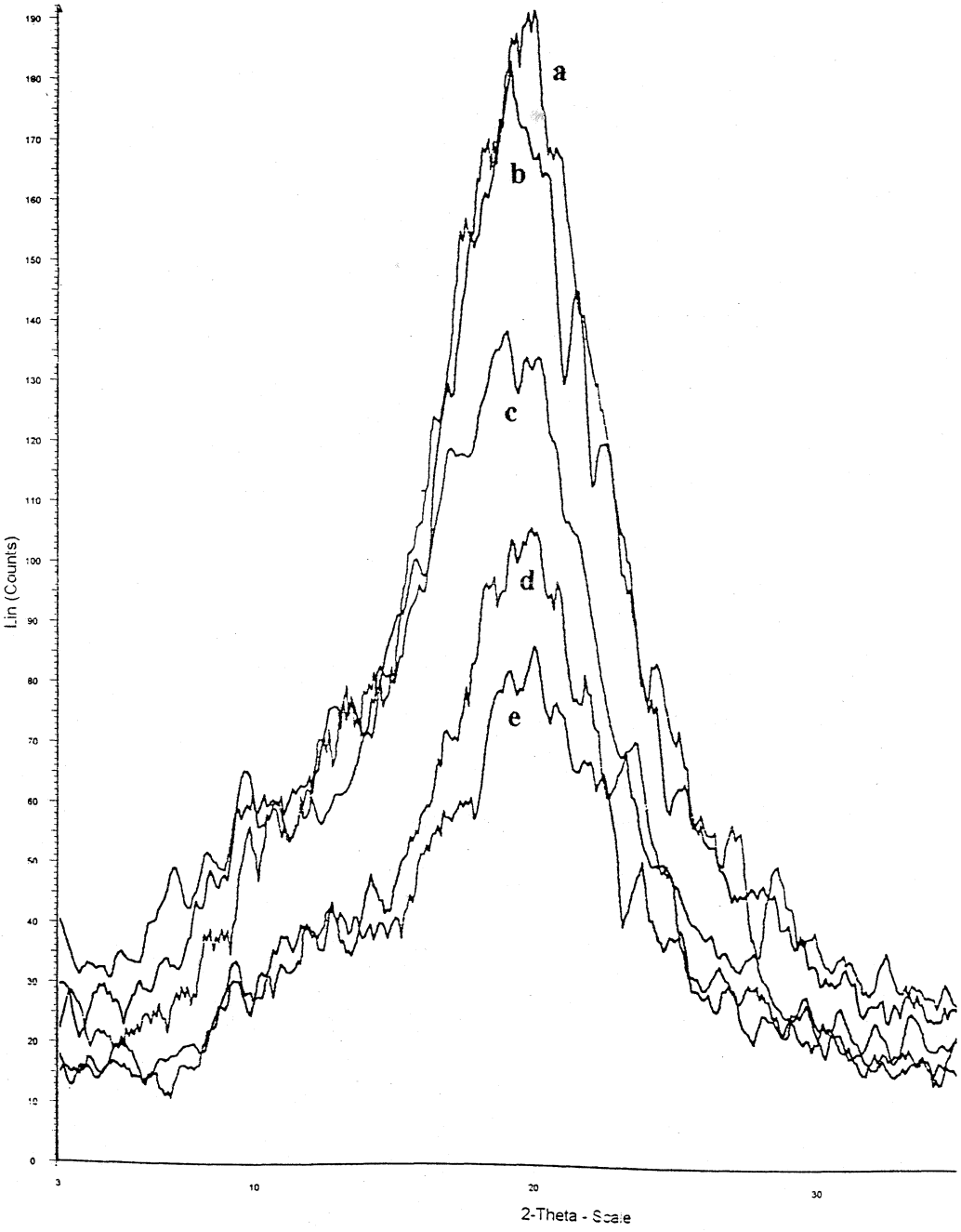


Figure 4. 10 Wide angle X-ray diffraction spectra of polymers (a) HFL15-PU (b) HFL18-PU (c) HFL13-PU (d) HFL17-PU (e) HFL3-PU

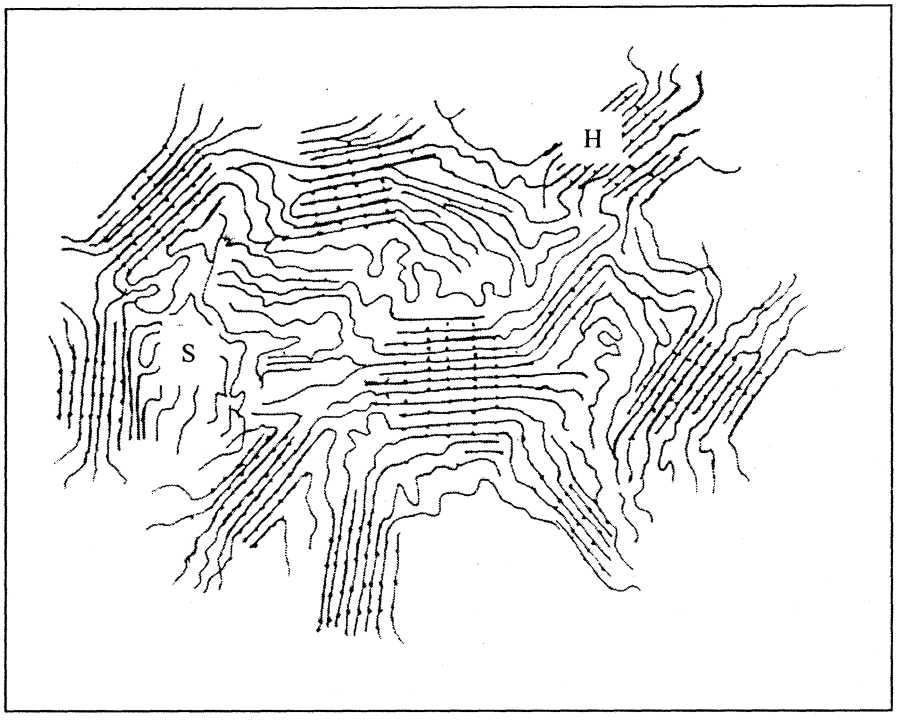


Figure 4.11 The diagramatic representation of microcrystallites (H-Hard segment, S-soft segment)

In the case of poly(urethane urea)s, as the virtual crosslink density increases the peak intensity at 19^0 angle increases. The WAXD peaks for HFL15-PU and HFL18-PU were found to have more intensity. This is attributed to the intense microcrystallisation resulting from the higher degree of virtual crossliking due to higher hard segment percentage (75-80%). Li *et al* (1988) have reported that segmented polybutadiene diol-based polyurethanes with low hard segment content exhibited a morphology of dispersed, short segment cylinders embedded in a matrix of polybutadiene soft segments. Another study by Ogata *et al* (1987) also supported the formation of lamellar morphology in polybutadiene-polyamide multiblock copolymers. However, the present poly(urethane urea)s and poly(ether urethane

urea)s are associated with microcrystallites structure in hard segment domain.

4.2.4 Viscoelastic Properties - Dynamic Mechanical Analyses

Dynamic mechanical analysis (DMA) measures the response of the polymers to a sinusoidal stress over a range of temperature and frequencies and is sensitive to chemical, physical stresses of polymers and their components. The complex modulus (E*) obtained from DMA is resolved into two components, i.e., storage modulus E' and loss modulus E". The storage modulus (E') represents the elastic component of a system and is equivalent to the energy stored in a cycle of deformation. The loss modulus (E") represents the viscous component of a material behaviour and it is equivalent to the energy dissipated through deformation or energy loss as heat in cycle of deformation. The tangent of phase angle, (tan δ) is a measure of the ratio of the energy lost to energy stored during cyclic deformation. For a perfectly elastic solid, the strain is exactly in phase with the stress. For a perfectly viscous liquid, the strain is 90^0 out of phase. In the case of a viscoelastic material, the strain is somewhere in between ($\pi/2 > \delta > 0$) (Ferry, 1980).

$$E^* = E' + iE'' \qquad E'' = / E^* / \sin\delta,$$

$$E' = / E^* / \cos\delta \qquad \tan\delta = E''/E'$$

For materials, which have a higher value of loss tangent, energy used to deform the material is dissipated as heat and cause changes in the polymeric structure by movements of polymeric segments or atomic grouping. The movements may not be completely reversible and may therefore result in permanent deformation of the material. However, for materials intended for fatigue applications, the loss modulus should be low (Miller, 1981), because the heat generated during flexing is directly

proportional to loss modulus. The relation between the loss modulus and heat generated is given by the equation;

$$Q = (7.5 \times 10^{-8}) \Sigma_0^2 E'' Vf$$

Where Q = Heat generated per second in the sample (calories)

Σ = Maximum strain amplitude, E"= Loss modulus (dyn/ cm^2)

V= Volume of the sample (cm^3) and f= Frequency of testing (Hz)

The storage modulus (E') and loss modulus (E") and the tan δ observed at the glass transition temperature of the present polymers are given in Table 4.6. The storage modulus and loss modulus values of poly(urethane urea)s and poly(ether urethane urea)s are found to increase with increase of hard segment content.

Table 4.6 Dynamic mechanical data of polymers

Polymer	Hard segment (%)	Tg (^{0}C)	Storage modulus E' (dyn/cm^2)	Loss modulus E" (dyn/cm^2)	Tan δ (E"/E')
Polyurethane urea					
HFL9-PU	57.5	-66	8.89×10^9	3.11×10^9	0.35
HFL13-PU	67.9	-67	1.06×10^{10}	3.49×10^9	0.33
HFL18-PU	75	-66	1.18×10^{10}	4.00×10^9	0.39
HFL15-PU	80	-65	1.25×10^{10}	4.24×10^9	0.40
Poly(ether urethane urea)					
HFL16-PU	57.5	-72	5.68×10^9	2.21×10^9	0.39
HFL17-PU	67.9	-66	1.31×10^{10}	2.49×10^9	0.20
Tecoflex 85 A	--	-65	1.32×10^{10}	2.80×10^9	0.21

The temperature at which the loss modulus reaches a maximum value is considered as glass transition temperature (Takahara et al 1991). The glass transition temperature (Tg) observed at around -65 ^{0}C reflects the glass transition of soft segments irrespective of the hard segment content (Table 4.6.). The loss moduli have

been found to be low. Under the physiological conditions, the heart valve leaflet may undergo flexing with small strain amplitude.

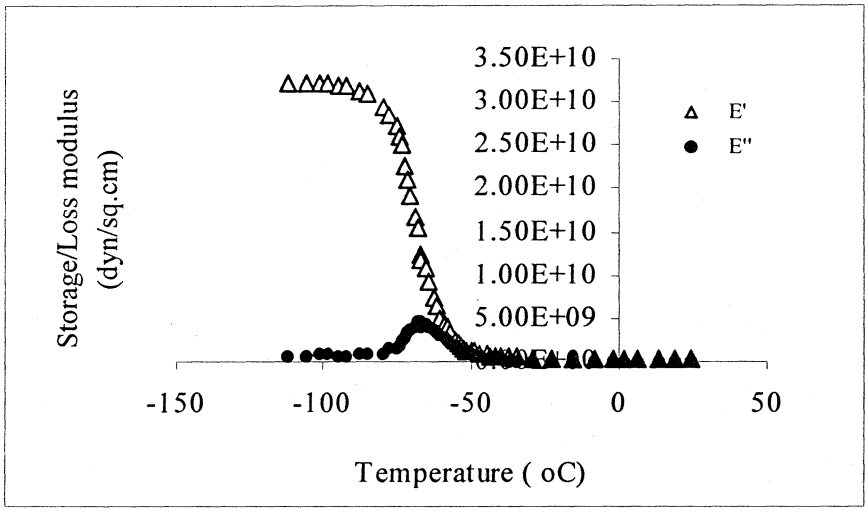


Figure 4.12 Dynamic mechanical properties of HFL18-PU at 35 Hz

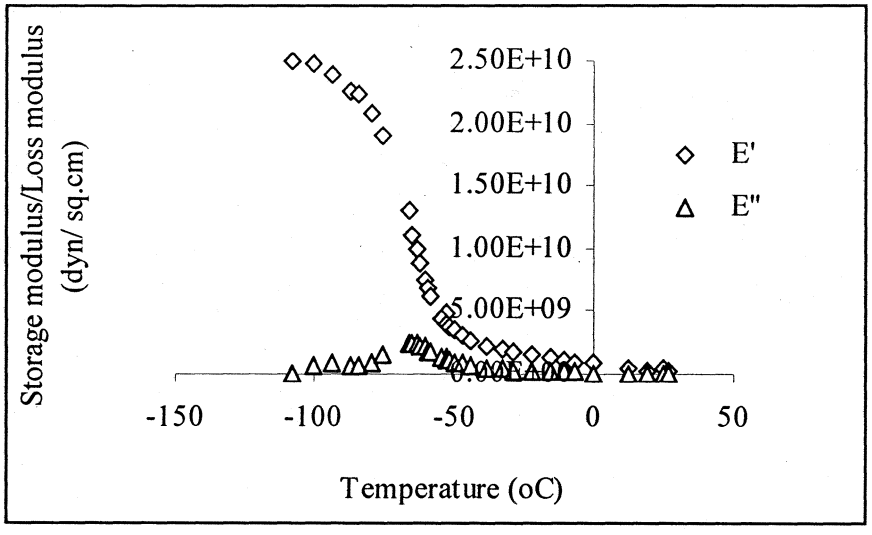


Figure 4.13 Dynamic mechanical properties of poly(ether urethane urea), HFL17-PU at 35 Hz

Under the conditions of small amplitude, and low ambient temperature of 37 ^{0}C and the possibility for large heat transfer to the surrounding physiological fluid, the heat generation during flexing becomes nearly equal to heat transfer. Therefore,

the chance for thermal failure during flexing is negligible. Due to the low loss modulus, the chances for the brittle failure during dynamic flexing are probably low. The representative DMA curves of poly(urethane urea), HFL18-PU and poly(ether urethane urea), HFL17-PU are given Figure 4.12 and Figure 4.13 respectively.

4.2.5 Determination of Mechanical Properties

The mechanical properties are extremely important requirements of elastomeric polymers used in leaflets of heart valve and pump diaphragm of blood pump. For example, the material flexibility expressed by the elastic modulus and its stability are important for the design and performance of blood pump and heart valve. Reliability and safety of a blood pump are closely related to the tensile strength, ultimate elongation and fatigue life of component polymer (Hayashi, 1985). The data on mechanical properties of virgin polymers are given in Table 4.7. Tensile data of virgin polymers reveal that all the polymers have low elastic modulus.

Table 4.7 Mechanical properties of polymers

Polymer	Ultimate tensile strength (MPa)	Ultimate elongation (%)	Elastic modulus (MPa)	Hardness Shore A
Polyurethane				
HFL1-PU	3.430±0.697	236.8±83.9	2.064±0.195	59±2
HFL3-PU	5.084±0.600	161.0±33.0	3.881±0.085	69±1
Poly(urethane urea)				
HFL9-PU	7.280±0.600	336.0±8.4	3.313±0.240	71±3
HFL13-PU	7.780±0.678	207.4±17.4	4.630±0.161	73±2
HFL18-PU	15.75±0.600	336.9±15.0	6.841±0.267	80±2
HFL15-PU	15.00±1.09	151.3±19.5	11.39±0.435	90±1
Poly ether urethane urea				
HFL16-PU	9.097±1.527	335.5±63.0	4.189±0.049	75±3
HFL17-PU	14.81±1.10	273.4±38.3	8.348±0.519	80±2

Polyurethanes used as vital components in biomechanically sensitive blood contact devices such membrane of blood pumps and cardiac assist devices and flexible leaflet of the artificial heart valve should have low elastic modulus, which can allow repeated cyclic flexing in biological conditions. Moreover, low elastic modulus polyurethanes with reduced bending stress can allow the membrane to flex more freely without producing adverse changes in blood flow.

Mechanical properties such as tensile strength, elongation (%), elastic modulus and hardness are influenced by many parameters such as molecular weight of the polyol, hard to soft segment ratio, crystallite formation and virtual crosslinking. Generally, as the hard segment content increases the virtual crosslink density increases and formation of microcrystallites also increases. The relation between crosslink density and tensile properties is given in Figure 4.14. It is observed that with the increase of virtual crosslink density, the tensile strength and elastic modulus increases. This may be attributed to the increased hard segment aggregation due to urea-urea interactions and also due to the increase in hard segment percentage or amine content. HFL15-PU has the highest elastic modulus in comparison with all other polymers due to the higher cross-link density and higher hard segment content (80%). Moreover, the higher percentage of cross-links in HFL15-PU has resulted in reduced elongation (%) in comparison with that of all other polymers. The required range of elastic modulus of polymers for use in cardiac assist devices such as trileaflet heart valve is 5-8 MPa. Accordingly, poly(urethane urea), HFL18-PU having elastic modulus 6.814 MPa may be a suitable candidate material for application in cardiac

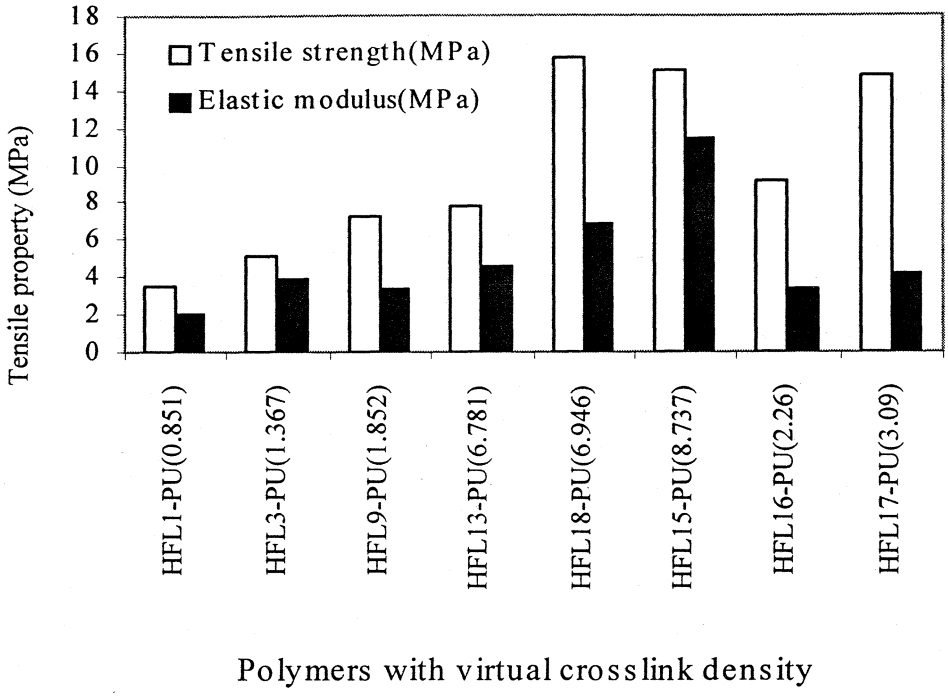


Figure 4.14 Virtual crosslink density Vs. Mechanical properties

Commercial polyurethanes, Elast-Eon®, (Elatomedic Pty Ltd, Australia) which has been claimed for use in cardiac applications, possess low elastic modulus in the range of 5-10 MPa. With the present poly(ether urethane urea)s also, the ultimate strength increased with increase in crosslink density. HFL16-PU and HFL17-PU showed higher value of tensile strength compared to poly(urethane urea)s, HFL9-PU and HFL13-PU of similar hard segment content. This increased tensile strength in poly(ether urethane urea)s is due to the strong phase-mixing interaction offered by the ether-urea and ether-urethane linkages. The ultimate tensile strength of the polyurethanes, HFL1-PU and HFL3-PU are not appreciably higher than that of the poly(ether urethane urea), HFL16-PU and poly(urethane urea), HFL9-PU though the polyurethanes have higher hard segment content. This is due to the lack of virtual crosslinking and microcrystallites in hard segment domains in polyurethanes.

The Hardness of the newly prepared polymers is also given in Table 4.7. All the polymers have hardness in the range of 70-90 Shore-A, which is in agreement with the hardness of commercial polyurethanes, e.g. 85A for Tecoflex®, 75A for Biomer®, 72A for Cardiothane®, 80A for Cardiomat®, 80-90A for Pellethane®, 72-85A for Elast-Eon® etc. The data on hardness of poly(urethane urea)s showed sharp increase in hardness with increase in the virtual crosslink density or hard segment content. Poly(ether urethane urea)s also showed a similar trend as discussed on tensile strength and crosslink density. The hardness of polyurethanes is relatively lower due to lack of virtual crosslinking and microcrystallies in hard segment domains.

4.2.6 Determination of Surface Properties

Surface water contact angle is a measure of the surface wetability of the polymer. Since a biomaterial which interfaces with blood has a surface tension close to that of water, measurement of contact angles in water phase is desirable (Kaelble *et al,* 1977). The lower the water contact angle, the higher is the tendency of water to spread over the surface (Rabek, 1980). Thus hydrophilic materials are expected to have lower contact angles than hydrophobic materials. The water contact angles measured for the present polymers by sessile drop method are given in Table 4.8. The surface free energy was obtained using contact angle-free energy conversion tables published elsewhere (Neumann, 1980).

It is observed that all the present polyurethanes, poly(ether urethane urea)s and poly(urethane urea)s are hydrophobic irrespective of the value of virtual crosslink density. High hydrophobic water contact angle was mainly due to the presence of

hydrophobic soft segment HTPBD. Polyurethane HFL1-PU (with polyol 37.5%) and HFL3-PU (with polyol 35.8%) have exhibited water contact angle of 87.75 and 87.25 deg respectively. However, poly(urethane urea) HFL9-PU (with polyol 42.5%) and poly(ether urethane urea) HFL16-PU (with polyol 42.5%) have showed contact angle 82.0 and 84.0 deg respectively.

Table 4.8 Surface properties of polymers

Polymer	Water contact angle (deg)	Surface energy (dynes/cm) γ_{SV}	γ_{SL}
Polyurethane			
HFL1-PU	87.75±1.6	33.86	23.77
HFL3-PU	87.25±1.5	30.71	26.92
Poly(urethane urea)			
HFL9-PU	82.0±1.2	33.66	23.77
HFL13-PU	83.0±1.7	33.23	24.40
HFL18-PU	85.4±1.5	31.97	25.06
HFL15-PU	85.0±1.3	31.97	25.66
Polyether urethane urea			
HFL16-PU	84.0±2.3	32.60	25.03
HFL17-PU	83.2±1.4	33.23	24.40

Hence it can be inferred that the introduction of more urea linkages in the present poly(ether urethane urea)s and poly(urethane urea)s could influence the surface property. However, all these polymers are catagorised as hydrophobic polymers. Since contact angle studies were conducted in air medium it could be inferred that hydrocarbon polyol is present at the air-surface. The hydrophobicity of the present polymers is a favorable factor for blood contact applications of polymers as it can enhance albumin adsorption and reduce platelet adhesion on the surface of

these polymers (Strizinar and Sefton, 1992; Marconi *et al*, 1995). Yoda (1998) has also reported the use of hydrophobic polyurethane polymer as a strategy to improve antithrombogenicity of polymer- surfaces.

4.2.7 Studies on Thermal Characteristics

The thermal characteristics of the poly(urethane urea)s were assessed using thermogravimetric analyses (TGA). TGA curves of the poly(urethane urea)s are given in Figure 4.15. The TGA curves of polyurethane and poly(ether urethane urea)s are given in Figure 4.16.

The thermal degradation of thrermoset systems proceeds by random scission and can be assigned a first order reaction (Mathew *et al,* 2000). Mahajan *et al* (2000) reported that thermal degradation occurs through a concerted reaction mechanism and first stage of degradation may be at C-NH bond, the weakest bond in polyurethane. The last stage of degradation is the oxidative degradation of backbone structure of polyurethane.

The TGA curves shows degradation of the present virtually crosslinked polymers through two stages. The thermogravimetric analyses data are given in Table 4.9. The temperature at which loss of weight starts for the first and second stages are given as $T_{d1,0}$ and $T_{d2,0}$. The temperature at which the half of weight reaches 50% is given as $T_{d,1/2}$. The onset temperature of the first step degradation ($T_{d1,0}$) of poly(urethane urea)s increases with increase of soft segment content. On the other hand the onset temperature of the second step degradation ($T_{d2,0}$) decreases with increase of soft segment content.

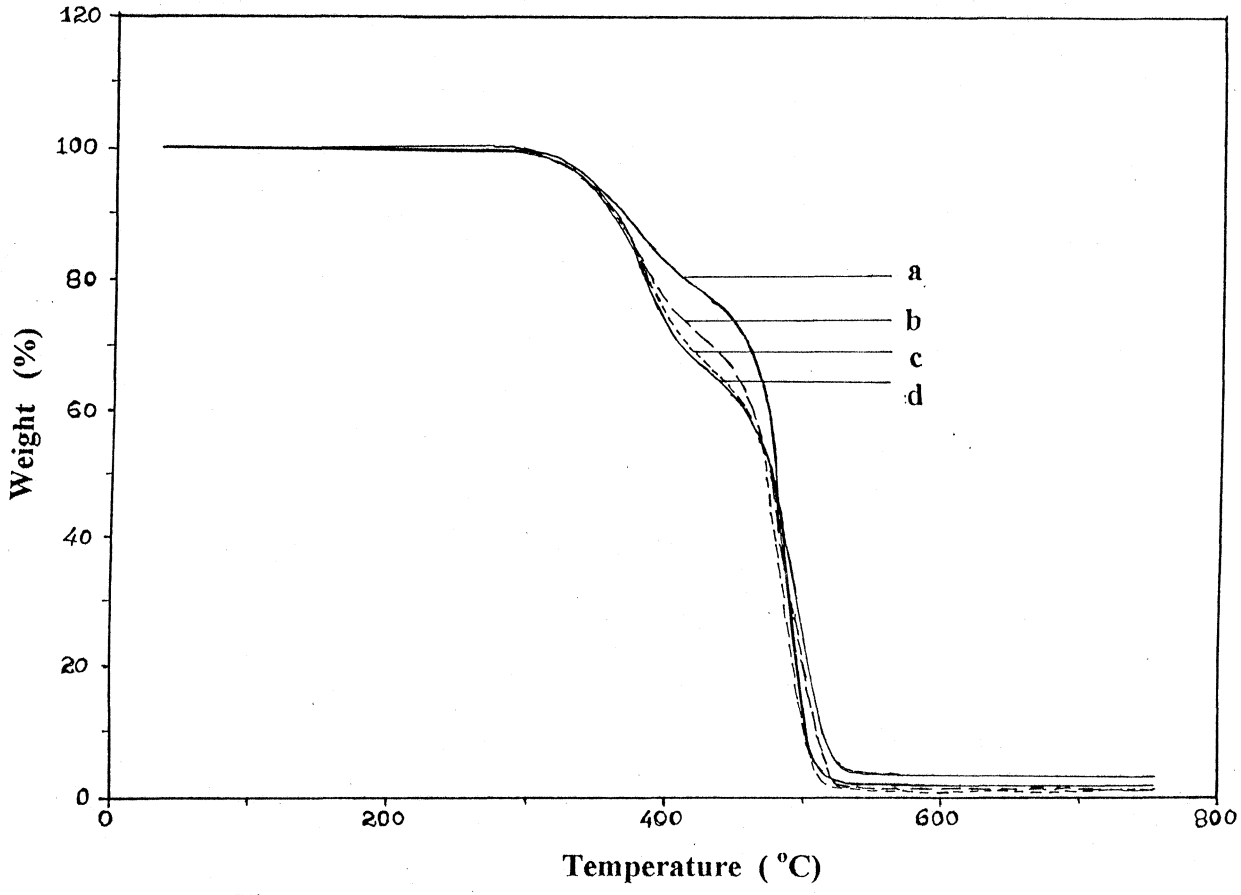


Figure 4. 15 TGA curves of poly(urethane urea)s
(a) HFL9-PU (b) HFL13-PU (c) HFL15-PU (d) HFL18-PU

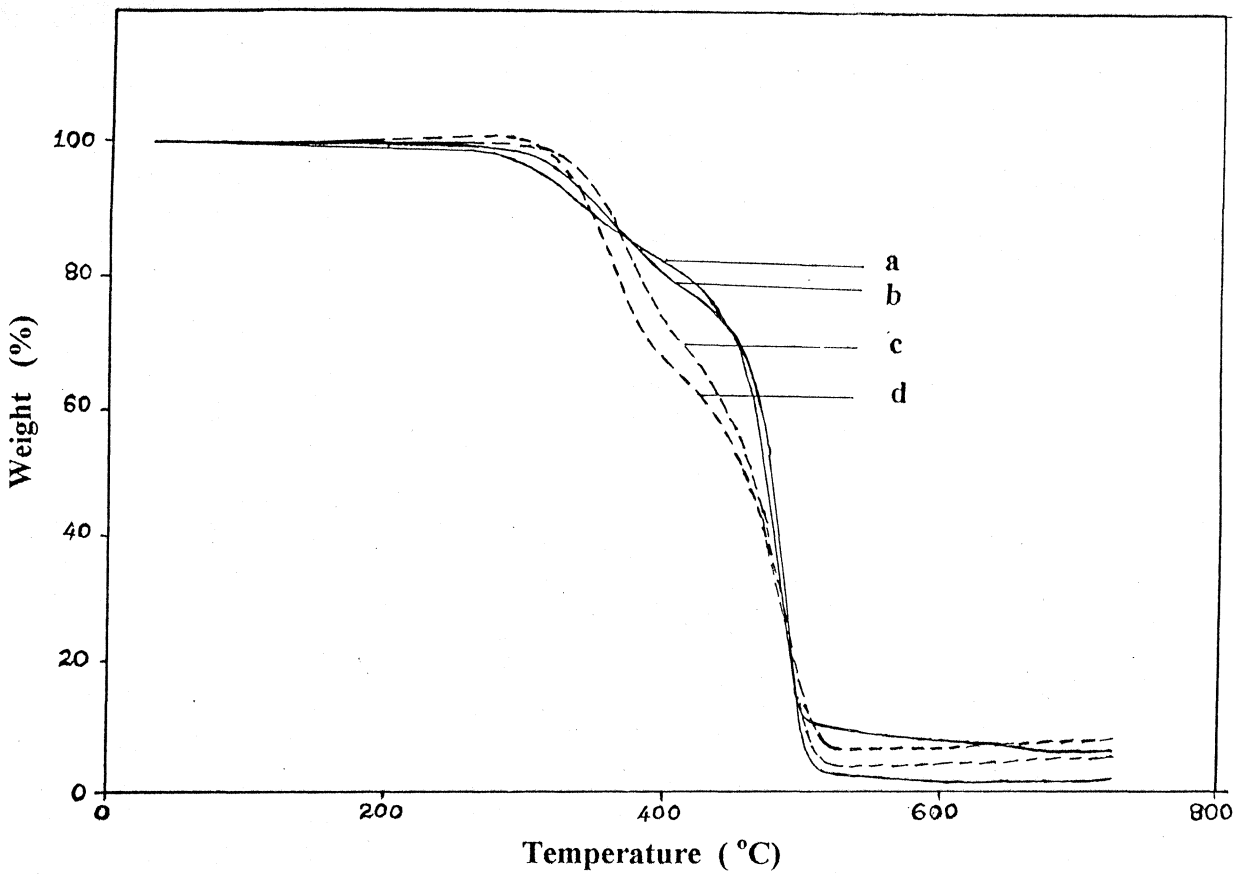


Figure 4.16 TGA curves of poly(ether urethane urea)s and polyurethanes (a) HFL3-PU (b) HFL1-PU (c) HFL16-PU (d) HFL17-PU

Table 4.9 Thermal characteristics of polymers

Polymer	Soft segment (%)	Td1,0 (^{0}C)	Weight loss in first step	Td2,0 (^{0}C)	Td 1/2 (^{0}C)
Polyurethane					
HFL1-PU	37.5	260	22	405	475
HFL3-PU	36.8	250	23	415	475
Poly(urethane urea)					
HFL9-PU	42.5	295	22	415	485
HFL13-PU	32.1	285	30	420	475
HFL18-PU	25	275	35	425	480
HFL15-PU	20	260	38	440	480
Poly(ether urethane urea)					
HFL16-PU	42.5	270	35	425	475
HFL17-PU	32.1	275	35	410	460

However, a reverse trend was observed in the case of poly(ether urethane urea)s. The weight loss (%) in the first step degradation decreased with increase of soft segment content (Table 4. 9). The general behaviour of thermal degradation resembles the thermal degradation of segmented polyurethanes.

4.3. Studies on the Biostability of Polyurethanes

Biodegradation of biomedical materials is a great concern for implants, which are intended for long-term applications. The biodegradation can induce changes in properties of the materials and also lead adverse effects to the surrounding physiological environment. Differences in the degree of degradation reported and in the mechanisms proposed for the degradation have resulted from the various

definitions assigned to 'biodegradation'. Lemm *et al* (1981) considered the changes in surface properties or loss of mechanical strength of implants as biodegradation. Potts defined 'biodegradable materials as those which, because of their chemical structure, are susceptible to be assimilated by micro-organisms such as fungi and bacteria' (Potts *et al*, 1973). Vert has stated that biodegradable polymeric implant systems and devices can be attacked by biological elements so that the integrity of the system is affected and, in some cases not necessarily, gives fragments or other degradation by-products (Vert *et al*, 1992). Huang *et al*, (1979) stated biodegradation as enzyme-induced degradation. Marchant *et al* (1984) presented biodegradation as 'occurring on many different structural levels, i.e., molecular, macromolecular, microscopic and macroscopic, depending on the mechanism'. Ratner *et al* (1988) adopted the classical polymer science definition of degradation that requires backbone chain breakage and change in molecular weight and developed an *in vitro* model to study the polyurethane susceptibility to degradation by exposing the polymer to enzymatic and oxidative treatments. ASTM D 671 defined fatigue failure as: (i) decrease of elastic modulus to 70% of the original value (ii) change of warping, crazing, cracking, formation of internal voids or deformation (ASTM 1999).

Many factors can have an impact on the biostability of polyurethanes. Hydrolytic and oxidative stability of the various segments and sequences in a polymer material is important, as is the hard-soft segment composition ratio. Chemical composition of the polyurethane is primarily attributed as the chief determinant of biostability. However, synthesis, processing and fabrication methods as well as storage and sterilisation conditions can also play a role.

Early studies on the biostability of segmented polyurethanes have generated contradictory results. Some groups reported long-term stability without evidence of biodegradation (Mirkovitch *et al,* 1962; Hunter *et al,* 1982). But most of the commercial polyurethanes are made of aromatic diisocyanates such as MDI or TDI. Degradation of these polyurethanes has been found to occur by different routes such as: sterilisation of the medical devices by gamma radiation, autoclave (steam) sterilisation or as the result of long-term exposure to body fluids. Degradation of aromatic polyurethanes can yield extractable 2,4-and 2,6- toluene diamines (TDA) and 4,4'-methylene dianiline (MDA) (Luu and Hutter, 2000; Shintani, 1995; Sepai *et al*, 1995). These are hydrolytic degradation products of 2,4- and 2,6-toluene diisocyanate (TDI) and 4,4'-methylene diisocyanate (MDI) based polyurethanes respectively. The toxicology profile of MDA has been extensively reviewed elsewhere (U.S Dept., 1998). Both TDA and MDA have been shown to be mutagenic in salmonella typhymorium test strains. MDA was found to cause cancer in both rat and mice with primary tumour sites in the liver, kidney and thyroid. Humans exposed to high dose of MDA, accidentally or occupationally, developed jaundice, skin rash, dyspnea, rhino-conjuctions and liver damage (U.S. Dept., 1998).

The present polyurethanes, poly(ether urethane urea)s and poly(urethane urea)s contain linkages ether, or -C=C- double bond in soft segments, urethane and urea functionalities in hard segments depending on the nature of polymer, that can be attacked by hydrolytic, oxidative agents or enzymes and result macromolecular degradation with dispersion *in vivo*.

4.3.1 Studies on the Influence of Biochemical Agents on the Degradation

Virtually crosslinked, amorphous polyurethane polymers can under go five processes due to the interactions of biochemical agents during aging in physiological media. (1) Rearrangement of urethane and urea hydrogen bonds; (2) vitrification of segments (3) reconstruction of glassy hard segments and microphase separation of crystalline hard segments; (4) mixing of hard and soft segments resulting in a phase-mixed state; (5) degradation of functional group involved in hydrogen bonding.

4.3.1.1 Evaluation of hydrolytic Stability in Ionic Media

Generally in polyurethanes, the urethane or carbamate linkage is susceptible to hydrolysis similar to that of an ester linkage of a substituted carbamic acid. Unlike in the hydrolytic degradation of carboxylic ester linkage, the hydrolytic degradation occurs less readily in urethane (Schollenberger and Stewart, 1971) and urea groups. However, under extraneous polymer processing conditions such as injection moulding at high temperature and extrusion in the presence of water, urethane linkage is prone to undergo thermohydrolytic cleavage (Stokes *et al*, 1995). Hydrolytic degradation of polyurethanes with different soft segments has been observed in the order : polyester polyol-based polyurethanes> polyether polyol based-polyurethanes> polycarbonate based polyurethanes> polyhydrocarbon polyol based polyurethanes> poly(urethane urea)s.

The possibility of hydrolytic degradation in the present polymers is probably centered on urethane linkages associated only with the hydroxy terminated poly butadiene soft segment and the urea groups in the hard segments. But the probable mechanism of hydrolysis of urethane linkages produces two new shorter chains, one

hydroxy teminated and other amine terminated as shown in equation 4.1.

$$\sim(O\text{-}(CH_2)_4)_n\text{-}O\text{-}CO\text{-}NH\text{-}R\text{-}CO\text{-}O\sim + H_2O \longrightarrow \sim(O\text{-}(CH_2)_4)_n\text{-}OH + H_2N\text{-}R\text{-}NH\text{-}CO\text{-}O\sim + CO_2 \quad (4.1)$$

Low molecular weight compounds containing free urea groups are also susceptible to hydrolysis. Due to the hydrophobicity of all the present polymers, the urethane linkages associated with the hydroxy terminated poly butadiene soft segment must be predominantly present on the surface. This surface–occupied urethane linkages may undergo hydrolytic degradation.

Aging studies on the present polymers in hydrolytic media *viz.* Ringer's solution and PBS revealed the hydrolytic stability of poly (ether urethane urea)s and poly(urethane urea)s . Poly (ether urethane urea)s and poly(urethane urea)s aged in hydrolytic media revealed no change in weight both in the wet and dry conditions. However, polyurethanes, HFL1-PU and HFL3-PU undergo loss of weight in both Ringer's solution and PBS media. HFL3-PU has showed 4.07±1.7 % weight loss in Ringers solution and 3.06± 0.68 % weight loss in PBS medium after 30 days *in vitro* aging. Similarly HFL1-PU also showed nearly 5 % weight loss in both hydrolytic media. Further, HFL3-PU undergoes appreciable increase of ultimate tensile stress (57%) due to aging in Ringers solution and 13% in PBS when comparison to its control virgin samples. The aged-samples of HFL1-PU also undergo appreciable loss of tensile properties in comparison with that of its control in both the media. This may be attributed to the low value of virtual crosslink density and also to the hydrolysis of urethane linkages. The mechanical and surface properties of polymers aged in Ringers solution are given in Table 4.10 and Table 4.11respectively. The mechanical and

surface properties of polymers aged in PBS are given in Table 4.12 and Table 4.13 respectively.

Table 4.10 Mechanical properties of aged polymers in Ringers solution

Polymer	Ultimate tensile strength (MPa)	Ultimate elongation (%)	Elastic modulus (MPa)	Hardness (Shore A)
Polyurethane				
HFL1-PU	3.12±0.33	19.150 ± 7	-	62
HFL3-PU	7.99±0.65	8.705 ± 2	-	85.5
Poly(urethane urea)				
HFL9-PU	8.483±1.3	363 ± 20	3.821 ± 0.3	79
HFL13-PU	6.371±0.6	190 ± 11	4.279 ± 0.12	81
HFL18-PU	16.56±2.1	331.9 ± 13	8.053 ± 0.21	93
HFL15-PU	17.75±2.6	146.66± 9	13.191 ± 0.7	91
Poly(ether urethane urea)				
HFL16-PU	7.696±1.19	315±13	3.819±0.014	79
HFL17-PU	9.703±1.1	173± 8	7.371±0.12	91

The tensile strain of aged HFL3-PU decreased from 151 % to ~8 % after aging in both media. Similarly tensile properties of HFL1-PU also changed drastically due to aging in Ringers solution and PBS media. The appreciable loss of tensile strain of HFL1-PU and HFL3-PU in hydrolytic media may be due to breakdown of urethane linkages present in between the soft-hard segments and the formation of HTPBD soft segment chain ends. Further, the hardness of aged HFL1-PU and HFL3-PU materials in both the media increased significantly compared to that in poly(urethane urea)s and poly(ether urethane urea)s. With the scission of urethane linkages the resultant polymer could have enriched with hard segment leading to the increase in hardness (24%). This inference is confirmed with the fact that water contact angle is decreased

appreciably in the case of aged samples of HFL1-PU and HFL3-PU in both the hydrolytic media.

Table 4.11 Surface properties of polymers aged in Ringers solution

Polymer	Water contact angle (deg)	Surface free energy γ_{SV}	γ_{SL}
Polyurethane			
HFL1-PU	53.87±1.7	50.62	8.00
HFL3-PU	47.5± 2.0	54.14	5.62
Poly(urethane urea)			
HFL9-PU	75±1.2	38.2	19.43
HFL13-PU	76±1.0	37.58	20.05
HFL18-PU	77±2.0	36.97	20.66
HFL15-PU	76±2.1	37.58	20.05
Poly(ether urethane urea)			
HFL16-PU	77±1.5	36.97	20.66
HFL17-PU	73±3.0	39.41	18.22

Table 4.12 Mechanical properties of aged polymers in PBS

Polymer	Ultimate tensile strength (MPa)	Ultimate elongation (%)	Elastic modulus (MPa)	Hardness (Shore A)
Polyurethane				
HFL1-PU	2.676±0.17	59.29 ± 8	-	59
HFL3-PU	5.741±2.1	8.09 ±2.8	-	85.5
Poly(urethane urea)				
HFL9-PU	9.172±0.23	388 ± 15	3.277±0.31	80
HFL13-PU	5.947±0.14	163.33±10	4.278±0.4	82
HFL18-PU	16.15±20.18	367.9±13	7.812±0.31	92
HFL15-PU	17.27±1.09	141.66±10	13.09±0.21	92
Poly(ether urethane urea)				
HFL16-PU	7.349±0.75	266.66	3.765±0.16	79
HFL17-PU	10.913±1.3	166	8.625±0.41	91

Table 4.13 Surface properties of polymers aged in PBS

Polymer	Water contact angle (deg)	Surface free energy (dynes/cm) γ_{SV}	γ_{SL}
Polyurethane			
HFL1-PU	63.44±2.1	45.31	12.40
HFL3-PU	47.3±2.0	54.72	5.27
Poly(urethane urea)			
HFL9-PU	76±2.2	37.58	20.05
HFL13-PU	79±1.2	35.73	21.90
HFL18-PU	72±2.0	40.02	17.61
HFL15-PU	77±1.7	36.97	20.66
Poly(ether urethane urea)			
HFL16-PU	77±2	36.97	20.66
HFL17-PU	75±1.5	38.20	19.43

The aged polyurethanes, HFL1-PU and HFL3-PU are hydrophilic as indicated by the low value of water contact angle after aging in hydrolytic media. Since the weight loss during aging is not very high (<5%), the fragmented oligomers may be entrapped mainly in the polymer.

Poly(ether urethane urea)s HFL16-PU and HFL17-PU, moderately phase-separated polymers, undergo some loss of tensile strength and elongation due to aging in hydrolytic ionic media (Tables 4.10 and 4.12). This is due to the rearrangement of hydrogen bonding and molecular structure during aging. For example the virtual crosslink density of aged HFL16-PU increased to 7.4223×10^{-4}, in comparison with crosslink density 2.26×10^{-4}, of the virgin polymer. The appreciable increase in crosslink density in HFL16-PU could be mediated through hydrogen bonding interaction with soft and hard segments leading to increased phase mixing. Such phase mixing may be responsible for the decreased tensile strength and elongation in

the aged polymer. The FTIR-ATR-spectra of the aged HFL16-PU exhibit a sharp peak at 1702 cm^{-1} for urethane carbonyl (bonded) in comparison with that of virgin polymer (Figure 4.17). The studies suggest the presence of urethane linkages on the surface due to the rearrangement of segments. The peaks for bonded urea amide I (at around 1631.67 cm^{-1}) and amide II (at around 1558.71 cm^{-1}) also remain intact. The spectra also reveal the continued presence of the peak for C-O-C in O=C-O-C of urethane linkage at 1246.39 cm^{-1}. The studies reveal appreciable hydrolytic stability of urethane and urea linkages in poly(ether urethane urea)s.

Poly(urethane urea)s are appreciably phase– separated in contrast to poly(ether urethane urea)s. In contrast to the inverse relation between the tensile behaviour of aged poly(ether urethane urea) and crosslink density, a direct relation between these parameters was observed with aged poly(urethane urea)s. The tensile properties of aged HFL13-PU slightly decreased in comparison to the virgin polymer. The ultimate tensile strength decreased by 18% and 23% after aging in Ringers solution and PBS respectively. The elastic modulus of HFL13-PU also decreased by 7.7% due to aging in both media. The percentage elongation decreased slightly. This decrease of tensile properties is attributed to the rearrangement of hydrogen bonding and reduction of cross-linking in the bulk of the polymer. The cross-link density of the aged HFL13-PU polymer is 4.8692×10^{-4} and 5.3239×10^{-4} in Ringer's solution and PBS media respectively. In contrast to the aged polymer HFL13-PU, the aged poly(urethane urea) HFL9-PU has attained higher tensile properties after the aging compared to the virgin polymer. The increase in tensile properties of HFL9-PU is due to formation of more physical cross-linking (hydrogen bonding) through urea-

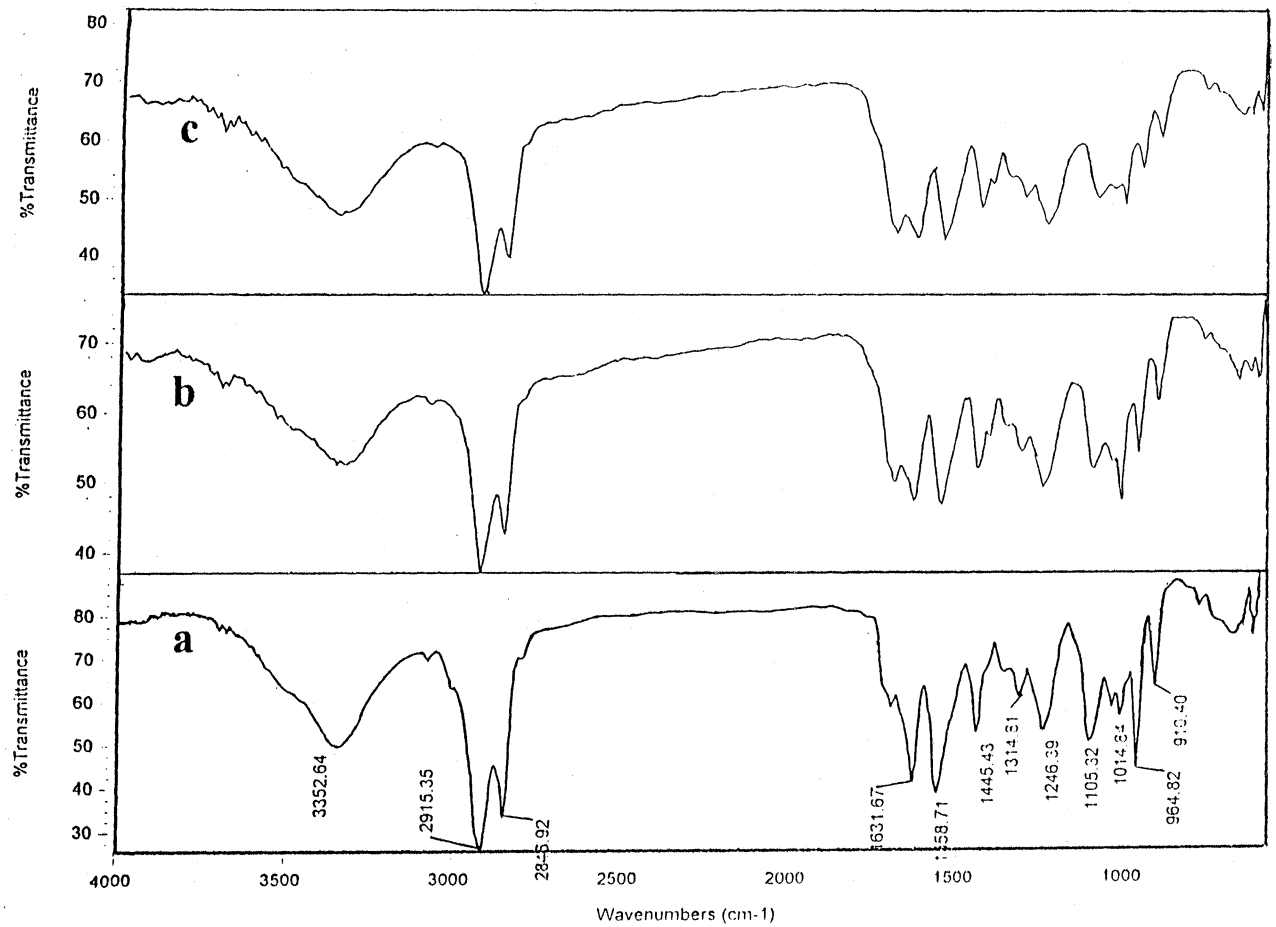


Figure 4.17 FT-IR spectrum of aged poly(ether urethane urea), HFL16-PU (a) virgin polymer (b) in PBS (c) in Ringers solution

urea linkage in the bulk of the polymer. The crosslink density of the aged-sample of HFL9-PU is 2.9141 x 10^{-4} in Ringer's solution. The decrease or increase of physical crosslink density in poly(urethane urea)s does not influence the phase-separation drastically. Therefore, the degree of physical crosslinking is directly proportional to tensile properties. The studies reveal that higher the crosslink density in the bulk of the aged poly (urethane urea), higher is the tensile properties and vice versa.

It has been reported that yield strength and elastic modulus of the polymers are independent when the molecular weight of these polymers reaches above 10000. For polyurethane, these properties are independent of molecular weight above 30,000-40,000 (Phua *et al*, 1987). In the present investigation there is no weight loss due to biodegradation in the case of poly(urethane urea)s and poly(ether urethane urea)s. The change in elastic modulus is attributed to the change in cross-link density resulted from the reorganisation of hard segments.

Elastic modulus of the elastomers is one of the important parameters that influence the performance of the implant in biomechanical environment. The elastic moduli of the present polymers fall in the range 3-8 MPa. The elastic modulus of virgin HFL18-PU is 6.841±0.267 MPa. The required range of elastic modulus for the polymers intended for cardiac valve prostheses is 5-8 MPa. It has been found that the change of elastic modulus of the aged polymers in Ringer's solution and PBS media is not drastic enough to alter the required range of elastic modulus. However, a slight increase was noticed in aged polymer HFL9-PU while a slight decrease was noticed in polymers HFL13-PU and HFL16-PU. The aging of HFL9-PU, HFL15-PU, HFL18-PU polymers in the hydrolytic media has resulted in increased-stiffness,

which is similar to that in physical aging or age stiffening as per 'Struik's rule on physical aging'. Struik (1978) has reported that without progressive stiffening due to physical aging, polymeric materials would not be able to resist mechanical load during long periods of time. Therefore increase of elastic modulus in HFL9-PU, HFL15-PU, HFL18-PU may have some relevance for long-term application.

The IR spectral studies of the aged poly(urethane urea) reveal in vitro biostability. With poly(urethane urea) HFL13-PU aged in hydrolytic media, except a mild peak at 1702cm^{-1} there is no change in the ATR - spectra (Figure 4.18). The peak for bonded urea amide I (at around 1631.69 cm^{-1}) and amide II (at around 1559.36 cm^{-1}) also remain intact after aging. The spectra also revealed the continued presence of the peak for C-O-C in O=C-O-C of urethane linkage and amide III urea at 1246.67 cm^{-1}. The spectra of aged HFL9-PU (Figure 4.19) reveal mild urethane carbonyl (1702 cm^{-1}) and urea carbonyl (bonded) at 1634.52 cm^{-1} peaks. The spectra also reveal the continued presence of the peak for C-O-C in O=C-O-C of urethane linkage and amide III urea at 1247.53 cm^{-1}. The FT-IR spectrum of HFL18-PU aged in hydrolytic media is given in Figure 4.20. The urea carbonyl (hydrogen bonded) peak appeared strongly at 1631.18 cm^{-1} for the sample aged in Ringers medium and at 1631.91 cm^{-1} for the samples aged PBS solution. A shoulder at the peak around 1700 cm-^{1} for the samples aged urethane carbonyl (bonded) also appeared as in virgin polymer. IR spectral data indicate that the poly(urethane urea)s are reasonably stable in hydrolytic media.

The effect of virtual crosslinking in polyurethanes, HFL1-PU and HFL3-PU due to urethane-urethane linkages in soft and hard segments on the degradation can be

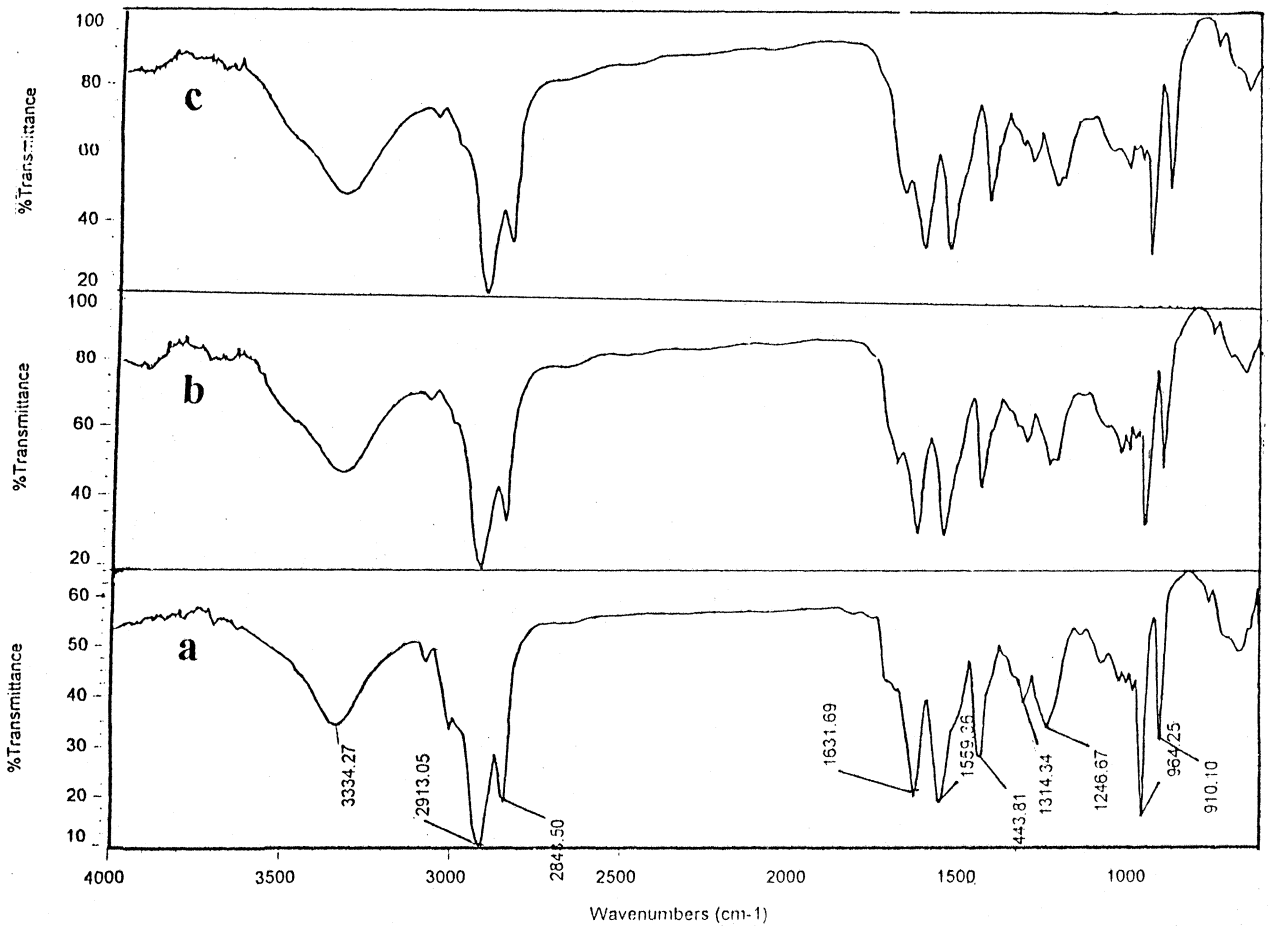


Figure 4.18 FT-IR spectrum of aged poly(urethane urea), HFL13-PU (a) virgin polymer (b) in PBS (c) in Ringers solution

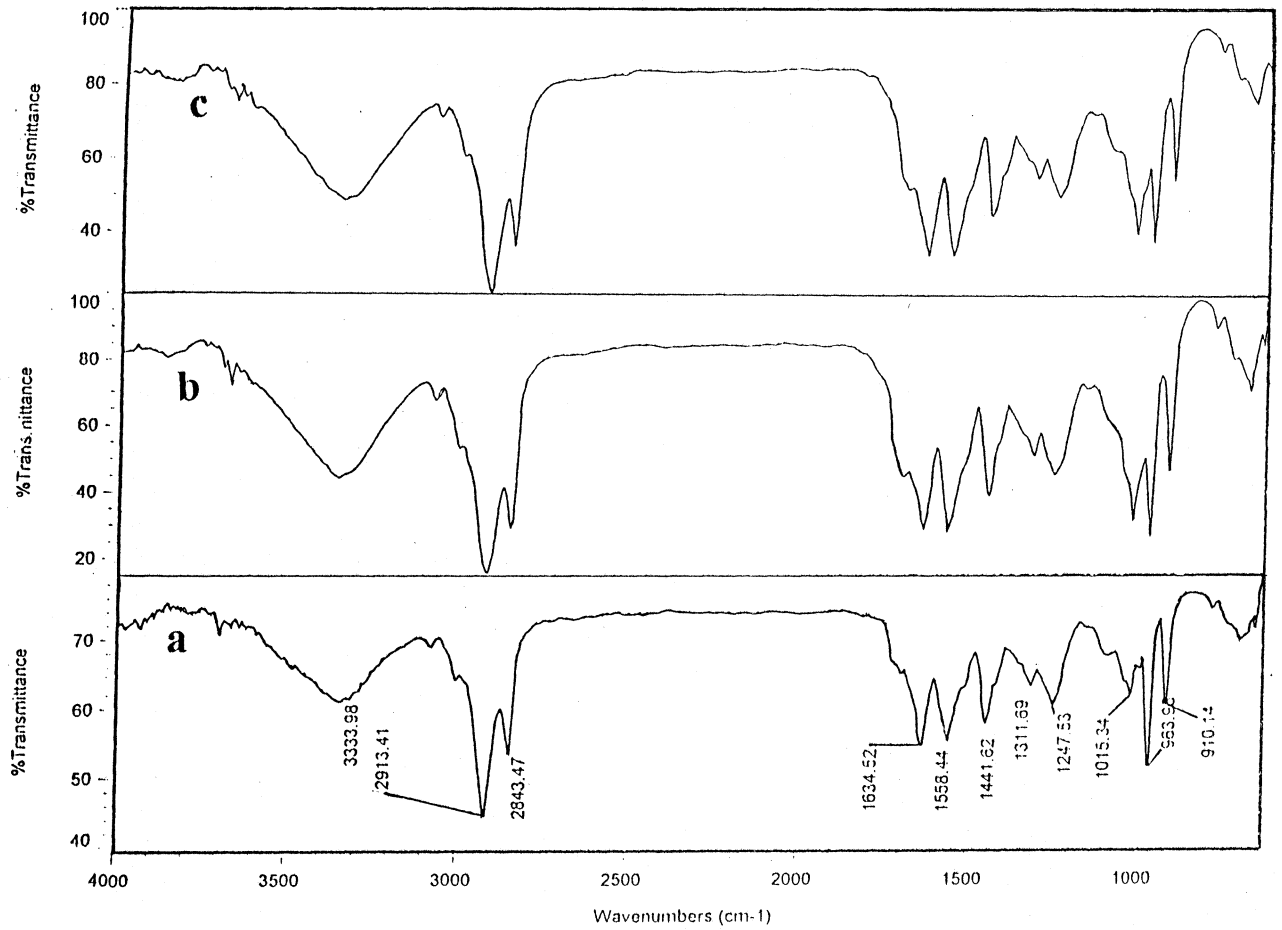


Figure 4.19 FT-IR spectrum of aged poly(urethane urea), HFL9-PU (a) virgin polymer (b) in PBS (c) in Ringers solution

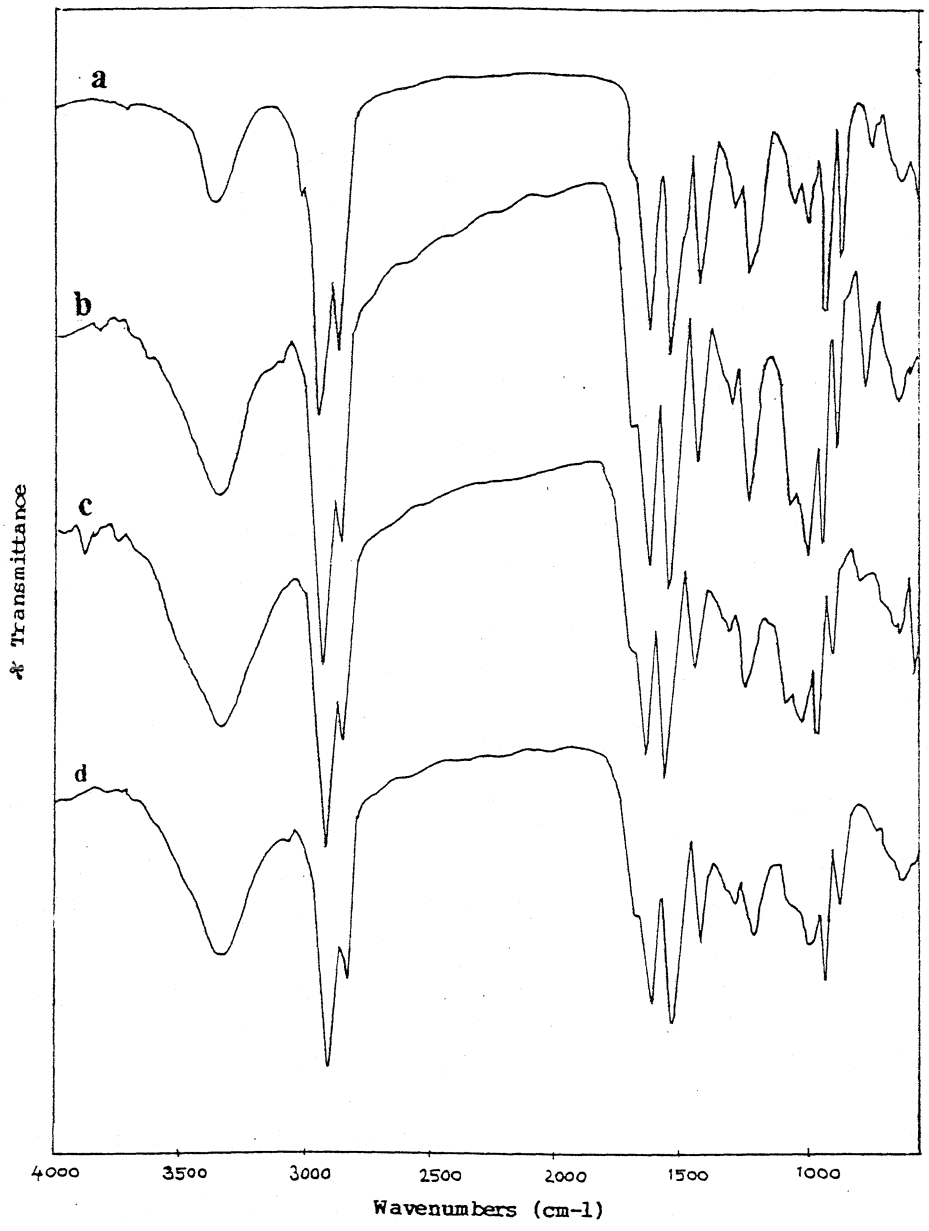


Figure 4.20 FT-IR spectrum of aged poly(urethane urea), HFL18-PU
(a) Virgin polymer (b) in PBS (c) in Ringers solution
(b) in Oxidation medium

explained as follows:

In the presence of aging media the virtual crosslinking may be enhanced by the presence of hydrogen bonding molecules like water. Water-assisted virtually crosslinked polyurethanes can be formed which may often become stronger when hydrated or saturated with hydrogen bonding liquids. As a result of these water-assisted hydrogen bonding the polyurethane backbone may first attain a coiled conformation as reported by Reed (1991). The presence of the coiled conformation allows the water, ions and other agents of hydrolysis to pass through it and finally collapsed in to fragments. Thus cleavage of the urethane bond liberates HTPBD fragments which were then entrapped in the polymer. The final polymer may be hydrophilic polyurethane enriched with hard segment. The present poly(urethane urea)s and poly (ether urethane urea)s are impermeable to aqueous physiological media.

Both the poly(ether urethane urea)s and poly(urethane urea)s exhibited slight decrease in surface water contact angle. Though the surfaces of virgin polymers are hydrophobic, the hydrophilic urea linkages would have migrated to the surface making it less hydrophobic during aging. Driving force for these types of migration and the reorganization of hard segment is the difference in the surface free energies of the hard and soft segments. Especially in aqueous hydrolytic media, the water molecules make hydrogen bonding with more hydrophilic urea and urethane linkages. Though the present poly(urethane urea)s have showed ~15% decrease in water contact angle in hydrolytic media, they are still hydrophobic polymers. The primary objective of this investigation was to develop biostable hydrophobic polymers.

Therefore the polymers HFL1-PU and HFL3-PU were not considered as candidate for further investigation on biostability.

The aging of poly(urethane urea)s and poly(ether urethane urea)s in PBS medium 37 ^{0}C for 6 months, revealed no significant effect. The weight of aged samples remained same as that of virgin polymer. The FT-IR spectral data (Figures 4.21 -4.23) reveal no change in the spectral responses of the aged polymer. The aging studies confirmed that the present poly(urethane urea)s and poly(ether urethane urea)s are appreciably stable in hydrolytic ionic medium.

4.3.1.2 Evaluation of Hydrolytic Stability in Hydrolytic Enzyme

Since medical implants survive in a predominantly hydrolytic environment *in vivo*, the importance of hydrolytic enzymes has been recognised in the mechanism of the degradation of segmented polyurethanes (Williams, 1992). Degranulation of neutrophils (PMN) which results in the release of reactive oxygen species and hydrolytic enzymes may be responsible to the biodegradation of implanted biomaterials (Anderson, 1993). It has been reported that organic synthetic polymers such as polyamides, polyurethanes and polyesters undergo enzymatic degradation (Williams, 1982; Vondracek, 1984). Enzymatic reactions are specific in nature. Major sites for enzymatic attack in polyurethanes and poly (urethane urea) s are the ester and urethane linkages in soft segments and urethane and urea linkages in hard segments. Poly(ether urethane)s are susceptible to degradation *in vivo* due to hydrolytic enzymes, mainly through the cleavage of urethane linkages. Recent reports showed that polycarbonate-based-polyurethanes (Tang *et al*, 2001) and poly(ether urethane)s (Wang *et al,* 1997) are prone to enzyme-induced degradation. The microscopic cracks

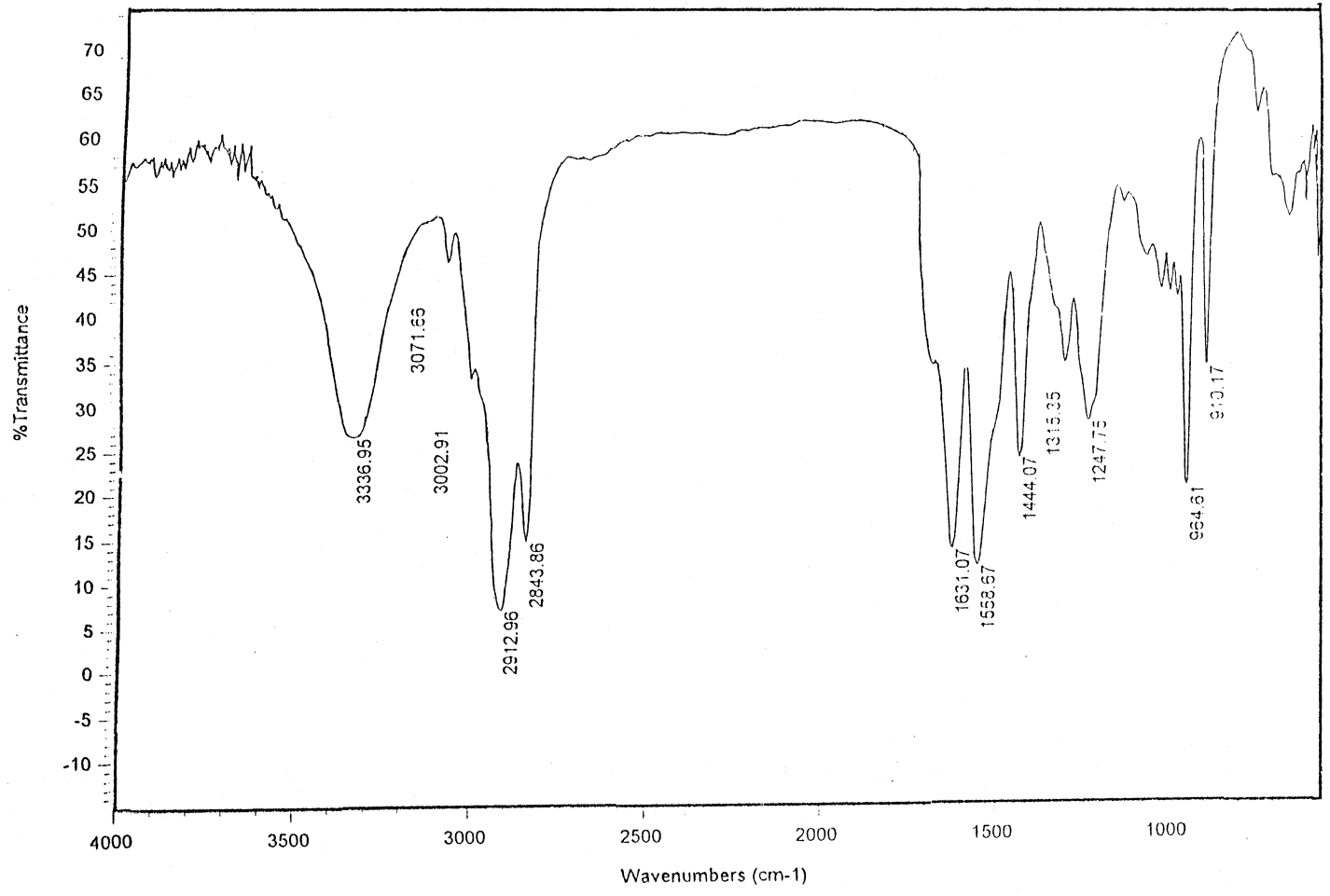


Figure 4. 21 FT-IR spectrum of HFL18-PU aged in PBS for 6 months

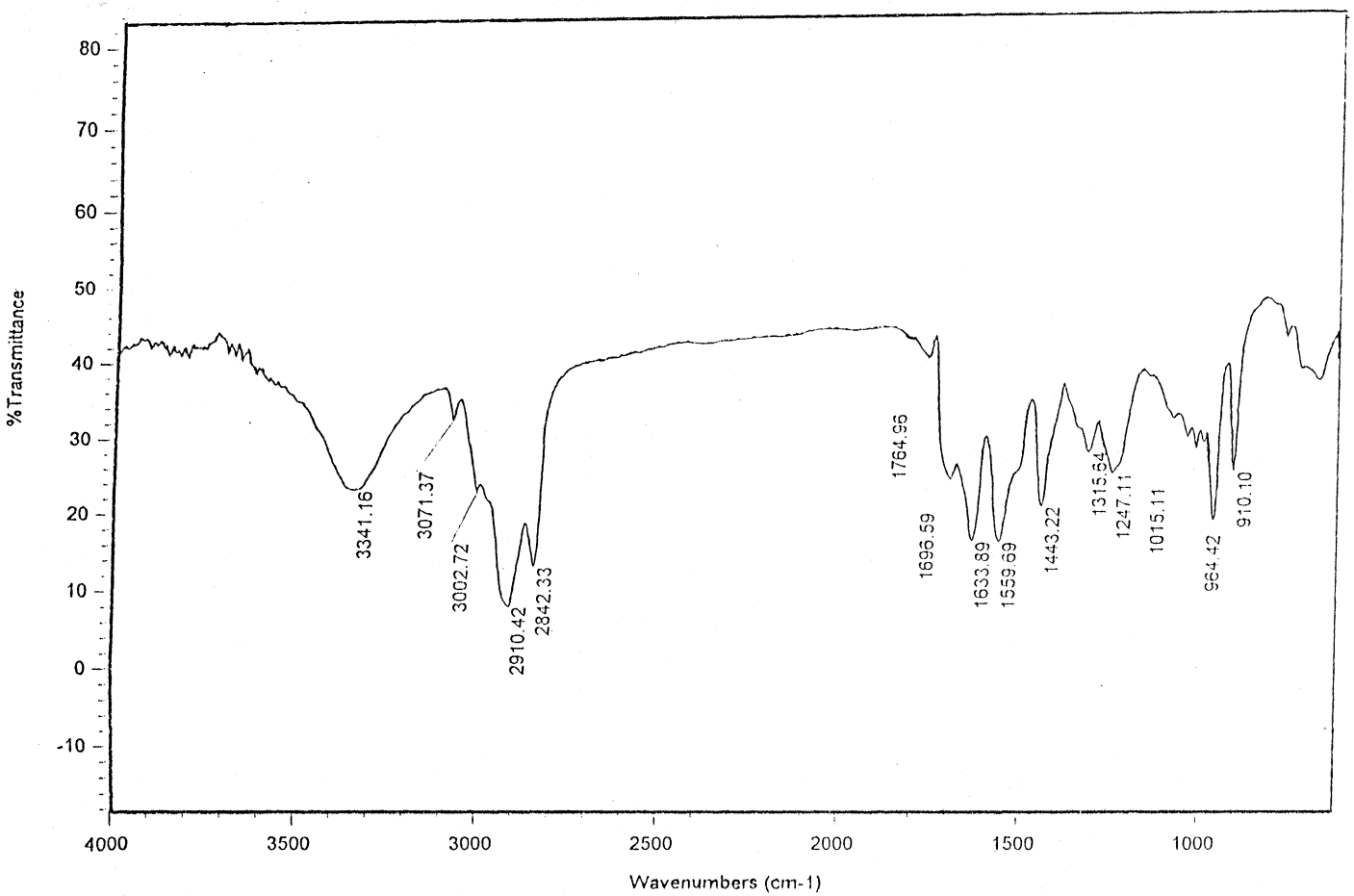


Figure 4. 22 FT-IR spectrum of HFL13-PU aged in PBS for 6 months

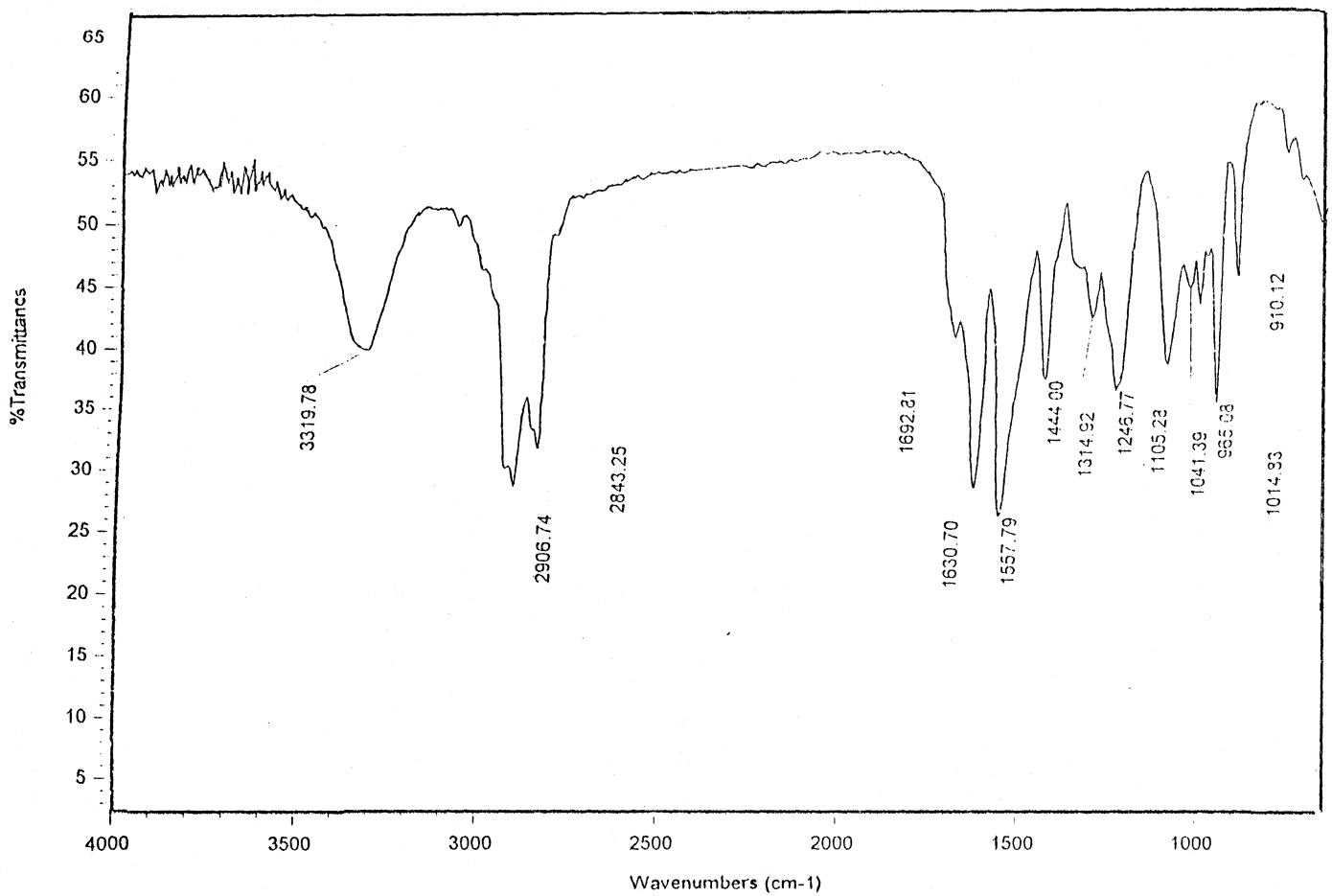


Figure 4. 23 FT-IR spectrum of HFL17-PU aged in PBS for 6 months

formed on the surface of pacemaker lead made of Pellethane® were attributed to the environmental stress cracking and enzymatic degradation (Stokes and Davis, 1985). Hydrolysis occurs through hydrolytic lysosomal enzymes produced by the cell-polymer interaction at the interface of polymer-cell (Marchant *et al*, 1988) Cell-polymer interaction *in vivo* leads to cellular activation and increased enzyme exocytosis by inflammatory cells (Anderson, 1988). It has been reported that, Biomer®, a poly(ether urethane urea) undergoes degradation *in vitro* in papain solution (Marchant *et al*, 1988; Marchant *et al*, 1987; Marchant *et al*, 1986; Zhao *et al,* 1990). FTIR-ATR studies have indicated the formation of primary aromatic amines due to hydrolytic degradation of Biomer® aged in papain solution (Sada Costa, 1981).

Papain is a thiol endopeptidase enzyme, which exhibits a similar enzymatic activity of lysosomal enzymes. Papain has the specificity to peptide bond (-NH-CO-) degradation. Since urethane (-NH-CO-O-) or urea (-NH-CO-NH-, diamide) linkage in poly(urethane urea)s is more or less similar to that of peptide (amide) in protein, studies based on papain-induced hydrolytic degradation is more appropriate. Takahara *et al* (1992) have showed more degradation of hydrophilic poly (ethylene oxide)-based polyurethane in the presence of papain *in vitro*. Moreover, the polyurethanes prepared from hydrophobic polyols, hydrogenated polybutadiene, hydroxy terminated polybutadiene and poly(dimethyl siloxane) also exhibited weight loss due to aging in papain solution (Takahara *et al*, 1992).

However, the data on aging studies of the present poly(urethane urea)s and poly(ether urethane urea)s in hydrolytic enzyme revealed the hydrolytic stability.

There is no weight loss due to enzymatic degradation for both poly(urethane urea)s and poly(etherurethane urea)s. The mechanical properties of present polymers aged in hydrolytic enzyme papain and its buffer medium are given in Table 4.14 and Table 4.15.

Table 4.14 Mechanical Properties of polymers after aging in papin enzyme

Polymer	Ultimate tensile strength (MPa)	Ultimate elongation (%)	Elastic modulus (MPa)	% change of elastic modulus
Poly(urethane urea)				
HFL9-PU	8.558 ± 0.7	379.37 ±66	3.785 ±0.08	+14.2
HFL13-PU	7.482 ±0.82	248.93 ±23.5	4.320 ±0.21	-7.0
HFL18-PU	13.94±0.40	231.5±32	7.258 ±0.08	+10.1
HFL15-PU	17.23±0.78	206.75±66.7	12.346±0.22	+8.4
Poly ether urethane urea				
HFL16-PU	9.694 ± 1.1	270.38 ±21	4.696 ±0.12	+12.1
HFL17-PU	15.88 ± 1.5	202.38±52	9.767±±0.37	+17

The elastic modulus of the present poly(urethane urea)s, HFL9-PU and HFL18-PU increased in comparison with its other two polymers HFL13-PU and HFL15-PU counter parts. Elastic modulus of the aged-poly(ether urethane urea), HFL16-PU in enzyme is increased. However, there is no significant change of elastic modulus in enzyme when compared to that in buffer. This indicates that the enzyme has little effect on the degradation of present poly(ether urethane urea)s and poly(urethane urea)s.

Interestingly, no weight loss was noticed in any of the polymer exposed to papain solution. Earlier investigators reported decrease of weight (~1.5%) for aromatic polyurethane based on polybutadiene soft segment (Mn =2000) and increase

in weight (~1.5%) for Biomer® (Takahara *et al*, 1992). Therefore the observed change in properties in the present investigation is due to the realignment of the virtual crosslinks in the polymer chains when aged in the media as discussed earlier.

Table 4.15 Mechanical Properties of polymers after aging in buffer (contol)

Polymer	Ultimate tensile strength (MPa)	Ultimate elongation (%)	Elastic modulus (MPa)	% change of elastic modulus
Poly(urethane urea)				
HFL9-PU	8.023 ±0.19	385.55±27	3.417±0.09	+3.13
HFL13-PU	6.545 ±0.63	216.23±13.5	3.988±0.06	-13.85
HFL18-PU	13.75±0.88	242±41±41	7.039±0.40	+8.84
HFL15-PU	16.23±0.66	191.9±13.1	12.29 ±0.16	+8.0
Poly ether urethane urea				
HFL16-PU	8.38±1.0	257.17±24.6	4.609±0.17	+10.03
HFL17-PU	17.11±1.2	212 ±41.1	9.942±0.21	+19.09

The IR spectral responses do not reveal any appreciable changes in the aged polymers in comparison with that aged in control buffer media (Figures 4.24- 4.29). The peaks for enzymatically hydrolysable urea and urethane linkages have appeared intact in aged polymers. Earlier, Angeline *et al* (1990) have reported long-term enzymatic degradation of Pellethane® in Papain under *in vitro* physiological condition and reported significant reduction in tensile strength and molecular weight. Enzymes and other swelling agents can effectively plasticize the polymer materials and reduce the modulus, tensile strength and elongation by affecting the phase-separated structure (Takahara *et al*, 1985a). The *in vitro* aging studies with papain enzyme and its buffer control reveal that the effect of hydrolytic enzyme papain on

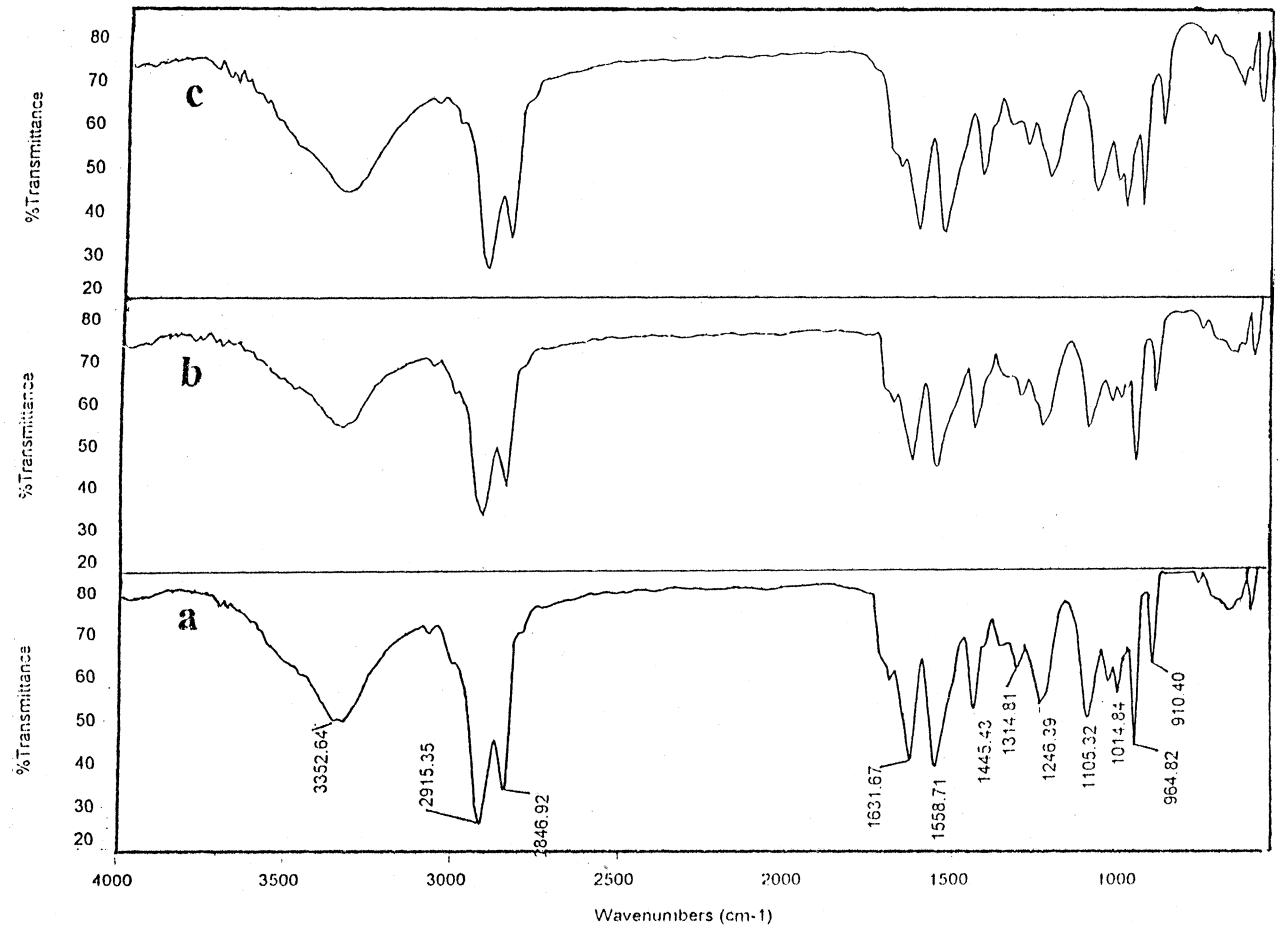


Figure 4. 24 FT IR spectrum of aged poly(ether urethane urea) ,HFL16-PU (a) virgin polymer (b) in papain (c) in buffer control

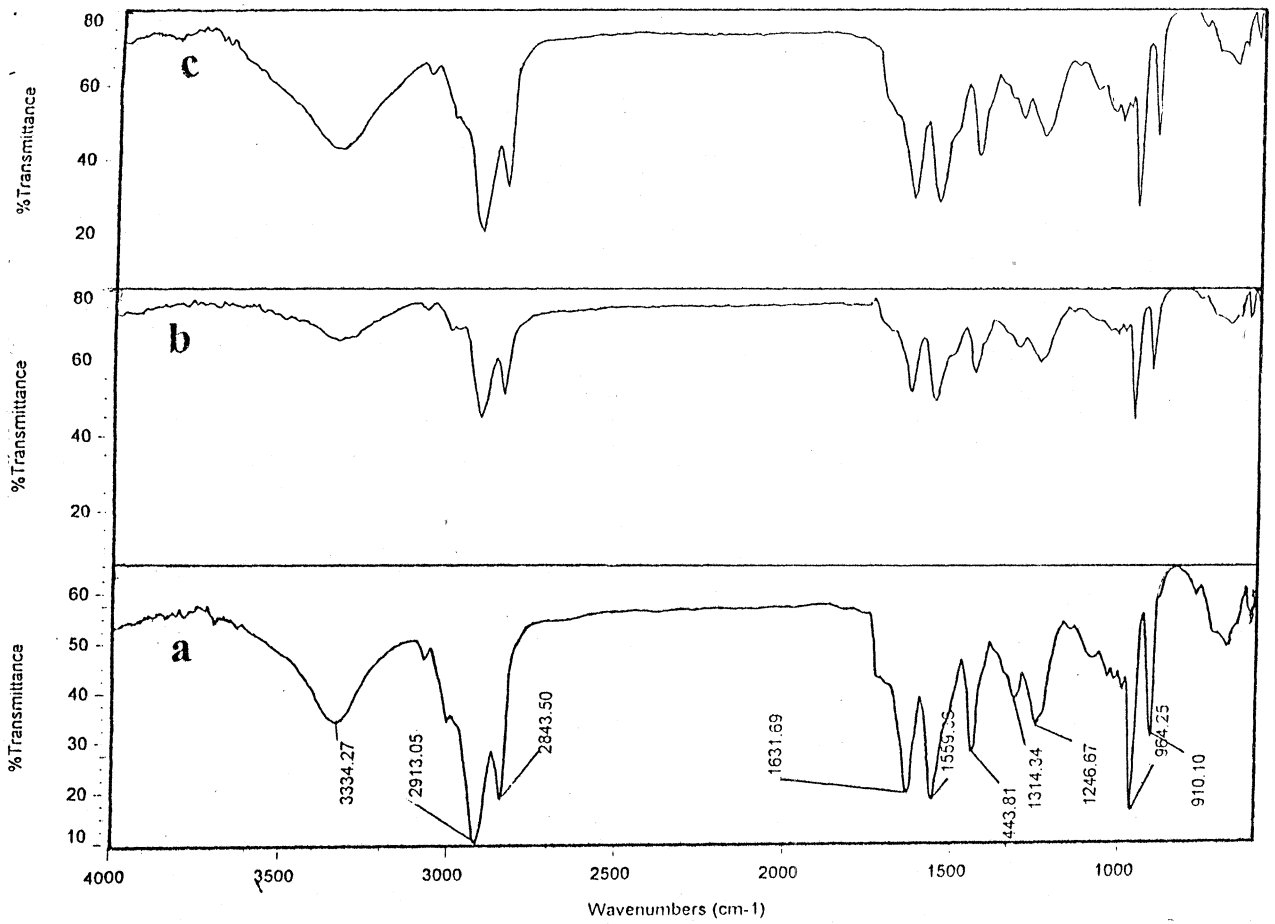


Figure 4. 25 FT IR spectrum of aged poly(urethane urea), HFL13-PU (a) virgin polymer (b) in papain (c) in buffer control

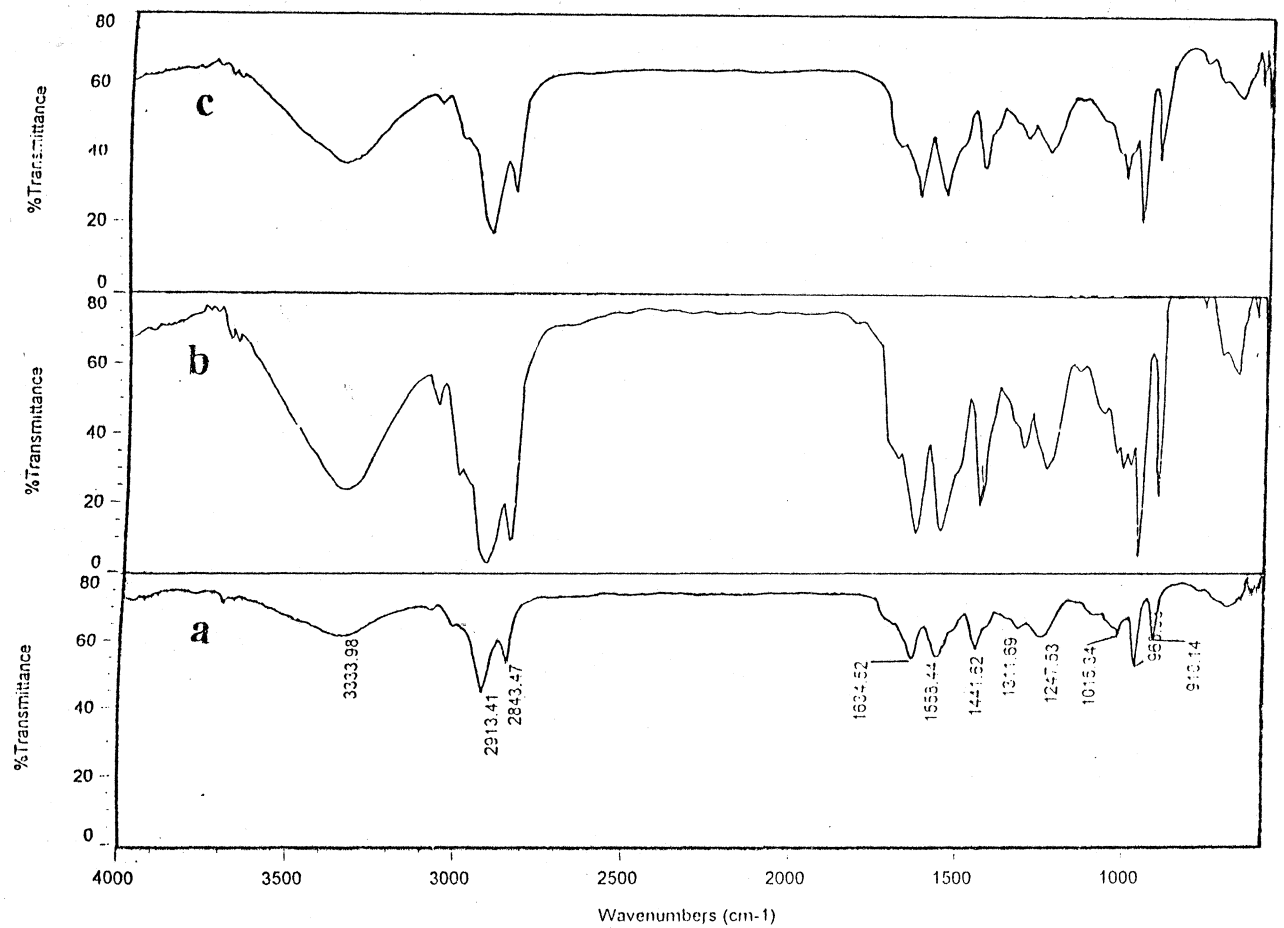


Figure 4. 26 FT IR spectrum of aged poly(urethane urea), HFL9-PU (a) virgin polymer (b) in papain (c) in buffer control

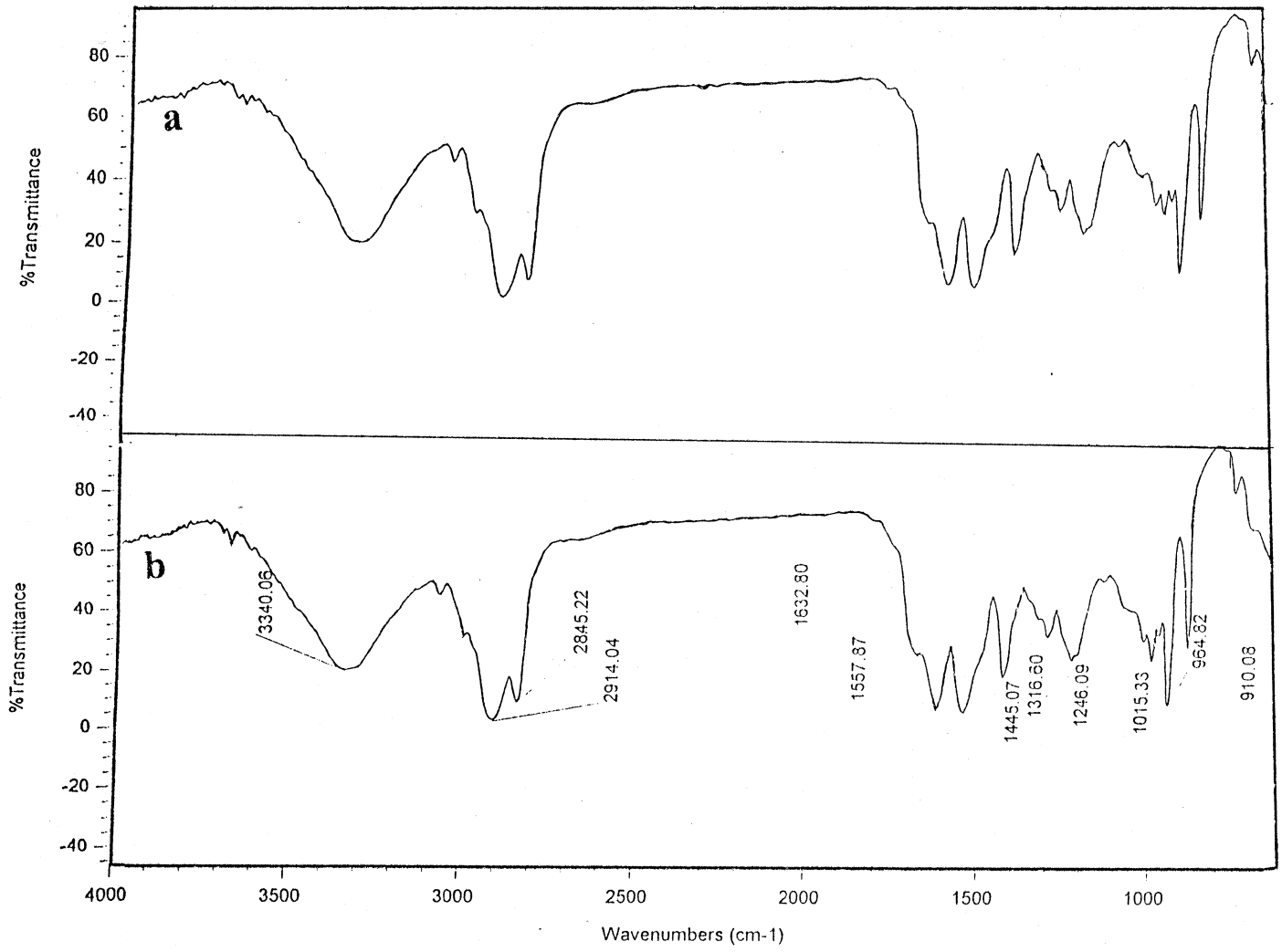


Figure 4. 27 FT IR spectrum of poly(urethane urea) HFL18-PU
(a) aged in papain (b) aged in buffer control

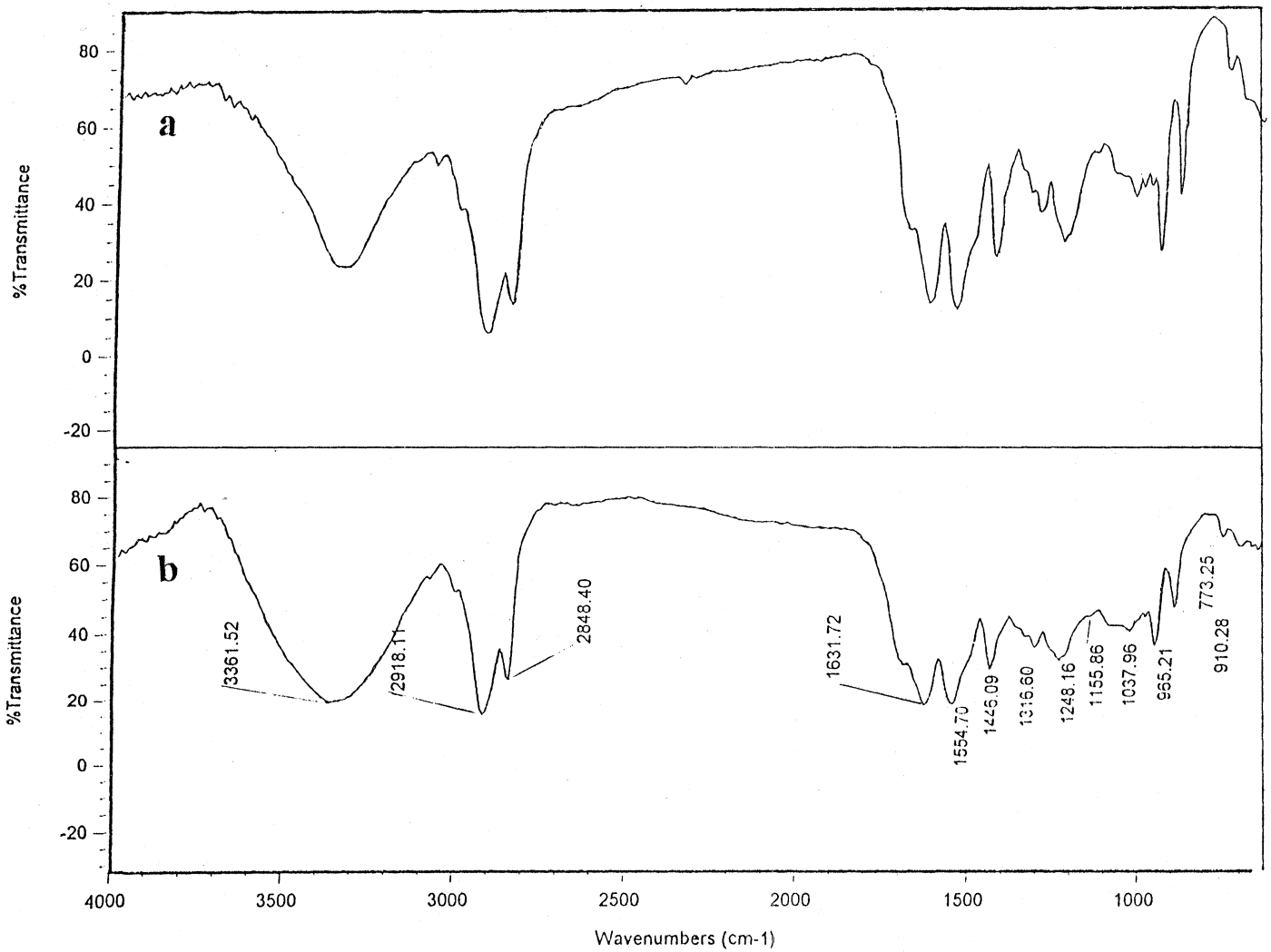


Figure 4. 28 FT IR spectrum of poly(urethane urea) HFL15-PU
(a) aged in papain (b) aged in buffer control

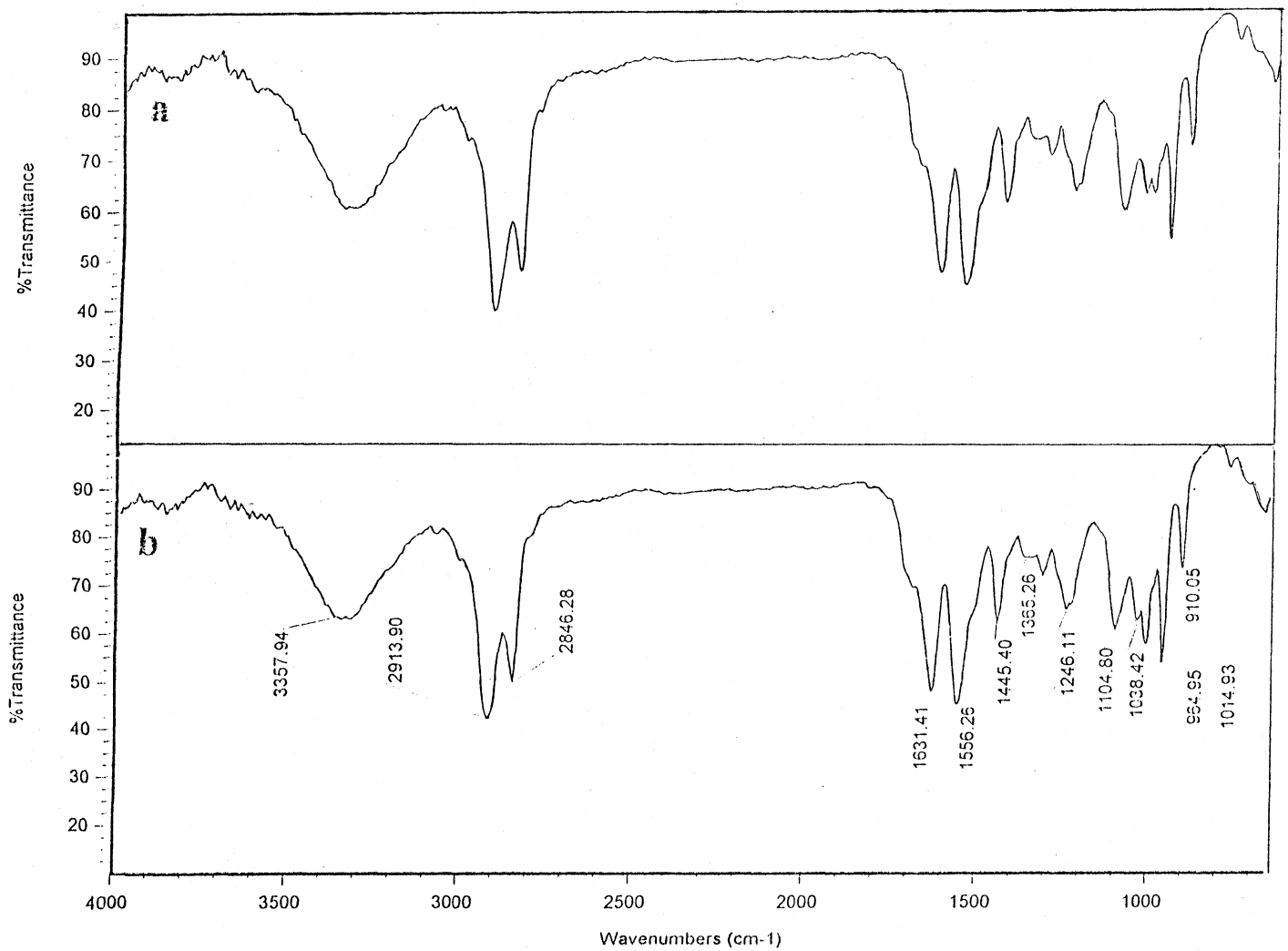


Figure 4. 29 FT IR spectrum of poly(ether urethane urea) HFL17-PU (a) aged in papain (b) aged in buffer control

the hydrolytic degradation of the present polymers is not very significant. This is due to the absence of substrate site-specific interaction of the enzyme, since the surface of the polyurethanes is more hydrophobic. Takahara *et al* (1992) has reported increased nitrogen content in segmented polyurethanes after treatment with enzyme solution, which may be due to the possible adsorption of papain on the polyurethanes or a possible increase in amine end groups at the surface of polyurethanes. The present IR spectrum also do not show any additional peak for surface-adsorbed papain or amine end groups for the present polymers. Anderson and co-workers suggested that rate of enzymatic degradation of polyurethane was very slow and that might be limited to the interface between the polymer surface and the aqueous environment (Marchant *et al*, 1988; Marchant *et al*, 1987). Moreover, the virtually crosslinked urea groups in hard segment of the poly(urethane urea)s are more resistant to the hydrolysis as suggested by Takahara *et al* (1992). He reported that the resistance of hard segment of Biomer® (chain extended with ethylene diamine) to hydrolysis was higher than that of polyurethanes having hard segment chain extended with diol. This was attributed to the presence of virtually crosslinked urea linkages in the hard segment of Biomer®.

It was also shown that the degree of hard segment micro-domain formation in polyurethane materials as well as its structure influences the ability of enzymes to degrade the polymers (Tang *et al*, 2001). Santerre and Labow (1997) have formulated a relationship between the formation of hard segment domain and the hydrolysis of urea/ urethane groups in poly(ether urethane urea)s. They showed that the polymer containing the highest number of hydrolytically labile urea and urethane bonds exhibited the least degradation. The ability of HFL18-PU having 75 % of hard

segment to form hard segment micro-domain may contribute to the formation of a protective structure for the hydrolysable hard segment linkages located within the micro domains as reported elsewhere (Santerre and Labow, 1997). It is hypothesized that the virtual crosslinking will facilitate the formation of protective micro-domain structure in the present poly(urethane urea)s. The biostability of poly(uethane urea)s can be improved by manipulating the degree of virtual crosslinking of the materials .

4.3.1.3 Evaluation of Stability in Lipid Media

Usually hydrophobic polymers have high tendency to absorb plasma lipids. It has been reported that hydrophobic silicon rubber heart poppets in prosthetic heart valves degrade *in vivo* due to absorption of lipid from blood (Cuddihy *et al,* 1976). Also, large reductions in the mechanical strength of segmented poly(urethane urea)s on exposure to lipid have been reported (Takahara *et al*, 1985 a). The lipids that are absorbed by polyurethane can also act as initiation sites for cellular oxidation and also for calcification. Thus lipid uptake by implants is of great concern as it is a life-limiting process. Interestingly, Takahara *et al* (1985a) have reported that poly(ether urethane urea)s having well ordered hard segment like that of Biomer® absorb small amounts of plasma lipid (lecithin) than that having disordered hard segment domain. But the reduction in fatigue strength of segmented poly(ether urethane urea)s after exposure to a lipid solution have been reported (Takahara *et al,* 1985 a). Degradation due to lipid sorption was predominant in polydimethyl siloxane (PDMS)-based polyurethanes compared to polyhydrocarbon-based counterpart. The absorbed lipid seemed to have a significant role on both the hard and soft microdomains of segmented polyurethanes (Takahara *et al,* 1992). Thus the degree of interaction

between the lipid and the polyurethane is an important factor in the retention of mechanical properties of polymer indented for use as long-term implants.

The interaction of polyurethane with plasma lipids depends on their solubility parameter. The solubility parameter of the lipids (Szycher, 1991a) such as cholesterol and esters (8.4-8.9), triglycerides (8.0-8.3) are low compared to that of phospholipids (>16), protein (>18) and water (23). Generally phospholipids have lesser chances for the adsorption /absorption by the polymers as their solubility parameters are very high. However Biomer® undergoes plasticization by cholesterol and cholesterol esters (Szycher, 1991a). The present swelling studies revealed that the solubility parameters of the newly prepared polymers are close to that of tetrahydrofuran (9.1). In the present aliphatic poly(urethane urea)s the soft segments may be responsible for the lipid uptake as the solubility parameter of (9.0) lies close to that of cholesterol and triesters of fatty acids.

Aging in Dulbecco's modified eagle medium (DMEM) containing cholesterol and in a lipid rich medium (palm oil) were carried out to investigate the extent of lipid adsorption and /or absorption in the poly(ether urethane urea)s and poly(urethane urea)s. The data given in Table 4.16 showed an increase in weight of the polymeric samples after aging in DMEM. Weight increase has been reported by previous investigators for biomedical polymers immersed in lipid solutions both *in vitro* and *in vivo* (Moacanin 1973; Swanson and Lebeau, 1972; Carmen and Mutha, 1972). But for present poly(urethane urea)s and poly(ether urethane urea)s the weight increase due to lipid uptake is only marginal in DMEM. However, in lipid rich palm oil medium (100% liquid), appreciable weight increase was noticed.

Table 4.16 Change in weight and mechanical properties of polymers aged in DMEM containing cholesterol

Polymer	Ultimate tensile strength (MPa)	Ultimate elongation (%)	Elastic modulus (MPa)	Increase in weight (%)
Poly(urethane urea)				
HFL9-PU	9.054±0.50	344.6±32.6	3.06±0.15	0.72
HFL13-PU	6.754±0.60	240.4±19.4	5.21±0.17	0.60
HFL18-PU	12.02±1.25	254.4±29	6.335±0.27	0.51
HFL15-PU	14.84±0.94	215.9±23.1	9.65±0.09	0.57
Poly ether urethane urea				
HFL16-PU	9.343±2.5	509.13±65	4.690±0.30	0.66
HFL17-PU	13.99±1.67	265.7±50.3	10.47±0.40	0.72

The percentage of increase in weight is proportionate to the percentage of polyol present in the polymers. The percentage of weight increase in polymers aged in palm oil medium is given in Table 4.17. HFL9-PU having 42.5% polyol content has shown the highest lipid uptake in palm oil; however the lipid uptake in DMEM medium is only marginal (<1%). These data are in good agreement with that reported for Biomer®, (~0.75%) aged in cholesterol lipid medium for 28 days (Takahara *et al*, 1992). Among the poly(ether urethane urea)s having mixed hydrophobic and hydrophilic polyols soft segments, HFL16-PU (42.5 % polyol) showed lesser absorption of lipid in palm oil when compared to HFL17-PU (32.1 % polyol). The lipid uptake is also related to the virtual crosslink density. Figure 4.30 shows the lipid uptake (%) *vs* virtual crosslink density. In palm oil medium, the percentage of weight gain decreases with increase of virtual crosslink density for both poly(urethane urea)s and poly(ether urethane urea)s.

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HFL15-PU	14.84±0.94	215.9±23.1	9.65±0.09	0.57
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HFL16-PU	9.343±2.5	509.13±65	4.690±0.30	0.66
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Table 4.17 Change in weight and mechanical properties of polymers aged in palm oil medium

Polymer	Ultimate tensile strength (MPa)	Ultimate elongation (%)	Elastic modulus (MPa)	Increase in weight (%)
Poly(urethane urea)				
HFL9-PU	4.347±0.33	218.92±41.5	2.401±0.11	59.48±1.44
HFL13-PU	4.869±0.66	168±10.74	3.518±0.19	36.81±0.71
HFL18-PU	8.724±0.79	173.08±32.8	6.039±0.28	30.59±0.17
HFL15-PU	12.29±0.71	177.08±20.5	8.77±0.36	25.32±0.48
Poly ether urethane urea				
HFL16-PU	4.491±1.4	301± 39.7	3.15±0.16	40.45±1.10
HFL17-PU	14.11±0.32	238.87±17	8.625±0.41	31.96±1.02

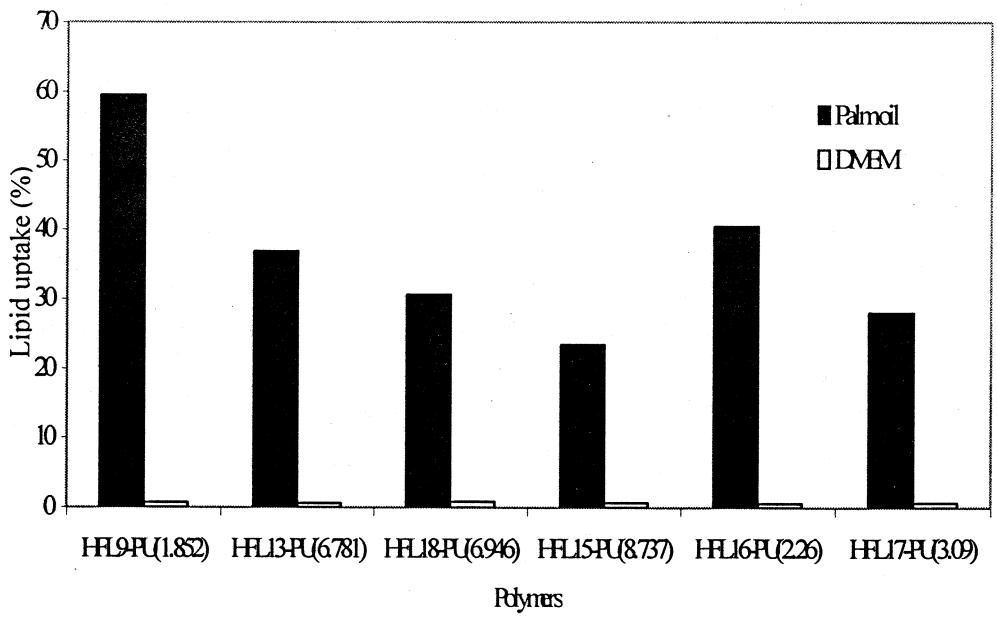

Figure 4.30 Virtual crosslink density Vs. Lipid uptake of polymers

The elastic modulus decreased for all the poly(ether urethane urea)s and poly(urethane urea)s aged in palm oil medium due to the plastizicing effect of absorbed lipid. Thus, there will be weakening or enhanced breaking of hydrogen bonding. The lipid absorption in HFL15-PU is the lowest in comparison to other

poly(urethane urea) polymers. This is attributed to the highest cross-link density formed with more urea linkages and also due to the lesser percentage of hydrophobic polyol in HFL15-PU.

In Dulbecco's medium the absorption of lipid by the present poly(ether urethane urea)s and poly(urethane urea)s is low though these polymers are hydrophobic in character. This may be attributed partially to the low concentration of lipid (1.6 g/l) in DMEM. Since DMEM contains all the major amino acids, it is assumed that the surface of the polymers may be enriched with amino acids and that result in the lower uptake of hydrophobic lipid. The tensile test of the aged samples revealed slight changes in mechanical properties. In the case of poly(urethane urea), HFL9-PU there is a slight increase in elastic modulus, while in HFL13-PU, HFL15-PU and HFL18-PU the elastic modulus slightly decreased (Table 4.17). Apart from the plasticizing effect of absorbed lipid, the change in tensile properties is attributed to the rearrangement of crosslinks resulted from different hydrogen bonding interactions as discussed earlier. The FT-IR specrta of the aged polymers are given in Figures 4.31- 4.33. The spectra showed the normal appearance of peaks for urethane carbonyl, urea carbonyl and urethane-ether linkages. The characteristic peaks for polybutadiene soft segment also appeared intact in all the aged polymers. There is no appearance of new peaks for lipid adsorbed permanently on the hydrophobic surface.

4.3.1.4 Evaluation of Biostability in vitro Oxidative Media

The use of hydroxy terminated polybutadiene (HTPBD) in biomedical field as biomaterial for the fabrication of long-term devices is not yet explored due to poor biostability due to oxidative degradation (Takahara *et al*, 1991) and lipid sorption

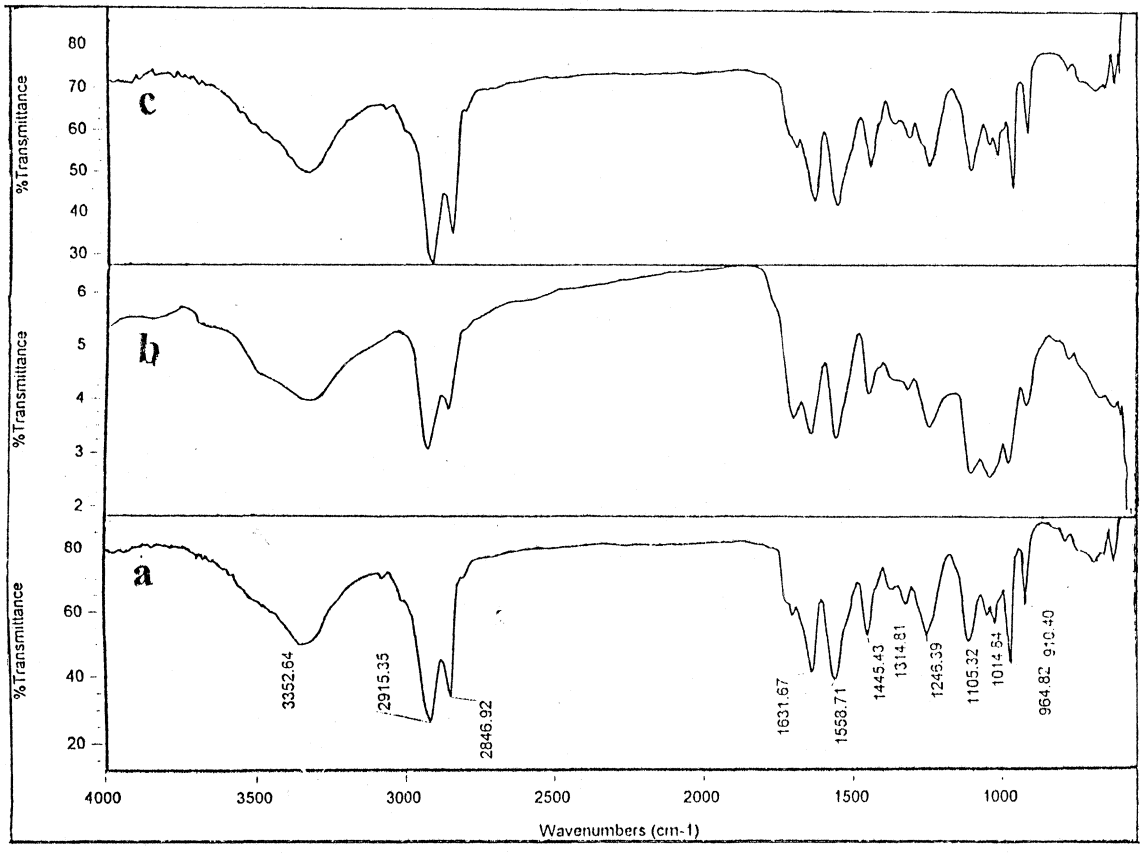

Figure 4.31 FT-IR spectrum of poly(ether urethane urea) HFL16-PU (a) virgin polymer (b) in oxidation medium (c) aged in DMEM

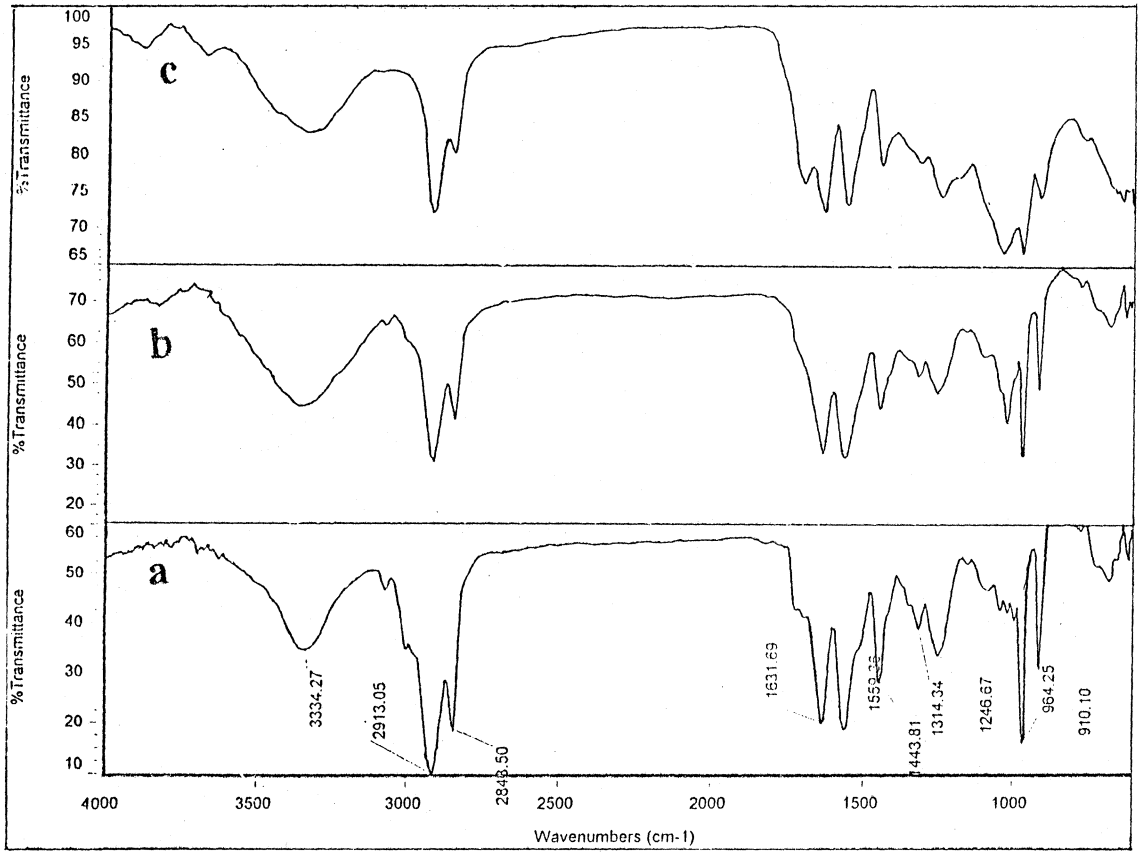

Figure 4.32 FT-IR spectrum of poly (urethane urea) HFL13-PU (a) virgin polymer (b) aged in DMEM (c) in oxidation medium

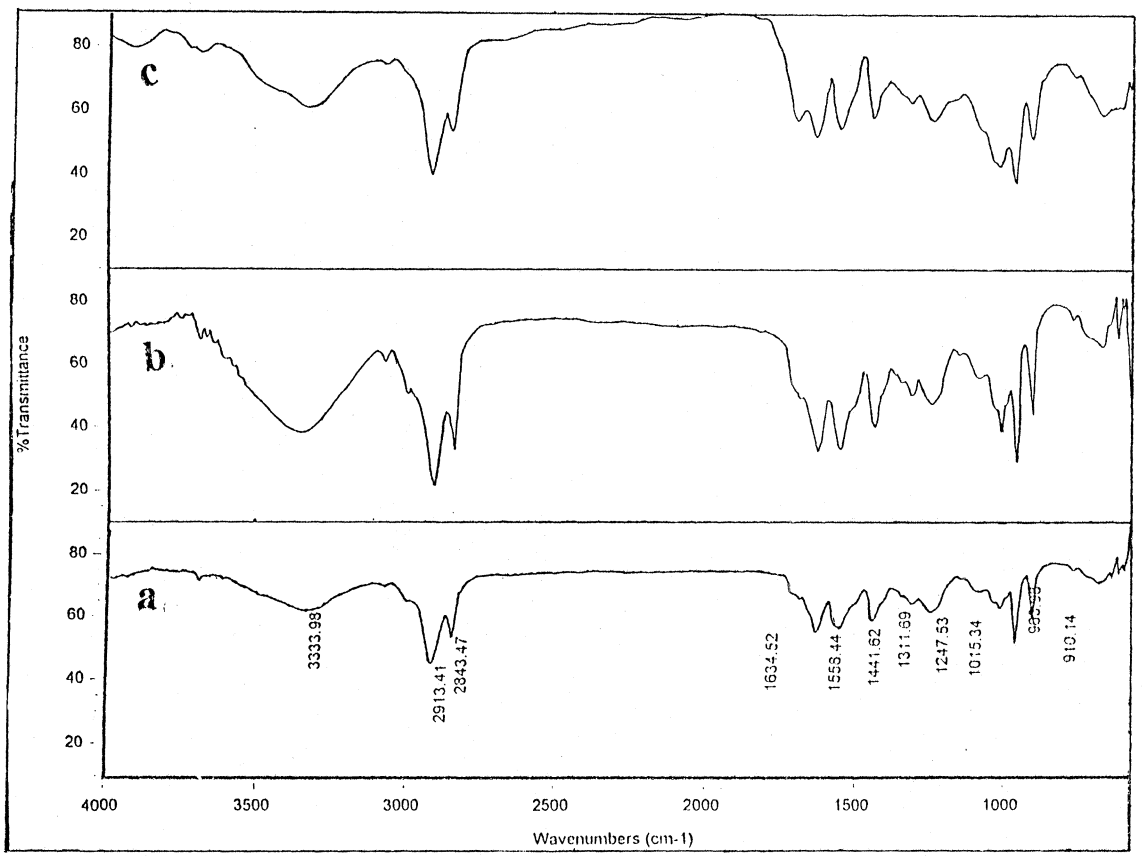

Figure 4.33 FT-IR spectrum of poly(urethane urea) HFL9-PU (a) virgin polymer (b) aged in DMEM (c) in oxidation medium

(Takahara *et al,* 1992). However, recent reports showed that polyurethane containing unsaturated diol (2-butene-1,4-diol) chain extender could improve the oxidation resistance and biostability (Hsu and Chen, 1999). Based on the hypothesis that metal ions having standard reduction potential ≥7V cause oxidative degradation of polyurethane (Stokes *et al*, 1985), aging studies in solution of 0.1 M silver nitrate and 0.1 M sodium lactate at 37 ^{0}C were carried out as per the method of Takahara *et al* (1991). Addition of organic acid, lactic acid or its sodium salt can accelerate the oxidative degradation. Possibly, a complex of the oxidative metal ion with the organic acid undergoes absorption more rapidly into the poly(urethane urea)s, thereby enhancing the effect of the cation. The interaction of the oxidising agent with species capable of surface activity or penetration into the polymer might be expected to accelerate the oxidative degradation.

The *in vitro* stability of poly(urethane urea)s and poly(ether urethane urea)s in oxidative medium was assessed from changes in mechanical properties and also from IR spectra. The mechanical properties of the aged polymer in oxidative medium are given in Table 4.18. None of the polymers showed weight loss due to oxidative degradation. However, analyses of aged samples for mechanical properties showed slight changes in tensile properties as in the previous cases. The tensile strength of poly(urethane urea), HFL18-PU was not increased appreciably; it undergoes increase of tensile strength only to an extent of 2.8%. The increase of elastic modulus is only 10.7%. HFL15-PU also showed change in tensile strength (2.9%) and elastic modulus (13%). The tensile strength of HFL9-PU and HFL13-PU was found to decrease by 40% and 23% respectively.

Table 4.18. Mechanical properties of polymers aged in oxidation medium

Polymer	Ultimate tensile strength (MPa)	Ultimate elongation (%)	Elastic modulus (MPa)	Hardness Shore A
Polyurethane				
HFL1-PU	3.51±0.2	26.1	-	86
HFL3-PU	5.89±0.31	52.27	-	89
Poly(urethane urea)				
HFL9-PU	4.36±0.272	296.66	2.136±0.01	78.75
HFL13-PU	5.919±0.733	171.66	4.287±0.02	75
HFL18-PU	16.2±1.01	334.80	7.574±0.10	92
HFL15-PU	15.44±0.66	177.60	10.77±0.21	93
Poly (ether urethane urea)				
HFL16-PU	11.14±1.46	429.45	3.765±0.16	87
HFL17-PU	14.11±0.32	238.87	8.625±0.41	90

However, the decrease of elastic modulus of HFL9-PU and HFL13-PU is only 12% and 6% respectively. HFL9-PU with 42.5% HTPBD and HFL13-PU with 32.1 % HTPBD can absorb the lactate more effectively and give plasticizing effect than other polymers. Since there is no weight loss in the aged polymers, the change of properties could also be due to reorganization of virtual crosslinking to a larger extent. The loss of tensile properties in HFL9-PU and HFL13-PU is due to break of virtual crosslinks (hydrogen bonds) in oxidation medium.

The mechanical properties of poly(ether urethane urea)s also changed only slightly. The tensile properties of HFL16-PU showed 22% increase in tensile strength and 7% decrease in tensile modulus whereas HFL17-PU showed 3.31% decrease in tensile modulus. The change of tensile properties and hardness observed with HFL16-PU and HFL17-PU is also due to the breaking and realignments of virtual crosslinks especially those associated with ether-urea and ether-urethane.

The surface properties of the aged polymer in oxidation medium are given in Table 4.19. There is no appreciable change in water contact angles of aged polymers in comparison to virgin polymers. The present poly(urethane urea)s and poly(ether urethane urea)s are still hydrophobic. The IR spectra (Figures 4.31 - 4.33) of the samples aged in oxidative medium showed intense peak for urethane carbonyl at 1705 cm^{-1} and urea carbonyl (bonded) at around 1631 cm^{-1}. There is no evidence of disappearance or decrease of peak intensity for CH=CH group (*trans* and *vinyl*) of HTPBD soft segment in poly(urethane urea) polymers, HFL9-PU, HFL13-PU, HFL15-PU and HFL18-PU.

Table 4.19 Surface properties of polymers aged in oxidation medium

Polymer	Water contact angle (deg)	Surface free energy (dynes/cm) γ_{SV}	γ_{SL}
Polyurethane			
HFL1-PU	74.0±1.5	38.81	18.82
HFL3-PU	72.2±1.3	40.02	17.61
Poly(urethane urea)			
HFL9-PU	82.8±0.8	33.23	24.40
HFL13-PU	75.2±1.4	38.20	19.43
HFL18-PU	81.7±1.2	33.86	23.77
HFL15-PU	80.6±1.6	34.49	23.14
Poly ether urethane urea			
HFL16-PU	80.1±0.8	35.11	22.52
HFL17-PU	81.2±1.1	34.49	23.14

The intensity of peak at around 964 cm^{-1} and at around 910 cm^{-1} (characteristic peak for poly butadiene) in the spectrum of aged polymer is same as that in the virgin polymer (Figures 4.31- 4.33). Similarly there is no change in the peak intensity for

-C-O-C- group of PTMG segment in poly(ether urethane urea)s HFL16-PU Figure 4.31) and HFL17-PU. The spectral studies of the aged polymer indicate that unsaturated double bonds of hydro carbon polyol units in the present polymers are not only resistant to significant oxidation but also protect ether soft segment of PTMG units associated with it. This is evident from the presence of ether peak at 1105.32 cm^{-1} in aged HFL16-PU (Figure 4.31). The resistance of HTPBD-PTMG based poly(ether urethane urea)s against oxidation is possibly by the protection of these soft segments by the cage-like hard segment domains also.

The aging of poly(urethane urea)s and poly(ether urethane urea)s in oxidation medium, $H_2O_2/CoCl_2$ solution at 37 ^0C for 6 months, revealed no significant effect on biostability. The weight of aged samples remained same as that of virgin polymer. The FT-IR spectral data (Figures 4.34 -4.36) reveal no change in the spectral responses of the aged polymer. The aging studies confirmed that the present poly(urethane urea)s and poly(ether urethane urea)s are appreciably stable in oxidation medium.

4.3.2 Studies on Accelerated Chemical Degradation of Polyurethanes

A recent report (Fare *et al*, 1999) revealed that accelerated media like concentrated nitric acid (HNO_3) or alkaline sodium hypochlorate (NaClO) can be used to evaluate degradation of polyurethanes even though such adverse conditions may not prevalent in the human body. Ismail and Aziz (2000) have also evaluated the hydrolytic stability of polyurethane elastomers by boiling the specimens under reflux conditions for 24 h in various media *viz.* deionised water, 2M hydrochloric acid (HCl) and 2M sodium hydroxide (NaOH) solution. The present studies on accelerated aging

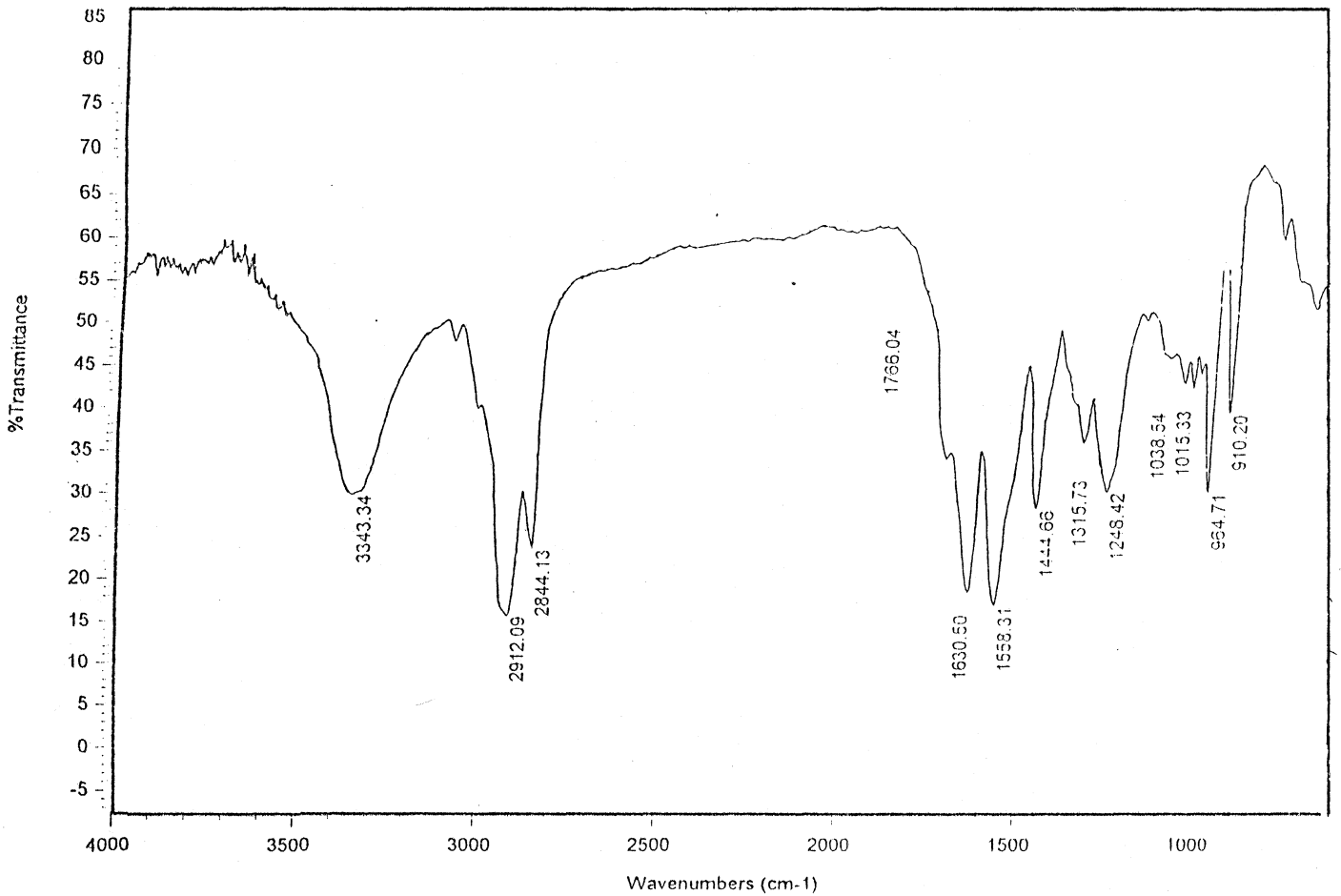

Figure 4.34 FT-IR spectrum of HFL18-PU aged in oxidation medium for 6 months

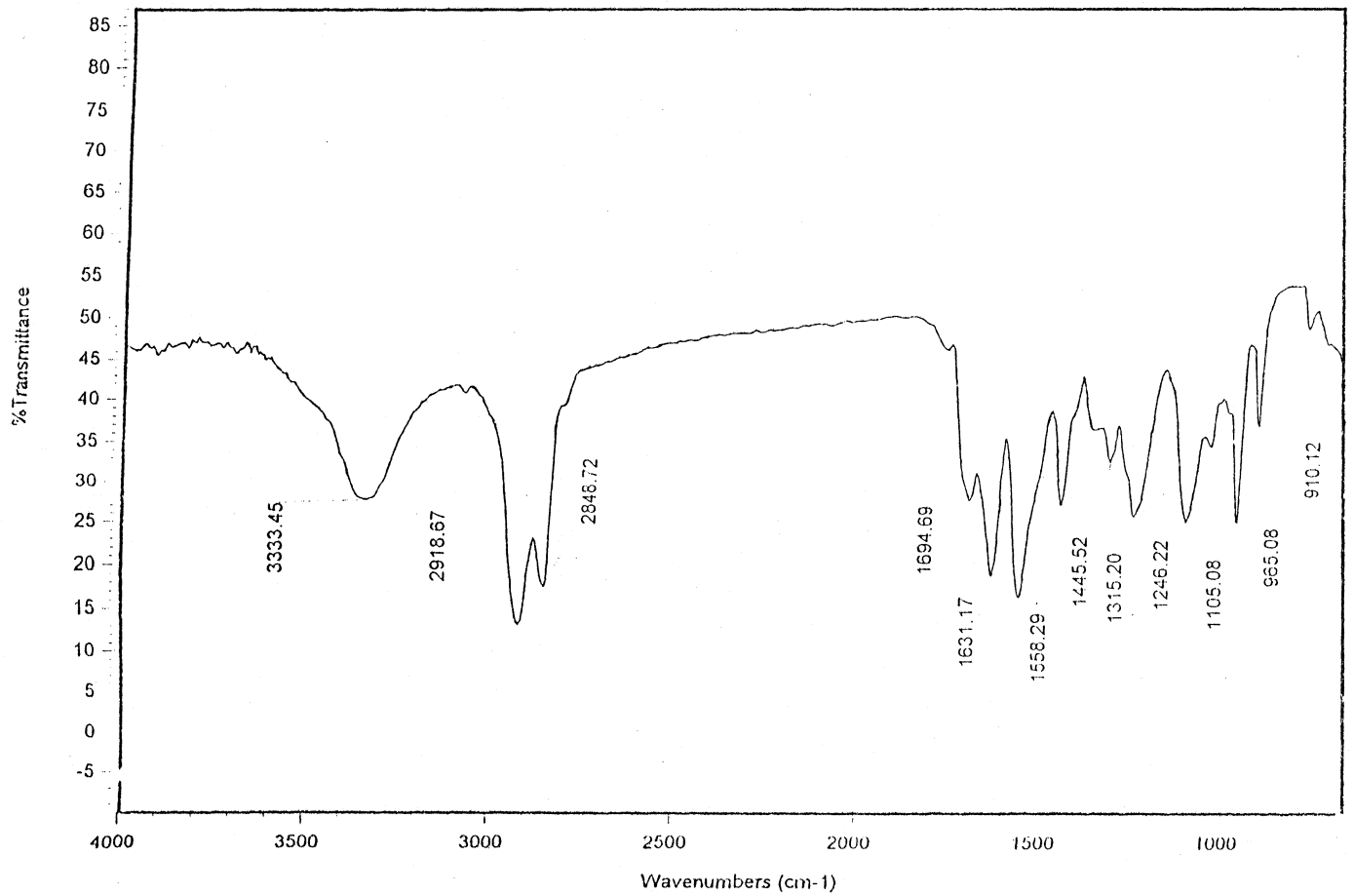

Figure 4.35 FT-IR spectrum of HFL16-PU aged in oxidation medium for 6 months

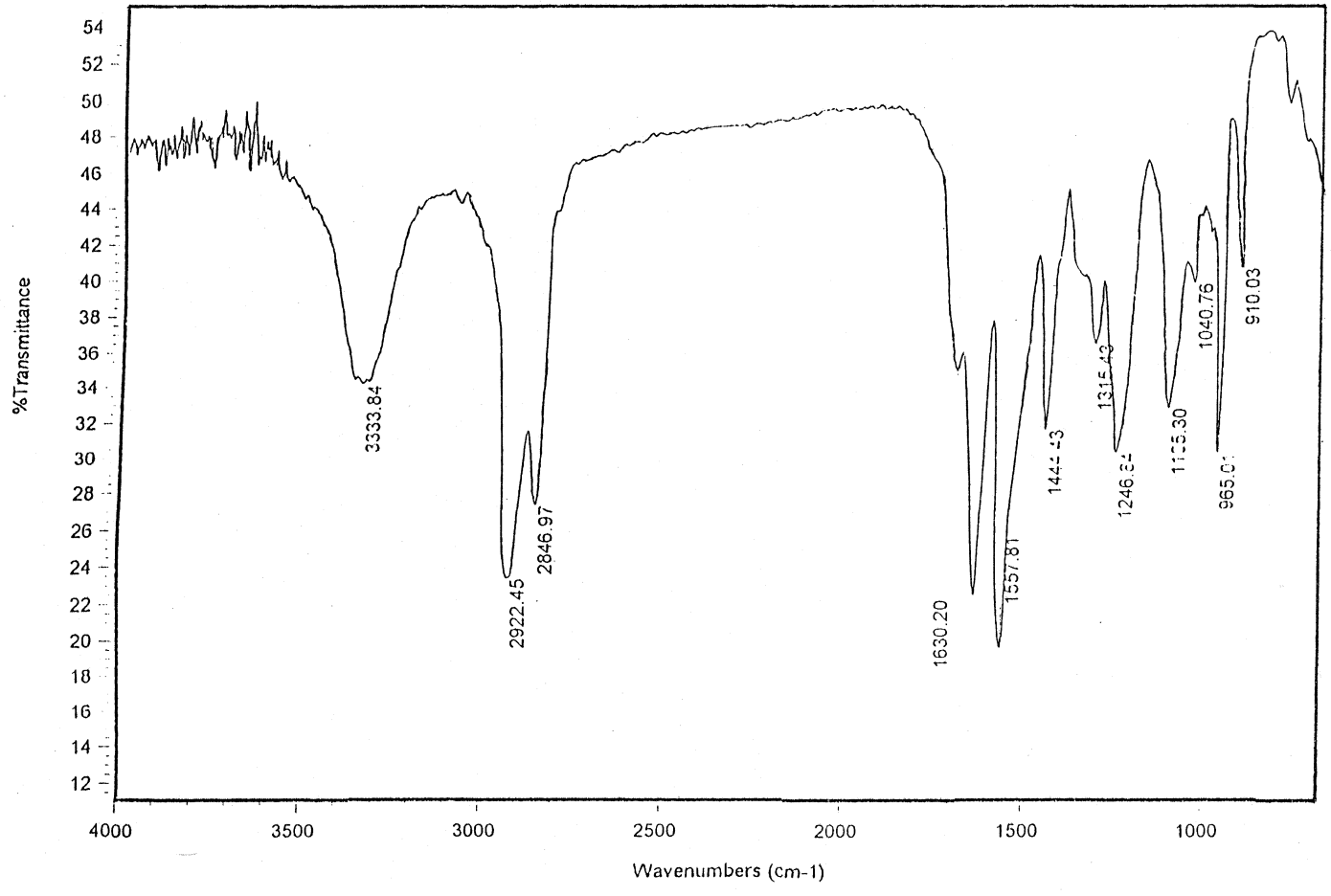

Figure 4.36 FT-IR spectrum of HFL17-PU aged in oxidation medium for 6 months

in boiling deionised water for 100 h and boiling alcoholic potassium hydroxide (0.5M) for 4 h was carried out as per the method reported elsewhere (Lemm, 1984). The accelerated aging revealed dimensional changes and weight loss in polyurethanes, HFL1-PU and HFL3-PU and also in poly(ether urethane urea)s, HFL16-PU and HFL17-PU. However, no degradation (weight loss) or dimensional change was observed with poly(urethane urea)s, HFL9-PU, HFL13-PU, HFL15-PU and HFL18-PU. The accelerated test in harsh and aggressive chemical conditions clearly indicates that the present poly(urethane urea)s would excellently be stable in physiological condition.

4.3.3 Studies on the Influence of Biomechanical Factors on the Degradation

4.3.3.1 Studies on the Synergistic Effect of Strain and Enzyme on Biodegradation

Biodegradation of segmented polyurethanes has been considered as the failure of polyurethane due to combined effect of stress and physiological fluids. A state of stress either externally-applied or processing-induced is a dominant factor in environmental stress cracking (ESC) of polyurethane biomaterials. Many tests were carried out to reproduce ESC *in vitro* and to develop test procedures to predict the functional performance in *vivo* physiological conditions.

The *in vitro* studies on the aging under induced-strain (20% in tension mode) in hydrolytic enzyme medium reveal interesting information. The aged polymers do not reveal any weight loss. The mechanical properties of aged polymers are given in Table 4.20. The polymer, which has been strained constantly, undergoes unidirectional reorganisation of polymer chains leading to increase of elastic modulus significantly. Similar studies by Fare *et al* (1999) using Corethane®[polycarbonate

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urethanes based on MDI/poly(1,6-hexyl-1,2-ethylcarbonate)/BD] and Coremer® [polycarbonate urethane urea based on MDI/poly(1,6-hexyl-1,2-ethylcarbonate)/ED] revealed increased rearrangements of hydrogen bonds between the carbonate and urethane/urea groups that took place under constant mechanical strain during the aging.

Table 4.20. Synergistic effect of strain (20%) and hydrolytic enzyme on properties of polymers

Polymer	Elastic modulus (MPa) and change of elastic modulus (%)			
	Buffer medium	(% change)	Papain enzyme	(% change)
Poly(urethane urea)				
HFL9-PU	3.977±0.54	+20.0	4.075±0.24	+23.0
HFL13-PU	5.469±0.22	+18.0	5.371±0.30	+16.1
HFL18-PU	7.823±0.42	+14.3	7.792±0.34	+13.9
HFL15-PU	12.88±0.36	+13.1	13.098±0.27	+15.0
Poly(ether urethane urea)				
HFL16-PU	5.047±0.19	+20.5	5.718±0.31	+36.5
HFL17-PU	10.719±0.32	+28.4	12.28±0.38	+47.1

In the present aliphatic poly(urethane urea)s, the rearrangement of hydrogen bonds within the hard segment might have undergone increased reorganisation. However, under repeated flexing environment of actual clinical situation, the possibility of formation of unidirectional reorganisation of polymer chains is negligible. Moreover the increase in elastic modulus observed could be reversed under a continuously flexing environment as hydrogen bonds can form and break repeatedly.

The FT-IR spectra of HFL18-PU and HFL17-PU aged under strained condition are given in Figures 4.37-4.38. The spectrum of HFL18-PU revealed appearance of strong peak at 1705 cm^{-1} for bonded urethane carbonyl and 1634 cm^{-1} for bonded urea carbonyl linkages. Similar spectral responses are also observed for HFL17-PU. IR studies revealed the absence of degradation in urethane or urea bonds and degradation products like primary amine, butyric acid or succinic acid as reported elsewhere (Fare *et al* , 1999). These studies indicate that the poly(urethane urea)s could be resistant to stress and strain-induced degradation.

4.3.3.2 Studies on Environmental Stress Corrosion Cracking

Early experience with poly(ether urethane)-insulated cardiac pacemaker revealed failure through mechanism, environmental stress corrosion cracking (ESC) (Stokes *et al*, 1987). ESC is commonly observed in vitro for plastics under the action of detergent (active chemical agent) and tensile stress (Skokes *et al,* 1987 Kambour 1973). Stress may be inherent due to the phase separated nature of polyurethanes or may be applied to a device during manufacture, implantation, or through intracorporeal movement. Environmental stress cracking is characterized by deep, ragged fracture or surface pitting within polyurethane often occurring perpendicular the direction of stress (Wiggins *et al,* 2001). The data on the environmental stress corrosion-cracking test of the present polymers are given in Table 4.21. The poly(urethane urea)s and poly(etherurethane urea)s aged under environmental stress corrosion environment in Ringers Solution and PBS media do not undergo any weight loss or ragged fracture. However, dimensional changes such as warping or hardening were observed with poly(ether urethane urea)s.

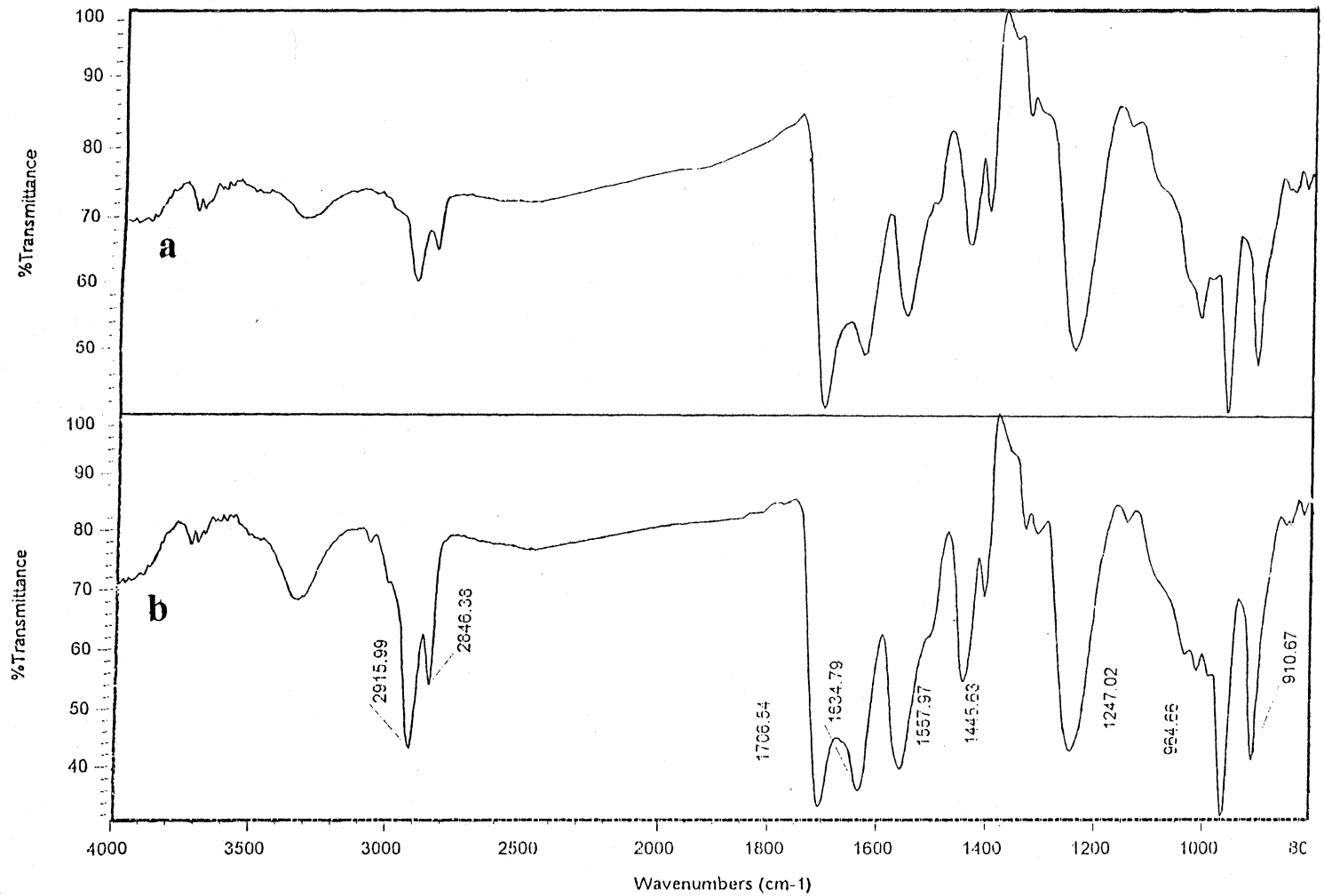


Figure 4.37 FT-IR spectrum of poly(urethane urea) HFL18-PU aged under strain (a) in papain enzyme (b) in buffer control

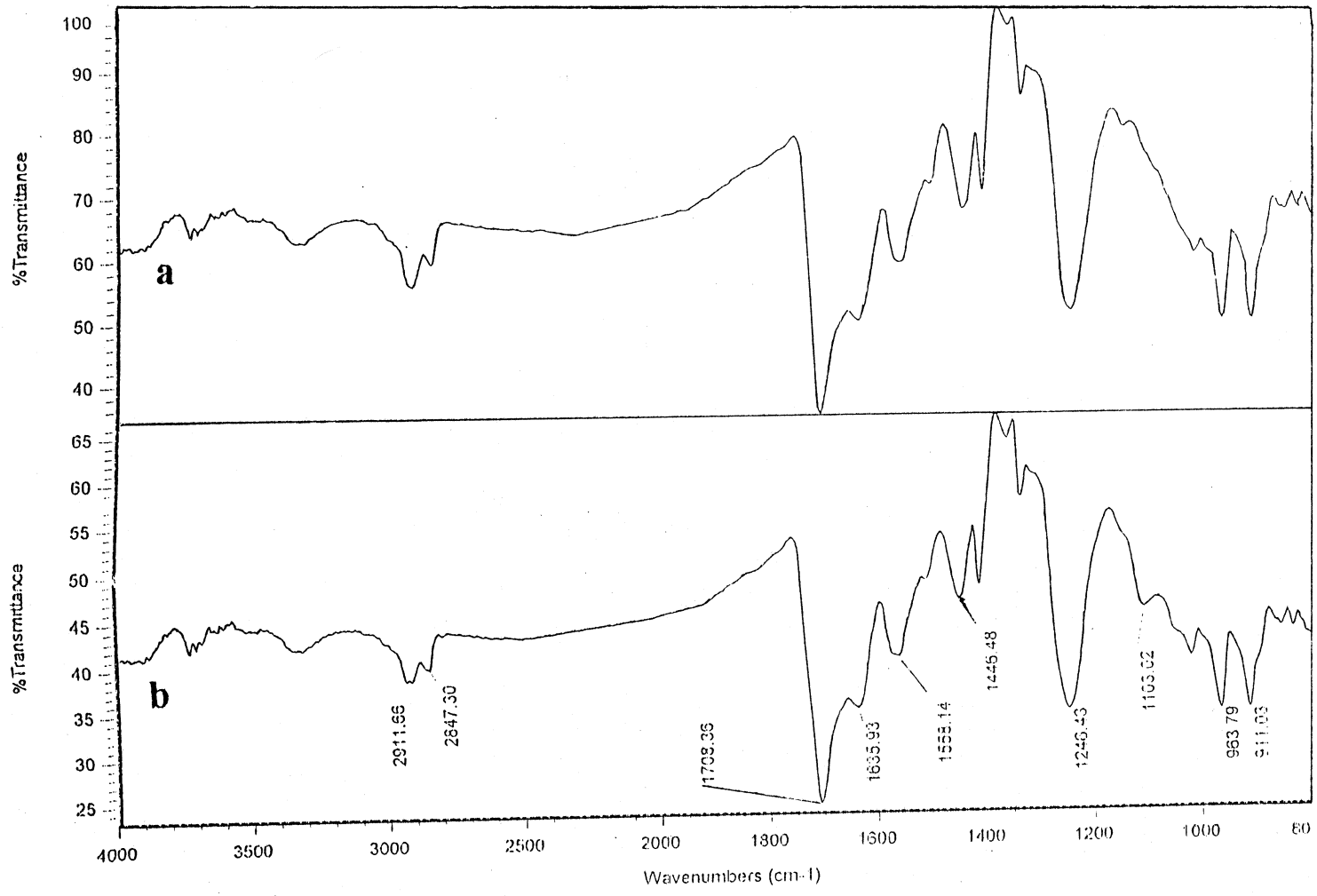


Figure 4.38 FT-IR spectrum of poly(ether urethane urea) HFL17-PU aged under strain (a) in papain enzyme (b) in buffer control

Table 4.21. Environmental stress cracking resistance data

Polymer	% of specimen failed Ringer's solution	(Remarks) PBS
Poly(urethane urea)		
HFL9-PU	Warped and harder	Brittle and warped
HFL13-PU	Warped and harder	Brittle and warped
HFL18-PU	0 No visible change	0 No visible change
HFL15-PU	0 No visible change	0 No visible change
Poly(ether urethane urea)s		
HFL16-PU	Brittle and warped	Warped and harder
HFL17-PU	Warped and harder	Warped and harder

These dimensional changes are due to the constantly-applied-bending stress. Poly(urethane urea) samples especially HFL18-PU and HFL15-PU remain intact without any visible change in dimension, flexibility and weight. There was no crack formation or whitening in any of the poly(urethane urea) samples. The resistance to environmental sress-corrosion of poly(urethane urea)s is attributed to the microphase-separated three-dimensional networks present in the hard segment domain of poly(urethane urea)s.

4.4 Studies on *in vitro* Calcification of Polyurethanes

Calcification is a severe problem with biomedical devices that are used in blood contact applications (Harasaki *et al*, 1979; Reul *et al*, 1980, Hennig *et al*, 1981; Nose *et al*, 1981; Coleman, 1981). Calcification is defined as the deposition of calcium compounds, such as either some calcium phosphate minerals consisting of

hydroxyapatite or other calcium salts. Calcium ions exist in a metastable equilibrium with dissolved phosphate species in the normal physiological condition. This equilibrium may shift on contact with an artificial implant, resulting in local calcium phosphate deposition. The precipitated calcium phosphate can be further converted into crystalline hydroxyapatite through enzymatic and hydrolytic processes (Randall, 1987; Harasaki *et al* , 1987).

Two types of ectopic calcification occur in human body: metastatic and dystropic calcification. The former is probably mediated by the presence of elevated calcium and/ or phosphate serum level and also by the materials properties, local haemodynamics and shear stress, local thermal effects, local surface charge, sorption of lipids, lipoproteins and hydrophobicity. The latter probably mediated by the cellular components, which undergo degeneration. But the absence of deposition of cellular components on smooth hydrophobic polyurethane surfaces prior to the calcification raises doubt on the role of dystrophic calcification. Moreover, patients having solubility product of calcium and phosphate more than 60 in serum may manifest a tendency to metastatic calcification. Biomaterials that calcify under the influence of physiological environment are no longer biocompatible and biodurable. The studies carried out *in vitro* conditions by Hossainy and Hubbell (1994) have proved that materials nature, structure, morphology, molecular weight mechanical stress and deformation of device play role for *in vitro* calcification of polymeric implants.

Chemical theories of biomaterial-associated metastatic-calcification emphasize the importance of the chemical composition of biomaterials. It has been

postulated that the formation of a crown ether complex between calcium ions and the polyether segment (PTMG) of the poly(ether urethane) could be the main factor for calcification of poly (ether urethane)s based on PTMG polyols. Among the polyether polyols having a range of molecular weight 650-3450, PTMG-1000 involved in the highest degree of calcification (Randall, 1987; Harasaki *et al,* 1987). Calcification leads to stiffening, perforations, ultimate degradation and fatigue failure of implants (Schoen *et al*, 1988; Levy *et al*, 1991; Chandy *et al*, 1994). Such a calcification occurs very often in bioprosthetic heart valves (Ferrans *et al,* 1980; Schoen, 1987), aortic homografts (Webb *et al*, 1988), polymeric blood pumps (Coleman, 1981; Harasaki *et al,* 1979), trileaflet polymeric heart valves (Hilbert *et al,* 1987;Wisman *et al,* 1982), and vasculargrafts and total artificial hearts. In blood pumps the calcification occurs around the areas of flexure in association with the diaphragm and pump housing (Schoen *et al,* 1988). In Biomer® -based trileaflet heart valves implanted in sheep in mitral position, calcification occurs around the areas of mechanical stress (Hilbert *et al,* 1987). Polyurethanes such as Biomer®, Mitrathane® and Pellathane® have been reported to calcify both *in vivo* and *in vitro* (Hilbert *et al,* 1987; Joshi *et al,* 1994). Other investigators have evaluated the calcification of modified polyurethanes. Han *et al* (1993) modified the surface of polyurethane valve fabricated from Pellethane® with polyethylene oxide and sulfonate groups and reported reduced calcification when compared with unmodified polyurethanes. Joshi *et al* (1994) have reported that modified polyurethane with phosphonate group reduces calcification *in vitro.* Recently Alferiev *et al* (2001) reported that bisphosphonate derivatized polyurethanes (Biospan, a polyureapolyurethane and Bionate, polycarbonate polyurethane) resist

calcification.

As far as polyurethanes are concerned, the effect of chemical composition (hard to soft segment ratio), amount of chain extender, molecular weight of soft segment, microphase separated structure and domain morphology do contribute to metastatic calcification. However, the effect of virtual crosslink density and resulting microcrystallite formation on the metastatic calcification of polyurethanes or poly(urethane urea)s is not yet investigated. Therefore, We hypothesised that the virtual crosslinking can influence calcification of polyurethanes.

Glansmacher *et al* (1987) performed *in vitro* studies on the calcification of polyurethane surfaces. Another *in vitro* model developed by Golomb and Wagner (1991) was found to be sensitive enough to diagnose the propensity of biomaterials to calcify and could serve as pre screening method for the selection of biomaterials. *In vitro* model might be used to explain the formation of calcium deposits and gross deposits in the absence of cellular involvement. The data on *in vitro* calcification of polyurethanes under stressed condition are given in Table 4.22.

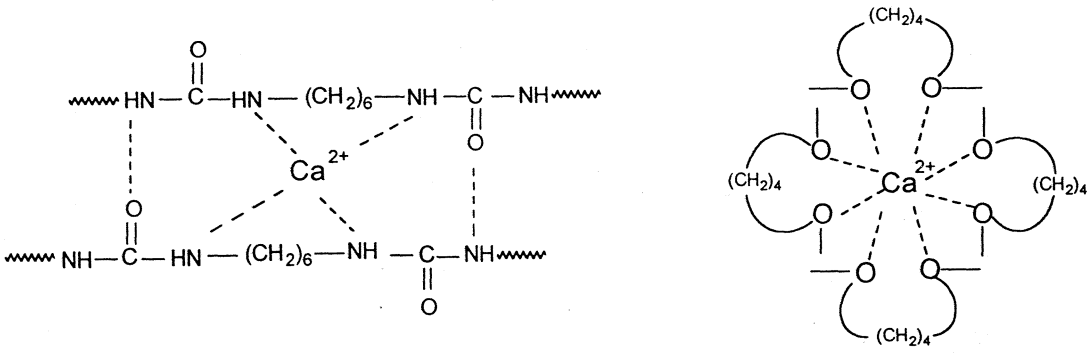
Comparing the calcification of polyurethane-ureas, HFL9-PU and HFL13-PU, higher degree of calcification was observed in the case of latter. This is attributed to the increased crosslink density of HFL13-PU. This is attributed to the increased presence of nitrogen of urea linkages, which can increase the calcium binding sites leading to the increased calcification (Bernacca *et al,* 1997*)*. Recent reports (Horkay *et al*, 2000) also predicts the role of Ca^{2+} ion in the reorganization of molecular chains by strong interaction between charged groups on the polymer chains and Ca^{2+}.

Table 4.22. Data on *in vitro* calcification of polymers

Polymer	Crosslink density (x 10^4)	Calcium deposited (mg/g)	Phosphorous deposited (mg/g)	Total mineralisation (mg/g)	Ca/P ratio
Poly(urethane urea)					
HFL9-PU	1.852	0.0733±0.02*	0.0384±0.01#	0.1117	1.90
HFL13-PU	6.781	0.3433±0.10*	0.1996±0.02*	0.5429	1.70
HFL18-PU	6.946	0.1646±0.02*	0.0487±0.01#	0.2133	3.379
HFL15-PU	8.737	0.1734±0.03	0.0810±0.03*	0.2544	2.141
Polyether urethane urea					
HFL16-PU	2.260	0.4238±0.13*	0.0417±0.01#	0.4655	10.2
HFL17-PU	3.090	0.2446±0.08#	0.0919±0.02*	0.3365	2.7
Tecoflex 85A	-	0.2382±0.10	0.0546±0.01	0.2928	4.4

Tecoflex 85A is a linear segmented polyurethane containing H_{12}MDI, PTMG and BD
*p values< 0.05 were considered as statistically significant when all values were compared to those of Tecoflex 85A.
#P was not significant

The even-numbered-methylene groups present in the diamine chain extender (HDA) units also promote the packing of polymer chains as reported by Takahara *et al* (1985 b). The mechanism of hard segment-mediated-calcification of poly(urethane urea)s and soft segment-mediated-calcification of poly(ether urethane urea)s are postulated as in Figure 4.39. However, the increase of calcification with increase of crosslink density has a limiting value beyond which a reverse phenomenon occurs. For HFL15-PU and HFL18-PU poly(urethane urea)s, though virtual crosslink density is more compared with that of HFL9-PU and HFL13-PU, calcification was found to be less. Since the poly(urethane urea)s, HFL15-PU and HFL18-PU have hard segment content 80 and 75 % respectively, they possess more ordered array of hard segment crystallites.



a . Hard segment mediated calcification

b. PTMG mediated calcification

Figure 4.39. Mechanism of calcification of poly(urethane urea)s and poly(ether urethane urea)s

Hence the availability of donor atoms (nitrogen) for calcium complexation becomes less leading to lesser calcification. The relation between virtual crosslink density and in vitro calcification of poly(urethane urea)s is given in Figure 4.40.

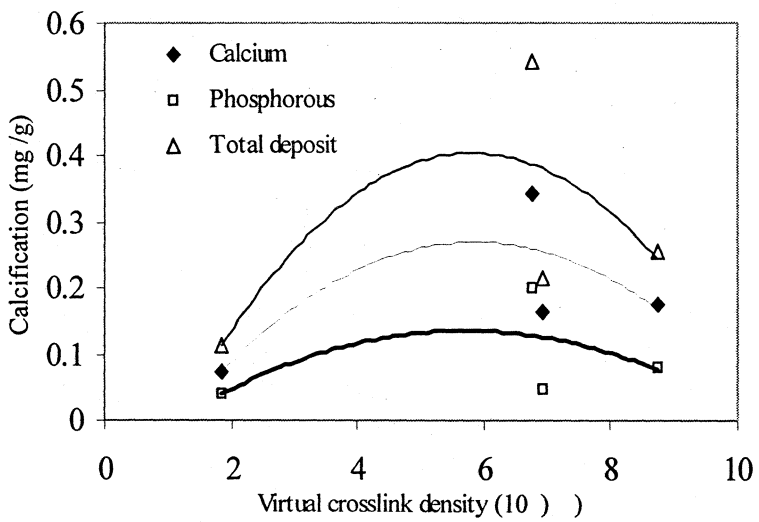


Figure 4.40 Calcification Vs. Virtual crosslink density in poly(urethane urea)s

As the virtual crosslinking increases the calcification increases to a maximum level due to hard segment mediated calcium complexation mechanism and exothermic reordering of hydrogen bonds and then decreases due to unavailability of free donor atoms (urea linkages) for calcium complexation. Since virtual crosslink density of poly(urethane urea)s is directly proportional to the amount of amine chain extender (hard segment %), *in vitro* calcification of poly(urethane urea)s is also proportional to the percentage of amine chain extender as shown in Figure 4 .41.

An interesting observation has been noticed in the case of present poly(etherurethane urea)s, which contain physical crosslinking not only through urea-urea linkages but also urethane-urea and ether-urea linkages. Such mixed physical crosslinking resulted in a moderately phase-mixed structure. Figure 4.42 shows the calcification vs. percentage of PTMG in poly(ether urethane urea)s.

Comparing the calcification of present poly(etherurethane urea)s, HFL16-PU and HFL17-PU containing PTMG soft segments, the former showed a higher deposition of calcium ions than the latter. This is attributed to the increased crown ether-like complex formation of calcium ions with the ether units of the polytetramethylene glycol, in addition to the hard segment-mediated-calcification. The decreased crosslink density in the former suggests the availability of free ether units of PTMG soft segment in HFL16-PU. Poly(ether urethane urea), HFL17-PU contains a lesser percentage of PTMG polyol (16.05%) and higher crosslink density in comparison with the counter part HFL16-PU (21.25%). Hence the combined effect of soft segment-mediated crown ether formation and hard segment-mediated complex formation is less pronounced in HFL17-PU.

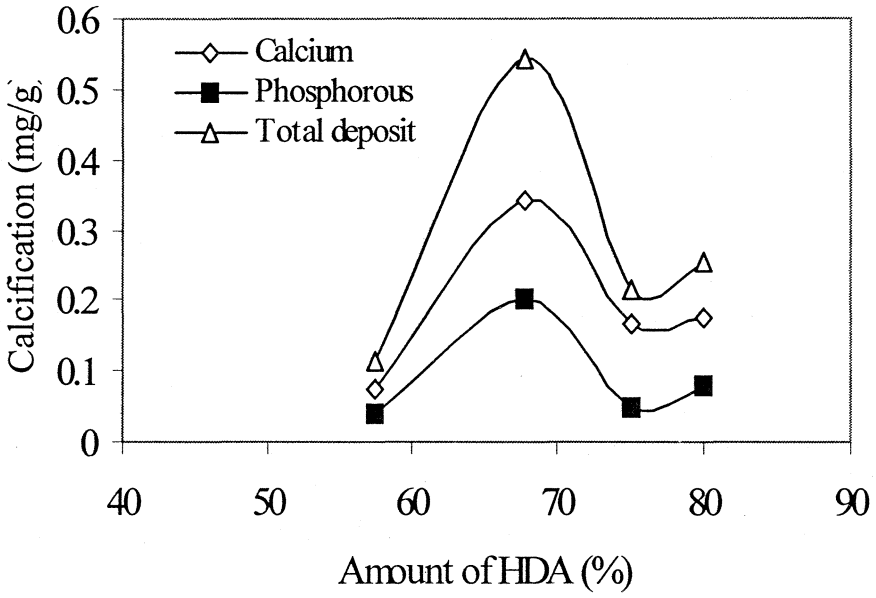


Figure 4.41 Calcification Vs. Amine chain extender (%) of poly(urethane urea)s

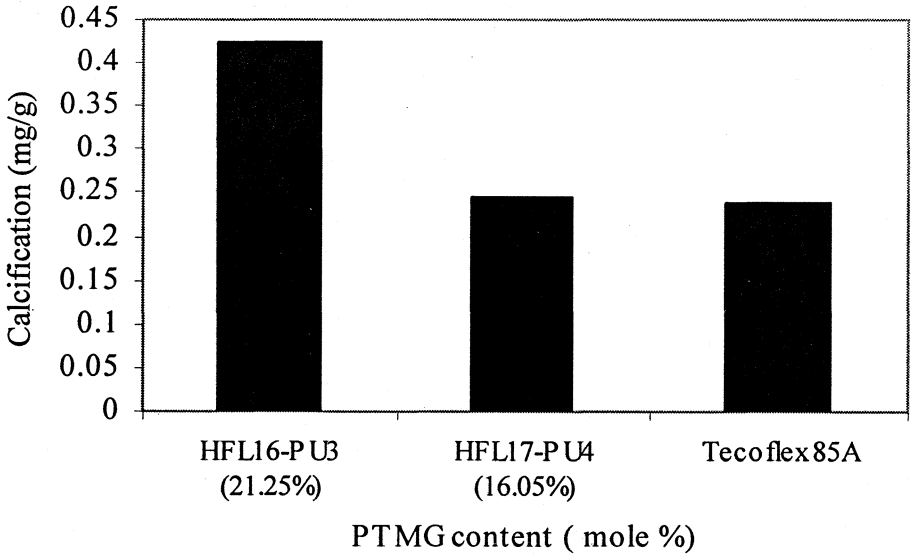


Figure 4.42 Variation of calcification in poly(ether urethane urea)s

Therefore, it is inferred that the higher the physical crosslinking in virtually crosslinked poly(ether urethane urea)s containing PTMG soft segment, the lesser is the calcification.

Jayabalan *et al* have reported that the higher the hard segment in the chemically crosslinked poly(ether urethane)s based on dicyclohexyl methane diisocyanate/ PTMG2000/ trimethylol propane, higher is the amount of calcium deposition (Shunmugakumar and Jayabalan, 1992). An inverse relationship between PTMG-2000 polyol concentration which can form crown ether with calcium and calcium deposition has been reported in these poly(ether urethane)s by the investigators. The reasons attributed to this trend by the investigators were highly phase–mixed structure of hard and soft segment and hydrophilicity. It has been reported that the higher the hard segment content, higher is the degree of phase mixing in chemically crosslinked poly(ether urethane)s (Shunmugakumar and Jayabalan,1992). Interestingly, the present virtually cross-linked poly(urethane urea)s HFL9-PU ,HFL13-PU, HFL18-PU and HFL15-PU are highly phase-separated and hydrophobic in nature.

The data on total deposition and ratio of calcium and phosphate are given in Table 4.22. Poly(urethane urea), HFL13-PU revealed higher total deposition of calcium and phosphate in comparison with that of HFL9-PU. However, both the poly(urethane urea)s, HFL9-PU and HFL13-PU reveal more or less similar calcium-phosphate ratio which lies within the physiological limit of calcium-phosphate ratio 1.67 of hydroxyapatite. The higher calcium-phosphate ratio observed in HFL16-PU in comparison with HFL17-PU is due to the excessive calcium deposition by crown

ether formation with PTMG-1000 polyol apart from the deposition as calcium phosphate. A similar situation has been observed in the case of Tecoflex® 85 A, poly(ether urethane) containing PTMG polyol.

The present studies revealed that all the newly developed poly(urethane urea)s, do not undergo excessive calcium deposition / calcification as that has been observed by various other investigators. Bernacca *et al* (1995; 1997) have reported calcification value 0.63-2.21 mg calcium/g of polymer for the Estane58201® [MDI/ PTMG/BD based poly (ether urethane)]. Hossainy and Hubbell (1994) have studied the effect of molecular weight between chemical cross-links (molecular weight of PEG) of PEG-based hydrogel on *in vivo* calcification. They reported higher value, 224 mg of Ca/g for hydrogel based on PEG (MW=1000) and lesser value, 0.23 mg of Ca/g for hydrogel based on PEG (MW =10,000) and classified the former as highly calcifying gel. In short, the studies on the *in vitro* calcification using stressed poly(urethane urea)s in metastable calcium phosphate solution proved that the virtual crosslinking play a role in the biomineralisation of polymeric implants.

4.5. Studies on Biocompatibility of Polyurethanes

Biocompatibility implies benign behaviour of materials in the dynamic physiologic environment and simultaneous benign response of the dynamic physiologic environment toward implants during their intended period of performance. This reciprocal relationship seems to be the *sine qua non* for long-term biocompatibility (Bruck, 1997). Commercially available polyurethanes still seems not quite appropriate to be used for high demanding long-term blood contact applications. There are various approaches to improve blood compatibility of polyurethanes.

It is well known that the blood vessel wall and blood components are negatively charged. Srinivasan and Sawyer (1970) have demonstrated that materials with negatively charged surface were antithrombogenic while those with positively charged surface were thrombogenic. Under normal conditions, the negatively charged groups on the blood vessel wall result in thromboresistance because of electrical repulsion. Thus studies of Weerwind *et al* (1998) have revealed that the antithrombigenic activity of heparin, a natural anticoagulant, is attributed to its negatively charged surface functional groups (carboxylate and sulfonate). Strategies reported for improving blood compatibility also include surface immobilisation techniques such as albumin coating (Marois *et al,* 1996) endothelial cell seeding (Pasic *et al,* 1996) and use of hydrophobic polyurethane that release nitric oxide (Mowery *et al,* 2000). However, none of these methods have found to be completely successful. One possible solution to the problem of thrombogenicity is the use of hydrophobic polymers as reported by Yoda (1998). To date many investigators focussed on improving the biocompatibility of polyurethanes derived from aromatic diisocyanate. However, only a few investigators have studied the biocompatibilitiy of polyurethanes composed of aliphatic diisocyanate, H_{12}MDI and HTPBD. In the present investigation, the biocompatibility of the new polyurethanes was assessed by both *in vitro* and *in vivo* methods as per internationally accepted standard protocols.

4.5.1. Evaluation of *in vitro* haemolysis

The lysis of red blood cells (RBC) and the consequent liberation of haemoglobin are called haemolysis. Damage to RBC can be resulted from surface interaction and from presence of leachants from polymers or from rheological

stresses. The percentage of haemolysis of the present hydrophobic polyurethanes, poly (urethane urea)s and poly(ether urethane urea)s is given in Table 4.23.

Table 4.23. Haemolytic potential of polymers

Polymer	Haemolysis (%)
Polyurethane	
HFL1-PU	0.42
HFL3-PU	1.08
Poly(urethane urea)	
HFL9-PU	0.52
HFL13-PU	1.28
HFL18-PU	0.47
HFL15-PU	0.20
Poly(ether urethane urea)	
HFL16-PU	0.13
HFL17-PU	0.13
Tecoflex 85A	0.34

The percentage of haemolysis is well below the acceptable limit of 5% (O'Leary and Guess, 1968). The haemolytic potential of the present polymers is comparable to that of commercial aliphatic poly(ether urethane), Tecoflex85A®. The results are in good agreement with that reported for the poly(urethane urea), Biomer®. Hung *et al* (1975) have also assayed the *in vitro* haemolytic activity of Biomer® and Biomer® containing additives *viz.* dibutyl tin compound and found that haemolysis was higher with samples containing additives. The study also reveals that the present polyurethanes are extremely pure without any oligomer or reactants present as leachants. The absence of haemolytic leachants in the present polymers is due to the successful extraction of materials in ethanol during purification step. Moreover, it also reveals that continuous ethanol extraction using a Soxhlet extractor

is an effective method to purify poly(urethane urea)s and to improve blood compatibility like methanol extraction of polyurethane could improve blood compatibility (Lelah *et al,* 1986a). McTernan (1970) has also reported minimal haemolysis with poly(urethane urea), Biomer®. Irrespective of the virtual crosslink density, and percentage of hydrophobic polyol, all the present polymers have found to possess low haemolytic activity. This indicates that virtual crosslink density of polyurethanes does not have much influence on the haemolysis data. The present new polymers can therefore be considered as non-haemolytic and suitable for blood contact applications. The non-haemolytic behaviour was further confirmed using blood-material interaction assay with human blood.

4.5.2 Evaluation of *in vitro* blood compatibility-Haematology

The blood-material interaction is mediated with the initial event of adsorption of blood protein. The data of RBC count and concentration of haemoglobin present in the whole blood at the start of the experiment and after 1h exposure to the polymers are given in Table 4.24. The present polymers do not show remarkable increase of haemoglobin at the end of 1h of exposure in comparison to the control blood. The RBC count also does not reveal remarkable change in comparison to the control blood. These data confirm that there is no significant cell lysis of red blood cells (haemolysis) even though the blood was shaken in the presence of solid polymer as discussed in haemolysis assay. However, studies reveal a slight variation (increase) in RBC count in all test samples including control blood. This may be due to the variation in the rate of erythrocyte sedimentation with time in the presence of polymers.

Table 4.24. RBC and Haemoglobin count due to blood-material interaction

Polymer	RBC count (x 10^{-6}/ mm^3)		Amount of haemoglobin (g/dl)	
	Initial	Final	Initial	Final
Poly(urethane urea)				
HFL9-PU	1.96±0.11	3.62±0.07	15.70±0.88	15.95±0.20
HFL13-PU	1.72±0.07	3.07±0.08	14.17±0.66	14.40±0.51
HFL18-PU	1.90±0.04	3.38±0.02	15.02±0.32	15.50±0.18
HFL15-PU	1.75±0.07	3.12±0.08	14.61±0.27	14.52±0.44
Poly(ether urethane urea)				
HFL16-PU	1.76±0.06	3.12±0.03	14.42±0.47	14.80±0.30
HFL17-PU	1.94±0.09	3.29±0.06	15.11±0.61	14.93±0.31
Tecoflex 85A	1.79±0.06	2.89±0.08	14.90±0.60	13.53±0.16
Control blood	1.89±0.05	3.46±0.02	15.20±0.14	14.80±0.05

Platelet adhesion and aggregation by synthetic materials limits their use for blood contact applications (Salzman *et al,* 1987). *In vitro* platelet interaction in whole blood exposed to material surface has been used to assess blood compatibility of biomaterial (Claire *et al,* 1993). The degree of interaction and blood compatibility of the new poly(urethane urea)s was evaluated by counting the number of platelets and white blood corpuscles (WBC) in the blood after the exposure to the material. The percentage retention of platelet and WBC after 1h exposure to human whole blood is given in Tables 4.25 and 4.26. The studies revealed that the present polymers do not induce leucopenia (Table 4.25). The absence of significant reduction of WBC suggests that these polymers may not induce complement activation.

Table 4.25 WBC count determined after whole blood-material interaction

Polymer	WBC count ($/mm^3$) Initial	After 1 h	WBC consumed/ mm^2	Retention (%)
Poly(urethane urea)				
HFL9-PU	4000±50	3930±150	0.175	98.3
HFL13-PU	5100±50	4830±100	0.675	94.7
HFL18-PU	4250±81	3706±97	1.360	87.2
HFL15-PU	5133±42	4230±96	2.257	82.4
Poly(ether urethane urea)				
HFL16-PU	5100±110	4800±50	0.750	94.1
HFL17-PU	3600±120	3500±110	0.250	97.2
Tecoflex 85A	5530±55	4600±35	2.325	83.2
Control blood	3950±110	3800±85	0.375	96.2

These polymers are comparatively better than the Tecoflex 85A®, polyurethane. The studies also suggest a correlation between the physical crosslink density and WBC consumption as shown in Figure 4.43. Among the poly(urethane urea)s, HFL9-PU having lower value of physical crosslink density, elicits a lower amount of WBC consumption in comparison with others (HFL13-PU). *i.e.,* Virtual crosslink density is directly proportional to WBC consumption for poly(urethane urea)s. An inverse trend was observed in the case of poly(ether urethane urea)s, HFL16-PU and HFL17-PU, which is attributed to the marginally phase-mixed morphology of these polymers. However, Tecoflex85A® exhibited the highest WBC consumption.

The degree of interaction between blood platelets (thrombocytes) and the polymers determine the thrombogenicity of the polymers. Thrombogenicity is mediated by (i) protein adsorption and deformation (ii) the attachment of platelets

with the substrate polymer (iii) centrifugal growth of filopodia and (iv) cytoplasmic webbing and flattening of the central mass.

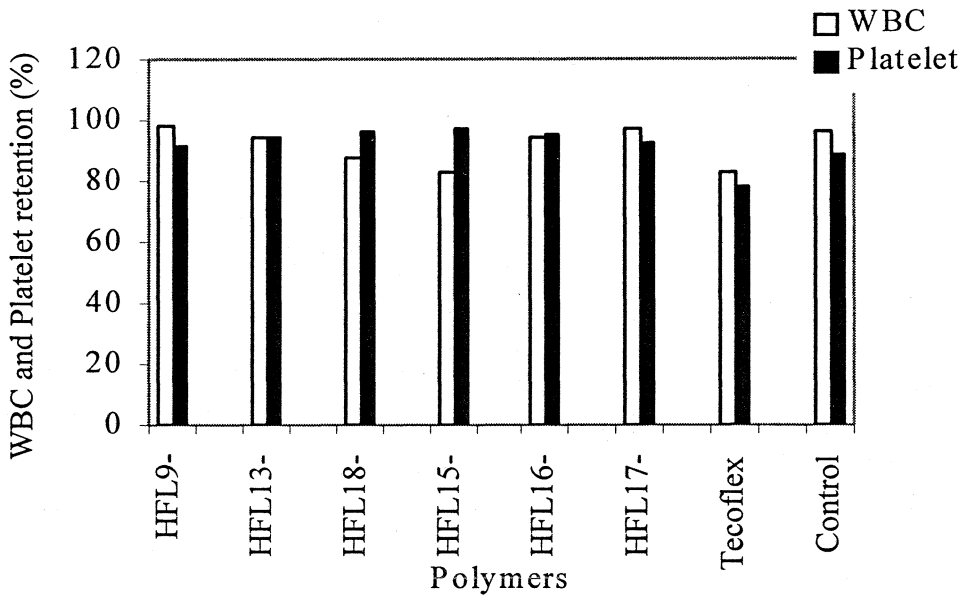


Figure 4.43 Retention percentage of WBC and Platelet after whole blood-material interaction

Since the deformation of adhered platelets proceeds in the above order with time, the estimation of platelets remaining in the blood after 1 hour can be used as a measure of non-thrombogenicity. Sa da Costa *et al* (1980; 1981) dealt on the relationship of surface composition of segmented polyurethane and platelet retention. Those polyurethanes (aromatic diisocyanate/ polyether polyols based) which contained highest fraction of ethereal carbon in the surface were found to have the lowest platelet retention. It was also reported that PEG and PEG/PPG based polyurethanes are more bland towards platelets than polyurethanes based on PTMG and Biomer®. Earlier investigators (Claire *et al*, 1993) have demonstrated improved blood compatibility with polymers having hydrocarbon–rich surface. Claire *et al*

(1993) have found that poly(ether urethane urea) having hydrocarbon moieties at the surface are more bland towards platelet adhesion and activation than Biomer®. The data of platelet count are given in the Table 4.26.

Table 4.26. Platelets count due to blood-material interaction

Polymer	Platelet count (x 10^{-2}/mm^3) Initial	After 1 h	Platelet consumed/ mm^2	Retention (%)
Poly(urethane urea)				
HFL9-PU	1495±90	1370±55	31.25	91.6
HFL13-PU	1300±56	1230±21	17.50	94.6
HFL18-PU	1483±22	1433±19	12.50	96.6
HFL15-PU	1336±45	1299±40	09.25	97.2
Poly(ether urethane urea)				
HFL16-PU	1293±49	1237±55	14.20	95.7
HFL17-PU	1480±91	1370±32	27.50	92.6
Tecoflex 85A	1480±90	1160±87	80.00	78.4
Control blood	1645±25	1445±28	47.00	88.4

Quite interestingly, the test poly(urethane urea)s and poly(ether urethane urea)s showed remarkably lower number of consumed platelets in comparison with the control (without test material) and Tecoflex85A®. Takahara *et al* (1985 b) have reported that the aromatic poly(urethane urea)s based on MDI/poly propylene glycol/diamine induced platelet deformation more with polymer based on diamine containing odd number of methylene groups and less with the polymer based on diamine containing even number of methylene groups. They have attributed this behaviour to the greater phase mixing in the former and greater phase separation in the latter. Merrill *et al* (1982a) have reported that aliphatic poly(ether urethane urea)

based on cyclohexyl diisocyanate (CHDI-PEG-ED) are more bland towards platelet adhesion which is due to the presence of PEG on the surface to a depth of 40 A^{o} .

Earlier investigators have predicted that the blood compatibility of polyurethanes must be correlated in terms of their surface properties. Since the interaction between a polyurethane and blood occurs at the blood-polymer interface, the surface characteristics obviously influence the blood response. The relationship between polyurethane surface-soft or hard segment concentration and thrombogenicity is of great interest. Several *ex vivo* (Lelah *et al,* 1983, Lelah *et al,* 1985) and *in vitro* (Sa da Costa *et al,* 1981, Merril *et al,* 1982 b) studies have indicated that surface-soft-segment concentration correlates with some measure of blood compatibility. Picha *et al* (1978) suggested that the degree of microphase separation was of importance in blood-polyurethane interactions; but the changes in the amount of polyether soft segment were not found to significantly affect platelet adhesion from anticoagulated blood. Hanson *et al* (1980) discovered a strong inverse correlation between platelet consumption per unit area and the surface fraction of carbon atoms forming C-H chemical bond. These results suggest that an increased concentration of PTMG soft segment on the surface leads to reduced blood compatibility. A possible explanation for these apparently conflicting findings was suggested by Takahara *et al* (1982). Takahara *et al* observed a minimum of *in vitro* platelet shape change, which occurs at an intermediate molecular weight of soft segment.

The correlation between virtual crosslink density and platelet retention is given in Figure 4.43. In the present investigation, a lower degree of platelet consumption in all the polymers was observed, which is attributed to the presence of

three dimensional net work of virtual cross links and micro phase-separated hard domain. In the present study the blood compatibility is correlated with virtual crosslink density or molecular weight between crosslinks of both poly(urethane urea)s and poly(ether urethane urea)s. As virtual crosslink density increases the platelet retention (%) increases for poly(urethane urea)s and vice versa. An inverse trend is observed with poly(ether urethane urea)s. It is interesting to note that all the present hydrophobic polymers are blood compatible.

4.5.3 Evaluation of cytotoxicity of polyurethane *in vitro*

Impurities (e.g., catalysts, solvents, monomers and oligomers) introduced in a polymeric biomaterial during synthesis affect the polymer-cell interaction. Impurities can also be deposited on the surface in the later stages, e.g., air-born particulate, dust, mold release agents etc. The presence of impurities on the surface makes it highly thrombogenic and cytotoxic. Leachable components or residual solvents in the polyurethane may cause necrosis of the neointima, while contamination can also kill the cells (Bruck, 1979).

4.5.3.1. Evaluation of cytocompatibility by direct contact method

The cytotoxicity studies carried out by direct contact method revealed no signs of cell toxicity with the present polymers. The microphotographs of L929 fibroblast cells grown in contact with the newly prepared poly(urethane urea)s are given in Figures 4.44 - 4.45. The microphotographs of cells grown in contact with poly(ether urethane urea)s are also given in Figure 4.46. The cells in physical contact with the present polymers at the interface (dark line) showed the same cellular morphology (spindle shape) as those present in negative control (polystyrene cell culture plate). Optical micrographs of L929 fibroblast cells with a positive control (Zinc diethyl

dithiocarbamate, a known cytotoxic material) and cells with Tecoflex 85A® are also given in Figure 4.47. The morphology of the cells in contact with cytotoxic material (positive control) has changed to round shape. Poly(urethane urea)s and poly(ether urethane urea)s have excellent cytocompatibility irrespective of the nature, composition and crosslink density.

4.5.3.2. Cell viability studies of poly(urethane urea)s-MTT test

The *in vitro* cytotoxicity analyses carried out using the extract of the material by MTT test method revealed high degree of cell viability. The percentage of cell viability for 100% and 50% extract of the poly(urethane urea)s and poly(ether urethane urea)s are given in Figure 4.48. The poly(urethane urea)s (HFL9-PU, HFL13-PU, HFL15-PU and HFL18-PU) and poly(ether urethane urea)s (HFL16-PU and HFL17-PU) showed appreciably high degree of formation of MTT formazan product in both 100 and 50% extract of the material. The extract of HFL17-PU has showed slightly less percentage of formation of formazan with 100% extract. The studies revealed that the polymers contain no leachables capable of inducing cell toxicity. Figure 4.49 is the representative microphotograph showing the morphology of the fibroblast cells after incubation with extract (100%) of polymer HFL18-PU. The normal and cellular morphology of fibroblast cells remains the same. The lack of cytotoxicity of fibroblasts in both direct and indirect (extract) close contact of poly(urethane ureas) and preservation of their normal and cellular morphology suggested that the newly prepared polymeric materials were non-cytotoxic to fibroblasts cells.

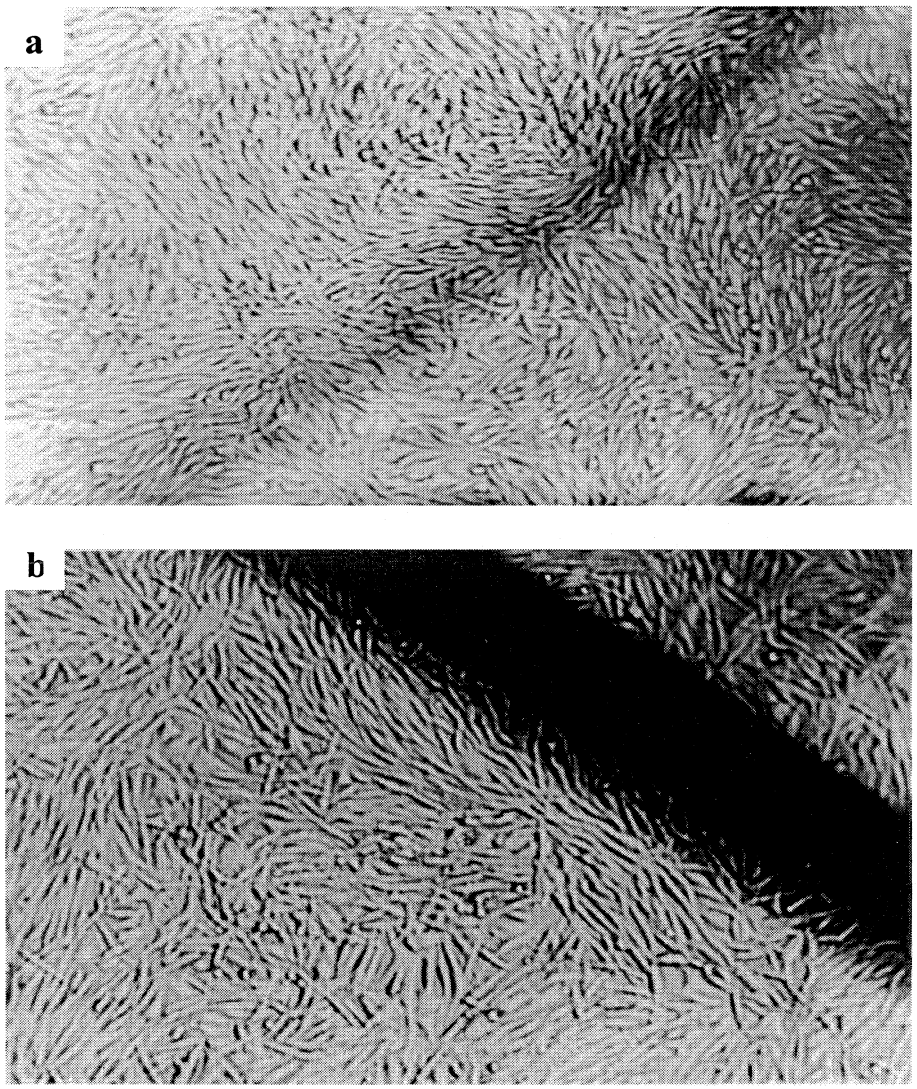


Figure 4.44. Optical microphotograph of fibroblast cells in contact with poly(urethane urea)s ; (a) HFL9-PU (b) HFL13-PU

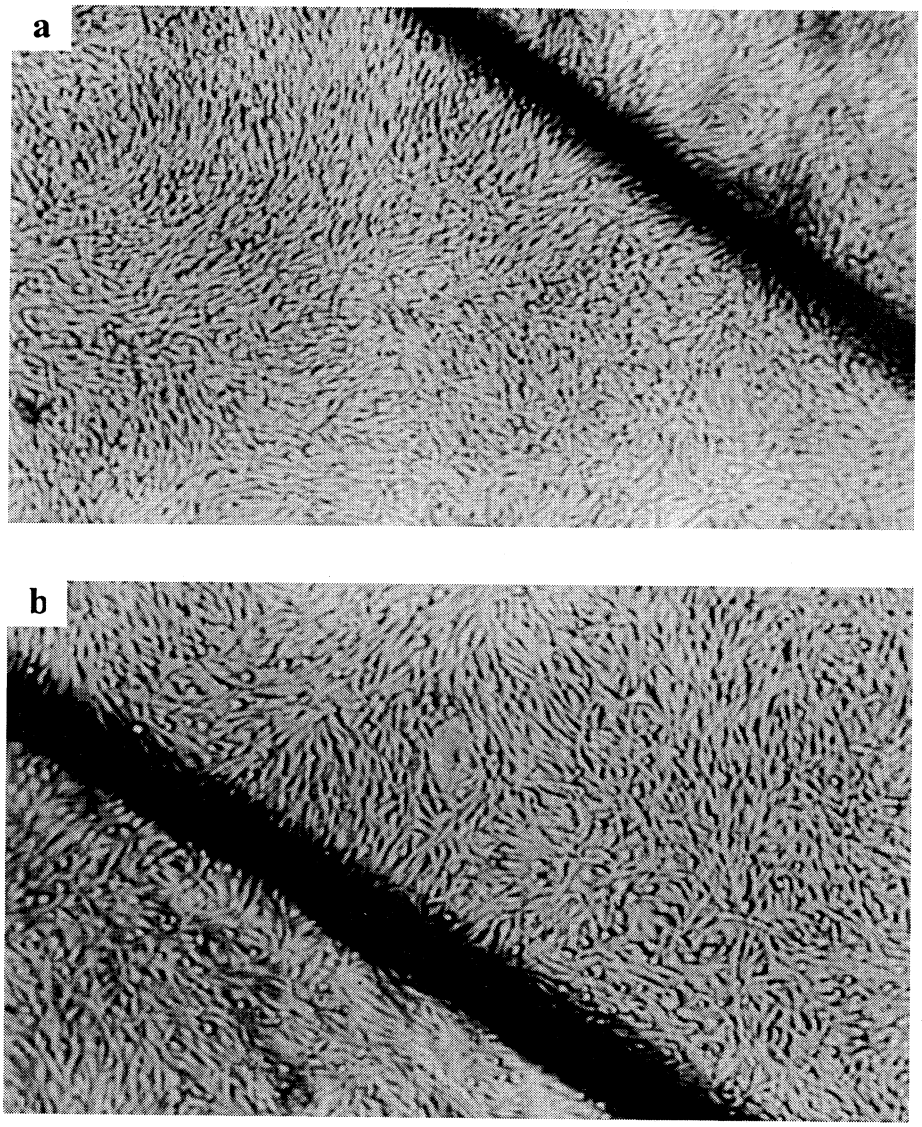


Figure 4.45. Optical microphotograph of fibroblast cells in contact with poly(urethane urea)s ; (a) HFL18-PU (b) HFL15-PU

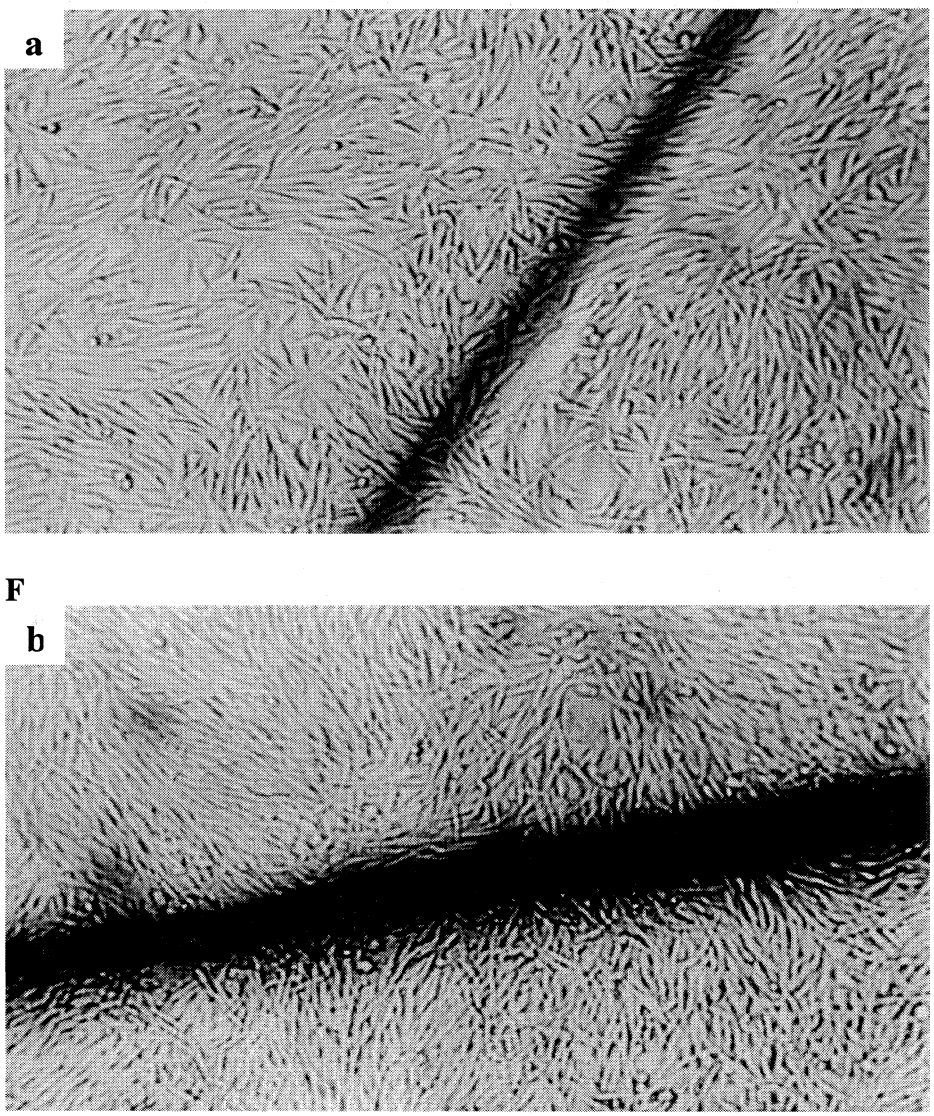


Figure 4.46. Optical microphotograph of fibroblast cells in contact with poly(ether urethane urea)s ; (a) HFL16-PU (b) HFL17-PU

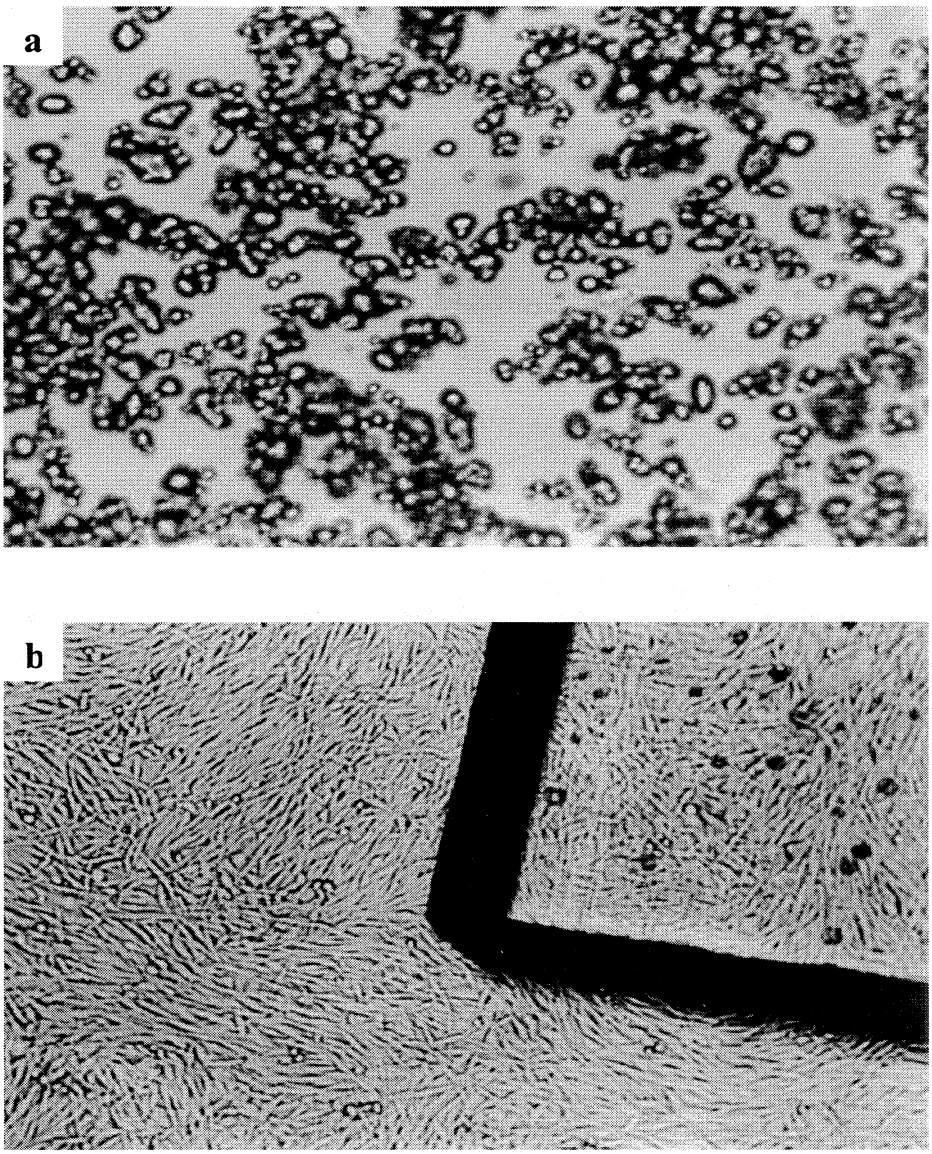


Figure 4.47. Optical microphotograph of fibroblast cells in contact with (a) Positive control and (b) Tecoflex 85 A

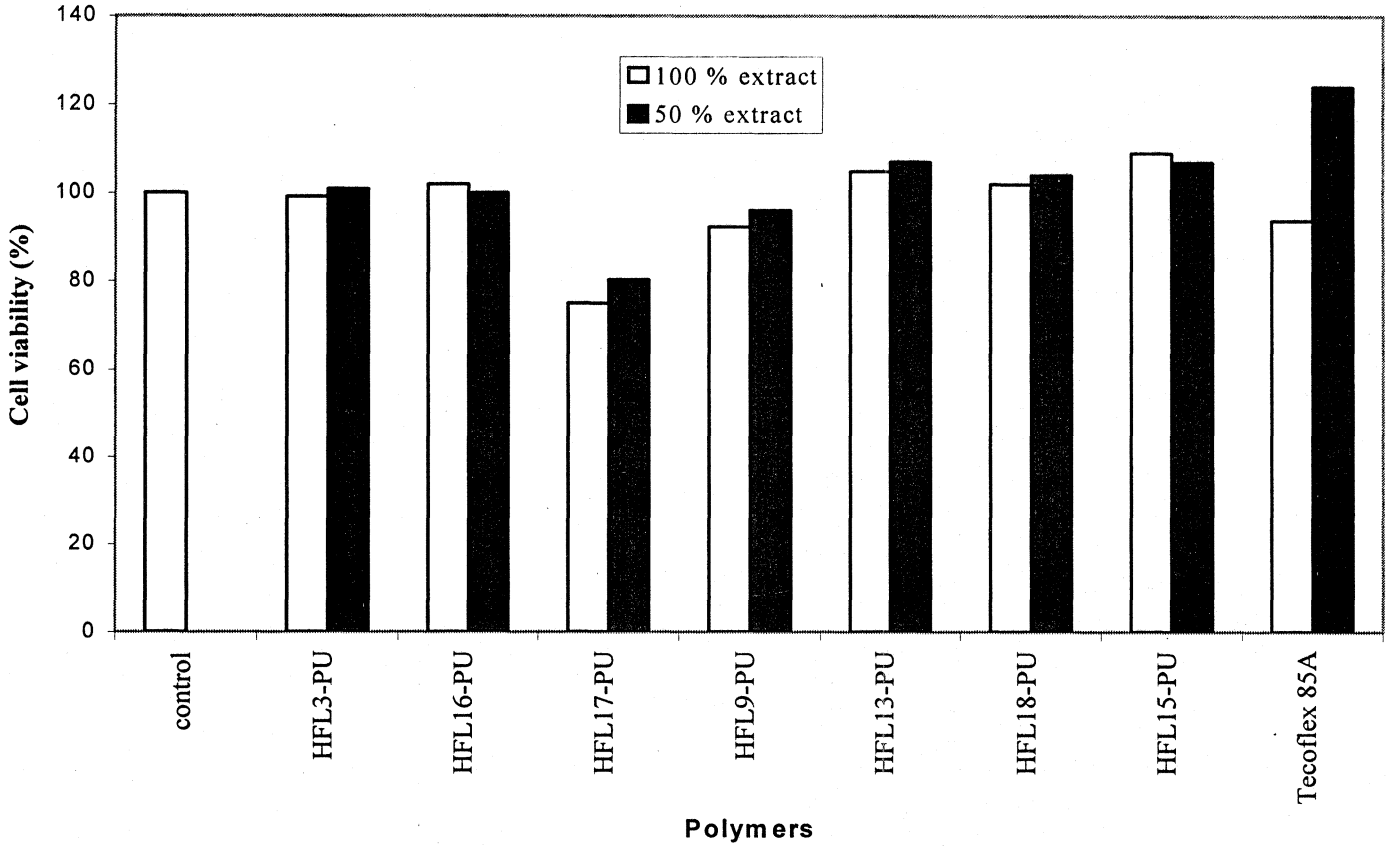


Figure 4.48 Cell viability of poly(urethaneurea)s and poly(ether urethane urea)s

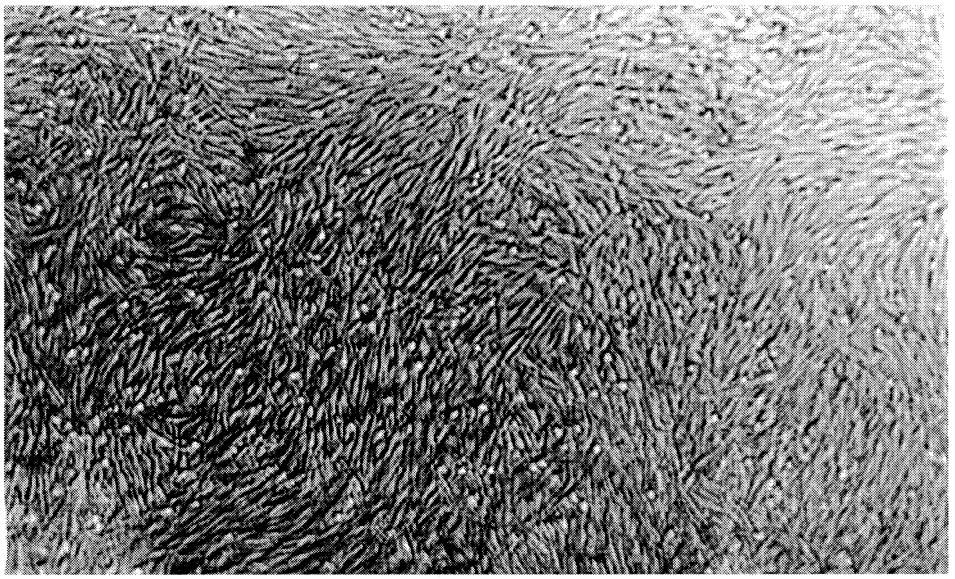


Figure 4.49 Microphotograph showing the morphology of the fibroblast cells after incubation with extract (100%) of HFL18-PU

4.5.4 *In vivo* Evaluation of Systemic Toxicity in Mice

This is one of the few systemic response tests to provide a more direct assessment of neuro-toxicity or other adverse systemic effects. This involves the simple direct intravenous and intra-peritoneal test of extracts of materials in mice. The systemic toxicity studies of the poly(urethane urea), HFL18-PU do not elicit adverse systemic response for observation period of immediate and at 4, 24, 48, 72 h and up to 7-days as given in Table 4.27. No toxic symptoms were noticed in test animals immediately after injection or at any days of the experimental period. None of the animals treated with material extracts showed any significantly greater biological reactions than the animals treated with control. So it was concluded that the extract

(both saline and cotton seed oil) of the candidate poly(urethane urea), HFL18-PU is non-toxic and the polymer meets the requirements of the test.

Table 4.27 Systemic toxicity result of candidate poly(urethane urea) HFL18-PU in mice

Observation	0.9 % NaCl extract	Cotton seed oil extract
Toxic symptoms	Nil	Nil
Feed & water consumption	Normal	Normal
Body weight	Normal	Normal
Mortality	Nil	Nil

4.5.5. *In vivo* Evaluation of Intracutaneous Irritation in Rabbit

Allergic contact dermatitis is not a life threatening, but a large number of people suffer from this reaction. This test in the protocol of biomaterial evaluation is to assess the allergic responses produced by the extract of material when applied through intracutaneous injection in rabbits. The signs of toxicity such as erythema and edema are recorded.

The *in vivo* intracutaneous irritation data of candidate poly(urethane urea) HFL18-PU are given in Table 4.28. The intracutaneous irritation test with extract of HFL18-PU in both the media did not reveal adverse local responses in rabbit. The test results indicate an average irritation score of 0.00 and 0.04 for the injection with extract of saline and cottonseed oil respectively. The irritation is well below the generally accepted limit (<1) as per ASTM standard F 749. Therefore, it is concluded that the saline and cotton seed oil extract of the poly(urethane urea) HFL18-PU do not

elicit any adverse local tissue responses and the material has passed the intracutaneous irritation test.

Table 4.28 Intracutaneous irritation test results of HFL18-PU in rabbit

Skin reaction	Scoring period	Irritation Score			
		Control		Test	
		NaCl Extract	Cotton seed oil	NaCl Extract	Cotton seed oil extract
1. Erthyma	Immediate	0	0	0	0
	24 h	0	0	0	0.50
	48 h	0	0	0	0
	72 h	0	0	0	0
Sub total		0	0	0	0.50
2. Edema	Immediate	0	0	0	0
	24 h	0	0	0	0
	48 h	0	0	0	0
	72 h	0	0	0	0
Sub total		0	0	0	0.00
Total Skin Irritatio score*		0	0	0 0.00	0.50 0.04

*Score value < 1 shows non-toxicity

This test also clearly indicated that the candidate polymer material is free from extractable low molecular fractions or catalysts. The *in vivo* toxicological screening studies revealed biocompatibility of candidate poly(urethane urea), HFL18-PU.

4.5.6 Evaluation of *in vivo* Biocompatibility -Intramuscular Implantation

Histocompatibility deals with biocompatibility in terms of whether the implant or its degradation products, if any, initiate adverse tissue responses in the host, or conversely whether deleterious changes in the chemical, physical and mechanical properties of the implant material are caused by the host environment. The tissue

response to an implant is a specialized version of inflammation and repair or the mammalian reaction to local injury.

In vivo biocompatibility was studied by intramuscular implantation of the candidate poly (urethane urea) HFL18-PU in rabbit animal model for 7, 30 and 90 days. Gross examination revealed that all the animals were in good health condition throughout the experimental period. The consumption of feed and water were normal during the observation period. When the animals were sacrificed at the end of 90 days, none of implantation site (both test and control) showed any macroscopic abnormalities such as haemorrhage, necrosis, discolouration and infection.

Poly(urethane urea), like any material implanted in body elicit initial inflammatory reactions following implantation. Inflammation begins when surgical injury causes migration of cells from the circulating blood to the site of polymer implantation, where a transient inflammatory exudate is formed. Monocytes that migrate from the vasculature to the implant site may adhere to the surface of the materials. Over the period of time, these adherent monocytes differentiate into macrophages, which can then fuse together to foreign body giant cells (Kao *et al,* 1994; Zhao *et al,* 1992). However, these inflammatory responses were minimum after 30 days.

The data on histopathological analyses of the tissue surrounding the candidate poly(urethane urea), HFL18-PU and control ultrahigh molecular weight polyethylene (UHMWPE) are given in Table 4.29. Inflammatory cells *viz.* polymorpho nuclear leukocytes, neutrophils, lymphocytes and macrophages were absent after 90 days .The plasma cells were also absent. The optical microphotographs (Figure 4.50) of tissues

surrounding the implant HFL18-PU also confirmed the *in vivo* biocompatibility. If the polymer were non-biocompatible there would have been prolonged inflammatory reactions and presence of foreign body giant cells.

Table 4.29 Histopathology results of HFL18-PU after intramuscular implantation in rabbit for 3 months.

Histopathology Parameters	Control (UHMWPE)	Test (HFL18-PU)
Necrosis	0	0
Inflammation		
Neutrophils	0	0
Macrophages	0	0
Lymphocytes	0	0
Plasma cells	0	0
Giant cells	0	0
Eosinophils	0	0
Foreign body debri	0	0
Fibroplasia	1-2+	0
Fibrocytes	2-3+	1+
Fatty infilteration	0	0
Calcification	0	0
Oedema	0	0
Any other	0	0

Scoring: 0 -item absent; 1+ to 3+ - item present mild to high degree

The degree of necrosis, edema and haemorrhage were also absent with the implant. It has been shown that the adherent macrophages and giant cells are responsible for the biodegradation in the form of cracking and surface pitting through the release of bioactive agents (Zhao et al 1991). The absence of foreign body giant cells in the present investigation reveals the absence of degraded polymeric particles. The present analyses also reveal the marginal presence of fibrocytes for optimum tissue reactivity. Calcification of tissues surrounding the candidate implant was absent

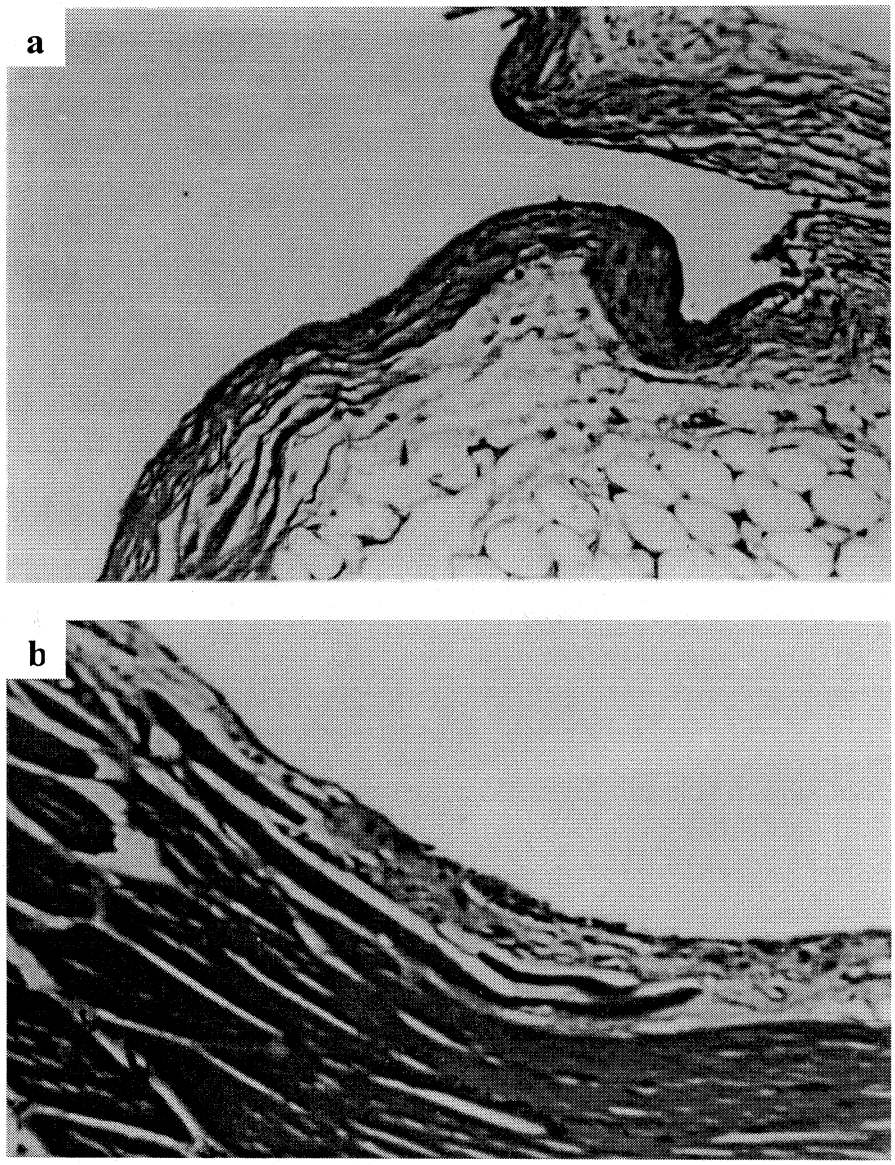


Figure 4.50 Optical microphotograph (150x) showingtissue compatibility (a) UHMWPE (control) (b)HFL18-PU after 90 days implantation in rabbit animal model

There is evidence of repair around the present implant site, with the formation of a thin collagenous capsule <5 to 5 μm thicknesses. There is no evidence of chronic inflammation in any of the sample.The analyses also reveal that the present candidate poly(urethane urea), HFL18-PU is better than or equal to the biocompatible control polymer (UHMWPE).

4.6. Studies on Long-term Performance and *in vivo* Biodurability

4.6.1 Studies on accelerated flexural fatigue tests on polyurethane.

The flexural endurance of the polymers was evaluated in a cantilever flexural fatigue-testing machine. The data of accelerated flexural fatigue test for poly(urethane urea), HFL18-PU is given in Table 4.30. Poly(ether urethane urea), HFL17-PU was also evaluated for comparison.

Table 4.30. Data on accelerated flexural fatigue test

Load on sample : 3.8432 Kg

Flexural Stress : 1.3975 Kg/ cm^2

Flexing rate : 1425 cycles/ min.

Humidity = 80 ± 2 deg. and Room Temperature = 31 ± 1 ^{0}C

Sl No	Polymer	Flex-life
1	HFL17-PU	351 x 10^6
2	HFL18-PU	721 x 10^6
3	Biomer*	320 x 10^6
4	Teccothane	361 x 10^6
5	Elast-Eon #	500 x 10^6

*Solution grade Biomer (PTMG based aromatic Polyether urethane urea)cast film

Aromatic polyurethane based on polydimethyl siloxane and polyhexamethylene oxide glycol

The human heart valve leaflets is normally associated with a peak pressure load to the equivalent of 1.95 Psi (156 g/cm^2) in normal human adult. In condition associated with arterial or ventricular hypertension, this load may be as much as 6 Psi. In the present investigation the load applied on the sample (fixed as cantilever) is 1397.5g/cm^2. With accelerated flexing rate of 1425 cycles/min. and higher load, the long-term performance is predicted in short-term.

The poly(urethane urea), HFL18-PU survived flexing of 721± 30 million cycles whereas the poly(ether urethane urea), HFL17-PU failed at 351 million cycles of flexing. Considering that the human heart valve leaflets undergo flexing for 40 million cycles per year, the HFL18-PU polymer would survive flexing for 18 years. Biomer® (initial modulus 2.5-5.5 MPa, hardness 75A) has flex life only about 320 million (Philips *et al,* 1980). Mackay *et al* (1996) developed polyurethane valve, which has survived 527 million cycles without failure, equivalent to approximately 13 year's service. Butterfield *et al* (2001) have demonstrated that the trileaflet valve fabricated from Teccothane® polyurethane survived only 360 million cycles. Heart valve made from Elast-Eon® by Aot Tech international has a claim of 500 million cycles only. Till date there is no aliphatic, hydrophobic poly(urethane urea) available for the development of cardiac devices especially blood compatible polymeric heart valve that can sustain biostability and cyclic flexing >700 million cycles.

The excellent performance of high flex life of HFL18-PU polymer is due to the virtual crosslinking in polymeric chain through hydrogen bonding between urea-urea, urea-urethane, and urethane-urethane linkages. The virtual crosslinking through

urea-urea hydrogen bonds enables long range ordering leading to crystalline regions. The Fourier transform infrared spectral analysis has confirmed the formation of hydrogen bonding interactions. The X-ray diffraction analysis also revealed semi crystalline nature of this polymer. Under the repeated flexing condition, the flexing endurance of the polymer is ensured with the hydrogen bonds. Since virtual crosslinking has 1/20 the strength of true covalent crosslink, the virtual crosslinking in the physically-crosslinked-poly(urethane urea)s can undergo breaking and re-forming in a number of times during the repeated cycles of flexing without adverse changes in the properties of the polyurethane. In pure crosslinked-polyurethanes having covalent crosslinking, the material under goes bond breaking leading to the change in physical and mechanical properties. Moreover, the low elastic modulus of the material enable the material to undergo repeated loading and unloading in a biomechanically sensitive environment. The high flex-life of poly (urethane urea), HFL18-PU was achieved with the effect of extensive hydrogen bonding interactions and low elastic modulus.

4.6.2 Studies on Endothelial Cell Response to Poly(urethane urea) HFL18-PU

The promotion of endothelialization is important for blood contacting devices, whether they are heart valves, vascular prosthesis or stents (Hsu *et al,* 2000). The biological layer that formed at the blood-contacting interface is referred to as a neointima or pseudointima. Neointima formation results from the in-growth of cellular material over a bed of fibrin. The formation of a stable, thin neointima is a desirable characteristic for long-term blood contacting implants. The vascular endothelium forms the most thromboresistant surface known (Pasic et al, 1996). If a neointima can develop on the implant surface, with a confluent layer of the

endothelium, it is reasonable to assume that the long-term thromborgenicity of the implant and the risks of thrombus formation associated with this can be significantly reduced.

The studies with endothelial cell cultures using human tissues will be an important adjunct to *in vivo* studies (Kirkpatrick *et al*, 1999; Hsu *et al* 2000). Hsu *et al* (2000) found that porous gelatin-modified polyurethane surface with static contact angle 82 (deg) improved the cell adhesion, spreading and proliferation. The use of suitable growth factor proteins on to the surface of the polymer can enhance the endothelialization still more. Attempts to improve the endothelialization of polyurethanes for blood contacting applications have been reported. Seeding the device surface with endothelial cells prior to implantation (Wachem *et al,* 1990), pre-treating the surfaces with growth factor and proteins (Soldani *et al,* 1991) and coating surface with extracellular matrix components to seeding (Bordenave *et a,l* 1993) are well known.

The endothelial response to the candidate material HFL18-PU was investigated using endothelial cells isolated from human umbilical vein. The material was exposed to the endothelial cells grown in Isocoves's medium enriched with 20 % fetal calf serum and incubated. The cells near the material were observed for any sign of adverse response. No morphological change or lysis of endothelial cells was observed when contacted with the material (Figure 4.51). The morphology of endothelial cells in the test materisl resembles to that (droplet-like) from negative control (cells in culture medium). The studies on cell seeding and culture of endothelial cells on the material reveal optimum growth of endothelial cells (≈30 %) on the candidate material.

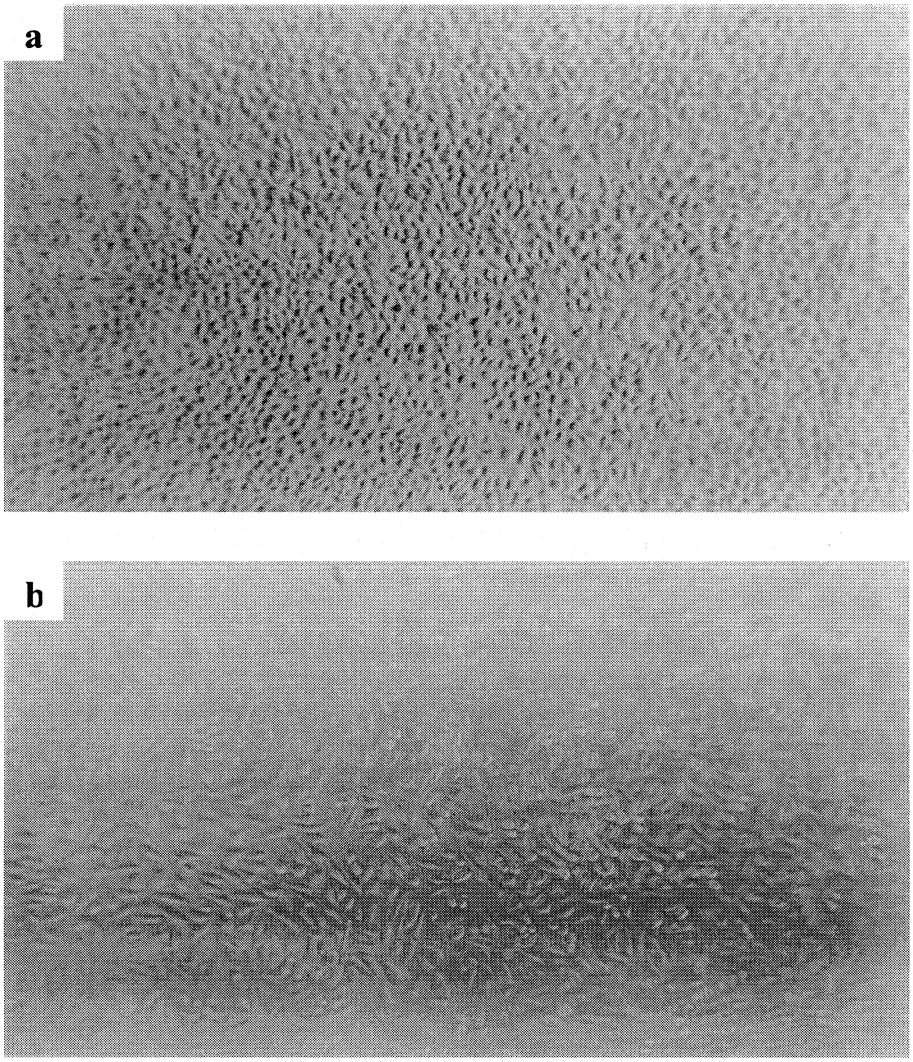


Figure 4.51 Optical microphotograph (100x) showing endothelial cell response of poly(urethane urea)s; (a) Negative control (cells without material) (b) HFL18-PU, candidate poly(urethane urea)

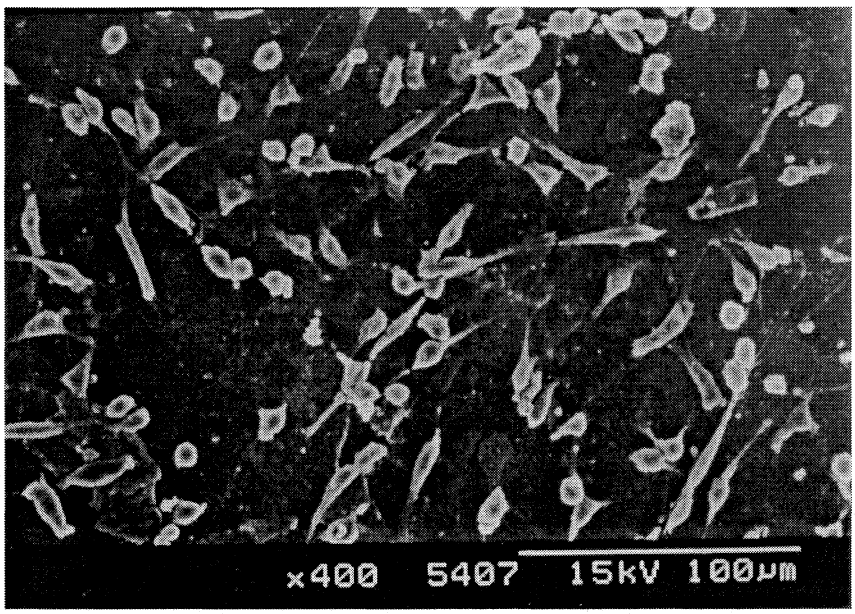


Figure 4 .52 Scanning electron micrograph (SEM) showing endothelial cell growth on surface of candidate polyurethane urea HFL18-PU due to endothelial cell seeding in 48 h

The scanning electron microscopic picture given in Figure 4.52 clearly shows the growth of endothelial cells. Although the surface contact angle of HFL18-PU shows hydrophobicity it initiates cell attachment and spreading as reported by Hsu et al (2000). The studies on the endothelial response and seeding indicated that the present poly(urethane urea)HFL18-PU has the potential to be used as a material for heart valve.

4.6.3. Studies on Long- Term biodurability of HFL18-PU in rat model

The studies on *in vivo* biodurability of the candidate poly(urethane urea), HFL18-PU reveal long-term stability in experimental animals without

biodegradation. The candidate poly(urethane urea) HFL18-PU was found to be compatible with tissue without eliciting any adverse tissue reaction. All the animals were in good health condition throughout the experimental period. The consumption of feed and water were normal during the observation period. When the animals were sacrificed at the end of 24 weeks, none of implantation site showed any macroscopic abnormalities such as haemorrhage, necrosis, discolouration and infection. The harvested samples showed no sign of colour change. There is no change in the weight of the sample after the 6 months of implantation. The properties of explanted (polyurethane urea), HFL18-PU are given in Table 4. 31

Table 4.31 Properties of polyurethane-urea HFL18-PU after implantation (6 months) in rat animal model

Sl. No.	Properties	Value
1.	Weight loss	0
2.	Colour change	Nil
3.	Surface pitting	Nil
4.	Ultimate tensile strength	16.43 ± 0.8 MPa
5.	Ultimate tensile strain	378 .12 ±38 %
6.	Elastic modulus	7.875 ± 0.25 MPa
7.	Water contact angle	77.2 ± 3 deg.
8.	Shore hardness	82.22 A

The mechanical properties of the harvested sample have revealed only marginal increase of ultimate tensile strength, ultimate tensile strain and elastic modulus. However the elastic modulus of the harvested sample lies within the required range of elastic modulus (5-8 MPa) intended for the polyurethane heart

valves. The hardness of the harvested sample is also retained without significant change from that of the virgin sample. The water contact angle of the harvested sample revealed that the hydrophobicity of the material retained event after 6 months implantation. The retention of hydrophobic nature and the lack of weight change in the implanted samples showed that there is no water uptake and lipid absorption by the poly(urethane urea) *in vivo*.

Earlier investigators have studied the chemical changes in implanted unstrained sheets of polyurethanes Pellethane 2363-80A®, Tecoflex EG80A® and Biomer® (McCarthy et al 1997). McCarthy et al (1997) reported the chemical changes associated with localized oxidation of the soft segment and hydrolysis of the urethane bonds joining the soft and hard segments. Tecoflex EG80A® was found to be susceptible to localized hydrolysis of the urethane bond within the aliphatic hard segment while Biomer® showed evidence of a significant non-specific bulk degradation in the implanted materials.

The ATR-FTIR studies of the present harvested sample revealed the biostability of the candidate poly(urethane urea), HFL18-PU (Figure 4.53). Explanted samples showed peaks at 3319 cm^{-1} (ν N-H, hydrogen bonded), 2917 and 2846 cm^{-1} (νC-H in CH_2), 1705 cm^{-1} (νC=O, urethane amide I hydrogen bonded), 1631 cm^{-1} (ν C=O, urea amide I hydrogen bonded), 1562 cm^{-1} (νC-N +δ N-H amide II), 1447 cm^{-1}(δ C-H in CH_2), 1248 cm^{-1} (urethane ether), 1206 cm^{-1} (νC-N+δN-H amide III), 1014 cm^{-1} (ν C-C), 965 cm^{-1} (νC-H in trans 1,4-polybutadiene), 910 cm^{-1} (νC-H vinyl polybutadiene) and 777 cm^{-1} (ρ C-H in -CH_2). There is no significant indication for the disappearance of peaks for urethane, urea and unsaturated double bonds of polyol

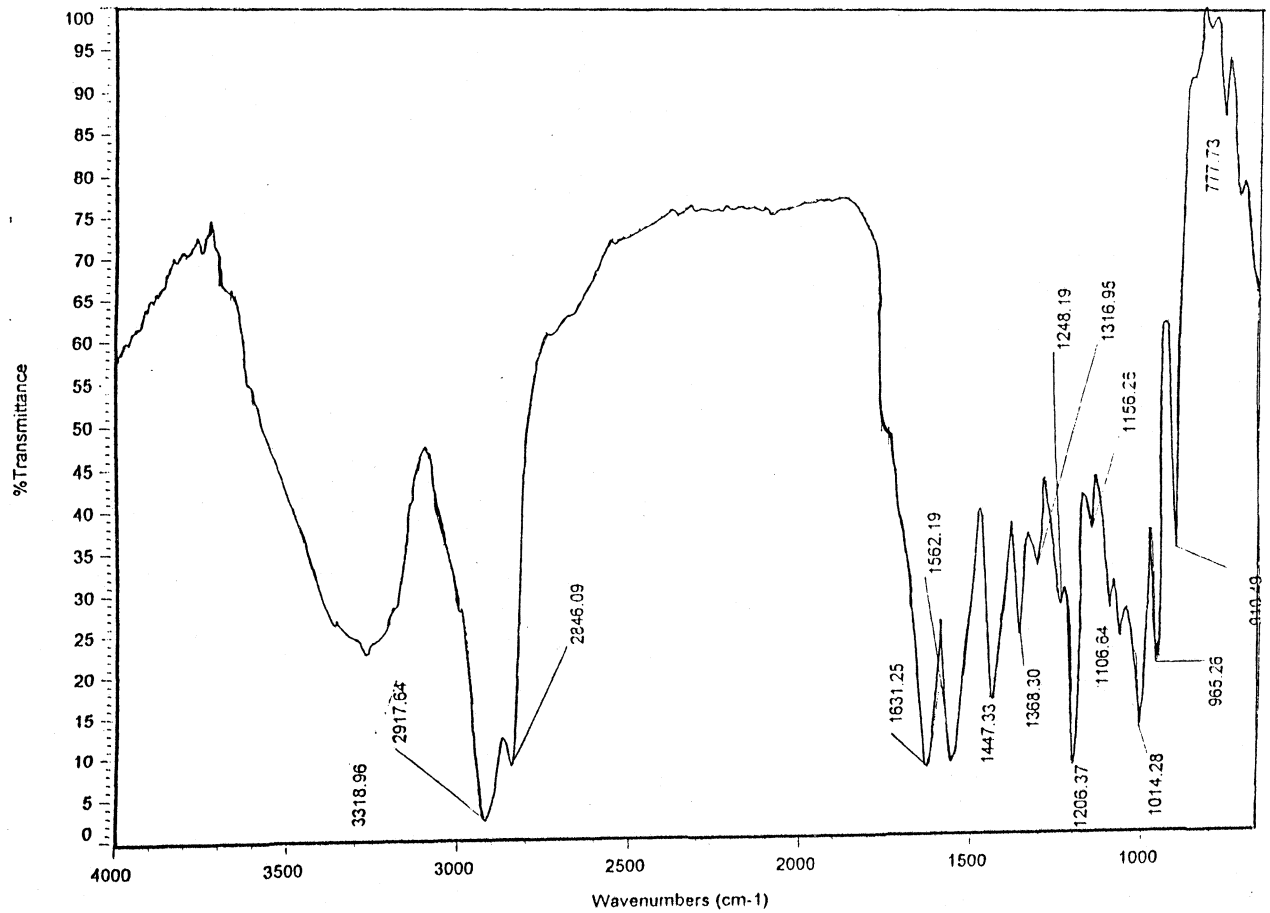


Figure 4.53 Infrared spectrum of candidate poly(urethane urea) HFL18-PU after 6 months subcutaneous implantation in rat

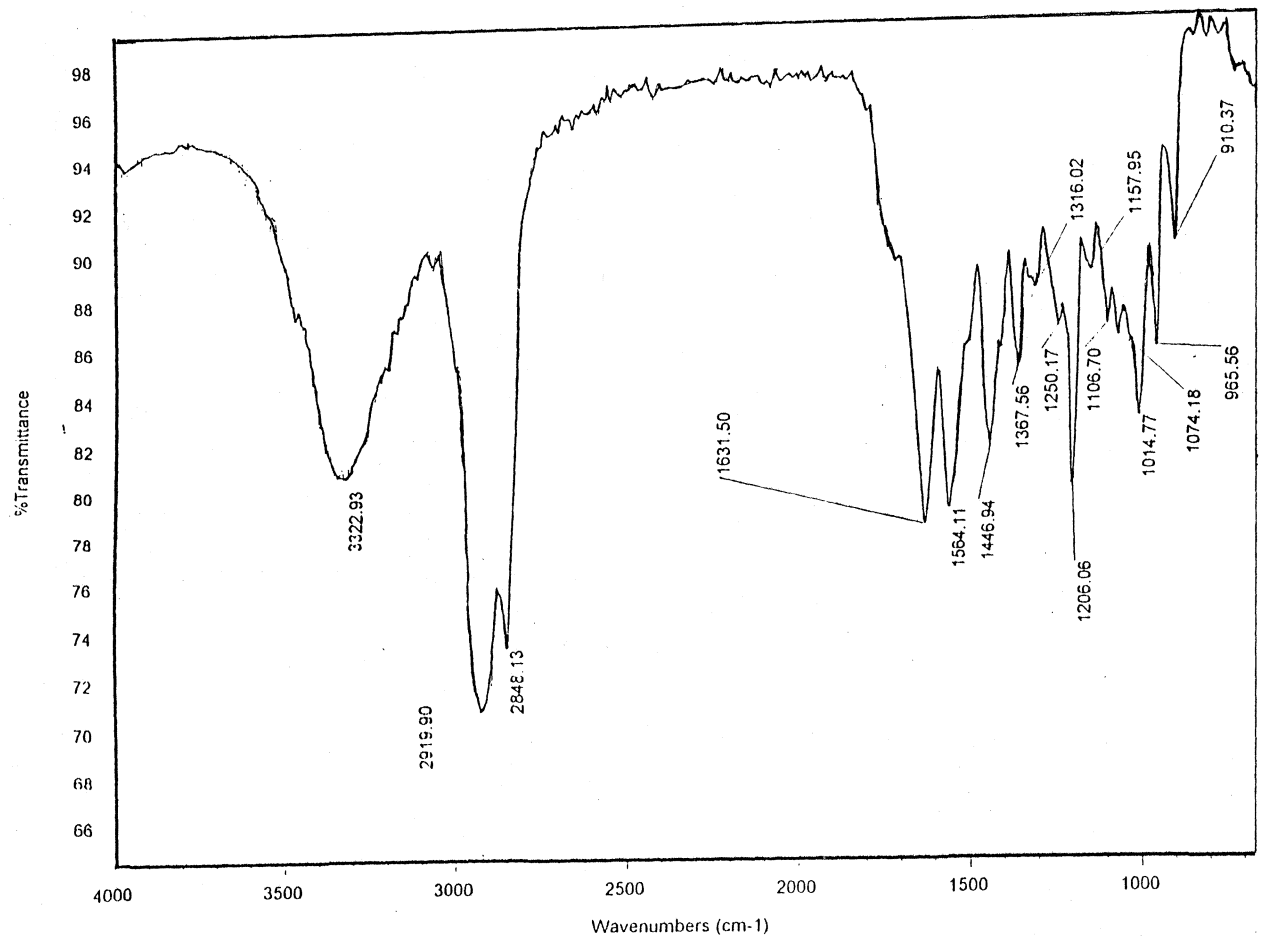


Figure 4.54 Infrared spectrum of candidate poly(urethane urea) HFL18-PU before 6 months subcutaneous implantation in rat

soft segment in comparison to the non-implanted control sample (Figure 4.54). There is no change in the spectral response for the hydrogen bonding interactions. All the peaks for urethane-urethane, urethane-urea and urea-urea hydrogen bonding interactions remain intact. There is no new peak corresponding to the formation of primary amine or carboxylic acid groups for fragmentation. The biostability of urea, urethane and unsaturated C=C double bonds can be attributed to higher virtual crosslinking that resulted from the extensive hydrogen bonding interactions and also to greater microphase separated structure. The ability of the poly(urethane urea) to form hard segment domains may contribute to the formation of a protective structure for the hydrolysable hard segment linkages located within the micro-domains. Since mechanical properties did not change appreciably it could be inferred that the flexing endurance resulted form the low elasticity and virtual crosslinking would not be lost in implanted material. Therefore, the material can perform biomechanically for a long-term.

The scanning electron microscopic (SEM) pictures of explanted HFL18-PU (after implantation) and virgin control (before implantation) are given in Figure 4.55. The SEM pictures reveal the absence of surface degradation either in the form of surface pitting or microcracks due to auto-oxidation or ESC as reported in the case of polyether and poly(ester urethane)s (Pinchuk 1994). Auto-oxidation and the resultant surface cracking appear to result from the foreign body response associated with biomaterials *in vivo* (Pinchuk, 1994, Stokes *et al,* 1995,Anderson 1988, Zhao *et al* 1991).

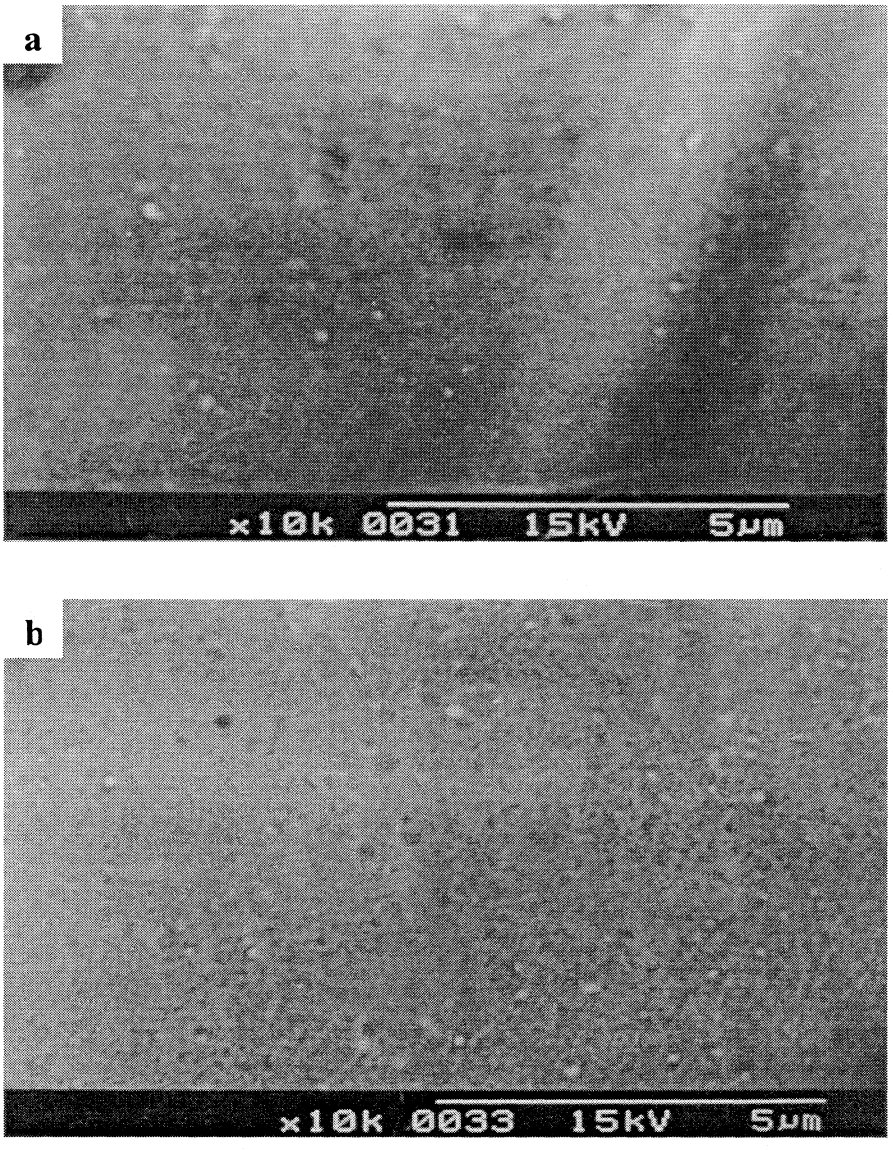


Figure 4.55 Scanning electron micrograph of candidate poly(urethane urea) HFL18-PU after 180 days subcutaneous implantation in rat animal model showing its biodurability (a) Virgin polymer (control) (B) After implantation.

Wang *et al* (2000) have reported the formation of micro-cracks in poly(ester urethane) samples implanted in rat for the period of 8 weeks. Wu *et al* (1999) have also reported the formation of pitting-corrosion in the poly(ether urethane urea) (Biolon ®) blood sacs implanted in calves after the period of 17 weeks.

In the present poly(urethane urea), there was no reduction in tensile properties or any surface deterioration even after 24 weeks of *in vivo* implantation. Christ *et al* (1992) demonstrated that aliphatic polyurethanes experience severe cracking after short (as little as 30 days) subcutaneous implantation in rabbits. Hergenrother et al (1993) compared H_{12}MDI based aliphatic polyurethane with aromatic MDI based polyurethanes and reported significant cracking in aliphatic polyurethane materials after 4 weeks (Hergenrother et al 1993). Contrary to these reports the present aliphatic poly(urethane urea) having virtual crosslinked structure through three-dimensional hydrogen bonding interactions revealed high biostability even after 6 months subcutaneous implantation in rat. The analyses of the harvested samples clearly establish that the candidate poly(urethane urea) HFL18-PU is excellently tissue compatible, biofunctional for long-term and also biodurable.

The objective of the present investigation is to develop high flex-life polyurethane for use in cardiovascular devices such as heart valve and membrane of left ventricular assist device. The required characteristics for a polymer for use in these devices are hydrophobicity, low elastic modulus (5-8 MPa), linear polyurethane with virtually crosslinked polymeric chain, aging stability in physiological environment, aging stability under stressed and strained conditions, long-term flexing endurance and biocompatibility. With these criteria, candidate polymeric group is

selected from a wide spectrum of polymers consisting polyurethane, poly (ether urethane urea), and poly (urethane urea). Poly (urethane urea) is selected as more promising than other two classes of polymers. Out of four poly(urethane urea)s further selection is done based on the aging stability in different conditions and mechanical property. Based on these studies, HFL18-PU is selected as candidate material since the elastic modulus of aged polymer is retained within the limit of 5-8 MPa. The flex-life of this polymer is very high to satisfy the long-term requirement for polymeric heart valve. The biocompatibility and biostabilty of HFL18-PU polymer also equally satisfies the requirement. The salient features of candidate high flex-life poly(urethane urea), HFL18-PU are given in Table 4.32 and Table 4.33 . Therefore biodurable poly(urethane urea) HFL18-PU is selected as the candidate polymer for the fabrication of cardiovascular device like polyurethane tri-leaflet heart valve (having identical anatomic and fluid dynamic characteristics).

Table 4.32 Biostability data of HFL18-PU poly(urethane urea)

Properties	Virgin polymer	Aged polymer under *in vitro* physiological media						Aged polymer *in vivo*
		PBS	Ringers solution	Oxidation medium	Lipid DMEM	Papain enzyme	enzyme buffer	
Weight change(%)	-	0	0	0	+0.89±0.03	0	0	0
Colour change	-	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Tensile strength (MPa)	15.75±0.6	16.15±0.18	16.56±2.1	16.2±1.01	12.02±1.25	13.94±0.40	13.75±0.88	16.43±0.8
Elongation(%)	337±15	368±13	332±16	334.8±13	254±29	231.5±32	242±41	378.12±38
Elastic modulus (MPa)	6.841±0.27	7.812±0.31	8.053±0.2	7.574±0.1	6.335±0.27	7.258±0.08	7.039±0.40	7.575±0.25
Hardness (Shore A)	80±2	92±2	93±1	92±2	77±4	78±4	82±2	82.2±2
Water contact angle (deg)	85.4±1.5	72±3	77±3	80±2	79±2	75±4	81±1	77.2±3

Table 4.33 Biological performance data of HFL18-PU polyurethane urea

Test assay	HFL18-PU	Control
Haemolysis *in vitro*	0.47 %	0.34% (Tecoflex 85 A)
Cell viability (MTT assay)	102% (with 100%extract)	100% (culture medium)
Blood-material interaction		
Platelet retention	97%	85 % (Tecoflex 85 A)
WBC retention	87%	83% (Tecoflex 85 A)
Bio calcification *in vitro*		
Calcium deposition	0.1646±0.02 mg/g	0.2382±0.10 mg/g (Tecoflex 85 A)
Phosphorous deposition	0.0487±0.01 mg/g	0.0546±0.01 mg/g (Tecoflex 85 A)
Systemic toxicity *in vivo*		
With NaCl extract	Nil	Nil (extraction medium)
With Cotton seed oil extract	Nil	Nil (extraction medium)
Intracutaneous irritation *in vivo*		
With NaCl extract	0.00	0.00 (extraction medium)
With Cotton seed oil extract	0.04	0.00 (extraction medium)
In vivo intramuscular implantation and histology	passed	Passed (UHMWPE)

CHAPTER V

SUMMARY, CONCLUSION AND FUTURE PROSPECTS

CHAPTER V

SUMMARY, CONCLUSION AND FUTURE PROSPECTS

5.1 Summary

Development of a new generation high flex life polyurethane with required elastic modulus, biocompatibility, blood compatibility, resistantance to calcification, and biodurability for the long term use as cardiac devices was our primary objective of this investigation

To achieve the objective we have developed a set of four virtually crosslinked poly(urethane urea) elastomers (HFL9-PU, HFL13-PU, HFL18-PU and HFL15-PU) using aliphatic organic compounds comprising a cycloaliphatic diisocyanate, an aliphatic unsaturated hydrocarbon polyol, and aliphatic diamine. The present virtually crosslinked polymers are unique; because, so far no virtually crosslinked poly(urethane urea) has been developed using a cycloaliphatic diisocyanate, dicyclohexyl methane diisocyanate (H_{12}MDI), and aliphatic unsaturated hydrocarbon polyol, hydroxy terminated polybutadiene (HTPBD) so far. Only poly(urethane urea)s made from aromatic diisocyanate, MDI and a polyether polyol or hydroxy terminated polybutadiene are reported so far. The new generation and virtually crosslinked poly(urethane urea)s were composed of soft segments of aliphatic

unsaturated hydrocarbon polyol, HTPBD and hard segments formed with $H_{12}MDI$ and aliphatic hexamethylene diamine (HDA) units. Two poly(ether urethane urea)s (HFL16-PU and HFL17-PU) and two polyurethanes (HFL1-PU and HFL3-PU) were also prepared to compare the properties of present poly(urethane urea)s. The poly(ether urethane urea)s contain mixture of poly tetramethylene oxide polyol (PTMG) and hydrocarbun polyol, HTPBD as soft segments and hard segment formed with $H_{12}MDI$ and aliphatic diamine, HDA. The polyurethanes (HFL1-PU and HFL3-PU) contain HTPBD as soft segment and hard segment formed with $H_{12}MDI$ and butane diol (BD) The present virtually crosslinked poly(urethane urea)s contains more urea linkages than urethane linkages due to the higher percentage of hard segment content.

We have designed the virtually crosslinked poly(urethane urea)s (HFL9-PU, HFL13-PU, HFL18-PU and HFL15-PU) by introducing hydrogen bonding interaction between urea and urea groups extensively. Infrared spectral analysis (ATR-FTIR) revealed hydrogen bonding interactions in the present virtually crosslinked poly(urethane urea) as evident from the peaks for the hydrogen bonded N-H (at around 3329 cm^{-1}), highly ordered urea C=O (at around 1632 cm^{-1}) and bonded urethane C=O (at 1702 cm^{-1}) groups. Such interaction produced a three-dimensional network of hydrogen bonds that favours short and long range ordering leading to the formation of micro crystallites in hard domains. We have demonstrated the presence of micro crystallite formation in hard segments through extensive hydrogen bonding interactions. The wide-angle X-ray diffraction analyses (WAXD) of poly(urethane urea) supports the formation of micro crystallites through virtual cross-linking. The more intense peak at 19^0 of 2θ scale corresponding to 4-5 A^0 d-

spacing is due to the presence of crystallites in poly(urethane urea) by short range ordering in hard segment domains due to hydrogen bonding interactions. In spite of the isomeric forms of the cycloaliphatic diisocyanate, H_{12}MDI used in the development of present poly(urethane urea), micro crystallite formation in hard segments through extensive hydrogen bonding interactions has been observed which were hitherto observed only in aromatic poly(urethane urea)s. In the present poly(urethane urea)s, the formation of microcrystallites increases with increase of virtual crosslink density. The FT-IR and WAXD analyses of poly(ether urethane urea) reveal mixed hydrogen bonding interaction between leading to moderately phase-mixed structure with lesser degree of microcrystallies in hard segment domain.

We have also demonstrated that virtual crosslinking in the present poly(urethane urea) elastomers (HFL9-PU, HFL13-PU, HFL18-PU and HFL15-PU) makes it insoluble in organic solvents (thermoset-like character). The swelling studies reveal that the present virtually crosslinked poly(urethane urea)s undergo only swelling which is attributed to virtual crosslinking. The data of crosslink density and the number average molecular weight between two crosslinks also supports the presence of virtual crosslinking. The virtual crosslink density in the poly(urethane urea)s increases with increase in the chain extender content (diamine %). The low value of molecular weight between two crosslinks showed the extensive physical crosslinking through hydrogen bonding. This virtual crosslinking enable polymers to behave like crosslinked polymers. This type of three-dimensional virtual cross-linking in hard domains and microphase separation influences the properties *in vitro* and *in vivo* significantly. The present poly(ether urethane urea)s HFL16-PU and

HFL17-PU, are also found to be virtually crosslinked though there is some kind of phase-mixing due to ether-urea and ether- urethane linkages.

We have investigated the mechanical and surface properties of the virtually crosslinked poly(urethane urea) elastomers (HFL9-PU, HFL13-PU, HFL18-PU and HFL15-PU). The data on mechanical properties revealed low elastic modulus with low hardness. Polyurethanes used as vital components in biomechanically sensitive blood contact devices such as flexible leaflet of artificial heart valve should have low elastic modulus which allow repeated cyclic flexing in biological conditions. Moreover, low elastic modulus with reduced bending stress allows the polyurethane membrane to flex more freely without producing adverse changes in blood flow. Therefore the elastic modulus of the present poly(urethane urea) lies in the required range of 5 -8 MPa. The candidate poly(urethane urea) HFL18-PU possesses 6.814 MPa elastic modulus. Water contact angle, a measure of wettability of the polymer surface, indicated that the surface of the present poly(urethane urea)s and poly(ether urethane urea)s are hydrophobic in nature. The hydophobicity is mainly due to the presence of aliphatic unsaturated hydrocarbon polyol soft segment on the air-material interface. The hydrophobicity is a favourable factor for blood contact applications of polymers as it can enhance albumin adsorption and reduce platelet adhesion.

Studies on *in vitro* aging of polymers in physiological media demonstrated that the extensive virtual crosslinking available in the present poly(urethane urea)s and poly(ether urethane urea)s protect the polymer against degradation in hydrolysis, oxidation, enzyme and lipid media. The *in vitro* hydrolytic stability of poly(urethane urea)s was investigated by aging the polymer in Ringer's solution, phosphate buffered saline (PBS) and papain enzyme. No weight loss was observed in any of the

poly(urethane urea)s and poly(ether urethane urea)s after the aging in any of these media. The marginal changes in tensile properties were noticed. The marginal changes noticed in tensile properties are due to the changes in the degree of hydrogen bonding and associated rearrangement of molecular structure. The ATR-FTIR spectra of the aged samples in Ringer's solution and PBS did not show any evidence of degradation; the peaks for urethane and urea groups are intact. The studies revealed that virtual cross-linking in the present poly(urethane urea)s protects the urethane and urea linkages from the hydrolytic degradation. However, the present polyurethanes, HFL1-PU and HFL3-PU, underwent weight loss and drastic change in mechanical and surface properties.

In papain enzyme medium also, the poly(urethane urea)s and poly(ether urethane urea)s were found to be intact as there is no weight loss or surface changes of the aged polymer. There was no significant change in properties of the aged polymer in enzyme and buffer (control). We have observed marginal increase of mechanical properties in most of poly(urethane urea)s in both control and enzyme media. The marginal increase of mechanical properties is due to physical aging as per Struik's law. Though the poly(urethane urea)s contained more urea site specific to the attack of papain enzyme (a thiol endo peptidase), the enzyme had no degradative interaction with the surface as evident from FT-IR spectra. However, surface contact angle of the aged polymer in hydrolytic media decreased slightly. This indicated the mild reorganization on the surface of the material without any adverse effect on bulk properties.

Studies on the *in vitro* aging of polymers in oxidative medium (0.1 M silver nitrate and 0.1 M sodium lactate) demonstrated that the extensive virtual crosslinking

in all poly(urethane urea)s and poly(ether urethane urea)s also protects the unsaturated double bond of hydroxy terminated poly butadiene soft segment against degradation. The aged samples of the present poly(urethane urea) and poly(ether urethane urea)s reveal no weight loss in the oxidation medium. The IR spectral analyses of the samples aged in the oxidation medium reveal no disappearance of peak or change in intensity of peak centered at 964 cm^{-1} and 910 cm^{-1} characteristic peak for unsaturated polybutadiene segment of the present polymers. Moreover, the ether peak at around 1105 cm^{-1} of poly(ether urethane urea)s also found to be intact. Similarly we have also demonstrated that all the new poly(urethane urea)s were found to stable in lipid medium though they are hydrophobic in nature. All the poly(urethane urea)s aged in Dulbecco's modified eagle medium containing cholesterol (serum level), showed marginal change in tensile properties, which is attributed to the platicizing effect of the absorbed lipid (<1 % lipid absorption). In lipid rich medium, palm oil (100% lipid), the uptake of lipid by the present poly(urethane urea)s decreases with increase of virtual crosslink density or diamine content.

Accelerated chemical degradation carried out in boiling water for 100 h and boiling alcoholic potassium hydroxide (0.5M) for 4 h, revealed weight loss and dimensional changes in polyurethanes, HFL1-PU and HFL3-PU and also in poly(ether urethane urea)s, HFL16-PU and HFL17-PU. However, no weight loss or dimensional change was observed with any of the poly(urethane urea)s, HFL9-PU, HFL13-PU, HFL18-PU and HFL15-PU. The accelerated test in harsh and aggressive chemical conditions clearly indicates that the present poly(urethane urea)s would excellently be stable in physiological condition.

The poly(urethane urea)s and poly(ether urethane urea)s aged under environmental stress corrosion environment in Ringers Solution and PBS media do not undergo any weight loss or ragged fracture in any of the polymers. However, dimensional changes such as warping or hardening were observed with poly(ether urethane urea)s. These dimensional changes are due to the constantly applied bending stress. Poly(urethane urea) samples especially HFL18-PU and HFL15-PU remain intact without any visible change in dimension, flexibility and weight. There was no crack formation or whitening in any of the poly(urethane urea) samples. The resistance to environmental stress-corrosion of poly(urethane urea)s is attributed to the microphase-separated three-dimensional networks present in the poly(urethane urea)s.

The *in vitro* studies on the aging under induced-strain (20% in tension mode) in hydrolytic enzyme medium reveal increase of elastic modulus significantly in both poly(ether urethane urea)s and poly(urethane urea)s. This is due to the unidirectional reorganisation of polymer chains in a constantly applied stress condition . However, under repeated flexing environment of actual clinical situation, the possibility of formation of unidirectional reorganisation of polymer chains is negligible. Moreover the increase in elastic modulus observed could be reversed under a continuously flexing environment as hydrogen bonds can form and break repeatedly.

The propensity for calcification of poly(urethane urea)s and poly(ether urethane urea)s was evaluated *in vitro* using metastable calcium phosphate solution. The effect of virtual crosslink density on the biomaterial-associated calcification was studied. In poly(urethane urea)s, the hard-segment-mediated calcification increases with increase of virtual crosslink density, and then decreases with increase of virtual

crosslink density. In poly(ether urethane urea)s, the combined mechanism of hard-segment-mediated calcification and PTMG-mediated calcification by crown-ether complexation is responsible for higher calcification compared to poly(urethane urea)s. The calcium deposition in poly(urethane urea), HFL18-PU containing urea linkages was found to be less when compared to that of commercial polyurethane Tecoflex 85 A® containing urethane linkages in hard segment. Therefore it is inferred that the present poly(urethane urea)s, HFL18-PU and HFL15-PU are not prone to calcification.

In vitro and *in vivo* studies on the biocompatibility of the present poly(urethane urea)s, HFL9-PU, HFL13-PU, HFL18-PU and HFL15-PU and poly(ether urethane urea)s, HFL16-PU and HFL17-PU were carried out in order to establish the compatibility of the present polymeric materials with living system. The haemolysis and blood-material interaction (haematology) revealed the blood compatibility of the present poly(urethane urea)s and poly(ether urethane urea)s. The percentage of haemolysis lies within the permissible limit (5%). Studies on blood-materials interaction with poly(urethane urea)s revealed an increase of platelet retention and a decreases of WBC retention with increase of virtual crosslink density. A reverse trend was observed for poly(ether urethane urea)s. The studies on *in vitro* blood - material interaction revealed 97 % platelet retention for poly(urethane urea) HFL18-PU after incubation in whole blood for 60 min when compared to the control blood (88 %)and commercial polyurethane Tecoflex 85A (85%) . There is no reduction in WBC, RBC count when compared to the control blood and commercial polyurethane Tecoflex 85A ®. Therefore poly(urethane urea) HFL18-PU is antithrombogenic, essential property for the heart valve.

Cytotoxicty and cell viability assays reveal that all the present polymers are compatible with fibroblast cells irrespective of the value of virtual crosslink density. The present polymers are non-cytotoxic as there was lack of cell lysis. The normal cellular morphology of fibroblast cells was preserved in comparison to positive control (cytotoxic material). MTT assay reveals very good cell viability with the extract of the polymers. *In vitro* haemolysis and cytocompatibility results for the present poly(urethane urea) especially HFL18-PU were comparable to or better than the commercial polyurethane Tecoflex 85A®. These studies indicated that the present polymers do not release any harmful leachant that can induce any adverse responses in body system.

Based on the physicochemical and mechanical properties, *in vitro* aging stability, biomechanical stability, stability against environmental stress corrosion, stability against harsh chemical degradation, resistance to calcification and *in vitro* cytocompatibilty, the candidate poly(urethane urea) (HFL18-PU) was selected for the evaluation of *in vivo* biocompatibility, *in vivo* biostabiltity and functional performance.

The biocompatibility of the candidate poly(urethane urea) (HFL18-PU) was further confirmed by *in vivo* toxicological tests as per international standards. Intracutaneous irritation test in rabbit animal model revealed that the material HFL18-PU possesses no leachant that can cause allergic responses *in vivo*. Systemic toxicity test in mice animal model confirmed this fact. There were no symptoms for oedema or erythema. *In vivo* intramuscular implantation of the material in rabbit model followed by histopathological analyses of the tissues surrounding the implant

confirmed the *in vivo* biocompatibility of the candidate poly(urethane urea) (HFL18-PU).

The functional performance of the candidate poly(urethane urea), HFL18-PU intended for the application in heart valve or LVAD was also evaluated. The new generation candidate poly(urethane urea) HFL18-PU is compatible to endothelial cells also. The studies on *in vitro* endothelial cell culture assay reveal normal cell growth in the presence of the extract of the candidate poly(urethane urea) without any cell lysis. Scanning electron microscopic (SEM) analysis revealed the spreading and growth of cells on the solid substrate of the candidate poly(urethane urea) due to cell proliferation

The studies on flex-life have demonstrated that the present virtually crosslinked poly(urethane urea) has satisfactory flex-life. The candidate poly(urethane urea) HFL18-PU survived flexing for a total 721 ± 30 million cycles in air before failure. This flex life is equivalent to approximate 18 years by taking the flexing of heart valve as 40 million cycles per year. The poly(urethane urea) HFL18-PU is superior to commercial polyurethane Elast-Eon that has flex life of 500 million cycles. We have proposed that the high flex life this material is attributed to the three dimensional virtual crosslinking through urea-urea hydrogen bonds.

We have demonstrated that the candidate poly(urethane urea), HFL18-PU is extremely stable *in vivo* for long-term. The long-term biostability of the material was evaluated with subcutaneous implantation in rat animal model for 180 days. The explanted material has shown no visible colour change and weight change due to degradation or lipid uptake. There is no appreciable change in mechanical properties (table 4). It has showed only marginal increase in ultimate tensile strength (4.32%),

ultimate elongation (12.23%). However, the elastic modulus (7.575 MPa) of explanted HFL18-PU lies within the required range of modulus (5-8 MPa) intended for polyurethane heart valve. The hardness of the harvested sample retained without significant change from that of virgin sample. The water contact angle values of explanted material revealed the retention of hydrophobic character. The degree of changes (10%) of mechanical properties observed with the explanted (implanted *in vivo*) sample are found to be similar to that of the sample aged in simulated physiological fluids (*in vitro* hydrolytic, oxidative and enzymatic). The biostability of the candidate material was ascertained by the SEM analysis of the explanted material. The SEM photomicrograph of the explanted candidate poly(urethane urea) presents no indication for surface pitting or microcracks due to biodegradation even after 24 weeks in rat model. Therefore we have proposed that the candidate poly(urethane urea) does not undergo any type of degradation, but undergoes only rearrangement of thermodynamically incompatible soft and hard segments leading to changes in hydrogen bonding interaction. The reorganization of hard segment occurs in such away as to form a long range ordering of hard segments, which enables the slight increase in tensile properties. The biodurability has been confirmed by the IR spectral analysis which clearly indicated the presence of peak at 965 cm^{-1} ,910 cm^{-1} and at 777 cm^{-1} for the unsaturated double bond of soft segment in explanted material Therefore, the possibility of crosslinking at the C=C bond of the aliphatic hydrocarbon polyol soft segment and the increase of mechanical properties as reported by Takahara *et al* (1991) for the polybutadiene diol based aromatic poly(urethane urea)s is not observed in the present candidate aliphatic poly(urethane urea). We have further proposed that the hard segment micro crystallite domain would protect the unsaturated double bond

in the candidate aliphatic poly(urethane urea)s through dynamic realignment mechanism.

5.2 Conclusion

In conclusion, the present investigation reveals the presence of micro crystallite formation in candidate poly(urethane urea) HFL18-PU in hard segments in spite of the isomeric forms of a cycloaliphatic diisocyanate due to extensive hydrogen bonding interactions, which were hitherto observed only in aromatic poly(urethane urea)s. No reports on the formation of microcrystallites in aliphatic poly(urethane urea) have appeared so far. The extensive hydrogen bonding between urea-urea groups in the present poly(urethane urea) impart virtual cross-linking leading to three-dimensional physical network in hard domain. The three-dimensional physical cross-linking of the candidate polymer enables to attain resistance to accelerated chemical degradation, high flex-life and biodurability. The present investigation also reveals that the unsaturated hydrocarbon polyol soft segment in the candidate polymer is excellently stable against oxidative degradation, which was generally observed till now in the currently available polyurethanes containing such polyether polyol or polyhydrocarbon polyols. The stability of candidate polymer in oxidizing environment is attributed to the protection of unsaturated groups by the three dimensional virtually crosslinked structure. The non-aromatic and completely aliphatic candidate polymer HFL18-PU has excellent flexing endurance and *in vivo* biostablity, biocompatibility, blood compatibility and resistance to calcification. The candidate polymer also enables optimum growth of endothelial cells. We report that

the present aliphatic poly(urethane urea) elastomer HFL18-PU is a new generation candidate material for the development of polymeric heart valve.

5.3 Future Prospects

The candidate poly(urethane urea) HFL18-PU is more promising polymer for the development of polyurethane mitral valve and membrane-based LVAD. The major of polyurethane valves so far developed was due to flexural fatigue failure, calcification and thrombosis. The candidate poly(urethane urea) HFL18-PU has excellent characteristics to withstand these events *in vivo*. Therefore the candidate poly(urethane urea) HFL18-PU could be explored for the development of heart valve.

The development of membrane-based LVAD was discontinued owing to the non availability of high flex-life polyurethane. Now with the development of candidate polyurethane urea, the development of membrane-based LVAD could be revived. Since the membrane-based LVAD has better advantages over the rotary pumps in respect to blood flow, the membrane-based LVAD could be a better cardiac-assist device.

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Appendix A Phosphate Buffered Saline (PBS)

Anhydrous Na_2HPO_4	-	28.4 g
$NaH_2PO_4 . H_2O$	-	27.6 g
0.9%saline solution	-	1000 ml

Appendix B Ringer's solution

Sodium chloride	-	9 g
Sodium hydrogen carbonate	-	0.42 g
Calcium chloride	-	0.24 g
Glucose	-	1 g
Distilled water	-	1000 mL

Appendix C HEPES Buffer solution

HEPES	-	23.83 g
Sodium azide	-	0.2 %
Mercaptoethanol	-	4.6878 g
EDTA	-	3.7224g
Distilled water	-	1000 mL

Appendix D

Dulbeccos modified eagle's medium (DMEM)

Calcium Chloride, Anhydrous	-	200 mg
Ferric Nitrate, Nonahydrate	-	0.10 mg
Potassium Chloride	-	400 mg
Magnesium Sulfate, Anhydrous	-	97.67 mg
Sodium Chloride	-	6400 mg
Sodium Phosphate, Monobasic, Monohydrate	-	125 mg
Dextrose	-	4500 mg
Phenol Red, Sodium Salt	-	15.93 mg
L-Arginine HCI	-	84 mg
L-Cystine 2HCI	-	62.57 mg
L-Glutamine	-	584 mg
L-Histidine HCI H_2O	-	42 mg
L-Isoleucine	-	104.8 mg
L-Leucine	-	104.8 mg
L-Lysine HCI	-	146.2 mg
L-Methionine	-	30 mg
L-Phenylalanine	-	66 mg
L-Serine	-	42 mg
L-Threonine	-	95.2 mg
L-Tryptophan	-	16 mg
L-Tyrosine 2Na, Dihydrate	-	103.79 mg
L-Valine	-	93.6 mg
D-Calcium Pantothenate	-	4 mg
Choline Chloride	-	4 mg
Folic Acid	-	4 mg
Myo-Inositol	-	7 mg
Niacinamide	-	4 mg
Pyridoxal HCI	-	4 mg
Riboflavin	-	0.4 mg
Thiamine HCI	-	4 mg
Sodium Bicarbonate	-	3700 mg

Appendix E
List of Abbreviations

ASTM	-	American standards for testing and materials
ATR	-	Attenuated total reflectance
BD	-	1, 4-butane diol
CPD	-	Critical point drying
DBTDL	-	Dibutyl tin dilaurate
DMA	-	Dynamic mechanical analysis
DMEM	-	Dulbecco's modified eagle medium
EDA	-	Ethylene diamine
EDTA	-	Ethylenediamine tetra acetate
ESC	-	Environmental stress cracking
FT-IR	-	Fourier transform infrared spectroscopy
HA	-	Hydroxyapatite
H_{12}MDI	-	4, 4'-methylene bis (p-cyclohexyl isocyanate)
HDA	-	1, 6-hexamethylene diamine
HDI	-	Hexamethylene diisocyanate
HPLC	-	High pressure liquid chromatography
HTPBD	-	Hydroxy terminated polybutadiene
LVAD	-	Left ventricular assist device
MDA	-	Methylene dianiline
MDI	-	4,4'-Methylene bis(p-phenyl isocyanate)
MIO	-	Metal induced oxidation
OD	-	Optical density
PBD	-	Polybutadiene
PPG	-	polypropylene glycol
PBS	-	Phosphate buffered saline
PDMS	-	Polydimethyl siloxane
PEG	-	Polyethylene glycol
PEO	-	Polyethyle oxide
PHMO	-	Polyhexamethylene oxide diol
PMMA	-	Polymethyl methacrylate
PPG	-	Polypropylene glycol
PPO	-	Polypropyle oxide
PTMG	-	Polytetramethylene glycol
PTMO	-	Polytetra methylene oxide diol
RBC	-	Red blood corpuscles
SEM	-	Scanning electron microscopy
TDA		Toluene dianiline
TDI	-	Toluene diisocyanate
TGA	-	Thermogravimetric analysis
THF	-	Tetrahydrofuran
TMP	-	Trimethylol propane
UHMWPE	-	Ultra high molecular weight polyethylene
USP	-	United states pharmacopoeia
WAXD	-	Wide angle X-ray diffraction

Appendix F

List of publications

1. Synthesis of hydrolytically stable low elastic modulus polyurethane-urea for biomedical applications, Jayabalan M., Lizymol P.P., Vinoy Thomas, *Polymer Int.*, **49**, 88 -92, 2000.

2. Studies on the effect of virtual cross-linking on the hydrolytic stability of aliphatic poly(urethane urea) for blood contact applications, Vinoy Thomas , Jayabalan M., *J. Biomed. Mater. Res.*, **56**, 144-157, 2001.

3. *In vitro* studies on the effect of physical crosslinking on the biological performance of aliphatic poly (urethane urea) for blood contact applications, Vinoy Thomas, Kumari T.V., Jayabalan M., *Biomacromolecules*, **2**, 588-596, 2001.

4. The effect of virtual cross-linking on the oxidative stability and lipid uptake of aliphatic poly (urethane urea), Vinoy Thomas, Jayabalan M., ***Biomaterials*** **, 23**, 273-282, 2001.

5. Synthesis and evaluation of aliphatic low elastic modulus polyurethane-urea for biomedical applications, Jayabalan M., Lizymol P.P., Vinoy Thomas, *Macromolecules - New Frontiers*, **2**, 607, 1998.

6. Studies on oxidative stability of poly(urethane urea) for blood contacting applications, Vinoy Thomas, Jayabalan M., *Biomedical Materials-New Frontiers, p* 15, 1998.

7. Studies on Biomechanical characteristics of polyurethane-urea for cardiac assist devices, Vinoy Thomas, Jayabalan M., *Biomedical Materials-New Frontiers,* p 23, 1998.

8. New generation high flex life aliphatic poly(urethane urea) for fabrication of polymeric tri-leaflet heart valve, Jayabalan M., Vinoy Thomas, (communicated).

9. Synergistic effect of mechanical strain and hydrolytic enzyme on the biodegradation of physically crosslinked poly(urethane urea)s due to aging , Vinoy Thomas, Jayabalan M., (communicated).

10. Dynamic mechanical and Thermal analyses of virtually crossliked aliphatic polyurethane urea based on hydroxy-terminated polybutadiene. Vinoy Thomas, Bhagavan S. S., Jayabalan M., (communicated).

Patent

Virtually-Crosslinked High Flex-Life Aliphatic Polyurethane-Urea Material for the Development of Tri-leaflet Polymeric Heart Valve, Indian Patent (Applied).